(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) **World Intellectual Property Organization**
International Bureau

(10) **International Publication Number**
WO 2020/081869 A1

(43) **International Publication Date**
23 April 2020 (23.04.2020)

(51) **International Patent Classification:**
A61K 35/14 (2015.01) *C12N 5/10* (2006.01)
A61K 35/28 (2015.01) *C12N 15/85* (2006.01)
C12N 5/0775 (2010.01) *C12N 15/86* (2006.01)
C12N 5/078 (2010.01)

(21) **International Application Number:** PCT/US2019/056824

(22) **International Filing Date:** 17 October 2019 (17.10.2019)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
62/747,109 17 October 2018 (17.10.2018) US
62/747,114 17 October 2018 (17.10.2018) US
62/843,180 03 May 2019 (03.05.2019) US

(71) **Applicant: SENTI BIOSCIENCES, INC.** [US/US]; 2 Corporate Drive, First Floor, South San Francisco, CA 94080 (US).

(72) **Inventors: LU, Timothy, Kuan-Ta**; 2 Corporate Drive, First Floor, South San Francisco, CA 94080 (US). **GORDLEY, Russell, Morrison**; 2 Corporate Drive, First Floor, South San Francisco, CA 94080 (US). **LIN, Jack, Tzu-Chiao**; 2 Corporate Drive, First Floor, South San Francisco, CA 94080 (US). **GARRISON, Brian, Scott**; 2 Corporate Drive, First Floor, South San Francisco, CA 94080 (US). **LEE, Philip, Janmin**; 2 Corporate Drive, First Floor, South San Francisco, CA 94080 (US). **GONZALEZ-JUNCA, Alba**; 2 Corporate Drive, First Floor, South San Francisco, CA 94080 (US). **WANG, Don-Hong**; 2 Corporate Drive, First Floor, South San Francisco, CA 94080 (US). **FRIMANNSSON, Daniel**; 2 Corporate Drive, First Floor, South San Francisco, CA 94080 (US).

(74) **Agent: NEWMAN, Zachary, Robert** et al.; Goodwin Procter LLP, 100 Northern Avenue, Boston, MA 02210 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME,

(54) **Title:** COMBINATORIAL CANCER IMMUNOTHERAPY

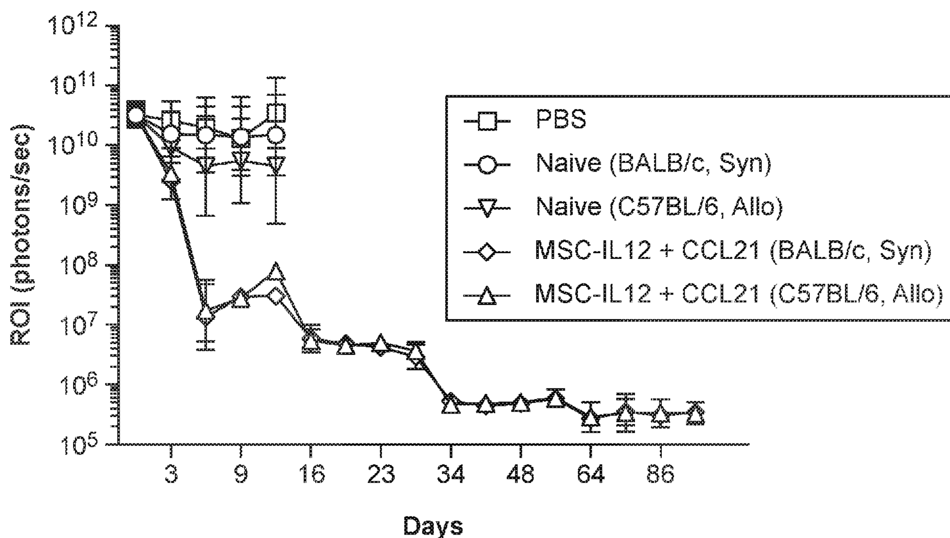


FIG. 1

(57) **Abstract:** Provided herein are methods and compositions for dynamically controlling and targeting multiple immunosuppressive mechanisms in cancer. Some aspects provide cells engineered to produce multiple effector molecules, each of which modulates a different immunosuppressive mechanisms of a tumor, as well as methods of using the cells to treat cancer, such as ovarian, breast, or colon cancer.

WO 2020/081869 A1

[Continued on next page]

MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

COMBINATORIAL CANCER IMMUNOTHERAPY

Cross Reference to Related Applications

This application claims the benefit of U.S. Provisional Application Nos. 62/747,109 filed on October 17, 2018; 62/747,114 filed on October 17, 2018; and 62/843,180 filed May 3, 2019, each of which is hereby incorporated in its entirety by reference.

Sequence Listing

The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated herein by reference in its entirety. Said ASCII copy, created on Month XX, 20XX, is named XXXXXUS_sequencelisting.txt, and is X,XXX,XXX bytes in size.

BACKGROUND

There are more than 22,000 new cases of ovarian cancer and more than 14,000 deaths each year in the United States (Siegel RL, et al. (2016) CA Cancer J Clin 66(1):7-30), with an estimated annual healthcare burden of greater than $600M (Dizon D MJ (2010) Gynecol Oncol 116(3)). Conventional approaches, such as chemotherapy (e.g., carboplatin/cisplatin and/or paclitaxel), are often unable to cure ovarian cancer. Approximately 70% of patients do not achieve remission on first-line chemotherapy, and 40-50% of patients that do have a remission will relapse within three years.

Treatment of other cancers, such as breast cancer and colon cancer, is associated with five-year survival rates of 85% and 65%, respectively. Therapies often include a combination of invasive surgeries and chemotherapies.

SUMMARY

Provided herein, in some embodiments, is a combinatorial cell-based immunotherapy for the targeted treatment of cancer, such as ovarian cancer, breast cancer, colon cancer, lung cancer, and pancreatic cancer. This combinatorial immunotherapy relies on engineered cell circuits that enable multifactorial modulation within and/or near a tumor (a "tumor microenvironment (TME)"). Despite exciting advancements in combinatorial immunotherapy, its efficacy against cancer has been limited due in part to the following challenges. It is difficult to deliver multiple

therapies simultaneously to achieve maximal efficacy without triggering significant side effects. It is also difficult in clinical trials to determine the appropriate dosing and timing of multiple systemically-administered and/or locally-injected therapies.

The combinatorial immunotherapy provided herein, however, is tumor-specific and effective yet limits systemic toxicity. This combinatorial immunotherapy delivers to a tumor microenvironment multiple immunomodulatory effector molecules from a single delivery vehicle. The design of the delivery vehicle is optimized to improve overall function in cancer therapy, including, but not limited to, optimization of the promoters, linkers, signal peptides, and order of the multiple immunomodulatory effector molecules.

Advantageously, cell circuits of the present disclosure are engineered in mesenchymal stem cells (MSCs), which are able to selectively home to tumors (including metastases), are able to produce a pro-inflammatory/immunostimulatory secretome and under certain conditions an anti-inflammatory secretome, and are hypoimmunogenic. These characteristics, among others, enable their use for allogenic cell therapies, for example, without significant safety issues, side effects, or rejection.

It has been increasingly recognized that tumors are a complex interplay between the tumor cells and the surrounding stroma, which includes the extracellular matrix, cancer-associated stromal cells (MSCs and fibroblasts), tumor vasculature, and the immune system. The TME suppresses anti-tumor immune responses through multiple mechanisms that target both the innate and adaptive immune system of the patient. For example, tumors can recruit and induce regulatory T cells that suppress the anti-tumor activity of conventional T cells by elaborating specific chemokines such as CCL22. Tumors can also express molecules that inhibit the activity of T cells and NK cells, such as immune checkpoints such as PD-L1. Thus, targeting a single pathway is likely insufficient for achieving robust efficacy against solid tumors.

Non-limiting examples of effector molecules encompassed by the present disclosure include cytokines, antibodies, chemokines, nucleotides, peptides, enzymes, and oncolytic viruses. For example, MSCs may be engineered to express (and typically secrete) at least one, two, three or more of the following effector molecules: IL-12, IL-16, IFN-β, IFN-γ, IL-2, IL-15, IL-7, IL-36γ, IL-18, IL-1β, IL-21, OX40-ligand, CD40L, anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-CTLA-4 antibodies, anti-TGFβ antibodies, anti-TNFR2, MIP1α (CCL3), MIP1β (CCL5), CCL21, CpG oligodeoxynucleotides, and anti-tumor peptides (e.g., anti-microbial

peptides having anti-tumor activity, see, e.g., Gaspar, D. et al. *Front Microbiol.* 2013; 4: 294; Chu, H. et al. PLoS One. 2015; 10(5): e0126390, and website:aps.unmc.edu/AP/main.php).

Provided for herein is an engineered cell comprising: a) a promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, E1 comprises a polynucleotide sequence encoding a first effector molecule, L comprises a linker polynucleotide sequence, S2 comprises a polynucleotide sequence encoding a second signal peptide, E2 comprises a polynucleotide sequence encoding a second effector molecule, and wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule., In some aspects, the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

In some aspects, the cell is a mesenchymal stem cell (MSC). In some aspects, the cell is a stem cell. In some aspects, the cell is an immune cell. In some aspects, the cell is a natural killer (NK) cell. In some aspects, the cell is a NKT cell. In some aspects, the cell is an innate lymphoid cell. In some aspects, the cell is a tumor-infiltrating lymphocyte (TIL). In some aspects, the cell is a mast cell. In some aspects, the cell is a eosinophil. In some aspects, the cell is a basophil. In some aspects, the cell is a monocyte. In some aspects, the cell is a macrophage. In some aspects, the cell is a neutrophil. In some aspects, the cell is a myeloid cell. In some aspects, the cell is a dendritic cell. In some aspects, the cell is a T cell. In some aspects, the cell is a CD8+ T cell. In some aspects, the cell is a CD4+ T cell. In some aspects, the cell is a cytotoxic T lymphocyte (CTL). In some aspects, the cell is a viral-specific T cell. In some aspects, the cell is a gamma-delta T cell. In some aspects, the cell is a T regulatory cell. In some aspects, the cell is a B cell.

In some aspects, the promoter comprises an exogenous promoter polynucleotide sequence. In some aspects, the promoter comprises an endogenous promoter. In some aspects,

the promoter is operably linked to the expression cassette such that the polynucleotides are capable of being transcribed as a single polynucleotide comprising the formula S1 – E1 – L – S2 – E2. In some aspects, the linker polynucleotide sequence is operably associated with the translation of the first effector molecule and the second effector molecule as separate polypeptides. In some aspects, the linker polynucleotide sequence encodes a 2A ribosome skipping tag. In some aspects, the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A. In some aspects, the linker polynucleotide sequence encodes a T2A ribosome skipping tag. In some aspects, the linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES). In some aspects, the linker polynucleotide sequence encodes a cleavable polypeptide. In some aspects, the cleavable polypeptide comprises a Furin recognition polypeptide sequence. In some aspects, the linker polynucleotide sequence further encodes a Gly-comprising. Ser-comprising, or Gly-Ser comprising polypeptide sequence, e.g., a Gly-Ser-Gly polypeptide sequenceGly-Ser-Gly polypeptide sequence. In some aspects, the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus.

In some aspects, the linker polynucleotide sequence encodes a second promoter, wherein the promoter is operably linked to the expression cassette such that a first polynucleotide comprising the formula S1 – E1 is capable of being transcribed, wherein the second promoter is operably linked to the expression cassette such that a second polynucleotide comprising the formula S2 – E2 is capable of being transcribed, and wherein the first and the second polynucleotide are separate polynucleotides. In some aspects, the promoter and the second promoter are identical. In some aspects, the promoter and the second promoter are different.

In some aspects, the engineered cell is HLA-typed with reference to a subject in need of therapeutic treatment. In some aspects, the engineered cell is a human cell. In some aspects, the human cell is an isolated cell from a subject, e.g., the subject who will receive the cell. . In some aspects, the isolated cell is isolated from a tissue consisting of the group of: bone marrow, adipose tissue, the umbilical cord, fetal liver, muscle, and lung tissue. In some aspects, the engineered cell is a cultured cell.

In some aspects, the engineered MSC comprises a cellular marker phenotype comprising the cellular markers CD105+, CD73+,and CD90+. In some aspects, the cellular marker

phenotype further comprises a phenotype lacking or substantially lacking one or more cellular markers selected from the group consisting of: CD45, CD34, CD14, CD11b, CD79α, CD19, HLA class II, and combinations thereof. In some aspects, the engineered MSC comprises a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD45-, CD34-, CD14-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD11b-, CD79α-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD19-, HLA class II-; or a cellular marker phenotype comprising CD73+, CD90+, CD105+, and CD166+, CD11b-, CD14-, CD19-, CD34-, CD45-, and HLA-DR-. In some aspects, the cellular marker phenotype is determined or has been determined by flow-cytometry.

In some aspects, the engineered cell comprises a T cell. In some aspects, the engineered cell comprises a NK cell. In some aspects, the engineered cell comprises a NKT cell.

In some aspects, the cellular marker phenotype further comprises a cellular marker comprising a cognate receptor or a cognate receptor ligand for the first effector molecule, the second effector molecule, or the first and second effector molecules expressed in the engineered cells. In some aspects, the receptor is selected from the group consisting of: IL12RB1, IL12RB2, CCL7, and combinations thereof.

In some aspects, the promoter and/or the second promoter comprises a constitutive promoter. In some aspects, the constitutive promoter is selected from the group consisting of: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb. In some aspects, the promoter comprises an SFFV promoter. In some aspects, the promoter and/or the second promoter comprises an inducible promoter. In some aspects, the inducible promoter is selected from the group consisting of: minP, NFkB response element, CREB response element, NFAT response element, SRF response element 1, SRF response element 2, AP1 response element, TCF-LEF response element promoter fusion, Hypoxia responsive element, SMAD binding element, STAT3 binding site, inducer molecule responsive promoters, and tandem repeats thereof.

In some aspects, the first signal peptide or the second signal peptide comprises a native signal peptide native to the first effector molecule or the second effector molecule, respectively. In some aspects, the first signal peptide or the second signal peptide comprises a non-native signal peptide non-native to the first effector molecule or the second effector molecule, respectively. In some aspects, the non-native signal peptide is selected from the group consisting

of: IL12, IL2, optimized IL2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, and IL21.

In some aspects, the first signal peptide and the second signal peptide are identical. In some aspects, the polynucleotide sequence encoding the first signal peptide comprises a codon optimized polynucleotide sequence. In some aspects, the first secretion polypeptide is a human IL12 signal peptide.

In some aspects, the polynucleotide sequence encoding the second signal peptide comprises a codon optimized polynucleotide sequence. In some aspects, the second secretion polypeptide is a human IL21 signal peptide.

In some aspects, the first effector molecule is selected from a therapeutic class, wherein the therapeutic class is selected from the group consisting of: a cytokine, a chemokine, a growth factor, a co-activation molecule, a tumor microenvironment modifier a, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.

In some aspects, the second effector molecule is selected from a therapeutic class, wherein the therapeutic class is selected from the group consisting of: a cytokine, a chemokine, a growth factor, a co-activation molecule, a tumor microenvironment modifier, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme. In some aspects, the therapeutic class of the first effector molecule and the second effector molecule are different.

In some aspects, the first effector molecule and/or the second effector molecule is a modified effector molecule. In some aspects, the first effector molecule and/or the second effector molecule is modified to comprises a cell membrane tethering domain. In some aspects, the cell membrane tethering domain comprises a transmembrane-intracellular domain or a transmembrane domain. In some aspects, the cell membrane tethering domain comprises a cell surface receptor, or a cell membrane-bound portion thereof. In some aspects, the modified effector molecule is a fusion protein that comprises the cell surface receptor, or a cell membrane-bound portion thereof. In some aspects, the modified effector molecule further comprises a linker between the effector molecule and the cell membrane tethering domain. In some aspects, when expressed the modified effector molecule is tethered to a cell membrane of the engineered cell.

In some aspects, the cytokine is selected from the group consisting of: IL12, IL7, IL21, IL18, IL15, Type I interferons, and Interferon-gamma. In some aspects, the IL12 cytokine is an IL12p70 fusion protein. In some aspects, the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, CXCL10-11 fusion, CCL19, CXCL9, and XCL1. In some aspects, the growth factor is selected from the group consisting of: Flt3L and GM-CSF. In some aspects, the co-activation molecule is selected from the group consisting of: 4-1BBL and CD40L. In some aspects, the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, TGFbeta inhibitors, immune checkpoint inhibitors, VEGF inhibitors, and HPGE2. In some aspects, the TGFbeta inhibitors are selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and combinations thereof. In some aspects, the immune checkpoint inhibitors comprise anti-PD-1 antibodies. In some aspects, the VEGF inhibitors comprise anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof.

In some aspects, the first effector molecule and the second effector molecule are human-derived effector molecules.

In some aspects, the first effector molecule comprises interlekin 12 (IL12), for example, p35 and p40 as a dimer that is generally referred to in the art as IL-12p70. In some aspects, the first effector molecule comprises an IL12p70 fusion protein. In some aspects, the IL12p70 fusion protein is a human IL12p70 fusion protein. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 137. In some aspects, the human IL12 comprises the p35 subunit inidicated in SEQ ID NO: 137. In some aspects, the human IL12 comprises the p40 subunit inidicated in SEQ ID NO: 137.

In some aspects, the second effector molecule comprises CCL21a. In some aspects, the CCL21a is a human CCL21a. In some aspects, the second effector molecule comprises IL7. In some aspects, the IL7 is a human IL7. In some aspects, the second effector molecule comprises IL21. In some aspects, the IL21 is a human IL21.

In some aspects, the expression cassette further comprises an E3 comprising a polynucleotide sequence encoding a third effector molecule. In some aspects, the third effector molecule comprises Flt3L. In some aspects, the third effector molecule comprises anti-PD1. For example, anti-PD1 can be an anti-PD1 antibody. In some aspects, the expression cassette further comprises an E4 comprising a polynucleotide sequence encoding a fourth effector molecule. In

some aspects, the fourth effector molecule comprises adenosine deaminase. In some aspects, the third effector molecule comprises adenosine deaminase. In some aspects, the third effector molecule comprises CD40L. In some aspects, the third effector molecule comprises a CXCL10-CXCL11 fusion protein. In some aspects, the third effector molecule comprises XCL1.

5 In some aspects, the second effector molecule comprises Flt3L. In some aspects, the second effector molecule comprises a CXCL10-CXCL11 fusion protein. In some aspects, the second effector molecule comprises anti-PD1. In some aspects, the second effector molecule comprises CD40L.

In some aspects, the first effector molecule comprises interferon-beta and the second 10 effector molecule comprises Flt3L.

In some aspects, the polynucleotide sequence encoding the first effector molecule comprises a codon optimized polynucleotide sequence. In some aspects, the polynucleotide sequence encoding the second effector molecule comprises a codon optimized polynucleotide sequence.

15 In some aspects, the engineered cell comprises a polynucleotide sequence encoding the promoter and the expression cassette. In some aspects, the exogenous polynucleotide sequence comprises the sequence shown in SEQ ID NO: 144.

In some aspects, the exogenous polynucleotide sequence is integrated into the genome of the engineered cell. In some aspects, the exogenous polynucleotide sequence comprises one or 20 more viral vector polynucleotide sequences.

In some aspects, the one or more viral vector polynucleotide sequences comprise lentiviral, retroviral, retrotransposon, or adenoviral polynucleotide sequences.

In some aspects, the expression cassette further comprises following E2, an additional exogenous polynucleotide sequence comprising a formula, oriented from 5’ to 3’, comprising:

25 $\mathbf{(L - S - E)_X}$

wherein S comprises a polynucleotide sequence encoding a signal peptide, E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20 wherein the promoter is operably linked to the expression cassette, and wherein for each X the corresponding signal peptide is operably associated with the effector 30 molecule.

Also provided for herein is an engineered cell comprising a construct, wherein the construct comprises: a) an SFFV promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

5 wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide; E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein; L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide

10 sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus; S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide; E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and wherein the SFFV promoter is operably linked to the expression

15 cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic

20 cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 137. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 138. In some aspects, polynucleotide sequence encoding the human IL12p70 fusion protein comprises the sequence

25 shown in SEQ ID NO: 136. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 142. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 143. In some aspects, polynucleotide sequence encoding the human IL21 comprises the sequence shown in SEQ ID NO: 141. In some aspects, the linker comprises the sequence shown in SEQ ID NO: 140. In some aspects, the linker polynucleotide sequence comprises the sequence

30 shown in SEQ ID NO: 139. In some aspects, the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144.

Also provided for herein is an engineered cell comprising a construct, wherein the construct comprises: a) an SFFV promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide; E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein; L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus ; S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide; E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered cell is a mesenchymal stem cell (MSC). In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 137. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 138. In some aspects, polynucleotide sequence encoding the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 136. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 142. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 143. In some aspects, polynucleotide sequence encoding the human IL21 comprises the sequence shown in SEQ ID NO: 141. In some aspects, the linker comprises the sequence shown in SEQ ID NO: 140. In some aspects, the linker polynucleotide sequence comprises the sequence shown in SEQ ID NO: 139. In some aspects, the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144.

Also provided for herein is an engineered cell comprising a construct, wherein the construct comprises: a) an SFFV promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide; E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein; L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus ; S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide; E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered cell is a mesenchymal stem cell (MSC), wherein the MSC comprises a cellular marker phenotype comprising CD73+, CD90+, CD105+, and CD166+, CD11b-, CD14-, CD19-, CD34-, CD45-, and HLA-DR-. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 137. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 138. In some aspects, polynucleotide sequence encoding the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 136. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 142. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 143. In some aspects, polynucleotide sequence encoding the human IL21 comprises the sequence shown in SEQ ID NO: 141. In some aspects, the linker comprises the sequence shown in SEQ ID NO: 140. In some aspects, the linker polynucleotide sequence comprises the sequence shown in SEQ ID NO: 139. In some aspects, the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144. In some aspects, the cellular marker phenotype is determined or has been determined by flow-cytometry.

Also provided for herein is an engineered MSC comprising a construct, wherein the construct comprises: a) an SFFV promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5’ to 3’, comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide; E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein; L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus ; S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide; E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered MSC comprises a cellular marker phenotype comprising CD73+, CD90+, CD105+, and CD166+, CD11b-, CD14-, CD19-, CD34-, CD45-, and HLA-DR-. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 137. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 138. In some aspects, polynucleotide sequence encoding the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 136. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 142. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 143. In some aspects, polynucleotide sequence encoding the human IL21 comprises the sequence shown in SEQ ID NO: 141. In some aspects, the linker comprises the sequence shown in SEQ ID NO: 140. In some aspects, the linker polynucleotide sequence comprises the sequence shown in SEQ ID NO: 139. In some aspects, the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144. In some aspects, the cellular marker phenotype is determined or has been determined by flow-cytometry.

Also provided for herein is an engineered cell comprising a construct, wherein the construct comprises: a) an SFFV promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide; E1 comprises a polynucleotide sequence

encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein; L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus ; S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide; E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 137. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 138. In some aspects, polynucleotide sequence encoding the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 136. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 142. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 143. In some aspects, polynucleotide sequence encoding the human IL21 comprises the sequence shown in SEQ ID NO: 141. In some aspects, the linker comprises the sequence shown in SEQ ID NO: 140. In some aspects, the linker polynucleotide sequence comprises the sequence shown in SEQ ID NO: 139. In some aspects, the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144. In some aspects, the cell is a mesenchymal stem cell (MSC). In some aspects, the cell is a natural killer (NK) cell. In some aspects, the cell is a NKT cell. In some aspects, the cell is an innate lymphoid cell. In some aspects, the cell is a tumor-infiltrating lymphocyte (TIL). In some aspects, the cell is a mast cell. In some aspects, the cell is a eosinophil. In some aspects, the cell is a basophil. In some aspects, the cell is a monocyte. In some aspects, the cell is a macrophage. In some aspects, the cell is a neutrophil. In some aspects, the cell is a myeloid cell. In some aspects, the cell is a dendritic cell. In some aspects, the cell is

a T cell. In some aspects, the cell is a CD8+ T cell. In some aspects, the cell is a CD4+ T cell. In some aspects, the cell is a cytotoxic T lymphocyte (CTL). In some aspects, the cell is a viral-specific T cell. In some aspects, the cell is a gamma-delta T cell. In some aspects, the cell is a T regulatory cell. In some aspects, the cell is a B cell. In some aspects, the cell is a human cell.

5 In some aspects, the engineered cell is HLA-typed with reference to a subject in need of therapeutic treatment. In some aspects, the engineered cell is a human cell. In some aspects, the human cell is an isolated cell from a subject, e.g., the subject who will receive the cell. . In some aspects, the isolated cell is isolated from a tissue consisting of the group of: bone marrow, adipose tissue, the umbilical cord, fetal liver, muscle, and lung tissue. In some aspects, the
10 engineered cell is a cultured cell.

In some aspects, the engineered MSC comprises a cellular marker phenotype comprising the cellular markers CD105+, CD73+, and CD90+. In some aspects, the cellular marker phenotype further comprises a phenotype lacking or substantially lacking one or more cellular markers selected from the group consisting of: CD45, CD34, CD14, CD11b, CD79α, CD19,
15 HLA class II, and combinations thereof. In some aspects, the engineered MSC comprises a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD45-, CD34-, CD14-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD11b-, CD79α-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD19-, HLA class II-; or a cellular marker phenotype comprising CD73+, CD90+, CD105+, and CD166+, CD11b-, CD14-, CD19-,
20 CD34-, CD45-, and HLA-DR-. In some aspects, the cellular marker phenotype is determined or has been determined by flow-cytometry.

In some aspects, the engineered cell comprises a T cell. In some aspects, the T cell is a CD8+ T cell, a CD4+ T cell, a cytotoxic T lymphocyte (CTL), a viral-specific T cell, a gamma-delta T cell, or a T regulatory cell. In some aspects, the engineered cell comprises a NK cell. In
25 some aspects, the engineered cell comprises a NKT cell. In some aspects, the engineered cell comprises a monocyte cell. In some aspects, the engineered cell comprises a macrophage. In some aspects, the engineered cell comprises a TIL.

In some aspects, the exogenous polynucleotide sequence is integrated into the genome of the engineered cell. In some aspects, the exogenous polynucleotide sequence comprises one or
30 more viral vector polynucleotide sequences. In some aspects, the one or more viral vector polynucleotide sequences comprise lentiviral, retroviral, retrotransposon, or adenoviral

polynucleotide sequences. In some aspects, the one or more viral vector polynucleotide sequences comprise lentiviral polynucleotide sequences.

In some aspects, the cell secretes each effector molecule. In some aspects, the first effector molecule is secreted at a ratio that is 10 fold higher relative to secretion of the second effector molecule.

In some aspects, the cell further comprises an antigen recognizing receptor. In some aspects, the antigen recognizing receptor recognizes an antigen selected from the group consisting of: 5T4, ADAM9, ADGRE2, AFP, AXL, B7-H3, B7-H4, B7-H6, C4.4, CA6, Cadherin 3, Cadherin 6, CCR1, CCR4, CD117, CD123, CD131, CD133, CD138, CD142, CD166, CD25, CD244, CD30, CD300LF, CD33, CD352, CD37, CD38, CD44, CD56, CD66e, CD70, CD71, CD74, CD79b, CD80, CD93, CEA, CEACAM5, Claudin18.2, CLEC12A, cMet, CSPG4, CTLA, DLK1, DLL3, DR5, EGFR, EMB, ENPP3, EpCAM, EphA2, Ephrin A4, ETBR, FGFR2, FGFR3, FRalpha, FRb, FLT3, GAPT, GCC, GD2, GFRa4, gpA33, GPC3, gpNBM, GPRC5, HER2, IL-1RAP, IL-13R, IL-13Ra, IL-13Ra2, IL-8, IL-15, IL1RAP, Integrin aV, KIT, L1CAM, LAMP1, LAT2, Lewis Y, LeY, LILRA2, LILRB2, LIV-1, LRRC, LY6E, MCSP, Mesothelin, MLC1, MS4A3, MUC1, MUC16, MUC1C, MYADM, NaPi2B, Nectin 4, NKG2D, NOTCH3, NY ESO 1, Ovarin, P-cadherin, pan-Erb2, PIEZO1, PRAM1, PSCA, PSMA, PTK7, ROR1, S Aures, SCT, SLAMF7, SLC22A16, SLC17A9, SLITRK6, SPNS3, SSTR2, STEAP1, Survivin, TDGF1, TIM1, TROP2, VSTM1, and WT1.

In some aspects, the antigen recognizing receptor comprises an antigen-binding domain. In some aspects, the antigen-binding domain comprises an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb). In some aspects, the antigen-binding domain comprises a single chain variable fragment (scFv). In some aspects, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some aspects, the VH and VL are separated by a peptide linker. In some aspects, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

In some aspects, the antigen recognizing receptor is a chimeric antigen receptor (CAR) or T cell receptor (TCR). In some aspects, the antigen recognizing receptor is a chimeric antigen receptor (CAR). In some aspects, the CAR comprises one or more intracellular signaling

domains, and the one or more intracellular signaling domains are selected from the group consisting of: a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain. In some aspects, the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of: a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain. In some aspects, the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain.

Also provided for herein is a population of cells, the population of cells comprising any of the engineered cells described herein. In some aspects, the population of cells is enriched for the engineered cells.

In some aspects, the first effector molecule, the second effector molecule, or the first and second effector molecules expressed in the engineered cells promotes increased growth, viability, or growth and viability relative to cells in the population that do not express the first effector molecule, the second effector molecule, or the first and second effector molecules. In some aspects, the first effector molecule is IL12 or an IL12p70 fusion protein. In some aspects, the population of cells enriched for the engineered cells express IL12 receptor β1 or increased levels thereof, IL12 receptor β2 or increased levels thereof, or IL12 receptor β1 and IL12 receptor β2 or increased levels thereof. In some aspects, the second effector molecule is IL21. In some aspects, the second effector molecule is CCL21. In some aspects, the population of cells enriched for the engineered cells express a CCL21 receptor or increased levels thereof. In some aspects, the CCL21 receptor is CCR7.

Also provided for herein is a method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the engineered cells or the population of cells described herein.

5 Also provided for herein is a method of stimulating (*e.g.*, inducing) an immune response, the method comprising administering to a subject a therapeutically effective dose of any of the engineered cells or the population of cells described herein.

Also provided for herein is a method of providing anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of 10 any of the engineered cells any of the engineered cells or the population of cells described herein.

Also provided for herein is a method of treating a subject having cancer, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the engineered cells or the population of cells described herein.

15 Also provided for herein is a method of reducing tumor volume in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the engineered cells or the population of cells described herein.

In some aspects, the engineered cell is derived from the subject. In some aspects, the engineered cell is allogeneic with reference to the subject.

20 In some aspects, the tumor is selected from the group consisting of: an adenocarcinoma, an acute myeloid leukemia (AML), an acute lymphoblastic B-cell leukemia (BALL), an acute lymphoblastic T-cell leukemia (TALL), a B-cell prolymphocytic leukemia, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a chronic lymphocytic leukemia, a chronic myeloid leukemia (CML), a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a 25 liver tumor, a lung tumor, a lymphoma, a melanoma, a mesothelioma, a myelodysplasia, an ovarian tumor, a pancreatic tumor, a plasma cell myeloma, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor. In some aspects, the tumor is an ovarian tumor. In some aspects, the tumor is a tumor located in a peritoneal space.

