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(54) **Titre : PROCEDES DE FABRICATION DE CELLULES EXPRIMANT UN RECEPTEUR ANTIGENIQUE CHIMERIQUE**
(54) **Title: METHODS OF MAKING CHIMERIC ANTIGEN RECEPTOR-EXPRESSING CELLS**

(57) **Abrégé/Abstract:**
The invention provides methods of making immune effector cells (for example, T cells, NK cells) that express a chimeric antigen receptor (CAR), and compositions generated by such methods.

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# METHODS OF MAKING CHIMERIC ANTIGEN RECEPTOR–EXPRESSING CELLS

## RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application 63/235,634 filed on August 20, 2021, the entire contents of which are hereby incorporated by reference.

## FIELD OF THE INVENTION

The present invention relates generally to methods of making immune effector cells (for example, T cells or NK cells) engineered to express a Chimeric Antigen Receptor (CAR), and compositions comprising the same.

## SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in XML format compliant with WIPO Standard ST.26 and is hereby incorporated by reference in its entirety. Said XLM copy, created on August 15, 2022, is named N2067-7191WO.XML and is 962.9 kb in size.

## BACKGROUND OF THE INVENTION

Adoptive cell transfer (ACT) therapy with T cells, especially with T cells transduced with Chimeric Antigen Receptors (CARs), has shown promise in several hematologic cancer trials. The manufacture of gene-modified T cells is currently a complex process. There exists a need for methods and processes to improve production of the CAR-expressing cell therapy product, enhance product quality, and maximize the therapeutic efficacy of the product.

## SUMMARY OF THE INVENTION

The present disclosure pertains to methods of making immune effector cells (for example, T cells or NK cells) engineered to express a CAR, and compositions generated using such methods. Also disclosed are methods of using such compositions for treating a disease, for example, cancer, in a subject.

In some aspects, the disclosure provides a method of making a population of cells (for example, T cells) that express a chimeric antigen receptor (CAR). The method comprises: (i) contacting (for example, binding) a population of cells (for example, T cells, for example, T

cells isolated from a frozen or fresh leukapheresis product) with a multispecific binding molecule comprising (A) an anti-CD3 binding domain, and (B) a costimulatory molecule binding domain (e.g., an anti-CD2 binding domain or an anti-CD28 binding domain), and (C) an Fc region comprising: a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system; a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system; a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system; a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system; a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system; a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system; (ii) contacting the population of cells (for example, T cells) with a nucleic acid molecule (for example, a DNA or RNA molecule) encoding the CAR, thereby providing a population of cells (for example, T cells) comprising the nucleic acid molecule, and (iii) harvesting the population of cells (for example, T cells) for storage (for example, reformulating the population of cells in cryopreservation media) or administration, wherein: (a) step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), for example, no later than 12, 13, 14, 15, 16, 17, or 18 hours after the beginning of step (i), for example, no later than 18 hours after the beginning of step (i), and step (iii) is performed no later than 26 hours after the beginning of step (i), for example, no later than 22, 23, 24, or 25 hours after the beginning of step (i), for example, no later than 24 hours after the beginning of step (i); (b) step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), for example, no later than 12, 13, 14, 15, 16, 17, or 18 hours after the beginning of step (i), for example, no later than 18 hours after the beginning of step (i), and step (iii) is performed no later than 30, 36, or 48 hours after the beginning of step (ii), for example, no later than 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 hours after the beginning of step (ii); or (c) the population of cells from step (iii) are not expanded, or expanded by no more than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (i). In some embodiments, the nucleic acid molecule in step (ii) is a DNA molecule. In

some embodiments, the nucleic acid molecule in step (ii) is an RNA molecule. In some embodiments, the nucleic acid molecule in step (ii) is on a viral vector, for example, a viral vector chosen from a lentivirus vector, an adenoviral vector, or a retrovirus vector. In some embodiments, the nucleic acid molecule in step (ii) is on a non-viral vector. In some embodiments, the nucleic acid molecule in step (ii) is on a plasmid. In some embodiments, the nucleic acid molecule in step (ii) is not on any vector. In some embodiments, step (ii) comprises transducing the population of cells (for example, T cells) with a viral vector comprising a nucleic acid molecule encoding the CAR.

In some aspects, the disclosure provides a multispecific binding molecule comprising (A) an anti-CD3 binding domain, (B) a costimulatory molecule binding domain (e.g., an anti-CD2 binding domain or an anti-CD28 binding domain); and optionally (C) an Fc region comprising: a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system; a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system; a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system; a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system; a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system; a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system.

In some embodiments, the anti-CD3 binding domain, e.g., an anti-CD3 scFv, is situated N-terminal of the costimulatory molecule binding domain, e.g., an anti-CD2 Fab or an anti-CD28 Fab. In some embodiments, the anti-CD3 binding domain, e.g., an anti-CD3 scFv, is situated C-terminal of the costimulatory molecule binding domain, e.g., an anti-CD2 Fab or an anti-CD28 Fab. In some embodiments, the Fc region comprises a CH2. In some embodiments, the Fc region comprises a CH3. In some embodiments, the Fc region is situated between the anti-CD3 binding domain and the costimulatory molecule binding domain. In some embodiments, the anti-CD3 binding domain is situated C-terminal of the CH2. In some embodiments, the anti-CD3 binding domain is situated N-terminal of the CH2.

In some embodiments of the compositions and methods herein, the multispecific binding molecule comprises: (i) a first polypeptide comprising from N-terminal to C-terminal:

VH of the anti-CD3 binding domain, VL of the anti-CD3 binding domain, VH of the costimulatory molecule binding domain, CH1, CH2, and CH3; and (ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the costimulatory molecule binding domain and CL. In some embodiments, the multispecific binding molecule comprises: (i) a first polypeptide comprising from N-terminal to C-terminal: VH of the costimulatory molecule binding domain, CH1, CH2, CH3, VH of the anti-CD3 binding domain, and VL of the anti-CD3 binding domain; and (ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the costimulatory molecule binding domain and CL. In some embodiments, the multispecific binding molecule comprises: (i) a first polypeptide comprising from N-terminal to C-terminal: VH of the costimulatory molecule binding domain, CH1, VH of the anti-CD3 binding domain, VL of the anti-CD3 binding domain, CH2, and CH3; and (ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the costimulatory molecule binding domain and CL. In some embodiments, the anti-CD3 binding domain comprises an scFv and the costimulatory molecule binding domain is part of a Fab fragment.

In some embodiments, the anti-CD3 binding domain comprises: (i) a variable heavy chain region (VH) comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, and a HCDR3, and a light chain variable region (VL) comprising a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3 of an anti-CD3 antibody molecule of Table 27 (for example the anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4)); and/or (ii) the amino acid sequence of any one of the VH and/or VL region of an anti-CD3 antibody molecule provided in Table 27 (for example the anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4)), or an amino acid sequence at least 95% identical thereto.

In some embodiments, the costimulatory molecule binding domain is an anti-CD2 antigen binding domain. In some embodiments, the anti-CD2 antigen binding domain comprises: (i) a VH comprising a HCDR1, a HCDR2, and a HCDR3, and a VL comprising a LCDR1, a LCDR2, and a LCDR3 of an anti-CD2 antibody molecule of Table 27 (for example the anti-CD2 (1)); and/or (ii) the amino acid sequence of any one of the VH and/or VL region of an anti-CD2 antibody molecule provided in Table 27 (for example the anti-CD2 (1)), or an amino acid sequence at least 95% identical thereto.

In some embodiments, the costimulatory molecule binding domain is an anti-CD28 antigen binding domain. In some embodiments, the anti-CD28 antigen binding domain

comprises: (i) a VH comprising a HCDR1, a HCDR2, and a HCDR3, and a VL comprising a LCDR1, a LCDR2, and a LCDR3 of an anti-CD28 antibody molecule of Table 27 (for example the anti-CD28 (1) or anti-CD28 (2)); and/or (ii) the amino acid sequence of any one of the VH and/or VL region of an anti-CD28 antibody molecule provided in Table 27 (for example the anti-CD28 (1) or anti-CD28 (2)), or an amino acid sequence at least 95% identical thereto.

In some embodiments, the anti-CD3 binding domain comprises an scFv. In some embodiments, the anti-CD3 binding domain comprises a VH linked to a VL by a peptide linker, e.g., a glycine-serine linker, e.g., a $(G_4S)_4$ linker. In some embodiments, the anti-CD3 binding domain comprises a VH and a VL, wherein the VH is N-terminal of the VL.

In some embodiments, the costimulatory molecule binding domain is part of a Fab fragment, e.g., a Fab fragment that is part of a polypeptide sequence that comprises an Fc domain, optionally wherein the Fc domain comprises an amino acid sequence provided in Table 28, or a sequence with at least 95% sequence identity thereto.

In some embodiments, the anti-CD3 binding domain is situated N-terminal of the costimulatory molecule binding domain. In some embodiments, the anti-CD3 binding domain is linked to the costimulatory molecule binding domain by a peptide linker, e.g., a glycine-serine linker, e.g., a (G4S)4 linker.

In some embodiments, the anti-CD3 binding domain is situated C-terminal of the costimulatory molecule binding domain, wherein optionally an Fc region is situated between the anti-CD3 binding domain and the costimulatory molecule binding domain.

In some embodiments, the multispecific binding molecule comprises a CH1. In some embodiments, the CH1 is C-terminal of the VH of the costimulatory molecule binding domain.

In some embodiments, the multispecific binding molecule comprises one or both of a CH2 and a CH3.

In some embodiments, the anti-CD3 binding domain is linked to the CH3 by a peptide linker, e.g., a glycine-serine linker, e.g., a (G4S)4 linker. In some embodiments, the anti-CD3 binding domain is situated C-terminal of the CH1. In some embodiments, the construct comprises a CH2, and the anti-CD3 binding domain is situated N-terminal of the CH2. In some embodiments, anti-CD3 binding domain is linked to the CH1 by a peptide linker, e.g., a glycine-serine linker, e.g., a (G4S)2 linker. In some embodiments, the anti-CD3 binding

domain is linked to the CH2 by a peptide linker, e.g., a glycine-serine linker, e.g., a (G4S)4 linker.

In some embodiments, the multispecific binding molecule further comprises a CL. In some embodiments, the CL is C-terminal of the VL of the costimulatory molecule binding domain. In some embodiments, the CL domain is linked to the CH1, e.g., via a disulfide bridge.

In some embodiments, the multispecific binding molecule comprises: (i) the amino acid sequence of any heavy chain provided in Table 28, or an amino acid sequence with at least 95% sequence identity thereto; and/or (ii) the amino acid sequence of any light chain provided in Table 28, or an amino acid sequence with at least 95% sequence identity thereto.

In some embodiments, this invention features a method of making a population of cells (for example, T cells) that express a chimeric antigen receptor (CAR), the method comprising: (i) contacting (for example, binding) a population of cells (for example, T cells, for example, T cells isolated from a frozen or fresh leukapheresis product) with (A) an agent that stimulates a CD3/TCR complex and/or (B) an agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells; (ii) contacting the population of cells (for example, T cells) with a nucleic acid molecule (for example, a DNA or RNA molecule) encoding the CAR, thereby providing a population of cells (for example, T cells) comprising the nucleic acid molecule, and (iii) harvesting the population of cells (for example, T cells) for storage (for example, reformulating the population of cells in cryopreservation media) or administration, wherein: (a) step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), for example, no later than 12, 13, 14, 15, 16, 17, or 18 hours after the beginning of step (i), for example, no later than 18 hours after the beginning of step (i), and step (iii) is performed no later than 26 hours after the beginning of step (i), for example, no later than 22, 23, 24, or 25 hours after the beginning of step (i), for example, no later than 24 hours after the beginning of step (i); (b) step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), for example, no later than 12, 13, 14, 15, 16, 17, or 18 hours after the beginning of step (i), for example, no later than 18 hours after the beginning of step (i), and step (iii) is performed no later than 30, 36, or 48 hours after the beginning of step (ii), for example, no later than 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 hours after the beginning of step (ii); or

(c) the population of cells from step (iii) are not expanded, or expanded by no more than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (i). In some embodiments, the nucleic acid molecule in step (ii) is a DNA molecule. In some embodiments, the nucleic acid molecule in step (ii) is an RNA molecule. In some embodiments, the nucleic acid molecule in step (ii) is on a viral vector, for example, a viral vector chosen from a lentivirus vector, an adenoviral vector, or a retrovirus vector. In some embodiments, the nucleic acid molecule in step (ii) is on a non-viral vector. In some embodiments, the nucleic acid molecule in step (ii) is on a plasmid. In some embodiments, the nucleic acid molecule in step (ii) is not on any vector. In some embodiments, step (ii) comprises transducing the population of cells (for example, T cells) with a viral vector comprising a nucleic acid molecule encoding the CAR. In some embodiments, step (ii) further comprises contacting the population of cells (for example , T cells) with shRNA that targets Tet2 comprising (A) a sense strand comprising a Tet2 target sequence and (B) an antisense strand complementary to the sense strand in whole or in part or a vector encoding the shRNA. In some embodiments, sense strand comprises the Tet2 target sequence GGGTAAGCCAAGAAAGAAA (SEQ ID NO: 418). In some embodiments, the anti-sense strand comprises the reverse complement thereof, i.e. TTTCTTTCTTGGCTTACCC (SEQ ID NO: 419). In some embodiments, the vector encoding the shRNA is the same or different from the vector encoding the CAR. In some embodiments, the vector encoding the shRNA sequence comprises promoter (such as but not limited to a U6 promoter), a sense strand comprising a Tet2 target sequence, a loop, an anti-sense strand complementary to the sense strand in whole or in part, and, optionally, a polyT tail, e.g. the sequences in **Table 29**. In some embodiments, step (ii) is performed together with step (i). In some embodiments, step (ii) is performed no later than 20 hours after the beginning of step (i). In some embodiments, step (ii) is performed no later than 12, 13, 14, 15, 16, 17, or 18 hours after the beginning of step (i). In some embodiments, step (ii) is performed no later than 18 hours after the beginning of step (i). In some embodiments, step (iii) is performed no later than 26 hours after the beginning of step (i). In some embodiments, step (iii) is performed no later than 22, 23, 24, or 25 hours after the beginning of step (i). In some embodiments, step (iii) is performed no later than 24 hours after the beginning of step (i). In some embodiments, step (iii) is performed no later than 30, 36, or

48 hours after the beginning of step (ii). In some embodiments, step (iii) is performed no later than 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 hours after the beginning of step (ii).

In some embodiments, the population of cells from step (iii) are not expanded. In some embodiments, the population of cells from step (iii) are expanded by no more than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (i). In some embodiments, the population of cells from step (iii) are expanded by no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (i).

In some embodiments, the agent that stimulates a CD3/TCR complex is an agent that stimulates CD3. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor is an agent that stimulates CD28, ICOS, CD27, HVEM, LIGHT, CD40, 4-1BB, OX40, DR3, GITR, CD30, TIM1, CD2, CD226, or any combination thereof. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor is an agent that stimulates CD28. In some embodiments, the agent that stimulates a CD3/TCR complex is chosen from an antibody (for example, a single-domain antibody (for example, a heavy chain variable domain antibody), a peptibody, a Fab fragment, or a scFv), a small molecule, or a ligand (for example, a naturally-existing, recombinant, or chimeric ligand). In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor is chosen from an antibody (for example, a single-domain antibody (for example, a heavy chain variable domain antibody), a peptibody, a Fab fragment, or a scFv), a small molecule, or a ligand (for example, a naturally-existing, recombinant, or chimeric ligand). In some embodiments, the agent that stimulates a CD3/TCR complex does not comprise a bead. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor does not comprise a bead. In some embodiments, the agent that stimulates a CD3/TCR complex comprises an anti-CD3 antibody. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor comprises an anti-CD28 antibody. In some embodiments, the agent that stimulates a CD3/TCR complex comprises an anti-CD3 antibody covalently attached to a colloidal polymeric nanomatrix. In some embodiments, the agent that stimulates CD3 comprises one or more of a CD3 or TCR

antigen binding domain, such as but not limited to an anti-CD3 or anti-TCR antibody or an antibody fragment comprising one or more CDRs, heavy chain, and/or light chain thereof – such as but not limited to an anti-CD3 or anti-TCR antibody provided in Table 27. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor comprises an anti-CD28 antibody covalently attached to a colloidal polymeric nanomatrix. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor is an agent that stimulates CD28, ICOS, CD27, CD25, 4-1BB, IL6RA, IL6RB, or CD2. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor comprises one or more of a CD28, ICOS, CD27, CD25, 4-1BB, IL6RB, and/or CD2 antigen binding domain, such as but not limited to an anti- CD28, anti-ICOS, anti-CD27, anti-CD25, anti-4-1BB, anti-IL6RA, anti-IL6RB, or anti-CD2 antibody or an antibody fragment comprising one or more CDRs, heavy chain, and/or light chain thereof – such as but not limited to an anti- CD28, anti-ICOS, anti-CD27, anti-CD25, anti-4-1BB, anti-IL6RA, anti-IL6RB, or anti-CD2 antibody provided in Table 27. In some embodiments, the agent that stimulates a CD3/TCR complex and the agent that stimulates a costimulatory molecule and/or growth factor receptor comprise T Cell TransAct™. In some embodiments, the agent that stimulates a CD3/TCR complex and the agent that stimulates a costimulatory molecule and/or growth factor receptor are comprised in a multispecific binding molecule. In some embodiments, the multispecific binding molecule comprises a CD3 antigen binding domain and a CD28 or CD2 antigen binding domain. In some embodiments, the multispecific binding molecules comprise one or more heavy and/or light chains – such as but not limited to the heavy and/or light chains provided in Table 28. In some embodiments, the multispecific binding molecule comprises a bispecific antibody. In some embodiments, the bispecific antibody is configured in any one of the schema provided in **FIG. 50A**. In some embodiments, the bispecific antibody is monovalent or bivalent. In some embodiments, the bispecific antibody comprises an Fc region. In some embodiments, the Fc region of the bispecific antibody is silenced. In some embodiments, the multispecific binding molecule comprises a plurality of bispecific antibodies. In some embodiments, one or more of the plurality of bispecific antibodies is monovalent. In some embodiments, one or more of the plurality of bispecific antibodies comprises an Fc region. In some embodiments, the Fc region of the one or more of the plurality of bispecific antibodies is silenced. In some embodiments, one or more

of the plurality of bispecific antibodies are conjugated together into a multimer. In some embodiments, the multimer is configured in any one of the schema provided in **FIG. 50B**.

In some embodiments, the agent that stimulates a CD3/TCR complex does not comprise hydrogel. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor does not comprise hydrogel. In some embodiments, the agent that stimulates a CD3/TCR complex does not comprise alginate. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor does not comprise alginate.

In some embodiments, the agent that stimulates a CD3/TCR complex comprises hydrogel. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor comprises hydrogel. In some embodiments, the agent that stimulates a CD3/TCR complex comprises alginate. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor comprises alginate. In some embodiments, the agent that stimulates a CD3/TCR complex or the agent that stimulates a costimulatory molecule and/or growth factor receptor comprises MagCloudz™ from Quad Technologies.

In some embodiments, step (i) increases the percentage of CAR-expressing cells in the population of cells from step (iii), for example, the population of cells from step (iii) shows a higher percentage of CAR-expressing cells (for example, at least 10, 20, 30, 40, 50, or 60% higher), compared with cells made by an otherwise similar method without step (i).

In some embodiments, the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) is the same as the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (i). In some embodiments, the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) differs by no more than 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12% from the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (i). In some embodiments, the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) differs by no more than 5 or 10% from the percentage of naïve cells, for example, naïve T cells, for

example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (i).

In some embodiments, the population of cells from step (iii) shows a higher percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells (for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40% higher), compared with cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i). In some embodiments, the population of cells from step (iii) shows a higher percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells (for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40% higher), compared with cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

In some embodiments, the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells from step (iii) is the same as the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the beginning of step (i). In some embodiments, the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells from step (iii) differs by no more than 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12% from the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the beginning of step (i). In some embodiments, the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells from step (iii) differs by no more than 5 or 10% from the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the beginning of step (i).

In some embodiments, the population of cells from step (iii) shows a lower percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells (for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40% lower), compared with cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8,

9, 10, 11, or 12 days after the beginning of step (i). In some embodiments, the population of cells from step (iii) shows a lower percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells (for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40% lower), compared with cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

In some embodiments, the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is increased, as compared to the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells at the beginning of step (i). In some embodiments, the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is increased, as compared to the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells at the beginning of step (i). In some embodiments, the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is higher than the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i). In some embodiments, the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is higher than the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i). In some embodiments, the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is higher than the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor

β+CCR7+CD62L+ T cells, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days. In some embodiments, the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is higher than the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

In some embodiments, the median GeneSetScore (Up TEM vs. Down TSCM) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 75, 100, or 125% from the median GeneSetScore (Up TEM vs. Down TSCM) of the population of cells at the beginning of step (i). In some embodiments, the median GeneSetScore (Up TEM vs. Down TSCM) of the population of cells from step (iii) is lower (for example, at least about 100, 150, 200, 250, or 300% lower) than the median GeneSetScore (Up TEM vs. Down TSCM) of cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i). In some embodiments, the median GeneSetScore (Up TEM vs. Down TSCM) of the population of cells from step (iii) is lower (for example, at least about 100, 150, 200, 250, or 300% lower) than the median GeneSetScore (Up TEM vs. Down TSCM) of cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days. In some embodiments, the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 100, 150, or 200% from the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells at the beginning of step (i). In some embodiments, the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells from step (iii) is lower (for example, at least about 50, 100, 125, 150, or 175% lower) than the median GeneSetScore (Up Treg vs. Down Teff) of cells made by an otherwise similar method

in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i). In some embodiments, the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells from step (iii) is lower (for example, at least about 50, 100, 125, 150, or 175% lower) than the median GeneSetScore (Up Treg vs. Down Teff) of cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days. In some embodiments, the median GeneSetScore (Down stemness) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 100, 150, 200, or 250% from the median GeneSetScore (Down stemness) of the population of cells at the beginning of step (i). In some embodiments, the median GeneSetScore (Down stemness) of the population of cells from step (iii) is lower (for example, at least about 50, 100, or 125% lower) than the median GeneSetScore (Down stemness) of cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i). In some embodiments, the median GeneSetScore (Down stemness) of the population of cells from step (iii) is lower (for example, at least about 50, 100, or 125% lower) than the median GeneSetScore (Down stemness) of cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days. In some embodiments, the median GeneSetScore (Up hypoxia) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 125, 150, 175, or 200% from the median GeneSetScore (Up hypoxia) of the population of cells at the beginning of step (i). In some embodiments, the median GeneSetScore (Up hypoxia) of the population of cells from step (iii) is lower (for example, at least about 40, 50, 60, 70, or 80% lower) than the median GeneSetScore (Up hypoxia) of cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i). In some embodiments, the median GeneSetScore (Up hypoxia) of the population of cells from step (iii) is lower (for example, at least about 40, 50, 60, 70, or 80% lower) than the median GeneSetScore (Up hypoxia) of cells made by an otherwise similar

method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days. In some embodiments, the median GeneSetScore (Up autophagy) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 180, 190, 200, or 210% from the median GeneSetScore (Up autophagy) of the population of cells at the beginning of step (i). In some embodiments, the median GeneSetScore (Up autophagy) of the population of cells from step (iii) is lower (for example, at least 20, 30, or 40% lower) than the median GeneSetScore (Up autophagy) of cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i). In some embodiments, the median GeneSetScore (Up autophagy) of the population of cells from step (iii) is lower (for example, at least 20, 30, or 40% lower) than the median GeneSetScore (Up autophagy) of cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

In some embodiments, the population of cells from step (iii), after being incubated with a cell expressing an antigen recognized by the CAR, secretes IL-2 at a higher level (for example, at least 2, 4, 6, 8, 10, 12, or 14-fold higher) than cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days, for example, as assessed using methods described in Example 8 with respect to FIGs. 29C-29D.

In some embodiments, the population of cells from step (iii), after being administered in vivo, persists longer or expands at a higher level (for example, at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, or 90% higher) (for example, as assessed using methods described in Example 1 with respect to FIG. 4C), compared with cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i). In some embodiments, the population of cells from step (iii), after being administered in vivo,

persists longer or expands at a higher level (for example, at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, or 90% higher) (for example, as assessed using methods described in Example 1 with respect to FIG. 4C), compared with cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

In some embodiments, the population of cells from step (iii), after being administered in vivo, shows a stronger anti-tumor activity (for example, a stronger anti-tumor activity at a low dose, for example, a dose no more than $0.15 \times 10^6$, $0.2 \times 10^6$, $0.25 \times 10^6$, or $0.3 \times 10^6$ viable CAR-expressing cells) than cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

In some embodiments, the population of cells from step (iii) are not expanded, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (i). In some embodiments, the population of cells from step (iii) decreases from the number of living cells in the population of cells at the beginning of step (i), for example, as assessed by the number of living cells. In some embodiments, the population of cells from step (iii) are expanded by no more than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (i). In some embodiments, the population of cells from step (iii) are not expanded, or expanded by less than 0.5, 1, 1.5, or 2 hours, for example, less than 1 or 1.5 hours, compared to the population of cells at the beginning of step (i).

In some embodiments, steps (i) and (ii) are performed in cell media (for example, serum-free media) comprising IL-2, IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)), IL-6 (for example, IL-6/sIL-6Ra), a LSD1 inhibitor, or a MALT1 inhibitor. In some embodiments, steps (i) and (ii) are performed in cell media (for example, serum-free media) comprising IL-7, IL-21, or a combination thereof. In some embodiments, steps (i) and (ii) are performed in cell media (for example, serum-free media) comprising IL-2, IL-15 (for example, hetIL-15

(IL15/sIL-15Ra)), IL-21, IL-7, IL-6 (for example, IL-6/sIL-6Ra), a LSD1 inhibitor, a MALT1 inhibitor, or a combination thereof. In some embodiments, step (i) is performed in cell media (for example, serum-free media) comprising IL-2, IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)), IL-6 (for example, IL-6/sIL-6Ra), a LSD1 inhibitor, or a MALT1 inhibitor. In some embodiments, step (ii) is performed in cell media (for example, serum-free media) comprising IL-2, IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)), IL-6 (for example, IL-6/sIL-6Ra), a LSD1 inhibitor, or a MALT1 inhibitor. In some embodiments, step (i) is performed in cell media (for example, serum-free media) comprising IL-7, IL-21, or a combination thereof. In some embodiments, step (ii) is performed in cell media (for example, serum-free media) comprising IL-7, IL-21, or a combination thereof. In some embodiments, step (i) is performed in cell media (for example, serum-free media) comprising IL-2, IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)), IL-21, IL-7, IL-6 (for example, IL-6/sIL-6Ra), a LSD1 inhibitor, a MALT1 inhibitor, or a combination thereof. In some embodiments, step (ii) is performed in cell media (for example, serum-free media) comprising IL-2, IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)), IL-21, IL-7, IL-6 (for example, IL-6/sIL-6Ra), a LSD1 inhibitor, a MALT1 inhibitor, or a combination thereof. In some embodiments, the cell media is a serum-free media comprising a serum replacement. In some embodiments, the serum replacement is CTS™ Immune Cell Serum Replacement (ICSR).

In some embodiments, the aforementioned methods further comprise prior to step (i): (iv) receiving a fresh leukapheresis product (or an alternative source of hematopoietic tissue such as a fresh whole blood product, a fresh bone marrow product, or a fresh tumor or organ biopsy or removal (for example, a fresh product from thymectomy)) from an entity, for example, a laboratory, hospital, or healthcare provider.

In some embodiments, the aforementioned methods further comprise prior to step (i): (v) isolating the population of cells (for example, T cells, for example, CD8+ and/or CD4+ T cells) contacted in step (i) from a fresh leukapheresis product (or an alternative source of hematopoietic tissue such as a fresh whole blood product, a fresh bone marrow product, or a fresh tumor or organ biopsy or removal (for example, a fresh product from thymectomy)). In some embodiments, step (iii) is performed no later than 35, 36, or 48 hours after the beginning of step (v), for example, no later than 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48 hours after the beginning of step (v), for example, no later than 30,

36, or 48 hours after the beginning of step (v). In some embodiments, the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the end of step (v).

In some embodiments, the aforementioned methods further comprise prior to step (i): receiving cryopreserved T cells isolated from a leukapheresis product (or an alternative source of hematopoietic tissue such as cryopreserved T cells isolated from whole blood, bone marrow, or tumor or organ biopsy or removal (for example, thymectomy)) from an entity, for example, a laboratory, hospital, or healthcare provider.

In some embodiments, the aforementioned methods further comprise prior to step (i): (iv) receiving a cryopreserved leukapheresis product (or an alternative source of hematopoietic tissue such as a cryopreserved whole blood product, a cryopreserved bone marrow product, or a cryopreserved tumor or organ biopsy or removal (for example, a cryopreserved product from thymectomy)) from an entity, for example, a laboratory, hospital, or healthcare provider.

In some embodiments, the aforementioned methods further comprise prior to step (i): (v) isolating the population of cells (for example, T cells, for example, CD8+ and/or CD4+ T cells) contacted in step (i) from a cryopreserved leukapheresis product (or an alternative source of hematopoietic tissue such as a cryopreserved whole blood product, a cryopreserved bone marrow product, or a cryopreserved tumor or organ biopsy or removal (for example, a cryopreserved product from thymectomy)). In some embodiments, step (iii) is performed no later than 35, 36, or 48 hours after the beginning of step (v), for example, no later than 27, 28, 29, 30, 31, 32, 33, 34, 35,36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48 hours after the beginning of step (v), for example, no later than 30, 36, or 38 hours after the beginning of step (v). In some embodiments, the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the end of step (v).

In some embodiments, this invention features a method of making a population of cells (for example, T cells) that express a chimeric antigen receptor (CAR), the method comprising: (1) contacting a population of cells (for example, T cells, for example, T cells isolated from a

frozen leukapheresis product) with a cytokine chosen from IL-2, IL-7, IL-15, IL-21, IL-6, or a combination thereof, (2) contacting the population of cells (for example, T cells) with a nucleic acid molecule (for example, a DNA or RNA molecule) encoding the CAR, thereby providing a population of cells (for example, T cells) comprising the nucleic acid molecule, and (3) harvesting the population of cells (for example, T cells) for storage (for example, reformulating the population of cells in cryopreservation media) or administration, wherein: (a) step (2) is performed together with step (1) or no later than 5 hours after the beginning of step (1), for example, no later than 1, 2, 3, 4, or 5 hours after the beginning of step (1), and step (3) is performed no later than 26 hours after the beginning of step (1), for example, no later than 22, 23, 24, or 25 hours after the beginning of step (1), for example, no later than 24 hours after the beginning of step (1), or (b) the population of cells from step (3) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (1). In some embodiments, the nucleic acid molecule in step (2) is a DNA molecule. In some embodiments, the nucleic acid molecule in step (2) is an RNA molecule. In some embodiments, the nucleic acid molecule in step (2) is on a viral vector, for example, a viral vector chosen from a lentivirus vector, an adenoviral vector, or a retrovirus vector. In some embodiments, the nucleic acid molecule in step (2) is on a non-viral vector. In some embodiments, the nucleic acid molecule in step (2) is on a plasmid. In some embodiments, the nucleic acid molecule in step (2) is not on any vector. In some embodiments, step (2) comprises contacting, optionally transducing, the population of cells (for example, T cells) with a viral vector comprising a nucleic acid molecule encoding the CAR. In some embodiments, step (2) further comprises contacting the population of cells (for example , T cells) with shRNA that targets Tet2 comprising (A) a sense strand comprising a Tet2 target sequence and (B) an antisense strand complementary to the sense strand in whole or in part or a vector encoding the shRNA. In some embodiments, sense strand comprises the Tet2 target sequence GGGTAAGCCAAGAAAGAAA (SEQ ID NO: 418). In some embodiments, the anti-sense strand comprises the reverse complement thereof, i.e. TTTCTTTCTTGGCTTACCC (SEQ ID NO: 419). In some embodiments, the vector encoding the shRNA is the same or different from the vector encoding the CAR. In some embodiments, the vector encoding the shRNA sequence comprises promoter (such as but not limited to a U6 promoter), a sense strand comprising a

Tet2 target sequence, a loop, an anti-sense strand complementary to the sense strand in whole or in part, and, optionally, a polyT tail, e.g. the sequences in **Table 29**.

In some embodiments, step (2) is performed together with step (1). In some embodiments, step (2) is performed no later than 5 hours after the beginning of step (1). In some embodiments, step (2) is performed no later than 1, 2, 3, 4, or 5 hours after the beginning of step (1). In some embodiments, step (3) is performed no later than 26 hours after the beginning of step (1). In some embodiments, step (3) is performed no later than 22, 23, 24, or 25 hours after the beginning of step (1). In some embodiments, step (3) is performed no later than 24 hours after the beginning of step (1).

In some embodiments, the population of cells from step (3) are not expanded, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (1). In some embodiments, the population of cells from step (3) are expanded by no more than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (1). In some embodiments, the population of cells from step (3) are expanded by no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (1).

In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-2. In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-7. In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)). In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-21. In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-6 (for example, IL-6/sIL-6Ra). In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-2 and IL-7. In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-2 and IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)). In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-2 and IL-21. In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-2 and IL-6 (for example, IL-6/sIL-6Ra). In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-7 and IL-15

(for example, hetIL-15 (IL15/sIL-15Ra)). In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-7 and IL-21. In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-7 and IL-6 (for example, IL-6/sIL-6Ra). In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)) and IL-21. In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)) and IL-6 (for example, IL-6/sIL-6Ra). In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-21 and IL-6 (for example, IL-6/sIL-6Ra). In some embodiments, step (1) comprises contacting the population of cells (for example, T cells) with IL-7, IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)), and IL-21.

In some embodiments, the population of cells from step (3) shows a higher percentage of naïve cells among CAR-expressing cells (for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40% higher), compared with cells made by an otherwise similar method which further comprises contacting the population of cells with, for example, an anti-CD3 antibody.

In some embodiments, the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (3) is the same as the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (1). In some embodiments, the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (3) differs by no more than 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12% from the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (1). In some embodiments, the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (3) differs by no more than 5 or 10% from the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (1). In some embodiments, the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (3) is increased as compared to the percentage of naïve cells, for example, naïve T cells, for example,

CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (1). In some embodiments, the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (3) is increased by at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20%, as compared to the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (1). In some embodiments, the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (3) is increased by at least 10 or 20%, as compared to the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (1).

In some embodiments, the population of cells from step (3) shows a higher percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells (for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40% higher), compared with cells made by an otherwise similar method in which step (3) is performed more than 26 hours after the beginning of step (1), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (1). In some embodiments, the population of cells from step (3) shows a higher percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells (for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40% higher), compared with cells made by an otherwise similar method which further comprises, after step (2) and prior to step (3), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

In some embodiments, the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells from step (3) is the same as the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the beginning of step (i). In some embodiments, the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells from step (3) differs by no more than 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12% from the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the beginning of step (i). In some embodiments, the percentage of central memory cells, for example, central memory T cells, for example, CD95+

central memory T cells, in the population of cells from step (3) differs by no more than 5 or 10% from the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the beginning of step (i). In some embodiments, the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells from step (3) is decreased as compared to the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the beginning of step (1). In some embodiments, the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells from step (3) is decreased by at least 10 or 20%, as compared to the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the beginning of step (1). In some embodiments, the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells from step (3) is decreased by at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20%, as compared to the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the beginning of step (1).

In some embodiments, the population of cells from step (3) shows a lower percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells (for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40% lower), compared with cells made by an otherwise similar method in which step (3) is performed more than 26 hours after the beginning of step (1), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (1). In some embodiments, the population of cells from step (3) shows a lower percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells (for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40% lower), compared with cells made by an otherwise similar method which further comprises, after step (2) and prior to step (3), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

In some embodiments, the population of cells from step (3), after being administered in vivo, persists longer or expands at a higher level (for example, at least 20, 25, 30, 35, 40, 45,

50, 55, 60, 65, 70, 75, 80, 85, or 90% higher) (for example, as assessed using methods described in Example 1 with respect to FIG. 4C), compared with cells made by an otherwise similar method in which step (3) is performed more than 26 hours after the beginning of step (1), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (1). In some embodiments, the population of cells from step (3), after being administered in vivo, persists longer or expands at a higher level (for example, at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, or 90% higher) (for example, as assessed using methods described in Example 1 with respect to FIG. 4C), compared with cells made by an otherwise similar method which further comprises, after step (2) and prior to step (3), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

In some embodiments, the population of cells from step (3) are not expanded, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (1). In some embodiments, the population of cells from step (3) are expanded by no more than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (1). In some embodiments, the population of cells from step (3) are expanded by no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (1). In some embodiments, the number of living cells in the population of cells from step (3) decreases from the number of living cells in the population of cells at the beginning of step (1), for example, as assessed by the number of living cells.

In some embodiments, the population of cells from step (3) are not expanded compared to the population of cells at the beginning of step (1), for example, as assessed by the number of living cells. In some embodiments, the population of cells from step (3) are expanded by less than 0.5, 1, 1.5, or 2 hours, for example, less than 1 or 1.5 hours, compared to the population of cells at the beginning of step (1).

In some embodiments, the population of cells is not contacted in vitro with (A) an agent that stimulates a CD3/TCR complex and/or (B) an agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells, or if contacted, the contacting step is less than 2 hours, for example, no more than 1 or 1.5 hours. In some embodiments, the agent that stimulates a CD3/TCR complex is an agent that stimulates CD3

(for example, an anti-CD3 antibody). In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor is an agent that stimulates CD28, ICOS, CD27, HVEM, LIGHT, CD40, 4-1BB, OX40, DR3, GITR, CD30, TIM1, CD2, CD226, or any combination thereof. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor is an agent that stimulates CD28. In some embodiments, the agent that stimulates a CD3/TCR complex or the agent that stimulates a costimulatory molecule and/or growth factor receptor is chosen from an antibody (for example, a single-domain antibody (for example, a heavy chain variable domain antibody), a peptibody, a Fab fragment, or a scFv), a small molecule, or a ligand (for example, a naturally-existing, recombinant, or chimeric ligand).

In some embodiments, steps (1) and/or (2) are performed in cell media comprising no more than 5, 4, 3, 2, 1, or 0% serum. In some embodiments, steps (1) and/or (2) are performed in cell media comprising no more than 2% serum. In some embodiments, steps (1) and/or (2) are performed in cell media comprising about 2% serum. In some embodiments, steps (1) and/or (2) are performed in cell media comprising a LSD1 inhibitor or a MALT1 inhibitor. In some embodiments, step (1) is performed in cell media comprising no more than 5, 4, 3, 2, 1, or 0% serum. In some embodiments, step (1) is performed in cell media comprising no more than 2% serum. In some embodiments, step (1) is performed in cell media comprising about 2% serum. In some embodiments, step (2) is performed in cell media comprising no more than 5, 4, 3, 2, 1, or 0% serum. In some embodiments, step (2) is performed in cell media comprising no more than 2% serum. In some embodiments, step (2) is performed in cell media comprising about 2% serum. In some embodiments, step (1) is performed in cell media comprising a LSD1 inhibitor or a MALT1 inhibitor. In some embodiments, step (2) is performed in cell media comprising a LSD1 inhibitor or a MALT1 inhibitor.

In some embodiments, the aforementioned methods further comprise prior to step (i): (iv) receiving a fresh leukapheresis product (or an alternative source of hematopoietic tissue such as a fresh whole blood product, a fresh bone marrow product, or a fresh tumor or organ biopsy or removal (for example, a fresh product from thymectomy)) from an entity, for example, a laboratory, hospital, or healthcare provider.

In some embodiments, the aforementioned methods further comprise prior to step (i): (v) isolating the population of cells (for example, T cells, for example, CD8+ and/or CD4+ T

cells) contacted in step (i) from a fresh leukapheresis product (or an alternative source of hematopoietic tissue such as a fresh whole blood product, a fresh bone marrow product, or a fresh tumor or organ biopsy or removal (for example, a fresh product from thymectomy)). In some embodiments, step (iii) is performed no later than 35, 36, or 48 hours after the beginning of step (v), for example, no later than 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48 hours after the beginning of step (v), for example, no later than 30, 36, or 48 hours after the beginning of step (v). In some embodiments, the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the end of step (v).

In some embodiments, the aforementioned methods further comprise prior to step (i): receiving cryopreserved T cells isolated from a leukapheresis product (or an alternative source of hematopoietic tissue such as cryopreserved T cells isolated from whole blood, bone marrow, or tumor or organ biopsy or removal (for example, thymectomy)) from an entity, for example, a laboratory, hospital, or healthcare provider.

In some embodiments, the aforementioned methods further comprise prior to step (i): (iv) receiving a cryopreserved leukapheresis product (or an alternative source of hematopoietic tissue such as a cryopreserved whole blood product, a cryopreserved bone marrow product, or a cryopreserved tumor or organ biopsy or removal (for example, a cryopreserved product from thymectomy)) from an entity, for example, a laboratory, hospital, or healthcare provider.

In some embodiments, the aforementioned methods further comprise prior to step (i): (v) isolating the population of cells (for example, T cells, for example, CD8+ and/or CD4+ T cells) contacted in step (i) from a cryopreserved leukapheresis product (or an alternative source of hematopoietic tissue such as a cryopreserved whole blood product, a cryopreserved bone marrow product, or a cryopreserved tumor or organ biopsy or removal (for example, a cryopreserved product from thymectomy)). In some embodiments, step (iii) is performed no later than 35, 36, or 48 hours after the beginning of step (v), for example, no later than 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48 hours after the beginning of step (v), for example, no later than 30, 36, or 48 hours after the beginning of step (v). In some embodiments, the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%,

for example, as assessed by the number of living cells, compared to the population of cells at the end of step (v).

In some embodiments, the population of cells at the beginning of step (i) or step (1) has been enriched for IL6R-expressing cells (for example, cells that are positive for IL6Rα and/or IL6Rβ). In some embodiments, the population of cells at the beginning of step (i) or step (1) comprises no less than 40, 45, 50, 55, 60, 65, or 70% of IL6R-expressing cells (for example, cells that are positive for IL6Rα and/or IL6Rβ).

In some embodiments, steps (i) and (ii) or steps (1) and (2) are performed in cell media comprising IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)). In some embodiments, IL-15 increases the ability of the population of cells to expand, for example, 10, 15, 20, or 25 days later. In some embodiments, IL-15 increases the percentage of IL6Rβ-expressing cells in the population of cells.

In some embodiments of the aforementioned methods, the methods are performed in a closed system. In some embodiments, T cell separation, activation, transduction, incubation, and washing are all performed in a closed system. In some embodiments of the aforementioned methods, the methods are performed in separate devices. In some embodiments, T cell separation, activation and transduction, incubation, and washing are performed in separate devices.

In some embodiments of the aforementioned methods, the methods further comprise adding an adjuvant or a transduction enhancement reagent in the cell culture medium to enhance transduction efficiency. In some embodiments, the adjuvant or transduction enhancement reagent comprises a cationic polymer. In some embodiments, the adjuvant or transduction enhancement reagent is chosen from: LentiBOOST™ (Sirion Biotech), vectofusin-1, F108 (Poloxamer 338 or Pluronic® F-38), protamine sulfate, hexadimethrine bromide (Polybrene), PEA, Pluronic F68, Pluronic F127, Synperonic or LentiTrans™. In some embodiments, the transduction enhancement reagent is LentiBOOST™ (Sirion Biotech). In some embodiments, the transduction enhancement reagent is F108 (Poloxamer 338 or Pluronic® F-38).

In some embodiments of the aforementioned methods, the transducing the population of cells (for example, T cells) with a viral vector comprises subjecting the population of cells and viral vector to a centrifugal force under conditions such that transduction efficiency is enhanced. In an embodiment, the cells are transduced by spinoculation.

In some embodiments of the aforementioned methods, cells (e.g., T cells) are activated and transduced in a cell culture flask comprising a gas-permeable membrane at the base that supports large media volumes without substantially compromising gas exchange. In some embodiments, cell growth is achieved by providing access, e.g., substantially uninterrupted access, to nutrients through convection.

In some embodiments of the aforementioned methods, the CAR comprises an antigen binding domain, a transmembrane domain, and an intracellular signaling domain.

In some embodiments, the antigen binding domain binds to an antigen chosen from: CD19, CD20, CD22, BCMA, mesothelin, EGFRvIII, GD2, Tn antigen, sTn antigen, Tn-O-Glycopeptides, sTn-O-Glycopeptides, PSMA, CD97, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, leguman, GD3, CD171, IL-11Ra, PSCA, MAD-CT-1, MAD-CT-2, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBBs (for example, ERBB2), Her2/neu, MUC1, EGFR, NCAM, Ephrin B2, CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, FAP, Legumain, HPV E6 or E7, ML-IAP, CLDN6, TSHR, GPRC5D, ALK, Polysialic acid, Fos-related antigen, neutrophil elastase, TRP-2, CYP1B1, sperm protein 17, beta human chorionic gonadotropin, AFP, thyroglobulin, PLAC1, globoH, RAGE1, MN-CA IX, human telomerase reverse transcriptase, intestinal carboxyl esterase, mut hsp 70-2, NA-17, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, NY-ESO-1, GPR20, Ly6k, OR51E2, TARP, GFRα4, or a peptide of any of these antigens presented on MHC. In some embodiments, the antigen binding domain comprises a CDR, VH, VL, scFv or a CAR sequence disclosed herein. In some embodiments, the antigen binding domain comprises a VH and a VL, wherein the VH and VL are connected by a linker, optionally wherein the linker comprises the amino acid sequence of SEQ ID NO: 63 or 104.

In some embodiments, the transmembrane domain comprises a transmembrane domain of a protein chosen from the alpha, beta or zeta chain of T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154. In some embodiments, the transmembrane domain comprises a

transmembrane domain of CD8. In some embodiments, the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof. In some embodiments, the nucleic acid molecule comprises a nucleic acid sequence encoding the transmembrane domain, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 17, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof.

In some embodiments, the antigen binding domain is connected to the transmembrane domain by a hinge region. In some embodiments, the hinge region comprises the amino acid sequence of SEQ ID NO: 2, 3, or 4, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof. In some embodiments, the nucleic acid molecule comprises a nucleic acid sequence encoding the hinge region, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 13, 14, or 15, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof.

In some embodiments, the intracellular signaling domain comprises a primary signaling domain. In some embodiments, the primary signaling domain comprises a functional signaling domain derived from CD3 zeta, TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (ICOS), FcεRI, DAP10, DAP12, or CD66d. In some embodiments, the primary signaling domain comprises a functional signaling domain derived from CD3 zeta. In some embodiments, the primary signaling domain comprises the amino acid sequence of SEQ ID NO: 9 or 10, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof. In some embodiments, the nucleic acid molecule comprises a nucleic acid sequence encoding the primary signaling domain, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 20 or 21, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof.

In some embodiments, the intracellular signaling domain comprises a costimulatory signaling domain. In some embodiments, the costimulatory signaling domain comprises a functional signaling domain derived from a MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), an activating NK cell receptor, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, 4-1BB (CD137), B7-H3, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80

(KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD28-OX40, CD28-4-1BB, or a ligand that specifically binds with CD83. In some embodiments, the costimulatory signaling domain comprises a functional signaling domain derived from 4-1BB. In some embodiments, the costimulatory signaling domain comprises the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof. In some embodiments, the nucleic acid molecule comprises a nucleic acid sequence encoding the costimulatory signaling domain, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 18, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof.

In some embodiments, the intracellular signaling domain comprises a functional signaling domain derived from 4-1BB and a functional signaling domain derived from CD3 zeta. In some embodiments, the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 (or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof) and the amino acid sequence of SEQ ID NO: 9 or 10 (or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof). In some embodiments, the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 and the amino acid sequence of SEQ ID NO: 9 or 10.

In some embodiments, the CAR further comprises a leader sequence comprising the amino acid sequence of SEQ ID NO: 1.

In some embodiments, this invention features a population of CAR-expressing cells (for example, autologous or allogeneic CAR-expressing T cells or NK cells) made by any of the aforementioned methods or any other method disclosed herein. In some embodiments,

disclosed herein is a pharmaceutical composition comprising a population of CAR-expressing cells disclosed herein and a pharmaceutically acceptable carrier.

In some embodiments, in the final CAR cell product manufactured using the methods described herein, the total amount of beads (e.g., CD4 beads, CD8 beads, and/or TransACT beads) is no more than 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, or 0.5% of the total amount of beads added during the manufacturing process.

In some embodiments, this invention features a population of CAR-expressing cells (for example, autologous or allogeneic CAR-expressing T cells or NK cells) comprising one or more of the following characteristics: (a) about the same percentage of naïve cells, for example, naïve T cells, for example, CD45RO- CCR7+ T cells, as compared to the percentage of naïve cells, for example, naïve T cells, for example, CD45RO- CCR7+ cells, in the same population of cells prior to being engineered to express the CAR; (b) a change within about 5% to about 10% of naïve cells, for example, naïve T cells, for example, CD45RO- CCR7+ T cells, for example, as compared to the percentage of naïve cells, for example, naïve T cells, for example, CD45RO- CCR7+ cells, in the same population of cells prior to being engineered to express the CAR; (c) an increased percentage of naïve cells, for example, naïve T cells, for example, CD45RO- CCR7+ T cells, for example, increased by at least 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, or 3-fold, as compared to the percentage of naïve cells, for example, naïve T cells, for example, CD45RO- CCR7+ cells, in the same population of cells prior to being engineered to express the CAR; (d) about the same percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, as compared to the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in the same population of cells prior to being engineered to express the CAR; (e) a change within about 5% to about 10% of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, as compared to the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in the same population of cells prior to being engineered to express the CAR; (f) a decreased percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, for example, decreased by at least 20, 25, 30, 35, 40, 45, or 50%, as compared to the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in the same population of cells prior to being engineered to express

the CAR; (g) about the same percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, as compared to the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the same population of cells prior to being engineered to express the CAR; (h) a change within about 5% to about 10% of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, as compared to the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the same population of cells prior to being engineered to express the CAR; or (i) an increased percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, as compared to the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the same population of cells prior to being engineered to express the CAR.

In some embodiments, this invention features a population of CAR-expressing cells (for example, autologous or allogeneic CAR-expressing T cells or NK cells), wherein: (a) the median GeneSetScore (Up TEM vs. Down TSCM) of the population of cells is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 75, 100, or 125% from the median GeneSetScore (Up TEM vs. Down TSCM) of the same population of cells prior to being engineered to express the CAR; (b) the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 100, 150, or 200% from the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells prior to being engineered to express the CAR; (c) the median GeneSetScore (Down stemness) of the population of cells is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 100, 150, 200, or 250% from the median GeneSetScore (Down stemness) of the population of cells prior to being engineered to express the CAR; (d) the median GeneSetScore (Up hypoxia) of the population of cells is about the same as or differs by no more than (for example, increased by no more than) about 125, 150, 175, or 200% from the median GeneSetScore (Up hypoxia) of the population of cells prior to being engineered to express the CAR; or (e) the median GeneSetScore (Up autophagy) of the population of cells is about the same as or differs by no more than (for example, increased by no more than) about 180, 190,

200, or 210% from the median GeneSetScore (Up autophagy) of the population of cells prior to being engineered to express the CAR.

In some embodiments, this invention features a method of increasing an immune response in a subject, comprising administering a population of CAR-expressing cells disclosed herein or a pharmaceutical composition disclosed herein to the subject, thereby increasing an immune response in the subject.

In some embodiments, disclosed herein is a method of treating a cancer in a subject, comprising administering a population of CAR-expressing cells disclosed herein or a pharmaceutical composition disclosed herein to the subject, thereby treating the cancer in the subject. In some embodiments, the cancer is a solid cancer, for example, chosen from: one or more of mesothelioma, malignant pleural mesothelioma, non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, large cell lung cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, esophageal adenocarcinoma , breast cancer, glioblastoma, ovarian cancer, colorectal cancer, prostate cancer, cervical cancer, skin cancer, melanoma, renal cancer, liver cancer, brain cancer, thymoma, sarcoma, carcinoma, uterine cancer, kidney cancer, gastrointestinal cancer, urothelial cancer, pharynx cancer, head and neck cancer, rectal cancer, esophagus cancer, or bladder cancer, or a metastasis thereof. In some embodiments, the cancer is a liquid cancer, for example, chosen from: chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), multiple myeloma, acute lymphoid leukemia (ALL), Hodgkin lymphoma, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), small lymphocytic leukemia (SLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL), DLBCL associated with chronic inflammation, chronic myeloid leukemia, myeloproliferative neoplasms, follicular lymphoma, pediatric follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma (extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue), Marginal zone lymphoma, myelodysplasia, myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, splenic marginal zone lymphoma, splenic lymphoma/leukemia, splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant, lymphoplasmacytic

lymphoma, a heavy chain disease, plasma cell myeloma, solitary plasmocytoma of bone, extraosseous plasmocytoma, nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, primary cutaneous follicle center lymphoma, lymphomatoid granulomatosis, primary mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, ALK+ large B-cell lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, B-cell lymphoma, acute myeloid leukemia (AML), or unclassifiable lymphoma.

In some embodiments, the method further comprises administering a second therapeutic agent to the subject. In some embodiments, the second therapeutic agent is an anti-cancer therapeutic agent, for example, a chemotherapy, a radiation therapy, or an immune-regulatory therapy. In some embodiments, the second therapeutic agent is IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)).

In some aspects, the disclosure features an antibody molecule that binds CD28, comprising a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, and a HCDR3, and a light chain variable region (VL) comprising a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3, wherein (i) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NO: 538, 539, 540, 530, 531, and 532, respectively; (ii) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NO: 541, 539, 540, 530, 531, and 532, respectively; (iii) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NO: 542, 543, 540, 533, 534, and 535, respectively; or (iv) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NO: 544, 545, 546, 536, 534, and 532, respectively.

In some aspects, the disclosure features an antibody molecule that binds CD28, comprising a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, and a HCDR3, and a light chain variable region (VL) comprising a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3, and an Fc region, wherein (i) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NO: 538, 539, 540, 530, 531, and 532,

respectively; (ii) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NO: 541, 539, 540, 530, 531, and 532, respectively; (iii) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NO: 542, 543, 540, 533, 534, and 535, respectively; or (iv) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NO: 544, 545, 546, 536, 534, and 532, respectively; and wherein the Fc region comprises: (a) a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system; (b) a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system; (c) a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system; (d) a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system; (e) a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system; (f) a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or (g) a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system

In some embodiments, the anti- CD28 antibody molecule described herein comprises: (i) a VH comprising the amino acid sequence of SEQ ID NO: 547 or 548, or a sequence with at least 95% sequence identity to SEQ ID NO: 547 or 548; and/or (ii) a VL comprising the amino acid sequence of SEQ ID NO: 537, or a sequence with at least 95% sequence identity thereto. In some embodiments, the anti- CD28 antibody molecule comprises: (i) a VH comprising the amino acid sequence of SEQ ID NO: 547 or a sequence with at least 95% sequence identity thereto, and a VL comprising the amino acid sequence of SEQ ID NO: 537, or a sequence with at least 95% sequence identity thereto; or (ii) a VH comprising the amino acid sequence of SEQ ID NO: 548 or a sequence with at least 95% sequence identity thereto, and a VL comprising the amino acid sequence of SEQ ID NO: 537, or a sequence with at least 95% sequence identity thereto. In some embodiments, the anti- CD28 antibody molecule is a human antibody, a full length antibody, a bispecific antibody, Fab, F(ab')2, Fv, or a single chain Fv fragment (scFv). In some embodiments, the antibody molecule comprises a heavy chain constant region selected from IgG1, IgG2, IgG3, and IgG4, and a light chain constant region chosen from the light chain constant regions of kappa or lambda.

In some aspects, the disclosure features an antibody molecule that (i) competes for binding to CD28 with an anti-CD28 antibody molecule described herein; and/or (ii) binds to the same epitope as, substantially the same epitope as, an epitope that overlaps with, or an epitope that substantially overlaps with, the epitope of an anti-CD28 antibody molecule described herein.

In some aspects, the disclosure features an antibody molecule comprising an Fc region that (i) competes for binding to CD28 with an anti-CD28 antibody molecule described herein; and/or (ii) binds to the same epitope as, substantially the same epitope as, an epitope that overlaps with, or an epitope that substantially overlaps with, the epitope of an anti-CD28 antibody molecule described herein, wherein the Fc region is silenced by a combination of amino acid substitutions selected from the group consisting of LALGA (L234A, L235A, and G237A), LALASKPA (L234A, L235A, S267K, and P329A), DAPASK (D265A, P329A, and S267K), GADAPA (G237A, D265A, and P329A), GADAPASK (G237A, D265A, P329A, and S267K), LALAPG (L234A, L235A, and P329G), and LALAPA (L234A, L235A, and P329A), wherein the amino acid residues are numbered according to the EU numbering system.

In some aspects, the disclosure features a multispecific binding molecule comprising: (i) an anti-CD3 binding domain, and (ii) a CD28 antigen binding domain comprising an anti-CD28 antibody molecule described herein. In some embodiments, the anti-CD3 binding domain comprises: (i) a HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 of an anti-CD3 antibody molecule of Table 27 (for example the anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4)); (ii) the amino acid sequence of any one of the VH and/or VL region of an anti-CD3 antibody molecule provided in Table 27 (for example the anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4)), or an amino acid sequence at least 95% identical thereto.

In some aspects, the disclosure features a multispecific binding molecule comprising: (i) an anti-CD3 binding domain, (ii) an anti-CD28 binding domain comprising a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, and a HCDR3, and a light chain variable region (VL) comprising a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3, wherein: (a)

the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 538, 539, 540, 530, 531, and 532, respectively; (b) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 541, 539, 540, 530, 531, and 532, respectively; (c) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 542, 543, 540, 533, 534, and 535, respectively; or (d) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 544, 545, 546, 536, 534, and 532, respectively; and (iii) an Fc region comprising: a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system; a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system; a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system; a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system; a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system; a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system.

In some aspects, the disclosure features a multispecific binding molecule, comprising a first binding domain and a second binding domain: (i) a first polypeptide comprising from N-terminal to C-terminal: VH of the first binding domain, VL of the first binding domain, VH of the second binding domain, CH1, an Fc region comprising a CH2 and a CH3; and (ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the second binding domain and CL; wherein the Fc region comprises: a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system; a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system; a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system; a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system; a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system; a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system. In some embodiments, the first

binding domain comprises an anti-CD3 binding domain and the second binding domain comprises a costimulatory molecule binding domain. In some embodiments, the first binding domain comprises a costimulatory molecule binding domain and the second binding domain comprises an anti-CD3 binding domain. In some embodiments, the costimulatory molecule binding domain comprises an anti-CD2 binding domain or an anti-CD28 binding domain.

In some aspects, the disclosure features a multispecific binding molecule comprising a first binding domain and a second binding domain: (i) a first polypeptide comprising from N-terminal to C-terminal: VH of the second binding domain, CH1, and an Fc region comprising a CH2 and a CH3, VH of the first binding domain, and VL of the first binding domain; and (ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the second binding domain and CL; wherein the Fc region comprises: a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system; a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system; a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system; a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system; a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system; a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system.

In some embodiments, the first binding domain comprises an anti-CD3 binding domain and the second binding domain comprises a costimulatory molecule binding domain. In some embodiments, the first binding domain comprises a costimulatory molecule binding domain and the second binding domain comprises an anti-CD3 binding domain. In some embodiments, the costimulatory molecule binding domain comprises an anti-CD2 binding domain or an anti-CD28 binding domain.

In some aspects, the disclosure features a multispecific binding molecule comprising a first binding domain and a second binding domain: (i) a first polypeptide comprising from N-terminal to C-terminal: VH of the second binding domain, CH1, VH of the first binding domain, VL of the first binding domain, an Fc region comprising a CH2 and a CH3; and (ii) a

second polypeptide comprising from N-terminal to C-terminal: VL of the second binding domain and CL; wherein the Fc region comprises: a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system; a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system; a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system; a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system; a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system; a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system. In some embodiments, the first binding domain comprises an anti-CD3 binding domain and the second binding domain comprises a costimulatory molecule binding domain. In some embodiments, the first binding domain comprises a costimulatory molecule binding domain and the second binding domain comprises an anti-CD3 binding domain. In some embodiments, the costimulatory molecule binding domain comprises an anti-CD2 binding domain or an anti-CD28 binding domain.

In some embodiments, the Fc region, e.g., the Fc region of a multispecific binding molecule described herein or to be used in a method described herein, is silenced by a combination of amino acid substitutions selected from the group consisting of LALGA (L234A, L235A, and G237A), LALASKPA (L234A, L235A, S267K, and P329A), DAPASK (D265A, P329A, and S267K), GADAPA (G237A, D265A, and P329A), GADAPASK (G237A, D265A, P329A, and S267K), LALAPG (L234A, L235A, and P329G), and LALAPA (L234A, L235A, and P329A), wherein the amino acid residues are numbered according to the EU numbering system.

In some embodiments, the Fc region, e.g., the Fc region of a multispecific binding molecule described herein or to be used in a method described herein, comprises: a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system; a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system; a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system; a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system; a D265A, P329A, and S267K mutation

(DAPASK), numbered according to the EU numbering system; a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system.

In some embodiments, a multispecific binding molecule comprises a heavy chain comprising the amino acid sequence of any of SEQ ID NOs: 794, 795, 798, 800, or 815-816 or an amino acid sequence having at least 95% sequence identity thereto; and/or a light chain comprising the amino acid sequence of any of SEQ ID NOs: 673, 796, 797, 799, or 801, or an amino acid sequence having at least 95% sequence identity thereto. In some embodiments, the multispecific binding molecule comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 794 or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 796, or an amino acid sequence having at least 95% sequence identity thereto. In some embodiments, the multispecific binding molecule comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 794 or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 797, or an amino acid sequence having at least 95% sequence identity thereto. In some embodiments, the multispecific binding molecule comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 795 or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 796, or an amino acid sequence having at least 95% sequence identity thereto. In some embodiments, the multispecific binding molecule comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 795 or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 797, or an amino acid sequence having at least 95% sequence identity thereto. In some embodiments, the multispecific binding molecule comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 798 or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 799, or an amino acid sequence having at least 95% sequence identity thereto. In some embodiments, the multispecific binding molecule comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 815 or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 799, or an amino acid

sequence having at least 95% sequence identity thereto. In some embodiments, the multispecific binding molecule comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 800 or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 801, or an amino acid sequence having at least 95% sequence identity thereto. In some embodiments, the multispecific binding molecule comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 816 or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 673, or an amino acid sequence having at least 95% sequence identity thereto. In some embodiments, the multispecific binding molecule comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 817 or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 673, or an amino acid sequence having at least 95% sequence identity thereto.

In some aspects, the disclosure features a method of activating cells (e.g., immune effector cells, e.g., T cells), comprising contacting (for example, binding) a population of cells (for example, T cells, for example, T cells isolated from a frozen or fresh leukapheresis product) with a multispecific binding molecule described herein.

In some aspects, the disclosure features a method of transducing cells (e.g., immune effector cells, e.g., T cells), comprising contacting (for example, binding) a population of cells (for example, T cells, for example, T cells isolated from a frozen or fresh leukapheresis product) with (i) a multispecific binding molecule described herein and (ii) a nucleic acid molecule, e.g., a nucleic acid molecule encoding a CAR.

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following enumerated embodiments.

**Enumerated Embodiments**

1. A method of making a population of cells (for example, T cells) that express a chimeric antigen receptor (CAR), the method comprising:

(i) contacting (for example, binding) a population of cells (for example, T cells, for example, T cells isolated from a frozen or fresh leukapheresis product) with a multispecific binding molecule comprising (A) an anti-CD3 binding domain, (B) a costimulatory molecule binding domain (e.g., an anti-CD2 binding domain or an anti-CD28 binding domain), and (C) an Fc region comprising:

a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system;

a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system;

a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system;

a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system;

a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system;

a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or

a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system;

(ii) contacting the population of cells (for example, T cells) with a nucleic acid molecule (for example, a DNA or RNA molecule) encoding the CAR, thereby providing a population of cells (for example, T cells) comprising the nucleic acid molecule, and

(iii) harvesting the population of cells (for example, T cells) for storage (for example, reformulating the population of cells in cryopreservation media) or administration, wherein:

(a) step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), for example, no later than 12, 13, 14, 15, 16, 17, or 18 hours after the beginning of step (i), for example, no later than 18 hours after the beginning of step (i), and

step (iii) is performed no later than 30 (for example, 26) hours after the beginning of step (i), for example, no later than 22, 23, 24, 25, 26, 27, 28, 29, or 30 hours after the beginning of step (i), for example, no later than 24 hours after the beginning of step (i),

(b) step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), for example, no later than 12, 13, 14, 15, 16, 17, or 18 hours after the beginning of step (i), for example, no later than 18 hours after the beginning of step (i), and

step (iii) is performed no later than 30 hours after the beginning of step (ii), for example, no later than 22, 23, 24, 25, 26, 27, 28, 29, or 30 hours after the beginning of step (ii), or

(c) the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (i),

optionally wherein the nucleic acid molecule in step (ii) is on a viral vector, optionally wherein the nucleic acid molecule in step (ii) is an RNA molecule on a viral vector, optionally wherein step (ii) comprises transducing the population of cells (for example, T cells) with a viral vector comprising a nucleic acid molecule encoding the CAR.

2. The method of embodiment 1, wherein:

(i) the anti-CD3 binding domain, e.g., an anti-CD3 scFv, is situated N-terminal of the costimulatory molecule binding domain, e.g., an anti-CD2 Fab or an anti-CD28 Fab; or

(ii) the anti-CD3 binding domain, e.g., an anti-CD3 scFv, is situated C-terminal of the costimulatory molecule binding domain, e.g., an anti-CD2 Fab or an anti-CD28 Fab.

3. The method of embodiment 1 or 2, wherein the Fc region comprises a CH2.

4. The method of any one of embodiments 1-3, wherein the Fc region comprises a CH3.

5. The method of any one of embodiments 1-4, wherein the anti-CD3 binding domain is situated C-terminal of the Fc region.

6. The method of any one of embodiments 1-4, wherein the anti-CD3 binding domain is situated N-terminal of the Fc region.

7. The method of any one of embodiments 1-6, wherein the Fc region is situated between the anti-CD3 binding domain and the costimulatory molecule binding domain.

8. The method of any one of embodiments 1-4 or 6, wherein the multispecific binding molecule comprises:

(i) a first polypeptide comprising from N-terminal to C-terminal: VH of the anti-CD3 binding domain, VL of the anti-CD3 binding domain, VH of the costimulatory molecule binding domain, CH1, CH2, and CH3; and

(ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the costimulatory molecule binding domain and CL.

9. The method of any one of embodiments 1-5 or 7, wherein the multispecific binding molecule comprises:

(i) a first polypeptide comprising from N-terminal to C-terminal: VH of the costimulatory molecule binding domain, CH1, CH2, CH3, VH of the anti-CD3 binding domain, and VL of the anti-CD3 binding domain; and

(ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the costimulatory molecule binding domain and CL.

10. The method of any one of embodiments 1-4 or 6, wherein the multispecific binding molecule comprises:

(i) a first polypeptide comprising from N-terminal to C-terminal: VH of the costimulatory molecule binding domain, CH1, VH of the anti-CD3 binding domain, VL of the anti-CD3 binding domain, CH2, and CH3; and

(ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the costimulatory molecule binding domain and CL.

11. The method of any one of embodiments 1-10, wherein the anti-CD3 binding domain comprises an scFv and the costimulatory molecule binding domain is part of a Fab fragment.

12. The method of any one of embodiments 1-11, wherein the anti-CD3 binding domain comprises:

(i) a variable heavy chain region (VH) comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, and a HCDR3, and a light chain variable region (VL) comprising a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3 of an anti-CD3 antibody molecule of Table 27 (for example the anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4)); and/or

(ii) the amino acid sequence of any VH and/or VL region of an anti-CD3 antibody molecule provided in Table 27 (for example the anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4)), or an amino acid sequence at least 95% identical thereto.

13. The method of any one of embodiments 1-12, wherein the costimulatory molecule binding domain is an anti-CD2 binding domain, optionally wherein the anti-CD2 binding domain comprises:

(i) a VH comprising a HCDR1, a HCDR2, and a HCDR3, and a VL comprising a LCDR1, a LCDR2, and a LCDR3 of an anti-CD2 antibody molecule of Table 27 (for example the anti-CD2 (1)); and/or

(ii) the amino acid sequence of any VH and/or VL region of an anti-CD2 antibody molecule provided in Table 27 (for example the anti-CD2 (1)), or an amino acid sequence at least 95% identical thereto.

14. The method of any one of embodiments 1-13, wherein the costimulatory molecule binding domain is an anti-CD28 binding domain, optionally wherein the anti-CD28 binding domain comprises:

(i) a VH comprising a HCDR1, a HCDR2, and a HCDR3, and a VL comprising a LCDR1, a LCDR2, and a LCDR3 of an anti-CD28 antibody molecule of Table 27 (for example the anti-CD28 (1) or anti-CD28 (2)); and/or

(ii) the amino acid sequence of any VH and/or VL region of an anti-CD28 antibody molecule provided in Table 27 (for example the anti-CD28 (1) or anti-CD28 (2)), or an amino acid sequence at least 95% identical thereto.

15. The method of any one of embodiments 1-14, wherein the anti-CD3 binding domain comprises:

(i) an scFv;

(ii) a VH linked to a VL by a peptide linker, e.g., a glycine-serine linker, e.g., a $(G_4S)_4$ linker; or

(iii) a VH and a VL, wherein the VH is N-terminal of the VL.

16. The method of any one of embodiments 1-15, wherein the costimulatory molecule binding domain is part of a Fab fragment, e.g., a Fab fragment that is part of a polypeptide sequence that comprises the Fc region.

17. The method of any one of embodiments 1-16, wherein the anti-CD3 binding domain is situated N-terminal of the costimulatory molecule binding domain, optionally wherein the anti-CD3 binding domain is linked to the costimulatory molecule binding domain by a peptide linker, e.g., a glycine-serine linker, e.g., a $(G_4S)_4$ linker.

18. The method of any one of embodiments 1-7 or 9-17, wherein the anti-CD3 binding domain is situated C-terminal of the costimulatory molecule binding domain.

19. The method of embodiment 18, wherein:

(i) the Fc region is situated between the anti-CD3 binding domain and the costimulatory molecule binding domain; and/or

(ii) the multispecific binding molecule comprises one or both of a CH2 and a CH3, optionally wherein the anti-CD3 binding domain is linked to the CH3 by a peptide linker, e.g., a glycine-serine linker, e.g., a $(G_4S)_4$ linker.

20. The method of embodiment 18, wherein:

(i) the multispecific binding molecule comprises a CH2, and the anti-CD3 binding domain is situated N-terminal of the CH2;

(ii) the anti-CD3 binding domain is linked to a CH1 by a peptide linker, e.g., a glycine-serine linker, e.g., a $(G_4S)_2$ linker; and/or

(iii) the anti-CD3 binding domain is linked to a CH2 by a peptide linker, e.g., a glycine-serine linker, e.g., a $(G_4S)_4$ linker.

21. The method of any one of embodiments 1-20, wherein step (i) increases the percentage of CAR-expressing cells in the population of cells from step (iii), for example, the population of cells from step (iii) shows a higher percentage of CAR-expressing cells (for example, at least 10, 20, 30, 40, 50, or 60% higher), compared with cells made by an otherwise similar method without step (i).

22. The method of any one of embodiments 1-21, wherein:

(a) the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) is the same as or differs by no more than 5 or 10% from the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (i);

(b) the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) is increased by, for example, at least 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, or 3-fold, as compared to the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (i);

(c) the percentage of CAR-expressing naïve T cells, for example, CAR-expressing CD45RA+ CD45RO- CCR7+ T cells in the population of cells increases during the duration of step (ii), for example, increases by, for example, at least 30, 35, 40, 45, 50, 55, or 60%, between 18-24 hours after the beginning of step (ii); or

(d) the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) does not decrease, or decreases by no more than 5 or 10%, as compared to the percentage of naïve cells, for example,

naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (i).

23. The method of any one of embodiments 1-22, wherein:

(a) the population of cells from step (iii) shows a higher percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells (for example, at least 10, 20, 30, or 40% higher), compared with cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(b) the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) is higher (for example, at least 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, or 3-fold higher) than the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(c) the percentage of CAR-expressing naïve T cells, for example, CAR-expressing CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) is higher (for example, at least 4, 6, 8, 10, or 12-fold higher) than the percentage of CAR-expressing naïve T cells, for example, CAR-expressing CD45RA+ CD45RO- CCR7+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(d) the population of cells from step (iii) shows a higher percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells (for example, at least 10, 20, 30, or 40% higher), compared with cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(e) the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) is higher (for example, at least 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, or 3-fold higher) than the percentage of naïve cells,

for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days; or

(f) the percentage of CAR-expressing naïve T cells, for example, CAR-expressing CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) is higher (for example, at least 4, 6, 8, 10, or 12-fold higher) than the percentage of CAR-expressing naïve T cells, for example, CAR-expressing CD45RA+ CD45RO- CCR7+ T cells, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

24. The method of any one of embodiments 1-23, wherein:

(a) the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells from step (iii) is the same as or differs by no more than 5 or 10% from the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the beginning of step (i);

(b) the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in the population of cells from step (iii) is reduced by at least 20, 25, 30, 35, 40, 45, or 50%, as compared to the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in the population of cells at the beginning of step (i);

(c) the percentage of CAR-expressing central memory T cells, for example, CAR-expressing CCR7+CD45RO+ cells, decreases during the duration of step (ii), for example, decreases by, for example, at least 8, 10, 12, 14, 16, 18, or 20%, between 18-24 hours after the beginning of step (ii); or

(d) the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in the population of cells from step (iii) does not increase, or increases by no more than 5 or 10%, as compared to the percentage of central memory cells,

for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in the population of cells at the beginning of step (i).

25. The method of any one of embodiments 1-24, wherein:

(a) the population of cells from step (iii) shows a lower percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells (for example, at least 10, 20, 30, or 40% lower), compared with cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(b) the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells in the population of cells from step (iii) is lower (for example, at least 20, 30, 40, or 50% lower) than the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(c) the percentage of CAR-expressing central memory T cells, for example, CAR-expressing CCR7+CD45RO+ T cells in the population of cells from step (iii) is lower (for example, at least 10, 20, 30, or 40% lower) than the percentage of CAR-expressing central memory T cells, for example, CAR-expressing CCR7+CD45RO+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(d) the population of cells from step (iii) shows a lower percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells (for example, at least 10, 20, 30, or 40% lower), compared with cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(e) the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells in the population of cells from step (iii) is lower (for

example, at least 20, 30, 40, or 50% lower) than the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days; or

(f) the percentage of CAR-expressing central memory T cells, for example, CAR-expressing CCR7+CD45RO+ T cells in the population of cells from step (iii) is lower (for example, at least 10, 20, 30, or 40% lower) than the percentage of CAR-expressing central memory T cells, for example, CAR-expressing CCR7+CD45RO+ T cells, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

26. The method of any one of embodiments 1-25, wherein:

(a) the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is increased, as compared to the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells at the beginning of step (i);

(b) the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is increased, as compared to the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells at the beginning of step (i);

(c) the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is higher than the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i); or

(d) the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of

cells from step (iii) is higher than the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(e) the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is higher than the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days; or

(f) the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is higher than the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

27. The method of any one of embodiments 1-26, wherein:

(a) the median GeneSetScore (Up TEM vs. Down TSCM) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 75, 100, or 125% from the median GeneSetScore (Up TEM vs. Down TSCM) of the population of cells at the beginning of step (i);

(b) the median GeneSetScore (Up TEM vs. Down TSCM) of the population of cells from step (iii) is lower (for example, at least about 100, 150, 200, 250, or 300% lower) than the median GeneSetScore (Up TEM vs. Down TSCM) of:

cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or

cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(c) the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 100, 150, or 200% from the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells at the beginning of step (i);

(d) the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells from step (iii) is lower (for example, at least about 50, 100, 125, 150, or 175% lower) than the median GeneSetScore (Up Treg vs. Down Teff) of:

cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or

cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(e) the median GeneSetScore (Down stemness) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 100, 150, 200, or 250% from the median GeneSetScore (Down stemness) of the population of cells at the beginning of step (i);

(f) the median GeneSetScore (Down stemness) of the population of cells from step (iii) is lower (for example, at least about 50, 100, or 125% lower) than the median GeneSetScore (Down stemness) of:

cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or

cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(g) the median GeneSetScore (Up hypoxia) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about

125, 150, 175, or 200% from the median GeneSetScore (Up hypoxia) of the population of cells at the beginning of step (i);

(h) the median GeneSetScore (Up hypoxia) of the population of cells from step (iii) is lower (for example, at least about 40, 50, 60, 70, or 80% lower) than the median GeneSetScore (Up hypoxia) of:

cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or

cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(j) the median GeneSetScore (Up autophagy) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 180, 190, 200, or 210% from the median GeneSetScore (Up autophagy) of the population of cells at the beginning of step (i); or

(k) the median GeneSetScore (Up autophagy) of the population of cells from step (iii) is lower (for example, at least 20, 30, or 40% lower) than the median GeneSetScore (Up autophagy) of:

cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or

cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

28. The method of any one of embodiments 1-27, wherein the population of cells from step (iii), after being incubated with a cell expressing an antigen recognized by the CAR, secretes IL-2 at a higher level (for example, at least 2, 4, 6, 8, 10, 12, or 14-fold higher) than cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or cells made by an otherwise similar method which further comprises, after step (ii)

and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days, for example, as assessed using methods described in Example 8 with respect to FIGs. 29C-29D.

29. The method of any one of embodiments 1-28, wherein the population of cells from step (iii), after being administered in vivo, persists longer or expands at a higher level (for example, as assessed using methods described in Example 1 with respect to FIG. 4C), compared with cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or compared with cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

30. The method of any one of embodiments 1-29, wherein the population of cells from step (iii), after being administered in vivo, shows a stronger anti-tumor activity (for example, a stronger anti-tumor activity at a low dose, for example, a dose no more than $0.15 \times 10^6$, $0.2 \times 10^6$, $0.25 \times 10^6$, or $0.3 \times 10^6$ viable CAR-expressing cells) than cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

31. The method of any one of embodiments 1-30, the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (i), optionally wherein the number of living cells in the population of cells from step (iii) decreases from the number of living cells in the population of cells at the beginning of step (i).

32. The method of any one of embodiments 1-31, wherein the population of cells from step (iii) are not expanded, or expanded by less than 2 hours, for example, less than 1 or 1.5 hours, compared to the population of cells at the beginning of step (i).

33. The method of any one of embodiments 1-32, wherein steps (i) and/or (ii) are performed in cell media (for example, serum-free media) comprising IL-2, IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)), IL-7, IL-21, IL-6 (for example, IL-6/sIL-6Ra), a LSD1 inhibitor, a MALT1 inhibitor, or a combination thereof.

34. The method of any one of embodiments 1-33, wherein steps (i) and/or (ii) are performed in serum-free cell media comprising a serum replacement.

35. The method of embodiment 30, wherein the serum replacement is CTS™ Immune Cell Serum Replacement (ICSR).

36. The method of any one of embodiments 1-35, further comprising prior to step (i):

(iv) (optionally) receiving a fresh leukapheresis product (or an alternative source of hematopoietic tissue such as a fresh whole blood product, a fresh bone marrow product, or a fresh tumor or organ biopsy or removal (for example, a fresh product from thymectomy)) from an entity, for example, a laboratory, hospital, or healthcare provider, and

(v) isolating the population of cells (for example, T cells, for example, CD8+ and/or CD4+ T cells) contacted in step (i) from a fresh leukapheresis product (or an alternative source of hematopoietic tissue such as a fresh whole blood product, a fresh bone marrow product, or a fresh tumor or organ biopsy or removal (for example, a fresh product from thymectomy)), optionally wherein:

step (iii) is performed no later than 35 hours after the beginning of step (v), for example, no later than 27, 28, 29, 30, 31, 32, 33, 34, or 35 hours after the beginning of step (v), for example, no later than 30 hours after the beginning of step (v), or

the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the end of step (v).

37. The method of any one of embodiments 1-36, further comprising prior to step (i): receiving cryopreserved T cells isolated from a leukapheresis product (or an alternative source of hematopoietic tissue such as cryopreserved T cells isolated from whole blood, bone marrow, or tumor or organ biopsy or removal (for example, thymectomy)) from an entity, for example, a laboratory, hospital, or healthcare provider.

38. The method of any one of embodiments 1-36, further comprising prior to step (i):

(iv) (optionally) receiving a cryopreserved leukapheresis product (or an alternative source of hematopoietic tissue such as a cryopreserved whole blood product, a cryopreserved bone marrow product, or a cryopreserved tumor or organ biopsy or removal (for example, a cryopreserved product from thymectomy)) from an entity, for example, a laboratory, hospital, or healthcare provider, and

(v) isolating the population of cells (for example, T cells, for example, CD8+ and/or CD4+ T cells) contacted in step (i) from a cryopreserved leukapheresis product (or an alternative source of hematopoietic tissue such as a cryopreserved whole blood product, a cryopreserved bone marrow product, or a cryopreserved tumor or organ biopsy or removal (for example, a cryopreserved product from thymectomy)), optionally wherein:

step (iii) is performed no later than 35 hours after the beginning of step (v), for example, no later than 27, 28, 29, 30, 31, 32, 33, 34, or 35 hours after the beginning of step (v), for example, no later than 30 hours after the beginning of step (v), or

the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the end of step (v).

39. The method of any one of embodiments 1-38, further comprising step (vi):

culturing a portion of the population of cells from step (iii) for at least 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, or 7 days, for example, at least 2 days and no more than 7 days, and measuring CAR expression level in the portion (for example, measuring the percentage of viable, CAR-expressing cells in the portion), optionally wherein:

step (iii) comprises harvesting and freezing the population of cells (for example, T cells) and step (vi) comprises thawing a portion of the population of cells from step (iii), culturing the portion for at least 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, or 7 days, for example, at least 2 days and no more than 7 days, and measuring CAR expression level in the portion (for example, measuring the percentage of viable, CAR-expressing cells in the portion).

40. The method of any one of embodiments 1-39, wherein step (ii) further comprises adding F108 during transduction and/or contacting the population of cells (for example, T cells) with an shRNA that targets Tet2.

41. The method of any one of embodiments 1-40, wherein the population of cells at the beginning of step (i) or step (1) has been enriched for IL6R-expressing cells (for example, cells that are positive for IL6Rα and/or IL6Rβ).

42. The method of any one of embodiments 1-41, wherein the population of cells at the beginning of step (i) or step (1) comprises no less than 50, 60, or 70% of IL6R-expressing cells (for example, cells that are positive for IL6Rα and/or IL6Rβ).

43. The method of any one of embodiments 1-42, wherein steps (i) and (ii) or steps (1) and (2) are performed in cell media comprising IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)).

44. The method of embodiment 43, wherein IL-15 increases the ability of the population of cells to expand, for example, 10, 15, 20, or 25 days later.

45. The method of embodiment 43, wherein IL-15 increases the percentage of IL6Rβ-expressing cells in the population of cells.

46. The method of any one of embodiments 1-45, wherein the CAR comprises an antigen binding domain, a transmembrane domain, and an intracellular signaling domain.

47. The method of embodiment 46, wherein the antigen binding domain binds to an antigen chosen from: CD19, CD20, CD22, BCMA, mesothelin, EGFRvIII, GD2, Tn antigen, sTn antigen, Tn-O-Glycopeptides, sTn-O-Glycopeptides, PSMA, CD97, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, leguman, GD3, CD171, IL-11Ra, PSCA, MAD-CT-1, MAD-CT-2, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBBs (for example, ERBB2), Her2/neu, MUC1, EGFR, NCAM, Ephrin B2, CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, FAP, Legumain, HPV E6 or E7, ML-IAP, CLDN6, TSHR, GPRC5D, ALK, Polysialic acid, Fos-related antigen, neutrophil elastase, TRP-2, CYP1B1, sperm protein 17, beta human chorionic gonadotropin, AFP, thyroglobulin, PLAC1, globoH, RAGE1, MN-CA IX, human telomerase reverse transcriptase, intestinal carboxyl esterase, mut hsp 70-2, NA-17, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, NY-ESO-1, GPR20, Ly6k, OR51E2, TARP, GFRα4, or a peptide of any of these antigens presented on MHC.

48. The method of embodiment 46 or 47, wherein the antigen binding domain comprises a CDR, VH, VL, scFv or CAR sequence disclosed herein, optionally wherein:

(a) the antigen binding domain binds to BCMA and comprises a CDR, VH, VL, scFv or CAR sequence disclosed in Tables 3-15, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto;

(b) the antigen binding domain binds to CD19 and comprises a CDR, VH, VL, scFv or CAR sequence disclosed in Table 2, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto;

(c) the antigen binding domain binds to CD20 and comprises a CDR, VH, VL, scFv or CAR sequence disclosed herein, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto; or

(d) the antigen binding domain binds to CD22 and comprises a CDR, VH, VL, scFv or CAR sequence disclosed herein, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto.

49. The method of any one of embodiments 46-48, wherein the antigen binding domain comprises a VH and a VL, wherein the VH and VL are connected by a linker, optionally wherein the linker comprises the amino acid sequence of SEQ ID NO: 63 or 104.

50. The method of any one of embodiments 46-49, wherein:

(a) the transmembrane domain comprises a transmembrane domain of a protein chosen from the alpha, beta or zeta chain of T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154,

(b) the transmembrane domain comprises a transmembrane domain of CD8,

(c) the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof, or

(d) the nucleic acid molecule comprises a nucleic acid sequence encoding the transmembrane domain, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 17, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof.

51. The method of any one of embodiments 46-50, wherein the antigen binding domain is connected to the transmembrane domain by a hinge region, optionally wherein:

(a) the hinge region comprises the amino acid sequence of SEQ ID NO: 2, 3, or 4, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof, or

(b) the nucleic acid molecule comprises a nucleic acid sequence encoding the hinge region, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 13, 14, or 15, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof.

52. The method of any one of embodiments 46-51, wherein the intracellular signaling domain comprises a primary signaling domain, optionally wherein the primary signaling domain comprises a functional signaling domain derived from CD3 zeta, TCR zeta, FcR gamma, FcR

beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (ICOS), FcεRI, DAP10, DAP12, or CD66d, optionally wherein:

(a) the primary signaling domain comprises a functional signaling domain derived from CD3 zeta,

(b) the primary signaling domain comprises the amino acid sequence of SEQ ID NO: 9 or 10, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof, or

(c) the nucleic acid molecule comprises a nucleic acid sequence encoding the primary signaling domain, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 20 or 21, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof.

53. The method of any one of embodiments 46-52, wherein the intracellular signaling domain comprises a costimulatory signaling domain, optionally wherein the costimulatory signaling domain comprises a functional signaling domain derived from a MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), an activating NK cell receptor, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, 4-1BB (CD137), B7-H3, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD28-OX40, CD28-4-1BB, or a ligand that specifically binds with CD83, optionally wherein:

(a) the costimulatory signaling domain comprises a functional signaling domain derived from 4-1BB,

(b) the costimulatory signaling domain comprises the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof, or

(c) the nucleic acid molecule comprises a nucleic acid sequence encoding the costimulatory signaling domain, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 18, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof.

54. The method of any one of embodiments 46-53, wherein the intracellular signaling domain comprises a functional signaling domain derived from 4-1BB and a functional signaling domain derived from CD3 zeta, optionally wherein the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 (or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof) and the amino acid sequence of SEQ ID NO: 9 or 10 (or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof), optionally wherein the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 and the amino acid sequence of SEQ ID NO: 9 or 10.

55. The method of any one of embodiments 46-54, wherein the CAR further comprises a leader sequence comprising the amino acid sequence of SEQ ID NO: 1.

56. A population of CAR-expressing cells (for example, autologous or allogeneic CAR-expressing T cells or NK cells) made by the method of any one of embodiments 1-55.

57. A pharmaceutical composition comprising the population of CAR-expressing cells of embodiment 56 and a pharmaceutically acceptable carrier.

58. A method of increasing an immune response in a subject, comprising administering the population of CAR-expressing cells of embodiment 56 or the pharmaceutical composition of embodiment 57 to the subject, thereby increasing an immune response in the subject.

59. A method of treating a cancer in a subject, comprising administering the population of CAR-expressing cells of embodiment 56 or the pharmaceutical composition of embodiment 57 to the subject, thereby treating the cancer in the subject.

60. The method of embodiment 59, wherein the cancer is a solid cancer, for example, chosen from: one or more of mesothelioma, malignant pleural mesothelioma, non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, large cell lung cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, esophageal adenocarcinoma , breast cancer, glioblastoma, ovarian cancer, colorectal cancer, prostate cancer, cervical cancer, skin cancer, melanoma, renal cancer, liver cancer, brain cancer, thymoma, sarcoma, carcinoma, uterine cancer, kidney cancer, gastrointestinal cancer, urothelial cancer, pharynx cancer, head and neck cancer, rectal cancer, esophagus cancer, or bladder cancer, or a metastasis thereof.

61. The method of embodiment 59, wherein the cancer is a liquid cancer, for example, chosen from: chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), multiple myeloma, acute lymphoid leukemia (ALL), Hodgkin lymphoma, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), small lymphocytic leukemia (SLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL), DLBCL associated with chronic inflammation, chronic myeloid leukemia, myeloproliferative neoplasms, follicular lymphoma, pediatric follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma (extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue), Marginal zone lymphoma, myelodysplasia, myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, splenic marginal zone lymphoma, splenic lymphoma/leukemia, splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant, lymphoplasmacytic lymphoma, a heavy chain disease, plasma cell myeloma, solitary plasmocytoma of bone, extraosseous plasmocytoma, nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, primary cutaneous follicle center lymphoma, lymphomatoid granulomatosis, primary mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, ALK+ large B-cell lymphoma, large B-cell lymphoma

arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, B-cell lymphoma, acute myeloid leukemia (AML), or unclassifiable lymphoma.

62. The method of any one of embodiments 58-61, further comprising administering a second therapeutic agent to the subject.

63. The method of any one of embodiments 58-62, wherein the population of CAR-expressing cells is administered at a dose determined based on the percentage of CAR-expressing cells measured in embodiment 39.

64. The population of CAR-expressing cells of embodiment 56 or the pharmaceutical composition of embodiment 57 for use in a method of increasing an immune response in a subject, said method comprising administering to the subject an effective amount of the population of CAR-expressing cells or an effective amount of the pharmaceutical composition.

65. The population of CAR-expressing cells of embodiment 56 or the pharmaceutical composition of embodiment 57 for use in a method of treating a cancer in a subject, said method comprising administering to the subject an effective amount of the population of CAR-expressing cells or an effective amount of the pharmaceutical composition.

66. A multispecific binding molecule comprising:

(i) an anti-CD3 binding domain,

(ii) an anti-CD28 binding domain comprising a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, and a HCDR3, and a light chain variable region (VL) comprising a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3 wherein:

(a) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 538, 539, 540, 530, 531, and 532, respectively;

(b) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 541, 539, 540, 530, 531, and 532, respectively;

(c) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 542, 543, 540, 533, 534, and 535, respectively; or

(d) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 544, 545, 546, 536, 534, and 532, respectively; and

(iii) an Fc region comprising:

a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system;

a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system;

a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system;

a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system;

a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system;

a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or

a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system.

67. The multispecific binding molecule of embodiment 66, wherein the anti-CD28 binding domain comprises:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 547 or 548, or a sequence with at least 95% sequence identity to SEQ ID NO: 547 or 548;

(ii) a VL comprising the amino acid sequence of SEQ ID NO: 537, or a sequence with at least 95% sequence identity thereto;

(iii) a VH comprising the amino acid sequence of SEQ ID NO: 547 or a sequence with at least 95% sequence identity thereto, and a VL comprising the amino acid sequence of SEQ ID NO: 537, or a sequence with at least 95% sequence identity thereto; or

(iv) a VH comprising the amino acid sequence of SEQ ID NO: 548 or a sequence with at least 95% sequence identity thereto, and a VL comprising the amino acid sequence of SEQ ID NO: 537, or a sequence with at least 95% sequence identity thereto.

68. The multispecific binding molecule of embodiment 66 or 67, which further comprises a light chain constant region chosen from the light chain constant regions of kappa or lambda.

69. The multispecific binding molecule of any one of embodiments 66-68, wherein the Fc region comprises a CH2, a CH3, or both a CH2 and CH3, optionally wherein the CH2 and/or CH3 are selected from IgG1, IgG2, IgG3, or IgG4.

70. The multispecific binding molecule of any one of embodiments 66-69, wherein the anti-CD3 binding domain comprises:

(i) a HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 of an anti-CD3 antibody molecule of Table 27 (for example the anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4)); or

(ii) the amino acid sequence of any VH and/or VL region of an anti-CD3 antibody molecule provided in Table 27 (for example the anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4)), or an amino acid sequence at least 95% identical thereto.

71. A multispecific binding molecule, comprising a first binding domain and a second binding domain, wherein the multispecific binding molecule comprises:

(i) a first polypeptide comprising from N-terminal to C-terminal: VH of the first binding domain, VL of the first binding domain, VH of the second binding domain, CH1 and an Fc region, which comprises a CH2 and a CH3; and

(ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the second binding domain and CL;

wherein the Fc region comprises:

a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system;

a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system;

a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system;

a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system;

a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system;

a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or

a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system.

72. A multispecific binding molecule, comprising a first binding domain and a second binding domain, wherein the multispecific binding molecule comprises:

(i) a first polypeptide comprising from N-terminal to C-terminal: VH of the second binding domain, CH1, an Fc region comprising a CH2 and a CH3, VH of the first binding domain, and VL of the first binding domain; and

(ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the second binding domain and CL;

wherein the Fc region comprises:

a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system;

a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system;

a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system;

a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system;

a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system;

a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or

a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system.

73. A multispecific binding molecule, comprising a first binding domain and a second binding domain, wherein the multispecific binding molecule comprises:

(i) a first polypeptide comprising from N-terminal to C-terminal: VH of the second binding domain, CH1, VH of the first binding domain, VL of the first binding domain, and an Fc region comprising a CH2 and a CH3; and

(ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the second binding domain and CL;

wherein the Fc region comprises:

a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system;

a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system;

a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system;

a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system;

a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system;

a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or

a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system.

74. The multispecific binding molecule of any one of embodiments 71-73, wherein the first binding domain comprises an anti-CD3 binding domain and the second binding domain comprises a costimulatory molecule binding domain.

75. The multispecific binding molecule of any one of embodiments 71-73, wherein the first binding domain comprises a costimulatory molecule binding domain and the second binding domain comprises an anti-CD3 binding domain.

76. The multispecific binding molecule of embodiment 74 or 75, wherein the costimulatory molecule binding domain comprises an anti-CD2 binding domain or an anti-CD28 binding domain.

77. The method of any one of embodiments 1-55, or the multispecific binding molecule of any one of embodiments 66-76, wherein the multispecific binding molecule comprises:

(i) a heavy chain comprising the amino acid sequence of any of SEQ ID NOs: 794, 795, 798, 800, or 815-817 or an amino acid sequence having at least 95% sequence identity thereto; and/or

(ii) a light chain comprising the amino acid sequence of any of SEQ ID NOs: 673, 796, 797, 799, or 801, or an amino acid sequence having at least 95% sequence identity thereto.

78. The method of any one of embodiments 1-55, or the multispecific binding molecule of any one of embodiments 66-77, wherein the multispecific binding molecule comprises:

(i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 794, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 796, or an amino acid sequence having at least 95% sequence identity thereto;

(ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 794, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 797, or an amino acid sequence having at least 95% sequence identity thereto;

(iii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 795, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 796, or an amino acid sequence having at least 95% sequence identity thereto;

(iv) a heavy chain comprising the amino acid sequence of SEQ ID NO: 795, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 797, or an amino acid sequence having at least 95% sequence identity thereto;

(v) a heavy chain comprising the amino acid sequence of SEQ ID NO: 798, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 799, or an amino acid sequence having at least 95% sequence identity thereto;

(vi) a heavy chain comprising the amino acid sequence of SEQ ID NO: 815, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 799, or an amino acid sequence having at least 95% sequence identity thereto;

(vii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 800, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 801, or an amino acid sequence having at least 95% sequence identity thereto;

(viii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 816, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 673, or an amino acid sequence having at least 95% sequence identity thereto; or

(ix) a heavy chain comprising the amino acid sequence of SEQ ID NO: 817, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 673, or an amino acid sequence having at least 95% sequence identity thereto.

79. A method of activating cells (e.g., immune effector cells, e.g., T cells), comprising contacting (for example, binding) a population of cells (for example, T cells, for example, T cells isolated from a frozen or fresh leukapheresis product) with the multispecific binding molecule of any one of embodiments 66-78.

80. A method of transducing cells (e.g., immune effector cells, e.g., T cells), comprising contacting (for example, binding) a population of cells (for example, T cells, for example, T cells isolated from a frozen or fresh leukapheresis product) with (i) the multispecific binding molecule of any one of embodiments 66-78, and (ii) a nucleic acid molecule, e.g., a nucleic acid molecule encoding a CAR.

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references (for example, sequence database reference numbers) mentioned herein are incorporated by reference in their entirety. For example, all GenBank, Unigene, and Entrez sequences referred to herein, for example, in any Table herein, are incorporated by reference. When one gene or protein references a plurality of sequence accession numbers, all of the sequence variants are encompassed.

In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Headings, sub-headings or numbered or lettered elements, for example, (a), (b), (i) etc., are presented merely for ease of reading. The use of headings or numbered or lettered elements in this document does not require the steps or elements be performed in alphabetical order or that the steps or elements are necessarily discrete from one another. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

## BRIEF DESCRIPTION OF THE FIGURES

**FIGs. 1A-1I**: When purified T cells were incubated with cytokines, the naïve cells were the predominant population transduced. FIG. 1A is a graph showing exemplary cytokine process. FIG. 1B is a pair of graphs showing the percentages of CD3+ CAR+ cells at each indicated time point after transduction. FIG. 1C is a set of graphs showing the transduction within the CD3+CCR7+CD45RO- population in a CD3/CD28 bead stimulated populations (left) compared to cytokines only populations (right) in two independent donors. For the sample referred to as "Short stim IL7+IL15" in FIG. 1C, the cells were stimulated with beads for 2 days and then they were removed in the presence of IL7 and IL15. FIGs. 1D, 1E, and 1F

are a set of flow cytometry graphs showing the transduction of T-cell subsets cultured with IL2 (FIG. 1D), IL15 (FIG. 1E), and IL7+IL15 (FIG. 1F) daily over a three-day period. FIG. 1G is a set of flow cytometry graphs showing the T cell differentiation on day 0 (left) and on day 1 (right) for CCR7 and CD45RO after stimulation with IL2 (upper right panel) or IL-15 (lower right panel). FIGs. 1H and 1I are a set of graphs showing the percentages of CD3+CCR7+RO-, CD3+CCR7+RO+, CD3+CCR7-RO+, and CD3+CCR7-RO- cells at day 0 or after 24-hour incubation with the indicated cytokines.

**FIGs. 2A-2D**: CARTs generated with one day of cytokine stimulation were functional. FIG. 2A: Purified T cells were transduced with a MOI of 1 and in all the cytokine conditions tested, the percentages of CAR-expressing cells observed at day 1 and day 10 were similar. The CARTs were generated within one day and expanded via CD3/CD28 beads after harvest for 9 days to mimic the in vivo setting. FIG. 2A is a pair of graphs showing the average percentages of CD3+ CAR+ cells under each condition for day 1 CARTs (left) and day 10 CARTs (right). FIG. 2B: The cytotoxicity capacity of the day 1 CARTs post expansion was measured using Nalm6 as the target cells. FIG. 2B is a graph showing % killing of CD19 positive Nalm6 cells by CARTs from each condition. Day 10 CARTs expanded using CD3/CD28 beads are marked as "Day 10." All the other samples were day 1 CARTs. FIG. 2C: The secretion of IFNg of the expanded day 1 CARTs in response to Nalm6 target cells was tested. FIG. 2C is a graph showing the amount of IFN-gamma secretion by CARTs from each condition in the presence of CD19 positive or CD19 negative target cells. FIG. 2D: The proliferative capacity of the day 1 CARTs was tested by measurement of the incorporation of EDU. FIG. 2D is a graph showing the average percentages of EDU-positive cells for each condition. Similar to FIG. 2B, day 10 CARTs are marked as "Day 10" and all the other samples were day 1 CARTs.

**FIGs. 3A-3B**: The impact of MOI and media composition on transduction on day 0. FIG. 3A: Purified T cells were transduced with a range of MOIs from 1 to 10 in the presence of IL15, IL2+IL15, IL2+IL7, or IL7+IL15. Regardless of cytokine used, a linear increase in transduction was observed. FIG. 3A is a set of graphs where the percentages of CD3+ CAR+ cells are plotted against MOIs for each condition tested. FIG. 3B: The composition of the media impacted the transduction in the cytokine process. FIG. 3B is a pair of graphs showing the percentages of CD3+ CAR+ cells on day 1 (left) or day 8 (right) for each condition tested. "2.50" indicates a MOI of 2.50. "5.00" indicates a MOI of 5.00.

**FIGs. 4A-4D**: CAR T cells generated within 24 hours can eliminate tumor. FIG. 4A: Purified T cells were transduced with an anti-CD19 CAR and 24 hours later were harvested. FIG. 4A is a set of flow cytometry plots showing the transduction of T cells with the anti-CD19 CAR that were cultured with IL2, IL15 and IL7+IL15, illustrating the transduction with each cytokine condition. FIG. 4B: A graph showing average viability which was above 80% in all the conditions tested. FIG. 4C: The expansion of the day 1 CARTs in the peripheral blood is increased *in vivo* as compared to their day 10 counterparts. The percentage of live CD45+CD11b-CD3+CAR+ cells at indicated time points after infusion for each condition tested. The day 10 CARTs are marked as "D10 1e6" or "D10 5e6" and all the other samples were day 1 CARTs. FIG. 4D: The day 1 CARTs could eliminate tumor in vivo although with a delayed kinetics as compared to the day 10 CARTs. FIG. 4D is a graph showing total flux at indicated time points after tumor inoculation for each condition tested. CARTs were administered 4 days after tumor inoculation. The day 10 CARTs are marked as "5e6 d. 10" and all the other samples were day 1 CARTs.

**FIGs. 5A-5B**: The cytokine process was scalable. FIG. 5A: The T cells were enriched on a CliniMACS® Prodigy® and the B cell compartment was reduced to less than 1%. FIG. 5A is a set of flow cytometry plots showing the staining of cells with an anti-CD3 antibody (left) or an anti-CD19 antibody and an anti-CD14 antibody (right) for leukopak cells (upper) or cells post CD4+CD8+ enrichment (lower). FIG. 5B: Purified T cells from a frozen apheresis were transduced with an anti-CD19 CAR in either a 24 well plate or a PL30 bag post enrichment. The CARTs were harvested 24 hours later. FIG. 5B is a set of flow cytometry plots showing staining for CD3 and CAR of cells manufactured in the presence of either IL2 or hetIL-15 (IL15/sIL-15Ra).

**FIGs. 6A-6C**: The CARTs manufactured by the activation process showed superior anti-tumor efficacy in vivo. FIGs. 6A and 6B are graphs where tumor burden is plotted against the indicated time point after tumor implantation. "d.1" indicates CARTs manufactured using the activation process. "d.9" indicates CARTs manufactured with a traditional 9-day expansion protocol, serving as a positive control in this study. FIG. 6C is a set of representative images showing bioluminescence from mice.

**FIGs. 7A-7B**: IL6Rα and IL6Rβ expressing cells were enriched in less differentiated T cell population. Fresh T cells were stained for indicated surface antigens and examined for expression levels of IL6Rα and IL6Rβ on CD4 (FIG. 7A) and CD8 (FIG. 7B) T cell subsets.

**FIGs. 8A and 8B**: Both IL6Rα and IL6Rβ expressing cells were enriched in less differentiated T cell population. Fresh T cells were stained for indicated surface antigens and examined for expression levels of indicated surface antigens on CD4 (FIG. 8A) and CD8 (FIG. 8B) T cell subsets.

**FIG. 9**: IL6Rα expressing cells expressed surface markers of less differentiated T cells. Fresh T cells were stained for indicated surface antigens and examined for expression levels of various surface antigens in IL6Rα high, middle, and low expressing cell subsets.

**FIG. 10**: IL6Rβ expressing cells expressed surface markers of less differentiated T cells. Fresh T cells were stained for indicated surface antigens and examined for expression levels of various surface antigens in IL6Rβ high, middle, and low expressing cell subsets.

**FIG. 11**: IL6Rα but not IL6Rβ expression was down-regulated following TCR engagement. T cells were activated with αCD3αCD28 beads at day 0 and then examined for expression levels of IL6Rα and IL6Rβ at indicated time points.

**FIG. 12**: Fold expansion of cytokine treated T cells after TCR engagement. T cells were activated with αCD3αCD28 beads at day 0 in the presence of indicated cytokines and then monitored for cell numbers at indicated time points.

**FIGs. 13A and 13B**: IL2, IL7, and IL15 treatment did not affect cell size and viability after TCR engagement. T cells were activated with αCD3αCD28 beads at day 0 in the presence of indicated cytokines and then monitored for cell size (FIG. 13A) and viability (FIG. 13B) at indicated time points.

**FIG. 14**: Expression kinetics of various surface molecules on CD4 T cells after cytokine treatment. T cells were activated with αCD3αCD28 beads at day 0 in the presence of indicated cytokines and then examined for expression of various surface molecules by flow cytometry at indicated time points.

**FIG. 15**: Expression kinetics of various surface molecules on CD8 T cells after cytokine treatment. T cells were activated with αCD3αCD28 beads at day 0 in the presence of indicated cytokines and then examined for expression of various surface molecules by flow cytometry at indicated time points.

**FIG. 16**: IL6Rβ expression was mainly restricted on CD27 expressing T cell subsets after TCR engagement. T cells were activated with αCD3αCD28 beads at day 0 in the presence of indicated cytokines and then examined for IL6Rβ expression by flow cytometry at day 15.

**FIG. 17**: IL6Rβ expression was mainly restricted on CD57 non-expressing T cell subsets after TCR engagement. T cells were activated with αCD3αCD28 beads at day 0 in the presence of indicated cytokines and then examined for IL6Rβ expression by flow cytometry at day 25.

**FIG. 18**: Common γ-chain cytokine treated T cells produced functional cytokines at day 25. T cells were activated with αCD3αCD28 beads at day 0 in the presence of indicated cytokines and then examined for percentages of IL2, IFNγ, and TNFα producing T cells by flow cytometry at day 25.

**FIGs. 19A and 19B**: BCMA CAR expression on Day 1 using ARM at MOI=2.5 in T cells from two healthy donors. FIG. 19A is a panel of histograms showing BCMA CAR expression as measured by flow cytometry. FIG. 19B is a table listing reagents/conditions used in the flow cytometry analysis.

**FIGs. 20A, 20B, and 20C**: *In vitro* CAR expression kinetics from day 1 to day 4 of cells manufactured using the ARM process. CARs were stably expressed on day 3. FIG. 20A is a panel of histograms showing CAR expression at the indicated time points measured by flow cytometry. FIGs. 20B and 20C are graphs showing CAR+% and MFI values over time, respectively.

**FIGs. 21A and 21B**: *In vivo* triage in a KMS-11-luc multiple myeloma xenograft mouse model. Each mouse received 1.5E6 of day 1 CART product. FIG. 21A is a panel of histograms showing the day 1 and day 7 CAR expression in the CART cells. FIG. 21B is a graph showing the tumor kinetics (BLI level) after CART treatment.

**FIGs. 22A, 22B, and 22C**: *In vivo* triage of BCMA CAR using dose titration in a KMS-11-luc multiple myeloma xenograft mouse model. FIG. 22A is a panel of histograms showing the CAR expression at day 1 and day 3. FIG. 22B is a graph showing tumor intake kinetics after CART treatment using two different doses: a dose of 1.5e5 CAR+ T cells and a dose of 5e4 CAR+ T cells. The doses of CAR+ cells were normalized based on the day 3 CAR expression. FIG. 22C is a graph showing body weight kinetics over the course of this study.

**FIGs. 23A, 23B, and 23C**. FIGs. 23A and 23B are graphs showing percentage of T cell expressing the CAR on their cell surface (FIG. 23A) and mean fluorescence intensity (MFI) of CD3+CAR+ cells (FIG. 23B) observed over time (replicate efficiencies are averaged from the two flow panels shown in FIG. 23C). FIG. 23C is a panel of flow cytometry plots showing gating strategy for surface CAR expression on viable CD3+ cells, as based on UTD samples. Numbers in the plots indicate percent CAR positive.

**FIGs. 24A and 24B**. FIG. 24A is a graph showing end-to-end composition of the starting material (Prodigy® product) and at harvest at various time points after culture initiation. Naive (n), central memory (cm), effector memory (em), and effector (eff) subsets were defined by CD4, CD8, CCR7, and CD45RO surface expression or lack thereof. CD4 composition is indicated. For each time point, the left bar shows cell composition of the overall CD3+ population (bulk) and the right bar shows cell composition of the CAR+ fraction. FIG. 24B is a panel of flow cytometry plots showing gating strategy applied on live CD3+ events to determine overall transduction efficiency (top row), CD4/CD8 composition (middle row), and memory subsets (bottom row) within the overall CD3+ population (bulk) and the CAR+ fraction.

**FIG. 25**. Kinetics of T cell subsets expressing surface CAR over time, expressed as number of viable cells in the respective subsets.

**FIG. 26**. Viable cell recovery (number of viable cells recovered at harvest versus number of viable cells seeded) 12 to 24 hours after culture initiation as determined from pre-wash counts.

**FIG. 27**. Viability of rapid CARTs harvested 12 to 24 hours after culture initiation, as determined pre-wash and post-wash at the time of harvest.

**FIGs. 28A, 28B, 28C, and 28D**. FIG. 28A is a graph showing composition of the starting material (healthy donor leukopak; LKPK) and the T cell-enriched product as analyzed by flow cytometry. Numbers indicate % of parent (live, single cells). T: T cells; mono: monocytes; B: B cells; CD56 (NK): NK cells. FIG. 28B is a panel of flow cytometry plots showing gating strategy on live CD3+ events used to determine transduction rate (forward scatter FSC vs. CAR) and T cell subsets (CD4 vs. CD8 and CCR7 vs. CD45RO). For ARM-CD19 CAR (CD19 CART cells manufactured using the Activated Rapid Manufacturing (ARM) process) and TM-CD19 CAR (CD19 CART cells manufactured using the traditional

manufacturing (TM) process), the left lower panels represent bulk cultures, while the right panels represent CAR+ T cells. "ARM-UTD" and "TM-UTD" refer to untransduced T cells (UTD) manufactured according to the ARM and the TM processes, respectively. Numbers in quadrants indicate % of parental population. Boxes in the TM-UTD and TM-CD19 CAR plots indicate skewing toward a $T_{CM}$ phenotype for the TM process. Boxes in the ARM-UTD and ARM-CD19 CAR plots indicate the maintenance of naïve-like cells by the ARM process. NA: not applicable. FIG. 28C is a graph showing end-to-end T cell composition of ARM-CD19 CAR and TM-CD19 CAR. Composition is shown for "bulk" and "CAR+" populations where applicable. The percentage of the respective populations refers to % of parental, either CD3+ or CAR+CD3+ as applicable. The % of CD4 cells of the respective bulk or CAR+ population is indicated. LKPK: Leukopak starting material; 4 and 8: CD4+ and CD8+, respectively; eff: effector; em: effector memory; cm: central memory; n: naïve-like. Data is representative of 3 full-scale runs with 3 different healthy donors (n= 3) and several small-scale runs used to optimize the process. FIG. 28D is a table showing the percentages shown in FIG. 28C.

**FIGs. 29A, 29B, 29C, and 29D**. Cytokine concentration in cell culture supernatants. IFN-γ (FIGs. 29A and 29B) and IL-2 (FIGs. 29C and 29D). FIGs. 29A and 29C: TM-CD19 CAR, ARM-CD19 CAR, and respective UTD were co-cultured with NALM6-WT (ALL), TMD-8 (DLBCL), or without cancer cells (T cells alone). Supernatant was collected 48h later. FIGs. 29B and 29D: ARM-CD19 CAR was cocultured with NALM6-WT, NALM6-19KO (CD19-negative) or alone. Supernatant was collected after 24h or 48h. To further assess antigen-specific cytokine secretion, ARM-CD19 CAR was cultured alone for 24h, washed and then co-cultured with target cells for 24h. Data shown is derived from 2 healthy donor T cells and is representative of 2 experiments with three donors total.

**FIGs. 30A, 30B, and 30C**. FIG. 30A is a graph outlining the xenograft mouse model to study the anti-tumor activity of ARM-CD19 CAR. FIG. 30B is a panel of flow cytometry plots showing determination of CAR expression on ARM-CD19 CAR cells from a sentinel vial. ARM-CD19 CAR cells were cultured for the time period described in the figure, prior to flow-cytometry analysis. Gating for CAR expression was based on an isotype control (Iso) staining. FIG. 30C is a graph showing in vivo efficacy of ARM-CD19 CAR in the xenograft mouse model. NSG mice were injected with the pre-B ALL line NALM6, expressing the luciferase reporter gene; the tumor burden is expressed as total body luminescence (p/s),

depicted as mean tumor burden with 95% confidence interval. On day 7 post tumor inoculation, mice were treated with ARM-CD19 CAR or TM-CD19 CAR at the respective doses (number of viable CAR+ T cells). High dose ARM-CD19 CAR group was terminated on day 33 due to onset of X-GVHD. Vehicle (PBS) and non-transduced T cells (UTD) served as negative controls. n=5 mice for all groups, except n=4 for ARM-UTD $1\times10^6$ dose and all TM-CD19 CAR dose groups. Five xenograft studies were run with CAR-T cells generated from 5 different healthy donors, three of which included a comparison to TM-CD19 CAR.

**FIGs. 31A, 31B, 31C, and 31D**. Plasma cytokine levels of NALM6 tumor-bearing mice treated with ARM-CD19 CAR or TM-CD19 CAR at respective CAR-T cell doses. Mice were bled and plasma cytokine measured by MSD assay. IFN-γ (FIGs. 31A and 31B) and IL-2 (FIGs. 31C and 31D) are shown for mice treated with CAR-T (FIGs. 31A and 31C) or ARM- and TM-UTD cells (FIGs. 31B and 31D). Bars within each dose represent the mean cytokine level within the group at different time points (from left: day 4, 7, 10, 12, 16, 19, 23, 26). Horizontal bars and numbers indicate the fold-change comparisons between ARM-CD19 CAR ($1\times10^6$ dose group) and TM-CD19 CAR ($0.5\times10^6$ dose group) described in the text: 3-fold for IFN-γ; and 10-fold for IL-2. Groups taken down due to tumor burden or body weight loss do not show the last time points. Plasma cytokine levels were measured for 2 studies. no tum: no tumor.

**FIG. 32**. Time course of total and CAR+ T cell concentrations in NALM6 tumor-bearing mice treated with PBS vehicle, UTD, TM-CD19 CAR, or ARM-CD19 CAR. Blood samples were taken at 4, 7, 14, 21 and 28 days post CAR-T cell injection. Total T cells (CD3+, upper) and CAR+ T cell (CD3+CAR+, lower) concentrations were analyzed by flow cytometry at designed time points, depicted as mean cells with 95% confidence interval.

**FIGs. 33A and 33B**. IL-6 protein levels in three-party co-culture supernatants in pg/mL. ARM-CD19 CAR/K562 co-cultured cells (FIG. 33A) or TM-CD19 CAR/K562 cell co-cultured cells (FIG. 33B), for 6 or 24 hours incubated at different ratios (1:1 and 1:2.5), were then added to PMA-differentiated THP-1 cells for another 24 hours. Results from CAR-T cells co-cultured with K562-CD19 cells, CAR-T cells co-cultured with K562-Mesothelin cells, and CAR-T cells alone are shown. 1:5 ratios are not shown for clarity. ARM-CD19 CAR only and TM-CD19 CAR only designated bars represent CAR-T cell cultures (6 h, 24 h) without target cells. Mean + SEM, duplicates of n= 1 (TM-CD19 CAR) and n= 3 (ARM-CD19 CAR).

**FIGs. 34A, 34B, and 34C**. ARM process preserves BCMA CAR+T cell stemness. PI61, R1G5 and BCMA10 CART cells manufactured using the ARM process were assessed for CAR expression at thaw (FIG. 34A) and 48h post-thaw (FIG. 34B). CCR7/CD45RO markers were also assessed for the 48h post-thaw product (FIG. 34C). Data shown is one representative from two experiments performed using two donor T cells.

**FIGs. 35A and 35B**. The TM process mainly resulted in central-memory T cells (TCM) (CD45RO+/CCR7+), while the naive-like T cell population is almost gone in the CAR+T cells with TM process. PI61, R1G5 and BCMA10 CART cells manufactured using the TM process were assessed for CAR expression at day 9 (FIG. 35A). CCR7/CD45RO markers were also assessed at day 9 post-thaw product (FIG. 35B). Data shown is one representative from two experiments performed using two donor T cells.

**FIGs. 36A, 36B, 36C, and 36D.** ARM processed BCMA CAR-T cells demonstrates BCMA-specific activation and secretes higher levels of IL2 and IFN-γ. IL-2 and IFN-γ concentrations in cell culture supernatants. PI61, R1G5 and BCMA10 CART cells manufactured using the ARM or TM process, and respective UTD were co-cultured with KMS-11 at 2.5:1 ratio. Supernatants were collected 20h later. For the ARM products, IFN-γ concentrations are shown in FIG. 36A and IL-2 concentrations are shown in FIG. 36B. For the TM products, IFN-γ concentrations are shown in FIG. 36C and IL-2 concentrations are shown in FIG. 36D. Data shown is one representative from two experiments performed using two donor T cells.

**FIGs. 37A, 37B, and 37C.** Single cell RNA-seq data for input cells (FIG. 37A), Day 1 cells (FIG. 37B), and Day 9 cells (FIG. 37C). The "nGene" graphs show the number of expressed genes per cell. The "nUMI" graphs show the number of unique molecular identifiers (UMIs) per cell.

**FIGs. 38A, 38B, 38C, and 38D.** T-Distributed Stochastic Neighbor Embedding (TSNE) plots comparing input cells (FIG. 38A), Day 1 cells (FIG. 38B), and Day 9 cells (FIG. 38C) for a proliferation signature, which was determined based on expression of genes *CCNB1*, *CCND1*, *CCNE1*, *PLK1*, and *MKI67*. Each dot represents a cell in that sample. Cells shown as light grey do not express the proliferation genes whereas dark shaded cells express one or more of the proliferation genes. FIG. 38D is a violin plot showing the distribution of gene set scores for a gene set comprised of genes that characterize a resting vs. activated T cell state for Day 1

cells, Day 9 cells, and input cells. In FIG. 38D, a higher gene set score (Up resting vs. Down activated) indicates an increasing resting T cell phenotype, whereas a lower gene set score (Up resting vs. Down activated) indicates an increasing activated T cell phenotype. Input cells were overall in more of a resting state compared to Day 9 and Day 1 cells. Day 1 cells show the greatest activation gene set score.

**FIGs. 39A, 39B, 39C, 39D and 39E.** Gene set analysis for input cells, Day 1 cells, and Day 9 cells. In FIG. 39A, a higher gene set score for the gene set "Up TEM vs. Down TSCM" indicates an increasing effector memory T cell (TEM) phenotype of the cells in that sample, whereas a lower gene set score indicates an increasing stem cell memory T cell (TSCM) phenotype. In FIG. 39B, a higher gene set score for the gene set "Up Treg vs. Down Teff" indicates an increasing regulatory T cell (Treg) phenotype, whereas a lower gene set score indicates an increasing effector T cell (Teff) phenotype. In FIG. 39C, a lower gene set score for the gene set "Down stemness" indicates an increasing stemness phenotype. In FIG. 39D, a higher gene set score for the gene set "Up hypoxia" indicates an increasing hypoxia phenotype. In FIG. 39E, a higher gene set score for the gene set "Up autophagy" indicates an increasing autophagy phenotype. Day 1 cells looked similar to the input cells in terms of memory, stem-like and differentiation signature. Day 9 cells, on the other hand, show a higher enrichment for metabolic stress.

**FIGs. 40A, 40B, and 40C.** Gene cluster analysis for input cells. FIGs. 40A-40C are violin plots showing the gene set scores from gene set analysis of the four clusters of the input cells. Each dot overlaying the violin plots in FIGs. 40A-40C represents a cell's gene set score. In FIG. 40A, a higher gene set score of the gene set "Up Treg vs. Down Teff" indicates an increasing Treg cell phenotype, whereas a lower gene set score of the gene set "Up Treg vs. Down Teff" indicates an increasing Teff cell phenotype. In FIG. 40B, a higher gene set score of the gene set "Progressively up in memory differentiation" indicates an increasing late memory T cell phenotype, whereas a lower gene set score of the gene set "Progressively up in memory differentiation" indicates an increasing early memory T cell phenotype. In FIG. 40C, a higher gene set score of the gene set "Up TEM vs. Down TN" indicates an increasing effector memory T cell phenotype, whereas a lower gene set score of the gene set "Up TEM vs. Down TN" indicates an increasing naïve T cell phenotype. The cells in Cluster 3 are shown to be in a later memory, further differentiated T cell state compared to the cells in Cluster 1 and Cluster 2

which are in an early memory, less differentiated T cell state. Cluster 0 appears to be in an intermediate T cell state. Taken together, this data shows that there is a considerable level of heterogeneity within input cells.

**FIGs. 41A, 41B, and 41C.** TCR sequencing and measuring clonotype diversity. Day 9 cells have flatter distribution of clonotype frequencies (higher diversity).

**FIG. 42** is a flow chart showing the design of a Phase I clinical trial testing BCMA CART cells manufactured using the ARM process in adult patients with relapsed and/or refractory multiple myeloma.

**FIG. 43** is a graph showing FACS analyses for ARM-BCMA CAR expression at different collection time points post viral addition in the presence or absence of AZT at two different concentrations (30μM and 100μM). Lentiviral vector was added 1h later prior to AZT treatment at the time of activation and cell seeding.

**FIGs. 44A and 44B** are graphs showing assessment of ARM-BCMA CAR for CAR expression at thaw (FIG. 44A) and 48h post-thaw and CCR7/CD45RO markers at 48h post-thaw product as well as day 9 for TM-BCMA CAR (FIG. 44B). Data shown is one representative from two experiments performed using T cells from two donors.

**FIGs. 45A and 45B** are graphs showing cytokine concentrations in cell culture supernatants. ARM-BCMA CAR and TM-BCMA CAR, and respective UTD were co-cultured with KMS-11. Supernatant was collected 24h later. Data shown is one representative from two experiments performed using T cells from two donors.

**FIG. 46** is a graph showing outline of xenograft efficacy study to test ARM-BCMA.

**FIG. 47** is a graph comparing the efficacy of ARM-BCMA CAR with that of TM-BCMA CAR in a xenograft model. NSG mice were injected with MM cell line KMS11, expressing the luciferase reporter gene. The tumor burden is expressed as total body luminescence (p/s), depicted as mean tumor burden +SEM. On day 8 post tumor inoculation, mice were treated with ARM-BCMA CAR or TM-BCMA CAR at the respective doses (number of viable CAR+ T cells). Vehicle (PBS) and UTD T cells served as negative controls. N=5 mice for all groups, except N=4 for ARM-BCMA CAR (1e4 cells), PBS, and UTD groups.

**FIGs. 48A, 48B, and 48C** are graphs showing plasma IFN-γ kinetics of mice treated with ARM-BCMA CAR or TM-BCMA CAR. Plasma IFN-γ levels of KMS11-luc tumor-

bearing mice treated with UTD, ARM-BCMA CAR, or TM-BCMA CAR at respective CAR-T doses. All IFN-γ levels were depicted as mean ± SEM. Mice were bled and plasma cytokine measured by Meso Scale Discovery (MSD) assay.

**FIG. 49** is a graph showing cellular kinetics of ARM-BCMA CAR and TM-BCMA CAR in vivo. Cellular kinetics in peripheral blood of KMS11 tumor-bearing mice treated with TM UTD, ARM UTD, ARM-BCMA CAR, and TM-BCMA CAR at different doses. Cell count is expressed as mean cell count +SD. On day 8 post tumor inoculation, mice were treated with ARM-BCMA CAR or TM-BCMA CAR at the respective doses (number of viable CAR+ T cells). Vehicle (PBS) and UTD T cells served as negative controls. Blood samples were taken at 7, 14, and 21 days post CAR-T injection and were analyzed by flow cytometry at designed time points. N=5 mice for all groups, except N=4 for ARM-BCMA CAR (1e4 cells), PBS, and UTD groups

**FIG. 50A-C** provide exemplary schema for bispecific antibodies, including single bispecific antibody schema (FIG. 50A), multimeric bispecific antibody schema (FIG. 50B), and a figure legend (FIG. 50C).

**FIGs. 51A-B** depict schema of the 17 different constructs comprising a CD3 antigen binding domain comprising a heavy and light chain derived from an anti-CD3 antibody and, in all but control Constructs 11, 14, and 17, an a CD28 or CD2 antigen binding domain, as noted, comprising a heavy and light chain derived from an anti-CD28 or CD2 antibody, respectively.

Construct 1 comprises an anti-CD3 scFv fused to an anti-CD2 Fab, which is further fused to an Fc region. Construct 1 comprises a first chain and a second chain. The first chain comprises, from the N-terminus to the C-terminus, anti-CD2 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL, (G4S)4 linker (SEQ ID NO: 63), anti-CD2 VH, CH1, CH2, and CH3.

Construct 2 comprises an anti-CD3 scFv fused to an anti-CD28 Fab, which is further fused to an Fc region. Construct 2 comprises a first chain and a second chain. The first chain comprises, from the N-terminus to the C-terminus, anti-CD28 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL, (G4S)4 linker (SEQ ID NO: 63), anti-CD28 VH, CH1, CH2, and CH3.

Construct 3 comprises an anti-CD2 Fab fused to an Fc region, which is further fused to an anti-CD3 scFv. Construct 3 comprises a first chain and a second chain. The first chain

comprises, from the N-terminus to the C-terminus, anti-CD2 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD2 VH, CH1, CH2, CH3, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL. Construct 4 comprises an anti-CD28 Fab fused to an Fc region, which is further fused to an anti-CD3 scFv. Construct 4 comprises a first chain and a second chain. The first chain comprises, from the N-terminus to the C-terminus, anti-CD28 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD28 VH, CH1, CH2, CH3, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL.

Construct 5 comprises an anti-CD2 Fab fused to an anti-CD3 scFv, which is further fused to an Fc region. Construct 5 comprises a first chain and a second chain. The first chain comprises, from the N-terminus to the C-terminus, anti-CD2 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD2 VH, CH1, (G4S)2 linker (SEQ ID NO: 5), anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL, (G4S)4 linker (SEQ ID NO: 63), CH2, and CH3. Construct 6 comprises an anti-CD28 Fab fused to an anti-CD3 scFv, which is further fused to an Fc region. Construct 6 comprises a first chain and a second chain. The first chain comprises, from the N-terminus to the C-terminus, anti-CD28 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD28 VH, CH1, (G4S)2 linker (SEQ ID NO: 5), anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL, (G4S)4 linker (SEQ ID NO: 63), CH2, and CH3.

Construct 7 comprises an anti-CD3 scFv fused to an Fc region, which is further fused to an anti-CD2 Fab. Construct 7 comprises a first chain and a second chain. The first chain comprises, from the N-terminus to the C-terminus, anti-CD2 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL, (G4S) linker (SEQ ID NO: 25), CH2, CH3, (G4S)4 linker (SEQ ID NO: 63), anti-CD2 VH, and CH1. Construct 8 comprises an anti-CD3 scFv fused to an Fc region, which is further fused to an anti-CD28 Fab. Construct 8 comprises a first chain and a second chain. The first chain comprises, from the N-terminus to the C-terminus, anti-CD28 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL, (G4S) linker (SEQ ID NO: 25), CH2, CH3, (G4S)4 linker (SEQ ID NO: 63), anti-CD28 VH, and CH1.

Construct 9 comprises an anti-CD2 Fab fused to a first Fc region and an anti-CD3 scFv fused to a second Fc region. Construct 9 comprises a first chain, a second chain, and a third chain. The first chain comprises, from the N-terminus to the C-terminus, anti-CD2 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD2 VH, CH1, CH2, and CH3. The third chain comprises, from the N-terminus to the C-terminus, anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL, (G4S) linker (SEQ ID NO: 25), CH2, and CH3. Construct 10 comprises an anti-CD28 Fab fused to a first Fc region and an anti-CD3 scFv fused to a second Fc region. Construct 10 comprises a first chain, a second chain, and a third chain. The first chain comprises, from the N-terminus to the C-terminus, anti-CD28 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD28 VH, CH1, CH2, and CH3. The third chain comprises, from the N-terminus to the C-terminus, anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL, (G4S) linker (SEQ ID NO: 25), CH2, and CH3.

Construct 11 comprises an anti-CD3 scFv fused to an Fc region. Construct 11 comprises a first chain and a second chain. The first chain comprises, from the N-terminus to the C-terminus, CH2 and CH3. The second chain comprises, from the N-terminus to the C-terminus, anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL, (G4S) linker (SEQ ID NO: 25), CH2, and CH3.

Construct 12 comprises an anti-CD2 Fab fused to a first Fc region and an anti-CD3 scFv fused to a second Fc region. Construct 12 comprises a first chain, a second chain, and a third chain. The first chain comprises, from the N-terminus to the C-terminus, anti-CD2 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD2 VH, CH1, CH2, and CH3. The third chain comprises, from the N-terminus to the C-terminus, anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL, (G4S) linker (SEQ ID NO: 25), CH2, CH3, (G4S)3 linker (SEQ ID NO: 104), and Matrilin1. Construct 13 comprises an anti-CD28 Fab fused to a first Fc region and an anti-CD3 scFv fused to a second Fc region. Construct 13 comprises a first chain, a second chain, and a third chain. The first chain comprises, from the N-terminus to the C-terminus, anti-CD28 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD28 VH, CH1, CH2, and CH3. The third chain comprises, from the N-terminus to the C-terminus, anti-CD3 VH, (G4S)4 linker

(SEQ ID NO: 63), anti-CD3 VL, (G4S) linker (SEQ ID NO: 25), CH2, CH3, (G4S)3 linker ((SEQ ID NO: 104), and Matrilin1.

Construct 14 comprises an anti-CD3 scFv fused to an Fc region. Construct 14 comprises a first chain and a second chain. The first chain comprises, from the N-terminus to the C-terminus, CH2 and CH3. The second chain comprises, from the N-terminus to the C-terminus, anti-CD3 VH, (G4S)4 (SEQ ID NO: 63), linker, anti-CD3 VL, (G4S) linker (SEQ ID NO: 25), CH2, CH3, (G4S)3 linker (SEQ ID NO: 104), and Matrilin1.

Construct 15 comprises an anti-CD2 Fab fused to a first Fc region and an anti-CD3 scFv fused to a second Fc region. Construct 15 comprises a first chain, a second chain, and a third chain. The first chain comprises, from the N-terminus to the C-terminus, anti-CD2 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD2 VH, CH1, CH2, and CH3. The third chain comprises, from the N-terminus to the C-terminus, anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL, (G4S) linker (SEQ ID NO: 25), CH2, CH3, (G4S) linker (SEQ ID NO: 25), and the coiled-coil domain of cartilage oligomeric matrix protein (COMPcc). Construct 16 comprises an anti-CD28 Fab fused to a first Fc region and an anti-CD3 scFv fused to a second Fc region. Construct 16 comprises a first chain, a second chain, and a third chain. The first chain comprises, from the N-terminus to the C-terminus, anti-CD28 VL and CL. The second chain comprises, from the N-terminus to the C-terminus, anti-CD28 VH, CH1, CH2, and CH3. The third chain comprises, from the N-terminus to the C-terminus, anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL, (G4S) linker (SEQ ID NO: 25), CH2, CH3, (G4S) linker (SEQ ID NO: 25), and COMPcc.

Construct 17 comprises an anti-CD3 scFv fused to an Fc region. Construct 17 comprises a first chain and a second chain. The first chain comprises, from the N-terminus to the C-terminus, CH2 and CH3. The second chain comprises, from the N-terminus to the C-terminus, anti-CD3 VH, (G4S)4 linker (SEQ ID NO: 63), anti-CD3 VL, (G4S) linker (SEQ ID NO: 25), CH2, CH3, (G4S) linker (SEQ ID NO: 25), and COMPcc.

**FIG. 52** provides images of T cells from brightfield microscopy on day 4, after use of 10 μg/mL of the constructs and positive control. The numbers at the upper left corner of each image refer to the name of the constructs tested. For example, "1" refers to Construct 1, "2" refers to Construct 2, and so on and so forth. "TA" stands for TransAct.

**FIG. 53** shows the results of the IFN gamma and IL-2 readouts from the MSD for each of the 17 constructs and TransAct. The numbers on the x-axis refer to the name of the constructs tested. For example, "1" refers to Construct 1, "2" refers to Construct 2, and so on and so forth. "TA" stands for TransAct.

**FIG. 54** shows percent transduction of an anti-CD19 CAR for each of the 17 constructs and TransAct. The numbers on the x-axis refer to the name of the constructs tested. For example, "1" refers to Construct 1, "2" refers to Construct 2, and so on and so forth. "TA" stands for TransAct.

**FIG. 55** depicts CAR transduction as a function of valency or costimulatory molecule targeted (CD2/CD28). In FIG. 55, F1, F2, F3, F4, F5, and F7 have a ligand valency of 2; F12 and F13 have a ligand valency of 3; and F15 and F16 have a ligand valency of 5. F1, F3, F5, F7, F12, and F15 bind to CD2, whereas F2, F4, F13, and F16 bind to CD28.

**FIGs. 56A-56D** show specific killing of tumor cells (calculated by subtracting average % kill in Nalm6 CD19KO cells from average % kill in Nalm6 WT cells) for CAR T-cells generated using Constructs 1 (F1), 3 (F3), 4 (F4), 5 (F5) versus TransAct ("TA"). "H" (in "3H" and "5H") indicates an antibody concentration of 10 μg/mL; "M" (in "1M," "3M," "4M," and "5M") indicates an antibody concentration of 1 μg/mL; and "L" (in "1L," "3L," "4L," and "5L") indicates an antibody concentration of 0.1 μg/mL.

**FIGs. 57A-B** show secreted cytokine levels of CAR T-cells generated using Construct 1 (F1), 3 (F3), 4 (F4), or 5 (F5) or TransAct, when co-cultured with Nalm6 WT cells ("ARM vs. Nalm6 WT") or Nalm6 CD19 knockout cells ("ARM vs. Nalm6 CD19 KO").

**FIG. 58** shows the tumor burden as a function of time in the Nalm6 xenograft mouse model treated with CAR transduced or untransduced cells from both donors.

**FIG. 59** shows both CAR+ and CD3+ counts (per 20 μL) from mice treated with CAR transduced or untransduced cells from both donors. These counts were obtained from week 2 blood samples subjected to FACS analysis.

**FIGs. 60A-60D** show anti-tumor activity (FIGs. 60A-60B) and in vivo CAR expansion (FIGs. 60C-60D) by donor.

**FIGs. 61A-61B** shows the binding information (FIG. 61A) and configuration (FIG. 61B) of second generation stimulatory constructs. "F5 ANTI-CD3 (2)" refers to an F5 construct with an anti-CD3 binder based on ANTI-CD3 (2).

**FIG. 62** shows the transduction efficiency of various stimulatory constructs, including those shown in FIG. 61A. "TA" stands for TransAct. TransAct was used at 0.1% by volume (1 μL for every 1000 μL of culture).

**FIGs. 63A-63B** show the binder information (FIG. 63A) and configuration (FIG. 63B) of third generation stimulatory constructs.

**FIG. 64** shows the transduction efficiency of various stimulatory constructs, including those shown in FIG. 63A. "TA" stands for TransAct.

**FIGs. 65A and 65B** show specific killing of Nalm6 cells (FIG. 65A) and non-specific killing of FcγR-bearing PL21 cells (FIG. 65B). "F3 Fc-silent" refers to NEG2042 and "F4 Fc-silent" refers to NEG2043 in FIG. 63A.

**FIGs. 66A and 66B**. FIG. 66A shows the percentages of T cells expressing an anti-CD19 CAR ("CAR19+"; upper), an anti-BCMA CAR ("BCMA+"; middle), or co-expressing both CARs ("BCMA+CAR19+"; lower) following co-transduction with two vectors. FIG. 66B shows the percentages of T cells expressing an anti-CD22 CAR ("CAR22+") or co-expressing anti-CD19 CAR and anti-CD22 CAR ("CAR19+22+") following transduction with a dual CAR-encoding vector, as determined at two different time points post-manufacturing.

**FIG. 67** shows the tumor reduction of CD19 CARs prepared using an Fc-silenced (LALASKPA) Anti-CD3 (4)/anti-CD28 (2) bispecific construct (SEQ ID NO: 794 and 796) , Anti-CD3 (2)/anti-CD28 (2) construct (SEQ ID NO: 798 and 799), Anti-CD3 (4)/anti-CD28 (1) construct (SEQ ID NO: 800 and 801), or TransAct ("TA") and compared to untransduced controls ("UTD") and PBS.

**FIGs. 68A and 68B** show specific killing of Nalm6 cells (FIG. 68A) and non-specific killing of FcγR-bearing PL21 cells (FIG. 68B). TA stands for TransAct. In FIG. 68B, the tumor cells were co-cultured with either CART cells ("CART") or T cells not expressing CAR (UTD).

**FIG. 69** show non-specific killing of FcγR-bearing PL21 cells. TA stands for TransAct.

**FIGs. 70A-70C** show BCMA CAR expression (FIG. 70A), T cell memory phenotype (FIG. 70B) and activation phenotype (FIG. 70C). BCMA CART cells were manufactured using Anti-CD3 (4)/anti-CD28 (2) bispecific (SEQ ID NO: 794 and 796) (5 μg/mL) or TransAct ("TA"). D0 stands for Day 0, D1 stands for Day 1, EFF stands for effector T cells,

EM stands for effector memory T cells, CM stands for central memory T cells, and N stands for naive T cells.

**FIGs. 71A-71F** show CD19 CAR expression (FIGs. 71A and 71D), T cell memory phenotype (FIGs. 71B and 71E) and activation phenotype (FIGs. 71C and 71F). FIGs. 71A-71C show data generated using T cells from a first donor and FIGs. 71D-71F show data generated using T cells from a second donor. D0 stands for Day 0, D1 stands for Day 1, EFF stands for effector T cells, EM stands for effector memory T cells, CM stands for central memory T cells, and N stands for naive T cells.

## DETAILED DESCRIPTION

### Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

The term "a" and "an" refers to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

The term "about" when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or in some instances ±10%, or in some instances ±5%, or in some instances ±1%, or in some instances ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

The compositions and methods of the present invention encompass polypeptides and nucleic acids having the sequences specified, or sequences substantially identical or similar thereto, *for example*, sequences at least 85%, 90%, or 95% identical or higher to the sequence specified. In the context of an amino acid sequence, the term "substantially identical" is used herein to refer to a first amino acid sequence that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity, for example, amino acid sequences that contain a common structural domain having at least about

85%, 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, *for example*, a sequence provided herein.

In the context of a nucleotide sequence, the term "substantially identical" is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity, for example, nucleotide sequences having at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, *for example*, a sequence provided herein.

The term "variant" refers to a polypeptide that has a substantially identical amino acid sequence to a reference amino acid sequence, or is encoded by a substantially identical nucleotide sequence. In some embodiments, the variant is a functional variant.

The term "functional variant" refers to a polypeptide that has a substantially identical amino acid sequence to a reference amino acid sequence, or is encoded by a substantially identical nucleotide sequence, and is capable of having one or more activities of the reference amino acid sequence.

The term cytokine (for example, IL-2, IL-7, IL-15, IL-21, or IL-6) includes full length, a fragment or a variant, for example, a functional variant, of a naturally-occurring cytokine (including fragments and functional variants thereof having at least 10%, 30%, 50%, or 80% of the activity, e.g., the immunomodulatory activity, of the naturally-occurring cytokine). In some embodiments, the cytokine has an amino acid sequence that is substantially identical (e.g., at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity) to a naturally-occurring cytokine, or is encoded by a nucleotide sequence that is substantially identical (e.g., at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity) to a naturally-occurring nucleotide sequence encoding a cytokine. In some embodiments, as understood in context, the cytokine further comprises a receptor domain, e.g., a cytokine receptor domain (e.g., an IL-15/IL-15R).

The term "Chimeric Antigen Receptor" or alternatively a "CAR" refers to a recombinant polypeptide construct comprising at least an extracellular antigen binding domain, a transmembrane domain and a cytoplasmic signaling domain (also referred to herein as "an

intracellular signaling domain") comprising a functional signaling domain derived from a stimulatory molecule as defined below. In some embodiments, the domains in the CAR polypeptide construct are in the same polypeptide chain, for example, comprise a chimeric fusion protein. In some embodiments, the domains in the CAR polypeptide construct are not contiguous with each other, for example, are in different polypeptide chains, for example, as provided in an RCAR as described herein.

In some embodiments, the cytoplasmic signaling domain comprises a primary signaling domain (for example, a primary signaling domain of CD3-zeta). In some embodiments, the cytoplasmic signaling domain further comprises one or more functional signaling domains derived from at least one costimulatory molecule as defined below. In some embodiments, the costimulatory molecule is chosen from 41BB (i.e., CD137), CD27, ICOS, and/or CD28. In some embodiments, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a stimulatory molecule. In some embodiments, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a costimulatory molecule and a functional signaling domain derived from a stimulatory molecule. In some embodiments, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising two functional signaling domains derived from one or more costimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule. In some embodiments, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising at least two functional signaling domains derived from one or more costimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule. In some embodiments the CAR comprises an optional leader sequence at the amino-terminus (N-terminus) of the CAR fusion protein. In some embodiments, the CAR further comprises a leader sequence at the N-terminus of the extracellular antigen recognition domain, wherein the leader sequence is optionally cleaved from the antigen recognition domain (for example, an scFv) during cellular processing and localization of the CAR to the cellular membrane.

A CAR that comprises an antigen binding domain (for example, an scFv, a single domain antibody, or TCR (for example, a TCR alpha binding domain or TCR beta binding domain)) that targets a specific tumor marker X, wherein X can be a tumor marker as described herein, is also referred to as XCAR. For example, a CAR that comprises an antigen binding domain that targets BCMA is referred to as BCMA CAR. The CAR can be expressed in any cell, for example, an immune effector cell as described herein (for example, a T cell or an NK cell).

The term "signaling domain" refers to the functional portion of a protein which acts by transmitting information within the cell to regulate cellular activity via defined signaling pathways by generating second messengers or functioning as effectors by responding to such messengers.

The term "antibody," as used herein, refers to a protein, or polypeptide sequence derived from an immunoglobulin molecule, which specifically binds with an antigen. Antibodies can be polyclonal or monoclonal, multiple or single chain, or intact immunoglobulins, and may be derived from natural sources or from recombinant sources. Antibodies can be tetramers of immunoglobulin molecules.

The term "antibody fragment" refers to at least one portion of an intact antibody, or recombinant variants thereof, and refers to the antigen binding domain, for example, an antigenic determining variable region of an intact antibody, that is sufficient to confer recognition and specific binding of the antibody fragment to a target, such as an antigen. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, and Fv fragments, scFv antibody fragments, linear antibodies, single domain antibodies such as sdAb (either VL or VH), camelid VHH domains, and multi-specific molecules formed from antibody fragments such as a bivalent fragment comprising two or more, for example, two, Fab fragments linked by a disulfide bridge at the hinge region, or two or more, for example, two isolated CDR or other epitope binding fragments of an antibody linked. An antibody fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, for example, Hollinger and Hudson, Nature Biotechnology 23:1126-1136, 2005). Antibody fragments can also be grafted into scaffolds based on polypeptides such as a fibronectin type III (Fn3) (see U.S. Patent No.: 6,703,199, which describes fibronectin polypeptide minibodies).

The term "scFv" refers to a fusion protein comprising at least one antibody fragment comprising a variable region of a light chain and at least one antibody fragment comprising a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked via a short flexible polypeptide linker, and capable of being expressed as a single chain polypeptide, and wherein the scFv retains the specificity of the intact antibody from which it is derived. Unless specified, as used herein an scFv may have the VL and VH variable regions in either order, for example, with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL. In some embodiments, the scFv may comprise the structure of $NH_2$-$V_L$-linker-$V_H$-COOH or $NH_2$-$V_H$-linker-$V_L$-COOH.

The terms "complementarity determining region" or "CDR," as used herein, refer to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. For example, in general, there are three CDRs in each heavy chain variable region (for example, HCDR1, HCDR2, and HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, and LCDR3). The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme), Al-Lazikani et al., (1997) JMB 273,927-948 ("Chothia" numbering scheme), or a combination thereof. In a combined Kabat and Chothia numbering scheme, in some embodiments, the CDRs correspond to the amino acid residues that are part of a Kabat CDR, a Chothia CDR, or both.

The portion of the CAR composition of the invention comprising an antibody or antibody fragment thereof may exist in a variety of forms, for example, where the antigen binding domain is expressed as part of a polypeptide chain including, for example, a single domain antibody fragment (sdAb), a single chain antibody (scFv), or for example, a human or humanized antibody (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426). In some embodiments, the antigen

binding domain of a CAR composition of the invention comprises an antibody fragment. In some embodiments, the CAR comprises an antibody fragment that comprises an scFv.