Also provided for herein is an engineered cell comprising: a) a promoter; and b) an 30 exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5’ to 3’, comprising:

(L – S – E)$_X$

wherein S comprises a polynucleotide sequence encoding a signal peptide, E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 2 to 20, wherein the promoter is operably linked to the expression cassette,
5 wherein for the first iteration of the (L – S – E) unit L is absent, and wherein for each X the corresponding signal peptide is operably associated with the effector molecule, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid
10 cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

Also provided for herein is a population of cells comprising one or more engineered cells, wherein the one or more engineered cells comprise: a) a promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from
15 5’ to 3’, comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, E1 comprises a polynucleotide sequence encoding a first effector molecule, L comprises a linker polynucleotide sequence, S2 comprises a polynucleotide sequence encoding a second signal peptide, E2
20 comprises a polynucleotide sequence encoding a second effector molecule, and wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate
25 lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

Also provided for herein is a population of cells comprising one or more engineered cells, wherein the one or more engineered cells comprise: a) a promoter; and b) an exogenous
30 polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5’ to 3’, comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, E1 comprises a polynucleotide sequence encoding a first effector molecule, L comprises a linker polynucleotide sequence, S2 comprises a polynucleotide sequence encoding a second signal peptide, E2 comprises a polynucleotide sequence encoding a second effector molecule, and wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the first effector molecule, the second effector molecule, or the first and second effector molecules expressed in the engineered cells promotes increased growth, viability, or growth and viability relative to cells in the population that do not express the first effector molecule, the second effector molecule, or the first and second effector molecules, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

In some aspects, the one or more engineered cells express a cognate receptor or cognate receptor ligand for the first effector molecule, the second effector molecule, or the first and second effector molecules expressed in the engineered cells. In some aspects, the first effector molecule is IL12 or an IL12p70 fusion protein. In some aspects, the second effector molecule is IL21. In some aspects, the second effector molecule is CCL21.

Also provided for herein is a population of cells comprising one or more engineered cells, wherein the one or more engineered cells comprise a construct, wherein the construct comprises: a) an SFFV promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide; E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein; L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide

sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus; S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide; E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

Also provided for herein is a population of cells comprising one or more engineered cells, wherein the one or more engineered cells comprise a construct, wherein the construct comprises: a) an SFFV promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide; E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein; L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus; S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide; E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the first effector molecule, the second effector molecule, or the first and second effector molecules expressed in the engineered cells promotes increased growth, viability, or growth and viability relative to cells

in the population that do not express the first effector molecule, the second effector molecule, or the first and second effector molecules, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 137. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 138. In some aspects, polynucleotide sequence encoding the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 136. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 142. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 143. In some aspects, polynucleotide sequence encoding the human IL21 comprises the sequence shown in SEQ ID NO: 141. In some aspects, the linker comprises the sequence shown in SEQ ID NO: 140. In some aspects, the linker polynucleotide sequence comprises the sequence shown in SEQ ID NO: 139. In some aspects, the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144.

Also provided for herein is a method of producing a population of cells enriched for one or more receptors or receptor ligands, comprising culturing one or more cells under conditions where the one or more cells are contacted with a first effector molecule, a second effector molecule, or a first and a second effector molecule, wherein the contacted cells express one or more cognate receptors or cognate receptor ligands for the first effector molecule, the second effector molecule, or the first and second effector molecules, and wherein the first effector molecule, the second effector molecule, or the first and the second effector molecules increase growth, viability, or growth and viability of the contacted cells relative to cells cultured in the absence of the first effector molecule, the second effector molecule, or the first and second effector molecules.

In some aspects, the first effector molecule, the second effector molecule, or the first and second effector molecules are heterologously expressed in one or more cells, and the one or more cells are contacted with the first effector molecule, the second effector molecule, or the first and second effector molecules in an autocrine manner. In some aspects, the first effector

molecule, the second effector molecule, or the first and second effector molecules are expressed in one or more additional cells, and the one or more cells are contacted with the first effector molecule, the second effector molecule, or the first and second effector molecules in an paracrine manner. In some aspects, the one or more additional cells are a feeder cells. In some aspects, the one or more cells are cultured in media.

In some aspects, the one or more cells are contacted with the first effector molecule, the second effector molecule, or the first and second effector molecules by addition of a soluble first effector molecule, a soluble second effector molecule, or a soluble first and second effector molecules to the media. In some aspects, the soluble first effector molecule and/or soluble second effector molecule is a recombinant effector molecule.

In some aspects, the one or more cells are cultured under adherent conditions. In some aspects, the one or more cells are adhered onto a surface. In some aspects, the adhered cells are contacted with the first effector molecule, the second effector molecule, or the first and second effector molecules by exposing the one or more cells to first effector molecule, the second effector molecule, or the first and second effector molecules is immobilized on the surface.

In some aspects, the first effector molecule is IL12 or an IL12p70 fusion protein. In some aspects, the population of cells is enriched for IL12 receptor β1 (IL12Rβ1), enriched for IL12 receptor β2 (IL12Rβ2), or enriched for IL12Rβ1 and IL12Rβ2. In some aspects, the population of MSCs comprises a cellular marker phenotype comprising the cellular markers CD105+, CD73+, CD90+, IL12Rβ1+, and IL12Rβ2+. In some aspects, the cellular marker phenotype further comprises a phenotype lacking or substantially lacking one or more cellular markers selected from the group consisting of: CD45, CD34, CD14, CD11b, CD79α, CD19, HLA class II, and combinations thereof.

In some aspects, the population of cells comprises a cell selected from the group consisting of: natural killer (NK) cells, NKT cells, innate lymphoid cells, mast cells, eosinophils, basophils, monocytes, macrophages, neutrophils, and dendritic cells, T cells, CD8+ T cells, CD4+ T cells, gamma-delta T cells, and T regulatory cells, and B cells. In some aspects, the population of cells comprises a T cell, a NK cell, a NKT cell, a monocyte, a macrophage, or a myeloid derived cell.

In some aspects, the second effector molecule is IL21. In some aspects, the second effector molecule is CCL21. In some aspects, the population of cells is enriched for CCR7.

In some aspects, the population of MSCs comprises a cellular marker phenotype comprising the cellular markers CD105+, CD73+, CD90+, IL12Rβ1+, IL12Rβ2+, and CCR7+. In some aspects, the cellular marker phenotype further comprises a phenotype lacking or substantially lacking one or more cellular markers selected from the group consisting of: CD45, CD34, CD14, CD11b, CD79α, CD19, HLA class II, and combinations thereof.

Also provided for herein is a population of cells enriched for one or more receptors or receptor ligands produced by any of the methods described herein.

Also provided for herein is one or more proteins expressed by a polynucleotide sequence, wherein the polynucleotide sequence comprising a promoter and an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, E1 comprises a polynucleotide sequence encoding a first effector molecule, L comprises a linker polynucleotide sequence, S2 comprises a polynucleotide sequence encoding a second signal peptide, E2 comprises a polynucleotide sequence encoding a second effector molecule, and wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule.

Also provided for herein is one or more proteins expressed by a polynucleotide sequence, wherein the polynucleotide sequence comprises an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, E1 comprises a polynucleotide sequence encoding a first effector molecule, L comprises a linker polynucleotide sequence, S2 comprises a polynucleotide sequence encoding a second signal peptide, E2 comprises a polynucleotide sequence encoding a second effector molecule, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule.

Also provided for herein is an isolated polynucleotide sequence comprising a promoter and an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, E1 comprises a polynucleotide sequence encoding a first effector molecule, L comprises a linker polynucleotide sequence, S2 comprises a polynucleotide sequence encoding a second signal peptide, E2 comprises a polynucleotide sequence encoding a second effector molecule, and wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule.

Also provided for herein is an isolated polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, E1 comprises a polynucleotide sequence encoding a first effector molecule, L comprises a linker polynucleotide sequence, S2 comprises a polynucleotide sequence encoding a second signal peptide, E2 comprises a polynucleotide sequence encoding a second effector molecule, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule.

In some aspects, the promoter comprises an exogenous promoter polynucleotide sequence. In some aspects, the promoter comprises an endogenous promoter. In some aspects, the promoter is operably linked to the expression cassette such that the polynucleotides are capable of being transcribed as a single polynucleotide comprising the formula S1 – E1 – L – S2 – E2.

In some aspects, the linker polynucleotide sequence is operably associated with the translation of the first effector molecule and the second effector molecule as separate polypeptides. In some aspects, the linker polynucleotide sequence encodes a 2A ribosome skipping tag. In some aspects, the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A. In some aspects, the linker polynucleotide sequence encodes a T2A ribosome skipping tag. In some aspects, the linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES).

In some aspects, the linker polynucleotide sequence encodes a cleavable polypeptide. In some aspects, the cleavable polypeptide comprises a Furin recognition polypeptide sequence. In

some aspects, the linker polynucleotide sequence further encodes a Gly-comprising. Ser-comprising, or Gly-Ser comprising polypeptide sequence, e.g., a Gly-Ser-Gly polypeptide sequenceGly-Ser-Gly polypeptide sequence. In some aspects, the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus.

In some aspects, the linker polynucleotide sequence encodes a second promoter, wherein the promoter is operably linked to the expression cassette such that a first polynucleotide comprising the formula S1 – E1 is capable of being transcribed, wherein the second promoter is operably linked to the expression cassette such that a second polynucleotide comprising the formula S2 – E2 is capable of being transcribed, and wherein the first and the second polynucleotide are separate polynucleotides. In some aspects, the promoter and the second promoter are identical. In some aspects, the promoter and the second promoter are different.

In some aspects, the promoter and/or the second promoter comprises a constitutive promoter. In some aspects, the constitutive promoter is selected from the group consisting of: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb. In some aspects, the promoter comprises an SFFV promoter. In some aspects, the promoter and/or the second promoter comprises an inducible promoter. In some aspects, the inducible promoter is selected from the group consisting of: minP, NFkB response element, CREB response element, NFAT response element, SRF response element 1, SRF response element 2, AP1 response element, TCF-LEF response element promoter fusion, Hypoxia responsive element, SMAD binding element, STAT3 binding site, inducer molecule responsive promoters, and tandem repeats thereof.

In some aspects, the first signal peptide or the second signal peptide comprises a native signal peptide native to the first effector molecule or the second effector molecule, respectively. In some aspects, the first signal peptide or the second signal peptide comprises a non-native signal peptide non-native to the first effector molecule or the second effector molecule, respectively. In some aspects, the non-native signal peptide is selected from the group consisting of: IL12, IL2, optimized IL2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, and IL21.

In some aspects, the first signal peptide and the second signal peptide are identical. In some aspects, the polynucleotide sequence encoding the first signal peptide comprises a codon optimized polynucleotide sequence.

In some aspects, the first secretion polypeptide is a human IL12 signal peptide. In some aspects, the polynucleotide sequence encoding the second signal peptide comprises a codon optimized polynucleotide sequence. In some aspects, the second secretion polypeptide is a human IL21 signal peptide.

In some aspects, the first effector molecule is selected from a therapeutic class, wherein the therapeutic class is selected from the group consisting of: a cytokine, a chemokine, a growth factor, a co-activation molecule, a tumor microenvironment modifier a, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme. In some aspects, the second effector molecule is selected from a therapeutic class, wherein the therapeutic class is selected from the group consisting of: a cytokine, a chemokine, a growth factor, a co-activation molecule, a tumor microenvironment modifier, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme. In some aspects, the therapeutic class of the first effector molecule and the second effector molecule are different. In some aspects, the first effector molecule and/or the second effector molecule is a modified effector molecule.

In some aspects, the first effector molecule and/or the second effector molecule is modified to comprises a cell membrane tethering domain. In some aspects, the cell membrane tethering domain comprises a transmembrane-intracellular domain or a transmembrane domain. In some aspects, the cell membrane tethering domain comprises a cell surface receptor, or a cell membrane-bound portion thereof. In some aspects, the modified effector molecule is a fusion protein that comprises the cell surface receptor, or a cell membrane-bound portion thereof. In some aspects, the modified effector molecule further comprises a linker between the effector molecule and the cell membrane tethering domain. In some aspects, when expressed in a cell, the modified effector molecule is tethered to a cell membrane of the cell.

In some aspects, the cytokine is selected from the group consisting of: IL12, IL7, IL21, IL18, IL15, Type I interferons, and Interferon-gamma. In some aspects, the IL12 cytokine is an IL12p70 fusion protein. In some aspects, the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, CXCL10-11 fusion, CCL19, CXCL9, and XCL1. In some aspects, the growth factor is selected from the group consisting of: Flt3L and GM-CSF. In

some aspects, the co-activation molecule is selected from the group consisting of: 4-1BBL and CD40L. In some aspects, the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, TGFbeta inhibitors, immune checkpoint inhibitors, VEGF inhibitors, and HPGE2. In some aspects, the TGFbeta inhibitors are selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and combinations thereof. In some aspects, the immune checkpoint inhibitors comprise anti-PD-1 antibodies. In some aspects, the VEGF inhibitors comprise anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof.

In some aspects, the first effector molecule and the second effector molecule are human-derived effector molecules.

In some aspects, the first effector molecule comprises IL12. In some aspects, the first effector molecule comprises an IL12p70 fusion protein. In some aspects, the IL12p70 fusion protein is a human IL12p70 fusion protein.

In some aspects, the second effector molecule comprises CCL21a. In some aspects, the CCL21a is a human CCL21a. In some aspects, the second effector molecule comprises IL7. In some aspects, the IL7 is a human IL7. In some aspects, the second effector molecule comprises IL21. In some aspects, the IL21 is a human IL21.

In some aspects, the expression cassette further comprises an E3 comprising a polynucleotide sequence encoding a third effector molecule. In some aspects, the third effector molecule comprises Flt3L. In some aspects, the third effector molecule comprises anti-PD1.

In some aspects, the expression cassette further comprises an E4 comprising a polynucleotide sequence encoding a fourth effector molecule. In some aspects, the fourth effector molecule comprises adenosine deaminase.

In some aspects, the third effector molecule comprises adenosine deaminase. In some aspects, the third effector molecule comprises CD40L. In some aspects, the third effector molecule comprises a CXCL10-CXCL11 fusion protein. In some aspects, the third effector molecule comprises XCL1.

In some aspects, the second effector molecule comprises Flt3L. In some aspects, the second effector molecule comprises a CXCL10-CXCL11 fusion protein. In some aspects, the second effector molecule comprises anti-PD1. In some aspects, the second effector molecule comprises CD40L.

In some aspects, the first effector molecule comprises interferon-beta and the second effector molecule comprises Flt3L.

In some aspects, the polynucleotide sequence encoding the first effector molecule comprises a codon optimized polynucleotide sequence. In some aspects, the polynucleotide sequence encoding the second effector molecule comprises a codon optimized polynucleotide sequence.

In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 137. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 138. In some aspects, polynucleotide sequence encoding the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 136. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 142. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 143. In some aspects, polynucleotide sequence encoding the human IL21 comprises the sequence shown in SEQ ID NO: 141. In some aspects, the linker comprises the sequence shown in SEQ ID NO: 140. In some aspects, the linker polynucleotide sequence comprises the sequence shown in SEQ ID NO: 139. In some aspects, the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144.

Also provided for herein is an exogenous polynucleotide sequence comprising an SFFV promoter and an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide; E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein; L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus; S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide; E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule.

In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 137. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 138. In some aspects, polynucleotide sequence encoding the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 136. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 142. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 143. In some aspects, polynucleotide sequence encoding the human IL21 comprises the sequence shown in SEQ ID NO: 141. In some aspects, the linker comprises the sequence shown in SEQ ID NO: 140. In some aspects, the linker polynucleotide sequence comprises the sequence shown in SEQ ID NO: 139. In some aspects, the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144.

Also provided for herein is an exogenous polynucleotide sequence comprising an SFFV promoter and an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide; E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein; L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus; S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide; E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule; wherein the promoter is operably linked to the expression cassette such that the polynucleotides are capable of being transcribed as a single polynucleotide comprising the formula S1 – E1 – L – S2 – E2; and wherein the polynucleotide sequence comprises the polynucleotide sequence shown in SEQ ID NO: 144.

In some aspects, the exogenous polynucleotide sequence is encoded by a nucleic acid selected from the group consisting of: a DNA, a cDNA, an RNA, an mRNA, and a naked plasmid.

Also provided for herein is an expression vector comprising any of the exogenous polynucleotide sequences described herein. In some aspects, the expression vector is a viral vector. In some aspects, the viral vector is a lentiviral vector.

Also provided for herein is a pharmaceutical composition comprising any of the exogenous polynucleotide sequences described herein, and a pharmaceutically acceptable carrier.

Also provided for herein is a pharmaceutical composition comprising any of engineered cells described herein, and a pharmaceutically acceptable carrier.

An isolated cell comprising any of the exogenous polynucleotide sequences described herein, any of the expression vectors described herein, or any of the pharmaceutical compositions described herein.

In some aspects, the isolated cell is selected from the group consisting of: a T cell, a CD8+ T cell, a CD4+ T cell, a gamma-delta T cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a viral-specific T cell, a Natural Killer T (NKT) cell, a Natural Killer (NK) cell, a B cell, a tumor-infiltrating lymphocyte (TIL), an innate lymphoid cell, a mast cell, an eosinophil, a basophil, a neutrophil, a myeloid cell, a macrophage, a monocyte, a dendritic cell, an erythrocyte, a platelet cell, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, an MSC, an induced pluripotent stem cell (iPSC), and an iPSC-derived cell.

In some aspects, the isolated cell is an MSC.

In some aspects, the exogenous polynucleotide sequence is integrated into the genome of the cell. In some aspects, the exogenous polynucleotide sequence comprises one or more viral vector polynucleotide sequences.

In some aspects, the one or more viral vector polynucleotide sequences comprise lentiviral, retroviral, retrotransposon, or adenoviral polynucleotide sequences. In some aspects, the one or more viral vector polynucleotide sequences comprise lentiviral polynucleotide sequences.

In some aspects, the engineered cell is HLA-typed with reference to a subject in need of therapeutic treatment. In some aspects, the engineered cell is a human cell. In some aspects, the human cell is an isolated cell from a subject, e.g., the subject who will receive the cell. In some aspects, the isolated cell is isolated from a tissue consisting of the group of: bone marrow,

adipose tissue, the umbilical cord, fetal liver, muscle, and lung tissue. In some aspects, the cell is a cultured cell.

In some aspects, the MSC comprises a cellular marker phenotype comprising the cellular markers CD105+, CD73+,and CD90+. In some aspects, the cellular marker phenotype further comprises a phenotype lacking or substantially lacking one or more cellular markers selected from the group consisting of: CD45, CD34, CD14, CD11b, CD79α, CD19, HLA class II, and combinations thereof. In some aspects, the MSC comprises a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD45-, CD34-, CD14-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD11b-, CD79α-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD19-, HLA class II-; or a cellular marker phenotype comprising CD73+, CD90+, CD105+, and CD166+, CD11b-, CD14-, CD19-, CD34-, CD45-, and HLA-DR-. In some aspects, the cellular marker phenotype is determined or has been determined by flow-cytometry.

In some aspects, the cellular marker phenotype further comprises a cellular marker comprising a cognate receptor or a cognate receptor ligand for the first effector molecule, the second effector molecule, or the first and second effector molecules expressed in the cell. In some aspects, the receptor is selected from the group consisting of: IL12RB1, IL12RB2, CCL7, and combinations thereof.

In some aspects, the cell secretes each effector molecule. In some aspects, the first effector molecule is secreted at a ratio that is 10 fold higher relative to secretion of the second effector molecule.

In some aspects, the cell further comprises an antigen recognizing receptor. In some aspects, the antigen recognizing receptor comprises an antigen-binding domain. In some aspects, the antigen-binding domain comprises an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb). In some aspects, the antigen-binding domain comprises a single chain variable fragment (scFv). In some aspects, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some aspects, the VH and VL are separated by a peptide linker. In some aspects, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

In some aspects, the antigen recognizing receptor is a chimeric antigen receptor (CAR) or T cell receptor (TCR). In some aspects, the antigen recognizing receptor is a chimeric antigen receptor (CAR). In some aspects, the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of: a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain. In some aspects, the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of: a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain. In some aspects, the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain.

Also provided for herein is a virus comprising any of the exogenous polynucleotide sequences described herein or any of the expression vectors described herein. In some aspects, the virus is selected from the group consisting of: a lentivirus, a retrovirus, a retrotransposon, and an adenovirus. In some aspects, the virus is a lentivirus.

Also provided for herein is a method of reducing tumor volume in a subject, the method comprising delivering to a subject having a tumor a composition comprising cells engineered to produce multiple effector molecules that modulate tumor-mediated immunosuppressive mechanisms, in an effective amount to reduce the volume of the tumor, wherein the engineered cells comprise: a) a promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5’ to 3’, comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, E1 comprises a polynucleotide sequence encoding a first effector molecule, L comprises a linker polynucleotide sequence, S2 comprises a polynucleotide sequence encoding a second signal peptide, E2 comprises a polynucleotide sequence encoding a second effector molecule, and wherein the
5 promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte,
10 macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

Also provided for herein is a method of reducing tumor volume in a subject, the method comprising delivering to a subject having a tumor a composition comprising cells engineered to produce IL12 and IL21, in an effective amount to reduce the volume of the tumor, wherein the
15 engineered cells comprise a construct, wherein the construct comprises: a) an SFFV promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the
20 first signal peptide is a human IL12 signal peptide; E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein; L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-
25 terminus; S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide; E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is
30 operably linked to the second effector molecule, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer

(NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

5 Also provided for herein is a method of stimulating (*e.g.*, inducing) an immune response, the method comprising delivering to a subject a composition comprising cells engineered to produce multiple effector molecules that modulate tumor-mediated immunosuppressive mechanisms, in an effective amount to induce an immune response, wherein the engineered cells comprise: a) a promoter; and b) an exogenous polynucleotide sequence comprising an 10 expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, E1 comprises a polynucleotide sequence encoding a first effector molecule, L comprises a linker polynucleotide sequence, S2 comprises a polynucleotide sequence encoding a second signal peptide, E2 15 comprises a polynucleotide sequence encoding a second effector molecule, and wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate 20 lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

Also provided for herein is a method of stimulating (*e.g.*, inducing) an immune response in a subject, the method comprising delivering to a subject a composition comprising cells 25 engineered to produce IL12 and IL21, in an effective amount to induce an immune response, wherein the engineered cells comprise a construct, wherein the construct comprises: a) an SFFV promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

30 wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide; E1 comprises a polynucleotide sequence

encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein; L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus; S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide; E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 137. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 138. In some aspects, polynucleotide sequence encoding the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 136. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 142. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 143. In some aspects, polynucleotide sequence encoding the human IL21 comprises the sequence shown in SEQ ID NO: 141. In some aspects, the linker comprises the sequence shown in SEQ ID NO: 140. In some aspects, the linker polynucleotide sequence comprises the sequence shown in SEQ ID NO: 139. In some aspects, the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144.

In some aspects, the method further comprises administering a checkpoint inhibitor. In some aspects, the checkpoint inhibitor is an anti-PD-1 antibody, anti-PD-1L antibody or an anti-CTLA-4 antibody. In some aspects, the method further comprises administering an anti-CD40 antibody.

In some aspects, the tumor is selected from the group consisting of: an adenocarcinoma, an acute myeloid leukemia (AML), an acute lymphoblastic B-cell leukemia (BALL), an acute

lymphoblastic T-cell leukemia (TALL), a B-cell prolymphocytic leukemia, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a chronic lymphocytic leukemia, a chronic myeloid leukemia (CML), a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a lymphoma, a melanoma, a mesothelioma, a myelodysplasia, an
5 ovarian tumor, a pancreatic tumor, a plasma cell myeloma, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor. In some aspects, the tumor is an ovarian tumor. In some aspects, the tumor is a tumor located in a peritoneal space.

In some aspects, the administering comprises systemic administration, intraperitoneal administration, or intratumoral administration.

10 In some aspects, the volume of the tumor is reduced by at least 25% relative to a control, optionally wherein the control is an unmodified cell. In some aspects, the volume of the tumor is reduced by at least 50% relative to a control, optionally wherein the control is an unmodified cell. In some aspects, the volume of the tumor is reduced by at least 75% relative to a control, optionally wherein the control is an unmodified cell.

15 Also provided for herein is a method of reducing tumor volume in a subject, the method comprising delivering to a subject having a tumor a composition capable of engineering an cell to produce multiple effector molecules that modulate tumor-mediated immunosuppressive mechanisms, in an effective amount to reduce the volume of the tumor, wherein each engineered cell comprises: a) a promoter; and b) an exogenous polynucleotide sequence comprising an
20 expression cassette described in a formula, oriented from 5’ to 3’, comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, E1 comprises a polynucleotide sequence encoding a first effector molecule, L comprises a linker polynucleotide sequence, S2 comprises a polynucleotide sequence encoding a second signal peptide, E2
25 comprises a polynucleotide sequence encoding a second effector molecule, and wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate
30 lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte,

macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

Also provided for herein is method of reducing tumor volume in a subject, the method comprising delivering to a subject having a tumor a composition capable of engineering a cell to produce IL12 and IL21, in an effective amount to reduce the volume of the tumor, wherein the engineered cell comprises a construct, wherein the construct comprises: a) an SFFV promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide; E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein; L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus; S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide; E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

Also provided for herein is a method of stimulating (*e.g.*, inducing) an immune response in a subject, the method comprising delivering to a subject a composition capable of engineering an cell to produce multiple effector molecules that modulate tumor-mediated immunosuppressive mechanisms, in an effective amount to induce an immune response, wherein

the engineered cell comprises: a) a promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, E1 comprises a polynucleotide sequence encoding a first effector molecule, L comprises a linker polynucleotide sequence, S2 comprises a polynucleotide sequence encoding a second signal peptide, E2 comprises a polynucleotide sequence encoding a second effector molecule, and wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

Also provided for herein is method of stimulating (*e.g.*, inducing) an immune response in a subject, the method comprising delivering to a subject a composition capable of engineering a cell to produce IL12 and IL21, in an effective amount to induce an immune respone, wherein the engineered cell comprises a construct, wherein the construct comprises: a) an SFFV promoter; and b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide; E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein; L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus; S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide; E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and wherein the SFFV promoter is operably linked to the expression cassette, the first

signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 137. In some aspects, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 138. In some aspects, polynucleotide sequence encoding the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 136. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 142. In some aspects, the human IL21 comprises the sequence shown in SEQ ID NO: 143. In some aspects, polynucleotide sequence encoding the human IL21 comprises the sequence shown in SEQ ID NO: 141. In some aspects, the linker comprises the sequence shown in SEQ ID NO: 140. In some aspects, the linker polynucleotide sequence comprises the sequence shown in SEQ ID NO: 139. In some aspects, the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144.

In some aspects, the composition comprises a delivery system selected from the group consisting of: a viral system, a transposon system, and a nuclease genomic editing system. In some aspects, the viral system is selected from the group consisting of: a lentivirus, a retrovirus, a retrotransposon, and an adenovirus. In some aspects, the nuclease genomic editing system is selected from the group consisting of: a zinc-finger system, a TALEN system, and a CRISPR system.

In some aspects, the tumor is selected from the group consisting of: an adenocarcinoma, an acute myeloid leukemia (AML), an acute lymphoblastic B-cell leukemia (BALL), an acute lymphoblastic T-cell leukemia (TALL), a B-cell prolymphocytic leukemia, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a chronic lymphocytic leukemia, a chronic myeloid leukemia (CML), a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a lymphoma, a melanoma, a mesothelioma, a myelodysplasia, an

ovarian tumor, a pancreatic tumor, a plasma cell myeloma, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

In some aspects, the administering comprises systemic administration, intraperitoneal administration, or intratumoral administration.

5

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows treatment using syngeneic and allogeneic MSCs expressing IL12p70/CCL21a in a CT26 model.

FIG. 2 shows rechallenge of tumor free mice with CT26 tumors previously treated using 10 syngeneic and allogeneic MSCs expressing IL12p70/CCL21a in a CT26 model.

FIG. 3 shows data indicating that intraperitoneally injected murine BM-derived MSCs (BM-MSCs) home to the tumor site of 4T1 breast cancer cells *in vivo*. Fluorescently labeled BM-MSCs (therapeutic cells) were injected into mice bearing 4T1 breast tumor cells. The breast tumor cells express a luciferase reporter. The first top two panels on the left show imaging of 15 therapeutic cells (BM-MSCs) in mice bearing tumors on day 1 and on day 7 after injection as indicated. The third top panel on the left shows imaging of tumor cells in mice bearing tumors on day 7 after injection. The bottom two panels on the left show imaging of therapeutic cells in normal mice not bearing tumors on day 1 and on day 7 after injection as indicated. A schematic showing the effect of tumors on homing of therapeutic cells is provided on the far right.

20 **FIG. 4** shows data indicating that engineered MSCs expressing IL-12 and CCL21a induced significant tumor growth delay in an orthotopic mouse model of breast cancer. The chart on the left shows the effects of engineered MSCs on 4T1 breast tumor growth in mice (n = 8). Each line in the chart represents tumor volume in mice receiving intraperitoneal injection of either control MSC growth media or engineered MSCs on day 0 and day 7. Mice received 25 intraperitoneal injection of engineered MSCs expressing IL-12 and engineered MSCs expressing CCL21a. Tumor volume was determined by caliper measurements every other day. Data represent mean ± SEM. $^{*}p< 0.05$, $^{**}p< 0.005$ as compared to control media group. The schematic on the right shows a timeline of treatment and the effect of engineered MSCs expressed combinatorial genes IL-12 and CCL21a on tumor burden in treated mice.

30 **FIG. 5A** includes data indicating that engineered MSCs expressing IFN-β, IFN-γ, IL-12, CCL21a, or combinations thereof inhibit tumor growth in an orthotopic mouse model of breast

cancer (4T1 triple negative breast carcinoma). Each effector was expressed by a different MSC, and the MSCs were combined (at a 1:1 ratio) for combinatorial treatment. Each chart shows the effect of engineered MSCs expressing the indicated immunotherapies alone or in combination on the growth of 4T1 breast tumors in mice (n = 6-8). Each line of **FIG. 5A** represents an individual mouse. The left graph of **FIG. 5B** shows the tumor weight for individual mice in each treatment on day 14. The right graph of **FIG. 5B** shows the tumor volume represented as mean ± SEM for mice receiving each treatment over time.

FIG. 6A includes data indicating that engineered MSCs expressing OX40L, TRAIL, IL15, cGAS, or combinations thereof do not inhibit tumor growth significantly in an orthotopic mouse model of breast cancer (4T1 triple negative breast carcinoma). Each effector was expressed by a different MSC, and the MSCs were combined (at a 1:1 ratio) for combinatorial treatment. Each chart shows the effect of engineered MSCs expressing the indicated immunotherapies alone or in combination on the growth of 4T1 breast tumors in mice (n = 6-8). Each line of **FIG. 6A** represents an individual mouse. The left graph of **FIG. 6B** shows the tumor weight for individual mice in each treatment. The right graph of **FIG. 6B** shows body weight represented as mean ± SEM for mice receiving each treatment over time.

FIG. 7A includes data indicating that engineered MSCs expressing IL-12 and CCL21a inhibit tumor growth in an orthotopic mouse model of breast cancer (4T1 triple negative breast carcinoma); however the addition of anti-CD40 antibody does not reduce tumor growth. Each effector was expressed by a different MSC, and the MSCs were combined (at a 1:1 ratio) for combinatorial treatment. Each chart shows the effect of engineered MSCs expressing the indicated immunotherapies alone or in combination on the growth of 4T1 breast tumors in mice (n = 6-8). Each line of **FIG. 7A** represents an individual mouse. **FIG. 7B** shows the tumor weight for individual mice in each treatment.

FIG. 8A includes data indicating that engineered MSCs expressing OX40L, TRAIL, IL15, HACvPD-1, or combinations thereof do not inhibit tumor growth significantly in an subcutaneous mouse model of breast cancer (4T1 triple negative breast carcinoma). Each effector was expressed by a different MSC, and the MSCs were combined (at a 1:1 ratio) for combinatorial treatment. Each chart shows the effect of engineered MSCs expressing the indicated immunotherapies alone or in combination on the growth of 4T1 breast tumors in mice (n = 6-8). Each line of **FIG. 8A** represents an individual mouse. The left graph of **FIG. 8B**

shows the tumor weight for individual mice in each treatment. The right graph of **FIG. 8B** shows body weight represented as mean ± SEM for mice receiving each treatment over time.

FIG. 9A includes data indicating that engineered MSCs expressing IL-12 and CCL21a inhibit tumor growth in an orthotopic mouse model of breast cancer (4T1 triple negative breast 5 carcinoma); however the combination of MSCs expressing CCL21a, IL-36 gamma and IL-7 does not reduce tumor growth. Some of the effector combinations tested, however, may cause toxicity. Each effector was expressed by a different MSC, and the MSCs were combined (at a 1:1 ratio) for combinatorial treatment. Each chart shows the effect of engineered MSCs expressing the indicated immunotherapies alone or in combination on the growth of 4T1 breast 10 tumors in mice (n = 6-8). Each line of **FIG. 9A** represents an individual mouse. **FIG. 9B** shows the tumor weight for individual mice in each treatment.