As used herein, the term “binding domain” or "antibody molecule" (also referred to herein as “anti-target binding domain”) refers to a protein, for example, an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable domain sequence. The term “binding domain” or “antibody molecule” encompasses antibodies and antibody fragments. In some embodiments, an antibody molecule is a multispecific antibody molecule, for example, it comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In some embodiments, a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope.

The terms “bispecific antibody” and “bispecific antibodies” refer to molecules that combine the antigen binding sites of two antibodies within a single molecule. Thus, a bispecific antibody is able to bind two different antigens simultaneously or sequentially. Methods for making bispecific antibodies are well known in the art. Various formats for combining two antibodies are also known in the art. Forms of bispecific antibodies of the invention include, but are not limited to, a diabody, a single-chain diabody, Fab dimerization (Fab-Fab), Fab-scFv, and a tandem antibody, as known to those of skill in the art.

The term “antibody heavy chain,” refers to the larger of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations, and which normally determines the class to which the antibody belongs.

The term “antibody light chain,” refers to the smaller of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations. Kappa (κ) and lambda (λ) light chains refer to the two major antibody light chain isotypes.

The term “recombinant antibody” refers to an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage or yeast expression system. The term should also be construed to mean an antibody which has

been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using recombinant DNA or amino acid sequence technology which is available and well known in the art.

The term "antigen" or "Ag" refers to a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA, which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an "antigen" as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to encode polypeptides that elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a "gene" at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample, or might be macromolecule besides a polypeptide. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a fluid with other biological components.

The term "multispecific binding molecule" refers to a molecule that specifically binds to at least two antigens and comprise two or more antigen-binding domains. The antigen-binding domains can each independently be an antibody fragment (*e.g.*, scFv, Fab, nanobody), a ligand, or a non-antibody derived binder (*e.g.*, fibronectin, Fynomer, DARPin).

The term "monovalent" as used herein in the context of a multispecific binding molecule, antibody (e.g., bispecific antibody), or antibody fragment refers to a multispecific binding molecule, antibody (e.g., bispecific antibody), or antibody fragment in which there is a single antigen binding domain for each antigen to which the multispecific binding molecule, antibody (e.g., bispecific antibody), or antibody fragment binds.

The term "bivalent" as used herein in the context of a multispecific binding molecule, antibody (e.g., bispecific antibody), or antibody fragment refers to a multispecific binding

molecule, antibody (e.g., bispecific antibody), or antibody fragment in which there are two antigen binding domains for each antigen to which the multispecific binding molecule, antibody (e.g., bispecific antibody), or antibody fragment binds.

The term "multimer" refers to an aggregate of a plurality of molecules (such as but not limited to antibodies (e.g. bispecific antibodies)), optionally conjugated to one another.

The term "conjugated to" refers to one or more molecules covalently or non-covalently bound together, optionally, directly or via linker.

The term "Fc silent" refers to an Fc domain that has been modified to have minimal interaction with effector cells, e.g., to reduce or eliminate the ability of a binding molecule to mediate antibody dependent cellular cytotoxicity (ADCC) and/or antibody dependent cellular phagocytosis (ADCP). Silenced effector functions may be obtained by mutation in the Fc region of the antibodies and have been described in the art, such as, but not limited to, LALA and N297A (Strohl, W., 2009, Curr. Opin. Biotechnol. vol. 20(6):685-691); and D265A (Baudino et al., 2008, J. Immunol. 181: 6664- 69) see also Heusser et al., WO2012065950. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat, et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). Examples of Fc silencing mutations include the LALA mutant comprising L234A and L235A mutation in the IgG1 Fc amino acid sequence, DAPA (D265A, P329A) (see, *e.g.*, US 6,737,056), N297A, DANAPA (D265A, N297A, and P329A), and/or LALADANAPS (L234A, L235A, D265A, N297A and P331S). Further, non-limiting exemplary embodiments of silencing mutations include LALGA (L234A, L235A, and G237A), LALASKPA (L234A, L235A, S267K, and P329A), DAPASK (D265A, P329A, and S267K), GADAPA (G237A, D265A, and P329A), GADAPASK (G237A, D265A, P329A, and S267K), LALAPG (L234A, L235A, and P329G), and LALAPA (L234A, L235A, and P329A), wherein the amino acid residues are numbered according to the EU numbering system. It is understood that the terms "LALA," "DAPA," "DANAPA," "LALADANAPS," "LALAGA", "LALASKPA", "DAPASK", "GADAPA", "GADAPASK", "LALAPG", and "LALAPA" represent shorthand terminology for the different combinations of substitutions described in this paragraph rather than contiguous amino acid sequences.

The term "CD3/TCR complex" refers to a complex on the T-cell surface comprising a TCR including a TCR alpha and TCR beta chain; CD3 including one CD3 gamma chain, one CD3 delta chain, and two CD3 epsilon chains; and a zeta domain. UniProt accession numbers P01848 (TCR alpha, constant domain), P01850 (TCR beta, constant domain 1), A0A5B9 (TCR beta, constant domain 2), P09693 (CD3 gamma), P04234 (CD3 delta), P07766 (CD3 epsilon) provide exemplary human sequences for these chains, with the exception of the zeta chain, responsible for intracellular signaling, which is discussed in further detail below. Further relevant accession numbers include A0A075B662 (murine TCR alpha, constant domain), A0A0A6YWV4 and/or A0A075B5J3 (murine TCR beta, constant domain 1), A0A075B5J4 (murine TCR beta, constant domain 2), P11942 (murine CD3 gamma), P04235 (murine CD3 delta), P22646 (murine CD3 epsilon).

The term "CD28" refers to a T-cell specific glycoprotein CD28, also referred to as Tp44, as well as all alternate names thereof, which functions as a costimulatory molecule. UniProt accession number P10747 provides exemplary human CD28 amino acid sequences (see also HGNC: 1653, Entrez Gene: 940, Ensembl: ENSG00000178562, and OMIM: 186760). Further relevant CD28 sequences include UniProt accession number P21041 (murine CD28).

The term "ICOS" refers to inducible T-cell costimulator, also referred to as AILIM, CVID1, CD278, as well as all alternate names thereof, which functions as a costimulatory molecule. UniProt accession number Q9Y6W8 provides exemplary human ICOS amino acid sequences (see also HGNC: 5351, Entrez Gene: 29851, Ensembl: ENSG00000163600, and OMIM: 604558). Further relevant ICOS sequences include UniProt accession number Q9WVS0 (murine ICOS).

The term "CD27" refers to T-cell activation antigen CD27, Tumor necrosis factor receptor superfamily member 7, T14, T-cell activation antigen S152, Tp55, as well as alternate names thereof, which functions as a costimulatory molecule. UniProt accession number P26842 provides exemplary human CD27 amino acid sequences (see also HGNC: 11922, Entrez Gene: 939, Ensembl: ENSG00000139193, and OMIM: 186711). Further relevant CD27 sequences include UniProt accession number P41272 (murine CD27).

The term "CD25" refers to IL-2 subunit alpha, TAC antigen, p55, insulin dependent diabetes mellitus 10, IMD21, P55, TCGFR, as well as alternate names thereof, which functions as a growth factor receptor. UniProt accession number P01589 provides exemplary human

CD25 amino acid sequences (see also HGNC: 6008, Entrez Gene: 3559, Ensembl: ENSG00000134460, and OMIM: 147730). Further relevant CD25 sequences include UniProt accession number P01590 (murine CD25).

The term "4-1BB" refers to CD137 or Tumor necrosis factor receptor superfamily member 9, as well as alternate names thereof, which functions as a costimulatory molecule. UniProt accession number Q07011 provides exemplary human 4-1BB amino acid sequences (see also HGNC: 11924, Entrez Gene: 3604, Ensembl: ENSG00000049249, and OMIM: 602250). Further relevant 4-1BB sequences include UniProt accession number P20334 (murine 4-1BB).

The term "IL6RA" refers to IL-6 receptor subunit alpha or CD126, as well as alternate names thereof, which functions as a growth factor receptor. UniProt accession number P08887 provides exemplary human IL6RA amino acid sequences (see also HGNC: 6019, Entrez Gene: 3570, Ensembl: ENSG00000160712, and OMIM: 147880 Further relevant IL6RA sequences include UniProt accession number P22272 (murine IL6RA).

The term "IL6RB" refers to IL-6 receptor subunit beta or CD130, as well as alternate names thereof, which functions as a growth factor receptor. UniProt accession number P40189 provides exemplary human IL6RB amino acid sequences. Further relevant IL6RB sequences include UniProt accession number Q00560 (murine IL6RB).

The term "CD2" refers to T-cell surface antigen T11/Leu-5/CD2, lymphocyte function antigen 2, T11, or erythrocyte/rosette/LFA-3 receptor, as well as alternate names thereof, , which functions as a growth factor receptor. UniProt accession number P06729 provides exemplary human CD2 amino acid sequences (see also HGNC: 1639, Entrez Gene: 914, Ensembl: ENSG00000116824, and OMIM: 186990). Further relevant CD2 sequences include UniProt accession number P08920 (murine CD2).

The terms "anti-tumor effect" and "anti-cancer effect" are used interchangeably and refer to a biological effect which can be manifested by various means, including but not limited to, for example, a decrease in tumor volume or cancer volume, a decrease in the number of tumor cells or cancer cells, a decrease in the number of metastases, an increase in life expectancy, a decrease in tumor cell proliferation or cancer cell proliferation, a decrease in tumor cell survival or cancer cell survival, or amelioration of various physiological symptoms associated with the cancerous condition. An "anti-tumor effect" or "anti-cancer effect" can also

be manifested by the ability of the peptides, polynucleotides, cells and antibodies of the invention in prevention of the occurrence of tumor or cancer in the first place.

The term "autologous" refers to any material derived from the same individual to whom it is later to be re-introduced into the individual.

The term "allogeneic" refers to any material derived from a different animal of the same species as the individual to whom the material is introduced. Two or more individuals are said to be allogeneic to one another when the genes at one or more loci are not identical. In some embodiments, allogeneic material from individuals of the same species may be sufficiently unlike genetically to interact antigenically.

The term "xenogeneic" refers to a graft derived from an animal of a different species.

The term "apheresis" as used herein refers to the art-recognized extracorporeal process by which the blood of a donor or patient is removed from the donor or patient and passed through an apparatus that separates out selected particular constituent(s) and returns the remainder to the circulation of the donor or patient, for example, by retransfusion. Thus, in the context of "an apheresis sample" refers to a sample obtained using apheresis.

The term "cancer" refers to a disease characterized by the rapid and uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers are described herein and include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like. In some embodiments cancers treated by the methods described herein include multiple myeloma, Hodgkin's lymphoma or non-Hodgkin's lymphoma.

The terms "tumor" and "cancer" are used interchangeably herein, for example, both terms encompass solid and liquid, for example, diffuse or circulating, tumors. As used herein, the term "cancer" or "tumor" includes premalignant, as well as malignant cancers and tumors.

"Derived from" as that term is used herein, indicates a relationship between a first and a second molecule. It generally refers to structural similarity between the first molecule and a second molecule and does not connotate or include a process or source limitation on a first molecule that is derived from a second molecule. For example, in the case of an intracellular signaling domain that is derived from a CD3zeta molecule, the intracellular signaling domain

retains sufficient CD3zeta structure such that is has the required function, namely, the ability to generate a signal under the appropriate conditions. It does not connotate or include a limitation to a particular process of producing the intracellular signaling domain, for example, it does not mean that, to provide the intracellular signaling domain, one must start with a CD3zeta sequence and delete unwanted sequence, or impose mutations, to arrive at the intracellular signaling domain.

The term "conservative sequence modifications" refers to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody or antibody fragment containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody or antibody fragment of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (for example, lysine, arginine, histidine), acidic side chains (for example, aspartic acid, glutamic acid), uncharged polar side chains (for example, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (for example, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (for example, threonine, valine, isoleucine) and aromatic side chains (for example, tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within a CAR of the invention can be replaced with other amino acid residues from the same side chain family and the altered CAR can be tested using the functional assays described herein.

The term "stimulation" in the context of stimulation by a stimulatory and/or costimulatory molecule refers to a response, for example, a primary or secondary response, induced by binding of a stimulatory molecule (for example, a TCR/CD3 complex) and/or a costimulatory molecule (for example, CD28 or 4-1BB) with its cognate ligand thereby mediating a signal transduction event, such as, but not limited to, signal transduction via the TCR/CD3 complex. Stimulation can mediate altered expression of certain molecules and/or reorganization of cytoskeletal structures, and the like.

The term "stimulatory molecule," refers to a molecule expressed by a T cell that provides the primary cytoplasmic signaling sequence(s) that regulate primary activation of the TCR complex in a stimulatory way for at least some aspect of the T cell signaling pathway. In some embodiments, the ITAM-containing domain within the CAR recapitulates the signaling of the primary TCR independently of endogenous TCR complexes. In some embodiments, the primary signal is initiated by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, and which leads to mediation of a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A primary cytoplasmic signaling sequence (also referred to as a "primary signaling domain") that acts in a stimulatory manner may contain a signaling motif which is known as immunoreceptor tyrosine-based activation motif or ITAM. Examples of an ITAM containing primary cytoplasmic signaling sequence that is of particular use in the invention includes, but is not limited to, those derived from TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS"), FcεRI and CD66d, DAP10 and DAP12. In a specific CAR of the invention, the intracellular signaling domain in any one or more CARS of the invention comprises an intracellular signaling sequence, for example, a primary signaling sequence of CD3-zeta. The term "antigen presenting cell" or "APC" refers to an immune system cell such as an accessory cell (for example, a B-cell, a dendritic cell, and the like) that displays a foreign antigen complexed with major histocompatibility complexes (MHC's) on its surface. T-cells may recognize these complexes using their T-cell receptors (TCRs). APCs process antigens and present them to T-cells.

An "intracellular signaling domain," as the term is used herein, refers to an intracellular portion of a molecule. In embodiments, the intracellular signal domain transduces the effector function signal and directs the cell to perform a specialized function. While the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

The intracellular signaling domain generates a signal that promotes an immune effector function of the CAR containing cell, for example, a CART cell. Examples of immune effector function, for example, in a CART cell, include cytolytic activity and helper activity, including the secretion of cytokines.

In some embodiments, the intracellular signaling domain can comprise a primary intracellular signaling domain. Exemplary primary intracellular signaling domains include those derived from the molecules responsible for primary stimulation, or antigen dependent simulation. In some embodiments, the intracellular signaling domain can comprise a costimulatory intracellular domain. Exemplary costimulatory intracellular signaling domains include those derived from molecules responsible for costimulatory signals, or antigen independent stimulation. For example, in the case of a CART, a primary intracellular signaling domain can comprise a cytoplasmic sequence of a T cell receptor, and a costimulatory intracellular signaling domain can comprise cytoplasmic sequence from co-receptor or costimulatory molecule.

A primary intracellular signaling domain can comprise a signaling motif which is known as an immunoreceptor tyrosine-based activation motif or ITAM. Examples of ITAM containing primary cytoplasmic signaling sequences include, but are not limited to, those derived from CD3 zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS"), FcεRI, CD66d, DAP10 and DAP12.

The term "zeta" or alternatively "zeta chain", "CD3-zeta" or "TCR-zeta" refers to CD247. Swiss-Prot accession number P20963 provides exemplary human CD3 zeta amino acid sequences. A "zeta stimulatory domain" or alternatively a "CD3-zeta stimulatory domain" or a "TCR-zeta stimulatory domain" refers to a stimulatory domain of CD3-zeta or a variant thereof (for example, a molecule having mutations, for example, point mutations, fragments, insertions, or deletions). In some embodiments, the cytoplasmic domain of zeta comprises residues 52 through 164 of GenBank Acc. No. BAG36664.1 or a variant thereof (for example, a molecule having mutations, for example, point mutations, fragments, insertions, or deletions). In some embodiments, the "zeta stimulatory domain" or a "CD3-zeta stimulatory domain" is the sequence provided as SEQ ID NO: 9 or 10, or a variant thereof (for example, a molecule having mutations, for example, point mutations, fragments, insertions, or deletions).

The term "costimulatory molecule" refers to the cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules are cell surface molecules other than antigen receptors or their ligands that are required for an efficient immune response. Costimulatory molecules include, but are not limited to an MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD28-OX40, CD28-4-1BB, and a ligand that specifically binds with CD83.

A costimulatory intracellular signaling domain refers to the intracellular portion of a costimulatory molecule.

The intracellular signaling domain can comprise the entire intracellular portion, or the entire native intracellular signaling domain, of the molecule from which it is derived, or a functional fragment thereof.

A "4-1BB costimulatory domain" refers to a costimulatory domain of 4-1BB, or a variant thereof (for example, a molecule having mutations, for example, point mutations, fragments, insertions, or deletions). In some embodiments, the "4-1BB costimulatory domain" is the sequence provided as SEQ ID NO: 7 or a variant thereof (for example, a molecule having mutations, for example, point mutations, fragments, insertions, or deletions).

"Immune effector cell," as that term is used herein, refers to a cell that is involved in an immune response, for example, in the promotion of an immune effector response. Examples of immune effector cells include T cells, for example, alpha/beta T cells and gamma/delta T cells,

B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloic-derived phagocytes.

"Immune effector function or immune effector response," as that term is used herein, refers to function or response, for example, of an immune effector cell, that enhances or promotes an immune attack of a target cell. For example, an immune effector function or response refers a property of a T or NK cell that promotes killing or the inhibition of growth or proliferation, of a target cell. In the case of a T cell, primary stimulation and costimulation are examples of immune effector function or response.

The term "effector function" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines.

The term "encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (for example, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene, cDNA, or RNA, encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or a RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

The term "effective amount" or "therapeutically effective amount" are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result.

The term "endogenous" refers to any material from or produced inside an organism, cell, tissue or system.

The term "exogenous" refers to any material introduced from or produced outside an organism, cell, tissue or system.

The term "expression" refers to the transcription and/or translation of a particular nucleotide sequence. In some embodiments, expression comprises translation of an mRNA introduced into a cell.

The term "transfer vector" refers to a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "transfer vector" includes an autonomously replicating plasmid or a virus. The term should also be construed to further include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, a polylysine compound, liposome, and the like. Examples of viral transfer vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

The term "expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, including cosmids, plasmids (for example, naked or contained in liposomes) and viruses (for example, lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

The term "lentivirus" refers to a genus of the Retroviridae family. Lentiviruses are unique among the retroviruses in being able to infect non-dividing cells; they can deliver a significant amount of genetic information into the DNA of the host cell, so they are one of the most efficient methods of a gene delivery vector. HIV, SIV, and FIV are all examples of lentiviruses.

The term "lentiviral vector" refers to a vector derived from at least a portion of a lentivirus genome, including especially a self-inactivating lentiviral vector as provided in Milone et al., Mol. Ther. 17(8): 1453–1464 (2009). Other examples of lentivirus vectors that

may be used in the clinic, include but are not limited to, for example, the LENTIVECTOR® gene delivery technology from Oxford BioMedica, the LENTIMAX™ vector system from Lentigen and the like. Nonclinical types of lentiviral vectors are also available and would be known to one skilled in the art.

The term "homologous" or "identity" refers to the subunit sequence identity between two polymeric molecules, for example, between two nucleic acid molecules, such as, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; for example, if a position in each of two DNA molecules is occupied by adenine, then they are homologous or identical at that position. The homology between two sequences is a direct function of the number of matching or homologous positions; for example, if half (for example, five positions in a polymer ten subunits in length) of the positions in two sequences are homologous, the two sequences are 50% homologous; if 90% of the positions (for example, 9 of 10), are matched or homologous, the two sequences are 90% homologous.

"Humanized" forms of non-human (for example, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies and antibody fragments thereof are human immunoglobulins (recipient antibody or antibody fragment) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, a humanized antibody/antibody fragment can comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications can further refine and optimize antibody or antibody fragment performance. In general, the humanized antibody or antibody fragment thereof will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or a significant portion of the FR regions are those of a human immunoglobulin sequence. The humanized antibody or antibody fragment can also comprise at least a portion of an

immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature, 321: 522-525, 1986; Reichmann et al., Nature, 332: 323-329, 1988; Presta, Curr. Op. Struct. Biol., 2: 593-596, 1992.

"Fully human" refers to an immunoglobulin, such as an antibody or antibody fragment, where the whole molecule is of human origin or consists of an amino acid sequence identical to a human form of the antibody or immunoglobulin.

The term "isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. "A" refers to adenosine, "C" refers to cytosine, "G" refers to guanosine, "T" refers to thymidine, and "U" refers to uridine.

The term "operably linked" or "transcriptional control" refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences can be contiguous with each other and, for example, where necessary to join two protein coding regions, are in the same reading frame.

The term "parenteral" administration of an immunogenic composition includes, for example, subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intrasternal injection, intratumoral, or infusion techniques.

The term "nucleic acid," "nucleic acid molecule," "polynucleotide," or "polynucleotide molecule" refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. In some embodiments, a "nucleic acid," "nucleic acid

molecule," "polynucleotide," or "polynucleotide molecule" comprise a nucleotide/nucleoside derivative or analog. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (for example, degenerate codon substitutions, for example, conservative substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions, for example, conservative substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

The terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

The term "promoter" refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

The term "promoter/regulatory sequence" refers to a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

The term "constitutive" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

The term "inducible" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

The term "tissue-specific" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide encodes or specified by a gene, causes the gene product to be produced in a cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

The terms "cancer associated antigen," "tumor antigen," "hyperproliferative disorder antigen," and "antigen associated with a hyperproliferative disorder" interchangeably refer to antigens that are common to specific hyperproliferative disorders. In some embodiments, these terms refer to a molecule (typically a protein, carbohydrate or lipid) that is expressed on the surface of a cancer cell, either entirely or as a fragment (for example, MHC/peptide), and which is useful for the preferential targeting of a pharmacological agent to the cancer cell. In some embodiments, a tumor antigen is a marker expressed by both normal cells and cancer cells, for example, a lineage marker, for example, CD19 on B cells. In some embodiments, a tumor antigen is a cell surface molecule that is overexpressed in a cancer cell in comparison to a normal cell, for instance, 1-fold over expression, 2-fold overexpression, 3-fold overexpression or more in comparison to a normal cell. In some embodiments, a tumor antigen is a cell surface molecule that is inappropriately synthesized in the cancer cell, for instance, a molecule that contains deletions, additions or mutations in comparison to the molecule expressed on a normal cell. In some embodiments, a tumor antigen will be expressed exclusively on the cell surface of a cancer cell, entirely or as a fragment (for example, MHC/peptide), and not synthesized or expressed on the surface of a normal cell. In some embodiments, the hyperproliferative disorder antigens of the present invention are derived from, cancers including but not limited to primary or metastatic melanoma, thymoma, lymphoma, sarcoma, lung cancer, liver cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, leukemias, uterine cancer, cervical cancer, bladder cancer, kidney cancer and adenocarcinomas such as breast cancer, prostate cancer (for

example, castrate-resistant or therapy-resistant prostate cancer, or metastatic prostate cancer), ovarian cancer, pancreatic cancer, and the like, or a plasma cell proliferative disorder, for example, asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), monoclonal gammopathy of undetermined significance (MGUS), Waldenstrom's macroglobulinemia, plasmacytomas (for example, plasma cell dyscrasia, solitary myeloma, solitary plasmacytoma, extramedullary plasmacytoma, and multiple plasmacytoma), systemic amyloid light chain amyloidosis, and POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome). In some embodiments, the CARs of the present invention include CARs comprising an antigen binding domain (for example, antibody or antibody fragment) that binds to a MHC presented peptide. Normally, peptides derived from endogenous proteins fill the pockets of Major histocompatibility complex (MHC) class I molecules and are recognized by T cell receptors (TCRs) on CD8 + T lymphocytes. The MHC class I complexes are constitutively expressed by all nucleated cells. In cancer, virus-specific and/or tumor-specific peptide/MHC complexes represent a unique class of cell surface targets for immunotherapy. TCR-like antibodies targeting peptides derived from viral or tumor antigens in the context of human leukocyte antigen (HLA)-A1 or HLA-A2 have been described (see, for example, Sastry et al., J Virol. 2011 85(5):1935-1942; Sergeeva et al., Blood, 2011 117(16):4262-4272; Verma et al., J Immunol 2010 184(4):2156-2165; Willemsen et al., Gene Ther 2001 8(21) :1601-1608; Dao et al., Sci Transl Med 2013 5(176) :176ra33 ; Tassev et al., Cancer Gene Ther 2012 19(2):84-100). For example, TCR-like antibody can be identified from screening a library, such as a human scFv phage displayed library.

The term "tumor-supporting antigen" or "cancer-supporting antigen" interchangeably refer to a molecule (typically a protein, carbohydrate or lipid) that is expressed on the surface of a cell that is, itself, not cancerous, but supports the cancer cells, for example, by promoting their growth or survival for example, resistance to immune cells. Exemplary cells of this type include stromal cells and myeloid-derived suppressor cells (MDSCs). The tumor-supporting antigen itself need not play a role in supporting the tumor cells so long as the antigen is present on a cell that supports cancer cells.

The term "flexible polypeptide linker" or "linker" as used in the context of an scFv refers to a peptide linker that consists of amino acids such as glycine and/or serine residues used alone or in combination, to link variable heavy and variable light chain regions together.

In some embodiments, the flexible polypeptide linker is a Gly/Ser linker and comprises the amino acid sequence (Gly-Gly-Gly-Ser)n, where n is a positive integer equal to or greater than 1 (SEQ ID NO: 41). For example, n=1, n=2, n=3. n=4, n=5 and n=6, n=7, n=8, n=9 and n=10 In some embodiments, the flexible polypeptide linkers include, but are not limited to, (Gly4 Ser)4 (SEQ ID NO: 27) or (Gly4 Ser)3 (SEQ ID NO: 28). In some embodiments, the linkers include multiple repeats of (Gly2Ser), (GlySer) or (Gly3Ser) (SEQ ID NO: 29). Also included within the scope of the invention are linkers described in WO2012/138475, incorporated herein by reference.

As used herein, a 5' cap (also termed an RNA cap, an RNA 7-methylguanosine cap or an RNA m7G cap) is a modified guanine nucleotide that has been added to the "front" or 5' end of a eukaryotic messenger RNA shortly after the start of transcription. The 5' cap consists of a terminal group which is linked to the first transcribed nucleotide. Its presence is critical for recognition by the ribosome and protection from RNases. Cap addition is coupled to transcription, and occurs co-transcriptionally, such that each influences the other. Shortly after the start of transcription, the 5' end of the mRNA being synthesized is bound by a cap-synthesizing complex associated with RNA polymerase. This enzymatic complex catalyzes the chemical reactions that are required for mRNA capping. Synthesis proceeds as a multi-step biochemical reaction. The capping moiety can be modified to modulate functionality of mRNA such as its stability or efficiency of translation.

As used herein, "in vitro transcribed RNA" refers to RNA that has been synthesized in vitro. In some embodiments the RNA is mRNA. Generally, the in vitro transcribed RNA is generated from an in vitro transcription vector. The in vitro transcription vector comprises a template that is used to generate the in vitro transcribed RNA.

As used herein, a "poly(A)" is a series of adenosines attached by polyadenylation to the mRNA. In some embodiments of a construct for transient expression, the poly(A) is between 50 and 5000 (SEQ ID NO: 30). In some embodiments the poly(A) is greater than 64. In some embodiments the poly(A)is greater than 100. In some embodiments the poly(A) is greater than 300. In some embodiments the poly(A) is greater than 400. poly(A) sequences can be modified chemically or enzymatically to modulate mRNA functionality such as localization, stability or efficiency of translation.

As used herein, "polyadenylation" refers to the covalent linkage of a polyadenylyl moiety, or its modified variant, to a messenger RNA molecule. In eukaryotic organisms, most messenger RNA (mRNA) molecules are polyadenylated at the 3' end. The 3' poly(A) tail is a long sequence of adenine nucleotides (often several hundred) added to the pre-mRNA through the action of an enzyme, polyadenylate polymerase. In higher eukaryotes, the poly(A) tail is added onto transcripts that contain a specific sequence, the polyadenylation signal. The poly(A) tail and the protein bound to it aid in protecting mRNA from degradation by exonucleases. Polyadenylation is also important for transcription termination, export of the mRNA from the nucleus, and translation. Polyadenylation occurs in the nucleus immediately after transcription of DNA into RNA, but additionally can also occur later in the cytoplasm. After transcription has been terminated, the mRNA chain is cleaved through the action of an endonuclease complex associated with RNA polymerase. The cleavage site is usually characterized by the presence of the base sequence AAUAAA near the cleavage site. After the mRNA has been cleaved, adenosine residues are added to the free 3' end at the cleavage site.

As used herein, "transient" refers to expression of a non-integrated transgene for a period of hours, days or weeks, wherein the period of time of expression is less than the period of time for expression of the gene if integrated into the genome or contained within a stable plasmid replicon in the host cell.

As used herein, the terms "treat", "treatment" and "treating" refer to the reduction or amelioration of the progression, severity and/or duration of a proliferative disorder, or the amelioration of one or more symptoms (preferably, one or more discernible symptoms) of a proliferative disorder resulting from the administration of one or more therapies (for example, one or more therapeutic agents such as a CAR of the invention). In specific embodiments, the terms "treat", "treatment" and "treating" refer to the amelioration of at least one measurable physical parameter of a proliferative disorder, such as growth of a tumor, not necessarily discernible by the patient. In other embodiments the terms "treat", "treatment" and "treating" - refer to the inhibition of the progression of a proliferative disorder, either physically by, for example, stabilization of a discernible symptom, physiologically by, for example, stabilization of a physical parameter, or both. In other embodiments the terms "treat", "treatment" and "treating" refer to the reduction or stabilization of tumor size or cancerous cell count.

The term "signal transduction pathway" refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. The phrase "cell surface receptor" includes molecules and complexes of molecules capable of receiving a signal and transmitting signal across the membrane of a cell.

The term "subject" is intended to include living organisms in which an immune response can be elicited (for example, mammals, for example, human).

The term, a "substantially purified" cell refers to a cell that is essentially free of other cell types. A substantially purified cell also refers to a cell which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of cells. In other instances, this term refers simply to cell that have been separated from the cells with which they are naturally associated in their natural state. In some embodiments, the cells are cultured in vitro. In some embodiments, the cells are not cultured in vitro.

The term "therapeutic" as used herein means a treatment. A therapeutic effect is obtained by reduction, suppression, remission, or eradication of a disease state.

The term "prophylaxis" as used herein means the prevention of or protective treatment for a disease or disease state.

The term "transfected" or "transformed" or "transduced" refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A "transfected" or "transformed" or "transduced" cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

The term "specifically binds," refers to an antibody, or a ligand, which recognizes and binds with a cognate binding partner (for example, a stimulatory and/or costimulatory molecule present on a T cell) protein present in a sample, but which antibody or ligand does not substantially recognize or bind other molecules in the sample.

"Regulatable chimeric antigen receptor (RCAR)," as used herein, refers to a set of polypeptides, typically two in the simplest embodiments, which when in an immune effector cell, provides the cell with specificity for a target cell, typically a cancer cell, and with intracellular signal generation. In some embodiments, an RCAR comprises at least an

extracellular antigen binding domain, a transmembrane domain and a cytoplasmic signaling domain (also referred to herein as "an intracellular signaling domain") comprising a functional signaling domain derived from a stimulatory molecule and/or costimulatory molecule as defined herein in the context of a CAR molecule. In some embodiments, the set of polypeptides in the RCAR are not contiguous with each other, for example, are in different polypeptide chains. In some embodiments, the RCAR includes a dimerization switch that, upon the presence of a dimerization molecule, can couple the polypeptides to one another, for example, can couple an antigen binding domain to an intracellular signaling domain. In some embodiments, the RCAR is expressed in a cell (for example, an immune effector cell) as described herein, for example, an RCAR-expressing cell (also referred to herein as "RCARX cell"). In some embodiments the RCARX cell is a T cell and is referred to as a RCART cell. In some embodiments the RCARX cell is an NK cell, and is referred to as a RCARN cell. The RCAR can provide the RCAR-expressing cell with specificity for a target cell, typically a cancer cell, and with regulatable intracellular signal generation or proliferation, which can optimize an immune effector property of the RCAR-expressing cell. In embodiments, an RCAR cell relies at least in part, on an antigen binding domain to provide specificity to a target cell that comprises the antigen bound by the antigen binding domain.

"Membrane anchor" or "membrane tethering domain", as that term is used herein, refers to a polypeptide or moiety, for example, a myristoyl group, sufficient to anchor an extracellular or intracellular domain to the plasma membrane.

"Switch domain," as that term is used herein, for example, when referring to an RCAR, refers to an entity, typically a polypeptide-based entity, that, in the presence of a dimerization molecule, associates with another switch domain. The association results in a functional coupling of a first entity linked to, for example, fused to, a first switch domain, and a second entity linked to, for example, fused to, a second switch domain. A first and second switch domain are collectively referred to as a dimerization switch. In embodiments, the first and second switch domains are the same as one another, for example, they are polypeptides having the same primary amino acid sequence and are referred to collectively as a homodimerization switch. In embodiments, the first and second switch domains are different from one another, for example, they are polypeptides having different primary amino acid sequences, and are referred to collectively as a heterodimerization switch. In embodiments, the switch is

intracellular. In embodiments, the switch is extracellular. In embodiments, the switch domain is a polypeptide-based entity, for example, FKBP or FRB-based, and the dimerization molecule is small molecule, for example, a rapalogue. In embodiments, the switch domain is a polypeptide-based entity, for example, an scFv that binds a myc peptide, and the dimerization molecule is a polypeptide, a fragment thereof, or a multimer of a polypeptide, for example, a myc ligand or multimers of a myc ligand that bind to one or more myc scFvs. In embodiments, the switch domain is a polypeptide-based entity, for example, myc receptor, and the dimerization molecule is an antibody or fragments thereof, for example, myc antibody.

"Dimerization molecule," as that term is used herein, for example, when referring to an RCAR, refers to a molecule that promotes the association of a first switch domain with a second switch domain. In embodiments, the dimerization molecule does not naturally occur in the subject or does not occur in concentrations that would result in significant dimerization. In embodiments, the dimerization molecule is a small molecule, for example, rapamycin or a rapalogue, for example, RAD001.

The term "low, immune enhancing, dose" when used in conjunction with an mTOR inhibitor, for example, an allosteric mTOR inhibitor, for example, RAD001 or rapamycin, or a catalytic mTOR inhibitor, refers to a dose of mTOR inhibitor that partially, but not fully, inhibits mTOR activity, for example, as measured by the inhibition of P70 S6 kinase activity. Methods for evaluating mTOR activity, for example, by inhibition of P70 S6 kinase, are discussed herein. The dose is insufficient to result in complete immune suppression but is sufficient to enhance the immune response. In some embodiments, the low, immune enhancing, dose of mTOR inhibitor results in a decrease in the number of PD-1 positive T cells and/or an increase in the number of PD-1 negative T cells, or an increase in the ratio of PD-1 negative T cells/PD-1 positive T cells. In some embodiments, the low, immune enhancing, dose of mTOR inhibitor results in an increase in the number of naive T cells. In some embodiments, the low, immune enhancing, dose of mTOR inhibitor results in one or more of the following:

an increase in the expression of one or more of the following markers: $CD62L^{high}$, $CD127^{high}$, $CD27^{+}$, and BCL2, for example, on memory T cells, for example, memory T cell precursors;

a decrease in the expression of KLRG1, for example, on memory T cells, for example, memory T cell precursors; and

an increase in the number of memory T cell precursors, for example, cells with any one or combination of the following characteristics: increased $CD62L^{high}$, increased $CD127^{high}$, increased $CD27^{+}$, decreased KLRG1, and increased BCL2;

wherein any of the changes described above occurs, for example, at least transiently, for example, as compared to a non-treated subject.

"Refractory" as used herein refers to a disease, for example, cancer, that does not respond to a treatment. In embodiments, a refractory cancer can be resistant to a treatment before or at the beginning of the treatment. In other embodiments, the refractory cancer can become resistant during a treatment. A refractory cancer is also called a resistant cancer.

"Relapsed" or "relapse" as used herein refers to the return or reappearance of a disease (for example, cancer) or the signs and symptoms of a disease such as cancer after a period of improvement or responsiveness, for example, after prior treatment of a therapy, for example, cancer therapy. The initial period of responsiveness may involve the level of cancer cells falling below a certain threshold, for example, below 20%, 1%, 10%, 5%, 4%, 3%, 2%, or 1%. The reappearance may involve the level of cancer cells rising above a certain threshold, for example, above 20%, 1%, 10%, 5%, 4%, 3%, 2%, or 1%. For example, for example, in the context of B-ALL, the reappearance may involve, for example, a reappearance of blasts in the blood, bone marrow (> 5%), or any extramedullary site, after a complete response. A complete response, in this context, may involve < 5% BM blast. More generally, in some embodiments, a response (for example, complete response or partial response) can involve the absence of detectable MRD (minimal residual disease). In some embodiments, the initial period of responsiveness lasts at least 1, 2, 3, 4, 5, or 6 days; at least 1, 2, 3, or 4 weeks; at least 1, 2, 3, 4, 6, 8, 10, or 12 months; or at least 1, 2, 3, 4, or 5 years.

Ranges: throughout this disclosure, various embodiments of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2,

2.7, 3, 4, 5, 5.3, and 6. As another example, a range such as 95-99% identity, includes something with 95%, 96%, 97%, 98%, or 99% identity, and includes subranges such as 96-99%, 96-98%, 96-97%, 97-99%, 97-98%, and 98-99% identity. This applies regardless of the breadth of the range.

A "gene editing system" as the term is used herein, refers to a system, for example, one or more molecules, that direct and effect an alteration, for example, a deletion, of one or more nucleic acids at or near a site of genomic DNA targeted by said system. Gene editing systems are known in the art and are described more fully below.

Administered "in combination", as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, for example, the two or more treatments are delivered after the subject has been diagnosed with the disorder and before the disorder has been cured or eliminated or treatment has ceased for other reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery". In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, for example, an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

The term "depletion" or "depleting", as used interchangeably herein, refers to the decrease or reduction of the level or amount of a cell, a protein, or macromolecule in a sample after a process, for example, a selection step, for example, a negative selection, is performed. The depletion can be a complete or partial depletion of the cell, protein, or macromolecule. In some embodiments, the depletion is at least a 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%,

40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% decrease or reduction of the level or amount of a cell, a protein, or macromolecule, as compared to the level or amount of the cell, protein or macromolecule in the sample before the process was performed.

As used herein, a "naïve T cell" refers to a T cell that is antigen-inexperienced. In some embodiments, an antigen-inexperienced T cell has encountered its cognate antigen in the thymus but not in the periphery. In some embodiments, naïve T cells are precursors of memory cells. In some embodiments, naïve T cells express both CD45RA and CCR7, but do not express CD45RO. In some embodiments, naïve T cells may be characterized by expression of CD62L, CD27, CCR7, CD45RA, CD28, and CD127, and the absence of CD95 or CD45RO isoform. In some embodiments, naïve T cells express CD62L, IL-7 receptor-α, IL-6 receptor, and CD132, but do not express CD25, CD44, CD69, or CD45RO. In some embodiments, naïve T cells express CD45RA, CCR7, and CD62L and do not express CD95 or IL-2 receptor β. In some embodiments, surface expression levels of markers are assessed using flow cytometry.

The term "central memory T cells" refers to a subset of T cells that in humans are CD45RO positive and express CCR7. In some embodiments, central memory T cells express CD95. In some embodiments, central memory T cells express IL-2R, IL-7R and/or IL-15R. In some embodiments, central memory T cells express CD45RO, CD95, IL-2 receptor β, CCR7, and CD62L. In some embodiments, surface expression levels of markers are assessed using flow cytometry.

The term "stem memory T cells," "stem cell memory T cells," "stem cell-like memory T cells," "memory stem T cells," "T memory stem cells," "T stem cell memory cells" or "TSCM cells" refers to a subset of memory T cells with stem cell-like ability, for example, the ability to self-renew and/or the multipotent capacity to reconstitute memory and/or effector T cell subsets. In some embodiments, stem memory T cells express CD45RA, CD95, IL-2 receptor β, CCR7, and CD62L. In some embodiments, surface expression levels of markers are assessed using flow cytometry. In some embodiments, exemplary stem memory T cells are disclosed in Gattinoni et al., Nat Med. 2017 January 06; 23(1): 18–27, herein incorporated by reference in its entirety.

For clarity purposes, unless otherwise noted, classifying a cell or a population of cells as "not expressing," or having an "absence of" or being "negative for" a particular marker may not necessarily mean an absolute absence of the marker. The skilled artisan can readily compare the cell against a positive and/or a negative control, and/or set a predetermined threshold, and classify the cell or population of cells as not expressing or being negative for the marker when the cell has an expression level below the predetermined threshold or a population of cells has an overall expression level below the predetermined threshold using conventional detection methods, e.g., using flow cytometry, for example, as described in the Examples herein. For example, representative gating strategies are shown in FIG. 1G. For example, CCR7 positive, CD45RO negative cells are shown in the top left quadrant in FIG. 1G.

As used herein, the term "GeneSetScore (Up TEM vs. Down TSCM)" of a cell refers to a score that reflects the degree at which the cell shows an effector memory T cell (TEM) phenotype vs. a stem cell memory T cell (TSCM) phenotype. A higher GeneSetScore (Up TEM vs. Down TSCM) indicates an increasing TEM phenotype, whereas a lower GeneSetScore (Up TEM vs. Down TSCM) indicates an increasing TSCM phenotype. In some embodiments, the GeneSetScore (Up TEM vs. Down TSCM) is determined by measuring the expression of one or more genes that are up-regulated in TEM cells and/or down-regulated in TSCM cells, for example, one or more genes selected from the group consisting of MXRA7, CLIC1, NAT13, TBC1D2B, GLCCI1, DUSP10, APOBEC3D, CACNB3, ANXA2P2, TPRG1, EOMES, MATK, ARHGAP10, ADAM8, MAN1A1, SLFN12L, SH2D2A, EIF2C4, CD58, MYO1F, RAB27B, ERN1, NPC1, NBEAL2, APOBEC3G, SYTL2, SLC4A4, PIK3AP1, PTGDR, MAF, PLEKHA5, ADRB2, PLXND1, GNAO1, THBS1, PPP2R2B, CYTH3, KLRF1, FLJ16686, AUTS2, PTPRM, GNLY, and GFPT2. In some embodiments, the GeneSetScore (Up TEM vs. Down TSCM) is determined for each cell using RNA-seq, for example, single-cell RNA-seq (scRNA-seq), for example, as exemplified in Example 10 with respect to FIG. 39A. In some embodiments, the GeneSetScore (Up TEM vs. Down TSCM) is calculated by taking the mean log normalized gene expression value of all of the genes in the gene set.

As used herein, the term "GeneSetScore (Up Treg vs. Down Teff)" of a cell refers to a score that reflects the degree at which the cell shows a regulatory T cell (Treg) phenotype vs. an effector T cell (Teff) phenotype. A higher GeneSetScore (Up Treg vs. Down Teff) indicates

an increasing Treg phenotype, whereas a lower GeneSetScore (Up Treg vs. Down Teff) indicates an increasing Teff phenotype. In some embodiments, the GeneSetScore (Up Treg vs. Down Teff) is determined by measuring the expression of one or more genes that are up-regulated in Treg cells and/or down-regulated in Teff cells, for example, one or more genes selected from the group consisting of C12orf75, SELPLG, SWAP70, RGS1, PRR11, SPATS2L, SPATS2L, TSHR, C14orf145, CASP8, SYT11, ACTN4, ANXA5, GLRX, HLA-DMB, PMCH, RAB11FIP1, IL32, FAM160B1, SHMT2, FRMD4B, CCR3, TNFRSF13B, NTNG2, CLDND1, BARD1, FCER1G, TYMS, ATP1B1, GJB6, FGL2, TK1, SLC2A8, CDKN2A, SKAP2, GPR55, CDCA7, S100A4, GDPD5, PMAIP1, ACOT9, CEP55, SGMS1, ADPRH, AKAP2, HDAC9, IKZF4, CARD17, VAV3, OBFC2A, ITGB1, CIITA, SETD7, HLA-DMA, CCR10, KIAA0101, SLC14A1, PTTG3P, DUSP10, FAM164A, PYHIN1, MYO1F, SLC1A4, MYBL2, PTTG1, RRM2, TP53INP1, CCR5, ST8SIA6, TOX, BFSP2, ITPRIPL1, NCAPH, HLA-DPB2, SYT4, NINJ2, FAM46C, CCR4, GBP5, C15orf53, LMCD1, MKI67, NUSAP1, PDE4A, E2F2, CD58, ARHGEF12, LOC100188949, FAS, HLA-DPB1, SELP, WEE1, HLA-DPA1, FCRL1, ICA1, CNTNAP1, OAS1, METTL7A, CCR6, HLA-DRB4, ANXA2P3, STAM, HLA-DQB2, LGALS1, ANXA2, PI16, DUSP4, LAYN, ANXA2P2, PTPLA, ANXA2P1, ZNF365, LAIR2, LOC541471, RASGRP4, BCAS1, UTS2, MIAT, PRDM1, SEMA3G, FAM129A, HPGD, NCF4, LGALS3, CEACAM4, JAKMIP1, TIGIT, HLA-DRA, IKZF2, HLA-DRB1, FANK1, RTKN2, TRIB1, FCRL3, and FOXP3. In some embodiments, the GeneSetScore (Up Treg vs. Down Teff) is determined using RNA-seq, for example, single-cell RNA-seq (scRNA-seq), for example, as exemplified in Example 10 with respect to FIG. 39B. In some embodiments, the GeneSetScore (Up Treg vs. Down Teff) is calculated by taking the mean log normalized gene expression value of all of the genes in the gene set.

As used herein, the term "GeneSetScore (Down stemness)" of a cell refers to a score that reflects the degree at which the cell shows a stemness phenotype. A lower GeneSetScore (Down stemness) indicates an increasing stemness phenotype. In some embodiments, the GeneSetScore (Down stemness) is determined by measuring the expression of one or more genes that are upregulated in a differentiating stem cell vs downregulated in a hematopoietic stem cell, for example, one or more genes selected from the group consisting of ACE, BATF, CDK6, CHD2, ERCC2, HOXB4, MEOX1, SFRP1, SP7, SRF, TAL1, and XRCC5. In some

embodiments, the GeneSetScore (Down stemness) is determined using RNA-seq, for example, single-cell RNA-seq (scRNA-seq), for example, as exemplified in Example 10 with respect to FIG. 39C. In some embodiments, the GeneSetScore (Down stemness) is calculated by taking the mean log normalized gene expression value of all of the genes in the gene set.

As used herein, the term "GeneSetScore (Up hypoxia)" of a cell refers to a score that reflects the degree at which the cell shows a hypoxia phenotype. A higher GeneSetScore (Up hypoxia) indicates an increasing hypoxia phenotype. In some embodiments, the GeneSetScore (Up hypoxia) is determined by measuring the expression of one or more genes that are up-regulated in cells undergoing hypoxia, for example, one or more genes selected from the group consisting of ABCB1, ACAT1, ADM, ADORA2B, AK2, AK3, ALDH1A1, ALDH1A3, ALDOA, ALDOC, ANGPT2, ANGPTL4, ANXA1, ANXA2, ANXA5, ARHGAP5, ARSE, ART1, BACE2, BATF3, BCL2L1, BCL2L2, BHLHE40, BHLHE41, BIK, BIRC2, BNIP3, BNIP3L, BPI, BTG1, C11orf2, C7orf68, CA12, CA9, CALD1, CCNG2, CCT6A, CD99, CDK1, CDKN1A, CDKN1B, CITED2, CLK1, CNOT7, COL4A5, COL5A1, COL5A2, COL5A3, CP, CTSD, CXCR4, D4S234E, DDIT3, DDIT4, 1-Dec, DKC1, DR1, EDN1, EDN2, EFNA1, EGF, EGR1, EIF4A3, ELF3, ELL2, ENG, ENO1, ENO3, ENPEP, EPO, ERRFI1, ETS1, F3, FABP5, FGF3, FKBP4, FLT1, FN1, FOS, FTL, GAPDH, GBE1, GLRX, GPI, GPRC5A, HAP1, HBP1, HDAC1, HDAC9, HERC3, HERPUD1, HGF, HIF1A, HK1, HK2, HLA-DQB1, HMOX1, HMOX2, HSPA5, HSPD1, HSPH1, HYOU1, ICAM1, ID2, IFI27, IGF2, IGFBP1, IGFBP2, IGFBP3, IGFBP5, IL6, IL8, INSIG1, IRF6, ITGA5, JUN, KDR, KRT14, KRT18, KRT19, LDHA, LDHB, LEP, LGALS1, LONP1, LOX, LRP1, MAP4, MET, MIF, MMP13, MMP2, MMP7, MPI, MT1L, MTL3P, MUC1, MXI1, NDRG1, NFIL3, NFKB1, NFKB2, NOS1, NOS2, NOS2P1, NOS2P2, NOS3, NR3C1, NR4A1, NT5E, ODC1, P4HA1, P4HA2, PAICS, PDGFB, PDK3, PFKFB1, PFKFB3, PFKFB4, PFKL, PGAM1, PGF, PGK1, PGK2, PGM1, PIM1, PIM2, PKM2, PLAU, PLAUR, PLIN2, PLOD2, PNN, PNP, POLM, PPARA, PPAT, PROK1, PSMA3, PSMD9, PTGS1, PTGS2, QSOX1, RBPJ, RELA, RIOK3, RNASEL, RPL36A, RRP9, SAT1, SERPINB2, SERPINE1, SGSM2, SIAH2, SIN3A, SIRPA, SLC16A1, SLC16A2, SLC20A1, SLC2A1, SLC2A3, SLC3A2, SLC6A10P, SLC6A16, SLC6A6, SLC6A8, SORL1, SPP1, SRSF6, SSSCA1, STC2, STRA13, SYT7, TBPL1, TCEAL1, TEK, TF, TFF3, TFRC, TGFA, TGFB1, TGFB3, TGFBI, TGM2, TH, THBS1, THBS2, TIMM17A, TNFAIP3, TP53, TPBG, TPD52, TPI1, TXN, TXNIP, UMPS,

VEGFA, VEGFB, VEGFC, VIM, VPS11, and XRCC6. In some embodiments, the GeneSetScore (Up hypoxia) is determined using RNA-seq, for example, single-cell RNA-seq (scRNA-seq), for example, as exemplified in Example 10 with respect to FIG. 39D. In some embodiments, the GeneSetScore (Up hypoxia) is calculated by taking the mean log normalized gene expression value of all of the genes in the gene set.

As used herein, the term "GeneSetScore (Up autophagy)" of a cell refers to a score that reflects the degree at which the cell shows an autophagy phenotype. A higher GeneSetScore (Up autophagy) indicates an increasing autophagy phenotype. In some embodiments, the GeneSetScore (Up autophagy) is determined by measuring the expression of one or more genes that are up-regulated in cells undergoing autophagy, for example, one or more genes selected from the group consisting of ABL1, ACBD5, ACIN1, ACTRT1, ADAMTS7, AKR1E2, ALKBH5, ALPK1, AMBRA1, ANXA5, ANXA7, ARSB, ASB2, ATG10, ATG12, ATG13, ATG14, ATG16L1, ATG16L2, ATG2A, ATG2B, ATG3, ATG4A, ATG4B, ATG4C, ATG4D, ATG5, ATG7, ATG9A, ATG9B, ATP13A2, ATP1B1, ATPAF1-AS1, ATPIF1, BECN1, BECN1P1, BLOC1S1, BMP2KL, BNIP1, BNIP3, BOC, C11orf2, C11orf41, C12orf44, C12orf5, C14orf133, C1orf210, C5, C6orf106, C7orf59, C7orf68, C8orf59, C9orf72, CA7, CALCB, CALCOCO2, CAPS, CCDC36, CD163L1, CD93, CDC37, CDKN2A, CHAF1B, CHMP2A, CHMP2B, CHMP3, CHMP4A, CHMP4B, CHMP4C, CHMP6, CHST3, CISD2, CLDN7, CLEC16A, CLN3, CLVS1, COX8A, CPA3, CRNKL1, CSPG5, CTSA, CTSB, CTSD, CXCR7, DAP, DKKL1, DNAAF2, DPF3, DRAM1, DRAM2, DYNLL1, DYNLL2, DZANK1, EI24, EIF2S1, EPG5, EPM2A, FABP1, FAM125A, FAM131B, FAM134B, FAM13B, FAM176A, FAM176B, FAM48A, FANCC, FANCF, FANCL, FBXO7, FCGR3B, FGF14, FGF7, FGFBP1, FIS1, FNBP1L, FOXO1, FUNDC1, FUNDC2, FXR2, GABARAP, GABARAPL1, GABARAPL2, GABARAPL3, GABRA5, GDF5, GMIP, HAP1, HAPLN1, HBXIP, HCAR1, HDAC6, HGS, HIST1H3A, HIST1H3B, HIST1H3C, HIST1H3D, HIST1H3E, HIST1H3F, HIST1H3G, HIST1H3H, HIST1H3I, HIST1H3J, HK2, HMGB1, HPR, HSF2BP, HSP90AA1, HSPA8, IFI16, IPPK, IRGM, IST1, ITGB4, ITPKC, KCNK3, KCNQ1, KIAA0226, KIAA1324, KRCC1, KRT15, KRT73, LAMP1, LAMP2, LAMTOR1, LAMTOR2, LAMTOR3, LARP1B, LENG9, LGALS8, LIX1, LIX1L, LMCD1, LRRK2, LRSAM1, LSM4, MAP1A, MAP1LC3A, MAP1LC3B, MAP1LC3B2, MAP1LC3C, MAP1S, MAP2K1, MAP3K12, MARK2, MBD5, MDH1, MEX3C, MFN1, MFN2, MLST8, MRPS10,

MRPS2, MSTN, MTERFD1, MTMR14, MTMR3, MTOR, MTSS1, MYH11, MYLK, MYOM1, NBR1, NDUFB9, NEFM, NHLRC1, NME2, NPC1, NR2C2, NRBF2, NTHL1, NUP93, OBSCN, OPTN, P2RX5, PACS2, PARK2, PARK7, PDK1, PDK4, PEX13, PEX3, PFKP, PGK2, PHF23, PHYHIP, PI4K2A, PIK3C3, PIK3CA, PIK3CB, PIK3R4, PINK1, PLEKHM1, PLOD2, PNPO, PPARGC1A, PPY, PRKAA1, PRKAA2, PRKAB1, PRKAB2, PRKAG1, PRKAG2, PRKAG3, PRKD2, PRKG1, PSEN1, PTPN22, RAB12, RAB1A, RAB1B, RAB23, RAB24, RAB33B, RAB39, RAB7A, RB1CC1, RBM18, REEP2, REP15, RFWD3, RGS19, RHEB, RIMS3, RNF185, RNF41, RPS27A, RPTOR, RRAGA, RRAGB, RRAGC, RRAGD, S100A8, S100A9, SCN1A, SERPINB10, SESN2, SFRP4, SH3GLB1, SIRT2, SLC1A3, SLC1A4, SLC22A3, SLC25A19, SLC35B3, SLC35C1, SLC37A4, SLC6A1, SLCO1A2, SMURF1, SNAP29, SNAPIN, SNF8, SNRPB, SNRPB2, SNRPD1, SNRPF, SNTG1, SNX14, SPATA18, SQSTM1, SRPX, STAM, STAM2, STAT2, STBD1, STK11, STK32A, STOM, STX12, STX17, SUPT3H, TBC1D17, TBC1D25, TBC1D5, TCIRG1, TEAD4, TECPR1, TECPR2, TFEB, TM9SF1, TMBIM6, TMEM203, TMEM208, TMEM39A, TMEM39B, TMEM59, TMEM74, TMEM93, TNIK, TOLLIP, TOMM20, TOMM22, TOMM40, TOMM5, TOMM6, TOMM7, TOMM70A, TP53INP1, TP53INP2, TRAPPC8, TREM1, TRIM17, TRIM5, TSG101, TXLNA, UBA52, UBB, UBC, UBQLN1, UBQLN2, UBQLN4, ULK1, ULK2, ULK3, USP10, USP13, USP30, UVRAG, VAMP7, VAMP8, VDAC1, VMP1, VPS11, VPS16, VPS18, VPS25, VPS28, VPS33A, VPS33B, VPS36, VPS37A, VPS37B, VPS37C, VPS37D, VPS39, VPS41, VPS4A, VPS4B, VTA1, VTI1A, VTI1B, WDFY3, WDR45, WDR45L, WIPI1, WIPI2, XBP1, YIPF1, ZCCHC17, ZFYVE1, ZKSCAN3, ZNF189, ZNF593, and ZNF681. In some embodiments, the GeneSetScore (Up autophagy) is determined using RNA-seq, for example, single-cell RNA-seq (scRNA-seq), for example, as exemplified in Example 10 with respect to FIG. 39E. In some embodiments, the GeneSetScore (Up autophagy) is calculated by taking the mean log normalized gene expression value of all of the genes in the gene set.

As used herein, the term "GeneSetScore (Up resting vs. Down activated)" of a cell refers to a score that reflects the degree at which the cell shows a resting T cell phenotype vs. an activated T cell phenotype. A higher GeneSetScore (Up resting vs. Down activated) indicates an increasing resting T cell phenotype, whereas a lower GeneSetScore (Up resting vs. Down activated) indicates an increasing activated T cell phenotype. In some embodiments, the

GeneSetScore (Up resting vs. Down activated) is determined by measuring the expression of one or more genes that are up-regulated in resting T cells and/or down-regulated in activated T cells, for example, one or more genes selected from the group consisting of ABCA7, ABCF3, ACAP2, AMT, ANKH, ATF7IP2, ATG14, ATP1A1, ATXN7, ATXN7L3B, BCL7A, BEX4, BSDC1, BTG1, BTG2, BTN3A1, C11orf21, C19orf22, C21orf2, CAMK2G, CARS2, CCNL2, CD248, CD5, CD55, CEP164, CHKB, CLK1, CLK4, CTSL1, DBP, DCUN1D2, DENND1C, DGKD, DLG1, DUSP1, EAPP, ECE1, ECHDC2, ERBB2IP, FAM117A, FAM134B, FAM134C, FAM169A, FAM190B, FAU, FLJ10038, FOXJ2, FOXJ3, FOXL1, FOXO1, FXYD5, FYB, HLA-E, HSPA1L, HYAL2, ICAM2, IFIT5, IFITM1, IKBKB, IQSEC1, IRS4, KIAA0664L3, KIAA0748, KLF3, KLF9, KRT18, LEF1, LINC00342, LIPA, LIPT1, LLGL2, LMBR1L, LPAR2, LTBP3, LYPD3, LZTFL1, MANBA, MAP2K6, MAP3K1, MARCH8, MAU2, MGEA5, MMP8, MPO, MSL1, MSL3, MYH3, MYLIP, NAGPA, NDST2, NISCH, NKTR, NLRP1, NOSIP, NPIP, NUMA1, PAIP2B, PAPD7, PBXIP1, PCIF1, PI4KA, PLCL2, PLEKHA1, PLEKHF2, PNISR, PPFIBP2, PRKCA, PRKCZ, PRKD3, PRMT2, PTP4A3, PXN, RASA2, RASA3, RASGRP2, RBM38, REPIN1, RNF38, RNF44, ROR1, RPL30, RPL32, RPLP1, RPS20, RPS24, RPS27, RPS6, RPS9, RXRA, RYK, SCAND2, SEMA4C, SETD1B, SETD6, SETX, SF3B1, SH2B1, SLC2A4RG, SLC35E2B, SLC46A3, SMAGP, SMARCE1, SMPD1, SNPH, SP140L, SPATA6, SPG7, SREK1IP1, SRSF5, STAT5B, SVIL, SYF2, SYNJ2BP, TAF1C, TBC1D4, TCF20, TECTA, TES, TMEM127, TMEM159, TMEM30B, TMEM66, TMEM8B, TP53TG1, TPCN1, TRIM22, TRIM44, TSC1, TSC22D1, TSC22D3, TSPYL2, TTC9, TTN, UBE2G2, USP33, USP34, VAMP1, VILL, VIPR1, VPS13C, ZBED5, ZBTB25, ZBTB40, ZC3H3, ZFP161, ZFP36L1, ZFP36L2, ZHX2, ZMYM5, ZNF136, ZNF148, ZNF318, ZNF350, ZNF512B, ZNF609, ZNF652, ZNF83, ZNF862, and ZNF91. In some embodiments, the GeneSetScore (Up resting vs. Down activated) is determined using RNA-seq, for example, single-cell RNA-seq (scRNA-seq), for example, as exemplified in Example 10 with respect to FIG. 38D. In some embodiments, the GeneSetScore (Up resting vs. Down activated) is calculated by taking the mean log normalized gene expression value of all of the genes in the gene set.

As used herein, the term "GeneSetScore (Progressively up in memory differentiation)" of a cell refers to a score that reflects the stage of the cell in memory differentiation. A higher GeneSetScore (Progressively up in memory differentiation) indicates an increasing late

memory T cell phenotype, whereas a lower GeneSetScore (Progressively up in memory differentiation) indicates an increasing early memory T cell phenotype. In some embodiments, the GeneSetScore (Up autophagy) is determined by measuring the expression of one or more genes that are up-regulated during memory differentiation, for example, one or more genes selected from the group consisting of MTCH2, RAB6C, KIAA0195, SETD2, C2orf24, NRD1, GNA13, COPA, SELT, TNIP1, CBFA2T2, LRP10, PRKCI, BRE, ANKS1A, PNPLA6, ARL6IP1, WDFY1, MAPK1, GPR153, SHKBP1, MAP1LC3B2, PIP4K2A, HCN3, GTPBP1, TLN1, C4orf34, KIF3B, TCIRG1, PPP3CA, ATG4D, TYMP, TRAF6, C17orf76, WIPF1, FAM108A1, MYL6, NRM, SPCS2, GGT3P, GALK1, CLIP4, ARL4C, YWHAQ, LPCAT4, ATG2A, IDS, TBC1D5, DMPK, ST6GALNAC6, REEP5, ABHD6, KIAA0247, EMB, TSEN54, SPIRE2, PIWIL4, ZSCAN22, ICAM1, CHD9, LPIN2, SETD8, ZC3H12A, ULBP3, IL15RA, HLA-DQB2, LCP1, CHP, RUNX3, TMEM43, REEP4, MEF2D, ABL1, TMEM39A, PCBP4, PLCD1, CHST12, RASGRP1, C1orf58, C11orf63, C6orf129, FHOD1, DKFZp434F142, PIK3CG, ITPR3, BTG3, C4orf50, CNNM3, IFI16, AK1, CDK2AP1, REL, BCL2L1, MVD, TTC39C, PLEKHA2, FKBP11, EML4, FANCA, CDCA4, FUCA2, MFSD10, TBCD, CAPN2, IQGAP1, CHST11, PIK3R1, MYO5A, KIR2DL3, DLG3, MXD4, RALGDS, S1PR5, WSB2, CCR3, TIPARP, SP140, CD151, SOX13, KRTAP5-2, NF1, PEA15, PARP8, RNF166, UEVLD, LIMK1, CACNB1, TMX4, SLC6A6, LBA1, SV2A, LLGL2, IRF1, PPP2R5C, CD99, RAPGEF1, PPP4R1, OSBPL7, FOXP4, SLA2, TBC1D2B, ST7, JAZF1, GGA2, PI4K2A, CD68, LPGAT1, STX11, ZAK, FAM160B1, RORA, C8orf80, APOBEC3F, TGFBI, DNAJC1, GPR114, LRP8, CD69, CMIP, NAT13, TGFB1, FLJ00049, ANTXR2, NR4A3, IL12RB1, NTNG2, RDX, MLLT4, GPRIN3, ADCY9, CD300A, SCD5, ABI3, PTPN22, LGALS1, SYTL3, BMPR1A, TBK1, PMAIP1, RASGEF1A, GCNT1, GABARAPL1, STOM, CALHM2, ABCA2, PPP1R16B, SYNE2, PAM, C12orf75, CLCF1, MXRA7, APOBEC3C, CLSTN3, ACOT9, HIP1, LAG3, TNFAIP3, DCBLD1, KLF6, CACNB3, RNF19A, RAB27A, FADS3, DLG5, APOBEC3D, TNFRSF1B, ACTN4, TBKBP1, ATXN1, ARAP2, ARHGEF12, FAM53B, MAN1A1, FAM38A, PLXNC1, GRLF1, SRGN, HLA-DRB5, B4GALT5, WIPI1, PTPRJ, SLFN11, DUSP2, ANXA5, AHNAK, NEO1, CLIC1, EIF2C4, MAP3K5, IL2RB, PLEKHG1, MYO6, GTDC1, EDARADD, GALM, TARP, ADAM8, MSC, HNRPLL, SYT11, ATP2B4, NHSL2, MATK, ARHGAP18, SLFN12L, SPATS2L, RAB27B, PIK3R3, TP53INP1, MBOAT1, GYG1, KATNAL1, FAM46C,

ZC3HAV1L, ANXA2P2, CTNNA1, NPC1, C3AR1, CRIM1, SH2D2A, ERN1, YPEL1, TBX21, SLC1A4, FASLG, PHACTR2, GALNT3, ADRB2, PIK3AP1, TLR3, PLEKHA5, DUSP10, GNAO1, PTGDR, FRMD4B, ANXA2, EOMES, CADM1, MAF, TPRG1, NBEAL2, PPP2R2B, PELO, SLC4A4, KLRF1, FOSL2, RGS2, TGFBR3, PRF1, MYO1F, GAB3, C17orf66, MICAL2, CYTH3, TOX, HLA-DRA, SYNE1, WEE1, PYHIN1, F2R, PLD1, THBS1, CD58, FAS, NETO2, CXCR6, ST6GALNAC2, DUSP4, AUTS2, C1orf21, KLRG1, TNIP3, GZMA, PRR5L, PRDM1, ST8SIA6, PLXND1, PTPRM, GFPT2, MYBL1, SLAMF7, FLJ16686, GNLY, ZEB2, CST7, IL18RAP, CCL5, KLRD1, and KLRB1. In some embodiments, the GeneSetScore (Progressively up in memory differentiation) is determined using RNA-seq, for example, single-cell RNA-seq (scRNA-seq), for example, as exemplified in Example 10 with respect to FIG. 40B. In some embodiments, the GeneSetScore (Progressively up in memory differentiation) is calculated by taking the mean log normalized gene expression value of all of the genes in the gene set.

As used herein, the term "GeneSetScore (Up TEM vs. Down TN)" of a cell refers to a score that reflects the degree at which the cell shows an effector memory T cell (TEM) phenotype vs. a naïve T cell (TN) phenotype. A higher GeneSetScore (Up TEM vs. Down TN) indicates an increasing TEM phenotype, whereas a lower GeneSetScore (Up TEM vs. Down TN) indicates an increasing TN phenotype. In some embodiments, the GeneSetScore (Up TEM vs. Down TN) is determined by measuring the expression of one or more genes that are up-regulated in TEM cells and/or down-regulated in TN cells, for example, one or more genes selected from the group consisting of MYO5A, MXD4, STK3, S1PR5, GLCCI1, CCR3, SOX13, KRTAP5-2, PEA15, PARP8, RNF166, UEVLD, LIMK1, SLC6A6, SV2A, KPNA2, OSBPL7, ST7, GGA2, PI4K2A, CD68, ZAK, RORA, TGFBI, DNAJC1, JOSD1, ZFYVE28, LRP8, OSBPL3, CMIP, NAT13, TGFB1, ANTXR2, NR4A3, RDX, ADCY9, CHN1, CD300A, SCD5, PTPN22, LGALS1, RASGEF1A, GCNT1, GLUL, ABCA2, CLDND1, PAM, CLCF1, MXRA7, CLSTN3, ACOT9, METRNL, BMPR1A, LRIG1, APOBEC3G, CACNB3, RNF19A, RAB27A, FADS3, ACTN4, TBKBP1, FAM53B, MAN1A1, FAM38A, GRLF1, B4GALT5, WIPI1, DUSP2, ANXA5, AHNAK, CLIC1, MAP3K5, ST8SIA1, TARP, ADAM8, MATK, SLFN12L, PIK3R3, FAM46C, ANXA2P2, CTNNA1, NPC1, SH2D2A, ERN1, YPEL1, TBX21, STOM, PHACTR2, GBP5, ADRB2, PIK3AP1, DUSP10, PTGDR, EOMES, MAF, TPRG1, NBEAL2, NCAPH, SLC4A4, FOSL2, RGS2, TGFBR3, MYO1F, C17orf66,

CYTH3, WEE1, PYHIN1, F2R, THBS1, CD58, AUTS2, FAM129A, TNIP3, GZMA, PRR5L, PRDM1, PLXND1, PTPRM, GFPT2, MYBL1, SLAMF7, ZEB2, CST7, CCL5, GZMK, and KLRB1. In some embodiments, the GeneSetScore (Up TEM vs. Down TN) is determined using RNA-seq, for example, single-cell RNA-seq (scRNA-seq), for example, as exemplified in Example 10 with respect to FIG. 40C. In some embodiments, the GeneSetScore (Up TEM vs. Down TN) is calculated by taking the mean log normalized gene expression value of all of the genes in the gene set.

In the context of GeneSetScore values (e.g., median GeneSetScore values), when a positive GeneSetScore is reduced by 100%, the value becomes 0. When a negative GeneSetScore is increased by 100%, the value becomes 0. For example, in FIG. 39A, the median GeneSetScore of the Day1 sample is -0.084; the median GeneSetScore of the Day9 sample is 0.035; and the median GeneSetScore of the input sample is -0.1. In FIG. 39A, increasing the median GeneSetScore of the input sample by 100% leads to a GeneSetScore value of 0; and increasing the median GeneSetScore of the input sample by 200% leads to a GeneSetScore value of 0.1. In FIG. 39A, decreasing the median GeneSetScore of the Day9 sample by 100% leads to a GeneSetScore value of 0; and decreasing the median GeneSetScore of the Day9 sample by 200% leads to a GeneSetScore value of -0.035.

As used herein, the term "bead" refers to a discrete particle with a solid surface, ranging in size from approximately 0.1 μm to several millimeters in diameter. Beads may be spherical (for example, microspheres) or have an irregular shape. Beads may comprise a variety of materials including, but not limited to, paramagnetic materials, ceramic, plastic, glass, polystyrene, methylstyrene, acrylic polymers, titanium, latex, Sepharose™, cellulose, nylon and the like. In some embodiments, the beads are relatively uniform, about 4.5 μm in diameter, spherical, superparamagnetic polystyrene beads, for example, coated, for example, covalently coupled, with a mixture of antibodies against CD3 (for example, CD3 epsilon) and CD28. In some embodiments, the beads are Dynabeads®. In some embodiments, both anti-CD3 and anti-CD28 antibodies are coupled to the same bead, mimicking stimulation of T cells by antigen presenting cells. The property of Dynabeads® and the use of Dynabeads® for cell isolation and expansion are well known in the art, for example, see, Neurauter et al., *Cell isolation and expansion using Dynabeads*, Adv Biochem Eng Biotechnol. 2007;106:41-73, herein incorporated by reference in its entirety.

As used herein, the term "nanomatrix" refers to a nanostructure comprising a matrix of mobile polymer chains. The nanomatrix is 1 to 500 nm, for example, 10 to 200 nm, in size. In some embodiments, the matrix of mobile polymer chains is attached to one or more agonists which provide activation signals to T cells, for example, agonist anti-CD3 and/or anti-CD28 antibodies. In some embodiments, the nanomatrix comprises a colloidal polymeric nanomatrix attached, for example, covalently attached, to an agonist of one or more stimulatory molecules and/or an agonist of one or more costimulatory molecules. In some embodiments, the agonist of one or more stimulatory molecules is a CD3 agonist (for example, an anti-CD3 agonistic antibody). In some embodiments, the agonist of one or more costimulatory molecules is a CD28 agonist (for example, an anti-CD28 agonistic antibody). In some embodiments, the nanomatrix is characterized by the absence of a solid surface, for example, as the attachment point for the agonists, such as anti-CD3 and/or anti-CD28 antibodies. In some embodiments, the nanomatrix is the nanomatrix disclosed in WO2014/048920A1 or as given in the MACS® GMP T Cell TransAct™ kit from Miltenyi Biotcc GmbH, herein incorporated by reference in their entirety. MACS® GMP T Cell TransAct™ consists of a colloidal polymeric nanomatrix covalently attached to humanized recombinant agonist antibodies against human CD3 and CD28.

Various embodiments of the compositions and methods herein are described in further detail below. Additional definitions are set out throughout the specification.

**Description**

Provided herein are methods of manufacturing immune effector cells (for example, T cells or NK cells) engineered to express a CAR, for example, a CAR described herein, compositions comprising such cells, and methods of using such cells for treating a disease, such as cancer, in a subject. In some embodiments, the methods disclosed herein may manufacture immune effector cells engineered to express a CAR in less than 24 hours. Without wishing to be bound by theory, the methods provided herein preserve the undifferentiated phenotype of T cells, such as naïve T cells, during the manufacturing process. These CAR-expressing cells with an undifferentiated phenotype may persist longer and/or expand better in vivo after infusion. In some embodiments, CART cells produced by the manufacturing methods provided herein comprise a higher percentage of stem cell memory T cells, compared to CART cells

produced by the traditional manufacturing process, e.g., as measured using scRNA-seq (e.g., as measured using methods described in Example 10 with respect to FIG. 39A). In some embodiments, CART cells produced by the manufacturing methods provided herein comprise a higher percentage of effector T cells, compared to CART cells produced by the traditional manufacturing process, e.g., as measured using scRNA-seq (e.g., as measured using methods described in Example 10 with respect to FIG. 39B). In some embodiments, CART cells produced by the manufacturing methods provided herein better preserve the stemness of T cells, compared to CART cells produced by the traditional manufacturing process, e.g., as measured using scRNA-seq (e.g., as measured using methods described in Example 10 with respect to FIG. 39C). In some embodiments, CART cells produced by the manufacturing methods provided herein show a lower level of hypoxia, compared to CART cells produced by the traditional manufacturing process, e.g., as measured using scRNA-seq (e.g., as measured using methods described in Example 10 with respect to FIG. 39D). In some embodiments, CART cells produced by the manufacturing methods provided herein show a lower level of autophagy, compared to CART cells produced by the traditional manufacturing process, e.g., as measured using scRNA-seq (e.g., as measured using methods described in Example 10 with respect to FIG. 39E).

In some embodiments, the methods disclosed herein do not involve using a bead, such as Dynabeads® (for example, CD3/CD28 Dynabeads®), and do not involve a de-beading step. In some embodiments, the CART cells manufactured by the methods disclosed herein may be administered to a subject with minimal ex vivo expansion, for example, less than 1 day, less than 12 hours, less than 8 hours, less than 6 hours, less than 4 hours, less than 3 hours, less than 2 hours, less than 1 hour, or no ex vivo expansion. Accordingly, the methods described herein provide a fast manufacturing process of making improved CAR-expressing cell products for use in treating a disease in a subject.

**Cytokine Process**

In some embodiments, the present disclosure provides methods of making a population of cells (for example, T cells) that express a chimeric antigen receptor (CAR) comprising: (1) contacting a population of cells with a cytokine chosen from IL-2, IL-7, IL-15, IL-21, IL-6, or a combination thereof, (2) contacting the population of cells (for example, T cells) with a nucleic acid molecule (for example, a DNA or RNA molecule) encoding the CAR, thereby providing a

population of cells (for example, T cells) comprising the nucleic acid molecule, and (3) harvesting the population of cells (for example, T cells) for storage (for example, reformulating the population of cells in cryopreservation media) or administration, wherein: (a) step (2) is performed together with step (1) or no later than 5 hours after the beginning of step (1), for example, no later than 1, 2, 3, 4, or 5 hours after the beginning of step (1), and step (3) is performed no later than 26 hours after the beginning of step (1), for example, no later than 22, 23, or 24 hours after the beginning of step (1), for example, no later than 24 hours after the beginning of step (1), or (b) the population of cells from step (3) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (1). In some embodiments, the nucleic acid molecule in step (2) is a DNA molecule. In some embodiments, the nucleic acid molecule in step (2) is an RNA molecule. In some embodiments, the nucleic acid molecule in step (2) is on a viral vector, for example, a viral vector chosen from a lentivirus vector, an adenoviral vector, or a retrovirus vector. In some embodiments, the nucleic acid molecule in step (2) is on a non-viral vector. In some embodiments, the nucleic acid molecule in step (2) is on a plasmid. In some embodiments, the nucleic acid molecule in step (2) is not on any vector. In some embodiments, step (2) comprises transducing the population of cells (for example, T cells) with a viral vector comprising a nucleic acid molecule encoding the CAR. In some embodiments, step (2) further comprises contacting the population of cells (for example , T cells) with shRNA that targets Tet2 comprising (A) a sense strand comprising a Tet2 target sequence and (B) an antisense strand complementary to the sense strand in whole or in part or a vector encoding the shRNA. In some embodiments, sense strand comprises the Tet2 target sequence GGGTAAGCCAAGAAAGAAA (SEQ ID NO: 418). In some embodiments, the anti-sense strand comprises the reverse complement thereof, i.e. TTTCTTTCTTGGCTTACCC (SEQ ID NO: 419). In some embodiments, the vector encoding the shRNA is the same or different from the vector encoding the CAR. In some embodiments, the vector encoding the shRNA sequence comprises promoter (such as but not limited to a U6 promoter), a sense strand comprising a Tet2 target sequence, a loop, an anti-sense strand complementary to the sense strand in whole or in part, and, optionally, a polyT tail, e.g. the sequences in **Table 29**.

In some embodiments, the population of cells (for example, T cells) is collected from an apheresis sample (for example, a leukapheresis sample) from a subject.

In some embodiments, the apheresis sample (for example, a leukapheresis sample) is collected from the subject and shipped as a frozen sample (for example, a cryopreserved sample) to a cell manufacturing facility. The frozen apheresis sample is then thawed, and T cells (for example, CD4+ T cells and/or CD8+ T cells) are selected from the apheresis sample, for example, using a cell sorting machine (for example, a CliniMACS® Prodigy® device). The selected T cells (for example, CD4+ T cells and/or CD8+ T cells) are then seeded for CART manufacturing using the cytokine process described herein. In some embodiments, at the end of the cytokine process, the CAR T cells are cryopreserved and later thawed and administered to the subject. In some embodiments, the selected T cells (for example, CD4+ T cells and/or CD8+ T cells) undergo one or more rounds of freeze-thaw before being seeded for CART manufacturing.

In some embodiments, the apheresis sample (for example, a leukapheresis sample) is collected from the subject and shipped as a fresh product (for example, a product that is not frozen) to a cell manufacturing facility. T cells (for example, CD4+ T cells and/or CD8+ T cells) are selected from the apheresis sample, for example, using a cell sorting machine (for example, a CliniMACS® Prodigy® device). The selected T cells (for example, CD4+ T cells and/or CD8+ T cells) are then seeded for CART manufacturing using the cytokine process described herein. In some embodiments, the selected T cells (for example, CD4+ T cells and/or CD8+ T cells) undergo one or more rounds of freeze-thaw before being seeded for CART manufacturing.

In some embodiments, the apheresis sample (for example, a leukapheresis sample) is collected from the subject. T cells (for example, CD4+ T cells and/or CD8+ T cells) are selected from the apheresis sample, for example, using a cell sorting machine (for example, a CliniMACS® Prodigy® device). The selected T cells (for example, CD4+ T cells and/or CD8+ T cells) are then shipped as a frozen sample (for example, a cryopreserved sample) to a cell manufacturing facility. The selected T cells (for example, CD4+ T cells and/or CD8+ T cells) are later thawed and seeded for CART manufacturing using the cytokine process described herein.

In some embodiments, after cells (for example, T cells) are seeded, one or more cytokines (for example, one or more cytokines chosen from IL-2, IL-7, IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)), IL-21, or IL-6 (for example, IL-6/sIL-6R)) as well as vectors (for example, lentiviral vectors) encoding a CAR are added to the cells. After incubation for 20-24 hours, the cells are washed and formulated for storage or administration.

Different from traditional CART manufacturing approaches, the cytokine process provided herein does not involve CD3 and/or CD28 stimulation, or ex vivo T cell expansion. T cells that are contacted with anti-CD3 and anti-CD28 antibodies and expanded extensively ex vivo tend to show differentiation towards a central memory phenotype. Without wishing to be bound by theory, the cytokine process provided herein preserves or increases the undifferentiated phenotype of T cells during CART manufacturing, generating a CART product that may persist longer after being infused into a subject.

In some embodiments, the population of cells is contacted with one or more cytokines (for example, one or more cytokines chosen from IL-2, IL-7, IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)), IL-21, or IL-6 (for example, IL-6/sIL-6Ra).

In some embodiments, the population of cells is contacted with IL-2. In some embodiments, the population of cells is contacted with IL-7. In some embodiments, the population of cells is contacted with IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)). In some embodiments, the population of cells is contacted with IL-21. In some embodiments, the population of cells is contacted with IL-6 (for example, IL-6/sIL-6Ra). In some embodiments, the population of cells is contacted with IL-2 and IL-7. In some embodiments, the population of cells is contacted with IL-2 and IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)). In some embodiments, the population of cells is contacted with IL-2 and IL-21. In some embodiments, the population of cells is contacted with IL-2 and IL-6 (for example, IL-6/sIL-6Ra). In some embodiments, the population of cells is contacted with IL-7 and IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)). In some embodiments, the population of cells is contacted with IL-7 and IL-21. In some embodiments, the population of cells is contacted with IL-7 and IL-6 (for example, IL-6/sIL-6Ra). In some embodiments, the population of cells is contacted with IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)) and IL-21. In some embodiments, the population of cells is contacted with IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)) and IL-6 (for example,

IL-6/sIL-6Ra). In some embodiments, the population of cells is contacted with IL-21 and IL-6 (for example, IL-6/sIL-6Ra). In some embodiments, the population of cells is contacted with IL-7, IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)), and IL-21. In some embodiments, the population of cells is further contacted with a LSD1 inhibitor. In some embodiments, the population of cells is further contacted with a MALT1 inhibitor.

In some embodiments, the population of cells is contacted with 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, or 300 U/ml of IL-2. In some embodiments, the population of cells is contacted with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 ng/ml of IL-7. In some embodiments, the population of cells is contacted with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 ng/ml of IL-15.

In some embodiments, the population of cells is contacted with a nucleic acid molecule encoding a CAR. In some embodiments, the population of cells is transduced with a DNA molecule encoding a CAR.

In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs simultaneously with contacting the population of cells with the one or more cytokines described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 hours after the beginning of contacting the population of cells with the one or more cytokines described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 5 hours after the beginning of contacting the population of cells with the one or more cytokines described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 4 hours after the beginning of contacting the population of cells with the one or more cytokines described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 3 hours after the beginning of contacting the population of cells with the one or more cytokines described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 2 hours after the beginning of contacting the population of cells with the one or more cytokines described above.

In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 1 hour after the beginning of contacting the population of cells with the one or more cytokines described above.

In some embodiments, the population of cells is harvested for storage or administration.

In some embodiments, the population of cells is harvested for storage or administration no later than 72, 60, 48, 36, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, or 18 hours after the beginning of contacting the population of cells with the one or more cytokines described above. In some embodiments, the population of cells is harvested for storage or administration no later than 26 hours after the beginning of contacting the population of cells with the one or more cytokines described above. In some embodiments, the population of cells is harvested for storage or administration no later than 25 hours after the beginning of contacting the population of cells with the one or more cytokines described above. In some embodiments, the population of cells is harvested for storage or administration no later than 24 hours after the beginning of contacting the population of cells with the one or more cytokines described above. In some embodiments, the population of cells is harvested for storage or administration no later than 23 hours after the beginning of contacting the population of cells with the one or more cytokines described above. In some embodiments, the population of cells is harvested for storage or administration no later than 22 hours after the beginning of contacting the population of cells with the one or more cytokines described above.

In some embodiments, the population of cells is not expanded ex vivo.

In some embodiments, the population of cells is expanded by no more than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, or 60%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the one or more cytokines described above. In some embodiments, the population of cells is expanded by no more than 5%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the one or more cytokines described above. In some embodiments, the population of cells is expanded by no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the one or more cytokines described above. In some embodiments, the population of cells is expanded by no more than 15%, for example, as

assessed by the number of living cells, compared to the population of cells before it is contacted with the one or more cytokines described above. In some embodiments, the population of cells is expanded by no more than 20%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the one or more cytokines described above. In some embodiments, the population of cells is expanded by no more than 25%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the one or more cytokines described above. In some embodiments, the population of cells is expanded by no more than 30%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the one or more cytokines described above. In some embodiments, the population of cells is expanded by no more than 35%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the one or more cytokines described above. In some embodiments, the population of cells is expanded by no more than 40%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the one or more cytokines described above.

In some embodiments, the population of cells is expanded by no more than 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20, 24, 36, or 48 hours, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the one or more cytokines described above.

In some embodiments, the population of cells is not contacted in vitro with an agent that stimulates a CD3/TCR complex (for example, an anti-CD3 antibody) and/or an agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells (for example, an anti-CD28 antibody), or if contacted, the contacting step is less than 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5 hours.

In some embodiments, the population of cells is contacted in vitro with an agent that stimulates a CD3/TCR complex (for example, an anti-CD3 antibody) and/or an agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells (for example, an anti-CD28 antibody) for 20, 21, 22, 23, 24, 25, 26, 27, or 28 hours.

In some embodiments, the population of cells manufactured using the cytokine process provided herein shows a higher percentage of naïve cells among CAR-expressing cells (for

example, at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, or 60% higher), compared with cells made by an otherwise similar method which further comprises contacting the population of cells with, for example, an agent that binds a CD3/TCR complex (for example, an anti-CD3 antibody) and/or an agent that binds a costimulatory molecule on the surface of the cells (for example, an anti-CD28 antibody).

In some embodiments, the cytokine process provided herein is conducted in cell media comprising no more than 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, or 8% serum. In some embodiments, the cytokine process provided herein is conducted in cell media comprising a LSD1 inhibitor, a MALT1 inhibitor, or a combination thereof.

**Activation Process**

In some embodiments, the present disclosure provides methods of making a population of cells (for example, T cells) that express a chimeric antigen receptor (CAR) comprising: (i) contacting a population of cells (for example, T cells, for example, T cells isolated from a frozen or fresh leukapheresis product) with (A) an agent that stimulates a CD3/TCR complex and/or (B) an agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells; (ii) contacting the population of cells (for example, T cells) with a nucleic acid molecule (for example, a DNA or RNA molecule) encoding the CAR, thereby providing a population of cells (for example, T cells) comprising the nucleic acid molecule, and (iii) harvesting the population of cells (for example, T cells) for storage (for example, reformulating the population of cells in cryopreservation media) or administration, wherein: (a) step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), for example, no later than 12, 13, 14, 15, 16, 17, or 18 hours after the beginning of step (i), for example, no later than 18 hours after the beginning of step (i), and step (iii) is performed no later than 26 hours after the beginning of step (i), for example, no later than 22, 23, or 24 hours after the beginning of step (i), for example, no later than 24 hours after the beginning of step (i); (b) step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), for example, no later than 12, 13, 14, 15, 16, 17, or 18 hours after the beginning of step (i), for example, no later than 18 hours after the beginning of step (i), and step (iii) is performed no later than 30, 36, or 48 hours after the beginning of step (ii), for example, no later than 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48 hours after the beginning of step (ii); or (c) the population of cells from step (iii)

are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (i). In some embodiments, the nucleic acid molecule in step (ii) is a DNA molecule. In some embodiments, the nucleic acid molecule in step (ii) is an RNA molecule. In some embodiments, the nucleic acid molecule in step (ii) is on a viral vector, for example, a viral vector chosen from a lentivirus vector, an adenoviral vector, or a retrovirus vector. In some embodiments, the nucleic acid molecule in step (ii) is on a non-viral vector. In some embodiments, the nucleic acid molecule in step (ii) is on a plasmid. In some embodiments, the nucleic acid molecule in step (ii) is not on any vector. In some embodiments, step (ii) comprises transducing the population of cells (for example, T cells) a viral vector comprising a nucleic acid molecule encoding the CAR. In some embodiments, step (2) further comprises contacting the population of cells (for example , T cells) with shRNA that targets Tet2 comprising (A) a sense strand comprising a Tet2 target sequence and (B) an antisense strand complementary to the sense strand in whole or in part or a vector encoding the shRNA. In some embodiments, sense strand comprises the Tet2 target sequence GGGTAAGCCAAGAAAGAAA. In some embodiments, the anti-sense strand comprises the reverse complement thereof, i.e. TTTCTTTCTTGGCTTACCC. In some embodiments, the vector encoding the shRNA is the same or different from the vector encoding the CAR. In some embodiments, the vector encoding the shRNA sequence comprises promoter (such as but not limited to a U6 promoter), a sense strand comprising a Tet2 target sequence, a loop, an anti-sense strand complementary to the sense strand in whole or in part, and, optionally, a polyT tail, e.g. the sequences in **Table 29**.

In some embodiments, the population of cells (for example, T cells) is collected from an apheresis sample (for example, a leukapheresis sample) from a subject.

In some embodiments, the apheresis sample (for example, a leukapheresis sample) is collected from the subject and shipped as a frozen sample (for example, a cryopreserved sample) to a cell manufacturing facility. Then the frozen apheresis sample is thawed, and T cells (for example, CD4+ T cells and/or CD8+ T cells) are selected from the apheresis sample, for example, using a cell sorting machine (for example, a CliniMACS® Prodigy® device). The selected T cells (for example, CD4+ T cells and/or CD8+ T cells) are then seeded for CART manufacturing using the activation process described herein. In some embodiments, the

selected T cells (for example, CD4+ T cells and/or CD8+ T cells) undergo one or more rounds of freeze-thaw before being seeded for CART manufacturing.

In some embodiments, the apheresis sample (for example, a leukapheresis sample) is collected from the subject and shipped as a fresh product (for example, a product that is not frozen) to a cell manufacturing facility. T cells (for example, CD4+ T cells and/or CD8+ T cells) are selected from the apheresis sample, for example, using a cell sorting machine (for example, a CliniMACS® Prodigy® device). The selected T cells (for example, CD4+ T cells and/or CD8+ T cells) are then seeded for CART manufacturing using the activation process described herein. In some embodiments, the selected T cells (for example, CD4+ T cells and/or CD8+ T cells) undergo one or more rounds of freeze-thaw before being seeded for CART manufacturing.

In some embodiments, the apheresis sample (for example, a leukapheresis sample) is collected from the subject. T cells (for example, CD4+ T cells and/or CD8+ T cells) are selected from the apheresis sample, for example, using a cell sorting machine (for example, a CliniMACS® Prodigy® device). The selected T cells (for example, CD4+ T cells and/or CD8+ T cells) are then shipped as a frozen sample (for example, a cryopreserved sample) to a cell manufacturing facility. The selected T cells (for example, CD4+ T cells and/or CD8+ T cells) are later thawed and seeded for CART manufacturing using the activation process described herein.

In some embodiments, cells (for example, T cells) are contacted with anti-CD3 and anti-CD28 antibodies for, for example, 12 hours, followed by transduction with a vector (for example, a lentiviral vector) encoding a CAR. 24 hours after culture initiation, the cells are washed and formulated for storage or administration.

Without wishing to be bound by theory, brief CD3 and CD28 stimulation may promote efficient transduction of self-renewing T cells. Compared to traditional CART manufacturing approaches, the activation process provided herein does not involve prolonged ex vivo expansion. Similar to the cytokine process, the activation process provided herein also preserves undifferentiated T cells during CART manufacturing.

In some embodiments, the population of cells is contacted with (A) an agent that stimulates a CD3/TCR complex and/or (B) an agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells.

In some embodiments, the agent that stimulates a CD3/TCR complex is an agent that stimulates CD3. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor is an agent that stimulates CD28, ICOS, CD27, HVEM, LIGHT, CD40, 4-1BB, OX40, DR3, GITR, CD30, TIM1, CD2, CD226, or any combination thereof. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor is an agent that stimulates CD28. In some embodiments, the agent that stimulates a CD3/TCR complex is chosen from an antibody (for example, a single-domain antibody (for example, a heavy chain variable domain antibody), a peptibody, a Fab fragment, or a scFv), a small molecule, or a ligand (for example, a naturally-existing, recombinant, or chimeric ligand). In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor is chosen from an antibody (for example, a single-domain antibody (for example, a heavy chain variable domain antibody), a peptibody, a Fab fragment, or a scFv), a small molecule, or a ligand (for example, a naturally-existing, recombinant, or chimeric ligand). In some embodiments, the agent that stimulates a CD3/TCR complex does not comprise a bead. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor does not comprise a bead. In some embodiments, the agent that stimulates a CD3/TCR complex comprises an anti-CD3 antibody. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor comprises an anti-CD28 antibody. In some embodiments, the agent that stimulates a CD3/TCR complex comprises an anti-CD3 antibody covalently attached to a colloidal polymeric nanomatrix. In some embodiments, the agent that stimulates CD3 comprises one or more of a CD3 or TCR antigen binding domain, such as but not limited to an anti-CD3 or anti-TCR antibody or an antibody fragment comprising one or more CDRs, heavy chain, and/or light chain thereof – such as but not limited to an anti-CD3 or anti-TCR antibody provided in Table 27. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor comprises an anti-CD28 antibody covalently attached to a colloidal polymeric nanomatrix. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor is an agent that stimulates CD28, ICOS, CD27, CD25, 4-1BB, IL6RA, IL6RB, or

CD2. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor comprises one or more of a CD28, ICOS, CD27, CD25, 4-1BB, IL6RB, and/or CD2 antigen binding domain, such as but not limited to an anti- CD28, anti-ICOS, anti-CD27, anti-CD25, anti-4-1BB, anti-IL6RA, anti-IL6RB, or anti-CD2 antibody or an antibody fragment comprising one or more CDRs, heavy chain, and/or light chain thereof – such as but not limited to an anti- CD28, anti-ICOS, anti-CD27, anti-CD25, anti-4-1BB, anti-IL6RA, anti-IL6RB, or anti-CD2 antibody provided in Table 27. In some embodiments, the agent that stimulates a CD3/TCR complex and the agent that stimulates a costimulatory molecule and/or growth factor receptor comprise T Cell TransAct™. In some embodiments, the agent that stimulates a CD3/TCR complex and the agent that stimulates a costimulatory molecule and/or growth factor receptor are comprised in a multispecific binding molecule. In some embodiments, the multispecific binding molecule comprises a CD3 antigen binding domain and a CD28 or CD2 antigen binding domain. In some embodiments, the multispecific binding molecules comprise one or more heavy and/or light chains – such as but not limited to the heavy and/or light chains provided in Table 28. In some embodiments, the multispecific binding molecule comprises a bispecific antibody. In some embodiments, the bispecific antibody is configured in any one of the schema provided in **FIG. 50A**. In some embodiments, the bispecific antibody is monovalent or bivalent. In some embodiments, the bispecific antibody comprises an Fc region. In some embodiments, the Fc region of the bispecific antibody is silenced. In some embodiments, the Fc region of the bispecific antibody is silenced by a combination of amino acid substitutions selected from the group consisting of LALA, DAPA, DANAPA, LALADANAPS, LALAGA, LALASKPA, DAPASK, GADAPA, GADAPASK, LALAPG, and LALAPA. In some embodiments, the multispecific binding molecule comprises a plurality of bispecific antibodies. In some embodiments, one or more of the plurality of bispecific antibodies is monovalent. In some embodiments, one or more of the plurality of bispecific antibodies comprises an Fc region. In some embodiments, the Fc region of the one or more of the plurality of bispecific antibodies is silenced. In some embodiments, one or more of the plurality of bispecific antibodies are conjugated together into a multimer. In some embodiments, the multimer is configured in any one of the schema provided in **FIG. 50B**.

In some embodiments, the matrix comprises or consists of a polymeric, for example, biodegradable or biocompatible inert material, for example, which is non-toxic to cells. In

some embodiments, the matrix is composed of hydrophilic polymer chains, which obtain maximal mobility in aqueous solution due to hydration of the chains. In some embodiments, the mobile matrix may be of collagen, purified proteins, purified peptides, polysaccharides, glycosaminoglycans, or extracellular matrix compositions. A polysaccharide may include for example, cellulose ethers, starch, gum arabic, agarose, dextran, chitosan, hyaluronic acid, pectins, xanthan, guar gum or alginate. Other polymers may include polyesters, polyethers, polyacrylates, polyacrylamides, polyamines, polyethylene imines, polyquaternium polymers, polyphosphazenes, polyvinylalcohols, polyvinylacetates, polyvinylpyrrolidones, block copolymers, or polyurethanes. In some embodiments, the mobile matrix is a polymer of dextran.

In some embodiments, the population of cells is contacted with a nucleic acid molecule encoding a CAR. In some embodiments, the population of cells is transduced with a DNA molecule encoding a CAR.

In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs simultaneously with contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0.5 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 20 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 19 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the

nucleic acid molecule encoding the CAR occurs no later than 18 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 17 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 16 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 15 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 14 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 14 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 13 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 12 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of

the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 11 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 10 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 9 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 8 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 7 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 6 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 5 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 4 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the

agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 3 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 2 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 1 hour after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, contacting the population of cells with the nucleic acid molecule encoding the CAR occurs no later than 30 minutes after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above.

In some embodiments, the population of cells is harvested for storage or administration.

In some embodiments, the population of cells is harvested for storage or administration no later than 72, 60, 48, 36, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, or 18 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, the population of cells is harvested for storage or administration no later than 26 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, the population of cells is harvested for storage or administration no later than 25 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of

the cells described above. In some embodiments, the population of cells is harvested for storage or administration no later than 24 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, the population of cells is harvested for storage or administration no later than 23 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, the population of cells is harvested for storage or administration no later than 22 hours after the beginning of contacting the population of cells with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above.

In some embodiments, the population of cells is not expanded ex vivo.

In some embodiments, the population of cells is expanded by no more than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, or 60%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, the population of cells is expanded by no more than 5%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, the population of cells is expanded by no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, the population of cells is expanded by no more than 15%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, the population of cells is expanded by no more than 20%, for

example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, the population of cells is expanded by no more than 25%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, the population of cells is expanded by no more than 30%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, the population of cells is expanded by no more than 35%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above. In some embodiments, the population of cells is expanded by no more than 40%, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the agent that stimulates a CD3/TCR complex and/or the agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells described above.

In some embodiments, the population of cells is expanded by no more than 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20, 24, 36, or 48 hours, for example, as assessed by the number of living cells, compared to the population of cells before it is contacted with the one or more cytokines described above.

In some embodiments, the activation process is conducted in serum free cell media. In some embodiments, the activation process is conducted in cell media comprising one or more cytokines chosen from: IL-2, IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)), or IL-6 (for example, IL-6/sIL-6Ra). In some embodiments, hetIL-15 comprises the amino acid sequence of

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIH
DTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSITCPPPM

SVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR DPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPS KSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQG (SEQ ID NO: 309). In some embodiments, hetIL-15 comprises an amino acid sequence having at least about 70, 75, 80, 85, 90, 95, or 99% identity to SEQ ID NO: 309. In some embodiments, the activation process is conducted in cell media comprising a LSD1 inhibitor. In some embodiments, the activation process is conducted in cell media comprising a MALT1 inhibitor. In some embodiments, the serum free cell media comprises a serum replacement. In some embodiments, the serum replacement is CTS™ Immune Cell Serum Replacement (ICSR). In some embodiments, the level of ICSR can be, for example, up to 5%, for example, about 1%, 2%, 3%, 4%, or 5%. Without wishing to be bound by theory, using cell media, for example, Rapid Media shown in Table 21 or Table 25, comprising ICSR, for example, 2% ICSR, may improve cell viability during a manufacture process described herein.

In some embodiments, the present disclosure provides methods of making a population of cells (for example, T cells) that express a chimeric antigen receptor (CAR) comprising: (a) providing an apheresis sample (for example, a fresh or cryopreserved leukapheresis sample) collected from a subject; (b) selecting T cells from the apheresis sample (for example, using negative selection, positive selection, or selection without beads); (c) seeding isolated T cells at, for example, $1 \times 10^6$ to $1 \times 10^7$ cells/mL; (d) contacting T cells with an agent that stimulates T cells, for example, an agent that stimulates a CD3/TCR complex and/or an agent that stimulates a costimulatory molecule and/or growth factor receptor on the surface of the cells (for example, contacting T cells with anti-CD3 and/or anti-CD28 antibody, for example, contacting T cells with TransAct); (e) contacting T cells with a nucleic acid molecule (for example, a DNA or RNA molecule) encoding the CAR (for example, contacting T cells with a virus comprising a nucleic acid molecule encoding the CAR) for, for example, 6-48 hours, for example, 20-28 hours; and (f) washing and harvesting T cells for storage (for example, reformulating T cells in cryopreservation media) or administration. In some embodiments, step (f) is performed no later than 30, 36, or 48 hours after the beginning of step (d) or (e), for example, no later than 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48 hours after the beginning of step (d) or (e).

In some embodiments of the aforementioned methods, the methods are performed in a closed system. In some embodiments, T cell separation, activation, transduction, incubation, and washing are all performed in a closed system. In some embodiments of the aforementioned methods, the methods are performed in separate devices. In some embodiments, T cell separation, activation and transduction, incubation, and washing are performed in separate devices.

In some embodiments of the aforementioned methods, the methods further comprise adding an adjuvant or a transduction enhancement reagent in the cell culture medium to enhance transduction efficiency. In some embodiments, the adjuvant or transduction enhancement reagent comprises a cationic polymer. In some embodiments, the adjuvant or transduction enhancement reagent is chosen from: LentiBOOST™ (Sirion Biotech), vectofusin-1, F108 (Poloxamer 338 or Pluronic® F-38), protamine sulfate, hexadimethrine bromide (Polybrene), PEA, Pluronic F68, Pluronic F127, Synperonic or LentiTrans™. In some embodiments, the transduction enhancement reagent is LentiBOOST™ (Sirion Biotech). In some embodiments, the transduction enhancement reagent is F108 (Poloxamer 338 or Pluronic® F-38)

In some embodiments of the aforementioned methods, the transducing the population of cells (for example, T cells) with a viral vector comprises subjecting the population of cells and viral vector to a centrifugal force under conditions such that transduction efficiency is enhanced. In an embodiment, the cells are transduced by spinoculation.

In some embodiments of the aforementioned methods, cells (e.g., T cells) are activated and transduced in a cell culture flask comprising a gas-permeable membrane at the base that supports large media volumes without substantially compromising gas exchange. In some embodiments, cell growth is achieved by providing access, e.g., substantially uninterrupted access, to nutrients through convection.

**Anti-CD28 Antibody Molecules**

In some embodiments, the anti-CD28 antibody, e.g., an anti-CD28 antibody to be used in a multispecific binding molecule described herein, comprises at least one antigen-binding region, e.g., a variable region or an antigen-binding fragment thereof, from anti-CD28 (2) as described in **Table 27.** In some embodiments, the anti-CD28 antibody molecule comprises one

or two variable regions from anti-CD28 (2), as described in **Table 27**. In some embodiments, the anti-CD28 antibody comprises a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, and a HCDR3, and a light chain variable region (VL) comprising a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 538, 539, 540, 530, 531, and 532, respectively; the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 541, 539, 540, 530, 531, and 532, respectively; the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 542, 543, 540, 533, 534, and 535, respectively; or the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 544, 545, 546, 536, 534, and 532, respectively.

In some embodiments, the anti-CD28 antibody molecule comprises a VH comprising the amino acid sequence of SEQ ID NO: 547 or 548, or a sequence having at least about 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 547 or 548. In some embodiments, the anti-CD28 antibody comprises a VL comprising the amino acid sequence of SEQ ID NO: 537, or a sequence having at least about 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the anti-CD28 antibody comprises a VH and a VL comprising the amino acid sequences of SEQ ID NOs: 547 and 537, respectively, or a sequence having at least about 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity to any of the aforesaid sequences. In some embodiments, the anti-CD28 antibody comprises a VH and a VL comprising the amino acid sequences of SEQ ID NOs: 548 and 537, respectively, or a sequence having at least about 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity to any of the aforesaid sequences.

It is understood that an anti-CD28 antibody described herein can be used in the context of a multispecific binding molecule, e.g., with an additional binding domain, e.g., an anti-CD3 binding domain described herein. It is also understood that anti-CD28 antibody described herein can be used in other contexts, e.g., as a monospecific antibody.

**Multispecific Binding Molecule Reagents**

In some embodiments, the agent that stimulates a CD3/TCR complex is an agent that stimulates CD3. In some embodiments, the agent that stimulates CD3 comprises one or more of a CD3 or TCR antigen binding domain, such as but not limited to an anti-CD3 or anti-TCR antibody or an antibody fragment comprising one or more CDRs, VH, heavy chain, VL, and/or light chain thereof.

Anti-CD3 antibody sequences and methods of making such antibodies are known in the art. Non-limiting examples of anti-CD3 antibody sequences, along with the relevant CDR, VH, and VL sequences are provided in **Table 27**. In some embodiments, the anti-CD3 binding domain comprises a VH and a VL comprising the amino acid sequences of SEQ ID NOs: 437 and 427, respectively. In some embodiments, the anti-CD3 binding domain comprises a VH and a VL comprising the amino acid sequences of SEQ ID NOs: 456 and 445, respectively. In some embodiments, the anti-CD3 binding domain comprises a VH and a VL comprising the amino acid sequences of SEQ ID NOs: 457 and 446, respectively. In some embodiments, the anti-CD3 binding domain comprises a VH and a VL comprising the amino acid sequences of SEQ ID NOs: 475 and 467, respectively. In some embodiments, the anti-CD3 binding domain comprises a VH and a VL comprising the amino acid sequences of SEQ ID NOs: 476 and 468, respectively. In some embodiments, the anti-CD3 binding domain comprises a VH and a VL comprising the amino acid sequences of SEQ ID NOs: 494 and 484, respectively.

Anti-TCR antibody sequences and methods of making such antibodies are known in the art. Non-limiting examples of anti-TCR antibody sequences, along with the relevant CDR, VH, and VL sequences are provided in **Table 27**.

In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor is an agent that stimulates CD28, ICOS, CD27, CD25, 4-1BB, IL6RA, IL6RB, or CD2. In some embodiments, the agent that stimulates a costimulatory molecule and/or growth factor receptor comprises one or more of a CD28, ICOS, CD27, CD25, 4-1BB, IL6RB, and/or CD2 antigen binding domain, such as but not limited to an anti-CD28, anti-ICOS, anti-CD27, anti-CD25, anti-4-1BB, anti-IL6RA, anti-IL6RB, or anti-CD2 antibody or an antibody fragment comprising one or more CDRs, heavy chain, and/or light chain thereof.

Anti-CD28 antibody sequences and methods of making such antibodies are known in the art. Non-limiting examples of anti-CD28 antibody sequences, along with the relevant CDR,

VH, VL, HC and LC sequences are provided in **Table 27**. Anti-ICOS antibody sequences and methods of making such antibodies are known in the art. Non-limiting examples of anti-ICOS antibody sequences, along with the relevant CDR, VH, VL, and LC sequences are provided in **Table 27**.

Anti-CD27 antibody sequences and methods of making such antibodies are known in the art. Non-limiting examples of anti-CD27 antibody sequences, along with the relevant CDR, VH, and VL sequences are provided in **Table 27**.

Anti-CD25 antibody sequences and methods of making such antibodies are known in the art. Non-limiting examples of anti-CD25 antibody sequences, along with the relevant CDR, VH, VL, HC, and LC sequences are provided in **Table 27**.

Anti-4-1BB antibody sequences and methods of making such antibodies are known in the art. Non-limiting examples of anti-4-IBB antibody sequences, along with the relevant CDR, VH, and VL sequences are provided in **Table 27**.

Anti-IL6RA antibody sequences and methods of making such antibodies are known in the art. Non-limiting examples of IL6RA antibody sequences, along with the relevant CDR, VH, and VL sequences are provided in **Table 27**.

Anti-IL6RB antibody sequences and methods of making such antibodies are known in the art. Non-limiting examples of IL6RB antibody sequences, along with the relevant CDR, VH, and VL sequences are provided in **Table 27**.

Anti-CD2 antibody sequences and methods of making such antibodies are known in the art. Non-limiting examples of anti-CD2 antibody sequences, along with the relevant CDR, VH, VL, HC and LC sequences are provided in **Table 27**.

**Table 27 – Exemplary antibody, CDR, heavy chain variable region (VH), light chain variable region (VL), heavy chain (HC), and light chain (LC) sequences by target antigen**

| SEQ ID NO | Description | Amino acid sequence |
|---|---|---|
| **ANTI-CD3 (1)** | | |
| SEQ ID NO: 420 (Combined) | LCDR1 | SASSSVSYMN |
| SEQ ID NO: 421 (Combined) | LCDR2 | DTSKLAS |
| SEQ ID NO: 422 (Combined) | LCDR3 | QQWSSNPFT |
| SEQ ID NO: 420 (Kabat) | LCDR1 | SASSSVSYMN |

| | | |
|---|---|---|
| SEQ ID NO: 421 (Kabat) | LCDR2 | DTSKLAS |
| SEQ ID NO: 422 (Kabat) | LCDR3 | QQWSSNPFT |
| SEQ ID NO: 423 (Chothia) | LCDR1 | SSSVSY |
| SEQ ID NO: 424 (Chothia) | LCDR2 | DTS |
| SEQ ID NO: 425 (Chothia) | LCDR3 | WSSNPF |
| SEQ ID NO: 426 (IMGT) | LCDR1 | SSVSY |
| SEQ ID NO: 424 (IMGT) | LCDR2 | DTS |
| SEQ ID NO: 422 (IMGT) | LCDR3 | QQWSSNPFT |
| SEQ ID NO: 427 | VL | DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQ QTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFT ISSLQPEDIATYYCQQWSSNPFTFGQGTKLQIT |
| SEQ ID NO: 428 (Combined) | HCDR1 | GYTFTRYTMH |
| SEQ ID NO: 429 (Combined) | HCDR2 | YINPSRGYTNYNQKVKD |
| SEQ ID NO: 430 (Combined) | HCDR3 | YYDDHYSLDY |
| SEQ ID NO: 431 (Kabat) | HCDR1 | RYTMH |
| SEQ ID NO: 429 (Kabat) | HCDR2 | YINPSRGYTNYNQKVKD |
| SEQ ID NO: 430 (Kabat) | HCDR3 | YYDDHYSLDY |
| SEQ ID NO: 432 (Chothia) | HCDR1 | GYTFTRY |
| SEQ ID NO: 433 (Chothia) | HCDR2 | NPSRGY |
| SEQ ID NO: 430 (Chothia) | HCDR3 | YYDDHYSLDY |
| SEQ ID NO: 434 (IMGT) | HCDR1 | GYTFTRYT |
| SEQ ID NO: 435 (IMGT) | HCDR2 | INPSRGYT |
| SEQ ID NO: 436 (IMGT) | HCDR3 | ARYYDDHYSLDY |
| SEQ ID NO: 437 | VH | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMH WVRQAPGKGLEWIGYINPSRGYTNYNQKVKDRFTIS RDNSKNTAFLQMDSLRPEDTGVYFCARYYDDHYSL DYWGQGTPVTVSS |
| **ANTI-CD3 (2)** | | |
| SEQ ID NO: 438 (Combined) | LCDR1 | GSSTGAVTSGNYPN |

| | | |
|---|---|---|
| SEQ ID NO: 439 (Combined) | LCDR2 | GTKFLAP |
| SEQ ID NO: 440 (Combined) | LCDR3 | VLWYSNRWV |
| SEQ ID NO: 438 (Kabat) | LCDR1 | GSSTGAVTSGNYPN |
| SEQ ID NO: 439 (Kabat) | LCDR2 | GTKFLAP |
| SEQ ID NO: 440 (Kabat) | LCDR3 | VLWYSNRWV |
| SEQ ID NO: 441 (Chothia) | LCDR1 | STGAVTSGNY |
| SEQ ID NO: 442 (Chothia) | LCDR2 | GTK |
| SEQ ID NO: 443 (Chothia) | LCDR3 | WYSNRW |
| SEQ ID NO: 444 (IMGT) | LCDR1 | TGAVTSGNY |
| SEQ ID NO: 442 (IMGT) | LCDR2 | GTK |
| SEQ ID NO: 440 (IMGT) | LCDR3 | VLWYSNRWV |
| SEQ ID NO: 445 | VL | QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPN WVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGK AALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLT VL |
| SEQ ID NO: 446 | VL | QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPN WVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGK AALTLSGVQPEDEAEYYCVLWYSNRWVFGCGTKLT VL |
| SEQ ID NO: 447 (Combined) | HCDR1 | GFTFNKYAMN |
| SEQ ID NO: 448 (Combined) | HCDR2 | RIRSKYNNYATYYADSVKD |
| SEQ ID NO: 449 (Combined) | HCDR3 | HGNFGNSYISYWAY |
| SEQ ID NO: 450 (Kabat) | HCDR1 | KYAMN |
| SEQ ID NO: 448 (Kabat) | HCDR2 | RIRSKYNNYATYYADSVKD |
| SEQ ID NO: 449 (Kabat) | HCDR3 | HGNFGNSYISYWAY |
| SEQ ID NO: 451 (Chothia) | HCDR1 | GFTFNKY |
| SEQ ID NO: 452 (Chothia) | HCDR2 | RSKYNNYA |
| SEQ ID NO: 449 (Chothia) | HCDR3 | HGNFGNSYISYWAY |
| SEQ ID NO: 453 (IMGT) | HCDR1 | GFTFNKYA |

| | | |
|---|---|---|
| SEQ ID NO: 454 (IMGT) | HCDR2 | IRSKYNNYAT |
| SEQ ID NO: 455 (IMGT) | HCDR3 | VRHGNFGNSYISYWAY |
| SEQ ID NO: 456 | VH | EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMN WVRQAPGKGLEWVARIRSKYNNYATYYADSVKDR FTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYISYWAYWGQGTLVTVSS |
| SEQ ID NO: 457 | VH | EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMN WVRQAPGKCLEWVARIRSKYNNYATYYADSVKDR FTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYISYWAYWGQGTLVTVSS |
| **ANTI-CD3 (3)** | | |
| SEQ ID NO: 458 (Combined) | LCDR1 | RSSTGAVTTSNYAN |
| SEQ ID NO: 459 (Combined) | LCDR2 | GTNKRAP |
| SEQ ID NO: 460 (Combined) | LCDR3 | ALWYSNLWV |
| SEQ ID NO: 458 (Kabat) | LCDR1 | RSSTGAVTTSNYAN |
| SEQ ID NO: 459 (Kabat) | LCDR2 | GTNKRAP |
| SEQ ID NO: 460 (Kabat) | LCDR3 | ALWYSNLWV |
| SEQ ID NO: 461 (Chothia) | LCDR1 | STGAVTTSNY |
| SEQ ID NO: 462 (Chothia) | LCDR2 | GTN |
| SEQ ID NO: 463 (Chothia) | LCDR3 | WYSNLW |
| SEQ ID NO: 464 (IMGT) | LCDR1 | TGAVTTSNY |
| SEQ ID NO: 465 (IMGT) | LCDR2 | GGT |
| SEQ ID NO: 466 (IMGT) | LCDR3 | ALWYSNL |
| SEQ ID NO: 467 | VL | QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYAN WVQQKPGQAPRGLIGGTNKRAPWTPARFSGSLLGD KAALTLSGAQPEDEAEYFCALWYSNLWVFGGGTKL TVL |
| SEQ ID NO: 468 | VL | QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYAN WVQQKPGQAPRGLIGGTNKRAPWTPARFSGSLLGD KAALTLSGAQPEDEAEYFCALWYSNLWVFGCGTKL TVL |
| SEQ ID NO: 469 (Combined) | HCDR1 | GFTFNTYAMN |
| SEQ ID NO: 448 (Combined) | HCDR2 | RIRSKYNNYATYYADSVKD |

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|---|---|---|
| SEQ ID NO: 470 (Combined) | HCDR3 | HGNFGNSYVSWFAY |
| SEQ ID NO: 471 (Kabat) | HCDR1 | TYAMN |
| SEQ ID NO: 448 (Kabat) | HCDR2 | RIRSKYNNYATYYADSVKD |
| SEQ ID NO: 470 (Kabat) | HCDR3 | HGNFGNSYVSWFAY |
| SEQ ID NO: 472 (Chothia) | HCDR1 | GFTFNTY |
| SEQ ID NO: 452 (Chothia) | HCDR2 | RSKYNNYA |
| SEQ ID NO: 470 (Chothia) | HCDR3 | HGNFGNSYVSWFAY |
| SEQ ID NO: 473 (IMGT) | HCDR1 | GFTFNTYA |
| SEQ ID NO: 454 (IMGT) | HCDR2 | IRSKYNNYAT |
| SEQ ID NO: 474 (IMGT) | HCDR3 | VRHGNFGNSYVSWFAY |
| SEQ ID NO: 475 | VH | EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMN WVRQASGKGLEWVGRIRSKYNNYATYYADSVKDR FTISRDDSKSTLYLQMNSLKTEDTAVYYCVRHGNFG NSYVSWFAYWGQGTLVTVSS |
| SEQ ID NO: 476 | VH | EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMN WVRQASGKCLEWVGRIRSKYNNYATYYADSVKDR FTISRDDSKSTLYLQMNSLKTEDTAVYYCVRHGNFG NSYVSWFAYWGQGTLVTVSS |
| **ANTI-CD3 (4)** | | |
| SEQ ID NO: 477 (Combined) | LCDR1 | RSSQSLVRSEGTTYFN |
| SEQ ID NO: 478 (Combined) | LCDR2 | RVSNRFS |
| SEQ ID NO: 479 (Combined) | LCDR3 | LQSSHFPWT |
| SEQ ID NO: 477 (Kabat) | LCDR1 | RSSQSLVRSEGTTYFN |
| SEQ ID NO: 478 (Kabat) | LCDR2 | RVSNRFS |
| SEQ ID NO: 479 (Kabat) | LCDR3 | LQSSHFPWT |
| SEQ ID NO: 480 (Chothia) | LCDR1 | SQSLVRSEGTTY |
| SEQ ID NO: 481 (Chothia) | LCDR2 | RVS |
| SEQ ID NO: 482 (Chothia) | LCDR3 | SSHFPW |
| SEQ ID NO: 483 (IMGT) | LCDR1 | QSLVRSEGTTY |

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|---|---|---|
| SEQ ID NO: 481 (IMGT) | LCDR2 | RVS |
| SEQ ID NO: 479 (IMGT) | LCDR3 | LQSSHFPWT |
| SEQ ID NO: 484 | VL | DILVTQTPVSLPVSLGGHVSISCRSSQSLVRSEGTTYF NWYLQKPGQSPQLLIYRVSNRFSGVPDRFSGSGSGT DFTLKISRVEPEDLGVYYCLQSSHFPWTFGGGTKLEL K |
| SEQ ID NO: 485 (Combined) | HCDR1 | GFTFSKQGMH |
| SEQ ID NO: 486 (Combined) | HCDR2 | MIYYDSSKMYYADTVKG |
| SEQ ID NO: 487 (Combined) | HCDR3 | FWWDLDFDH |
| SEQ ID NO: 488 (Kabat) | HCDR1 | KQGMH |
| SEQ ID NO: 486 (Kabat) | HCDR2 | MIYYDSSKMYYADTVKG |
| SEQ ID NO: 487 (Kabat) | HCDR3 | FWWDLDFDH |
| SEQ ID NO: 489 (Chothia) | HCDR1 | GFTFSKQ |
| SEQ ID NO: 490 (Chothia) | HCDR2 | YYDSSK |
| SEQ ID NO: 487 (Chothia) | HCDR3 | FWWDLDFDH |
| SEQ ID NO: 491 (IMGT) | HCDR1 | GFTFSKQG |
| SEQ ID NO: 492 (IMGT) | HCDR2 | IYYDSSKM |
| SEQ ID NO: 493 (IMGT) | HCDR3 | ASFWWDLDFDH |
| SEQ ID NO: 494 | VH | EVKLVESGGDLVQPGDSLTLSCVASGFTFSKQGMH WIRQAPKKGLEWIAMIYYDSSKMYYADTVKGRFTIS RDNSKNTLYLEMNSLRSEDTAMYYCASFWWDLDF DHWGQGVMVTVSS |
| **ANTI-TCR** | | |
| SEQ ID NO: 495 (Combined) | LCDR1 | SATSSVSYMH |
| SEQ ID NO: 421 (Combined) | LCDR2 | DTSKLAS |
| SEQ ID NO: 496 (Combined) | LCDR3 | QQWSSNPLT |
| SEQ ID NO: 495 (Kabat) | LCDR1 | SATSSVSYMH |
| SEQ ID NO: 421 (Kabat) | LCDR2 | DTSKLAS |
| SEQ ID NO: 496 (Kabat) | LCDR3 | QQWSSNPLT |

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|---|---|---|
| SEQ ID NO: 497 (Chothia) | LCDR1 | TSSVSY |
| SEQ ID NO: 424 (Chothia) | LCDR2 | DTS |
| SEQ ID NO: 498 (Chothia) | LCDR3 | WSSNPL |
| SEQ ID NO: 426 (IMGT) | LCDR1 | SSVSY |
| SEQ ID NO: 424 (IMGT) | LCDR2 | DTS |
| SEQ ID NO: 496 (IMGT) | LCDR3 | QQWSSNPLT |
| SEQ ID NO: 499 | VL | QIVLTQSPAIMSASPGEKVTMTCSATSSVSYMHWYQ QKSGTSPKRWIYDTSKLASGVPARFSGSGSGTSYSLT ISSMEAEDAATYYCQQWSSNPLTFGAGTKLELK |
| SEQ ID NO: 500 (Combined) | HCDR1 | GYKFTSYVMH |
| SEQ ID NO: 501 (Combined) | HCDR2 | YINPYNDVTKYNEKFKG |
| SEQ ID NO: 502 (Combined) | HCDR3 | GSYYDYDGFVY |
| SEQ ID NO: 503 (Kabat) | HCDR1 | SYVMH |
| SEQ ID NO: 501 (Kabat) | HCDR2 | YINPYNDVTKYNEKFKG |
| SEQ ID NO: 502 (Kabat) | HCDR3 | GSYYDYDGFVY |
| SEQ ID NO: 504 (Chothia) | HCDR1 | GYKFTSY |
| SEQ ID NO: 505 (Chothia) | HCDR2 | NPYNDV |
| SEQ ID NO: 502 (Chothia) | HCDR3 | GSYYDYDGFVY |
| SEQ ID NO: 506 (IMGT) | HCDR1 | GYKFTSYV |
| SEQ ID NO: 507 (IMGT) | HCDR2 | INPYNDVT |
| SEQ ID NO: 508 (IMGT) | HCDR3 | ARGSYYDYDGFVY |
| SEQ ID NO: 509 | VH | EVQLQQSGPELVKPGASVKMSCKASGYKFTSYVMH WVKQKPGQGLEWIGYINPYNDVTKYNEKFKGKATL TSDKSSSTAYMELSSLTSEDSAVHYCARGSYYDYDG FVYWGQGTLVTVSA |
| **ANTI-CD28 (1)** | | |
| SEQ ID NO: 510 (Combined) | LCDR1 | HASQNIYVWLN |
| SEQ ID NO: 511 (Combined) | LCDR2 | KASNLHT |
| SEQ ID NO: 512 (Combined) | LCDR3 | QQGQTYPYT |

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|---|---|---|
| SEQ ID NO: 510 (Kabat) | LCDR1 | HASQNIYVWLN |
| SEQ ID NO: 511 (Kabat) | LCDR2 | KASNLHT |
| SEQ ID NO: 512 (Kabat) | LCDR3 | QQGQTYPYT |
| SEQ ID NO: 513 (Chothia) | LCDR1 | SQNIYVW |
| SEQ ID NO: 514 (Chothia) | LCDR2 | KAS |
| SEQ ID NO: 515 (Chothia) | LCDR3 | GQTYPY |
| SEQ ID NO: 516 (IMGT) | LCDR1 | QNIYVW |
| SEQ ID NO: 514 (IMGT) | LCDR2 | KAS |
| SEQ ID NO: 512 (IMGT) | LCDR3 | QQGQTYPYT |
| SEQ ID NO: 517 | VL | DIQMTQSPSSLSASVGDRVTITCHASQNIYVWLNWY QQKPGKAPKLLIYKASNLHTGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQGQTYPYTFGGGTKVEIK |
| SEQ ID NO: 518 | LC | DIQMTQSPSSLSASVGDRVTITCHASQNIYVWLNWY QQKPGKAPKLLIYKASNLHTGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQGQTYPYTFGGGTKVEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| SEQ ID NO: 519 (Combined) | HCDR1 | GYTFTSYYIH |
| SEQ ID NO: 520 (Combined) | HCDR2 | CIYPGNVNTNYNEKFKD |
| SEQ ID NO: 521 (Combined) | HCDR3 | SHYGLDWNFDV |
| SEQ ID NO: 522 (Kabat) | HCDR1 | SYYIH |
| SEQ ID NO: 520 (Kabat) | HCDR2 | CIYPGNVNTNYNEKFKD |
| SEQ ID NO: 521 (Kabat) | HCDR3 | SHYGLDWNFDV |
| SEQ ID NO: 523 (Chothia) | HCDR1 | GYTFTSY |
| SEQ ID NO: 524 (Chothia) | HCDR2 | YPGNVN |
| SEQ ID NO: 521 (Chothia) | HCDR3 | SHYGLDWNFDV |
| SEQ ID NO: 525 (IMGT) | HCDR1 | GYTFTSYY |
| SEQ ID NO: 526 (IMGT) | HCDR2 | IYPGNVNT |

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|---|---|---|
| SEQ ID NO: 527 (IMGT) | HCDR3 | TRSHYGLDWNFDV |
| SEQ ID NO: 528 | VH | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIH WVRQAPGQGLEWIGCIYPGNVNTNYNEKFKDRATL TVDTSISTAYMELSRLRSDDTAVYFCTRSHYGLDWN FDVWGQGTTVTVSS |
| SEQ ID NO: 529 | HC | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIH WVRQAPGQGLEWIGCIYPGNVNTNYNEKFKDRATL TVDTSISTAYMELSRLRSDDTAVYFCTRSHYGLDWN FDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD KRVEPKSC |
| **ANTI-CD28 (2)** | | |
| SEQ ID NO: 530 (Combined) | LCDR1 | SGSSSNIVSNYVN |
| SEQ ID NO: 531 (Combined) | LCDR2 | DNNKRPS |
| SEQ ID NO: 532 (Combined) | LCDR3 | QSYAIGSYSVV |
| SEQ ID NO: 530 (Kabat) | LCDR1 | SGSSSNIVSNYVN |
| SEQ ID NO: 531 (Kabat) | LCDR2 | DNNKRPS |
| SEQ ID NO: 532 (Kabat) | LCDR3 | QSYAIGSYSVV |
| SEQ ID NO: 533 (Chothia) | LCDR1 | SSSNIVSNY |
| SEQ ID NO: 534 (Chothia) | LCDR2 | DNN |
| SEQ ID NO: 535 (Chothia) | LCDR3 | YAIGSYSV |
| SEQ ID NO: 536 (IMGT) | LCDR1 | SSNIVSNY |
| SEQ ID NO: 534 (IMGT) | LCDR2 | DNN |
| SEQ ID NO: 532 (IMGT) | LCDR3 | QSYAIGSYSVV |
| SEQ ID NO: 537 | VL | DIVLTQPPSVSGAPGQRVTISCSGSSSNIVSNYVNWY QQLPGTAPKLLIYDNNKRPSGVPDRFSGSKSGTSASL AITGLQSEDEADYYCQSYAIGSYSVVFGGGTKLTVL |
| SEQ ID NO: 538 (Combined) | HCDR1 | GFTFSTYGMS |
| SEQ ID NO: 539 (Combined) | HCDR2 | SIFYTGSSTYYADSVKG |
| SEQ ID NO: 540 (Combined) | HCDR3 | IGYAGDSKYAI |
| SEQ ID NO: 541 (Kabat) | HCDR1 | TYGMS |

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|---|---|---|
| SEQ ID NO: 539 (Kabat) | HCDR2 | SIFYTGSSTYYADSVKG |
| SEQ ID NO: 540 (Kabat) | HCDR3 | IGYAGDSKYAI |
| SEQ ID NO: 542 (Chothia) | HCDR1 | GFTFSTY |
| SEQ ID NO: 543 (Chothia) | HCDR2 | FYTGSS |
| SEQ ID NO: 540 (Chothia) | HCDR3 | IGYAGDSKYAI |
| SEQ ID NO: 544 (IMGT) | HCDR1 | GFTFSTYG |
| SEQ ID NO: 545 (IMGT) | HCDR2 | IFYTGSST |
| SEQ ID NO: 546 (IMGT) | HCDR3 | ARIGYAGDSKYAI |
| SEQ ID NO: 547 | VH | QVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMS WVRQAPGKGLEWVSSIFYTGSSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCARIGYAGDSK YAIWGQGTLVTVSS |
| SEQ ID NO: 548 | VH | EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMSW VRQAPGKGLEWVSSIFYTGSSTYYADSVKGRFTISRD NSKNTLYLQMNSLRAEDTAVYYCARIGYAGDSKYA IWGQGTLVTVSS |
| **ANTI-ICOS** | | |
| SEQ ID NO: 549 (Combined) | LCDR1 | KSSQSLLSGSFNYLT |
| SEQ ID NO: 550 (Combined) | LCDR2 | YASTRHT |
| SEQ ID NO: 551 (Combined) | LCDR3 | HHHYNAPPT |
| SEQ ID NO: 549 (Kabat) | LCDR1 | KSSQSLLSGSFNYLT |
| SEQ ID NO: 550 (Kabat) | LCDR2 | YASTRHT |
| SEQ ID NO: 551 (Kabat) | LCDR3 | HHHYNAPPT |
| SEQ ID NO: 552 (Chothia) | LCDR1 | SQSLLSGSFNY |
| SEQ ID NO: 553 (Chothia) | LCDR2 | YAS |
| SEQ ID NO: 554 (Chothia) | LCDR3 | HYNAPP |
| SEQ ID NO: 555 (IMGT) | LCDR1 | QSLLSGSFNY |
| SEQ ID NO: 553 (IMGT) | LCDR2 | YAS |
| SEQ ID NO: 551 (IMGT) | LCDR3 | HHHYNAPPT |

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|---|---|---|
| SEQ ID NO: 556 | VL | DIVMTQSPDSLAVSLGERATINCKSSQSLLSGSFNYL TWYQQKPGQPPKLLIFYASTRHTGVPDRFSGSGSGT DFTLTISSLQAEDVAVYYCHHHYNAPPTFGPGTKVDI K |
| SEQ ID NO: 557 | LC | DIVMTQSPDSLAVSLGERATINCKSSQSLLSGSFNYL TWYQQKPGQPPKLLIFYASTRHTGVPDRFSGSGSGT DFTLTISSLQAEDVAVYYCHHHYNAPPTFGPGTKVDI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| SEQ ID NO: 558 (Combined) | HCDR1 | GFTFSDYWMD |
| SEQ ID NO: 559 (Combined) | HCDR2 | NIDEDGSITEYSPFVKG |
| SEQ ID NO: 560 (Combined) | HCDR3 | WGRFGFDS |
| SEQ ID NO: 561 (Kabat) | HCDR1 | DYWMD |
| SEQ ID NO: 559 (Kabat) | HCDR2 | NIDEDGSITEYSPFVKG |
| SEQ ID NO: 560 (Kabat) | HCDR3 | WGRFGFDS |
| SEQ ID NO: 562 (Chothia) | HCDR1 | GFTFSDY |
| SEQ ID NO: 563 (Chothia) | HCDR2 | DEDGSI |
| SEQ ID NO: 560 (Chothia) | HCDR3 | WGRFGFDS |
| SEQ ID NO: 564 (IMGT) | HCDR1 | GFTFSDYW |
| SEQ ID NO: 565 (IMGT) | HCDR2 | IDEDGSIT |
| SEQ ID NO: 566 (IMGT) | HCDR3 | TRWGRFGFDS |
| SEQ ID NO: 567 | VH | EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMD WVRQAPGKGLVWVSNIDEDGSITEYSPFVKGRFTISR DNAKNTLYLQMNSLRAEDTAVYYCTRWGRFGFDS WGQGTLVTVSS |
| **ANTI-CD27** | | |
| SEQ ID NO: 568 (Combined) | LCDR1 | RASQGISRWLA |
| SEQ ID NO: 55 (Combined) | LCDR2 | AASSLQS |
| SEQ ID NO: 569 (Combined) | LCDR3 | QQYNTYPRT |
| SEQ ID NO: 568 (Kabat) | LCDR1 | RASQGISRWLA |
| SEQ ID NO: 55 (Kabat) | LCDR2 | AASSLQS |
| SEQ ID NO: 569 (Kabat) | LCDR3 | QQYNTYPRT |

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|---|---|---|
| SEQ ID NO: 570 (Chothia) | LCDR1 | SQGISRW |
| SEQ ID NO: 58 (Chothia) | LCDR2 | AAS |
| SEQ ID NO: 571 (Chothia) | LCDR3 | YNTYPR |
| SEQ ID NO: 572 (IMGT) | LCDR1 | QGISRW |
| SEQ ID NO: 58 (IMGT) | LCDR2 | AAS |
| SEQ ID NO: 569 (IMGT) | LCDR3 | QQYNTYPRT |
| SEQ ID NO: 573 | VL | DIQMTQSPSSLSASVGDRVTITCRASQGISRWLAWY QQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQYNTYPRTFGQGTKVEIK |
| SEQ ID NO: 574 (Combined) | HCDR1 | GFTFSSYDMH |
| SEQ ID NO: 575 (Combined) | HCDR2 | VIWYDGSNKYYADSVKG |
| SEQ ID NO: 576 (Combined) | HCDR3 | GSGNWGFFDY |
| SEQ ID NO: 577 (Kabat) | HCDR1 | SYDMH |
| SEQ ID NO: 575 (Kabat) | HCDR2 | VIWYDGSNKYYADSVKG |
| SEQ ID NO: 576 (Kabat) | HCDR3 | GSGNWGFFDY |
| SEQ ID NO: 47 (Chothia) | HCDR1 | GFTFSSY |
| SEQ ID NO: 578 (Chothia) | HCDR2 | WYDGSN |
| SEQ ID NO: 576 (Chothia) | HCDR3 | GSGNWGFFDY |
| SEQ ID NO: 579 (IMGT) | HCDR1 | GFTFSSYD |
| SEQ ID NO: 580 (IMGT) | HCDR2 | IWYDGSNK |
| SEQ ID NO: 581 (IMGT) | HCDR3 | ARGSGNWGFFDY |
| SEQ ID NO: 582 | VH | QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYDMH WVRQAPGGLEWVAVIWYDGSNKYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCARGSGNWGFF DYWGQGTLVTVSS |
| **ANTI-CD25 (1)** | | |
| SEQ ID NO: 583 (Combined) | LCDR1 | SASSSRSYMQ |
| SEQ ID NO: 421 (Combined) | LCDR2 | DTSKLAS |
| SEQ ID NO: 584 (Combined) | LCDR3 | HQRSSYT |

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|---|---|---|
| SEQ ID NO: 583 (Kabat) | LCDR1 | SASSSRSYMQ |
| SEQ ID NO: 421 (Kabat) | LCDR2 | DTSKLAS |
| SEQ ID NO: 584 (Kabat) | LCDR3 | HQRSSYT |
| SEQ ID NO: 585 (Chothia) | LCDR1 | SSSRSY |
| SEQ ID NO: 424 (Chothia) | LCDR2 | DTS |
| SEQ ID NO: 586 (Chothia) | LCDR3 | RSSY |
| SEQ ID NO: 587 (IMGT) | LCDR1 | SSRSY |
| SEQ ID NO: 424 (IMGT) | LCDR2 | DTS |
| SEQ ID NO: 584 (IMGT) | LCDR3 | HQRSSYT |
| SEQ ID NO: 588 | VL | QIVSTQSPAIMSASPGEKVTMTCSASSSRSYMQWYQ QKPGTSPKRWIYDTSKLASGVPARFSGSGSGTSYSLT ISSMEAEDAATYYCHQRSSYTFGGGTKLEIK |
| SEQ ID NO: 589 (Combined) | HCDR1 | GYSFTRYWMH |
| SEQ ID NO: 590 (Combined) | HCDR2 | AIYPGNSDTSYNQKFEG |
| SEQ ID NO: 591 (Combined) | HCDR3 | DYGYYFDF |
| SEQ ID NO: 592 (Kabat) | HCDR1 | RYWMH |
| SEQ ID NO: 590 (Kabat) | HCDR2 | AIYPGNSDTSYNQKFEG |
| SEQ ID NO: 591 (Kabat) | HCDR3 | DYGYYFDF |
| SEQ ID NO: 593 (Chothia) | HCDR1 | GYSFTRY |
| SEQ ID NO: 594 (Chothia) | HCDR2 | YPGNSD |
| SEQ ID NO: 591 (Chothia) | HCDR3 | DYGYYFDF |
| SEQ ID NO: 595 (IMGT) | HCDR1 | GYSFTRYW |
| SEQ ID NO: 596 (IMGT) | HCDR2 | IYPGNSDT |
| SEQ ID NO: 597 (IMGT) | HCDR3 | SRDYGYYFDF |
| SEQ ID NO: 598 | VH | EVQLQQSGTVLARPGASVKMSCKASGYSFTRYWMH WIKQRPGQGLEWIGAIYPGNSDTSYNQKFEGKAKLT AVTSASTAYMELSSLTHEDSAVYYCSRDYGYYFDF WGQGTTLTVSS |
| **ANTI-CD25 (2)** | | |

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|---|---|---|
| SEQ ID NO: 599 (Combined) | LCDR1 | KASQSVDYDGDSYMN |
| SEQ ID NO: 600 (Combined) | LCDR2 | AASNLES |
| SEQ ID NO: 601 (Combined) | LCDR3 | QQSNEDPYT |
| SEQ ID NO: 599 (Kabat) | LCDR1 | KASQSVDYDGDSYMN |
| SEQ ID NO: 600 (Kabat) | LCDR2 | AASNLES |
| SEQ ID NO: 601 (Kabat) | LCDR3 | QQSNEDPYT |
| SEQ ID NO: 602 (Chothia) | LCDR1 | SQSVDYDGDSY |
| SEQ ID NO: 58 (Chothia) | LCDR2 | AAS |
| SEQ ID NO: 603 (Chothia) | LCDR3 | SNEDPY |
| SEQ ID NO: 604 (IMGT) | LCDR1 | QSVDYDGDSY |
| SEQ ID NO: 58 (IMGT) | LCDR2 | AAS |
| SEQ ID NO: 601 (IMGT) | LCDR3 | QQSNEDPYT |
| SEQ ID NO: 605 | VL | DIVLTQSPLSLPVTLGQPASISCKASQSVDYDGDSYM NWYQQRPGQSPRLLIYAASNLESGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYCQQSNEDPYTFGGGTKVE IK |
| SEQ ID NO: 606 | LC | DIVLTQSPLSLPVTLGQPASISCKASQSVDYDGDSYM NWYQQRPGQSPRLLIYAASNLESGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYCQQSNEDPYTFGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| SEQ ID NO: 607 (Combined) | HCDR1 | GYAFTNYLIE |
| SEQ ID NO: 608 (Combined) | HCDR2 | VINPGSGGTNYNEKFKG |
| SEQ ID NO: 609 (Combined) | HCDR3 | WRGDGYYAYFDV |
| SEQ ID NO: 610 (Kabat) | HCDR1 | NYLIE |
| SEQ ID NO: 608 (Kabat) | HCDR2 | VINPGSGGTNYNEKFKG |
| SEQ ID NO: 609 (Kabat) | HCDR3 | WRGDGYYAYFDV |
| SEQ ID NO: 611 (Chothia) | HCDR1 | GYAFTNY |
| SEQ ID NO: 612 (Chothia) | HCDR2 | NPGSGG |

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|---|---|---|
| SEQ ID NO: 609 (Chothia) | HCDR3 | WRGDGYYAYFDV |
| SEQ ID NO: 613 (IMGT) | HCDR1 | GYAFTNYL |
| SEQ ID NO: 614 (IMGT) | HCDR2 | INPGSGGT |
| SEQ ID NO: 615 (IMGT) | HCDR3 | ARWRGDGYYAYFDV |
| SEQ ID NO: 616 | VH | EVQLVQSGAEVKKPGESLKISCKGSGYAFTNYLIEW VRQMPGKGLEWMGVINPGSGGTNYNEKFKGQVTIS ADKSISTAYLQWSSLKASDTAMYYCARWRGDGYY AYFDVWGQGTTVTVSS |
| SEQ ID NO: 617 | HC | EVQLVQSGAEVKKPGESLKISCKGSGYAFTNYLIEW VRQMPGKGLEWMGVINPGSGGTNYNEKFKGQVTIS ADKSISTAYLQWSSLKASDTAMYYCARWRGDGYY AYFDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSC |
| **ANTI-4-1BB** | | |
| SEQ ID NO: 618 (Combined) | LCDR1 | RASQSVSSYLA |
| SEQ ID NO: 619 (Combined) | LCDR2 | DASNRAT |
| SEQ ID NO: 620 (Combined) | LCDR3 | QQRSNWPPALT |
| SEQ ID NO: 618 (Kabat) | LCDR1 | RASQSVSSYLA |
| SEQ ID NO: 619 (Kabat) | LCDR2 | DASNRAT |
| SEQ ID NO: 620 (Kabat) | LCDR3 | QQRSNWPPALT |
| SEQ ID NO: 621 (Chothia) | LCDR1 | SQSVSSY |
| SEQ ID NO: 622 (Chothia) | LCDR2 | DAS |
| SEQ ID NO: 623 (Chothia) | LCDR3 | RSNWPPAL |
| SEQ ID NO: 624 (IMGT) | LCDR1 | QSVSSY |
| SEQ ID NO: 622 (IMGT) | LCDR2 | DAS |
| SEQ ID NO: 620 (IMGT) | LCDR3 | QQRSNWPPALT |
| SEQ ID NO: 625 | VL | EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQ QKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTI SSLEPEDFAVYYCQQRSNWPPALTFCGGTKVEIK |
| SEQ ID NO: 626 (Combined) | HCDR1 | GGSFSGYYWS |

| | | |
|---|---|---|
| SEQ ID NO: 627 (Combined) | HCDR2 | EINHGGYVTYNPSLES |
| SEQ ID NO: 628 (Combined) | HCDR3 | DYGPGNYDWYFDL |
| SEQ ID NO: 629 (Kabat) | HCDR1 | GYYWS |
| SEQ ID NO: 627 (Kabat) | HCDR2 | EINHGGYVTYNPSLES |
| SEQ ID NO: 628 (Kabat) | HCDR3 | DYGPGNYDWYFDL |
| SEQ ID NO: 630 (Chothia) | HCDR1 | GGSFSGY |
| SEQ ID NO: 631 (Chothia) | HCDR2 | NHGGY |
| SEQ ID NO: 628 (Chothia) | HCDR3 | DYGPGNYDWYFDL |
| SEQ ID NO: 632 (IMGT) | HCDR1 | GGSFSGYY |
| SEQ ID NO: 633 (IMGT) | HCDR2 | INHGGYV |
| SEQ ID NO: 634 (IMGT) | HCDR3 | ARDYGPGNYDWYFDL |
| SEQ ID NO: 635 | VH | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWS WIRQSPEKGLEWIGEINHGGYVTYNPSLESRVTISVD TSKNQFSLKLSSVTAADTAVYYCARDYGPGNYDWY FDLWGRGTLVTVSS |
| **ANTI-IL6RA** | | |
| SEQ ID NO: 636 (Combined) | LCDR1 | RASQDISSYLN |
| SEQ ID NO: 637 (Combined) | LCDR2 | YTSRLHS |
| SEQ ID NO: 300 (Combined) | LCDR3 | QQGNTLPYT |
| SEQ ID NO: 636 (Kabat) | LCDR1 | RASQDISSYLN |
| SEQ ID NO: 637 (Kabat) | LCDR2 | YTSRLHS |
| SEQ ID NO: 300 (Kabat) | LCDR3 | QQGNTLPYT |
| SEQ ID NO: 638 (Chothia) | LCDR1 | SQDISSY |
| SEQ ID NO: 639 (Chothia) | LCDR2 | YTS |
| SEQ ID NO: 640 (Chothia) | LCDR3 | GNTLPY |
| SEQ ID NO: 641 (IMGT) | LCDR1 | QDISSY |
| SEQ ID NO: 639 (IMGT) | LCDR2 | YTS |

| | | |
|---|---|---|
| SEQ ID NO: 300 (IMGT) | LCDR3 | QQGNTLPYT |
| SEQ ID NO: 642 | VL | DIQMTQSPSSLSASVGDRVTITCRASQDISSYLNWYQ QKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDFTFTI SSLQPEDIATYYCQQGNTLPYTFGQGTKVEIK |
| SEQ ID NO: 643 (Combined) | HCDR1 | GYSITSDHAWS |
| SEQ ID NO: 644 (Combined) | HCDR2 | YISYSGITTYNPSLKS |
| SEQ ID NO: 645 (Combined) | HCDR3 | SLARTTAMDY |
| SEQ ID NO: 646 (Kabat) | HCDR1 | SDHAWS |
| SEQ ID NO: 644 (Kabat) | HCDR2 | YISYSGITTYNPSLKS |
| SEQ ID NO: 645 (Kabat) | HCDR3 | SLARTTAMDY |
| SEQ ID NO: 647 (Chothia) | HCDR1 | GYSITSDH |
| SEQ ID NO: 648 (Chothia) | HCDR2 | SYSGI |
| SEQ ID NO: 645 (Chothia) | HCDR3 | SLARTTAMDY |
| SEQ ID NO: 649 (IMGT) | HCDR1 | GYSITSDHA |
| SEQ ID NO: 650 (IMGT) | HCDR2 | ISYSGIT |
| SEQ ID NO: 651 (IMGT) | HCDR3 | ARSLARTTAMDY |
| SEQ ID NO: 652 | VH | QVQLQESGPGLVRPSQTLSLTCTVSGYSITSDHAWS WVRQPPGRGLEWIGYISYSGITTYNPSLKSRVTMLR DTSKNQFSLRLSSVTAADTAVYYCARSLARTTAMD YWGQGSLVTVSS |
| **ANTI-IL6RB** | | |
| SEQ ID NO: 54 (Combined) | LCDR1 | RASQSISSYLN |
| SEQ ID NO: 55 (Combined) | LCDR2 | AASSLQS |
| SEQ ID NO: 653 (Combined) | LCDR3 | QQSYSTPPIT |
| SEQ ID NO: 54 (Kabat) | LCDR1 | RASQSISSYLN |
| SEQ ID NO: 55 (Kabat) | LCDR2 | AASSLQS |
| SEQ ID NO: 653 (Kabat) | LCDR3 | QQSYSTPPIT |
| SEQ ID NO: 57 (Chothia) | LCDR1 | SQSISSY |
| SEQ ID NO: 58 (Chothia) | LCDR2 | AAS |
| SEQ ID NO: 654 (Chothia) | LCDR3 | SYSTPPI |

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|---|---|---|
| SEQ ID NO: 60 (IMGT) | LCDR1 | QSISSY |
| SEQ ID NO: 58 (IMGT) | LCDR2 | AAS |
| SEQ ID NO: 653 (IMGT) | LCDR3 | QQSYSTPPIT |
| SEQ ID NO: 655 | VL | DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQ QKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQSYSTPPITFGQGTRLEIK |
| SEQ ID NO: 656 (Combined) | HCDR1 | GFTFSDYYMT |
| SEQ ID NO: 657 (Combined) | HCDR2 | YISSSGTNKYNADSVKG |
| SEQ ID NO: 658 (Combined) | HCDR3 | DPPWGMDV |
| SEQ ID NO: 659 (Kabat) | HCDR1 | DYYMT |
| SEQ ID NO: 657 (Kabat) | HCDR2 | YISSSGTNKYNADSVKG |
| SEQ ID NO: 658 (Kabat) | HCDR3 | DPPWGMDV |
| SEQ ID NO: 562 (Chothia) | HCDR1 | GFTFSDY |
| SEQ ID NO: 660 (Chothia) | HCDR2 | SSSGTN |
| SEQ ID NO: 658 (Chothia) | HCDR3 | DPPWGMDV |
| SEQ ID NO: 661 (IMGT) | HCDR1 | GFTFSDYY |
| SEQ ID NO: 662 (IMGT) | HCDR2 | ISSSGTNK |
| SEQ ID NO: 663 (IMGT) | HCDR3 | VRDPPWGMDV |
| SEQ ID NO: 664 | VH | QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMT WIRQTPGKGLDWVSYISSSGTNKYNADSVKGRFTIS RDNAKNSLYLQMNSLRAEDTAVYYCVRDPPWGMD VWGQGTTVTVSS |
| **ANTI-CD2 (1)** | | |
| SEQ ID NO: 665 (Combined) | LCDR1 | RSSQSLLHSSGNTYLN |
| SEQ ID NO: 666 (Combined) | LCDR2 | LVSKLES |
| SEQ ID NO: 667 (Combined) | LCDR3 | MQFTHYPYT |
| SEQ ID NO: 665 (Kabat) | LCDR1 | RSSQSLLHSSGNTYLN |
| SEQ ID NO: 666 (Kabat) | LCDR2 | LVSKLES |
| SEQ ID NO: 667 (Kabat) | LCDR3 | MQFTHYPYT |
| SEQ ID NO: 668 (Chothia) | LCDR1 | SQSLLHSSGNTY |

| | | |
|---|---|---|
| SEQ ID NO: 669 (Chothia) | LCDR2 | LVS |
| SEQ ID NO: 670 (Chothia) | LCDR3 | FTHYPY |
| SEQ ID NO: 671 (IMGT) | LCDR1 | QSLLHSSGNTY |
| SEQ ID NO: 669 (IMGT) | LCDR2 | LVS |
| SEQ ID NO: 667 (IMGT) | LCDR3 | MQFTHYPYT |
| SEQ ID NO: 672 | VL | DVVMTQSPPSLLVTLGQPASISCRSSQSLLHSSGNTY LNWLLQRPGQSPQPLIYLVSKLESGVPDRFSGSGSGT DFTLKISGVEAEDVGVYYCMQFTHYPYTFGQGTKLE IK |
| SEQ ID NO: 673 | LC | DVVMTQSPPSLLVTLGQPASISCRSSQSLLHSSGNTY LNWLLQRPGQSPQPLIYLVSKLESGVPDRFSGSGSGT DFTLKISGVEAEDVGVYYCMQFTHYPYTFGQGTKLE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| SEQ ID NO: 674 (Combined) | HCDR1 | GYIFTEYYMY |
| SEQ ID NO: 675 (Combined) | HCDR2 | RIDPEDGSIDYVEKFKK |
| SEQ ID NO: 676 (Combined) | HCDR3 | GKFNYRFAY |
| SEQ ID NO: 677 (Kabat) | HCDR1 | EYYMY |
| SEQ ID NO: 675 (Kabat) | HCDR2 | RIDPEDGSIDYVEKFKK |
| SEQ ID NO: 676 (Kabat) | HCDR3 | GKFNYRFAY |
| SEQ ID NO: 678 (Chothia) | HCDR1 | GYIFTEY |
| SEQ ID NO: 679 (Chothia) | HCDR2 | DPEDGS |
| SEQ ID NO: 676 (Chothia) | HCDR3 | GKFNYRFAY |
| SEQ ID NO: 680 (IMGT) | HCDR1 | GYIFTEYY |
| SEQ ID NO: 681 (IMGT) | HCDR2 | IDPEDGSI |
| SEQ ID NO: 682 (IMGT) | HCDR3 | ARGKFNYRFAY |
| SEQ ID NO: 683 | VH | QVQLVQSGAEVQRPGASVKVSCKASGYIFTEYYMY WVRQAPGQGLELVGRIDPEDGSIDYVEKFKKKVTLT ADTSSSTAYMELSSLTSDDTAVYYCARGKFNYRFAY WGQGTLVTVSS |
| SEQ ID NO: 684 | HC | QVQLVQSGAEVQRPGASVKVSCKASGYIFTEYYMY WVRQAPGQGLELVGRIDPEDGSIDYVEKFKKKVTLT |

| | | |
|---|---|---|
| | | ADTSSSTAYMELSSLTSDDTAVYYCARGKFNYRFAY<br>WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL<br>GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG<br>LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK<br>VEPKSC |

In some embodiments, the agent that stimulates a CD3/TCR complex and the agent that stimulates a costimulatory molecule and/or growth factor receptor are comprised in a multispecific binding molecule. Thus, contemplated herein are multispecific binding molecules comprising an agent that stimulates a CD3/TCR complex and an agent that stimulates a costimulatory molecule and/or growth factor receptor, such as but not limited to a multispecific binding molecule comprising a CD3 antigen binding domain and one or more of a CD28, ICOS, CD27, CD25, 4-1BB, IL6RA, IL6RB, and/or CD2 antigen binding domain. Non-limiting examples of such binding domains, as noted above, are provided above, for example in **Table 27** and the publications incorporated by reference herein.

In some embodiments, the multispecific binding molecule comprises a CD3 antigen binding domain and a CD28 or CD2 antigen binding domain. In some embodiments, the CD3 antigen binding domain is an anti-CD3 antibody, optionally the anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4) provided in **Table 27**, or an antibody fragment comprising one or more CDRs, VH, and/or VL thereof. In some embodiments, the CD28 antigen binding domain is an anti-CD28 antibody, optionally the anti-CD28 (1) or anti-CD28 (2) provided in **Table 27**, or an antibody fragment comprising one or more CDRs, VH, heavy chain, VL, and/or light chain thereof. In some embodiments, the CD2 antigen binding domain is an anti-CD2 antibody, optionally the anti-CD2 (1), provided in **Table 27**, or an antibody fragment comprising one or more CDRs, VH, heavy chain, VL, and/or light chain thereof.

In some embodiments, the multispecific binding molecules comprise one or more heavy and/or light chains. Non-limiting exemplary heavy and light chain sequences that may be comprised in these multispecific binding molecules are provided in **Table 28** below. Non-limiting exemplary combinations thereof are suggested in **Table 28** based on the categorization of the recited heavy and/or light chains as within a Construct. This Construct organization provides examples of configurations of heavy and/or light chains but further combinations and permutations thereof are also possible.

In some embodiments, an Anti-CD3 (4)/anti-CD28 (2) bispecific construct is also referred to as Construct A herein. In some embodiments, an Anti-CD3 (2)/anti-CD28 (2) construct is referred to as Construct B herein. In some embodiments, an Anti-CD3 (4)/anti-CD28 (1) construct is referred to as Constuct C herein.

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**Table 28 – Exemplary Fc, heavy chain (HC), and light chain (LC) sequences**

| SEQ ID No: | Chain | Amino acid sequence |
|---|---|---|
| 701 | Wild type Fc | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK |
| 702 | Fc DANAPA | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV AVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSV LTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK |
| 673 | NEG2042 LC | DVVMTQSPPSLLVTLGQPASISCRSSQSLLHSSGNTYLNWLLQR PGQSPQPLIYLVSKLESGVPDRFSGSGSGTDFTLKISGVEAEDV GVYYCMQFTHYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC |
| 703 | NEG2042 HC | QVQLVQSGAEVQRPGASVKVSCKASGYIFTEYYMYWVRQAP GQGLELVGRIDPEDGSIDYVEKFKKKVTLTADTSSSTAYMELSS LTSDDTAVYYCARGKFNYRFAYWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAST YRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGSGGGGSQVQ LVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKG LEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLR PEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGGGG SGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYMN WYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSL QPEDIATYYCQQWSSNPFTFGQGTKLQIT |

| | | |
|---|---|---|
| 518 | NEG2043 LC | DIQMTQSPSSLSASVGDRVTITCHASQNIYVWLNWYQQKPGK APKLLIYKASNLHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQGQTYPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 704 | NEG2043 HC | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPG QGLEWIGCIYPGNVNTNYNEKFKDRATLTVDTSISTAYMELSR LRSDDTAVYFCTRSHYGLDWNFDVWGQGTTVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGSGGGGSQ VQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPG KGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDS LRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQIT |
| 705 | NEG2044 LC | DIVLTQPPSVSGAPGQRVTISCSGSSSNIVSNYVNWYQQLPGTA PKLLIYDNNKRPSGVPDRFSGSKSGTSASLAITGLQSEDEADYY CQSYAIGSYSVVFGGGTKLTVLGQGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC |
| 706 | NEG2044 HC | QVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMSWVRQAPG KGLEWVSSIFYTGSSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARIGYAGDSKYAIWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGSGGGGSQ VQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPG KGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDS LRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQIT |
| 673 | NEG2050 LC | DVVMTQSPPSLLVTLGQPASISCRSSQSLLHSSGNTYLNWLLQR PGQSPQPLIYLVSKLESGVPDRFSGSGSGTDFTLKISGVEAEDV GVYYCMQFTHYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC |

| | | |
|---|---|---|
| 707 | NEG2050 HC | QVQLVQSGAEVQRPGASVKVSCKASGYIFTEYYMYWVRQAP<br>GQGLELVGRIDPEDGSIDYVEKFKKKVTLTADTSSSTAYMELSS<br>LTSDDTAVYYCARGKFNYRFAYWGQGTLVTVSSASTKGPSVF<br>PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT<br>FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK<br>RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE<br>VTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAST<br>YRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAKG<br>QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN<br>GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV<br>MHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGSGGGGSEVQL<br>VESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKCLE<br>WVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNL<br>KTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGG<br>SGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTG<br>AVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLG<br>GKAALTLSGVQPEDEAEYYCVLWYSNRWVFGCGTKLTVL |
| 673 | NEG2051 LC | DVVMTQSPPSLLVTLGQPASISCRSSQSLLHSSGNTYLNWLLQR<br>PGQSPQPLIYLVSKLESGVPDRFSGSGSGTDFTLKISGVEAEDV<br>GVYYCMQFTHYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKS<br>GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK<br>DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG<br>EC |
| 708 | NEG2051 HC | QVQLVQSGAEVQRPGASVKVSCKASGYIFTEYYMYWVRQAP<br>GQGLELVGRIDPEDGSIDYVEKFKKKVTLTADTSSSTAYMELSS<br>LTSDDTAVYYCARGKFNYRFAYWGQGTLVTVSSASTKGPSVF<br>PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT<br>FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK<br>RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE<br>VTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAST<br>YRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAKG<br>QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN<br>GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV<br>MHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGSGGGGSEVQL<br>VESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKCLE<br>WVGRIRSKYNNYATYYADSVKDRFTISRDDSKSTLYLQMNSL<br>KTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGG<br>SGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTS<br>NYANWVQQKPGQAPRGLIGGTNKRAPWTPARFSGSLLGDKA<br>ALTLSGAQPEDEAEYFCALWYSNLWVFGCGTKLTVL |
| 518 | NEG2052 LC | DIQMTQSPSSLSASVGDRVTITCHASQNIYVWLNWYQQKPGK<br>APKLLIYKASNLHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY<br>CQQGQTYPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS<br>VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY<br>SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |

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| 709 | NEG2052 HC | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPG<br>QGLEWIGCIYPGNVNTNYNEKFKDRATLTVDTSISTAYMELSR<br>LRSDDTAVYFCTRSHYGLDWNFDVWGQGTTVTVSSASTKGPS<br>VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV<br>HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV<br>DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR<br>TPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY<br>ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISK<br>AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS<br>CSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGSGGGGSE<br>VQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPG<br>KCLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQ<br>MNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSS<br>GGGGSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTC<br>GSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFS<br>GSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGCGTKLT<br>VL |
| 518 | NEG2053 LC | DIQMTQSPSSLSASVGDRVTITCHASQNIYVWLNWYQQKPGK<br>APKLLIYKASNLHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY<br>CQQGQTYPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS<br>VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY<br>SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 710 | NEG2053 HC | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPG<br>QGLEWIGCIYPGNVNTNYNEKFKDRATLTVDTSISTAYMELSR<br>LRSDDTAVYFCTRSHYGLDWNFDVWGQGTTVTVSSASTKGPS<br>VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV<br>HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV<br>DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR<br>TPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY<br>ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISK<br>AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS<br>CSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGSGGGGSE<br>VQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASG<br>KCLEWVGRIRSKYNNYATYYADSVKDRFTISRDDSKSTLYLQ<br>MNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS<br>GGGGSGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLTCRSSTGA<br>VTTSNYANWVQQKPGQAPRGLIGGTNKRAPWTPARFSGSLLG<br>DKAALTLSGAQPEDEAEYFCALWYSNLWVFGCGTKLTVL |
| 673 | Construct 1<br>Light Chain | DVVMTQSPPSLLVTLGQPASISCRSSQSLLHSSGNTYLNWLLQR<br>PGQSPQPLIYLVSKLESGVPDRFSGSGSGTDFTLKISGVEAEDV<br>GVYYCMQFTHYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKS<br>GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK<br>DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG<br>EC |

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| 711 | Construct 1 Heavy Chain | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAP GKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITGGGGSGGGGSG GGGSGGGGSQVQLVQSGAEVQRPGASVKVSCKASGYIFTEYY MYWVRQAPGQGLELVGRIDPEDGSIDYVEKFKKKVTLTADTS SSTAYMELSSLTSDDTAVYYCARGKFNYRFAYWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| 518 | Construct 2 Light Chain | DIQMTQSPSSLSASVGDRVTITCHASQNIYVWLNWYQQKPGK APKLLIYKASNLHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQGQTYPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 712 | Construct 2 Heavy Chain | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAP GKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITGGGGSGGGGSG GGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYY IHWVRQAPGQGLEWIGCIYPGNVNTNYNEKFKDRATLTVDTSI STAYMELSRLRSDDTAVYFCTRSHYGLDWNFDVWGQGTTVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| 673 | Construct 3 Light Chain | DVVMTQSPPSLLVTLGQPASISCRSSQSLLHSSGNTYLNWLLQR PGQSPQPLIYLVSKLESGVPDRFSGSGSGTDFTLKISGVEAEDV GVYYCMQFTHYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC |

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| 713 | Construct 3 Heavy Chain | QVQLVQSGAEVQRPGASVKVSCKASGYIFTEYYMYWVRQAP GQGLELVGRIDPEDGSIDYVEKFKKKVTLTADTSSSTAYMELSS LTSDDTAVYYCARGKFNYRFAYWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGSGGGGSQVQ LVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKG LEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLR PEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGGGG SGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYMN WYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSL QPEDIATYYCQQWSSNPFTFGQGTKLQIT |
| 518 | Construct 4 Light Chain | DIQMTQSPSSLSASVGDRVTITCHASQNIYVWLNWYQQKPGK APKLLIYKASNLHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQGQTYPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 714 | Construct 4 Heavy Chain | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPG QGLEWIGCIYPGNVNTNYNEKFKDRATLTVDTSISTAYMELSR LRSDDTAVYFCTRSHYGLDWNFDVWGQGTTVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGSGGGGSQV QLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSL RPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGGG GSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYM NWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTIS SLQPEDIATYYCQQWSSNPFTFGQGTKLQIT |
| 673 | Construct 5 Light Chain | DVVMTQSPPSLLVTLGQPASISCRSSQSLLHSSGNTYLNWLLQR PGQSPQPLIYLVSKLESGVPDRFSGSGSGTDFTLKISGVEAEDV GVYYCMQFTHYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC |

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| 715 | Construct 5 Heavy Chain | QVQLVQSGAEVQRPGASVKVSCKASGYIFTEYYMYWVRQAP GQGLELVGRIDPEDGSIDYVEKFKKKVTLTADTSSSTAYMELSS LTSDDTAVYYCARGKFNYRFAYWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCGGGGSGGGGSQVQLVQSGGGVVQPGRSLRLSCKAS GYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKVKD RFTISRDNSKNTAFLQMDSLRPEDTGVYFCARYYDDHYSLDY WGQGTPVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSL SASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKL ASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFG QGTKLQITGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| 518 | Construct 6 Light Chain | DIQMTQSPSSLSASVGDRVTITCHASQNIYVWLNWYQQKPGK APKLLIYKASNLHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQGQTYPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 716 | Construct 6 Heavy Chain | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPG QGLEWIGCIYPGNVNTNYNEKFKDRATLTVDTSISTAYMELSR LRSDDTAVYFCTRSHYGLDWNFDVWGQGTTVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCGGGGSGGGGSQVQLVQSGGGVVQPGRSLRLSCK ASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKV KDRFTISRDNSKNTAFLQMDSLRPEDTGVYFCARYYDDHYSLD YWGQGTPVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSS LSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKL ASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFG QGTKLQITGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| 673 | Construct 7 Light Chain | DVVMTQSPPSLLVTLGQPASISCRSSQSLLHSSGNTYLNWLLQR PGQSPQPLIYLVSKLESGVPDRFSGSGSGTDFTLKISGVEAEDV GVYYCMQFTHYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC |

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| 717 | Construct 7 Heavy Chain | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAP GKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITGGGGSDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVQRPGAS VKVSCKASGYIFTEYYMYWVRQAPGQGLELVGRIDPEDGSIDY VEKFKKKVTLTADTSSSTAYMELSSLTSDDTAVYYCARGKFN YRFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC |
| 518 | Construct 8 Light Chain | DIQMTQSPSSLSASVGDRVTITCHASQNIYVWLNWYQQKPGK APKLLIYKASNLHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQGQTYPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 718 | Construct 8 Heavy Chain | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAP GKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITGGGGSDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGAS VKVSCKASGYTFTSYYIHWVRQAPGQGLEWIGCIYPGNVNTN YNEKFKDRATLTVDTSISTAYMELSRLRSDDTAVYFCTRSHYG LDWNFDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC |
| 673 | Construct 9 Light Chain | DVVMTQSPPSLLVTLGQPASISCRSSQSLLHSSGNTYLNWLLQR PGQSPQPLIYLVSKLESGVPDRFSGSGSGTDFTLKISGVEAEDV GVYYCMQFTHYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC |

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| 719 | Construct 9 Heavy Chain 1 | QVQLVQSGAEVQRPGASVKVSCKASGYIFTEYYMYWVRQAP GQGLELVGRIDPEDGSIDYVEKFKKKVTLTADTSSSTAYMELSS LTSDDTAVYYCARGKFNYRFAYWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSV MHEALHNRFTQKSLSLSPGK |
| 720 | Construct 9 Heavy Chain 2 | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAP GKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITGGGGSDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK |
| 518 | Construct 10 Light Chain | DIQMTQSPSSLSASVGDRVTITCHASQNIYVWLNWYQQKPGK APKLLIYKASNLHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQGQTYPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 721 | Construct 10 Heavy Chain 1 | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPG QGLEWIGCIYPGNVNTNYNEKFKDRATLTVDTSISTAYMELSR LRSDDTAVYFCTRSHYGLDWNFDVWGQGTTVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSC SVMHEALHNRFTQKSLSLSPGK |
| 720 | Construct 10 Heavy Chain 2 | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAP GKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITGGGGSDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK |

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| 722 | Construct 11 Heavy Chain 1 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV CTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH NRFTQKSLSLSPGK |
| 720 | Construct 11 Heavy Chain 2 | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAP GKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITGGGGSDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK |
| 673 | Construct 12 Light Chain | DVVMTQSPPSLLVTLGQPASISCRSSQSLLHSSGNTYLNWLLQR PGQSPQPLIYLVSKLESGVPDRFSGSGSGTDFTLKISGVEAEDV GVYYCMQFTHYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC |
| 719 | Construct 12 Heavy Chain 1 | QVQLVQSGAEVQRPGASVKVSCKASGYIFTEYYMYWVRQAP GQGLELVGRIDPEDGSIDYVEKFKKKVTLTADTSSSTAYMELSS LTSDDTAVYYCARGKFNYRFAYWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSV MHEALHNRFTQKSLSLSPGK |
| 723 | Construct 12 Heavy Chain 2 | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAP GKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITGGGGSDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGGGGGSGGGGSGGGGSEEDPCACESLVKFQAKVEGLL QALTRKLEAVSKRLAILENTVV |

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| 518 | Construct 13 Light Chain | DIQMTQSPSSLSASVGDRVTITCHASQNIYVWLNWYQQKPGK APKLLIYKASNLHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQGQTYPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 721 | Construct 13 Heavy Chain 1 | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPG QGLEWIGCIYPGNVNTNYNEKFKDRATLTVDTSISTAYMELSR LRSDDTAVYFCTRSHYGLDWNFDVWGQGTTVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSC SVMHEALHNRFTQKSLSLSPGK |
| 723 | Construct 13 Heavy Chain 2 | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAP GKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITGGGGSDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGGGGGSGGGGSGGGGSEEDPCACESLVKFQAKVEGLL QALTRKLEAVSKRLAILENTVV |
| 722 | Construct 14 Heavy Chain 1 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV CTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH NRFTQKSLSLSPGK |
| 723 | Construct 14 Heavy Chain 2 | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAP GKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITGGGGSDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGGGGGSGGGGSGGGGSEEDPCACESLVKFQAKVEGLL QALTRKLEAVSKRLAILENTVV |

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| 673 | Construct 15 Light Chain | DVVMTQSPPSLLVTLGQPASISCRSSQSLLHSSGNTYLNWLLQR PGQSPQPLIYLVSKLESGVPDRFSGSGSGTDFTLKISGVEAEDV GVYYCMQFTHYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC |
| 719 | Construct 15 Heavy Chain 1 | QVQLVQSGAEVQRPGASVKVSCKASGYIFTEYYMYWVRQAP GQGLELVGRIDPEDGSIDYVEKFKKKVTLTADTSSSTAYMELSS LTSDDTAVYYCARGKFNYRFAYWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSV MHEALHNRFTQKSLSLSPGK |
| 724 | Construct 15 Heavy Chain 2 | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAP GKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITGGGGSDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGGGGGSDLGPQMLRELQETNAALQDVRELLRQQVREI TFLKNTVMECDACGMQQ |
| 518 | Construct 16 Light Chain | DIQMTQSPSSLSASVGDRVTITCHASQNIYVWLNWYQQKPGK APKLLIYKASNLHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQGQTYPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 721 | Construct 16 Heavy Chain 1 | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPG QGLEWIGCIYPGNVNTNYNEKFKDRATLTVDTSISTAYMELSR LRSDDTAVYFCTRSHYGLDWNFDVWGQGTTVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSC SVMHEALHNRFTQKSLSLSPGK |

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| 724 | Construct 16 Heavy Chain 2 | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAP GKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITGGGGSDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGGGGGSDLGPQMLRELQETNAALQDVRELLRQQVREI TFLKNTVMECDACGMQQ | |
| 722 | Construct 17 Heavy Chain 1 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV CTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH NRFTQKSLSLSPGK |
| 724 | Construct 17 Heavy Chain 2 | QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAP GKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYSLDYWGQGTPVTVSSGGGGSGG GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITGGGGSDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGGGGGSDLGPQMLRELQETNAALQDVRELLRQQVREI TFLKNTVMECDACGMQQ |

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| SEQ ID NO: 794 | Anti-CD3 (4)/anti-CD28 (2) bispecific heavy chain | QVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMSWVR QAPGKGLEWVSSIFYTGSSTYYADSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCARIGYAGDSKYAIWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAP EAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALAAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGS EVKLVESGGDLVQPGDSLTLSCVASGFTFSKQGMHWIRQ APKKGLEWIAMIYYDSSKMYYADTVKGRFTISRDNSKNT LYLEMNSLRSEDTAMYYCASFWWDLDFDHWGQGVMVT VSSGGGGSGGGGSGGGGSGGGGSDILVTQTPVSLPVSLG GHVSISCRSSQSLVRSEGTTYFNWYLQKPGQSPQLLIYRV SNRFSGVPDRFSGSGSGTDFTLKISRVEPEDLGVYYCLQSS HFPWTFGGGTKLELK |
| SEQ ID NO: 795 | Anti-CD3 (4)/anti-CD28 (2) bispecific alternative heavy chain | EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMSWVRQ APGKGLEWVSSIFYTGSSTYYADSVKGRFTISRDNSKNTL YLQMNSLRAEDTAVYYCARIGYAGDSKYAIWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALAAPIEKTISKAKGQPREPQVYT LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGS EVKLVESGGDLVQPGDSLTLSCVASGFTFSKQGMHWIRQ APKKGLEWIAMIYYDSSKMYYADTVKGRFTISRDNSKNT LYLEMNSLRSEDTAMYYCASFWWDLDFDHWGQGVMVT VSSGGGGSGGGGSGGGGSGGGGSDILVTQTPVSLPVSLG GHVSISCRSSQSLVRSEGTTYFNWYLQKPGQSPQLLIYRV SNRFSGVPDRFSGSGSGTDFTLKISRVEPEDLGVYYCLQSS HFPWTFGGGTKLELK |
| SEQ ID NO: 796 | Anti-CD3 (4)/anti-CD28 (2) bispecific light chain 1 | DIVLTQPPSVSGAPGQRVTISCSGSSSNIVSNYVNWYQQL PGTAPKLLIYDNNKRPSGVPDRFSGSKSGTSASLAITGLQS EDEADYYCQSYAIGSYSVVFGGGTKLTVLGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC |

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| SEQ ID NO: 797 | Anti-CD3 (4)/anti-CD28 (2) bispecific light chain 2 | DIVLTQPPSVSGAPGQRVTISCSGSSSNIVSNYVNWYQQL PGTAPKLLIYDNNKRPSGVPDRFSGSKSGTSASLAITGLQS EDEADYYCQSYAIGSYSVVFGGGTKLTVLGQPKAAPSVT LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQV THEGSTVEKTVAPTECS |
| SEQ ID NO: 798 | Anti-CD3 (2)/anti-CD28 (2) heavy chain | QVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMSWVR QAPGKGLEWVSSIFYTGSSTYYADSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCARIGYAGDSKYAIWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAP EAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALAAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVR QAPGKCLEWVARIRSKYNNYATYYADSVKDRFTISRDDS KNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAY WGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSQTVVTQE PSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAP RGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEA EYYCVLWYSNRWVFGCGTKLTVL |
| SEQ ID NO: 815 | Anti-CD3 (2)/anti-CD28 (2) alternative heavy chain | EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMSWVRQ APGKGLEWVSSIFYTGSSTYYADSVKGRFTISRDNSKNTL YLQMNSLRAEDTAVYYCARIGYAGDSKYAIWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALAAPIEKTISKAKGQPREPQVYT LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVR QAPGKCLEWVARIRSKYNNYATYYADSVKDRFTISRDDS KNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAY WGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSQTVVTQE PSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAP RGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEA EYYCVLWYSNRWVFGCGTKLTVL |

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| SEQ ID NO: 799 | Anti-CD3 (2)/anti-CD28 (2) light chain | DIVLTQPPSVSGAPGQRVTISCSGSSSNIVSNYVNWYQQL PGTAPKLLIYDNNKRPSGVPDRFSGSKSGTSASLAITGLQS EDEADYYCQSYAIGSYSVVFGGGTKLTVLGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC |
| SEQ ID NO: 800 | Anti-CD3 (4)/anti-CD28 (1) heavy chain | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVR QAPGQGLEWIGCIYPGNVNTNYNEKFKDRATLTVDTSIST AYMELSRLRSDDTAVYFCTRSHYGLDWNFDVWGQGTT VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALAAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGGGSEVKLVESGGDLVQPGDS LTLSCVASGFTFSKQGMHWIRQAPKKGLEWIAMIYYDSS KMYYADTVKGRFTISRDNSKNTLYLEMNSLRSEDTAMY YCASFWWDLDFDHWGQGVMVTVSSGGGGSGGGGSGG GGSGGGGSDILVTQTPVSLPVSLGGHVSISCRSSQSLVRSE GTTYFNWYLQKPGQSPQLLIYRVSNRFSGVPDRFSGSGSG TDFTLKISRVEPEDLGVYYCLQSSHFPWTFGGGTKLELK |
| SEQ ID NO: 801 | Anti-CD3 (4)/anti-CD28 (1) light chain | DIQMTQSPSSLSASVGDRVTITCHASQNIYVWLNWYQQK PGKAPKLLIYKASNLHTGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQGQTYPYTFGGGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC |

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| SEQ ID NO: 816 | Anti-CD3 (1)/anti-CD2 (1) heavy chain GADAPASK | QVQLVQSGAEVQRPGASVKVSCKASGYIFTEYYMYWVR<br>QAPGQGLELVGRIDPEDGSIDYVEKFKKKVTLTADTSSST<br>AYMELSSLTSDDTAVYYCARGKFNYRFAYWGQGTLVTV<br>SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT<br>VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT<br>QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL<br>GAPSVFLFPPKPKDTLMISRTPEVTCVVVAVKHEDPEVKF<br>NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD<br>WLNGKEYKCKVSNKALAAPIEKTISKAKGQPREPQVYTL<br>PPSRREEMKNQVSLTCLVKGFYPSDIAVEWESNGQPENN<br>YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH<br>EALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGSQ<br>VQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQ<br>APGKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNT<br>AFLQMDSLRPEDTGVYFCARYYDDHYSLDYWGQGTPVT<br>VSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVG<br>DRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLAS<br>GVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFT<br>FGQGTKLQIT |
| SEQ ID NO: 817 | Anti-CD3 (1)/anti-CD2 (1) heavy chain LALAPG | QVQLVQSGAEVQRPGASVKVSCKASGYIFTEYYMYWVR<br>QAPGQGLELVGRIDPEDGSIDYVEKFKKKVTLTADTSSST<br>AYMELSSLTSDDTAVYYCARGKFNYRFAYWGQGTLVTV<br>SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT<br>VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT<br>QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEA<br>AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK<br>FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD<br>WLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTL<br>PPSRREEMKNQVSLTCLVKGFYPSDIAVEWESNGQPENN<br>YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH<br>EALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGSQ<br>VQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQ<br>APGKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNT<br>AFLQMDSLRPEDTGVYFCARYYDDHYSLDYWGQGTPVT<br>VSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVG<br>DRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLAS<br>GVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFT<br>FGQGTKLQIT |
| SEQ ID NO: 802 | Fc LALAPG | DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTC<br>VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST<br>YRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK<br>AKGQPREPQVYTLPPSRREEMKNQVSLTCLVKGFYPSDIA<br>VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPGK |

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| SEQ ID NO: 803 | Fc DAPA | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| SEQ ID NO: 804 | Fc LALASKPA | DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| SEQ ID NO: 805 | Fc GADAPASK | DKTHTCPPCPAPELLGAPSVFLFPPKPKDTLMISRTPEVTC VVVAVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTIS KAKGQPREPQVYTLPPSRREEMKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |

In some embodiments, the multispecific binding molecule comprises a bispecific antibody. In some embodiments, the bispecific antibody is configured in any one of the schema provided in **FIG. 50A, FIGs. 51A-51B,** and **FIGs. 61A-61B**, and **FIGs. 63A-63B**. In some embodiments, the bispecific antibody is monovalent or bivalent. In some embodiments, the bispecific antibody comprises an Fc region. In some embodiments, the Fc region of the bispecific antibody is silenced. In some embodiments, the Fc region of the bispecific antibody is silenced by a combination of amino acid substitutions selected from the group consisting of LALA, DAPA, DANAPA, LALADANAPS, LALAGA, LALASKPA, DAPASK, GADAPA, GADAPASK, LALAPG, and LALAPA.

In some embodiments, the multispecific binding molecule comprises a plurality of bispecific antibodies. In some embodiments, one or more of the plurality of bispecific antibodies is monovalent. In some embodiments, one or more of the plurality of bispecific antibodies comprises an Fc region. In some embodiments, the Fc region of the one or more of the plurality of bispecific antibodies is silenced. In some embodiments, one or more of the plurality of bispecific antibodies are conjugated together into a multimer. In some embodiments, the multimer is configured in any one of the schema provided in **FIG. 50B** and **FIG. 51B**.

In some embodiments, a multispecific binding molecule described herein comprises an Fc region, e.g., wherein the Fc region is Fc silent. In some embodiments, the Fc region comprises a mutation at one or more of (e.g., all of) D265, N297, and P329, wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises the mutations D265A, N297A, and P329A (DANAPA), wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises a mutation at one or more of (e.g., all of) L234A, L235A, and G237A, wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises the mutations L234A, L235A, and G237A (LALAGA), wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises a mutation at one or more of (e.g., all of) L234A, L235A, S267K, and P329A, wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises the mutations L234A, L235A, S267K, and P329A (LALASKPA), wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises a mutation at one or more of (e.g., all of) D265A, P329A, and S267K, wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises the mutations D265A, P329A, and S267K (DAPASK), wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises a mutation at one or more of (e.g., all of) G237A, D265A, and P329A, wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises the mutations G237A, D265A, and P329A (GADAPA), wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises a mutation at one or more of (e.g., all of) G237A, D265A, P329A, and S267K, wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises the mutations G237A, D265A, P329A, and S267K (GADAPASK), wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises a mutation at one or more of (e.g., all of) L234A, L235A, and P329G, wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises the

mutations L234A, L235A, and P329G (LALAPG), wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises a mutation at one or more of (e.g., all of) L234A, L235A, and P329A, wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the Fc region comprises the mutations L234A, L235A, and P329A (LALAPA), wherein the amino acid residues are numbered according to the EU numbering system.

In some embodiments, a multispecific binding molecule described herein comprises a first binding domain and a second binding domain. For instance, the first binding domain may be an anti-CD3 binding domain and the second binding domain may be a costimulatory molecule binding domain, or the first binding domain may be a costimulatory molecule binding domain and the second binding domain may be an anti-CD3 binding domain. In some embodiments, the costimulatory molecule binding domain binds to CD2, CD28, CD25, CD27, IL6Rb, ICOS, or 41BB. In some embodiments, the costimulatory molecule binding domain activates CD2, CD28, CD25, CD27, IL6Rb, ICOS, or 41BB. In some embodiments, a multispecific binding molecule described herein comprises an Fc region that is mutated to have reduced binding to Fc receptor or reduced ADCC, ADCP, or CDC activity, e.g., an Fc region comprising the mutations D265A, N297A, and P329A (DANAPA); L234A, L235A, and G237A (LALAGA); L234A, L235A, S267K, and P329A (LALASKPA); D265A, P329A, and S267K (DAPASK); G237A, D265A, and P329A (GADAPA); G237A, D265A, P329A, and S267K (GADAPASK); L234A, L235A, and P329G (LALAPG); or L234A, L235A, and P329A (LALAPA), wherein the amino acid residues are numbered according to the EU numbering system.

In some embodiments, the first binding domain (e.g., an scFv) is N-terminal of the VH of the second binding domain (e.g., a Fab fragment), e.g., linked via a peptide linker. In some embodiments, the multispecific binding molecule further comprises one or more of (e.g., all of) a CH1, CH2, and CH3, e.g., in order from N-terminal to C-terminal. In some embodiments, a polypeptide of the multispecific binding molecule comprises the following sequences, from N-terminal to C-terminal: VH of the first binding domain, first peptide linker (e.g., a (G4S)4 linker), VL of first binding domain, second peptide linker (e.g., a (G4S)4 linker), VH of the second binding domain, CH1, CH2, and CH3. In some embodiments, a polypeptide of the multispecific binding molecule comprises the following sequences: from N-terminal to C-

terminal: VL of the second binding domain and CL. In some embodiments, the multispecific binding molecule comprises an Fc region that is mutated to have reduced binding to Fc receptor or reduced ADCC, ADCP, or CDC activity, e.g., an Fc region comprising the mutations D265A, N297A, and P329A (DANAPA) ; L234A, L235A, and G237A (LALAGA); L234A, L235A, S267K, and P329A (LALASKPA); D265A, P329A, and S267K (DAPASK); G237A, D265A, and P329A (GADAPA); G237A, D265A, P329A, and S267K (GADAPASK); L234A, L235A, and P329G (LALAPG); or L234A, L235A, and P329A (LALAPA), wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the first binding fragment comprises an anti-CD3 binding domain, e.g., an anti-CD3 scFv, e.g., comprising an anti-CD3 sequence disclosed in Table 27. In some embodiments, the second binding domain comprises a costimulatory molecule binding domain, e.g., an anti-CD2 binding domain, e.g., an anti-CD2 Fab, e.g., comprising an anti-CD2 sequence disclosed in Table 27. In some embodiments, the second binding domain comprises a costimulatory molecule binding domain, e.g., an anti-CD28 binding domain, e.g., an anti-CD28 Fab, e.g., comprising an anti-CD28 sequence disclosed in Table 27. In some embodiments, the first binding fragment comprises a costimulatory molecule binding domain, e.g., an anti-CD2 or anti-CD28 binding domain, e.g., an anti-CD2 or anti-CD28 scFv, e.g., comprising an anti-CD2 or anti-CD28 sequence disclosed in Table 27. In some embodiments, the second binding domain comprises an anti-CD3 binding domain, e.g., an anti-CD3 Fab, e.g., comprising an anti-CD3 sequence disclosed in Table 27. Examples of such multispecific binding molecules are depicted as the top left construct in **FIG. 50A**; Construct 1 or Construct 2 in **FIG. 51A**; and Construct 1 or Construct 2 in Table 28.

In some embodiments, the first binding domain (e.g., a Fab fragment) is N-terminal to a second binding domain (e.g., an scFv), e.g., wherein an Fc region is situated between the first and second binding domain. In some embodiments, the Fc region is mutated to have reduced binding to Fc receptor or reduced ADCC, ADCP, or CDC activity, e.g., an Fc region comprising the mutations D265A, N297A, and P329A (DANAPA); L234A, L235A, and G237A (LALAGA); L234A, L235A, S267K, and P329A (LALASKPA); D265A, P329A, and S267K (DAPASK); G237A, D265A, and P329A (GADAPA); G237A, D265A, P329A, and S267K (GADAPASK); L234A, L235A, and P329G (LALAPG); or L234A, L235A, and P329A (LALAPA), wherein the amino acid residues are numbered according to the EU

numbering system. In some embodiments, the multispecific binding molecule further comprises one or more of (e.g., all of) a CH1, CH2, and CH3, e.g., in order from N-terminal to C-terminal. In some embodiments, a polypeptide of the multispecific binding molecule comprises the following sequences, from N-terminal to C-terminal: VH of the first binding domain, CH1, CH2, CH3, first peptide linker (e.g., a (G4S)4 linker), VH of second binding domain, second peptide linker (e.g., a (G4S)4 linker), and VL of the second binding domain. In some embodiments, a polypeptide of the multispecific binding molecule comprises the following sequences: from N-terminal to C-terminal: VL of the first binding domain and CL. In some embodiments, the first binding domain comprises a costimulatory molecule binding domain, e.g., an anti-CD2 binding domain, e.g., an anti-CD2 Fab, e.g., comprising an anti-CD2 sequence disclosed in Table 27. In some embodiments, the first binding domain comprises a costimulatory molecule binding domain, e.g., an anti-CD28 binding domain, e.g., an anti-CD28 Fab, e.g., comprising an anti-CD28 sequence disclosed in Table 27, e.g., anti-CD28 (1) or anti-CD28 (2). In some embodiments, the second binding domain comprises an anti-CD3 binding domain, e.g., an anti-CD3 scFv, e.g., comprising an anti-CD3 sequence disclosed in Table 27, e.g., anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4). In some embodiments, the first binding domain comprises an anti-CD3 binding domain, e.g., an anti-CD3 Fab, e.g., comprising an anti-CD3 sequence disclosed in Table 27, e.g., anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4). In some embodiments, the second binding domain comprises a costimulatory molecule binding domain, e.g., an anti-CD2 or anti-CD28 binding domain, e.g., an anti-CD2 or anti-CD28 scFv, e.g., comprising an anti-CD2 or anti-CD28 sequence disclosed in Table 27. Examples of such multispecific binding molecules are depicted as the second construct from the left in the top row of **FIG. 50A**; Construct 3 or Construct 4 in **FIG. 51A**; and Construct 3, Construct 4, Anti-CD3 (4)/anti-CD28 (2) bispecific construct, Anti-CD3 (2)/anti-CD28 (2) construct, or Anti-CD3 (4)/anti-CD28 (1) construct in Table 28.

In some embodiments, the first binding domain (e.g., a Fab fragment) is N terminal to a second binding domain (e.g., a scFv), e.g., via a peptide linker. In some embodiments, the multispecific binding molecule further comprises one or more of (e.g., all of) a CH1, CH2, and CH3, e.g., in order from N-terminal to C-terminal. In some embodiments, a polypeptide of the multispecific binding molecule comprises the following sequences, from N-terminal to C-terminal: VH of the first binding domain, CH1, first peptide linker (e.g., a (G4S)2 linker), VH

of the second binding domain, second peptide linker (e.g., a (G4S)4 linker), VL of the second binding domain, third peptide linker (e.g., a (G4S)4 linker), CH2, and CH3. In some embodiments, a polypeptide of the multispecific binding molecule comprises the following sequences: from N-terminal to C-terminal: VL of the first binding domain and CL. In some embodiments, the multispecific binding molecule comprises an Fc region that is mutated to have reduced binding to Fc receptor or reduced ADCC, ADCP, or CDC activity, e.g., an Fc region comprising the mutations D265A, N297A, and P329A (DANAPA); L234A, L235A, and G237A (LALAGA); L234A, L235A, S267K, and P329A (LALASKPA); D265A, P329A, and S267K (DAPASK); G237A, D265A, and P329A (GADAPA); G237A, D265A, P329A, and S267K (GADAPASK); L234A, L235A, and P329G (LALAPG); or L234A, L235A, and P329A (LALAPA), wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the first binding domain comprises a costimulatory molecule binding domain, e.g., an anti-CD2 binding domain, e.g., an anti-CD2 Fab, e.g., comprising an anti-CD2 sequence disclosed in Table 27. In some embodiments, the first binding domain comprises a costimulatory molecule binding domain, e.g., an anti-CD28 binding domain, e.g., an anti-CD28 Fab, e.g., comprising an anti-CD28 sequence disclosed in Table 27, e.g., anti-CD28 (1) or anti-CD28 (2). In some embodiments, the first binding domain comprises a costimulatory molecule binding domain, e.g., an anti-CD25 binding domain (for example, an anti-CD25 Fab), an anti-CD27 binding domain (for example, an anti-CD27 Fab), an anti-IL6Rb binding domain (for example, an anti-IL6Rb Fab), an anti-ICOS binding domain (for example, an anti-ICOS Fab), or an anti-41BB binding domain (for example, an anti-41BB Fab). In some embodiments, the second binding domain comprises an anti-CD3 binding domain, e.g., an anti-CD3 scFv, e.g., comprising an anti-CD3 sequence disclosed in Table 27, e.g., anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4). In some embodiments, the first binding domain comprises an anti-CD3 binding domain, e.g., an anti-CD3 Fab, e.g., comprising an anti-CD3 sequence disclosed in Table 27, e.g., anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4). In some embodiments, the second binding domain comprises a costimulatory molecule binding domain, e.g., an anti-CD2 binding domain (for example, an anti-CD2 scFv), an anti-CD28 binding domain (for example, an anti-CD28 scFv), an anti-CD25 binding domain (for example, an anti-CD25 scFv), an anti-CD27 binding domain (for example, an anti-CD27 scFv), an anti-IL6Rb binding domain (for example, an anti-IL6Rb scFv), an anti-ICOS binding

domain (for example, an anti-ICOS scFv), or an anti-41BB binding domain (for example, an anti-41BB scFv). Examples of such multispecific binding molecules are depicted as the third construct from the left in the top row of **FIG. 50A**; Construct 5 or Construct 6 in **FIG. 51A**; and Construct 5 or Construct 6 in Table 28.

In some embodiments, the first binding domain (e.g., an scFv) is N-terminal to a second binding domain (e.g., a Fab fragment), e.g., wherein an Fc region is situated between the first and second binding domain. In some embodiments, the Fc region is mutated to have reduced binding to Fc receptor or reduced ADCC, ADCP, or CDC activity, e.g., an Fc region comprising the mutations D265A, N297A, and P329A (DANAPA); L234A, L235A, and G237A (LALAGA); L234A, L235A, S267K, and P329A (LALASKPA); D265A, P329A, and S267K (DAPASK); G237A, D265A, and P329A (GADAPA); G237A, D265A, P329A, and S267K (GADAPASK); L234A, L235A, and P329G (LALAPG); or L234A, L235A, and P329A (LALAPA), wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, the multispecific binding molecule further comprises one or more of (e.g., all of) a CH2, CH3, and CH1, e.g., in order from N-terminal to C-terminal. In some embodiments, a polypeptide of the multispecific binding molecule comprises the following sequences, from N-terminal to C-terminal: VH of the first binding domain, first peptide linker (e.g., a (G4S)4 linker), VL of the first binding domain, second peptide linker (e.g., a (G4S) linker), CH2, CH3, third peptide linker (e.g., a (G4S)4 linker), VH of the second binding domain, and CH1. In some embodiments, a polypeptide of the multispecific binding molecule comprises the following sequences: from N-terminal to C-terminal: VL of the second binding domain and CL. In some embodiments, the first binding domain comprises an anti-CD3 binding domain, e.g., an anti-CD3 scFv, e.g., comprising an anti-CD3 sequence disclosed in Table 27. In some embodiments, the second binding domain comprises a costimulatory molecule binding domain, e.g., an anti-CD2 binding domain, e.g., an anti-CD2 Fab, e.g., comprising an anti-CD2 sequence disclosed in Table 27. In some embodiments, the second binding domain comprises a costimulatory molecule binding domain, e.g., an anti-CD28 binding domain, e.g., an anti-CD28 Fab, e.g., comprising an anti-CD28 sequence disclosed in Table 27. In some embodiments, the first binding domain comprises a costimulatory molecule binding domain, e.g., an anti-CD2 or anti-CD28 binding domain, e.g., an anti-CD2 or anti-CD28 scFv, e.g., comprising an anti-CD2 or anti-CD28 sequence disclosed

in Table 27. In some embodiments, the second binding domain comprises an anti-CD3 binding domain, e.g., an anti-CD3 Fab, e.g., comprising an anti-CD3 sequence disclosed in Table 27. Examples of such multispecific binding molecules are depicted as the rightmost construct in the top row of **FIG. 50A**; Construct 7 or Construct 8 in **FIG. 51A**; and Construct 7 or Construct 8 in Table 28.

In some embodiments, the first binding domain (e.g., a Fab fragment) is situated N terminal to a first Fc region. In some embodiments, the multispecific binding molecule comprises one or more of (e.g., all of) a first CH1, a first CH2, and a first CH3, e.g., in order from N-terminal to C-terminal. In some embodiments, the second binding domain (e.g., an scFv) is situated N terminal to a second Fc region, e.g., in a second polypeptide chain. In some embodiments, the multispecific binding molecule comprises, e.g., in the second polypeptide chain, one or more of (e.g., both of) a second CH2 and a second CH3, e.g., in order from N-terminal to C-terminal. In some embodiments, the multispecific binding molecule comprises a heterodimeric antibody molecule, such as for instance, wherein the first and second Fc regions comprise knob-into-hole mutations. In some embodiments, the first Fc region binds the second Fc region more strongly than the first Fc region binds another copy of the first Fc region. In some embodiments, a first polypeptide of the multispecific binding molecule comprises the following sequences, from N-terminal to C-terminal: VH of the first binding domain, a first CH1, a first CH2, and a first CH3. In some embodiments, a second polypeptide of the multispecific binding molecule comprises the following sequences, from N-terminal to C-terminal: VH of the second binding domain, a first peptide linker (e.g., a (G4S) linker), VL of the second binding domain, a second CH2, and a second CH3. In some embodiments, a third polypeptide of the multispecific binding molecule comprises the following sequences: from N-terminal to C-terminal: VL of the first binding domain and CL. In some embodiments, the second polypeptide of the multispecific binding molecule further comprises a homomultimerization domain, e.g., a Matrilin1 protein or the coiled-coil domain of cartilage oligomeric matrix protein (COMPcc), C-terminal to the second CH3, e.g., via a peptide linker (e.g., a (G4S)4 linker, a (G4S) linker, or a (G4S)3 linker). In some embodiments, the multispecific binding molecule comprises two, three, four, or five copies of the first binding domain and the same number of copies of the second binding domain, e.g., as depicted in **FIG. 50B**. In some embodiments, the first binding domain comprises a costimulatory molecule

binding domain, e.g., an anti-CD2 binding domain (for example, an anti-CD2 Fab). In some embodiments, the first binding domain comprises a costimulatory molecule binding domain, e.g., an anti-CD28 binding domain (for example, an anti-CD28 Fab). In some embodiments, the second binding domain comprises an anti-CD3 binding domain, e.g., an anti-CD3 scFv. Examples of such multispecific binding molecules are depicted as the leftmost construct in the bottom row of **FIG. 50A**; constructs in **FIG. 50B**, Construct 9, Construct 10, Construct 12, Construct 13, Construct 15, and Construct 16 in **FIG. 51B**; and Construct 9, Construct 10, Construct 12, Construct 13, Construct 15, and Construct 16 in Table 28.

In some embodiments, a binding molecule described herein comprises a binding domain. In some embodiments the binding domain (e.g., an scFv) is situated N terminal to an Fc region. In some embodiments, the binding molecule comprises a heterodimeric antibody molecule, such as for instance, wherein the first and second Fc regions comprise knob-into-hole mutations. In some embodiments, the first Fc region binds the second Fc region more strongly than the first Fc region binds another copy of the first Fc region. In some embodiments, the binding molecule comprises one or more of (e.g., all of) a CH2 and a CH3, e.g., in order from N-terminal to C-terminal. In some embodiments, a second polypeptide of the binding molecule comprises the following sequences, from N-terminal to C-terminal: VH of the binding domain, first peptide linker (e.g., a (G4S)4 linker), VL of the binding domain, second peptide linker (e.g., (G4S)4 linker or (G4S) linker), CH2, and CH3. In some embodiments, the second polypeptide of the binding molecule further comprises a homomultimerization domain, e.g., a Matrilin1 protein or the coiled-coil domain of cartilage oligomeric matrix protein (COMPcc), C-terminal to the second CH3, e.g., via a peptide linker (e.g., (G4S)4 linker, (GS4)3 linker, or (G4S) linker). In some embodiments, the binding molecule comprises two, three, four, or five copies of the binding, e.g., as depicted in **FIG. 50B**. In some embodiments, the binding domain comprises an anti-CD3 binding domain, e.g., an anti-CD3 scFv. In some embodiments, a costimulatory molecule binding domain is absent. Examples of such binding molecules are depicted as the rightmost construct in the bottom row of **FIG. 50A**; Construct 11, Construct 14, and Construct 17 in **FIG. 51B**; and Construct 11, Construct 14, and Construct 17 in Table 28.

In some embodiments, the multispecific binding protein comprises an anti-CD28 binding domain, e.g., an anti-CD28 Fab, e.g., comprising an anti-CD28 (2) sequence of Table 27 (or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%

sequence identity thereto), and an anti-CD3 scFv, e.g., comprising an anti-CD3 (4) sequence of Table 27 (or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto). In some embodiments, the multispecific binding protein comprises an Fc region, wherein the anti-CD28 Fab is fused to the Fc region, which is further fused to the anti-CD3 scFv. In some embodiments, the Fc region comprises L234A, L235A, S267K, and P329A mutations (LALASKPA), numbered according to the Eu numbering system. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 794 or 795, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 794 or 795. In some embodiments, the multispecific binding protein comprises a light chain comprising the amino acid sequence of SEQ ID NO: 796 or 797, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 796 or 797. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 794 or 795, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 794 or 795, and a light chain comprising the amino acid sequence of SEQ ID NO: 796 or 797, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 796 or 797. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 794, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 796, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 794, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 797, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 795, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 796, or an amino acid sequence at least 70%, 75%, 80%, 85%,

90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 795, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 797, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

In some embodiments, the multispecific binding protein comprises an anti-CD28 binding domain, e.g., an anti-CD28 Fab, e.g., comprising an anti-CD28 (2) sequence of Table 27 (or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto), and an anti-CD3 scFv, e.g., comprising an anti-CD3 (2) sequence of Table 27 (or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto). In some embodiments, the multispecific binding protein comprises an Fc region, wherein the anti-CD28 Fab is fused to the Fc region, which is further fused to the anti-CD3 scFv. In some embodiments, the Fc region comprises L234A, L235A, S267K, and P329A mutations (LALASKPA), numbered according to the Eu numbering system. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 798 or 815, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 798 or 815. In some embodiments, the multispecific binding protein comprises a light chain comprising the amino acid sequence of SEQ ID NO: 799, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 798, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 799, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 815, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 799, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

In some embodiments, the multispecific binding protein comprises an anti-CD28 binding domain, e.g., an anti-CD28 Fab, e.g., comprising an anti-CD28 (1) sequence of Table 27 (or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto) and an anti-CD3 scFv, e.g., comprising an anti-CD3 (4) sequence of Table 27 (or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto). In some embodiments, the multispecific binding protein comprises an Fc region, wherein the anti-CD28 Fab is fused to the Fc region, which is further fused to the anti-CD3 scFv. In some embodiments, the Fc region comprises L234A, L235A, S267K, and P329A mutations (LALASKPA), numbered according to the Eu numbering system. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 800, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some embodiments, the multispecific binding protein comprises a light chain comprising the amino acid sequence of SEQ ID NO: 801, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 800, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 801, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

In some embodiments, the multispecific binding protein comprises an anti-CD2 binding domain, e.g., comprising an anti-CD2 (1) sequence of Table 27 (or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto) and an anti-CD3 binding domain, comprising an anti-CD3 (1) sequence of Table 27 (or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto). In some embodiments, the multispecific binding protein comprises an Fc region. In some embodiments, the Fc region comprises a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 816, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some embodiments, the multispecific binding protein

comprises a light chain comprising the amino acid sequence of SEQ ID NO: 673, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 816, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 673, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

In some embodiments, the multispecific binding protein comprises an anti-CD2 binding domain, e.g., comprising an anti-CD2 (1) sequence of Table 27 (or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto) and an anti-CD3 binding domain, e.g., comprising an anti-CD3 (1) sequence of Table 27 (or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto). In some embodiments, the multispecific binding protein comprises an Fc region. In some embodiments, the Fc region comprises a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 817, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some embodiments, the multispecific binding protein comprises a light chain comprising the amino acid sequence of SEQ ID NO: 673, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some embodiments, the multispecific binding protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 817, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 673, or an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

It is understood that in many of the embodiments herein, a multispecific binding molecule comprises two or more polypeptide chains that are covalently linked to each other, e.g., via a disulfide bridge. However, in some embodiments, the two or more polypeptide chains of the multispecific binding molecule may be noncovalently bound to each other.

It is also understood that a Fab fragment may be present as part of a larger protein, for instance, a Fab fragment may be fused with CH2 and CH3 and thus be part of full length antibody.

The multispecific binding molecule comprising an agent that stimulates a CD3/TCR complex and an agent that stimulates a costimulatory molecule and/or growth factor receptor disclosed herein is contemplated for use in the manufacturing embodiments disclosed herein, e.g., traditional manufacture or activated rapid manufacture.

In some embodiments, the multispecific binding molecule is a multispecific binding molecule described, e.g., in WO2021173985, the contents of which are hereby incorporated by reference in their entirety.

**Fc Variants**

In some embodiments, a multispecific binding molecule described herein comprises an Fc region, e.g., as described herein. In some embodiments, the Fc region is a wild type Fc region, e.g., a wild type human Fc region. In some embodiments, the Fc region comprises a variant, e.g., an Fc region comprising an addition, substitution, or deletion of at least one amino acid residue in the Fc region which results in, e.g., reduced or ablated affinity for at least one Fc receptor.

In some embodiments, the Fc region of an antibody interacts with a number of receptors or ligands including Fc Receptors (e.g., FcγRI, FcγRIIA, FcγRIIIA), the complement protein CIq, and other molecules such as proteins A and G. These interactions promote a variety of effector functions and downstream signaling events including: antibody dependent cell-mediated cytotoxicity (ADCC), Antibody-dependent cellular phagocytosis (ADCP) and complement dependent cytotoxicity (CDC).

In some embodiments, a multispecific binding molecule described herein comprising a variant Fc region has reduced, e.g., ablated, affinity for an Fc receptor, e.g., an Fc receptor described herein. In some embodiments, the reduced affinity is compared to an otherwise similar antibody with a wild type Fc region.

In some embodiments, a multispecific binding molecule described herein comprising a variant Fc region has one or more of the following properties: (1) reduced effector function (e.g., reduced ADCC, ADCP and/or CDC); (2) reduced binding to one or more Fc receptors;

and/or (3) reduced binding to C1q complement. In some embodiments, the reduction in any one, or all of properties (1)-(3) is compared to an otherwise similar antibody with a wildtype Fc region.

Exemplary Fc region variants are provided in **Table 34** and also disclosed in Saunders O, (2019) *Frontiers in Immunology;* vol 10, article1296, the entire contents of which is hereby incorporated by reference.

In some embodiments, a multispecific binding molecule described herein comprises any one or all, or any combination of Fc region variants, e.g., mutations, disclosed in **Table 34**. In some embodiments, the Fc region of a multispecific binding molecule described herein is silenced. In some embodiments, the Fc region of a multispecific binding protein described herein is silenced by a combination of amino acid substitutions selected from the group consisting of LALA, DAPA, DANAPA, LALADANAPS, LALAGA, LALASKPA, DAPASK, GADAPA, GADAPASK, LALAPG, and LALAPA (numbered according to the Eu numbering system).

In some embodiments, a multispecific binding molecule described herein comprises any one or all, or any combination of a mutant comprising a L234, e.g., L234A and/or L235, e.g., L234A, mutation (LALA) in the IgG1 Fc amino acid sequence, numbered according to the Eu numbering system; D265, e.g., D265A and/or P329, e.g., P329A (DAPA); N297, e.g., N297A; DANAPA (D265A, N297A, and P329A), numbered according to the Eu numbering system; and/or L234, e.g. L234A, L235, e.g., L235A, D265, e.g., D265A, N297, e.g., N297A, and P331, e.g., P331S (LALADANAPS), wherein the amino acid residues are numbered according to the EU numbering system. In some embodiments, a multispecific binding molecule described herein comprises any one or all, or any combination of a mutant comprising a L234, e.g. L234A, L235, e.g. L235A, and G237, e.g. G237A, mutation (LALAGA) in the IgG1 Fc amino acid sequence, numbered according to the Eu numbering system; L234, e.g. L234A, L235, e.g. L235A, S267, e.g. S267K, and P239, e.g. P329A (LALASKPA), numbered according to the Eu numbering system; D265, e.g. D265A, P239, e.g. P329A, and S267, e.g. S267K (DAPASK), numbered according to the Eu numbering system; G237, e.g. G237A, D265, e.g. D265A, and P329, e.g. P329A (GADAPA), numbered according to the Eu numbering system; G236, e.g. G237A, D265, e.g. D265A, P329, e.g. P329A, and S267, e.g. S267K (GADAPASK), numbered according to the Eu numbering system; L234, e.g. L234A, L

235, e.g. L235A, and P329, e.g. P329G (LALAPG), numbered according to the Eu numbering system; and/or L234, e.g. L234A, L235, e.g. L235A, and P329, e.g. P329A (LALAPA), wherein the amino acid residues are numbered according to the EU numbering system.

In some embodiments, the Fc region of a multispecific binding protein described herein comprises a mutation that results in reduced binding to an Fc receptor or reduced ADCC, ADCP, or CDC activity, e.g., an Fc region comprising: a D265 (e.g., D265A), N297 (e.g., N297A), and P329 (e.g., P329A) mutation (DANAPA), numbered according to the Eu numbering system; an L234 (e.g., L234A), L235 (e.g., L235A), and G237 (G237A) mutation (LALAGA), numbered according to the Eu numbering system; an L234 (L234A), L235 (e.g., L235A), S267 (e.g., S267K), and P329 (e.g., P329A) mutation (LALASKPA), numbered according to the Eu numbering system; a D265 (e.g., D265A), P329 (e.g., P329A), and S267 (e.g., S267K) mutation (DAPASK), numbered according to the Eu numbering system; a G237 (e.g., G237A), a D265 (e.g., D265A), and P329 (P329A) mutation (GADAPA), numbered according to the Eu numbering system; a G237 (e.g., G237A), D265 (e.g., D265A), P329 (e.g., P329A), and S267 (e.g., S267K) mutation (GADAPASK), numbered according to the Eu numbering system; an L234 (e.g., L234A), L235 (e.g., L235A), and P329 (e.g., P329G) mutation (LALAPG), numbered according to the Eu numbering system; or an L234 (e.g., L234A), L235 (e.g., L235A), and P329 (e.g., P329A) mutation (LALAPA), numbered according to the Eu numbering system.

In some embodiments, the Fc region comprises a mutation at one, two, three or all of positions L234 (e.g. L234A), L235 (e.g. L235A), S267 (e.g. S267K), and P239 (e.g. P329A), numbered according to the Eu numbering system. In some embodiments, the Fc region comprises a mutation at L234 (e.g. L234A), L235 (e.g. L235A), S267 (e.g. S267K), and P239 (e.g. P329A) (LALASKPA), numbered according to the EU numbering system. In some embodiments, the Fc region comprises a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system.

**Table 34: Exemplary Fc modifications**

| Modification or mutation | Altered effector function |
|---|---|
| Leu235Glu | ADCC; |
| Leu234Ala/Leu235Ala (LALA) | ADCC; ADCP; CDC |
| Ser228Pro/Leu235Glu | |
| Leu234Ala/Leu235Ala/Pro329Gly | ADCP |
| Pro331Ser/Leu234Glu/Leu235Phe | CDC |

| | |
|---|---|
| Asp265Ala | ADCC, ADCP |
| Gly237Ala | ADCP |
| Glu318Ala | ADCP |
| Glu233Pro | |
| Gly236Arg/Leu328Arg | ADCC |
| His268Gln/Val309Leu/Ala330Ser/Pro331Ser | ADCC; ADCP; CDC |
| Val234Ala/Gly237Ala/Pro238Ser/ His268Ala/Val309Leu/Ala330Ser/Pro331Ser | ADCC; ADCP; CDC |
| Leu234Ala/L235Ala/Gly237Ala/P238Ser/ His268Ala/Ala330Ser/Pro331Ser | ADCC; CDC |
| Ala330Leu | CDC |
| Asp270Ala | CDC |
| Lys322Ala | CDC |
| Pro329Ala | CDC |
| Pro331Ala | CDC |
| Val264Ala | CDC |
| High mannose glycosylation | CDC |
| Phe241Ala | CDC |
| Asn297Ala or Gly or Gln | ADCC; ADCP; CDC |
| S228P/Phe234Ala/Leu235Ala | ADCC; CDC |

Further non-limiting exemplary Fc modifications include LALAGA (L234A, L235A, and G237A), LALASKPA (L234A, L235A, S267K, and P329A), DAPASK (D265A, P329A, and S267K), GADAPA (G237A, D265A, and P329A), GADAPASK (G237A, D265A, P329A, and S267K), LALAPG (L234A, L235A, and P329G), and LALAPA (L234A, L235A, and P329A), wherein the amino acid residues are numbered according to the EU numbering system.

Non-limiting exemplary Fc regions featuring these and other silencing modifications disclosed herein are provided in Table 28 above. Additional non-limiting exemplary Fc regions featuring these and other silencing modifications disclosed herein are provided in Table 35 below.

**Table 35 – Additional exemplary silenced Fc sequences**

| SEQ ID No: | Chain | Amino acid sequence |
|---|---|---|
| SEQ ID NO: 806 | Human IgG1 Fc variant L234A/L235A/P329A (LALAPA) Amino acid sequence | APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL AAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK |
| SEQ ID NO: 807 | Human IgG1 Fc variant L234A/L235A/G237A (LALAGA) Amino acid sequence | APEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK |
| SEQ ID NO: 808 | Human IgG1 Fc variant L234A/L235A/P329G (LALAPG) Amino acid sequence | APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL GAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK |
| SEQ ID NO: 809 | Human IgG1 Fc variant D265A/P329A (DAPA) Amino acid sequence | APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVA VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALA APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK |
| SEQ ID NO: 810 | Human IgG1 Fc variant L234A/L235A/S267K/ P329A (LALASKPA) Amino acid sequence | APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVKHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL AAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK |
| SEQ ID NO: 811 | Human IgG1 Fc variant D265A/P329A/S267K (DAPASK) Amino acid sequence | APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVA VKHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALA APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK |

| | | |
|---|---|---|
| SEQ ID NO: 812 | Human IgG1 Fc variant G237A/D265A/P329A (GADAPA) Amino acid sequence | APELLGAPSVFLFPPKPKDTLMISRTPEVTCVVVA VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALA APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK |
| SEQ ID NO: 813 | Human IgG1 Fc variant G237A/D265A/P329A/ S267K (GADAPASK) Amino acid sequence | APELLGAPSVFLFPPKPKDTLMISRTPEVTCVVVA VKHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALA APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK |
| SEQ ID NO: 814 | Human IgG1 Fc variant D265A/N297A/P329A (DANAPA) Amino acid sequence | APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVA VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALA APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK |

**Population of CAR-Expressing Cells Manufactured by the Processes Disclosed Herein**

In some embodiments, the disclosure features an immune effector cell (for example, T cell or NK cell), for example, made by any of the manufacturing methods described herein, engineered to express a CAR, wherein the engineered immune effector cell exhibits an antitumor property. In some embodiments, the CAR comprises an antigen binding domain, a transmembrane domain, and an intracellular signaling domain. An exemplary antigen is a cancer associated antigen described herein. In some embodiments, the cell (for example, T cell or NK cell) is transformed with the CAR and the CAR is expressed on the cell surface. In some embodiments, the cell (for example, T cell or NK cell) is transduced with a viral vector encoding the CAR. In some embodiments, the viral vector is a retroviral vector. In some embodiments, the viral vector is a lentiviral vector. In some such embodiments, the cell may stably express the CAR. In some embodiments, the cell (for example, T cell or NK cell) is transfected with a nucleic acid, for example, mRNA, cDNA, or DNA, encoding a CAR. In some such embodiments, the cell may transiently express the CAR.

In some embodiments, provided herein is a population of cells (for example, immune effector cells, for example, T cells or NK cells) made by any of the manufacturing processes

described herein (for example, the cytokine process, or the activation process described herein), engineered to express a CAR.

In some embodiments, the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells at the end of the manufacturing process (for example, at the end of the cytokine process or the activation process described herein) (1) is the same as, (2) differs, for example, by no more than 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15%, from, or (3) is increased, for example, by at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25%, as compared to, the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of the manufacturing process (for example, at the beginning of the cytokine process or the activation process described herein). In some embodiments, the population of cells at the end of the manufacturing process (for example, at the end of the cytokine process or the activation process described herein) shows a higher percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells (for example, at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50% higher), compared with cells made by an otherwise similar method which lasts, for example, more than 26 hours (for example, which lasts more than 5, 6, 7, 8, 9, 10, 11, or 12 days) or which involves expanding the population of cells in vitro for, for example, more than 3 days (for example, expanding the population of cells in vitro for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days).

In some embodiments, the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells at the end of the manufacturing process (for example, at the end of the cytokine process or the activation process described herein) is not less than 20, 25, 30, 35, 40, 45, 50, 55, or 60%.

In some embodiments, the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the end of the manufacturing process (for example, at the end of the cytokine process or the activation process described herein) (1) is the same as, (2) differs, for example, by no more than 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15% from, or (3) is decreased, for example, by at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25%, as compared to,

the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the beginning of the manufacturing process (for example, at the beginning of the cytokine process or the activation process described herein). In some embodiments, the population of cells at the end of the manufacturing process (for example, at the end of the cytokine process or the activation process described herein) shows a lower percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells (for example, at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50% lower), compared with cells made by an otherwise similar method which lasts, for example, more than 26 hours (for example, which lasts more than 5, 6, 7, 8, 9, 10, 11, or 12 days) or which involves expanding the population of cells in vitro for, for example, more than 3 days (for example, expanding the population of cells in vitro for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days).

In some embodiments, the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the end of the manufacturing process (for example, at the end of the cytokine process or the activation process described herein) is no more than 40, 45, 50, 55, 60, 65, 70, 75, or 80%.

In some embodiments, the population of cells at the end of the manufacturing process (for example, at the end of the cytokine process or the activation process described herein) after being administered in vivo, persists longer or expands at a higher level (for example, at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, or 90% higher) (for example, as assessed using methods described in Example 1 with respect to FIG. 4C), compared with cells made by an otherwise similar method which lasts, for example, more than 26 hours (for example, which lasts more than 5, 6, 7, 8, 9, 10, 11, or 12 days) or which involves expanding the population of cells in vitro for, for example, more than 3 days (for example, expanding the population of cells in vitro for 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days).

In some embodiments, the population of cells has been enriched for IL6R-expressing cells (for example, cells that are positive for IL6Rα and/or IL6Rβ) prior to the beginning of the manufacturing process (for example, prior to the beginning of the cytokine process or the activation process described herein). In some embodiments, the population of cells comprises, for example, no less than 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, or 80% of IL6R-expressing cells

(for example, cells that are positive for IL6Rα and/or IL6Rβ) at the beginning of the manufacturing process (for example, at the beginning of the cytokine process or the activation process described herein).

**Pharmaceutical Composition**

Furthermore, the present disclosure provides CAR-expressing cell compositions and their use in medicaments or methods for treating, among other diseases, cancer or any malignancy or autoimmune diseases involving cells or tissues which express a tumor antigen as described herein. In some embodiments, provided herein are pharmaceutical compositions comprising a CAR-expressing cell, for example, a plurality of CAR-expressing cells, made by a manufacturing process described herein (for example, the cytokine process, or the activation process described herein), in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients.

**Chimeric Antigen Receptor (CAR)**

The present invention provides immune effector cells (for example, T cells or NK cells) that are engineered to contain one or more CARs that direct the immune effector cells to cancer. This is achieved through an antigen binding domain on the CAR that is specific for a cancer associated antigen. There are two classes of cancer associated antigens (tumor antigens) that can be targeted by the CARs described herein: (1) cancer associated antigens that are expressed on the surface of cancer cells; and (2) cancer associated antigens that themselves are intracellular, however, fragments (peptides) of such antigens are presented on the surface of the cancer cells by MHC (major histocompatibility complex).

Accordingly, an immune effector cell, for example, obtained by a method described herein, can be engineered to contain a CAR that targets one of the following cancer associated antigens (tumor antigens): CD19, CD123, CD22, CD30, CD171, CS-1, CLL-1, CD33, EGFRvIII , GD2, GD3, BCMA, Tn Ag, PSMA, ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3, KIT, IL-13Ra2, Mesothelin, IL-11Ra, PSCA, VEGFR2, LewisY, CD24, PDGFR-beta, PRSS21, SSEA-4, CD20, Folate receptor alpha, ERBB2 (Her2/neu), MUC1, EGFR, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, Fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, TSHR, GPRC5D, CXORF61, CD97, CD179a,

ALK, Plysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-1a, legumain, HPV E6,E7, MAGE-A1, MAGE A1, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, prostein, survivin and telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin B1, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, and mut hsp70-2.

Sequences of non-limiting examples of various components that can be part of a CAR molecule described herein are listed in **Table 1**, where "aa" stands for amino acids, and "na" stands for nucleic acids that encode the corresponding peptide.

**Table 1. Sequences of various components of CAR**

| SEQ ID NO | Description | Sequence |
|---|---|---|
| SEQ ID NO: 11 | EF-1α promoter (na) | CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACAT<br>CGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGC<br>AATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAA<br>CTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCC<br>GAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGC<br>CGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAAC<br>ACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCT<br>CTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCC<br>ACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGG<br>GTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAA<br>GGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCT<br>GGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTT<br>CGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTT<br>AAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCA<br>AGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGG<br>TATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCG<br>TGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCG<br>AGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAG<br>CTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGT<br>GTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGG<br>CACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCC<br>CTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCG<br>GGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAG<br>GGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACG<br>GAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCG<br>AGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGG<br>TTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAG<br>ACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCC<br>TTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTC |

| | | |
|---|---|---|
| | | TCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATT TCAGGTGTCGTGA |
| SEQ ID NO: 1 | Leader (aa) | MALPVTALLLPLALLLHAARP |
| SEQ ID NO: 12 | Leader (na) | ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTC TGCTGCTGCATGCCGCTAGACCC |
| SEQ ID NO: 199 | Leader (na) | ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTC TTCTGCTCCACGCCGCTCGGCCC |
| SEQ ID NO: 2 | CD 8 hinge (aa) | TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACD |
| SEQ ID NO: 13 | CD8 hinge (na) | ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCC CACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGC GTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGG GGCTGGACTTCGCCTGTGAT |
| SEQ ID NO: 3 | Ig4 hinge (aa) | ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN VFSCSVMHEALHNHYTQKSLSLSLGKM |
| SEQ ID NO: 14 | Ig4 hinge (na) | GAGAGCAAGTACGGCCCTCCCTGCCCCCCTTGCCCTGCC CCCGAGTTCCTGGGCGGACCCAGCGTGTTCCTGTTCCCC CCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCC GAGGTGACCTGTGTGGTGGTGGACGTGTCCCAGGAGGAC CCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAG GTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGTT CAATAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCT GCACCAGGACTGGCTGAACGGCAAGGAATACAAGTGTA AGGTGTCCAACAAGGGCCTGCCCAGCAGCATCGAGAAA ACCATCAGCAAGGCCAAGGGCCAGCCTCGGGAGCCCCA GGTGTACACCCTGCCCCCTAGCCAAGAGGAGATGACCAA GAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTA CCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCC AGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGG ACAGCGACGGCAGCTTCTTCCTGTACAGCCGGCTGACCG TGGACAAGAGCCGGTGGCAGGAGGGCAACGTCTTTAGC TGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACC CAGAAGAGCCTGAGCCTGTCCCTGGGCAAGATG |
| SEQ ID NO: 4 | IgD hinge (aa) | RWPESPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRG GEEKKKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAVQD LWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEG LLERHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHPSLPPQ RLMALREPAAQAPVKLSLNLLASSDPPEAASWLLCEVSGFS PPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFWAWSVLR VPAPPSPQPATYTCVVSHEDSRTLLNASRSLEVSYVTDH |
| SEQ ID NO: 15 | IgD hinge (na) | AGGTGGCCCGAAAGTCCCAAGGCCCAGGCATCTAGTGTT CCTACTGCACAGCCCCAGGCAGAAGGCAGCCTAGCCAA AGCTACTACTGCACCTGCCACTACGCGCAATACTGGCCG TGGCGGGGAGGAGAAGAAAAAGGAGAAAGAGAAAGAA GAACAGGAAGAGAGGGAGACCAAGACCCCTGAATGTCC ATCCCATACCCAGCCGCTGGGCGTCTATCTCTTGACTCCC |

| | | |
|---|---|---|
| | | GCAGTACAGGACTTGTGGCTTAGAGATAAGGCCACCTTT<br>ACATGTTTCGTCGTGGGCTCTGACCTGAAGGATGCCCAT<br>TTGACTTGGGAGGTTGCCGGAAAGGTACCCACAGGGGG<br>GGTTGAGGAAGGGTTGCTGGAGCGCCATTCCAATGGCTC<br>TCAGAGCCAGCACTCAAGACTCACCCTTCCGAGATCCCT<br>GTGGAACGCCGGGACCTCTGTCACATGTACTCTAAATCA<br>TCCTAGCCTGCCCCCACAGCGTCTGATGGCCCTTAGAGA<br>GCCAGCCGCCCAGGCACCAGTTAAGCTTAGCCTGAATCT<br>GCTCGCCAGTAGTGATCCCCCAGAGGCCGCCAGCTGGCT<br>CTTATGCGAAGTGTCCGGCTTTAGCCCGCCCAACATCTT<br>GCTCATGTGGCTGGAGGACCAGCGAGAAGTGAACACCA<br>GCGGCTTCGCTCCAGCCCGGCCCCCACCCCAGCCGGGTT<br>CTACCACATTCTGGGCCTGGAGTGTCTTAAGGGTCCCAG<br>CACCACCTAGCCCCCAGCCAGCCACATACACCTGTGTTG<br>TGTCCCATGAAGATAGCAGGACCCTGCTAAATGCTTCTA<br>GGAGTCTGGAGGTTTCCTACGTGACTGACCATT |
| SEQ ID NO: 6 | CD8 Transmembrane (aa) | IYIWAPLAGTCGVLLLSLVITLYC |
| SEQ ID NO: 17 | CD8 Transmembrane (na) | ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTC<br>CTTCTCCTGTCACTGGTTATCACCCTTTACTGC |
| SEQ ID NO: 7 | 4-1BB intracellular domain (aa) | KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCE<br>L |
| SEQ ID NO: 18 | 4-1BB intracellular domain (na) | AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACA<br>ACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAG<br>ATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGA<br>GGATGTGAACTG |
| SEQ ID NO: 8 | CD27 (aa) | QRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKP<br>EPACSP |
| SEQ ID NO: 19 | CD27 (na) | AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACAT<br>GAACATGACTCCCCGCCGCCCCGGGCCCACCCGCAAGCA<br>TTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTA<br>TCGCTCC |
| SEQ ID NO: 9 | CD3-zeta (aa) (Q/K mutant) | RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRR<br>GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKG<br>ERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR |
| SEQ ID NO: 20 | CD3-zeta (na) (Q/K mutant) | AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTA<br>CAAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCT<br>AGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGAC<br>GTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGG<br>AAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAA<br>AGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGA<br>AAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTT<br>TACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGAC<br>GCCCTTCACATGCAGGCCCTGCCCCCTCGC |
| SEQ ID NO: 10 | CD3-zeta (aa) (NCBI Reference Sequence NM_000734.3) | RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR<br>GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKG<br>ERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR |

| | | |
|---|---|---|
| SEQ ID NO: 21 | CD3-zeta (na) (NCBI Reference Sequence NM_000734.3) | AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTA CCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCT AGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGAC GTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGG AAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAA AGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGA AAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTT TACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGAC GCCCTTCACATGCAGGCCCTGCCCCCTCGC |
| SEQ ID NO: 36 | CD28 Intracellular domain (amino acid sequence) | RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYR S |
| SEQ ID NO: 37 | CD28 Intracellular domain (nucleotide sequence) | AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACAT GAACATGACTCCCCGCCGCCCCGGGCCCACCCGCAAGCA TTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTA TCGCTCC |
| SEQ ID NO: 38 | ICOS Intracellular domain (amino acid sequence) | T K K K Y S S S V H D P N G E Y M F M R A V N T A K K S R L T D V T L |
| SEQ ID NO: 39 | ICOS Intracellular domain (nucleotide sequence) | ACAAAAAAGAAGTATTCATCCAGTGTGCACGACCCTAAC GGTGAATACATGTTCATGAGAGCAGTGAACACAGCCAA AAAATCCAGACTCACAGATGTGACCCTA |
| SEQ ID NO: 5 | GS hinge/linker (aa) | GGGGSGGGGS |
| SEQ ID NO: 16 | GS hinge/linker (na) | GGTGGCGGAGGTTCTGGAGGTGGAGGTTCC |
| SEQ ID NO: 40 | GS hinge/linker (na) | GGTGGCGGAGGTTCTGGAGGTGGGGGTTCC |
| SEQ ID NO: 25 | linker | GGGGS |
| SEQ ID NO: 26 | linker | (Gly-Gly-Gly-Gly-Ser)n, where n = 1-6, for example, GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS |
| SEQ ID NO: 27 | linker | GGGGSGGGGSGGGGSGGGGS |
| SEQ ID NO: 28 | linker | GGGGSGGGGSGGGGS |
| SEQ ID NO: 29 | linker | GGGS |
| SEQ ID NO: 41 | linker | (Gly-Gly-Gly-Ser)n where n is a positive integer equal to or greater than 1 |
| SEQ ID NO: 42 | linker | (Gly-Gly-Gly-Ser)n, where n = 1-10, for example, GGGSGGGSGG GSGGGSGGGS GGGSGGGSGG GSGGGSGGGS |
| SEQ ID NO: 43 | linker | GSTSGSGKPGSGEGSTKG |
| SEQ ID NO: 30 | poly(A) | $(A)_{5000}$<br>This sequence may encompass 50-5000 adenines. |
| SEQ ID NO: 31 | polyT | $(T)_{100}$ |

| | | |
|---|---|---|
| SEQ ID NO: 32 | polyT | $(T)_{5000}$<br>This sequence may encompass 50-5000 thymines. |
| SEQ ID NO: 33 | poly(A) | $(A)_{5000}$<br>This sequence may encompass 100-5000 adenines. |
| SEQ ID NO: 34 | poly(A) | $(A)_{400}$<br>This sequence may encompass 100-400 adenines. |
| SEQ ID NO: 35 | poly(A) | $(A)_{2000}$<br>This sequence may encompass 50-2000 adenines. |
| SEQ ID NO: 22 | PD1 CAR (aa) | pgwfldspdrpwnpptfspallvvtegdnatftcsfsntsesfvlnwyrmspsnqtdklaaf pedrsqpgqdcrfrvtqlpngrdfhmsvvrarrndsgtylcgaislapkaqikeslraelrvt erraevptahpspsprpaggfqtlvtttpaprpptpaptiasqplslrpeacrpaaggavhtrg ldfacdiyiwaplagtcgvlllslvitlyckrgrkkllyifkqpfmrpvqttqeedgcscrfpe eeeggcelrvkfsrsadapaykqgqnqlynelnlgrreeydvldkrrgrdpemggkprrk npqeglynelqkdkmaeayseigmkgerrrgkghdglyqglstatkdtydalhmqalpp r |
| SEQ ID NO: 23 | PD-1 CAR (na) (PD1 ECD underlined) | atggccctccctgtcactgccctgcttctcccccctcgcactcctgctccacgccgctagaccac ccggatggtttctggactctccggatcgcccgtggaatcccccaaccttctcaccggcactctt ggttgtgactgagggcgataatgcgaccttcacgtgctcgttctccaacacctccgaatcattc gtgctgaactggtaccgcatgagcccgtcaaaccagaccgacaagctcgccgcgtttccgga agatcggtcgcaaccgggacaggattgtcggttccgcgtgactcaactgccgaatggcagag acttccacatgagcgtggtccgcgctaggcgaaacgactccgggacctacctgtgcggagc catctcgctggcgcctaaggcccaaatcaaagagagcttgagggccgaactgagagtgacc gagcgcagagctgaggtgccaactgcacatccatccccatcgcctcggcctgcggggcagt ttcagaccctggtcacgaccactccggcgccgcgcccaccgactccggccccaactatcgc gagccagcccctgtcgctgaggccggaagcatgccgccctgccgccggaggtgctgtgcat acccggggattggacttcgcatgcgacatctacatttgggctcctctcgccggaacttgtggcg tgctccttctgtccctggtcatcaccctgtactgcaagcggggtcggaaaaagcttctgtacattt tcaagcagcccttcatgaggcccgtgcaaaccacccaggaggaggacggttgctcctgccg gttccccgaagaggaagaaggaggttgcgagctgcgcgtgaagttctcccggagcgccgac gccccgcctataagcagggccagaaccagctgtacaacgaactgaacctgggacggcgg gaagagtacgatgtgctggacaagcggcgcggccgggaccccgaaatgggcgggaagcc tagaagaaagaaccctcaggaaggcctgtataacgagctgcagaaggacaagatggccga ggcctactccgaaattgggatgaagggagagcggcggaggggaaaggggcacgacggcc tgtaccaaggactgtccaccgccaccaaggacacatacgatgccctgcacatgcaggcctt ccccctcgc |
| SEQ ID NO: 24 | PD-1 CAR (aa) with signal (PD1 ECD underlined) | Malpvtalllplalllhaarppgwfldspdrpwnpptfspallvvtegdnatftcsfsntsesf vlnwyrmspsnqtdklaafpedrsqpgqdcrfrvtqlpngrdfhmsvvrarrndsgtylc gaislapkaqikeslraelrvterraevptahpspsprpaggfqtlvtttpaprpptpaptiasq plslrpeacrpaaggavhtrgldfacdiyiwaplagtcgvlllslvitlyckrgrkkllyifkqp fmrpvqttqeedgcscrfpeeeeggcelrvkfsrsadapaykqgqnqlynelnlgrreeyd vldkrrgrdpemggkprrknpqeglynelqkdkmaeayseigmkgerrrgkghdglyq glstatkdtydalhmqalppr |

*Bispecific CARs*

In some embodiments a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence which

has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In some embodiments the first and second epitopes are on the same antigen, for example, the same protein (or subunit of a multimeric protein). In some embodiments the first and second epitopes overlap. In some embodiments the first and second epitopes do not overlap. In some embodiments the first and second epitopes are on different antigens, for example, different proteins (or different subunits of a multimeric protein). In some embodiments a bispecific antibody molecule comprises a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a first epitope and a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a second epitope. In some embodiments a bispecific antibody molecule comprises a half antibody having binding specificity for a first epitope and a half antibody having binding specificity for a second epitope. In some embodiments a bispecific antibody molecule comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In some embodiments a bispecific antibody molecule comprises a scFv, or fragment thereof, have binding specificity for a first epitope and a scFv, or fragment thereof, have binding specificity for a second epitope.

In certain embodiments, the antibody molecule is a multi-specific (for example, a bispecific or a trispecific) antibody molecule. Protocols for generating bispecific or heterodimeric antibody molecules, and various configurations for bispecific antibody molecules, are described in, for example, paragraphs 455-458 of WO2015/142675, filed March 13, 2015, which is incorporated by reference in its entirety.

In some embodiments, the bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence, for example, a scFv, which has binding specificity for CD19, for example, comprises a scFv as described herein, or comprises the light chain CDRs and/or heavy chain CDRs from a scFv described herein, and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope on a different antigen.

*Chimeric TCR*

In some embodiments, the antibodies and antibody fragments of the present invention (for example, CD19 antibodies and fragments) can be grafted to one or more constant domain of a T cell receptor ("TCR") chain, for example, a TCR alpha or TCR beta chain, to create a chimeric TCR. Without being bound by theory, it is believed that chimeric TCRs will signal through the TCR complex upon antigen binding. For example, an scFv as disclosed herein, can be grafted to the constant domain, for example, at least a portion of the extracellular constant domain, the transmembrane domain and the cytoplasmic domain, of a TCR chain, for example, the TCR alpha chain and/or the TCR beta chain. As another example, an antibody fragment, for example a VL domain as described herein, can be grafted to the constant domain of a TCR alpha chain, and an antibody fragment, for example a VH domain as described herein, can be grafted to the constant domain of a TCR beta chain (or alternatively, a VL domain may be grafted to the constant domain of the TCR beta chain and a VH domain may be grafted to a TCR alpha chain). As another example, the CDRs of an antibody or antibody fragment may be grafted into a TCR alpha and/or beta chain to create a chimeric TCR. For example, the LCDRs disclosed herein may be grafted into the variable domain of a TCR alpha chain and the HCDRs disclosed herein may be grafted to the variable domain of a TCR beta chain, or vice versa. Such chimeric TCRs may be produced, for example, by methods known in the art (For example, Willemsen RA et al, Gene Therapy 2000; 7: 1369–1377; Zhang T et al, Cancer Gene Ther 2004; 11: 487–496; Aggen et al, Gene Ther. 2012 Apr;19(4):365-74).

*Non-Antibody Scaffolds*

In embodiments, the antigen binding domain comprises a non-antibody scaffold, for example, a fibronectin, ankyrin, domain antibody, lipocalin, small modular immuno-pharmaceutical, maxybody, Protein A, or affilin. The non-antibody scaffold has the ability to bind to target antigen on a cell. In embodiments, the antigen binding domain is a polypeptide or fragment thereof of a naturally occurring protein expressed on a cell. In some embodiments, the antigen binding domain comprises a non-antibody scaffold. A wide variety of non-antibody scaffolds can be employed so long as the resulting polypeptide includes at least one binding region which specifically binds to the target antigen on a target cell.

Non-antibody scaffolds include: fibronectin (Novartis, MA), ankyrin (Molecular Partners AG, Zurich, Switzerland), domain antibodies (Domantis, Ltd., Cambridge, MA, and

Ablynx nv, Zwijnaarde, Belgium), lipocalin (Pieris Proteolab AG, Freising, Germany), small modular immuno-pharmaceuticals (Trubion Pharmaceuticals Inc., Seattle, WA), maxybodies (Avidia, Inc., Mountain View, CA), Protein A (Affibody AG, Sweden), and affilin (gamma-crystallin or ubiquitin) (Scil Proteins GmbH, Halle, Germany).

In some embodiments the antigen binding domain comprises the extracellular domain, or a counter-ligand binding fragment thereof, of molecule that binds a counterligand on the surface of a target cell.

The immune effector cells can comprise a recombinant DNA construct comprising sequences encoding a CAR, wherein the CAR comprises an antigen binding domain (for example, antibody or antibody fragment, TCR or TCR fragment) that binds specifically to a tumor antigen, for example, a tumor antigen described herein, and an intracellular signaling domain. The intracellular signaling domain can comprise a costimulatory signaling domain and/or a primary signaling domain, for example, a zeta chain. As described elsewhere, the methods described herein can include transducing a cell, for example, from the population of T regulatory-depleted cells, with a nucleic acid encoding a CAR, for example, a CAR described herein.

In some embodiments, a CAR comprises a scFv domain, wherein the scFv may be preceded by an optional leader sequence such as provided in SEQ ID NO: 1, and followed by an optional hinge sequence such as provided in SEQ ID NO:2 or SEQ ID NO:36 or SEQ ID NO:38, a transmembrane region such as provided in SEQ ID NO:6, an intracellular signaling domain that includes SEQ ID NO:7 or SEQ ID NO:16 and a CD3 zeta sequence that includes SEQ ID NO:9 or SEQ ID NO:10, for example, wherein the domains are contiguous with and in the same reading frame to form a single fusion protein.

In some embodiments, an exemplary CAR constructs comprise an optional leader sequence (for example, a leader sequence described herein), an extracellular antigen binding domain (for example, an antigen binding domain described herein), a hinge (for example, a hinge region described herein), a transmembrane domain (for example, a transmembrane domain described herein), and an intracellular stimulatory domain (for example, an intracellular stimulatory domain described herein). In some embodiments, an exemplary CAR construct comprises an optional leader sequence (for example, a leader sequence described herein), an

extracellular antigen binding domain (for example, an antigen binding domain described herein), a hinge (for example, a hinge region described herein), a transmembrane domain (for example, a transmembrane domain described herein), an intracellular costimulatory signaling domain (for example, a costimulatory signaling domain described herein) and/or an intracellular primary signaling domain (for example, a primary signaling domain described herein).

An exemplary leader sequence is provided as SEQ ID NO: 1. An exemplary hinge/spacer sequence is provided as SEQ ID NO: 2 or SEQ ID NO:36 or SEQ ID NO:38. An exemplary transmembrane domain sequence is provided as SEQ ID NO:6. An exemplary sequence of the intracellular signaling domain of the 4-1BB protein is provided as SEQ ID NO: 7. An exemplary sequence of the intracellular signaling domain of CD27 is provided as SEQ ID NO:16. An exemplary CD3zeta domain sequence is provided as SEQ ID NO: 9 or SEQ ID NO:10.

In some embodiments, the immune effector cell comprises a recombinant nucleic acid construct comprising a nucleic acid molecule encoding a CAR, wherein the nucleic acid molecule comprises a nucleic acid sequence encoding an antigen binding domain, wherein the sequence is contiguous with and in the same reading frame as the nucleic acid sequence encoding an intracellular signaling domain. An exemplary intracellular signaling domain that can be used in the CAR includes, but is not limited to, one or more intracellular signaling domains of, for example, CD3-zeta, CD28, CD27, 4-1BB, and the like. In some instances, the CAR can comprise any combination of CD3-zeta, CD28, 4-1BB, and the like.

The nucleic acid sequences coding for the desired molecules can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the nucleic acid molecule, by deriving the nucleic acid molecule from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the nucleic acid of interest can be produced synthetically, rather than cloned.

Nucleic acids encoding a CAR can be introduced into the immune effector cells using, for example, a retroviral or lentiviral vector construct.

Nucleic acids encoding a CAR can also be introduced into the immune effector cell using, for example, an RNA construct that can be directly transfected into a cell. A method for generating mRNA for use in transfection involves *in vitro* transcription (IVT) of a template with specially designed primers, followed by poly(A) addition, to produce a construct containing 3' and 5' untranslated sequence ("UTR") (for example, a 3' and/or 5' UTR described herein), a 5' cap (for example, a 5' cap described herein) and/or Internal Ribosome Entry Site (IRES) (for example, an IRES described herein), the nucleic acid to be expressed, and a poly(A) tail, typically 50-2000 bases in length (for example, described in the Examples, for example, SEQ ID NO:35). RNA so produced can efficiently transfect different kinds of cells. In some embodiments, the template includes sequences for the CAR. In some embodiments, an RNA CAR vector is transduced into a cell, for example, a T cell by electroporation.

**Antigen binding domain**

In some embodiments, a plurality of the immune effector cells, for example, the population of T regulatory-depleted cells, include a nucleic acid encoding a CAR that comprises a target-specific binding element otherwise referred to as an antigen binding domain. The choice of binding element depends upon the type and number of ligands that define the surface of a target cell. For example, the antigen binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state. Thus, examples of cell surface markers that may act as ligands for the antigen binding domain in a CAR described herein include those associated with viral, bacterial and parasitic infections, autoimmune disease and cancer cells.

In some embodiments, the portion of the CAR comprising the antigen binding domain comprises an antigen binding domain that targets a tumor antigen, for example, a tumor antigen described herein.

The antigen binding domain can be any domain that binds to the antigen including but not limited to a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, and a functional fragment thereof, including but not limited to a single-domain antibody such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived nanobody, and to an alternative

scaffold known in the art to function as antigen binding domain, such as a recombinant fibronectin domain, a T cell receptor (TCR), or a fragment there of, for example, single chain TCR, and the like. In some instances, it is beneficial for the antigen binding domain to be derived from the same species in which the CAR will ultimately be used in. For example, for use in humans, it may be beneficial for the antigen binding domain of the CAR to comprise human or humanized residues for the antigen binding domain of an antibody or antibody fragment.

**CD19 CAR**

In some embodiments, the CAR-expressing cell described herein is a CD19 CAR-expressing cell (for example, a cell expressing a CAR that binds to human CD19).

In some embodiments, the antigen binding domain of the CD19 CAR has the same or a similar binding specificity as the FMC63 scFv fragment described in Nicholson et al. Mol. Immun. 34 (16-17): 1157-1165 (1997). In some embodiments, the antigen binding domain of the CD19 CAR includes the scFv fragment described in Nicholson et al. *Mol. Immun.* 34 (16-17): 1157-1165 (1997).

In some embodiments, the CD19 CAR includes an antigen binding domain (for example, a humanized antigen binding domain) according to Table 3 of WO2014/153270, incorporated herein by reference. WO2014/153270 also describes methods of assaying the binding and efficacy of various CAR constructs.

In some embodiments, the parental murine scFv sequence is the CAR19 construct provided in PCT publication WO2012/079000 (incorporated herein by reference). In some embodiments, the anti-CD19 binding domain is a scFv described in WO2012/079000.

In some embodiments, the CAR molecule comprises the fusion polypeptide sequence provided as SEQ ID NO: 12 in PCT publication WO2012/079000, which provides an scFv fragment of murine origin that specifically binds to human CD19.

In some embodiments, the CD19 CAR comprises an amino acid sequence provided as SEQ ID NO: 12 in PCT publication WO2012/079000.

In some embodiments, the amino acid sequence is:

Diqmtqttsslsaslgdrvtiscrasqdiskylnwyqqkpdgtvklliyhtsrlhsgvpsrfsgsgsgtdysltisnleqediat yfcqqgntlpytfgggtkleitggggsggggsggggsevklqesgpglvapsqslsvtctvsgvslpdygvswirqpprkglewlgv

iwgsettyynsalksrltiikdnsksqvflkmnslqtddtaiyycakhyyyggsyamdywgqgtsvtvsstttpaprpptpaptiasq plslrpeacrpaaggavhtrgldfacdiyiwaplagtcgvlllslvitlyckrgrkkllyifkqpfmrpvqttqeedgcscrfpeeeeggc elrvkfsrsadapaykqgqnqlynelnlgrreeydvldkrrgrdpemggkprrknpqeglynelqkdkmaeayseigmkgerrrg kghdglyqglstatkdtydalhmqalppr (SEQ ID NO: 292), or a sequence substantially homologous thereto.

In some embodiments, the CD19 CAR has the USAN designation TISAGENLECLEUCEL-T. In embodiments, CTL019 is made by a gene modification of T cells is mediated by stable insertion via transduction with a self-inactivating, replication deficient Lentiviral (LV) vector containing the CTL019 transgene under the control of the EF-1 alpha promoter. CTL019 can be a mixture of transgene positive and negative T cells that are delivered to the subject on the basis of percent transgene positive T cells.

In other embodiments, the CD19 CAR comprises an antigen binding domain (for example, a humanized antigen binding domain) according to Table 3 of WO2014/153270, incorporated herein by reference.

Humanization of murine CD19 antibody is desired for the clinical setting, where the mouse-specific residues may induce a human-anti-mouse antigen (HAMA) response in patients who receive CART19 treatment, i.e., treatment with T cells transduced with the CAR19 construct. The production, characterization, and efficacy of humanized CD19 CAR sequences is described in International Application WO2014/153270 which is herein incorporated by reference in its entirety, including Examples 1-5 (p. 115-159).

In some embodiments, the CAR molecule is a humanized CD19 CAR comprising the amino acid sequence of:

EIVMTQSPATLSLSPGERATLSCRASQDISKYLNWYQQKPGQAPRLLIYHTSRLHSGIPA RFSGSGSGTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGQGTKLEIKGGGGSGGGGSGG GGSQVQLQESGPGLVKPSETLSLTCTVSGVSLPDYGVSWIRQPPGKGLEWIGVIWGSET TYYQSSLKSRVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYYGGSYAMDYWGQ GTLVTVSS (SEQ ID NO: 293)

In some embodiments, the CAR molecule is a humanized CD19 CAR comprising the amino acid sequence of:

EIVMTQSPATLSLSPGERATLSCRASQDISKYLNWYQQKPGQAPRLLIYHTSRLHSGIPA
RFSGSGSGTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGQGTKLEIKGGGGSGGGGSGG
GGSQVQLQESGPGLVKPSETLSLTCTVSGVSLPDYGVSWIRQPPGKGLEWIGVIWGSET
TYYQSSLKSRVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYYGGSYAMDYWGQ
GTLVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPL
AGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO: 294)

Any known CD19 CAR, for example, the CD19 antigen binding domain of any known CD19 CAR, in the art can be used in accordance with the present disclosure. For example, LG-740; CD19 CAR described in the US Pat. No. 8,399,645; US Pat. No. 7,446,190; Xu et al., Leuk Lymphoma. 2013 54(2):255-260(2012); Cruz et al., Blood 122(17):2965-2973 (2013); Brentjens et al., Blood, 118(18):4817-4828 (2011); Kochenderfer et al., Blood 116(20):4099-102 (2010); Kochenderfer et al., Blood 122 (25):4129-39(2013); and 16th Annu Meet Am Soc Gen Cell Ther (ASGCT) (May 15-18, Salt Lake City) 2013, Abst 10.

Exemplary CD19 CARs include CD19 CARs described herein or an anti-CD19 CAR described in Xu et al. Blood 123.24(2014):3750-9; Kochenderfer et al. Blood 122.25(2013):4129-39, Cruz et al. Blood 122.17(2013):2965-73, NCT00586391, NCT01087294, NCT02456350, NCT00840853, NCT02659943, NCT02650999, NCT02640209, NCT01747486, NCT02546739, NCT02656147, NCT02772198, NCT00709033, NCT02081937, NCT00924326, NCT02735083, NCT02794246, NCT02746952, NCT01593696, NCT02134262, NCT01853631, NCT02443831, NCT02277522, NCT02348216, NCT02614066, NCT02030834, NCT02624258, NCT02625480, NCT02030847, NCT02644655, NCT02349698, NCT02813837, NCT02050347, NCT01683279, NCT02529813, NCT02537977, NCT02799550, NCT02672501, NCT02819583, NCT02028455, NCT01840566, NCT01318317, NCT01864889, NCT02706405, NCT01475058, NCT01430390, NCT02146924, NCT02051257, NCT02431988, NCT01815749, NCT02153580, NCT01865617, NCT02208362, NCT02685670, NCT02535364, NCT02631044, NCT02728882, NCT02735291, NCT01860937, NCT02822326, NCT02737085, NCT02465983,

NCT02132624, NCT02782351, NCT01493453, NCT02652910, NCT02247609, NCT01029366, NCT01626495, NCT02721407, NCT01044069, NCT00422383, NCT01680991, NCT02794961, or NCT02456207, each of which is incorporated herein by reference in its entirety.

In some embodiments, CD19 CARs comprise a sequence, for example, a CDR, VH, VL, scFv, or full-CAR sequence, disclosed in Table 2, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto.

**Table 2. Amino acid sequences of exemplary anti-CD19 molecules**

| SEQ ID NO | Region | Sequence |
|---|---|---|
| **CTL019** | | |
| 295 | HCDR1 (Kabat) | DYGVS |
| 296 | HCDR2 (Kabat) | VIWGSETTYYNSALKS |
| 297 | HCDR3 (Kabat) | HYYYGGSYAMDY |
| 298 | LCDR1 (Kabat) | RASQDISKYLN |
| 299 | LCDR2 (Kabat) | HTSRLHS |
| 300 | LCDR3 (Kabat) | QQGNTLPYT |
| 301 | CTL019 Full amino acid sequence | MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRA SQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTD YSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGG SGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNS LQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSTTTPAPR PPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLA GTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCS CRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR |
| 302 | CTL019 Full nucleotide sequence | ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCT GCTCCACGCCGCCAGGCCGGACATCCAGATGACACAGACTAC ATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCACCATCAGT TGCAGGGCAAGTCAGGACATTAGTAAATATTTAAATTGGTATC AGCAGAAACCAGATGGAACTGTTAAACTCCTGATCTACCATAC ATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGT GGGTCTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAGC AAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAATACGCT TCCGTACACGTTCGGAGGGGGGACCAAGCTGGAGATCACAGG TGGCGGTGGCTCGGGCGGTGGTGGGTCGGGTGGCGGCGGATC TGAGGTGAAACTGCAGGAGTCAGGACCTGGCCTGGTGGCGCC |

| | | |
|---|---|---|
| | | CTCACAGAGCCTGTCCGTCACATGCACTGTCTCAGGGGTCTCA<br>TTACCCGACTATGGTGTAAGCTGGATTCGCCAGCCTCCACGAA<br>AGGGTCTGGAGTGGCTGGGAGTAATATGGGGTAGTGAAACCA<br>CATACTATAATTCAGCTCTCAAATCCAGACTGACCATCATCAA<br>GGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTG<br>CAAACTGATGACACAGCCATTTACTACTGTGCCAAACATTATT<br>ACTACGGTGGTAGCTATGCTATGGACTACTGGGGCCAAGGAAC<br>CTCAGTCACCGTCTCCTCAACCACGACGCCAGCGCCGCGACCA<br>CCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGC<br>GCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACA<br>CGAGGGGGCTGGACTTCGCCTGTGATATCTACATCTGGGCGCC<br>CTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCA<br>CCCTTTACTGCAAACGGGGCAGAAAGAAACTCCTGTATATATT<br>CAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGA<br>AGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGG<br>ATGTGAACTGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCC<br>CGCGTACAAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAA<br>TCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACG<br>TGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGA<br>ACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGA<br>TGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCC<br>GGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTA<br>CAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCT<br>GCCCCCTCGC |
| 303 | CTL019 scFv domain | DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVK<br>LLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN<br>TLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPS<br>QSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYY<br>NSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGS<br>YAMDYWGQGTSVTVSS |
| **Humanized CAR2** | | |
| 295 | HCDR1 (Kabat) | DYGVS |
| 304 | HCDR2 (Kabat) | VIWGSETTYYQSSLKS |
| 297 | HCDR3 (Kabat) | HYYYGGSYAMDY |
| 298 | LCDR1 (Kabat) | RASQDISKYLN |
| 299 | LCDR2 (Kabat) | HTSRLHS |
| 300 | LCDR3 (Kabat) | QQGNTLPYT |
| 293 | CAR2 scFv domain - aa (Linker is underlined) | EIVMTQSPATLSLSPGERATLSCRASQDISKYLNWYQQKPGQAPR<br>LLIYHTSRLHSGIPARFSGSGSGTDYTLTISSLQPEDFAVYFCQQGN<br>TLPYTFGQGTKLEIK<u>GGGGSGGGGSGGGGS</u>QVQLQESGPGLVKP<br>SETLSLTCTVSGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETTYY<br>QSSLKSRVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYYGG<br>SYAMDYWGQGTLVTVSS |
| 305 | CAR2 scFv | atggccctccctgtcaccgccctgctgcttccgctggctcttctgctccacgccgctcggcccgaaattgt |

| | | |
|---|---|---|
| | domain - nt | gatgacccagtcacccgccactcttagcctttcacccggtgagcgcgcaaccctgtcttgcagagcctcc caagacatctcaaaataccttaattggtatcaacagaagcccggacaggctcctcgccttctgatctacca caccagccggctccattctggaatccctgccaggttcagcggtagcggatctgggaccgactacaccct cactatcagctcactgcagccagaggacttcgctgtctatttctgtcagcaagggaacaccctgccctac acctttggacagggcaccaagctcgagattaaaggtggaggtggcagcggaggaggtgggtccggc ggtggaggaagccaggtccaactccaagaaagcggaccgggtcttgtgaagccatcagaaactctttc actgacttgtactgtgagcggagtgtctctccccgattacggggtgtcttggatcagacagccaccgggg aagggtctggaatggattggagtgatttggggctctgagactacttactaccaatcatccctcaagtcacg cgtcaccatctcaaaggacaactctaagaatcaggtgtcactgaaactgtcatctgtgaccgcagccgac accgccgtgtactattgcgctaagcattactattatggcgggagctacgcaatggattactggggacagg gtactctggtcaccgtgtccagccaccaccatcatcaccatcaccat |
| 306 | CAR 2 - Full - aa | MALPVTALLLPLALLLHAARPEIVMTQSPATLSLSPGERATLSCRA SQDISKYLNWYQQKPGQAPRLLIYHTSRLHSGIPARFSGSGSGTD YTLTISSLQPEDFAVYFCQQGNTLPYTFGQGTKLEIKGGGGSGGG GSGGGGSQVQLQESGPGLVKPSETLSLTCTVSGVSLPDYGVSWIR QPPGKGLEWIGVIWGSETTYYQSSLKSRVTISKDNSKNQVSLKLS SVTAADTAVYYCAKHYYYGGSYAMDYWGQGTLVTVSSTTTPAP RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPL AGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRRE EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR |
| 307 | CAR 2 - Full - nt | atggccctccctgtcaccgccctgctgcttccgctggctcttctgctccacgccgctcggcccgaaattgt gatgacccagtcacccgccactcttagcctttcacccggtgagcgcgcaaccctgtcttgcagagcctcc caagacatctcaaaataccttaattggtatcaacagaagcccggacaggctcctcgccttctgatctacca caccagccggctccattctggaatccctgccaggttcagcggtagcggatctgggaccgactacaccct cactatcagctcactgcagccagaggacttcgctgtctatttctgtcagcaagggaacaccctgccctac acctttggacagggcaccaagctcgagattaaaggtggaggtggcagcggaggaggtgggtccggc ggtggaggaagccaggtccaactccaagaaagcggaccgggtcttgtgaagccatcagaaactctttc actgacttgtactgtgagcggagtgtctctccccgattacggggtgtcttggatcagacagccaccgggg aagggtctggaatggattggagtgatttggggctctgagactacttactaccaatcatccctcaagtcacg cgtcaccatctcaaaggacaactctaagaatcaggtgtcactgaaactgtcatctgtgaccgcagccgac accgccgtgtactattgcgctaagcattactattatggcgggagctacgcaatggattactggggacagg gtactctggtcaccgtgtccagcaccactaccccagcaccgaggccacccaccccggctcctaccatc gcctcccagcctctgtccctgcgtccggaggcatgtagacccgcagctggtggggccgtgcataccgg gggtcttgacttcgcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttc actcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacccttcatgag gcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagaggaggaggaaggcggc tgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcctacaagcaggggcagaaccagc tctacaacgaactcaatcttggtcggagagaggagtacgacgtgctggacaagcggagaggacggga cccagaaatgggcgggaagccgcgcagaaagaatccccaagagggcctgtacaacgagctccaaaaa ggataagatggcagaagcctatagcgagattggtatgaaaggggaacgcagaagaggcaaaggcca cgacggactgtaccagggactcagcaccgccaccaaggacacctatgacgctcttcacatgcaggccc tgccgcctcgg |
| 349 | CAR 2A– Full amino acid sequence; signal peptide underlined | MALPVTALLLPLALLLHAARPEIVMTQSPATLSLSPGERATLSCRASQDIS KYLNWYQQKPGQAPRLLIYHTSRLHSGIPARFSGSGSGTDYTLTISSLQPE DFAVYFCQQGNTLPYTFGQGTKLEIKGGGGSGGGGSGGGGSQVQLQES GPGLVKPSETLSLTCTVSGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETT YYQSSLKSRVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHYYYGGSY AMDYWGQGTLVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV HTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNE |

| | | |
|---|---|---|
| | | LNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEA YSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR |
| 225 | CAR 2A – amino acid sequence; no signal peptide | EIVMTQSPATLSLSPGERATLSCRASQDISKYLNWYQQKPGQAPRLLIYH TSRLHSGIPARFSGSGSGTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGQG TKLEIKGGGGSGGGGSGGGGSQVQLQESGPGLVKPSETLSLTCTVSGVS LPDYGVSWIRQPPGKGLEWIGVIWGSETTYYQSSLKSRVTISKDNSKNQ VSLKLSSVTAADTAVYYCAKHYYYGGSYAMDYWGQGTLVTVSSTTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG TCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR |
| 354 | CAR 2A full nucleic acid sequence; signal peptide and stop codon underlined | atggccctccctgtcaccgccctgctgcttccgctggctcttctgctccacgccgctcggcccgaaattgtgatgacc cagtcacccgccactcttagcctttcacccggtgagcgcgcaaccctgtcttgcagagcctcccaagacatctcaaa ataccttaattggtatcaacagaagcccggacaggctcctcgccttctgatctaccacaccagccggctccattctgg aatccctgccaggttcagcggtagcggatctgggaccgactacaccctcactatcagctcactgcagccagagga cttcgctgtctatttctgtcagcaagggaacaccctgccctacacctttggacagggcaccaagctcgagattaaag gtggaggtggcagcggaggaggtgggtccggcggtggaggaagccaggtccaactccaagaaagcggaccg ggtcttgtgaagccatcagaaactctttcactgacttgtactgtgagcggagtgtctctccccgattacggggtgtctt ggatcagacagccaccggggaagggtctggaatggattggagtgatttggggctctgagactacttactaccaatc atccctcaagtcacgcgtcaccatctcaaaggacaactctaagaatcaggtgtcactgaaactgtcatctgtgaccg cagccgacaccgccgtgtactattgcgctaagcattactattatggcgggagctacgcaatggattactggggaca gggtactctggtcaccgtgtccagcaccactaccccagcaccgaggccacccaccccggctcctaccatcgcctc ccagcctctgtccctgcgtccggaggcatgtagacccgcagctggtggggccgtgcataccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcactcgtgatcactctttactgt aagcgcggtcggaagaagctgctgtacatctttaagcaacccttcatgaggcctgtgcagactactcaagaggagg acggctgttcatgccggttcccagaggaggaggaaggcggctgcgaactgcgcgtgaaattcagccgcagcgca gatgctccagcctaccagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaatccccaagagg gcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgagattggtatgaaaggggaacgcaga agaggcaaaggccacgacggactgtaccagggactcagcaccgccaccaaggacacctatgacgctcttcacat gcaggccctgccgcctcggtaa |
| 355 | CAR 2A nucleic acid sequence; signal peptide underlined; no stop codon | atggccctccctgtcaccgccctgctgcttccgctggctcttctgctccacgccgctcggcccgaaattgtgatgacc cagtcacccgccactcttagcctttcacccggtgagcgcgcaaccctgtcttgcagagcctcccaagacatctcaaa ataccttaattggtatcaacagaagcccggacaggctcctcgccttctgatctaccacaccagccggctccattctgg aatccctgccaggttcagcggtagcggatctgggaccgactacaccctcactatcagctcactgcagccagagga cttcgctgtctatttctgtcagcaagggaacaccctgccctacacctttggacagggcaccaagctcgagattaaag gtggaggtggcagcggaggaggtgggtccggcggtggaggaagccaggtccaactccaagaaagcggaccg ggtcttgtgaagccatcagaaactctttcactgacttgtactgtgagcggagtgtctctccccgattacggggtgtctt ggatcagacagccaccggggaagggtctggaatggattggagtgatttggggctctgagactacttactaccaatc atccctcaagtcacgcgtcaccatctcaaaggacaactctaagaatcaggtgtcactgaaactgtcatctgtgaccg cagccgacaccgccgtgtactattgcgctaagcattactattatggcgggagctacgcaatggattactggggaca gggtactctggtcaccgtgtccagcaccactaccccagcaccgaggccacccaccccggctcctaccatcgcctc ccagcctctgtccctgcgtccggaggcatgtagacccgcagctggtggggccgtgcataccggggtcttgacttc gcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcactcgtgatcactctttactgt aagcgcggtcggaagaagctgctgtacatctttaagcaacccttcatgaggcctgtgcagactactcaagaggagg acggctgttcatgccggttcccagaggaggaggaaggcggctgcgaactgcgcgtgaaattcagccgcagcgca gatgctccagcctaccagcaggggcagaaccagctctacaacgaactcaatcttggtcggagagaggagtacga cgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaagaatccccaagagg gcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgagattggtatgaaaggggaacgcaga agaggcaaaggccacgacggactgtaccagggactcagcaccgccaccaaggacacctatgacgctcttcacat gcaggccctgccgcctcgg |
| 356 | CAR 2A nucleic acid | gaaattgtgatgacccagtcacccgccactcttagcctttcacccggtgagcgcgcaaccctgtcttgcagagcctc ccaagacatctcaaaatacctttaattggtatcaacagaagcccggacaggctcctcgccttctgatctaccacacca gccggctccattctggaatccctgccaggttcagcggtagcggatctgggaccgactacaccctcactatcagctc actgcagccagaggacttcgctgtctatttctgtcagcaagggaacaccctgccctacacctttggacagggcacca |

| | | |
|---|---|---|
| | sequence; no signal peptide; stop codon underlined | agctcgagattaaaggtggaggtggcagcggaggaggtgggtccggcggtggaggaagccaggtccaactcca agaaagcggaccgggtcttgtgaagccatcagaaactctttcactgacttgtactgtgagcggagtgtctctccccg attacggggtgtcttggatcagacagccaccggggaagggtctggaatggattggagtgatttggggctctgagac tacttactaccaatcatccctcaagtcacgcgtcaccatctcaaaggacaactctaagaatcaggtgtcactgaaact gtcatctgtgaccgcagccgacaccgccgtgtactattgcgctaagcattactattatggcgggagctacgcaatgg attactggggacagggtactctggtcaccgtgtccagcaccactaccccagcaccgaggccacccaccccggctc ctaccatcgcctcccagcctctgtccctgcgtccggaggcatgtagacccgcagctggtggggccgtgcataccc ggggtcttgacttcgcctgcgatatctacatttgggcccctctggctggtacttgcggggtcctgctgctttcactcgtg atcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacccttcatgaggcctgtgcagact actcaagaggaggacggctgttcatgccggttcccagaggaggaggaaggcggctgcgaactgcgcgtgaaatt cagccgcagcgcagatgctccagcctaccagcaggggcagaaccagctctacaacgaactcaatcttggtcgga gagaggagtacgacgtgctggacaagcggagaggacgggacccagaaatgggcgggaagccgcgcagaaa gaatccccaagagggcctgtacaacgagctccaaaaggataagatggcagaagcctatagcgagattggtatgaa aggggaacgcagaagaggcaaaggccacgacggactgtaccagggactcagcaccgccaccaaggacaccta tgacgctcttcacatgcaggccctgccgcctcggtaa |
| SEQ ID NO: 417 | CAR 2A nucleic acid sequence; no signal peptide; no stop codon | gaaattgtgatgacccagtcacccgccactcttagcctttcacccggtgagcgcgcaaccctgtcttgca gagcctcccaagacatctcaaaataccttaattggtatcaacagaagcccggacaggctcctcgccttct gatctaccacaccagccggctccattctggaatccctgccaggttcagcggtagcggatctgggaccga ctacaccctcactatcagctcactgcagccagaggacttcgctgtctatttctgtcagcaagggaacaccc tgccctacacctttggacagggcaccaagctcgagattaaaggtggaggtggcagcggaggaggtgg gtccggcggtggaggaagccaggtccaactccaagaaagcggaccgggtcttgtgaagccatcagaa actctttcactgacttgtactgtgagcggagtgtctctccccgattacggggtgtcttggatcagacagcca ccggggaagggtctggaatggattggagtgatttggggctctgagactacttactaccaatcatccctca agtcacgcgtcaccatctcaaaggacaactctaagaatcaggtgtcactgaaactgtcatctgtgaccgc agccgacaccgccgtgtactattgcgctaagcattactattatggcgggagctacgcaatggattactgg ggacagggtactctggtcaccgtgtccagcaccactaccccagcaccgaggccacccaccccggctc ctaccatcgcctcccagcctctgtccctgcgtccggaggcatgtagacccgcagctggtggggccgtg cataccggggtcttgacttcgcctgcgatatctacatttgggcccctctggctggtacttgcggggtcct gctgctttcactcgtgatcactctttactgtaagcgcggtcggaagaagctgctgtacatctttaagcaacc cttcatgaggcctgtgcagactactcaagaggaggacggctgttcatgccggttcccagaggaggagg aaggcggctgcgaactgcgcgtgaaattcagccgcagcgcagatgctccagcctaccagcaggggc agaaccagctctacaacgaactcaatcttggtcggagagaggagtacgacgtgctggacaagcggag aggacgggacccagaaatgggcgggaagccgcgcagaaagaatccccaagagggcctgtacaacg agctccaaaaggataagatggcagaagcctatagcgagattggtatgaaaggggaacgcagaagagg caaaggccacgacggactgtaccagggactcagcaccgccaccaaggacacctatgacgctcttcac atgcaggccctgccgcctcgg |
| SEQ ID NO: 250 | Anti-CD19 VH | QVQLQESGPGLVKPSETLSLTCTVSGVSLPDYGVSWIRQPPGKGLEWIGV IWGSETTYYQSSLKSRVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHY YYGGSYAMDYWGQGTLVTVSS |
| SEQ ID NO: 251 | Anti-CD19 VL | EIVMTQSPATLSLSPGERATLSCRASQDISKYLNWYQQKPGQAPRLLIYH TSRLHSGIPARFSGSGSGTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGQG TKLEIK |
| SEQ ID NO: 331 | VH | QVQLQESGPGLVKPSETLSLTCTVSGVSLPDYGVSWIRQPPGKCLEWIGV IWGSETTYYQSSLKSRVTISKDNSKNQVSLKLSSVTAADTAVYYCAKHY YYGGSYAMDYWGQGTLVTVSS |
| SEQ ID NO: 332 | VL | EIVMTQSPATLSLSPGERATLSCRASQDISKYLNWYQQKPGQAPRLLIYH TSRLHSGIPARFSGSGSGTDYTLTISSLQPEDFAVYFCQQGNTLPYTFGCG TKLEIK |

**BCMA CAR**

In some embodiments, the CAR-expressing cell described herein is a BCMA CAR-expressing cell (for example, a cell expressing a CAR that binds to human BCMA). Exemplary BCMA CARs can include sequences disclosed in Table 1 or 16 of WO2016/014565, incorporated herein by reference. The BCMA CAR construct can include an optional leader sequence; an optional hinge domain, for example, a CD8 hinge domain; a transmembrane domain, for example, a CD8 transmembrane domain; an intracellular domain, for example, a 4-1BB intracellular domain; and a functional signaling domain, for example, a CD3 zeta domain. In certain embodiments, the domains are contiguous and in the same reading frame to form a single fusion protein. In other embodiments, the domains are in separate polypeptides, for example, as in an RCAR molecule as described herein.

In some embodiments, the BCMA CAR molecule includes one or more CDRs, VH, VL, scFv, or full-length sequences of BCMA-1, BCMA-2, BCMA-3, BCMA-4, BCMA-5, BCMA-6, BCMA-7, BCMA-8, BCMA-9, BCMA-10, BCMA-11, BCMA-12, BCMA-13, BCMA-14, BCMA-15, 149362, 149363, 149364, 149365, 149366, 149367, 149368, 149369, BCMA_EBB-C1978-A4, BCMA_EBB-C1978-G1, BCMA_EBB-C1979-C1, BCMA_EBB-C1978-C7, BCMA_EBB-C1978-D10, BCMA_EBB-C1979-C12, BCMA_EBB-C1980-G4, BCMA_EBB-C1980-D2, BCMA_EBB-C1978-A10, BCMA_EBB-C1978-D4, BCMA_EBB-C1980-A2, BCMA_EBB-C1981-C3, BCMA_EBB-C1978-G4, A7D12.2, C11D5.3, C12A3.2, or C13F12.1 disclosed in WO2016/014565, or a sequence substantially (for example, 95-99%) identical thereto.

Additional exemplary BCMA-targeting sequences that can be used in the anti-BCMA CAR constructs are disclosed in WO 2017/021450, WO 2017/011804, WO 2017/025038, WO 2016/090327, WO 2016/130598, WO 2016/210293, WO 2016/090320, WO 2016/014789, WO 2016/094304, WO 2016/154055, WO 2015/166073, WO 2015/188119, WO 2015/158671, US 9,243,058, US 8,920,776, US 9,273,141, US 7,083,785, US 9,034,324, US 2007/0049735, US 2015/0284467, US 2015/0051266, US 2015/0344844, US 2016/0131655, US 2016/0297884, US 2016/0297885, US 2017/0051308, US 2017/0051252, US 2017/0051252, WO 2016/020332, WO 2016/087531, WO 2016/079177, WO 2015/172800, WO 2017/008169, US 9,340,621, US 2013/0273055, US 2016/0176973, US 2015/0368351, US 2017/0051068, US 2016/0368988, and US 2015/0232557, herein incorporated by reference in their entirety. In

some embodiments, additional exemplary BCMA CAR constructs are generated using the VH and VL sequences from PCT Publication WO2012/0163805 (the contents of which are hereby incorporated by reference in its entirety).

In some embodiments, BCMA CARs comprise a sequence, for example, a CDR, VH, VL, scFv, or full-CAR sequence, disclosed in Tables 3-15, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the antigen binding domain comprises a human antibody or a human antibody fragment. In some embodiments, the human anti-BCMA binding domain comprises one or more (for example, all three) LC CDR1, LC CDR2, and LC CDR3 of a human anti-BCMA binding domain described herein (for example, in Tables 3-10 and 12-15), and/or one or more (for example, all three) HC CDR1, HC CDR2, and HC CDR3 of a human anti-BCMA binding domain described herein (for example, in Tables 3-10 and 12-15). In some embodiments, the human anti-BCMA binding domain comprises a human VL described herein (for example, in Tables 3, 7, and 12) and/or a human VH described herein (for example, in Tables 3, 7, and 12). In some embodiments, the anti-BCMA binding domain is a scFv comprising a VL and a VH of an amino acid sequence of Tables 3, 7, and 12. In some embodiments, the anti-BCMA binding domain (for example, an scFv) comprises: a VL comprising an amino acid sequence having at least one, two or three modifications (for example, substitutions, for example, conservative substitutions) but not more than 30, 20 or 10 modifications (for example, substitutions, for example, conservative substitutions) of an amino acid sequence provided in Tables 3, 7, and 12, or a sequence with 95-99% identity with an amino acid sequence of Tables 3, 7, and 12, and/or a VH comprising an amino acid sequence having at least one, two or three modifications (for example, substitutions, for example, conservative substitutions) but not more than 30, 20 or 10 modifications (for example, substitutions, for example, conservative substitutions) of an amino acid sequence provided in Tables 3, 7, and 12, or a sequence with 95-99% identity to an amino acid sequence of Tables 3, 7, and 12.