FIGs. 10A-10B include data from a GFP dose escalation study for toxicity and screening. **FIG. 10A** shows that engineered MSCs expressing GFP do not elicit toxicity. Each effector was expressed by a different MSC, and the MSCs were combined (at a 1:1 ratio) for 15 combinatorial treatment. Each chart shows the effect of engineered MSCs expressing the indicated immunotherapies alone or in combination on the growth of 4T1 breast tumors in mice (n = 6-8). Each line of **FIG. 10A** represents an individual mouse. **FIG. 10B** shows the tumor weight for individual mice in each treatment.

FIG. 11A shows that engineered human MSCs do not home to mouse 4T1 tumors. **FIG.** 20 **11B** shows the tumor weight for individual mice in each treatment. Efficacy was determined by tumor volume from caliper measurement every other day.

FIG. 12 includes data showing that IL-12 and CCL21a can reduce tumor expansion.

FIG. 13A includes data indicating that engineered MSCs expressing IL-12 and CCL21 are sufficient to inhibit tumor growth in an orthotopic mouse model of breast cancer (4T1 triple 25 negative breast carcinoma), and the addition of a checkpoint inhibitor (anti-PD-1 antibody or anti-CTLA-4 antibody) did not increase efficacy. Each effector was expressed by a different MSC, and the MSCs were combined (at a 1:1 ratio) for combinatorial treatment, and the checkpoint inhibitor was injected separately. Each chart shows the effect of engineered MSCs expressing the indicated immunotherapies alone or in combination on the growth of 4T1 breast 30 tumors in mice (n = 6-8). Each line of **FIG. 13A** represents an individual mouse. **FIG. 13B** shows the tumor weight for individual mice in each treatment.

FIG. 14 shows data indicating that engineered MSCs expressing IL-12 and CCL21a induced significant tumor growth delay in a mouse model of colorectal cancer. The graph on the left shows the effects of engineered MSCs on CT26 colorectal tumor growth in mice (n = 8). Each line in the chart represents tumor volume in mice receiving intraperitoneal injection of either control MSC growth media or engineered MSCs on day 0 and day 7. Mice received intraperitoneal injection of engineered MSCs expressing IL-12 and engineered MSCs expressing CCL21a. Tumor volume was determined by caliper measurements every other day. Data represent mean ± SEM. *p< 0.05, **p< 0.005 as compared to control media group. The schematic on the right shows a timeline of treatment and the effect of engineered MSCs expressed combinatorial genes IL-12 and CCL21a on tumor burden in treated mice.

FIG. 15 is a graph showing tumor growth kinetics in the CT26 mouse model to determine optimal time for dosing the engineered MSC cells.

FIGs. 16A-16B include data indicating the effects of engineered MSCs expressing IL-12 and CCL21a combined with anti-CD40 or anti-CTLA4 antibodies on average tumor growth in a syngeneic mouse model of colon cancer. Mice bearing CT26 colon tumors were treated with one of seven treatments (n=5-6 per treatment group). MSC-IL-12+MSC-CCL21a indicates treatment with engineered cells expressing IL-12 and with engineered cells expressing CCL21a (at a 1:1 ratio) for combinatorial treatment. The left graph of **FIG. 16B** shows the tumor weight for individual mice in each treatment. The right graph of **FIG. 16B** shows the tumor volume represented as mean ± SEM for mice receiving each treatment over time.

FIGs. 17A-17B include data from a dose-dependent long-term survival study. **FIG. 17A** shows the tumor volume of the individual group. **FIG. 17B** shows body weight (top), tumor volume (bottom), and survival rate (right).

FIG. 18A includes data indicating that engineered MSCs expressing IL-12, CCL21a, and either IL15 or HACvPD-1 inhibit tumor growth significantly in a moue model colorectal cancer. Each effector was expressed by a different MSC, and the MSCs were combined (at a 1:1 ratio) for combinatorial treatment. Each chart shows the effect of engineered MSCs expressing the indicated immunotherapies alone or in combination on the growth of CT26 colorectal tumors in mice (n = 6-8). Each line of **FIG. 18A** represents an individual mouse. **FIG. 18B** shows the tumor weight for individual mice in each treatment. **FIG. 18C** is a representative graph of the infiltrating immune population within the tumor microenvironment. **FIG. 18D** shows the

percentage of regulatory T cells (Treg) in the total CD3 population. There was a significant decrease in the numbers of Tregs in the tumor microenvironment treated with engineered MSC-IL2 and CCL21a. **FIG. 18E** correlates the percentage of immune infiltration with tumor weight. Samples with high lymphocytes (CD3+) were found to correlate with low tumor weight, while
5 samples with high myeloid (CD11b+) infiltration were correlated with higher tumor burden.

FIG. 19 shows the tumor volume for individual mice in each treatment. Efficacy was determined by tumor volume from caliper measurement every other day.

FIG. 20 shows the tumor weight for individual mice in each treatment. Efficacy was determined by tumor volume from caliper measurement every other day.

10 **FIGs. 21A-21B** show the kinetics of CT26-LUC (luciferase) tumor growth in the intraperitoneal space. A CT26 cell line was injected at day 0 and three (3) mice were harvested at day 7, day 10, day 14, and day 18 to determine the kinetics of tumor growth. The first row of **FIG. 21A** measures the mice body weight and ROI with an IVIS imager to monitor tumor burden. The second row monitors the tumor weight and the ROI of the tumor of individual mice
15 in each group. The third row correlates the tumor weight with either whole body ROI or tumor ROI. **FIG. 21B** shows the immune profile of three (3) mice in the day 18 group to better characterize the tumor microenvironment.

FIG. 22A includes data indicating that engineered MSCs expressing IL-12 and CCL21a inhibit tumor growth in a subcutaneous mouse model of colorectal cancer; however the
20 combination of MSCs expressing CCL21a and IL-36 gamma or IL-7 does not reduce tumor growth. Each effector was expressed by a different MSC, and the MSCs were combined (at a 1:1 ratio) for combinatorial treatment. Each chart shows the effect of engineered MSCs expressing the indicated immunotherapies alone or in combination on the growth of CT26 colon tumors in mice (n = 6-8). Each line of **FIG. 22A** represents an individual mouse. **FIG. 22B**
25 shows the tumor weight for individual mice in each treatment group.

FIGs. 23A-23B include tumor immune infiltrate statistics from the experiment represented by **FIGs. 22A-22B**. Three mice were selected from PBS, Naïve MSC, and MSC-IL12+MSC-CCL21a (combo) group to run flow cytometry to immune profile tumor microenvironment. **FIG. 23A** shows a significant increase in infiltrating CD3 and CD8
30 cytotoxic T population in the combo group compared to the group dosed with naïve MSC. **FIG.**

23B shows a significant reduction in granulocytic myeloid-derived suppressor cells (gMDSCs) and macrophage population in the combo group compared to group treated with Naïve MSC.

FIGs. 24A-24B include data relating to immune percentage and tumor weight, relating to the experiments represented by **FIGs. 22A-22B**. **FIG. 24A** and **FIG. 24B** show that samples with more CD3+ and CD8+ T cells (top left and center graph) correlate strongly with a decrease in tumor weight. These figures also show that samples with fewer CD11b myeloid cells, including macrophage, dendritic cells, and MDSC, display lower tumor burden (lower center and right graph of **FIG. 24A** and upper row of **FIG. 24B**).

FIGs. 25A-25B include data from MSC-IL-12+CCL21a therapy in intraperitoneal and subcutaneous colorectal cancer mouse models. Three different lots of a lentiviral transduced line was tested for MSC-IL12 and CCL21a (TLOO8-3/4, TL019-01/02, and TL022-01/02; each TL number represents one lot). **FIG. 25A** shows that all three lots of MSC-IL12 + MSC-CCL21a can reduce tumor burden in both subcutaneous and intraperitoneal model (first 5 graphs are from the SC model and last 3 are from the IP model). Tumors from all mice were collected on day 11. **FIG. 25B** shows the average tumor weight from each group.

FIG. 26A includes data indicating that engineered combination treatment MSC-IL-12+MSC-CCL21a, or MSC-CCL21a+MSC-IFN-β, inhibit tumor growth in a subcutaneous mouse model of colorectal cancer; however the combination of MSCs expressing CCL21a and s41BBL does not reduce tumor growth. Each effector was expressed by a different MSC, and the MSCs were combined (at a 1:1 ratio) for combinatorial treatment. Each chart shows the effect of engineered MSCs expressing the indicated immunotherapies alone or in combination on the growth of CT26 tumors in mice (n = 6-8). Each line of **FIG. 26A** represents an individual mouse. **FIG. 26B** shows the tumor weight for individual mice in each treatment. MSC-IL12 + MSC-CCL21a shows best efficacy compared to mice injected with naïve MSC. Treatment efficacy was also observed in the group treated with MSC-IFNb + MSC-CCL21a.

FIGs. 27A-27B provide additional data from the experiment represented by **FIGs. 26A-26B**. **FIGs. 27A-27B** are graphs that show immune profiles of each group treated with indicated engineered MSC. A consistent decrease in macrophage population was observed after treating with MSC-IL12 + MSC-CCL21a (**FIG. 27A**). A general trend of increased infiltration in CD3+ population and decreased infiltration in CD11b+ population was also observed when compared to group treated with MSC-IL12 + MSC-CCL21a against naïve MSC (**FIG. 27A** and **FIG. 27B**).

FIGs. 28A-28B also provide additional data from the experiment represented by **FIGs. 26A-26B**. **FIG. 28A-28B** show the correlation of immune infiltration with tumor weight. Samples with low macrophage and dendritic cells have lower tumor burden (**FIG. 28B**, top center and top right).

5 **FIG. 29** shows graphs combining the *in vivo* data from the colorectal cancer models above (**FIG. 22A** and **FIG. 26A**). The combined CT26 data from **FIG. 22A** and **FIG. 26A** capture three groups: Tumor only (PBS), treated with naïve MSC, and treated with MSC-IL12 + MSC-CCL21a.

FIGs. 30A-30C also show combined data from **FIG. 22A** and **FIG. 26A**. The graphs 10 show the average number of immune infiltration from the flow cytometry experiment data. Statistical significance was observed in CD8+T from **FIG. 30A**, demonstrating the ability of MSC-IL12 + MSC-CCL21a to repolarize tumor microenvironment and allow more cytotoxic T cell infiltration. Furthermore, there was a reduction in CD11b+ myeloid population infiltration in the groups that were treated by MSC-IL12 + MSC-CCL21a (**FIG. 30B**). The data collected 15 show that the dendritic cells and the macrophage population was statistical significance.

FIG. 31 shows the vector map of pL17D.

FIG. 32 shows MSCs engineered to express different effector molecules either alone or in combination and their efficacy in reducing CT26 tumor burden in an IP tumor model as assessed by BLI levels.

20 **FIG. 33** shows MSCs engineered to express different effector molecules either alone or in combination and their efficacy in reducing B16F10 tumor burden in an IP tumor model as assessed by BLI levels.

FIG. 34 shows lentiviral expression vector map for expression of human IL12 (p70) and human CCL21a from a single lentiviral expression vector.

25 **FIG. 35** shows production engineered hMSCs of both hIL12 (**Fig. 35A**) and hCCL21a (**Fig. 35B**), as assessed by cytokine ELISA.

FIG. 36 shows a transwell assay demonstrating functional T cell modulation by hIL12 produced from MSCs as assessed by IFNγ production.

FIG. 37 shows homing to tumors by MSCs in IP tumor-bearing mice tumors as assessed 30 by bioluminescence imaging. **FIG. 37A-D** shows homing in a CT26 tumor model (images shown in **Fig. 37A**, quantification summary of images in **Fig. 37B**), quantitative real time PCR

(**Fig. 37C**), and fluorescence microscopy against firefly luciferase (**Fig. 37D**). **FIG. 37E** shows homing in a B16F10 tumor model (quantification summary of images).

FIG. 38 shows IL12p70 expressing MSCs leading to reduction in tumor burden as assessed by BLI (top panels and bottom left panel) and a complete elimination of detectable intraperitoneal tumors by tumor weight (bottom right panel) in a CT26 IP model.

FIG. 39 shows IL12p70 expressing MSCs leading to reduction in tumor burden as assessed by BLI (top panels and bottom left panel) and a complete elimination of detectable intraperitoneal tumors by tumor weight (bottom right panel) in a B16F10 IP model.

FIG. 40 shows IL12p70/CCL21a expressing MSCs leading to reduction in tumor burden as assessed by BLI (top panels and bottom left panel) and a complete elimination of detectable intraperitoneal tumors by tumor weight (bottom right panel) in a CT26 IP model. **Fig. 40A** shows the mean tumor burden as assessed by BLI for PBS treated (circle), MSC-Flag-Myc ("Naïve MSC" square), and IL12p70/CCL21a expressing MSCs (triangle). **Fig. 40B** shows the tumor burden in individual mice as assessed by BLI for PBS treated, MSC-Flag-Myc ("Naïve MSC"), and IL12p70/CCL21a expressing MSCs (left, middle, and right panels, respectively). **Fig. 40C** shows treatment with IL12p70/CCL21a expressing MSCs led to prolonged survival (100% survival greater than 90 days), while control treated mice all died or were euthanized by Day 20.

FIG. 41 shows treatment with IL12p70 expressing MSCs led to prolonged survival

FIG. 42 shows relative growth of genetically engineered MSCs across different MOIs (95000, 9500, 950, or uninfected) in three separate donors (**Fig. 42A**, Donor 1; **Fig. 42B**, Donor 2; **Fig. 42C**, Donor 3).

FIG. 43 shows two independent human BM-MSC cell lines from 2 different donors (top and bottom row, respectively) that were transduced with constructs containing various promoters driving EGFP expression. Percent GFP (left panels) and MFI (right panels) of engineered cells at day 25 post transduction is shown.

FIG. 44 shows two independent human BM-MSC cell lines from 2 different donors that were transduced with constructs containing various promoters driving EGFP expression. Shown is EGFP MFI tracked over time (day 7 to day 28 post-transduction) for either the two independent human BM-MSC cell lines individually (left panel) or with data from the two independent human BM-MSC cell lines combined (right panel).

FIG. 45 shows secretion of IL-12p70 by engineered MSCs as assessed by ELISA.

FIG. 46 shows secretion of IL-21 by engineered MSCs as assessed by ELISA.

FIG. 47 shows the ratio of secreted IL-12p70 to IL-21 by engineered MSCs as assessed by ELISA.

5 **FIG. 48** shows results of a functional reporter assay for IL-12p70 using HEK-293T cells with a STAT4-SEAP reporter to assess cytokine production and secretion by engineered MSCs.

FIG. 49 shows a results of a functional reporter assay for IL-21 using intracellular phospho-flow to quantify phospho-STAT1 (left panel) and phospho-STAT3 (right panel) in NK-92 human natural killer cells to assess cytokine production and secretion by engineered MSCs.

10 **FIG. 50** shows results of a functional reporter assay for IL-12 using a IL21R-U2OS IL21R/IL2RG dimerization reporter to assess cytokine production and secretion by engineered MSCs.

FIG. 51A shows MSCs engineered to express different effector molecules either alone or in combination and their efficacy in reducing CT26 tumor burden in an IP tumor model as 15 assessed by BLI levels.

FIG. 51B shows MSCs engineered to express different effector molecules either alone or in combination and their efficacy in reducing B16F10 tumor burden in an IP tumor model as assessed by BLI levels.

FIG. 52 shows efficacy of treatment using IL12p70-expressing MSCs, IL21-expressing 20 MSCs, and the combination of IL12p70 and IL21-expressing MSCs as assessed by BLI (**Fig. 52A** left panel) and by tumor weight (**Fig. 52A** right panel) in a CT26 model. **Fig. 52B** demonstrates the BLI luciferase measurements of individual mice.

FIG. 53 shows efficacy of treatment using a lower dose of IL12p70-expressing MSCs, and the combination of IL12p70 and IL21-expressing MSCs as assessed by BLI **Fig. 53A**; 25 individual BLI measurements of mice - left panel; summary of BLI measurments – right panel). **Fig. 53B** shows survival curves of the treatment groups.

FIG. 54 shows efficacy of treatment using IL12p70-expressing MSCs, IL21-expressing MSCs, and the combination of IL12p70 and IL21-expressing MSCs as assessed by BLI (**Fig. 54** left panel) and by tumor weight (**Fig. 54** right panel) in a B16F10 model.

Fig. 55 demonstrates the BLI luciferase measurements of individual mice of following treatment using IL12p70-expressing MSCs, IL21-expressing MSCs, and the combination of IL12p70 and IL21-expressing MSCs in a B16F10 model.

Fig. 56 shows survival curves of the treatment groups receiving IL12p70-expressing MSCs, IL21-expressing MSCs, the combination of IL12p70 and IL21-expressing MSCs, anti-PD1, or , the combination of IL12p70 and anti-PD1.

Fig. 57 shows survival curves of mice following tumor rechallenge. **Fig. 57A** shows naïve untreated mice. **Fig. 57B** shows that previously received the treatment of IL12-expressing MSCs alone. **Fig. 57C** shows mice that previously received the combination treatment of IL12-expressing MSCs and IL21-expressing MSCs.

FIG. 58 shows dose-dependent efficacy of treatment using mMSCs engineered to express murine IL12 (p70) and murine IL21 from a single lentiviral expression vector in a CT26 tumor model. **Fig. 58A** shows summarized BLI assessment of efficacy normalized day 17 vs day 7. **Fig. 58B** and **Fig. 58C** show BLI measurements over time for individual mice. **Fig. 58D** shows survival curves of the treatment groups.

FIG. 59 shows dose-dependent efficacy of treatment using mMSCs engineered to express murine IL12 (p70) and murine IL21 from a single lentiviral expression vector in a B16F10 tumor model. **Fig. 59A** shows summarized BLI assessment of efficacy normalized day 17 vs day 7. **Fig. 59B** and **Fig. 59C** show BLI measurements over time for individual mice. **Fig. 59D** shows BLI measurements over time for individual mice for multiple administrations of higher doses. **Fig. 59E** shows survival curves of the treatment groups.

FIG. 60 shows dose-dependent efficacy of treatment using mMSCs engineered to express murine IL12 (p70) and murine IL21 from a single lentiviral expression vector in a MC-38 tumor model. **Fig. 60A** shows summarized BLI assessment of efficacy normalized day 18 vs day 9. **Fig. 60B** shows BLI measurements over time for individual mice. **Fig. 60C** shows survival curves of the treatment groups.

Fig. 61 shows preferential homing of human MSCs. **Fig. 61A** shows summarized luciferase quantification. **Fig. 61B** shows representative images of luciferase signal in organs.

Fig. 62A shows production of human IL12 (left panel) and human IL21 (right panel) in the peritoneal fluid (left column for each respective time point) and serum (right column for each respective time point) in a OVCAR8 model.

Fig. 62B shows transient production of murine IL12 (left panel) and murine IL21 (right panel) in the peritoneal fluid (left column for each respective time point) and serum (right column for each respective time point) in a CT26 model.

Fig. 63 shows efficacy of mice either treated with MSCs engineered to produce cytokines or treated with recombinant cytokine therapy in a CT26 model. **Fig. 63A** shows survival curves of MSC-IL12 vs rIL12. **Fig. 63B** shows survival curves of MSC-IL21 vs rIL21. **Fig. 63C** shows survival curves of MSC-IL12/IL21 vs rIL12+rIL21. **Fig. 63D** and **Fig. 63E** show BLI assessments of tumor burden for mice either treated with MSCs engineered to produce cytokines or treated with recombinant cytokine therapy.

Fig. 64 shows efficacy of mice either treated with MSCs engineered to produce cytokines or treated with recombinant cytokine therapy in a B16F10 model. **Fig. 63A** show tumor weight assessments of tumor burden for mice either treated with MSCs engineered to produce cytokines or treated with recombinant cytokine therapy **Fig. 64B** shows survival curves of treatment groups.

Fig. 65 shows the immune profile of mice following treatment with MSCs producing both IL12 and IL21 in a CT26 IP tumor model. Results shown are multicolor flow cytometry analysis used to characterize immune infiltrates in response to treatment. **Fig. 65A** and **Fig. 65B** shows T-cell subsets and activation markers (CD3, CD4, CD8, CD8/CD38+, CD8/IFNg+, CD8/Gzmb+, NK/Gzmb+ and ratio CD8:Tregs-FoxP3). **Fig. 65C** shows the immune profile of antigen-presenting cells such as dendritic cells.

DETAILED DESCRIPTION

Mesenchymal stem cells (MSCs) (also referred to as mesenchymal stromal cells, multipotent stromal cells, marrow stromal cells, or multipotent mesenchymal stromal cells) are a subset of non-hematopoietic adult stem cells that originate from the mesoderm. They possess self-renewal ability and multilineage differentiation into not only mesoderm lineages, such as chondrocytes, osteocytes and adipocytes, but also ectodermic cells and endodermic cells. MSCs, free of both ethical concerns and teratoma formation, are the major stem cell type used for cell therapy for treatment of both immune diseases and non-immune diseases. They can be easily isolated from the bone marrow, adipose tissue, the umbilical cord, fetal liver, muscle, and lung and can be successfully expanded *in vitro*. MSCs can be defined by cell surface marker phenotype including a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD45-, CD34-, CD14-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD11b-, CD79α-; or a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD19-, HLA class II-, as discussed in greater detail in Dominici, *et al.* (Cytotherapy. 2006;8(4):315-7), incorporated by reference for all purposes. Further, when MSCs are delivered exogenously and systemically to humans and animals, they tend to home to (migrate directly to) damaged tissue sites with inflammation, including tumor microenvironments and metastatic regions. The inflammation-directed MSC homing involves several important cell trafficking-related molecules, including chemokines, adhesion molecules, and matrix metalloproteinases (MMPs).

Provided herein are methods of engineering cells, such as MSCs, to produce effector molecules that modulate different tumor-mediated immunosuppressive mechanisms. These MSCs are referred to herein as “engineered MSCs.” These MSCs, which typically contain engineered nucleic acid, do not occur in nature. In some embodiments, the MSCs are engineered to include a nucleic acid comprising a promoter operably linked to a nucleotide sequence encoding an effector molecule, for example, one that stimulates an immune response.

Also provided herein are methods of engineering cells such as immune cells, including, but not limited to natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell, to produce effector molecules. These cells, including both MSCs and immune cells, are referred to herein as “engineered cells.”

These cells, which typically contain engineered nucleic acid, do not occur in nature. In some embodiments, the cells are engineered to include a nucleic acid comprising a promoter operably linked to a nucleotide sequence encoding an effector molecule, for example, one that stimulates an immune response.

An "effector molecule," refers to a molecule (e.g., a nucleic acid such as DNA or RNA, or a protein (polypeptide) or peptide) that binds to another molecule and modulates the biological activity of that molecule to which it binds. For example, an effector molecule may act as a ligand to increase or decrease enzymatic activity, gene expression, or cell signaling. Thus, in some embodiments, an effector molecule modulates (activates or inhibits) different immunomodulatory mechanisms. By directly binding to and modulating a molecule, an effector molecule may also indirectly modulate a second, downstream molecule. In some embodiments, an effector molecule is a secreted molecule, while in other embodiments, an effector molecule is bound to the cell surface or remains intracellular. For example, effector molecules include intracellular transcription factors, microRNA, and shRNAs that modify the internal cell state to, for example, enhance immunomodulatory activity, homing properties, or persistence of the cell. Non-limiting examples of effector molecules include cytokines, chemokines, enzymes that modulate metabolite levels, antibodies or decoy molecules that modulate cytokines, homing molecules, and/or integrins.

The term "modulate" encompasses maintenance of a biological activity, inhibition (partial or complete) of a biological activity, and stimulation/activation (partial or complete) of a biological activity. The term also encompasses decreasing or increasing (e.g., enhancing) a biological activity. Two different effector molecules are considered to "modulate different tumor-mediated immunosuppressive mechanisms" when one effector molecule modulates a tumor-mediated immunosuppressive mechanism (e.g., stimulates T cell signaling) that is different from the tumor-mediated immunosuppressive mechanism modulated by the other effector molecule (e.g., stimulates antigen presentation and/or processing).

Modulation by an effector molecule may be direct or indirect. Direct modulation occurs when an effector molecule binds to another molecule and modulates activity of that molecule. Indirect modulation occurs when an effector molecule binds to another molecule, modulates activity of that molecule, and as a result of that modulation, the activity of yet another molecule (to which the effector molecule is not bound) is modulated.

In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism by at least one effector molecule results in an increase in an immunostimulatory and/or anti-tumor immune response (e.g., systemically or in the tumor microenvironment) by at least 10% (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or 200%). For example, modulation of a tumor-mediated immunosuppressive mechanism may result in an increase in an immunostimulatory and/or anti-tumor immune response by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%. In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism results in an increase in an immunostimulatory and/or anti-tumor immune response 10-20%, 10-30%, 10-40%, 10-50%, 10-60%, 10-70%, 10-80%, 10-90%, 10-100%, 10-200%, 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-100%, 20-200%, 50-60%, 50-70%, 50-80%, 50-90%, 50-100%, or 50-200%. It should be understood that "an increase" in an immunostimulatory and/or anti-tumor immune response, for example, systemically or in a tumor microenvironment, is relative to the immunostimulatory and/or anti-tumor immune response that would otherwise occur, in the absence of the effector molecule(s).

In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism by at least one effector molecule results in an increase in an immunostimulatory and/or anti-tumor immune response (e.g., systemically or in the tumor microenvironment) by at least 2 fold (e.g., 2, 3, 4, 5, 10, 25, 20, 25, 50, or 100 fold). For example, modulation of a tumor-mediated immunosuppressive mechanism may result in an increase in an immunostimulatory and/or anti-tumor immune response by at least 3 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 50 fold, or at least 100 fold. In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism results in an increase in an immunostimulatory and/or anti-tumor immune response by 2-10, 2-20, 2-30, 2-40, 2-50, 2-60, 2-70, 2-80, 2-90, or 2-100 fold.

Non-limiting examples of immunostimulatory and/or anti-tumor immune mechanisms include T cell signaling, activity and/or recruitment, antigen presentation and/or processing, natural killer cell-mediated cytotoxic signaling, activity and/or recruitment, dendritic cell differentiation and/or maturation, immune cell recruitment, pro-inflammatory macrophage signaling, activity and/or recruitment, stroma degradation, immunostimulatory metabolite production, stimulator of interferon genes (STING) signaling (which increases the secretion of IFN and Th1 polarization, promoting an anti-tumor immune response), and/or Type I interferon

signaling. An effector molecule may stimulate at least one (one or more) of the foregoing immunostimulatory mechanisms, thus resulting in an increase in an immunostimulatory response. Changes in the foregoing immunostimulatory and/or anti-tumor immune mechanisms may be assessed, for example, using *in vitro* assays for T cell proliferation or cytotoxicity, *in vitro* antigen presentation assays, expression assays (e.g., of particular markers), and/or cell secretion assays (e.g., of cytokines).

In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism by at least one effector molecule results in a decrease in an immunosuppressive response (e.g., systemically or in the tumor microenvironment) by at least 10% (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or 200%). For example, modulation of a tumor-mediated immunosuppressive mechanism may result in a decrease in an immunosuppressive response by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%. In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism results in a decrease in an immunosuppressive response 10-20%, 10-30%, 10-40%, 10-50%, 10-60%, 10-70%, 10-80%, 10-90%, 10-100%, 10-200%, 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-100%, 20-200%, 50-60%, 50-70%, 50-80%, 50-90%, 50-100%, or 50-200%. It should be understood that "a decrease" in an immunosuppressive response, for example, systemically or in a tumor microenvironment, is relative to the immunosuppressive response that would otherwise occur, in the absence of the effector molecule(s).

In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism by at least one effector molecule results in a decrease in an immunosuppressive response (e.g., systemically or in the tumor microenvironment) by at least 2 fold (e.g., 2, 3, 4, 5, 10, 25, 20, 25, 50, or 100 fold). For example, modulation of a tumor-mediated immunosuppressive mechanism may result in a decrease in an immunosuppressive response by at least 3 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 50 fold, or at least 100 fold. In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism results in a decrease in an immunosuppressive response by 2-10, 2-20, 2-30, 2-40, 2-50, 2-60, 2-70, 2-80, 2-90, or 2-100 fold.

Non-limiting examples of immunosuppressive mechanisms include negative costimulatory signaling, pro-apoptotic signaling of cytotoxic cells (e.g., T cells and/or NK cells),

T regulatory (Treg) cell signaling, tumor checkpoint molecule production/maintenance, myeloid-derived suppressor cell signaling, activity and/or recruitment, immunosuppressive factor/metabolite production, and/or vascular endothelial growth factor signaling. An effector molecule may inhibit at least one (one or more) of the foregoing immunosuppressive mechanisms, thus resulting in a decrease in an immunosuppressive response. Changes in the foregoing immunosuppressive mechanisms may be assessed, for example, by assaying for an increase in T cell proliferation and/or an increase in IFNγ production (negative co-stimulatory signaling, T_{reg} cell signaling and/or MDSC); Annexin V/PI flow staining (pro-apoptotic signaling); flow staining for expression, e.g., PDL1 expression (tumor checkpoint molecule production/maintenance); ELISA, LUMINEX®, RNA via qPCR, enzymatic assays, e.g., IDO tryptophan catabolism (immunosuppressive factor/metabolite production); and phosphorylation of PI3K, Akt, p38 (VEGF signaling).

In some embodiments, cells, such as MSCs, are engineered to express membrane-tethered anti-CD3 and/or anti-CD28 agonist extracellular domains.

In some embodiments, cells, such as MSCs, are engineered to produce at least two (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) effector molecules, each of which modulates a different tumor-mediated immunosuppressive mechanism. In other embodiments, cells are engineered to produce at least one effector molecule that is not natively produced by the cells. Such an effector molecule may, for example, complement the function of effector molecules natively produced by the cells

In some embodiments, effector molecules function additively: the effect of two effector molecules, for example, may be equal to the sum of the effect of the two effector molecules functioning separately. In other embodiments, effector molecules function synergistically: the effect of two effector molecules, for example, may be greater than the combined function of the two effector molecules. The present disclosure also encompasses additivity and synergy between an effector molecule(s) and the immune cell (e.g., MSC) from which they are produced.

Effector molecules that modulate tumor-mediated immunosuppressive mechanisms and/or modify tumor microenvironments may be, for example, secreted factors (e.g., cytokines, chemokines, antibodies, and/or decoy receptors that modulate extracellular mechanisms involved in the immune system), inhibitors (e.g., antibodies, antibody fragments, ligand TRAP and/or small blocking peptides), intracellular factors that control cell state (e.g., microRNAs and/or

transcription factors that modulate the state of cells to enhance pro-inflammatory properties), factors packaged into exosomes (e.g., microRNAs, cytosolic factors, and/or extracellular factors), surface displayed factors (e.g., checkpoint inhibitors, TRAIL), and and/or metabolic genes (e.g., enzymes that produce/modulate or degrade metabolites or amino acids).

5 In some embodiments, effector molecules may be selected from the following non-limiting classes of molecules: cytokines, antibodies, chemokines, nucleotides, peptides, and enzymes. Non-limiting examples of the foregoing classes of effector molecules are listed in Table 1 and specific sequences encoding exemplary effector molecules are listed in Table 6. Effector molecules can be human, such as those listed in Table 1 or Table 6 or human 10 equivalents of murine effector molecules listed in Table 1 or Table 6. Effector molecules can be human-derived, such as the endogenous human effector molecule or an effector molecule modified and/or optimized for function, *e.g.*, codon optimized to improve expression, modified to improve stability, or modified at its signal sequence (*see* below). Various programs and algorithms for optimizing function are known to those skilled in the art and can be selected 15 based on the improvement desired, such as codon optimization for a specific species (*e.g.*, human, mouse, bacteria, etc.).