**Table 3: Amino acid and nucleic acid sequences of exemplary PALLAS-derived anti-BCMA mlecules**

| SEQ ID NO | Name/ Description | Sequence |
|---|---|---|
| R1B6 | | |

| | | |
|---|---|---|
| SEQ ID NO: 44 | HCDR1 (Kabat) | SYAMS |
| SEQ ID NO: 45 | HCDR2 (Kabat) | AISGSGGSTYYADSVKG |
| SEQ ID NO: 46 | HCDR3 (Kabat) | REWVPYDVSWYFDY |
| SEQ ID NO: 47 | HCDR1 (Chothia) | GFTFSSY |
| SEQ ID NO: 48 | HCDR2 (Chothia) | SGSGGS |
| SEQ ID NO: 46 | HCDR3 (Chothia) | REWVPYDVSWYFDY |
| SEQ ID NO: 49 | HCDR1 (IMGT) | GFTFSSYA |
| SEQ ID NO: 50 | HCDR2 (IMGT) | ISGSGGST |
| SEQ ID NO: 51 | HCDR3 (IMGT) | ARREWVPYDVSWYFDY |
| SEQ ID NO: 52 | VH | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGL EWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCARREWVPYDVSWYFDYWGQGTLVTVSS |
| SEQ ID NO: 53 | DNA VH | GAAGTGCAGTTGCTGGAGTCAGGCGGAGGACTGGTGCAGCCCG GAGGATCGCTTCGCTTGAGCTGCGCAGCCTCAGGCTTTACCTTC TCCTCCTACGCCATGTCCTGGGTCAGACAGGCTCCCGGGAAGG GACTGGAATGGGTGTCCGCCATTAGCGGTTCCGGCGGAAGCAC TTACTATGCCGACTCTGTGAAGGGCCGCTTCACTATCTCCCGGG ACAACTCCAAGAACACCCTGTATCTCCAAATGAATTCCCTGAGG GCCGAAGATACCGCGGTGTACTACTGCGCTAGACGGGAGTGGG TGCCCTACGATGTCAGCTGGTACTTCGACTACTGGGGACAGGGC ACTCTCGTGACTGTGTCCTCC |
| SEQ ID NO: 54 | LCDR1 (Kabat) | RASQSISSYLN |
| SEQ ID NO: 55 | LCDR2 (Kabat) | AASSLQS |
| SEQ ID NO: 56 | LCDR3 (Kabat) | QQSYSTPLT |
| SEQ ID NO: 57 | LCDR1 (Chothia) | SQSISSY |
| SEQ ID NO: 58 | LCDR2 (Chothia) | AAS |
| SEQ ID NO: 59 | LCDR3 (Chothia) | SYSTPL |
| SEQ ID NO: 60 | LCDR1 (IMGT) | QSISSY |
| SEQ ID NO: 58 | LCDR2 (IMGT) | AAS |
| SEQ ID NO: 56 | LCDR3 (IMGT) | QQSYSTPLT |

| | | |
|---|---|---|
| SEQ ID NO: 61 | VL | DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYS TPLTFGQGTKVEIK |
| SEQ ID NO: 62 | DNA VL | GACATTCAAATGACTCAGTCCCCGTCCTCCCTCTCCGCCTCCGT GGGAGATCGCGTCACGATCACGTGCAGGGCCAGCCAGAGCATC TCCAGCTACCTGAACTGGTACCAGCAGAAGCCAGGGAAGGCAC CGAAGCTCCTGATCTACGCCGCTAGCTCGCTGCAGTCCGGCGTC CCTTCACGGTTCTCGGGATCGGGCTCAGGCACCGACTTCACCCT GACCATTAGCAGCCTGCAGCCGGAGGACTTCGCGACATACTAC TGTCAGCAGTCATACTCCACCCCTCTGACCTTCGGCCAAGGGAC CAAAGTGGAGATCAAG |
| SEQ ID NO: 63 | Linker | GGGGSGGGGSGGGGSGGGGS |
| SEQ ID NO: 64 | scFv (VH-linker-VL) | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGL EWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCARREWVPYDVSWYFDYWGQGTLVTVSSGGGGSGGGGS GGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYSTPLTFGQGTKVEIK |
| SEQ ID NO: 65 | DNA scFv | GAAGTGCAGTTGCTGGAGTCAGGCGGAGGACTGGTGCAGCCCG GAGGATCGCTTCGCTTGAGCTGCGCAGCCTCAGGCTTTACCTTC TCCTCCTACGCCATGTCCTGGGTCAGACAGGCTCCCGGGAAGG GACTGGAATGGGTGTCCGCCATTAGCGGTTCCGGCGGAAGCAC TTACTATGCCGACTCTGTGAAGGGCCGCTTCACTATCTCCCGGG ACAACTCCAAGAACACCCTGTATCTCCAAATGAATTCCCTGAGG GCCGAAGATACCGCGGTGTACTACTGCGCTAGACGGGAGTGGG TGCCCTACGATGTCAGCTGGTACTTCGACTACTGGGGACAGGGC ACTCTCGTGACTGTGTCCTCCGGTGGTGGTGGATCGGGGGGTGG TGGTTCGGGCGGAGGAGGATCTGGAGGAGGAGGGTCGGACATT CAAATGACTCAGTCCCCGTCCTCCCTCTCCGCCTCCGTGGGAGA TCGCGTCACGATCACGTGCAGGGCCAGCCAGAGCATCTCCAGC TACCTGAACTGGTACCAGCAGAAGCCAGGGAAGGCACCGAAGC TCCTGATCTACGCCGCTAGCTCGCTGCAGTCCGGCGTCCCTTCA CGGTTCTCGGGATCGGGCTCAGGCACCGACTTCACCCTGACCAT TAGCAGCCTGCAGCCGGAGGACTTCGCGACATACTACTGTCAG CAGTCATACTCCACCCCTCTGACCTTCGGCCAAGGGACCAAAGT GGAGATCAAG |
| SEQ ID NO: 66 | Full CAR amino acid sequence | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGL EWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCARREWVPYDVSWYFDYWGQGTLVTVSSGGGGSGGGGS GGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYSTPLTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRP EACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKF SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR |
| SEQ ID NO: 67 | Full CAR DNA sequence | GAAGTGCAGTTGCTGGAGTCAGGCGGAGGACTGGTGCAGCCCG GAGGATCGCTTCGCTTGAGCTGCGCAGCCTCAGGCTTTACCTTC TCCTCCTACGCCATGTCCTGGGTCAGACAGGCTCCCGGGAAGG |

| | | |
|---|---|---|
| | | GACTGGAATGGGTGTCCGCCATTAGCGGTTCCGGCGGAAGCAC<br>TTACTATGCCGACTCTGTGAAGGGCCGCTTCACTATCTCCCGGG<br>ACAACTCCAAGAACACCCTGTATCTCCAAATGAATTCCCTGAGG<br>GCCGAAGATACCGCGGTGTACTACTGCGCTAGACGGGAGTGGG<br>TGCCCTACGATGTCAGCTGGTACTTCGACTACTGGGGACAGGGC<br>ACTCTCGTGACTGTGTCCTCCGGTGGTGGTGGATCGGGGGGTGG<br>TGGTTCGGGCGGAGGAGGATCTGGAGGAGGAGGGTCGGACATT<br>CAAATGACTCAGTCCCCGTCCTCCCTCTCCGCCTCCGTGGGAGA<br>TCGCGTCACGATCACGTGCAGGGCCAGCCAGAGCATCTCCAGC<br>TACCTGAACTGGTACCAGCAGAAGCCAGGGAAGGCACCGAAGC<br>TCCTGATCTACGCCGCTAGCTCGCTGCAGTCCGGCGTCCCTTCA<br>CGGTTCTCGGGATCGGGCTCAGGCACCGACTTCACCCTGACCAT<br>TAGCAGCCTGCAGCCGGAGGACTTCGCGACATACTACTGTCAG<br>CAGTCATACTCCACCCCTCTGACCTTCGGCCAAGGGACCAAAGT<br>GGAGATCAAGACCACTACCCCAGCACCGAGGCCACCCACCCCG<br>GCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGC<br>ATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTT<br>GACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTAC<br>TTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAA<br>GCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCA<br>TGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATG<br>CCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTG<br>AAATTCAGCCGCAGCGCAGATGCTCCAGCCTACCAGCAGGGGC<br>AGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGA<br>GTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAAT<br>GGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTA<br>CAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAG<br>ATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGAC<br>GGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATG<br>ACGCTCTTCACATGCAGGCCCTGCCGCCTCGG |
| **R1F2** | | |
| SEQ ID NO: 44 | HCDR1 (Kabat) | SYAMS |
| SEQ ID NO: 45 | HCDR2 (Kabat) | AISGSGGSTYYADSVKG |
| SEQ ID NO: 68 | HCDR3 (Kabat) | REWWYDDWYLDY |
| SEQ ID NO: 47 | HCDR1 (Chothia) | GFTFSSY |
| SEQ ID NO: 48 | HCDR2 (Chothia) | SGSGGS |
| SEQ ID NO: 68 | HCDR3 (Chothia) | REWWYDDWYLDY |
| SEQ ID NO: 49 | HCDR1 (IMGT) | GFTFSSYA |
| SEQ ID NO: 50 | HCDR2 (IMGT) | ISGSGGST |
| SEQ ID NO: 69 | HCDR3 (IMGT) | ARREWWYDDWYLDY |

| | | |
|---|---|---|
| SEQ ID NO: 70 | VH | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGL EWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCARREWWYDDWYLDYWGQGTLVTVSS |
| SEQ ID NO: 71 | DNA VH | GAAGTGCAGTTGCTGGAGTCAGGCGGAGGACTGGTGCAGCCCG GAGGATCGCTTCGCTTGAGCTGCGCAGCCTCAGGCTTTACCTTC TCCTCCTACGCCATGTCCTGGGTCAGACAGGCTCCCGGGAAGG GACTGGAATGGGTGTCCGCCATTAGCGGTTCCGGCGGAAGCAC TTACTATGCCGACTCTGTGAAGGGCCGCTTCACTATCTCCCGGG ACAACTCCAAGAACACCCTGTATCTCCAAATGAATTCCCTGAGG GCCGAAGATACCGCGGTGTACTACTGCGCTAGACGGGAGTGGT GGTACGACGATTGGTACCTGGACTACTGGGGACAGGGCACTCT CGTGACTGTGTCCTCC |
| SEQ ID NO: 54 | LCDR1 (Kabat) | RASQSISSYLN |
| SEQ ID NO: 55 | LCDR2 (Kabat) | AASSLQS |
| SEQ ID NO: 56 | LCDR3 (Kabat) | QQSYSTPLT |
| SEQ ID NO: 57 | LCDR1 (Chothia) | SQSISSY |
| SEQ ID NO: 58 | LCDR2 (Chothia) | AAS |
| SEQ ID NO: 59 | LCDR3 (Chothia) | SYSTPL |
| SEQ ID NO: 60 | LCDR1 (IMGT) | QSISSY |
| SEQ ID NO: 58 | LCDR2 (IMGT) | AAS |
| SEQ ID NO: 56 | LCDR3 (IMGT) | QQSYSTPLT |
| SEQ ID NO: 61 | VL | DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYS TPLTFGQGTKVEIK |
| SEQ ID NO: 62 | DNA VL | GACATTCAAATGACTCAGTCCCCGTCCTCCCTCTCCGCCTCCGT GGGAGATCGCGTCACGATCACGTGCAGGGCCAGCCAGAGCATC TCCAGCTACCTGAACTGGTACCAGCAGAAGCCAGGGAAGGCAC CGAAGCTCCTGATCTACGCCGCTAGCTCGCTGCAGTCCGGCGTC CCTTCACGGTTCTCGGGATCGGGCTCAGGCACCGACTTCACCCT GACCATTAGCAGCCTGCAGCCGGAGGACTTCGCGACATACTAC TGTCAGCAGTCATACTCCACCCCTCTGACCTTCGGCCAAGGGAC CAAAGTGGAGATCAAG |
| SEQ ID NO: 63 | Linker | GGGGSGGGGSGGGGSGGGGS |
| SEQ ID NO: 72 | scFv (VH-linker-VL) | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGL EWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCARREWWYDDWYLDYWGQGTLVTVSSGGGGSGGGGSG GGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQ QKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQSYSTPLTFGQGTKVEIK |

| | | |
|---|---|---|
| SEQ ID NO: 73 | DNA scFv | GAAGTGCAGTTGCTGGAGTCAGGCGGAGGACTGGTGCAGCCCG<br>GAGGATCGCTTCGCTTGAGCTGCGCAGCCTCAGGCTTTACCTTC<br>TCCTCCTACGCCATGTCCTGGGTCAGACAGGCTCCCGGGAAGG<br>GACTGGAATGGGTGTCCGCCATTAGCGGTTCCGGCGGAAGCAC<br>TTACTATGCCGACTCTGTGAAGGGCCGCTTCACTATCTCCCGGG<br>ACAACTCCAAGAACACCCTGTATCTCCAAATGAATTCCCTGAGG<br>GCCGAAGATACCGCGGTGTACTACTGCGCTAGACGGGAGTGGT<br>GGTACGACGATTGGTACCTGGACTACTGGGGACAGGGCACTCT<br>CGTGACTGTGTCCTCCGGTGGTGGTGGATCGGGGGGTGGTGGTT<br>CGGGCGGAGGAGGATCTGGAGGAGGAGGGTCGGACATTCAAA<br>TGACTCAGTCCCCGTCCTCCCTCTCCGCCTCCGTGGGAGATCGC<br>GTCACGATCACGTGCAGGGCCAGCCAGAGCATCTCCAGCTACC<br>TGAACTGGTACCAGCAGAAGCCAGGGAAGGCACCGAAGCTCCT<br>GATCTACGCCGCTAGCTCGCTGCAGTCCGGCGTCCCTTCACGGT<br>TCTCGGGATCGGGCTCAGGCACCGACTTCACCCTGACCATTAGC<br>AGCCTGCAGCCGGAGGACTTCGCGACATACTACTGTCAGCAGT<br>CATACTCCACCCCTCTGACCTTCGGCCAAGGGACCAAAGTGGA<br>GATCAAG |
| SEQ ID NO: 74 | Full CAR amino acid sequence | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGL<br>EWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED<br>TAVYYCARREWWYDDWYLDYWGQGTLVTVSSGGGGSGGGGSG<br>GGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQ<br>QKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFA<br>TYYCQQSYSTPLTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPE<br>ACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCK<br>RGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS<br>RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK<br>PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ<br>GLSTATKDTYDALHMQALPPR |
| SEQ ID NO: 75 | Full CAR DNA sequence | GAAGTGCAGTTGCTGGAGTCAGGCGGAGGACTGGTGCAGCCCG<br>GAGGATCGCTTCGCTTGAGCTGCGCAGCCTCAGGCTTTACCTTC<br>TCCTCCTACGCCATGTCCTGGGTCAGACAGGCTCCCGGGAAGG<br>GACTGGAATGGGTGTCCGCCATTAGCGGTTCCGGCGGAAGCAC<br>TTACTATGCCGACTCTGTGAAGGGCCGCTTCACTATCTCCCGGG<br>ACAACTCCAAGAACACCCTGTATCTCCAAATGAATTCCCTGAGG<br>GCCGAAGATACCGCGGTGTACTACTGCGCTAGACGGGAGTGGT<br>GGTACGACGATTGGTACCTGGACTACTGGGGACAGGGCACTCT<br>CGTGACTGTGTCCTCCGGTGGTGGTGGATCGGGGGGTGGTGGTT<br>CGGGCGGAGGAGGATCTGGAGGAGGAGGGTCGGACATTCAAA<br>TGACTCAGTCCCCGTCCTCCCTCTCCGCCTCCGTGGGAGATCGC<br>GTCACGATCACGTGCAGGGCCAGCCAGAGCATCTCCAGCTACC<br>TGAACTGGTACCAGCAGAAGCCAGGGAAGGCACCGAAGCTCCT<br>GATCTACGCCGCTAGCTCGCTGCAGTCCGGCGTCCCTTCACGGT<br>TCTCGGGATCGGGCTCAGGCACCGACTTCACCCTGACCATTAGC<br>AGCCTGCAGCCGGAGGACTTCGCGACATACTACTGTCAGCAGT<br>CATACTCCACCCCTCTGACCTTCGGCCAAGGGACCAAAGTGGA<br>GATCAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCT<br>CCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATG<br>TAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGAC<br>TTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTG<br>CGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGC |

| | | |
|---|---|---|
| | | GCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATG<br>AGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCC<br>GGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAA<br>ATTCAGCCGCAGCGCAGATGCTCCAGCCTACCAGCAGGGGCAG<br>AACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGT<br>ACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGG<br>GCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACA<br>ACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGAT<br>TGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGG<br>ACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGAC<br>GCTCTTCACATGCAGGCCCTGCCGCCTCGG |
| **R1G5** | | |
| SEQ ID NO: 44 | HCDR1 (Kabat) | SYAMS |
| SEQ ID NO: 45 | HCDR2 (Kabat) | AISGSGGSTYYADSVKG |
| SEQ ID NO: 76 | HCDR3 (Kabat) | REWWGESWLFDY |
| SEQ ID NO: 47 | HCDR1 (Chothia) | GFTFSSY |
| SEQ ID NO: 48 | HCDR2 (Chothia) | SGSGGS |
| SEQ ID NO: 76 | HCDR3 (Chothia) | REWWGESWLFDY |
| SEQ ID NO: 49 | HCDR1 (IMGT) | GFTFSSYA |
| SEQ ID NO: 50 | HCDR2 (IMGT) | ISGSGGST |
| SEQ ID NO: 77 | HCDR3 (IMGT) | ARREWWGESWLFDY |
| SEQ ID NO: 78 | VH | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGL<br>EWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED<br>TAVYYCARREWWGESWLFDYWGQGTLVTVSS |
| SEQ ID NO: 79 | DNA VH | GAAGTGCAGTTGCTGGAGTCAGGCGGAGGACTGGTGCAGCCCG<br>GAGGATCGCTTCGCTTGAGCTGCGCAGCCTCAGGCTTTACCTTC<br>TCCTCCTACGCCATGTCCTGGGTCAGACAGGCTCCCGGGAAGG<br>GACTGGAATGGGTGTCCGCCATTAGCGGTTCCGGCGGAAGCAC<br>TTACTATGCCGACTCTGTGAAGGGCCGCTTCACTATCTCCCGGG<br>ACAACTCCAAGAACACCCTGTATCTCCAAATGAATTCCCTGAGG<br>GCCGAAGATACCGCGGTGTACTACTGCGCTAGACGGGAGTGGT<br>GGGGAGAAAGCTGGCTGTTCGACTACTGGGGACAGGGCACTCT<br>CGTGACTGTGTCCTCC |
| SEQ ID NO: 54 | LCDR1 (Kabat) | RASQSISSYLN |
| SEQ ID NO: 55 | LCDR2 (Kabat) | AASSLQS |
| SEQ ID NO: 56 | LCDR3 (Kabat) | QQSYSTPLT |
| SEQ ID NO: 57 | LCDR1 (Chothia) | SQSISSY |

| | | |
|---|---|---|
| SEQ ID NO: 58 | LCDR2 (Chothia) | AAS |
| SEQ ID NO: 59 | LCDR3 (Chothia) | SYSTPL |
| SEQ ID NO: 60 | LCDR1 (IMGT) | QSISSY |
| SEQ ID NO: 58 | LCDR2 (IMGT) | AAS |
| SEQ ID NO: 56 | LCDR3 (IMGT) | QQSYSTPLT |
| SEQ ID NO: 61 | VL | DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYS TPLTFGQGTKVEIK |
| SEQ ID NO: 62 | DNA VL | GACATTCAAATGACTCAGTCCCCGTCCTCCCTCTCCGCCTCCGT GGGAGATCGCGTCACGATCACGTGCAGGGCCAGCCAGAGCATC TCCAGCTACCTGAACTGGTACCAGCAGAAGCCAGGGAAGGCAC CGAAGCTCCTGATCTACGCCGCTAGCTCGCTGCAGTCCGGCGTC CCTTCACGGTTCTCGGGATCGGGCTCAGGCACCGACTTCACCCT GACCATTAGCAGCCTGCAGCCGGAGGACTTCGCGACATACTAC TGTCAGCAGTCATACTCCACCCCTCTGACCTTCGGCCAAGGGAC CAAAGTGGAGATCAAG |
| SEQ ID NO: 63 | Linker | GGGGSGGGGSGGGGSGGGGS |
| SEQ ID NO: 80 | scFv (VH-linker-VL) | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGL EWVSAISGSGGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCARREWWGESWLFDYWGQGTLVTVSSGGGGSGGGGSGG GGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCQQSYSTPLTFGQGTKVEIK |
| SEQ ID NO: 81 | DNA scFv | GAAGTGCAGTTGCTGGAGTCAGGCGGAGGACTGGTGCAGCCCG GAGGATCGCTTCGCTTGAGCTGCGCAGCCTCAGGCTTTACCTTC TCCTCCTACGCCATGTCCTGGGTCAGACAGGCTCCCGGGAAGG GACTGGAATGGGTGTCCGCCATTAGCGGTTCCGGCGGAAGCAC TTACTATGCCGACTCTGTGAAGGGCCGCTTCACTATCTCCCGGG ACAACTCCAAGAACACCCTGTATCTCCAAATGAATTCCCTGAGG GCCGAAGATACCGCGGTGTACTACTGCGCTAGACGGGAGTGGT GGGGAGAAAGCTGGCTGTTCGACTACTGGGGACAGGGCACTCT CGTGACTGTGTCCTCCGGTGGTGGTGGATCGGGGGGTGGTGGTT CGGGCGGAGGAGGATCTGGAGGAGGAGGGTCGGACATTCAAA TGACTCAGTCCCCGTCCTCCCTCTCCGCCTCCGTGGGAGATCGC GTCACGATCACGTGCAGGGCCAGCCAGAGCATCTCCAGCTACC TGAACTGGTACCAGCAGAAGCCAGGGAAGGCACCGAAGCTCCT GATCTACGCCGCTAGCTCGCTGCAGTCCGGCGTCCCTTCACGGT TCTCGGGATCGGGCTCAGGCACCGACTTCACCCTGACCATTAGC AGCCTGCAGCCGGAGGACTTCGCGACATACTACTGTCAGCAGT CATACTCCACCCCTCTGACCTTCGGCCAAGGGACCAAAGTGGA GATCAAG |
| SEQ ID NO: 82 | Full CAR amino acid sequence | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGL EWVSAISGSGGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCARREWWGESWLFDYWGQGTLVTVSSGGGGSGGGGSGG GGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQ |

| | | |
|---|---|---|
| | | KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFAT<br>YYCQQSYSTPLTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEA<br>CRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKR<br>GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSR<br>SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP<br>RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG<br>LSTATKDTYDALHMQALPPR |
| SEQ ID NO: 83 | Full CAR DNA sequence | GAAGTGCAGTTGCTGGAGTCAGGCGGAGGACTGGTGCAGCCCG<br>GAGGATCGCTTCGCTTGAGCTGCGCAGCCTCAGGCTTTACCTTC<br>TCCTCCTACGCCATGTCCTGGGTCAGACAGGCTCCCGGGAAGG<br>GACTGGAATGGGTGTCCGCCATTAGCGGTTCCGGCGGAAGCAC<br>TTACTATGCCGACTCTGTGAAGGGCCGCTTCACTATCTCCCGGG<br>ACAACTCCAAGAACACCCTGTATCTCCAAATGAATTCCCTGAGG<br>GCCGAAGATACCGCGGTGTACTACTGCGCTAGACGGGAGTGGT<br>GGGGAGAAAGCTGGCTGTTCGACTACTGGGGACAGGGCACTCT<br>CGTGACTGTGTCCTCCGGTGGTGGTGGATCGGGGGGTGGTGGTT<br>CGGGCGGAGGAGGATCTGGAGGAGGAGGGTCGGACATTCAAA<br>TGACTCAGTCCCCGTCCTCCCTCTCCGCCTCCGTGGGAGATCGC<br>GTCACGATCACGTGCAGGGCCAGCCAGAGCATCTCCAGCTACC<br>TGAACTGGTACCAGCAGAAGCCAGGGAAGGCACCGAAGCTCCT<br>GATCTACGCCGCTAGCTCGCTGCAGTCCGGCGTCCCTTCACGGT<br>TCTCGGGATCGGGCTCAGGCACCGACTTCACCCTGACCATTAGC<br>AGCCTGCAGCCGGAGGACTTCGCGACATACTACTGTCAGCAGT<br>CATACTCCACCCCTCTGACCTTCGGCCAAGGGACCAAAGTGGA<br>GATCAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCT<br>CCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATG<br>TAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGAC<br>TTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTG<br>CGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGC<br>GCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATG<br>AGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCC<br>GGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAA<br>ATTCAGCCGCAGCGCAGATGCTCCAGCCTACCAGCAGGGGCAG<br>AACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGT<br>ACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGG<br>GCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACA<br>ACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGAT<br>TGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGG<br>ACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGAC<br>GCTCTTCACATGCAGGCCCTGCCGCCTCGG |

**Table 4: Kabat CDRs of exemplary PALLAS-derived anti-BCMA molecules**

| Kabat | HCDR1 | HCDR2 | HCDR3 | LCDR1 | LCDR2 | LCDR3 |
|---|---|---|---|---|---|---|
| R1B6 | SYAMS (SEQ ID NO: 44) | AISGSGGSTY YADSVKG (SEQ ID NO: 45) | REWVPYDVS WYFDY (SEQ ID NO: 46) | RASQSISS YLN (SEQ ID NO: 54) | AASSL QS (SEQ ID NO: 55) | QQSYSTP LT (SEQ ID NO: 56) |

| | | | | | | |
|---|---|---|---|---|---|---|
| R1F2 | SYAMS (SEQ ID NO: 44) | AISGSGGSTY YADSVKG (SEQ ID NO: 45) | REWWYDD WYLDY (SEQ ID NO: 68) | RASQSISS YLN (SEQ ID NO: 54) | AASSL QS (SEQ ID NO: 55) | QQSYSTP LT (SEQ ID NO: 56) |
| R1G5 | SYAMS (SEQ ID NO: 44) | AISGSGGSTY YADSVKG (SEQ ID NO: 45) | REWWGESW LFDY (SEQ ID NO: 76) | RASQSISS YLN (SEQ ID NO: 54) | AASSL QS (SEQ ID NO: 55) | QQSYSTP LT (SEQ ID NO: 56) |
| Consensus | SYAMS (SEQ ID NO: 44) | AISGSGGSTY YADSVKG (SEQ ID NO: 45) | REW$X_1X_2X_3X_4X_5X_6$W$X_7X_8$D Y, wherein $X_1$ is absent or V; $X_2$ is absent or P; $X_3$ is W or Y; $X_4$ is G, Y, or D; $X_5$ is E, D, or V; $X_6$ is S or D; $X_7$ is L or Y; and $X_8$ is F or L (SEQ ID NO: 84) | RASQSISS YLN (SEQ ID NO: 54) | AASSL QS (SEQ ID NO: 55) | QQSYSTP LT (SEQ ID NO: 56) |

**Table 5: Chothia CDRs of exemplary PALLAS-derived anti-BCMA molecules**

| Chothia | HCDR1 | HCDR2 | HCDR3 | LCDR1 | LCDR2 | LCDR3 |
|---|---|---|---|---|---|---|
| R1B6 | GFTFSSY (SEQ ID NO: 47) | SGSGGS (SEQ ID NO: 48) | REWVPYDVS WYFDY (SEQ ID NO: 46) | SQSISSY (SEQ ID NO: 57) | AAS (SEQ ID NO: 58) | SYSTPL (SEQ ID NO: 59) |
| R1F2 | GFTFSSY (SEQ ID NO: 47) | SGSGGS (SEQ ID NO: 48) | REWWYDD WYLDY (SEQ ID NO: 68) | SQSISSY (SEQ ID NO: 57) | AAS (SEQ ID NO: 58) | SYSTPL (SEQ ID NO: 59) |
| R1G5 | GFTFSSY (SEQ ID NO: 47) | SGSGGS (SEQ ID NO: 48) | REWWGESW LFDY (SEQ ID NO: 76) | SQSISSY (SEQ ID NO: 57) | AAS (SEQ ID NO: 58) | SYSTPL (SEQ ID NO: 59) |
| Consensus | GFTFSSY (SEQ ID NO: 47) | SGSGGS (SEQ ID NO: 48) | REW$X_1X_2X_3X_4X_5X_6$W$X_7X_8$D Y, wherein $X_1$ is absent or V; $X_2$ is absent or P; $X_3$ is W or Y; $X_4$ is G, Y, or D; $X_5$ is E, D, or V; $X_6$ is S or D; $X_7$ is L or Y; and $X_8$ is F or L (SEQ ID NO: 84) | SQSISSY (SEQ ID NO: 57) | AAS (SEQ ID NO: 58) | SYSTPL (SEQ ID NO: 59) |

**Table 6: IMGT CDRs of exemplary PALLAS-derived anti-BCMA molecules**

| IMGT | HCDR1 | HCDR2 | HCDR3 | LCDR1 | LCDR2 | LCDR3 |
|---|---|---|---|---|---|---|
| R1B6 | GFTFSSYA (SEQ ID NO: 49) | ISGSGGST (SEQ ID NO: 50) | ARREWVPY DVSWYFDY (SEQ ID NO: 51) | QSISSY (SEQ ID NO: 60) | AAS (SEQ ID NO: 58) | QQSYSTP LT (SEQ ID NO: 56) |
| R1F2 | GFTFSSYA (SEQ ID NO: 49) | ISGSGGST (SEQ ID NO: 50) | ARREWWYD DWYLDY (SEQ ID NO: 69) | QSISSY (SEQ ID NO: 60) | AAS (SEQ ID NO: 58) | QQSYSTP LT (SEQ ID NO: 56) |
| R1G5 | GFTFSSYA (SEQ ID NO: 49) | ISGSGGST (SEQ ID NO: 50) | ARREWWGE SWLFDY (SEQ ID NO: 77) | QSISSY (SEQ ID NO: 60) | AAS (SEQ ID NO: 58) | QQSYSTP LT (SEQ ID NO: 56) |
| Consensus | GFTFSSYA (SEQ ID NO: 49) | ISGSGGST (SEQ ID NO: 50) | ARREW$X_1X_2$ $X_3X_4X_5X_6WX_7$ $X_8$DY, wherein $X_1$ is absent or V; $X_2$ is absent or P; $X_3$ is W or Y; $X_4$ is G, Y, or D; $X_5$ is E, D, or V; $X_6$ is S or D; $X_7$ is L or Y; and $X_8$ is F or L (SEQ ID NO: 85) | QSISSY (SEQ ID NO: 60) | AAS (SEQ ID NO: 58) | QQSYSTP LT (SEQ ID NO: 56) |

**Table 7: Amino acid and nucleic acid sequences of exemplary B cell-derived anti-BCMA molecules**

| **SEQ ID NO** | **Name/ Description** | **Sequence** |
|---|---|---|
| **PI61** | | |
| SEQ ID NO: 86 | HCDR1 (Kabat) | SYGMH |
| SEQ ID NO: 87 | HCDR2 (Kabat) | VISYDGSNKYYADSVKG |
| SEQ ID NO: 88 | HCDR3 (Kabat) | SGYALHDDYYGLDV |
| SEQ ID NO: 47 | HCDR1 (Chothia) | GFTFSSY |
| SEQ ID NO: 89 | HCDR2 (Chothia) | SYDGSN |
| SEQ ID NO: 88 | HCDR3 (Chothia) | SGYALHDDYYGLDV |
| SEQ ID NO: 90 | HCDR1 (IMGT) | GFTFSSYG |
| SEQ ID NO: 91 | HCDR2 (IMGT) | ISYDGSNK |

| | | |
|---|---|---|
| SEQ ID NO: 92 | HCDR3 (IMGT) | GGSGYALHDDYYGLDV |
| SEQ ID NO: 93 | VH | QVQLQESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL EWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCGGSGYALHDDYYGLDVWGQGTLVTVSS |
| SEQ ID NO: 94 | DNA VH | CAAGTGCAGCTGCAGGAATCCGGTGGCGGAGTCGTGCAGCCTGG AAGGAGCCTGAGACTCTCATGCGCCGCGTCAGGGTTCACCTTTT CCTCCTACGGGATGCATTGGGTCAGACAGGCCCCCGGAAAGGGA CTCGAATGGGTGGCTGTGATCAGCTACGACGGCTCCAACAAGTA CTACGCCGACTCCGTGAAAGGCCGGTTCACTATCTCCCGGGACA ACTCCAAGAACACGCTGTATCTGCAAATGAATTCACTGCGCGCG GAGGATACCGCTGTGTACTACTGCGGTGGCTCCGGTTACGCCCT GCACGATGACTATTACGGCCTTGACGTCTGGGGCCAGGGAACCC TCGTGACTGTGTCCAGC |
| SEQ ID NO: 95 | LCDR1 (Kabat) | TGTSSDVGGYNYVS |
| SEQ ID NO: 96 | LCDR2 (Kabat) | DVSNRPS |
| SEQ ID NO: 97 | LCDR3 (Kabat) | SSYTSSSTLYV |
| SEQ ID NO: 98 | LCDR1 (Chothia) | TSSDVGGYNY |
| SEQ ID NO: 99 | LCDR2 (Chothia) | DVS |
| SEQ ID NO: 100 | LCDR3 (Chothia) | YTSSSTLY |
| SEQ ID NO: 101 | LCDR1 (IMGT) | SSDVGGYNY |
| SEQ ID NO: 99 | LCDR2 (IMGT) | DVS |
| SEQ ID NO: 97 | LCDR3 (IMGT) | SSYTSSSTLYV |
| SEQ ID NO: 102 | VL | QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAP KLMIYDVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSY TSSSTLYVFGSGTKVTVL |
| SEQ ID NO: 103 | DNA VL | CAGAGCGCACTGACTCAGCCGGCATCCGTGTCCGGTAGCCCCGG ACAGTCGATTACCATCTCCTGTACCGGCACCTCCTCCGACGTGG GAGGGTACAACTACGTGTCGTGGTACCAGCAGCACCCAGGAAA GGCCCCTAAGTTGATGATCTACGATGTGTCAAACCGCCCGTCTG GAGTCTCCAACCGGTTCTCCGGCTCCAAGTCCGGCAACACCGCC AGCCTGACCATTAGCGGGCTGCAAGCCGAGGATGAGGCCGACT ACTACTGCTCGAGCTACACATCCTCGAGCACCCTCTACGTGTTCG GCTCGGGGACTAAGGTCACCGTGCTG |
| SEQ ID NO: 104 | Linker | GGGGSGGGGSGGGGS |
| SEQ ID NO: 105 | scFv (VH-linker-VL) | QVQLQESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL EWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCGGSGYALHDDYYGLDVWGQGTLVTVSSGGGGSGGGGS GGGGSQSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQH |

| | | |
|---|---|---|
| | | PGKAPKLMIYDVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEAD<br>YYCSSYTSSSTLYVFGSGTKVTVL |
| SEQ ID NO: 106 | DNA scFv | CAAGTGCAGCTGCAGGAATCCGGTGGCGGAGTCGTGCAGCCTGG<br>AAGGAGCCTGAGACTCTCATGCGCCGCGTCAGGGTTCACCTTTT<br>CCTCCTACGGGATGCATTGGGTCAGACAGGCCCCCGGAAAGGGA<br>CTCGAATGGGTGGCTGTGATCAGCTACGACGGCTCCAACAAGTA<br>CTACGCCGACTCCGTGAAAGGCCGGTTCACTATCTCCCGGGACA<br>ACTCCAAGAACACGCTGTATCTGCAAATGAATTCACTGCGCGCG<br>GAGGATACCGCTGTGTACTACTGCGGTGGCTCCGGTTACGCCCT<br>GCACGATGACTATTACGGCCTTGACGTCTGGGGCCAGGGAACCC<br>TCGTGACTGTGTCCAGCGGTGGAGGAGGTTCGGGCGGAGGAGG<br>ATCAGGAGGGGGTGGATCGCAGAGCGCACTGACTCAGCCGGCA<br>TCCGTGTCCGGTAGCCCCGGACAGTCGATTACCATCTCCTGTACC<br>GGCACCTCCTCCGACGTGGGAGGGTACAACTACGTGTCGTGGTA<br>CCAGCAGCACCCAGGAAAGGCCCCTAAGTTGATGATCTACGATG<br>TGTCAAACCGCCCGTCTGGAGTCTCCAACCGGTTCTCCGGCTCCA<br>AGTCCGGCAACACCGCCAGCCTGACCATTAGCGGGCTGCAAGCC<br>GAGGATGAGGCCGACTACTACTGCTCGAGCTACACATCCTCGAG<br>CACCCTCTACGTGTTCGGCTCGGGGACTAAGGTCACCGTGCTG |
| SEQ ID NO: 107 | Full CAR amino acid sequence | QVQLQESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL<br>EWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAED<br>TAVYYCGGSGYALHDDYYGLDVWGQGTLVTVSSGGGGSGGGGS<br>GGGGSQSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQH<br>PGKAPKLMIYDVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEAD<br>YYCSSYTSSSTLYVFGSGTKVTVLTTTPAPRPPTPAPTIASQPLSLRP<br>EACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCK<br>RGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSR<br>SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR<br>RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS<br>TATKDTYDALHMQALPPR |
| SEQ ID NO: 108 | Full CAR DNA sequence | CAAGTGCAGCTGCAGGAATCCGGTGGCGGAGTCGTGCAGCCTGG<br>AAGGAGCCTGAGACTCTCATGCGCCGCGTCAGGGTTCACCTTTT<br>CCTCCTACGGGATGCATTGGGTCAGACAGGCCCCCGGAAAGGGA<br>CTCGAATGGGTGGCTGTGATCAGCTACGACGGCTCCAACAAGTA<br>CTACGCCGACTCCGTGAAAGGCCGGTTCACTATCTCCCGGGACA<br>ACTCCAAGAACACGCTGTATCTGCAAATGAATTCACTGCGCGCG<br>GAGGATACCGCTGTGTACTACTGCGGTGGCTCCGGTTACGCCCT<br>GCACGATGACTATTACGGCCTTGACGTCTGGGGCCAGGGAACCC<br>TCGTGACTGTGTCCAGCGGTGGAGGAGGTTCGGGCGGAGGAGG<br>ATCAGGAGGGGGTGGATCGCAGAGCGCACTGACTCAGCCGGCA<br>TCCGTGTCCGGTAGCCCCGGACAGTCGATTACCATCTCCTGTACC<br>GGCACCTCCTCCGACGTGGGAGGGTACAACTACGTGTCGTGGTA<br>CCAGCAGCACCCAGGAAAGGCCCCTAAGTTGATGATCTACGATG<br>TGTCAAACCGCCCGTCTGGAGTCTCCAACCGGTTCTCCGGCTCCA<br>AGTCCGGCAACACCGCCAGCCTGACCATTAGCGGGCTGCAAGCC<br>GAGGATGAGGCCGACTACTACTGCTCGAGCTACACATCCTCGAG<br>CACCCTCTACGTGTTCGGCTCGGGGACTAAGGTCACCGTGCTGA<br>CCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATC<br>GCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGC<br>AGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCG<br>ATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTCCTGC |

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| | | TGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGA AGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAG ACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGA GGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGC GCAGATGCTCCAGCCTACCAGCAGGGGCAGAACCAGCTCTACAA CGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACA AGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAG AAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGAT AAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAAC GCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAG CACCGCCACCAAGGACACCTATGACGCTCTTCACATGCAGGCCC TGCCGCCTCGG |
| **B61-02** | | |
| SEQ ID NO: 86 | HCDR1 (Kabat) | SYGMH |
| SEQ ID NO: 109 | HCDR2 (Kabat) | VISYKGSNKYYADSVKG |
| SEQ ID NO: 88 | HCDR3 (Kabat) | SGYALHDDYYGLDV |
| SEQ ID NO: 47 | HCDR1 (Chothia) | GFTFSSY |
| SEQ ID NO: 110 | HCDR2 (Chothia) | SYKGSN |
| SEQ ID NO: 88 | HCDR3 (Chothia) | SGYALHDDYYGLDV |
| SEQ ID NO: 90 | HCDR1 (IMGT) | GFTFSSYG |
| SEQ ID NO: 111 | HCDR2 (IMGT) | ISYKGSNK |
| SEQ ID NO: 92 | HCDR3 (IMGT) | GGSGYALHDDYYGLDV |
| SEQ ID NO: 112 | VH | QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL EWVAVISYKGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCGGSGYALHDDYYGLDVWGQGTLVTVSS |
| SEQ ID NO: 113 | DNA VH | CAAGTGCAGCTTGTCGAATCGGGAGGCGGAGTGGTGCAGCCTGG ACGATCGCTCCGGCTCTCATGTGCCGCGAGCGGATTCACCTTCTC GAGCTACGGCATGCACTGGGTCAGACAAGCCCCAGGAAAGGGC CTGGAATGGGTGGCTGTCATCTCGTACAAGGGCTCAAACAAGTA CTACGCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGCGATA ACTCCAAGAATACCCTCTATCTGCAAATGAACAGCCTGAGGGCC GAGGATACTGCAGTGTACTACTGCGGGGGTTCAGGCTACGCGCT GCACGACGACTACTACGGATTGGACGTCTGGGGCCAAGGAACTC TTGTGACCGTGTCCTCT |
| SEQ ID NO: 95 | LCDR1 (Kabat) | TGTSSDVGGYNYVS |
| SEQ ID NO: 114 | LCDR2 (Kabat) | EVSNRLR |
| SEQ ID NO: 115 | LCDR3 (Kabat) | SSYTSSSALYV |

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| SEQ ID NO: 98 | LCDR1 (Chothia) | TSSDVGGYNY |
| SEQ ID NO: 116 | LCDR2 (Chothia) | EVS |
| SEQ ID NO: 117 | LCDR3 (Chothia) | YTSSSALY |
| SEQ ID NO: 101 | LCDR1 (IMGT) | SSDVGGYNY |
| SEQ ID NO: 116 | LCDR2 (IMGT) | EVS |
| SEQ ID NO: 115 | LCDR3 (IMGT) | SSYTSSSALYV |
| SEQ ID NO: 118 | VL | QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAP KLMIYEVSNRLRGVSNRFSGSKSGNTASLTISGLQAEDEADYYCSS YTSSSALYVFGSGTKVTVL |
| SEQ ID NO: 119 | DNA VL | CAGAGCGCGCTGACTCAGCCTGCCTCCGTGAGCGGTTCGCCGGG ACAGTCCATTACCATTTCGTGCACCGGGACCTCCTCCGACGTGG GAGGCTACAACTACGTGTCCTGGTACCAGCAGCATCCCGGAAAG GCCCCGAAGCTGATGATCTACGAAGTGTCGAACAGACTGCGGGG AGTCTCCAACCGCTTTTCCGGGTCCAAGTCCGGCAACACCGCCA GCCTGACCATCAGCGGGCTCCAGGCAGAAGATGAGGCTGACTAT TACTGCTCCTCCTACACGTCAAGCTCCGCCCTCTACGTGTTCGGG TCCGGGACCAAAGTCACTGTGCTG |
| SEQ ID NO: 63 | Linker | GGGGSGGGGSGGGGSGGGGS |
| SEQ ID NO: 120 | scFv (VH-linker-VL) | QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL EWVAVISYKGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCGGSGYALHDDYYGLDVWGQGTLVTVSSGGGGSGGGGS GGGGSGGGGSQSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVS WYQQHPGKAPKLMIYEVSNRLRGVSNRFSGSKSGNTASLTISGLQA EDEADYYCSSYTSSSALYVFGSGTKVTVL |
| SEQ ID NO: 121 | DNA scFv | CAAGTGCAGCTTGTCGAATCGGGAGGCGGAGTGGTGCAGCCTGG ACGATCGCTCCGGCTCTCATGTGCCGCGAGCGGATTCACCTTCTC GAGCTACGGCATGCACTGGGTCAGACAAGCCCCAGGAAAGGGC CTGGAATGGGTGGCTGTCATCTCGTACAAGGGCTCAAACAAGTA CTACGCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGCGATA ACTCCAAGAATACCCTCTATCTGCAAATGAACAGCCTGAGGGCC GAGGATACTGCAGTGTACTACTGCGGGGGTTCAGGCTACGCGCT GCACGACGACTACTACGGATTGGACGTCTGGGGCCAAGGAACTC TTGTGACCGTGTCCTCTGGTGGAGGCGGATCAGGGGGTGGCGGA TCTGGGGGTGGTGGTTCCGGGGGAGGAGGATCGCAGAGCGCGC TGACTCAGCCTGCCTCCGTGAGCGGTTCGCCGGGACAGTCCATT ACCATTTCGTGCACCGGGACCTCCTCCGACGTGGGAGGCTACAA CTACGTGTCCTGGTACCAGCAGCATCCCGGAAAGGCCCCGAAGC TGATGATCTACGAAGTGTCGAACAGACTGCGGGGAGTCTCCAAC CGCTTTTCCGGGTCCAAGTCCGGCAACACCGCCAGCCTGACCAT CAGCGGGCTCCAGGCAGAAGATGAGGCTGACTATTACTGCTCCT CCTACACGTCAAGCTCCGCCCTCTACGTGTTCGGGTCCGGGACC AAAGTCACTGTGCTG |

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| SEQ ID NO: 122 | Full CAR amino acid sequence | QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL<br>EWVAVISYKGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAED<br>TAVYYCGGSGYALHDDYYGLDVWGQGTLVTVSSGGGGSGGGGS<br>GGGGSGGGGSQSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVS<br>WYQQHPGKAPKLMIYEVSNRLRGVSNRFSGSKSGNTASLTISGLQA<br>EDEADYYCSSYTSSSALYVFGSGTKVTVLTTTPAPRPPTPAPTIASQP<br>LSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIT<br>LYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR<br>VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM<br>GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL<br>YQGLSTATKDTYDALHMQALPPR |
| SEQ ID NO: 123 | Full CAR DNA sequence | CAAGTGCAGCTTGTCGAATCGGGAGGCGGAGTGGTGCAGCCTGG<br>ACGATCGCTCCGGCTCTCATGTGCCGCGAGCGGATTCACCTTCTC<br>GAGCTACGGCATGCACTGGGTCAGACAAGCCCCAGGAAAGGGC<br>CTGGAATGGGTGGCTGTCATCTCGTACAAGGGCTCAAACAAGTA<br>CTACGCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGCGATA<br>ACTCCAAGAATACCCTCTATCTGCAAATGAACAGCCTGAGGGCC<br>GAGGATACTGCAGTGTACTACTGCGGGGGTTCAGGCTACGCGCT<br>GCACGACGACTACTACGGATTGGACGTCTGGGGCCAAGGAACTC<br>TTGTGACCGTGTCCTCTGGTGGAGGCGGATCAGGGGGTGGCGGA<br>TCTGGGGGTGGTGGTTCCGGGGGAGGAGGATCGCAGAGCGCGC<br>TGACTCAGCCTGCCTCCGTGAGCGGTTCGCCGGGACAGTCCATT<br>ACCATTTCGTGCACCGGGACCTCCTCCGACGTGGGAGGCTACAA<br>CTACGTGTCCTGGTACCAGCAGCATCCCGGAAAGGCCCCGAAGC<br>TGATGATCTACGAAGTGTCGAACAGACTGCGGGGAGTCTCCAAC<br>CGCTTTTCCGGGTCCAAGTCCGGCAACACCGCCAGCCTGACCAT<br>CAGCGGGCTCCAGGCAGAAGATGAGGCTGACTATTACTGCTCCT<br>CCTACACGTCAAGCTCCGCCCTCTACGTGTTCGGGTCCGGGACC<br>AAAGTCACTGTGCTGACCACTACCCCAGCACCGAGGCCACCCAC<br>CCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGA<br>GGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTC<br>TTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTA<br>CTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTA<br>AGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTC<br>ATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATG<br>CCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTG<br>AAATTCAGCCGCAGCGCAGATGCTCCAGCCTACCAGCAGGGGCA<br>GAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGT<br>ACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGG<br>CGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAAC<br>GAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTG<br>GTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACT<br>GTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTC<br>TTCACATGCAGGCCCTGCCGCCTCGG |
| **B61-10** | | |
| SEQ ID NO: 86 | HCDR1 (Kabat) | SYGMH |
| SEQ ID NO: 109 | HCDR2 (Kabat) | VISYKGSNKYYADSVKG |
| SEQ ID NO: 88 | HCDR3 (Kabat) | SGYALHDDYYGLDV |

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|---|---|---|
| SEQ ID NO: 47 | HCDR1 (Chothia) | GFTFSSY |
| SEQ ID NO: 110 | HCDR2 (Chothia) | SYKGSN |
| SEQ ID NO: 88 | HCDR3 (Chothia) | SGYALHDDYYGLDV |
| SEQ ID NO: 90 | HCDR1 (IMGT) | GFTFSSYG |
| SEQ ID NO: 111 | HCDR2 (IMGT) | ISYKGSNK |
| SEQ ID NO: 92 | HCDR3 (IMGT) | GGSGYALHDDYYGLDV |
| SEQ ID NO: 112 | VH | QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL EWVAVISYKGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCGGSGYALHDDYYGLDVWGQGTLVTVSS |
| SEQ ID NO: 113 | DNA VH | CAAGTGCAGCTTGTCGAATCGGGAGGCGGAGTGGTGCAGCCTGG ACGATCGCTCCGGCTCTCATGTGCCGCGAGCGGATTCACCTTCTC GAGCTACGGCATGCACTGGGTCAGACAAGCCCCAGGAAAGGGC CTGGAATGGGTGGCTGTCATCTCGTACAAGGGCTCAAACAAGTA CTACGCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGCGATA ACTCCAAGAATACCCTCTATCTGCAAATGAACAGCCTGAGGGCC GAGGATACTGCAGTGTACTACTGCGGGGGTTCAGGCTACGCGCT GCACGACGACTACTACGGATTGGACGTCTGGGGCCAAGGAACTC TTGTGACCGTGTCCTCT |
| SEQ ID NO: 95 | LCDR1 (Kabat) | TGTSSDVGGYNYVS |
| SEQ ID NO: 114 | LCDR2 (Kabat) | EVSNRLR |
| SEQ ID NO: 97 | LCDR3 (Kabat) | SSYTSSSTLYV |
| SEQ ID NO: 98 | LCDR1 (Chothia) | TSSDVGGYNY |
| SEQ ID NO: 116 | LCDR2 (Chothia) | EVS |
| SEQ ID NO: 100 | LCDR3 (Chothia) | YTSSSTLY |
| SEQ ID NO: 101 | LCDR1 (IMGT) | SSDVGGYNY |
| SEQ ID NO: 116 | LCDR2 (IMGT) | EVS |
| SEQ ID NO: 97 | LCDR3 (IMGT) | SSYTSSSTLYV |
| SEQ ID NO: 124 | VL | QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAP KLMIYEVSNRLRGVSNRFSGSKSGNTASLTISGLQAEDEADYYCSS YTSSSTLYVFGSGTKVTVL |
| SEQ ID NO: 125 | DNA VL | CAGAGCGCGCTGACTCAGCCTGCCTCCGTGAGCGGTTCGCCGGG ACAGTCCATTACCATTTCGTGCACCGGGACCTCCTCCGACGTGG GAGGCTACAACTACGTGTCCTGGTACCAGCAGCATCCCGGAAAG GCCCCGAAGCTGATGATCTACGAAGTGTCGAACAGACTGCGGGG AGTCTCCAACCGCTTTTCCGGGTCCAAGTCCGGCAACACCGCCA |

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| | | GCCTGACCATCAGCGGGCTCCAGGCAGAAGATGAGGCTGACTAT<br>TACTGCTCCTCCTACACGTCAAGCTCCACCCTCTACGTGTTCGGG<br>TCCGGGACCAAAGTCACTGTGCTG |
| SEQ ID NO: 63 | Linker | GGGGSGGGGSGGGGSGGGGS |
| SEQ ID NO: 126 | scFv (VH-linker-VL) | QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL<br>EWVAVISYKGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAED<br>TAVYYCGGSGYALHDDYYGLDVWGQGTLVTVSSGGGGSGGGGS<br>GGGGSGGGGSQSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVS<br>WYQQHPGKAPKLMIYEVSNRLRGVSNRFSGSKSGNTASLTISGLQA<br>EDEADYYCSSYTSSSTLYVFGSGTKVTVL |
| SEQ ID NO: 127 | DNA scFv | CAAGTGCAGCTTGTCGAATCGGGAGGCGGAGTGGTGCAGCCTGG<br>ACGATCGCTCCGGCTCTCATGTGCCGCGAGCGGATTCACCTTCTC<br>GAGCTACGGCATGCACTGGGTCAGACAAGCCCCAGGAAAGGGC<br>CTGGAATGGGTGGCTGTCATCTCGTACAAGGGCTCAAACAAGTA<br>CTACGCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGCGATA<br>ACTCCAAGAATACCCTCTATCTGCAAATGAACAGCCTGAGGGCC<br>GAGGATACTGCAGTGTACTACTGCGGGGGTTCAGGCTACGCGCT<br>GCACGACGACTACTACGGATTGGACGTCTGGGGCCAAGGAACTC<br>TTGTGACCGTGTCCTCTGGTGGAGGCGGATCAGGGGGTGGCGGA<br>TCTGGGGGTGGTGGTTCCGGGGGAGGAGGATCGCAGAGCGCGC<br>TGACTCAGCCTGCCTCCGTGAGCGGTTCGCCGGGACAGTCCATT<br>ACCATTTCGTGCACCGGGACCTCCTCCGACGTGGGAGGCTACAA<br>CTACGTGTCCTGGTACCAGCAGCATCCCGGAAAGGCCCCGAAGC<br>TGATGATCTACGAAGTGTCGAACAGACTGCGGGGAGTCTCCAAC<br>CGCTTTTCCGGGTCCAAGTCCGGCAACACCGCCAGCCTGACCAT<br>CAGCGGGCTCCAGGCAGAAGATGAGGCTGACTATTACTGCTCCT<br>CCTACACGTCAAGCTCCACCCTCTACGTGTTCGGGTCCGGGACC<br>AAAGTCACTGTGCTG |
| SEQ ID NO: 128 | Full CAR amino acid sequence | QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL<br>EWVAVISYKGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAED<br>TAVYYCGGSGYALHDDYYGLDVWGQGTLVTVSSGGGGSGGGGS<br>GGGGSGGGGSQSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVS<br>WYQQHPGKAPKLMIYEVSNRLRGVSNRFSGSKSGNTASLTISGLQA<br>EDEADYYCSSYTSSSTLYVFGSGTKVTVLTTTPAPRPPTPAPTIASQP<br>LSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIT<br>LYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR<br>VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM<br>GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL<br>YQGLSTATKDTYDALHMQALPPR |
| SEQ ID NO: 129 | Full CAR DNA sequence | CAAGTGCAGCTTGTCGAATCGGGAGGCGGAGTGGTGCAGCCTGG<br>ACGATCGCTCCGGCTCTCATGTGCCGCGAGCGGATTCACCTTCTC<br>GAGCTACGGCATGCACTGGGTCAGACAAGCCCCAGGAAAGGGC<br>CTGGAATGGGTGGCTGTCATCTCGTACAAGGGCTCAAACAAGTA<br>CTACGCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGCGATA<br>ACTCCAAGAATACCCTCTATCTGCAAATGAACAGCCTGAGGGCC<br>GAGGATACTGCAGTGTACTACTGCGGGGGTTCAGGCTACGCGCT<br>GCACGACGACTACTACGGATTGGACGTCTGGGGCCAAGGAACTC<br>TTGTGACCGTGTCCTCTGGTGGAGGCGGATCAGGGGGTGGCGGA<br>TCTGGGGGTGGTGGTTCCGGGGGAGGAGGATCGCAGAGCGCGC<br>TGACTCAGCCTGCCTCCGTGAGCGGTTCGCCGGGACAGTCCATT |

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| | | ACCATTTCGTGCACCGGGACCTCCTCCGACGTGGGAGGCTACAA<br>CTACGTGTCCTGGTACCAGCAGCATCCCGGAAAGGCCCCGAAGC<br>TGATGATCTACGAAGTGTCGAACAGACTGCGGGGAGTCTCCAAC<br>CGCTTTTCCGGGTCCAAGTCCGGCAACACCGCCAGCCTGACCAT<br>CAGCGGGCTCCAGGCAGAAGATGAGGCTGACTATTACTGCTCCT<br>CCTACACGTCAAGCTCCACCCTCTACGTGTTCGGGTCCGGGACC<br>AAAGTCACTGTGCTGACCACTACCCCAGCACCGAGGCCACCCAC<br>CCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGA<br>GGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTC<br>TTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTA<br>CTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTA<br>AGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTC<br>ATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATG<br>CCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTG<br>AAATTCAGCCGCAGCGCAGATGCTCCAGCCTACCAGCAGGGGCA<br>GAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGT<br>ACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGG<br>CGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAAC<br>GAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTG<br>GTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACT<br>GTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTC<br>TTCACATGCAGGCCCTGCCGCCTCGG |

**Table 8: Kabat CDRs of exemplary B cell-derived anti-BCMA molecules**

| Kabat | HCDR1 | HCDR2 | HCDR3 | LCDR1 | LCDR2 | LCDR3 |
|---|---|---|---|---|---|---|
| PI61 | SYGMH (SEQ ID NO: 86) | VISYDGSN KYYADSV KG (SEQ ID NO: 87) | SGYALHDD YYGLDV (SEQ ID NO: 88) | TGTSSDV GGYNYV S (SEQ ID NO: 95) | DVSNRPS (SEQ ID NO: 96) | SSYTSSS TLYV (SEQ ID NO: 97) |
| B61-02 | SYGMH (SEQ ID NO: 86) | VISYKGSN KYYADSV KG (SEQ ID NO: 109) | SGYALHDD YYGLDV (SEQ ID NO: 88) | TGTSSDV GGYNYV S (SEQ ID NO: 95) | EVSNRLR (SEQ ID NO: 114) | SSYTSSS ALYV (SEQ ID NO: 115) |
| B61-10 | SYGMH (SEQ ID NO: 86) | VISYKGSN KYYADSV KG (SEQ ID NO: 109) | SGYALHDD YYGLDV (SEQ ID NO: 88) | TGTSSDV GGYNYV S (SEQ ID NO: 95) | EVSNRLR (SEQ ID NO: 114) | SSYTSSS TLYV (SEQ ID NO: 97) |
| Consensus | SYGMH (SEQ ID NO: 86) | VISYXGSN KYYADSV KG, wherein X is D or K (SEQ ID NO: 130) | SGYALHDD YYGLDV (SEQ ID NO: 88) | TGTSSDV GGYNYV S (SEQ ID NO: 95) | $X_1$VSNR$X_2X_3$, wherein $X_1$ is D or E; $X_2$ is P or L; and $X_3$ is S or R (SEQ ID NO: 131) | SSYTSSS XLYV, wherein X is T or A (SEQ ID NO: 132) |

**Table 9: Chothia CDRs of exemplary B cell-derived anti-BCMA molecules**

| Chothia | HCDR1 | HCDR2 | HCDR3 | LCDR1 | LCDR2 | LCDR3 |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| PI61 | GFTFSSY (SEQ ID NO: 47) | SYDGSN (SEQ ID NO: 89) | SGYALHDDY YGLDV (SEQ ID NO: 88) | TSSDVGG YNY (SEQ ID NO: 98) | DVS (SEQ ID NO: 99) | YTSSSTLY (SEQ ID NO: 100) |
| B61-02 | GFTFSSY (SEQ ID NO: 47) | SYKGSN (SEQ ID NO: 110) | SGYALHDDY YGLDV (SEQ ID NO: 88) | TSSDVGG YNY (SEQ ID NO: 98) | EVS (SEQ ID NO: 116) | YTSSSAL Y (SEQ ID NO: 117) |
| B61-10 | GFTFSSY (SEQ ID NO: 47) | SYKGSN (SEQ ID NO: 110) | SGYALHDDY YGLDV (SEQ ID NO: 88) | TSSDVGG YNY (SEQ ID NO: 98) | EVS (SEQ ID NO: 116) | YTSSSTLY (SEQ ID NO: 100) |
| Consensus | GFTFSSY (SEQ ID NO: 47) | SYXGSN, wherein X is D or K (SEQ ID NO: 133) | SGYALHDDY YGLDV (SEQ ID NO: 88) | TSSDVGG YNY (SEQ ID NO: 98) | XVS, wherein X is D or E (SEQ ID NO: 134) | YTSSSXL Y, wherein X is T or A (SEQ ID NO: 135) |

**Table 10: IMGT CDRs of exemplary B cell-derived anti-BCMA molecules**

| IMGT | HCDR1 | HCDR2 | HCDR3 | LCDR1 | LCDR2 | LCDR3 |
|---|---|---|---|---|---|---|
| PI61 | GFTFSSYG (SEQ ID NO: 90) | ISYDGSN K (SEQ ID NO: 91) | GGSGYALHDD YYGLDV (SEQ ID NO: 92) | SSDVGGY NY (SEQ ID NO: 101) | DVS (SEQ ID NO: 99) | SSYTSSSTL YV (SEQ ID NO: 97) |
| B61-02 | GFTFSSYG (SEQ ID NO: 90) | ISYKGSN K (SEQ ID NO: 111) | GGSGYALHDD YYGLDV (SEQ ID NO: 92) | SSDVGGY NY (SEQ ID NO: 101) | EVS (SEQ ID NO: 116) | SSYTSSSA LYV (SEQ ID NO: 115) |
| B61-10 | GFTFSSYG (SEQ ID NO: 90) | ISYKGSN K (SEQ ID NO: 111) | GGSGYALHDD YYGLDV (SEQ ID NO: 92) | SSDVGGY NY (SEQ ID NO: 101) | EVS (SEQ ID NO: 116) | SSYTSSSTL YV (SEQ ID NO: 97) |
| Consensus | GFTFSSYG (SEQ ID NO: 90) | ISYXGSN K, wherein X is D or K (SEQ ID NO: 136) | GGSGYALHDD YYGLDV (SEQ ID NO: 92) | SSDVGGY NY (SEQ ID NO: 101) | XVS, wherein X is D or E (SEQ ID NO: 134) | SSYTSSSX LYV, wherein X is T or A (SEQ ID NO: 132) |

**Table 11: Amino acid and nucleic acid sequences of exemplary anti-BCMA molecules based on PI61**

| Identification | Protein sequence | DNA sequence (5'-3') |
|---|---|---|
| Signal peptide | MALPVTALLLPLALLLHAA RP (SEQ ID NO: 1) | Atggccctccctgtcaccgctctgttgctgccgcttgctctgctg ctccacgcagcgcgaccg (SEQ ID NO: 252) |
| PI61 VH | QVQLQESGGGVVQPGRSLRLS CAASGFTFSSYGMHWVRQAP GKGLEWVAVISYDGSNKYYA DSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCGGSGYA LHDDYYGLDVWGQGTLVTVS S (SEQ ID NO: 93) | CAGGTACAATTGCAGGAGTCTGGAGGCGG TGTGGTGCAACCCGGTCGCAGCTTGCGCCT GAGTTGTGCTGCGTCTGGATTTACATTTTC ATCTTACGGAATGCATTGGGTACGCCAGG CACCGGGGAAAGGCCTTGAATGGGTGGCT GTAATTTCATACGATGGTTCCAACAAATAC TATGCTGACTCAGTCAAGGGTCGATTTACA ATTAGTCGGGACAACTCCAAGAACACCCT TTATCTTCAAATGAATTCCCTTAGAGCAGA GGATACGGCGGTCTATTACTGTGGTGGCA |

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|---|---|---|
| | | GTGGTTATGCACTTCATGATGATTACTATG GCTTGGATGTCTGGGGGCAAGGGACGCTT GTAACTGTATCCTCT (SEQ ID NO: 260) |
| PI61 VL | QSALTQPASVSGSPGQSITISCT GTSSDVGGYNYVSWYQQHPG KAPKLMIYDVSNRPSGVSNRFS GSKSGNTASLTISGLQAEDEAD YYCSSYTSSSTLYVFGSGTKVT VL (SEQ ID NO: 102) | CAATCTGCTCTGACTCAACCAGCAAGCGT ATCAGGGTCACCGGGACAGAGTATTACCA TAAGTTGCACGGGGACCTCTAGCGATGTA GGGGGGTATAATTATGTATCTTGGTATCAA CAACACCCCGGGAAAGCCCCTAAATTGAT GATCTACGACGTGAGCAATCGACCTAGTG GCGTATCAAATCGCTTCTCTGGTAGCAAGA GTGGGAATACGGCGTCCCTTACTATTAGCG GATTGCAAGCAGAAGATGAGGCCGATTAC TACTGCAGCTCCTATACTAGCTCTTCTACA TTGTACGTCTTTGGGAGCGGAACAAAAGT AACAGTACTC (SEQ ID NO: 261) |
| Linker | GGGGSGGGGSGGGGS (SEQ ID NO: 104) | |
| ScFv PI61 | QVQLQESGGGVVQPGRSLR LSCAASGFTFSSYGMHWVR QAPGKGLEWVAVISYDGSN KYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYY CGGSGYALHDDYYGLDVW GQGTLVTVSSGGGGSGGGG SGGGGSQSALTQPASVSGSP GQSITISCTGTSSDVGGYNY VSWYQQHPGKAPKLMIYD VSNRPSGVSNRFSGSKSGNT ASLTISGLQAEDEADYYCSS YTSSSTLYVFGSGTKVTVL (SEQ ID NO: 105) | CaggtacaattgcaggagtctggaggcggtgtgGtgcaacc cggtcgcagcttgcgcctgagttgtGctgcgtctggatttacatt ttcatcttacggaAtgcattgggtacgccaggcaccggggaa aggcCttgaatgggtggctgtaatttcatacgatggtTccaac aaatactatgctgactcagtcaagggtCgatttacaattagtcg ggacaactccaagaacAccctttatcttcaaatgaattcccttag agcaGaggatacggcggtctattactgtggtggcagtGgttat gcacttcatgatgattactatggcttgGatgtctgggggcaagg gacgcttgtaactgtaTcctctggtggtggtggtagtggtggg ggaggcTccggcggtggcggctctcaatctgctctgactCaa ccagcaagcgtatcagggtcaccgggacagAgtattaccata agttgcacggggacctctagcGatgtaggggggtataattatg tatcttggtatCaacaacaccccgggaaagcccctaaattgatg AtctacgacgtgagcaatcgacctagtggcgtaTcaaatcgc ttctctggtagcaagagtgggaatAcggcgtcccttactattag cggattgcaagcaGaagatgaggccgattactactgcagctc ctatActagctcttctacattgtacgtctttgggagcggaacaaa agtaacagtactc (SEQ ID NO: 253) |
| Transmembrane domain and hinge | TTTPAPRPPTPAPTIASQPLS LRPEACRPAAGGAVHTRGL DFACDIYIWAPLAGTCGVLL LSLVITLYC (SEQ ID NO: 202) | AcaacaacacctgccccgagaccgcctacaccaGccccga ctattgccagccagcctctgagcctcAggcctgaggcctgtag gcccgcagcgggcggcGcagttcatacacggggcttggattt cgcttgtGatatttatatttgggctcctttggcggggacaTgtgg cgtgctgcttctgtcacttgttattacactgtactgt (SEQ ID NO: 254) |
| 4-1BB | KRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEGG CEL (SEQ ID NO: 7) | AaacgcgggcgaaaaaaattgctgtatatttttAagcagccat ttatgaggcccgttcagacgacgCaggaggaggacggttgct cttgcaggttcccagaagaggaagaaggggctgtgaattg (SEQ ID NO: 255) |
| CD3zeta | RVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEG LYNELQKDKMAEAYSEIGM KGERRRGKGHDGLYQGLST ATKDTYDALHMQALPPR (SEQ ID NO: 10) | CgggttaaattttcaagatccgcagacgctccaGcataccaac agggacaaaaccaactctataacGagctgaatcttggaagaa gggaggaatatgatGtgctggataaacggcgcggtagagatc cggagAtgggcggaaaaccaaggcgaaaaaaccctcagG agggactctacaacgaactgcagaaagacaaaAtggcggag gcttattccgaaataggcatgaagGgcgagcggaggcgagg gaaagggcacgacggaCtgtatcaaggcctctcaaccgcga |

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|---|---|---|
| | | ctaaggatAcgtacgacgccctgcacatgcaggccctgcctc cgaga (SEQ ID NO: 256) |
| PI61 full CAR construct | MALPVTALLLPLALLLHAA RPQVQLQESGGGVVQPGRS LRLSCAASGFTFSSYGMHW VRQAPGKGLEWVAVISYDG SNKYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVY YCGGSGYALHDDYYGLDV WGQGTLVTVSSGGGGSGG GGSGGGGSQSALTQPASVS GSPGQSITISCTGTSSDVGGY NYVSWYQQHPGKAPKLMI YDVSNRPSGVSNRFSGSKSG NTASLTISGLQAEDEADYYC SSYTSSSTLYVFGSGTKVTV LTTTPAPRPPTPAPTIASQPL SLRPEACRPAAGGAVHTRG LDFACDIYIWAPLAGTCGVL LLSLVITLYCKRGRKKLLYI FKQPFMRPVQTTQEEDGCS CRFPEEEEGGCELRVKFSRS ADAPAYQQGQNQLYNELN LGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTY DALHMQALPPR (SEQ ID NO: 257) | ATGGCCCTCCCTGTCACCGCTCTGTTG CTGCCGCTTGCTCTGCTGCTCCACGCA GCGCGACCGCAGGTACAATTGCAGGA GTCTGGAGGCGGTGTGGTGCAACCCG GTCGCAGCTTGCGCCTGAGTTGTGCTG CGTCTGGATTTACATTTTCATCTTACGG AATGCATTGGGTACGCCAGGCACCGG GGAAAGGCCTTGAATGGGTGGCTGTA ATTTCATACGATGGTTCCAACAAATAC TATGCTGACTCAGTCAAGGGTCGATTT ACAATTAGTCGGGACAACTCCAAGAA CACCCTTTATCTTCAAATGAATTCCCTT AGAGCAGAGGATACGGCGGTCTATTA CTGTGGTGGCAGTGGTTATGCACTTCA TGATGATTACTATGGCTTGGATGTCTG GGGGCAAGGGACGCTTGTAACTGTATC CTCTGGTGGTGGTGGTAGTGGTGGGGG AGGCTCCGGCGGTGGCGGCTCTCAATC TGCTCTGACTCAACCAGCAAGCGTATC AGGGTCACCGGGACAGAGTATTACCA TAAGTTGCACGGGGACCTCTAGCGATG TAGGGGGGTATAATTATGTATCTTGGT ATCAACAACACCCCGGGAAAGCCCCT AAATTGATGATCTACGACGTGAGCAAT CGACCTAGTGGCGTATCAAATCGCTTC TCTGGTAGCAAGAGTGGGAATACGGC GTCCCTTACTATTAGCGGATTGCAAGC AGAAGATGAGGCCGATTACTACTGCA GCTCCTATACTAGCTCTTCTACATTGTA CGTCTTTGGGAGCGGAACAAAAGTAA CAGTACTCACAACAACACCTGCCCCGA GACCGCCTACACCAGCCCCGACTATTG CCAGCCAGCCTCTGAGCCTCAGGCCTG AGGCCTGTAGGCCCGCAGCGGGCGGC GCAGTTCATACACGGGGCTTGGATTTC GCTTGTGATATTTATATTTGGGCTCCTT TGGCGGGGACATGTGGCGTGCTGCTTC TGTCACTTGTTATTACACTGTACTGTA AACGCGGGCGAAAAAAATTGCTGTAT ATTTTTAAGCAGCCATTTATGAGGCCC GTTCAGACGACGCAGGAGGAGGACGG TTGCTCTTGCAGGTTCCCAGAAGAGGA AGAAGGGGGCTGTGAATTGCGGGTTA AATTTTCAAGATCCGCAGACGCTCCAG CATACCAACAGGGACAAAACCAACTC TATAACGAGCTGAATCTTGGAAGAAG GGAGGAATATGATGTGCTGGATAAAC GGCGCGGTAGAGATCCGGAGATGGGC GGAAAACCAAGGCGAAAAAACCCTCA |

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|---|---|---|
| | | GGAGGGACTCTACAACGAACTGCAGA AAGACAAAATGGCGGAGGCTTATTCC GAAATAGGCATGAAGGGCGAGCGGAG GCGAGGGAAAGGGCACGACGGACTGT ATCAAGGCCTCTCAACCGCGACTAAGG ATACGTACGACGCCCTGCACATGCAGG CCCTGCCTCCGAGA (SEQ ID NO: 258) |
| PI61 full CAR construct (Nucleic acid with signal peptide and stop codons) | | ATGGCCCTCCCTGTCACCGCTCTGTTGCTGCCGC TTGCTCTGCTGCTCCACGCAGCGCGACCGCAGGT ACAATTGCAGGAGTCTGGAGGCGGTGTGGTGCAA CCCGGTCGCAGCTTGCGCCTGAGTTGTGCTGCGT CTGGATTTACATTTTCATCTTACGGAATGCATTG GGTACGCCAGGCACCGGGGAAAGGCCTTGAATGG GTGGCTGTAATTTCATACGATGGTTCCAACAAAT ACTATGCTGACTCAGTCAAGGGTCGATTTACAAT TAGTCGGGACAACTCCAAGAACACCCTTTATCTT CAAATGAATTCCCTTAGAGCAGAGGATACGGCGG TCTATTACTGTGGTGGCAGTGGTTATGCACTTCA TGATGATTACTATGGCTTGGATGTCTGGGGGCAA GGGACGCTTGTAACTGTATCCTCTGGTGGTGGTG GTAGTGGTGGGGGAGGCTCCGGCGGTGGCGGCTC TCAATCTGCTCTGACTCAACCAGCAAGCGTATCA GGGTCACCGGGACAGAGTATTACCATAAGTTGCA CGGGGACCTCTAGCGATGTAGGGGGGTATAATTA TGTATCTTGGTATCAACAACACCCCGGGAAAGCC CCTAAATTGATGATCTACGACGTGAGCAATCGAC CTAGTGGCGTATCAAATCGCTTCTCTGGTAGCAA GAGTGGGAATACGGCGTCCCTTACTATTAGCGGA TTGCAAGCAGAAGATGAGGCCGATTACTACTGCA GCTCCTATACTAGCTCTTCTACATTGTACGTCTT TGGGAGCGGAACAAAAGTAACAGTACTCACAACA ACACCTGCCCCGAGACCGCCTACACCAGCCCCGA CTATTGCCAGCCAGCCTCTGAGCCTCAGGCCTGA GGCCTGTAGGCCCGCAGCGGGCGGCGCAGTTCAT ACACGGGGCTTGGATTTCGCTTGTGATATTTATA TTTGGGCTCCTTTGGCGGGGACATGTGGCGTGCT GCTTCTGTCACTTGTTATTACACTGTACTGTAAA CGCGGGCGAAAAAAATTGCTGTATATTTTTAAGC AGCCATTTATGAGGCCCGTTCAGACGACGCAGGA GGAGGACGGTTGCTCTTGCAGGTTCCCAGAAGAG GAAGAAGGGGGCTGTGAATTGCGGGTTAAATTTT CAAGATCCGCAGACGCTCCAGCATACCAACAGGG ACAAAACCAACTCTATAACGAGCTGAATCTTGGA AGAAGGGAGGAATATGATGTGCTGGATAAACGGC GCGGTAGAGATCCGGAGATGGGCGGAAAACCAAG GCGAAAAAACCCTCAGGAGGGACTCTACAACGAA CTGCAGAAAGACAAAATGGCGGAGGCTTATTCCG AAATAGGCATGAAGGGCGAGCGGAGGCGAGGGAA AGGGCACGACGGACTGTATCAAGGCCTCTCAACC GCGACTAAGGATACGTACGACGCCCTGCACATGC AGGCCCTGCCTCCGAGATGATAA (SEQ ID NO: 416) |
| PI61 mature CAR protein | QVQLQESGGGVVQPGRSLR LSCAASGFTFSSYGMHWVR QAPGKGLEWVAVISYDGSN KYYADSVKGRFTISRDNSK | caggtacaattgcaggagtctggaggcggtgtggtgcaacccggtc gcagcttgcgcctgagttgtgctgcgtctggatttacattttcatcttacg gaatgcattgggtacgccaggcaccggggaaaggccttgaatgggt ggctgtaatttcatacgatggttccaacaaatactatgctgactcagtca |

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|---|---|---|
| | NTLYLQMNSLRAEDTAVYY CGGSGYALHDDYYGLDVW GQGTLVTVSSGGGGSGGGG SGGGGSQSALTQPASVSGSP GQSITISCTGTSSDVGGYNY VSWYQQHPGKAPKLMIYD VSNRPSGVSNRFSGSKSGNT ASLTISGLQAEDEADYYCSS YTSSSTLYVFGSGTKVTVLT TTPAPRPPTPAPTIASQPLSL RPEACRPAAGGAVHTRGLD FACDIYIWAPLAGTCGVLLL SLVITLYCKRGRKKLLYIFK QPFMRPVQTTQEEDGCSCR FPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLG RREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKD KMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDA LHMQALPPR (SEQ ID NO: 107) | agggtcgatttacaattagtcgggacaactccaagaacacccttatctt caaatgaattcccttagagcagaggatacggcggtctattactgtggtg gcagtggttatgcacttcatgatgattactatggcttggatgtctggggg caagggacgcttgtaactgtatcctctggtggtggtggtagtggtggg ggaggctccggcggtggcggctctcaatctgctctgactcaaccagc aagcgtatcagggtcaccgggacagagtattaccataagttgcacgg ggacctctagcgatgtaggggggtataattatgtatcttggtatcaaca acaccccgggaaagcccctaaattgatgatctacgacgtgagcaatc gacctagtggcgtatcaaatcgcttctctggtagcaagagtgggaata cggcgtcccttactattagcggattgcaagcagaagatgaggccgatt actactgcagctcctatactagctcttctacattgtacgtctttgggagcg gaacaaaagtaacagtactcacaacaacacctgccccgagaccgcct acaccagccccgactattgccagccagcctctgagcctcaggcctga ggcctgtaggcccgcagcgggcggcgcagttcatacacggggcttg gatttcgcttgtgatatttatatttgggctcctttggcggggacatgtggc gtgctgcttctgtcacttgttattacactgtactgtaaacgcgggcgaaa aaaattgctgtatatttttaagcagccatttatgaggcccgttcagacga cgcaggaggaggacggttgctcttgcaggttcccagaagaggaaga agggggctgtgaattgcgggttaaattttcaagatccgcagacgctcc agcataccaacagggacaaaaccaactctataacgagctgaatcttg gaagaagggaggaatatgatgtgctggataaacggcgcggtagaga tccggagatgggcggaaaaccaaggcgaaaaaaccctcaggaggg actctacaacgaactgcagaaagacaaaatggcggaggcttattccg aaataggcatgaagggcgagcggaggcgagggaaagggcacgac ggactgtatcaaggcctctcaaccgcgactaaggatacgtacgacgc cctgcacatgcaggccctgcctccgaga (SEQ ID NO: 259) |

**Table 12: Amino acid and nucleic acid sequences of exemplary hybridoma-derived anti-BCMA molecules**

| SEQ ID NO | Name/ Description | Sequence |
|---|---|---|
| **Hy03** | | |
| SEQ ID NO: 137 | HCDR1 (Kabat) | GFWMS |
| SEQ ID NO: 138 | HCDR2 (Kabat) | NIKQDGSEKYYVDSVRG |
| SEQ ID NO: 139 | HCDR3 (Kabat) | ALDYYGMDV |
| SEQ ID NO: 140 | HCDR1 (Chothia) | GFTFSGF |
| SEQ ID NO: 141 | HCDR2 (Chothia) | KQDGSE |
| SEQ ID NO: 139 | HCDR3 (Chothia) | ALDYYGMDV |
| SEQ ID NO: 142 | HCDR1 (IMGT) | GFTFSGFW |
| SEQ ID NO: 143 | HCDR2 (IMGT) | IKQDGSEK |
| SEQ ID NO: 144 | HCDR3 (IMGT) | ARALDYYGMDV |

| | | |
|---|---|---|
| SEQ ID NO: 145 | VH | EVQLVESGGGLVQPGGSLRLSCAASGFTFSGFWMSWVRQAPGKG LEWVANIKQDGSEKYYVDSVRGRFTISRDNAKNSLYLQMNSLRAE DTAVYYCARALDYYGMDVWGQGTTVTVSS |
| SEQ ID NO: 146 | DNA VH | GAAGTGCAACTGGTGGAGAGCGGTGGAGGGCTTGTCCAGCCCG GAGGATCGCTGCGGCTGTCCTGTGCTGCGTCCGGGTTCACCTTC TCCGGCTTCTGGATGTCCTGGGTCAGACAGGCACCGGGAAAGG GCCTCGAATGGGTGGCCAACATCAAGCAGGATGGCTCCGAGAA GTACTACGTCGACTCCGTGAGAGGCCGCTTCACCATCTCCCGGG ACAACGCCAAGAACTCGCTGTACCTCCAAATGAATAGCCTCAG GGCGGAAGATACTGCTGTGTATTACTGCGCACGCGCCCTTGACT ACTACGGCATGGACGTCTGGGGCCAAGGGACCACTGTGACCGT GTCTAGC |
| SEQ ID NO: 147 | LCDR1 (Kabat) | RSSQSLLDSDDGNTYLD |
| SEQ ID NO: 148 | LCDR2 (Kabat) | TLSYRAS |
| SEQ ID NO: 149 | LCDR3 (Kabat) | TQRLEFPSIT |
| SEQ ID NO: 150 | LCDR1 (Chothia) | SQSLLDSDDGNTY |
| SEQ ID NO: 151 | LCDR2 (Chothia) | TLS |
| SEQ ID NO: 152 | LCDR3 (Chothia) | RLEFPSI |
| SEQ ID NO: 153 | LCDR1 (IMGT) | QSLLDSDDGNTY |
| SEQ ID NO: 151 | LCDR2 (IMGT) | TLS |
| SEQ ID NO: 149 | LCDR3 (IMGT) | TQRLEFPSIT |
| SEQ ID NO: 154 | VL | DIVMTQTPLSLPVTPGEPASISCRSSQSLLDSDDGNTYLDWYLQKP GQSPRLLIYTLSYRASGVPDRFSGSGSGTDFTLKISRVEAEDVGLYY CTQRLEFPSITFGQGTRLEIK |
| SEQ ID NO: 155 | DNA VL | GATATCGTGATGACCCAGACTCCCCTGTCCCTGCCTGTGACTCC CGGAGAACCAGCCTCCATTTCCTGCCGGTCCTCCCAGTCCCTGC TGGACAGCGACGACGGCAACACTTACCTGGACTGGTACTTGCA GAAGCCGGGCCAATCGCCTCGCCTGCTGATCTATACCCTGTCAT ACCGGGCCTCAGGAGTGCCTGACCGCTTCTCGGGATCAGGGAG CGGGACCGATTTCACCCTGAAAATTTCCCGAGTGGAAGCCGAG GACGTCGGACTGTACTACTGCACCCAGCGCCTCGAATTCCCGTC GATTACGTTTGGACAGGGTACCCGGCTTGAGATCAAG |
| SEQ ID NO: 63 | Linker | GGGGSGGGGSGGGGSGGGGS |
| SEQ ID NO: 156 | scFv (VH-linker-VL) | EVQLVESGGGLVQPGGSLRLSCAASGFTFSGFWMSWVRQAPGKG LEWVANIKQDGSEKYYVDSVRGRFTISRDNAKNSLYLQMNSLRAE DTAVYYCARALDYYGMDVWGQGTTVTVSSGGGGSGGGGSGGG GSGGGGSDIVMTQTPLSLPVTPGEPASISCRSSQSLLDSDDGNTYLD WYLQKPGQSPRLLIYTLSYRASGVPDRFSGSGSGTDFTLKISRVEA EDVGLYYCTQRLEFPSITFGQGTRLEIK |

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|---|---|---|
| SEQ ID NO: 157 | DNA scFv | GAAGTGCAACTGGTGGAGAGCGGTGGAGGGCTTGTCCAGCCCG<br>GAGGATCGCTGCGGCTGTCCTGTGCTGCGTCCGGGTTCACCTTC<br>TCCGGCTTCTGGATGTCCTGGGTCAGACAGGCACCGGGAAAGG<br>GCCTCGAATGGGTGGCCAACATCAAGCAGGATGGCTCCGAGAA<br>GTACTACGTCGACTCCGTGAGAGGCCGCTTCACCATCTCCCGGG<br>ACAACGCCAAGAACTCGCTGTACCTCCAAATGAATAGCCTCAG<br>GGCGGAAGATACTGCTGTGTATTACTGCGCACGCGCCCTTGACT<br>ACTACGGCATGGACGTCTGGGGCCAAGGGACCACTGTGACCGT<br>GTCTAGCGGAGGCGGAGGTTCAGGGGGCGGTGGATCAGGCGGA<br>GGAGGATCGGGGGGTGGTGGATCGGATATCGTGATGACCCAGA<br>CTCCCCTGTCCCTGCCTGTGACTCCCGGAGAACCAGCCTCCATT<br>TCCTGCCGGTCCTCCCAGTCCCTGCTGGACAGCGACGACGGCAA<br>CACTTACCTGGACTGGTACTTGCAGAAGCCGGGCCAATCGCCTC<br>GCCTGCTGATCTATACCCTGTCATACCGGGCCTCAGGAGTGCCT<br>GACCGCTTCTCGGGATCAGGGAGCGGGACCGATTTCACCCTGA<br>AAATTTCCCGAGTGGAAGCCGAGGACGTCGGACTGTACTACTG<br>CACCCAGCGCCTCGAATTCCCGTCGATTACGTTTGGACAGGGTA<br>CCCGGCTTGAGATCAAG |
| SEQ ID NO: 158 | Full CAR amino acid sequence | EVQLVESGGGLVQPGGSLRLSCAASGFTFSGFWMSWVRQAPGKG<br>LEWVANIKQDGSEKYYVDSVRGRFTISRDNAKNSLYLQMNSLRAE<br>DTAVYYCARALDYYGMDVWGQGTTVTVSSGGGGSGGGGSGGG<br>GSGGGGSDIVMTQTPLSLPVTPGEPASISCRSSQSLLDSDDGNTYLD<br>WYLQKPGQSPRLLIYTLSYRASGVPDRFSGSGSGTDFTLKISRVEA<br>EDVGLYYCTQRLEFPSITFGQGTRLEIKTTTPAPRPPTPAPTIASQPL<br>SLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIT<br>LYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR<br>VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM<br>GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG<br>LYQGLSTATKDTYDALHMQALPPR |
| SEQ ID NO: 159 | Full CAR DNA sequence | GAAGTGCAACTGGTGGAGAGCGGTGGAGGGCTTGTCCAGCCCG<br>GAGGATCGCTGCGGCTGTCCTGTGCTGCGTCCGGGTTCACCTTC<br>TCCGGCTTCTGGATGTCCTGGGTCAGACAGGCACCGGGAAAGG<br>GCCTCGAATGGGTGGCCAACATCAAGCAGGATGGCTCCGAGAA<br>GTACTACGTCGACTCCGTGAGAGGCCGCTTCACCATCTCCCGGG<br>ACAACGCCAAGAACTCGCTGTACCTCCAAATGAATAGCCTCAG<br>GGCGGAAGATACTGCTGTGTATTACTGCGCACGCGCCCTTGACT<br>ACTACGGCATGGACGTCTGGGGCCAAGGGACCACTGTGACCGT<br>GTCTAGCGGAGGCGGAGGTTCAGGGGGCGGTGGATCAGGCGGA<br>GGAGGATCGGGGGGTGGTGGATCGGATATCGTGATGACCCAGA<br>CTCCCCTGTCCCTGCCTGTGACTCCCGGAGAACCAGCCTCCATT<br>TCCTGCCGGTCCTCCCAGTCCCTGCTGGACAGCGACGACGGCAA<br>CACTTACCTGGACTGGTACTTGCAGAAGCCGGGCCAATCGCCTC<br>GCCTGCTGATCTATACCCTGTCATACCGGGCCTCAGGAGTGCCT<br>GACCGCTTCTCGGGATCAGGGAGCGGGACCGATTTCACCCTGA<br>AAATTTCCCGAGTGGAAGCCGAGGACGTCGGACTGTACTACTG<br>CACCCAGCGCCTCGAATTCCCGTCGATTACGTTTGGACAGGGTA<br>CCCGGCTTGAGATCAAGACCACTACCCCAGCACCGAGGCCACC<br>CACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTC<br>CGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCG<br>GGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGG<br>CTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTT |

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|---|---|---|
| | | ACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCA<br>ACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGC<br>TGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAAC<br>TGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACCA<br>GCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGG<br>AGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGAC<br>CCAGAAATGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAG<br>GGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCT<br>ATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAG<br>GCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGA<br>CACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG |
| **Hy52** | | |
| SEQ ID NO: 160 | HCDR1 (Kabat) | SFRMN |
| SEQ ID NO: 161 | HCDR2 (Kabat) | SISSSSSYIYYADSVKG |
| SEQ ID NO: 162 | HCDR3 (Kabat) | WLSYYGMDV |
| SEQ ID NO: 163 | HCDR1 (Chothia) | GFTFSSF |
| SEQ ID NO: 164 | HCDR2 (Chothia) | SSSSSY |
| SEQ ID NO: 162 | HCDR3 (Chothia) | WLSYYGMDV |
| SEQ ID NO: 165 | HCDR1 (IMGT) | GFTFSSFR |
| SEQ ID NO: 166 | HCDR2 (IMGT) | ISSSSSYI |
| SEQ ID NO: 167 | HCDR3 (IMGT) | ARWLSYYGMDV |
| SEQ ID NO: 168 | VH | EVQLVESGGGLVKPGGSLRLSCAASGFTFSSFRMNWVRQAPGKGL<br>EWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDT<br>AVYYCARWLSYYGMDVWGQGTTVTVSS |
| SEQ ID NO: 169 | DNA VH | GAAGTGCAACTGGTGGAGAGCGGTGGAGGGCTTGTCAAGCCCG<br>GAGGATCGCTGCGGCTGTCCTGTGCTGCGTCCGGGTTCACCTTC<br>TCCTCGTTCCGCATGAACTGGGTCAGACAGGCACCGGGAAAGG<br>GCCTCGAATGGGTGTCCTCAATCTCATCGTCCTCGTCCTACATC<br>TACTACGCCGACTCCGTGAAAGGCCGCTTCACCATCTCCCGGGA<br>CAACGCCAAGAACTCGCTGTACCTCCAAATGAATAGCCTCAGG<br>GCGGAAGATACTGCTGTGTATTACTGCGCACGCTGGCTTTCCTA<br>CTACGGCATGGACGTCTGGGGCCAAGGGACCACTGTGACCGTG<br>TCTAGC |
| SEQ ID NO: 147 | LCDR1 (Kabat) | RSSQSLLDSDDGNTYLD |
| SEQ ID NO: 170 | LCDR2 (Kabat) | TLSFRAS |
| SEQ ID NO: 171 | LCDR3 (Kabat) | MQRIGFPIT |
| SEQ ID NO: 150 | LCDR1 (Chothia) | SQSLLDSDDGNTY |

| | | |
|---|---|---|
| SEQ ID NO: 151 | LCDR2 (Chothia) | TLS |
| SEQ ID NO: 172 | LCDR3 (Chothia) | RIGFPI |
| SEQ ID NO: 153 | LCDR1 (IMGT) | QSLLDSDDGNTY |
| SEQ ID NO: 151 | LCDR2 (IMGT) | TLS |
| SEQ ID NO: 171 | LCDR3 (IMGT) | MQRIGFPIT |
| SEQ ID NO: 173 | VL | DIVMTQTPLSLPVTPGEPASISCRSSQSLLDSDDGNTYLDWYLQKP GQSPQLLIYTLSFRASGVPDRFSGSGSGTDFTLKIRRVEAEDVGVY YCMQRIGFPITFGQGTRLEIK |
| SEQ ID NO: 174 | DNA VL | GATATCGTGATGACCCAGACTCCCCTGTCCCTGCCTGTGACTCC CGGAGAACCAGCCTCCATTTCCTGCCGGTCCTCCCAGTCCCTGC TGGACAGCGACGACGGCAACACTTACCTGGACTGGTACTTGCA GAAGCCGGGCCAATCGCCTCAGCTGCTGATCTATACCCTGTCAT TCCGGGCCTCAGGAGTGCCTGACCGCTTCTCGGGATCAGGGAG CGGGACCGATTTCACCCTGAAAATTAGGCGAGTGGAAGCCGAG GACGTCGGAGTGTACTACTGCATGCAGCGCATCGGCTTCCCGAT TACGTTTGGACAGGGTACCCGGCTTGAGATCAAG |
| SEQ ID NO: 63 | Linker | GGGGSGGGGSGGGGSGGGGS |
| SEQ ID NO: 175 | scFv (VH-linker-VL) | EVQLVESGGGLVKPGGSLRLSCAASGFTFSSFRMNWVRQAPGKGL EWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDT AVYYCARWLSYYGMDVWGQGTTVTVSSGGGGSGGGGSGGGGS GGGGSDIVMTQTPLSLPVTPGEPASISCRSSQSLLDSDDGNTYLDW YLQKPGQSPQLLIYTLSFRASGVPDRFSGSGSGTDFTLKIRRVEAED VGVYYCMQRIGFPITFGQGTRLEIK |
| SEQ ID NO: 176 | DNA scFv | GAAGTGCAACTGGTGGAGAGCGGTGGAGGGCTTGTCAAGCCCG GAGGATCGCTGCGGCTGTCCTGTGCTGCGTCCGGGTTCACCTTC TCCTCGTTCCGCATGAACTGGGTCAGACAGGCACCGGGAAAGG GCCTCGAATGGGTGTCCTCAATCTCATCGTCCTCGTCCTACATC TACTACGCCGACTCCGTGAAAGGCCGCTTCACCATCTCCCGGGA CAACGCCAAGAACTCGCTGTACCTCCAAATGAATAGCCTCAGG GCGGAAGATACTGCTGTGTATTACTGCGCACGCTGGCTTTCCTA CTACGGCATGGACGTCTGGGGCCAAGGGACCACTGTGACCGTG TCTAGCGGAGGCGGAGGTTCAGGGGGCGGTGGATCAGGCGGAG GAGGATCGGGGGGTGGTGGATCGGATATCGTGATGACCCAGAC TCCCCTGTCCCTGCCTGTGACTCCCGGAGAACCAGCCTCCATTT CCTGCCGGTCCTCCCAGTCCCTGCTGGACAGCGACGACGGCAA CACTTACCTGGACTGGTACTTGCAGAAGCCGGGCCAATCGCCTC AGCTGCTGATCTATACCCTGTCATTCCGGGCCTCAGGAGTGCCT GACCGCTTCTCGGGATCAGGGAGCGGGACCGATTTCACCCTGA AAATTAGGCGAGTGGAAGCCGAGGACGTCGGAGTGTACTACTG CATGCAGCGCATCGGCTTCCCGATTACGTTTGGACAGGGTACCC GGCTTGAGATCAAG |
| SEQ ID NO: 177 | Full CAR amino acid sequence | EVQLVESGGGLVKPGGSLRLSCAASGFTFSSFRMNWVRQAPGKGL EWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDT AVYYCARWLSYYGMDVWGQGTTVTVSSGGGGSGGGGSGGGGS GGGGSDIVMTQTPLSLPVTPGEPASISCRSSQSLLDSDDGNTYLDW |

| | | |
|---|---|---|
| | | YLQKPGQSPQLLIYTLSFRASGVPDRFSGSGSGTDFTLKIRRVEAED<br>VGVYYCMQRIGFPITFGQGTRLEIKTTTPAPRPPTPAPTIASQPLSLR<br>PEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC<br>KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKF<br>SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG<br>KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY<br>QGLSTATKDTYDALHMQALPPR |
| SEQ ID NO: 178 | Full CAR DNA sequence | GAAGTGCAACTGGTGGAGAGCGGTGGAGGGCTTGTCAAGCCCG<br>GAGGATCGCTGCGGCTGTCCTGTGCTGCGTCCGGGTTCACCTTC<br>TCCTCGTTCCGCATGAACTGGGTCAGACAGGCACCGGGAAAGG<br>GCCTCGAATGGGTGTCCTCAATCTCATCGTCCTCGTCCTACATC<br>TACTACGCCGACTCCGTGAAAGGCCGCTTCACCATCTCCCGGGA<br>CAACGCCAAGAACTCGCTGTACCTCCAAATGAATAGCCTCAGG<br>GCGGAAGATACTGCTGTGTATTACTGCGCACGCTGGCTTTCCTA<br>CTACGGCATGGACGTCTGGGGCCAAGGGACCACTGTGACCGTG<br>TCTAGCGGAGGCGGAGGTTCAGGGGGCGGTGGATCAGGCGGAG<br>GAGGATCGGGGGGTGGTGGATCGGATATCGTGATGACCCAGAC<br>TCCCCTGTCCCTGCCTGTGACTCCCGGAGAACCAGCCTCCATTT<br>CCTGCCGGTCCTCCCAGTCCCTGCTGGACAGCGACGACGGCAA<br>CACTTACCTGGACTGGTACTTGCAGAAGCCGGGCCAATCGCCTC<br>AGCTGCTGATCTATACCCTGTCATTCCGGGCCTCAGGAGTGCCT<br>GACCGCTTCTCGGGATCAGGGAGCGGGACCGATTTCACCCTGA<br>AAATTAGGCGAGTGGAAGCCGAGGACGTCGGAGTGTACTACTG<br>CATGCAGCGCATCGGCTTCCCGATTACGTTTGGACAGGGTACCC<br>GGCTTGAGATCAAGACCACTACCCCAGCACCGAGGCCACCCAC<br>CCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGG<br>AGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGG<br>TCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTG<br>GTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACT<br>GTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACC<br>CTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGT<br>TCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGC<br>GCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACCAGCA<br>GGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGA<br>GAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCA<br>GAAATGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGC<br>CTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATA<br>GCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCC<br>ACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACAC<br>CTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG |

**Table 13: Kabat CDRs of exemplary hybridoma-derived anti-BCMA molecules**

| Kabat | HCDR1 | HCDR2 | HCDR3 | LCDR1 | LCDR2 | LCDR3 |
|---|---|---|---|---|---|---|
| Hy03 | GFWMS (SEQ ID NO: 137) | NIKQDGSEK YYVDSVRG (SEQ ID NO: 138) | ALDYYGMD V (SEQ ID NO: 139) | RSSQSLLDS DDGNTYLD (SEQ ID NO: 147) | TLSYRA S (SEQ ID NO: 148) | TQRLEFP SIT (SEQ ID NO: 149) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Hy52 | SFRMN (SEQ ID NO: 160) | SISSSSSYIYY ADSVKG (SEQ ID NO: 161) | WLSYYGMD V (SEQ ID NO: 162) | RSSQSLLDS DDGNTYLD (SEQ ID NO: 147) | TLSFRAS (SEQ ID NO: 170) | MQRIGFP IT (SEQ ID NO: 171) |
| Consensus | $X_1FX_2MX_3$, wherein $X_1$ is G or S; $X_2$ is W or R; and $X_3$ is S or N (SEQ ID NO: 179) | $X_1IX_2X_3X_4X_5S$ $X_6X_7YYX_8DS$ $VX_9G$, wherein $X_1$ is N or S; $X_2$ is K or S; $X_3$ is Q or S; $X_4$ is D or S; $X_5$ is G or S; $X_6$ is E or Y; $X_7$ is K or I; $X_8$ is V or A; and $X_9$ is R or K (SEQ ID NO: 180) | $X_1LX_2YYGM$ DV, wherein $X_1$ is A or W; and $X_2$ is D or S (SEQ ID NO: 181) | RSSQSLLDS DDGNTYLD (SEQ ID NO: 147) | TLSXRA S, wherein X is Y or F (SEQ ID NO: 182) | $X_1QRX_2X_3FPX_4IT$, wherein $X_1$ is T or M; $X_2$ is L or I; $X_3$ is E or G; and $X_4$ is S or absent (SEQ ID NO: 183) |

**Table 14: Chothia CDRs of exemplary hybridoma-derived anti-BCMA molecules**

| Chothia | HCDR1 | HCDR2 | HCDR3 | LCDR1 | LCDR2 | LCDR3 |
|---|---|---|---|---|---|---|
| Hy03 | GFTFSGF (SEQ ID NO: 140) | KQDGSE (SEQ ID NO: 141) | ALDYYGMD V (SEQ ID NO: 139) | SQSLLDSD DGNTY (SEQ ID NO: 150) | TLS (SEQ ID NO: 151) | RLEFPSI (SEQ ID NO: 152) |
| Hy52 | GFTFSSF (SEQ ID NO: 163) | SSSSSY (SEQ ID NO: 164) | WLSYYGMD V (SEQ ID NO: 162) | SQSLLDSD DGNTY (SEQ ID NO: 150) | TLS (SEQ ID NO: 151) | RIGFPI (SEQ ID NO: 172) |
| Consensus | GFTFSXF, wherein X is G or S (SEQ ID NO: 184) | $X_1X_2X_3X_4SX_5$, wherein $X_1$ is K or S; $X_2$ is Q or S; $X_3$ is D or S; $X_4$ is G or S; and $X_5$ is E or Y (SEQ ID NO: 185) | $X_1LX_2YYGM$ DV, wherein $X_1$ is A or W; and $X_2$ is D or S (SEQ ID NO: 181) | SQSLLDSD DGNTY (SEQ ID NO: 150) | TLS (SEQ ID NO: 151) | $RX_1X_2FP$ $X_3I$, wherein $X_1$ is L or I; $X_2$ is E or G; and $X_3$ is S or absent (SEQ ID NO: 186) |

**Table 15: IMGT CDRs of exemplary hybridoma-derived anti-BCMA molecules**

| IMGT | HCDR1 | HCDR2 | HCDR3 | LCDR1 | LCDR2 | LCDR3 |
|---|---|---|---|---|---|---|
| Hy03 | GFTFSGF W (SEQ ID NO: 142) | IKQDGSEK (SEQ ID NO: 143) | ARALDYYG MDV (SEQ ID NO: 144) | QSLLDSDD GNTY (SEQ ID NO: 153) | TLS (SEQ ID NO: 151) | TQRLEFPS IT (SEQ ID NO: 149) |
| Hy52 | GFTFSSFR (SEQ ID NO: 165) | ISSSSSYI (SEQ ID NO: 166) | ARWLSYYG MDV (SEQ ID NO: 167) | QSLLDSDD GNTY (SEQ ID NO: 153) | TLS (SEQ ID NO: 151) | MQRIGFPI T (SEQ ID NO: 171) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Consensus | GFTFSX$_1$F X$_2$, wherein X$_1$ is G or S; and X$_2$ is W or R (SEQ ID NO: 187) | IX$_1$X$_2$X$_3$X$_4$SX$_5$X$_6$, wherein X$_1$ is K or S; X$_2$ is Q or S; X$_3$ is D or S; X$_4$ is G or S; X$_5$ is E or Y; and X$_6$ is K or I (SEQ ID NO: 188) | ARX$_1$LX$_2$YY GMDV, wherein X$_1$ is A or W; and X$_2$ is D or S (SEQ ID NO: 189) | QSLLDSDD GNTY (SEQ ID NO: 153) | TLS (SEQ ID NO: 151) | X$_1$QRX$_2$X$_3$ FPX$_4$IT, wherein X$_1$ is T or M; X$_2$ is L or I; X$_3$ is E or G; and X$_4$ is S or absent (SEQ ID NO: 183) |

In some embodiments, the human anti-BCMA binding domain comprises a HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3.

In certain embodiments, the CAR molecule described herein or the anti-BCMA binding domain described herein includes:

(1) one, two, or three light chain (LC) CDRs chosen from:

(i) a LC CDR1 of SEQ ID NO: 54, LC CDR2 of SEQ ID NO: 55 and LC CDR3 of SEQ ID NO: 56; and/or

(2) one, two, or three heavy chain (HC) CDRs from one of the following:

(i) a HC CDR1 of SEQ ID NO: 44, HC CDR2 of SEQ ID NO: 45 and HC CDR3 of SEQ ID NO: 84; (ii) a HC CDR1 of SEQ ID NO: 44, HC CDR2 of SEQ ID NO: 45 and HC CDR3 of SEQ ID NO: 46; (iii) a HC CDR1 of SEQ ID NO: 44, HC CDR2 of SEQ ID NO: 45 and HC CDR3 of SEQ ID NO: 68; or (iv) a HC CDR1 of SEQ ID NO: 44, HC CDR2 of SEQ ID NO: 45 and HC CDR3 of SEQ ID NO: 76.

In certain embodiments, the CAR molecule described herein or the anti-BCMA binding domain described herein includes:

(1) one, two, or three light chain (LC) CDRs from one of the following:

(i) a LC CDR1 of SEQ ID NO: 95, LC CDR2 of SEQ ID NO: 131 and LC CDR3 of SEQ ID NO: 132; (ii) a LC CDR1 of SEQ ID NO: 95, LC CDR2 of SEQ ID NO: 96 and LC CDR3 of SEQ ID NO: 97; (iii) a LC CDR1 of SEQ ID NO: 95, LC CDR2 of SEQ ID NO: 114 and LC CDR3 of SEQ ID NO: 115; or (iv) a LC CDR1 of SEQ ID NO: 95, LC CDR2 of SEQ ID NO: 114 and LC CDR3 of SEQ ID NO: 97; and/or

(2) one, two, or three heavy chain (HC) CDRs from one of the following:

(i) a HC CDR1 of SEQ ID NO: 86, HC CDR2 of SEQ ID NO: 130 and HC CDR3 of SEQ ID NO: 88; (ii) a HC CDR1 of SEQ ID NO: 86, HC CDR2 of SEQ ID NO: 87 and HC

CDR3 of SEQ ID NO: 88; or (iii) a HC CDR1 of SEQ ID NO: 86, HC CDR2 of SEQ ID NO: 109 and HC CDR3 of SEQ ID NO: 88.

In certain embodiments, the CAR molecule described herein or the anti-BCMA binding domain described herein includes:

(1) one, two, or three light chain (LC) CDRs from one of the following:

(i) a LC CDR1 of SEQ ID NO: 147, LC CDR2 of SEQ ID NO: 182 and LC CDR3 of SEQ ID NO: 183; (ii) a LC CDR1 of SEQ ID NO: 147, LC CDR2 of SEQ ID NO: 148 and LC CDR3 of SEQ ID NO: 149; or (iii) a LC CDR1 of SEQ ID NO: 147, LC CDR2 of SEQ ID NO: 170 and LC CDR3 of SEQ ID NO: 171; and/or

(2) one, two, or three heavy chain (HC) CDRs from one of the following:

(i) a HC CDR1 of SEQ ID NO: 179, HC CDR2 of SEQ ID NO: 180 and HC CDR3 of SEQ ID NO: 181; (ii) a HC CDR1 of SEQ ID NO: 137, HC CDR2 of SEQ ID NO: 138 and HC CDR3 of SEQ ID NO: 139; or (iii) a HC CDR1 of SEQ ID NO: 160, HC CDR2 of SEQ ID NO: 161 and HC CDR3 of SEQ ID NO: 162.

In some embodiments, the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of SEQ ID NOs: 44, 45, 84, 54, 55, and 56, respectively. In some embodiments, the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of SEQ ID NOs: 44, 45, 46, 54, 55, and 56, respectively. In some embodiments, the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of SEQ ID NOs: 44, 45, 68, 54, 55, and 56, respectively. In some embodiments, the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of SEQ ID NOs: 44, 45, 76, 54, 55, and 56, respectively.

In some embodiments, the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of SEQ ID NOs: 47, 48, 84, 57, 58, and 59, respectively. In some embodiments, the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of SEQ ID NOs: 47, 48, 46, 57, 58, and 59, respectively. In some embodiments, the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of SEQ ID NOs: 47, 48, 68, 57, 58, and 59, respectively. In some embodiments, the HC CDR1, HC CDR2, HC CDR3, LC

CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of SEQ ID NOs: 47, 48, 76, 57, 58, and 59, respectively.

In some embodiments, the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of SEQ ID NOs: 49, 50, 85, 60, 58, and 56, respectively. In some embodiments, the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of SEQ ID NOs: 49, 50, 51, 60, 58, and 56, respectively. In some embodiments, the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of SEQ ID NOs: 49, 50, 69, 60, 58, and 56, respectively. In some embodiments, the HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3 comprise the amino acid sequences of SEQ ID NOs: 49, 50, 77, 60, 58, and 56, respectively.

In some embodiments, the human anti-BCMA binding domain comprises a scFv comprising a VH (for example, a VH described herein) and VL (for example, a VL described herein). In some embodiments, the VH is attached to the VL via a linker, for example, a linker described herein, for example, a linker described in Table 1. In some embodiments, the human anti-BCMA binding domain comprises a $(Gly_4\text{-}Ser)n$ linker, wherein n is 1, 2, 3, 4, 5, or 6, preferably 3 or 4 (SEQ ID NO: 26). The light chain variable region and heavy chain variable region of a scFv can be, for example, in any of the following orientations: light chain variable region-linker-heavy chain variable region or heavy chain variable region-linker-light chain variable region.

In some embodiments, the anti-BCMA binding domain is a fragment, for example, a single chain variable fragment (scFv). In some embodiments, the anti-BCMA binding domain is a Fv, a Fab, a (Fab')2, or a bi-functional (for example bi-specific) hybrid antibody (for example, Lanzavecchia et al., Eur. J. Immunol. 17, 105 (1987)). In some embodiments, the antibodies and fragments thereof of the invention binds a BCMA protein with wild-type or enhanced affinity.

In some instances, scFvs can be prepared according to method known in the art (see, for example, Bird et al., (1988) Science 242:423-426 and Huston et al., (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). ScFv molecules can be produced by linking VH and VL regions together using flexible polypeptide linkers. The scFv molecules comprise a linker (for example, a Ser-Gly linker) with an optimized length and/or amino acid composition. The linker length

can greatly affect how the variable regions of a scFv fold and interact. In fact, if a short polypeptide linker is employed (for example, between 5-10 amino acids) intrachain folding is prevented. Interchain folding is also required to bring the two variable regions together to form a functional epitope binding site. For examples of linker orientation and size see, for example, Hollinger et al. 1993 Proc Natl Acad. Sci. U.S.A. 90:6444-6448, U.S. Patent Application Publication Nos. 2005/0100543, 2005/0175606, 2007/0014794, and PCT publication Nos. WO2006/020258 and WO2007/024715, is incorporated herein by reference.

An scFv can comprise a linker of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, or more amino acid residues between its VL and VH regions. The linker sequence may comprise any naturally occurring amino acid. In some embodiments, the linker sequence comprises amino acids glycine and serine. In some embodiments, the linker sequence comprises sets of glycine and serine repeats such as $(Gly_4Ser)n$, where n is a positive integer equal to or greater than 1 (SEQ ID NO: 25). In some embodiments, the linker can be $(Gly_4Ser)_4$ (SEQ ID NO: 27) or $(Gly_4Ser)_3$(SEQ ID NO: 28). Variation in the linker length may retain or enhance activity, giving rise to superior efficacy in activity studies.

**CD20 CAR**

In some embodiments, the CAR-expressing cell described herein is a CD20 CAR-expressing cell (for example, a cell expressing a CAR that binds to human CD20). In some embodiments, the CD20 CAR-expressing cell includes an antigen binding domain according to WO2016164731 and WO2018067992, incorporated herein by reference. Exemplary CD20-binding sequences or CD20 CAR sequences are disclosed in, for example, Tables 1-5 of WO2018067992. In some embodiments, the CD20 CAR comprises a CDR, variable region, scFv, or full-length sequence of a CD20 CAR disclosed in WO2018067992 or WO2016164731.

**CD22 CAR**

In some embodiments, the CAR-expressing cell described herein is a CD22 CAR-expressing cell (for example, a cell expressing a CAR that binds to human CD22). In some embodiments, the CD22 CAR-expressing cell includes an antigen binding domain according to WO2016164731 and WO2018067992, incorporated herein by reference. Exemplary CD22-binding sequences or CD22 CAR sequences are disclosed in, for example, Tables 6A, 6B, 7A,

7B, 7C, 8A, 8B, 9A, 9B, 10A, and 10B of WO2016164731 and Tables 6-10 of WO2018067992. In some embodiments, the CD22 CAR sequences comprise a CDR, variable region, scFv or full-length sequence of a CD22 CAR disclosed in WO2018067992 or WO2016164731.

In embodiments, the CAR molecule comprises an antigen binding domain that binds to CD22 (CD22 CAR). In some embodiments, the antigen binding domain targets human CD22. In some embodiments, the antigen binding domain includes a single chain Fv sequence as described herein.

The sequences of human CD22 CAR are provided below. In some embodiments, a human CD22 CAR is CAR22-65.

Human CD22 CAR scFv sequence

EVQLQQSGPGLVKPSQTLSLTCAISGDSMLSNSDTWNWIRQSPSRGLEWLGRTYHRST WYDDYASSVRGRVSINVDTSKNQYSLQLNAVTPEDTGVYYCARVRLQDGNSWSDAF DVWGQGTMVTVSSGGGGSGGGGSGGGGSQSALTQPASASGSPGQSVTISCTGTSSDV GGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEA DYYCSSYTSSSTLYVFGTGTQLTVL (SEQ ID NO: 285)

Human CD22 CAR heavy chain variable region

EVQLQQSGPGLVKPSQTLSLTCAISGDSMLSNSDTWNWIRQSPSRGLEWLGRTYHRST WYDDYASSVRGRVSINVDTSKNQYSLQLNAVTPEDTGVYYCARVRLQDGNSWSDAF DVWGQGTMVTVSS (SEQ ID NO 286)

Human CD22 CAR light chain variable region

QSALTQPASASGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPS GVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTLYVFGTGTQLTVL (SEQ ID NO 287)

**Table 16 Heavy Chain Variable Domain CDRs of CD22 CAR (CAR22-65)**

| Candidate | HCDR1 | SEQ ID NO: | HCDR2 | SEQ ID NO: | HCDR3 | SEQ ID NO: |
|---|---|---|---|---|---|---|
| CAR22-65 Combined | GDSML SNSDT WN | 288 | RTYHRSTWYDDYA SSVRG | 290 | VRLQDGNSWSD AFDV | 291 |
| CAR22-65 Kabat | SNSDT WN | 289 | RTYHRSTWYDDYA SSVRG | 290 | VRLQDGNSWSD AFDV | 291 |

**Table 17 Light Chain Variable Domain CDRs of CD22 CAR (CAR22-65). The LC CDR sequences in this table have the same sequence under the Kabat or combined definitions.**

| Candidate | LCDR1 | SEQ ID NO: | LCDR2 | SEQ ID NO: | LCDR3 | SEQ ID NO: |
|---|---|---|---|---|---|---|
| CAR22-65 Combined | TGTSSDVGGYNYVS | 95 | DVSNRPS | 96 | SSYTSSSTLYV | 97 |

In some embodiments, the antigen binding domain comprises a HC CDR1, a HC CDR2, and a HC CDR3 of any heavy chain binding domain amino acid sequences listed in **Table 16**. In embodiments, the antigen binding domain further comprises a LC CDR1, a LC CDR2, and a LC CDR3. In embodiments, the antigen binding domain comprises a LC CDR1, a LC CDR2, and a LC CDR3 amino acid sequences listed in **Table 17.**

In some embodiments, the antigen binding domain comprises one, two or all of LC CDR1, LC CDR2, and LC CDR3 of any light chain binding domain amino acid sequences listed in **Table 17**, and one, two or all of HC CDR1, HC CDR2, and HC CDR3 of any heavy chain binding domain amino acid sequences listed in **Table 16**.

In some embodiments, the CDRs are defined according to the Kabat numbering scheme, the Chothia numbering scheme, or a combination thereof.

The order in which the VL and VH domains appear in the scFv can be varied (i.e., VL-VH, or VH-VL orientation), and where any of one, two, three or four copies of the "G4S" subunit (SEQ ID NO: 25), in which each subunit comprises the sequence GGGGS (SEQ ID NO: 25) (for example, $(G4S)_3$ (SEQ ID NO: 28) or $(G4S)_4$ (SEQ ID NO: 27)), can connect the variable domains to create the entirety of the scFv domain. Alternatively, the CAR construct can include, for example, a linker including the sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 43). Alternatively, the CAR construct can include, for example, a linker including the sequence LAEAAAK (SEQ ID NO: 308). In some embodiments, the CAR construct does not include a linker between the VL and VH domains.

These clones all contained a Q/K residue change in the signal domain of the co-stimulatory domain derived from CD3zeta chain.

**EGFR CAR**

In some embodiments, the CAR-expressing cell described herein is an EGFR CAR-expressing cell (for example, a cell expressing a CAR that binds to human EGFR). In some embodiments, the CAR-expressing cell described herein is an EGFRvIII CAR-expressing cell (for example, a cell expressing a CAR that binds to human EGFRvIII). Exemplary EGFRvIII CARs can include sequences disclosed in WO2014/130657, for example, Table 2 of WO2014/130657, incorporated herein by reference.

Exemplary EGFRvIII-binding sequences or EGFR CAR sequences may comprise a CDR, a variable region, an scFv, or a full-length CAR sequence of a EGFR CAR disclosed in WO2014/130657.

**Mesothelin CAR**

In some embodiments, the CAR-expressing cell described herein is a mesothelin CAR-expressing cell (for example, a cell expressing a CAR that binds to human mesothelin). Exemplary mesothelin CARs can include sequences disclosed in WO2015090230 and WO2017112741, for example, Tables 2, 3, 4, and 5 of WO2017112741, incorporated herein by reference.

**Other exemplary CARs**

In other embodiments, the CAR-expressing cells can specifically bind to CD123, for example, can include a CAR molecule (for example, any of the CAR1 to CAR8), or an antigen binding domain according to Tables 1-2 of WO 2014/130635, incorporated herein by reference. The amino acid and nucleotide sequences encoding the CD123 CAR molecules and antigen binding domains (for example, including one, two, three VH CDRs; and one, two, three VL CDRs according to Kabat or Chothia), are specified in WO 2014/130635. In other embodiments, the CAR-expressing cells can specifically bind to CD123, for example, can include a CAR molecule (for example, any of the CAR123-1 to CAR123-4 and hzCAR123-1 to hzCAR123-32), or an antigen binding domain according to Tables 2, 6, and 9 of WO2016/028896, incorporated herein by reference. The amino acid and nucleotide sequences encoding the CD123 CAR molecules and antigen binding domains (for example, including one, two, three VH CDRs; and one, two, three VL CDRs according to Kabat or Chothia), are specified in WO2016/028896.

In some embodiments, the CAR molecule comprises a CLL1 CAR described herein, for example, a CLL1 CAR described in US2016/0051651A1, incorporated herein by reference. In embodiments, the CLL1 CAR comprises an amino acid, or has a nucleotide sequence shown in US2016/0051651A1, incorporated herein by reference. In other embodiments, the CAR-expressing cells can specifically bind to CLL-1, for example, can include a CAR molecule, or an antigen binding domain according to Table 2 of WO2016/014535, incorporated herein by reference. The amino acid and nucleotide sequences encoding the CLL-1 CAR molecules and antigen binding domains (for example, including one, two, three VH CDRs; and one, two, three VL CDRs according to Kabat or Chothia), are specified in WO2016/014535.

In some embodiments, the CAR molecule comprises a CD33 CAR described herein, e.ga CD33 CAR described in US2016/0096892A1, incorporated herein by reference. In embodiments, the CD33 CAR comprises an amino acid, or has a nucleotide sequence shown in US2016/0096892A1, incorporated herein by reference. In other embodiments, the CAR-expressing cells can specifically bind to CD33, for example, can include a CAR molecule (for example, any of CAR33-1 to CAR-33-9), or an antigen binding domain according to Table 2 or 9 of WO2016/014576, incorporated herein by reference. The amino acid and nucleotide sequences encoding the CD33 CAR molecules and antigen binding domains (for example, including one, two, three VH CDRs; and one, two, three VL CDRs according to Kabat or Chothia), are specified in WO2016/014576.

In some embodiments, the antigen binding domain comprises one, two three (for example, all three) heavy chain CDRs, HC CDR1, HC CDR2 and HC CDR3, from an antibody described herein (for example, an antibody described in WO2015/142675, US-2015-0283178-A1, US-2016-0046724-A1, US2014/0322212A1, US2016/0068601A1, US2016/0051651A1, US2016/0096892A1, US2014/0322275A1, or WO2015/090230, incorporated herein by reference), and/or one, two, three (for example, all three) light chain CDRs, LC CDR1, LC CDR2 and LC CDR3, from an antibody described herein (for example, an antibody described in WO2015/142675, US-2015-0283178-A1, US-2016-0046724-A1, US2014/0322212A1, US2016/0068601A1, US2016/0051651A1, US2016/0096892A1, US2014/0322275A1, or WO2015/090230, incorporated herein by reference). In some embodiments, the antigen binding domain comprises a heavy chain variable region and/or a variable light chain region of an antibody listed above.

In embodiments, the antigen binding domain is an antigen binding domain described in WO2015/142675, US-2015-0283178-A1, US-2016-0046724-A1, US2014/0322212A1, US2016/0068601A1, US2016/0051651A1, US2016/0096892A1, US2014/0322275A1, or WO2015/090230, incorporated herein by reference.

In embodiments, the antigen binding domain targets BCMA and is described in US-2016-0046724-A1. In embodiments, the antigen binding domain targets CD19 and is described in US-2015-0283178-A1. In embodiments, the antigen binding domain targets CD123 and is described in US2014/0322212A1, US2016/0068601A1. In embodiments, the antigen binding domain targets CLL1 and is described in US2016/0051651A1. In embodiments, the antigen binding domain targets CD33 and is described in US2016/0096892A1.

Exemplary target antigens that can be targeted using the CAR-expressing cells, include, but are not limited to, CD19, CD123, EGFRvIII, CD33, mesothelin, BCMA, and GFR ALPHA-4, among others, as described in, for example, WO2014/153270, WO 2014/130635, WO2016/028896, WO 2014/130657, WO2016/014576, WO 2015/090230, WO2016/014565, WO2016/014535, and WO2016/025880, each of which is herein incorporated by reference in its entirety.

In other embodiments, the CAR-expressing cells can specifically bind to GFR ALPHA-4, for example, can include a CAR molecule, or an antigen binding domain according to Table 2 of WO2016/025880, incorporated herein by reference. The amino acid and nucleotide sequences encoding the GFR ALPHA-4 CAR molecules and antigen binding domains (for example, including one, two, three VH CDRs; and one, two, three VL CDRs according to Kabat or Chothia), are specified in WO2016/025880.

In some embodiments, the antigen binding domain of any of the CAR molecules described herein (for example, any of CD19, CD123, EGFRvIII, CD33, mesothelin, BCMA, and GFR ALPHA-4) comprises one, two three (for example, all three) heavy chain CDRs, HC CDR1, HC CDR2 and HC CDR3, from an antibody listed above, and/or one, two, three (for example, all three) light chain CDRs, LC CDR1, LC CDR2 and LC CDR3, from an antigen binding domain listed above. In some embodiments, the antigen binding domain comprises a heavy chain variable region and/or a variable light chain region of an antibody listed or described above.

In some embodiments, the antigen binding domain comprises one, two three (for example, all three) heavy chain CDRs, HC CDR1, HC CDR2 and HC CDR3, from an antibody listed above, and/or one, two, three (for example, all three) light chain CDRs, LC CDR1, LC CDR2 and LC CDR3, from an antibody listed above. In some embodiments, the antigen binding domain comprises a heavy chain variable region and/or a variable light chain region of an antibody listed or described above.

In some embodiments, the tumor antigen is a tumor antigen described in International Application WO2015/142675, filed March 13, 2015, which is herein incorporated by reference in its entirety. In some embodiments, the tumor antigen is chosen from one or more of: CD19; CD123; CD22; CD30; CD171; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33; epidermal growth factor receptor variant III (EGFRvIII); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlcp(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAcα-Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDGalp(1-4)bDGlcp(1-1)Cer); transglutaminase 5 (TGS5);

high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycoceramide (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; surviving; telomerase; prostate carcinoma tumor antigen-1 (PCTA-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b;

CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1).

In some embodiments, the antigen binding domain comprises one, two three (for example, all three) heavy chain CDRs, HC CDR1, HC CDR2 and HC CDR3, from an antibody listed above, and/or one, two, three (for example, all three) light chain CDRs, LC CDR1, LC CDR2 and LC CDR3, from an antibody listed above. In some embodiments, the antigen binding domain comprises a heavy chain variable region and/or a variable light chain region of an antibody listed or described above.

In some embodiments, the anti-tumor antigen binding domain is a fragment, for example, a single chain variable fragment (scFv). In some embodiments, the anti-a cancer associate antigen as described herein binding domain is a Fv, a Fab, a (Fab')2, or a bi-functional (for example bi-specific) hybrid antibody (for example, Lanzavecchia et al., Eur. J. Immunol. 17, 105 (1987)). In some embodiments, the antibodies and fragments thereof of the invention binds a cancer associate antigen as described herein protein with wild-type or enhanced affinity.

In some instances, scFvs can be prepared according to a method known in the art (see, for example, Bird et al., (1988) Science 242:423-426 and Huston et al., (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). ScFv molecules can be produced by linking VH and VL regions together using flexible polypeptide linkers. The scFv molecules comprise a linker (for example, a Ser-Gly linker) with an optimized length and/or amino acid composition. The linker length can greatly affect how the variable regions of a scFv fold and interact. In fact, if a short polypeptide linker is employed (for example, between 5-10 amino acids) intrachain folding is prevented. Interchain folding is also required to bring the two variable regions together to form a functional epitope binding site. For examples of linker orientation and size see, for example, Hollinger et al. 1993 Proc Natl Acad. Sci. U.S.A. 90:6444-6448, U.S. Patent Application

Publication Nos. 2005/0100543, 2005/0175606, 2007/0014794, and PCT publication Nos. WO2006/020258 and WO2007/024715, which are incorporated herein by reference.

An scFv can comprise a linker of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, or more amino acid residues between its VL and VH regions. The linker sequence may comprise any naturally occurring amino acid. In some embodiments, the linker sequence comprises amino acids glycine and serine. In some embodiments, the linker sequence comprises sets of glycine and serine repeats such as $(Gly_4Ser)n$, where n is a positive integer equal to or greater than 1 (SEQ ID NO: 25). In some embodiments, the linker can be $(Gly_4Ser)_4$ (SEQ ID NO: 27) or $(Gly_4Ser)_3$(SEQ ID NO: 28). Variation in the linker length may retain or enhance activity, giving rise to superior efficacy in activity studies.

In some embodiments, the antigen binding domain is a T cell receptor ("TCR"), or a fragment thereof, for example, a single chain TCR (scTCR). Methods to make such TCRs are known in the art. See, for example, Willemsen RA et al, Gene Therapy 7: 1369–1377 (2000); Zhang T et al, Cancer Gene Ther 11: 487–496 (2004); Aggen et al, Gene Ther. 19(4):365-74 (2012) (references are incorporated herein by its entirety). For example, scTCR can be engineered that contains the Vα and Vβ genes from a T cell clone linked by a linker (for example, a flexible peptide). This approach is very useful to cancer associated target that itself is intracellular, however, a fragment of such antigen (peptide) is presented on the surface of the cancer cells by MHC.

**Transmembrane domain**

With respect to the transmembrane domain, in various embodiments, a CAR can be designed to comprise a transmembrane domain that is attached to the extracellular domain of the CAR. A transmembrane domain can include one or more additional amino acids adjacent to the transmembrane region, for example, one or more amino acid associated with the extracellular region of the protein from which the transmembrane was derived (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the extracellular region) and/or one or more additional amino acids associated with the intracellular region of the protein from which the transmembrane protein is derived (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the intracellular region). In some embodiments, the transmembrane domain is one that is

associated with one of the other domains of the CAR is used. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins, for example, to minimize interactions with other members of the receptor complex. In some embodiments, the transmembrane domain is capable of homodimerization with another CAR on the CAR-expressing cell, for example, CART cell, surface. In some embodiments the amino acid sequence of the transmembrane domain may be modified or substituted so as to minimize interactions with the binding domains of the native binding partner present in the same CAR-expressing cell, for example, CART.

The transmembrane domain may be derived either from a natural or from a recombinant source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. In some embodiments the transmembrane domain is capable of signaling to the intracellular domain(s) whenever the CAR has bound to a target. A transmembrane domain of particular use in this invention may include at least the transmembrane region(s) of, for example, the alpha, beta or zeta chain of T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8 (for example, CD8 alpha, CD8 beta), CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. In some embodiments, a transmembrane domain may include at least the transmembrane region(s) of a costimulatory molecule, for example, MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG

(CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

In some instances, the transmembrane domain can be attached to the extracellular region of the CAR, for example, the antigen binding domain of the CAR, via a hinge, for example, a hinge from a human protein. For example, in some embodiments, the hinge can be a human Ig (immunoglobulin) hinge, for example, an IgG4 hinge, or a CD8a hinge. In some embodiments, the hinge or spacer comprises (for example, consists of) the amino acid sequence of SEQ ID NO: 2. In some embodiments, the transmembrane domain comprises (for example, consists of) a transmembrane domain of SEQ ID NO: 6.

In some embodiments, the hinge or spacer comprises an IgG4 hinge. For example, in some embodiments, the hinge or spacer comprises a hinge of SEQ ID NO: 3. In some embodiments, the hinge or spacer comprises a hinge encoded by the nucleotide sequence of SEQ ID NO: 14.

In some embodiments, the hinge or spacer comprises an IgD hinge. For example, in some embodiments, the hinge or spacer comprises a hinge of the amino acid sequence of SEQ ID NO: 4. In some embodiments, the hinge or spacer comprises a hinge encoded by the nucleotide sequence of SEQ ID NO:15.

In some embodiments, the transmembrane domain may be recombinant, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. In some embodiments, a triplet of phenylalanine, tryptophan and valine can be found at each end of a recombinant transmembrane domain.

Optionally, a short oligo- or polypeptide linker, between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the cytoplasmic region of the CAR. A glycine-serine doublet provides a particularly suitable linker. For example, in some embodiments, the linker comprises the amino acid sequence of SEQ ID NO: 5. In some embodiments, the linker is encoded by a nucleotide sequence of SEQ ID NO: 16.

In some embodiments, the hinge or spacer comprises a KIR2DS2 hinge.

**Cytoplasmic domain**

The cytoplasmic domain or region of a CAR of the present invention includes an intracellular signaling domain. An intracellular signaling domain is generally responsible for

activation of at least one of the normal effector functions of the immune cell in which the CAR has been introduced.

Examples of intracellular signaling domains for use in the CAR of the invention include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any recombinant sequence that has the same functional capability.

It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary and/or costimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation through the TCR (primary intracellular signaling domains) and those that act in an antigen-independent manner to provide a secondary or costimulatory signal (secondary cytoplasmic domain, for example, a costimulatory domain).

A primary signaling domain regulates primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary intracellular signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

Examples of ITAM containing primary intracellular signaling domains that are of particular use in the invention include those of TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as “ICOS”), FcεRI, DAP10, DAP12, and CD66d. In some embodiments, a CAR of the invention comprises an intracellular signaling domain, for example, a primary signaling domain of CD3-zeta.

In some embodiments, a primary signaling domain comprises a modified ITAM domain, for example, a mutated ITAM domain which has altered (for example, increased or decreased) activity as compared to the native ITAM domain. In some embodiments, a primary signaling domain comprises a modified ITAM-containing primary intracellular signaling domain, for example, an optimized and/or truncated ITAM-containing primary intracellular signaling domain. In some embodiments, a primary signaling domain comprises one, two, three, four or more ITAM motifs.

Further examples of molecules containing a primary intracellular signaling domain that are of particular use in the invention include those of DAP10, DAP12, and CD32.

The intracellular signaling domain of the CAR can comprise the primary signaling domain, for example, CD3-zeta signaling domain, by itself or it can be combined with any other desired intracellular signaling domain(s) useful in the context of a CAR of the invention. For example, the intracellular signaling domain of the CAR can comprise a primary signaling domain, for example, CD3 zeta chain portion, and a costimulatory signaling domain. The costimulatory signaling domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or its ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such molecules include MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83, and the like. For example, CD27 costimulation has been demonstrated to enhance expansion, effector function, and survival of human CART cells in vitro and augments human T cell persistence and antitumor activity in vivo (Song et al. Blood. 2012; 119(3):696-706). The intracellular signaling sequences within the cytoplasmic portion of the CAR of the invention may be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, for example, between 2 and 10 amino acids (for example, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) in length may form the linkage between intracellular signaling sequence. In some embodiments, a glycine-serine

doublet can be used as a suitable linker. In some embodiments, a single amino acid, for example, an alanine, a glycine, can be used as a suitable linker.

In some embodiments, the intracellular signaling domain is designed to comprise two or more, for example, 2, 3, 4, 5, or more, costimulatory signaling domains. In some embodiments, the two or more, for example, 2, 3, 4, 5, or more, costimulatory signaling domains, are separated by a linker molecule, for example, a linker molecule described herein. In some embodiments, the intracellular signaling domain comprises two costimulatory signaling domains. In some embodiments, the linker molecule is a glycine residue. In some embodiments, the linker is an alanine residue.

In some embodiments, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In some embodiments, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of 4-1BB. In some embodiments, the signaling domain of 4-1BB is a signaling domain of SEQ ID NO: 7. In some embodiments, the signaling domain of CD3-zeta is a signaling domain of SEQ ID NO: 9 (mutant CD3zeta) or SEQ ID NO: 10 (wild type human CD3zeta).

In some embodiments, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD27. In some embodiments, the signaling domain of CD27 comprises the amino acid sequence of SEQ ID NO: 8. In some embodiments, the signaling domain of CD27 is encoded by the nucleic acid sequence of SEQ ID NO: 19.

In some embodiments, the intracellular is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In some embodiments, the signaling domain of CD28 comprises the amino acid sequence of SEQ ID NO: 36. In some embodiments, the signaling domain of CD28 is encoded by the nucleic acid sequence of SEQ ID NO: 37.
In some embodiments, the intracellular is designed to comprise the signaling domain of CD3-zeta and the signaling domain of ICOS. In some embodiments, the signaling domain of ICOS comprises the amino acid sequence of SEQ ID NO: 38. In some embodiments, the signaling domain of ICOS is encoded by the nucleic acid sequence of SEQ ID NO: 39.

**Co-expression of CAR with Other Molecules or Agents**

**Co-expression of a Second CAR**

# DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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# JUMBO APPLICATIONS/PATENTS

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NOTE POUR LE TOME / VOLUME NOTE:

**What is claimed is:**

1. A method of making a population of cells (for example, T cells) that express a chimeric antigen receptor (CAR), the method comprising:

(i) contacting (for example, binding) a population of cells (for example, T cells, for example, T cells isolated from a frozen or fresh leukapheresis product) with a multispecific binding molecule comprising (A) an anti-CD3 binding domain, (B) a costimulatory molecule binding domain (e.g., an anti-CD2 binding domain or an anti-CD28 binding domain), and (C) an Fc region comprising:

a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system;

a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system;

a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system;

a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system;

a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system;

a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or

a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system;

(ii) contacting the population of cells (for example, T cells) with a nucleic acid molecule (for example, a DNA or RNA molecule) encoding the CAR, thereby providing a population of cells (for example, T cells) comprising the nucleic acid molecule, and

(iii) harvesting the population of cells (for example, T cells) for storage (for example, reformulating the population of cells in cryopreservation media) or administration, wherein:

(a) step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), for example, no later than 12, 13, 14, 15, 16, 17, or 18 hours after the beginning of step (i), for example, no later than 18 hours after the beginning of step (i), and

step (iii) is performed no later than 30 (for example, 26) hours after the beginning of step (i), for example, no later than 22, 23, 24, 25, 26, 27, 28, 29, or 30 hours after the beginning of step (i), for example, no later than 24 hours after the beginning of step (i),

(b) step (ii) is performed together with step (i) or no later than 20 hours after the beginning of step (i), for example, no later than 12, 13, 14, 15, 16, 17, or 18 hours after the beginning of step (i), for example, no later than 18 hours after the beginning of step (i), and

step (iii) is performed no later than 30 hours after the beginning of step (ii), for example, no later than 22, 23, 24, 25, 26, 27, 28, 29, or 30 hours after the beginning of step (ii), or

(c) the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (i),

optionally wherein the nucleic acid molecule in step (ii) is on a viral vector, optionally wherein the nucleic acid molecule in step (ii) is an RNA molecule on a viral vector, optionally wherein step (ii) comprises transducing the population of cells (for example, T cells) with a viral vector comprising a nucleic acid molecule encoding the CAR.

2. The method of claim 1, wherein:

(i) the anti-CD3 binding domain, e.g., an anti-CD3 scFv, is situated N-terminal of the costimulatory molecule binding domain, e.g., an anti-CD2 Fab or an anti-CD28 Fab; or

(ii) the anti-CD3 binding domain, e.g., an anti-CD3 scFv, is situated C-terminal of the costimulatory molecule binding domain, e.g., an anti-CD2 Fab or an anti-CD28 Fab.

3. The method of claim 1 or 2, wherein the Fc region comprises a CH2.

4. The method of any one of claims 1-3, wherein the Fc region comprises a CH3.

5. The method of any one of claims 1-4, wherein the anti-CD3 binding domain is situated C-terminal of the Fc region.

6. The method of any one of claims 1-4, wherein the anti-CD3 binding domain is situated N-terminal of the Fc region.

7. The method of any one of claims 1-6, wherein the Fc region is situated between the anti-CD3 binding domain and the costimulatory molecule binding domain.

8. The method of any one of claims 1-4 or 6, wherein the multispecific binding molecule comprises:

(i) a first polypeptide comprising from N-terminal to C-terminal: VH of the anti-CD3 binding domain, VL of the anti-CD3 binding domain, VH of the costimulatory molecule binding domain, CH1, CH2, and CH3; and

(ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the costimulatory molecule binding domain and CL.

9. The method of any one of claims 1-5 or 7, wherein the multispecific binding molecule comprises:

(i) a first polypeptide comprising from N-terminal to C-terminal: VH of the costimulatory molecule binding domain, CH1, CH2, CH3, VH of the anti-CD3 binding domain, and VL of the anti-CD3 binding domain; and

(ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the costimulatory molecule binding domain and CL.

10. The method of any one of claims 1-4 or 6, wherein the multispecific binding molecule comprises:

(i) a first polypeptide comprising from N-terminal to C-terminal: VH of the costimulatory molecule binding domain, CH1, VH of the anti-CD3 binding domain, VL of the anti-CD3 binding domain, CH2, and CH3; and

(ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the costimulatory molecule binding domain and CL.

11. The method of any one of claims 1-10, wherein the anti-CD3 binding domain comprises an scFv and the costimulatory molecule binding domain is part of a Fab fragment.

12. The method of any one of claims 1-11, wherein the anti-CD3 binding domain comprises:

(i) a variable heavy chain region (VH) comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, and a HCDR3, and a light chain variable region (VL) comprising a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3 of an anti-CD3 antibody molecule of Table 27 (for example the anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4)); and/or

(ii) the amino acid sequence of any VH and/or VL region of an anti-CD3 antibody molecule provided in Table 27 (for example the anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4)), or an amino acid sequence at least 95% identical thereto.

13. The method of any one of claims 1-12, wherein the costimulatory molecule binding domain is an anti-CD2 binding domain, optionally wherein the anti-CD2 binding domain comprises:

(i) a VH comprising a HCDR1, a HCDR2, and a HCDR3, and a VL comprising a LCDR1, a LCDR2, and a LCDR3 of an anti-CD2 antibody molecule of Table 27 (for example the anti-CD2 (1)); and/or

(ii) the amino acid sequence of any VH and/or VL region of an anti-CD2 antibody molecule provided in Table 27 (for example the anti-CD2 (1)), or an amino acid sequence at least 95% identical thereto.

14. The method of any one of claims 1-13, wherein the costimulatory molecule binding domain is an anti-CD28 binding domain, optionally wherein the anti-CD28 binding domain comprises:

(i) a VH comprising a HCDR1, a HCDR2, and a HCDR3, and a VL comprising a LCDR1, a LCDR2, and a LCDR3 of an anti-CD28 antibody molecule of Table 27 (for example the anti-CD28 (1) or anti-CD28 (2)); and/or

(ii) the amino acid sequence of any VH and/or VL region of an anti-CD28 antibody molecule provided in Table 27 (for example the anti-CD28 (1) or anti-CD28 (2)), or an amino acid sequence at least 95% identical thereto.

15. The method of any one of claims 1-14, wherein the anti-CD3 binding domain comprises:

(i) an scFv;

(ii) a VH linked to a VL by a peptide linker, e.g., a glycine-serine linker, e.g., a $(G_4S)_4$ linker; or

(iii) a VH and a VL, wherein the VH is N-terminal of the VL.

16. The method of any one of claims 1-15, wherein the costimulatory molecule binding domain is part of a Fab fragment, e.g., a Fab fragment that is part of a polypeptide sequence that comprises the Fc region.

17. The method of any one of claims 1-16, wherein the anti-CD3 binding domain is situated N-terminal of the costimulatory molecule binding domain, optionally wherein the anti-CD3 binding domain is linked to the costimulatory molecule binding domain by a peptide linker, e.g., a glycine-serine linker, e.g., a $(G_4S)_4$ linker.

18. The method of any one of claims 1-7 or 9-17, wherein the anti-CD3 binding domain is situated C-terminal of the costimulatory molecule binding domain.

19. The method of claim 18, wherein:

(i) the Fc region is situated between the anti-CD3 binding domain and the costimulatory molecule binding domain; and/or

(ii) the multispecific binding molecule comprises one or both of a CH2 and a CH3, optionally wherein the anti-CD3 binding domain is linked to the CH3 by a peptide linker, e.g., a glycine-serine linker, e.g., a $(G_4S)_4$ linker.

20. The method of claim 18, wherein:

(i) the multispecific binding molecule comprises a CH2, and the anti-CD3 binding domain is situated N-terminal of the CH2;

(ii) the anti-CD3 binding domain is linked to a CH1 by a peptide linker, e.g., a glycine-serine linker, e.g., a $(G_4S)_2$ linker; and/or

(iii) the anti-CD3 binding domain is linked to a CH2 by a peptide linker, e.g., a glycine-serine linker, e.g., a $(G_4S)_4$ linker.

21. The method of any one of claims 1-20, wherein step (i) increases the percentage of CAR-expressing cells in the population of cells from step (iii), for example, the population of cells from step (iii) shows a higher percentage of CAR-expressing cells (for example, at least 10, 20,

30, 40, 50, or 60% higher), compared with cells made by an otherwise similar method without step (i).

22. The method of any one of claims 1-21, wherein:

(a) the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) is the same as or differs by no more than 5 or 10% from the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (i);

(b) the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) is increased by, for example, at least 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, or 3-fold, as compared to the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (i);

(c) the percentage of CAR-expressing naïve T cells, for example, CAR-expressing CD45RA+ CD45RO- CCR7+ T cells in the population of cells increases during the duration of step (ii), for example, increases by, for example, at least 30, 35, 40, 45, 50, 55, or 60%, between 18-24 hours after the beginning of step (ii); or

(d) the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) does not decrease, or decreases by no more than 5 or 10%, as compared to the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ cells, in the population of cells at the beginning of step (i).

23. The method of any one of claims 1-22, wherein:

(a) the population of cells from step (iii) shows a higher percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells (for example, at least 10, 20, 30, or 40% higher), compared with cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(b) the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) is higher (for example, at

least 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, or 3-fold higher) than the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(c) the percentage of CAR-expressing naïve T cells, for example, CAR-expressing CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) is higher (for example, at least 4, 6, 8, 10, or 12-fold higher) than the percentage of CAR-expressing naïve T cells, for example, CAR-expressing CD45RA+ CD45RO- CCR7+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(d) the population of cells from step (iii) shows a higher percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells (for example, at least 10, 20, 30, or 40% higher), compared with cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(e) the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) is higher (for example, at least 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, or 3-fold higher) than the percentage of naïve cells, for example, naïve T cells, for example, CD45RA+ CD45RO- CCR7+ T cells, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days; or

(f) the percentage of CAR-expressing naïve T cells, for example, CAR-expressing CD45RA+ CD45RO- CCR7+ T cells, in the population of cells from step (iii) is higher (for example, at least 4, 6, 8, 10, or 12-fold higher) than the percentage of CAR-expressing naïve T cells, for example, CAR-expressing CD45RA+ CD45RO- CCR7+ T cells, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

24. The method of any one of claims 1-23, wherein:

(a) the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells from step (iii) is the same as or differs by no more than 5 or 10% from the percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells, in the population of cells at the beginning of step (i);

(b) the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in the population of cells from step (iii) is reduced by at least 20, 25, 30, 35, 40, 45, or 50%, as compared to the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in the population of cells at the beginning of step (i);

(c) the percentage of CAR-expressing central memory T cells, for example, CAR-expressing CCR7+CD45RO+ cells, decreases during the duration of step (ii), for example, decreases by, for example, at least 8, 10, 12, 14, 16, 18, or 20%, between 18-24 hours after the beginning of step (ii); or

(d) the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in the population of cells from step (iii) does not increase, or increases by no more than 5 or 10%, as compared to the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in the population of cells at the beginning of step (i).

25. The method of any one of claims 1-24, wherein:

(a) the population of cells from step (iii) shows a lower percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells (for example, at least 10, 20, 30, or 40% lower), compared with cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(b) the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells in the population of cells from step (iii) is lower (for example, at least 20, 30, 40, or 50% lower) than the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the

beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(c) the percentage of CAR-expressing central memory T cells, for example, CAR-expressing CCR7+CD45RO+ T cells in the population of cells from step (iii) is lower (for example, at least 10, 20, 30, or 40% lower) than the percentage of CAR-expressing central memory T cells, for example, CAR-expressing CCR7+CD45RO+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(d) the population of cells from step (iii) shows a lower percentage of central memory cells, for example, central memory T cells, for example, CD95+ central memory T cells (for example, at least 10, 20, 30, or 40% lower), compared with cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(e) the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells in the population of cells from step (iii) is lower (for example, at least 20, 30, 40, or 50% lower) than the percentage of central memory cells, for example, central memory T cells, for example, CCR7+CD45RO+ T cells, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days; or

(f) the percentage of CAR-expressing central memory T cells, for example, CAR-expressing CCR7+CD45RO+ T cells in the population of cells from step (iii) is lower (for example, at least 10, 20, 30, or 40% lower) than the percentage of CAR-expressing central memory T cells, for example, CAR-expressing CCR7+CD45RO+ T cells, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

26. The method of any one of claims 1-25, wherein:

(a) the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is increased, as compared to the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells at the beginning of step (i);

(b) the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is increased, as compared to the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells at the beginning of step (i);

(c) the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is higher than the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i); or

(d) the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is higher than the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i);

(e) the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is higher than the percentage of stem memory T cells, for example, CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days; or

(f) the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in the population of cells from step (iii) is higher than the percentage of CAR-expressing stem memory T cells, for example, CAR-expressing CD45RA+CD95+IL-2 receptor β+CCR7+CD62L+ T cells, in cells

made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

27. The method of any one of claims 1-26, wherein:

(a) the median GeneSetScore (Up TEM vs. Down TSCM) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 75, 100, or 125% from the median GeneSetScore (Up TEM vs. Down TSCM) of the population of cells at the beginning of step (i);

(b) the median GeneSetScore (Up TEM vs. Down TSCM) of the population of cells from step (iii) is lower (for example, at least about 100, 150, 200, 250, or 300% lower) than the median GeneSetScore (Up TEM vs. Down TSCM) of:

cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or

cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(c) the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 100, 150, or 200% from the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells at the beginning of step (i);

(d) the median GeneSetScore (Up Treg vs. Down Teff) of the population of cells from step (iii) is lower (for example, at least about 50, 100, 125, 150, or 175% lower) than the median GeneSetScore (Up Treg vs. Down Teff) of:

cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or

cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(e) the median GeneSetScore (Down stemness) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 25, 50, 100, 150, 200, or 250% from the median GeneSetScore (Down stemness) of the population of cells at the beginning of step (i);

(f) the median GeneSetScore (Down stemness) of the population of cells from step (iii) is lower (for example, at least about 50, 100, or 125% lower) than the median GeneSetScore (Down stemness) of:

cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or

cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(g) the median GeneSetScore (Up hypoxia) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 125, 150, 175, or 200% from the median GeneSetScore (Up hypoxia) of the population of cells at the beginning of step (i);

(h) the median GeneSetScore (Up hypoxia) of the population of cells from step (iii) is lower (for example, at least about 40, 50, 60, 70, or 80% lower) than the median GeneSetScore (Up hypoxia) of:

cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or

cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days;

(j) the median GeneSetScore (Up autophagy) of the population of cells from step (iii) is about the same as or differs by no more than (for example, increased by no more than) about 180, 190, 200, or 210% from the median GeneSetScore (Up autophagy) of the population of cells at the beginning of step (i); or

(k) the median GeneSetScore (Up autophagy) of the population of cells from step (iii) is lower (for example, at least 20, 30, or 40% lower) than the median GeneSetScore (Up autophagy) of:

cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or

cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

28. The method of any one of claims 1-27, wherein the population of cells from step (iii), after being incubated with a cell expressing an antigen recognized by the CAR, secretes IL-2 at a higher level (for example, at least 2, 4, 6, 8, 10, 12, or 14-fold higher) than cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days, for example, as assessed using methods described in Example 8 with respect to FIGs. 29C-29D.

29. The method of any one of claims 1-28, wherein the population of cells from step (iii), after being administered in vivo, persists longer or expands at a higher level (for example, as assessed using methods described in Example 1 with respect to FIG. 4C), compared with cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or compared with cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

30. The method of any one of claims 1-29, wherein the population of cells from step (iii), after being administered in vivo, shows a stronger anti-tumor activity (for example, a stronger anti-tumor activity at a low dose, for example, a dose no more than $0.15 \times 10^6$, $0.2 \times 10^6$, $0.25 \times 10^6$,

or 0.3 x $10^6$ viable CAR-expressing cells) than cells made by an otherwise similar method in which step (iii) is performed more than 26 hours after the beginning of step (i), for example, more than 5, 6, 7, 8, 9, 10, 11, or 12 days after the beginning of step (i), or cells made by an otherwise similar method which further comprises, after step (ii) and prior to step (iii), expanding the population of cells (for example, T cells) in vitro for more than 3 days, for example, for 5, 6, 7, 8 or 9 days.

31. The method of any one of claims 1-30, the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the beginning of step (i), optionally wherein the number of living cells in the population of cells from step (iii) decreases from the number of living cells in the population of cells at the beginning of step (i).

32. The method of any one of claims 1-31, wherein the population of cells from step (iii) are not expanded, or expanded by less than 2 hours, for example, less than 1 or 1.5 hours, compared to the population of cells at the beginning of step (i).

33. The method of any one of claims 1-32, wherein steps (i) and/or (ii) are performed in cell media (for example, serum-free media) comprising IL-2, IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)), IL-7, IL-21, IL-6 (for example, IL-6/sIL-6Ra), a LSD1 inhibitor, a MALT1 inhibitor, or a combination thereof.

34. The method of any one of claims 1-33, wherein steps (i) and/or (ii) are performed in serum-free cell media comprising a serum replacement.

35. The method of claim 30, wherein the serum replacement is CTS™ Immune Cell Serum Replacement (ICSR).

36. The method of any one of claims 1-35, further comprising prior to step (i):

(iv) (optionally) receiving a fresh leukapheresis product (or an alternative source of hematopoietic tissue such as a fresh whole blood product, a fresh bone marrow product, or a

fresh tumor or organ biopsy or removal (for example, a fresh product from thymectomy)) from an entity, for example, a laboratory, hospital, or healthcare provider, and

(v) isolating the population of cells (for example, T cells, for example, CD8+ and/or CD4+ T cells) contacted in step (i) from a fresh leukapheresis product (or an alternative source of hematopoietic tissue such as a fresh whole blood product, a fresh bone marrow product, or a fresh tumor or organ biopsy or removal (for example, a fresh product from thymectomy)), optionally wherein:

step (iii) is performed no later than 35 hours after the beginning of step (v), for example, no later than 27, 28, 29, 30, 31, 32, 33, 34, or 35 hours after the beginning of step (v), for example, no later than 30 hours after the beginning of step (v), or

the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the end of step (v).

37. The method of any one of claims 1-36, further comprising prior to step (i): receiving cryopreserved T cells isolated from a leukapheresis product (or an alternative source of hematopoietic tissue such as cryopreserved T cells isolated from whole blood, bone marrow, or tumor or organ biopsy or removal (for example, thymectomy)) from an entity, for example, a laboratory, hospital, or healthcare provider.

38. The method of any one of claims 1-36, further comprising prior to step (i):

(iv) (optionally) receiving a cryopreserved leukapheresis product (or an alternative source of hematopoietic tissue such as a cryopreserved whole blood product, a cryopreserved bone marrow product, or a cryopreserved tumor or organ biopsy or removal (for example, a cryopreserved product from thymectomy)) from an entity, for example, a laboratory, hospital, or healthcare provider, and

(v) isolating the population of cells (for example, T cells, for example, CD8+ and/or CD4+ T cells) contacted in step (i) from a cryopreserved leukapheresis product (or an alternative source of hematopoietic tissue such as a cryopreserved whole blood product, a cryopreserved bone marrow product, or a cryopreserved tumor or organ biopsy or removal (for example, a cryopreserved product from thymectomy)), optionally wherein:

step (iii) is performed no later than 35 hours after the beginning of step (v), for example, no later than 27, 28, 29, 30, 31, 32, 33, 34, or 35 hours after the beginning of step (v), for example, no later than 30 hours after the beginning of step (v), or

the population of cells from step (iii) are not expanded, or expanded by no more than 5, 10, 15, 20, 25, 30, 35, or 40%, for example, no more than 10%, for example, as assessed by the number of living cells, compared to the population of cells at the end of step (v).

39. The method of any one of claims 1-38, further comprising step (vi):

culturing a portion of the population of cells from step (iii) for at least 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, or 7 days, for example, at least 2 days and no more than 7 days, and measuring CAR expression level in the portion (for example, measuring the percentage of viable, CAR-expressing cells in the portion), optionally wherein:

step (iii) comprises harvesting and freezing the population of cells (for example, T cells) and step (vi) comprises thawing a portion of the population of cells from step (iii), culturing the portion for at least 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, or 7 days, for example, at least 2 days and no more than 7 days, and measuring CAR expression level in the portion (for example, measuring the percentage of viable, CAR-expressing cells in the portion).

40. The method of any one of claims 1-39, wherein step (ii) further comprises adding F108 during transduction and/or contacting the population of cells (for example, T cells) with an shRNA that targets Tet2.

41. The method of any one of claims 1-40, wherein the population of cells at the beginning of step (i) or step (1) has been enriched for IL6R-expressing cells (for example, cells that are positive for IL6Rα and/or IL6Rβ).

42. The method of any one of claims 1-41, wherein the population of cells at the beginning of step (i) or step (1) comprises no less than 50, 60, or 70% of IL6R-expressing cells (for example, cells that are positive for IL6Rα and/or IL6Rβ).

43. The method of any one of claims 1-42, wherein steps (i) and (ii) or steps (1) and (2) are performed in cell media comprising IL-15 (for example, hetIL-15 (IL15/sIL-15Ra)).

44. The method of claim 43, wherein IL-15 increases the ability of the population of cells to expand, for example, 10, 15, 20, or 25 days later.

45. The method of claim 43, wherein IL-15 increases the percentage of IL6Rβ-expressing cells in the population of cells.

46. The method of any one of claims 1-45, wherein the CAR comprises an antigen binding domain, a transmembrane domain, and an intracellular signaling domain.

47. The method of claim 46, wherein the antigen binding domain binds to an antigen chosen from: CD19, CD20, CD22, BCMA, mesothelin, EGFRvIII, GD2, Tn antigen, sTn antigen, Tn-O-Glycopeptides, sTn-O-Glycopeptides, PSMA, CD97, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, leguman, GD3, CD171, IL-11Ra, PSCA, MAD-CT-1, MAD-CT-2, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBBs (for example, ERBB2), Her2/neu, MUC1, EGFR, NCAM, Ephrin B2, CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, FAP, Legumain, HPV E6 or E7, ML-IAP, CLDN6, TSHR, GPRC5D, ALK, Polysialic acid, Fos-related antigen, neutrophil elastase, TRP-2, CYP1B1, sperm protein 17, beta human chorionic gonadotropin, AFP, thyroglobulin, PLAC1, globoH, RAGE1, MN-CA IX, human telomerase reverse transcriptase, intestinal carboxyl esterase, mut hsp 70-2, NA-17, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, NY-ESO-1, GPR20, Ly6k, OR51E2, TARP, GFRα4, or a peptide of any of these antigens presented on MHC.

48. The method of claim 46 or 47, wherein the antigen binding domain comprises a CDR, VH, VL, scFv or CAR sequence disclosed herein, optionally wherein:

(a) the antigen binding domain binds to BCMA and comprises a CDR, VH, VL, scFv or CAR sequence disclosed in Tables 3-15, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto;

(b) the antigen binding domain binds to CD19 and comprises a CDR, VH, VL, scFv or CAR sequence disclosed in Table 2, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto;

(c) the antigen binding domain binds to CD20 and comprises a CDR, VH, VL, scFv or CAR sequence disclosed herein, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto; or

(d) the antigen binding domain binds to CD22 and comprises a CDR, VH, VL, scFv or CAR sequence disclosed herein, or a sequence having at least 80%, 85%, 90%, 95%, or 99% identity thereto.

49. The method of any one of claims 46-48, wherein the antigen binding domain comprises a VH and a VL, wherein the VH and VL are connected by a linker, optionally wherein the linker comprises the amino acid sequence of SEQ ID NO: 63 or 104.

50. The method of any one of claims 46-49, wherein:

(a) the transmembrane domain comprises a transmembrane domain of a protein chosen from the alpha, beta or zeta chain of T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154,

(b) the transmembrane domain comprises a transmembrane domain of CD8,

(c) the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof, or

(d) the nucleic acid molecule comprises a nucleic acid sequence encoding the transmembrane domain, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 17, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof.

51. The method of any one of claims 46-50, wherein the antigen binding domain is connected to the transmembrane domain by a hinge region, optionally wherein:

(a) the hinge region comprises the amino acid sequence of SEQ ID NO: 2, 3, or 4, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof, or

(b) the nucleic acid molecule comprises a nucleic acid sequence encoding the hinge region, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO:

13, 14, or 15, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof.

52. The method of any one of claims 46-51, wherein the intracellular signaling domain comprises a primary signaling domain, optionally wherein the primary signaling domain comprises a functional signaling domain derived from CD3 zeta, TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (ICOS), FcεRI, DAP10, DAP12, or CD66d, optionally wherein:

(a) the primary signaling domain comprises a functional signaling domain derived from CD3 zeta,

(b) the primary signaling domain comprises the amino acid sequence of SEQ ID NO: 9 or 10, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof, or

(c) the nucleic acid molecule comprises a nucleic acid sequence encoding the primary signaling domain, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 20 or 21, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof.

53. The method of any one of claims 46-52, wherein the intracellular signaling domain comprises a costimulatory signaling domain, optionally wherein the costimulatory signaling domain comprises a functional signaling domain derived from a MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), an activating NK cell receptor, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, 4-1BB (CD137), B7-H3, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR,

LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD28-OX40, CD28-4-1BB, or a ligand that specifically binds with CD83, optionally wherein:

(a) the costimulatory signaling domain comprises a functional signaling domain derived from 4-1BB,

(b) the costimulatory signaling domain comprises the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof, or

(c) the nucleic acid molecule comprises a nucleic acid sequence encoding the costimulatory signaling domain, wherein the nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO: 18, or a nucleic acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof.

54. The method of any one of claims 46-53, wherein the intracellular signaling domain comprises a functional signaling domain derived from 4-1BB and a functional signaling domain derived from CD3 zeta, optionally wherein the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 (or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof) and the amino acid sequence of SEQ ID NO: 9 or 10 (or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereof), optionally wherein the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 and the amino acid sequence of SEQ ID NO: 9 or 10.

55. The method of any one of claims 46-54, wherein the CAR further comprises a leader sequence comprising the amino acid sequence of SEQ ID NO: 1.

56. A population of CAR-expressing cells (for example, autologous or allogeneic CAR-expressing T cells or NK cells) made by the method of any one of claims 1-55.

57. A pharmaceutical composition comprising the population of CAR-expressing cells of claim 56 and a pharmaceutically acceptable carrier.

58. A method of increasing an immune response in a subject, comprising administering the population of CAR-expressing cells of claim 56 or the pharmaceutical composition of claim 57 to the subject, thereby increasing an immune response in the subject.

59. A method of treating a cancer in a subject, comprising administering the population of CAR-expressing cells of claim 56 or the pharmaceutical composition of claim 57 to the subject, thereby treating the cancer in the subject.

60. The method of claim 59, wherein the cancer is a solid cancer, for example, chosen from: one or more of mesothelioma, malignant pleural mesothelioma, non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, large cell lung cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, esophageal adenocarcinoma , breast cancer, glioblastoma, ovarian cancer, colorectal cancer, prostate cancer, cervical cancer, skin cancer, melanoma, renal cancer, liver cancer, brain cancer, thymoma, sarcoma, carcinoma, uterine cancer, kidney cancer, gastrointestinal cancer, urothelial cancer, pharynx cancer, head and neck cancer, rectal cancer, esophagus cancer, or bladder cancer, or a metastasis thereof.

61. The method of claim 59, wherein the cancer is a liquid cancer, for example, chosen from: chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), multiple myeloma, acute lymphoid leukemia (ALL), Hodgkin lymphoma, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), small lymphocytic leukemia (SLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL), DLBCL associated with chronic inflammation, chronic myeloid leukemia, myeloproliferative neoplasms, follicular lymphoma, pediatric follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma (extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue), Marginal zone lymphoma, myelodysplasia, myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, splenic marginal zone lymphoma, splenic lymphoma/leukemia, splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant, lymphoplasmacytic lymphoma, a heavy chain disease, plasma cell myeloma, solitary plasmocytoma of bone, extraosseous plasmocytoma, nodal marginal zone lymphoma,

pediatric nodal marginal zone lymphoma, primary cutaneous follicle center lymphoma, lymphomatoid granulomatosis, primary mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, ALK+ large B-cell lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, B-cell lymphoma, acute myeloid leukemia (AML), or unclassifiable lymphoma.

62. The method of any one of claims 58-61, further comprising administering a second therapeutic agent to the subject.

63. The method of any one of claims 58-62, wherein the population of CAR-expressing cells is administered at a dose determined based on the percentage of CAR-expressing cells measured in claim 39.

64. The population of CAR-expressing cells of claim 56 or the pharmaceutical composition of claim 57 for use in a method of increasing an immune response in a subject, said method comprising administering to the subject an effective amount of the population of CAR-expressing cells or an effective amount of the pharmaceutical composition.

65. The population of CAR-expressing cells of claim 56 or the pharmaceutical composition of claim 57 for use in a method of treating a cancer in a subject, said method comprising administering to the subject an effective amount of the population of CAR-expressing cells or an effective amount of the pharmaceutical composition.

66. A multispecific binding molecule comprising:

(i) an anti-CD3 binding domain,

(ii) an anti-CD28 binding domain comprising a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, and a HCDR3, and a light chain variable region (VL) comprising a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3 wherein:

(a) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 538, 539, 540, 530, 531, and 532, respectively;

(b) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 541, 539, 540, 530, 531, and 532, respectively;

(c) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 542, 543, 540, 533, 534, and 535, respectively; or

(d) the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the amino acid sequences of SEQ ID NOs: 544, 545, 546, 536, 534, and 532, respectively; and

(iii) an Fc region comprising:

a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system;

a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system;

a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system;

a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system;

a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system;

a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or

a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system.

67. The multispecific binding molecule of claim 66, wherein the anti-CD28 binding domain comprises:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 547 or 548, or a sequence with at least 95% sequence identity to SEQ ID NO: 547 or 548;

(ii) a VL comprising the amino acid sequence of SEQ ID NO: 537, or a sequence with at least 95% sequence identity thereto;

(iii) a VH comprising the amino acid sequence of SEQ ID NO: 547 or a sequence with at least 95% sequence identity thereto, and a VL comprising the amino acid sequence of SEQ ID NO: 537, or a sequence with at least 95% sequence identity thereto; or

(iv) a VH comprising the amino acid sequence of SEQ ID NO: 548 or a sequence with at least 95% sequence identity thereto, and a VL comprising the amino acid sequence of SEQ ID NO: 537, or a sequence with at least 95% sequence identity thereto.