Table 1. Exemplary Effector Molecules

Effector name	**Category**	**Function**
anti-CD40 or CD40 Ligand	Agonist antibody	Stimulates B-cells and antigen presenting cells.
Flt3L	Ligand agonist	Stimulates myeloid cells and antigen presenting cells
CXCL10-11 fusion	Chemokine	Attracts T-cells
TGFb blocking peptides	Antagonist peptides	Inhibit TGFb pathway, TME modifier
Adenosine deaminase (ADA)	TME modifier	Degradation of suppressive adenosine in the TME
Kyneurinase	TME modifier	Degradation of kyneurine
HPGE2	TME modifier	Degradation of PGE2
CXCL13	Chemokine	Attracts B-cells
anti PD-1/PD-L1	Agonist antibody	Remove checkpoint
anti-CTLA-4	Agonist antibody	Remove checkpoint
anti-VEGF	Antagonist antibody	Neutralizes an immunosuppressive/angiogenesis factor

Effector name	Category	Function
anti-TNFa	Antagonist antibody	Neutralizes cytokine/pro-tumor factor
anti-IL-10	Antagonist antibody	Neutralizes immunosuppressive cytokine
anti-SDF1/CXCL12	Antagonist antibody	Neutralizes pro-tumor chemokine
(TβRII)2 trap	Capture trap	Neutralizes an immunosuppressive cytokine
CCL21	Chemokine	Attracts leukocytes/NK
CCL1	Chemokine	Attracts leukocytes/NK
CCL17	Chemokine	Attracts leukocytes/NK
CCL19	Chemokine	Attracts leukocytes/NK
CCL21	Chemokine	Attracts leukocytes/NK
CCL20	Chemokine	Attracts leukocytes/NK
CCL21a	Chemokine	Attracts leukocytes/NK
MIP1b (CCL5)	Chemokine	Attracts leukocytes/NK
CXCL10	Chemokine	Attracts leukocytes/NK
CXCL11	Chemokine	Attracts leukocytes/NK
CCL2	Chemokine	Attracts monocytes
MIP-1alpha (CCL3)	Chemokine	Attracts leukocytes/NK
XCL1	Chemokine	Attracts leukocytes/NK
IFNbeta	Cytokine	T cell response, tumor cell killing
IFNgamma	Cytokine	T cell response, tumor cell killing
IL-12	Cytokine	T cells, NK cells
IL-1beta	Cytokine	T cells, NK cells
IL-15	Cytokine	Stimulates T-cells and NK
IL-2	Cytokine	Stimulates T-cells and NK
IL-21	Cytokine	Stimulates T-cells
IL-24	Cytokine	Stimulates T-cells
IL36-gamma	Cytokine	Stimulates T-cells
IL-7	Cytokine	Stimulates T-cells
IL-22	Cytokine	Stimulates T-cells
IL-18	Cytokine	Stimulates T-cells
Granzymes/Perforin	Enzyme	Direct tumor cell killing
OX86 (anti-OX40)	ligand	Stimulates T-cells
anti-TGFbeta	Neutralizing antibody	Neutralizes an Immunosuppressive cytokine
TRAIL	Receptor/ligand	Direct tumor cell killing
FASL (CD49L)	Receptor/ligand	Direct tumor cell killing
OX40-L	Receptor/Ligand	Stimulates T-cells
cGAS	secreted molecule	Stimulates antigen-presenting cells

Effector name	Category	Function
41BBL	secreted molecule	Co-activation of T-cells
CD40L	secreted molecule	Stimulates T-cells
GM-CSF	secreted molecule	Growth factor for monocytes
STING	secreted molecule	Stimulates antigen-presenting cells
HAC-V 'microbody'_PD1	Antagonist antibody	inhibits checkpoint
yCD	Pro-drug	Converts to cytotoxic molecule upon activation
CpG/Nucleotides	Nucleotides	STING agonist

In some embodiments, cells, such as MSCs, comprise an engineered nucleic acid that comprises a promoter operably linked to a nucleotide sequence encoding an effector molecule. In some embodiments, an engineered nucleic acid comprises a promoter operably linked to a
5 nucleotide sequence encoding at least 2 effector molecules. For example, the engineered nucleic acid may comprise a promoter operably linked to a nucleotide sequence encoding at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 8, at least 9, or at least 10 effector molecules. In some embodiments, an engineered nucleic acid comprises a promoter operably linked to a nucleotide sequence encoding 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more effector molecules.
10 Engineered cells, such as engineered MSCs, in some embodiments, are engineered to include at least two engineered nucleic acids, each comprising a promoter operably linked to a nucleotide sequence encoding at least one (e.g., 1, 2 or 3) effector molecule. For example, the cells may be engineered to comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 8, at least 9, or at least 10, engineered nucleic acids, each comprising a
15 promoter operably linked to a nucleotide sequence encoding at least one (e.g., 1, 2 or 3) effector molecule. In some embodiments, the cells are engineered to comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, or more engineered nucleic acids, each comprising a promoter operably linked to a nucleotide sequence encoding at least one (e.g., 1, 2 or 3) effector molecule.

20 An "engineered nucleic acid" is a nucleic acid that does not occur in nature. It should be understood, however, that while an engineered nucleic acid as a whole is not naturally-occurring, it may include nucleotide sequences that occur in nature. In some embodiments, an engineered nucleic acid comprises nucleotide sequences from different organisms (*e.g.,* from different species). For example, in some embodiments, an engineered nucleic acid includes a

murine nucleotide sequence, a bacterial nucleotide sequence, a human nucleotide sequence, and/or a viral nucleotide sequence. The term “engineered nucleic acids” includes recombinant nucleic acids and synthetic nucleic acids. A “recombinant nucleic acid” refers to a molecule that is constructed by joining nucleic acid molecules and, in some embodiments, can replicate in a live cell. A “synthetic nucleic acid” refers to a molecule that is amplified or chemically, or by other means, synthesized. Synthetic nucleic acids include those that are chemically modified, or otherwise modified, but can base pair with naturally- occurring nucleic acid molecules. Recombinant nucleic acids and synthetic nucleic acids also include those molecules that result from the replication of either of the foregoing. Engineered nucleic acid of the present disclosure may be encoded by a single molecule (*e.g.*, included in the same plasmid or other vector) or by multiple different molecules (*e.g.*, multiple different independently-replicating molecules).

Engineered nucleic acid of the present disclosure may be produced using standard molecular biology methods (*see*, *e.g.*, Green and Sambrook, Molecular Cloning, A Laboratory Manual, 2012, Cold Spring Harbor Press). In some embodiments, engineered nucleic acid constructs are produced using GIBSON ASSEMBLY® Cloning (see, *e.g.*, Gibson, D.G. *et al.* Nature Methods, 343-345, 2009; and Gibson, D.G. *et al.* Nature Methods, 901-903, 2010, each of which is incorporated by reference herein). GIBSON ASSEMBLY® typically uses three enzymatic activities in a single-tube reaction: 5' exonuclease, the ′Y extension activity of a DNA polymerase and DNA ligase activity. The 5 ' exonuclease activity chews back the 5 ' end sequences and exposes the complementary sequence for annealing. The polymerase activity then fills in the gaps on the annealed regions. A DNA ligase then seals the nick and covalently links the DNA fragments together. The overlapping sequence of adjoining fragments is much longer than those used in Golden Gate Assembly, and therefore results in a higher percentage of correct assemblies. In some embodiments, engineered nucleic acid constructs are produced using IN-FUSION® cloning (Clontech).

A “promoter” refers to a control region of a nucleic acid sequence at which initiation and rate of transcription of the remainder of a nucleic acid sequence are controlled. A promoter may also contain sub-regions at which regulatory proteins and molecules may bind, such as RNA polymerase and other transcription factors. Promoters may be constitutive, inducible, repressible, tissue-specific or any combination thereof. A promoter drives expression or drives transcription of the nucleic acid sequence that it regulates. Herein, a promoter is considered to

be "operably linked" when it is in a correct functional location and orientation in relation to a nucleic acid sequence it regulates to control ("drive") transcriptional initiation and/or expression of that sequence.

A promoter may be one naturally associated with a gene or sequence, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment of a given gene or sequence. Such a promoter can be referred to as "endogenous." In some embodiments, a coding nucleic acid sequence may be positioned under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with the encoded sequence in its natural environment. Such promoters may include promoters of other genes; promoters isolated from any other cell; and synthetic promoters or enhancers that are not "naturally occurring" such as, for example, those that contain different elements of different transcriptional regulatory regions and/or mutations that alter expression through methods of genetic engineering that are known in the art. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including polymerase chain reaction (PCR) (see, e.g., U.S. Pat. No. 4,683,202 and U.S. Pat. No. 5,928,906).

Promoters of an engineered nucleic acid may be "inducible promoters," which refer to promoters that are characterized by regulating (*e.g.,* initiating or activating) transcriptional activity when in the presence of, influenced by or contacted by a signal. The signal may be endogenous or a normally exogenous condition (*e.g.,* light), compound (*e.g.,* chemical or non-chemical compound) or protein (e.g., cytokine) that contacts an inducible promoter in such a way as to be active in regulating transcriptional activity from the inducible promoter. Activation of transcription may involve directly acting on a promoter to drive transcription or indirectly acting on a promoter by inactivation a repressor that is preventing the promoter from driving transcription. Conversely, deactivation of transcription may involve directly acting on a promoter to prevent transcription or indirectly acting on a promoter by activating a repressor that then acts on the promoter.

A promoter is "responsive to" or "modulated by" a local tumor state (e.g., inflammation or hypoxia) or signal if in the presence of that state or signal, transcription from the promoter is activated, deactivated, increased, or decreased. In some embodiments, the promoter comprises a response element. A "response element" is a short sequence of DNA within a promoter region

that binds specific molecules (e.g., transcription factors) that modulate (regulate) gene expression from the promoter. Response elements that may be used in accordance with the present disclosure include, without limitation, a phloretin-adjustable control element (PEACE), a zinc-finger DNA-binding domain (DBD), an interferon-gamma-activated sequence (GAS)
5 (Decker, T. et al. *J Interferon Cytokine Res*. 1997 Mar;17(3):121-34, incorporated herein by reference), an interferon-stimulated response element (ISRE) (Han, K. J. et al. *J Biol Chem*. 2004 Apr 9;279(15):15652-61, incorporated herein by reference), a NF-kappaB response element (Wang, V. et al. Cell Reports. 2012; 2(4): 824-839, incorporated herein by reference), and a STAT3 response element (Zhang, D. et al. *J of Biol Chem*. 1996; 271: 9503-9509,
10 incorporated herein by reference). Other response elements are encompassed herein. Response elements can also contain tandem repeats (*e.g.*, consecutive repeats of the same nucleotide sequence encoding the response element) to generally increase sensitivity of the response element to its cognate binding molecule. Tandem repeats can be labeled 2X, 3X, 4X, 5X, *etc.* to denote the number of repeats present.
15 Non-limiting examples of responsive promoters (also referred to as "inducible promoters") (e.g., TGF-beta responsive promoters) are listed in Table 2, which shows the design of the promoter and transcription factor, as well as the effect of the inducer molecule towards the transcription factor (TF) and transgene transcription (T) is shown (B, binding; D, dissociation; n.d., not determined) (A, activation; DA, deactivation; DR, derepression) (see Horner, M. &
20 Weber, W. *FEBS Letters* 586 (2012) 20784-2096m, and references cited therein). Other non-limiting examples of inducible promoters include those presented in Table 3.

Table 2. Examples of Responsive Promoters.

System	Promoter and operator	Transcription factor (TF)	Inducer molecule	Response to inducer	
				TF	T
Transcriptional activator-responsive promoters					
AIR	PAIR (OalcA-PhCMVmin)	AlcR	Acetaldehyde	n.d.	A
ART	PART (OARG-PhCMVmin)	ArgR-VP16	l-Arginine	B	A
BIT	PBIT3 (OBirA3-PhCMVmin)	BIT (BirA-VP16)	Biotin	B	A
Cumate – activator	PCR5 (OCuO6-PhCMVmin)	cTA (CymR-VP16)	Cumate	D	DA

System	Promoter and operator	Transcription factor (TF)	Inducer molecule	Response to inducer	
Cumate – reverse activator	PCR5 (OCuO6-PhCMVmin)	rcTA (rCymR-VP16)	Cumate	B	A
E-OFF	PETR (OETR-PhCMVmin)	ET (E-VP16)	Erythromycin	D	DA
NICE-OFF	PNIC (ONIC-PhCMVmin)	NT (HdnoR-VP16)	6-Hydroxy-nicotine	D	DA
PEACE	PTtgR1 (OTtgR-PhCMVmin)	TtgA1 (TtgR-VP16)	Phloretin	D	DA
PIP-OFF	PPIR (OPIR-Phsp70min)	PIT (PIP-VP16)	Pristinamycin I	D	DA
QuoRex	PSCA (OscbR-PhCMVmin)PSPA (OpapRI-PhCMVmin)	SCA (ScbR-VP16)	SCB1	D	DA
Redox	PROP (OROP-PhCMVmin)	REDOX (REX-VP16)	NADH	D	DA
TET-OFF	PhCMV∗-1 (OtetO7-PhCMVmin)	tTA (TetR-VP16)	Tetracycline	D	DA
TET-ON	PhCMV∗-1 (OtetO7-PhCMVmin)	rtTA (rTetR-VP16)	Doxycycline	B	A
TIGR	PCTA (OrheO-PhCMVmin)	CTA (RheA-VP16)	Heat	D	DA
TraR	O7x(tra box)-PhCMVmin	p65-TraR	3-Oxo-C8-HSL	B	A
VAC-OFF	P1VanO2 (OVanO2-PhCMVmin)	VanA1 (VanR-VP16)	Vanillic acid	D	DA
Transcriptional repressor-responsive promoters					
Cumate - repressor	PCuO (PCMV5-OCuO)	CymR	Cumate	D	DR
E-ON	PETRON8 (PSV40-OETR8)	E-KRAB	Erythromycin	D	DR
NICE-ON	PNIC (PSV40-ONIC8)	NS (HdnoR-KRAB)	6-Hydroxy-nicotine	D	DR
PIP-ON	PPIRON (PSV40-OPIR3)	PIT3 (PIP-KRAB)	Pristinamycin I	D	DR
Q-ON	PSCAON8 (PSV40-OscbR8)	SCS (ScbR-KRAB)	SCB1	D	DR
TET-ON<comma> repressor-based	OtetO-PHPRT	tTS-H4 (TetR-HDAC4)	Doxycycline	D	DR
T-REX	PTetO (PhCMV-OtetO2)	TetR	Tetracycline	D	DR
UREX	PUREX8 (PSV40-OhucO8)	mUTS (KRAB-HucR)	Uric acid	D	DR
VAC-ON	PVanON8 (PhCMV-OVanO8)	VanA4 (VanR-KRAB)	Vanillic acid	D	DR
Hybrid promoters					
QuoRexPIP-ON(NOT IF gate)	OscbR8-OPIR3-PhCMVmin	SCAPIT3	SCB1Pristinamycin I	DD	DAD R
QuoRexE-ON(NOT IF gate)	OscbR-OETR8-PhCMVmin	SCAE-KRAB	SCB1Erythromycin	DD	DAD R
TET-OFFE-ON(NOT IF gate)	OtetO7-OETR8-PhCMVmin	tTAE-KRAB	TetracyclineErythrom ycin	DD	DAD R
TET-OFFPIP-ONE-ON	OtetO7-OPIR3-OETR8-PhCMVmin	tTAPIT3E-KRAB	TetracyclinePristinam ycin IErythromycin	DDD	DAD RDR

Table 3. Exemplary Inducible Promoters

Name	DNA SEQUENCE	Source
minimal promoter; minP	AGAGGGTATATAATGGAAGCTCGACTTCCAG (SEQ ID NO: 1)	EU581860.1 (Promega)
NFkB response element protein promoter; 5x NFkB-RE	GGGAATTTCCGGGGACTTTCCGGGAATTTCCGGGGACTTTCCGGGAATTTCC (SEQ ID NO: 2)	EU581860.1 (Promega)
CREB response element protein promoter; 4x CRE	CACCAGACAGTGACGTCAGCTGCCAGATCCCATGGCCGTCATACTGTGACGTCTTTCAGACACCCCATTGACGTCAATGGGAGAA (SEQ ID NO: 3)	DQ904461.1 (Promega)
NFAT response element protein promoter; 3x NFAT binding sites	GGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGT (SEQ ID NO: 4)	DQ904462.1 (Promega)
SRF response element protein promoter; 5x SRE	AGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCT (SEQ ID NO: 5)	FJ773212.1 (Promega)
SRF response element protein promoter 2; 5x SRF-RE	AGTATGTCCATATTAGGACATCTACCATGTCCATATTAGGACATCTACTATGTCCATATTAGGACATCTTGTATGTCCATATTAGGACATCTAAAATGTCCATATTAGGACATCT (SEQ ID NO: 6)	FJ773213.1 (Promega)
AP1 response element protein promoter; 6x AP1-RE	TGAGTCAGTGACTCAGTGAGTCAGTGACTCAGTGAGTCAGTGACTCAG (SEQ ID NO: 7)	JQ858516.1 (Promega)
TCF-LEF response element protein promoter; 8x TCF-LEF-RE	AGATCAAAGGGTTTAAGATCAAAGGGCTTAAGATCAAAGGGTATAAGATCAAAGGGCCTAAGATCAAAGGGACTAAGATCAAAGGGTTTAAGATCAAAGGGCTTAAGATCAAAGGGCCTA (SEQ ID NO: 8)	JX099537.1 (Promega)
SBEx4	GTCTAGACGTCTAGACGTCTAGACGTCTAGAC (SEQ ID NO: 9)	Addgene Cat No: 16495
SMAD2/3 - CAGACA x4	CAGACACAGACACAGACACAGACA (SEQ ID NO: 10)	Jonk et al. (J Biol Chem. 1998 Aug 14;273(33):21145-52.
STAT3 binding site	Ggatccggtactcgagatctgcgatctaagtaagcttggcattccggtactgttggtaaagccac (SEQ ID NO: 11)	Addgene Sequencing Result #211335

Other non-limiting examples of promoters include the cytomegalovirus (CMV) promoter, the elongation factor 1-alpha (EF1a) promoter, the elongation factor (EFS) promoter, the MND promoter (a synthetic promoter that contains the U3 region of a modified MoMuLV LTR with myeloproliferative sarcoma virus enhancer), the phosphoglycerate kinase (PGK) promoter, the spleen focus-forming virus (SFFV) promoter, the simian virus 40 (SV40) promoter, and the ubiquitin C (UbC) promoter (see Table 4).

Table 4. Exemplary Constitutive Promoters

Name	DNA SEQUENCE
CMV	GTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTA GTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCC GCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATG TTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTAT TTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTAC GCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGT ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCG CTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGG TTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTT GTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCC CATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAG AGCTC (SEQ ID NO: 12)
EF1a	GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGA AGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGG GGTAAACTGGGAAAGTGATGCCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTG GGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAAC GGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTG GCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGC AGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCG AGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCC TGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCG CTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGC TTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGT ATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCAC ATGTTCGGCGAGGCGGGGCCTGCGAGCGCGACCACCGAGAATCGGACGGGGG TAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGTCCTCGCGCCGCCGTGTAT CGCCCCGCCCCGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCG GAAAGATGGCCGCTTCCCGGTCCTGCTGCAGGGAGCTCAAAATGGAGGACGC GGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCT TTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCC AGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGG GGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAA GTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAG TTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTT CCATTTCAGGTGTCGTGA (SEQ ID NO: 13)
EFS	GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCAC AGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGA AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTT TCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCG CATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGT TGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCC GTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCC TTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTG CTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGC TGTGACCGGCGCCTAC (SEQ ID NO: 14)
MND	TTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCCACCTGTAGGTTTG GCAAGCTAGGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAA CAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGTT GGAACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCC CCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCA GTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGAC CCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCG CTTCTGCTCCCCGAGCTCAATAAAAGAGCCCA (SEQ ID NO: 15)

PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGCGCAGGGACGCG GCTGCTCTGGGCGTGGTTCCGGGAAACGCAGCGGCGCCGACCCTGGGTCTCG CACATTCTTCACGTCCGTTCGCAGCGTCACCCGGATCTTCGCCGCTACCCTTGT GGGCCCCCCGGCGACGCTTCCTGCTCCGCCCCTAAGTCGGGAAGGTTCCTTGC GGTTCGCGGCGTGCCGGACGTGACAAACGGAAGCCGCACGTCTCACTAGTAC CCTCGCAGACGGACAGCGCCAGGGAGCAATGGCAGCGCGCCGACCGCGATGG GCTGTGGCCAATAGCGGCTGCTCAGCGGGGCGCGCCGAGAGCAGCGGCCGGG AAGGGGCGGTGCGGGAGGCGGGGTGTGGGGCGGTAGTGTGGGCCCTGTTCCT GCCCGCGCGGTGTTCCGCATTCTGCAAGCCTCCGGAGCGCACGTCGGCAGTCG GCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCAG (SEQ ID NO: 16)
SFFV	GTAACGCCATTTTGCAAGGCATGGAAAAATACCAAACCAAGAATAGAGAAGT TCAGATCAAGGGCGGGTACATGAAAATAGCTAACGTTGGGCCAAACAGGATA TCTGCGGTGAGCAGTTTCGGCCCCGGCCCGGGGCCAAGAACAGATGGTCACC GCAGTTTCGGCCCCGGCCCGAGGCCAAGAACAGATGGTCCCCAGATATGGCC CAACCCTCAGCAGTTTCTTAAGACCCATCAGATGTTTCCAGGCTCCCCCAAGG ACCTGAAATGACCCTGCGCCTTATTTGAATTAACCAATCAGCCTGCTTCTCGC TTCTGTTCGCGCGCTTCTGCTTCCCGAGCTCTATAAAAGAGCTCACAACCCCTC ACTCGGCGCGCCAGTCCTCCGACAGACTGAGTCGCCCGGG (SEQ ID NO: 17)
SV40	CTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAG GCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAA GTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAG TCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCC CAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGA GGCCGAGGCCGCCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTT TTGGAGGCCTAGGCTTTTGCAAAAAGCT (SEQ ID NO: 18)
UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCTCCTCACGGCGAGCGCTG CCACGTCAGACGAAGGGCGCAGGAGCGTTCCTGATCCTTCCGCCCGGACGCT CAGGACAGCGGCCCGCTGCTCATAAGACTCGGCCTTAGAACCCCAGTATCAG CAGAAGGACATTTTAGGACGGGACTTGGGTGACTCTAGGGCACTGGTTTTCTT TCCAGAGAGCGGAACAGGCGAGGAAAAGTAGTCCCTTCTCGGCGATTCTGCG GAGGGATCTCCGTGGGGCGGTGAACGCCGATGATTATATAAGGACGCGCCGG GTGTGGCACAGCTAGTTCCGTCGCAGCCGGGATTTGGGTCGCGGTTCTTGTTT GTGGATCGCTGTGATCGTCACTTGGTGAGTTGCGGGCTGCTGGGCTGGCCGGG GCTTTCGTGGCCGCCGGGCCGCTCGGTGGGACGGAAGCGTGTGGAGAGACCG CCAAGGGCTGTAGTCTGGGTCCGCGAGCAAGGTTGCCCTGAACTGGGGGTTG GGGGGAGCGCACAAAATGGCGGCTGTTCCCGAGTCTTGAATGGAAGACGCTT GTAAGGCGGGCTGTGAGGTCGTTGAAACAAGGTGGGGGGCATGGTGGGCGGC AAGAACCCAAGGTCTTGAGGCCTTCGCTAATGCGGGAAAGCTCTTATTCGGGT GAGATGGGCTGGGGCACCATCTGGGGACCCTGACGTGAAGTTTGTCACTGAC TGGAGAACTCGGGTTTGTCGTCTGGTTGCGGGGGCGGCAGTTATGCGGTGCCG TTGGGCAGTGCACCCGTACCTTTGGGAGCGCGCGCCTCGTCGTGTCGTGACGT CACCCGTTCTGTTGGCTTATAATGCAGGGTGGGGCCACCTGCCGGTAGGTGTG CGGTAGGCTTTTCTCCGTCGCAGGACGCAGGGTTCGGGCCTAGGGTAGGCTCT CCTGAATCGACAGGCGCCGGACCTCTGGTGAGGGGAGGGATAAGTGAGGCGT CAGTTTCTTTGGTCGGTTTTATGTACCTATCTTCTTAAGTAGCTGAAGCTCCGG TTTTGAACTATGCGCTCGGGGTTGGCGAGTGTGTTTTGTGAAGTTTTTTAGGCA CCTTTTGAAATGTAATCATTTGGGTCAATATGTAATTTTCAGTGTTAGACTAGT AAAGCTTCTGCAGGTCGACTCTAGAAAATTGTCCGCTAAATTCTGGCCGTTTT TGGCTTTTTTGTTAGAC (SEQ ID NO: 19)
hEF1aV1	GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGA GAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGC GCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCG AGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTT TTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCC GCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTC CACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGG

	GTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTG AGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGC ACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTT TGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGG GCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACG GGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGC GCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTC TGGTGCCTGGTCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGG CTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGG CCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGG CGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCG CTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGT TCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGC GATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTG GCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGT TCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTG TCGTGA (SEQ ID NO: 20)
hCAGG	ACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATAT GGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC CAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACG CCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTG CCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGA CGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTA TGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCAT GGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTC CCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGG GGCGGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCG AGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAG CGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGC CCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGACGCTGCC TTCGCCCCGTGCCCCGCTCCGCCGCCGCCTCGCGCCGCCCGCCCCGGCTC TGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCT CCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGC TGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGC GGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCG GCTCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCT TTGTGCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCC CCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTG TGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTCGGTCGGGCTGCA ACCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGG GTGCGGGGCTCCGTACGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGG GGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGC CGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCCGGCGG CTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGA GAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGG GAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGC GCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCC GCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGC TGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGA CCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCT ACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCAAA GAATTC (SEQ ID NO: 21)
hEF1aV2	Gggcagagcgcacatcgcccacagtccccgagaagttggggggaggggtcggcaattgaaccggtgcctag agaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactggctccgcctttttcccgagggtgggggaga accgtatataagtgcagtagtcgccgtgaacgttctttttcgcaacgggtttgccgccagaacacag (SEQ ID NO: 22)

hACTb	CCACTAGTTCCATGTCCTTATATGGACTCATCTTTGCCTATTGCGACACACAC TCAATGAACACCTACTACGCGCTGCAAAGAGCCCCGCAGGCCTGAGGTGCC CCCACCTCACCACTCTTCCTATTTTTGTGTAAAAATCCAGCTTCTTGTCACCA CCTCCAAGGAGGGGGAGGAGGAGGAAGGCAGGTTCCTCTAGGCTGAGCCG AATGCCCCTCTGTGGTCCCACGCCACTGATCGCTGCATGCCCACCACCTGG GTACACACAGTCTGTGATTCCCGGAGCAGAACGGACCCTGCCCACCCGGTC TTGTGTGCTACTCAGTGGACAGACCCAAGGCAAGAAAGGGTGACAAGGACA GGGTCTTCCCAGGCTGGCTTTGAGTTCCTAGCACCGCCCCGCCCCCAATCC TCTGTGGCACATGGAGTCTTGGTCCCCAGAGTCCCCCAGCGGCCTCCAGAT GGTCTGGGAGGGCAGTTCAGCTGTGGCTGCGCATAGCAGACATACAACGGA CGGTGGGCCCAGACCCAGGCTGTGTAGACCCAGCCCCCCCGCCCCGCAGT GCCTAGGTCACCCACTAACGCCCCAGGCCTGGTCTTGGCTGGGCGTGACTG TTACCCTCAAAAGCAGGCAGCTCCAGGGTAAAAGGTGCCCTGCCCTGTAGA GCCCACCTTCCTTCCCAGGGCTGCGGCTGGGTAGGTTTGTAGCCTTCATCA CGGGCCACCTCCAGCCACTGGACCGCTGGCCCCTGCCCTGTCCTGGGGAG TGTGGTCCTGCGACTTCTAAGTGGCCGCAAGCCACCTGACTCCCCCAACAC CACACTCTACCTCTCAAGCCCAGGTCTCTCCCTAGTGACCCACCCAGCACAT TTAGCTAGCTGAGCCCCACAGCCAGAGGTCCTCAGGCCCTGCTTTCAGGGC AGTTGCTCTGAAGTCGGCAAGGGGGAGTGACTGCCTGGCCACTCCATGCCC TCCAAGAGCTCCTTCTGCAGGAGCGTACAGAACCCAGGGCCCTGGCACCCG TGCAGACCCTGGCCCACCCCACCTGGGCGCTCAGTGCCCAAGAGATGTCCA CACCTAGGATGTCCCGCGGTGGGTGGGGGGCCCGAGAGACGGGCAGGCC GGGGGCAGGCCTGGCCATGCGGGGCCGAACCGGGCACTGCCCAGCGTGG GGCGCGGGGGCCACGGCGCGCGCCCCCAGCCCCCGGGCCCAGCACCCCA AGGCGGCCAACGCCAAAACTCTCCCTCCTCCTCTTCCTCAATCTCGCTCTCG CTCTTTTTTTTTTTCGCAAAAGGAGGGGAGAGGGGGTAAAAAAATGCTGCAC TGTGCGGCGAAGCCGGTGAGTGAGCGGCGCGGGGCCAATCAGCGTGCGC CGTTCCGAAAGTTGCCTTTTATGGCTCGAGCGGCCGCGGCGGCGCCCTATA AAACCCAGCGGCGCGACGCGCCACCACCGCCGAGACCGCGTCCGCCCCG CGAGCACAGAGCCTCGCCTTTGCCGATCCGCCGCCCGTCCACACCCGCCG CCAGgtaagcccggccagccgaccggggcaggcggctcacggcccggccgcaggcggccgcggcccct tcgcccgtgcagagccgccgtctgggccgcagcgggggcgcatgggggggaaccggaccgccgtgggg ggcgcgggagaagcccctgggcctccggagatgggggacaccccacgccagttcggaggcgcgaggccgc gctcgggaggcgcgctccgggggtgccgctctcggggcgggggcaaccggcggggtctttgtctgagccggg ctcttgccaatggggatcgcagggtgggcgcggcggagcccccgccaggcccggtgggggctggggcgccat tgcgcgtgcgcgctggtcctttgggcgctaactgcgtgcgcgctgggaattggcgctaattgcgcgtgcgcgctgg gactcaaggcgctaactgcgcgtgcgttctggggcccggggtgccgcggcctgggctggggcgaaggcgggc tcggccggaaggggtggggtcgccgcggctcccgggcgcttgcgcgcacttcctgcccgagccgctggccgcc cgagggtgtggccgctgcgtgcgcgcgcgccgacccggcgctgtttgaaccgggcggaggcggggctggcgc ccggttgggagggggttggggcctggcttcctgccgcgcgccgcggggacgcctccgaccagtgtttgccttttat ggtaataacgcggccggcccggcttcctttgtccccaatctgggcgcgcgccggcgcccccctggcggcctaagg actcggcgcgccggaagtggccagggcgggggcgacctcggctcacagcgcgcccggctat (SEQ ID NO: 23)
heIF4A1	GTTGATTTCCTTCATCCCTGGCACACGTCCAGGCAGTGTCGAATCCATCTCT GCTACAGGGGAAAACAAATAACATTTGAGTCCAGTGGAGACCGGGAGCAGA AGTAAAGGGAAGTGATAACCCCCAGAGCCCGGAAGCCTCTGGAGGCTGAGA CCTCGCCCCCCTTGCGTGATAGGGCCTACGGAGCCACATGACCAAGGCACT GTCGCCTCCGCACGTGTGAGAGTGCAGGGCCCCAAGATGGCTGCCAGGCC TCGAGGCCTGACTCTTCTATGTCACTTCCGTACCGGCGAGAAAGGCGGGCC CTCCAGCCAATGAGGCTGCGGGGCGGGCCTTCACCTTGATAGGCACTCGA GTTATCCAATGGTGCCTGCGGGCCGGAGCGACTAGGAACTAACGTCATGCC GAGTTGCTGAGCGCCGGCAGGCGGGGCCGGGGCGGCCAAACCAATGCGA TGGCCGGGGCGGAGTCGGGCGCTCTATAAGTTGTCGATAGGCGGGCACTC CGCCCTAGTTTCTAAGGACCATG (SEQ ID NO: 24)
hGAPDH	AGTTCCCCAACTTTCCCGCCTCTCAGCCTTTGAAAGAAAGAAAGGGGAGGG GGCAGGCCGCGTGCAGTCGCGAGCGGTGCTGGGCTCCGGCTCCAATTCCC

	CATCTCAGTCGCTCCCAAAGTCCTTCTGTTTCATCCAAGCGTGTAAGGGTCC CCGTCCTTGACTCCCTAGTGTCCTGCTGCCCACAGTCCAGTCCTGGGAACC AGCACCGATCACCTCCCATCGGGCCAATCTCAGTCCCTTCCCCCCTACGTC GGGGCCCACACGCTCGGTGCGTGCCCAGTTGAACCAGGCGGCTGCGGAAA AAAAAAAGCGGGGAGAAAGTAGGGCCCGGCTACTAGCGGTTTTACGGGCG CACGTAGCTCAGGCCTCAAGACCTTGGGCTGGGACTGGCTGAGCCTGGCG GGAGGCGGGGTCCGAGTCACCGCCTGCCGCCGCGCCCCCGGTTTCTATAA ATTGAGCCCGCAGCCTCCCGCTTCGCTCTCTGCTCCTCCTGTTCGACAGTCA GCCGCATCTTCTTTTGCGTCGCCAGgtgaagacgggcggagagaaacccgggaggctagg gacggcctgaaggcggcaggggcgggcgcaggccggatgtgttcgcgccgctgcggggtgggcccgggcg gcctccgcattgcaggggcgggcggaggacgtgatgcggcgcgggctgggcatggaggcctggtgggggag gggaggggaggcgtgggtgtcggccggggccactaggcgctcactgttctctccctccgcgcagCCGAGC CACATCGCTGAGACAC (SEQ ID NO: 25)
hGRP78	AGTGCGGTTACCAGCGGAAATGCCTCGGGGTCAGAAGTCGCAGGAGAGATA GACAGCTGCTGAACCAATGGGACCAGCGGATGGGGCGGATGTTATCTACCA TTGGTGAACGTTAGAAACGAATAGCAGCCAATGAATCAGCTGGGGGGGCGG AGCAGTGACGTTTATTGCGGAGGGGGCCGCTTCGAATCGGCGGCGGCCAG CTTGGTGGCCTGGGCCAATGAACGGCCTCCAACGAGCAGGGCCTTCACCAA TCGGCGGCCTCCACGACGGGGCTGGGGGAGGGTATATAAGCCGAGTAGGC GACGGTGAGGTCGACGCCGGCCAAGACAGCACAGACAGATTGACCTATTGG GGTGTTTCGCGAGTGTGAGAGGGAAGCGCCGCGGCCTGTATTTCTAGACCT GCCCTTCGCCTGGTTCGTGGCGCCTTGTGACCCCGGGCCCCTGCCGCCTG CAAGTCGGAAATTGCGCTGTGCTCCTGTGCTACGGCCTGTGGCTGGACTGC CTGCTGCTGCCCAACTGGCTGGCAC (SEQ ID NO: 26)
hGRP94	TAGTTTCATCACCACCGCCACCCCCCCGCCCCCCCGCCATCTGAAAGGGTT CTAGGGGATTTGCAACCTCTCTCGTGTGTTTCTTCTTTCCGAGAAGCGCCGC CACACGAGAAAGCTGGCCGCGAAAGTCGTGCTGGAATCACTTCCAACGAAA CCCCAGGCATAGATGGGAAAGGGTGAAGAACACGTTGCCATGGCTACCGTT TCCCCGGTCACGGAATAAACGCTCTCTAGGATCCGGAAGTAGTTCCGCCGC GACCTCTCTAAAAGGATGGATGTGTTCTCTGCTTACATTCATTGGACGTTTTC CCTTAGAGGCCAAGGCCGCCCAGGCAAAGGGGCGGTCCCACGCGTGAGGG GCCCGCGGAGCCATTTGATTGGAGAAAAGCTGCAAACCCTGACCAATCGGA AGGAGCCACGCTTCGGGCATCGGTCACCGCACCTGGACAGCTCCGATTGGT GGACTTCCGCCCCCCCTCACGAATCCTCATTGGGTGCCGTGGGTGCGTGGT GCGGCGCGATTGGTGGGTTCATGTTTCCCGTCCCCCGCCCGCGAGAAGTG GGGGTGAAAAGCGGCCCGACCTGCTTGGGGTGTAGTGGGCGGACCGCGC GGCTGGAGGTGTGAGGATCCGAACCCAGGGGTGGGGGGTGGAGGCGGCT CCTGCGATCGAAGGGGACTTGAGACTCACCGGCCGCACGTC (SEQ ID NO: 27)
hHSP70	GGGCCGCCCACTCCCCCTTCCTCTCAGGGTCCCTGTCCCCTCCAGTGAATC CCAGAAGACTCTGGAGAGTTCTGAGCAGGGGGCGGCACTCTGGCCTCTGAT TGGTCCAAGGAAGGCTGGGGGGCAGGACGGGAGGCGAAAACCCTGGAATA TTCCCGACCTGGCAGCCTCATCGAGCTCGGTGATTGGCTCAGAAGGGAAAA GGCGGGTCTCCGTGACGACTTATAAAAGCCCAGGGGCAAGCGGTCCGGAT AACGGCTAGCCTGAGGAGCTGCTGCGACAGTCCACTACCTTTTTCGAGAGT GACTCCCGTTGTCCCAAGGCTTCCCAGAGCGAACCTGTGCGGCTGCAGGCA CCGGCGCGTCGAGTTTCCGGCGTCCGGAAGGACCGAGCTCTTCTCGCGGA TCCAGTGTTCCGTTTCCAGCCCCCAATCTCAGAGCGGAGCCGACAGAGAGC AGGGAACCC (SEQ ID NO: 28)
hKINb	GCCCCACCCCCGTCCGCGTTACAACCGGGAGGCCCGCTGGGTCCTGCACC GTCACCCTCCTCCCTGTGACCGCCCACCTGATACCCAAACAACTTTCTCGCC CCTCCAGTCCCCAGCTCGCCGAGCGCTTGCGGGGAGCCACCCAGCCTCAG TTTCCCCAGCCCCGGGCGGGGCGAGGGGCGATGACGTCATGCCGGCGCG CGGCATTGTGGGGCGGGGCGAGGCGGGGCGCCGGGGGGAGCAACACTGA GACGCCATTTTCGGCGGCGGGAGCGGCGCAGGCGGCCGAGCGGGACTGG CTGGGTCGGCTGGGCTGCTGGTGCGAGGAGCCGCGGGGCTGTGCTCGGC

	GGCCAAGGGGACAGCGCGTGGGTGGCCGAGGATGCTGCGGGGCGGTAGC TCCGGCGCCCCTCGCTGGTGACTGCTGCGCCGTGCCTCACACAGCCGAGG CGGGCTCGGCGCACAGTCGCTGCTCCGCGCTCGCGCCCGGCGGCGCTCC AGGTGCTGACAGCGCGAGAGAGCGCGGCCTCAGGAGCAACAC (SEQ ID NO: 29)
hUBIb	TTCCAGAGCTTTCGAGGAAGGTTTCTTCAACTCAAATTCATCCGCCTGATAAT TTTCTTATATTTTCCTAAAGAAGGAAGAGAAGCGCATAGAGGAGAAGGGAAA TAATTTTTTAGGAGCCTTTCTTACGGCTATGAGGAATTTGGGGCTCAGTTGAA AAGCCTAAACTGCCTCTCGGGAGGTTGGGCGCGGCGAACTACTTTCAGCGG CGCACGGAGACGGCGTCTACGTGAGGGGTGATAAGTGACGCAACACTCGTT GCATAAATTTGCGCTCCGCCAGCCCGGAGCATTTAGGGGCGGTTGGCTTTG TTGGGTGAGCTTGTTTGTGTCCCTGTGGGTGGACGTGGTTGGTGATTGGCA GGATCCTGGTATCCGCTAACAGgtactggcccacagccgtaaagacctgcgggggcgtgagag gggggaatgggtgaggtcaagctggaggcttcttggggttgggtgggccgctgaggggaggggagggcgag gtgacgcgacacccggcctttctgggagagtgggccttgttgacctaagggggggcgagggcagttggcacgcg cacgcgccgacagaaactaacagacattaaccaacagcgattccgtcgcgtttacttgggaggaaggcggaa aagaggtagtttgtgtggcttctggaaaccctaaatttggaatcccagtatgagaatggtgtcccttcttgtgtttcaat gggatttttacttcgcgagtcttgtgggtttggttttgttttcagtttgcctaacaccgtgcttaggtttgaggcagattgga gttcggtcgggggagtttgaatatccggaacagttagtggggaaagctgtggacgcttggtaagagagcgctctg gattttccgctgttgacgttgaaaccttgaatgacgaatttcgtattaagtgacttagccttgtaaaattgaggggagg cttgcggaatattaacgtatttaaggcattttgaaggaatagttgctaattttgaagaatattaggtgtaaaagcaag aaatacaatgatcctgaggtgacacgcttatgttttacttttaaactagGTCACC (SEQ ID NO: 30)

In some embodiments, a promoter of the present disclosure is modulated by signals within a tumor microenvironment. A tumor microenvironment is considered to modulate a promoter if, in the presence of the tumor microenvironment, the activity of the promoter is increased or decreased by at least 10%, relative to activity of the promoter in the absence of the tumor microenvironment. In some embodiments, the activity of the promoter is increased or decreased by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, relative to activity of the promoter in the absence of the tumor microenvironment. For example, the activity of the promoter is increased or decreased by 10-20%, 10-30%, 10-40%, 10-50%, 10-60%, 10-70%, 10-80%, 10-90%, 10-100%, 10-200%, 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-100%, 20-200%, 50-60%, 50-70%, 50-80%, 50-90%, 50-100%, or 50-200%, relative to activity of the promoter in the absence of the tumor microenvironment.

In some embodiments, the activity of the promoter is increased or decreased by at least 2 fold (e.g., 2, 3, 4, 5, 10, 25, 20, 25, 50, or 100 fold), relative to activity of the promoter in the absence of the tumor microenvironment. For example, the activity of the promoter is increased or decreased by at least 3 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 50 fold, or at least 100 fold, relative to activity of the promoter in the absence of the tumor microenvironment. In some embodiments, the activity of the promoter is increased or decreased

by 2-10, 2-20, 2-30, 2-40, 2-50, 2-60, 2-70, 2-80, 2-90, or 2-100 fold, relative to activity of the promoter in the absence of the tumor microenvironment.

In some embodiments, a promoter of the present disclosure is activated under a hypoxic condition. A "hypoxic condition" is a condition where the body or a region of the body is deprived of adequate oxygen supply at the tissue level. Hypoxic conditions can cause inflammation (e.g., the level of inflammatory cytokines increase under hypoxic conditions). In some embodiments, the promoter that is activated under hypoxic condition is operably linked to a nucleotide encoding an effector molecule that decreases the expression of activity of inflammatory cytokines, thus reducing the inflammation caused by the hypoxic condition. In some embodiments, the promoter that is activated under hypoxic conditions comprises a hypoxia responsive element (HRE). A "hypoxia responsive element (HRE)" is a response element that responds to hypoxia-inducible factor (HIF). The HRE, in some embodiments, comprises a consensus motif NCGTG (where N is either A or G).

In some embodiments, engineered cells produce multiple effector molecules. For example, cells may be engineered to produce 2-20 different effector molecules. In some embodiments, Cells engineered to produce 2-20, 2-19, 2-18, 2-17, 2-16, 2-15, 2-14, 2-13, 2-12, 2-11, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-20, 3-19, 3-18, 3-17, 3-16, 3-15, 3-14, 3-13, 3-12, 3-11, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-20, 4-19, 4-18, 4-17, 4-16, 4-15, 4-14, 4-13, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-20, 5-19, 5-18, 5-17, 5-16, 5-15, 5-14, 5-13, 5-12, 5-11, 5-10, 5-9, 5-8, 5-7, 5-6, 6-20, 6-19, 6-18, 6-17, 6-16, 6-15, 6-14, 6-13, 6-12, 6-11, 6-10, 6-9, 6-8, 6-7, 7-20, 7-19, 7-18, 7-17, 7-16, 7-15, 7-14, 7-13, 7-12, 7-11, 7-10, 7-9, 7-8, 8-20, 8-19, 8-18, 8-17, 8-16, 8-15, 8-14, 8-13, 8-12, 8-11, 8-10, 8-9, 9-20, 9-19, 9-18, 9-17, 9-16, 9-15, 9-14, 9-13, 9-12, 9-11, 9-10, 10-20, 10-19, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 10-11, 11-20, 11-19, 11-18, 11-17, 11-16, 11-15, 11-14, 11-13, 11-12, 12-20, 12-19, 12-18, 12-17, 12-16, 12-15, 12-14, 12-13, 13-20, 13-19, 13-18, 13-17, 13-16, 13-15, 13-14, 14-20, 14-19, 14-18, 14-17, 14-16, 14-15, 15-20, 15-19, 15-18, 15-17, 15-16, 16-20, 16-19, 16-18, 16-17, 17-20, 17-19, 17-18, 18-20, 18-19, or 19-20 effector molecules. In some embodiments, cells are engineered to produce 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 effector molecules.

In some embodiments, exogenous sequences can be multicistronic, *i.e.*, more than one separate polypeptide (*e.g.*, multiple effector molecules) can be produced from a single mRNA transcript. Exogenous sequences can be multicistronic through the use of various linkers, *e.g.*, a

polynucleotide sequence encoding a first effector molecule can be linked to a nucleotide sequence encoding a second effector molecule, such as in a first gene:linker:second gene 5' to 3' orientation. A linker can encode a 2A ribosome skipping element, such as T2A. Other 2A ribosome skipping elements include, but are not limited to, E2A, P2A, and F2A. 2A ribosome skipping elements allow production of separate polypeptides encoded by the first and second genes are produced during translation. A linker can encode a cleavable linker polypeptide sequence, such as a Furin cleavage site or a TEV cleavage site, wherein following expression the cleavable linker polypeptide is cleaved such that separate polypeptides encoded by the first and second genes are produced. A cleavable linker can include a polypeptide sequence, such as such a flexible linker (*e.g.*, a Gly-Ser-Gly sequence), that further promotes cleavage.

A linker can encode an Internal Ribosome Entry Site (IRES), such that separate polypeptides encoded by the first and second genes are produced during translation. A linker can encode a splice acceptor, such as a viral splice acceptor.

A linker can be a combination of linkers, such as a Furin-2A linker that can produce separate polypeptides through 2A ribosome skipping followed by further cleavage of the Furin site to allow for complete removal of 2A residues. In some embodiments, a combination of linkers can include a Furin sequence, a flexible linker, and 2A linker. Accordingly, in some embodiments, the linker is a Furin-Gly-Ser-Gly-2A fusion polypeptide. In some embodiments, a linker of the present disclosure is a Furin-Gly-Ser-Gly-T2A fusion polypeptide.

In general, a multicistronic system can use any number or combination of linkers, to express any number of genes or portions thereof (*e.g.*, an exogenous sequence can encode a first, a second, and a third effector molecule, each separated by linkers such that separate polypeptides encoded by the first, second, and third effector molecules are produced).

Exogenous sequences can use multiple promoters to express genes from multiple ORFs, *i.e.*, more than one separate mRNA transcript can be produced from the exogenous sequence. For example, a first promoter can be operably linked to a polynucleotide sequence encoding a first effector molecule, and a second promoter can be operably linked to a polynucleotide sequence encoding a second effector molecule.

"Linkers," as used herein can refer to polypeptides that link a first polypeptide sequence and a second polypeptide sequence, the multicistronic linkers described above, or the additional promoters that are operably linked to additional ORFs described above.

Engineered cells, such as MSCs, of the present disclosure typically produce multiple effector molecules, at least two of which modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, at least one of the effector molecules stimulates an inflammatory pathway in the tumor microenvironment, and at least one of the effector molecules inhibits a negative regulator of inflammation in the tumor microenvironment.

A "tumor microenvironment" is the cellular environment in which a tumor exists, including surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, lymphocytes, signaling molecules and the extracellular matrix (ECM) (see, e.g., Pattabiraman, D.R. & Weinberg, R.A. *Nature Reviews Drug Discovery* 13, 497–512 (2014); Balkwill, F.R. et al. *J Cell Sci* 125, 5591-5596, 2012; and Li, H. et al. *J Cell Biochem* 101(4), 805-15, 2007).

In some embodiments, cells are engineered to produce at least one homing molecule. "Homing," refers to active navigation (migration) of a cell to a target site (e.g., a cell, tissue (e.g., tumor), or organ). A "homing molecule" refers to a molecule that directs cells to a target site. In some embodiments, a homing molecule functions to recognize and/or initiate interaction of a cell to a target site. Non-limiting examples of homing molecules include CXCR1, CCR9, CXCR2, CXCR3, CXCR4, CCR2, CCR4, FPR2, VEGFR, IL6R, CXCR1, CSCR7, and PDGFR.

In some embodiments, a homing molecule is a chemokine receptor (cell surface molecule that binds to a chemokine). Chemokines are small cytokines or signaling proteins secreted by cells that can induce directed chemotaxis in cells. Chemokines can be classified into four main subfamilies: CXC, CC, CX3C and XC, all of which exert biological effects by binding selectively to chemokine receptors located on the surface of target cells. In some embodiments, cells are engineered to produce CXCR4, a chemokine receptor which allows cells to home along a chemokine gradient towards a stromal cell-derived factor 1 (also known as SDF1, C-X-C motif chemokine 12, and CXCL12)-expressing cell, tissue, or tumor. Non-limiting examples of chemokine receptors that may be produced by the engineered cells of the present disclosure include: CXC chemokine receptors (e.g., CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, and CXCR7), CC chemokine receptors (CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, and CCR11), CX3C chemokine receptors (e.g., CX3CR1, which binds to CX3CL1), and XC chemokine receptors (e.g., XCR1). In some embodiments, a chemokine

receptor is a G protein-linked transmembrane receptor, or a member of the tumor necrosis factor (TNF) receptor superfamily (including but not limited to TNFRSF1A, TNFRSF1B). In some embodiments, cells are engineered to produce CXCL8, CXCL9, and/or CXCL10, 11 or a fusion protein that encompass CXCL10 and CXCL11 (promote T-cell recruitment), CCL3 and/or CXCL5, CCL21 (Th1 recruitment and polarization). In some embodiments cells are engineered to produce CXCL13 to promote B-cell recruitment.

In some embodiments, cells are engineered to produce G-protein coupled receptors (GPCRs) that detect N-formylated-containing oligopeptides (including but not limited to FPR2 and FPRL1).

In some embodiments, cells are engineered to produce receptors that detect interleukins (including but not limited to IL6R).

In some embodiments, cells are engineered to produce receptors that detect growth factors secreted from other cells, tissues, or tumors (including but not limited to FGFR, PDGFR, EGFR, and receptors of the VEGF family, including but not limited to VEGF-C and VEGF-D).

In some embodiments, a homing molecule is an integrin. Integrins are transmembrane receptors that facilitate cell-extracellular matrix (ECM) adhesion. Integrins are obligate heterodimers having two subunits: α (alpha) and β (beta). The α subunit of an integrin may be, without limitation: ITGA1, ITGA2, ITGA3, ITGA4, ITGA5, ITGA6, IGTA7, ITGA8, ITGA9, IGTA10, IGTA11, ITGAD, ITGAE, ITGAL, ITGAM, ITGAV, ITGA2B, ITGAX. The β subunit of an integrin may be, without limitation: ITGB1, ITGB2, ITGB3, ITGB4, ITGB5, ITGB6, ITGB7, and ITGB8. Cells of the present disclosure may be engineered to produce any combination of the integrin α and β subunits.

In some embodiments, a homing molecule is a matrix metalloproteinase (MMP). MMPs are enzymes that cleave components of the basement membrane underlying the endothelial cell wall. Non-limiting examples of MMPs include MMP-2, MMP-9, and MMP. In some embodiments, cells are engineered to produce an inhibitor of a molecule (e.g., protein) that inhibits MMPs. For example, cells may be engineered to express an inhibitor (e.g., an RNAi molecule) of membrane type 1 MMP (MT1-MMP) or TIMP metallopeptidase inhibitor 1 (TIMP-1).

In some embodiments, a homing molecule is a ligand that binds to selectin (e.g., hematopoietic cell E-/L-selectin ligand (HCELL), Dykstra et al., Stem Cells. 2016 Oct;34(10):2501-2511) on the endothelium of a target tissue, for example.

The term "homing molecule" also encompasses transcription factors that regulate the production of molecules that improve/enhance homing of cells.

In some embodiments, cell homing is increased by locally irradiating a tumor/cancer cells in a subject. Radiological tissue damage aids in cell homing, as well as endogenous T cell homing to that damaged tissue.

Examples of Engineered Cells

Cells (e.g., MSCs) as provided herein are engineered to produce multiple effector molecules, at least two of which modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, at least one (e.g., 1, 2, 3, 4, 5, or more) effector molecule stimulates at least one immunostimulatory mechanism in the tumor microenvironment, or inhibits at least one immunosuppressive mechanism in the tumor microenvironment. In some embodiments, at least one (e.g., 1, 2, 3, 4, 5, or more) effector molecule inhibits at least one immunosuppressive mechanism in the tumor microenvironment, and at least one effector molecule (e.g., 1, 2, 3, 4, 5, or more) inhibits at least one immunosuppressive mechanism in the tumor microenvironment. In yet other embodiments, at least two (e.g., 2, 3, 4, 5, or more) effector molecules stimulate at least one immunostimulatory mechanism in the tumor microenvironment. In still other embodiments, at least two (e.g., 1, 2, 3, 4, 5, or more) effector molecules inhibit at least one immunosuppressive mechanism in the tumor microenvironment.

In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that stimulates T cell signaling, activity and/or recruitment. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that stimulates antigen presentation and/or processing. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that stimulates natural killer cell-mediated cytotoxic signaling, activity and/or recruitment. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that stimulates dendritic cell differentiation and/or maturation. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that stimulates immune cell recruitment. In some embodiments, a cell (e.g.,

MSC) is engineered to produce at least one effector molecule that stimulates M1 macrophage signaling, activity and/or recruitment. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that stimulates Th1 polarization. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that stimulates stroma degradation. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that stimulates immunostimulatory metabolite production. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that stimulates Type I interferon signaling. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that inhibits negative costimulatory signaling. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that inhibits pro-apoptotic signaling (e.g., via TRAIL) of anti-tumor immune cells. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that inhibits T regulatory (T_{reg}) cell signaling, activity and/or recruitment. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that inhibits tumor checkpoint molecules. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that activates stimulator of interferon genes (STING) signaling. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that inhibits myeloid-derived suppressor cell signaling, activity and/or recruitment. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that degrades immunosuppressive factors/metabolites. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that inhibits vascular endothelial growth factor signaling. In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule that directly kills tumor cells (e.g., granzyme, perforin, oncolytic viruses, cytolytic peptides and enzymes, anti-tumor antibodies, e.g., that trigger ADCC).

In some embodiments, at least one effector molecule: stimulates T cell signaling , activity and/or recruitment, stimulates antigen presentation and/or processing, stimulates natural killer cell-mediated cytotoxic signaling , activity and/or recruitment, stimulates dendritic cell differentiation and/or maturation, stimulates immune cell recruitment, stimulates macrophage signaling, stimulates stroma degradation, stimulates immunostimulatory metabolite production, or stimulates Type I interferon signaling; and at least one effector molecule inhibits negative costimulatory signaling, inhibits pro-apoptotic signaling of anti-tumor immune cells, inhibits T

regulatory (Treg) cell signaling, activity and/or recruitment, inhibits tumor checkpoint molecules, activates stimulator of interferon genes (STING) signaling, inhibits myeloid-derived suppressor cell signaling, activity and/or recruitment, degrades immunosuppressive factors/metabolites, inhibits vascular endothelial growth factor signaling, or directly kills tumor cells.

In some embodiments, a cell (e.g., MSC) is engineered to produce at least one effector molecule selected from IL-12, IFN-β, IFN-γ, IL-2, IL-15, IL-7, IL-36γ, IL-18, IL-1β, OX40-ligand, and CD40L; and/or at least one effector molecule selected from anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-CTLA-4 antibodies, and anti-IL-35 antibodies; and/or at least one effector molecule selected from MIP1α (CCL3), MIP1β (CCL5), and CCL21; and/or at least one effector molecule selected from CpG oligodeoxynucleotides; and/or at least one effector molecule selected from microbial peptides.

In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and at least one effector molecule selected from cytokines, antibodies, chemokines, nucleotides, peptides, enzymes, and stimulators of interferon genes (STINGs). In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and at least one cytokine or receptor/ligand (e.g., IL-12, , IFN-γ, IL-2, IL-15, IL-7, IL-36γ, IL-18, IL-1β, OX40-ligand, and/or CD40L).

In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and at least one cytokine or receptor/ligand (e.g., IL-12, , IFN-γ, IL-2, IL-15, IL-7, IL-36γ, IL-18, IL-1β, OX40-ligand, and/or CD40L).

In some embodiments the cytokine is produced as an engineered fusion protein with an antibody, antibody-fragment, or receptor that self-binds to the cytokine to induce cell-specific targeted binding such as with IL-2 fused to an antibody fragment preventing it from binding to Treg cells and preferentially binding to CD8 and NK cells. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and at least one antibody (e.g., anti-PD-1 antibody, anti-PD-L1 antibody, anti-CTLA-4 antibody, anti-VEGF, anti-TGF-β, anti-IL-10, anti-TNF-α, and/or anti-IL-35 antibody). In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and at least one chemokine (MIP1α (CCL3), MIP1β (CCL5), and/or CCL21). In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and at least one nucleotide (e.g., a CpG oligodeoxynucleotide). In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and at least one peptide (e.g., an anti-tumor peptide). In some embodiments, a

cell (e.g., MSC) is engineered to produce IFN-β and at least one enzyme. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and at least one STING activator. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and at least one effector with direct anti-tumor activity (e.g., oncolytic virus).

5 In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and MIP1-α. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and MIP1-β. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and CXCL9. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and CXCL10. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and CXCL11. In some 10 embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and CCL21. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL36-γ, IL-18, CD40L and/or 41BB-L. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and MIP1-α. In 15 some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and MIP1-β. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and CXCL9. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and CXCL10. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and CXCL11. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and CCL21. In some 20 embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL36-γ, IL-18, CD40L and/or 41BB-L. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce IL-12 and MIP1-α. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-12 and MIP1-β. In some 25 embodiments, a cell (e.g., MSC) is engineered to produce IL-12 and CXCL9. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-12 and CXCL10. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-12 and CXCL11. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-12 and CCL21. In some embodiments, the cell is engineered to further produce IFN-β, IFN-γ, IL-2, IL-7, IL-15, IL36-γ,

IL-18, CD40L and/or 41BB-L. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce TNF-related apoptosis-inducing ligand (TRAIL) and MIP1-α. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and MIP1-β. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and CXCL9. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and CXCL10. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and CXCL11. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and CCL21. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL36-γ, IL-18, CD40L and/or 41BB-L. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce a stimulator of interferon gene (STING) and MIP1-α. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and MIP1-β. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and CXCL9. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and CXCL10. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and CXCL11. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and CCL21. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL36-γ, IL-18, CD40L and/or 41BB-L. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and MIP1-α. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and MIP1-β. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and CXCL9. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and CXCL10. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and CXCL11. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and CCL21. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL36-γ, IL-18, and/or 41BB-L. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and MIP1-α. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and MIP1-β. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and CXCL9. In some embodiments, a cell (e.g., MSC) is engineered to 5 produce cytosine deaminase and CXCL10. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and CXCL11. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and CCL21. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL36-γ, IL-18, CD40L, and/or 41BB-L. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, 10 andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and IL-12. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and IFN-γ. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and IL-2. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and IL-7. In some 15 embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and IL-15. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and IL-36γ. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and IL-18. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and CD40L. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and 41BB-L. In some 20 embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, CXCL10-11 fusion, CXCL13 and/or CCL21. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and IL-12. In 25 some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and IFN-γ. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and IL-2. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and IL-7. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and IL-15. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and IL-36γ. In some 30 embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and IL-18. In some

embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and CD40L. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and 41BB-L. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce TNF-related apoptosis-inducing ligand (TRAIL) and IL-12. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and IFN-γ. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and IL-2. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and IL-7. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and IL-15. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and IL-36γ. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and IL-18. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and CD40L. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and 41BB-L. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce a stimulator of interferon gene (STING) and IL-12. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and IFN-γ. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and IL-2. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and IL-7. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and IL-15. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and IL-36γ. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and IL-18. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and CD40L. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and 41BB-L. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and IL-12. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and IFN-γ. In some

embodiments, a cell (e.g., MSC) is engineered to produce CD40L and IL-2. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and IL-7. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and IL-15. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and IL-36γ. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and IL-18. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and 41BB-L. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and IL-12. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and IFN-γ. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and IL-2. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and IL-7. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and IL-15. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and IL-36γ. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and IL-18. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and CD40L. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and 41BB-L. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-α and IL-12. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-α and MIP1-γ. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-α and IL-2. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-α and IL-7. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-α and IL-15. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-α and IL-36γ. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-α and IL-18. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-α and CD40L. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-α and 41BB-L. In some

embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-β and IL-12. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-β and MIP1-γ. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-β and IL-2. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-β and IL-7. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-β and IL-15. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-β and IL-36γ. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-β and IL-18. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-β and CD40L. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-β and 41BB-L. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL9 and IL-12. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL9 and IFN-γ. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL9 and IL-2. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL9 and IL-7. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL9 and IL-15. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL9 and IL-36γ. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL9 and IL-18. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL9 and CD40L. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL9 and 41BB-L. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce a CXCL10 and IL-12. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL10 and IFN-γ. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL10 and IL-2. In some

embodiments, a cell (e.g., MSC) is engineered to produce CXCL10 and IL-7. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL10 and IL-15. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL10 and IL-36γ. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL10 and IL-18. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL10 and CD40L. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL10 and 41BB-L. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL11 and IL-12. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL11 and IFN-γ. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL11 and IL-2. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL11 and IL-7. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL11 and IL-15. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL11 and IL-36γ. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL11 and IL-18. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL11 and 41BB-L. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce CCL21 and IL-12. In some embodiments, a cell (e.g., MSC) is engineered to produce CCL21 and IFN-γ. In some embodiments, a cell (e.g., MSC) is engineered to produce CCL21 and IL-2. In some embodiments, a cell (e.g., MSC) is engineered to produce CCL21 and IL-7. In some embodiments, a cell (e.g., MSC) is engineered to produce CCL21 and IL-15. In some embodiments, a cell (e.g., MSC) is engineered to produce CCL21 and IL-36γ. In some embodiments, a cell (e.g., MSC) is engineered to produce CCL21 and IL-18. In some embodiments, a cell (e.g., MSC) is engineered to produce CCL21 and CD40L. In some embodiments, a cell (e.g., MSC) is engineered to produce CCL21 and 41BB-L. In some embodiments, the cell is engineered to further produce IFN- α, IFN-β, TRAIL, STING, CD40L,

and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce anti-CD40 antibody, andti-CTLA4 antibody, anti-PD-L1 antibody, and/or OX40L.

In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-α and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CXCL21. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL-36γ, IL-18, CD40L, and/or 41BB-L.

In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-β and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CXCL21. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL-36γ, IL-18, CD40L, and/or 41BB-L.

In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce TRAIL and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CXCL21. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL-36γ, IL-18, CD40L, and/or 41BB-L.

In some embodiments, a cell (e.g., MSC) is engineered to produce STING and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce STING and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CXCL21. In some embodiments, the cell is

engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL-36γ, IL-18, CD40L, and/or 41BB-L.

In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CXCL21. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL-36γ, IL-18, CD40L, and/or 41BB-L.

In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce cytosine deaminase and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CXCL21. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL-36γ, IL-18, CD40L, and/or 41BB-L.

In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-α and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-α and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-α and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-α and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL-36γ, IL-18, CD40L, and/or 41BB-L.

In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-β and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-β and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-β and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce MIP1-β

and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL-36γ, IL-18, CD40L, and/or 41BB-L.

5 In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL9 and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL9 and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL9 and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL9 and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, 10 IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL-36γ, IL-18, CD40L, and/or 41BB-L.