68. The multispecific binding molecule of claim 66 or 67, which further comprises a light chain constant region chosen from the light chain constant regions of kappa or lambda.

69. The multispecific binding molecule of any one of claims 66-68, wherein the Fc region comprises a CH2, a CH3, or both a CH2 and CH3, optionally wherein the CH2 and/or CH3 are selected from IgG1, IgG2, IgG3, or IgG4.

70. The multispecific binding molecule of any one of claims 66-69, wherein the anti-CD3 binding domain comprises:

(i) a HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 of an anti-CD3 antibody molecule of Table 27 (for example the anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4)); or

(ii) the amino acid sequence of any VH and/or VL region of an anti-CD3 antibody molecule provided in Table 27 (for example the anti-CD3 (1), anti-CD3 (2), anti-CD3 (3), or anti-CD3 (4)), or an amino acid sequence at least 95% identical thereto.

71. A multispecific binding molecule, comprising a first binding domain and a second binding domain, wherein the multispecific binding molecule comprises:

(i) a first polypeptide comprising from N-terminal to C-terminal: VH of the first binding domain, VL of the first binding domain, VH of the second binding domain, CH1 and an Fc region, which comprises a CH2 and a CH3; and

(ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the second binding domain and CL;

wherein the Fc region comprises:

a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system;

a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system;

a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system;

a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system;

a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system;

a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or

a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system.

72. A multispecific binding molecule, comprising a first binding domain and a second binding domain, wherein the multispecific binding molecule comprises:

(i) a first polypeptide comprising from N-terminal to C-terminal: VH of the second binding domain, CH1, an Fc region comprising a CH2 and a CH3, VH of the first binding domain, and VL of the first binding domain; and

(ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the second binding domain and CL;

wherein the Fc region comprises:

a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system;

a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system;

a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system;

a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system;

a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system;

a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or

a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system.

73. A multispecific binding molecule, comprising a first binding domain and a second binding domain, wherein the multispecific binding molecule comprises:

(i) a first polypeptide comprising from N-terminal to C-terminal: VH of the second binding domain, CH1, VH of the first binding domain, VL of the first binding domain, and an Fc region comprising a CH2 and a CH3; and

(ii) a second polypeptide comprising from N-terminal to C-terminal: VL of the second binding domain and CL;

wherein the Fc region comprises:

a L234A, L235A, S267K, and P329A mutation (LALASKPA), numbered according to the EU numbering system;

a L234A, L235A, and P329G mutation (LALAPG), numbered according to the EU numbering system;

a G237A, D265A, P329A, and S267K mutation (GADAPASK), numbered according to the EU numbering system;

a L234A, L235A, and G237A mutation (LALGA), numbered according to the EU numbering system;

a D265A, P329A, and S267K mutation (DAPASK), numbered according to the EU numbering system;

a G237A, D265A, and P329A mutation (GADAPA), numbered according to the EU numbering system; or

a L234A, L235A, and P329A mutation (LALAPA), numbered according to the EU numbering system.

74. The multispecific binding molecule of any one of claims 71-73, wherein the first binding domain comprises an anti-CD3 binding domain and the second binding domain comprises a costimulatory molecule binding domain.

75. The multispecific binding molecule of any one of claims 71-73, wherein the first binding domain comprises a costimulatory molecule binding domain and the second binding domain comprises an anti-CD3 binding domain.

76. The multispecific binding molecule of claim 74 or 75, wherein the costimulatory molecule binding domain comprises an anti-CD2 binding domain or an anti-CD28 binding domain.

77. The method of any one of claims 1-55, or the multispecific binding molecule of any one of claims 66-76, wherein the multispecific binding molecule comprises:

(i) a heavy chain comprising the amino acid sequence of any of SEQ ID NOs: 794, 795, 798, 800, or 815-817 or an amino acid sequence having at least 95% sequence identity thereto; and/or

(ii) a light chain comprising the amino acid sequence of any of SEQ ID NOs: 673, 796, 797, 799, or 801, or an amino acid sequence having at least 95% sequence identity thereto.

78. The method of any one of claims 1-55, or the multispecific binding molecule of any one of claims 66-77, wherein the multispecific binding molecule comprises:

(i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 794, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 796, or an amino acid sequence having at least 95% sequence identity thereto;

(ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 794, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 797, or an amino acid sequence having at least 95% sequence identity thereto;

(iii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 795, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain

comprising the amino acid sequence of SEQ ID NO: 796, or an amino acid sequence having at least 95% sequence identity thereto;

(iv) a heavy chain comprising the amino acid sequence of SEQ ID NO: 795, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 797, or an amino acid sequence having at least 95% sequence identity thereto;

(v) a heavy chain comprising the amino acid sequence of SEQ ID NO: 798, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 799, or an amino acid sequence having at least 95% sequence identity thereto;

(vi) a heavy chain comprising the amino acid sequence of SEQ ID NO: 815, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 799, or an amino acid sequence having at least 95% sequence identity thereto;

(vii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 800, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 801, or an amino acid sequence having at least 95% sequence identity thereto;

(viii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 816, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 673, or an amino acid sequence having at least 95% sequence identity thereto; or

(ix) a heavy chain comprising the amino acid sequence of SEQ ID NO: 817, or an amino acid sequence having at least 95% sequence identity thereto, and a light chain comprising the amino acid sequence of SEQ ID NO: 673, or an amino acid sequence having at least 95% sequence identity thereto.

79. A method of activating cells (e.g., immune effector cells, e.g., T cells), comprising contacting (for example, binding) a population of cells (for example, T cells, for example, T cells isolated from a frozen or fresh leukapheresis product) with the multispecific binding molecule of any one of claims 66-78.

80. A method of transducing cells (e.g., immune effector cells, e.g., T cells), comprising contacting (for example, binding) a population of cells (for example, T cells, for example, T cells isolated from a frozen or fresh leukapheresis product) with (i) the multispecific binding molecule of any one of claims 66-78, and (ii) a nucleic acid molecule, e.g., a nucleic acid molecule encoding a CAR.

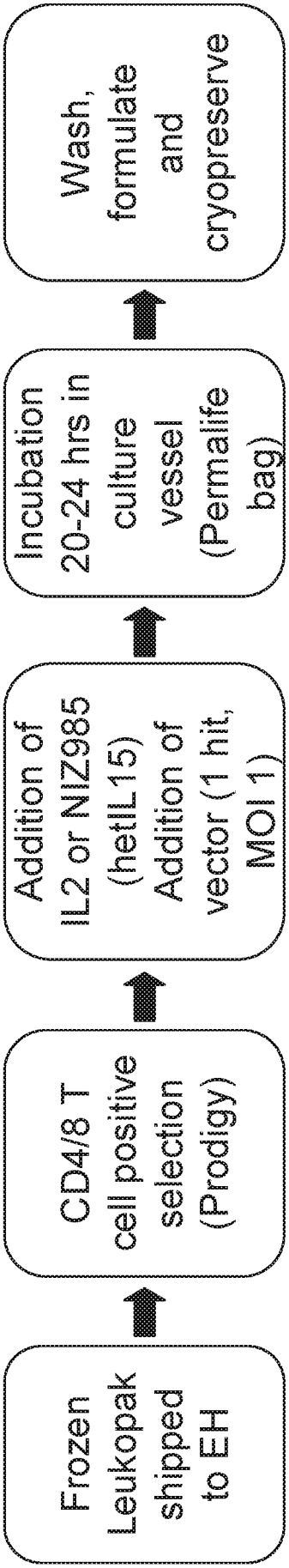

Frozen Leukopak shipped to EH
CD4/8 T cell positive selection (Prodigy)
Addition of IL2 or NIZ985 (hetIL15) Addition of vector (1 hit, MOI 1)
Incubation 20-24 hrs in culture vessel (Permalife bag)
Wash, formulate and cryopreserve

FIG. 1A

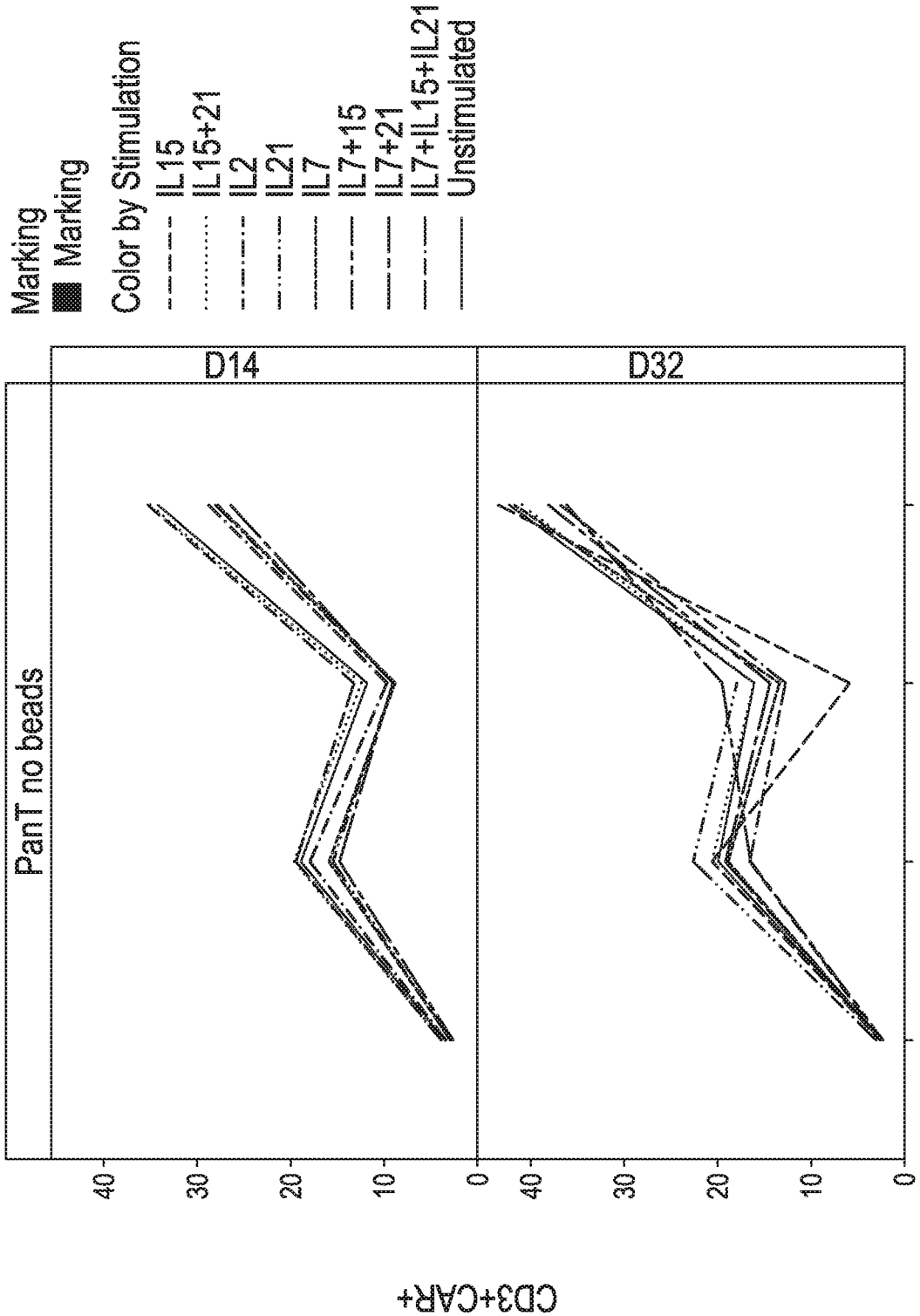

Marking
Marking
Color by Stimulation
IL15
IL15+21
IL2
IL21
IL7
IL7+15
IL7+21
IL7+IL15+IL21
Unstimulated
PanT no beads
D14
D32
CD3+CAR+
Day
0
10
20
30
40
1
2
3
4

FIG. 1B

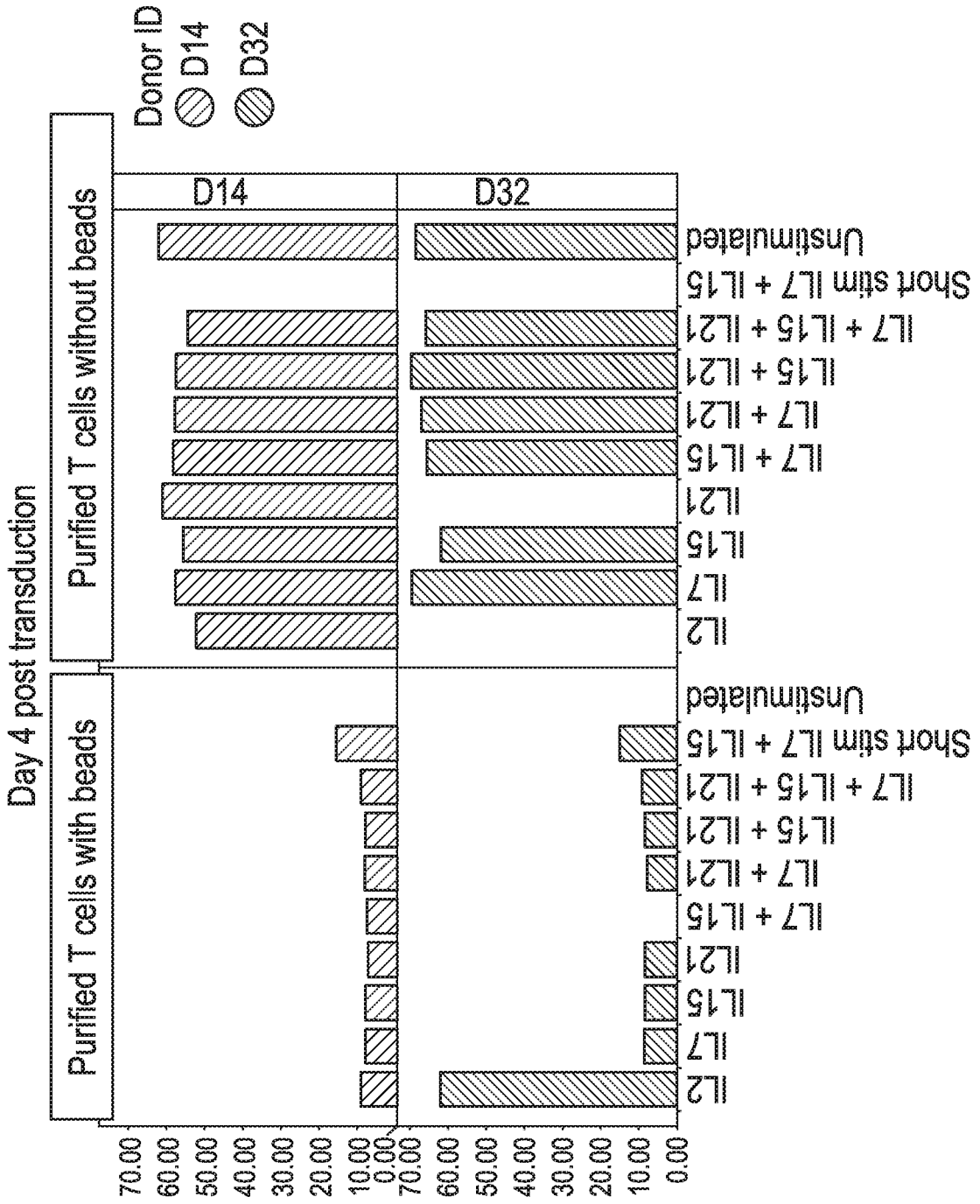

Day 4 post transduction
Purified T cells with beads
Purified T cells without beads
Donor ID
D14
D32
D14
D32
Unstimulated
Short stim IL7 + IL15
IL7 + IL15 + IL21
IL15 + IL21
IL7 + IL21
IL7 + IL15
IL21
IL15
IL7
IL2
Unstimulated
Short stim IL7 + IL15
IL7 + IL15 + IL21
IL15 + IL21
IL7 + IL21
IL7 + IL15
IL21
IL15
IL7
IL2
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Stimulation

FIG. 1C

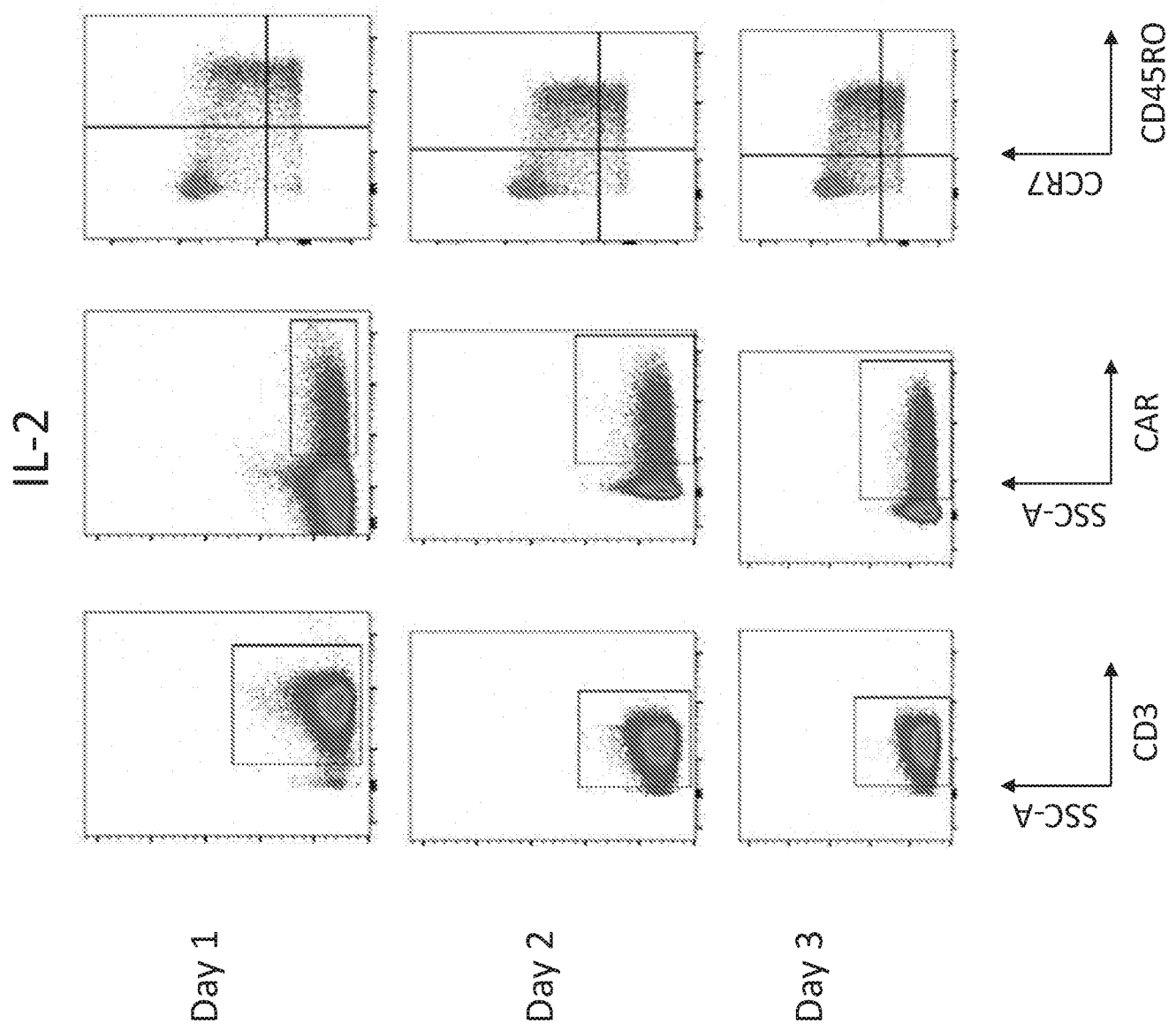



FIG. 1D

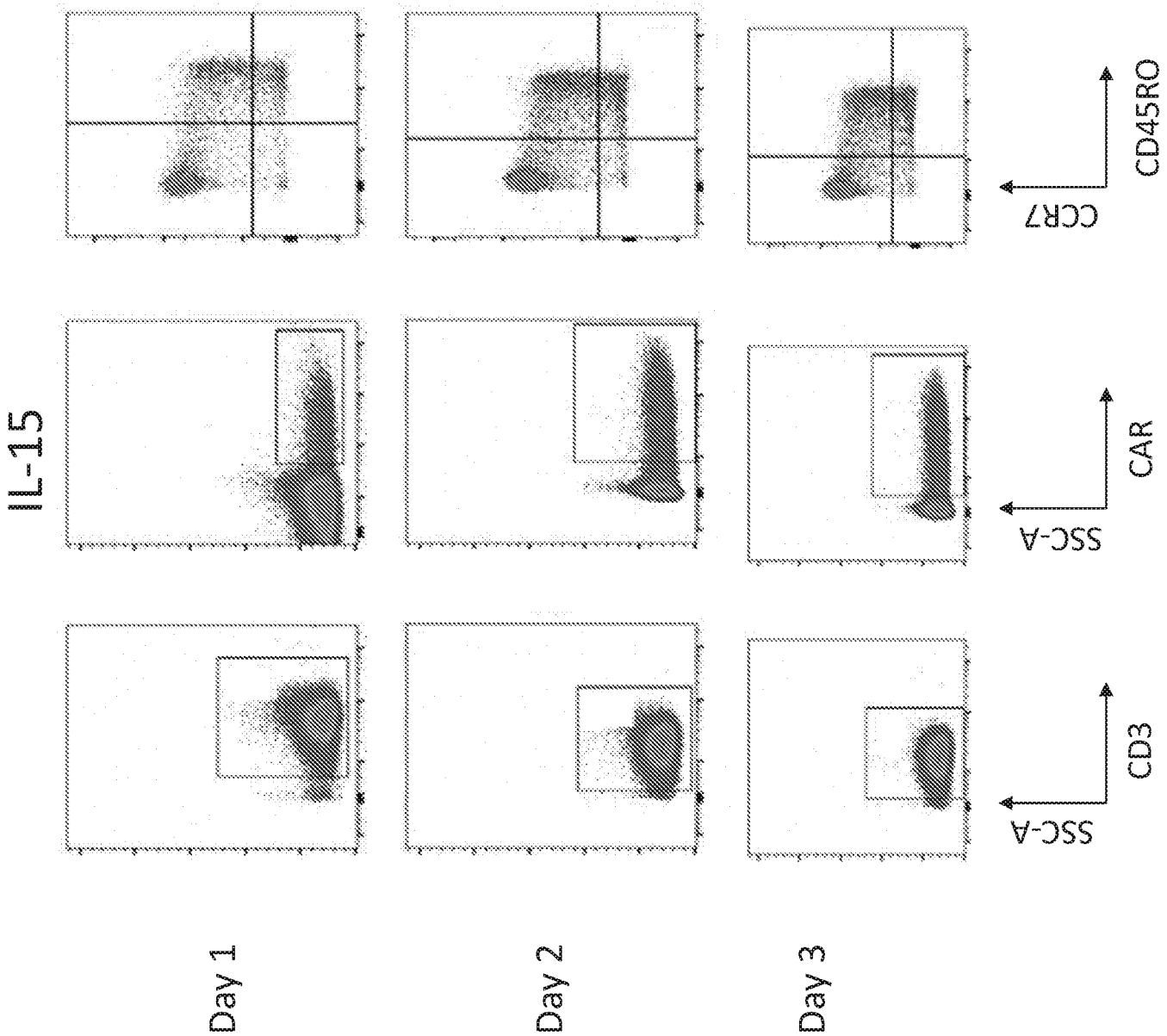



FIG. 1E

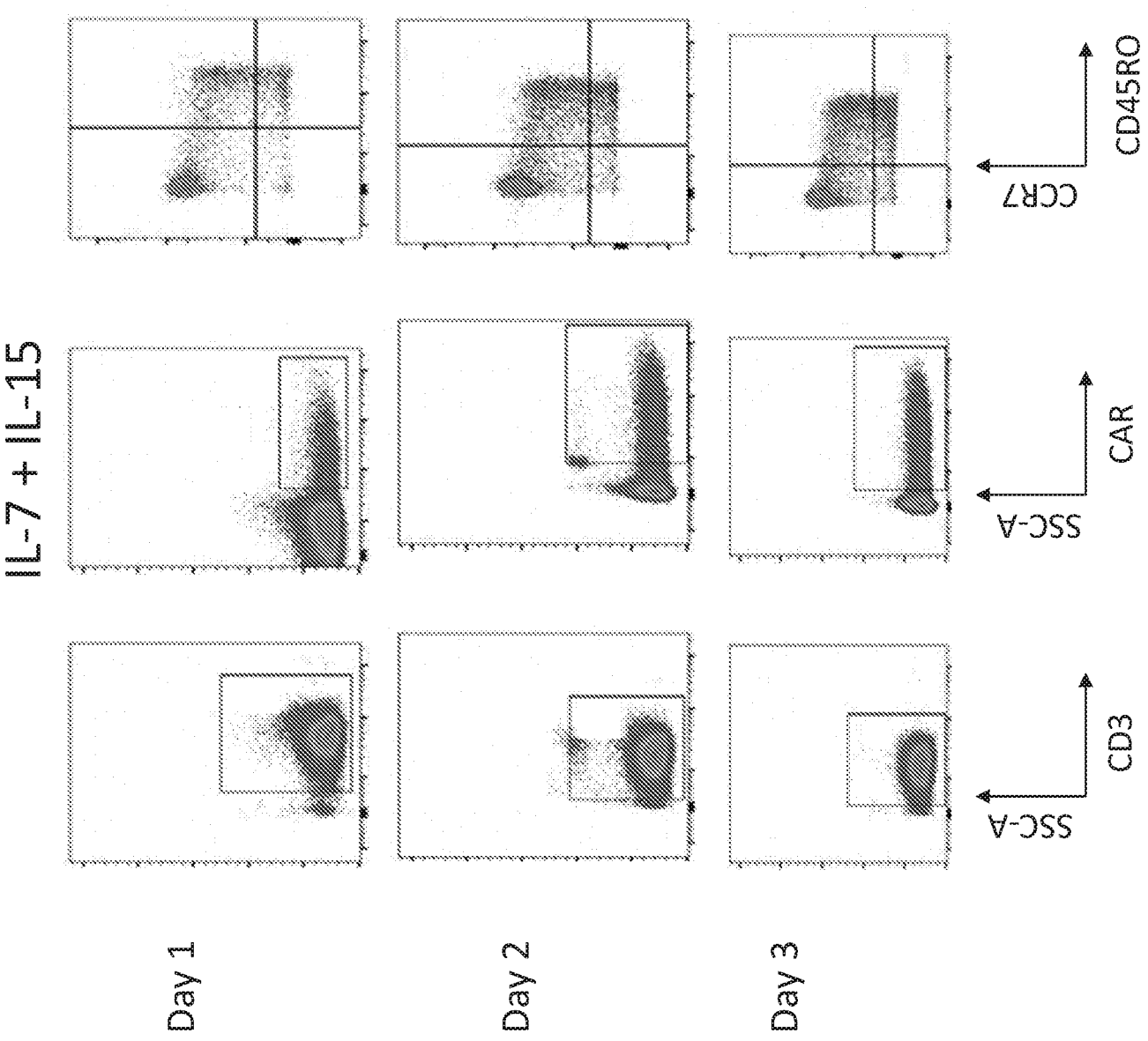



FIG. 1F

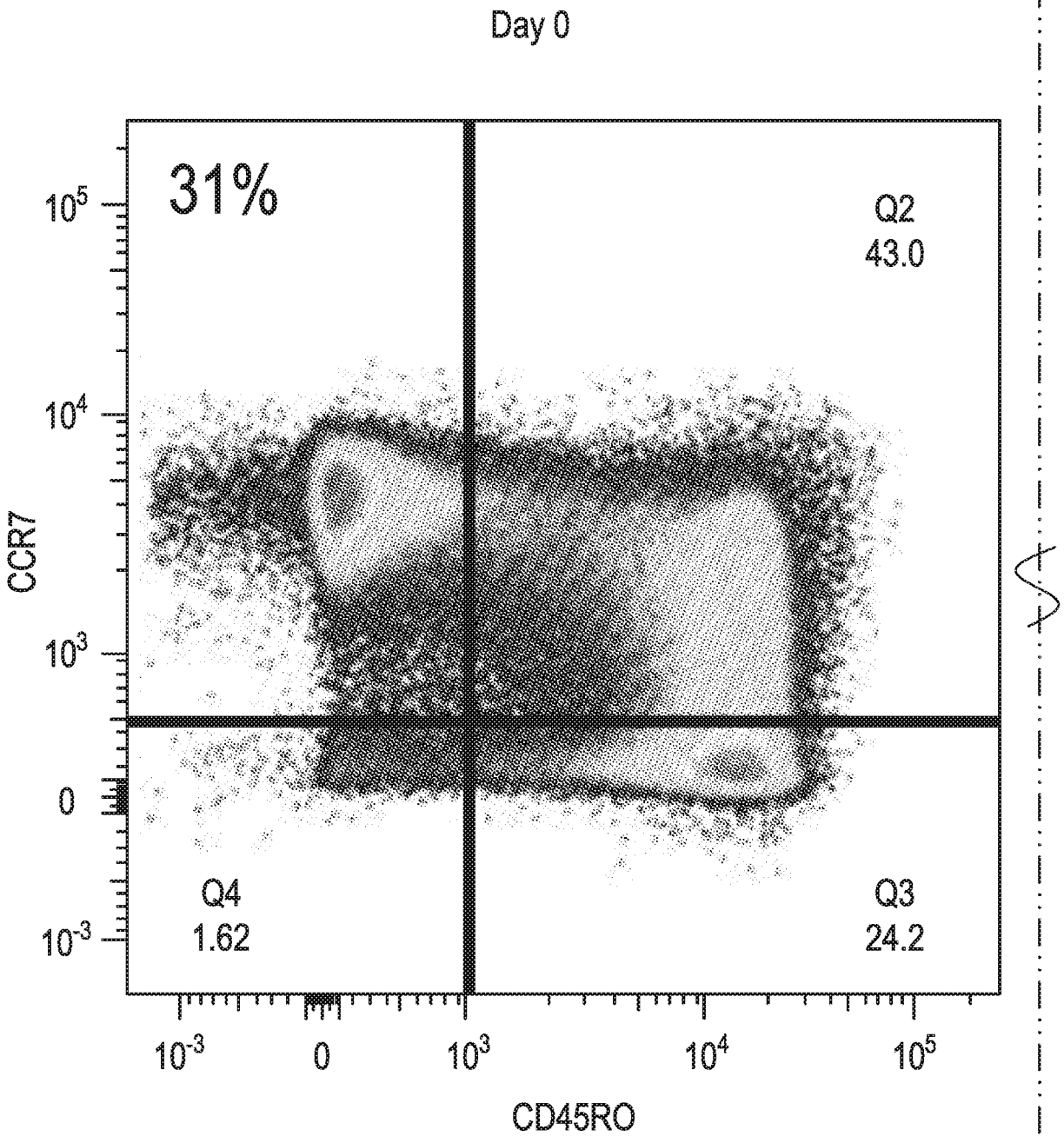

Day 0
31%
Q2
43.0
Q4
1.62
Q3
24.2
CCR7
CD45RO
$10^5$
$10^4$
$10^3$
0
$10^{-3}$
$10^{-3}$
0
$10^3$
$10^4$
$10^5$

FIG. 1G (part 1)

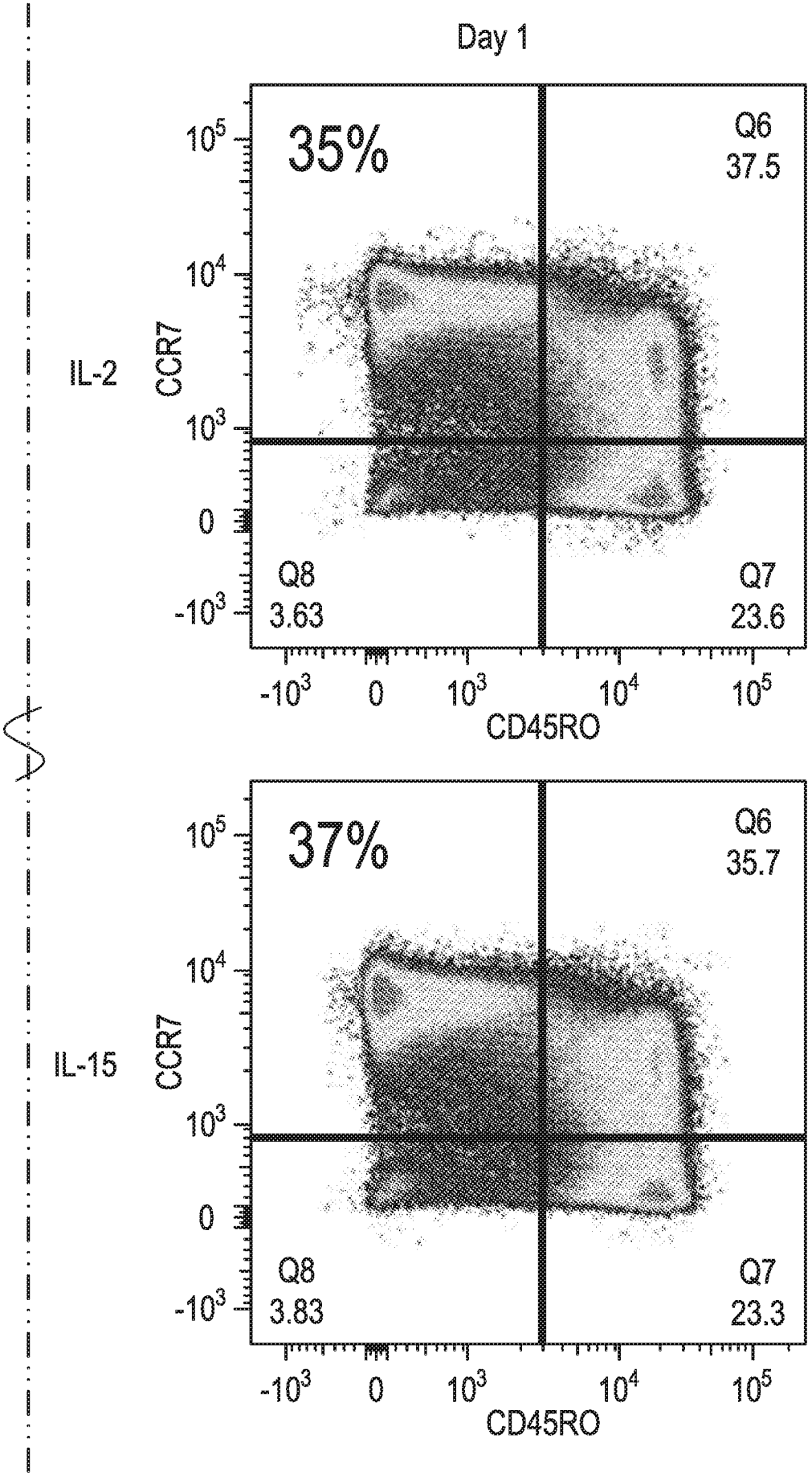

Day 1
IL-2
35%
Q6
37.5
Q8
3.63
Q7
23.6
CCR7
CD45RO
IL-15
37%
Q6
35.7
Q8
3.83
Q7
23.3
CCR7
CD45RO

FIG. 1G (part 2)

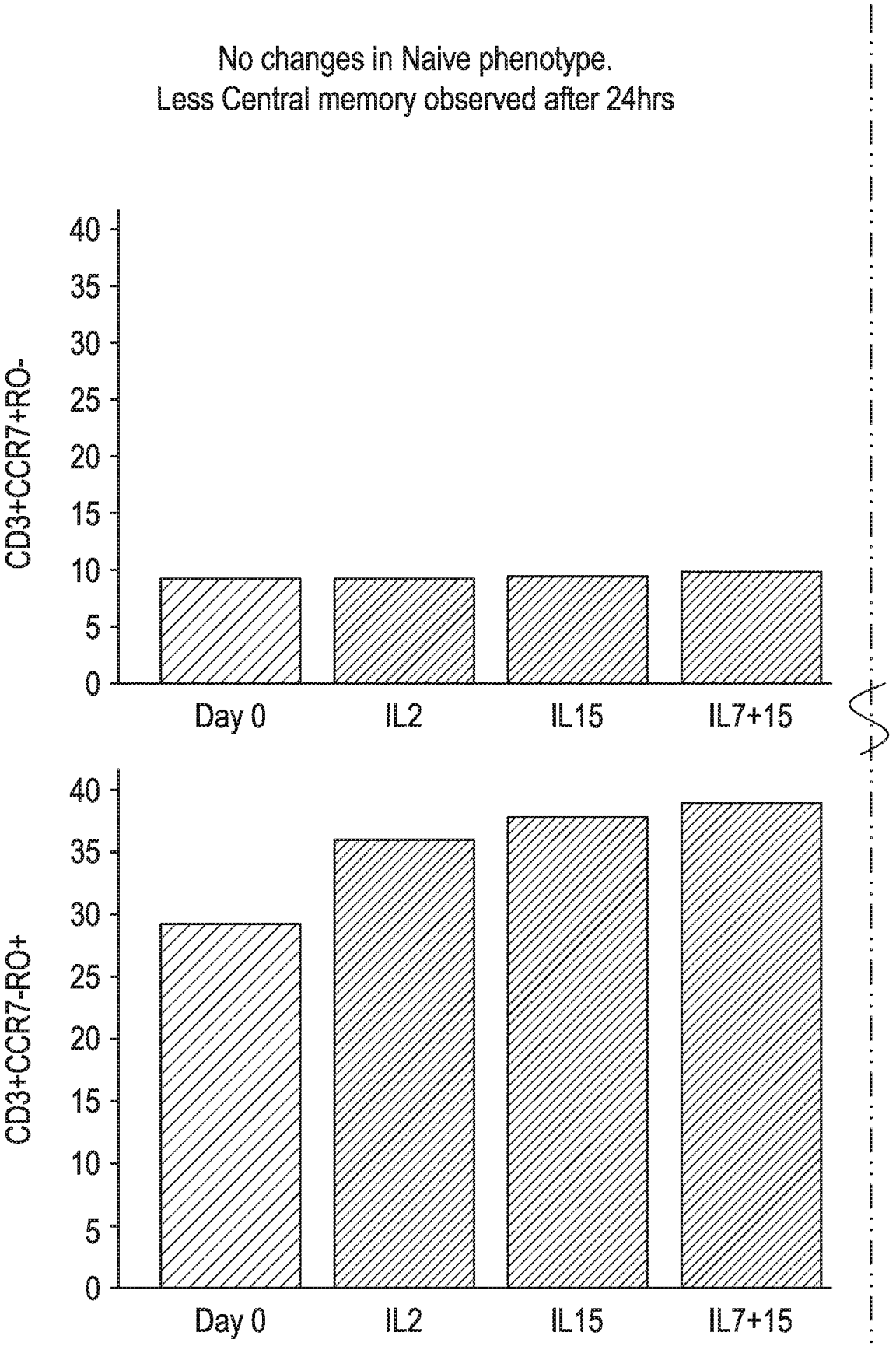

No changes in Naive phenotype.
Less Central memory observed after 24hrs
CD3+CCR7+RO-
40
35
30
25
20
15
10
5
0
Day 0
IL2
IL15
IL7+15
CD3+CCR7-RO+
40
35
30
25
20
15
10
5
0
Day 0
IL2
IL15
IL7+15

FIG. 1H (part 1)

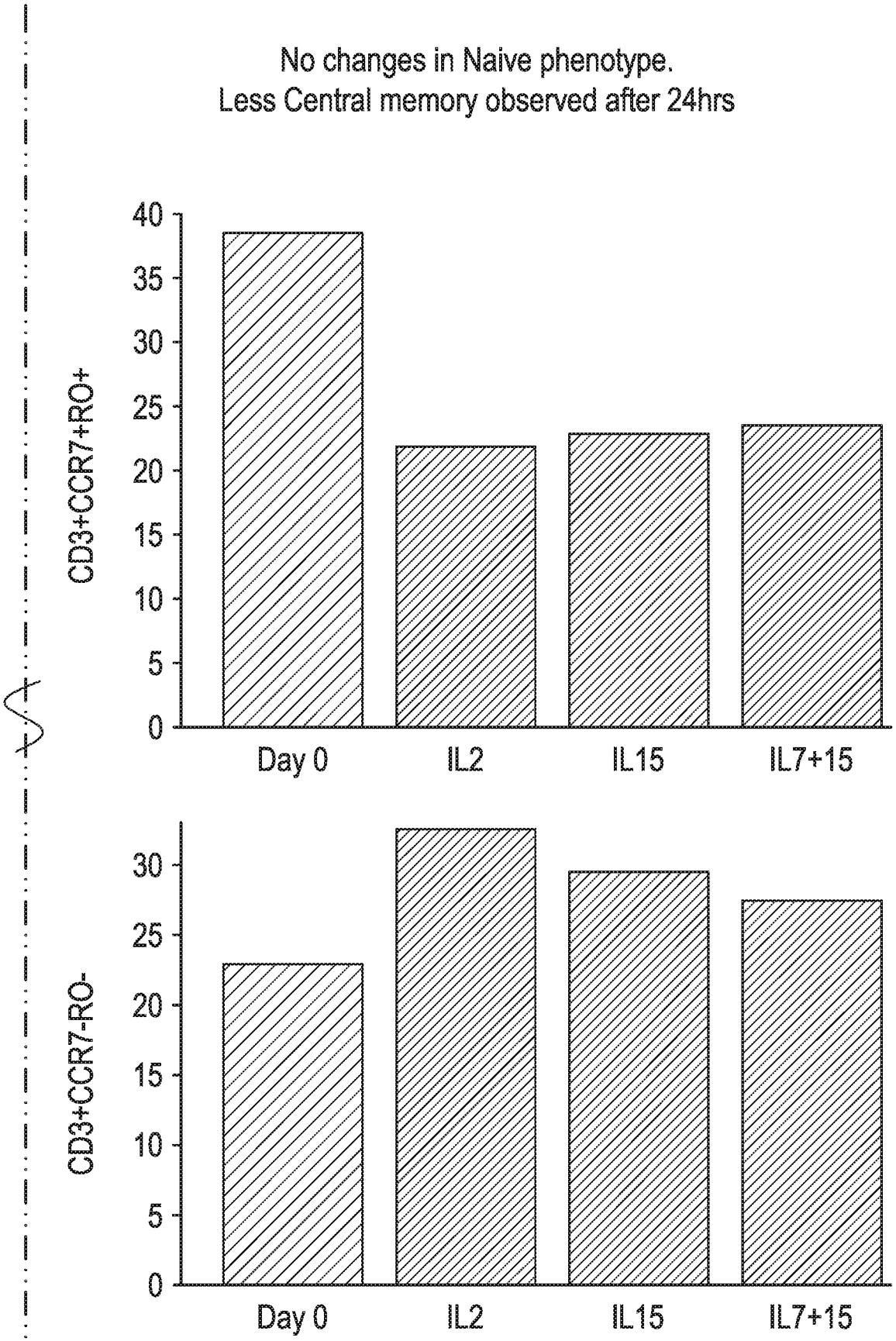

No changes in Naive phenotype.
Less Central memory observed after 24hrs
CD3+CCR7+RO+
40
35
30
25
20
15
10
5
0
Day 0
IL2
IL15
IL7+15
CD3+CCR7-RO-
30
25
20
15
10
5
0
Day 0
IL2
IL15
IL7+15

FIG. 1H (part 2)

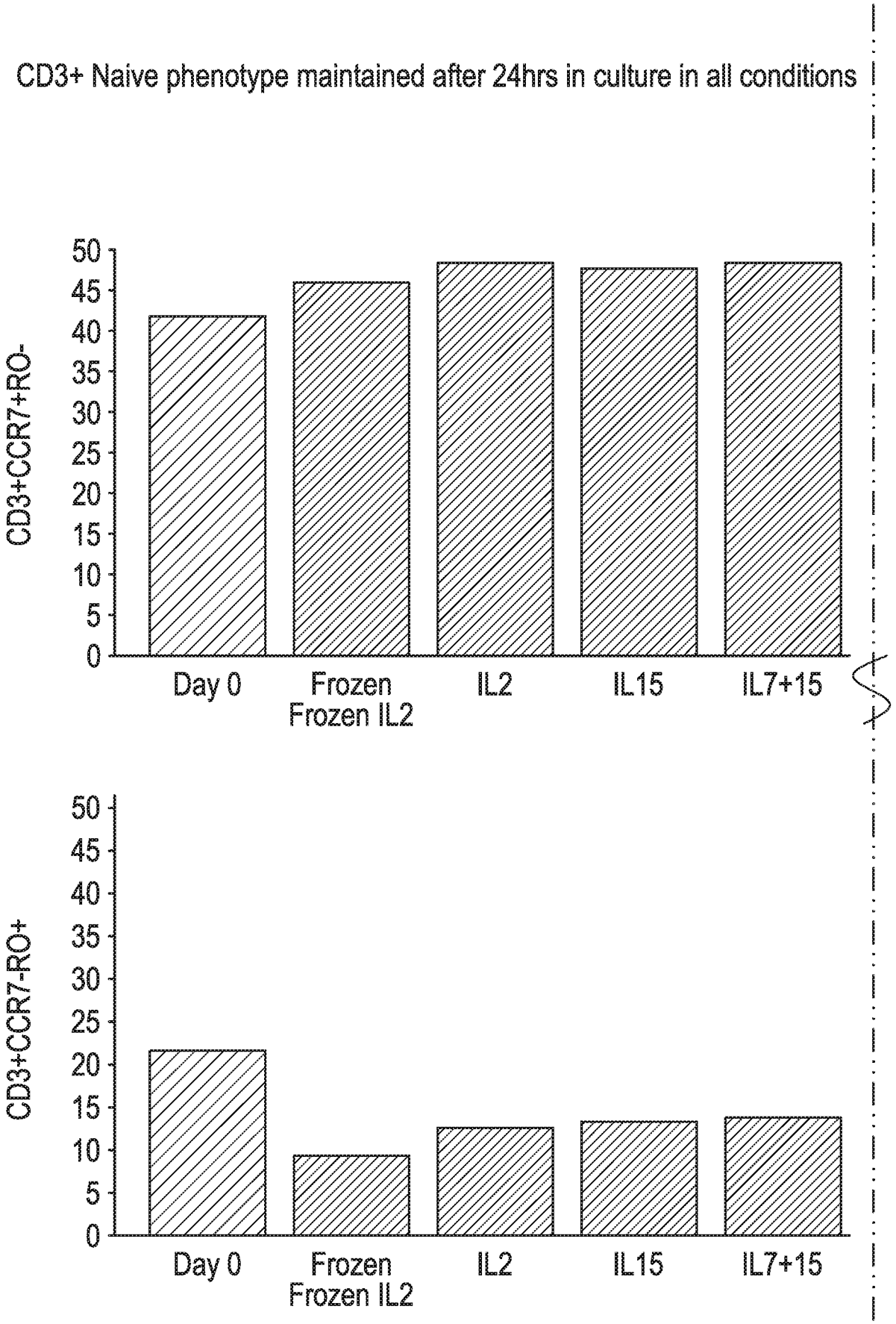

CD3+ Naive phenotype maintained after 24hrs in culture in all conditions
CD3+CCR7+RO-
50
45
40
35
30
25
20
15
10
5
0
Day 0
Frozen
Frozen IL2
IL2
IL15
IL7+15
CD3+CCR7-RO+
50
45
40
35
30
25
20
15
10
5
0
Day 0
Frozen
Frozen IL2
IL2
IL15
IL7+15

FIG. 1I (part 1)

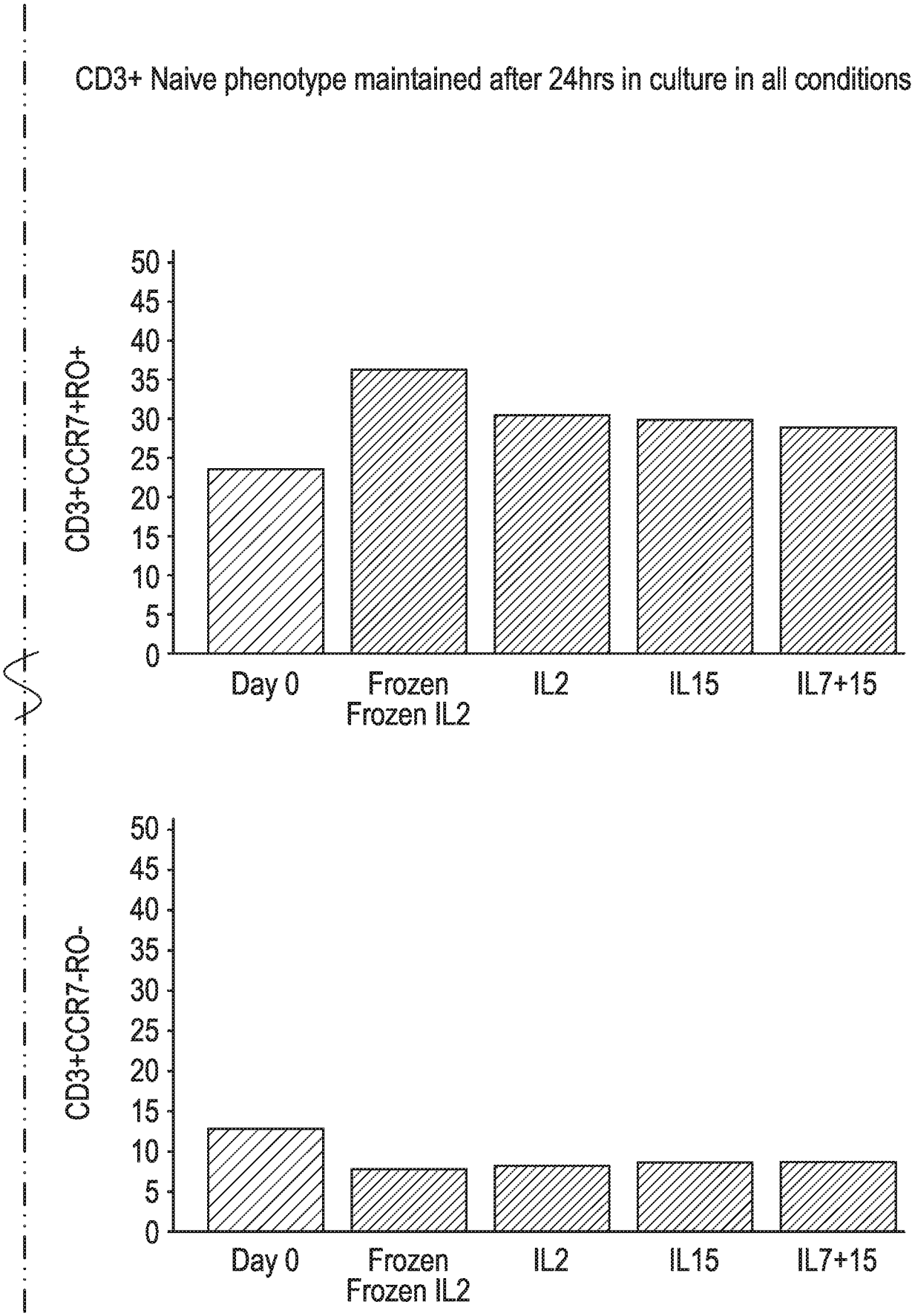

CD3+ Naive phenotype maintained after 24hrs in culture in all conditions
CD3+CCR7+RO+
50
45
40
35
30
25
20
15
10
5
0
Day 0
Frozen
Frozen IL2
IL2
IL15
IL7+15
CD3+CCR7-RO-
50
45
40
35
30
25
20
15
10
5
0
Day 0
Frozen
Frozen IL2
IL2
IL15
IL7+15

Fig. 1I (part 2)

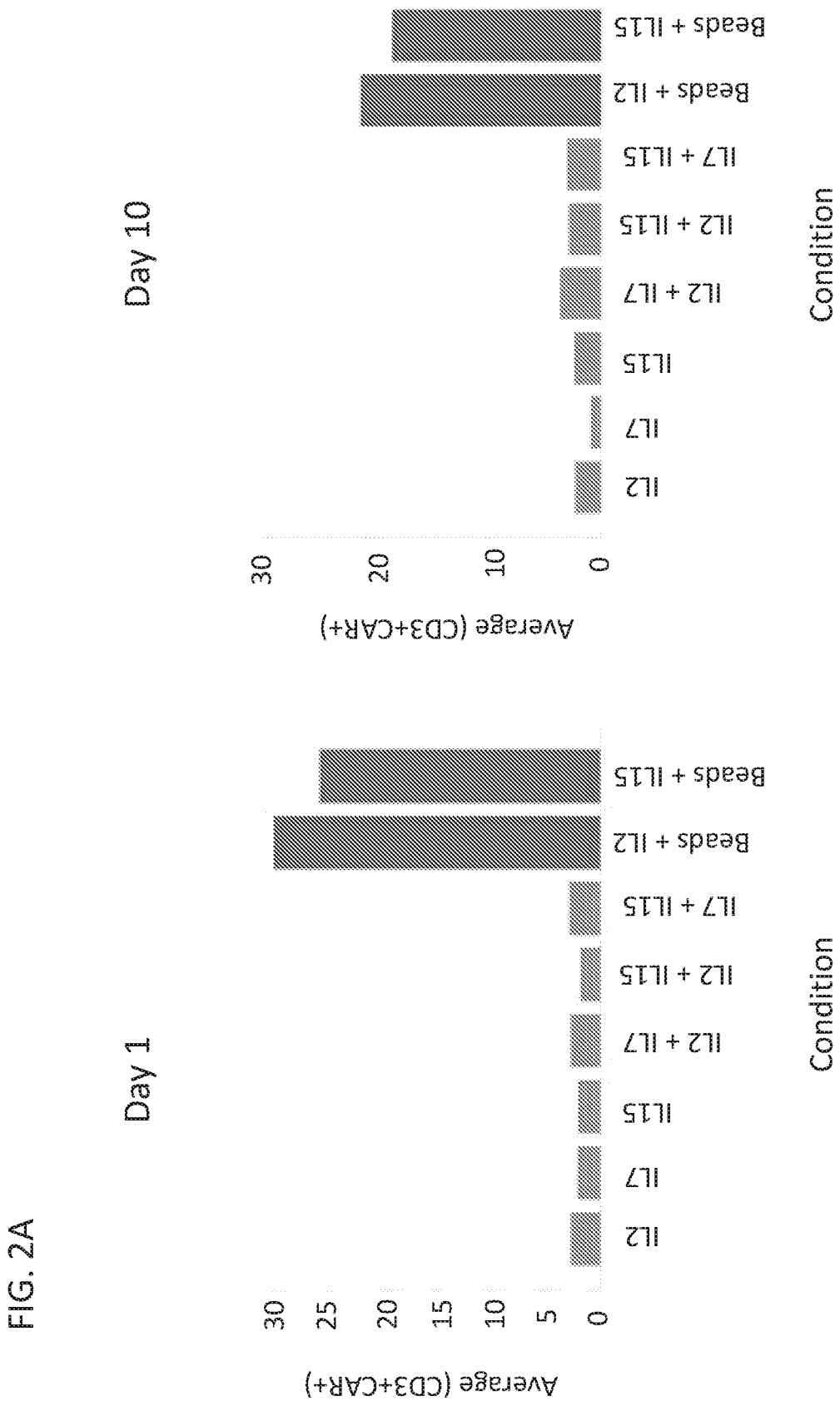

Day 1
Average (CD3+CAR+)
0
5
10
15
20
25
30
IL2
IL7
IL15
IL2 + IL7
IL2 + IL15
IL7 + IL15
Beads + IL2
Beads + IL15
Condition
Day 10
Average (CD3+CAR+)
0
10
20
30
IL2
IL7
IL15
IL2 + IL7
IL2 + IL15
IL7 + IL15
Beads + IL2
Beads + IL15
Condition

FIG. 2A

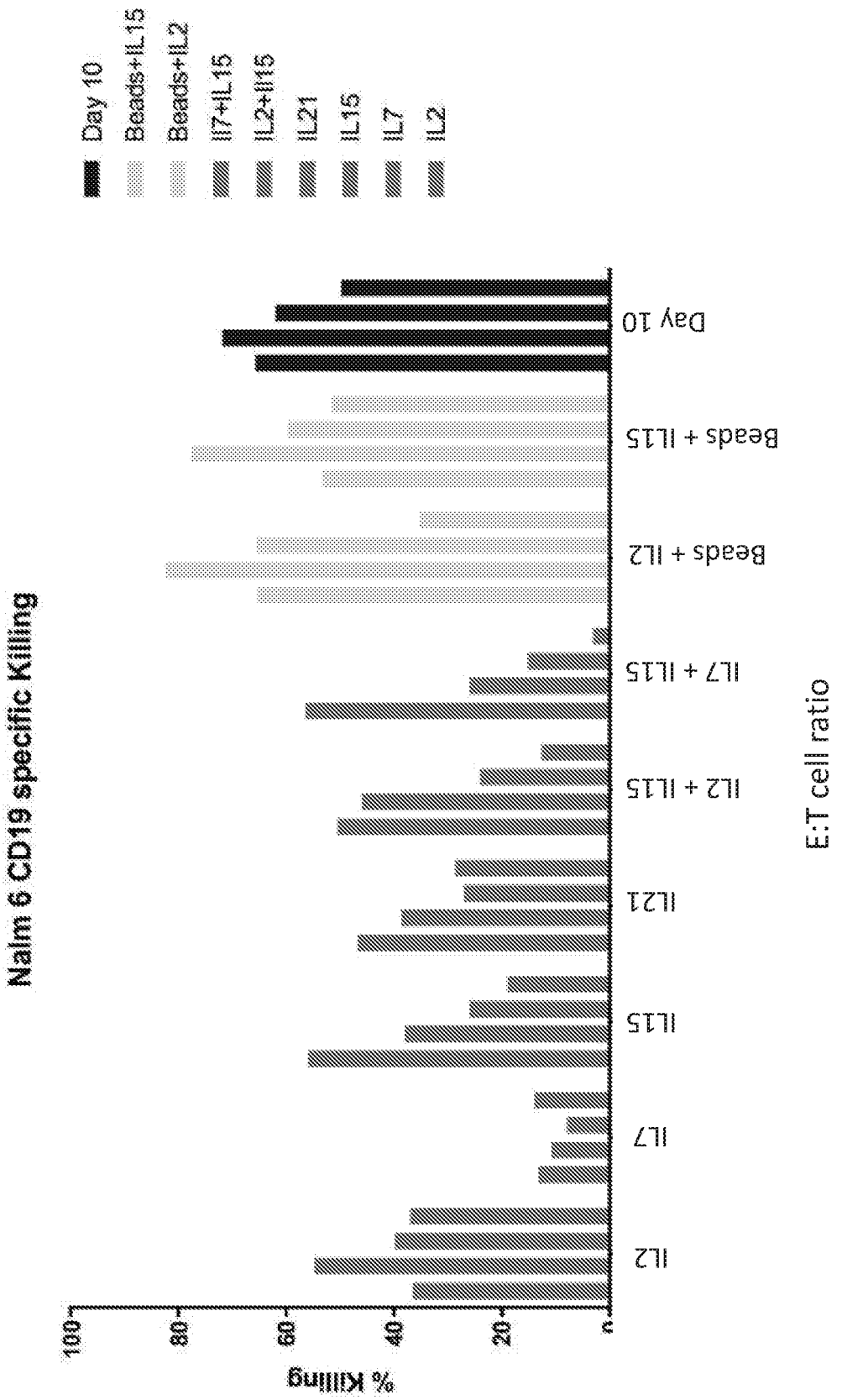

Day 10
Beads+IL15
Beads+IL2
Il7+IL15
IL2+Il15
IL21
IL15
IL7
IL2
Nalm 6 CD19 specific Killing
% Killing
100
80
60
40
20
0
IL2
IL7
IL15
IL21
IL2 + IL15
IL7 + IL15
Beads + IL2
Beads + IL15
Day 10
E:T cell ratio

FIG. 2B

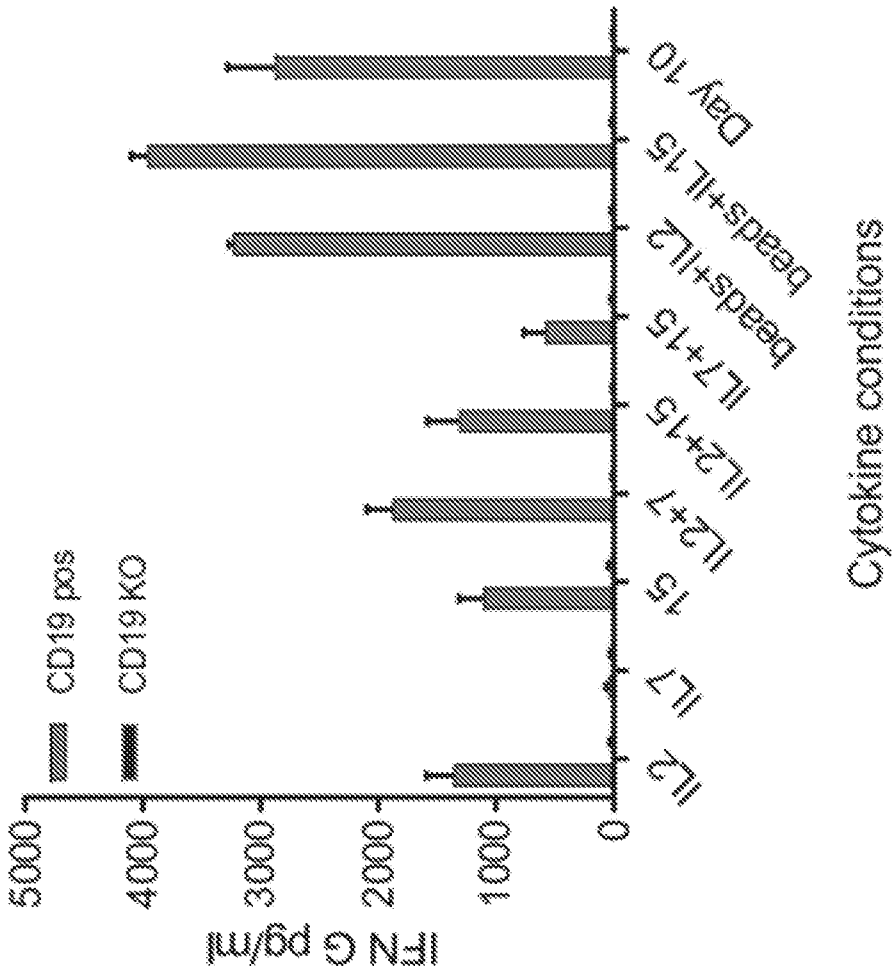

IFN G pg/ml
5000
4000
3000
2000
1000
0
CD19 pos
CD19 KO
IL2
IL7
15
IL2+7
IL2+15
IL7+15
beads+IL2
beads+IL15
Day 10
Cytokine conditions

FIG. 2C

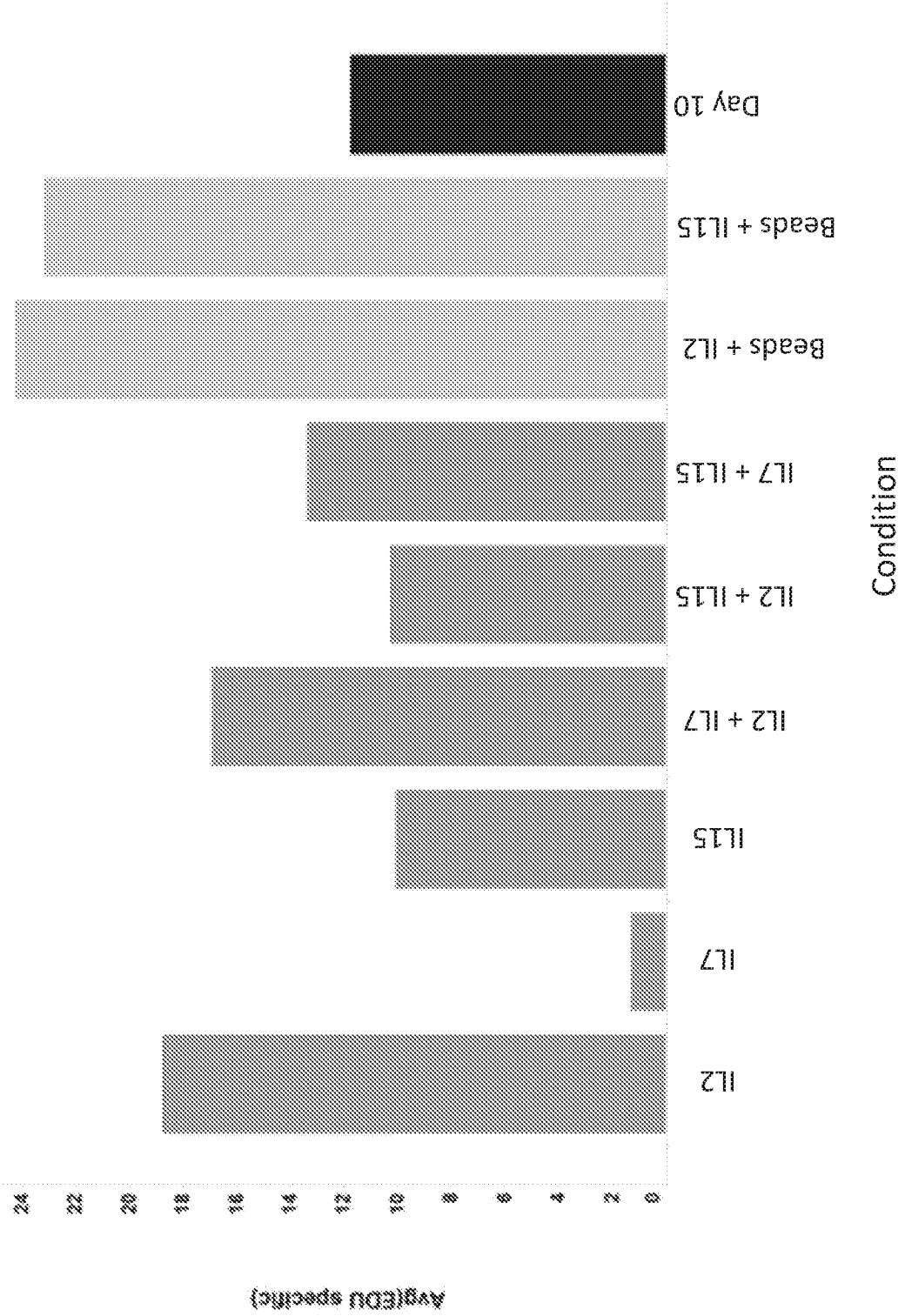

Avg(EDU specific)
0
2
4
6
8
10
12
14
16
18
20
22
24
IL2
IL7
IL15
IL2 + IL7
IL2 + IL15
IL7 + IL15
Beads + IL2
Beads + IL15
Day 10
Condition

FIG. 2D

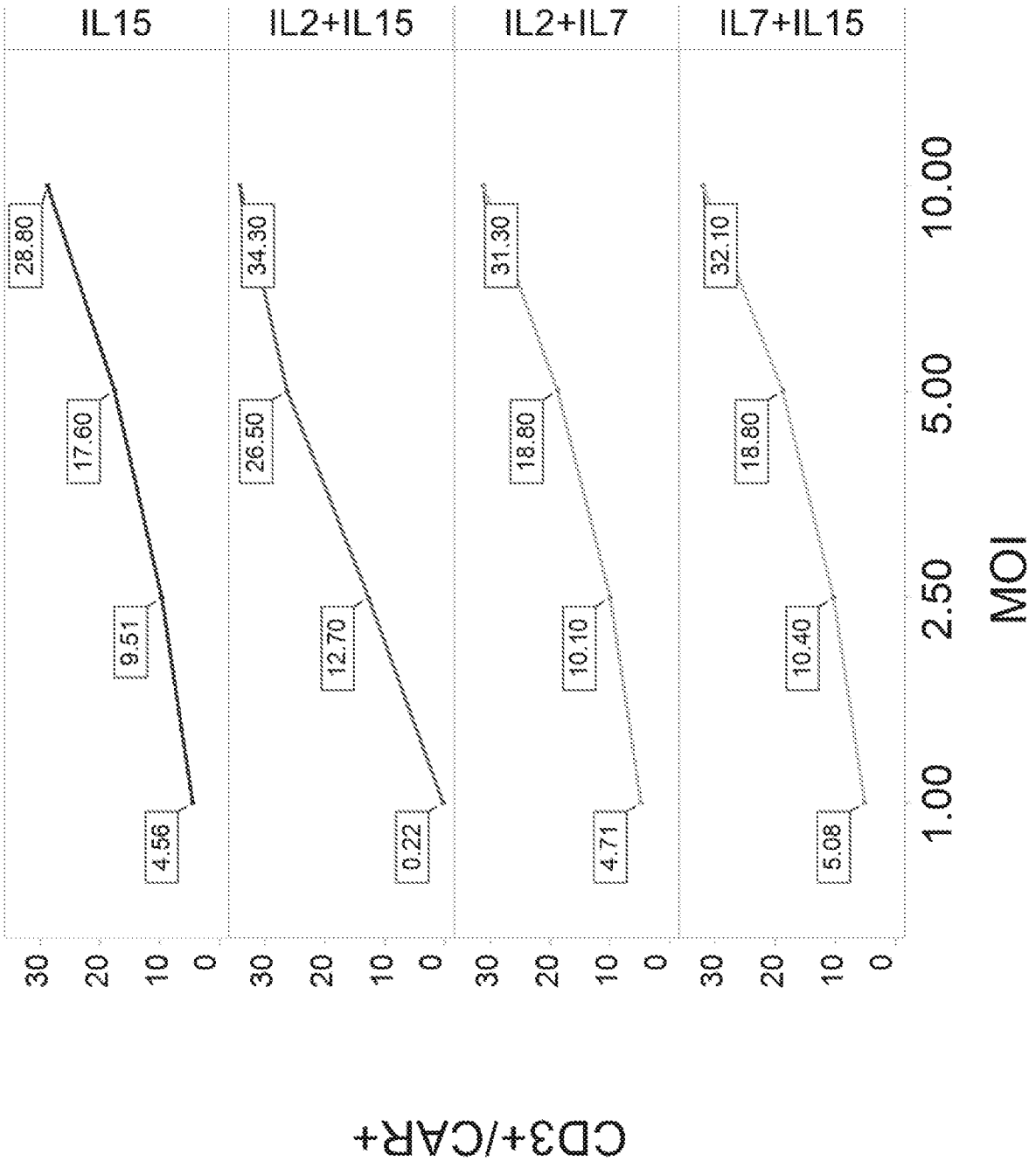

IL15
IL2+IL15
IL2+IL7
IL7+IL15
28.80
17.60
9.51
4.56
34.30
26.50
12.70
0.22
31.30
18.80
10.10
4.71
32.10
18.80
10.40
5.08
0
10
20
30
1.00
2.50
5.00
10.00
MOI
CD3+/CAR+

FIG. 3A

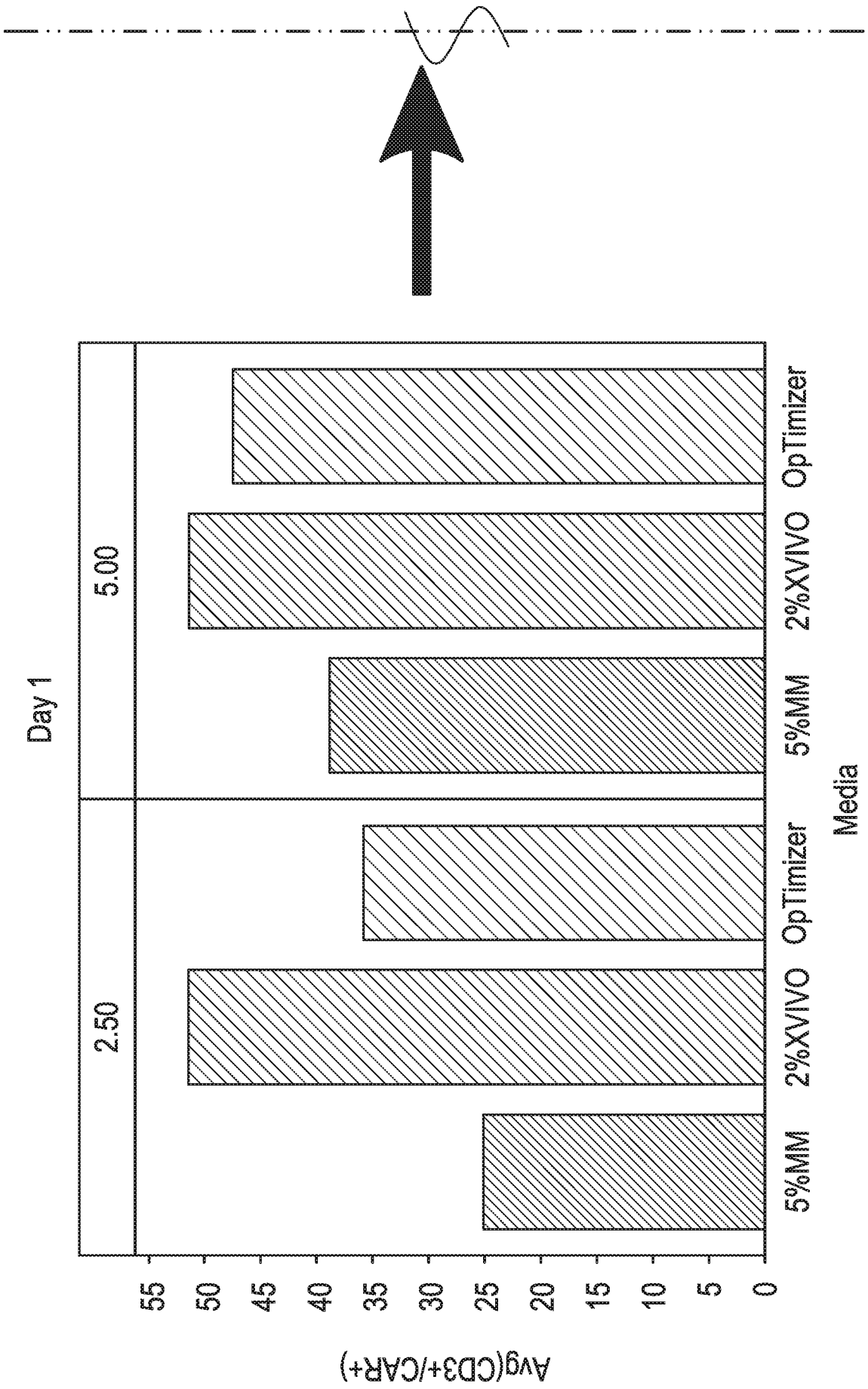

Day 1
2.50
5.00
Avg(CD3+/CAR+)
0
5
10
15
20
25
30
35
40
45
50
55
5%MM
2%XVIVO
OpTimizer
5%MM
2%XVIVO
OpTimizer
Media

FIG. 3B (part 1)

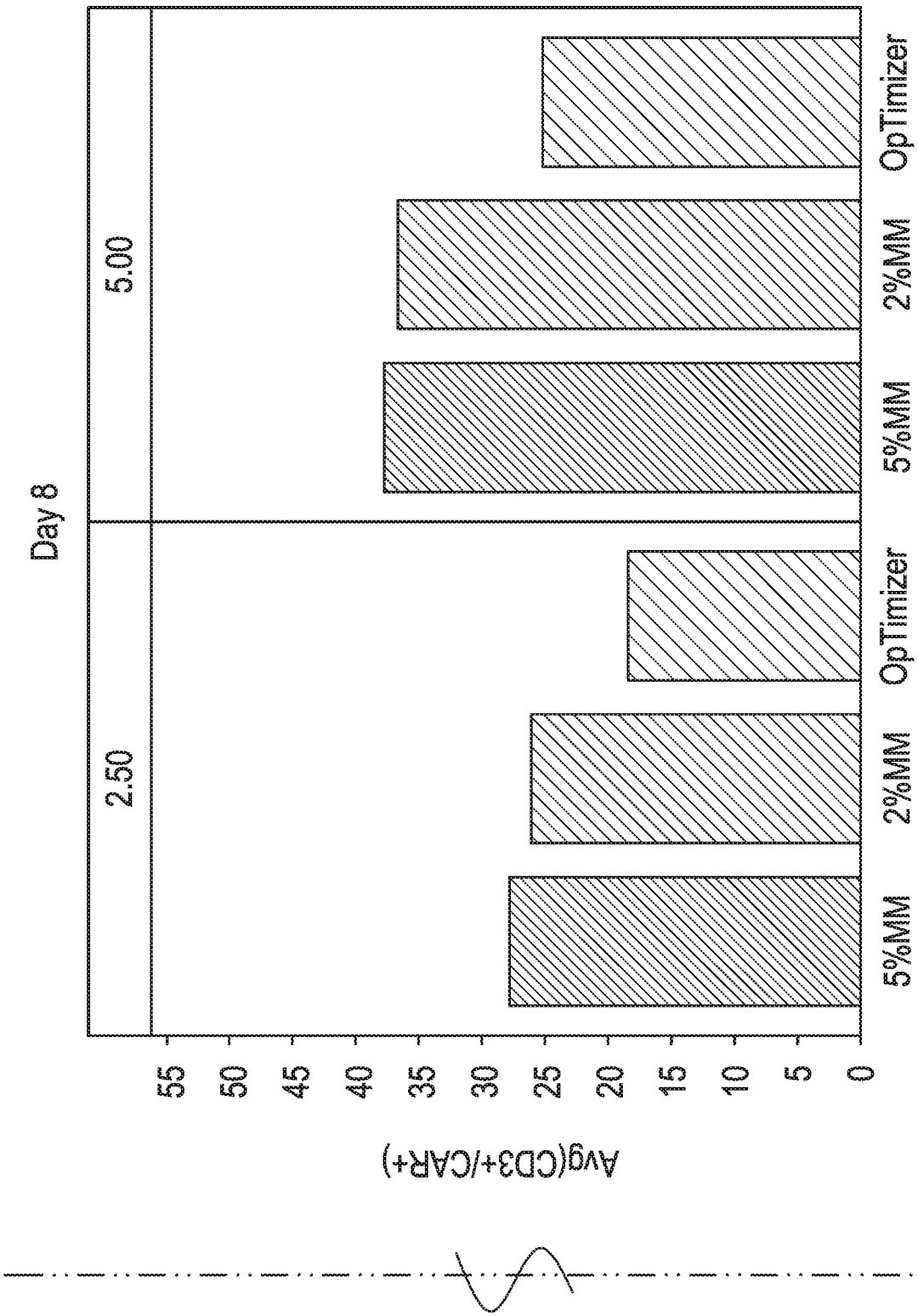

Day 8
2.50
5.00
Avg(CD3+/CAR+)
0
5
10
15
20
25
30
35
40
45
50
55
5%MM
2%MM
OpTimizer
5%MM
2%MM
OpTimizer
Media

FIG. 3B (part 2)

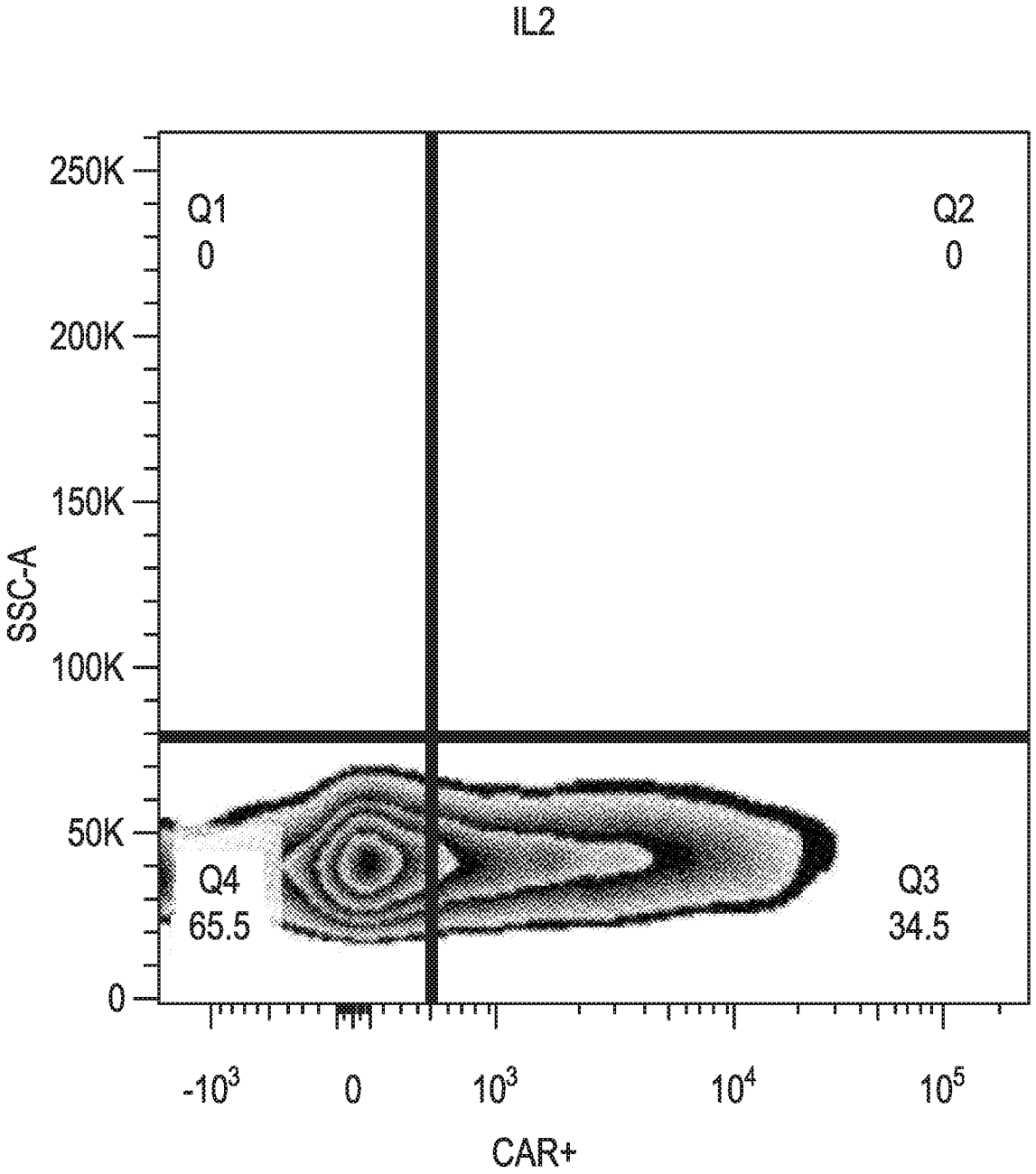

IL2
250K
200K
150K
100K
50K
0
SSC-A
Q1
0
Q2
0
Q4
65.5
Q3
34.5
-10^3
0
10^3
10^4
10^5
CAR+

FIG. 4A (part 1)

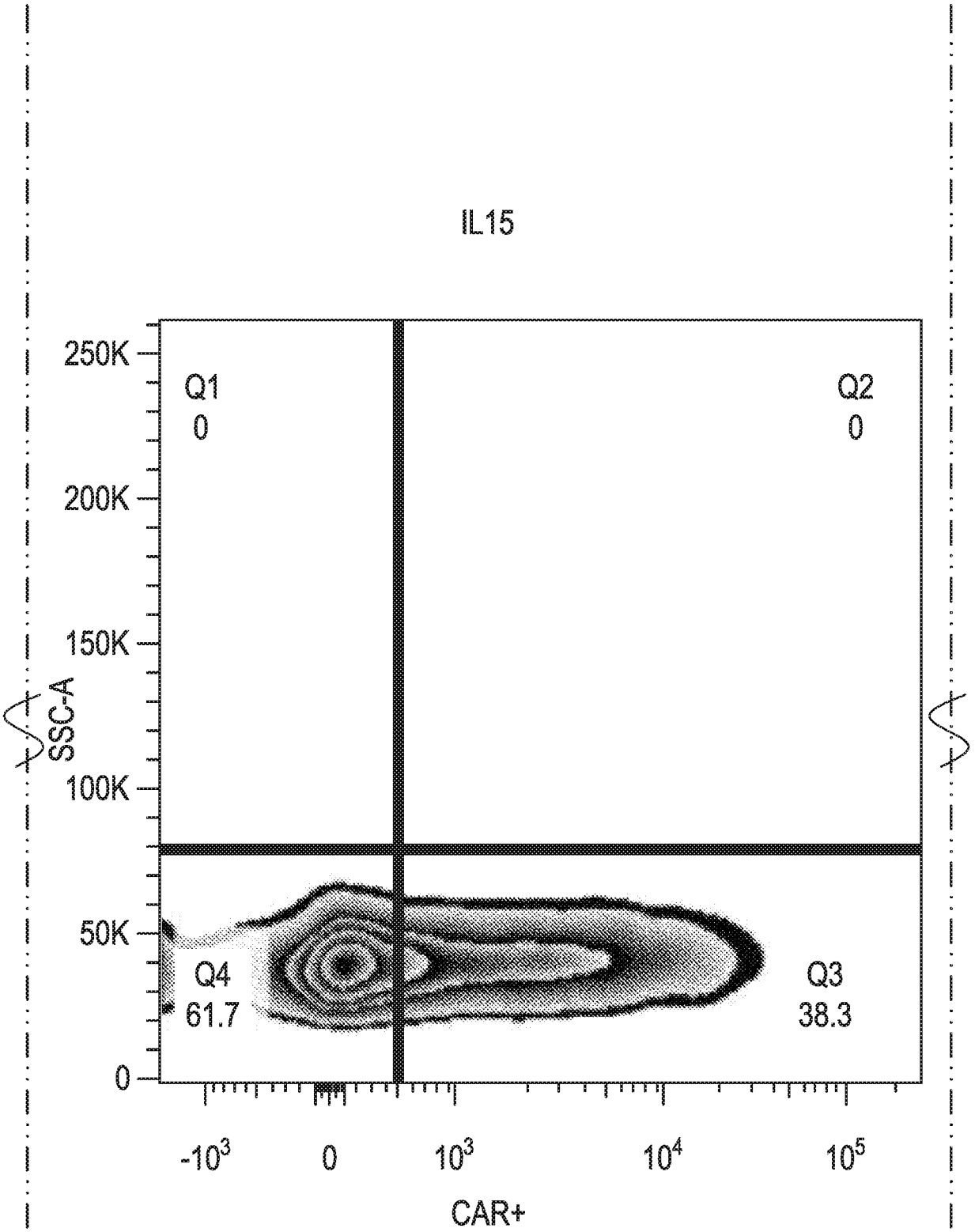

IL15
250K
200K
150K
100K
50K
0
SSC-A
Q1
0
Q2
0
Q4
61.7
Q3
38.3
-10^3
0
10^3
10^4
10^5
CAR+

FIG. 4A (part 2)

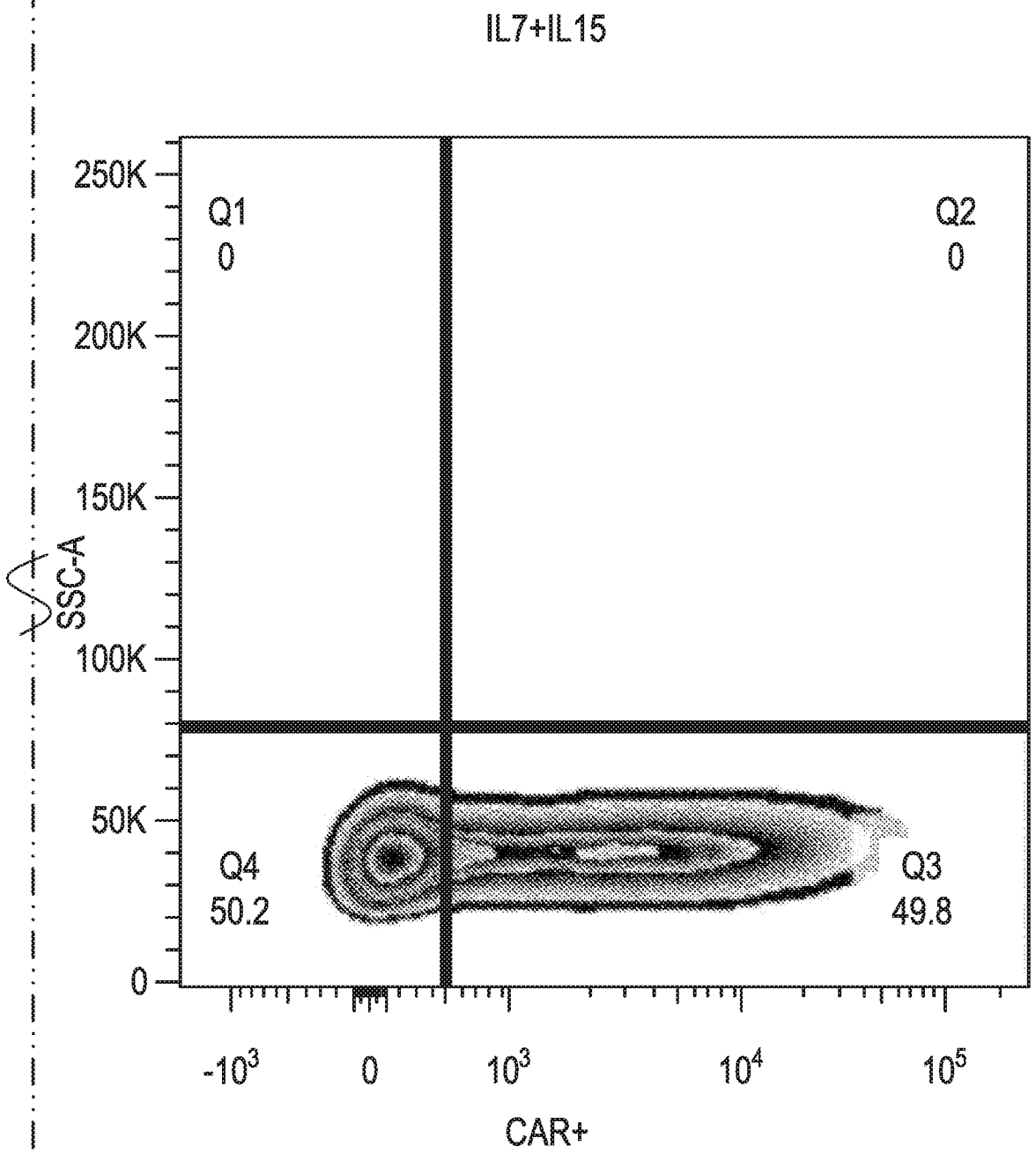

IL7+IL15
250K
200K
150K
100K
50K
0
SSC-A
Q1
0
Q2
0
Q4
50.2
Q3
49.8
-10^3
0
10^3
10^4
10^5
CAR+

FIG. 4A (part 3)

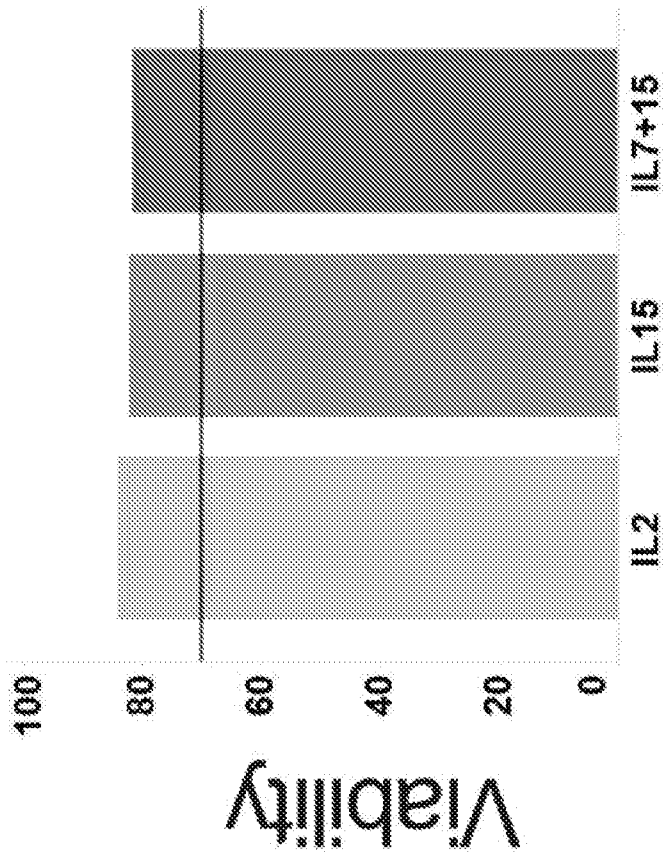



FIG. 4B

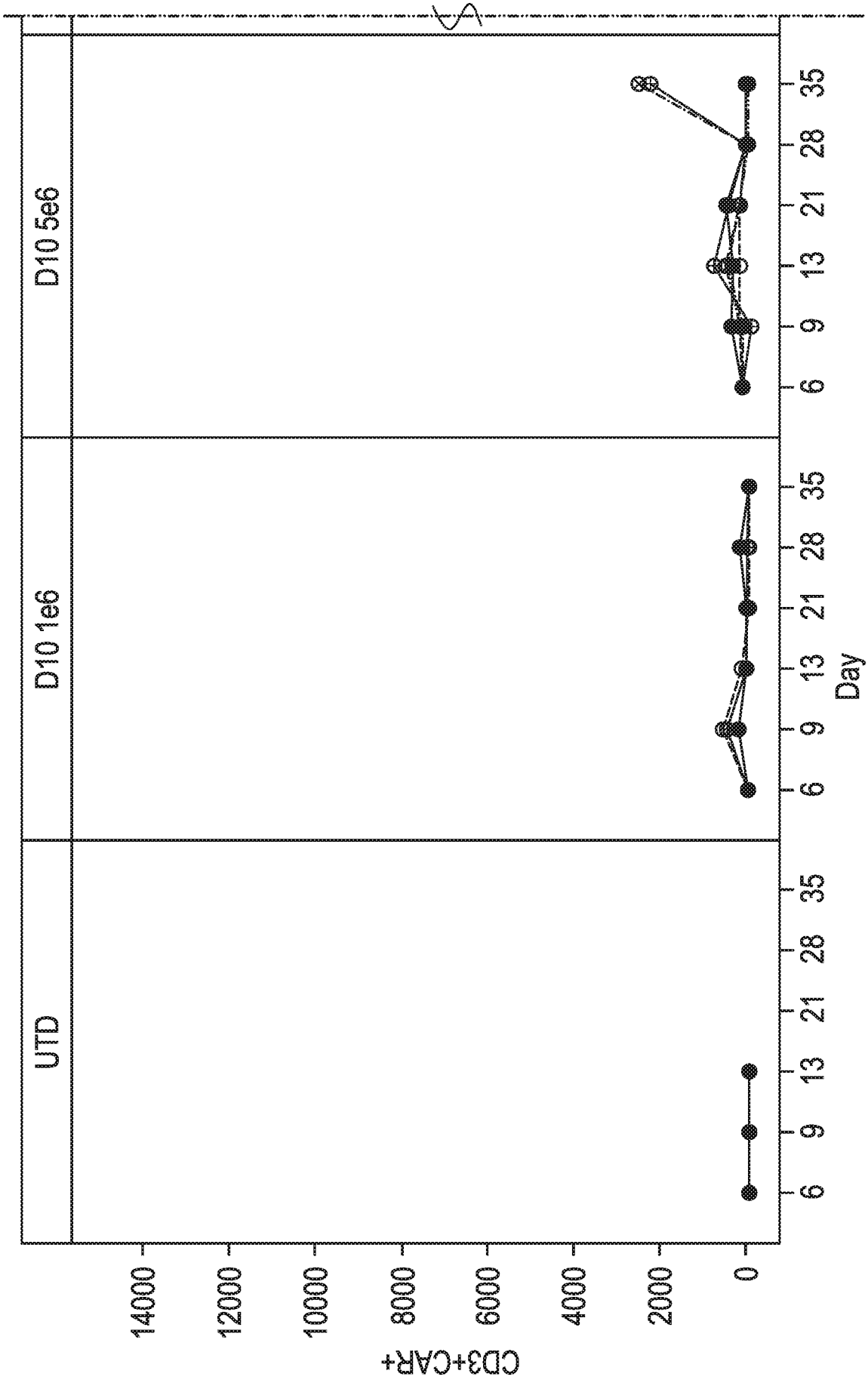

UTD
D10 1e6
D10 5e6
CD3+CAR+
0
2000
4000
6000
8000
10000
12000
14000
6
9
13
21
28
35
Day

FIG. 4C (part 1)

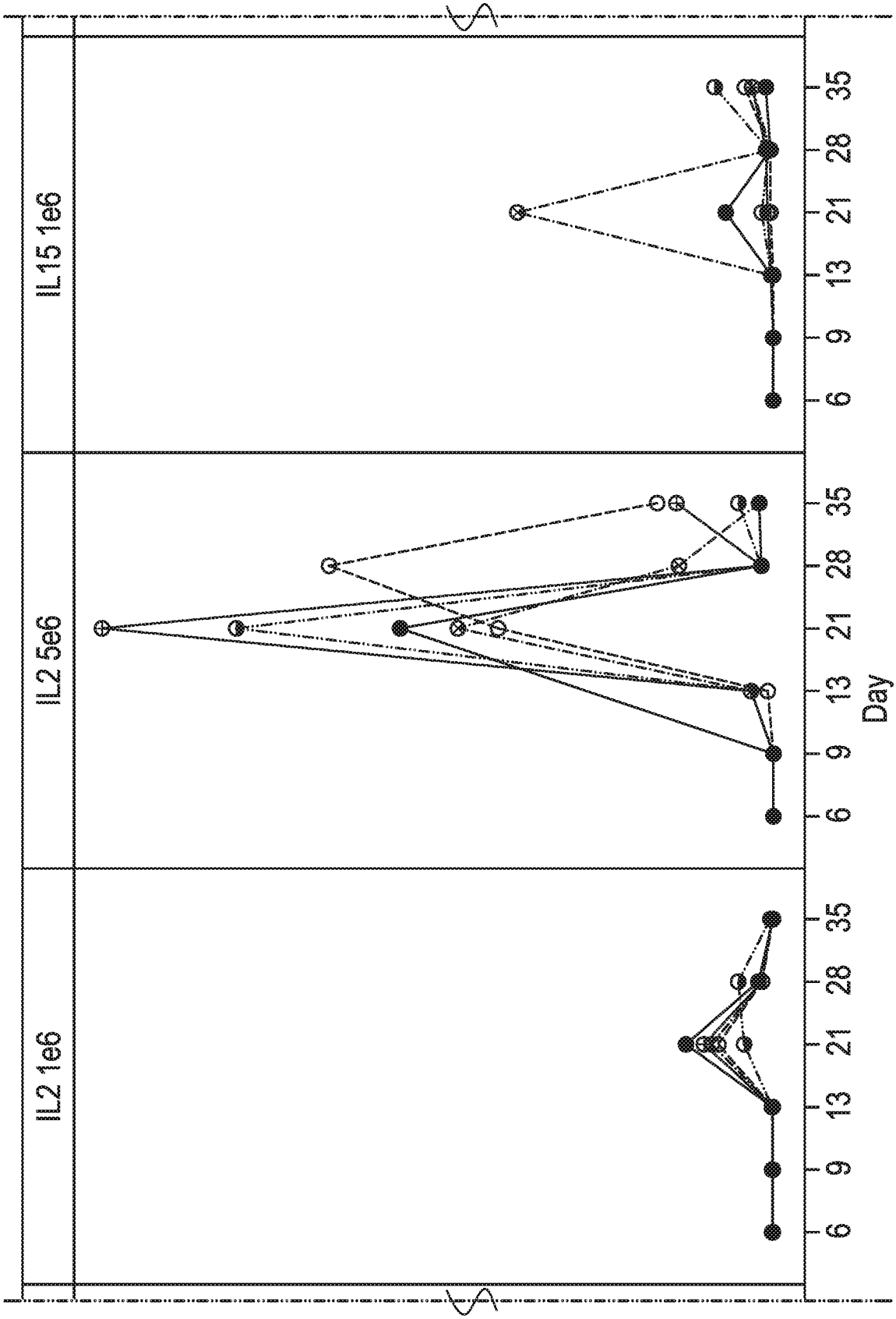



FIG. 4C (part 2)

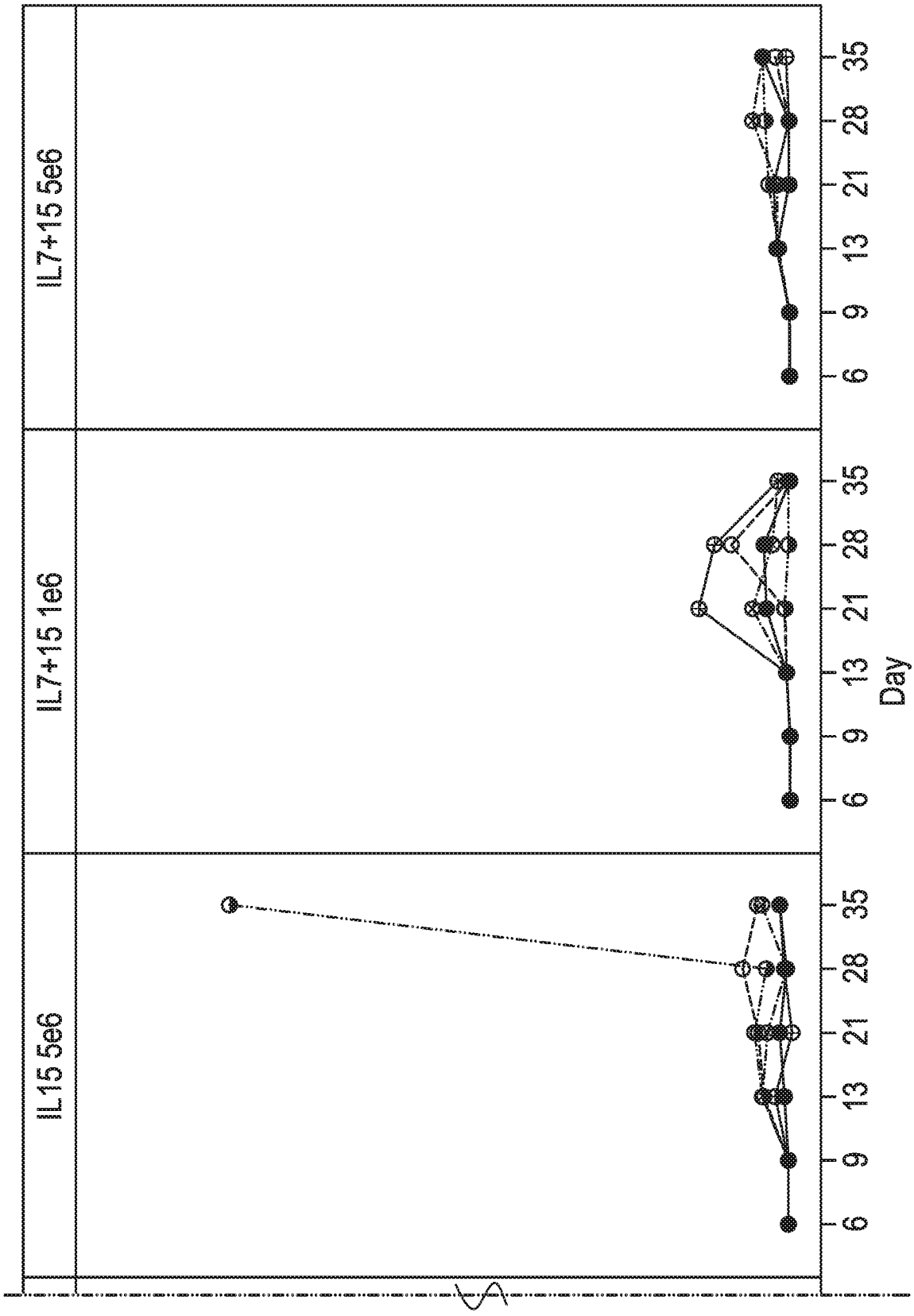



FIG. 4C (part 3)

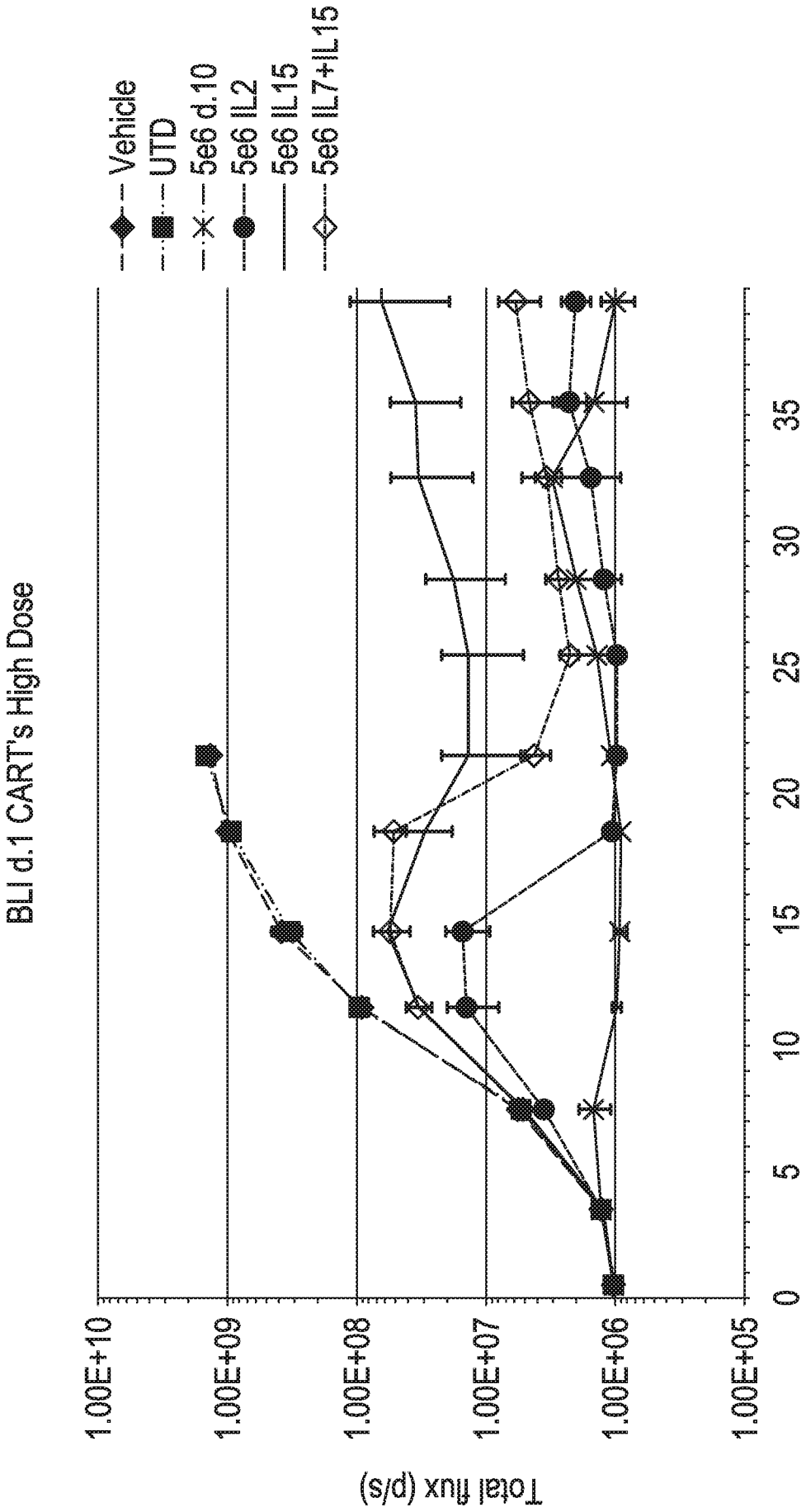

BLI d.1 CART's High Dose
Vehicle
UTD
5e6 d.10
5e6 IL2
5e6 IL15
5e6 IL7+IL15
Total flux (p/s)
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
0
5
10
15
20
25
30
35
Day

FIG. 4D

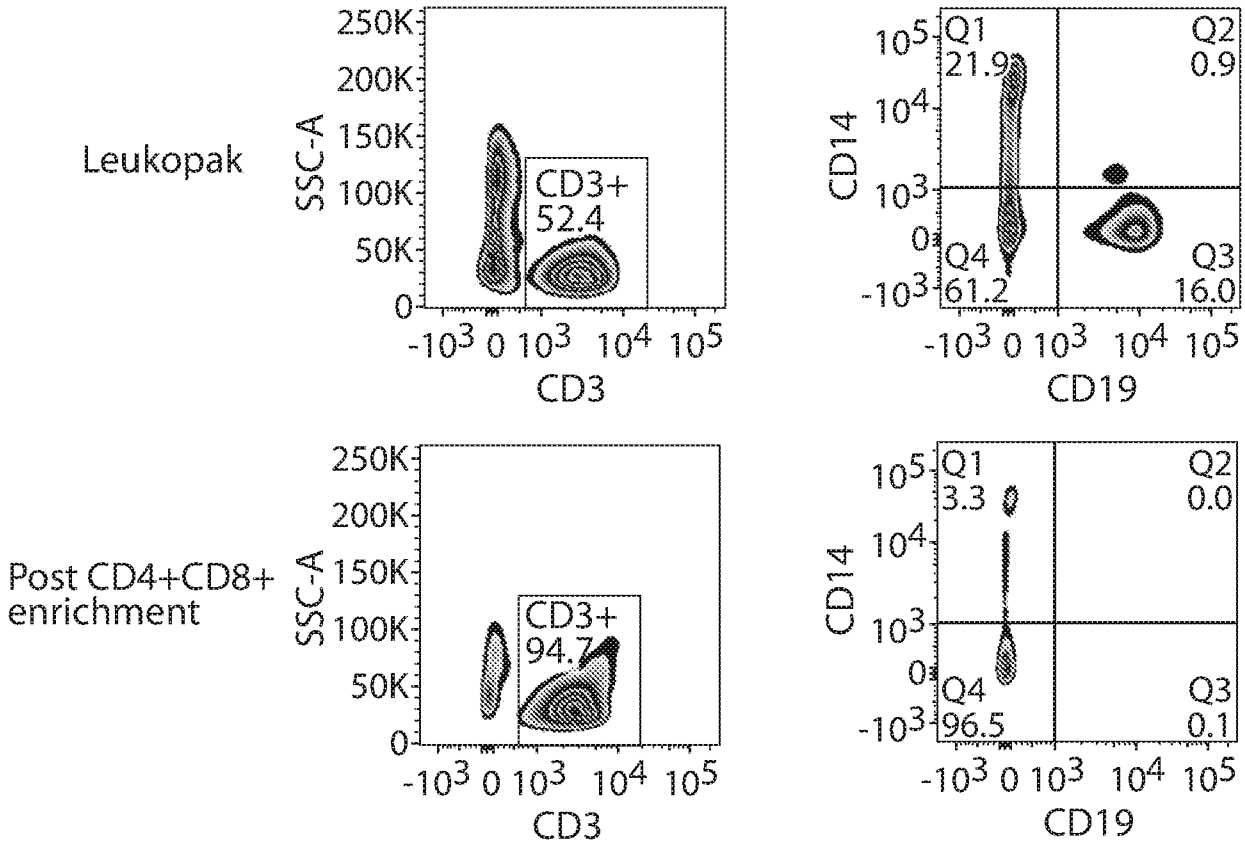

Leukopak
SSC-A
250K
200K
150K
100K
50K
0
CD3+
52.4
-10^3 0 10^3 10^4 10^5
CD3
CD14
Q1
21.9
Q2
0.9
Q4
61.2
Q3
16.0
CD19
Post CD4+CD8+
enrichment
CD3+
94.7
Q1
3.3
Q2
0.0
Q4
96.5
Q3
0.1

FIG. 5A

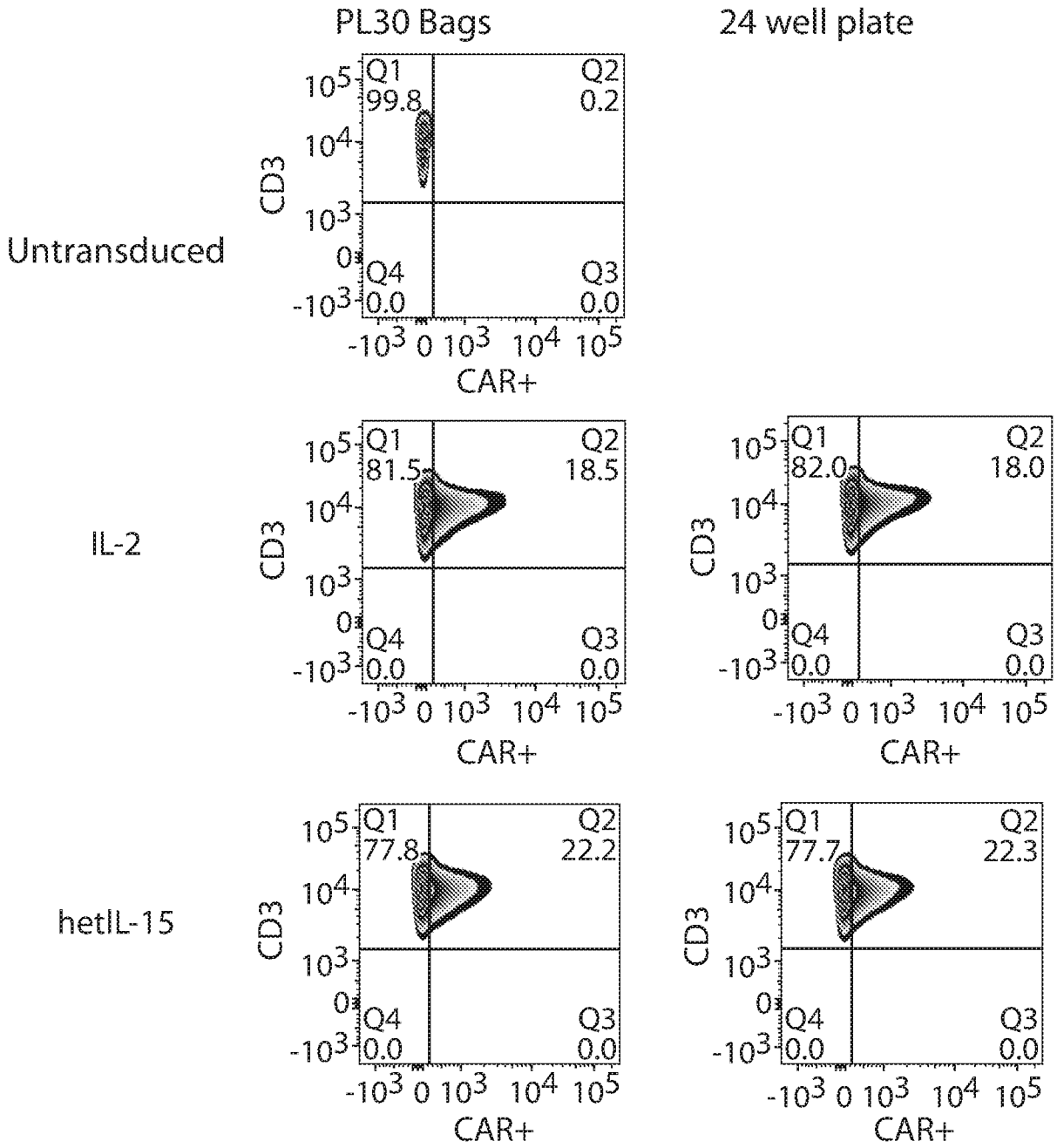

PL30 Bags
24 well plate
Untransduced
IL-2
hetIL-15
CD3
CAR+
Q1
99.8
Q2
0.2
Q4
0.0
Q3
0.0
Q1
81.5
Q2
18.5
Q4
0.0
Q3
0.0
Q1
82.0
Q2
18.0
Q4
0.0
Q3
0.0
Q1
77.8
Q2
22.2
Q4
0.0
Q3
0.0
Q1
77.7
Q2
22.3
Q4
0.0
Q3
0.0

FIG. 5B

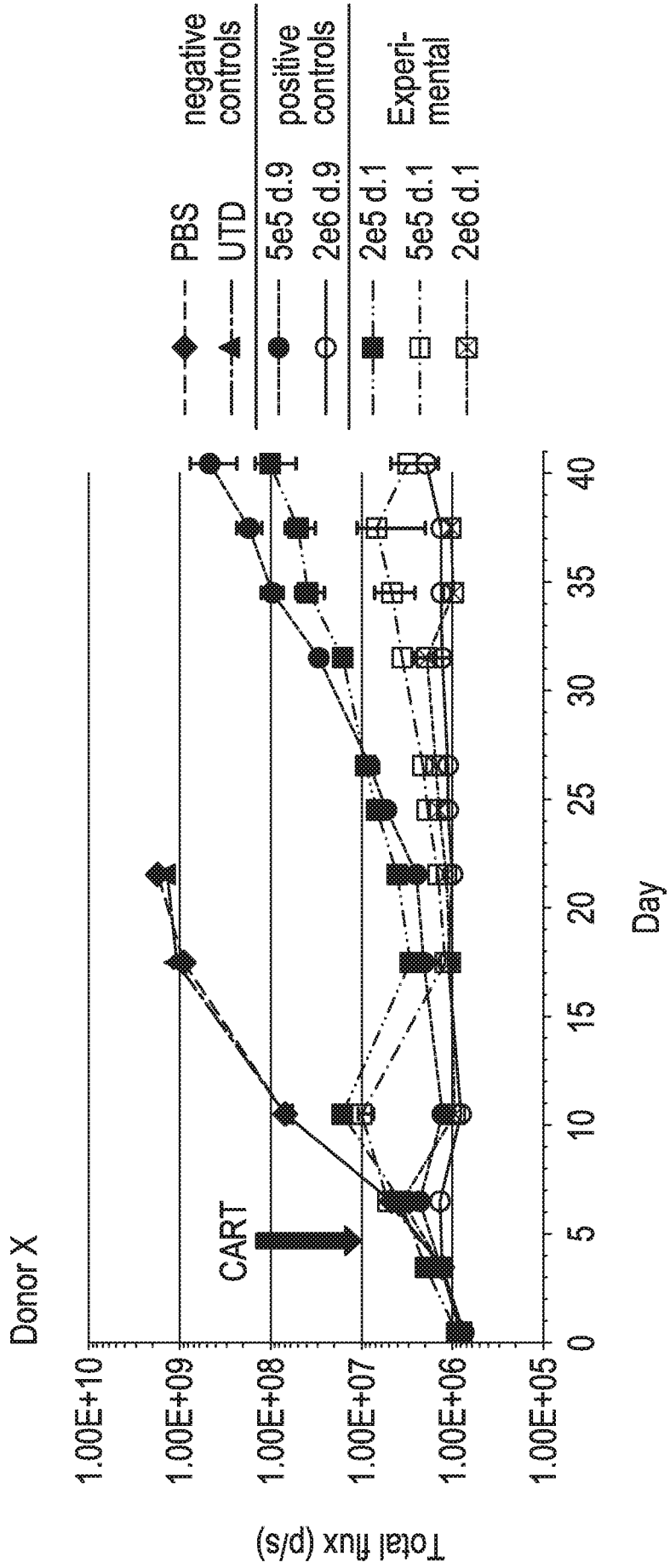

Donor X
CART
Total flux (p/s)
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
0
5
10
15
20
25
30
35
40
Day
PBS
UTD
5e5 d.9
2e6 d.9
2e5 d.1
5e5 d.1
2e6 d.1
negative controls
positive controls
Experi-mental

FIG. 6A

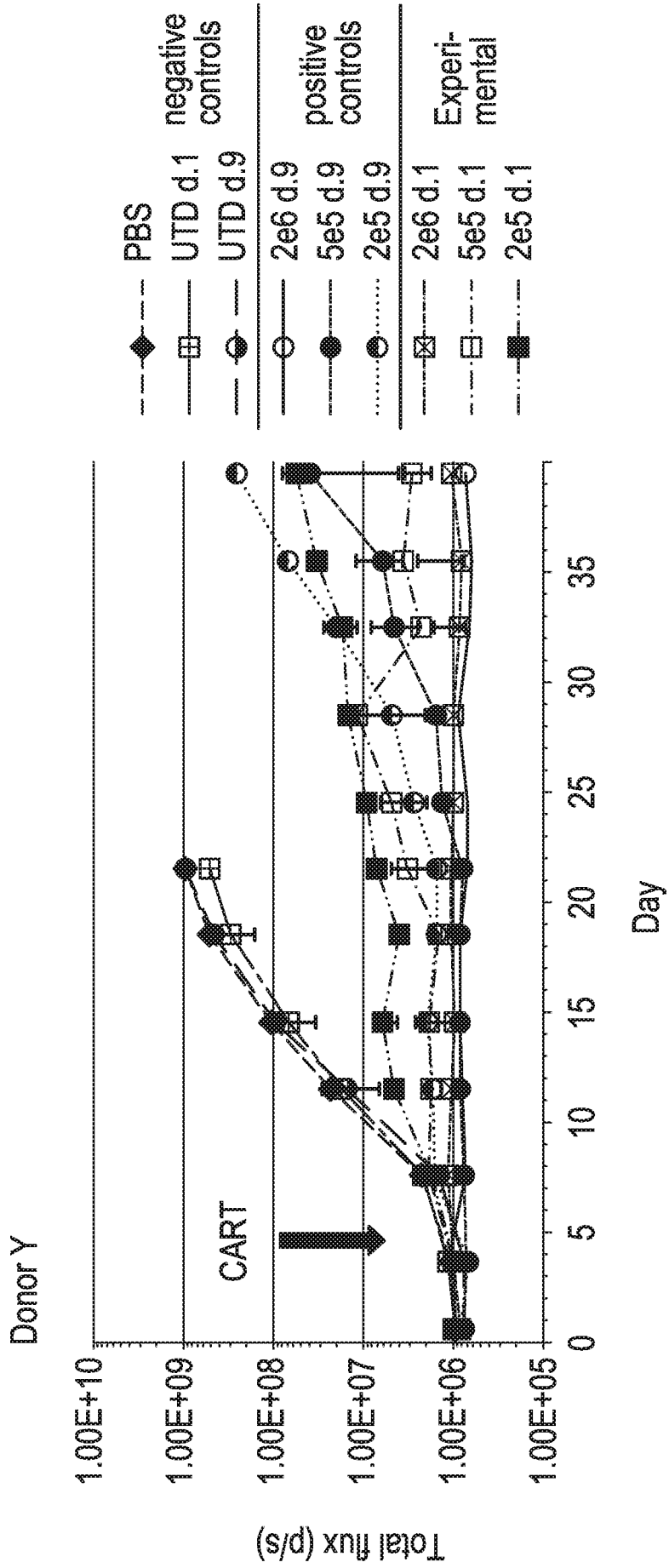
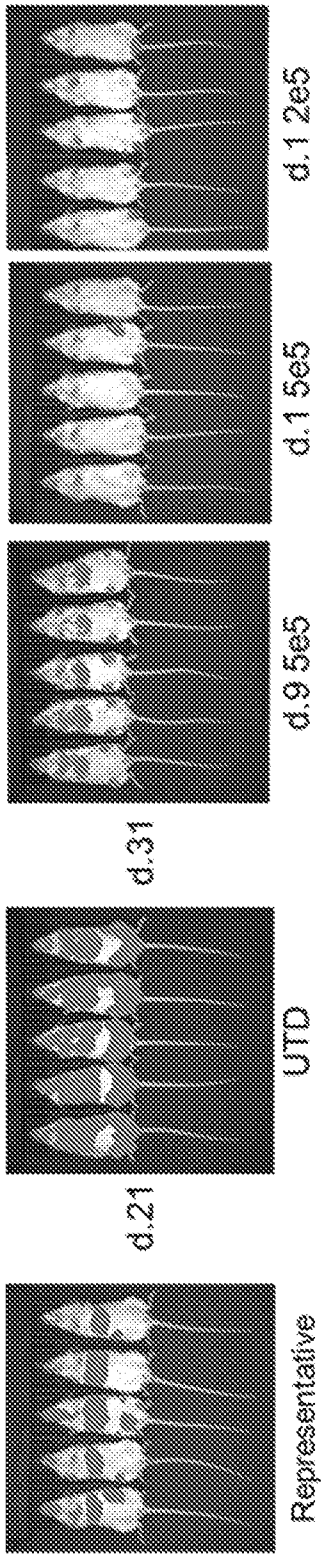

PBS
UTD d.1
UTD d.9
negative controls
2e6 d.9
5e5 d.9
2e5 d.9
positive controls
2e6 d.1
5e5 d.1
2e5 d.1
Experi-mental
Donor Y
CART
Total flux (p/s)
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
0
5
10
15
20
25
30
35
Day

FIG. 6B

FIG. 6C


Representative images – Donor 2
Representative Pre-Randomization
d.21
UTD
d.31
d.9 5e5
d.1 5e5
d.1 2e5

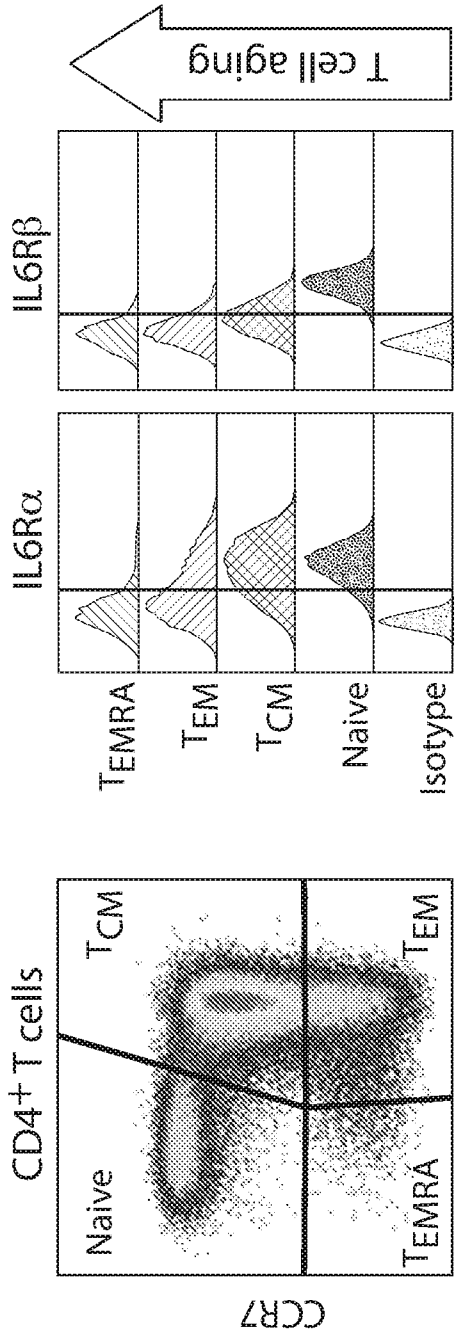

CD4+ T cells
Naive
TCM
TEM
TEMRA
CCR7
CD45RO
IL6Rα
IL6Rβ
TEMRA
TEM
TCM
Naive
Isotype
T cell aging

FIG. 7A

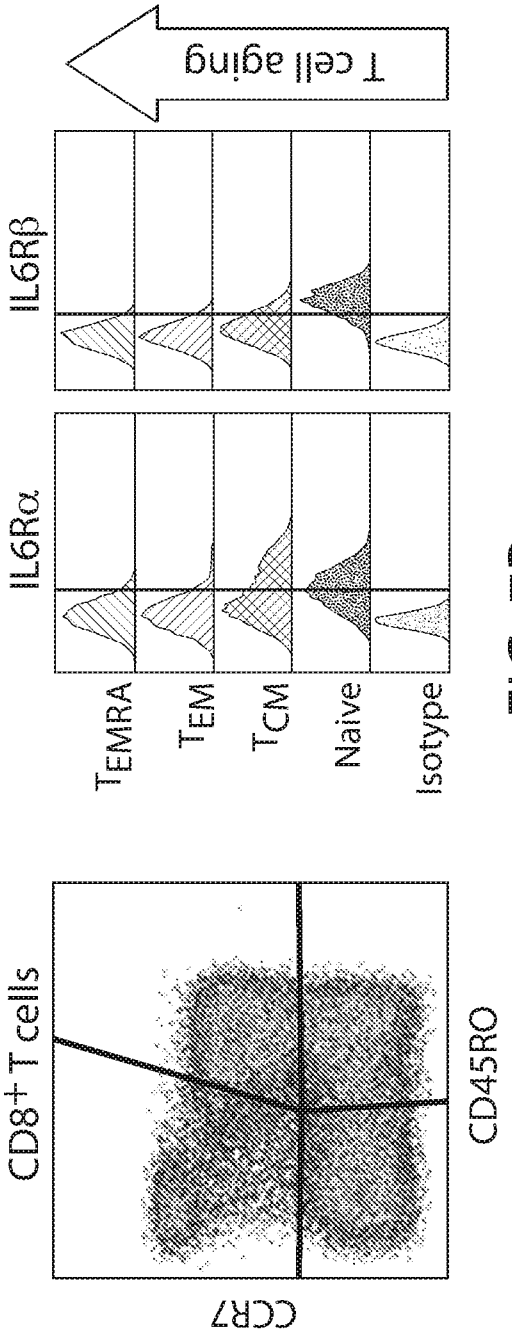

CD8+ T cells
CCR7
CD45RO
IL6Rα
IL6Rβ
TEMRA
TEM
TCM
Naive
Isotype
T cell aging

FIG. 7B

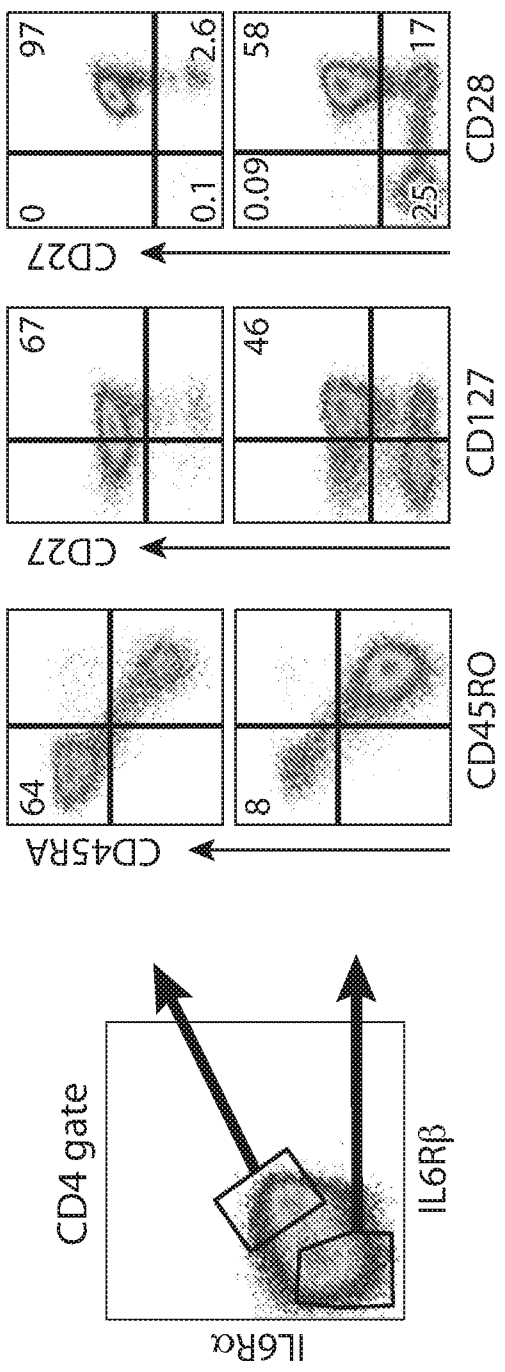

CD4 gate
IL6Rα
IL6Rβ
CD45RA
CD45RO
CD27
CD127
CD28
64
8
67
46
97
0
2.6
0.1
58
0.09
17
25

FIG. 8A

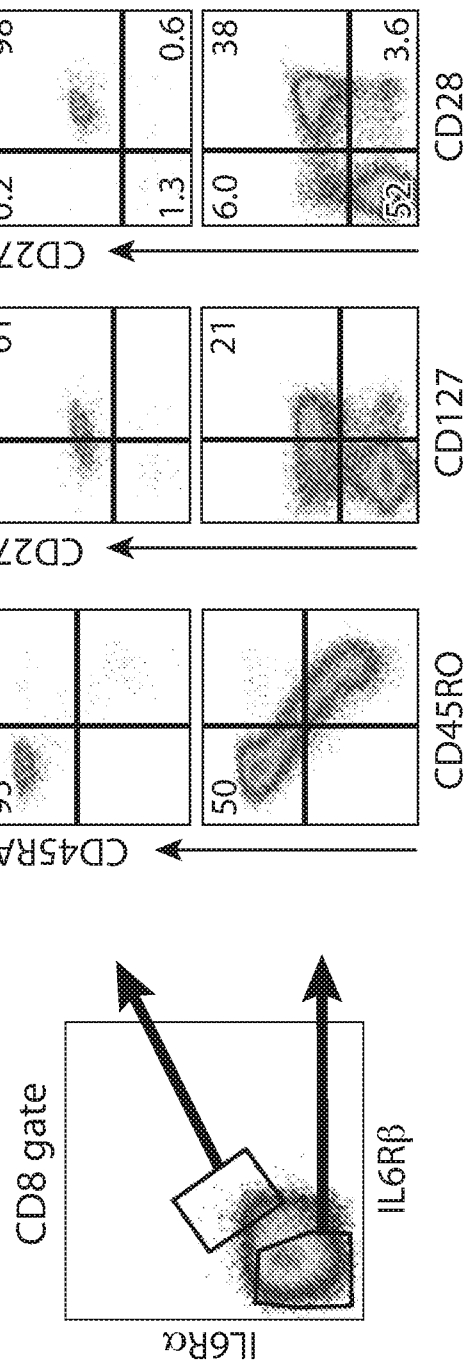

CD8 gate
IL6Rα
IL6Rβ
CD45RA
CD45RO
CD27
CD127
CD28
95
50
61
21
98
0.2
0.6
1.3
38
6.0
3.6
52

FIG. 8B

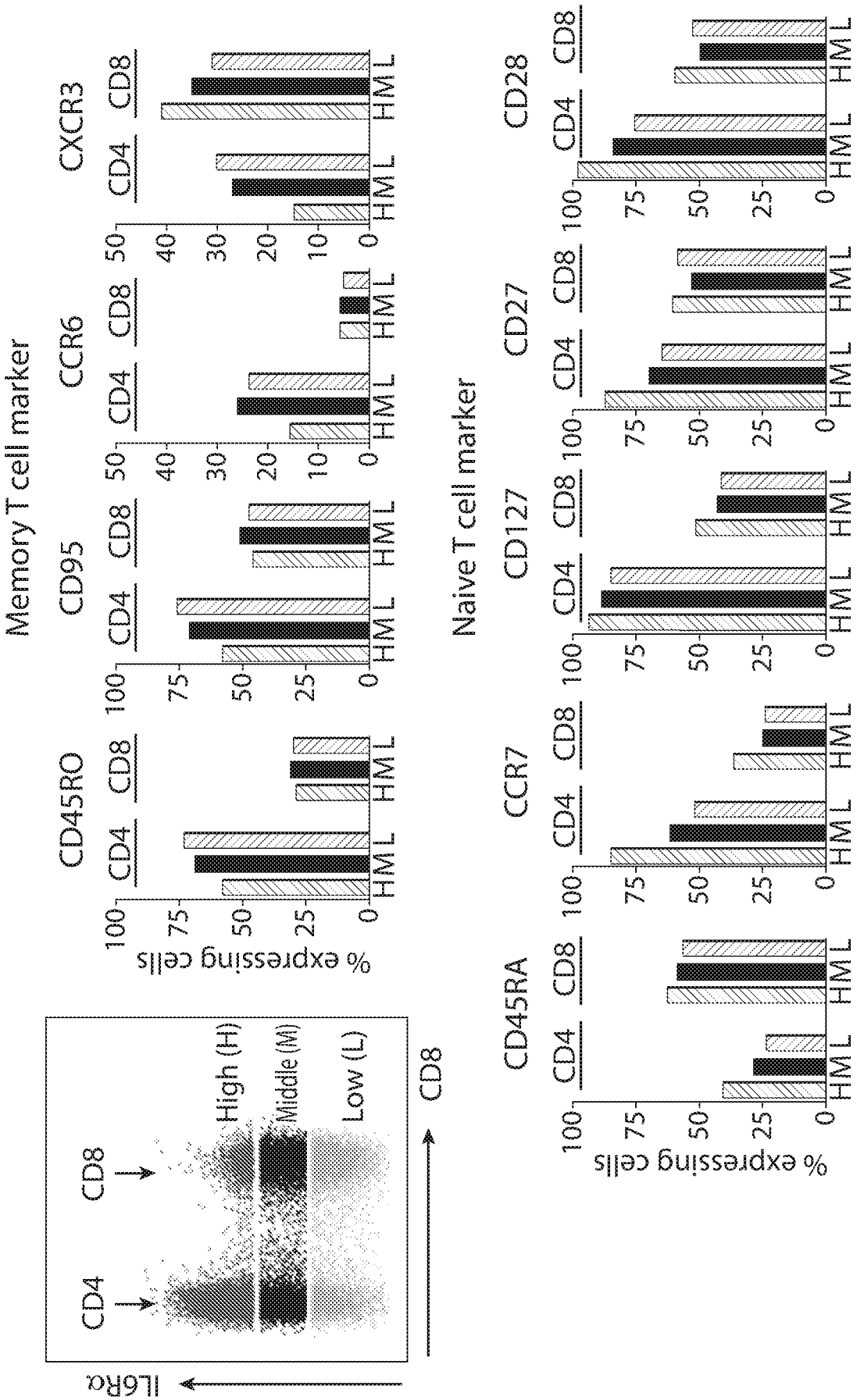

IL6Rα
CD4
CD8
High (H)
Middle (M)
Low (L)
CD8
% expressing cells
Memory T cell marker
CD45RO
CD95
CCR6
CXCR3
Naive T cell marker
CD45RA
CCR7
CD127
CD27
CD28
CD4
CD8
H M L

FIG. 9

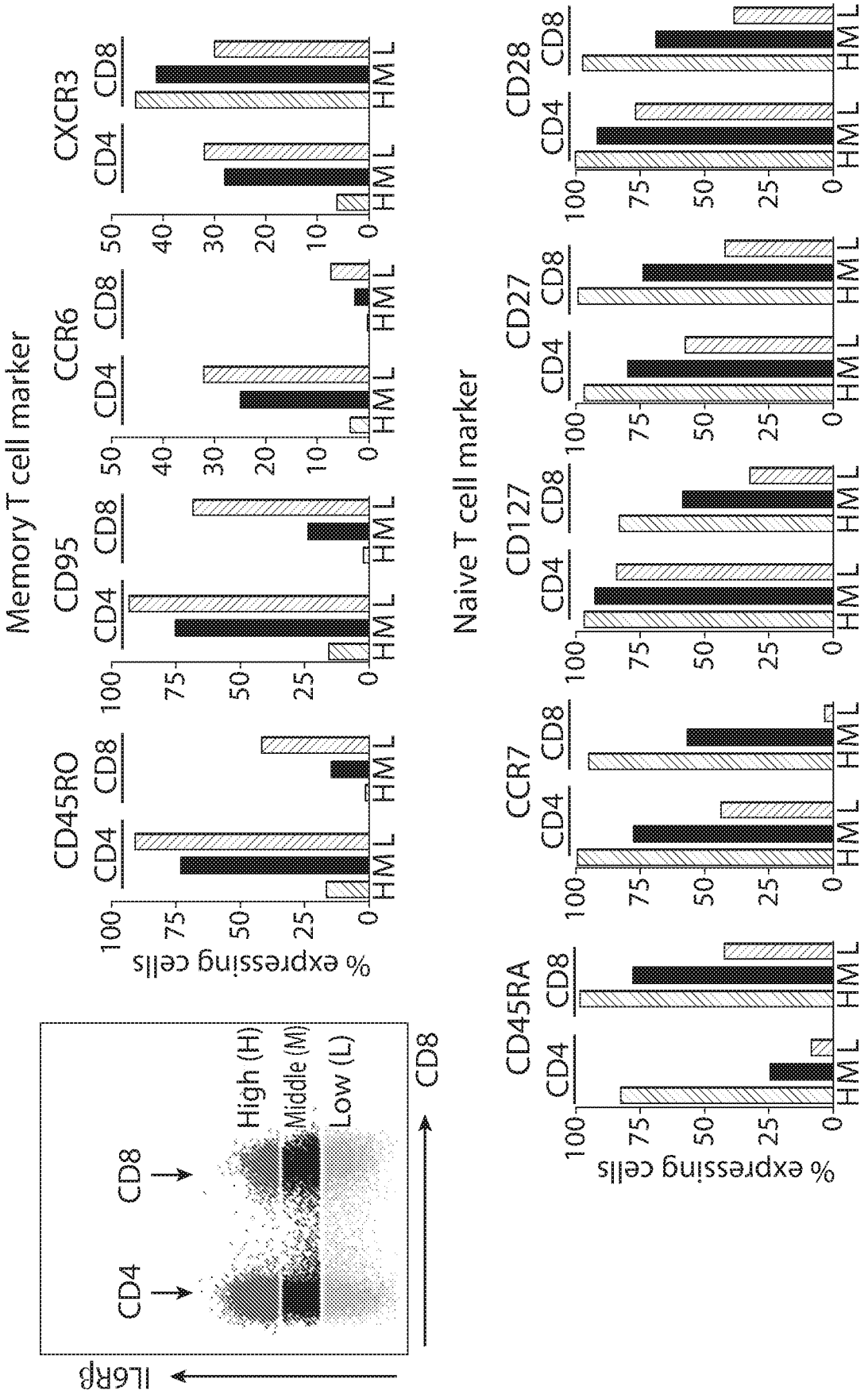
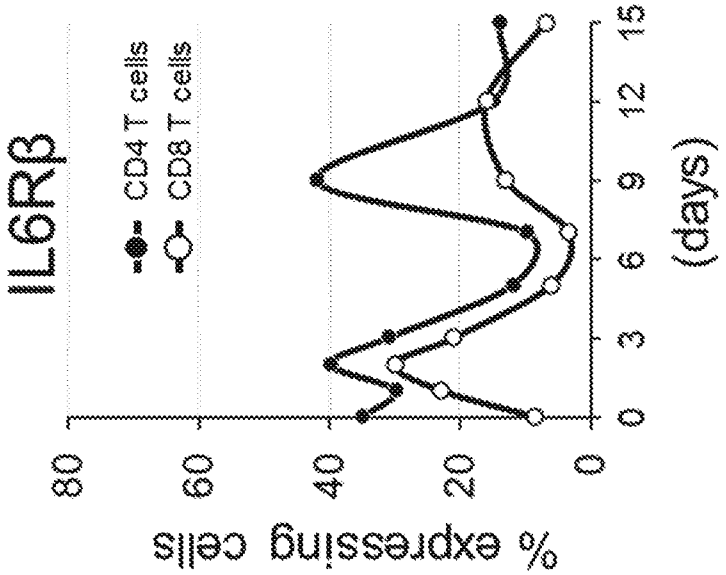

Memory T cell marker
CD45RO
CD95
CCR6
CXCR3
% expressing cells
CD4
CD8
H M L
Naive T cell marker
CD45RA
CCR7
CD127
CD27
CD28
IL6Rβ
High (H)
Middle (M)
Low (L)

FIG. 10


IL6Rβ
CD4 T cells
CD8 T cells
% expressing cells
0
20
40
60
80
0
3
6
9
12
15
(days)

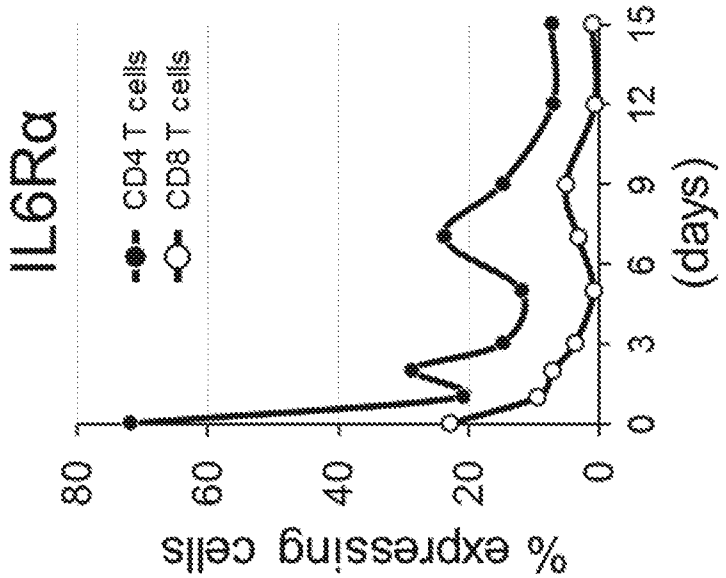

IL6Rα
CD4 T cells
CD8 T cells
% expressing cells
0
20
40
60
80
0
3
6
9
12
15
(days)