In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL10 and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL10 and 15 OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL10 and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL10 and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL-36γ, IL-18, CD40L, and/or 20 41BB-L.

In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL11 and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL11 and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL11 and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce CXCL11 25 and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL-36γ, IL-18, CD40L, and/or 41BB-L.

In some embodiments, a cell (e.g., MSC) is engineered to produce CCL21 and anti-PD-30 L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce CCL21 and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce CCL21 and anti-

CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce CCL21 and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce IL-12, IFN-γ, IL-2, IL-7, IL-15, IL-36γ, IL-18, CD40L, and/or 41BB-L.

In some embodiments, a cell (e.g., MSC) is engineered to produce IL-12 and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-12 and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-12 and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-12 and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21.

In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-γ and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-γ and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-γ and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IFN-γ and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21.

In some embodiments, a cell (e.g., MSC) is engineered to produce IL-2 and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-2 and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-2 and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-2 and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21.

In some embodiments, a cell (e.g., MSC) is engineered to produce IL-7 and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-7 and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-7 and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-7 and anti-

CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21.

In some embodiments, a cell (e.g., MSC) is engineered to produce IL-15 and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-15 and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-15 and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-15 and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21.

In some embodiments, a cell (e.g., MSC) is engineered to produce IL-36-γ and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-36-γ and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-36-γ and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-36-γ and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21.

In some embodiments, a cell (e.g., MSC) is engineered to produce IL-18 and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-18 and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-18 and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce IL-18 and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21.

In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce CD40L and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α,

IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21.

In some embodiments, a cell (e.g., MSC) is engineered to produce 41BB-L and anti-PD-L1 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce 41BB-L and OX40L. In some embodiments, a cell (e.g., MSC) is engineered to produce 41BB-L and anti-CTLA4 antibody. In some embodiments, a cell (e.g., MSC) is engineered to produce 41BB-L and anti-CD47 antibody. In some embodiments, the cell is engineered to further produce IFN-α, IFN-β, TRAIL, STING, CD40L, and/or cytosine deaminase. In some embodiments, the cell is engineered to further produce MIP1-α, MIP1-β, CXCL9, CXCL10, CXCL11, and/or CCL21.

Secretion Signals

In general, the one or more effector molecules comprise a secretion signal peptide (also referred to as a signal peptide or signal sequence) at the effector molecule's N-terminus that direct newly synthesized proteins destined for secretion or membrane insertion to the proper protein processing pathways. The secretion signal peptide operably associated with a effector molecule can be a native secretion signal peptide native secretion signal peptide(*e.g.*, the secretion signal peptide generally endogenously associated with the given effector molecule). The secretion signal peptide operably associated with a effector molecule can be a non-native secretion signal peptide native secretion signal peptide. Non-native secretion signal peptides can promote improved expression and function, such as maintained secretion, in particular environments, such as tumor microenvironments. Non-limiting examples of non-native secretion signal peptide are shown in Table 5.

Table 5. Exemplary Signal Secretion Peptides

Name	Protein SEQUENCE	Source (Uniprot)	DNA SEQUENCE
IL-12	MCHQQLVISWFSL VFLASPLVA (SEQ ID NO: 112)	P29460	ATGTGTCACCAGCAGCTCGTTATATC CTGGTTTAGTTTGGTGTTTCTCGCTTC ACCCCTGGTGGCA (SEQ ID NO: 31)
IL-12 (Codon Optimized)	MCHQQLVISWFSL VFLASPLVA (SEQ ID NO: 112)	-	ATGTGCCATCAGCAACTCGTCATCTC CTGGTTCTCCCTTGTGTTCCTCGCTTC CCCTCTGGTCGCC (SEQ ID NO: 32)
IL-2 (Optimized)	MQLLSCIALILALV (SEQ ID NO: 113)	-	ATGCAACTGCTGTCATGTATCGCACT CATCCTGGCGCTGGTA (SEQ ID NO: 33)
IL-2 (Native)	MYRMQLLSCIALSL ALVTNS (SEQ ID NO: 114)	P60568	ATGTATCGGATGCAACTTTTGAGCTG CATCGCATTGTCTCTGGCGCTGGTGA CAAATTCC (SEQ ID NO: 34)

Trypsinogen-2	MNLLLILTFVAAAVA (SEQ ID NO: 115)	P07478	ATGAATCTCTTGCTCATACTTACGTTTGTCGCTGCTGCCGTTGCG (SEQ ID NO: 35)
Gaussia Luciferase	MGVKVLFALICIAVAEA (SEQ ID NO: 116)	-	ATGGGCGTGAAGGTCTTGTTTGCCCTTATCTGCATAGCTGTTGCGGAGGCG (SEQ ID NO: 36)
CD5	MPMGSLQPLATLYLLGMLVASCLG (SEQ ID NO: 117)	P06127	ATGCCGATGGGGAGCCTTCAACCTTTGGCAACGCTTTATCTTCTGGGGATGTTGGTTGCTAGTTGCCTTGGG (SEQ ID NO: 37)
IgKVII (mouse)	METDTLLLWVLLLWVPGSTGD (SEQ ID NO: 118)		ATGGAAACTGACACGTTGTTGCTGTGGGTATTGCTCTTGTGGGTCCCAGGATCTACGGGCGAC (SEQ ID NO: 38)
IgKVII (human)	MDMRVPAQLLGLLLLWLRGARC (SEQ ID NO: 119)	P01597	ATGGATATGAGGGTTCCCGCCCAGCTTTTGGGGCTGCTTTTGTTGTGGCTTCGAGGGGCTCGGTGT (SEQ ID NO: 39)
VSV-G	MKCLLYLAFLFIGVNC (SEQ ID NO: 120)	-	ATGAAGTGTCTGTTGTACCTGGCGTTTCTGTTCATTGGTGTAAACTGT (SEQ ID NO: 40)
Prolactin	MNIKGSPWKGSLLLLLVSNLLLCQSVAP (SEQ ID NO: 121)	P01236	ATGAATATCAAAGGAAGTCCGTGGAAGGGTAGTCTCCTGCTGCTCCTCGTATCTAACCTTCTCCTTTGTCAATCCGTGGCACCC (SEQ ID NO: 41)
Serum albumin preproprotein	MKWVTFISLLFLFSSAYS (SEQ ID NO: 122)	P02768	ATGAAATGGGTAACATTCATATCACTTCTCTTTCTGTTCAGCTCTGCGTATTCT (SEQ ID NO: 42)
Azurocidin preproprotein	MTRLTVLALLAGLLASSRA (SEQ ID NO: 123)	20160	ATGACAAGGCTTACTGTTTTGGCTCTCCTCGCTGGACTCTTGGCTTCCTCCCGAGCA (SEQ ID NO: 43)
Osteonectin (BM40)	MRAWIFFLLCLAGRALA (SEQ ID NO: 124)	P09486	ATGAGGGCTTGGATTTTTTTTCTGCTCTGCCTTGCCGGTCGAGCCCTGGCG (SEQ ID NO: 44)
CD33	MPLLLLLPLLWAGALA (SEQ ID NO: 125)	P20138	ATGCCTCTTCTGCTTTTGCTTCCTCTTTTGTGGGCAGGTGCCCTCGCA (SEQ ID NO: 45)
IL-6	MNSFSTSAFGPVAFSLGLLLVLPAAFPAP (SEQ ID NO: 126)	P05231	ATGAACTCTTTCTCAACCTCTGCGTTTGGTCCGGTCGCTTTCTCCCTTGGGCTCCTGCTTGTCTTGCCAGCAGCGTTTCCTGCGCCA (SEQ ID NO: 46)
IL-8	MTSKLAVALLAAFLISAALC (SEQ ID NO: 127)	P10145	ATGACAAGTAAACTGGCGGTAGCCTTGCTCGCGGCCTTTTTGATTTCCGCAGCCCTTTGT (SEQ ID NO: 47)
CCL2	MKVSAALLCLLLIAATFIPQGLA (SEQ ID NO: 128)	P13500	ATGAAGGTAAGTGCAGCGTTGCTTTGCCTTCTCCTCATTGCAGCGACCTTTATTCCTCAAGGGCTGGCC (SEQ ID NO: 48)
TIMP2	MGAAARTLRLALGLLLLATLLRPADA (SEQ ID NO: 129)	P16035	ATGGGAGCGGCAGCTAGAACACTTCGACTTGCCCTTGGGCTCTTGCTCCTTGCAACCCTCCTTAGACCTGCCGACGCA (SEQ ID NO: 49)
VEGFB	MSPLLRRLLLAALLQLAPAQA (SEQ ID NO: 130)	P49765	ATGTCACCGTTGTTGCGGAGATTGCTGTTGGCCGCACTTTTGCAACTGGCTCCTGCTCAAGCC (SEQ ID NO: 50)

Osteoprotegerin	MNNLLCCALVFLDI SIKWTTQ (SEQ ID NO: 131)	O00300	ATGAATAACCTGCTCTGTTGTGCGCT CGTGTTCCTGGACATTTCTATAAAAT GGACAACGCAA (SEQ ID NO: 51)
Serpin E1	MQMSPALTCLVLG LALVFGEGSA (SEQ ID NO: 132)	P05121	ATGCAAATGTCTCCTGCCCTTACCTG TCTCGTACTTGGTCTTGCGCTCGTATT TGGAGAGGGATCAGCC (SEQ ID NO: 52)
GROalpha	MARAALSAAPSNP RLLRVALLLLLLVA AGRRAAG (SEQ ID NO: 133)	P09341	ATGGCAAGGGCTGCACTCAGTGCTG CCCCGTCTAATCCCAGATTGCTTCGA GTTGCATTGCTTCTTCTGTTGCTGGTT GCAGCTGGTAGGAGAGCAGCGGGT (SEQ ID NO: 53)
CXCL12	MNAKVVVVLVLVL TALCLSDG (SEQ ID NO: 134)	P48061	ATGAATGCAAAAGTCGTGGTCGTGCT GGTTTTGGTTCTGACGGCGTTGTGTC TTAGTGATGGG (SEQ ID NO: 54)
IL-21 (Codon Optimized)	MERIVICLMVIFLGT LVHKSSS (SEQ ID NO: 135)	Q9HBE4	ATGGAACGCATTGTGATCTGCCTGAT GGTCATCTTCCTGGGCACCTTAGTGC ACAAGTCGAGCAGC (SEQ ID NO: 55)

Cell Types

The present disclosure refers to mesenchymal stem cells (MSCs) (e.g., human MSCs) engineered to produce multiple effector molecules. An engineered cell (engineered to produce effector molecules), as provided herein, may also be selected from natural killer (NK) cells, NKT cells, innate lymphoid cells, mast cells, eosinophils, basophils, macrophages, neutrophils, and dendritic cells, T cells (e.g., CD8+ T cells, CD4+ T cells, gamma-delta T cells, and T regulatory cells ($CD4^+$, $FOXP3^+$, $CD25^+$)) and B cells. It should be understood, however, that any reference to MSC engineering can also be applied to other cell types (e.g., cell types of the immune system).

In some embodiments, an engineered cell (*e.g.*, MSC) is from (e.g., obtained from or derived from) bone marrow. In some embodiments, an engineered mesenchymal stem cell is from (e.g., obtained from or derived from) adipose tissue. In some embodiments, an engineered mesenchymal stem cell is from (e.g., obtained from or derived from) an umbilical cord. In some embodiments, engineered mesenchymal stem cell is from a pluripotent stem cell (e.g., an embryonic stem cell or an induced pluripotent stem cell).

Thus, the present disclosure provides a T cell (e.g., CD8+ T cell, CD4+ T cell, gamma-delta T cell, or T regulatory cell ($CD4^+$, $FOXP3^+$, $CD25^+$)) engineered to produce multiple effector molecules, at least two of which modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, a B cell is engineered to produce multiple effector

molecules, at least two of which modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, a NK cell is engineered to produce multiple effector molecules, at least two of which modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, a NKT cell is engineered to produce multiple effector molecules, at least two of which modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, an innate lymphoid cell is engineered to produce multiple effector molecules, at least two of which modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, a mast cell is engineered to produce multiple effector molecules, at least two of which modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, an eosinophil is engineered to produce multiple effector molecules, at least two of which modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, a basophil is engineered to produce multiple effector molecules, at least two of which modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, a macrophage is engineered to produce multiple effector molecules, at least two of which modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, a neutrophil is engineered to produce multiple effector molecules, at least two of which modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, a dendritic cell is engineered to produce multiple effector molecules, at least two of which modulate different tumor-mediated immunosuppressive mechanisms.

In some embodiments, at least one of the effector molecules stimulates an immunostimulatory mechanism in the tumor microenvironment and/or inhibits an immunosuppressive mechanism in the tumor microenvironment.

In some embodiments, at least one of the effector molecules (a) stimulates T cell signaling, activity and/or recruitment, (b) stimulates antigen presentation and/or processing, (c) stimulates natural killer cell-mediated cytotoxic signaling, activity and/or recruitment, (d) stimulates dendritic cell differentiation and/or maturation, (e) stimulates immune cell recruitment, (f) stimulates pro-inflammatory macrophage signaling, activity and/or recruitment or inhibits anti-inflammatory macrophage signaling, activity and/or recruitment, (g) stimulates stroma degradation, (h) stimulates immunostimulatory metabolite production, (i) stimulates Type I interferon signaling, (j) inhibits negative costimulatory signaling, (k) inhibits pro-apoptotic

signaling of anti-tumor immune cells, (l) inhibits T regulatory (T_{reg}) cell signaling, activity and/or recruitment, (m) inhibits tumor checkpoint molecules, (n) stimulates stimulator of interferon genes (STING) signaling, (o) inhibits myeloid-derived suppressor cell signaling, activity and/or recruitment, (p) degrades immunosuppressive factors/metabolites, (q) inhibits
5 vascular endothelial growth factor signaling, and/or (r) directly kills tumor cells.

Methods

Also provided herein are methods that include culturing the engineered MSCs (or other engineered immune cell) of the present disclosure. Methods of culturing MSCs are known. In
10 some embodiments, MSCs are culture in growth medium (e.g., MSCGM human Mesenchymal Stem Cell Growth BULLETKIT™ Medium (serum containing), THERAPEAK™ MSCGM-CD™ Mesenchymal Stem Cell Chemically Defined Medium (serum free), or RoosterBio xeno-free MSC media). Methods of culturing other cells, such as immune cells, are known to those skilled in the art.
15 Further provided herein are methods that include delivering, or administering, to a subject (e.g., a human subject) engineered cells as provided herein to produce *in vivo* at least one effector molecule produced by the cells. In some embodiments, the cells are administered via intravenous, intraperitoneal, intratracheal, subcutaneous, intratumoral, oral, anal, intranasal (e.g., packed in a delivery particle), or arterial (e.g., internal carotid artery) routes. Thus, the cells may
20 be administered systemically or locally (e.g., to a TME).
The engineered cells or polynucleotides described herein can be in a composition containing a pharmaceutically acceptable carrier, e.g., an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.9% saline, 0.3% glycine, hyaluronic acid and the like. These compositions can be sterilized by conventional, well known sterilization techniques, or can be
25 sterile filtered. The resulting aqueous solutions can be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate,
30 sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

Some methods comprise selecting a subject (or patient population) having a tumor (or cancer) and treating that subject with engineered cells.

The engineered cells of the present disclosure may be used, in some instances, to treat cancer, such as ovarian cancer. Other cancers are described herein. For example, the engineered cells may be used to treat bladder tumors, brain tumors, breast tumors, cervical tumors, colorectal tumors, esophageal tumors, gliomas, kidney tumors, liver tumors, lung tumors, melanomas, ovarian tumors, pancreatic tumors, prostate tumors, skin tumors, thyroid tumors, and/or uterine tumors.

The methods provided herein also include delivering a preparation of engineered cells, such as engineered cells. A preparation, in some embodiments, is a substantially pure preparation, containing, for example, less than 5% (e.g., less than 4%, 3%, 2%, or 1%) of cells other than cells. A preparation may comprise $1x10^5$ cells/kg to $1x10^7$ cells/kg, such as engineered cells.

The methods provided herein also include delivering a composition *in vivo* capable of producing the engineered cells described herein, such as delivering a lentivirus *in vivo.* Other *in vivo* delivery mechanisms and systems can also be used, including those known for use in human therapy, such as viral delivery systems (*e.g.*, retroviral or adenoviral systems), transposons (*e.g.*, Sleeping Beauty and PiggyBac transposon systems), integrated using PhiC31 into genomic pseudosites, or using nucleases, such as zinc fingers (ZFs), clustered regularly interspaced short palindromic repeats (CRISPR), or transcription activator-like effector nucleases (TALENs).

Table 6: Sequences encoding exemplary effector molecules
IL12 (Human) (SEQ ID NO: 56)
ATGTGCCATCAGCAGCTTGTCATATCTTGGTTTCACTTGTATTCCTGGCCAGCCCTTTGGTTGCGAT CTGGGAGCTCAAGAAGGATGTGTACGTTGTAGAGCTGGACTGGTACCCCGATGCTCCCGGTGAGAT GGTCGTTTTGACATGTGACACTCCAGAAGAGGACGGTATTACGTGGACTCTGGACCAGTCCTCCGA AGTTCTTGGTTCTGGTAAGACTCTGACTATCCAGGTGAAAGAATTTGGGGATGCGGGACAATACAC ATGCCACAAGGGAGGCGAGGTGTTGTCTCATAGTTTGCTGCTTCTCCACAAGAAAGAGGATGGAAT CTGGAGCACCGACATACTCAAGGATCAAAAGGAACCCAAAAATAAGACATTTCTGCGATGTGAGG CTAAGAACTATAGTGGCCGCTTCACTTGTTGGTGGCTGACTACCATCAGCACAGATCTCACGTTTTC AGTAAAAAGTAGTAGAGGTTCAAGTGATCCTCAAGGGGTAACGTGCGGTGCTGCAACACTGTCTGC TGAACGCGTAAGAGGAGATAATAAGGAGTACGAGTATTCCGTAGAATGCCAAGAGGACAGTGCTT GTCCTGCGGCCGAGGAGTCTCTCCCAATAGAAGTGATGGTGGACGCGGTGCATAAACTGAAATATG AGAACTACACAAGCAGTTTTTTTATAAGAGATATCATCAAGCCCGATCCGCCGAAGAATTTGCAAC TTAAACCGCTTAAAAACTCACGCCAGGTTGAAGTATCCTGGGAGTATCCGGATACATGGTCAACAC CACACAGCTATTTTTCCCTTACCTTCTGTGTGCAGGTCCAAGGGAAGAGCAAAAGGGAGAAGAAGG ACAGGGTATTCACTGATAAAACTTCCGCGACGGTCATCTGCCGAAAAAACGCTAGTATATCTGTAC GGGCGCAGGATAGGTACTATAGTTCTTCTTGGTCTGAGTGGGCCTCAGTTCCGTGCTCTGGGGGAGG

AAGTGGAGGAGGGTCCGGCGGTGGAAGCGGGGGAGGGAGTCGCAACTTGCCAGTGGCTACACCAG ATCCAGGCATGTTTCCATGTCTGCATCATTCCCAGAATCTCCTGAGAGCGGTGTCAAATATGCTCCA AAAAGCGAGACAAACACTGGAATTTTACCCGTGTACCAGTGAGGAGATTGATCACGAGGACATAA CCAAGGACAAGACCTCAACTGTAGAAGCGTGTTTGCCGCTGGAGTTGACTAAGAATGAGTCCTGCC TCAATTCCAGAGAAACTTCATTCATTACTAACGGCAGTTGTCTTGCATCCCGGAAAACGTCCTTTAT GATGGCCCTTTGCCTTAGTTCAATTTACGAGGATCTTAAAATGTATCAAGTGGAGTTTAAAACCATG AATGCTAAACTTCTTATGGACCCCAAACGACAAATTTTTCTGGATCAGAATATGCTTGCCGTGATAG ACGAACTCATGCAGGCGCTTAATTTTAACTCCGAAACAGTTCCACAAAAATCTAGCCTTGAAGAAC CTGATTTTTATAAAACGAAGATTAAACTGTGTATCCTGCTGCATGCCTTTCGCATCCGAGCTGTCAC AATCGATAGGGTTATGTCCTACCTTAACGCGAGCtaG
IL12p70 (Human; codon optimized; bold denotes signal sequence) (SEQ ID NO: 57)
ATGTGCCATCAGCAACTCGTCATCTCCTGGTTCTCCCTTGTGTTCCTCGCTTCCCCTCTGGTCGC **C**ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAGCTGGATTGGTACCCGGACGCCCCTGGA GAAATGGTCGTGCTGACTTGCGATACGCCAGAAGAGGACGGCATAACCTGGACCCTGGATCAGA GCTCCGAGGTGCTCGGAAGCGGAAAGACCCTGACCATTCAAGTCAAGGAGTTCGGCGACGCGG GCCAGTACACTTGCCACAAGGGTGGCGAAGTGCTGTCCCACTCCCTGCTGCTGCTGCACAAGAA AGAGGATGGAATCTGGTCCACTGACATCCTCAAGGACCAAAAAGAACCGAAGAACAAGACCTTCC TCCGCTGCGAAGCCAAGAACTACAGCGGTCGGTTCACCTGTTGGTGGCTGACGACAATCTCCAC CGACCTGACTTTCTCCGTGAAGTCGTCACGGGGATCAAGCGATCCTCAGGGCGTGACCTGTGGA GCCGCCACTCTGTCCGCCGAGAGAGTCAGGGGAGACAACAAGGAATATGAGTACTCCGTGGAAT GCCAGGAGGACAGCGCCTGCCCTGCCGCGGAAGAGTCCCTGCCTATCGAGGTCATGGTCGATG CCGTGCATAAGCTGAAATACGAGAACTACACTTCCTCCTTCTTTATCCGCGACATCATCAAGCCTG ACCCCCCCAAGAACTTGCAGCTGAAGCCACTCAAGAACTCCCGCCAAGTGGAAGTGTCTTGGGA ATATCCAGACACTTGGAGCACCCCGCACTCATACTTCTCGCTCACTTTCTGTGTGCAAGTGCAGG GAAAGTCCAAACGGGAGAAGAAAGACCGGGTGTTCACCGACAAAACCTCCGCCACTGTGATTTGT CGGAAGAACGCGTCAATCAGCGTCCGGGCGCAGGATAGATACTACTCGTCCTCCTGGAGCGAAT GGGCCAGCGTGCCTTGTTCCGGTGGCGGATCAGGCGGAGGTTCAGGAGGAGGCTCCGGAGGAG GTTCCCGGAACCTCCCTGTGGCAACCCCCGACCCTGGAATGTTCCCGTGCCTACACCACTCCCA AAACCTCCTGAGGGCTGTGTCGAACATGTTGCAGAAGGCCCGCCAGACCCTTGAGTTCTACCCCT GCACCTCGGAAGAAATTGATCACGAGGACATCACCAAGGACAAGACCTCGACCGTGGAAGCCTG CCTGCCGCTGGAACTGACCAAGAACGAATCGTGTCTGAACTCCCGCGAGACAAGCTTTATCACTA ACGGCAGCTGCCTGGCGTCGAGAAAGACCTCATTCATGATGGCGCTCTGTCTTTCCTCGATCTAC GAAGATCTGAAGATGTATCAGGTCGAGTTCAAGACCATGAACGCCAAGCTGCTCATGGACCCGAA GCGGCAGATCTTCCTGGACCAGAATATGCTCGCCGTGATTGATGAACTGATGCAGGCCCTGAATT TCAACTCCGAGACTGTGCCTCAAAAGTCCAGCCTGGAAGAACCGGACTTCTACAAGACCAAGATC AAGCTGTGCATCCTGTTGCACGCTTTCCGCATTCGAGCCGTGACCATTGACCGCGTGATGTCCTA CCTGAACGCCAGT
IL12 (Mouse) (SEQ ID NO: 58)
ATGTGTCCACAGAAGCTGACAATAAGTTGGTTTGCCATTGTCCTCCTGGTGAGCCCACTCATGGCAA TGTGGGAACTCGAAAAGGATGTCTACGTGGTAGAAGTAGATTGGACTCCAGACGCGCCAGGGGAG ACAGTGAATTTGACATGTGACACACCAGAAGAAGATGACATTACATGGACATCTGACCAACGCCAT GGCGTAATAGGGAGTGGGAAAACACTCACGATCACAGTTAAAGAGTTCTTGGATGCTGGTCAATAT ACTTGCCATAAAGGCGGCGAGACACTCAGCCACTCACATTTGCTTTTGCATAAAAAAGAGAATGGC ATTTGGAGCACTGAAATACTTAAGAACTTTAAGAACAAGACATTTCTCAAGTGTGAGGCCCCTAAT TACAGCGGCAGGTTCACGTGCTCATGGCTGGTCCAGCGCAACATGGACCTCAAGTTTAACATAAAA TCTTCTTCCTCTTCACCTGACTCCAGAGCTGTTACTTGCGGCATGGCTTCTCTGAGCGCAGAAAAAG TAACGTTGGATCAAAGAGACTACGAAAAGTACTCTGTTTCTTGTCAAGAGGATGTTACGTGCCCGA CGGCCGAAGAAACGCTTCCAATTGAACTCGCGTTGGAAGCTCGCCAACAAAACAAGTATGAAAACT ACAGTACAAGCTTCTTTATACGGGATATAATTAAACCCGATCCCCCCAAGAACTTGCAAATGAAAC CACTTAAGAACAGCCAGGTGGAAGTTTCCTGGGAGTATCCAGACTCATGGAGTACTCCTCACAGCT ATTTTTCTCTGAAATTCTTTGTAAGGATACAACGGAAGAAAGAGAAGATGAAAGAGACCGAGGAG GGTTGTAATCAGAAGGGAGCGTTTCTCGTGGAGAAAACGTCTACCGAAGTCCAATGTAAAGGTGGC AATGTGTGCGTCCAAGCTCAGGATAGATACTATAATTCAAGTTGCTCCAAGTGGGCCTGTGTTCCAT GCCGCGTTCGGAGCGGGGGAGGTAGCGGAGGAGGTAGTGGGGGTGGGTCAGGAGGAGGGAGTCG AGTTATCCCGGTGTCAGGCCCCGCACGCTGCTTGAGCCAGAGTCGCAACCTCCTTAAGACAACAGA TGACATGGTGAAAACAGCACGCGAAAAGCTTAAACACTACTCTTGTACGGCGGAGGATATTGATCA

CGAGGATATTACCCGAGACCAAACTAGCACTTTGAAAACCTGTCTGCCCCTTGAACTTCATAAAAA TGAGAGCTGTCTGGCTACACGAGAGACGTCAAGTACGACTAGGGGCAGCTGTCTCCCGCCGCAAAA GACAAGCCTCATGATGACGCTCTGTTTGGGTTCCATTTACGAGGACTTGAAAATGTATCAAACGGA GTTCCAGGCTATAAATGCGGCGTTGCAGAACCATAACCATCAACAAATTATACTTGATAAAGGCAT GTTGGTGGCGATTGATGAACTCATGCAGAGTCTCAATCACAACGGGGAAACGTTGAGACAGAAACC CCCAGTCGGTGAAGCGGACCCATATCGAGTAAAAATGAAGCTCTGCATTCTGCTTCACGCATTCAG CACTAGAGTTGTTACCATCAACCGGGTAATGGGATATCTCTCCAGTGCGtaG
IL21 (Human; codon optimized; bold denotes signal sequence) (SEQ ID NO: 59)
ATGGAACGCATTGTGATCTGCCTGATGGTCATCTTCCTGGGCACCTTAGTGCACAAGTCGAGCA GCCAGGGACAGGACAGGCACATGATTAGAATGCGCCAGCTCATCGATATCGTGGACCAGTTGAA GAACTACGTGAACGACCTGGTGCCCGAGTTCCTGCCGGCCCCCGAAGATGTGGAAACCAATTGC GAATGGTCGGCATTTTCCTGCTTTCAAAAGGCACAGCTCAAGTCCGCTAACACCGGGAACAACGA ACGGATCATCAACGTGTCCATCAAAAAGCTGAAGCGGAAGCCTCCCTCCACCAACGCCGGACGG AGGCAGAAGCATAGGCTGACTTGCCCGTCATGCGACTCCTACGAGAAGAAGCCGCCGAAGGAGT TCCTGGAGCGGTTCAAGTCGCTCCTGCAAAAGATGATTCATCAGCACCTGTCCTCCCGGACTCAT GGGTCTGAGGATTCA
IL12p70_T2A_IL21 (Human; codon optimized; bold denotes signal sequences) (SEQ ID NO: 60)
ATGTGCCATCAGCAACTCGTCATCTCCTGGTTCTCCCTTGTGTTCCTCGCTTCCCCTCTGGTCGC CATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAGCTGGATTGGTACCCGGACGCCCCTGGA GAAATGGTCGTGCTGACTTGCGATACGCCAGAAGAGGACGGCATAACCTGGACCCTGGATCAGA GCTCCGAGGTGCTCGGAAGCGGAAAGACCCTGACCATTCAAGTCAAGGAGTTCGGCGACGCGG GCCAGTACACTTGCCACAAGGGTGGCGAAGTGCTGTCCCACTCCCTGCTGCTGCTGCACAAGAA AGAGGATGGAATCTGGTCCACTGACATCCTCAAGGACCAAAAAGAACCGAAGAACAAGACCTTCC TCCGCTGCGAAGCCAAGAACTACAGCGGTCGGTTCACCTGTTGGTGGCTGACGACAATCTCCAC CGACCTGACTTTCTCCGTGAAGTCGTCACGGGGATCAAGCGATCCTCAGGGCGTGACCTGTGGA GCCGCCACTCTGTCCGCCGAGAGAGTCAGGGGAGACAACAAGGAATATGAGTACTCCGTGGAAT GCCAGGAGGACAGCGCCTGCCCTGCCGCGGAAGAGTCCCTGCCTATCGAGGTCATGGTCGATG CCGTGCATAAGCTGAAATACGAGAACTACACTTCCTCCTTCTTTATCCGCGACATCATCAAGCCTG ACCCCCCCAAGAACTTGCAGCTGAAGCCACTCAAGAACTCCCGCCAAGTGGAAGTGTCTTGGGA ATATCCAGACACTTGGAGCACCCCGCACTCATACTTCTCGCTCACTTTCTGTGTGCAAGTGCAGG GAAAGTCCAAACGGGAGAAGAAAGACCGGGTGTTCACCGACAAAACCTCCGCCACTGTGATTTGT CGGAAGAACGCGTCAATCAGCGTCCGGGCGCAGGATAGATACTACTCGTCCTCCTGGAGCGAAT GGGCCAGCGTGCCTTGTTCCGGTGGCGGATCAGGCGGAGGTTCAGGAGGAGGCTCCGGAGGAG GTTCCCGGAACCTCCCTGTGGCAACCCCCGACCCTGGAATGTTCCCGTGCCTACACCACTCCCA AAACCTCCTGAGGGCTGTGTCGAACATGTTGCAGAAGGCCCGCCAGACCCTTGAGTTCTACCCCT GCACCTCGGAAGAAATTGATCACGAGGACATCACCAAGGACAAGACCTCGACCGTGGAAGCCTG CCTGCCGCTGGAACTGACCAAGAACGAATCGTGTCTGAACTCCCGCGAGACAAGCTTTATCACTA ACGGCAGCTGCCTGGCGTCGAGAAAGACCTCATTCATGATGGCGCTCTGTCTTTCCTCGATCTAC GAAGATCTGAAGATGTATCAGGTCGAGTTCAAGACCATGAACGCCAAGCTGCTCATGGACCCGAA GCGGCAGATCTTCCTGGACCAGAATATGCTCGCCGTGATTGATGAACTGATGCAGGCCCTGAATT TCAACTCCGAGACTGTGCCTCAAAAGTCCAGCCTGGAAGAACCGGACTTCTACAAGACCAAGATC AAGCTGTGCATCCTGTTGCACGCTTTCCGCATTCGAGCCGTGACCATTGACCGCGTGATGTCCTA CCTGAACGCCAGTAGACGGAAACGCGGAAGCGGAGAGGGCAGAGGCTCGCTGCTTACATGCGG GGACGTGGAAGAGAACCCCGGTCCG**ATGGAACGCATTGTGATCTGCCTGATGGTCATCTTCCTG GGCACCTTAGTGCACAAGTCGAGCAGC**CAGGGACAGGACAGGCACATGATTAGAATGCGCCAG CTCATCGATATCGTGGACCAGTTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTCCTGCCGG CCCCCGAAGATGTGGAAACCAATTGCGAATGGTCGGCATTTTCCTGCTTTCAAAAGGCACAGCTC AAGTCCGCTAACACCGGGAACAACGAACGGATCATCAACGTGTCCATCAAAAAGCTGAAGCGGAA GCCTCCCTCCACCAACGCCGGACGGAGGCAGAAGCATAGGCTGACTTGCCCGTCATGCGACTCC TACGAGAAGAAGCCGCCGAAGGAGTTCCTGGAGCGGTTCAAGTCGCTCCTGCAAAAGATGATTC ATCAGCACCTGTCCTCCCGGACTCATGGGTCTGAGGATTCA
IL-12_2A_CCL21a (Human) (SEQ ID NO: 61)
ATGTGCCATCAGCAGCTTGTCATATCTTGGTTTTCACTTGTATTCCTGGCCAGCCCTTTGGTTGCGAT CTGGGAGCTCAAGAAGGATGTGTACGTTGTAGAGCTGGACTGGTACCCCGATGCTCCCGGTGAGAT GGTCGTTTTGACATGTGACACTCCAGAAGAGGACGGTATTACGTGGACTCTGGACCAGTCCTCCGA

AGTTCTTGGTTCTGGTAAGACTCTGACTATCCAGGTGAAAGAATTTGGGGATGCGGGACAATACAC ATGCCACAAGGGAGGCGAGGTGTTGTCTCATAGTTTGCTGCTTCTCCACAAGAAAGAGGATGGAAT CTGGAGCACCGACATACTCAAGGATCAAAAGGAACCCAAAAATAAGACATTTCTGCGATGTGAGG CTAAGAACTATAGTGGCCGCTTCACTTGTTGGTGGCTGACTACCATCAGCACAGATCTCACGTTTTC AGTAAAAAGTAGTAGAGGTTCAAGTGATCCTCAAGGGGTAACGTGCGGTGCTGCAACACTGTCTGC TGAACGCGTAAGAGGAGATAATAAGGAGTACGAGTATTCCGTAGAATGCCAAGAGGACAGTGCTT GTCCTGCGGCCGAGGAGTCTCTCCCAATAGAAGTGATGGTGGACGCGGTGCATAAACTGAAATATG AGAACTACACAAGCAGTTTTTTATAAGAGATATCATCAAGCCCGATCCGCCGAAGAATTTGCAAC TTAAACCGCTTAAAAACTCACGCCAGGTTGAAGTATCCTGGGAGTATCCGGATACATGGTCAACAC CACACAGCTATTTTTCCCTTACCTTCTGTGTGCAGGTCCAAGGGAAGAGCAAAAGGGAGAAGAAGG ACAGGGTATTCACTGATAAAACTTCCGCGACGGTCATCTGCCGAAAAAACGCTAGTATATCTGTAC GGGCGCAGGATAGGTACTATAGTTCTTCTTGGTCTGAGTGGGCCTCAGTTCCGTGCTCTGGGGGAGG AAGTGGAGGAGGGTCCGGCGGTGGAAGCGGGGGAGGGAGTCGCAACTTGCCAGTGGCTACACCAG ATCCAGGCATGTTTCCATGTCTGCATCATTCCCAGAATCTCCTGAGAGCGGTGTCAAATATGCTCCA AAAAGCGAGACAAACACTGGAATTTTACCCGTGTACCAGTGAGGAGATTGATCACGAGGACATAA CCAAGGACAAGACCTCAACTGTAGAAGCGTGTTTGCCGCTGGAGTTGACTAAGAATGAGTCCTGCC TCAATTCCAGAGAAACTTCATTCATTACTAACGGCAGTTGTCTTGCATCCCGGAAAACGTCCTTTAT GATGGCCCTTTGCCTTAGTTCAATTTACGAGGATCTTAAAATGTATCAAGTGGAGTTTAAAACCATG AATGCTAAACTTCTTATGGACCCCAAACGACAAATTTTTCTGGATCAGAATATGCTTGCCGTGATAG ACGAACTCATGCAGGCGCTTAATTTTAACTCCGAAACAGTTCCACAAAAATCTAGCCTTGAAGAAC CTGATTTTTATAAAACGAAGATTAAACTGTGTATCCTGCTGCATGCCTTTCGCATCCGAGCTGTCAC AATCGATAGGGTTATGTCCTACCTTAACGCGAGCCGGCGCAAGAGGGGTTCCGGAGAGGGAAGGG GTAGTCTGCTCACCTGCGGCGATGTTGAAGAAAATCCTGGTCCCATGGCGCAAAGTCTGGCTCTTTC ACTCCTGATCCTGGTCTTGGCCTTCGGGATTCCGAGGACCCAAGGAAGTGATGGTGGCGCCCAAGA TTGTTGCCTTAAATACAGCCAGCGGAAAATACCCGCGAAAGTGGTCAGGAGTTATAGAAAACAGGA GCCTTCCCTGGGTTGTAGTATCCCCGCCATACTTTTCCTCCCGAGAAAACGGAGCCAGGCCGAACTG TGCGCTGACCCTAAGGAACTTTGGGTGCAACAACTTATGCAACACCTGGATAAGACACCTTCTCCTC AAAAGCCAGCTCAGGGCTGCCGAAAAGATAGAGGCGCCTCAAAAACCGGAAAAAAGGGCAAAGG TTCTAAAGGATGTAAGCGGACTGAACGCTCTCAAACGCCTAAAGGGCCGtaG
IL-12_2A_CCL21a (Mouse) (SEQ ID NO: 62)
ATGTGTCCACAGAAGCTGACAATAAGTTGGTTTGCCATTGTCCTCCTGGTGAGCCCACTCATGGCAA TGTGGGAACTCGAAAAGGATGTCTACGTGGTAGAAGTAGATTGGACTCCAGACGCGCCAGGGGAG ACAGTGAATTTGACATGTGACACACCAGAAGAAGATGACATTACATGGACATCTGACCAACGCCAT GGCGTAATAGGGAGTGGGAAAACACTCACGATCACAGTTAAAGAGTTCTTGGATGCTGGTCAATAT ACTTGCCATAAAGGCGGCGAGACACTCAGCCACTCACATTTGCTTTTGCATAAAAAAGAGAATGGC ATTTGGAGCACTGAAATACTTAAGAACTTTAAGAACAAGACATTTCTCAAGTGTGAGGCCCCTAAT TACAGCGGCAGGTTCACGTGCTCATGGCTGGTCCAGCGCAACATGGACCTCAAGTTTAACATAAAA TCTTCTTCCTCTTCACCTGACTCCAGAGCTGTTACTTGCGGCATGGCTTCTCTGAGCGCAGAAAAAG TAACGTTGGATCAAAGAGACTACGAAAAGTACTCTGTTTCTTGTCAAGAGGATGTTACGTGCCCGA CGGCCGAAGAAACGCTTCCAATTGAACTCGCGTTGGAAGCTCGCCAACAAAACAAGTATGAAAACT ACAGTACAAGCTTCTTTATACGGGATATAATTAAACCCGATCCCCCCAAGAACTTGCAAATGAAAC CACTTAAGAACAGCCAGGTGGAAGTTTCCTGGGAGTATCCAGACTCATGGAGTACTCCTCACAGCT ATTTTTCTCTGAAATTCTTTGTAAGGATACAACGGAAGAAAGAGAAGATGAAAGAGACCGAGGAG GGTTGTAATCAGAAGGGAGCGTTTCTCGTGGAGAAAACGTCTACCGAAGTCCAATGTAAAGGTGGC AATGTGTGCGTCCAAGCTCAGGATAGATACTATAATTCAAGTTGCTCCAAGTGGGCCTGTGTTCCAT GCCGCGTTCGGAGCGGGGGAGGTAGCGGAGGAGGTAGTGGGGGTGGGTCAGGAGGAGGGAGTCG AGTTATCCCGGTGTCAGGCCCCGCACGCTGCTTGAGCCAGAGTCGCAACCTCCTTAAGACAACAGA TGACATGGTGAAAACAGCACGCGAAAAGCTTAAACACTACTCTTGTACGGCGGAGGATATTGATCA CGAGGATATTACCCGAGACCAAACTAGCACTTTGAAAACCTGTCTGCCCCTTGAACTTCATAAAAA TGAGAGCTGTCTGGCTACACGAGAGACGTCAAGTACGACTAGGGGCAGCTGTCTCCCGCCGCAAAA GACAAGCCTCATGATGACGCTCTGTTTGGGTTCCATTTACGAGGACTTGAAAATGTATCAAACGGA GTTCCAGGCTATAAATGCGGCGTTGCAGAACCATAACCATCAACAAATTATACTTGATAAAGGCAT GTTGGTGGCGATTGATGAACTCATGCAGAGTCTCAATCACAACGGGGAAACGTTGAGACAGAAACC CCCAGTCGGTGAAGCGGACCCATATCGAGTAAAAATGAAGCTCTGCATTCTGCTTCACGCATTCAG CACTAGAGTTGTTACCATCAACCGGGTAATGGGATATCTCTCCAGTGCGCGGCGCAAGAGGGGTTC CGGAGAGGGAAGGGGTAGTCTGCTCACCTGCGGCGATGTTGAAGAAAATCCTGGTCCCATGGCGCA

AATGATGACCCTTTCCCTGCTGAGTCTTGTCCTCGCGCTCTGCATCCCGTGGACGCAGGGGTCTGAT GGGGGGGGCCAAGACTGTTGCCTGAAGTATTCACAAAAAAAGATACCGTACTCTATTGTCAGAGGG TACAGGAAGCAAGAACCCTCCTTGGGTTGCCCTATACCAGCAATTCTTTTCTCCCCACGCAAGCATT CCAAACCAGAACTGTGTGCGAACCCCGAGGAGGGTTGGGTACAGAACTTGATGCGAAGGCTTGACC AGCCCCCAGCCCCTGGCAAGCAGTCACCTGGGTGCAGAAAAAACAGAGGTACTTCAAAGAGCGGC AAGAAAGGCAAAGGGAGTAAAGGATGTAAAAGAACGGAGCAGACCCAGCCTTCACGAGGCtaG
CCL21a_2A_IL-12 (Mouse) (SEQ ID NO: 63)
ATGGCGCAAATGATGACCCTTTCCCTGCTGAGTCTTGTCCTCGCGCTCTGCATCCCGTGGACGCAGG GGTCTGATGGGGGGGGCCAAGACTGTTGCCTGAAGTATTCACAAAAAAAGATACCGTACTCTATTG TCAGAGGGTACAGGAAGCAAGAACCCTCCTTGGGTTGCCCTATACCAGCAATTCTTTTCTCCCCACG CAAGCATTCCAAACCAGAACTGTGTGCGAACCCCGAGGAGGGTTGGGTACAGAACTTGATGCGAA GGCTTGACCAGCCCCCAGCCCCTGGCAAGCAGTCACCTGGGTGCAGAAAAAACAGAGGTACTTCAA AGAGCGGCAAGAAAGGCAAAGGGAGTAAAGGATGTAAAAGAACGGAGCAGACCCAGCCTTCACG AGGCCGGCGCAAGAGGGGTTCCGGAGAGGGAAGGGGTAGTCTGCTCACCTGCGGCGATGTTGAAG AAAATCCTGGTCCCATGTGTCCACAGAAGCTGACAATAAGTTGGTTTGCCATTGTCCTCCTGGTGAG CCCACTCATGGCAATGTGGGAACTCGAAAAGGATGTCTACGTGGTAGAAGTAGATTGGACTCCAGA CGCGCCAGGGGAGACAGTGAATTTGACATGTGACACACCAGAAGAAGATGACATTACATGGACAT CTGACCAACGCCATGGCGTAATAGGGAGTGGGAAAACACTCACGATCACAGTTAAAGAGTTCTTGG ATGCTGGTCAATATACTTGCCATAAAGGCGGCGAGACACTCAGCCACTCACATTTGCTTTTGCATAA AAAAGAGAATGGCATTTGGAGCACTGAAATACTTAAGAACTTTAAGAACAAGACATTTCTCAAGTG TGAGGCCCCTAATTACAGCGGCAGGTTCACGTGCTCATGGCTGGTCCAGCGCAACATGGACCTCAA GTTTAACATAAAATCTTCTTCCTCTTCACCTGACTCCAGAGCTGTTACTTGCGGCATGGCTTCTCTGA GCGCAGAAAAAGTAACGTTGGATCAAAGAGACTACGAAAAGTACTCTGTTTCTTGTCAAGAGGATG TTACGTGCCCGACGGCCGAAGAAACGCTTCCAATTGAACTCGCGTTGGAAGCTCGCCAACAAAACA AGTATGAAAACTACAGTACAAGCTTCTTTATACGGGATATAATTAAACCCGATCCCCCCAAGAACT TGCAAATGAAACCACTTAAGAACAGCCAGGTGGAAGTTTCCTGGGAGTATCCAGACTCATGGAGTA CTCCTCACAGCTATTTTTCTCTGAAATTCTTTGTAAGGATACAACGGAAGAAAGAGAAGATGAAAG AGACCGAGGAGGGTTGTAATCAGAAGGGAGCGTTTCTCGTGGAGAAAACGTCTACCGAAGTCCAAT GTAAAGGTGGCAATGTGTGCGTCCAAGCTCAGGATAGATACTATAATTCAAGTTGCTCCAAGTGGG CCTGTGTTCCATGCCGCGTTCGGAGCGGGGGAGGTAGCGGAGGAGGTAGTGGGGGTGGGTCAGGA GGAGGGAGTCGAGTTATCCCGGTGTCAGGCCCCGCACGCTGCTTGAGCCAGAGTCGCAACCTCCTT AAGACAACAGATGACATGGTGAAAACAGCACGCGAAAAGCTTAAACACTACTCTTGTACGGCGGA GGATATTGATCACGAGGATATTACCCGAGACCAAACTAGCACTTTGAAAACCTGTCTGCCCCTTGA ACTTCATAAAAATGAGAGCTGTCTGGCTACACGAGAGACGTCAAGTACGACTAGGGGCAGCTGTCT CCCGCCGCAAAAGACAAGCCTCATGATGACGCTCTGTTTGGGTTCCATTTACGAGGACTTGAAAAT GTATCAAACGGAGTTCCAGGCTATAAATGCGGCGTTGCAGAACCATAACCATCAACAAATTATACT TGATAAAGGCATGTTGGTGGCGATTGATGAACTCATGCAGAGTCTCAATCACAACGGGGAAACGTT GAGACAGAAACCCCCCAGTCGGTGAAGCGGACCCATATCGAGTAAAAATGAAGCTCTGCATTCTGCT TCACGCATTCAGCACTAGAGTTGTTACCATCAACCGGGTAATGGGATATCTCTCCAGTGCGtaG
IL7 (Mouse) (SEQ ID NO: 64)
ATGTTTCATGTGTCCTTCAGGTACATATTTGGTATCCCACCACTTATATTGGTGCTCTTGCCTGTAAC CAGCTCTGAATGTCATATAAAAGACAAGGAGGGCAAAGCATACGAGTCCGTATTGATGATCTCAAT CGATGAACTTGACAAGATGACAGGGACCGATTCTAATTGTCCAAATAACGAGCCAAACTTCTTTCG GAAACACGTGTGTGATGATACAAAAGAAGCTGCTTTTCTTAACAGAGCTGCCAGAAAACTCAAGCA GTTCCTCAAGATGAATATATCCGAGGAATTTAACGTGCATCTCCTCACAGTATCTCAGGGAACTCAA ACCCTTGTAAACTGCACTTCTAAGGAGGAGAAGAATGTCAAAGAGCAGAAGAAAAATGATGCATG TTTTTTGAAACGGCTGTTGAGGGAGATCAAAACATGCTGGAATAAAATCCTCAAGGGCTCAATTtaG
IL15 (Human) (SEQ ID NO: 65)
ATGGAAACAGACACATTGCTGCTTTGGGTATTGTTGCTCTGGGTGCCTGGATCAACAGGAAACTGG GTAAACGTAATTTCAGATCTGAAGAAGATCGAGGACCTTATTCAATCCATGCACATCGATGCCACT CTCTACACCGAAAGCGACGTTCACCCATCTTGCAAGGTGACCGCTATGAAATGTGAATTGTTGGAA CTTCAGGTAATTTCTCTGGAGAGCGGCGATGCCTCAATACATGACACCGTTGAAAATCTTATCATCC TTGCTAATGATTCACTCTCTAGTAATGGGAACGTAACAGAGAGCGGGTGTAAGGAGTGTGAAGAAC TGGAGGAGAAAAACATTAAGGAATTTTTGCAGTCATTCGTCCATATAGTGCAAATGTTCATAAACA CTTCCAGAAGAAAGCGAGGCTCTGGGGAGGGGCGAGGCTCTCTGCTGACCTGTGGGGATGTAGAA GAGAATCCAGGTCCCATGGACCGGCTGACCAGCTCATTCCTGCTTCTGATTGTGCCAGCCTACGTGC

TCTCCATCACATGTCCTCCCCCAATGAGCGTCGAGCATGCTGACATCTGGGTGAAGTCATACTCCTT GTACAGCAGAGAGAGATACATTTGTAATTCCGGATTCAAGCGCAAGGCCGGCACCTCCTCTCTGAC AGAGTGCGTCCTTAACAAAGCAACCAACGTAGCACATTGGACCACACCATCCTTGAAGTGCATACG AGAACCTAAATCTTGCGATAAGACTCATACTTGTCCACCTTGTCCAGCCCCAGAACTGCTTGGCGGA CCCTCAGTATTTTTGTTCCCACCAAAGCCAAAAGACACACTCATGATATCCAGAACTCCTGAGGTGA CCTGTGTCGTTGTAGACGTTTCCCACGAAGATCCTGAAGTAAAATTCAACTGGTACGTGGATGGGGT CGAAGTCCATAACGCCAAGACTAAACCAAGGGAGGAACAGTATAACTCTACTTACCGAGTAGTTTC TGTGTTGACCGTGCTGCACCAGGACTGGTTGAACGGGAAGGAGTACAAATGCAAGGTGAGCAATA AAGCTCTGCCCGCACCAATCGAAAAGACAATATCTAAGGCCAAGGGGCAGCCACGAGAGCCCCAG GTATACACACTGCCACCCTCACGCGATGAATTGACTAAGAACCAGGTTTCCCTGACCTGTCTTGTAA AAGGTTTCTACCCTTCCGACATAGCTGTTGAGTGGGAAAGTAACGGGCAGCCAGAGAACAATTACA AGACAACTCCACCCGTTCTTGATAGCGATGGATCATTTTTCTGTATTCCAAACTCACTGTCGATAA AAGTCGCTGGCAGCAAGGCAATGTTTTAGCTGCTCAGTCATGCACGAAGCACTGCATAATCACTA CACACAAAAAAGTTTGTCCCTTAGCCCTGGTAAGtaG
IL15 (Human) (SEQ ID NO: 66)
ATGTACTCAATGCAGTTGGCCTCCTGTGTAACATTGACCTTGGTCCTCTTGGTCAACAGCAATTGGA TCGATGTACGCTACGACTTGGAGAAGATTGAGTCCCTTATACAGAGTATACACATAGATACAACCT TGTATACTGACAGTGACTTCCATCCCAGCTGTAAAGTGACTGCAATGAACTGTTTTTGTTGGAGTT GCAAGTAATTCTGCATGAATACAGCAACATGACCCTCAATGAAACCGTTAGGAATGTCCTTTATCTC GCAAATTCTACTCTGAGTAGCAATAAGAATGTTGCCGAAAGCGGCTGCAAGGAGTGCGAAGAACTG GAGGAAAAAACTTTCACCGAGTTTCTCCAGAGTTTCATCAGAATTGTCCAAATGTTCATTAATACAA GTAGTGGTGGTGGGAGCGGGGGTGGAGGCAGTGGGGGAGGTGGGAGCGGAGGTGGAGGGTCCGG AGGGGGGAGCCTTCAAGGCACTACTTGTCCTCCACCCGTATCCATCGAGCACGCCGATATTCGAGTT AAAAATTATAGTGTTAATAGCAGAGAACGATACGTCTGCAACTCAGGGTTTAAGAGAAAGGCCGG AACTTCAACTCTCATAGAATGCGTGATTAATAAGAATACTAACGTCGCACATTGGACTACTCCCAGT CTCAAGTGCATACGCGATCCATCTCTCGCTCATTACTCACCAGTACCTACAGTGGTTACTCCTAAGG TGACCTCTCAGCCCGAATCACCATCTCCCAGCGCAAAAGAGCCTGAGGCCTTTTCTCCTAAATCAGA CACTGCTATGACTACAGAAACAGCCATAATGCCAGGAAGCCGGCTGACACCATCTCAAACTACCAG CGCAGGCACAACTGGGACTGGCTCCCACAAAAGCTCACGCGCACCAAGTCTCGCCGCAACAATGAC ATTGGAGCCTACAGCCAGCACATCTCTTAGAATCACAGAAATTTCTCCCCACAGTAGCAAGATGAC CAAGGTGGCAATTAGTACCAGCGTCCTTCTTGTAGGAGCTGGAGTTGTGATGGCATTTTTGGCATGG TATATCAAAAGCAGGtaG
IL15 (Mouse) (SEQ ID NO: 67)
ATGAAGATCCTCAAGCCATACATGCGAAACACTAGTATTAGCTGTTACTTGTGTTTTCTGCTGAATA GTCATTTTTTGACTGAAGCAGGAATCCATGTATTTATACTCGGTTGTGTGTCTGTAGGTCTGCCAAA GACTGAGGCTAATTGGATTGACGTGCGCTATGATCTTGAAAAAATAGAGTCCTTGATTCAATCAAT ACACATCGATACCACTCTCTACACCGACAGTGATTTCCATCCTTCCTGCAAGGTAACAGCTATGAAT TGCTTCCTCCTGGAGCTCCAAGTCATTCTCCATGAGTACTCCAACATGACTTTGAACGAAACTGTAA GAAACGTATTGTATCTGGCTAATAGCACCTTGTCTAGTAACAAAAATGTGGCAGAGAGCGGCTGCA AAGAATGTGAAGAATTGGAAGAGAAAACATTTACAGAGTTCCTGCAATCCTTTATTCGCATCGTCC AAATGTTTATCAATACCTCTtaG
IL15 (Mouse) (SEQ ID NO: 68)
ATGTATTCCATGCAACTTGCCAGTTGTGTAACCCTTACTCTCGTCCTGCTCGTTAATTCCGCTGGTGC TAACTGGATAGATGTTCGATACGATCTGGAAAAGATTGAGTCCCTTATCCAATCCATTCATATAGAT ACCACCCTTTATACTGACAGCGACTTCCATCCTTCTTGCAAGGTGACCGCTATGAATTGTTTCCTGCT GGAACTCCAAGTTATTCTGCATGAATACTCTAATATGACACTTAACGAGACCGTAAGAAATGTTCTC TATCTCGCTAATAGTACTTTGAGCTCAAATAAGAACGTGGCCGAGTCTGGGTGTAAGGAATGCGAA GAGCTGGAAGAAAAGACATTCACCGAGTTTCTCCAGTCTTTCATACGGATTGTGCAGATGTTTATCA ACACATCAGATTACAAAGACGACGATGATAAGtaG
IL18 (Mouse) (SEQ ID NO: 69)
ATGGCAGCCATGTCTGAGGACTCTTGTGTGAACTTTAAAGAAATGATGTTCATAGACAATACACTCT ACTTTATACCTGAGGAGAATGGAGATTTGGAATCTGACAACTTTGGCAGGCTGCATTGCACTACCG CAGTTATCCGAAACATCAACGATCAGGTACTGTTTGTTGATAAAAGACAACCTGTATTCGAGGACA TGACCGACATAGATCAGTCTGCCTCAGAGCCCCAGACTAGGCTTATCATCTATATGTACAAGGACA GCGAAGTACGAGGCCTGGCTGTTACACTCTCAGTCAAAGACTCTAAGATGAGCACCCTGTCATGCA

AGAACAAAATTATCAGTTTTGAGGAGATGGACCCACCTGAAAACATAGATGACATTCAGTCAGACCTCATTTTTTTTCAAAAGCGGGTACCAGGACACAACAAAATGGAATTTGAATCATCACTCTACGAAGGACATTTCCTTGCATGCCAGAAAGAGGATGACGCATTCAAATTGATCCTGAAAAAAAAGGACGAAAATGGTGATAAATCAGTCATGTTTACATTGACCAATCTTCACCAAAGTtaG
IL18 (Mouse) (SEQ ID NO: 70)
ATGGCTGCAATGTCTGAAGATAGCTGTGTCAACTTTAAGGAGATGATGTTCATTGATAATACTTTGTACTTTATACCTGAAGAAAATGGAGACCTTGAGTCAGACAACTTCGGGAGACTGCACTGCACAACTGCCGTTATCCGAAACATAAATGATCAAGTATTGTTCGTGGACAAAAGACAACCAGTCTTTGAGGATATGACAGACATCGACCAATCCGCATCTGAACCTCAGACTAGGCTGATCATCTATATGTACGCCGACTCCGAAGTAAGAGGCCTTGCTGTGACACTTAGTGTTAAGGATAGTAAGATGAGCACACTGTCCTGTAAGAATAAGATTATATCTTTTGAAGAGATGGACCCTCCCGAGAACATAGATGACATCCAGAGCGACTTGATCTTCTTTCAGAAGCGAGTGCCAGGCCATAACAAGATGGAATTTGAATCATCTCTTTATGAAGGCCATTTCCTCGCATGTCAAAAGGAGGACGATGCCTTCAAGCTCATTCTGAAAAAAAAAGACGAGAACGGTGATAAGAGCGTGATGTTCACTCTGACAAATCTGCACCAGTCAtaG
IL18 (Human) (SEQ ID NO: 71)
ATGTATCGCATGCAACTCCTGTCCTGCATTGCTCTGAGCTTGGCTTTGGTAACCAACTCATACTTCGGGAAACTGGAGAGTAAACTCTCCGTAATCAGGAATCTTAATGACCAAGTATTGTTTATTGACCAGGGCAACCGCCCGTTGTTCGAGGATATGACTGATTCTGACTGTCGGGATAACGCTCCGAGAACTATCTTTATCATTTCAATGTACAAGGACAGCCAACCGCGGGGTATGGCTGTGACAATCAGTGTCAAATGTGAGAAGATTTCCACGCTGTCCTGCGAAAACAAGATAATTTCTTTCAAAGAAATGAACCCCCCTGACAATATAAAGGATACAAAGAGTGATATCATCTTCTTTCAGAGGTCCGTGCCCGGCCACGATAATAAGATGCAATTTGAAAGTTCATCTTATGAGGGGTACTTTTTGGCATGCGAGAAAGAAAGGGATCTCTTCAAGTTGATCCTGAAGAAGGAGGACGAATTGGGCGACCGCTCCATCATGTTCACAGTCCAGAACGAGGACtaG
IL18 (Human) (SEQ ID NO: 72)
ATGTACCGCATGCAGCTCCTGAGTTGTATTGCCCTTTCCCTCGCTCTCGTTACCAATTCTTACTTCGGTAAGCTTGCCTCTAAACTCTCTGTTATTAGGAACTTGAACGACCAAGTCCTTTTCATAGACCAAGGGAACAGACCACTGTTTGAAGATATGACGGATAGCGATTGCCGAGATAATGCCCCTAGGACGATTTTTATCATTAGTATGTATGCGGACTCTCAACCGAGGGGGATGGCCGTTACTATAAGTGTGAAATGCGAGAAAATATCAACGCTCAGTTGTGAGAACAAAATCATAAGTTTCAAGGAGATGAATCCACCTGATAACATCAAAGACACTAAGTCTGATATTATATTTTTCCAACGAAGTGTTCCGGGACACGATAACAAAATGCAATTTGAGAGCTCCTCATACGAGGGCTACTTCCTCGCGTGTGAGAAAGAAAGGGATTTGTTTAAGCTTATCCTCAAGAAAGAGGACGAGTTGGGGGATCGGAGCATAATGTTTACCGTACAGAATGAGGACtaG
IL21 (Mouse) (SEQ ID NO: 73)
ATGGAGCGGACACTCGTGTGTCTTGTCGTAATTTTTCTCGGGACAGTCGCACACAAGTCCTCACCCCAGGGTCCTGATCGCCTTCTCATACGCCTCCGACATTTGATCGACATTGTAGAGCAGCTCAAAATTTACGAGAATGACCTCGATCCCGAGCTTTTGAGTGCTCCCCAAGACGTTAAGGGTCATTGCGAGCACGCAGCTTTTGCTTGCTTCCAGAAGGCCAAGTTGAAACCAAGCAACCCTGGTAATAATAAGACTTTCATCATCGACTTGGTCGCCCAACTCCGAAGGAGGCTGCCTGCCCGGCGCGGAGGAAAAAAACAAAAGCATATTGCAAAGTGTCCTTCATGTGATTCATACGAAAAGCGGACTCCCAAAGAGTTCTTGGAAAGGTTGAAATGGCTTCTTCAGAAGATGATTCATCAACATTTGTCAtaG
IFN-beta (Human) (SEQ ID NO: 74)
ATGACCAACAAATGCCTTTTGCAAATTGCCCTGCTTTTGTGTTTTAGCACTACCGCATTGAGCATGTCATATAACCTCCTCGGCTTCCTTCAGAGATCATCAAACTTTCAGTGTCAGAAACTGCTTTGGCAACTTAATGGCAGGCTCGAATATTGTCTGAAAGATCGGATGAATTTCGACATTCCAGAAGAAATAAAACAGCTTCAACAATTCCAGAAAGAGGACGCCGCCCTGACTATTTACGAGATGCTCCAGAATATCTTCGCCATTTTCCGGCAGGACAGCTCATCCACGGGGTGGAATGAGACTATTGTAGAAAATCTTCTGGCTAATGTGTACCATCAAATTAATCACCTCAAAACGGTGCTTGAGGAAAAACTTGAAAAGGAAGATTTCACACGGGGCAAGTTGATGTCCTCCCTGCACCTTAAACGATACTACGGCAGGATTCTTCATTACTTGAAGGCTAAGGAGTATAGCCATTGCGCGTGGACAATTGTACGGGTAGAAATACTGCGAAACTTTTATTTCATCAACCGGCTCACTGGATACCTTAGAAATtaG
IFN-beta (Mouse) (SEQ ID NO: 75)
ATGAACAATCGGTGGATACTCCACGCCGCATTTCTCCTCTGCTTTAGCACGACGGCCCTGTCCATCAACTACAAACAGCTTCAGTTGCAGGAGCGGACTAACATAAGGAAGTGCCAGGAACTGCTGGAACAG