FIG. 11

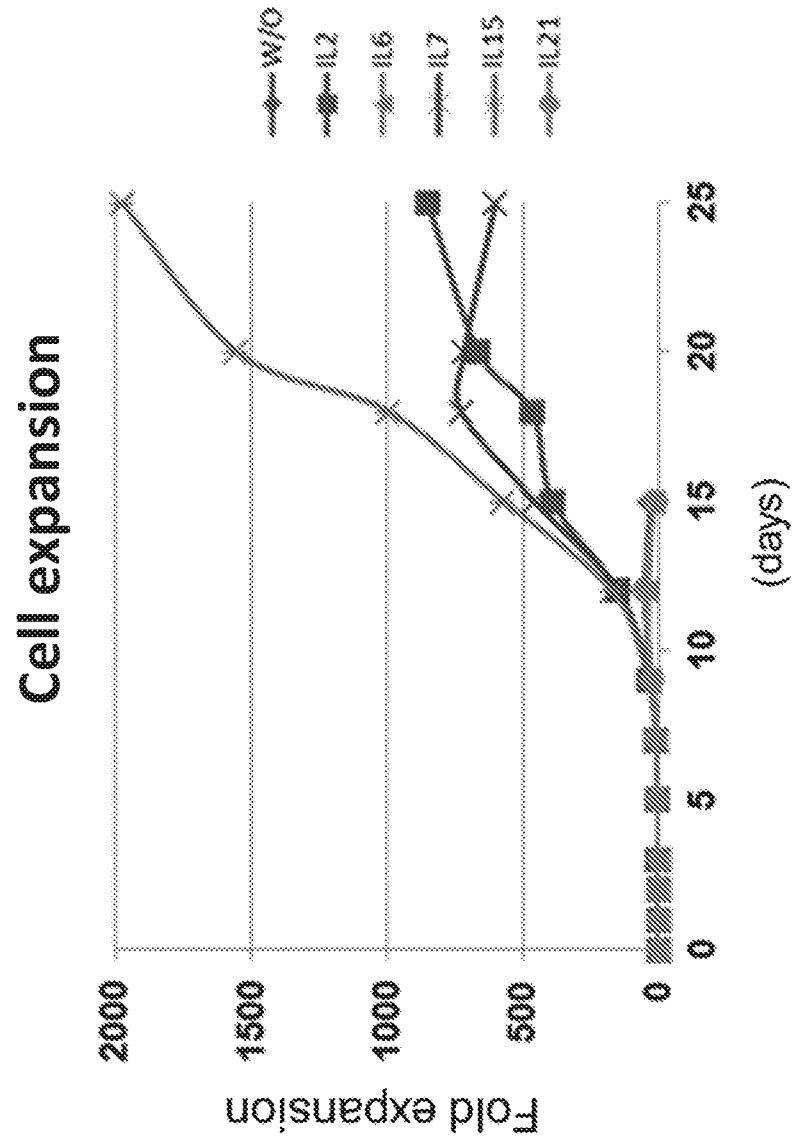

Cell expansion
W/O
IL2
IL6
IL7
IL15
IL21
Fold expansion
2000
1500
1000
500
0
0
5
10
15
20
25
(days)

FIG. 12

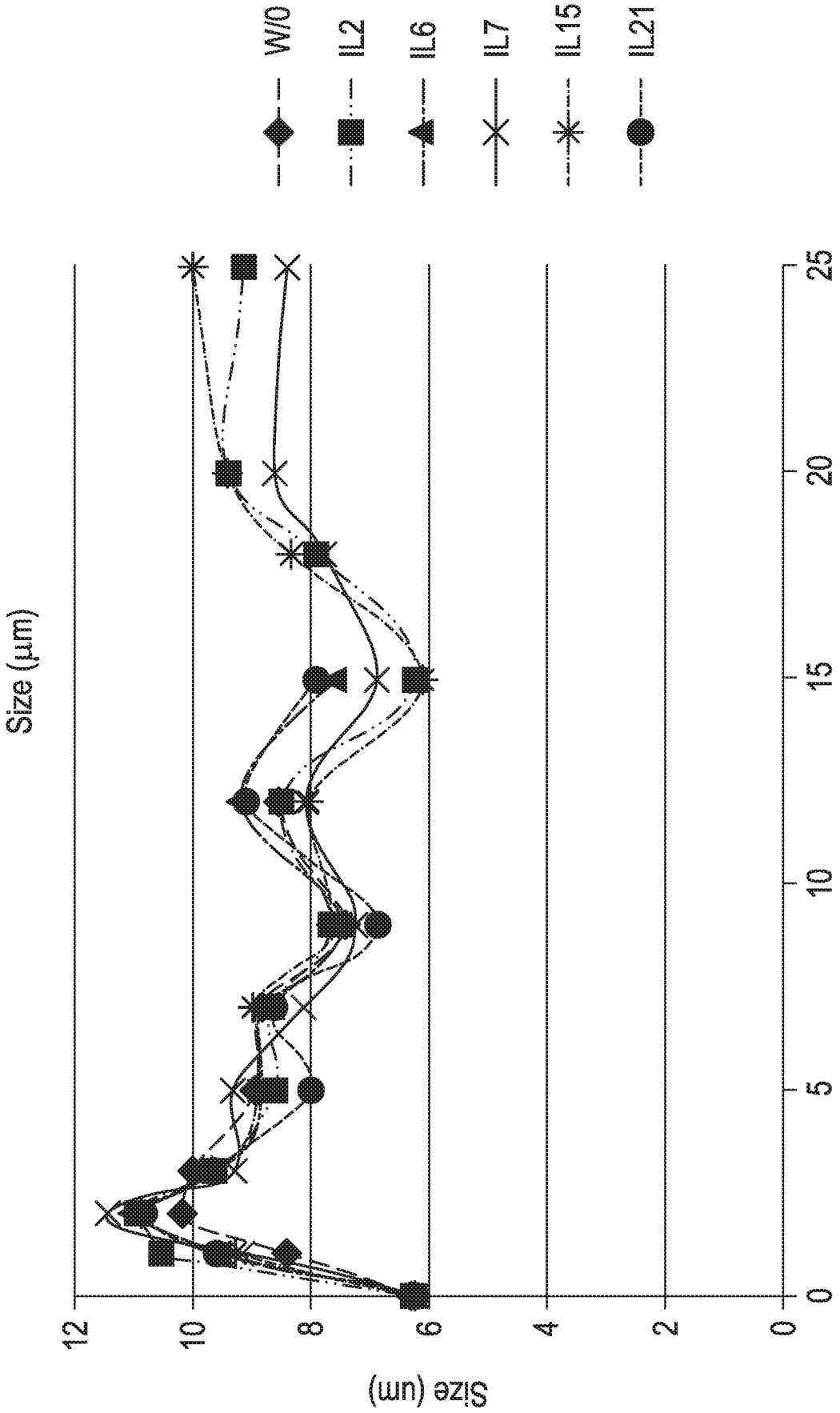

W/O
IL2
IL6
IL7
IL15
IL21
Size (µm)
Size (um)
0
2
4
6
8
10
12
0
5
10
15
20
25
(days)

FIG. 13A

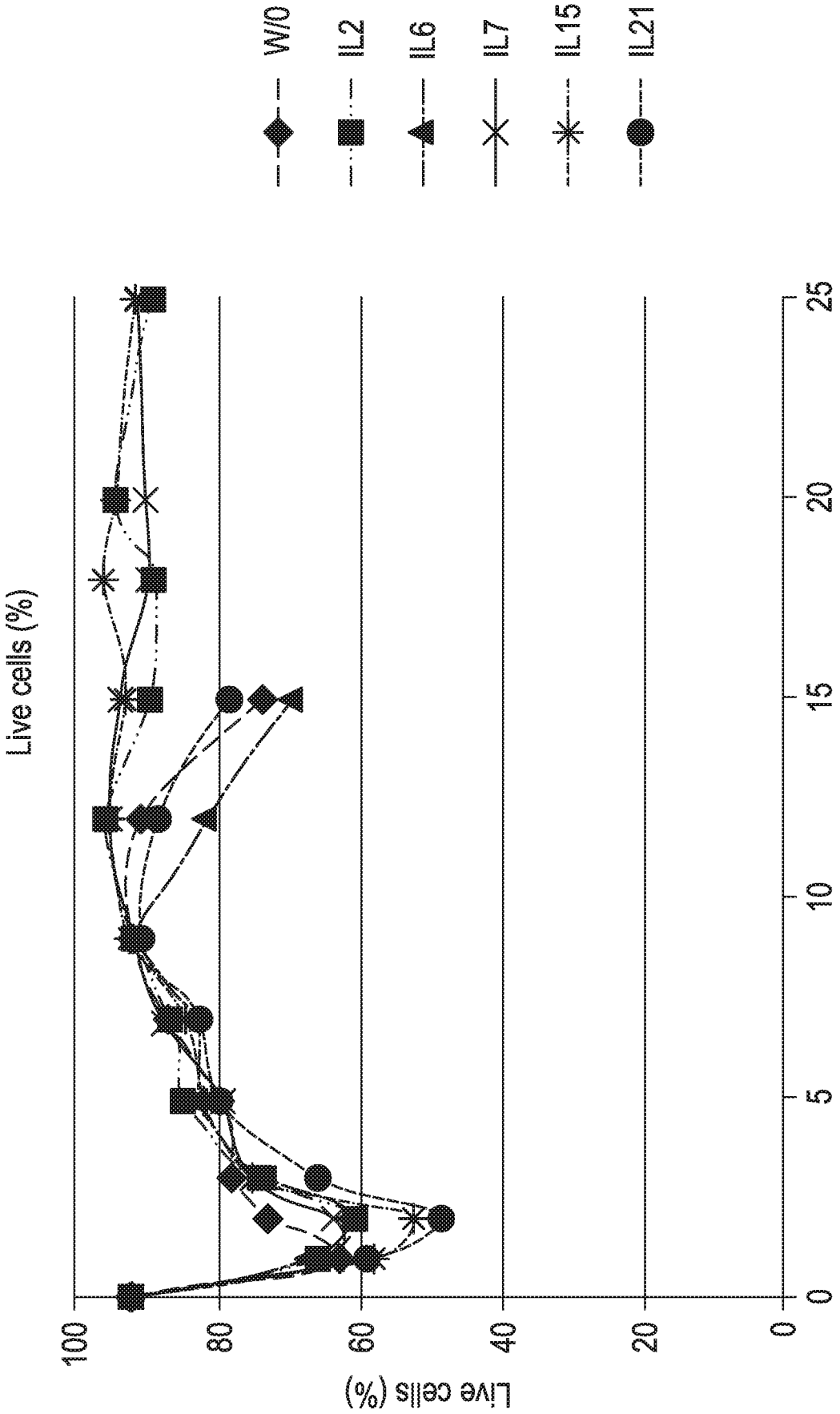

W/0
IL2
IL6
IL7
IL15
IL21
Live cells (%)
100
80
60
40
20
0
0
5
10
15
20
25
(days)
Live cells (%)

FIG. 13B

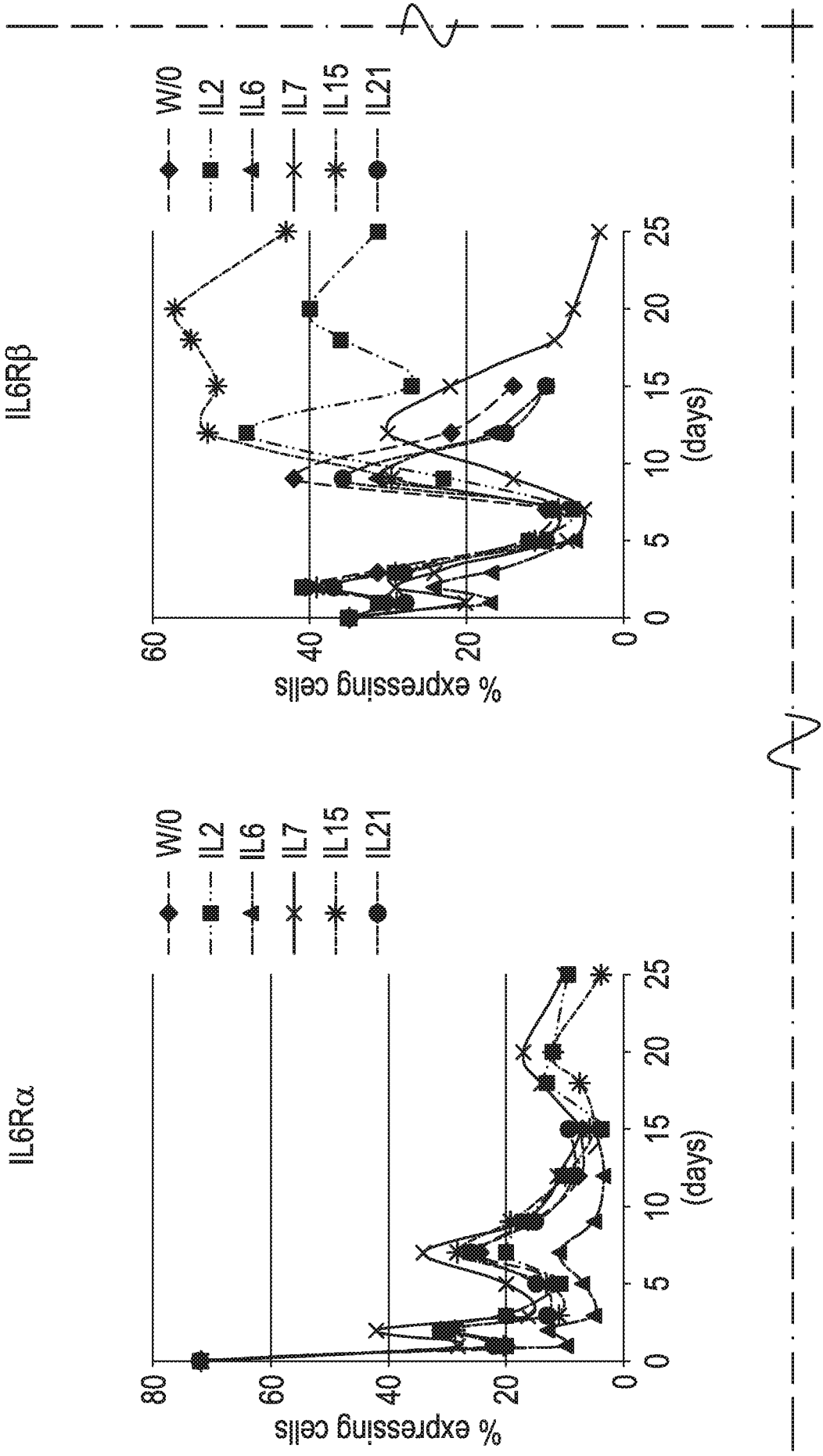

IL6Rβ
W/0
IL2
IL6
IL7
IL15
IL21
% expressing cells
0
20
40
60
0
5
10
15
20
25
(days)
IL6Rα
W/0
IL2
IL6
IL7
IL15
IL21
% expressing cells
0
20
40
60
80
0
5
10
15
20
25
(days)

FIG. 14 (part 1)

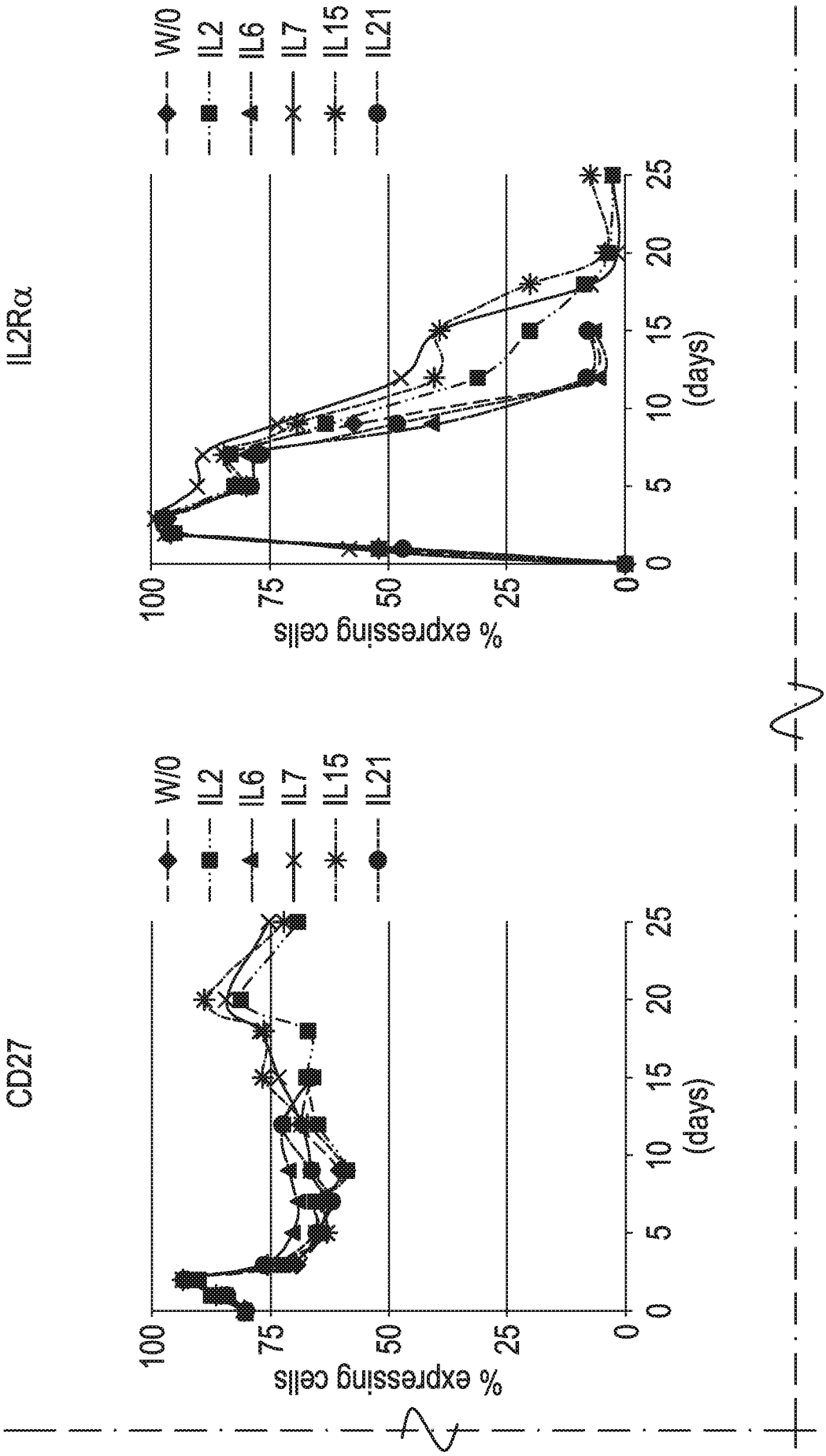

IL2Rα
W/0
IL2
IL6
IL7
IL15
IL21
% expressing cells
100
75
50
25
0
0
5
10
15
20
25
(days)
CD27
W/0
IL2
IL6
IL7
IL15
IL21
% expressing cells
100
75
50
25
0
0
5
10
15
20
25
(days)

FIG. 14 (part 2)

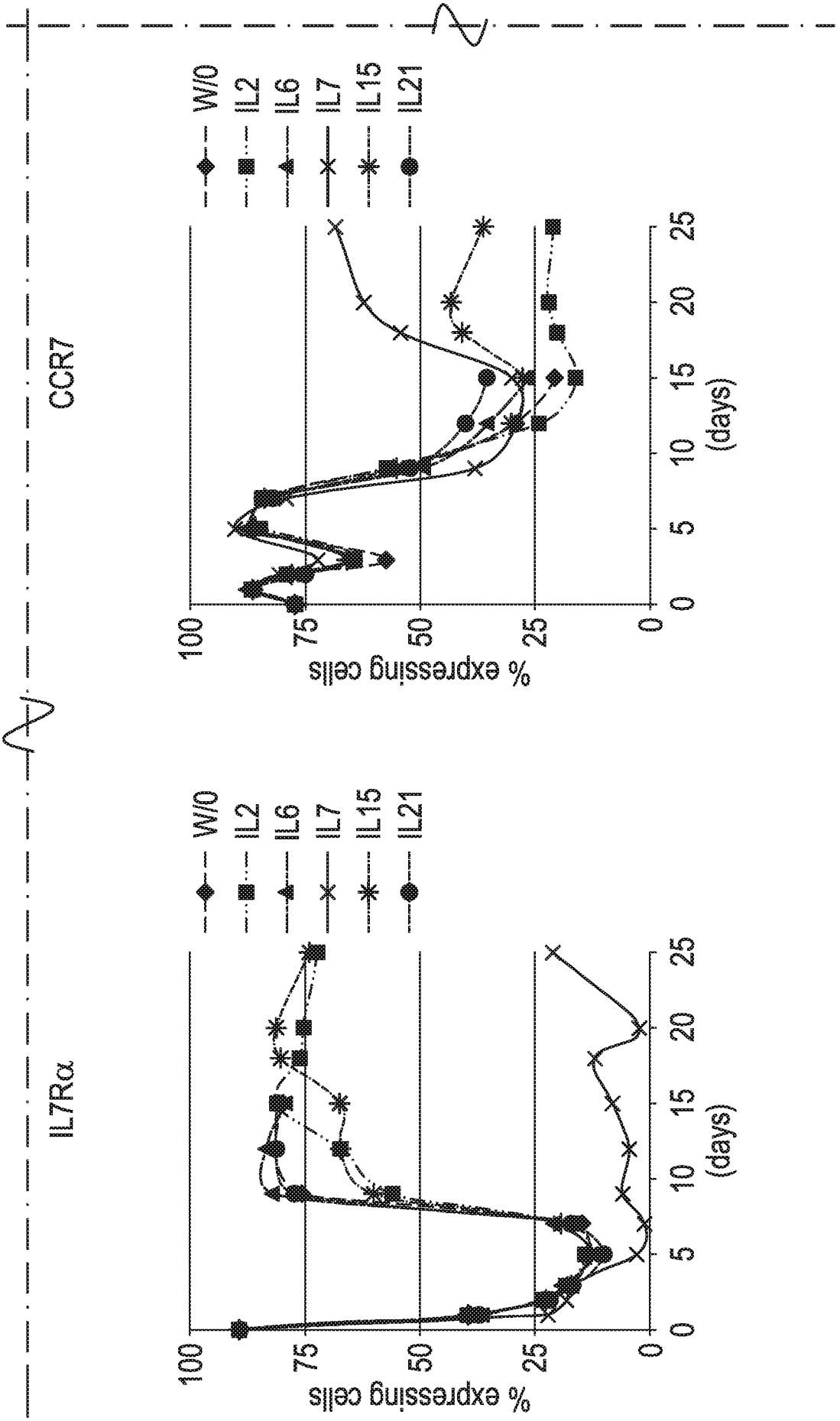

CCR7
IL7Rα
W/0
IL2
IL6
IL7
IL15
IL21
% expressing cells
100
75
50
25
0
0
5
10
15
20
25
(days)

FIG. 14 (part 3)

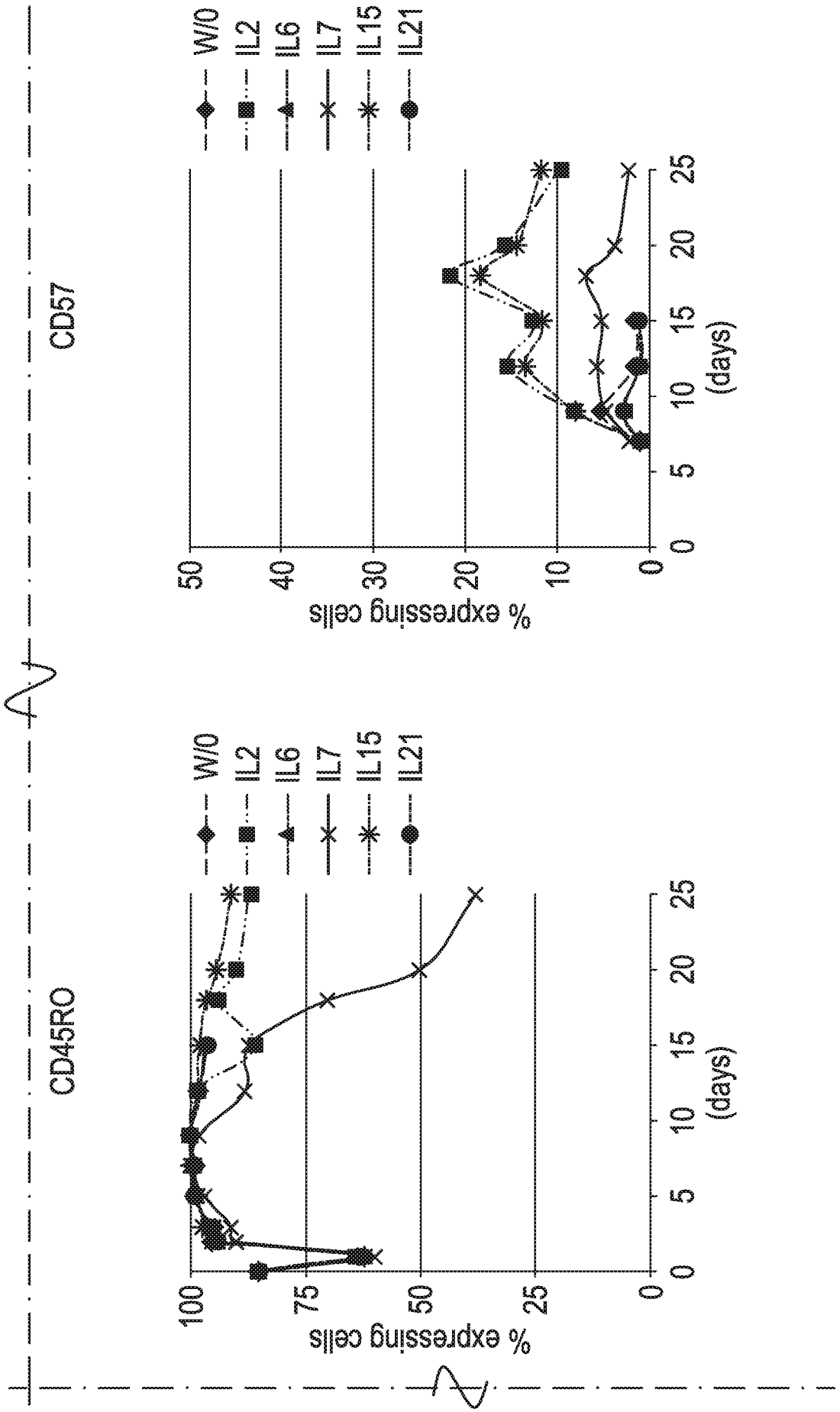

CD45RO
CD57
W/0
IL2
IL6
IL7
IL15
IL21
% expressing cells
(days)
0
5
10
15
20
25
0
25
50
75
100
0
10
20
30
40
50

FIG. 14 (part 4)

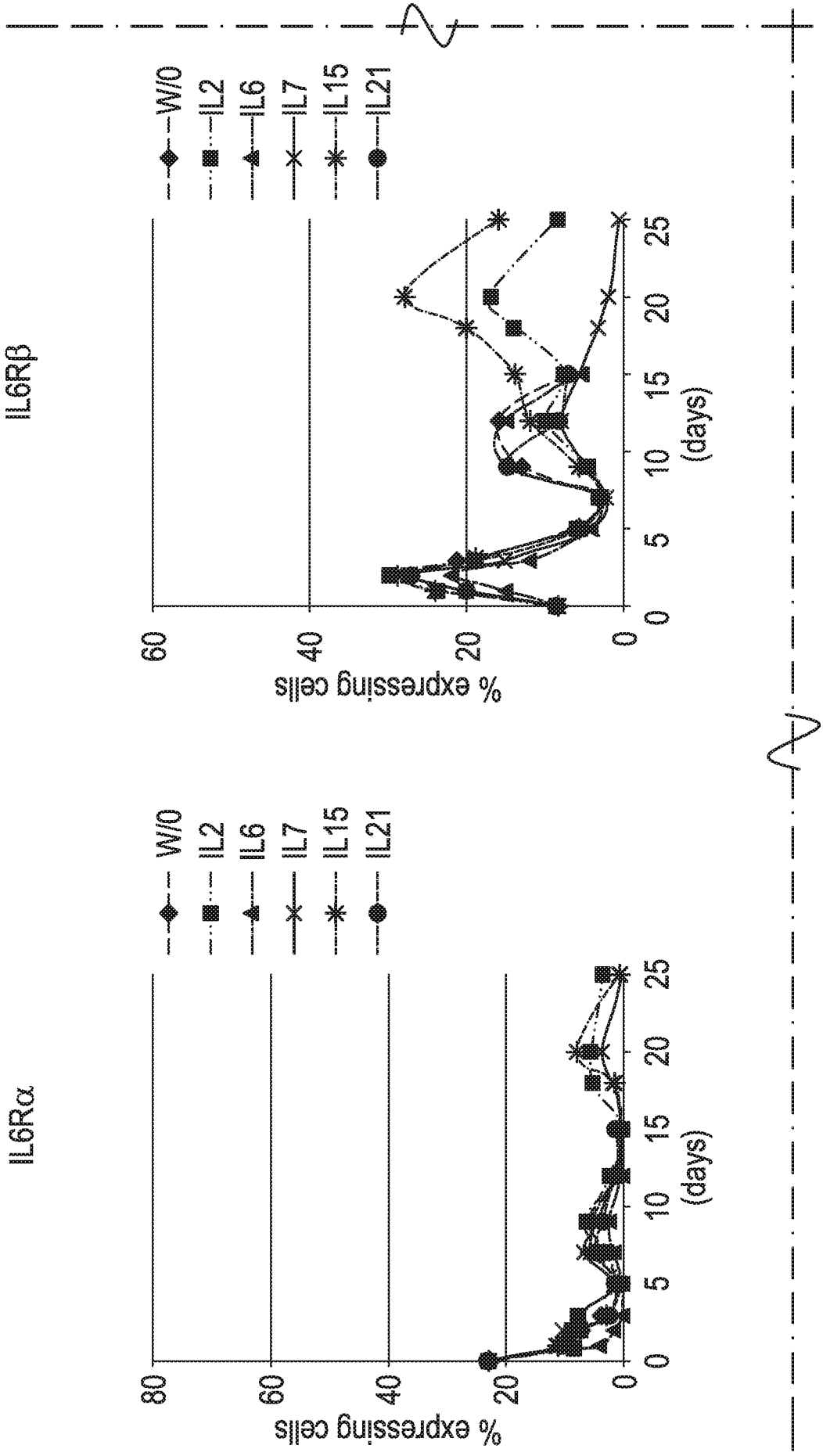

IL6Rβ
IL6Rα
W/0
IL2
IL6
IL7
IL15
IL21
% expressing cells
(days)
0
20
40
60
80
0
5
10
15
20
25

FIG. 15 (part 1)

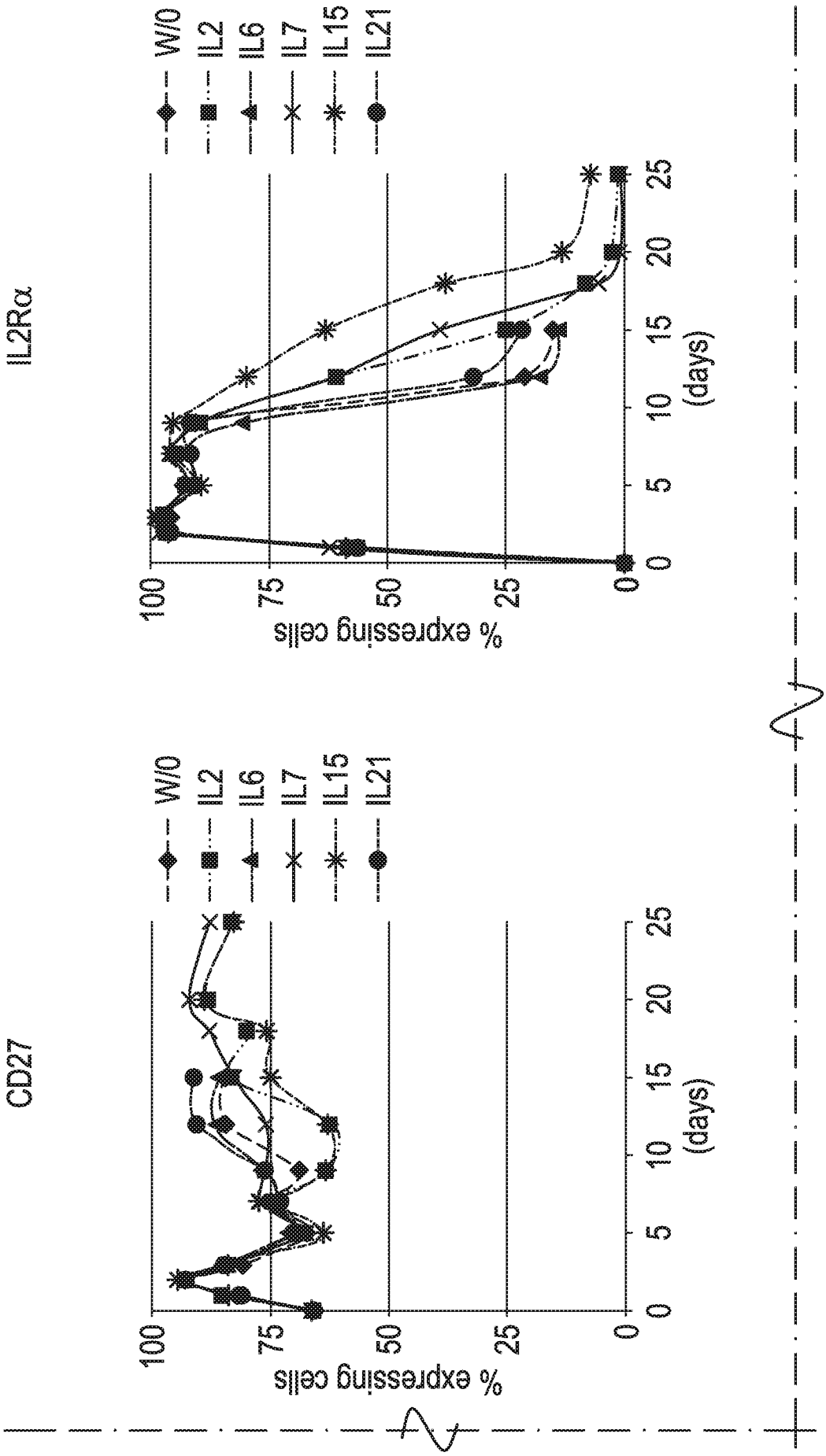

IL2Rα
W/0
IL2
IL6
IL7
IL15
IL21
% expressing cells
100
75
50
25
0
0
5
10
15
20
25
(days)
CD27
W/0
IL2
IL6
IL7
IL15
IL21
% expressing cells
100
75
50
25
0
0
5
10
15
20
25
(days)

FIG. 15 (part 2)

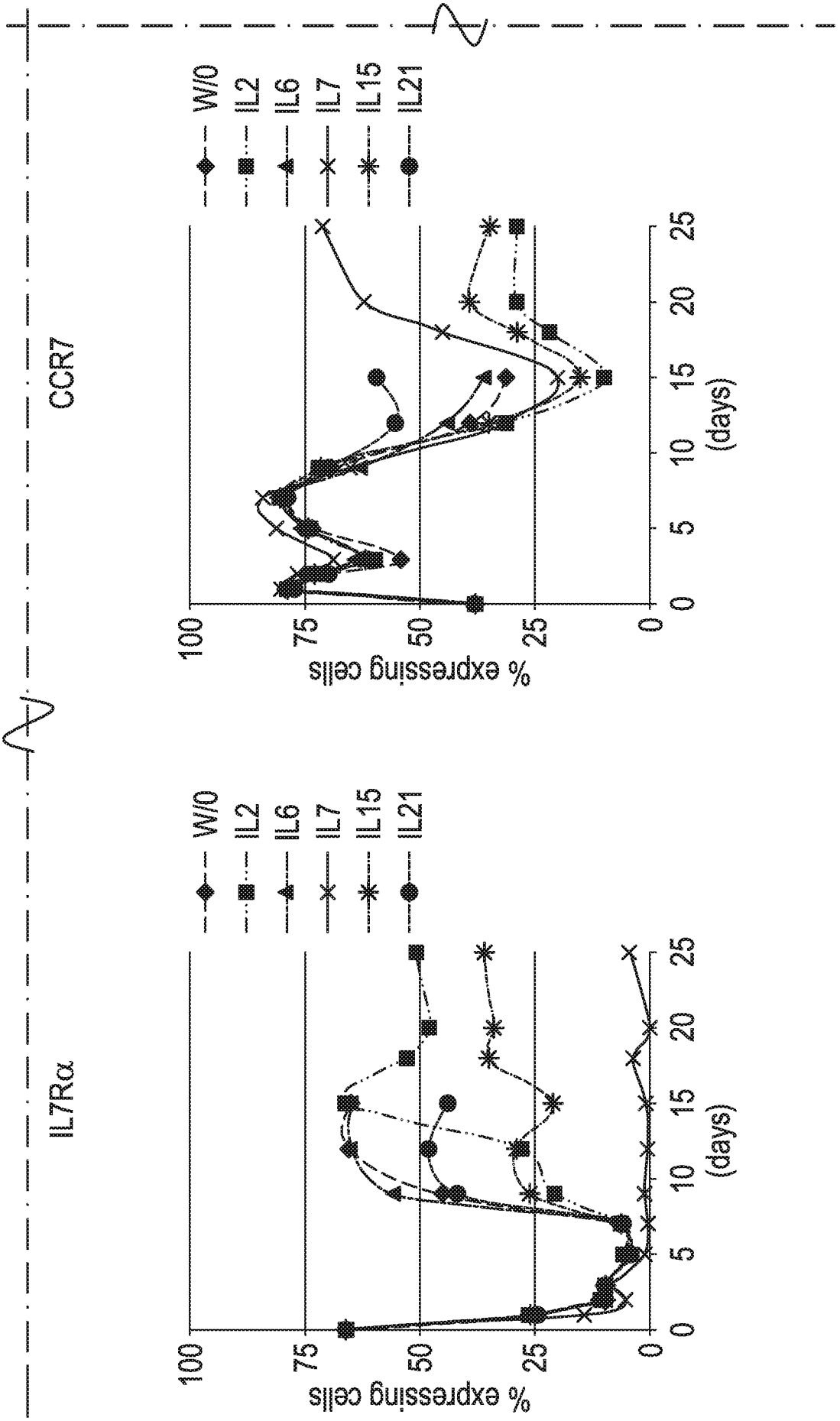

CCR7
IL7Rα
W/0
IL2
IL6
IL7
IL15
IL21
% expressing cells
100
75
50
25
0
0
5
10
15
20
25
(days)

FIG. 15 (part 3)

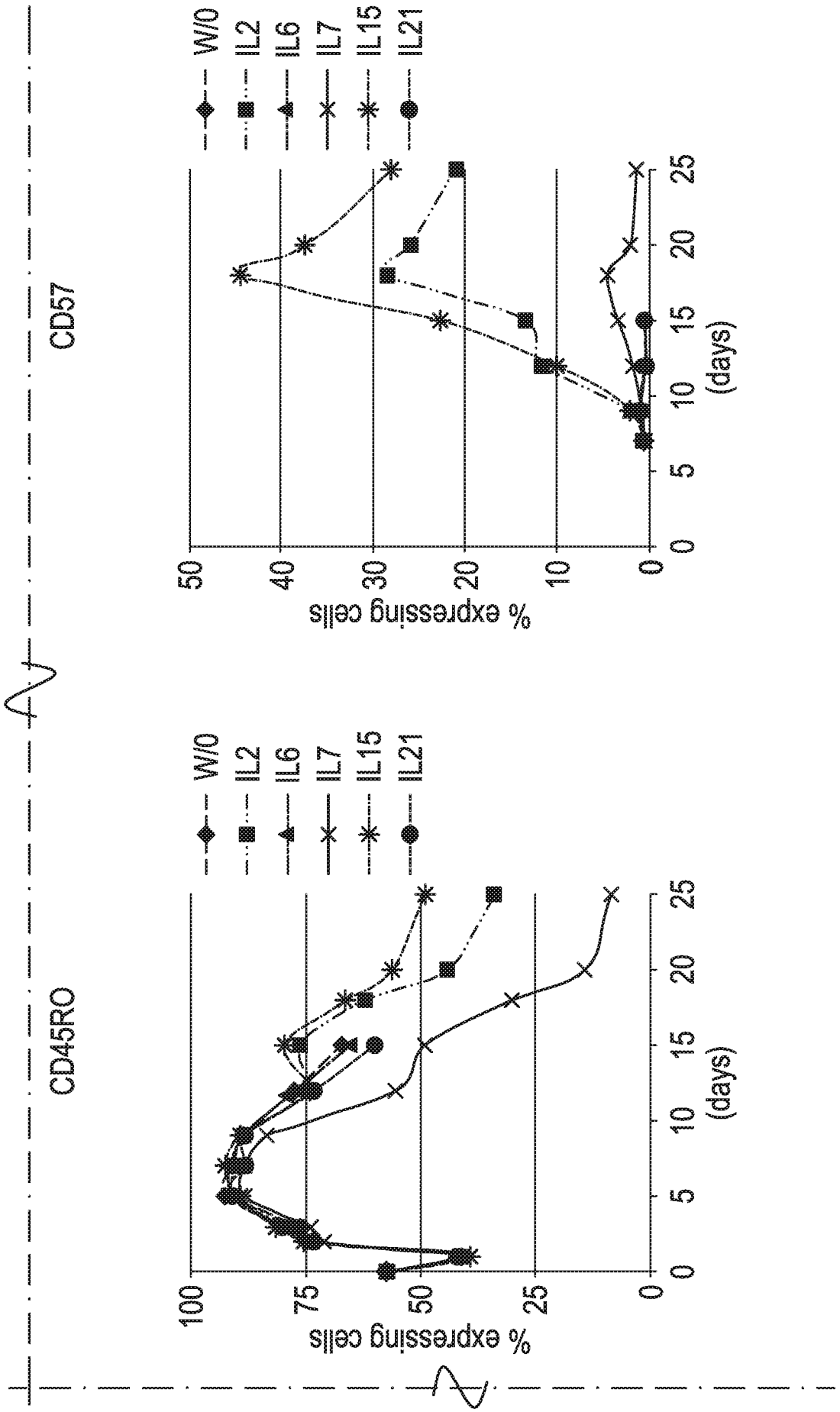

CD57
W/0
IL2
IL6
IL7
IL15
IL21
% expressing cells
0
10
20
30
40
50
0
5
10
15
20
25
(days)
CD45RO
W/0
IL2
IL6
IL7
IL15
IL21
% expressing cells
0
25
50
75
100
0
5
10
15
20
25
(days)

FIG. 15 (part 4)

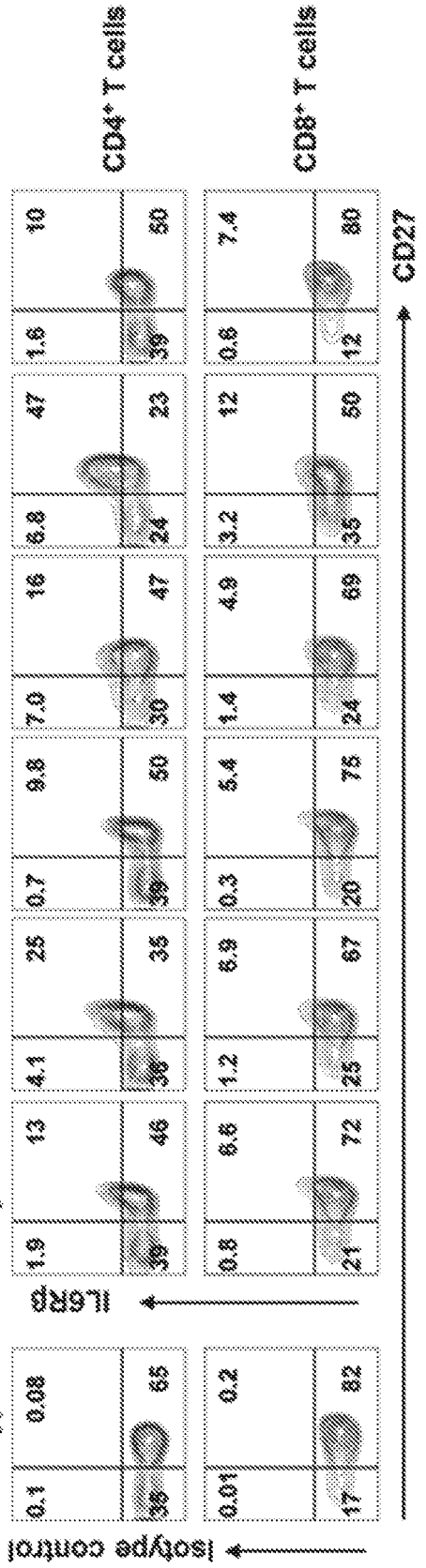

CD4+ T cells
CD8+ T cells
w/o cytokine
+ IL2
+ IL6
+ IL7
+ IL15
+ IL21
isotype
isotype control
IL6RB
CD27

FIG. 16

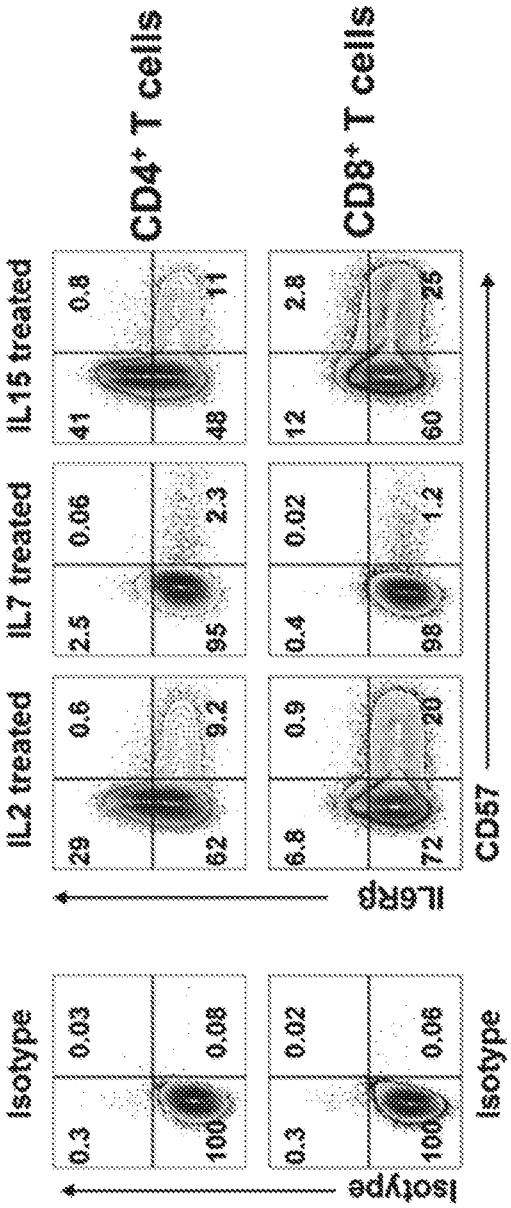
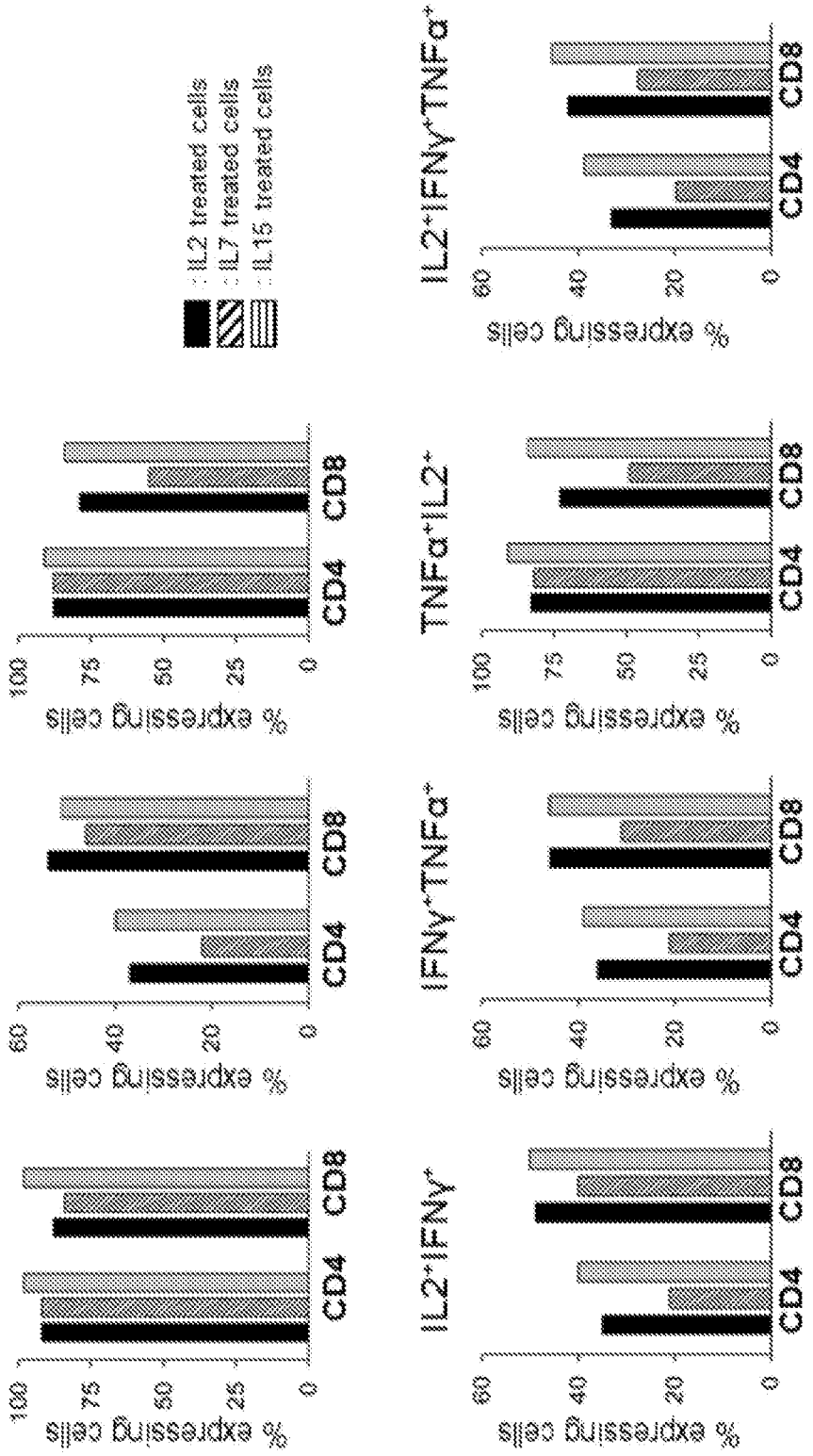

CD4+ T cells
CD8+ T cells
IL15 treated
IL7 treated
IL2 treated
Isotype
IL7Rβ
CD57
0.8
11
41
48
2.8
25
12
60
0.06
2.3
2.5
95
0.02
1.2
0.4
98
0.6
9.2
29
62
0.9
20
6.8
72
0.03
0.08
0.3
100
0.02
0.06
0.3
100

FIG. 17

FIG. 18


: IL2 treated cells
: IL7 treated cells
: IL15 treated cells
IL2+
IFNγ+
TNFα+
IL2+IFNγ+
TNFα+IL2+
IFNγ+TNFα+
IL2+IFNγ+TNFα+
% expressing cells
CD4
CD8

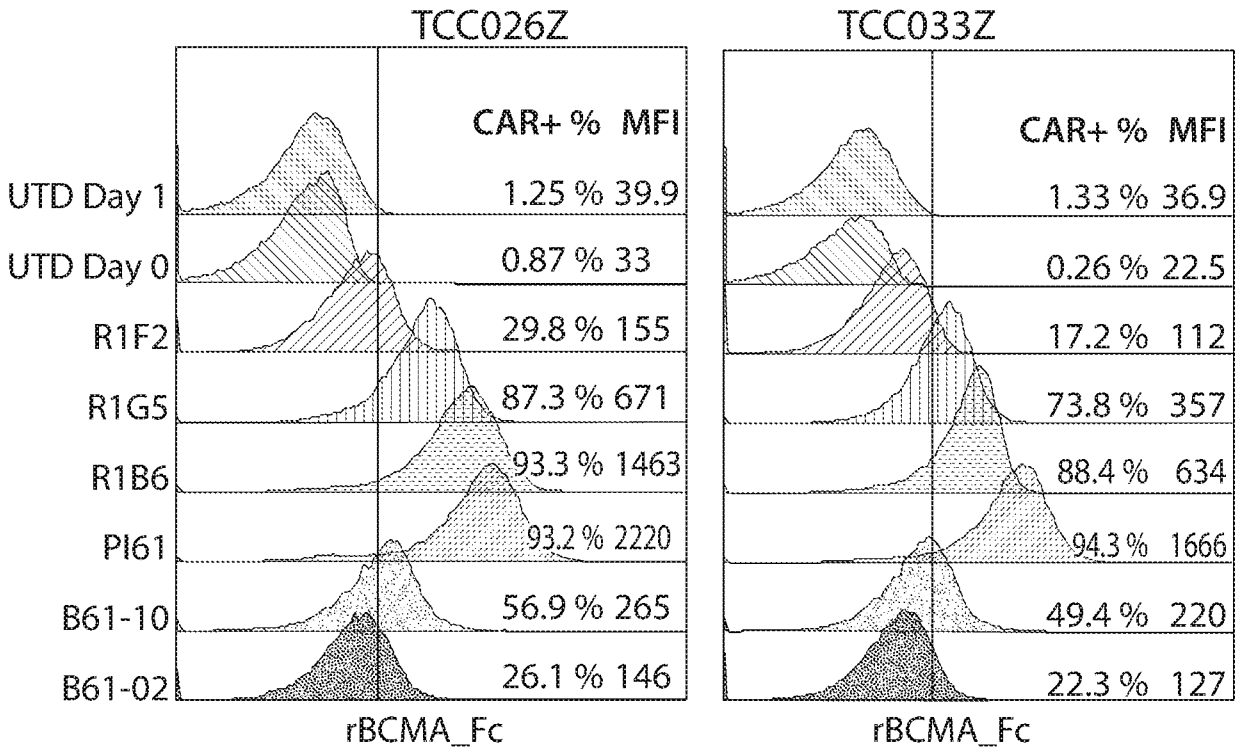

TCC026Z
TCC033Z
CAR+ % MFI
CAR+ % MFI
UTD Day 1
1.25 % 39.9
1.33 % 36.9
UTD Day 0
0.87 % 33
0.26 % 22.5
R1F2
29.8 % 155
17.2 % 112
R1G5
87.3 % 671
73.8 % 357
R1B6
93.3 % 1463
88.4 % 634
PI61
93.2 % 2220
94.3 % 1666
B61-10
56.9 % 265
49.4 % 220
B61-02
26.1 % 146
22.3 % 127
rBCMA_Fc
rBCMA_Fc

FIG. 19A

FACS Panel

| Stain | Dilution | Voltage |
|---|---|---|
| FSC | | 430 |
| SSC | | 255 |
| CD3 BUV395 | 1/200 | 330 |
| CD4 PerCP Cy5.5 | 1/100 | 430 |
| rBCMA Alexa 647 | 1/380 (3ug/ml) | 440 |
| CD8 APC H7 | 1/200 | 490 |
| Live/Dead Aqua | 1/500 | 400 |

FIG. 19B

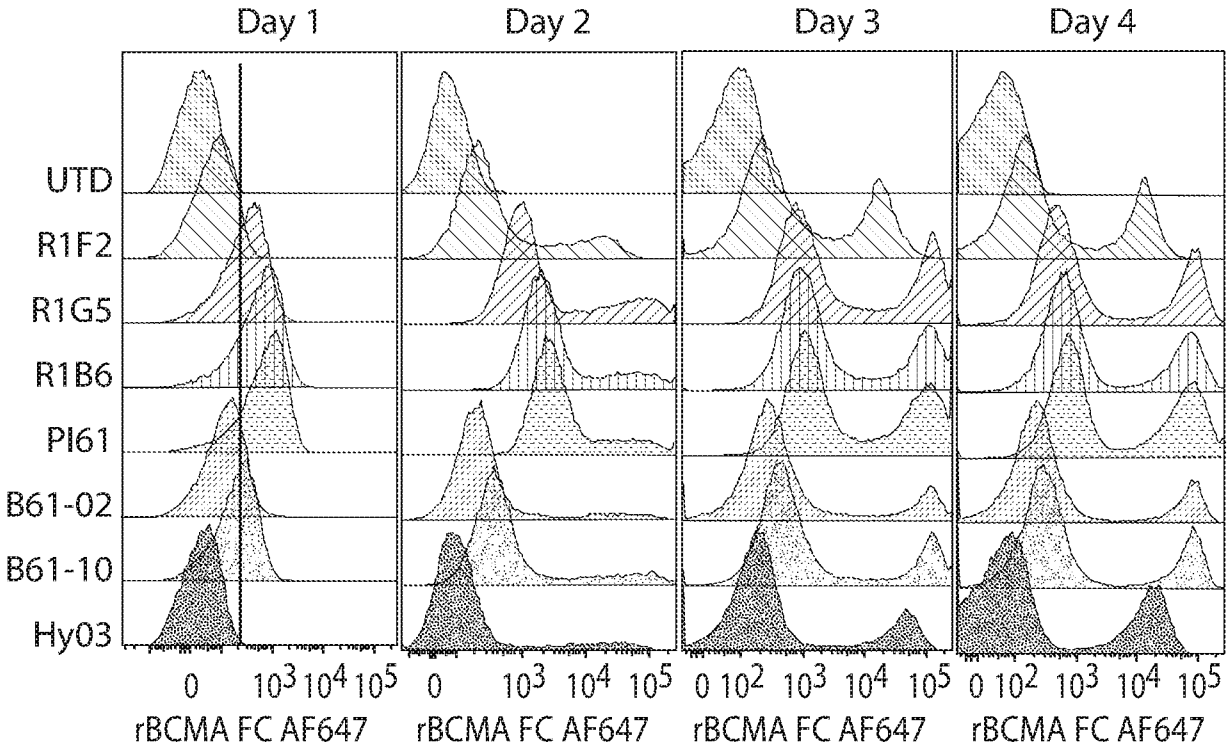

Day 1
Day 2
Day 3
Day 4
UTD
R1F2
R1G5
R1B6
PI61
B61-02
B61-10
Hy03
0 10^3 10^4 10^5
0 10^3 10^4 10^5
0 10^2 10^3 10^4 10^5
0 10^2 10^3 10^4 10^5
rBCMA FC AF647
rBCMA FC AF647
rBCMA FC AF647
rBCMA FC AF647

FIG. 20A

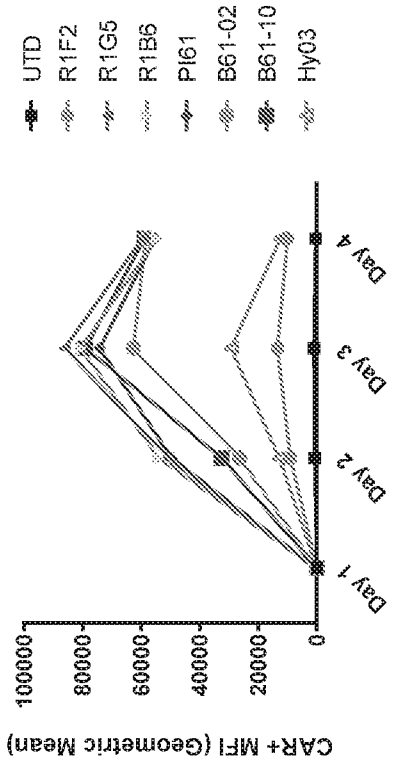



FIG. 20C

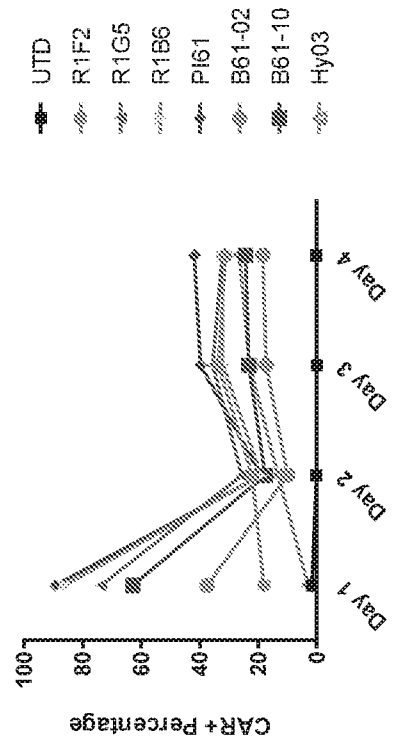



FIG. 20B

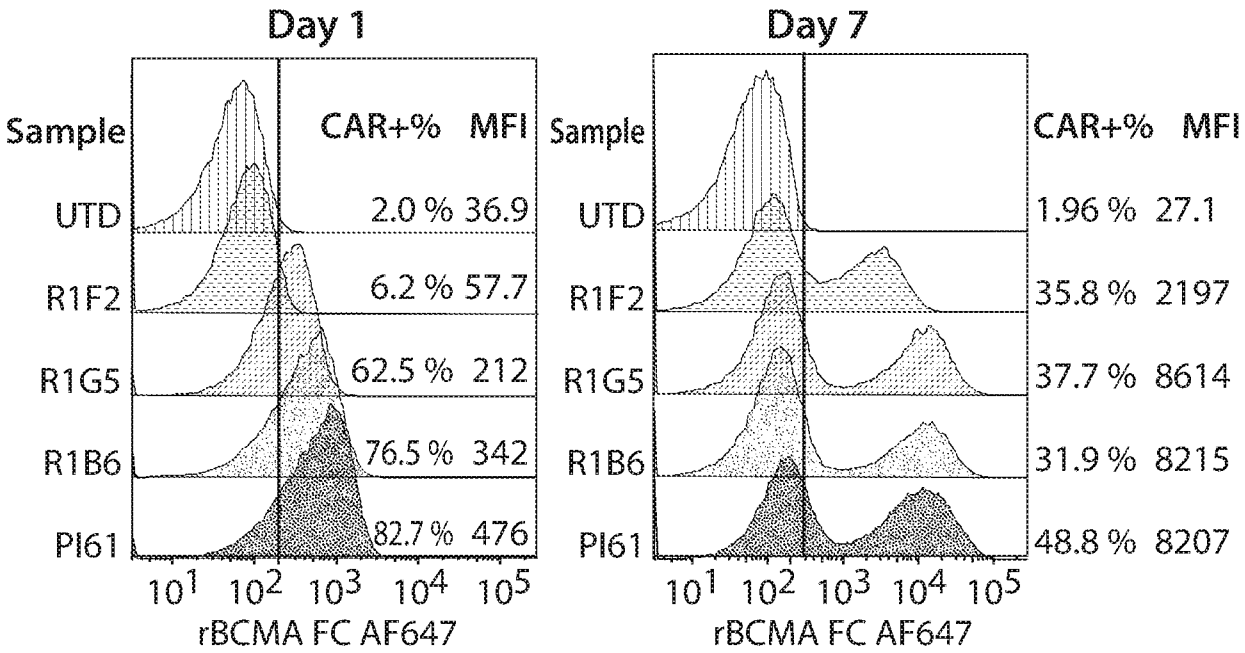

Day 1
Sample
CAR+%
MFI
UTD
2.0 %
36.9
R1F2
6.2 %
57.7
R1G5
62.5 %
212
R1B6
76.5 %
342
PI61
82.7 %
476
$10^1$ $10^2$ $10^3$ $10^4$ $10^5$
rBCMA FC AF647
Day 7
Sample
CAR+%
MFI
UTD
1.96 %
27.1
R1F2
35.8 %
2197
R1G5
37.7 %
8614
R1B6
31.9 %
8215
PI61
48.8 %
8207
$10^1$ $10^2$ $10^3$ $10^4$ $10^5$
rBCMA FC AF647

FIG. 21A

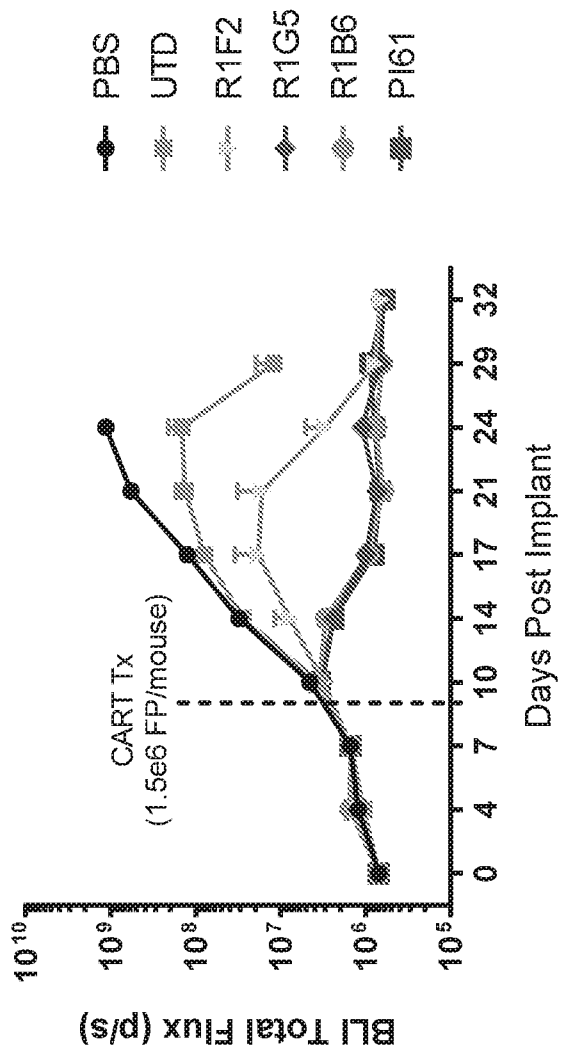

Tumor Kinetics
PBS
UTD
R1F2
R1G5
R1B6
PI61
CART Tx
(1.5e6 FP/mouse)
BLI Total Flux (p/s)
Days Post Implant
0
4
7
10
14
17
21
24
29
32

FIG. 21B

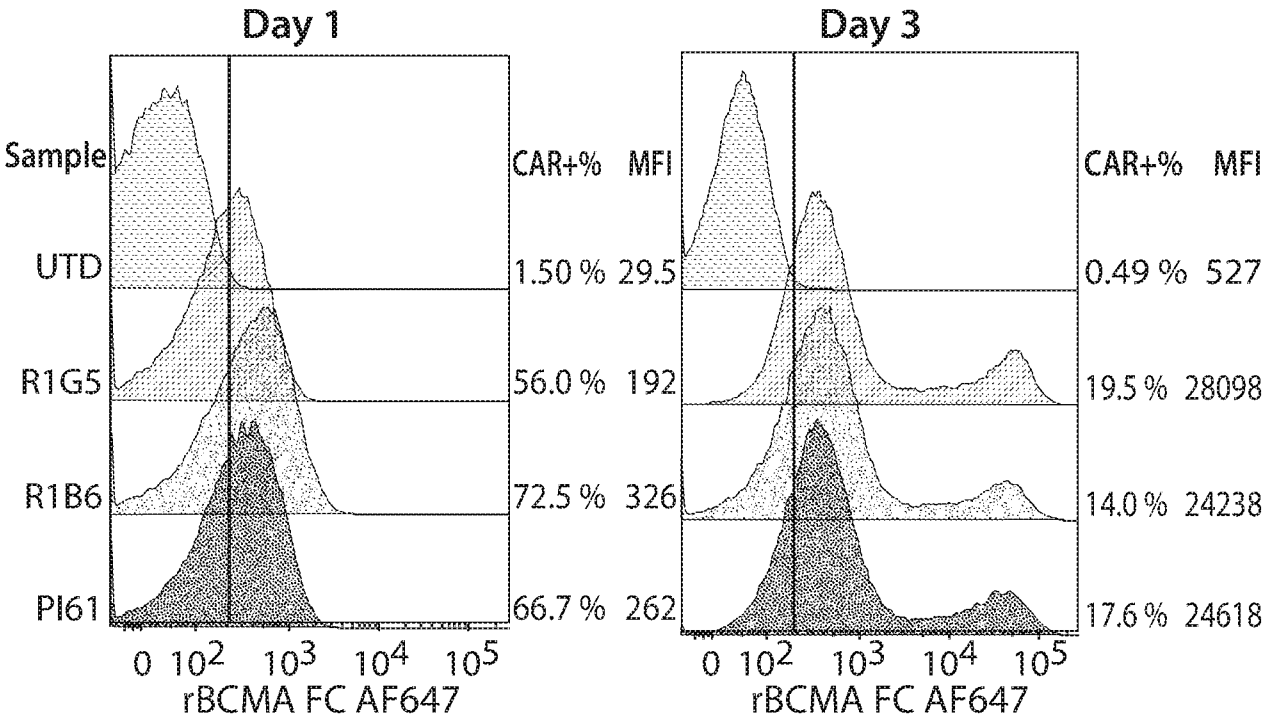

Day 1
Day 3
Sample
UTD
R1G5
R1B6
PI61
CAR+%
MFI
1.50 %
29.5
56.0 %
192
72.5 %
326
66.7 %
262
0.49 %
527
19.5 %
28098
14.0 %
24238
17.6 %
24618
0
10^2
10^3
10^4
10^5
rBCMA FC AF647

FIG. 22A

FIG. 22B

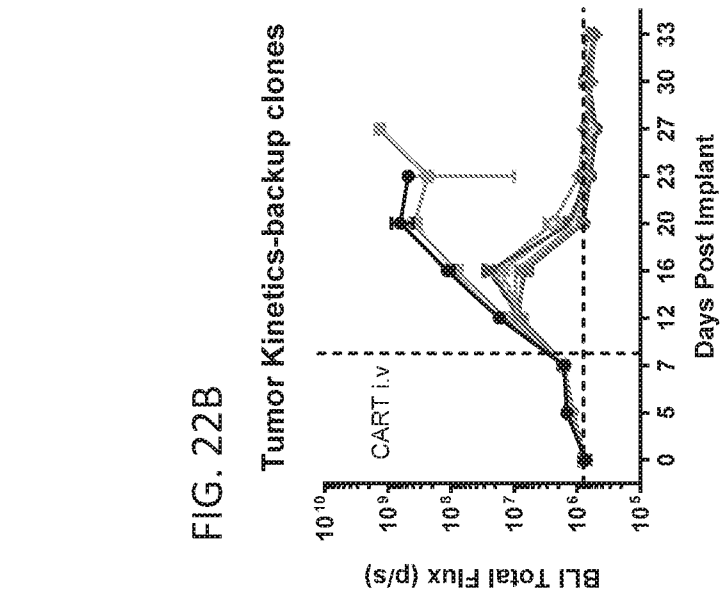
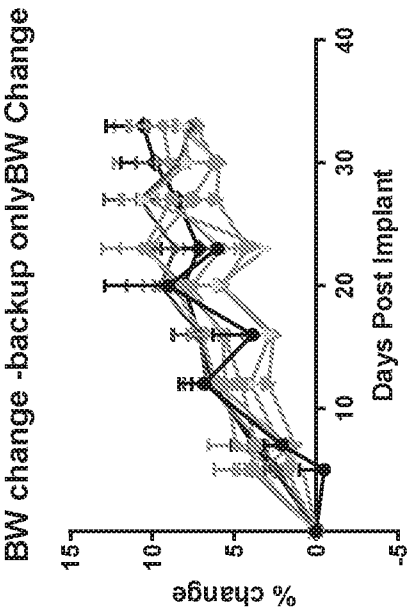



FIG. 22C



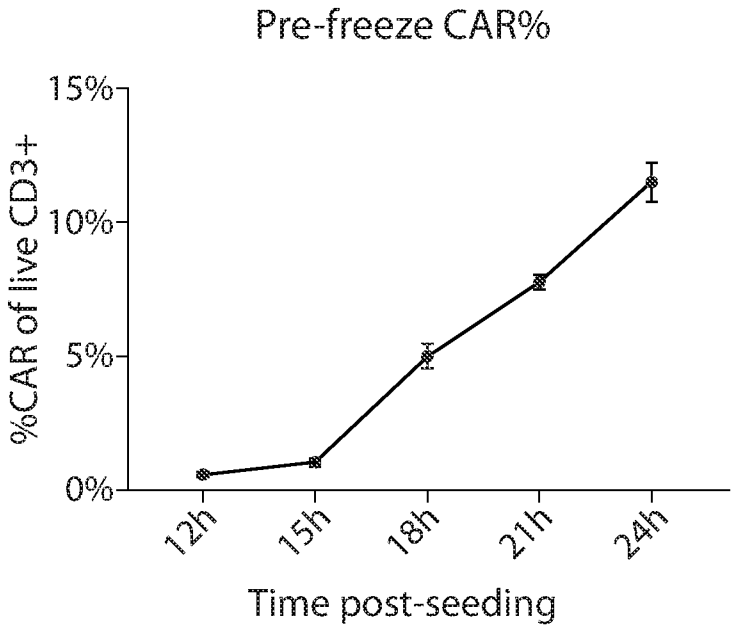

Pre-freeze CAR%
%CAR of live CD3+
15%
10%
5%
0%
12h
15h
18h
21h
24h
Time post-seeding

FIG. 23A

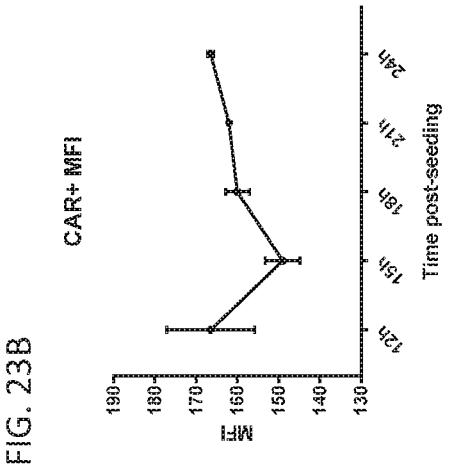

CAR+ MFI
MFI
190
180
170
160
150
140
130
12h
15h
18h
21h
24h
Time post-seeding

FIG. 23B

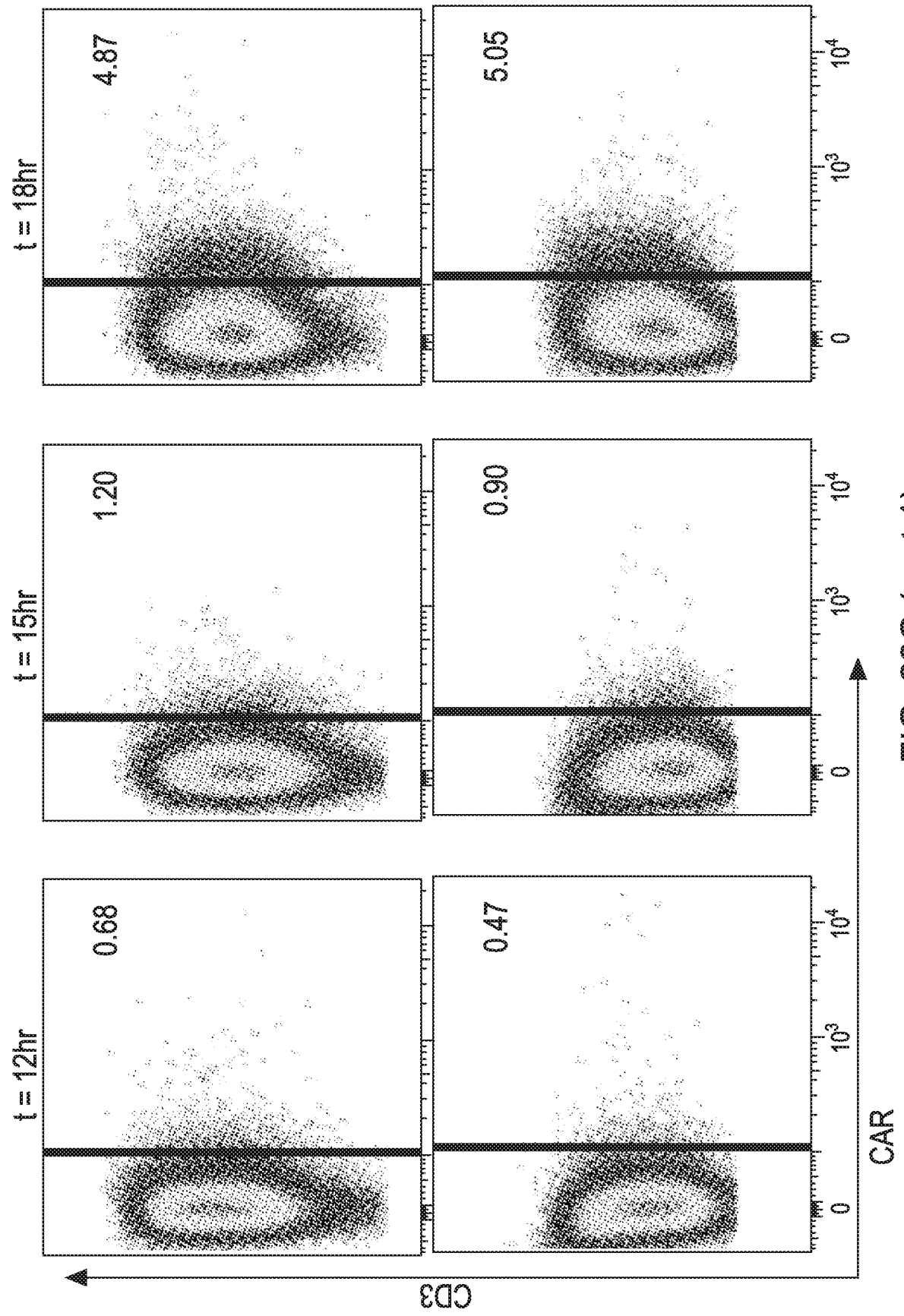



FIG. 23C (part 1)

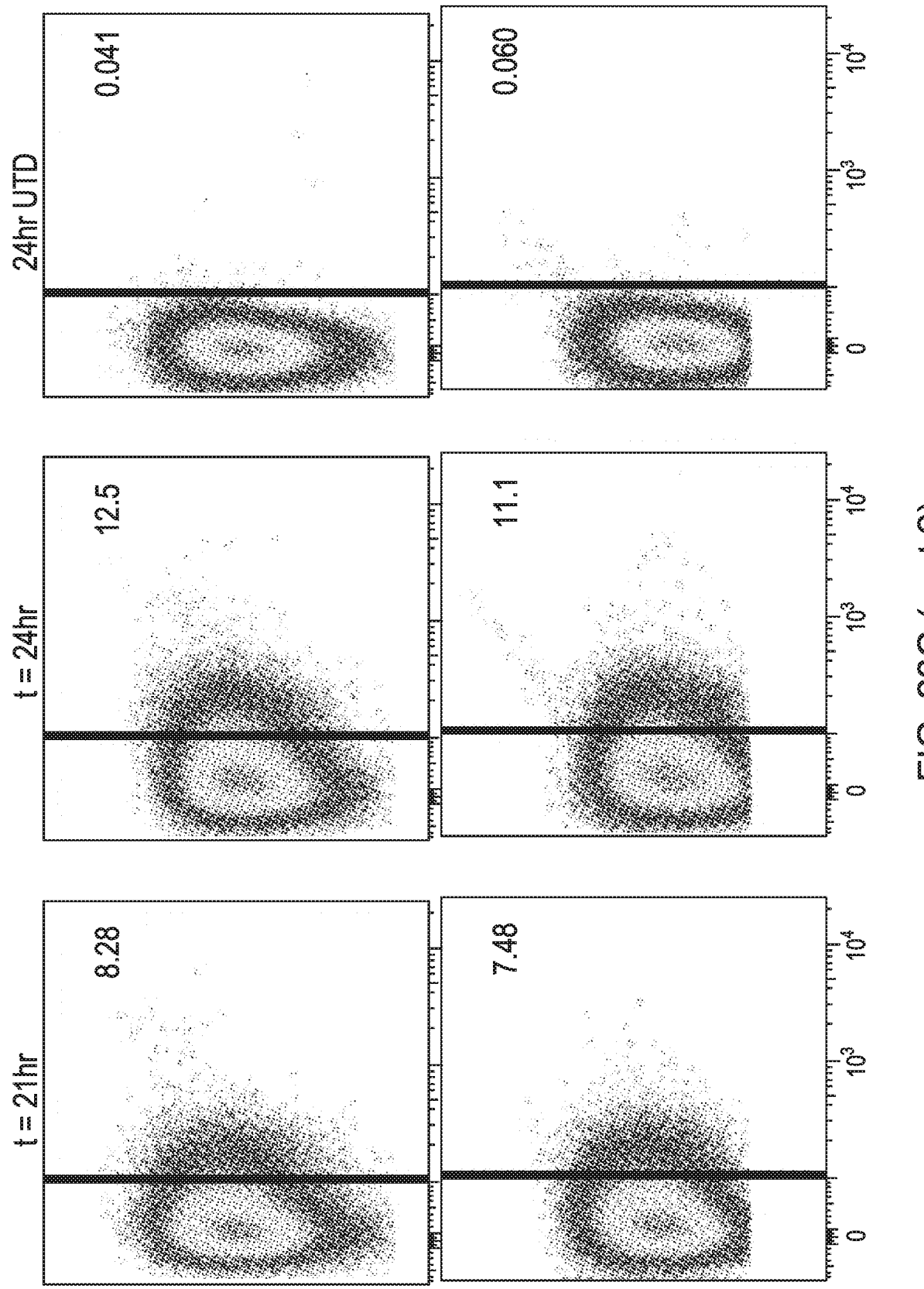



FIG. 23C (part 2)

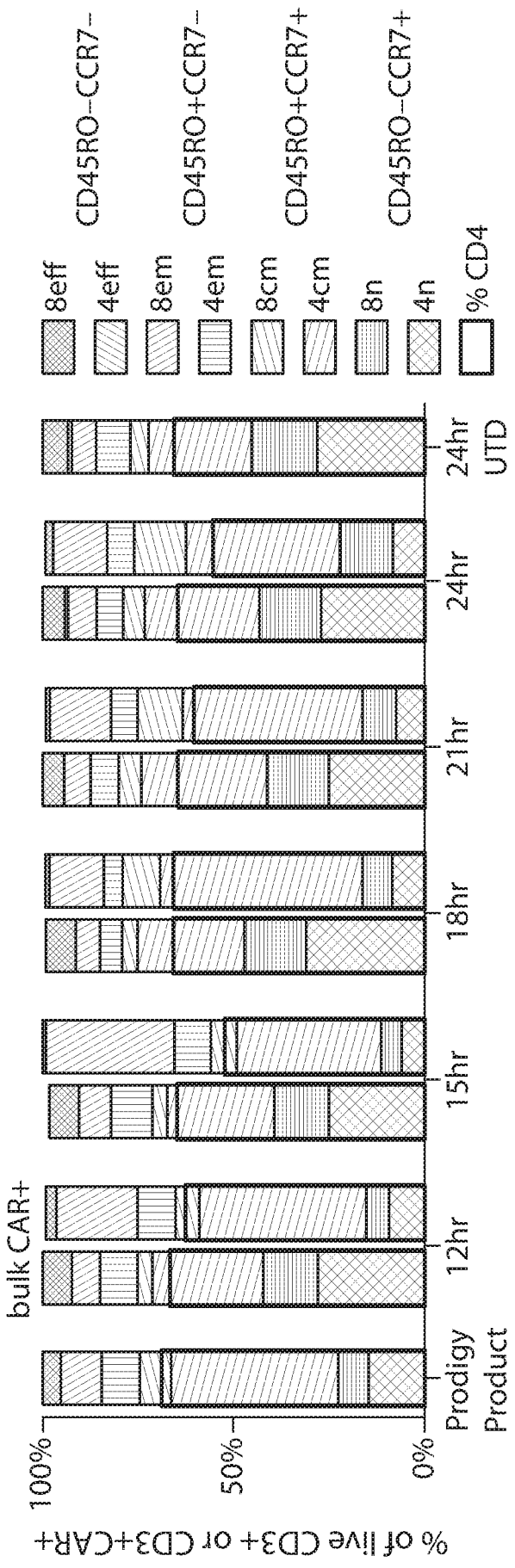

% of live CD3+ or CD3+CAR+
100%
50%
0%
Prodigy Product
bulk CAR+
12hr
15hr
18hr
21hr
24hr
24hr UTD
Time post-seeding
Day 0
% CD4
4n
8n
4cm
8cm
4em
8em
4eff
8eff
CD45RO−CCR7+
CD45RO+CCR7+
CD45RO+CCR7−
CD45RO−CCR7−

FIG. 24A

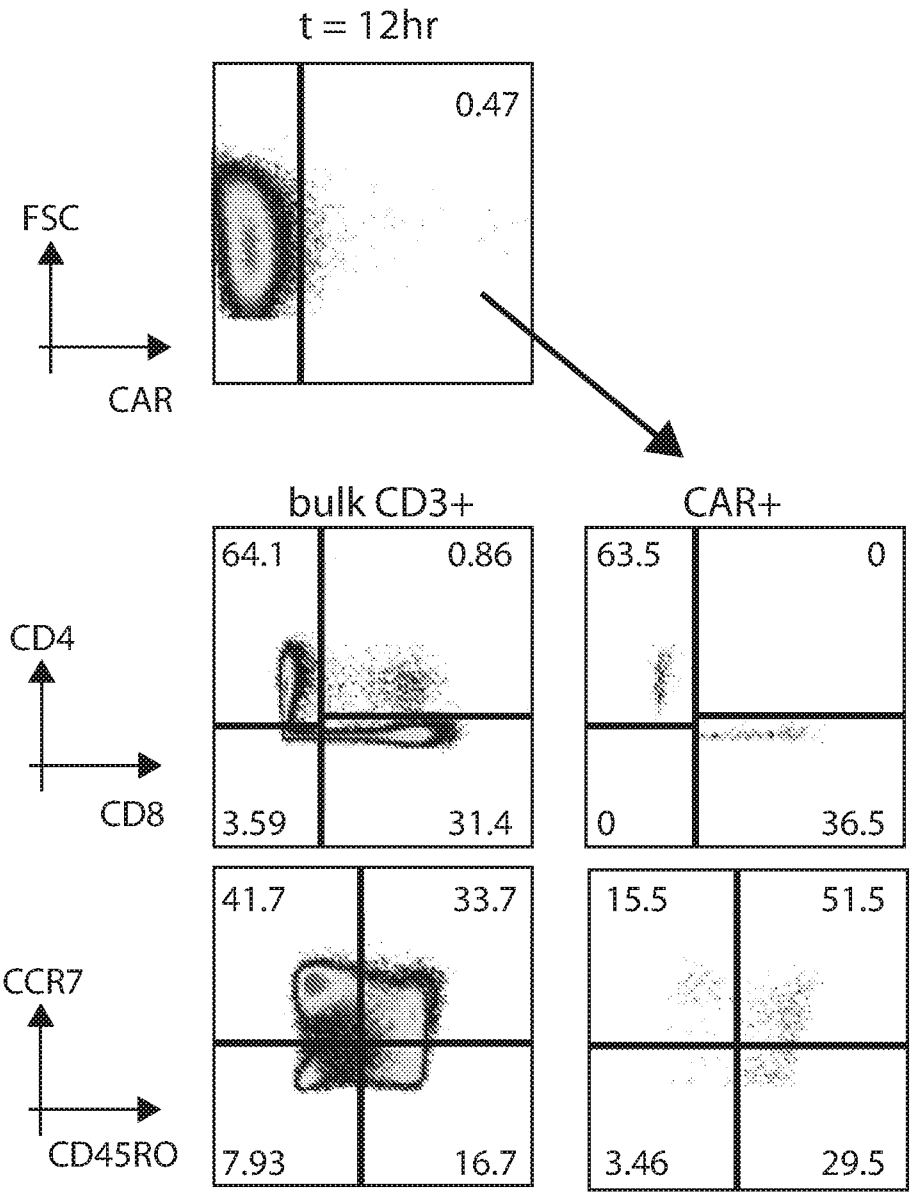

t = 12hr
0.47
FSC
CAR
bulk CD3+
CAR+
64.1
0.86
63.5
0
CD4
CD8
3.59
31.4
0
36.5
41.7
33.7
15.5
51.5
CCR7
CD45RO
7.93
16.7
3.46
29.5

FIG. 24B-1

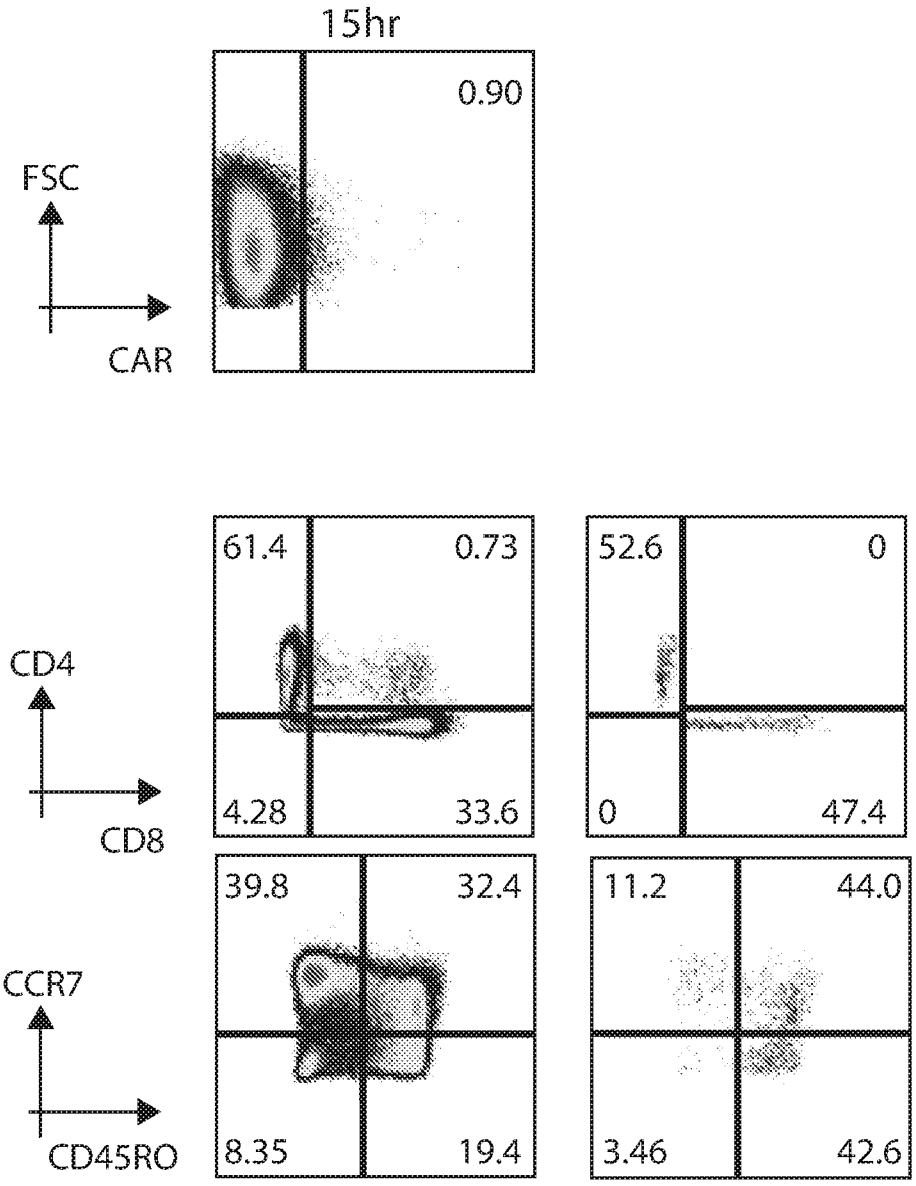

15hr
0.90
FSC
CAR
61.4
0.73
4.28
33.6
52.6
0
0
47.4
CD4
CD8
39.8
32.4
8.35
19.4
11.2
44.0
3.46
42.6
CCR7
CD45RO

FIG. 24B-2

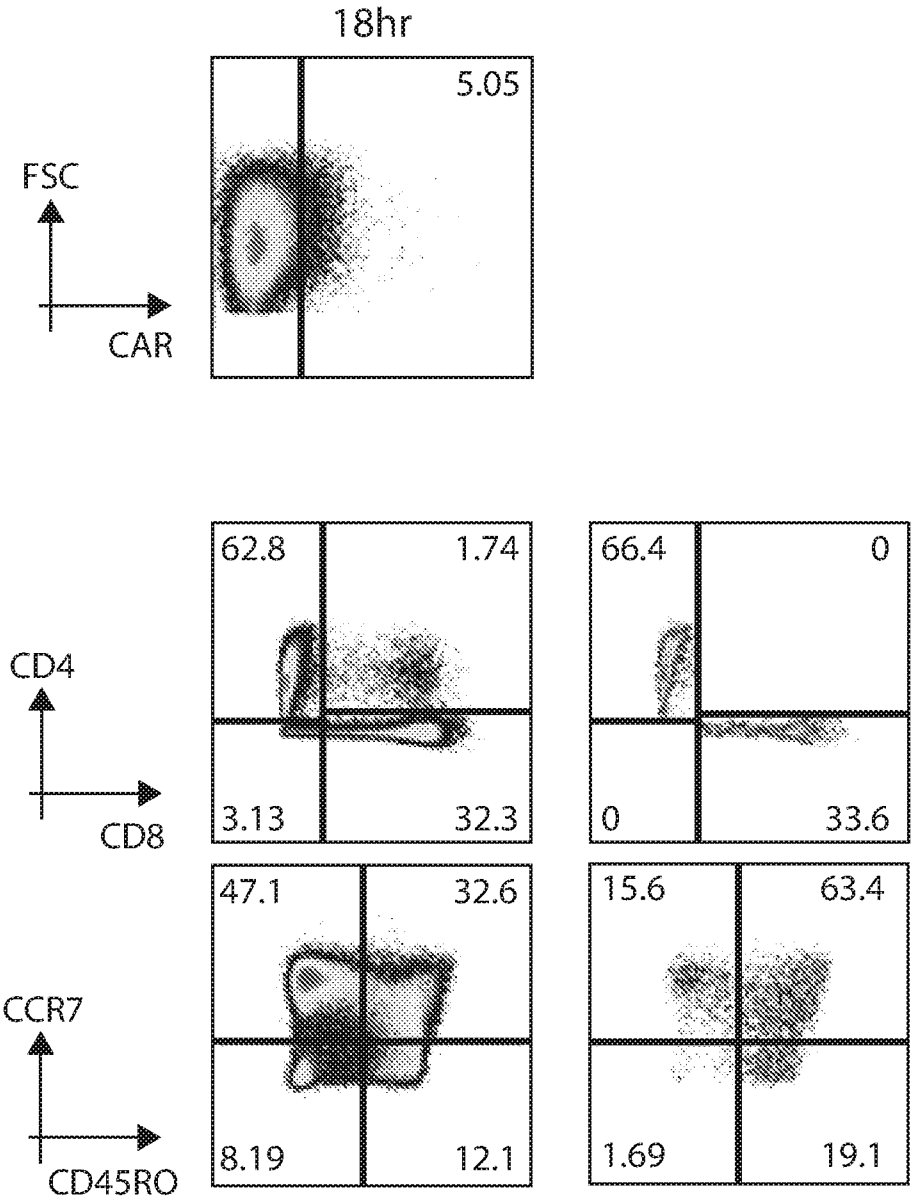

18hr
5.05
FSC
CAR
62.8
1.74
3.13
32.3
66.4
0
0
33.6
CD4
CD8
47.1
32.6
8.19
12.1
15.6
63.4
1.69
19.1
CCR7
CD45RO

FIG. 24B-3

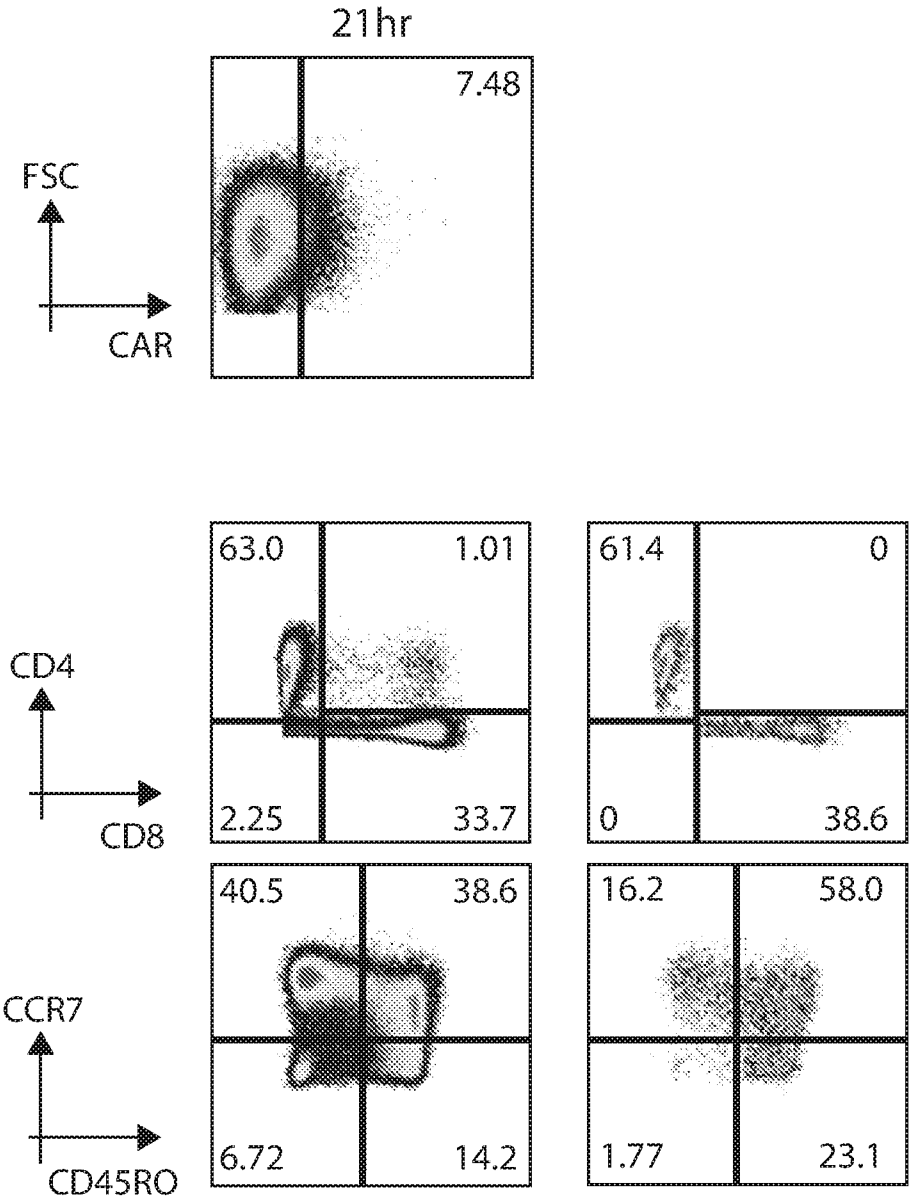

21hr
7.48
FSC
CAR
63.0
1.01
2.25
33.7
61.4
0
0
38.6
CD4
CD8
40.5
38.6
6.72
14.2
16.2
58.0
1.77
23.1
CCR7
CD45RO

FIG. 24B-4

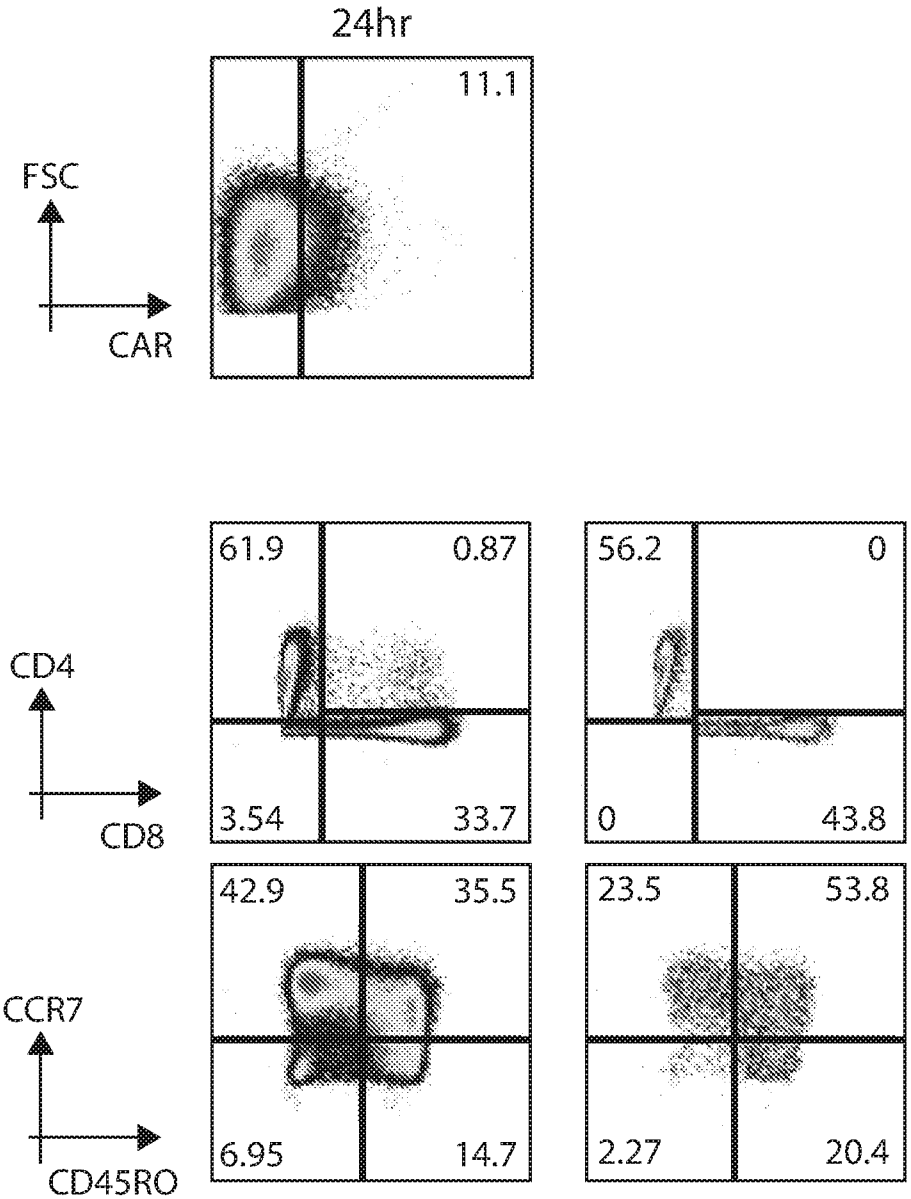

24hr
11.1
FSC
CAR
61.9
0.87
3.54
33.7
56.2
0
0
43.8
CD4
CD8
42.9
35.5
6.95
14.7
23.5
53.8
2.27
20.4
CCR7
CD45RO

FIG. 24B-5

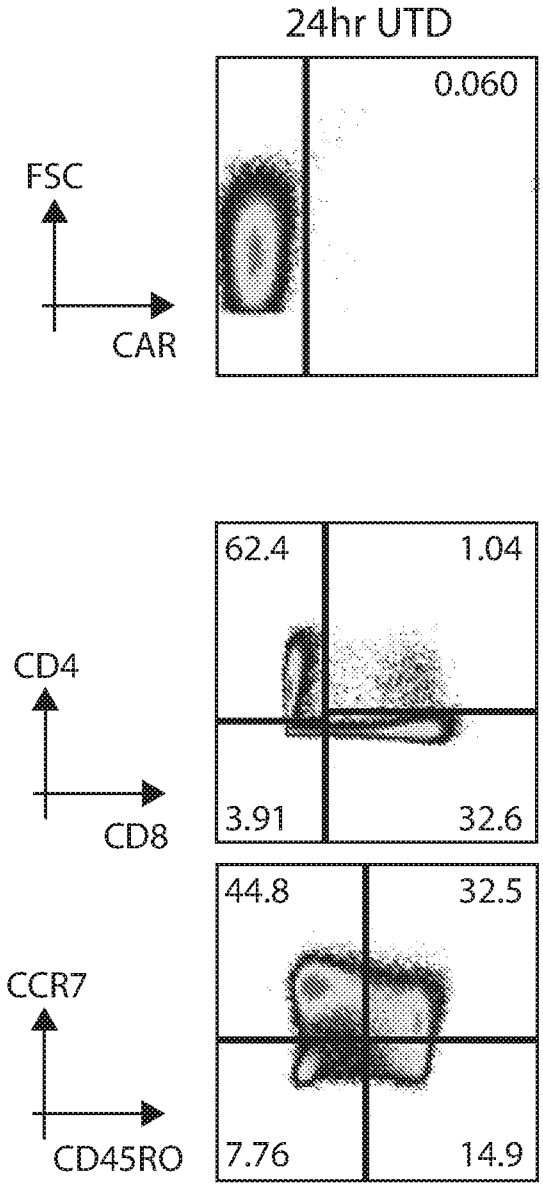

24hr UTD
0.060
FSC
CAR
62.4
1.04
CD4
CD8
3.91
32.6
44.8
32.5
CCR7
CD45RO
7.76
14.9

FIG. 24B-6

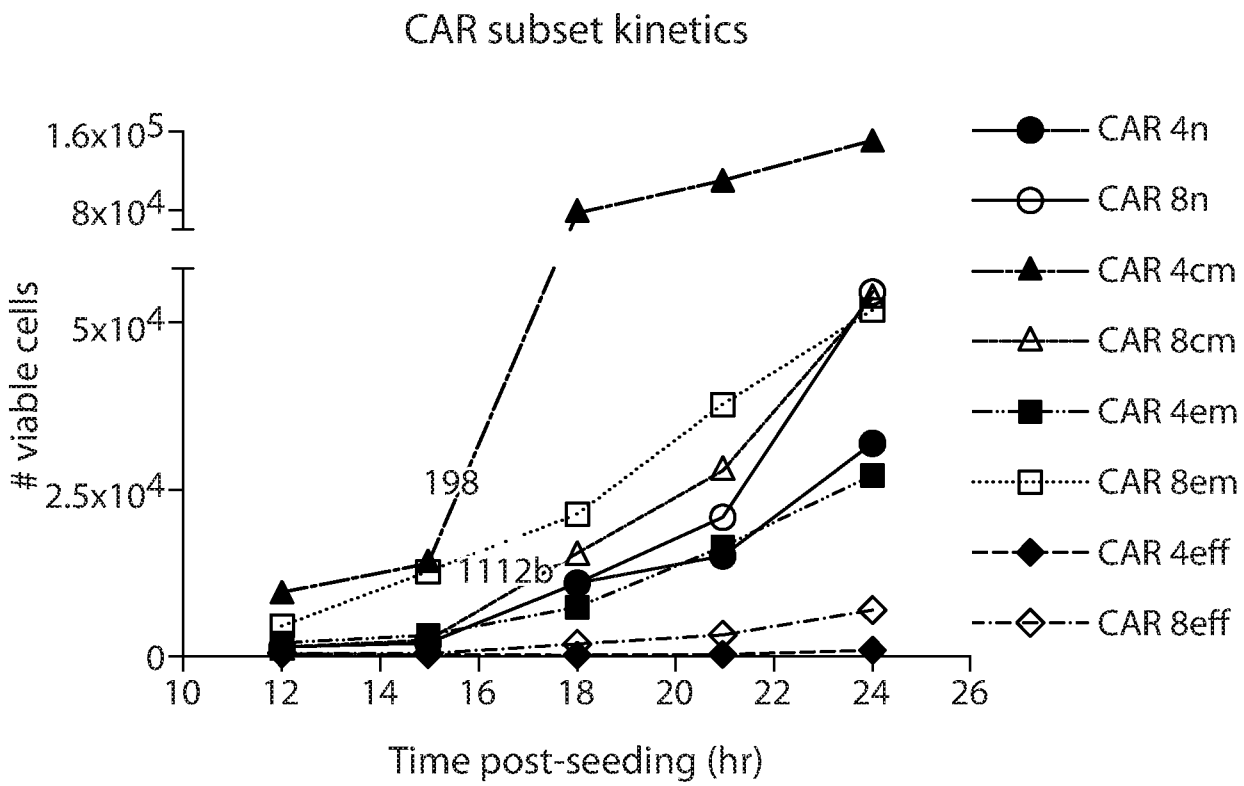

CAR subset kinetics
1.6x10^5
8x10^4
5x10^4
2.5x10^4
0
# viable cells
198
1112b
10
12
14
16
18
20
22
24
26
Time post-seeding (hr)
CAR 4n
CAR 8n
CAR 4cm
CAR 8cm
CAR 4em
CAR 8em
CAR 4eff
CAR 8eff

FIG. 25

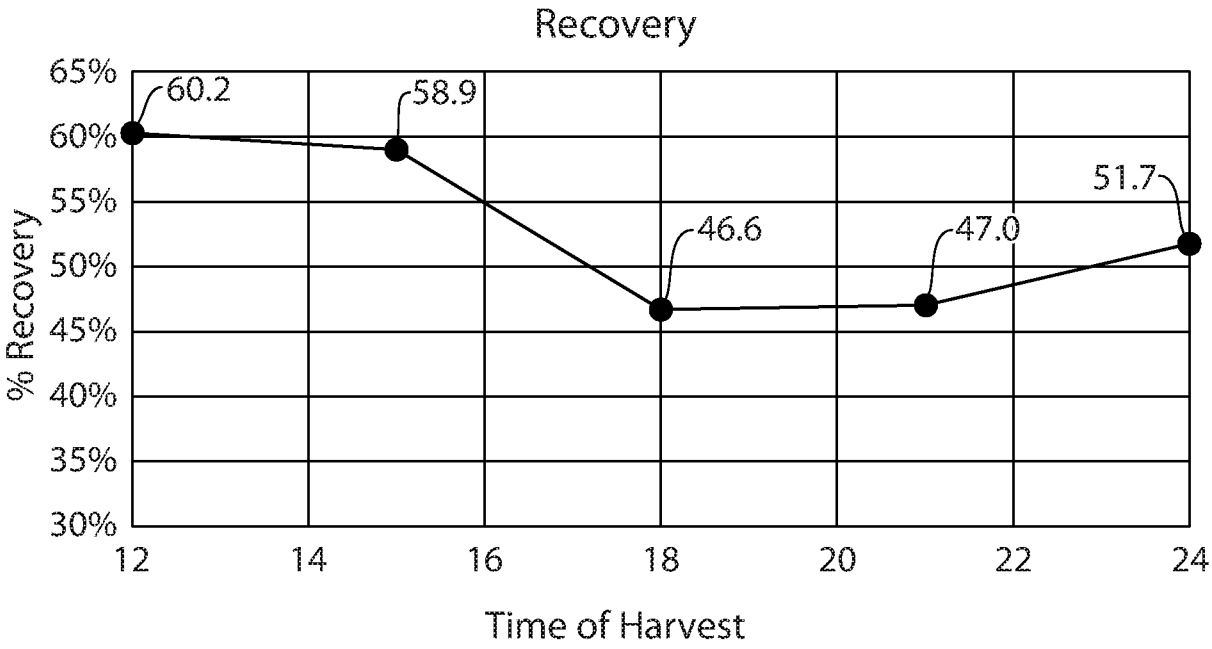

Recovery
65%
60%
55%
50%
45%
40%
35%
30%
% Recovery
60.2
58.9
46.6
47.0
51.7
12
14
16
18
20
22
24
Time of Harvest

FIG. 26

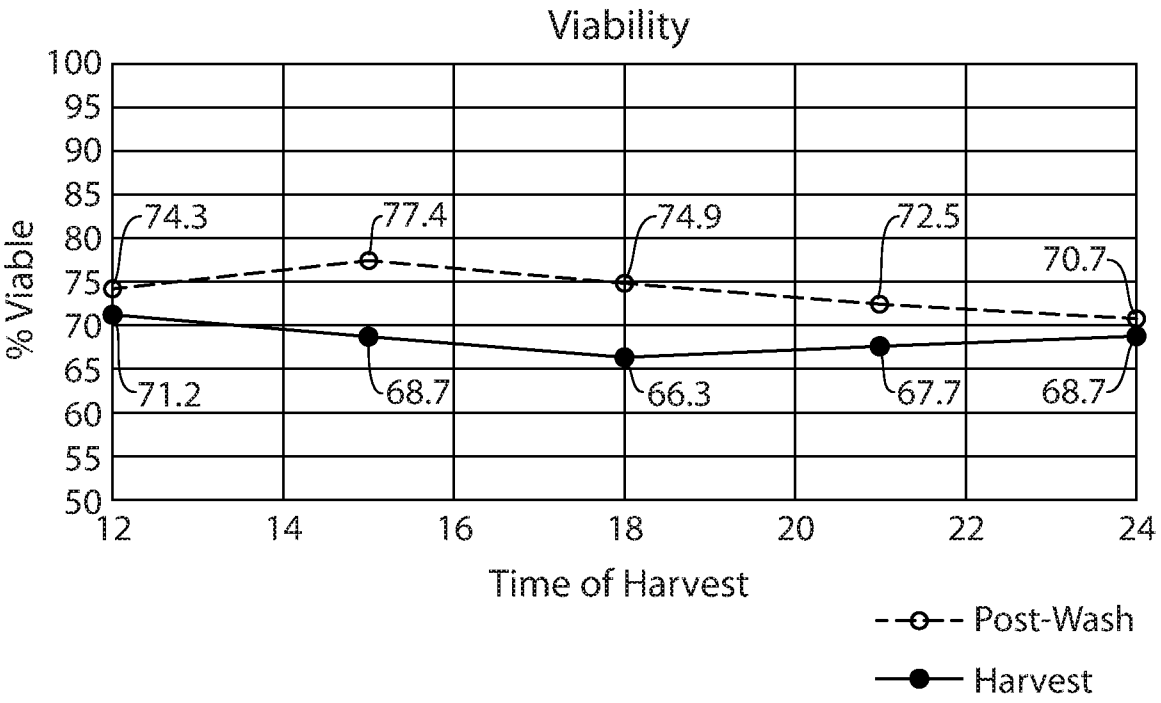

Viability
% Viable
100
95
90
85
80
75
70
65
60
55
50
12
14
16
18
20
22
24
Time of Harvest
74.3
77.4
74.9
72.5
70.7
71.2
68.7
66.3
67.7
68.7
Post-Wash
Harvest

FIG. 27

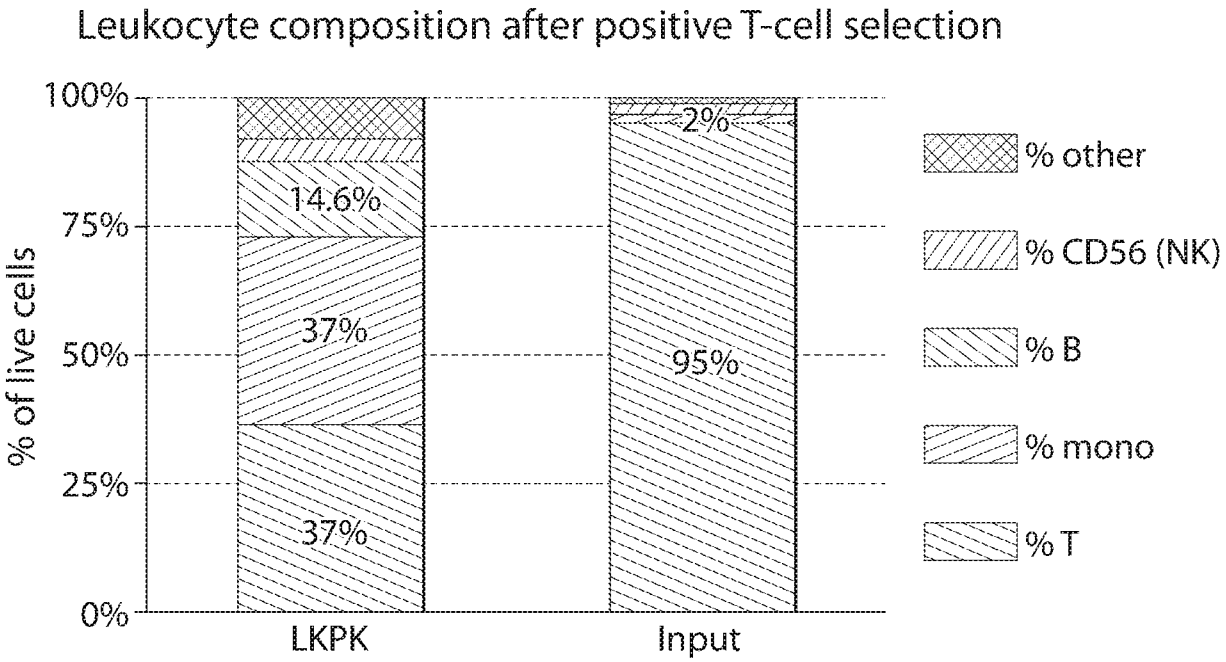

Leukocyte composition after positive T-cell selection
100%
75%
50%
25%
0%
% of live cells
14.6%
37%
37%
2%
95%
LKPK
Input
% other
% CD56 (NK)
% B
% mono
% T

FIG. 28A

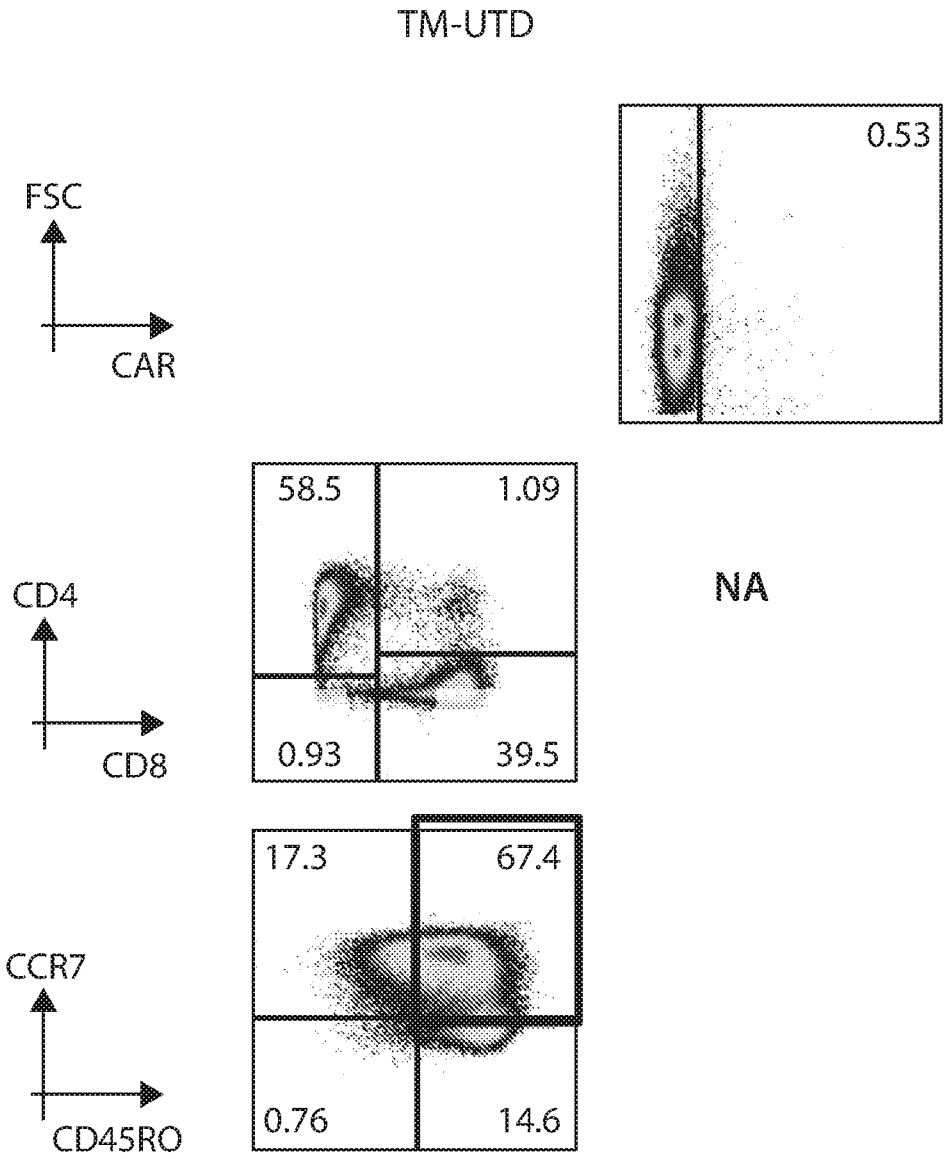

TM-UTD
FSC
CAR
0.53
CD4
CD8
58.5
1.09
0.93
39.5
NA
CCR7
CD45RO
17.3
67.4
0.76
14.6

FIG. 28B-1

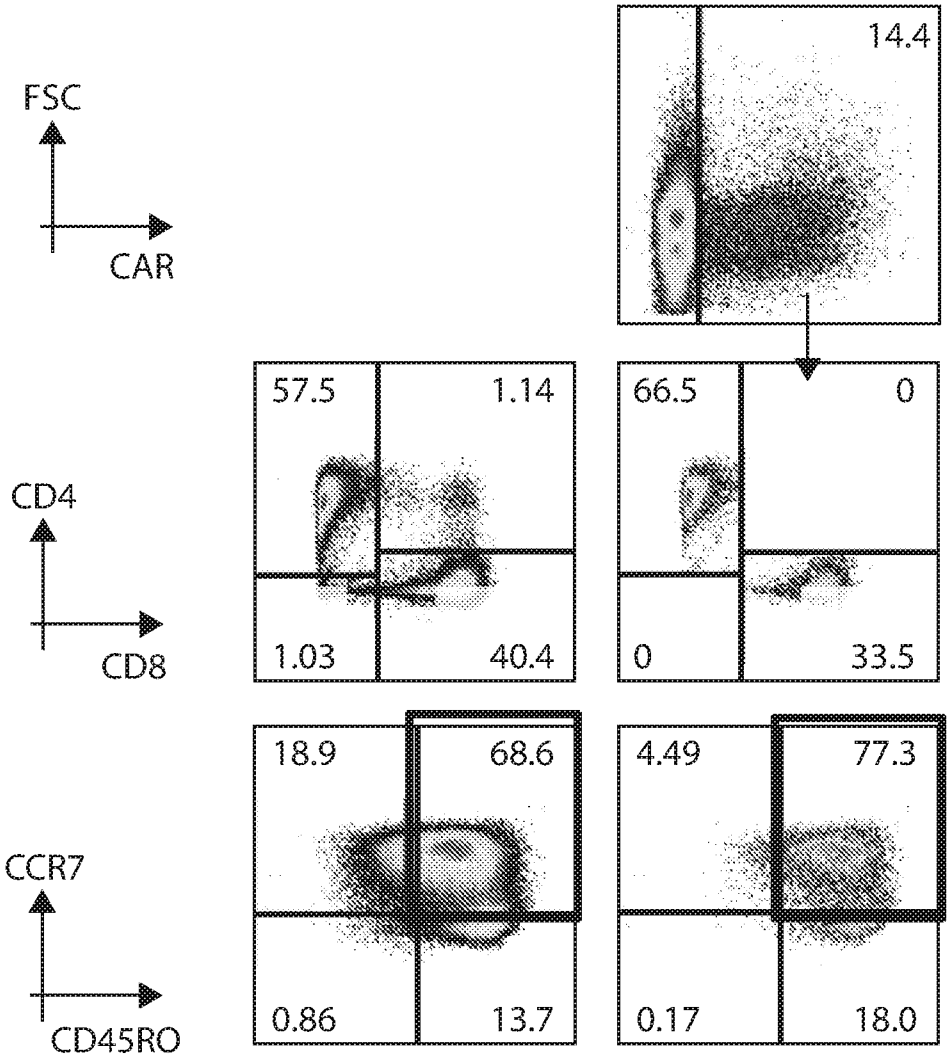

TM-CD19 CAR
FSC
CAR
14.4
CD4
CD8
57.5
1.14
1.03
40.4
66.5
0
0
33.5
CCR7
CD45RO
18.9
68.6
0.86
13.7
4.49
77.3
0.17
18.0

FIG. 28B-2

ARM-UTD

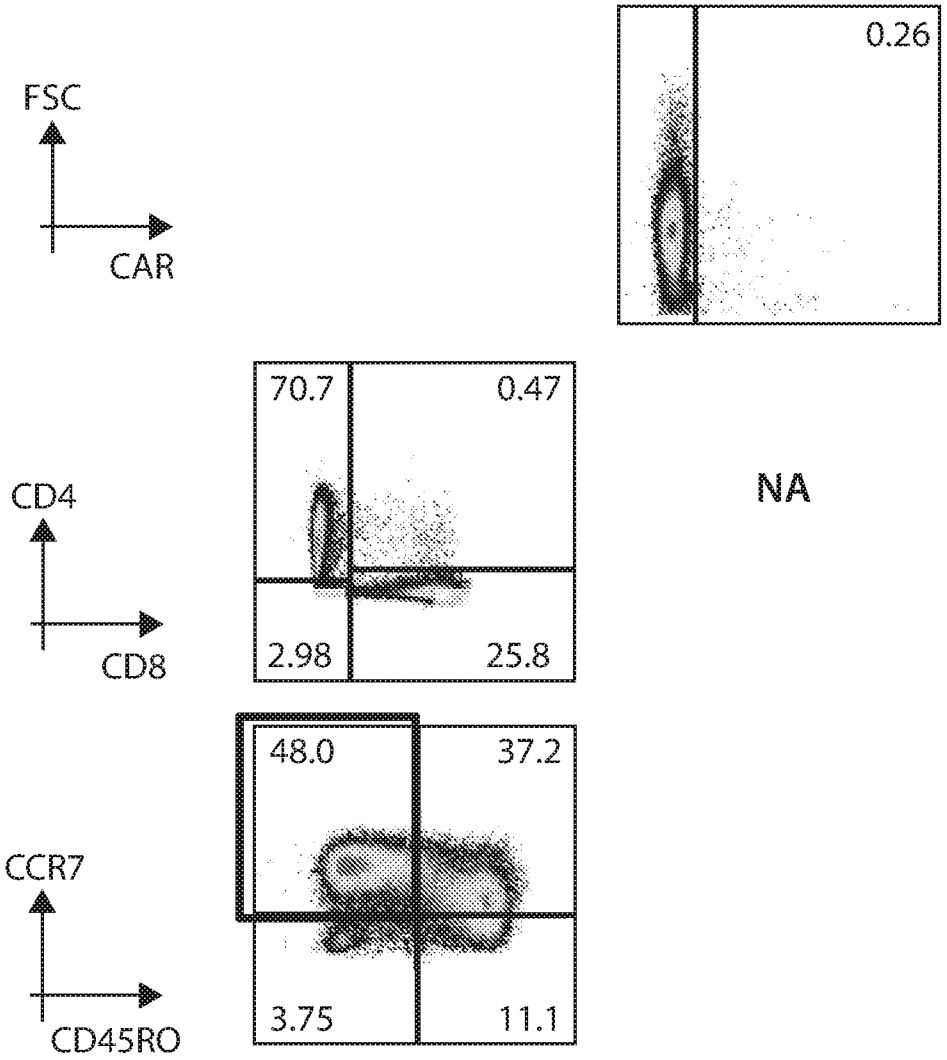



FIG. 28B-3

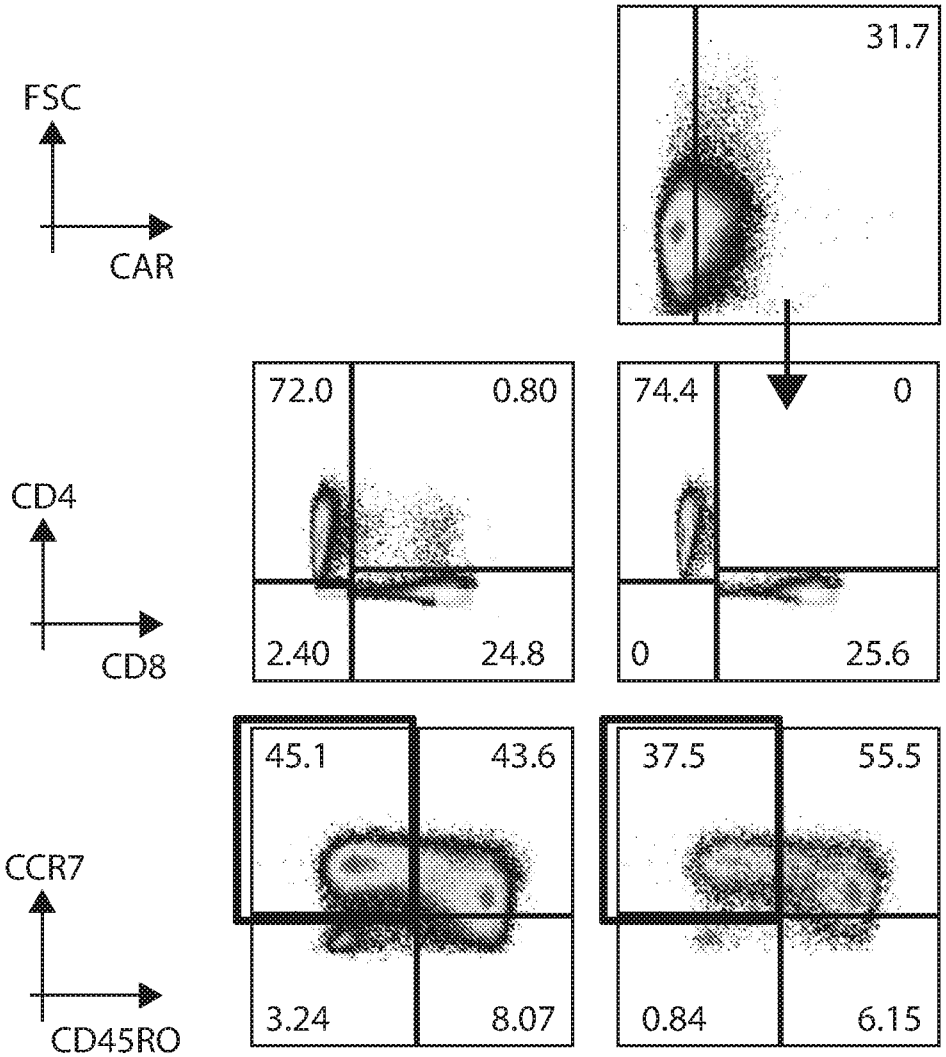

ARM-CD19 CAR
FSC
CAR
31.7
CD4
CD8
72.0
0.80
2.40
24.8
74.4
0
0
25.6
CCR7
CD45RO
45.1
43.6
3.24
8.07
37.5
55.5
0.84
6.15

FIG. 28B-4

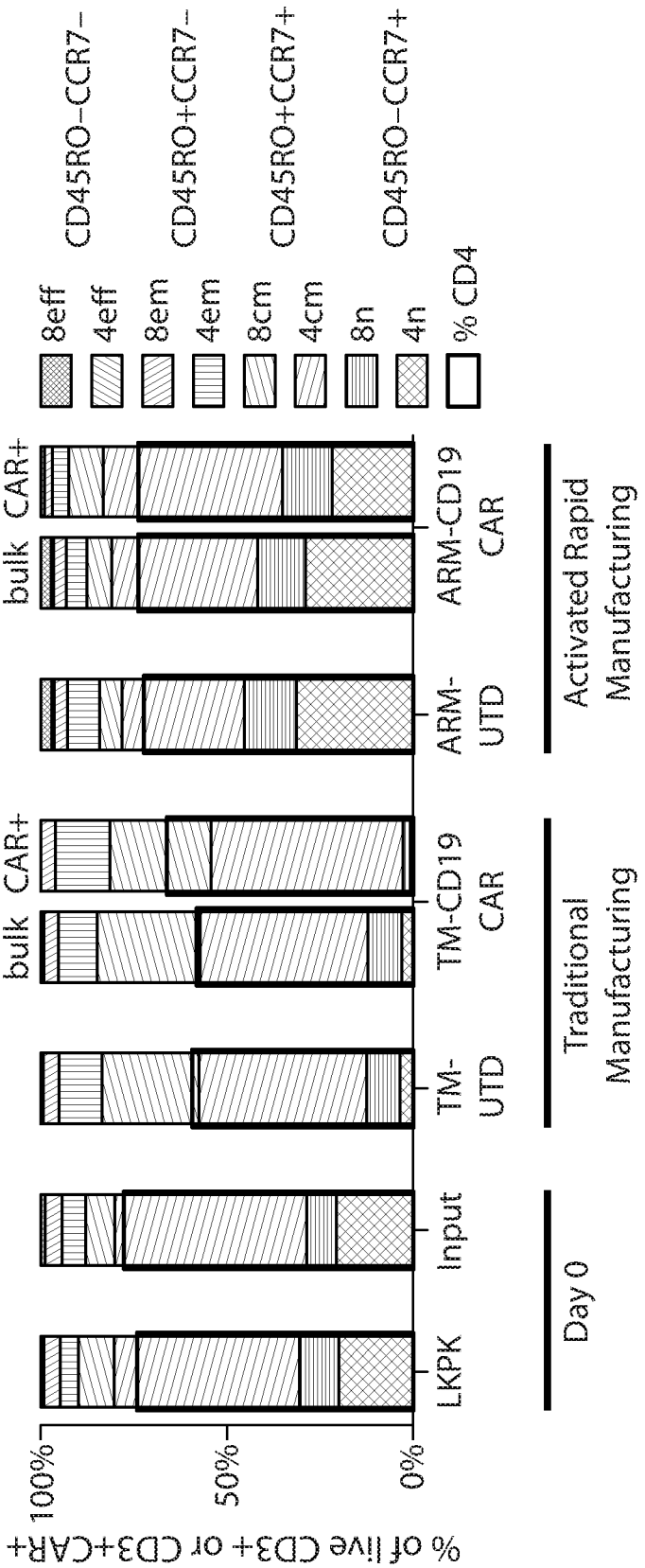

% of live CD3+ or CD3+CAR+
100%
50%
0%
LKPK
Input
TM-UTD
bulk
CAR+
TM-CD19 CAR
ARM-UTD
bulk
CAR+
ARM-CD19 CAR
Day 0
Traditional Manufacturing
Activated Rapid Manufacturing
8eff
4eff
8em
4em
8cm
4cm
8n
4n
% CD4
CD45RO−CCR7−
CD45RO+CCR7−
CD45RO+CCR7+
CD45RO−CCR7+

FIG. 28C

| | LKPK | Input | TM-UTD | TM-CD19 CAR | | ARM-UTD | ARM-CD19 CAR | |
|---|---|---|---|---|---|---|---|---|
| | | | | bulk | CAR+ | | bulk | CAR+ |
| Teff | 0.8 | 1.4 | 0.8 | 0.9 | 0.2 | 3.8 | 3.2 | 0.8 |
| Tem | 8.7 | 10.7 | 17.3 | 13.7 | 18.0 | 11.1 | 8.1 | 6.2 |
| Tcm | 59.5 | 59.4 | 67.4 | 68.6 | 77.3 | 37.2 | 43.6 | 55.5 |
| Tn | 30.9 | 28.6 | 14.6 | 16.9 | 4.5 | 48.0 | 45.1 | 37.5 |

FIG. 28D

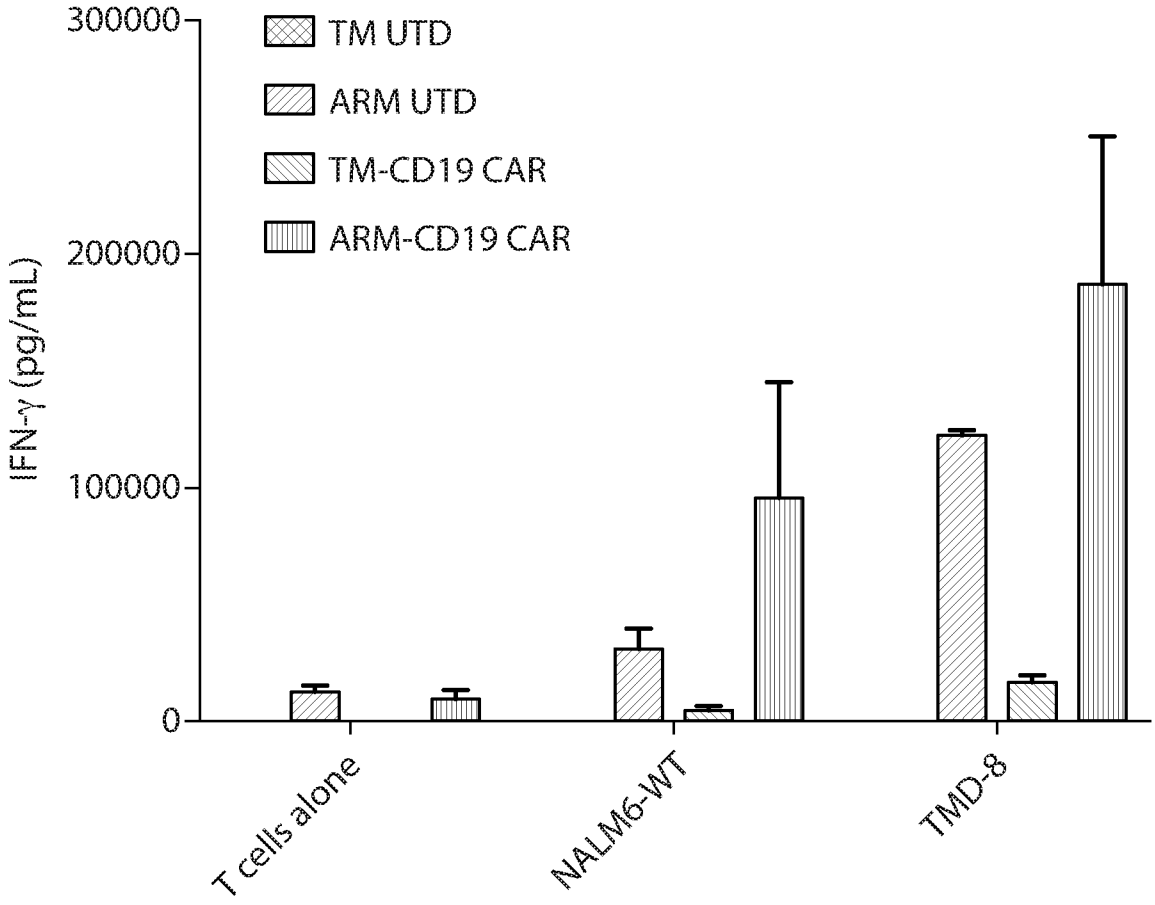

300000
200000
100000
0
IFN-γ (pg/mL)
TM UTD
ARM UTD
TM-CD19 CAR
ARM-CD19 CAR
T cells alone
NALM6-WT
TMD-8

FIG. 29A

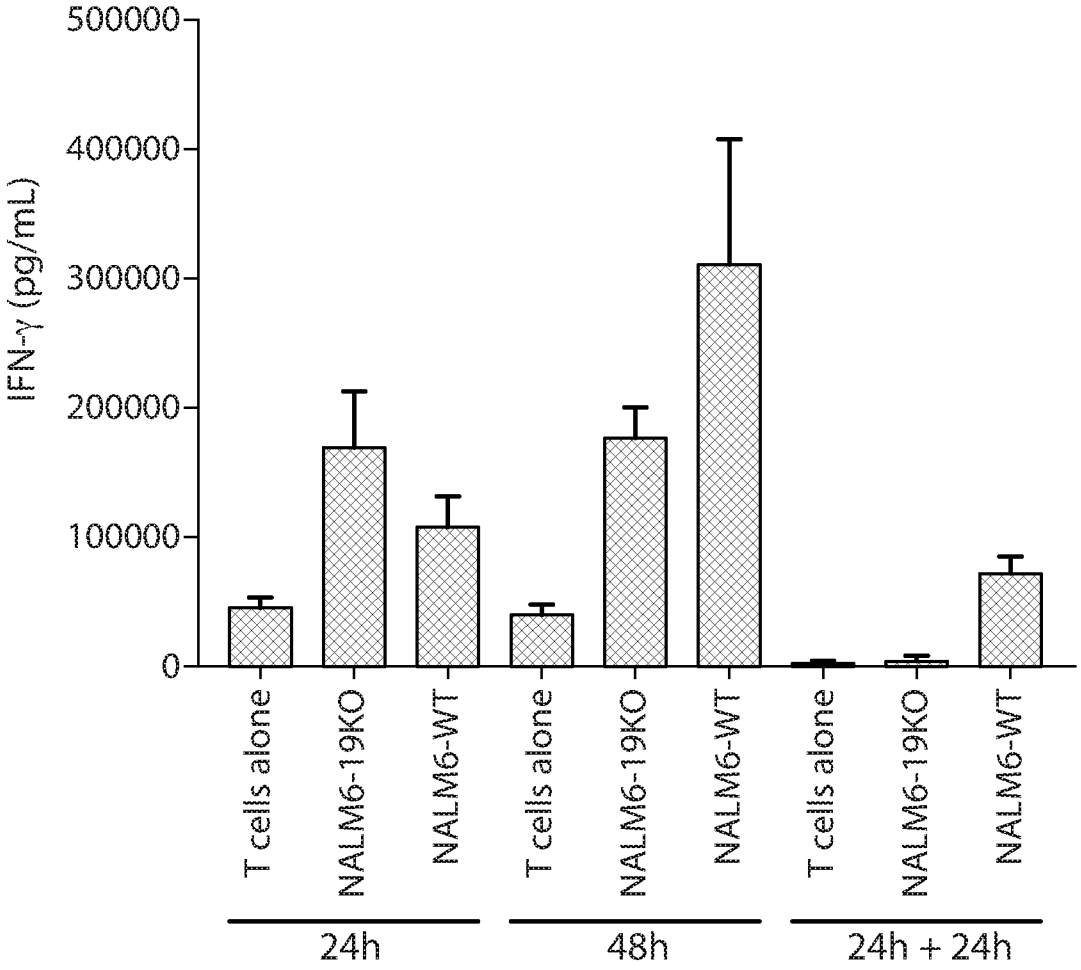

500000
400000
300000
200000
100000
0
IFN-γ (pg/mL)
T cells alone
NALM6-19KO
NALM6-WT
T cells alone
NALM6-19KO
NALM6-WT
T cells alone
NALM6-19KO
NALM6-WT
24h
48h
24h + 24h

FIG. 29B

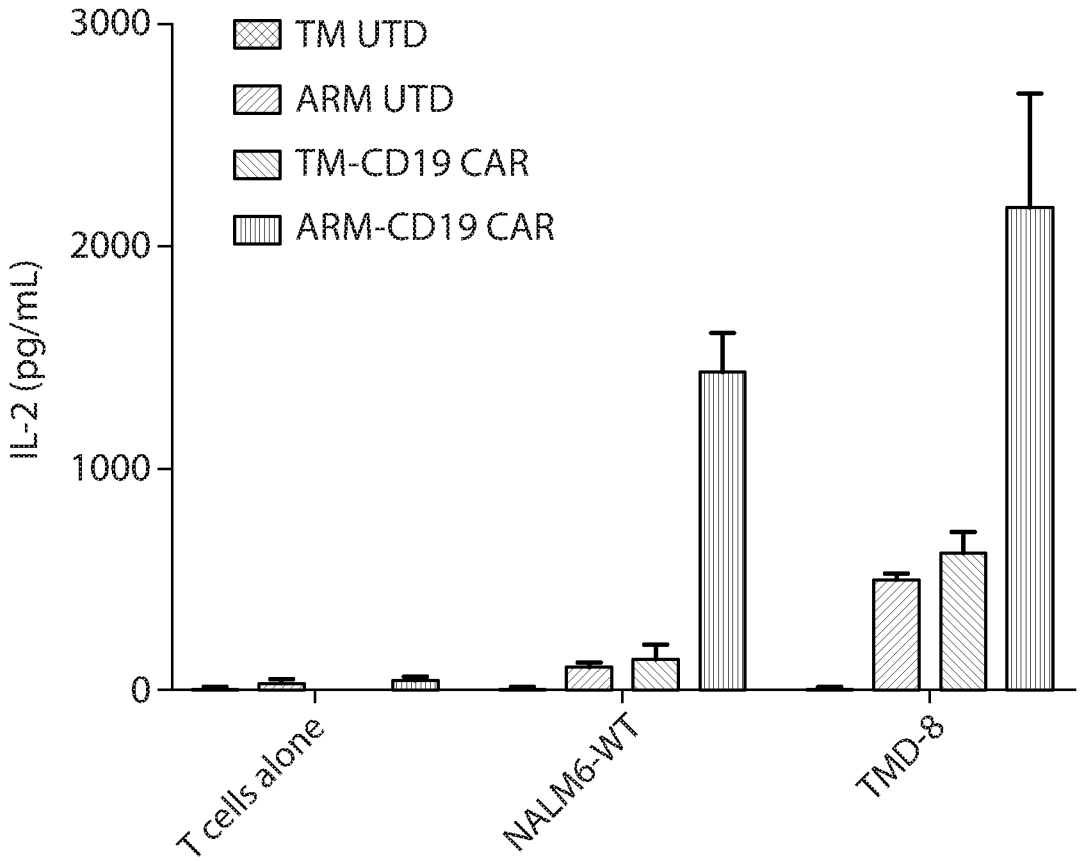

3000
2000
1000
0
IL-2 (pg/mL)
TM UTD
ARM UTD
TM-CD19 CAR
ARM-CD19 CAR
T cells alone
NALM6-WT
TMD-8

FIG. 29C

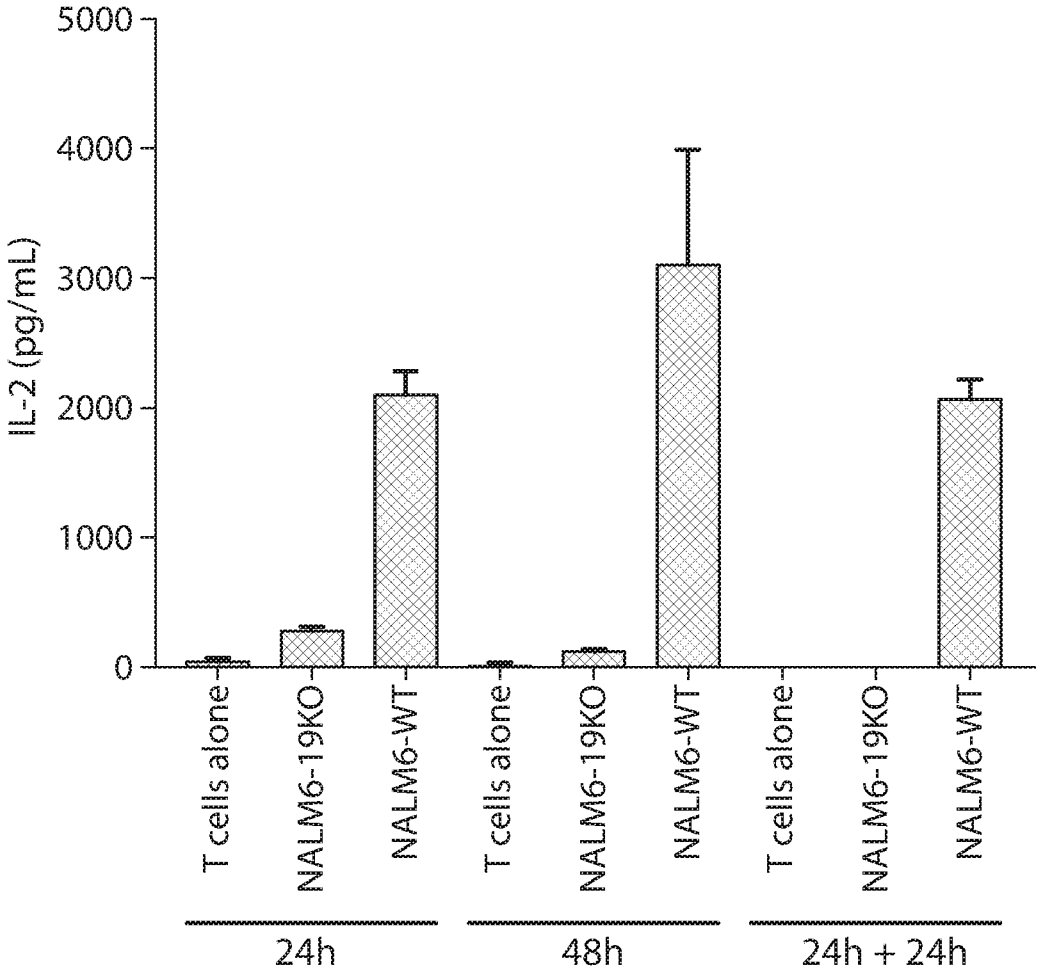

5000
4000
3000
2000
1000
0
IL-2 (pg/mL)
T cells alone
NALM6-19KO
NALM6-WT
T cells alone
NALM6-19KO
NALM6-WT
T cells alone
NALM6-19KO
NALM6-WT
24h
48h
24h + 24h

FIG. 29D

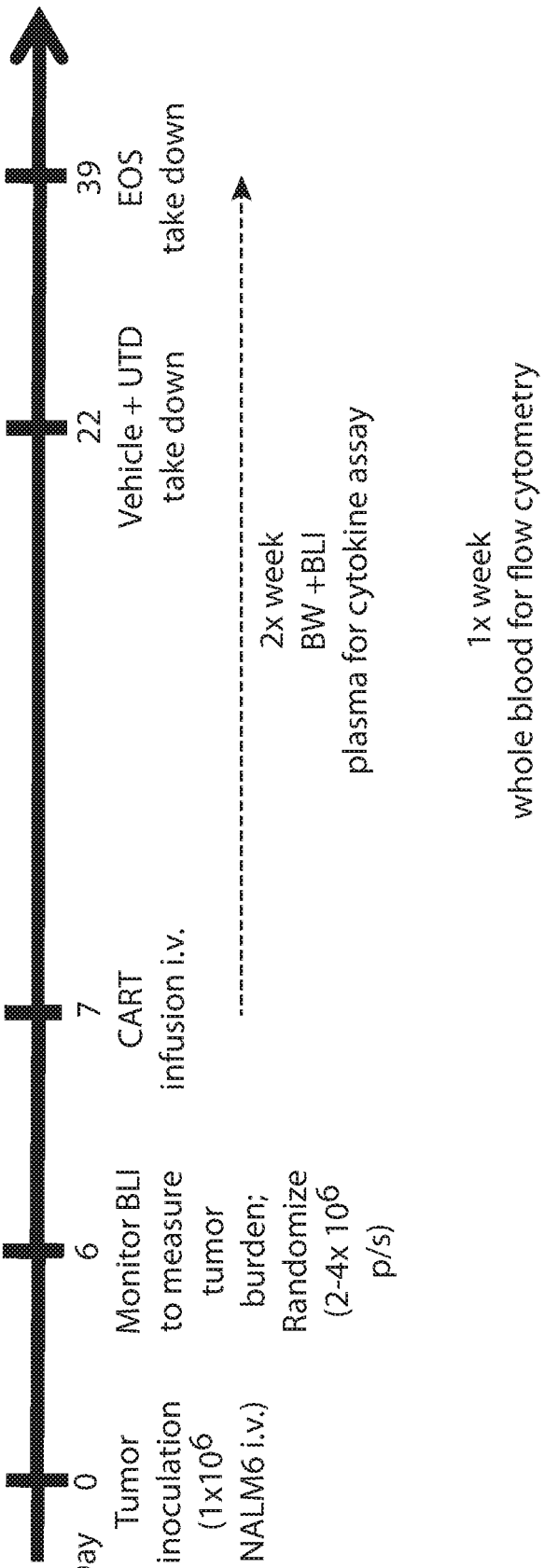

Day 0
Tumor inoculation (1x10^6 NALM6 i.v.)
6
Monitor BLI to measure tumor burden; Randomize (2-4x 10^6 p/s)
7
CART infusion i.v.
22
Vehicle + UTD take down
39
EOS take down
2x week
BW +BLI
plasma for cytokine assay
1x week
whole blood for flow cytometry

FIG. 30A

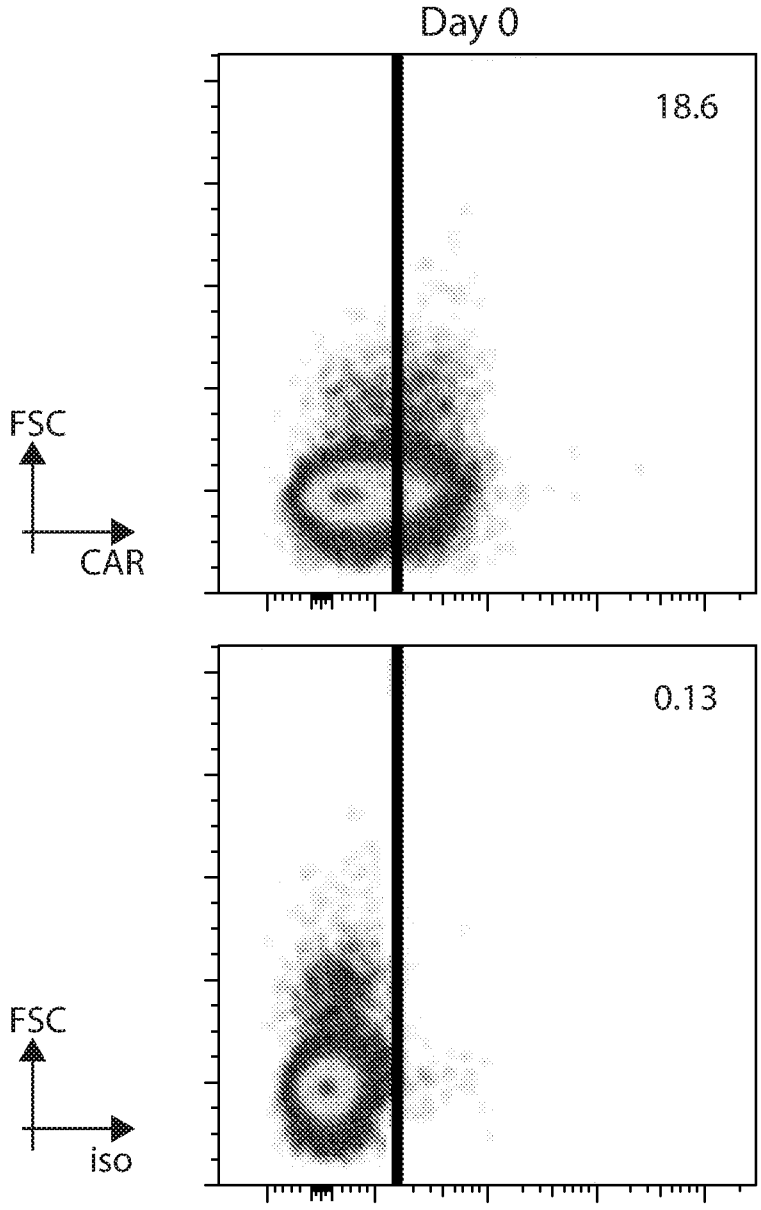



FIG. 30B-1

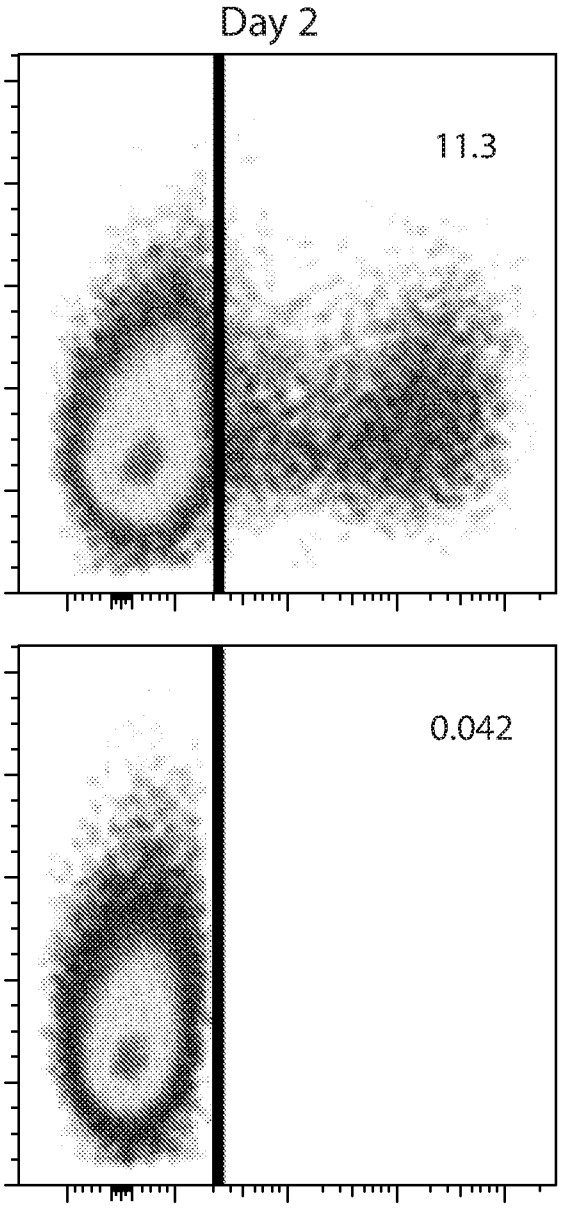



FIG. 30B-2

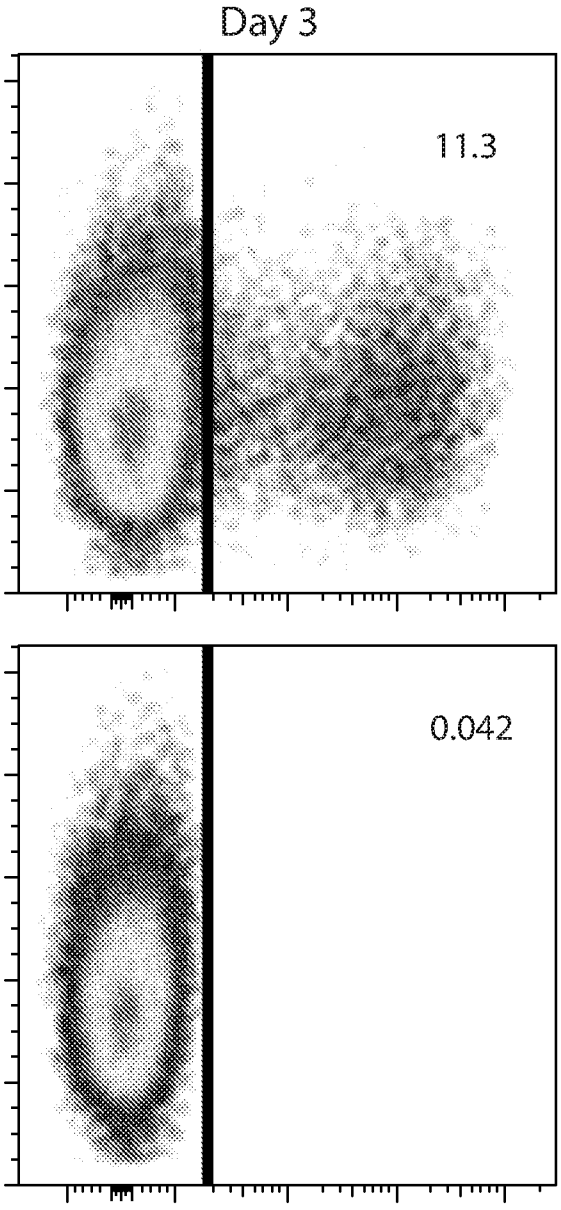



FIG. 30B-3

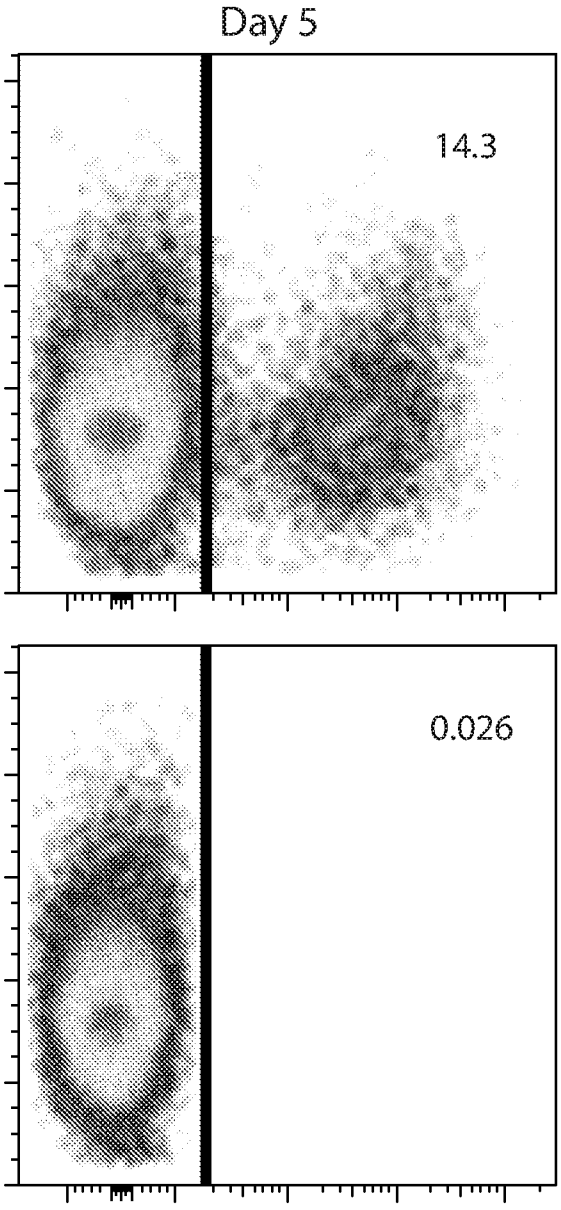



FIG. 30B-4

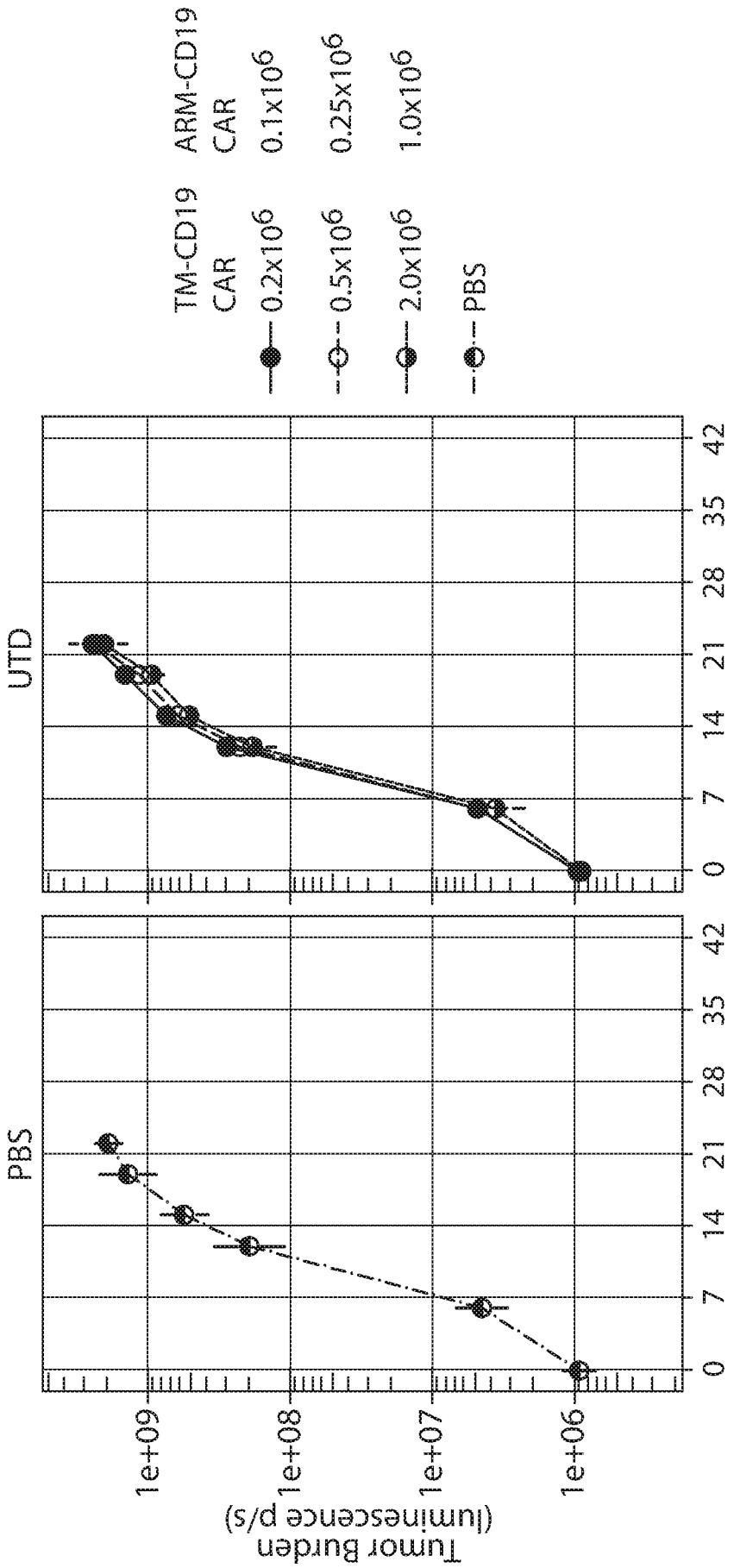

TM-CD19 CAR
0.2x10^6
0.5x10^6
2.0x10^6
PBS
ARM-CD19 CAR
0.1x10^6
0.25x10^6
1.0x10^6
UTD
PBS
Tumor Burden (luminescence p/s)
1e+09
1e+08
1e+07
1e+06
0
7
14
21
28
35
42
Days post tumor implantation

FIG. 30C-1

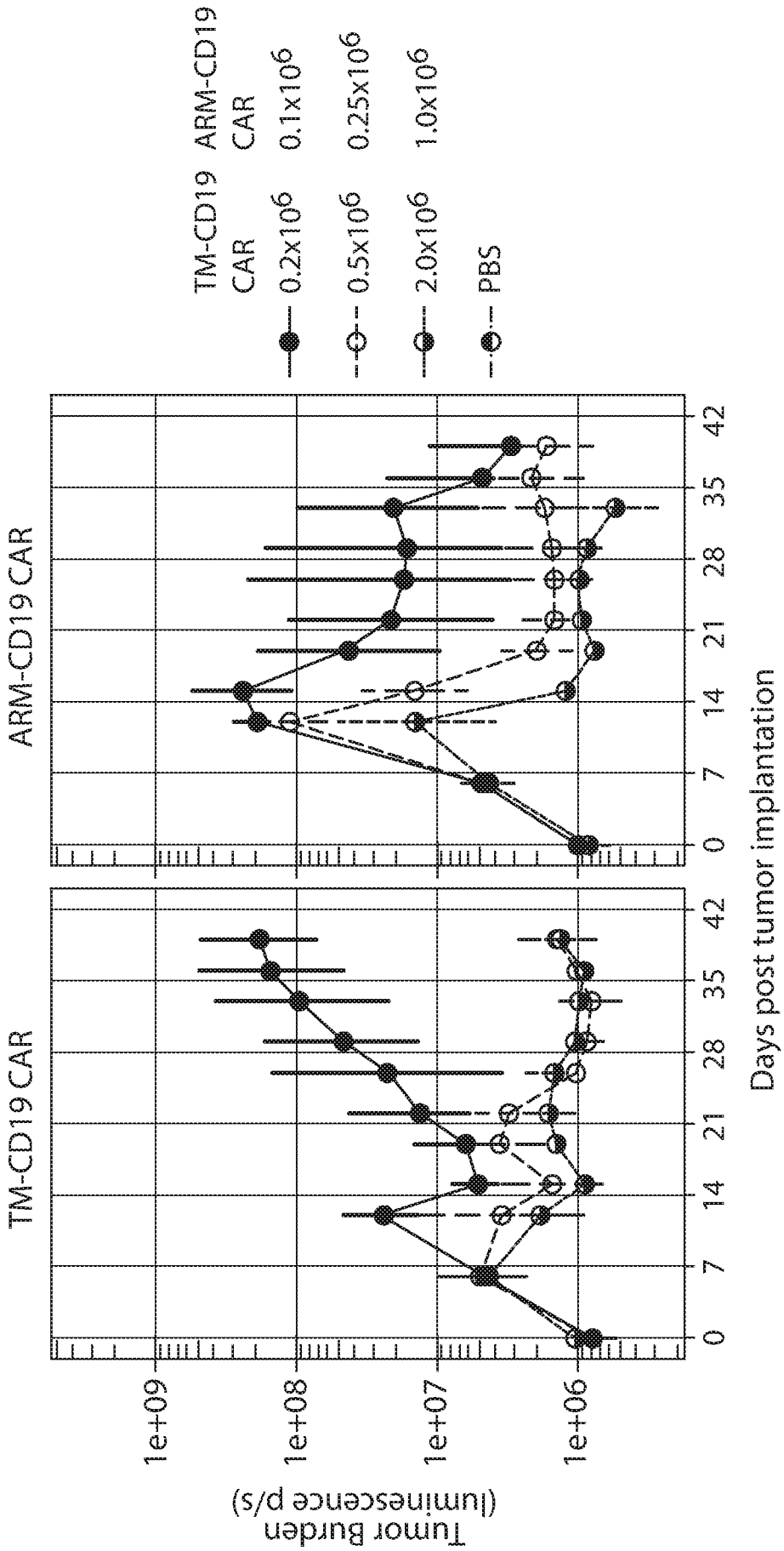

TM-CD19 CAR
ARM-CD19 CAR
TM-CD19 CAR 0.2x10^6
0.5x10^6
2.0x10^6
ARM-CD19 CAR 0.1x10^6
0.25x10^6
1.0x10^6
PBS
Tumor Burden (luminescence p/s)
1e+09
1e+08
1e+07
1e+06
0
7
14
21
28
35
42
Days post tumor implantation

FIG. 30C-2

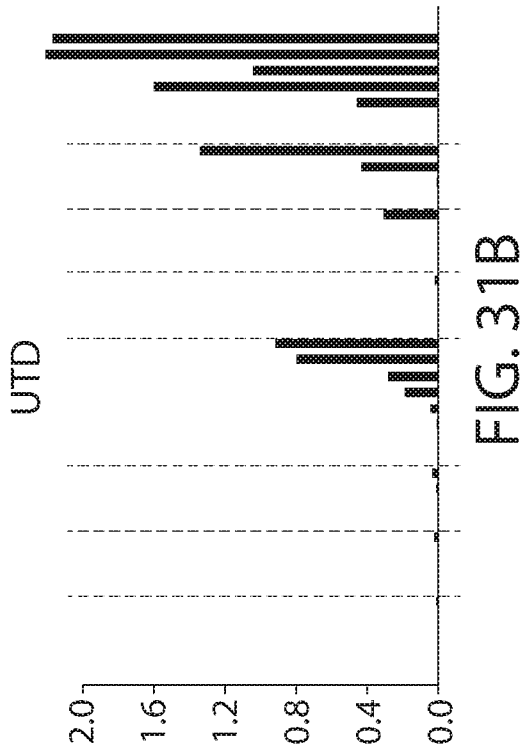



FIG. 31B

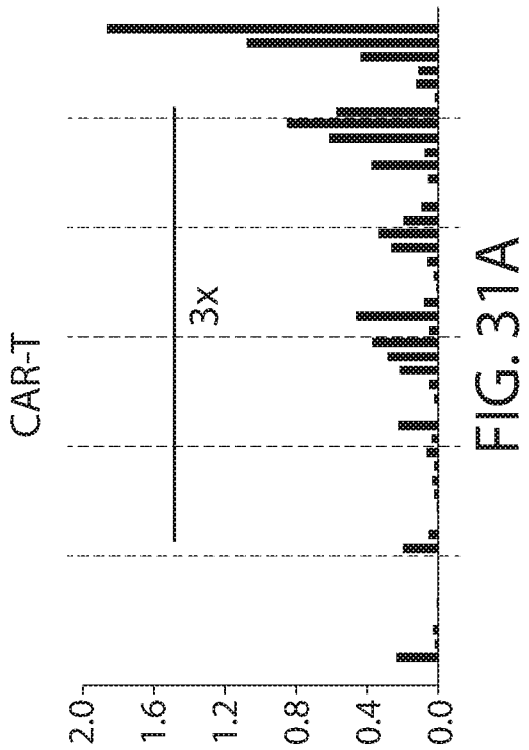



FIG. 31A

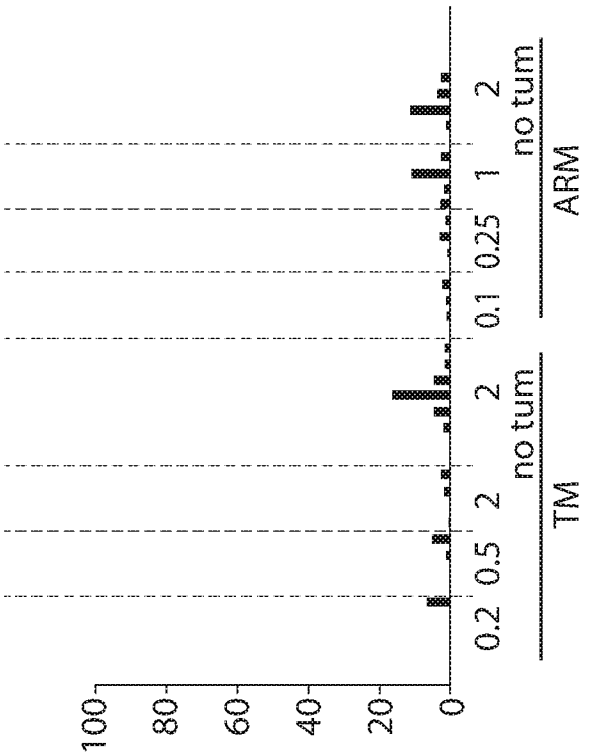



FIG. 31D

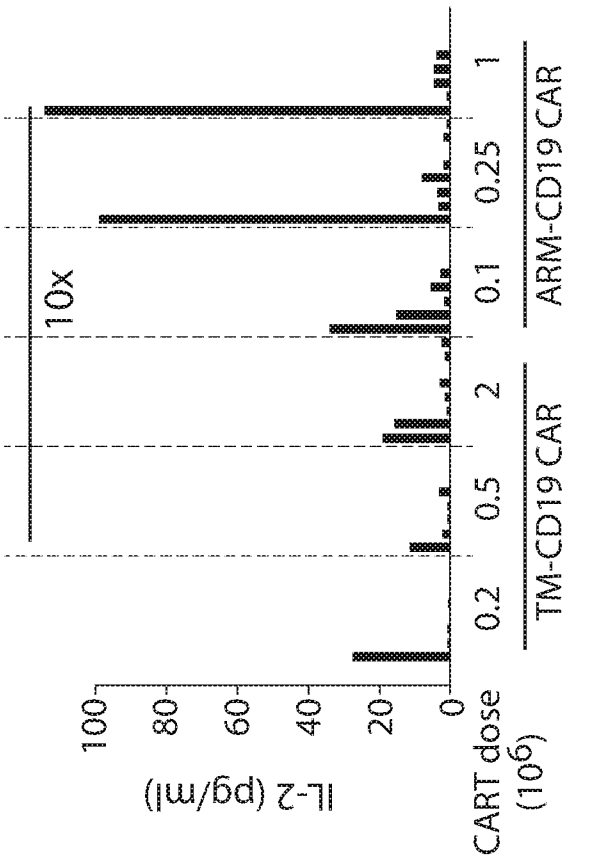



FIG. 31C

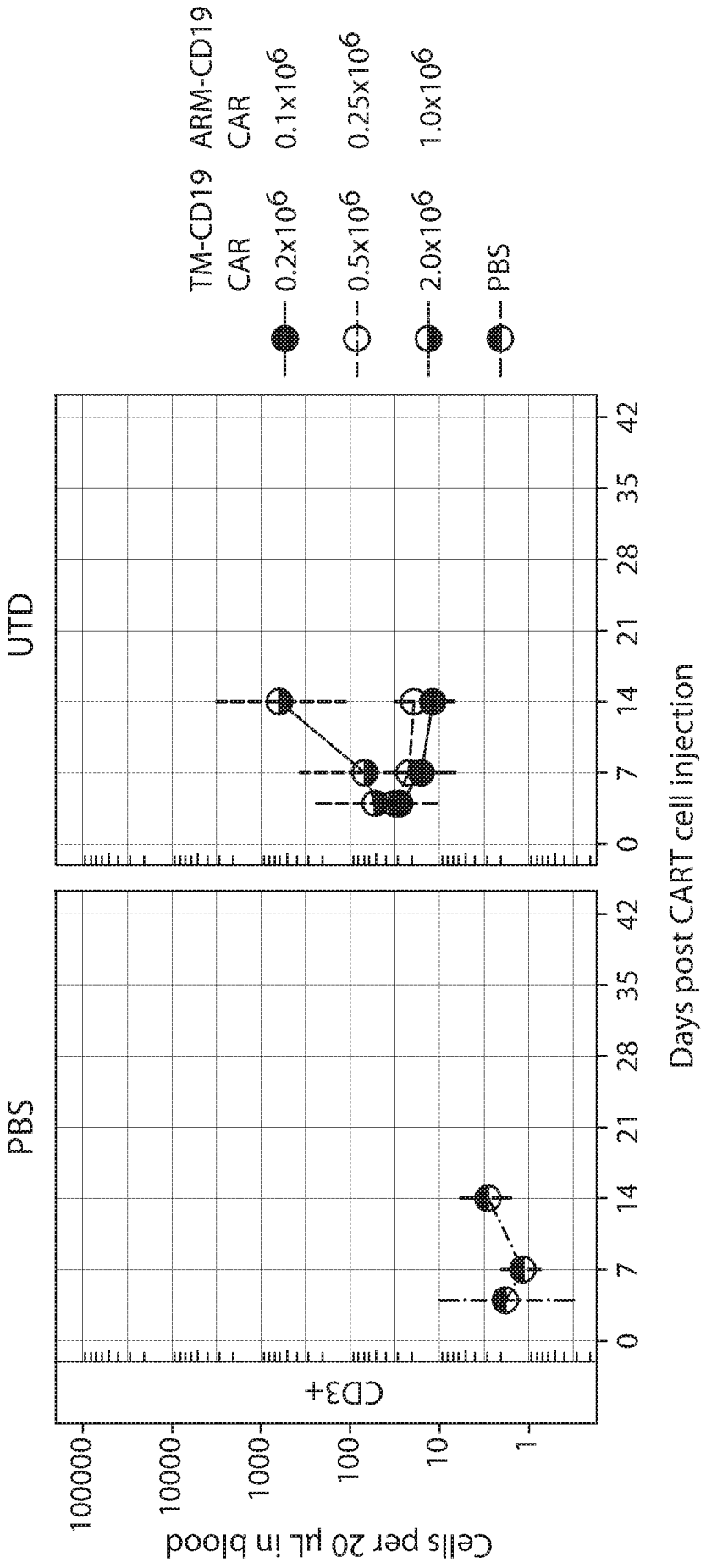

TM-CD19 CAR
0.2x10^6
0.5x10^6
2.0x10^6
PBS
ARM-CD19 CAR
0.1x10^6
0.25x10^6
1.0x10^6
UTD
PBS
CD3+
Cells per 20 µL in blood
100000
10000
1000
100
10
1
0
7
14
21
28
35
42
Days post CART cell injection

FIG. 32-1

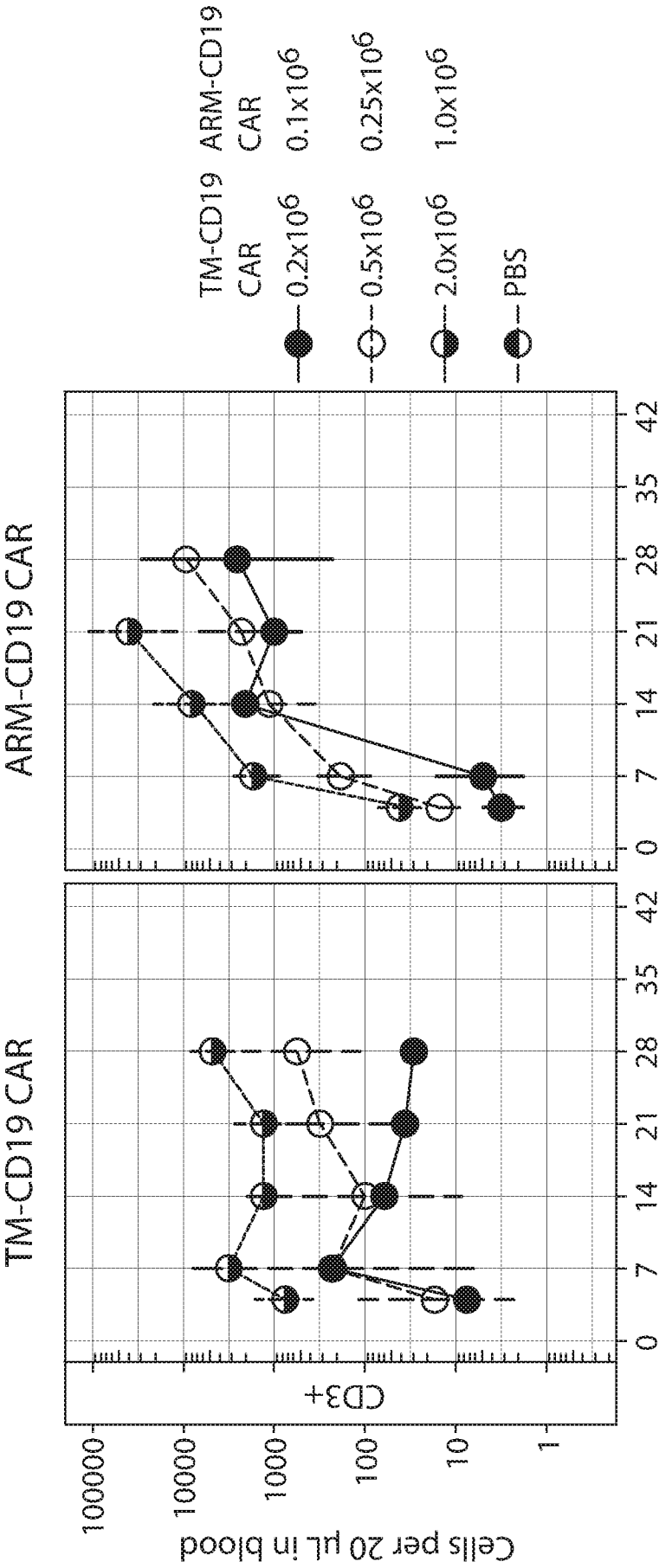

TM-CD19 CAR
ARM-CD19 CAR
CD3+
Cells per 20 µL in blood
100000
10000
1000
100
10
1
0
7
14
21
28
35
42
Days post CART cell injection
TM-CD19 CAR
0.2x10^6
0.5x10^6
2.0x10^6
PBS
ARM-CD19 CAR
0.1x10^6
0.25x10^6
1.0x10^6

FIG. 32-2

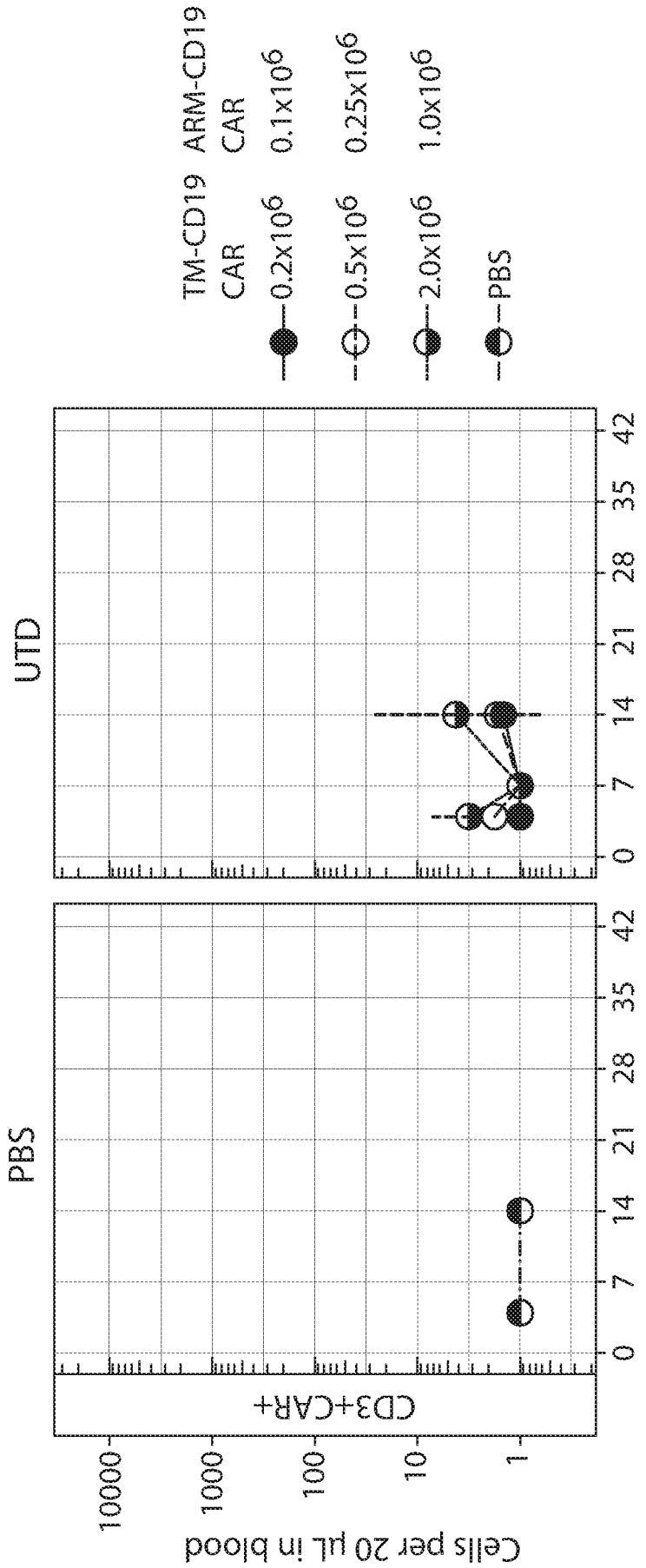

TM-CD19 CAR
0.2x10^6
0.5x10^6
2.0x10^6
PBS
ARM-CD19 CAR
0.1x10^6
0.25x10^6
1.0x10^6
UTD
PBS
CD3+CAR+
Cells per 20 µL in blood
10000
1000
100
10
1
0
7
14
21
28
35
42
Days post CART cell injection

FIG. 32-3

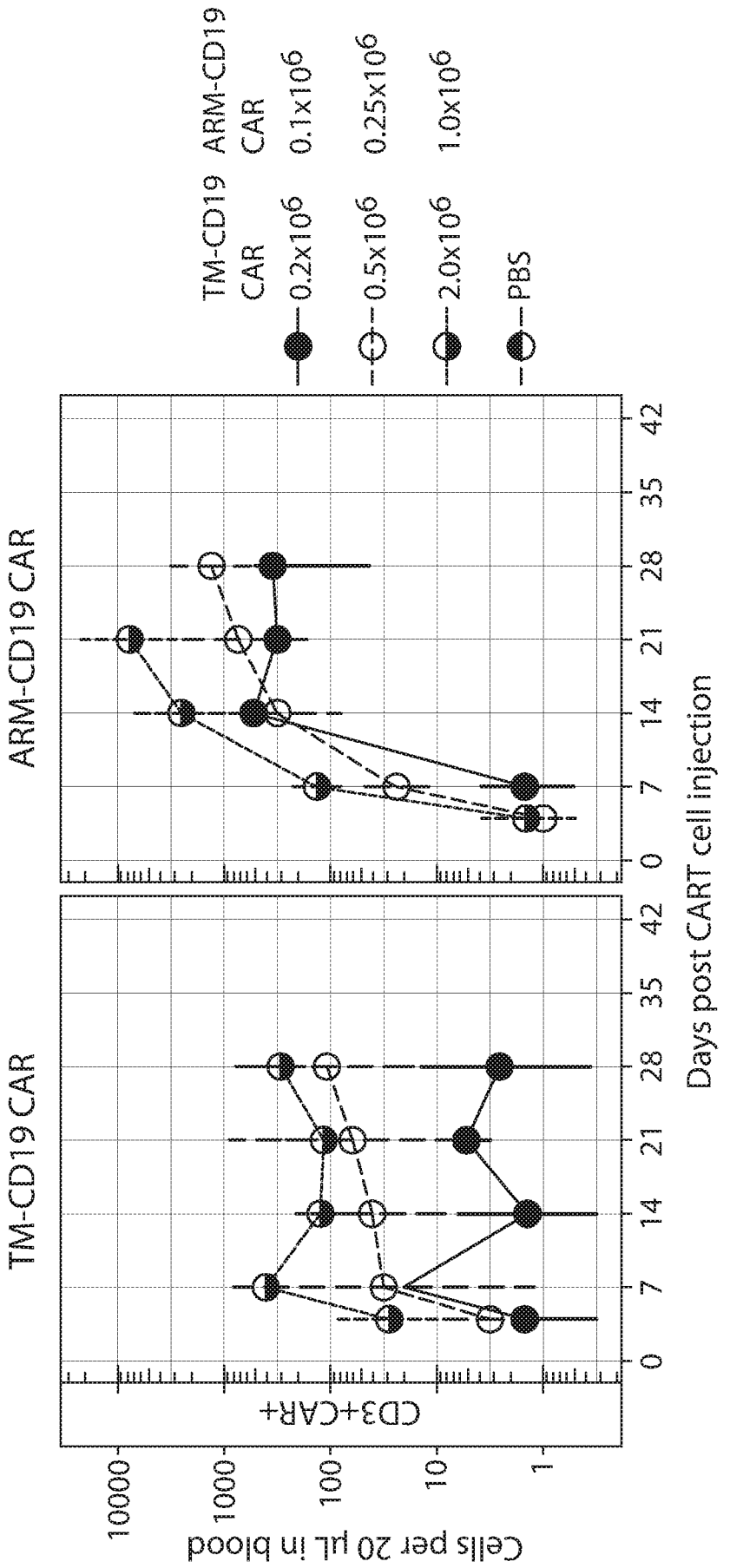

TM-CD19 CAR
ARM-CD19 CAR
TM-CD19 CAR
0.2x10^6
0.5x10^6
2.0x10^6
PBS
ARM-CD19 CAR
0.1x10^6
0.25x10^6
1.0x10^6
Cells per 20 µL in blood
CD3+CAR+
10000
1000
100
10
1
0
7
14
21
28
35
42
Days post CART cell injection

FIG. 32-4

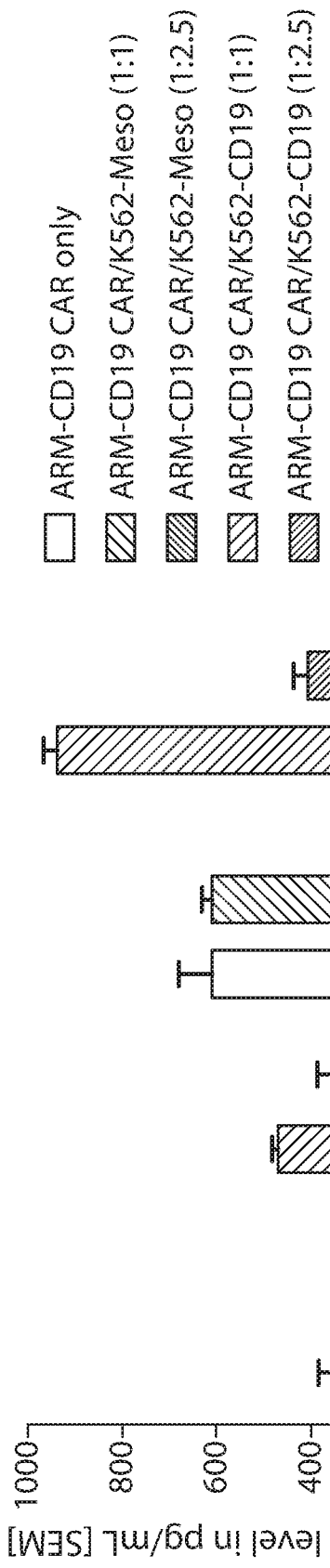

ARM-CD19 CAR only
ARM-CD19 CAR/K562-Meso (1:1)
ARM-CD19 CAR/K562-Meso (1:2.5)
ARM-CD19 CAR/K562-CD19 (1:1)
ARM-CD19 CAR/K562-CD19 (1:2.5)
ARM-CD19 CAR/K562 cell co-culture
IL-6 protein level in pg/mL [SEM]
1000
800
600
400
200
0
6h
24h

FIG. 33A

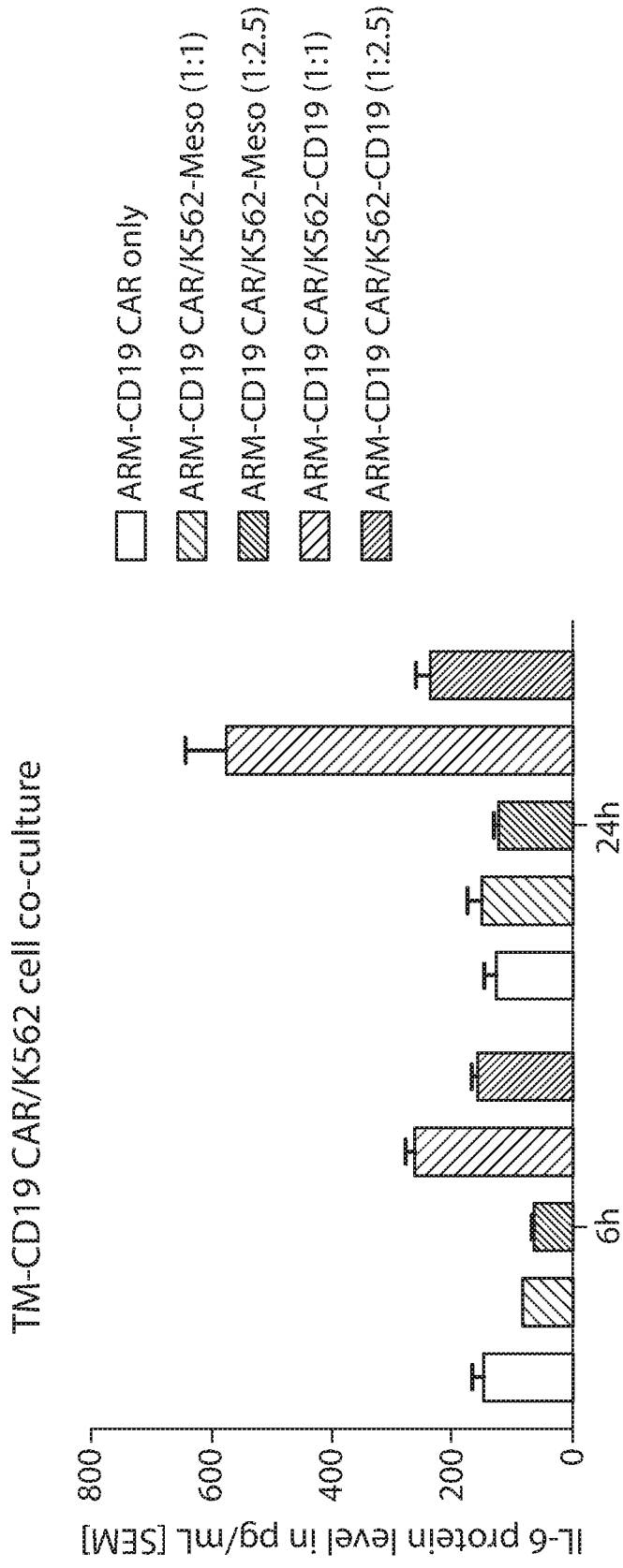

ARM-CD19 CAR only
ARM-CD19 CAR/K562-Meso (1:1)
ARM-CD19 CAR/K562-Meso (1:2.5)
ARM-CD19 CAR/K562-CD19 (1:1)
ARM-CD19 CAR/K562-CD19 (1:2.5)
TM-CD19 CAR/K562 cell co-culture
IL-6 protein level in pg/mL [SEM]
800
600
400
200
0
6h
24h

FIG. 33B

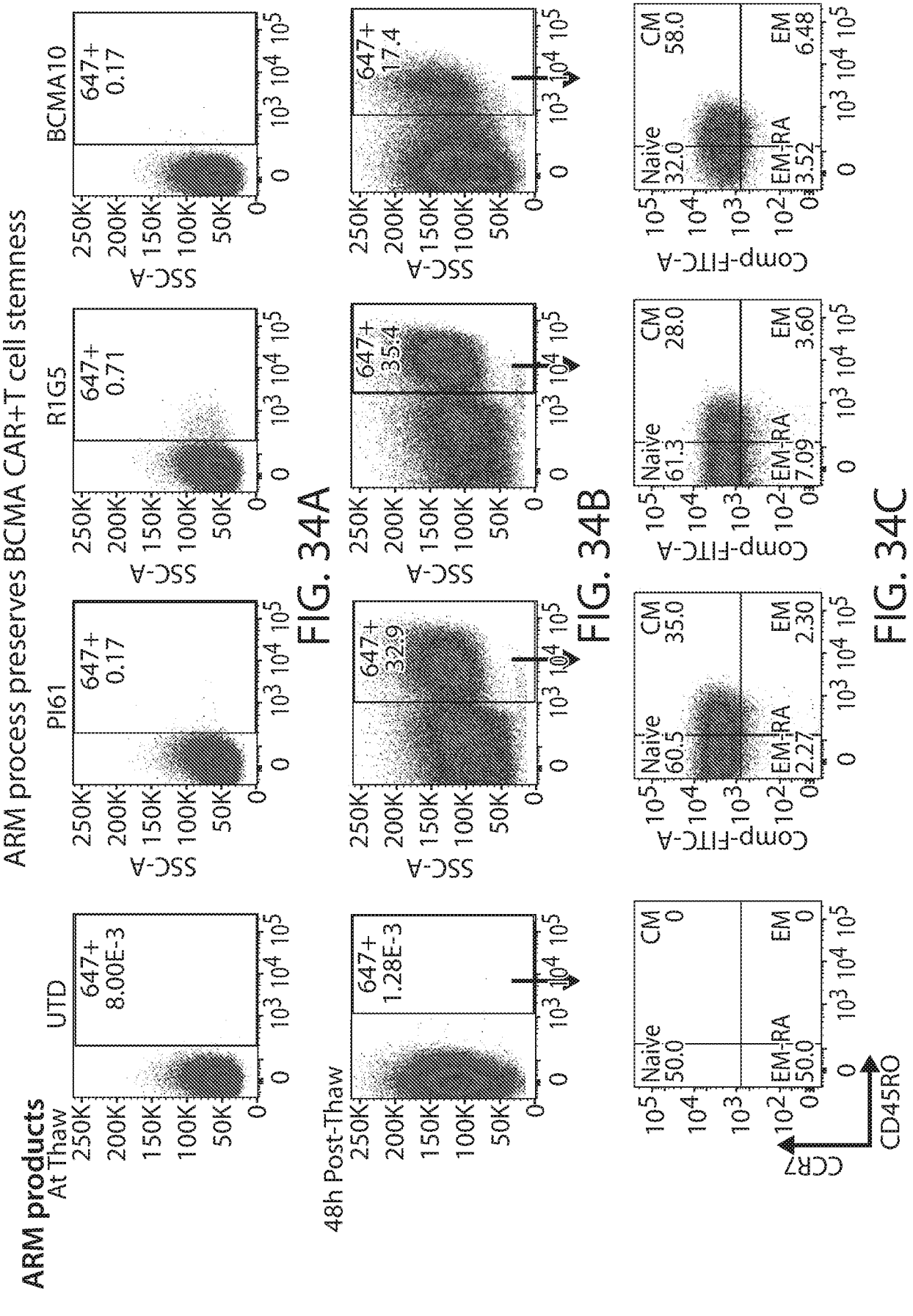



FIG. 34A

FIG. 34B

FIG. 34C

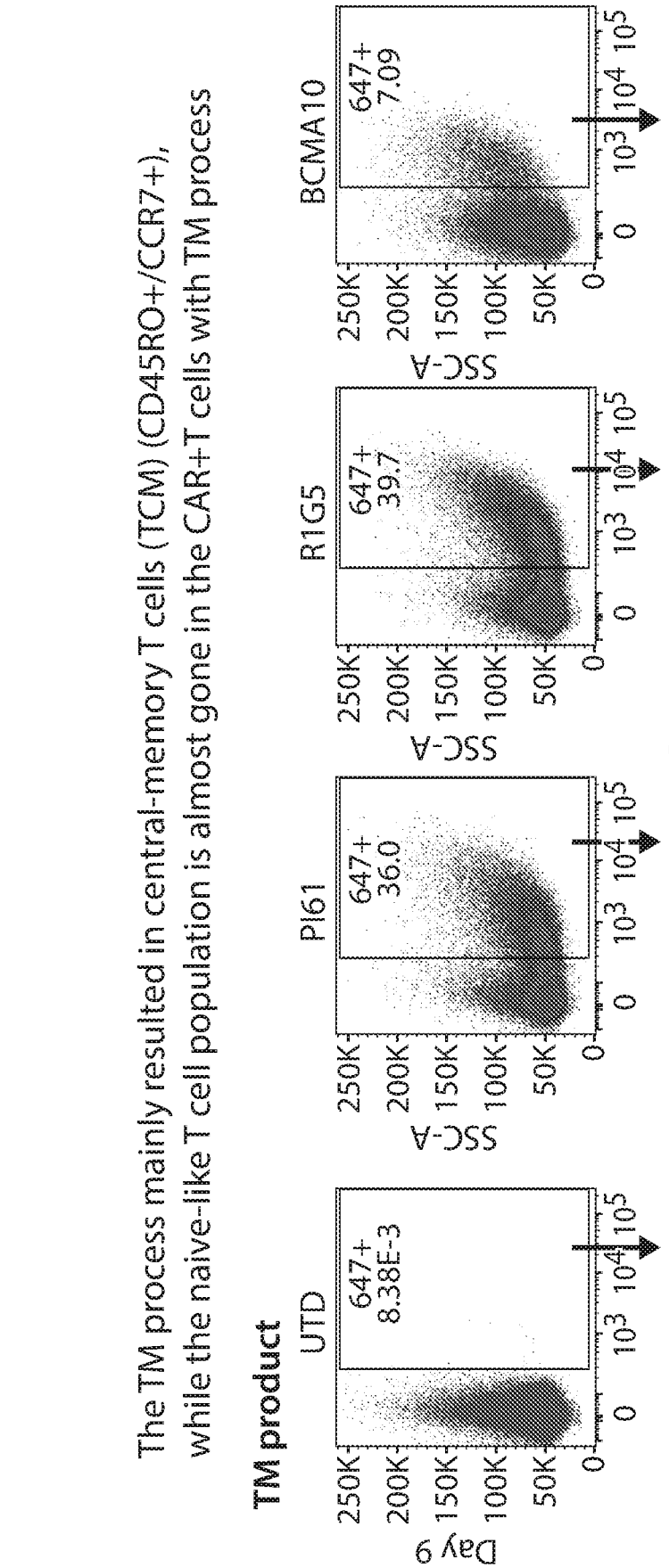



FIG. 35A

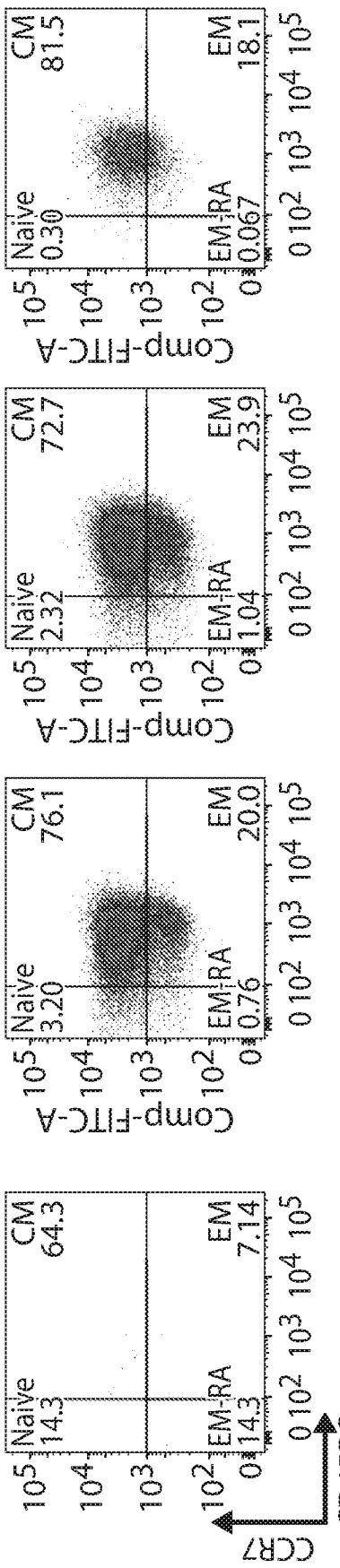



FIG. 35B

ARM processed BCMA CAR-T cells demonstrates BCMA-specific activation and secretes higher levels of IL2 and IFNg

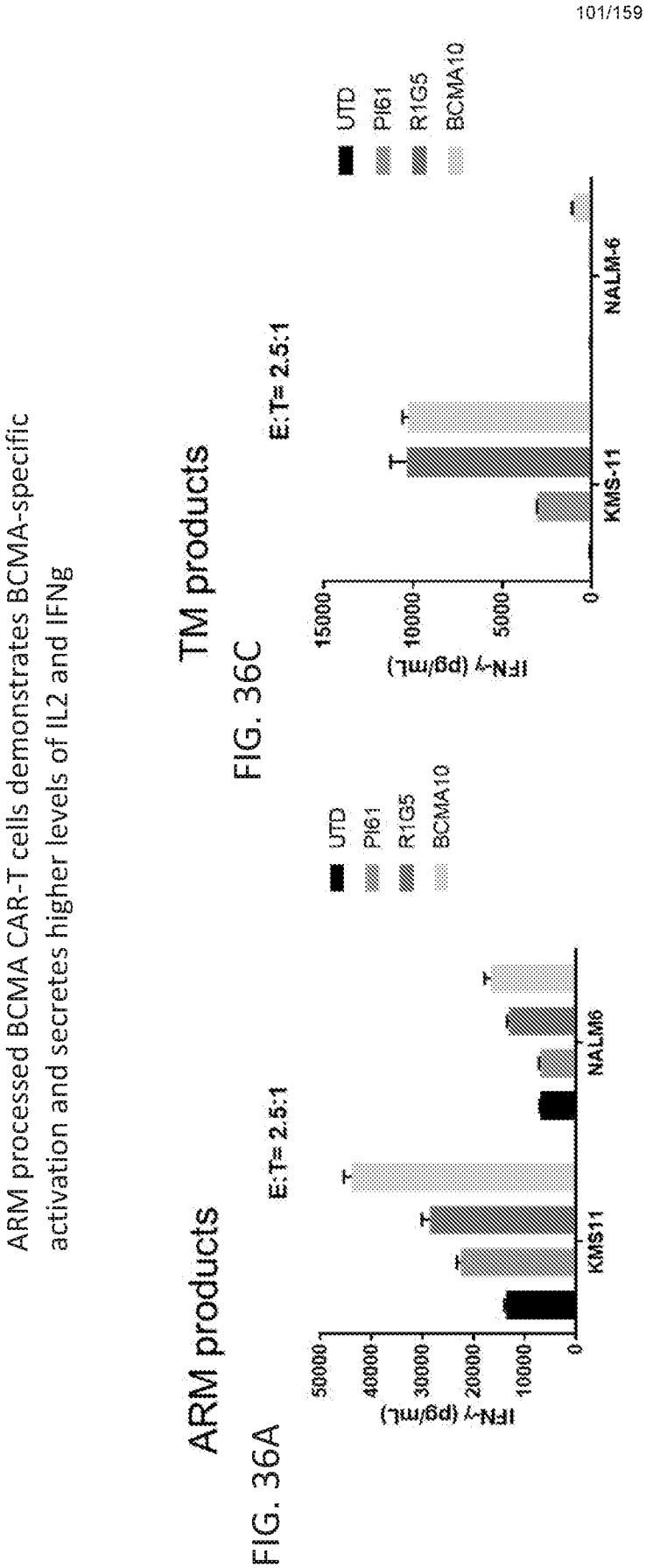



FIG. 36A

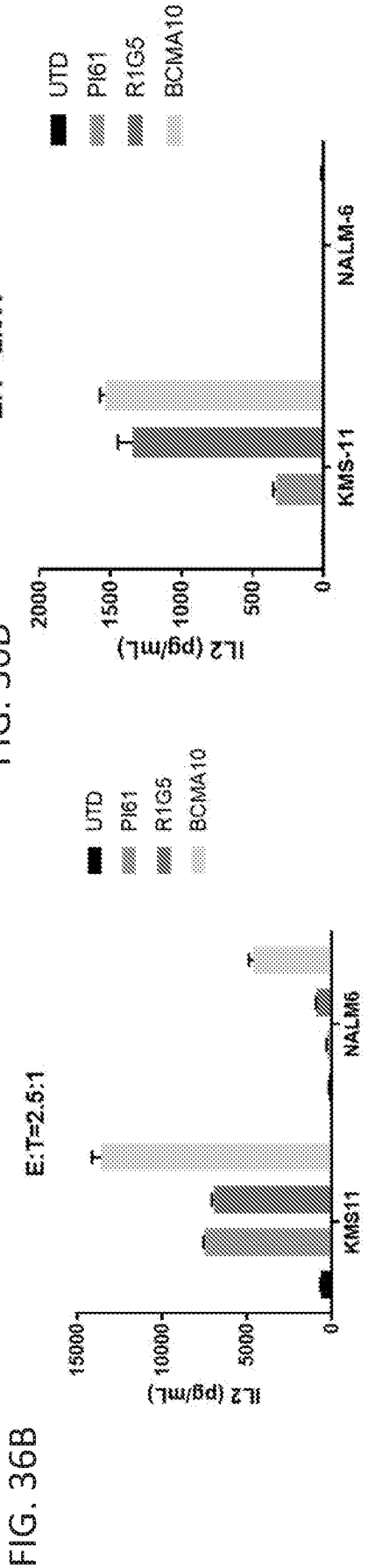



FIG. 36C




FIG. 36B




FIG. 36D

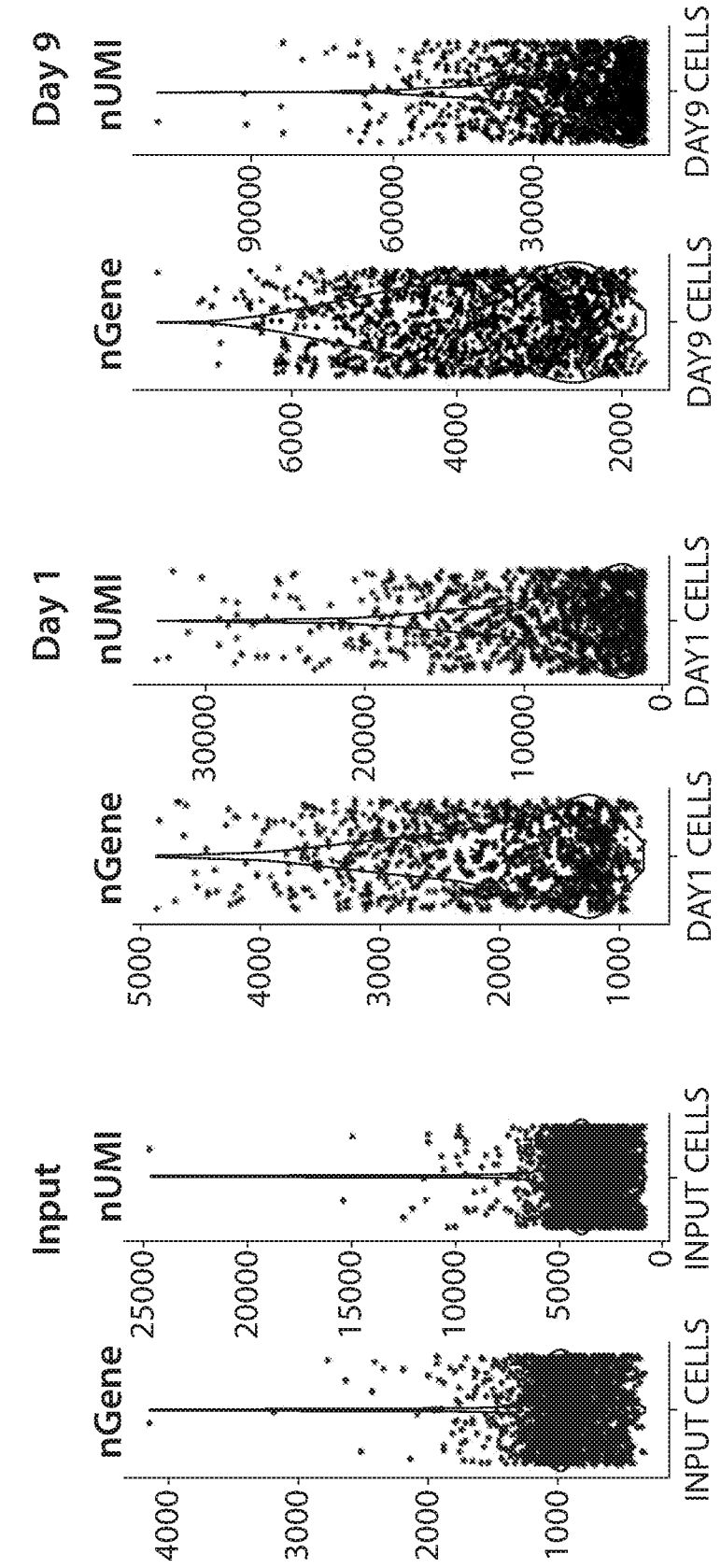



FIG. 37A




FIG. 37B




FIG. 37C

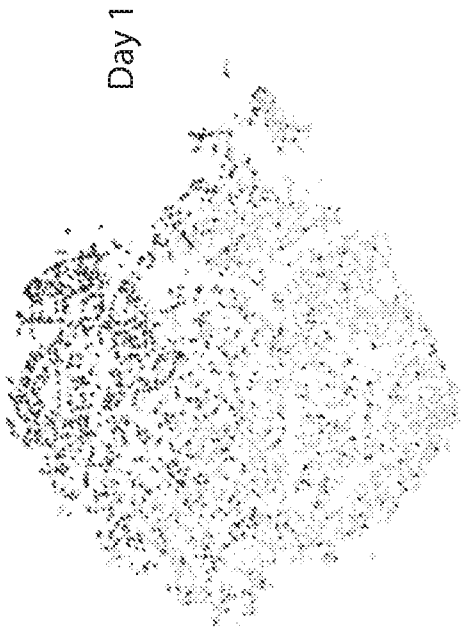



FIG. 38B


FIG. 38A

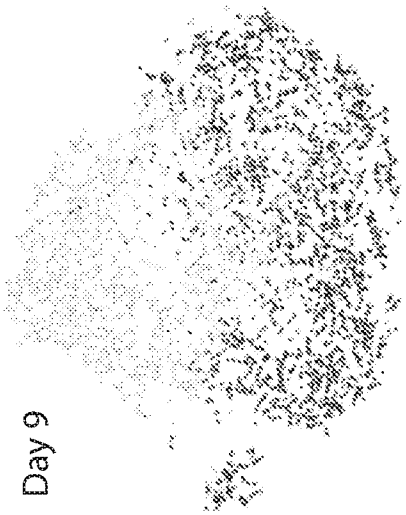



FIG. 38C

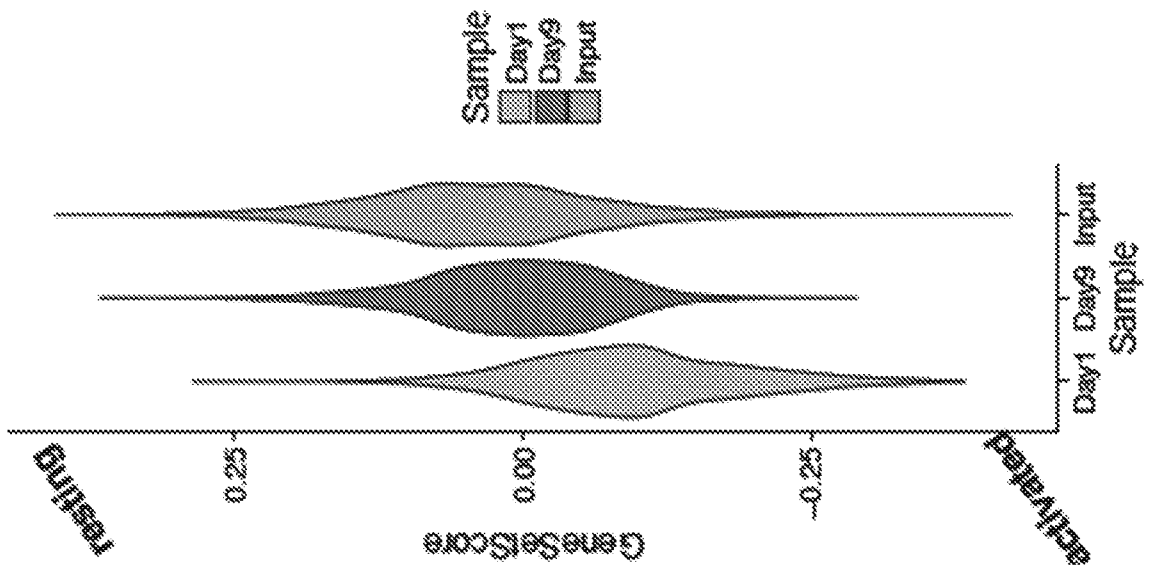
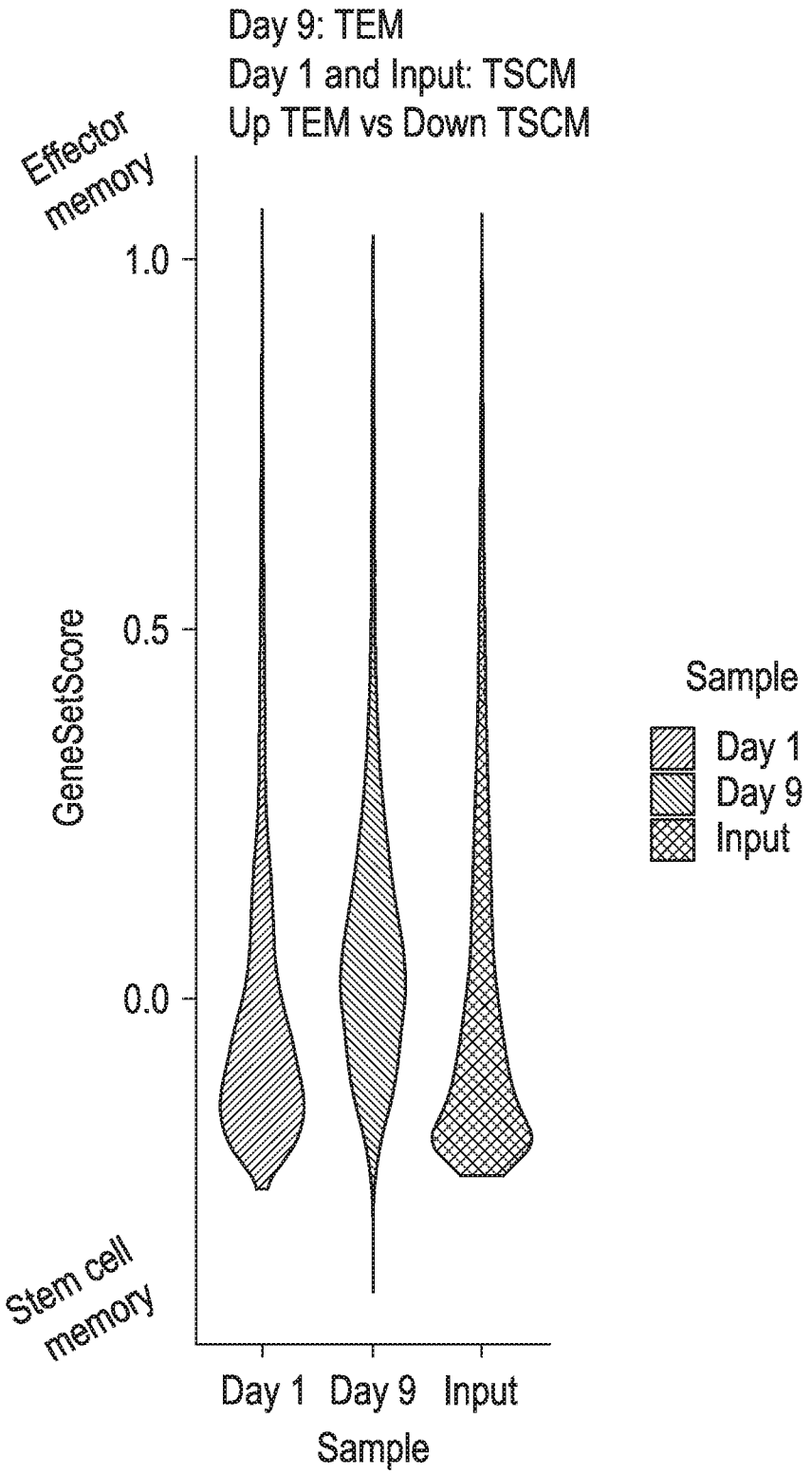
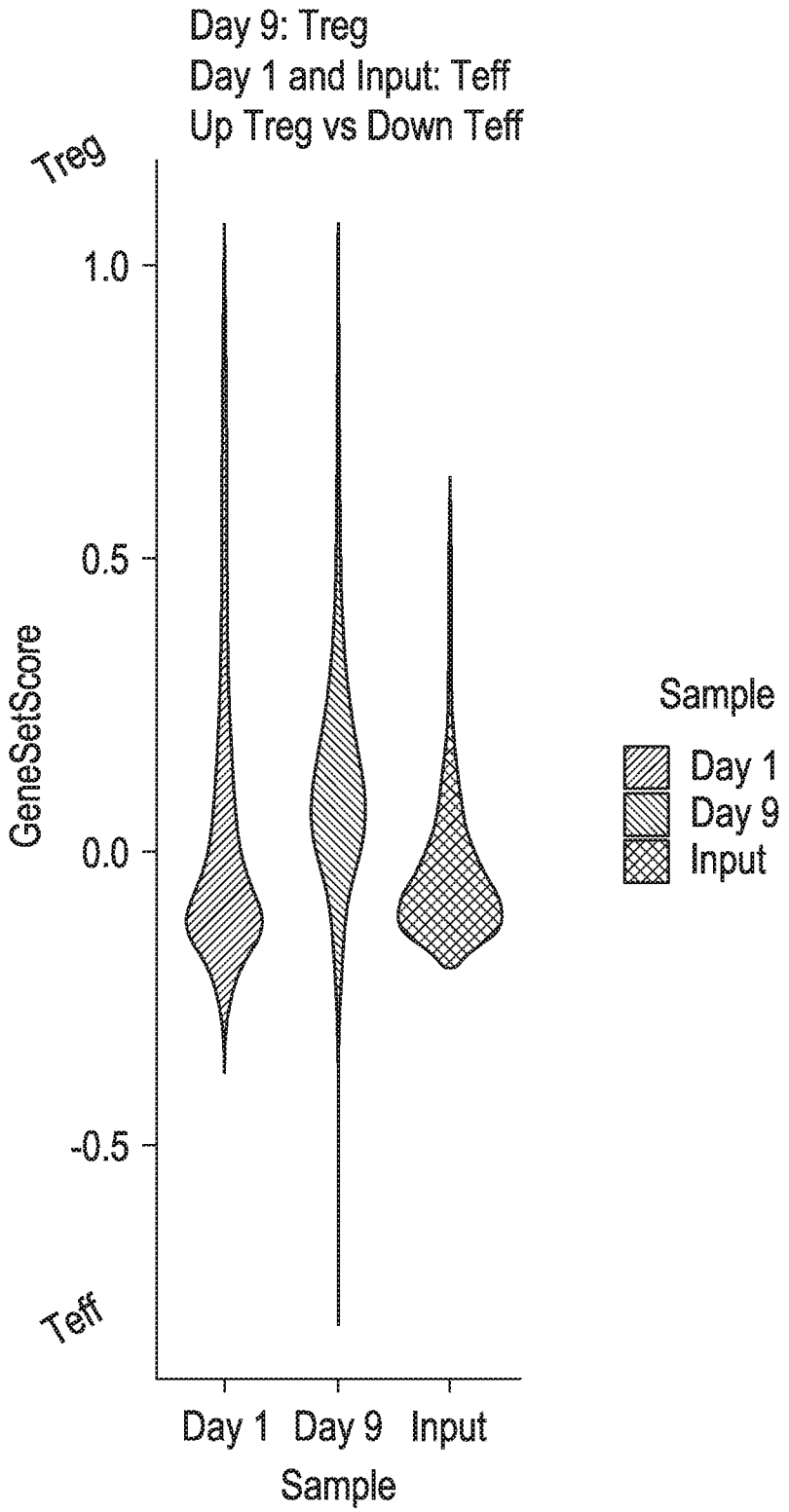

Sample
Day1
Day9
Input
resting
activated
0.25
0.00
−0.25
GeneSetScore
Day1 Day9 Input
Sample

FIG. 38D



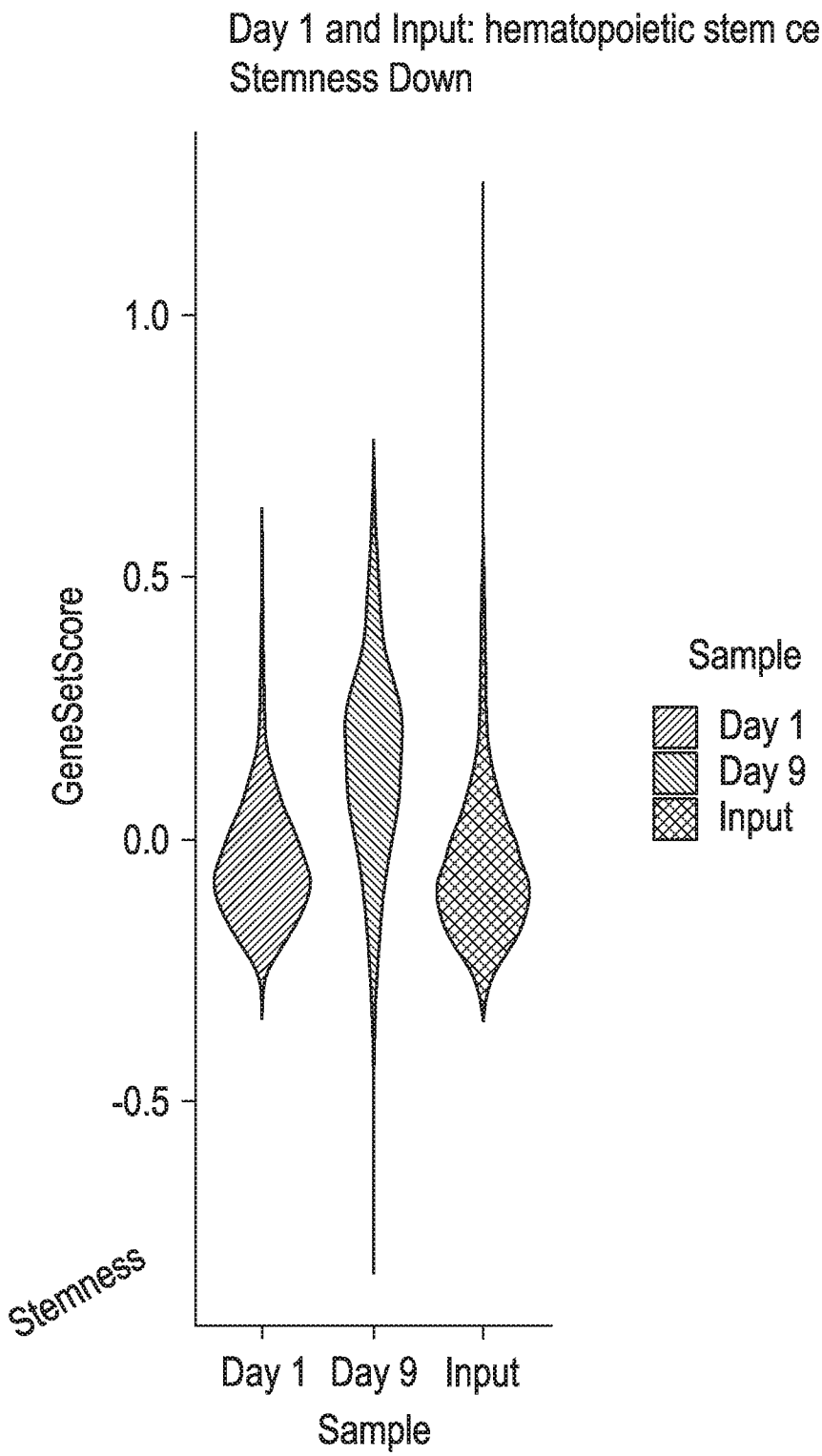

Medians:
D1: -0.084
D9: 0.035
Input: -0.1

FIG. 39A



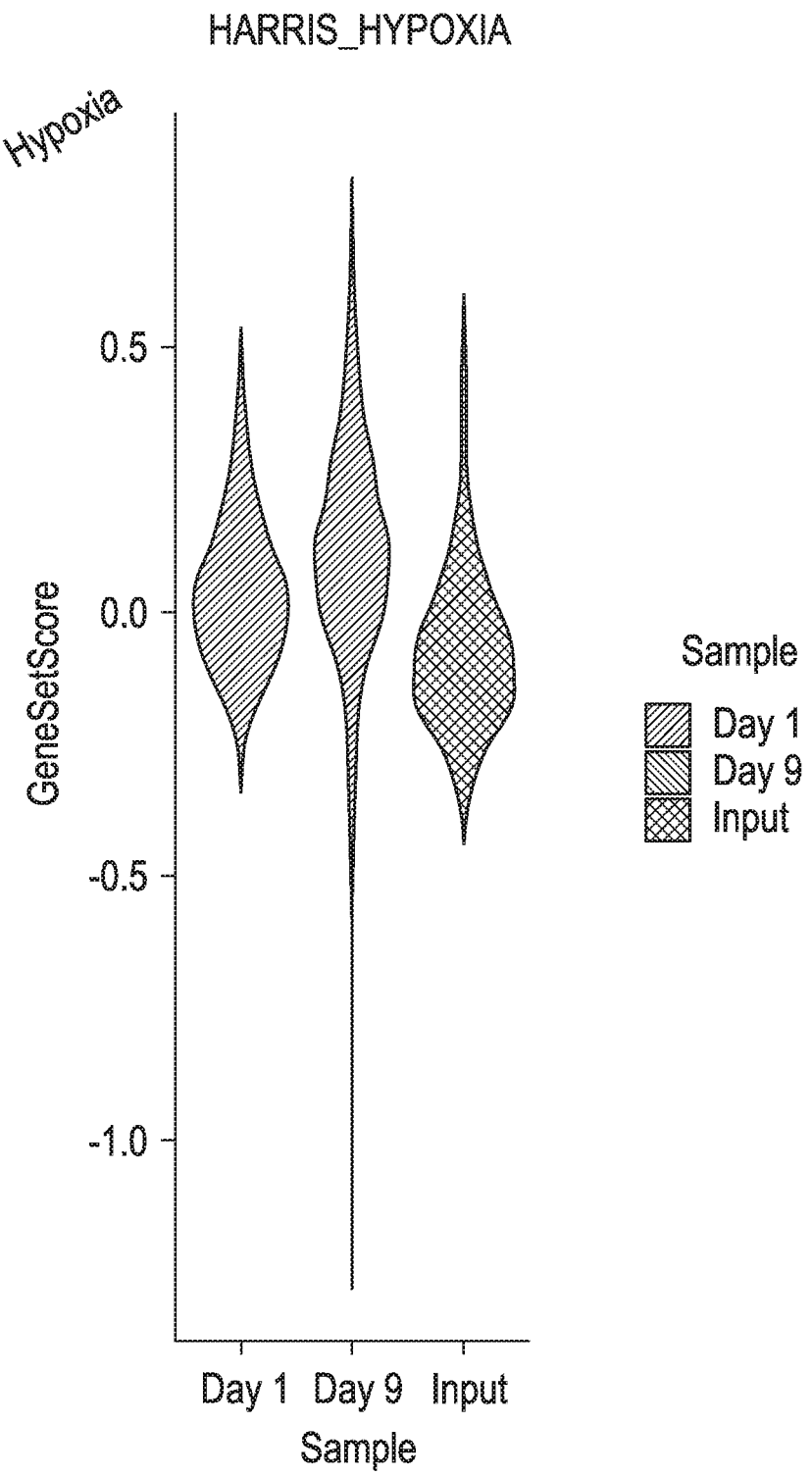

Medians:
D1: -0.082
D9: 0.087
Input: -0.071

FIG. 39B



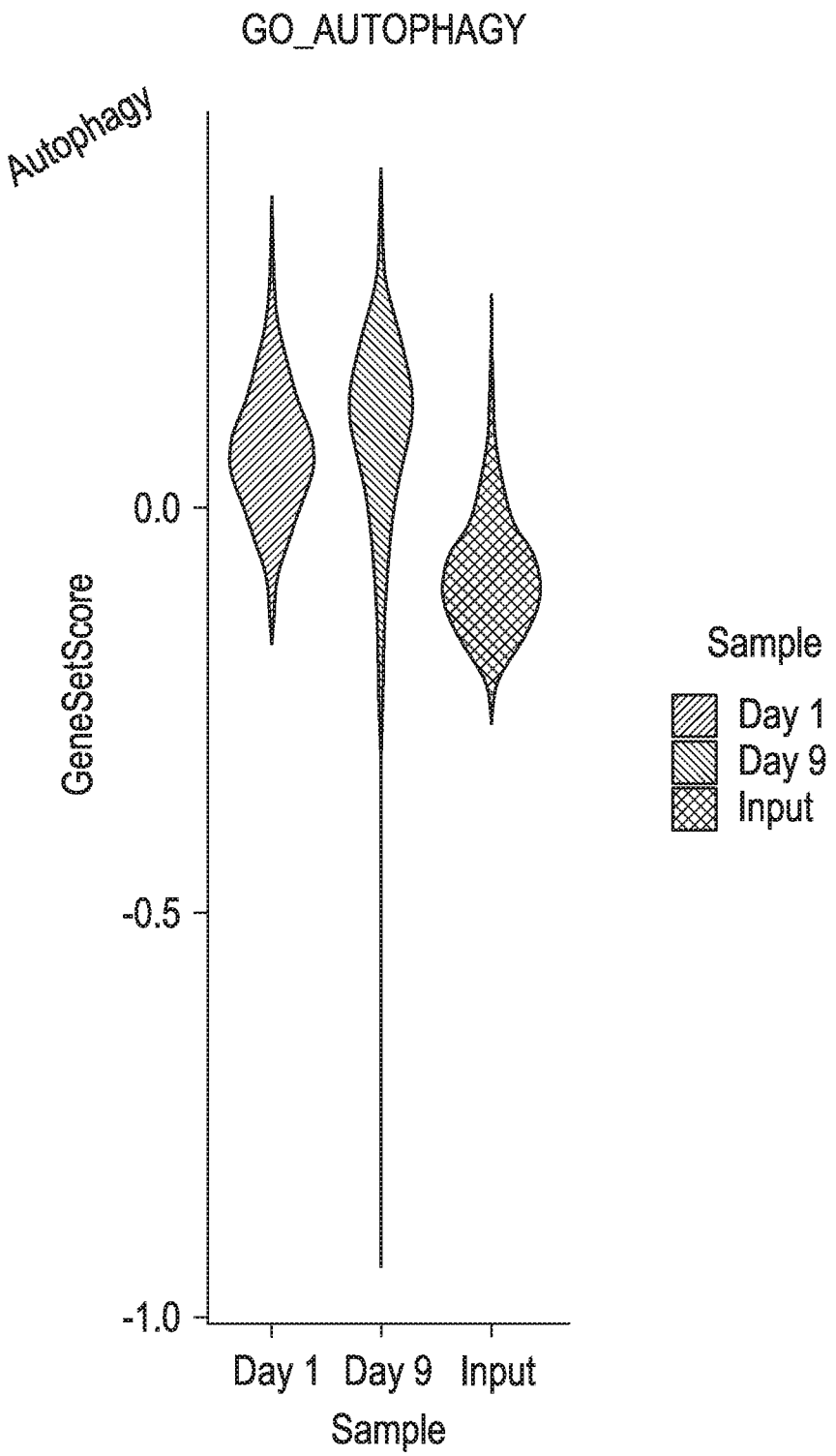

Medians:
D1: -0.062
D9: 0.14
Input: -0.081

FIG. 39C




Medians:
D1: 0.019
D9: 0.11
Input: -0.096

FIG. 39D




Medians:
D1: 0.066
D9: 0.11
Input: -0.09

FIG. 39E

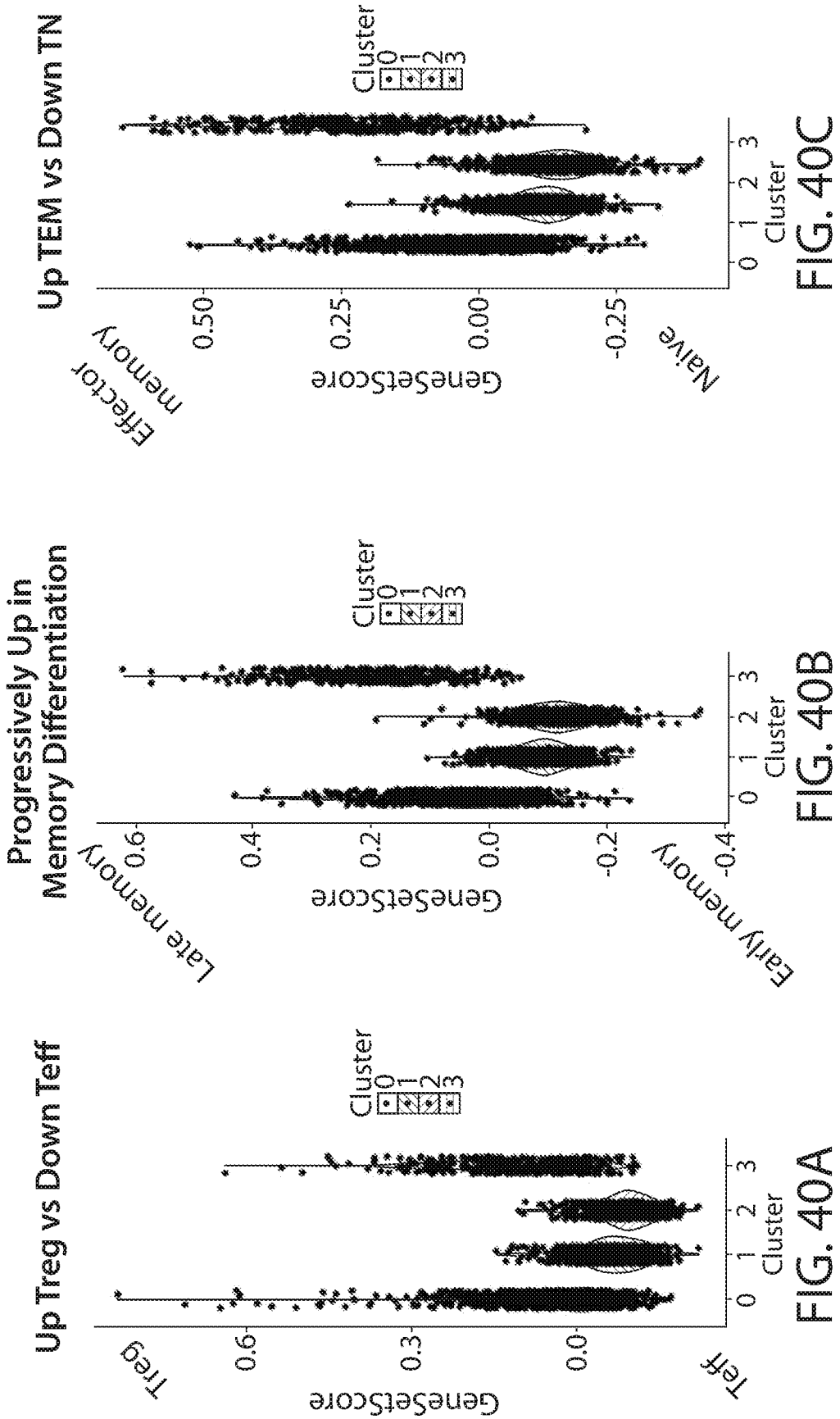



FIG. 40A




FIG. 40B




FIG. 40C

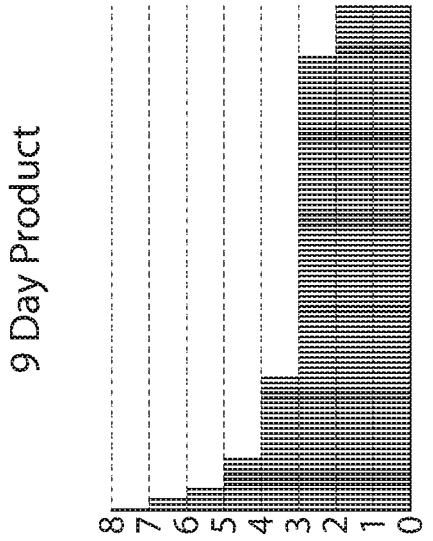



FIG. 41C

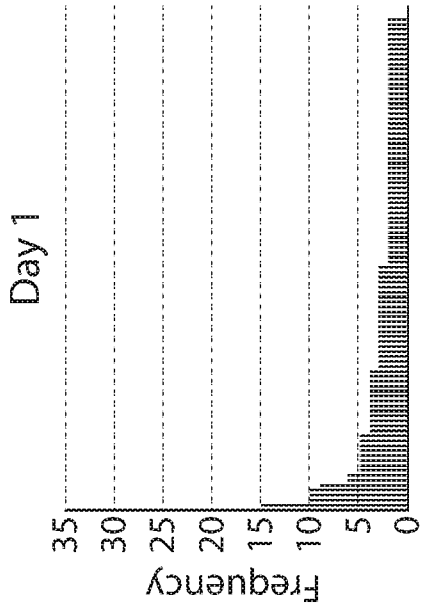



FIG. 41B

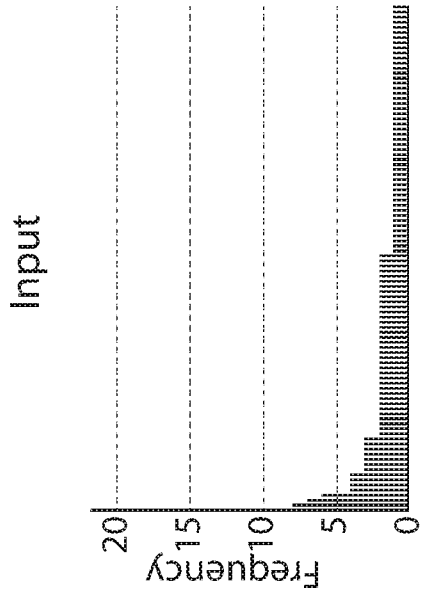
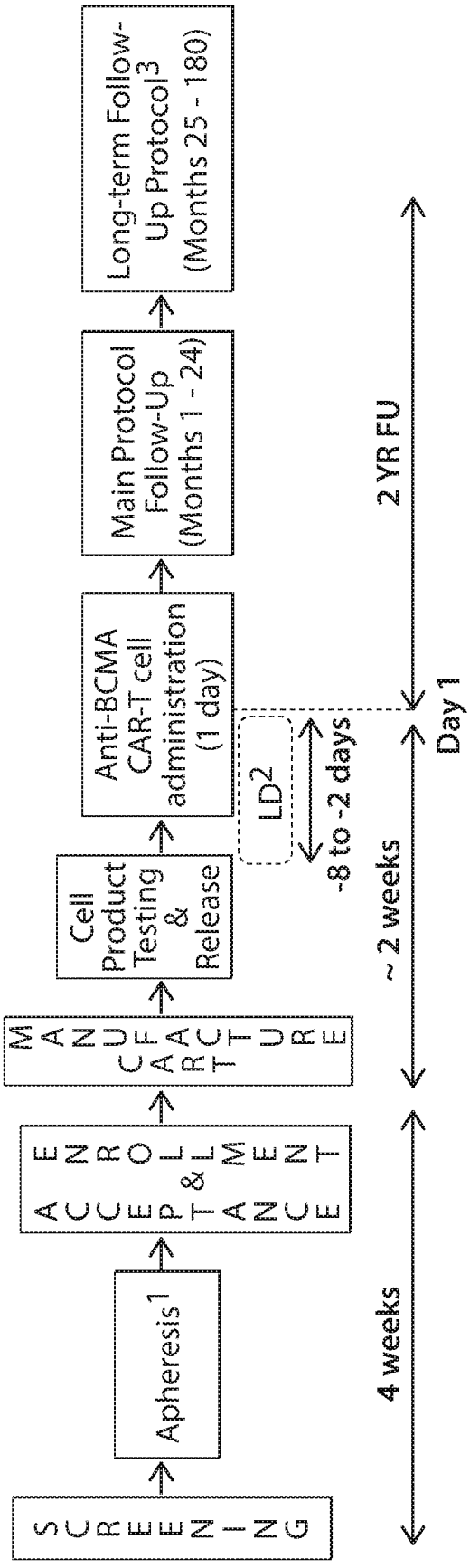



FIG. 41A




1 Historical apheresis collection may be used

2 Lymphodepletion (LD) of Fludarabine/Cyclophosphamide, delivered over 3 continuous days within the Day -8 to Day -2 period prior to anti- BCMA CAR-T cell administration

3 Long term follow-up protocol conducted under a separate protocol per Health Authority guidance.

FIG. 42

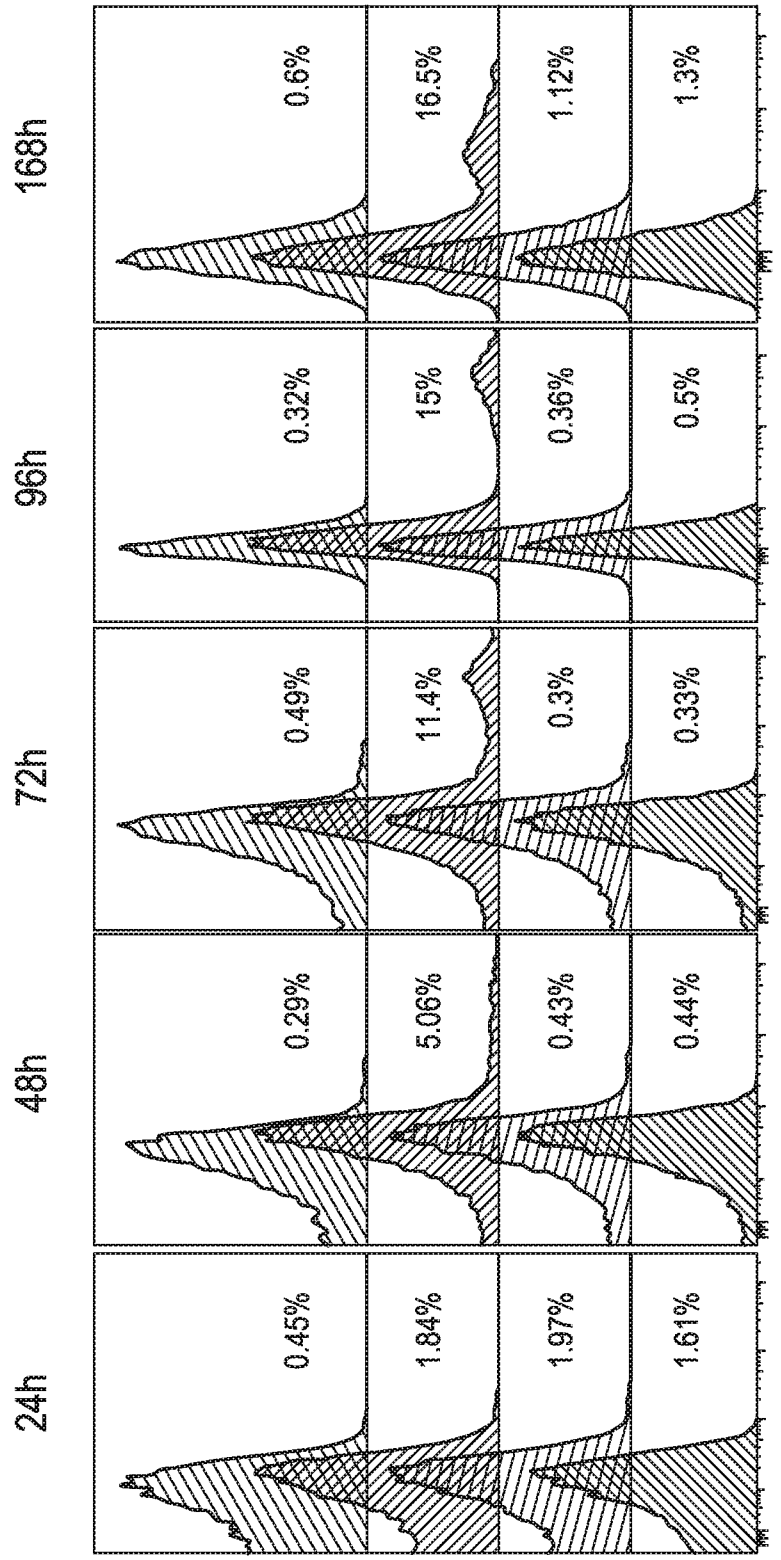

Post viral addition
24h
48h
72h
96h
168h
Samples
UTD
ARM-BCMA CAR
ARM-BCMA CAR+ 30µM AZT
ARM-BCMA CAR+ 100µM AZT
0.45%
1.84%
1.97%
1.61%
0.29%
5.06%
0.43%
0.44%
0.49%
11.4%
0.3%
0.33%
0.32%
15%
0.36%
0.5%
0.6%
16.5%
1.12%
1.3%
BCMA-Fc-Alexa647

FIG. 43

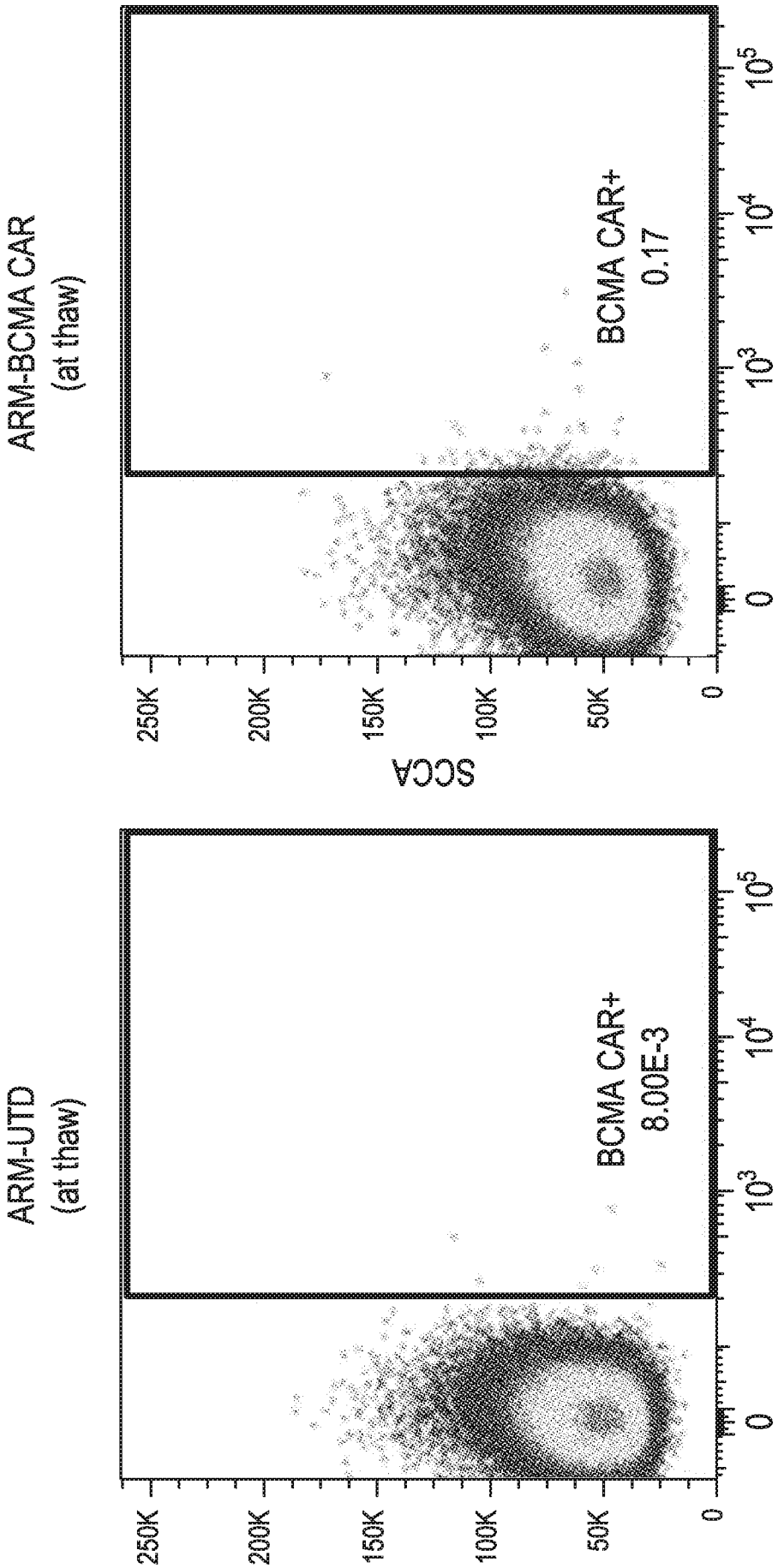

ARM-UTD
(at thaw)
BCMA CAR+
8.00E-3
ARM-BCMA CAR
(at thaw)
BCMA CAR+
0.17
SSCA
250K
200K
150K
100K
50K
0
$10^3$
$10^4$
$10^5$

FIG. 44A

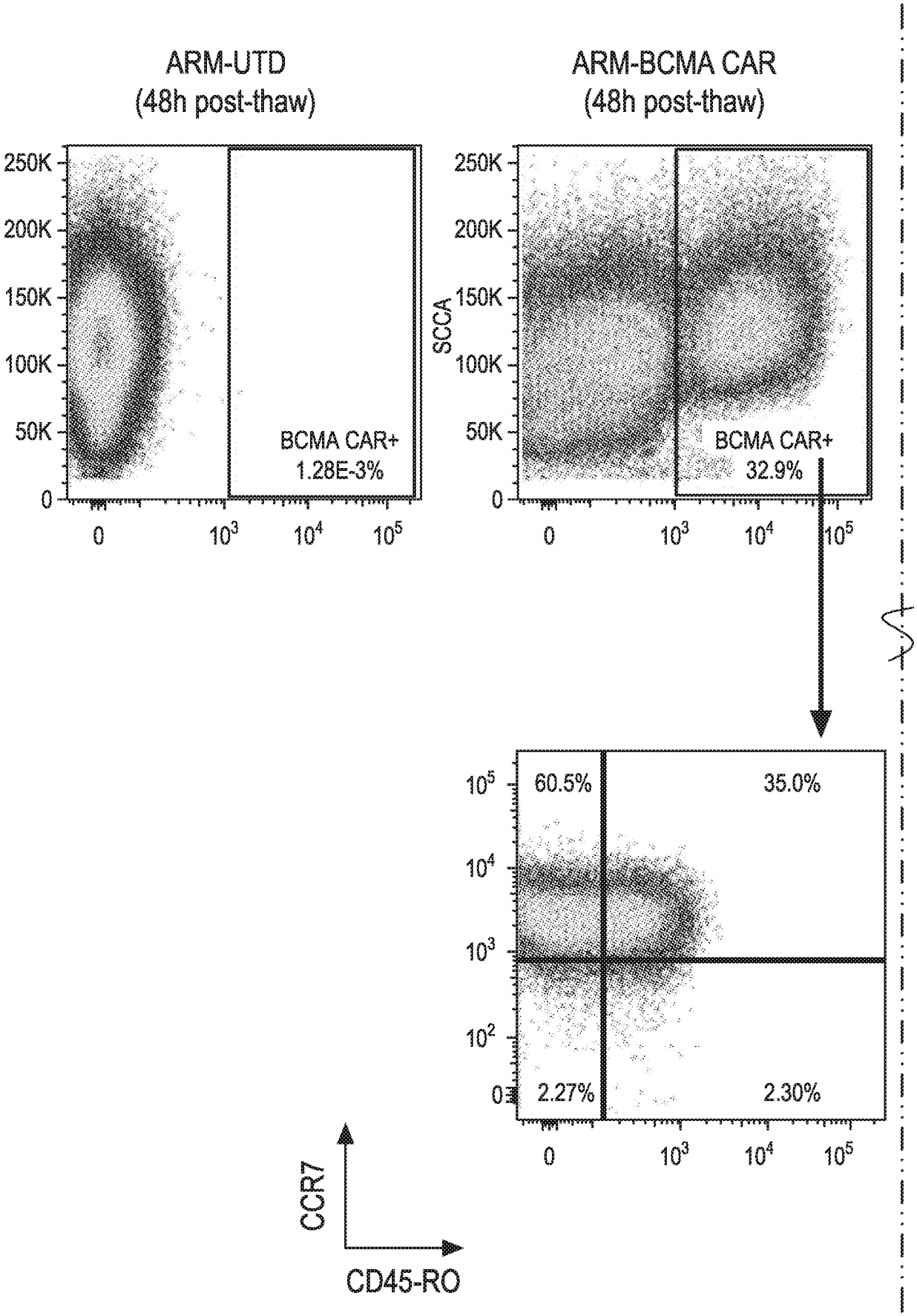



FIG. 44B (part 1)

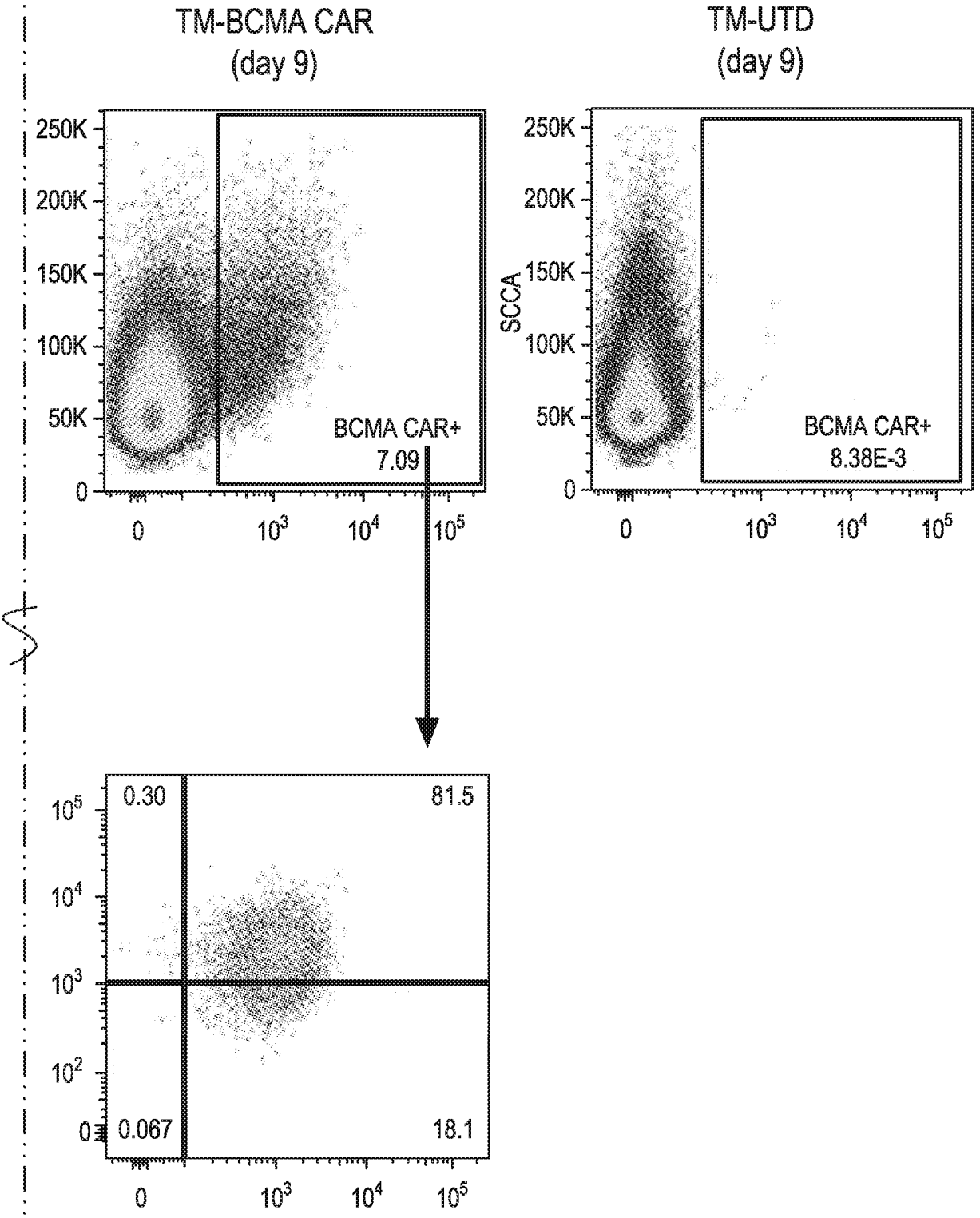



FIG. 44B (part 2)

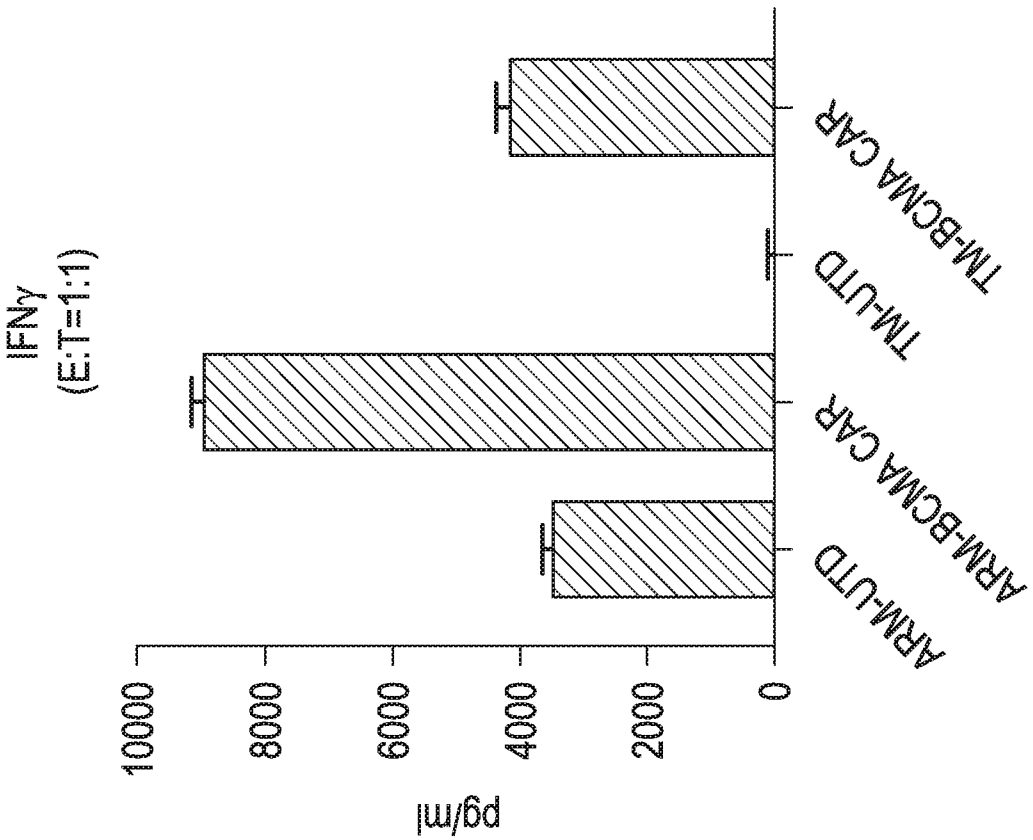



FIG. 45B

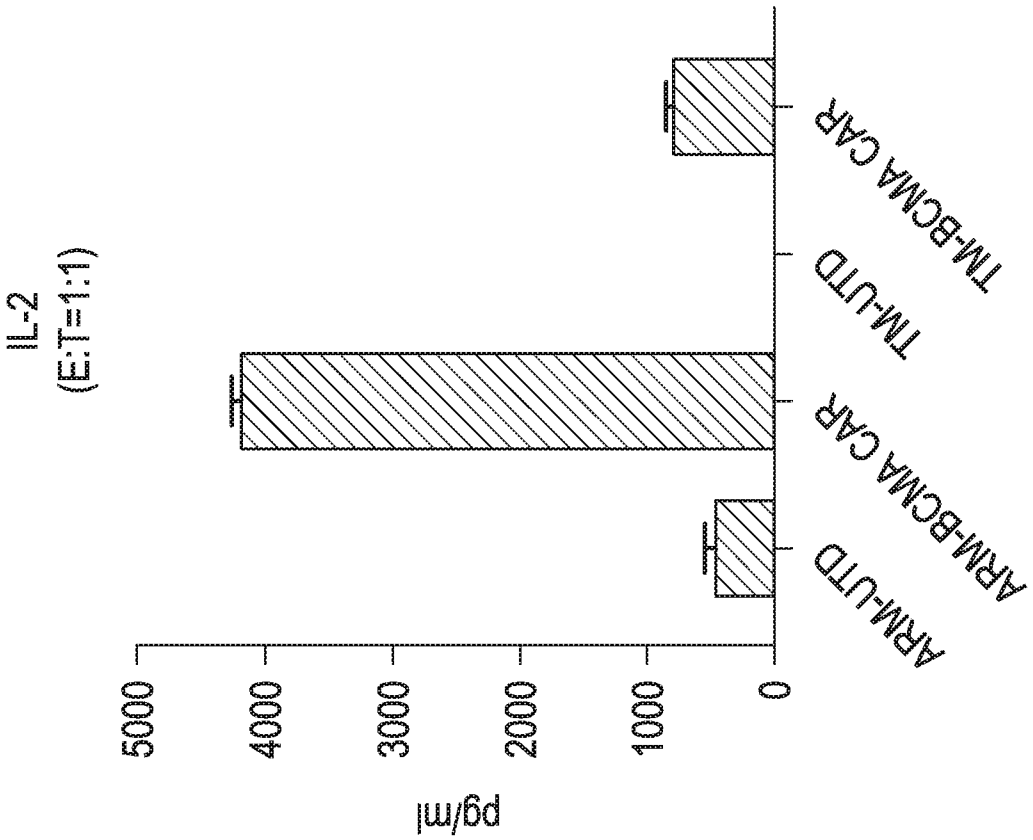
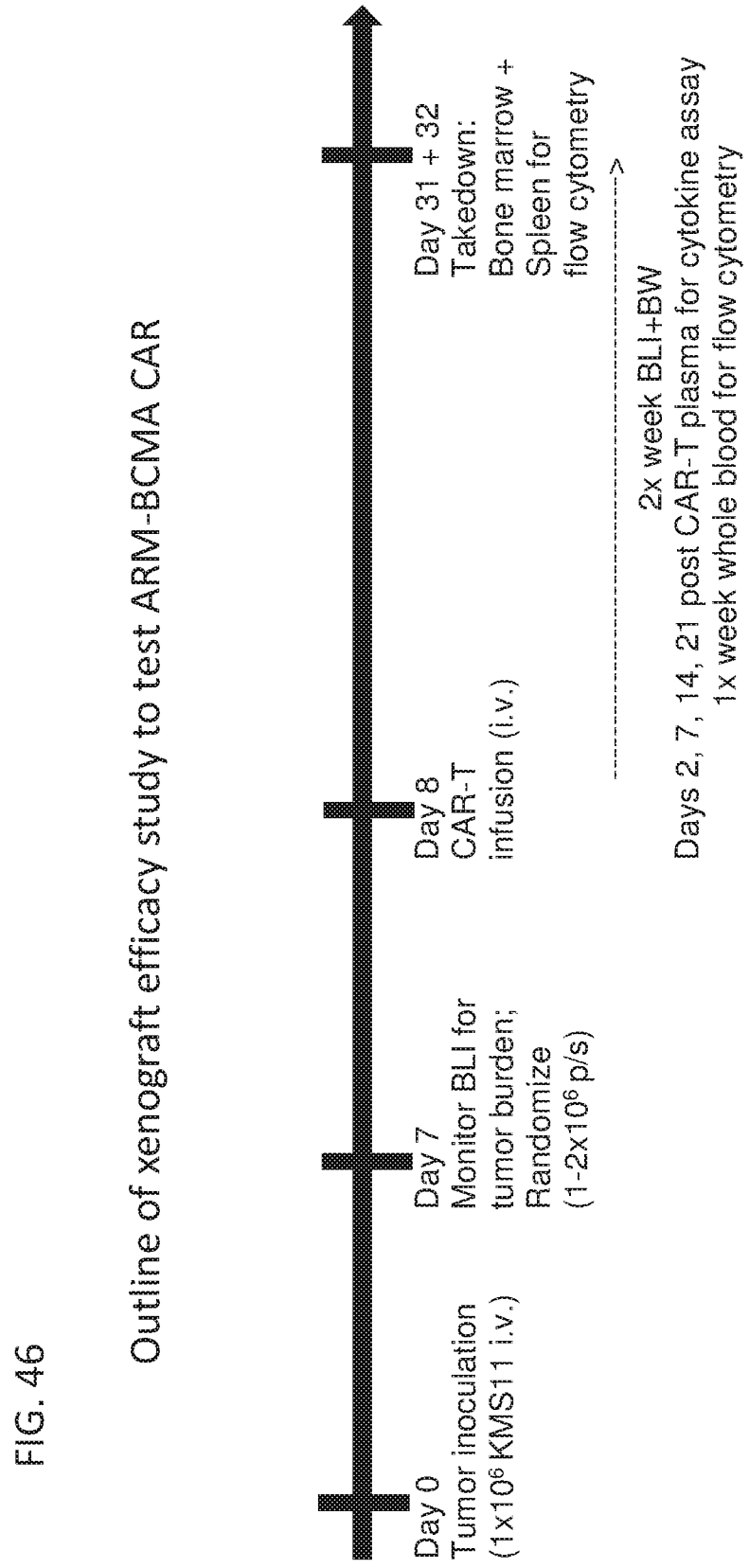



FIG. 45A

FIG. 46


Outline of xenograft efficacy study to test ARM-BCMA CAR
Day 0
Tumor inoculation
(1x10^6 KMS11 i.v.)
Day 7
Monitor BLI for
tumor burden;
Randomize
(1-2x10^6 p/s)
Day 8
CAR-T
infusion (i.v.)
Day 31 + 32
Takedown:
Bone marrow +
Spleen for
flow cytometry
2x week BLI+BW
Days 2, 7, 14, 21 post CAR-T plasma for cytokine assay
1x week whole blood for flow cytometry

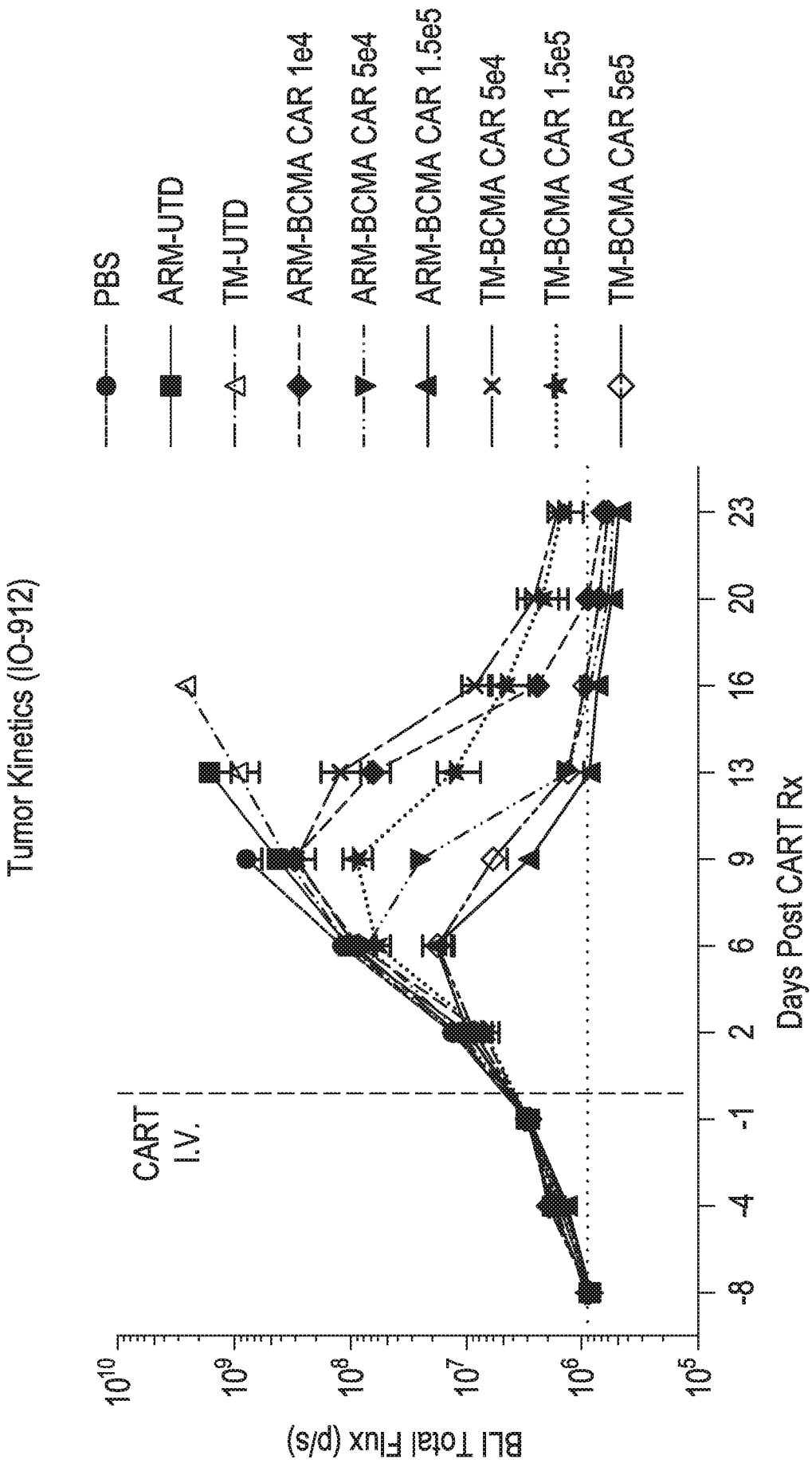

Tumor Kinetics (IO-912)
PBS
ARM-UTD
TM-UTD
ARM-BCMA CAR 1e4
ARM-BCMA CAR 5e4
ARM-BCMA CAR 1.5e5
TM-BCMA CAR 5e4
TM-BCMA CAR 1.5e5
TM-BCMA CAR 5e5
CART I.V.
BLI Total Flux (p/s)
$10^{10}$
$10^{9}$
$10^{8}$
$10^{7}$
$10^{6}$
$10^{5}$
-8
-4
-1
2
6
9
13
16
20
23
Days Post CART Rx

FIG. 47

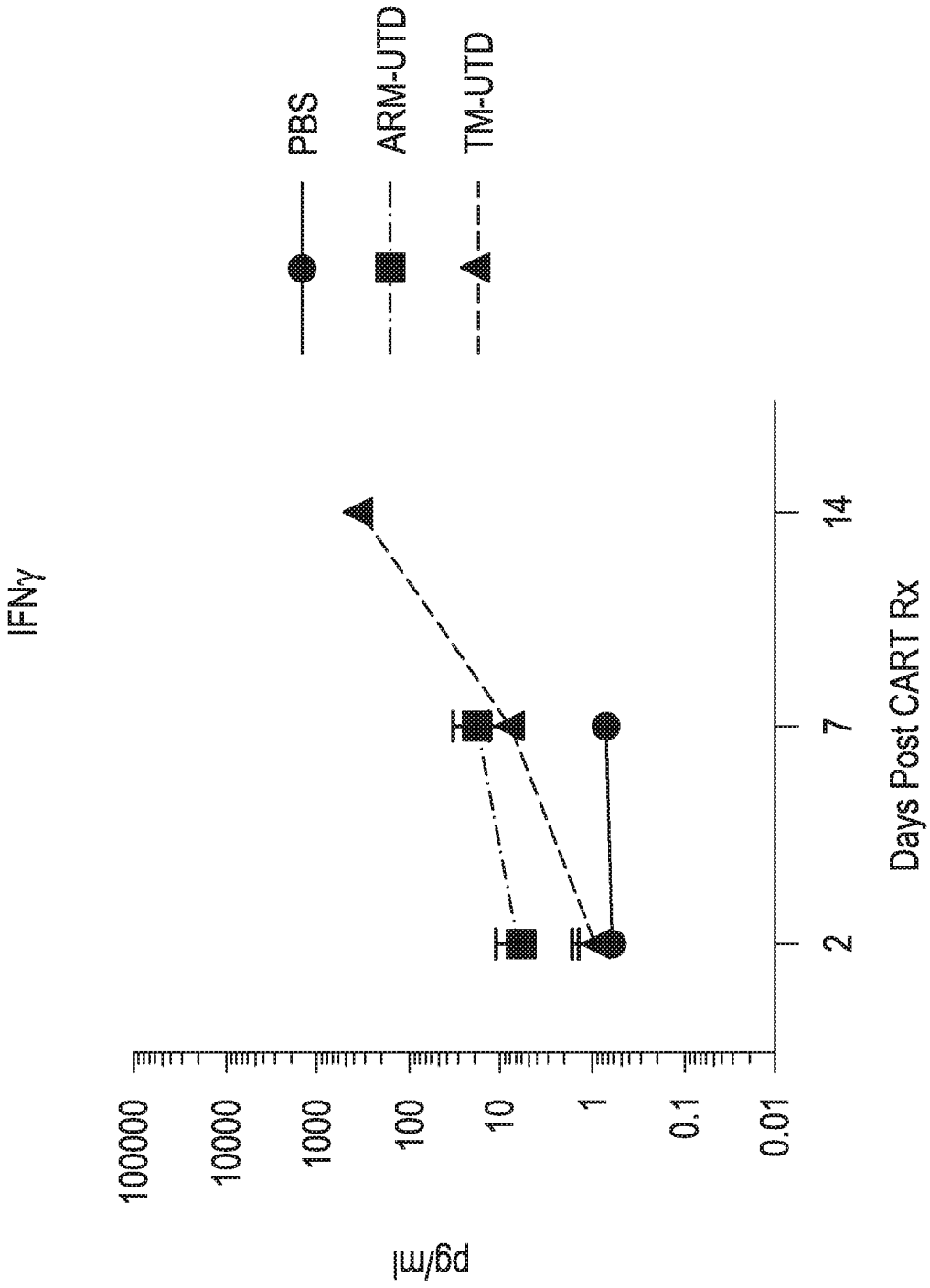

IFNγ
PBS
ARM-UTD
TM-UTD
100000
10000
1000
100
10
1
0.1
0.01
pg/ml
2
7
14
Days Post CART Rx

FIG. 48A

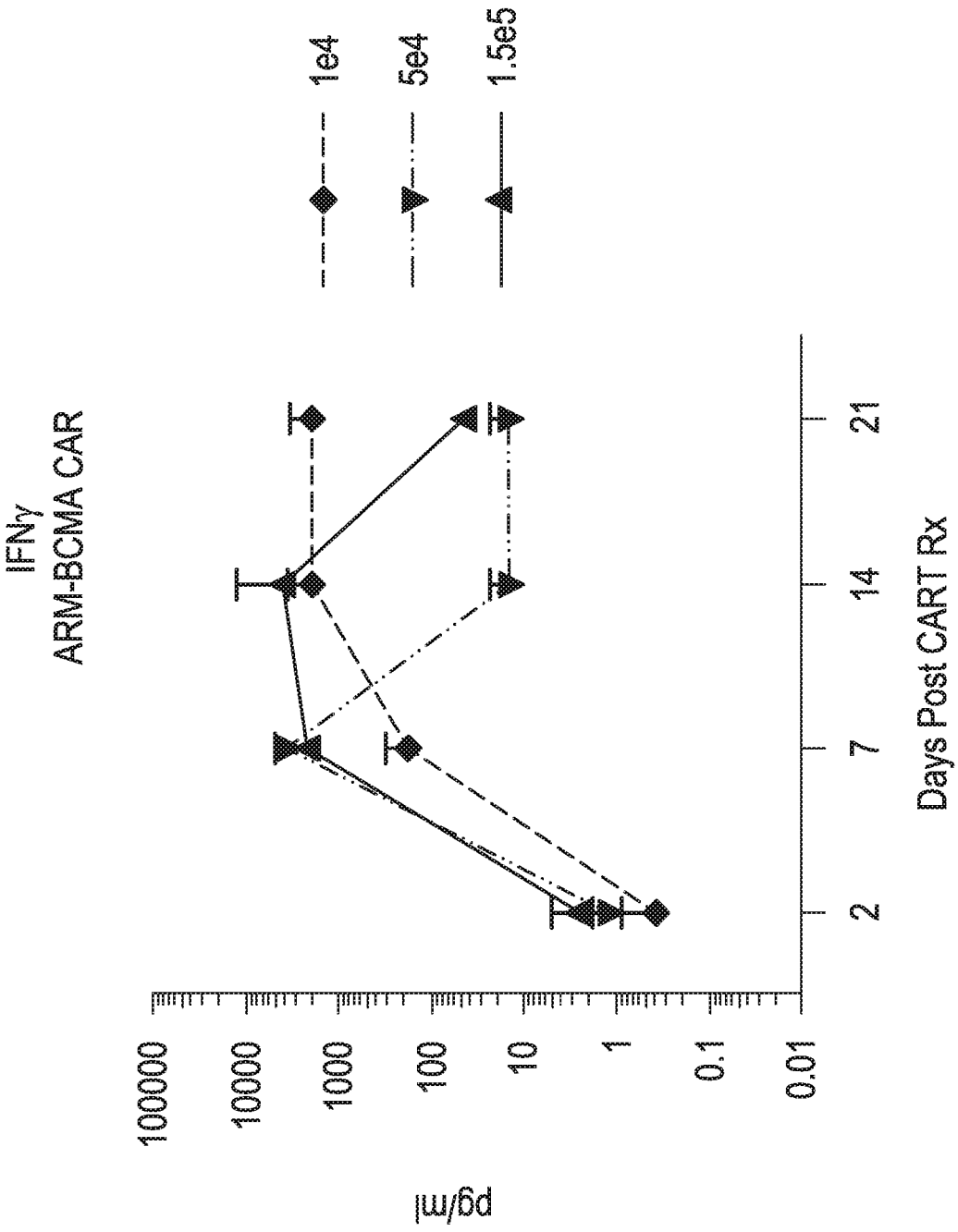

IFNγ
ARM-BCMA CAR
1e4
5e4
1.5e5
pg/ml
100000
10000
1000
100
10
1
0.1
0.01
2
7
14
21
Days Post CART Rx

FIG. 48B

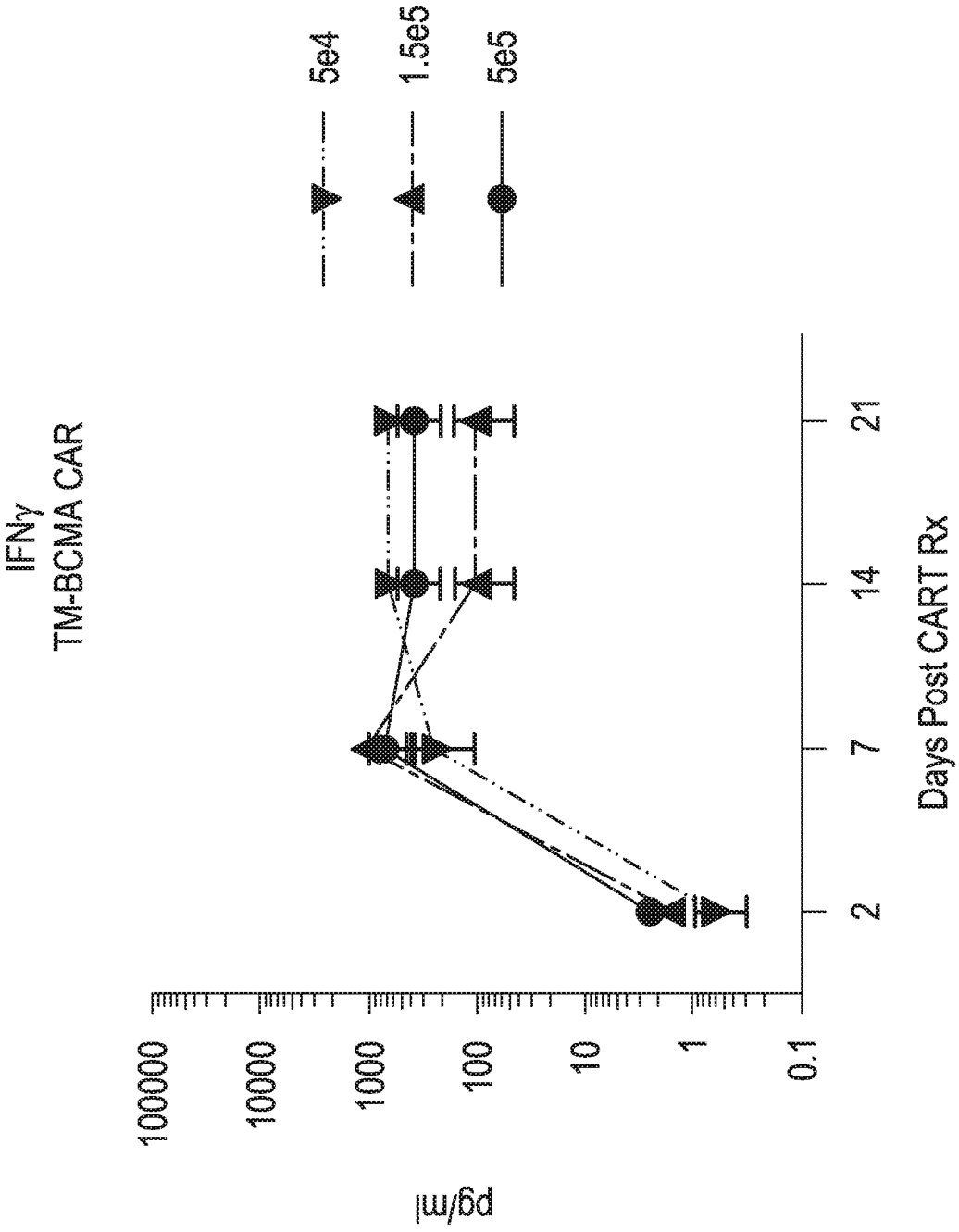

IFNγ
TM-BCMA CAR
5e4
1.5e5
5e5
pg/ml
100000
10000
1000
100
10
1
0.1
2
7
14
21
Days Post CART Rx

FIG. 48C

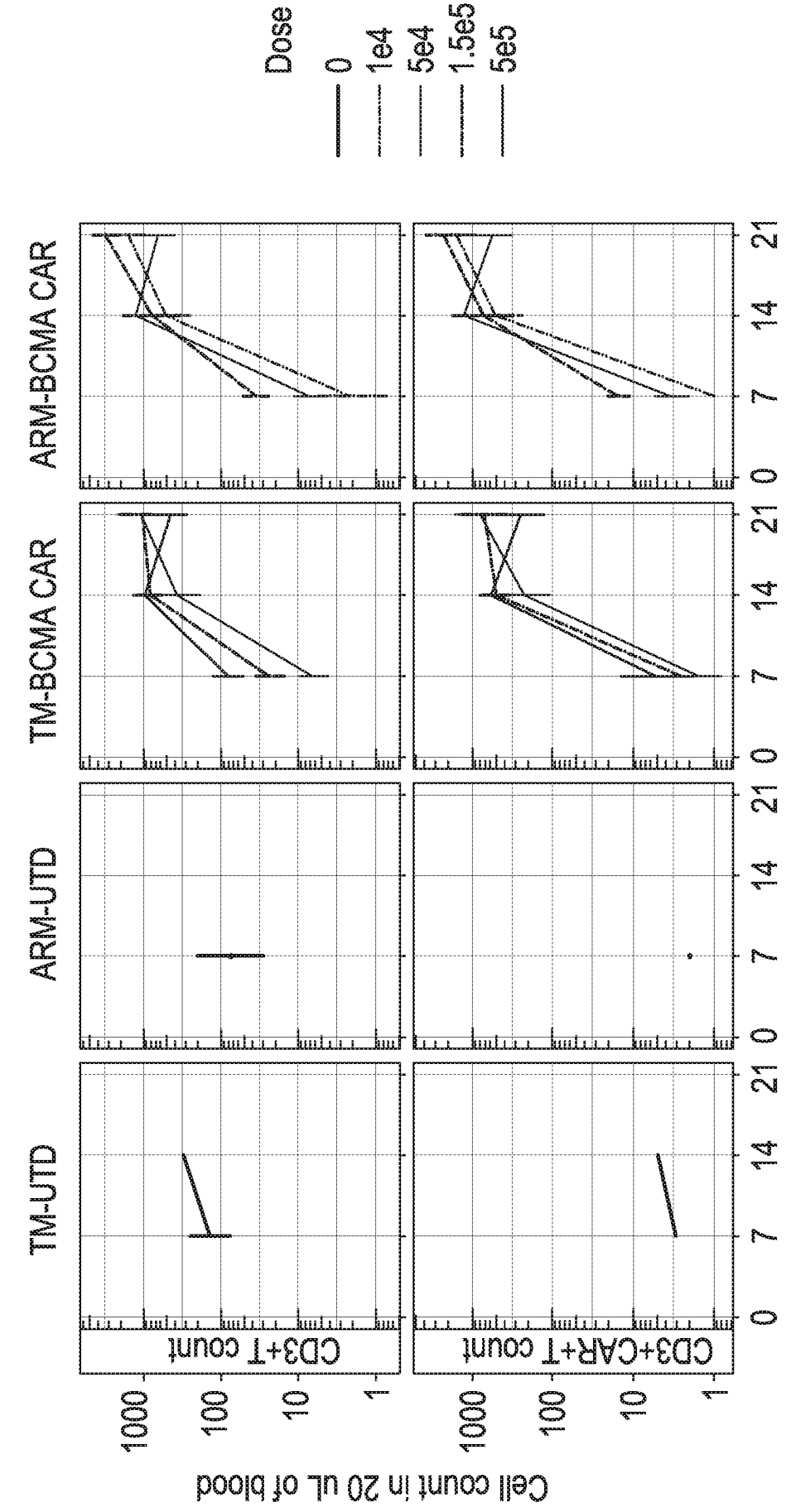

Dose
0
1e4
5e4
1.5e5
5e5
TM-UTD
ARM-UTD
TM-BCMA CAR
ARM-BCMA CAR
CD3+T count
CD3+CAR+T count
Cell count in 20 uL of blood
1000
100
10
1
0
7
14
21
Days post CART dose

FIG. 49

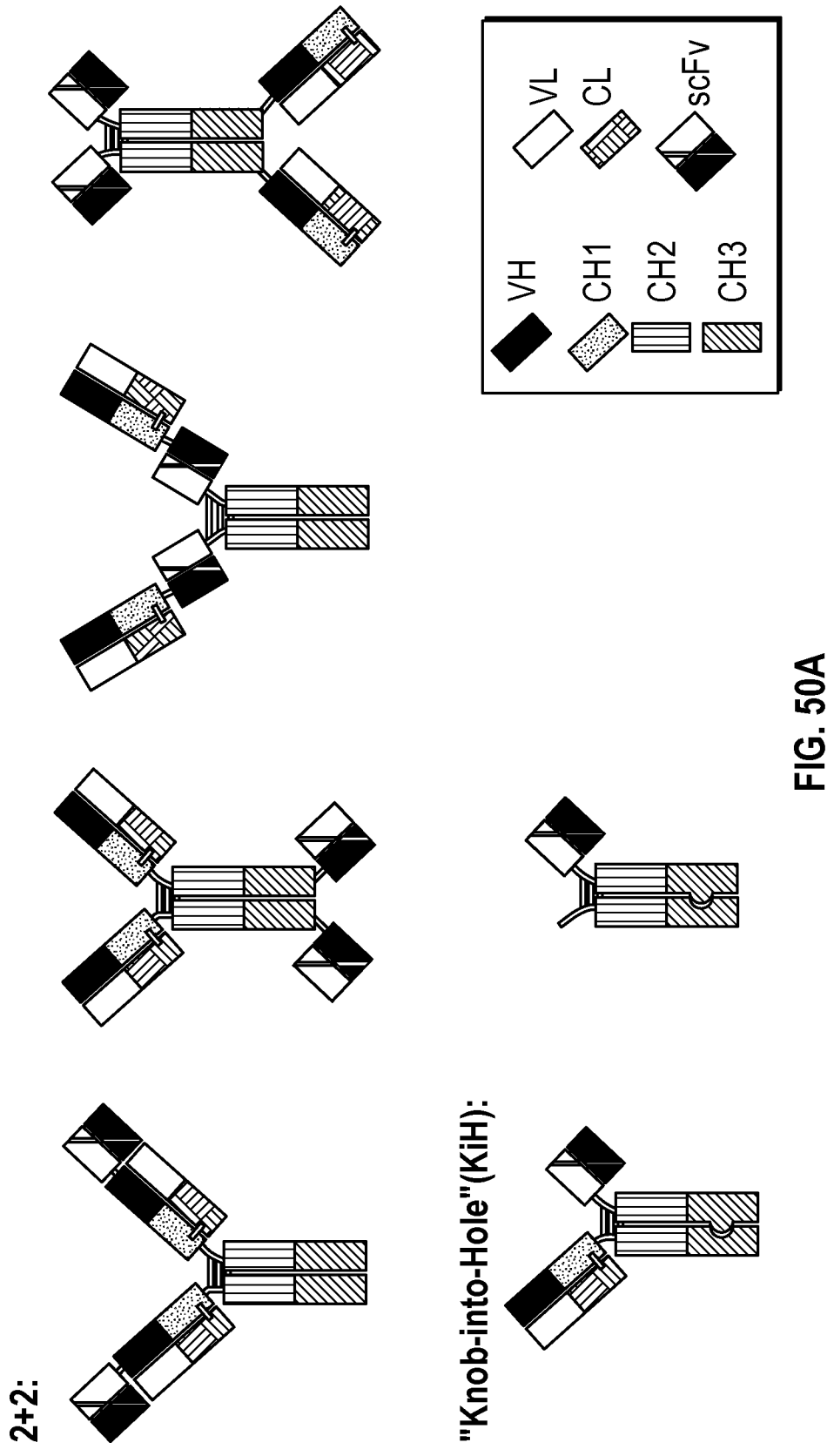



FIG. 50A

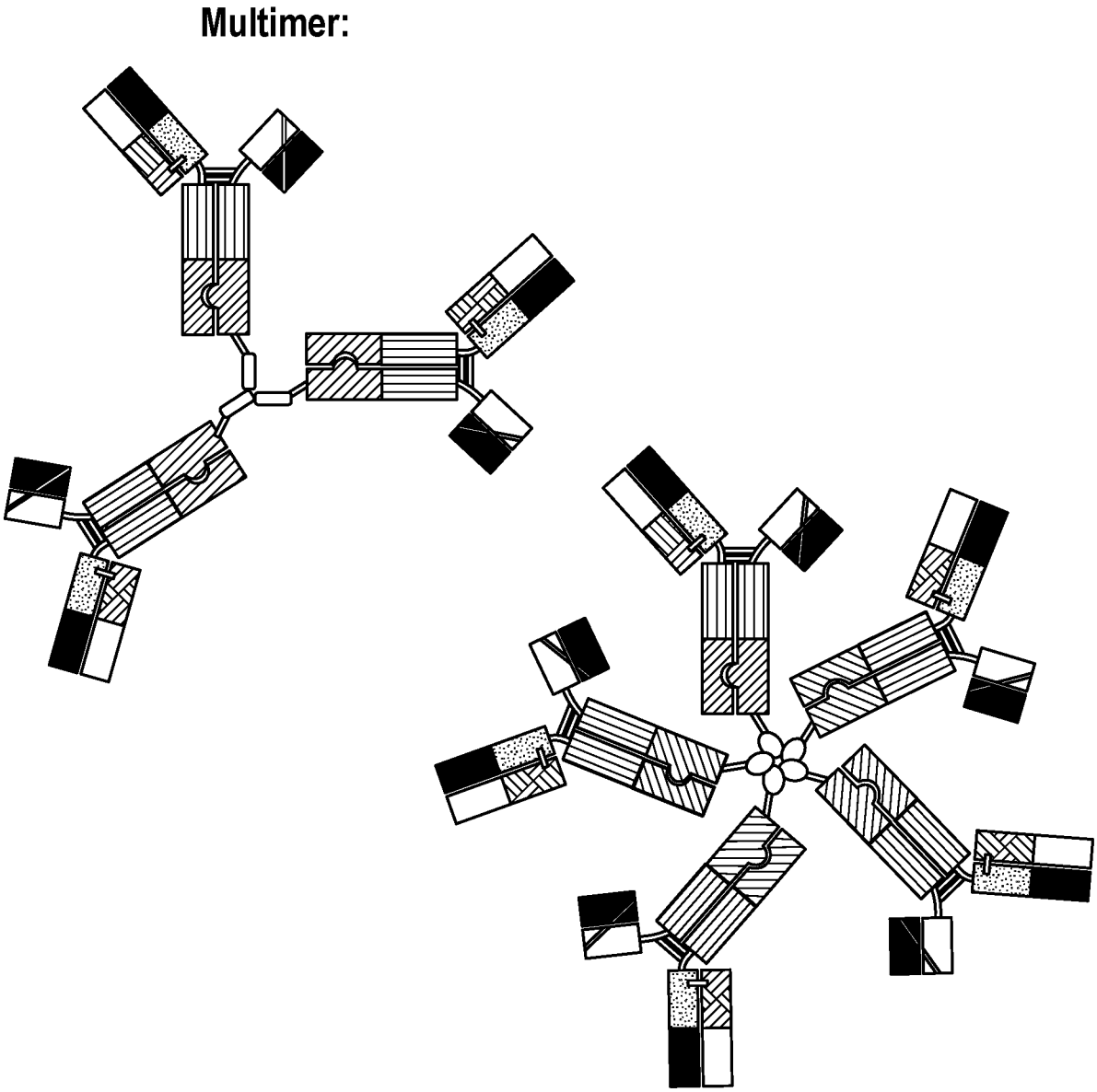



FIG. 50B

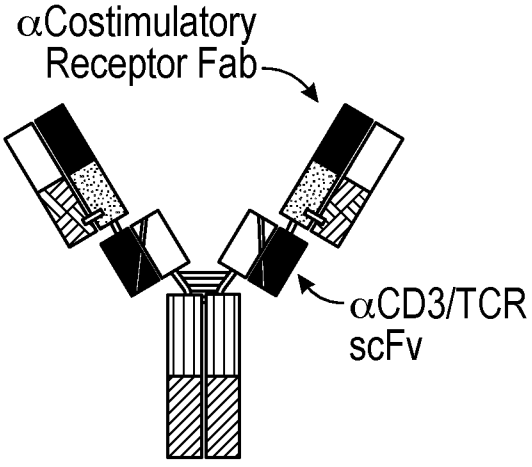



FIG. 50C

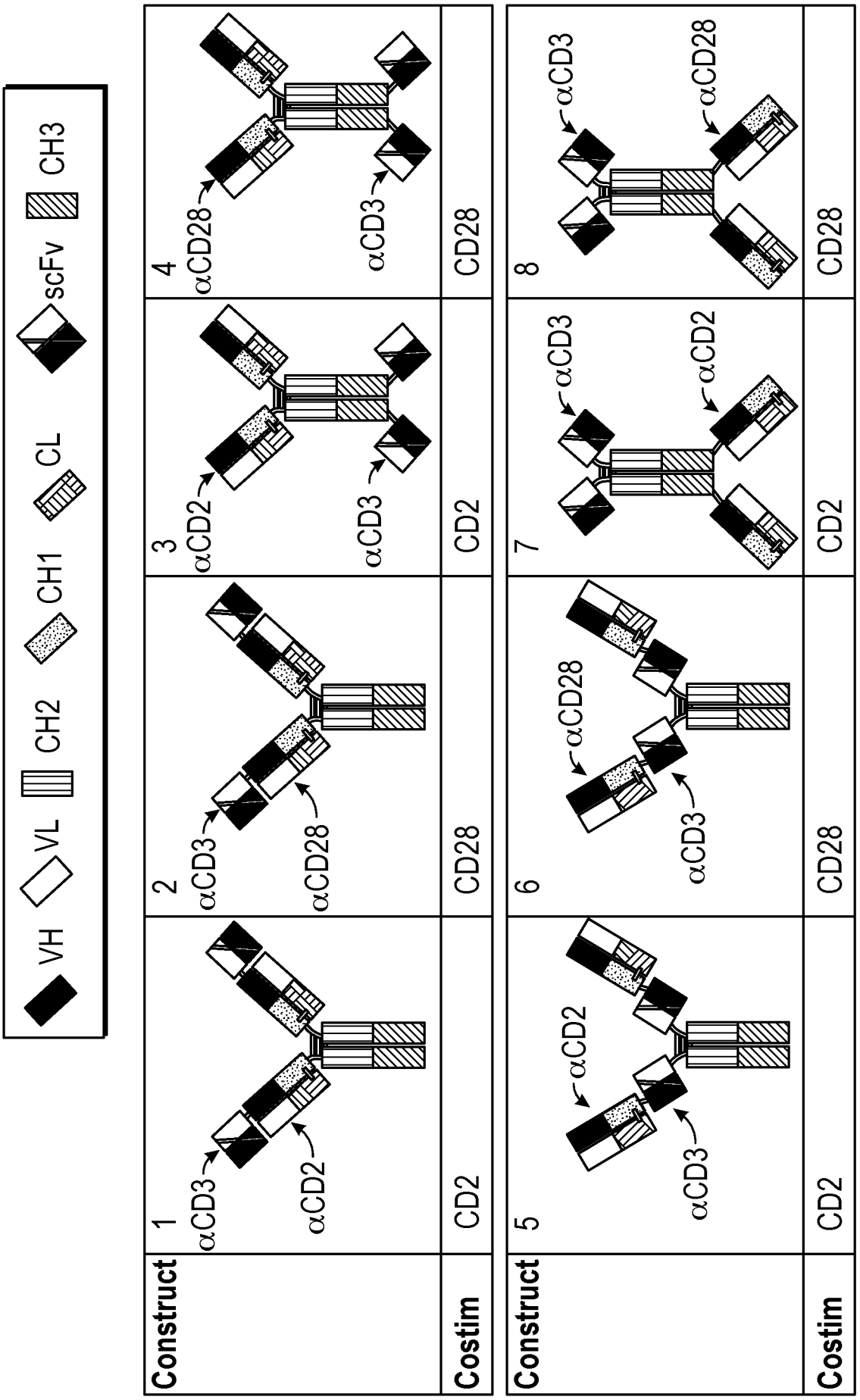

VH
VL
CH2
CH1
CL
scFv
CH3
Construct
Costim
1
αCD3
αCD2
CD2
2
αCD3
αCD28
CD28
3
αCD2
αCD3
CD2
4
αCD28
αCD3
CD28
5
αCD2
αCD3
CD2
6
αCD28
αCD3
CD28
7
αCD3
αCD2
CD2
8
αCD3
αCD28
CD28

FIG. 51A

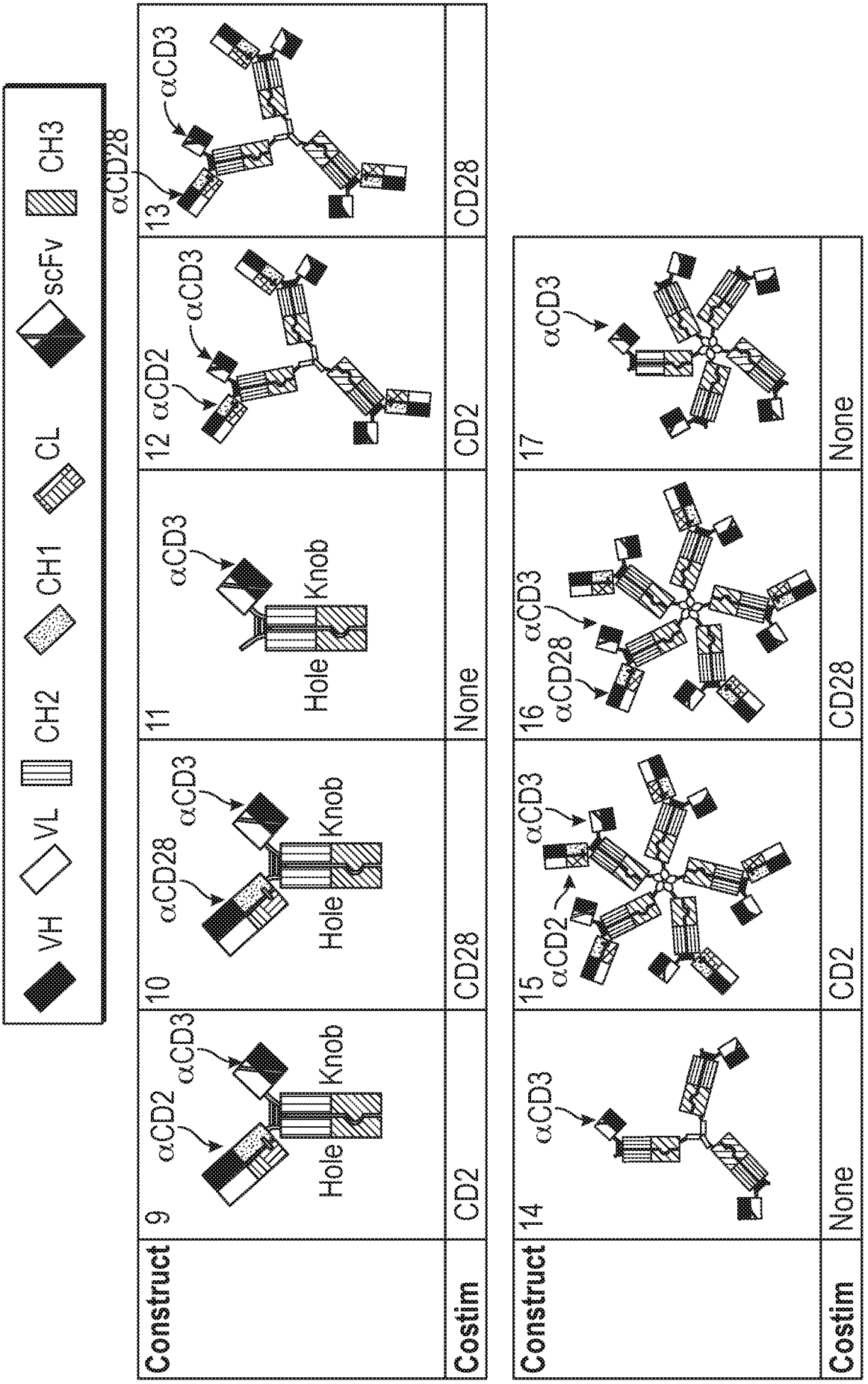

VH
VL
CH2
CH1
CL
scFv
CH3
αCD28
Construct
Costim
9
αCD2
αCD3
Hole
Knob
CD2
10
αCD28
αCD3
Hole
Knob
CD28
11
αCD3
Hole
Knob
None
12
αCD2
αCD3
CD2
13
αCD3
CD28
14
αCD3
None
15
αCD2
αCD3
CD2
16
αCD28
αCD3
CD28
17
αCD3
None

FIG. 51B

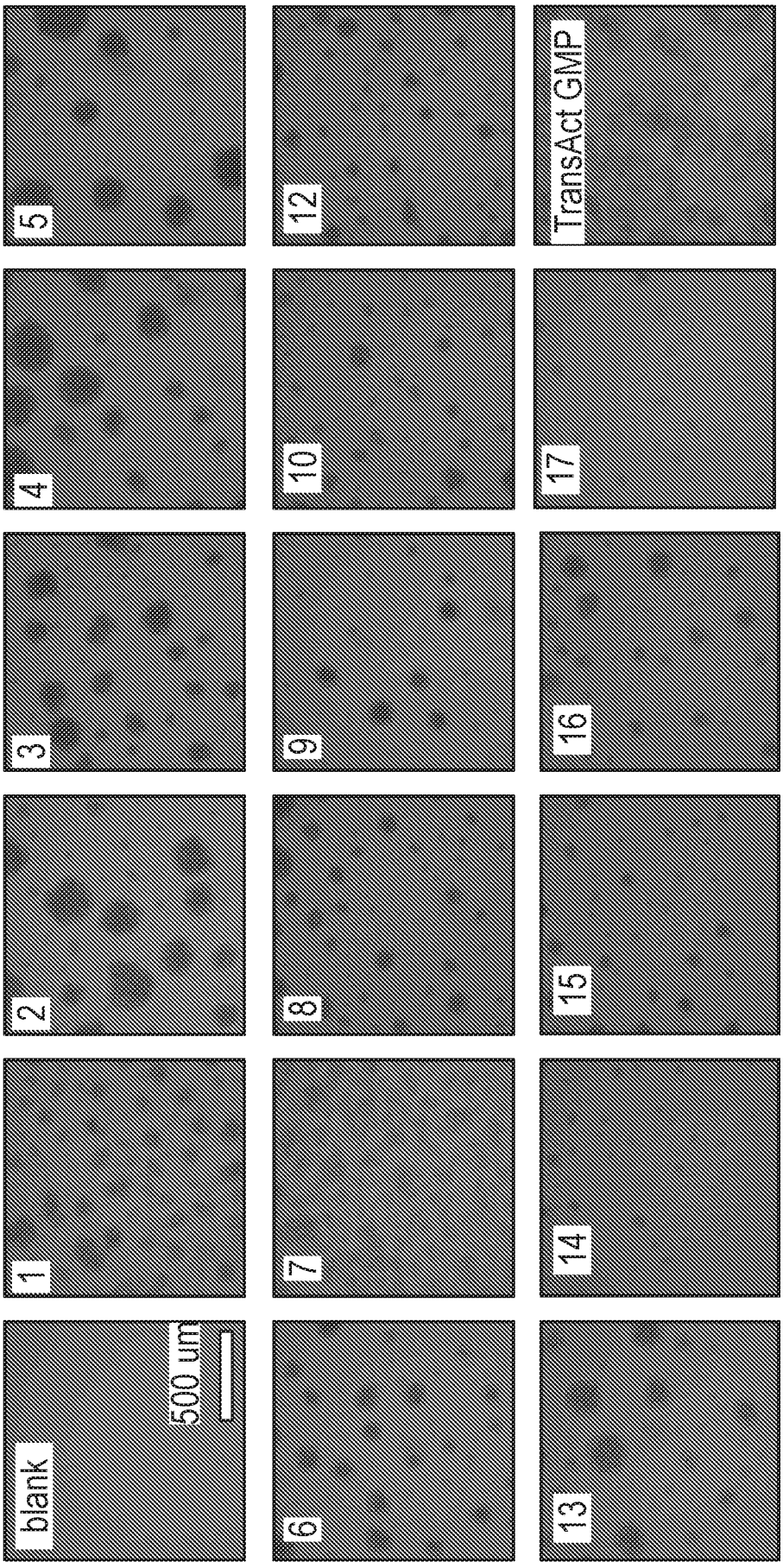



FIG. 52

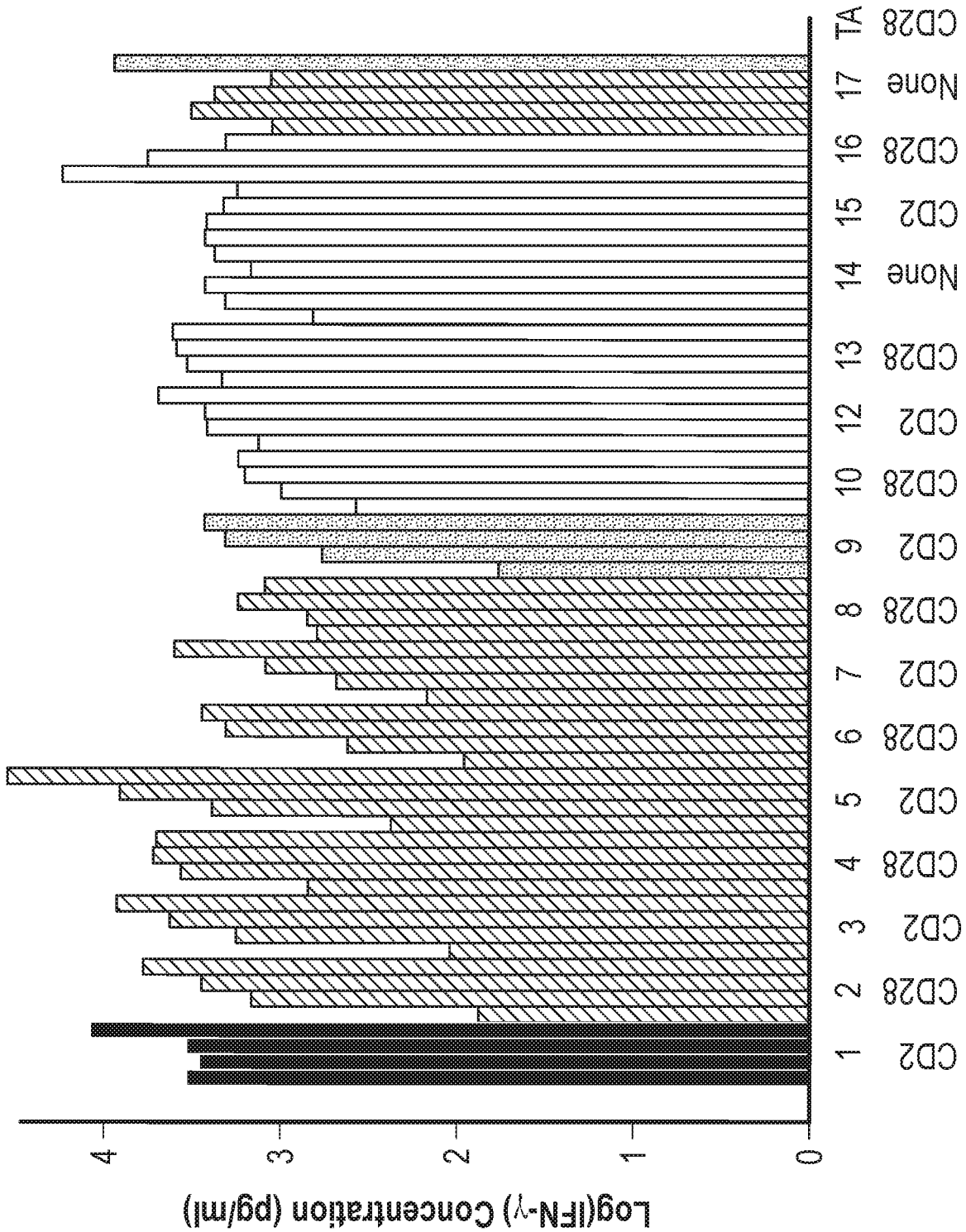

Log(IFN-γ) Concentration (pg/ml)
0
1
2
3
4
CD2
CD28
CD2
CD28
CD2
CD28
CD2
CD28
CD2
CD28
None
CD2
CD28
None
CD2
CD28
TA
1
2
3
4
5
6
7
8
9
10
12
13
14
15
16
17

FIG. 53

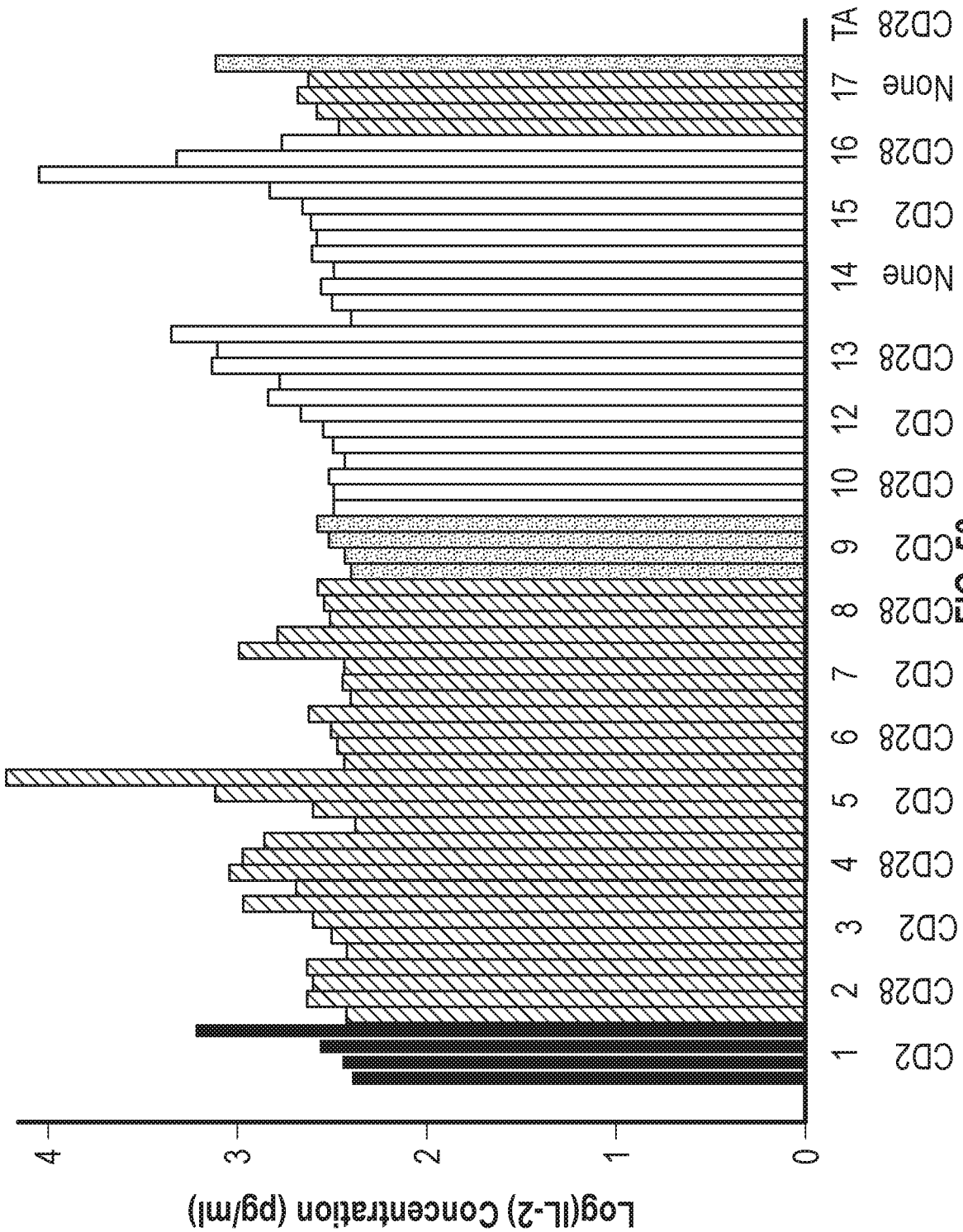



FIG. 53
(Continued)

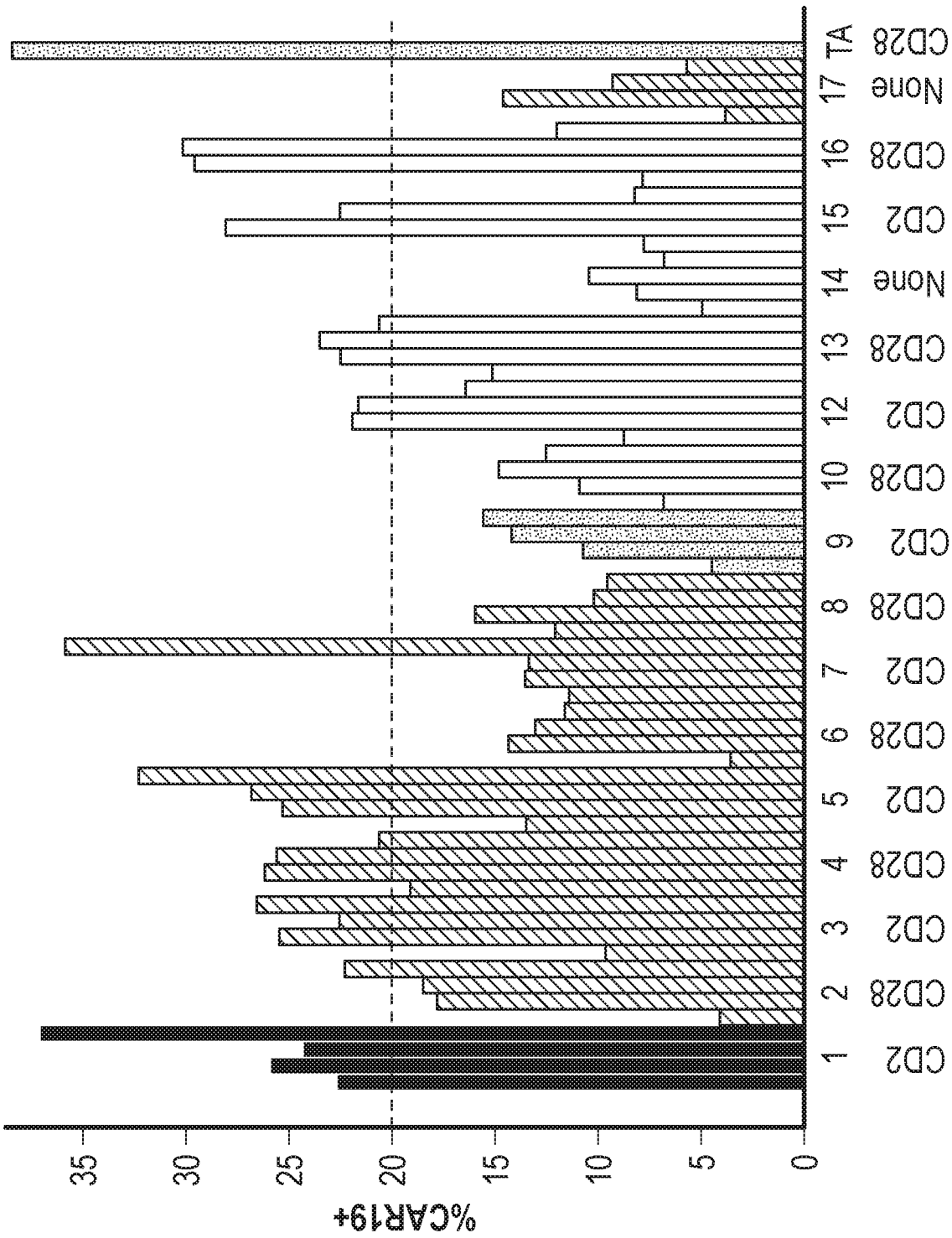

%CAR19+
0
5
10
15
20
25
30
35
1 CD2
2 CD28
3 CD2
4 CD28
5 CD2
6 CD28
7 CD2
8 CD28
9 CD2
10 CD28
12 CD2
13 CD28
14 None
15 CD2
16 CD28
17 None
TA CD28

FIG. 54

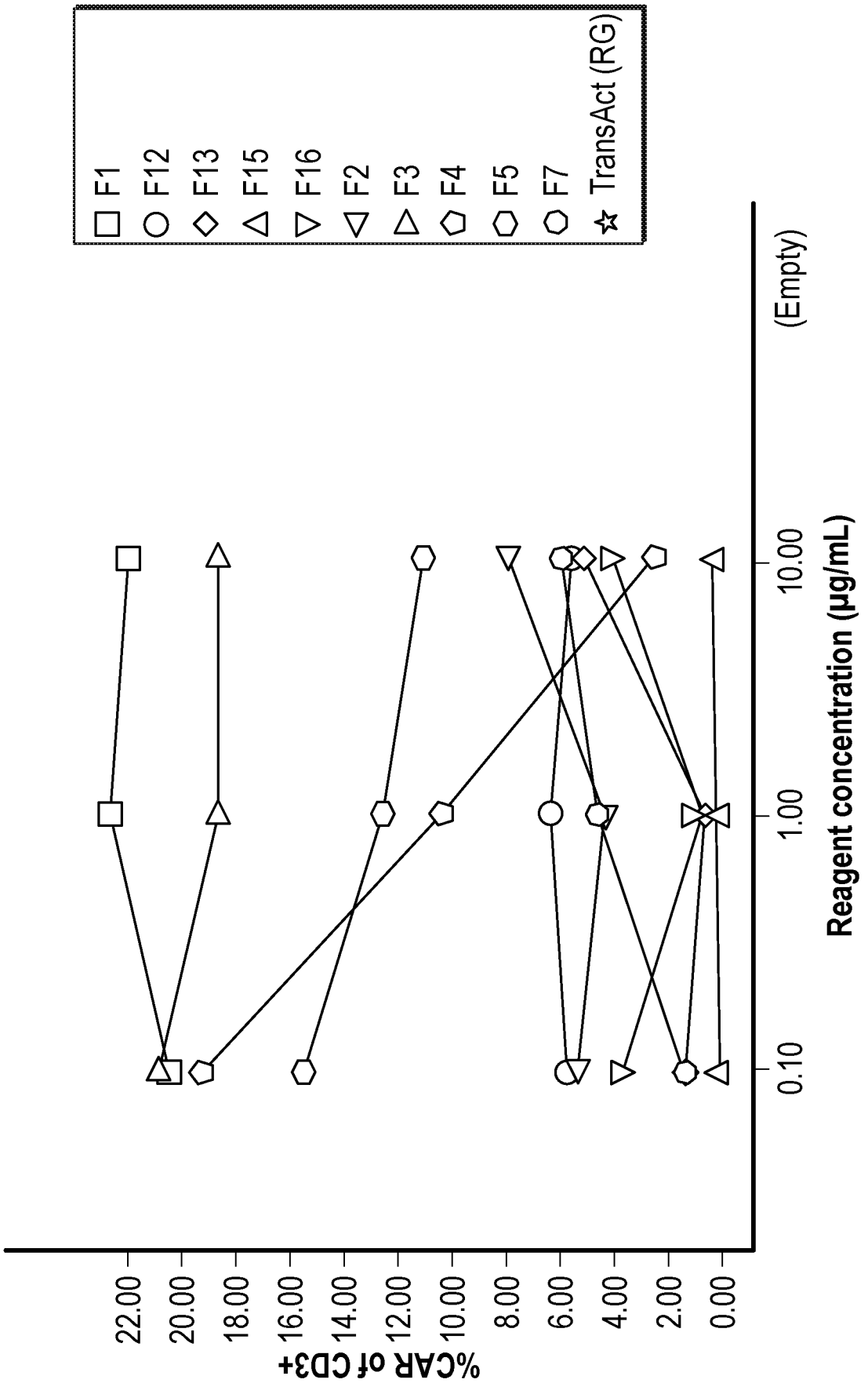

F1
F12
F13
F15
F16
F2
F3
F4
F5
F7
TransAct (RG)
%CAR of CD3+
22.00
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
0.10
1.00
10.00
(Empty)
Reagent concentration (µg/mL)

FIG. 55

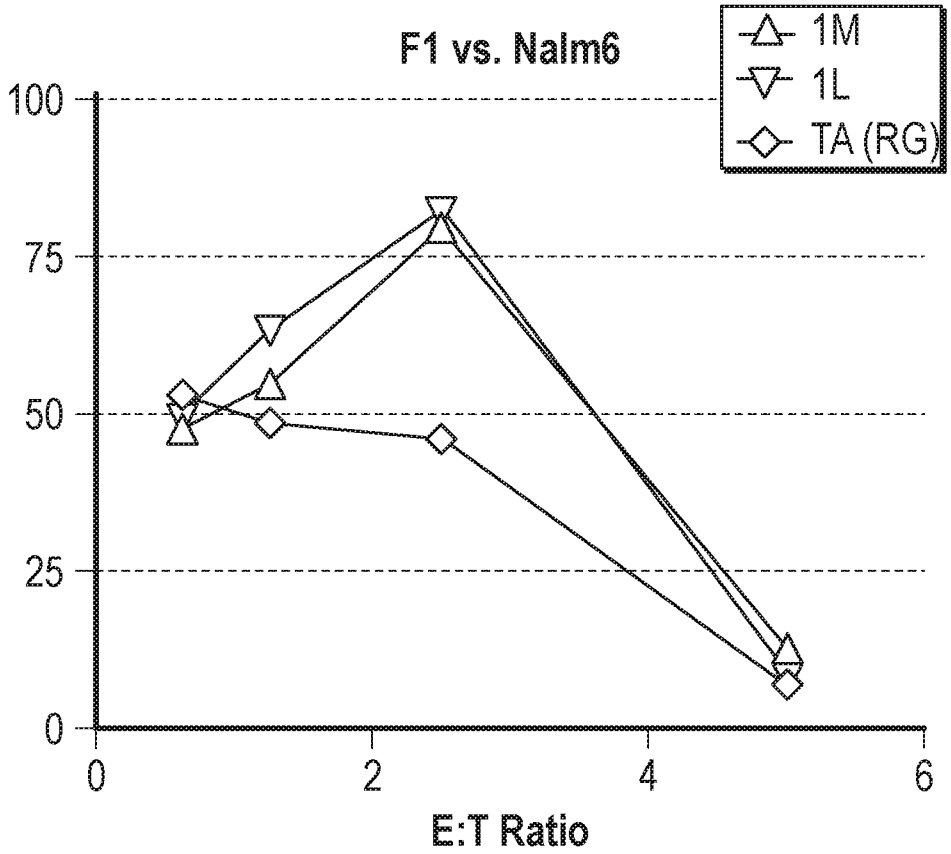



FIG. 56A

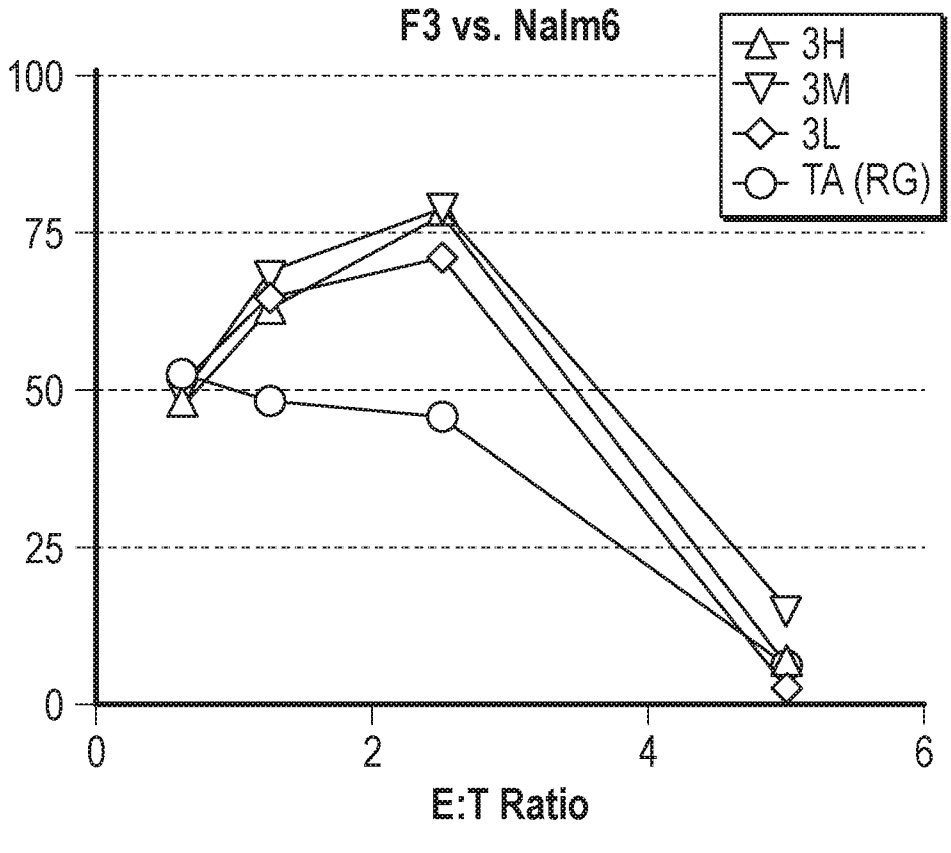



FIG. 56B

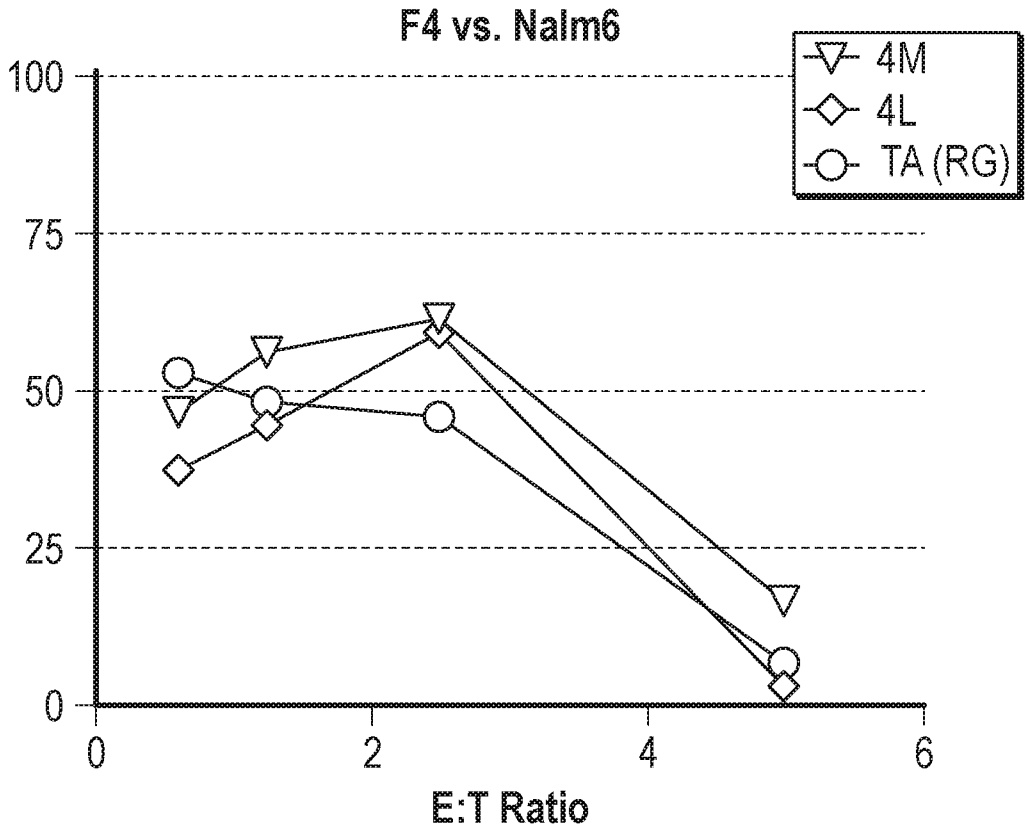



FIG. 56C

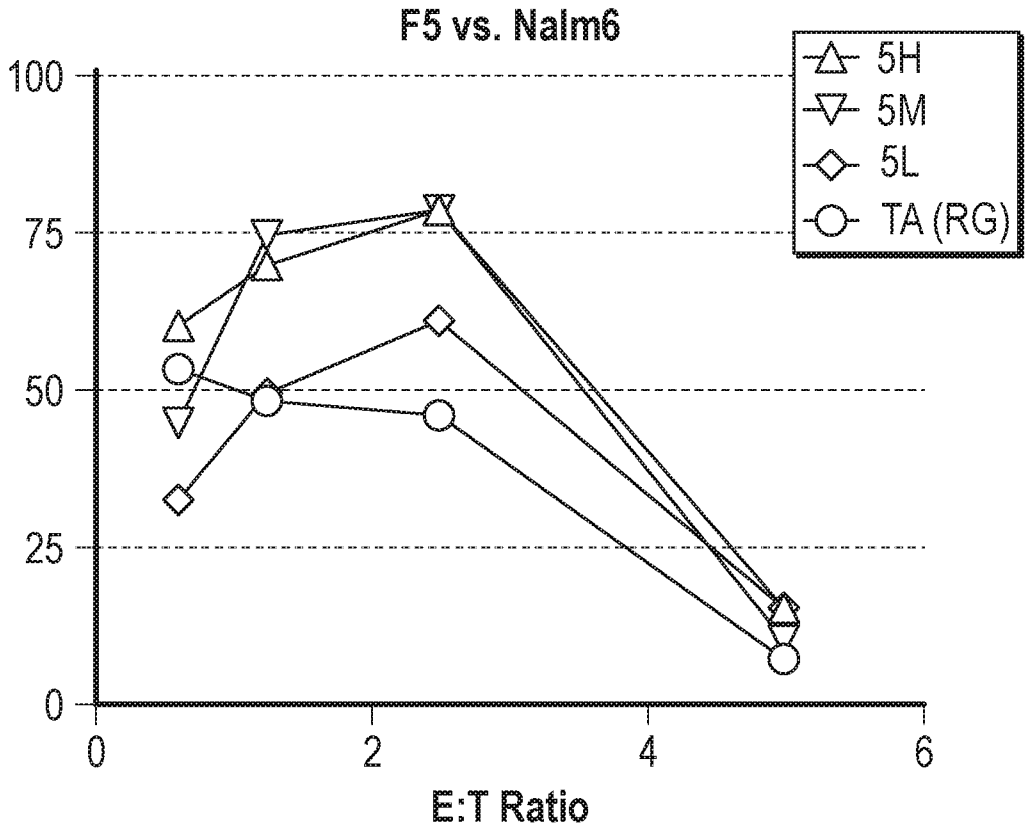



FIG. 56D

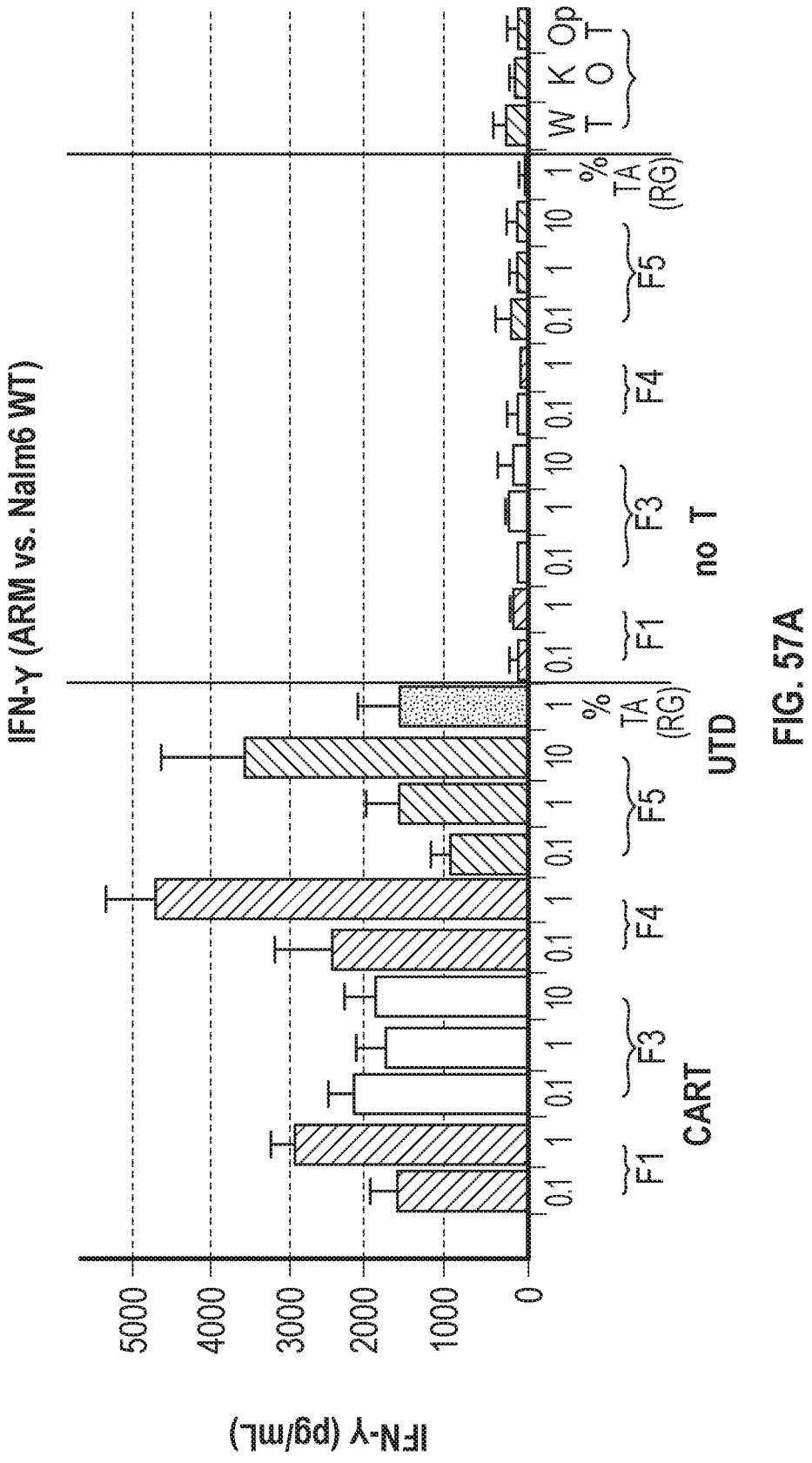

IFN-γ (ARM vs. Nalm6 WT)
IFN-γ (pg/mL)
5000
4000
3000
2000
1000
0
CART
UTD
no T
F1
F3
F4
F5
TA (RG)
%
0.1
1
10
WT
KO
Op T

FIG. 57A

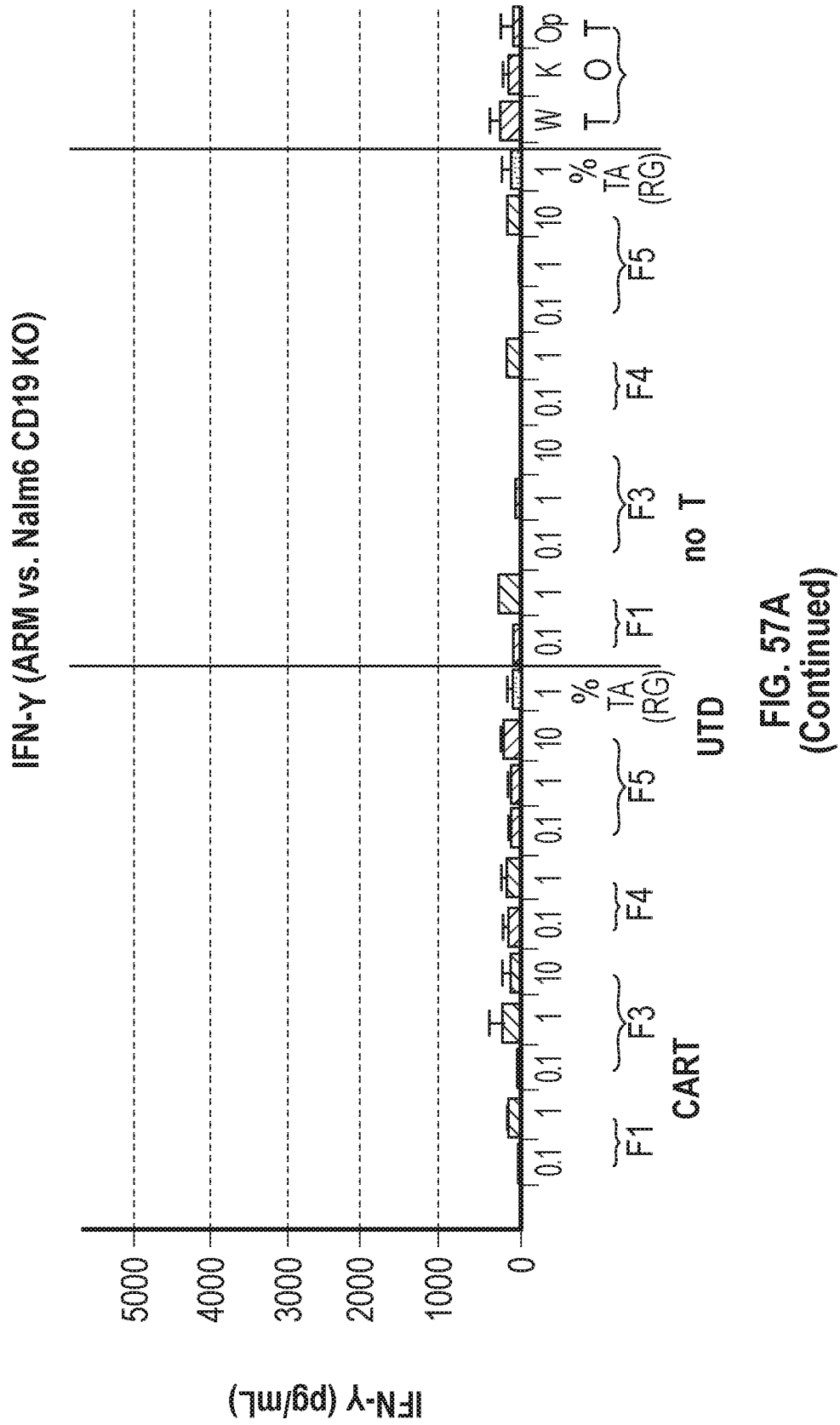



FIG. 57A
(Continued)

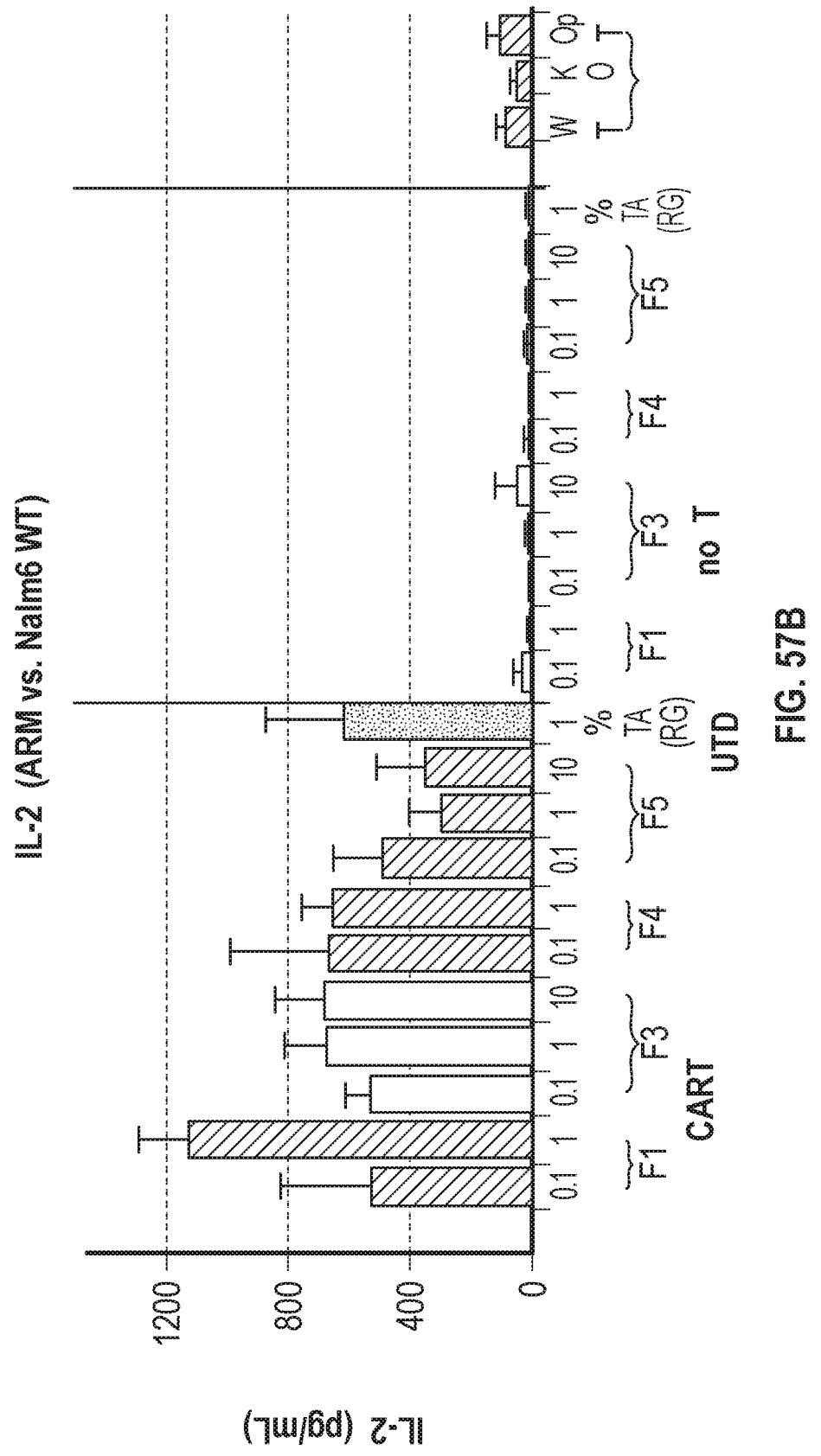

IL-2 (ARM vs. Nalm6 WT)
IL-2 (pg/mL)
1200
800
400
0
CART
UTD
no T
F1
F3
F4
F5
TA (RG)
%
0.1
1
10
W
K
Op
T
O

FIG. 57B

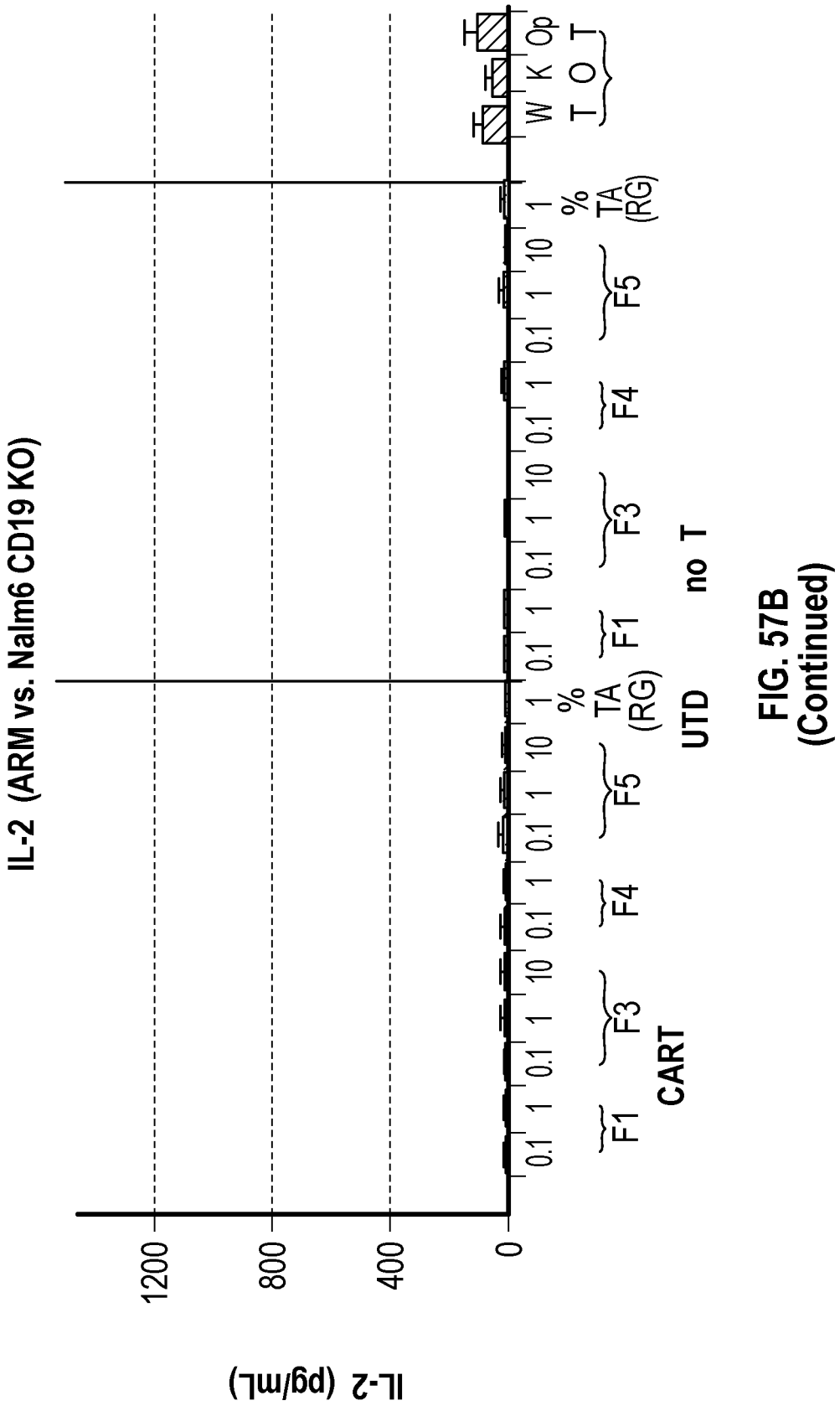



FIG. 57B
(Continued)

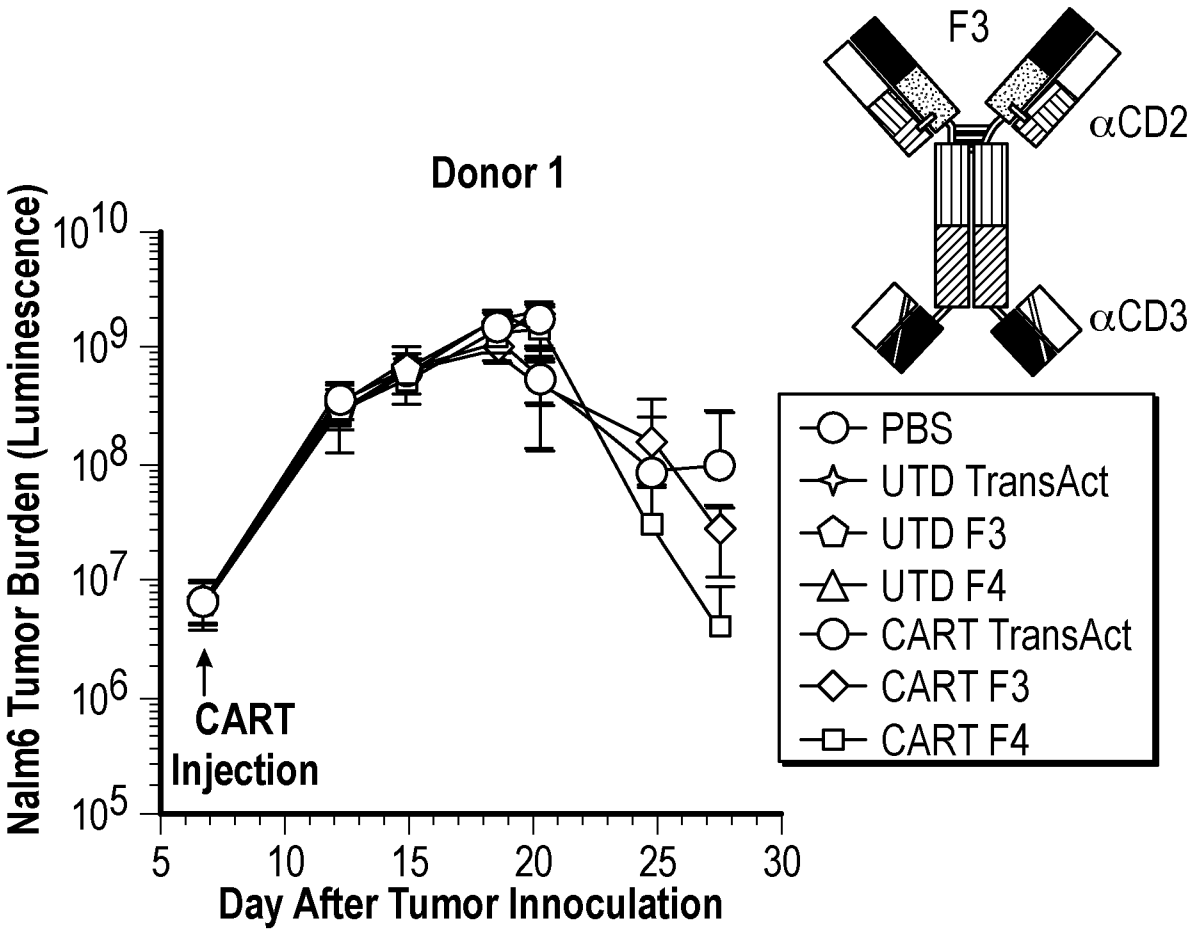

Donor 1
Nalm6 Tumor Burden (Luminescence)
$10^{10}$
$10^{9}$
$10^{8}$
$10^{7}$
$10^{6}$
$10^{5}$
CART Injection
5
10
15
20
25
30
Day After Tumor Innoculation
F3
αCD2
αCD3
PBS
UTD TransAct
UTD F3
UTD F4
CART TransAct
CART F3
CART F4

FIG. 58

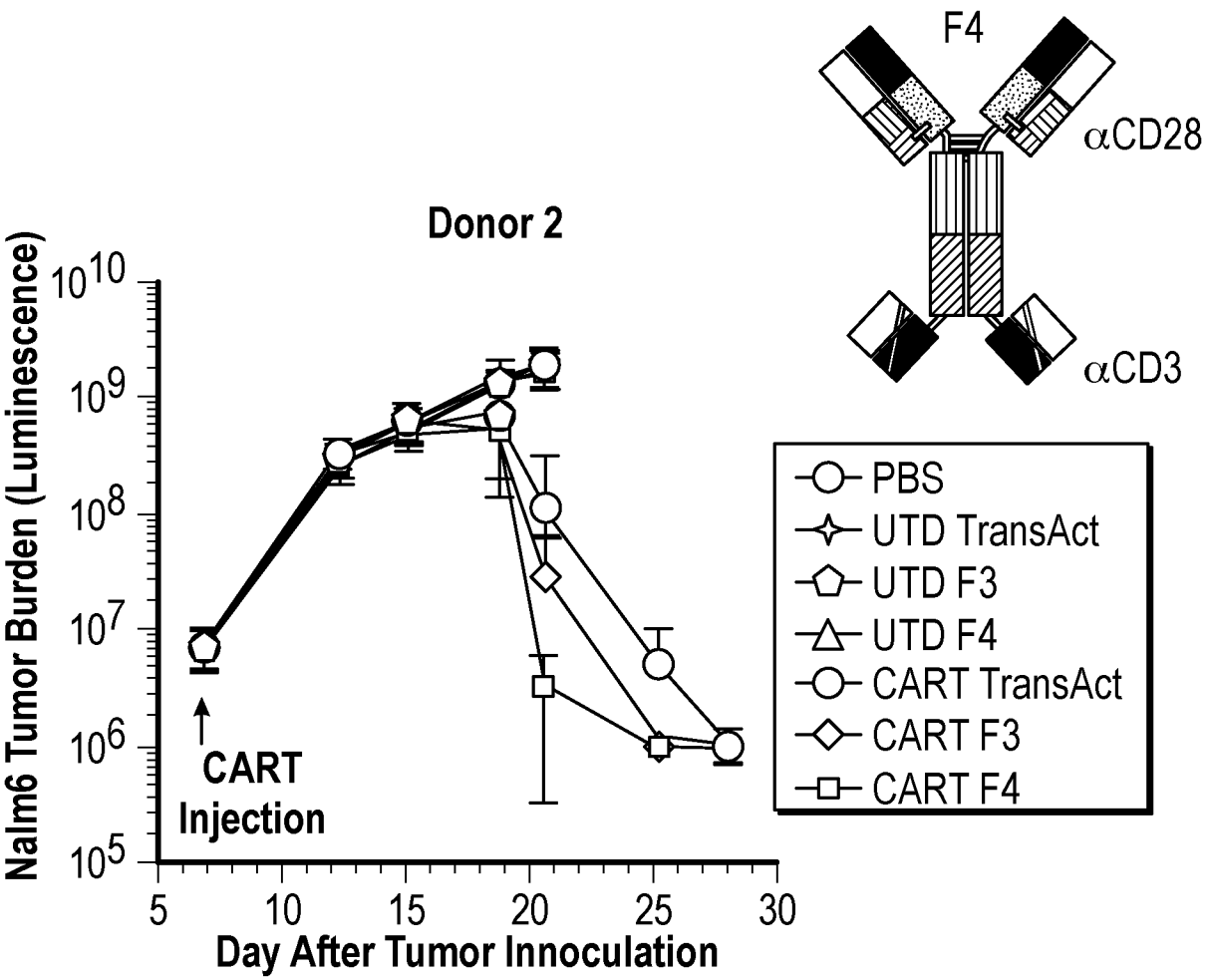



FIG. 58
(Continued)

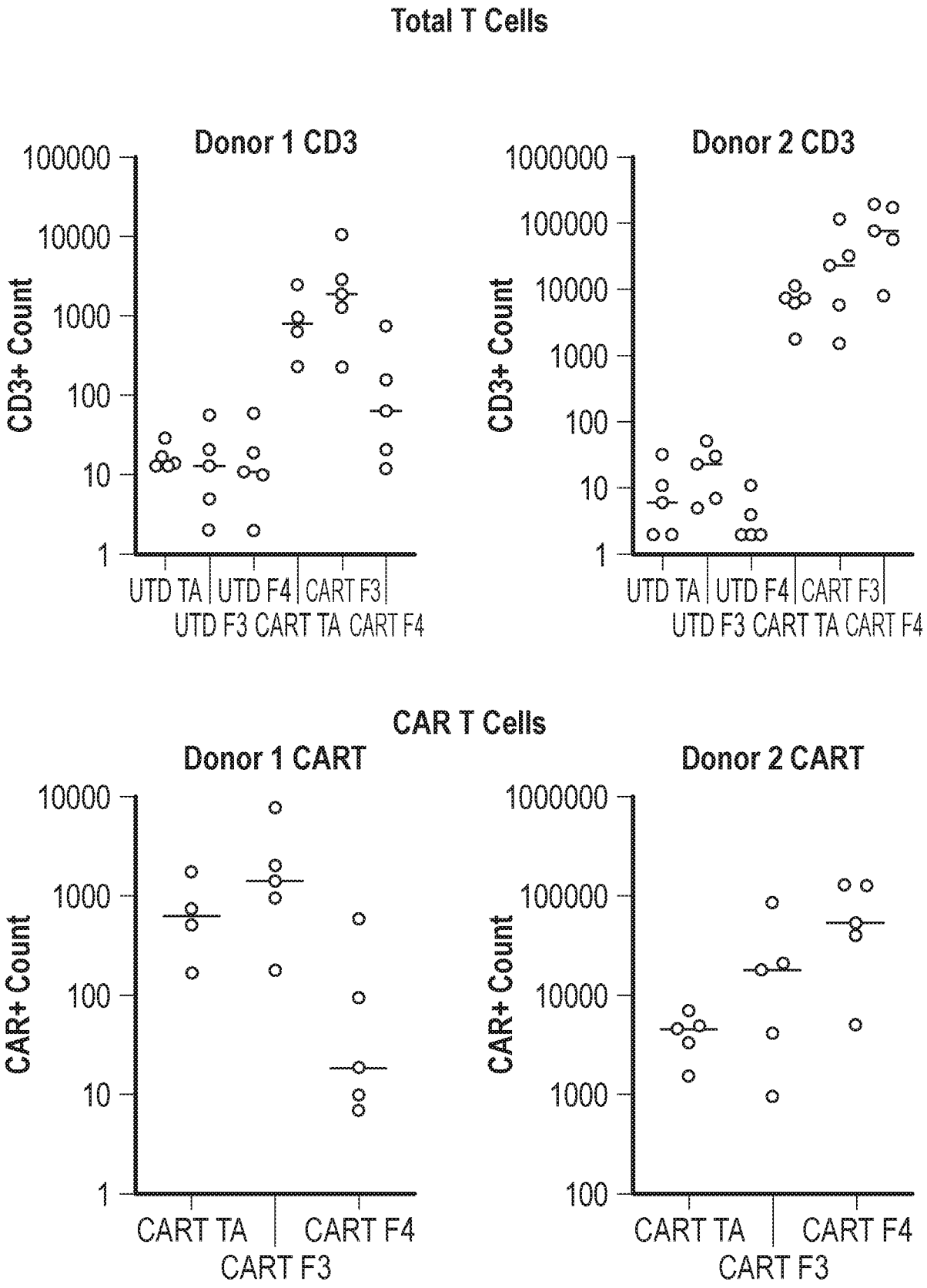

Total T Cells
Donor 1 CD3
CD3+ Count
100000
10000
1000
100
10
1
UTD TA
UTD F4
CART F3
UTD F3
CART TA
CART F4
Donor 2 CD3
CD3+ Count
1000000
100000
10000
1000
100
10
1
UTD TA
UTD F4
CART F3
UTD F3
CART TA
CART F4
CAR T Cells
Donor 1 CART
CAR+ Count
10000
1000
100
10
1
CART TA
CART F4
CART F3
Donor 2 CART
CAR+ Count
1000000
100000
10000
1000
100
CART TA
CART F4
CART F3

FIG. 59

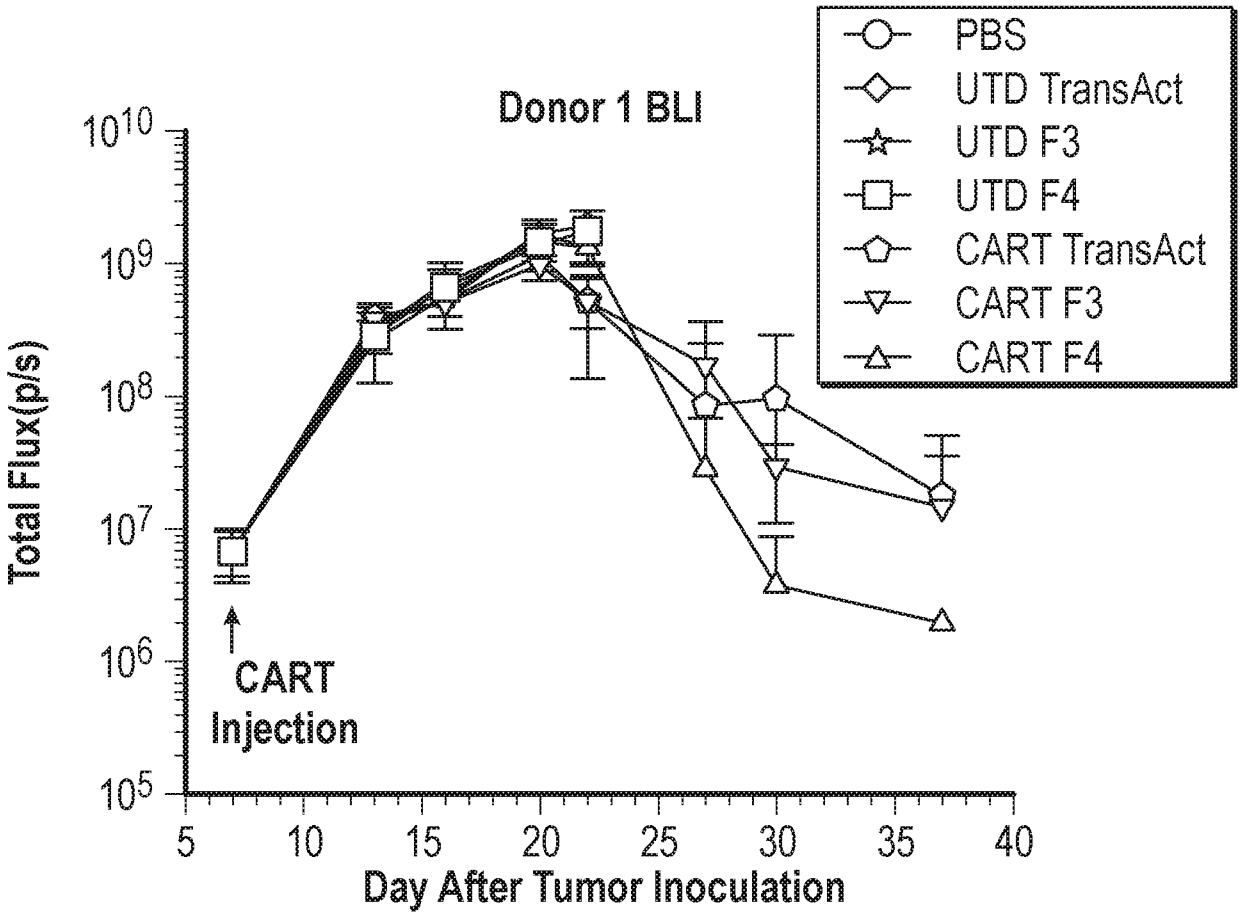

Donor 1 BLI
PBS
UTD TransAct
UTD F3
UTD F4
CART TransAct
CART F3
CART F4
Total Flux(p/s)
$10^{10}$
$10^{9}$
$10^{8}$
$10^{7}$
$10^{6}$
$10^{5}$
CART
Injection
5
10
15
20
25
30
35
40
Day After Tumor Inoculation

FIG. 60A

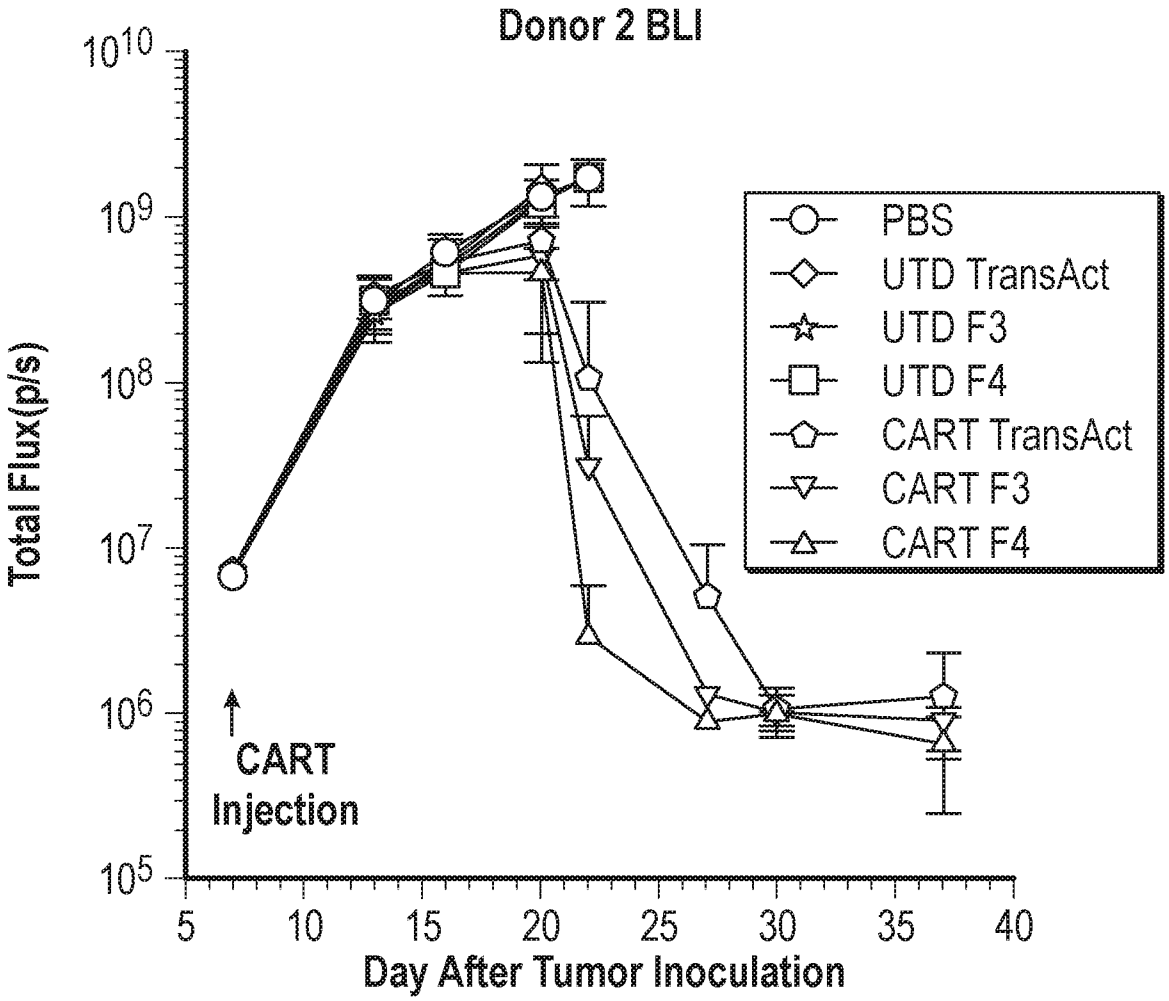

Donor 2 BLI
Total Flux(p/s)
$10^{10}$
$10^{9}$
$10^{8}$
$10^{7}$
$10^{6}$
$10^{5}$
5
10
15
20
25
30
35
40
Day After Tumor Inoculation
CART
Injection
PBS
UTD TransAct
UTD F3
UTD F4
CART TransAct
CART F3
CART F4

FIG. 60B

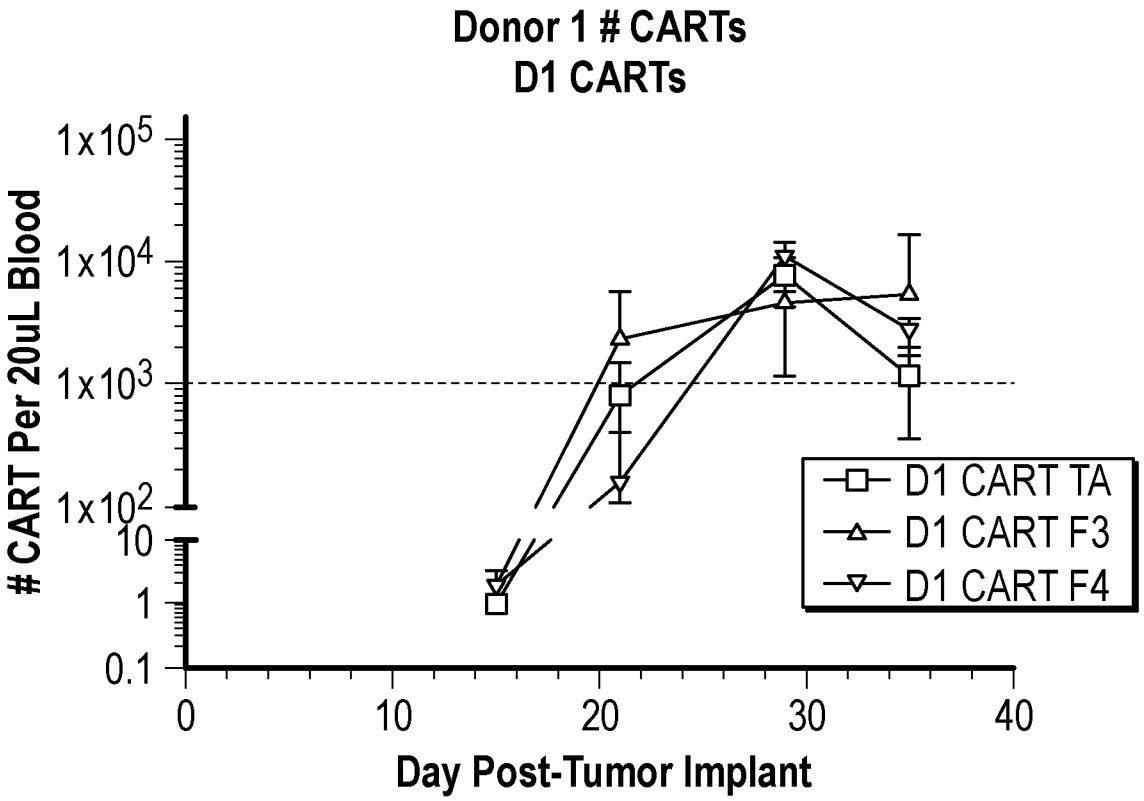



FIG. 60C

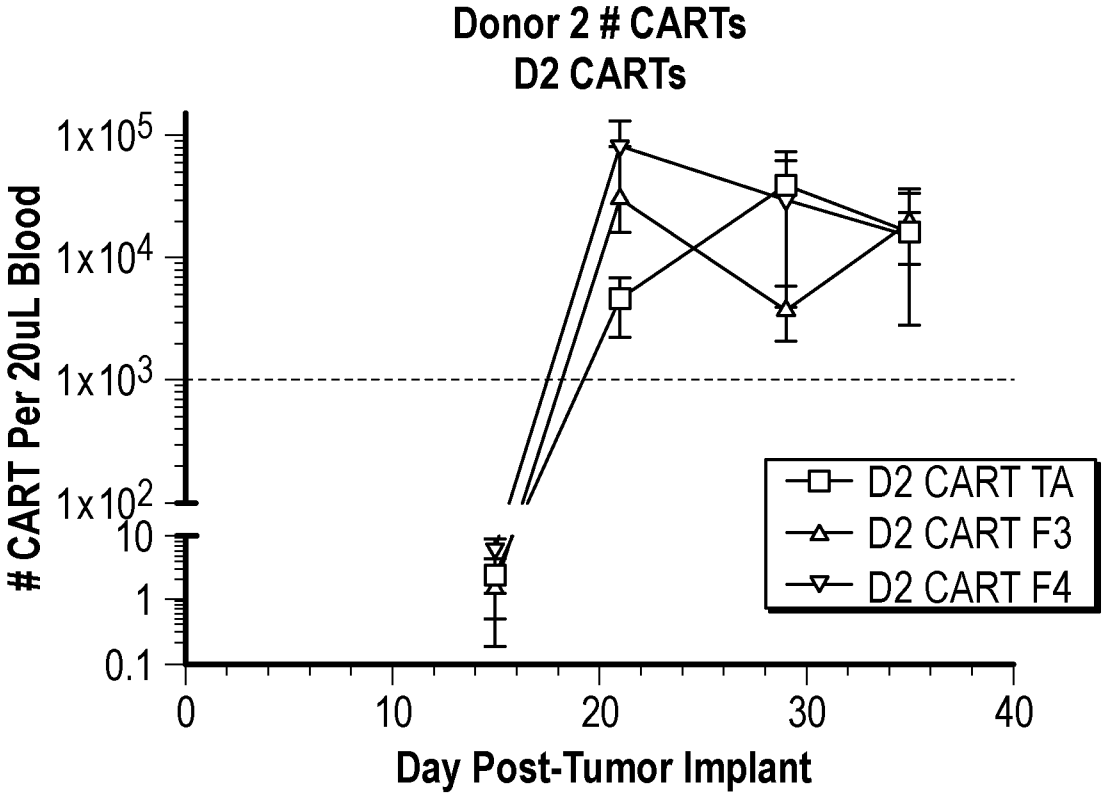



FIG. 60D

| Co-Stimulatory Binders | CD3 Binder | Name | |
|---|---|---|---|
| CD25 | ANTI-CD3 (1) | F5 CD25 | Alternative Costim |
| IL6Rb | ANTI-CD3 (1) | F5 IL6Rb | |
| CD27 | ANTI-CD3 (1) | F5 CD27 | |
| 41BB | ANTI-CD3 (1) | F5 41BB | |
| ICOS | ANTI-CD3 (1) | F5 ICOS | |
| CD2 | ANTI-CD3 (2) | F5 ANTI-CD3 (2) | Alternative CD3 Binder |

FIG. 61A

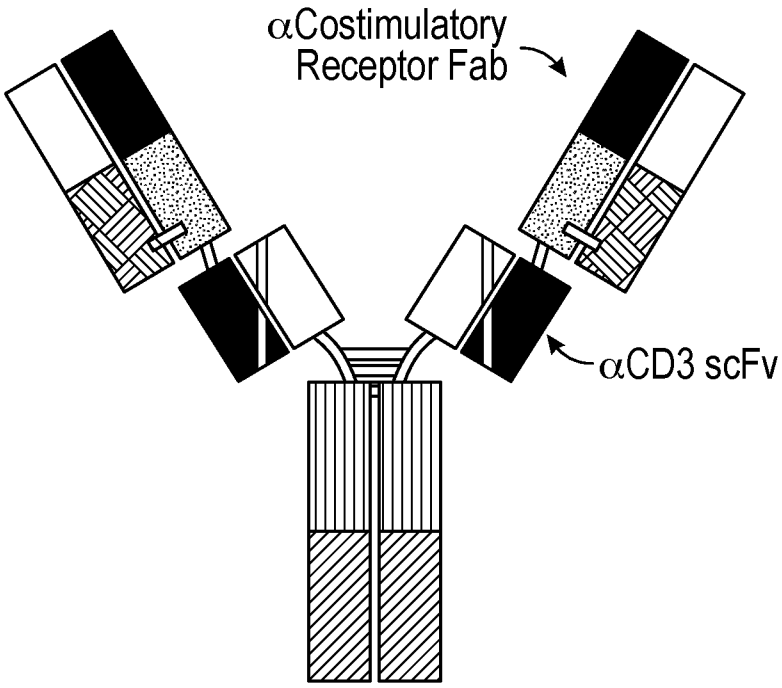



FIG. 61B

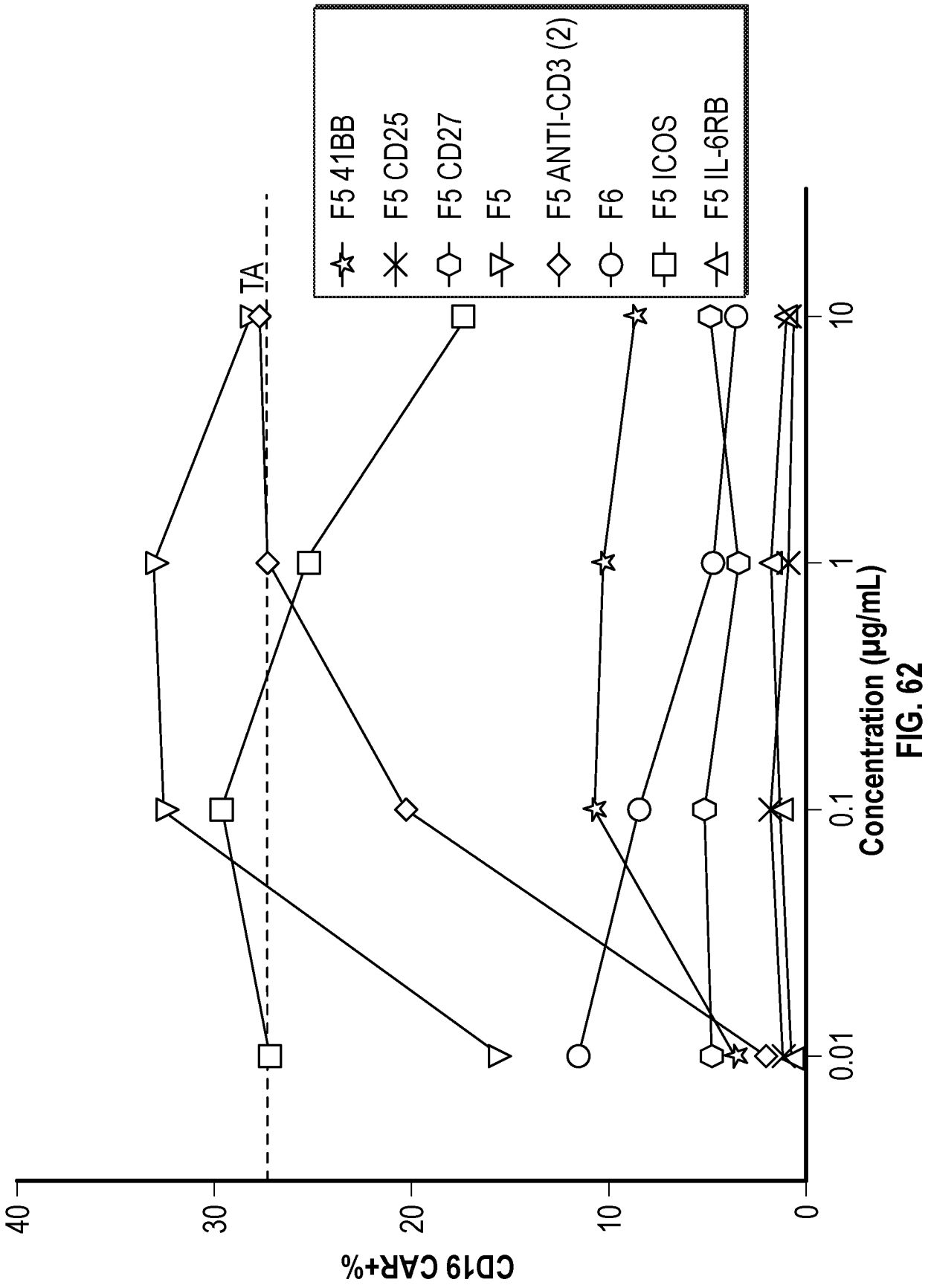

F5 41BB
F5 CD25
F5 CD27
F5
F5 ANTI-CD3 (2)
F6
F5 ICOS
F5 IL-6RB
TA
CD19 CAR+%
0
10
20
30
40
0.01
0.1
1
10
Concentration (µg/mL)

FIG. 62

| Variants | ANTI-CD3 Arm | ANTI-Costim Arm | |
|---|---|---|---|
| NEG2042 | ANTI-CD3 (1) | CD2 | |
| NEG2043 | ANTI-CD3 (1) | ANTI-CD28 (1) | |
| NEG2044 | ANTI-CD3 (1) | ANTI-CD28 (2) | |
| NEG2050 | ANTI-CD3 (2) | CD2 | Alternative CD3 Binders |
| NEG2051 | ANTI-CD3 (3) | CD2 | |
| NEG2052 | ANTI-CD3 (2) | ANTI-CD28 (1) | |
| NEG2053 | ANTI-CD3 (3) | ANTI-CD28 (1) | |
| F5-Not Fc Silenced | ANTI-CD3 (1) | CD2 | |
| Transact | | CD28 | |

FIG. 63A

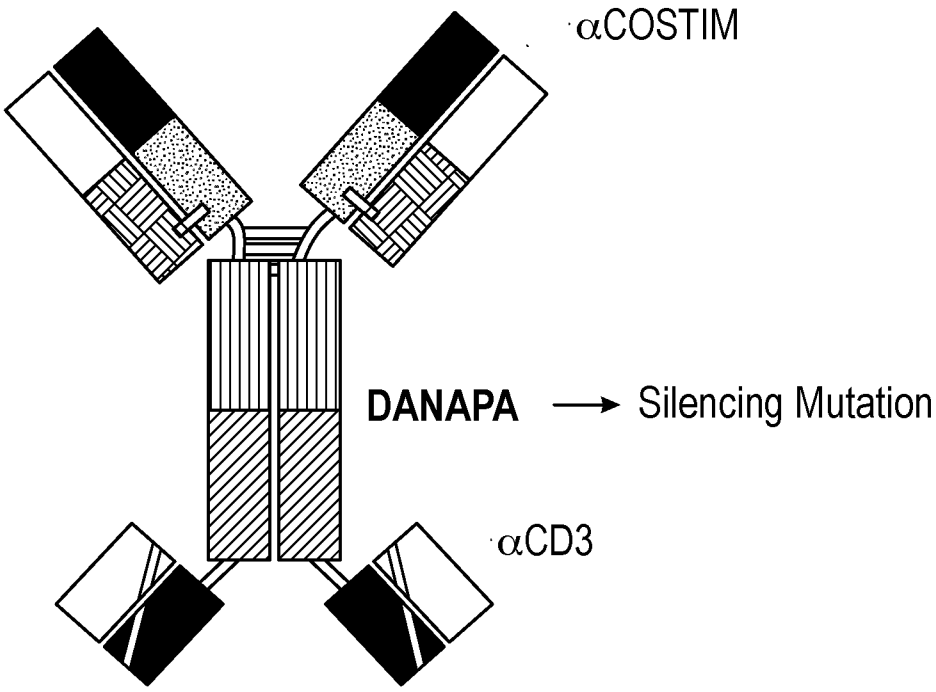



FIG. 63B

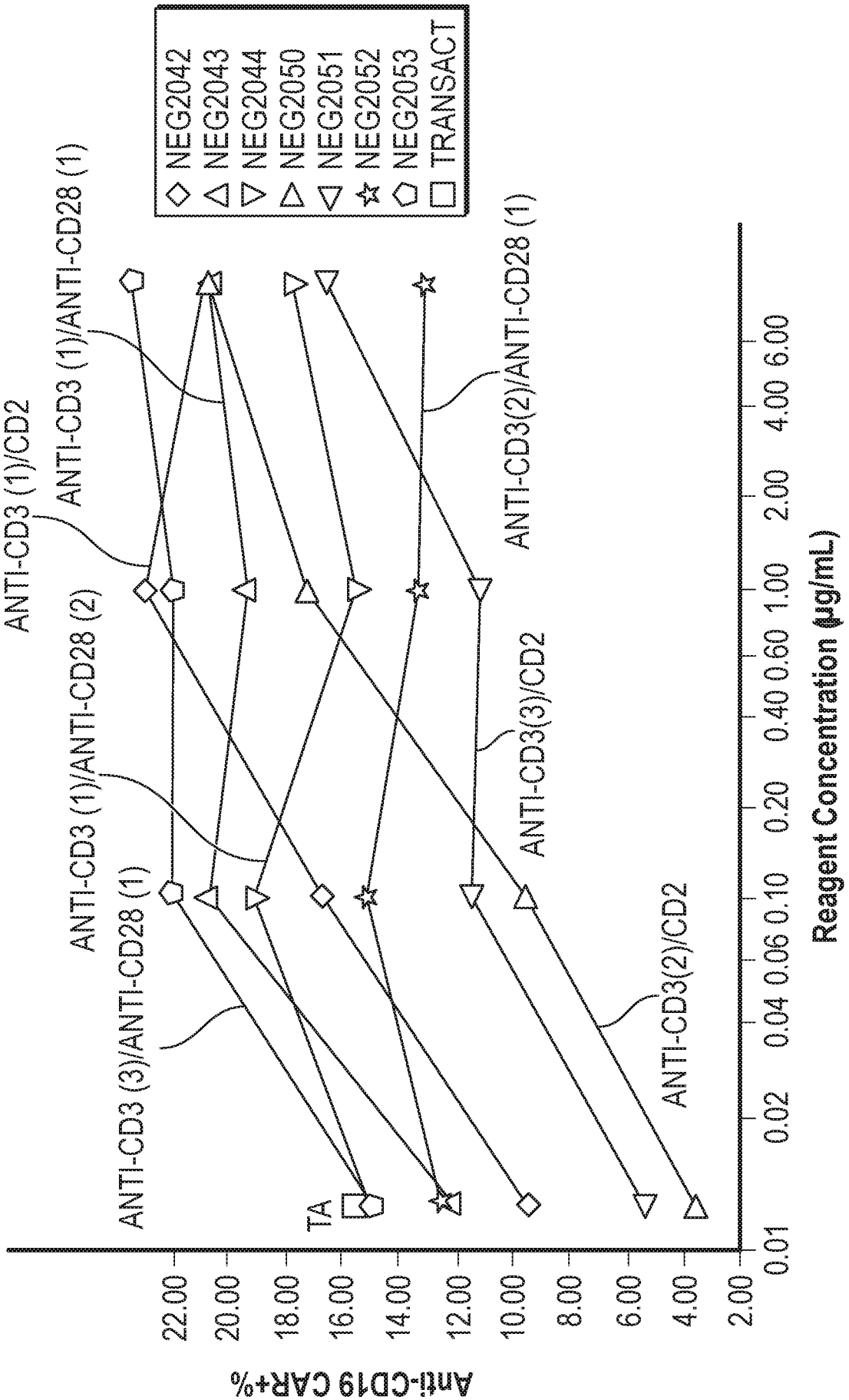

NEG2042
NEG2043
NEG2044
NEG2050
NEG2051
NEG2052
NEG2053
TRANSACT
ANTI-CD3 (1)/CD2
ANTI-CD3 (1)/ANTI-CD28 (1)
ANTI-CD3 (1)/ANTI-CD28 (2)
ANTI-CD3 (3)/ANTI-CD28 (1)
ANTI-CD3(2)/ANTI-CD28 (1)
ANTI-CD3(3)/CD2
ANTI-CD3(2)/CD2
TA
Anti-CD19 CAR+%
22.00
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.01
0.02
0.04
0.06
0.10
0.20
0.40
0.60
1.00
2.00
4.00
6.00
Reagent Concentration (µg/mL)

FIG. 64

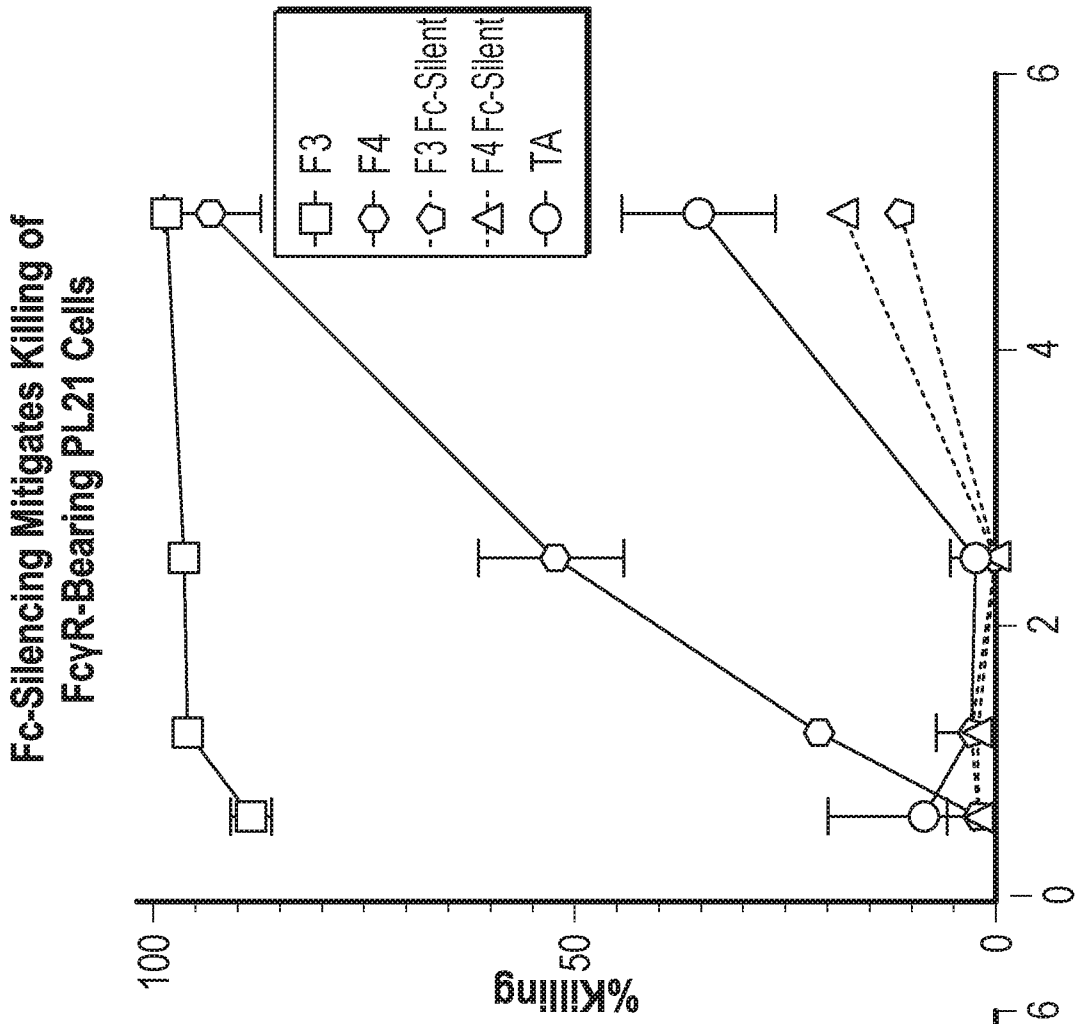



FIG. 65B

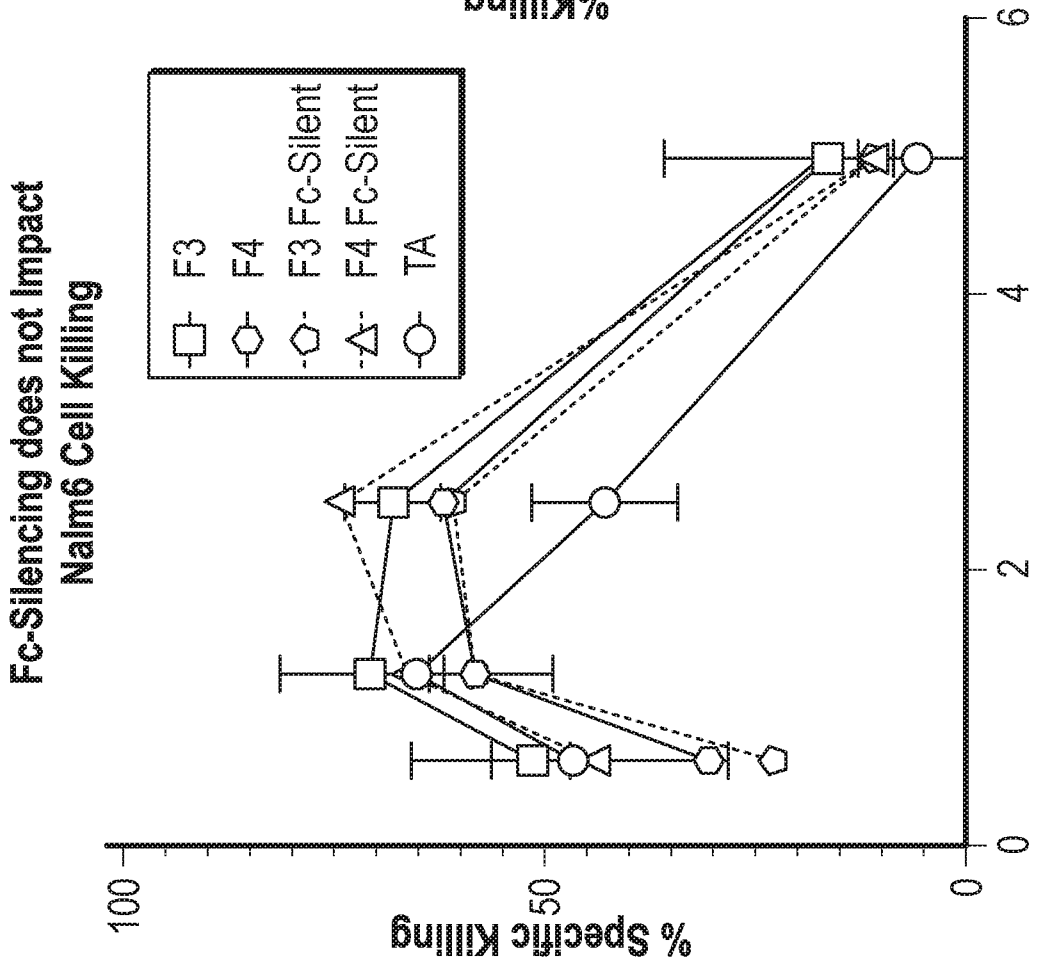



FIG. 65A

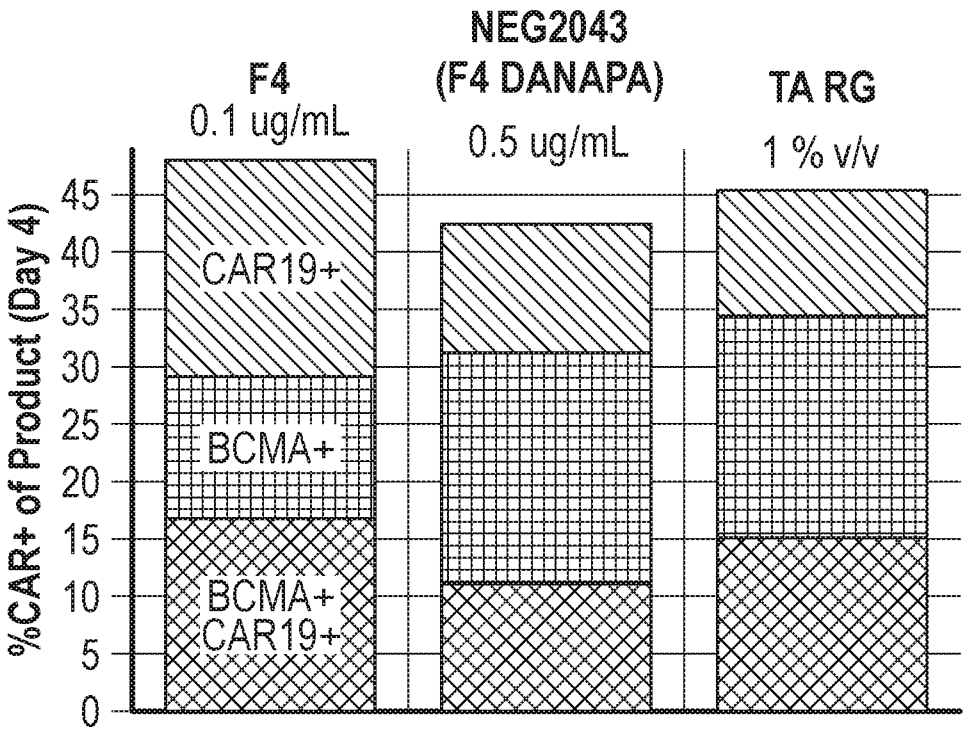



FIG. 66A

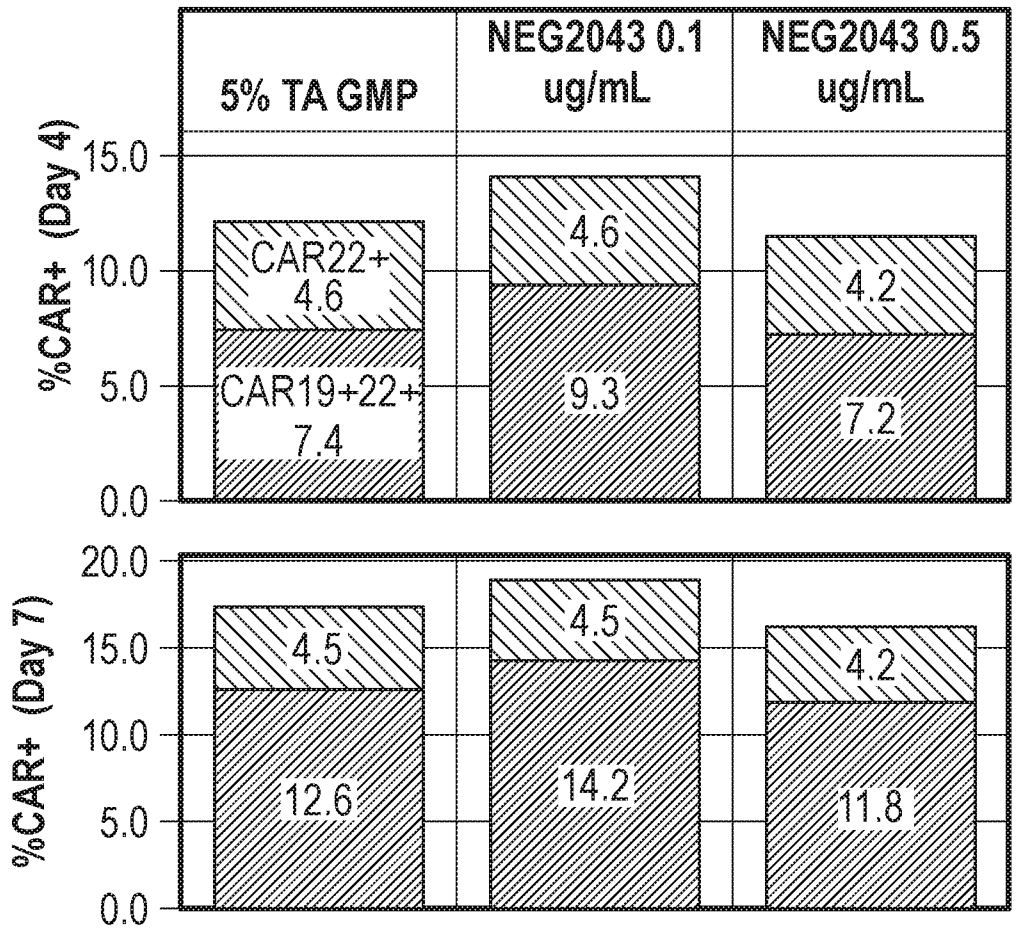



FIG. 66B

FIG. 67

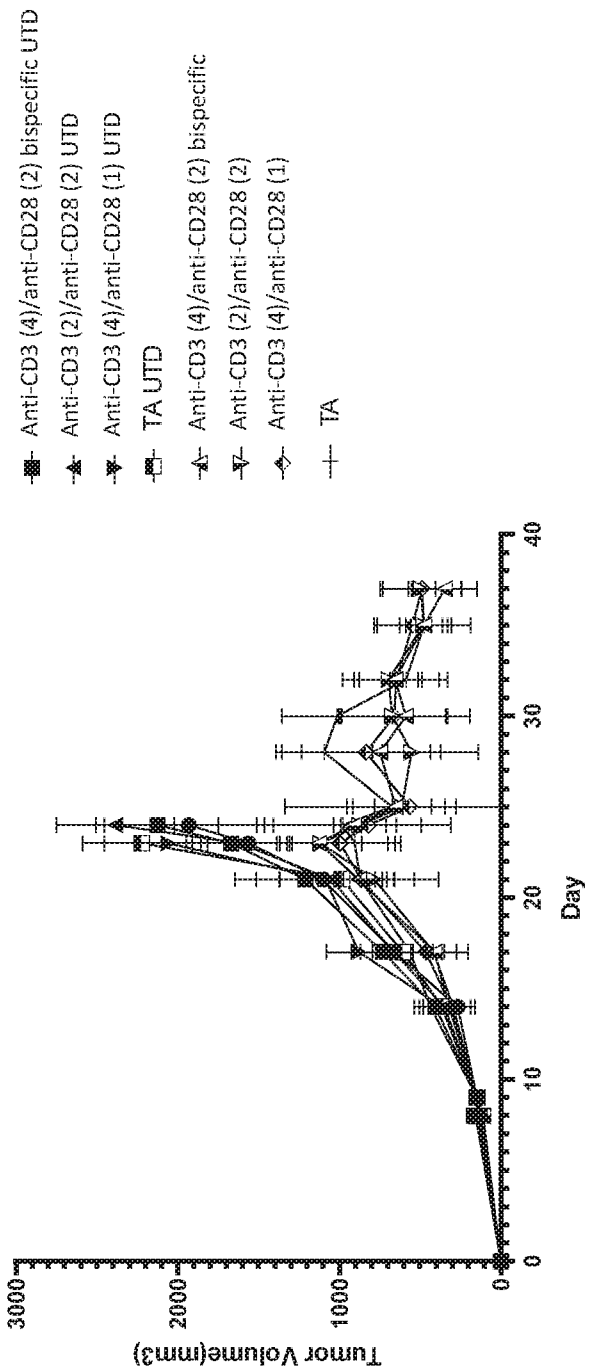

PBS
Anti-CD3 (4)/anti-CD28 (2) bispecific UTD
Anti-CD3 (2)/anti-CD28 (2) UTD
Anti-CD3 (4)/anti-CD28 (1) UTD
TA UTD
Anti-CD3 (4)/anti-CD28 (2) bispecific
Anti-CD3 (2)/anti-CD28 (2)
Anti-CD3 (4)/anti-CD28 (1)
TA
Tumor Volume(mm3)
3000
2000
1000
0
0
10
20
30
40
Day

FIG. 68A

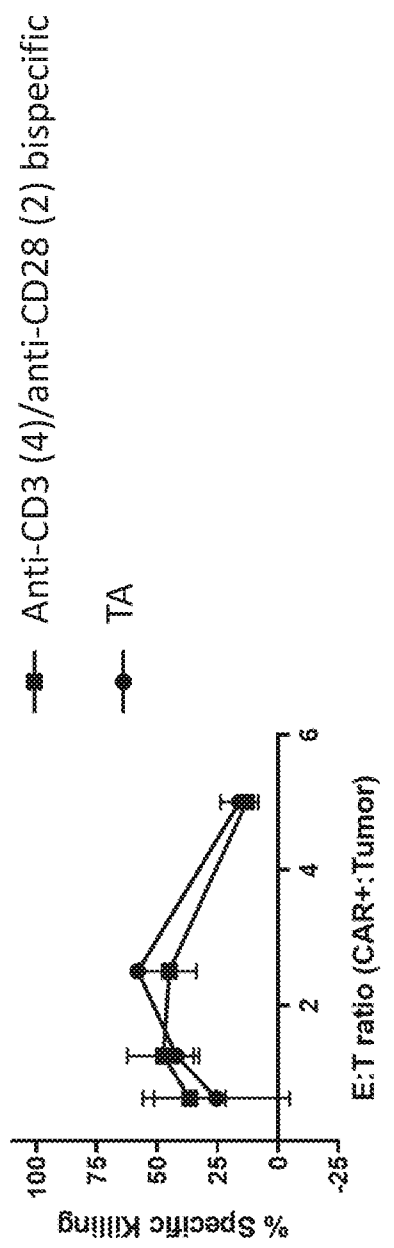
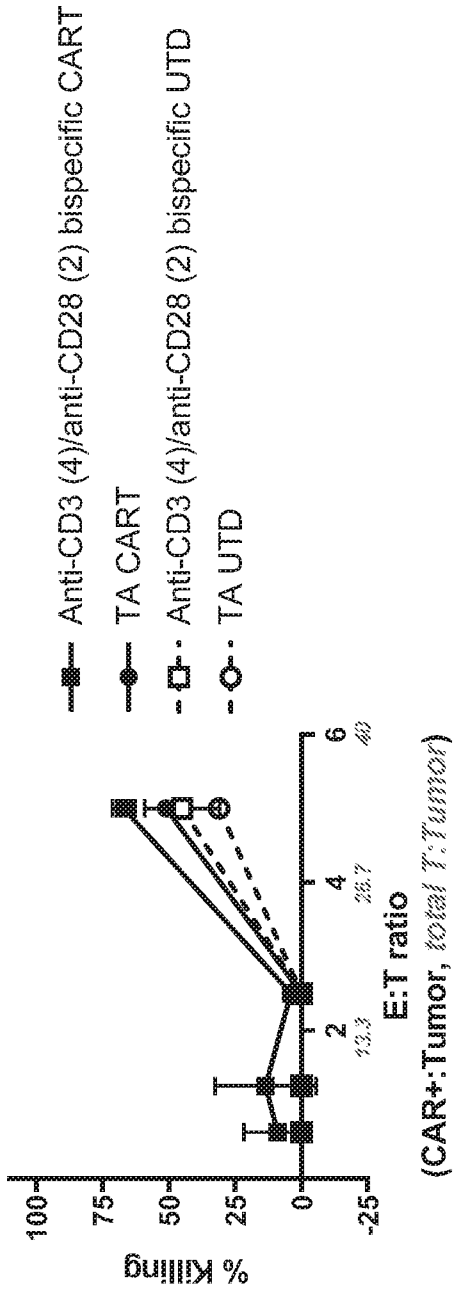



FIG. 68B



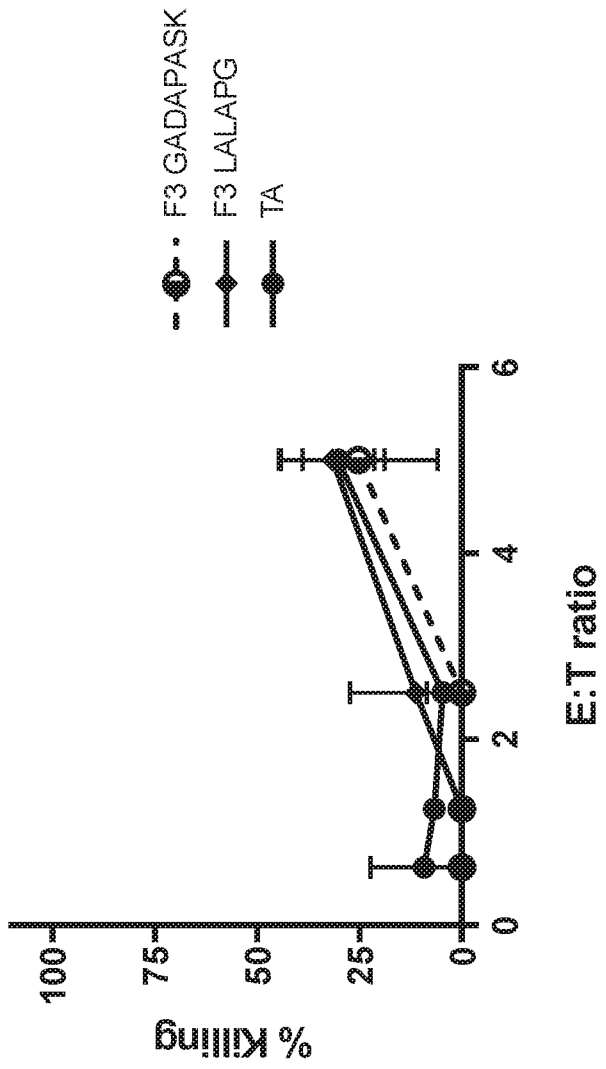

PL21
F3 GADAPASK
F3 LALAPG
TA
% killing
100
75
50
25
0
0
2
4
6
E:T ratio

FIG. 69

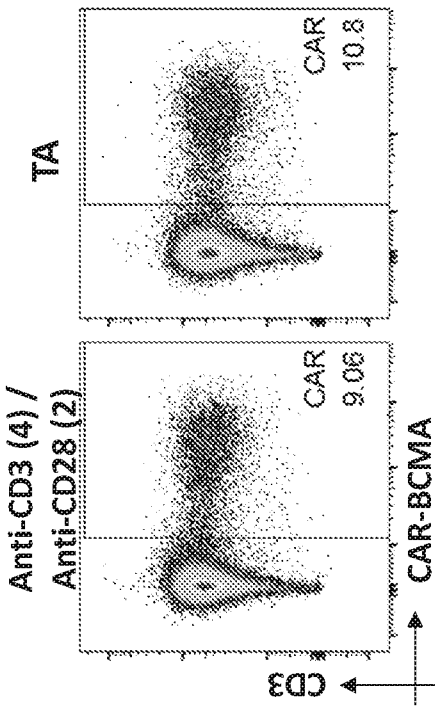



FIG. 70A

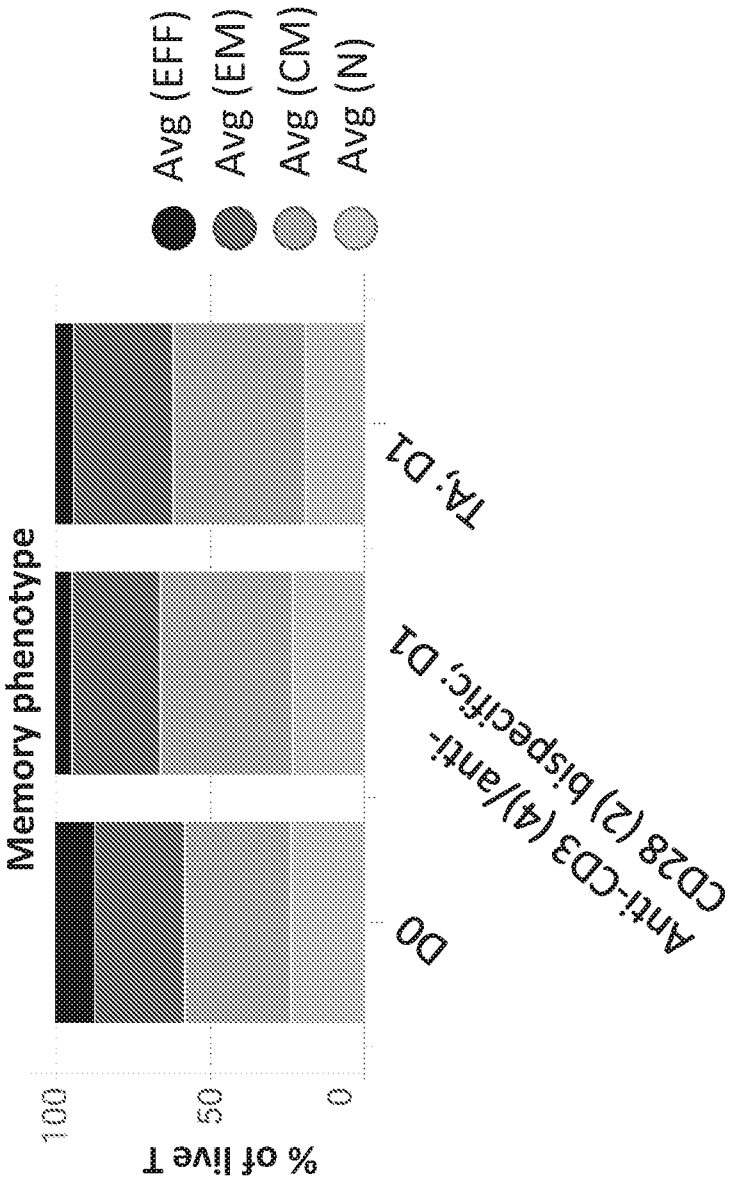



FIG. 70B

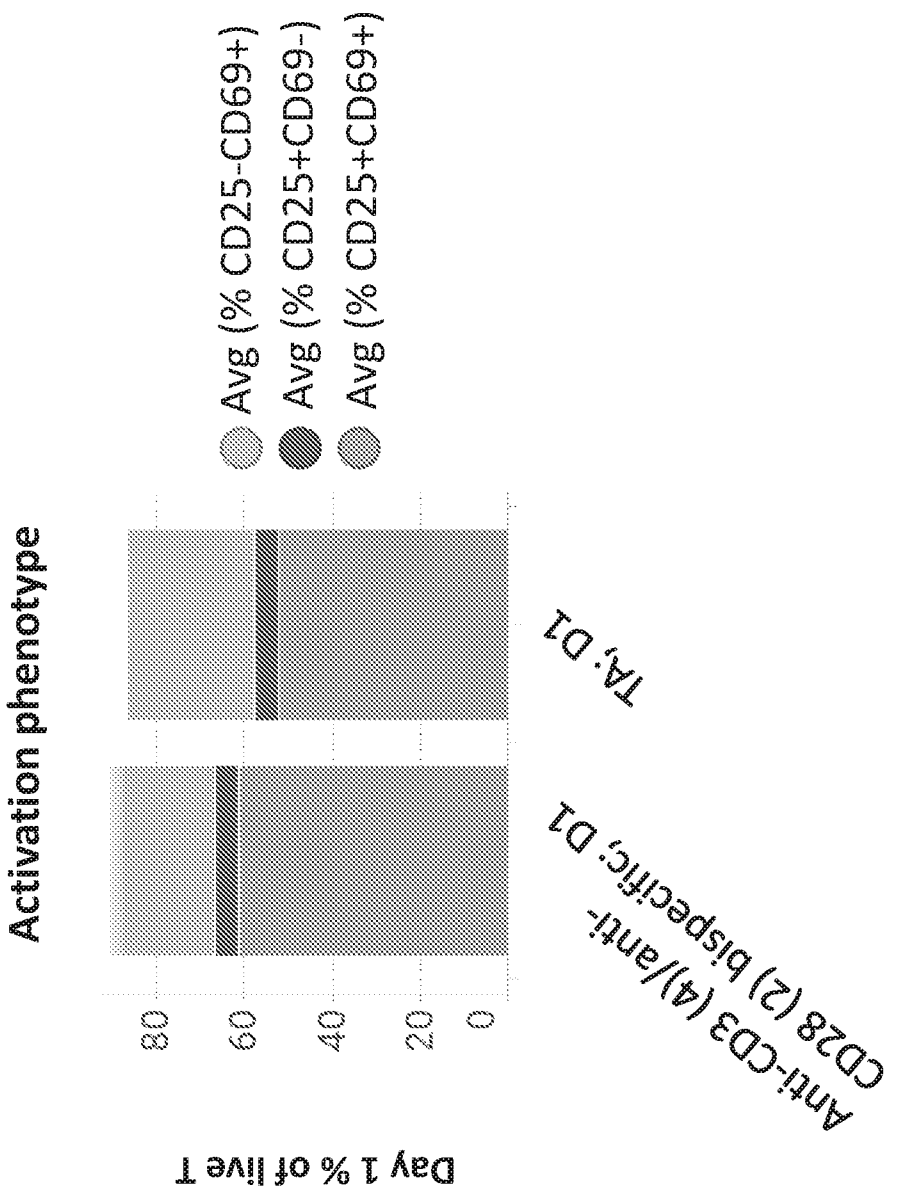

Activation phenotype
Avg (% CD25-CD69+)
Avg (% CD25+CD69-)
Avg (% CD25+CD69+)
Day 1 % of live T
0
20
40
60
80
Anti-CD3 (4)/anti-CD28 (2) bispecific; D1
TA; D1

FIG. 70C

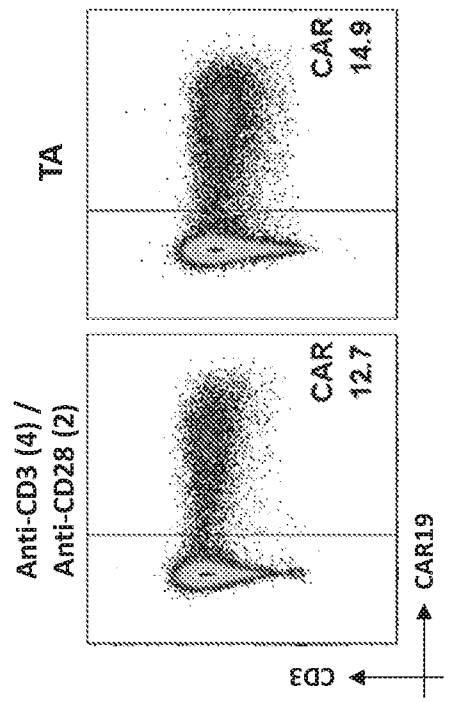



FIG. 71A

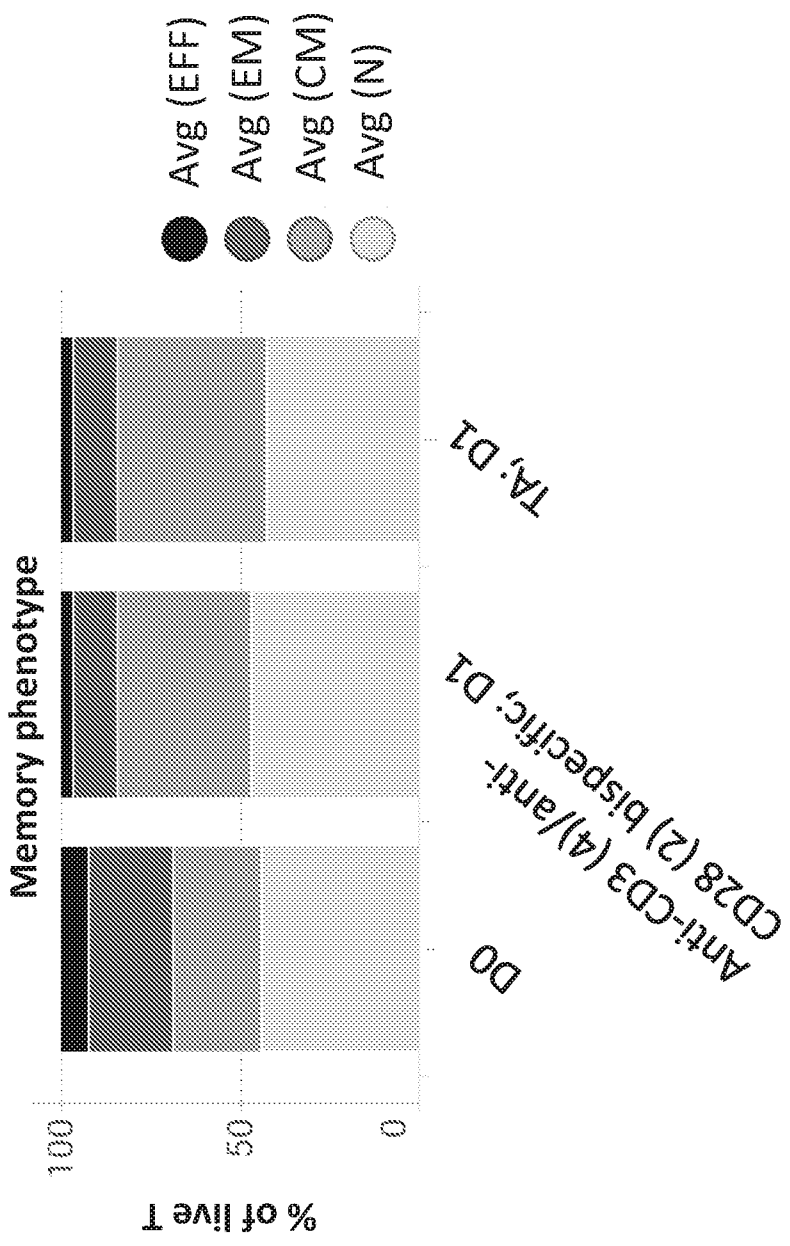



FIG. 71B

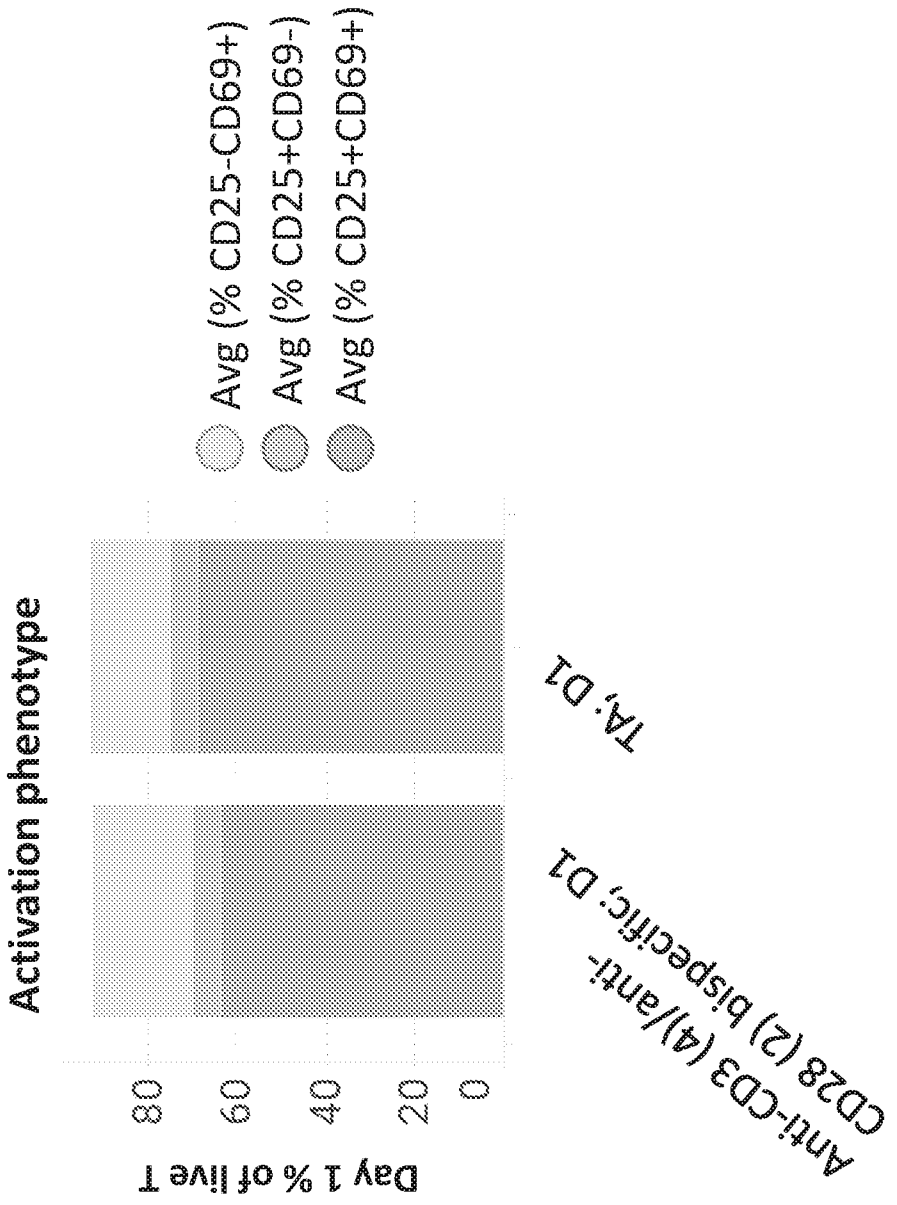

Activation phenotype
Day 1 % of live T
80
60
40
20
0
Avg (% CD25-CD69+)
Avg (% CD25+CD69-)
Avg (% CD25+CD69+)
TA: D1
Anti-CD3 (4)/anti-CD28 (2) bispecific: D1

FIG. 71C

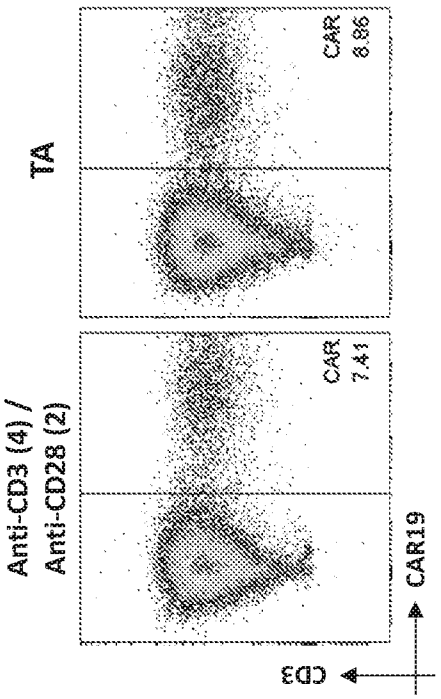



FIG. 71D

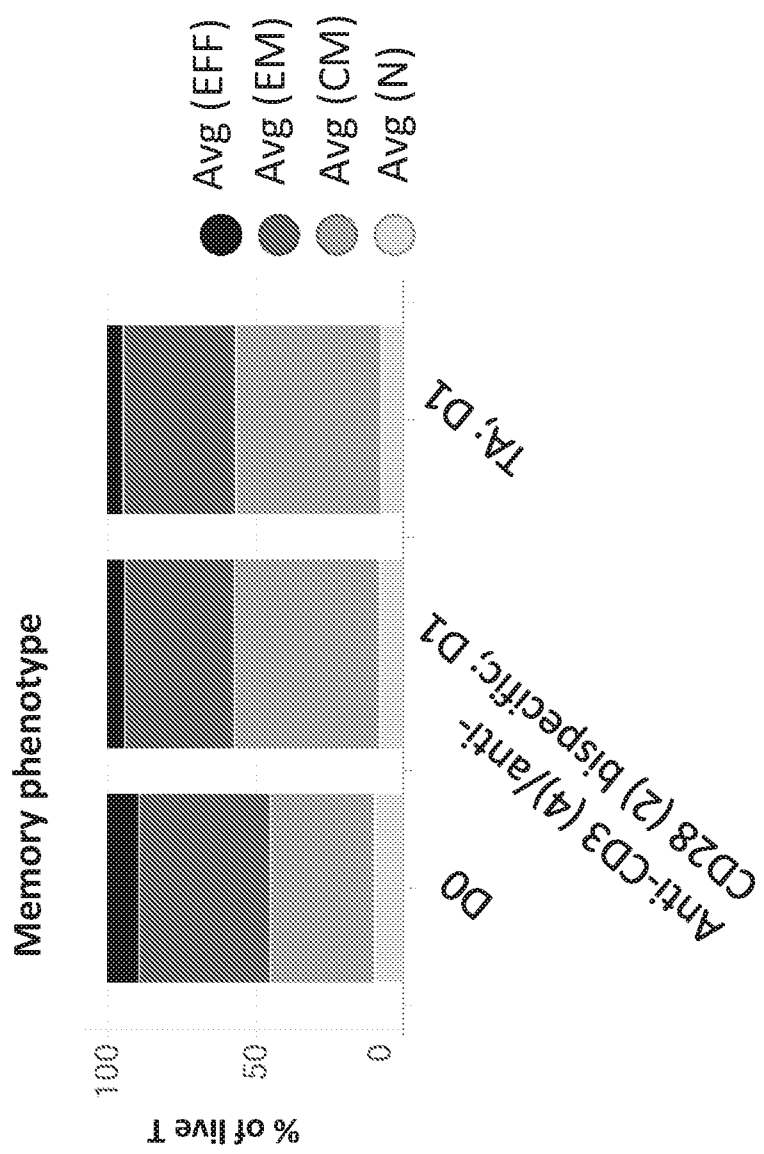



FIG. 71E

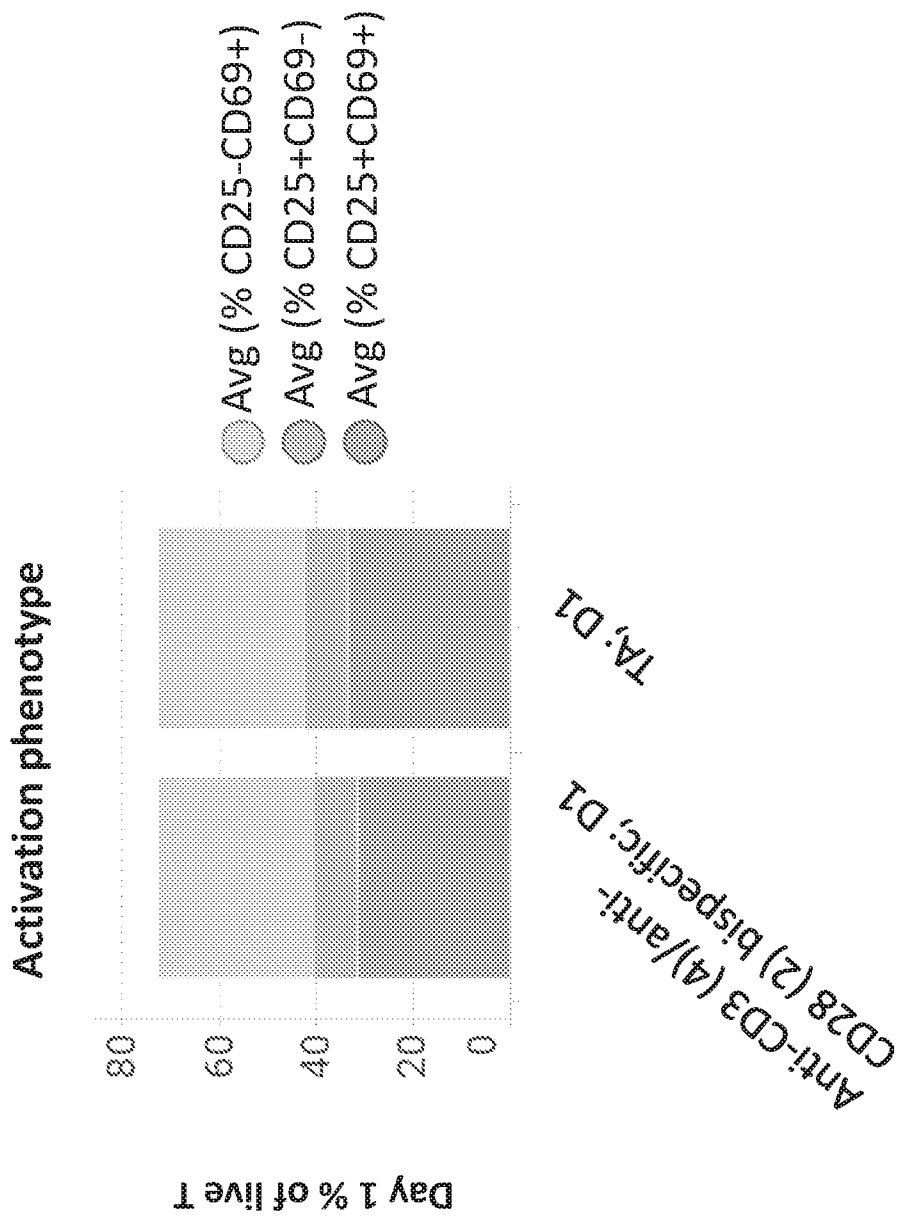

Activation phenotype
Avg (% CD25-CD69+)
Avg (% CD25+CD69-)
Avg (% CD25+CD69+)
Day 1 % of live T
0
20
40
60
80
TA: D1
Anti-CD3 (4)/anti-CD28 (2) bispecific: D1

FIG. 71F