CTTAATGGTAAAATTAATCTTACATACCGAGCTGACTTCAAAATTCCTATGGAAATGACCGAGAAG ATGCAGAAATCCTACACGGCATTCGCCATCCAGGAAATGCTCCAGAACGTATTTCTCGTGTTCCGCA ATAATTTCTCTTCTACGGGTTGGAACGAAACCATTGTTGTTAGACTGCTTGACGAACTGCATCAGCA AACCGTGTTCCTTAAAACCGTGCTTGAGGAGAAGCAGGAGGAGCGCCTGACTTGGGAGATGTCTAG TACCGCACTTCACTTGAAATCCTACTACTGGCGCGTTCAGCGGTATCTGAAGCTGATGAAGTATAAC TCATACGCCTGGATGGTAGTGCGCGCAGAGATCTTCAGAAACTTTCTTATCATCCGGCGACTGACCC GAAACTTTCAGAATtaG
IFN-gamma (Human) (SEQ ID NO: 76)
ATGAAGTACACTAGCTATATATTGGCCTTCCAGCTTTGCATCGTATTGGGTAGCCTCGGATGCTATT GCCAAGACCCGTATGTCAAAGAAGCCGAAAATCTCAAAAAGTATTTCAATGCCGGACACTCAGACG TCGCGGATAACGGTACACTGTTTCTTGGCATCCTGAAAAATTGGAAGGAAGAGAGTGACAGAAAAA TAATGCAGTCACAAATAGTGTCCTTTTACTTTAAGCTGTTCAAAAATTTCAAGGATGACCAAAGTAT CCAGAAGAGTGTTGAAACTATCAAAGAGGACATGAATGTGAAATTCTTTAACAGTAATAAGAAGA AGCGCGATGACTTCGAGAAACTCACTAATTACAGCGTAACGGATCTTAACGTCCAACGCAAGGCAA TCCACGAGCTTATACAGGTAATGGCTGAGCTTAGTCCCGCAGCCAAGACAGGGAAGAGAAAAAGG TCTCAAATGCTTTTTCGGGGCCGGCGAGCTTCACAAtaG
IFN-gamma (Mouse) (SEQ ID NO: 77)
ATGAACGCTACGCATTGCATCCTCGCACTCCAATTGTTCCTCATGGCTGTGTCAGGGTGTTACTGTC ACGGTACTGTCATAGAAAGCCTCGAATCCCTGAATAACTATTTTAACAGTAGCGGTATAGATGTAG AAGAAAAGTCTCTCTTTCTTGACATCTGGAGGAATTGGCAAAAGGATGGAGACATGAAGATTCTCC AATCTCAGATTATATCATTTTACTTGAGGCTTTTTGAGGTTCTGAAGGATAACCAGGCGATCAGCAA TAATATCAGCGTAATTGAATCTCACCTTATTACAACATTTTTCTCAAATTCCAAGGCAAAGAAAGAT GCTTTCATGTCTATCGCGAAATTTGAGGTGAACAATCCTCAGGTACAAAGGCAAGCCTTTAACGAG CTGATTAGAGTTGTACATCAGTTGTTGCCCGAAAGTAGTCTTAGAAAACGCAAACGGAGCCGATGCt aG
IFN-alpha (Mouse) (SEQ ID NO: 78)
ATGGCAAGGTTGTGCGCTTTTCTCATGGTACTGGCTGTGCTCTCCTATTGGCCTACTTGTTCTCTGGG ATGCGACTTGCCACAGACCCACAATCTGCGGAATAAGAGGGCTCTGACTCTGCTGGTGCAAATGAG ACGGCTCTCTCCACTTAGCTGTTTGAAAGATAGAAAGGATTTCGGGTTCCCCCAGGAGAAGGTGGA TGCCCAGCAGATCAAGAAGGCACAGGCTATCCCCGTCCTTTCCGAGCTGACCCAGCAAATTTTGAA CATCTTTACAAGTAAGGATAGTTCAGCTGCATGGAATACCACACTTTTGGATTCTTTTTGTAACGAT CTGCATCAGCAGCTGAACGATCTCCAGGGATGCCTGATGCAGCAAGTCGGCGTGCAAGAATTTCCA CTCACCCAGGAGGACGCTCTGCTCGCAGTGCGAAAGTATTTCACCGAATTACCGTGTACCTCCGGG AGAAAAAGCATTCACCCTGCGCTTGGGAAGTAGTCAGGGCCGAAGTATGGAGAGCCCTTAGTAGCT CCGCTAATGTACTGGGCCGGTTGCGGGAAGAGAAAtaG
CCL21 (Human) (SEQ ID NO: 79)
ATGGCGCAAAGTCTGGCTCTTTCACTCCTGATCCTGGTCTTGGCCTTCGGGATTCCGAGGACCCAAG GAAGTGATGGTGGCGCCCAAGATTGTTGCCTTAAATACAGCCAGCGGAAAATACCCGCGAAAGTGG TCAGGAGTTATAGAAAACAGGAGCCTTCCCTGGGTTGTAGTATCCCCGCCATACTTTTCCTCCCGAG AAAACGGAGCCAGGCCGAACTGTGCGCTGACCCTAAGGAACTTTGGGTGCAACAACTTATGCAACA CCTGGATAAGACACCTTCTCCTCAAAAGCCAGCTCAGGGCTGCCGAAAAGATAGAGGCGCCTCAAA AACCGGAAAAAAGGGCAAAGGTTCTAAAGGATGTAAGCGGACTGAACGCTCTCAAACGCCTAAAG GGCCGtaG
CCL21a (Mouse) (SEQ ID NO: 80)
ATGGCGCAAATGATGACCCTTTCCCTGCTGAGTCTTGTCCTCGCGCTCTGCATCCCGTGGACGCAGG GGTCTGATGGGGGGGGCCAAGACTGTTGCCTGAAGTATTCACAAAAAAAGATACCGTACTCTATTG TCAGAGGGTACAGGAAGCAAGAACCCTCCTTGGGTTGCCCTATACCAGCAATTCTTTTCTCCCCACG CAAGCATTCCAAACCAGAACTGTGTGCGAACCCCGAGGAGGGTTGGGTACAGAACTTGATGCGAA GGCTTGACCAGCCCCCAGCCCCTGGCAAGCAGTCACCTGGGTGCAGAAAAAACAGAGGTACTTCAA AGAGCGGCAAGAAAGGCAAAGGGAGTAAAGGATGTAAAAGAACGGAGCAGACCCAGCCTTCACG AGGCtaG
Tail-less CCL21 (Human) (SEQ ID NO: 81)
ATGGCGCAAAGTCTGGCTCTTTCACTCCTGATCCTGGTCTTGGCCTTCGGGATTCCGAGGACCCAAG GAAGTGATGGTGGCGCCCAAGATTGTTGCCTTAAATACAGCCAGCGGAAAATACCCGCGAAAGTGG TCAGGAGTTATAGAAAACAGGAGCCTTCCCTGGGTTGTAGTATCCCCGCCATACTTTTCCTCCCGAG

AAAACGGAGCCAGGCCGAACTGTGCGCTGACCCTAAGGAACTTTGGGTGCAACAACTTATGCAACA CCTGGATAAGACACCTTCTCCTCAAAAGCCAGCTCAGGGCtaG
Tail-less CCL21 (Mouse) (SEQ ID NO: 82)
ATGGCGCAAATGATGACCCTTTCCCTGCTGAGTCTTGTCCTCGCGCTCTGCATCCCGTGGACGCAGG GGTCTGATGGGGGGGGCCAAGACTGTTGCCTGAAGTATTCACAAAAAAAGATACCGTACTCTATTG TCAGAGGGTACAGGAAGCAAGAACCCTCCTTGGGTTGCCCTATACCAGCAATTCTTTTCTCCCCACG CAAGCATTCCAAACCAGAACTGTGTGCGAACCCCGAGGAGGGTTGGGTACAGAACTTGATGCGAA GGCTTGACCAGCCCCCAGCCCCTGGCAAGCAGTCACCTGGGtaG
CCL19 (Mouse) (SEQ ID NO: 83)
ATGGCACCCCGCGTCACACCCTTGCTTGCTTTTTCTCTGCTTGTCCTCTGGACCTTCCCCGCTCCTAC CCTTGGAGGAGCCAATGATGCCGAGGATTGCTGCCTGAGTGTTACACAAAGGCCAATACCAGGGAA TATAGTGAAGGCATTCCGGTATCTGCTCAATGAAGATGGGTGCAGAGTCCCCGCAGTTGTCTTTACA ACATTGCGAGGTTACCAGCTTTGTGCTCCCCCAGACCAGCCTTGGGTAGATCGCATTATTCGCCGGT TGAAGAAGAGCTCAGCAAAGAATAAGGGCAATTCCACACGGAGAAGCCCCGTCTCCtaG
CCL19 (Mouse) (SEQ ID NO: 84)
ATGAAATCAGCAGTCCTTTTCTTGCTCGGGATTATTTTTCTGGAACAATGTGGAGTGAGGGGAACAC TCGTAATAAGAAACGCTCGGTGCTCATGCATATCAACATCACGGGGCACTATCCACTACAAATCCC TGAAGGATCTGAAGCAGTTCGCCCCAAGCCCTAACTGTAACAAGACCGAAATTATCGCAACTCTCA AAAATGGAGATCAGACTTGTCTTGACCCAGATTCAGCAAATGTCAAGAAGCTGATGAAAGAGTGGG AAAAGAAGATTTCACAAAAAAAAAAGCAAAAACGCGGCAAGAAACATCAAAAGAACATGAAAAA CAGGAAACCTAAGACTCCCCAGTCAAGGAGAAGATCCCGCAAGACAACCtaG
CXCL11 (Mouse) (SEQ ID NO: 85)
ATGAACAGAAAAGTTACCGCTATAGCACTTGCTGCCATAATATGGGCCACCGCAGCTCAAGGGTTC CTGATGTTCAAGCAGGGCCGATGCCTCTGCATTGGCCCTGGAATGAAGGCCGTGAAAATGGCCGAA ATAGAAAAAGCTAGTGTCATATACCCCTCTAACGGTTGCGATAAAGTCGAGGTTATAGTCACAATG AAAGCTCATAAACGCCAACGCTGCCTCGACCCCCGGTCTAAGCAGGCTAGGCTCATAATGCAAGCA ATCGAGAAGAAAAACTTTCTTAGACGGCAAAACATGtaG
CXCL10 (Mouse) (SEQ ID NO: 86)
ATGAACCCATCTGCCGCCGTTATTTTCTGTCTGATACTCCTTGGGCTGAGTGGCACACAAGGCATAC CCCTCGCCCGCACAGTCCGGTGTAATTGTATACATATTGACGACGGCCCTGTTAGAATGCGGGCCAT CGGTAAGCTGGAGATTATACCAGCAAGCCTTAGTTGTCCCAGGGTTGAAATCATAGCAACTATGAA AAAAAACGACGAACAAAGATGTTTGAATCCCGAGAGCAAGACAATCAAAAACCTTATGAAAGCAT TTAGTCAAAAACGCTCTAAACGCGCTCCAtaG
CXCL10 (Human) (SEQ ID NO: 87)
ATGAATCAGACGGCAATCCTTATATGCTGCCTTATATTCCTTACTCTCTCAGGGATACAAGGGGTAC CACTTTCTCGGACTGTTCGCTGCACTTGCATTTCAATATCTAACCAACCTGTAAATCCGCGGAGCCT GGAAAAATTGGAGATTATACCTGCTTCTCAATTCTGCCCTCGGGTGGAAATCATCGCCACTATGAAG AAGAAGGGCGAGAAAAGGTGTCTGAATCCAGAGTCAAAGGCAATCAAAAACCTGCTGAAAGCGGT GTCAAAGGAACGGTCCAAGAGATCACCCtaG
CXCL11-CXCL10 (Mouse) (SEQ ID NO: 88)
ATGAACAGGAAAGTAACAGCCATTGCATTGGCTGCCATCATCTGGGCCACCGCAGCACAGGGTTTT CTGATGTTTAAGCAAGGGCGCTGTCTCTGTATAGGCCCAGGCATGAAGGCCGTGAAGATGGCAGAG ATTGAGAAGGCATCTGTGATTTATCCTTCTAACGGGTGCGATAAAGTCGAAGTTATTGTGACAATGA AGGCACACAAACGCCAACGGTGTTTGGACCCACGATCTAAACAGGCAAGATTGATTATGCAAGCCA TCGAGAAAAAGAACTTTCTCCGAAGGCAAAATATGATCCCTTTGGCTCGGACAGTGCGGTGTAACT GTATTCACATCGACGATGGGCCAGTACGGATGAGAGCAATAGGAAAGCTCGAAATCATACCCGCCT CATTGTCTTGTCCCAGGGTGGAAATAATCGCCACTATGAAAAAGAACGATGAACAGAGGTGTCTCA ACCCAGAGAGTAAGACTATCAAGAACCTTATGAAGGCATTCAGTCAGAAGAGGTCAAAGCGAGCA CCAtaG
XCL1 (Human) (SEQ ID NO: 89)
ATGAGACTTCTCATATTGGCGCTTCTCGGGATATGTTCTCTTACGGCATACATAGTTGAGGGGGTGG GATCTGAGGTTAGCGATAAACGAACTTGTGTTAGTCTTACAACACAGAGGCTTCCAGTCTCCAGGA TAAAAACATATACGATAACTGAGGGATCTCTCAGAGCGGTCATCTTCATAACGAAGAGGGGCCTGA AGGTCTGTGCTGACCCACAAGCGACTTGGGTAAGGGACGTTGTGCGGAGCATGGACAGGAAGAGC

AATACTCGCAACAACATGATCCAAACCAAACCTACGGGCACCCAACAGTCAACCAATACTGCGGTAACATTGACGGGGtaG
XCL1 (Mouse) (SEQ ID NO: 90)
ATGCGCCTCCTTCTGCTGACTTTTCTGGGTGTATGTTGCCTGACACCCTGGGTCGTAGAAGGAGTAGGAACCGAGGTTCTGGAAGAGTCCTCATGTGTAAACTTGCAGACACAACGACTCCCCGTCCAAAAAATCAAGACCTATATAATCTGGGAGGGGGCAATGCGGGCCGTCATTTTCGTGACTAAACGAGGTCTCAAAATCTGCGCCGACCCCGAGGCTAAGTGGGTGAAGGCAGCCATTAAGACCGTGGATGGGAGAGCCAGCACCAGAAAGAACATGGCCGAAACAGTACCTACTGGCGCACAGCGGTCAACCTCAACTGCTATAACCTTGACAGGAtaG
m_sCD40L #1 (SEQ ID NO: 91)
ATGGAGACTGACACTCTGCTTCTGTGGGTGTTGCTGCTGTGGGTGCCTGGCAGTACAGGCGATATGCAACGAGGTGACGAGGACCCTCAAATCGCCGCCCATGTAGTCTCTGAAGCTAATAGCAACGCTGCATCCGTCTTGCAGTGGGCAAAGAAAGGCTACTATACTATGAAGTCCAACTTGGTAATGCTTGAAAACGGCAAGCAGTTGACTGTCAAGAGAGAGGGACTTTATTACGTCTATACCCAAGTCACATTCTGTAGCAATCGAGAACCCTCCTCACAGAGGCCTTTTATAGTGGGACTCTGGCTTAAACCAAGTAGCGGCTCTGAGCGCATACTGTTGAAAGCCGCAAACACACACAGCTCTTCCCAACTCTGCGAGCAGCAATCCGTGCATCTCGGTGGAGTATTTGAGCTTCAAGCCGGTGCCTCAGTGTTTGTGAACGTCACTGAGGCCTCCCAGGTCATACATCGAGTTGGGTTCAGCTCCTTCGGCTTGCTCAAGCTCtaG
m_sCD40L #2 (SEQ ID NO: 92)
ATGGAAACTGATACATTGCTGCTCTGGGTTTTGCTGCTCTGGGTGCCTGGGAGTACAGGCGACATGAGGAGGCAGTTCGAGGATCTCGTTAAGGATATTACCCTTAATAAGGAGGAGAAGAAAGAAAACTCTTTTGAGATGCAACGAGGGGACGAAGATCCTCAGATCGCTGCTCACGTGGTCTCTGAAGCTAACAGCAACGCCGCTTCTGTCCTCCAGTGGGCCAAGAAAGGTTATTACACCATGAAATCAAACCTTGTAATGCTTGAAAACGGGAAACAGCTTACAGTGAAGAGGGAAGGTCTTTACTACGTCTATACCCAGGTAACCTTCTGCTCAAACAGAGAACCATCAAGCCAGAGGCCATTCATAGTGGGGCTCTGGCTCAAACCTTCCAGTGGCAGCGAGAGAATCTTGTTGAAAGCTGCTAATACACATAGTAGTAGCCAGCTTTGCGAGCAACAGTCAGTCCACCTCGGGGGGGTGTTTGAGTTGCAAGCAGGGGCCTCAGTATTCGTGAATGTCACTGAGGCTTCCCAGGTAATTCACAGGGTAGGCTTTAGTTCATTCGGTTTGCTGAAGCTTtaG
m_sCD40L #3 (SEQ ID NO: 93)
ATGCGAAGAATGCAGCTTCTGCTCCTTATTGCTCTGAGTCTCGCCCTTGTCACCAACTCCGGGGACAGAATGAAACAAATCGAGGACAAAATTGAAGAAATACTGAGTAAAATATATCACATCGAAAACGAAATTGCACGCATTAAGAAATTGATTGGCGAACGCACCAGTGGCGGCTCTGGTGGCACCGGAGGTTCAGGCGGGACCGGGGGCTCTGACAAAGTCGAAGAGGAGGTTAACCTTCATGAGGACTTTGTGTTCATCAAGAAGCTGAAACGGTGCAATAAAGGAGAAGGTTCTTTGAGCCTCCTTAATTGCGAAGAGATGCGACGACAGTTCGAGGATCTGGTTAAGGACATTACACTTAATAAGGAAGAGAAAAAGGAGAACTCTTTCGAAATGCAGCGCGGCGATGAAGATCCCCAGATAGCCGCCCATGTCGTCTCTGAGGCCAACTCTAACGCAGCATCCGTCCTCCAGTGGGCTAAGAAAGGATATTATACTATGAAAAGCAATTTGGTCATGCTCGAAAACGGTAAACAGCTCACTGTTAAGAGAGAAGGCCTCTATTACGTATATACTCAAGTAACTTTCTGTTCTAATAGGGAACCCTCCTCTCAAAGACCTTTTATCGTAGGACTCTGGTTGAAACCAAGTAGCGGTAGTGAAAGGATTCTGCTCAAAGCAGCTAATACTCACTCCAGCAGTCAACTGTGCGAACAACAAAGCGTTCACCTCGGGGGCGTCTTTGAACTTCAGGCAGGTGCCAGTGTTTTCGTCAACGTAACAGAAGCATCCCAGGTAATTCATCGAGTAGGGTTTTCTAGCTTTGGTTTGCTGAAGCTGtaG
anti-CD40_FGK4.5 (SEQ ID NO: 94)
ATGGAAACTGATCGCCTGTTGCTCTGGGTACTTCTTCTGTGGGTGCCTGGGTCCACTGGTGACACTGTACTTACACAATCACCCGCTTTGGCCGTTTCTCCTGGTGAACGGGTCACAATTAGTTGCCGAGCTTCCGATTCTGTATCTACTCTTATGCATTGGTATCAACAAAAACCTGGTCAGCAGCCAAAATTGCTCATTTATCTTGCTAGTCACTTGGAGTCCGGCGTACCTGCTCGATTCAGCGGTAGTGGGTCTGGCACAGATTTCACTTTGACCATAGATCCCGTGGAGGCCGATGACACTGCAACCTACTATTGCCAGCAATCCTGGAACGACCCTTGGACTTTCGGCGGCGGCACCAAGCTGGAACTCAAGCGAGCAGATGCTGCCCCAACCGTTAGTATATTCCCACCCTCAACCGAACAACTCGCCACAGGAGGCGCTAGTGTCGTGTGTCTTATGAACAATTTCTATCCACGAGACATTAGCGTCAAGTGGAAAATTGATGGGACAGAAAGGCGAGATGGAGTTTTGGATTCAGTAACAGACCAGGATTCAAAGGATTCTACCTATAGCATGAGCTCCACCTTGAGCCTGACCAAAGCTGATTATGAATCTCATAACCTGTATACTTGTGAAGTGGTGCATAAGACTTCTAGCTCACCAGTGGTTAAATCTTTTAACCGCAACGAATGTCGGCGCAAGAGGGGTTCCGGAGAGGGAAGGGGTAGTCTGCTCACCTGCGGCGATGTTGAAGAAAATCCTGGTCCCATGGACATTCGGCTCTCTTTGGTAT

TCCTGGTACTTTTTATAAAGGGGGTGCAATGTGAAGTCCAGCTCGTGGAAAGCGGTGGGGGCCTGG TTCAGCCCGGTCGCAGCCTTAAACTTAGTTGCGCAGCATCCGGATTTACATTTTCTGACTATAACAT GGCCTGGGTTCGACAGGCACCCAAAAAAGGGCTGGAGTGGGTCGCAACTATCATATACGATGGTTC CCGGACATACTATAGAGATTCAGTGAAGGGGCGCTTTACAATAAGCAGGGACAATGCTAAGTCTAC CTTGTATCTTCAGATGGACTCCCTGAGGAGCGAAGATACAGCAACATATTATTGTGCTACAAACCG CTGGTTGCTGCTTCATTATTTCGACTACTGGGGTCAGGGCGTCATGGTAACTGTATCAAGCGCCGAG ACCACAGCCCCTTCTGTATATCCATTGGCACCAGGTACTGCTCTGAAATCCAACTCAATGGTAACCC TTGGATGTCTGGTTAAGGGTTATTTTCCCGAGCCCGTCACAGTTACTTGGAACTCTGGGGCCCTTTCT AGCGGAGTCCATACCTTTCCCGCCGTTTTGCAGAGTGGTCTGTACACCCTTACCTCAAGCGTCACAG TTCCATCTAGCACATGGAGCTCCCAGGCAGTAACTTGTAATGTGGCCCATCCAGCCTCCTCAACTAA GGTAGATAAAAAGATCGTTCCCAGAGAATGCAATCCATGTGGATGCACCGGGTCTGAGGTCAGCAG TGTGTTCATTTTCCCACCCAAGACTAAAGATGTATTGACTATTACTCTTACACCCAAAGTAACCTGC GTGGTGGTTGATATTAGTCAAAATGATCCCGAGGTACGGTTCTCTTGGTTTATCGACGACGTCGAAG TACATACAGCTCAGACACACGCTCCCGAGAAACAAAGCAATTCCACTCTTAGGAGCGTGTCCGAGT TGCCAATCGTACATAGGGATTGGCTTAATGGCAAGACCTTTAAGTGTAAGGTCAATTCAGGGGCAT TCCCCGCACCAATAGAGAAGAGTATAAGCAAACCCGAGGGGACACCCAGAGGTCCACAGGTCTAT ACAATGGCTCCCCCCAAGGAAGAGATGACCCAAAGTCAAGTCTCAATTACATGTATGGTGAAGGGC TTTTATCCACCCGACATATACACTGAGTGGAAGATGAATGGACAGCCCCAAGAGAATTATAAAAAC ACTCCCCCTACCATGGACACCGACGGGTCCTATTTTCTTTATAGTAAATTGAACGTGAAAAAGGAG ACCTGGCAACAAGGCAACACTTTCACCTGCTCCGTTCTTCACGAGGGCCTGCATAATCATCATACCG AAAAGTCTCTCAGTCATTCTCCAGGTAAGtaG
CD40L_2 (Human) (SEQ ID NO: 95)
ATGGAAACAGATACGTTGCTGTTGTGGGTACTTCTCCTTTGGGTCCCTGGCAGCACAGGGGACGAG AATAGTTTCGAAATGCAGAAGGGCGACCAGAACCCACAGATCGCGGCTCACGTTATATCAGAAGCA AGTAGTAAGACCACTTCCGTACTTCAGTGGGCTGAAAAAGGATATTACACCATGTCCAACAATCTC GTGACACTGGAGAACGGTAAACAACTTACGGTGAAACGACAGGGCCTCTATTACATCTACGCTCAG GTGACATTCTGCTCAAATAGGGAGGCTTCTAGTCAAGCGCCCTTCATCGCCAGCCTGTGCCTCAAAT CTCCCGGCCGGTTCGAACGAATCCTGTTGCGAGCGGCCAATACCCATAGCTCAGCTAAACCTTGCG GCCAGCAGAGTATTCATCTTGGTGGTGTGTTTGAACTTCAGCCGGGAGCATCTGTGTTCGTCAACGT AACGGACCCTAGCCAAGTGTCTCATGGGACAGGTTTTACATCCTTCGGACTCCTCAAGTTGtaG
Flt3L (Human) (SEQ ID NO: 96)
ATGACAGTTCTCGCGCCAGCTTGGAGTCCCACCACATACTTGCTTTTGCTTCTGCTTCTGTCCTCTGG CCTGAGTGGGACCCAAGATTGTTCCTTTCAACATTCCCCAATTAGTTCTGATTTTGCAGTGAAGATT AGAGAGCTCTCAGACTATCTGCTGCAAGATTATCCTGTCACAGTCGCTTCAAACCTGCAAGACGAA GAGCTCTGCGGTGCCTTGTGGCGGTTGGTCTTGGCTCAAAGATGGATGGAGAGACTGAAAACCGTA GCAGGCAGCAAGATGCAGGGTCTCCTGGAAAGGGTGAACACGGAAATCCATTTTGTGACCAAGTGC GCGTTCCAGCCCCCACCGAGTTGTCTCCGGTTTGTTCAAACGAATATATCCCGGTTGCTCCAGGAAA CCTCAGAACAACTGGTGGCTTTGAAACCCTGGATCACAAGACAAAACTTTAGTCGGTGCCTCGAAC TCCAGTGCCAACCAGATTCTTCTACACTTCCCCCCCCGTGGTCCCCGCGCCCGTTGGAAGCAACGGC CCCAtaG
TGFb TRAP (Human) (SEQ ID NO: 97)
ATGGCCTGGAGTCCTCTGTTTCTGACTCTTATAACTCACTGTGCCGGCAGTTGGGCTATACCCCCTC ATGTACAGAAGTCTGTAAACAACGACATGATTGTAACCGACAATAATGGCGCAGTGAAATTCCCAC AACTGTGTAAGTTCTGTGATGTACGGTTTAGTACATGCGACAATCAAAAAAGCTGTATGTCTAACTG CTCTATTACATCCATATGTGAAAAACCTCAGGAGGTGTGTGTTGCCGTTTGGCGAAAAAATGATGA GAATATCACACTGGAGACAGTATGTCATGACCCTAAACTGCCATACCATGATTTCATACTGGAGGA CGCCGCCAGTCCTAAGTGCATTATGAAAGAGAAAAAGAAACCCGGTGAAACATTCTTTATGTGCTC TTGTAGCTCTGACGAGTGTAACGACAACATTATATTCAGCGAGGAGTACAATACAAGCAACCCCGA TATACCACCTCACGTACAAAAAAGTGTCAACAACGATATGATTGTTACCGACAATAACGGAGCTGT TAAGTTTCCTCAGTTGTGCAAGTTCTGCGATGTACGATTCTCTACCTGCGACAACCAAAAGTCATGT ATGTCTAACTGTTCCATAACCTCCATCTGCGAGAAGCCCCAGGAAGTCTGCGTCGCCGTGTGGCGG AAAAACGACGAGAATATCACTCTTGAAACCGTTTGTCATGATCCTAAACTGCCCTATCACGACTTTA TTCTGGAAGATGCTGCTTCCCCTAAGTGTATCATGAAAGAAAAGAAGAAACCTGGGGAGACATTCT TTATGTGTTCATGCTCCTCCGATGAGTGTAACGACAATATCATCTTCTCTGAGGAATACAACACTTC TAACCCTGATtaG

Fresolimumab (Human) (SEQ ID NO: 98)
ATGGCCTGGTCCCCTCTTTTTCTGACCCTCATCACACACTGTGCAGGCTCATGGGCTGAGACCGTCT TGACCCAGTCCCCAGGAACTTTGTCTCTGTCTCCTGGTGAAAGAGCTACCCTTAGTTGTCGAGCCTC TCAGTCCCTTGGTTCTAGCTATCTCGCTTGGTACCAGCAAAAGCCAGGCCAGGCCCCACGACTGCTG ATCTACGGAGCATCTTCACGGGCTCCCGGCATTCCCGATCGATTTTCCGGATCTGGTAGTGGTACAG ATTTCACACTGACCATATCTCGCCTGGAGCCCGAGGACTTTGCTGTTTATTATTGTCAGCAGTACGC CGATTCTCCTATCACTTTTGGACAGGGAACCCGCCTGGAGATTAAGCGCACAGTAGCAGCTCCATCC GTCTTTATCTTTCCACCATCAGATGAACAGCTCAAGAGTGGGACCGCAAGTGTAGTATGCCTGCTGA ACAATTTTTACCCTAGAGAGGCCAAAGTGCAGTGGAAGGTGGATAACGCCCTCCAGAGTGGCAATA GTCAAGAAAGTGTTACTGAGCAAGATAGTAAGGACTCTACATACTCTTTGAGTTCTACTTTGACCCT GTCAAAAGCAGATTATGAAAAACATAAGGTGTATGCATGTGAAGTTACACACCAAGGGTTGTCCTC TCCAGTTACAAAATCTTTTAATAGAGGAGAGTGCCGCCGCAAACGCGGTAGTGGAGAAGGTCGAGG CTCACTCTTGACCTGTGGCGACGTGGAAGAAAATCCCGGTCCTATGGATTGGACTTGGAGGGTATTT TGTCTTTTGGCAGTAACACCTGGAGCTCACCCCCAAGTACAGCTCGTCCAATCTGGTGCCGAGGTTA AAAAGCCTGGAAGTTCAGTGAAGGTCTCTTGCAAGGCATCTGGATACACCTTTTCATCTAACGTCAT ATCCTGGGTACGGCAAGCCCCAGGACAGGGACTTGAGTGGATGGGAGGGGTCATCCCCATCGTGGA CATTGCTAATTACGCTCAGCGATTCAAAGGGCGGGTTACTATAACTGCCGACGAGTCTACCTCAACT ACCTACATGGAGTTGTCCTCTCTCCGCTCCGAGGACACTGCTGTATATTACTGTGCCAGCACTCTCG GGTTGGTGTTGGATGCCATGGACTATTGGGGACAAGGAACCCTGGTGACAGTTAGCTCCGCAAGCA CTAAAGGCCCTTCTGTTTTTCCCTTGGCACCTTGTAGTAGGTCTACCTCTGAGTCTACAGCAGCACTT GGATGCTTGGTTAAGGACTATTTTCCCGAGCCAGTTACAGTCTCTTGGAACAGTGGTGCCCTCACAA GTGGGGTTCATACCTTCCCCGCAGTCCTCCAGAGTAGTGGCCTTTACAGCCTCTCATCAGTTGTGAC TGTTCCTAGTTCATCACTCGGTACTAAGACATATACATGTAACGTAGACCACAAGCCAAGCAACAC AAAAGTAGACAAACGAGTCGAATCTAAGTATGGACCCCCTTGTCCCTCCTGTCCTGCTCCCGAGTTC CTTGGGGGCCCTTCCGTGTTCTTGTTTCCTCCCAAGCCCAAGGATACCCTCATGATCTCACGAACCC CAGAGGTAACATGTGTGGTTGTTGACGTAAGTCAGGAAGATCCCGAAGTGCAATTTAATTGGTACG TGGATGGCGTCGAAGTCCATAACGCTAAAACAAAACCCCGAGAGGAACAATTCAATTCCACATATC GGGTGGTGAGTGTATTGACCGTTCTTCACCAAGATTGGCTGAACGGCAAGGAGTATAAGTGTAAAG TAAGCAACAAAGGTCTGCCAAGTAGCATAGAAAAAACAATATCTAAAGCTAAGGGCCAACCAAGG GAACCACAAGTATATACATTGCCCCCCTCTCAGGAAGAGATGACAAAGAATCAAGTTAGCCTGACC TGTTTGGTAAAGGGGTTCTATCCCTCAGATATAGCAGTCGAGTGGGAATCTAACGGCCAGCCCGAG AATAATTATAAAACAACCCCCCCTGTGTTGGACTCAGACGGCAGCTTCTTTCTCTATTCACGGCTCA CTGTTGATAAGTCCCGATGGCAGGAGGGGAATGTTTTCAGCTGTAGCGTGATGCACGAAGCTCTCC ACAACCACTATACACAGAAAAGTTTGTCTTTGTCCCTTGGAAAAtaG
TGFb neutralizing peptide (Human) (SEQ ID NO: 99)
ATGAGTACATCCTTTCCAGAGCTGGATCTGGAGAATTTTGAGTATGACGACAGTGCCGAAGCCTGC TACCTCGGGGACATAGTCGCATTCGGGACAATCTTTTTGTCTGTATTTTACGCCCTGGTGTTTACATT TGGCCTGGTTGGAAATCTGTTGGTCGTACTCGCTCTCACCAATTCCCGAAAACCCAAAAGTATAACA GACATATACCTGTTGAATCTGGCACTGAGTGACCTTTTGTTCGTCGCCACCCTTCCTTTTTGGACACA CTACCTTATCAGTCACGAGGGGCTTCATAATGCTATGTGCAAGCTCACTACTGCCTTCTTCTTTATCG GATTCTTCGGGGGTATCTTTTTTATCACAGTTATTAGCATTGACCGATACCTTGCCATAGTGCTCGCA GCCAACTCAATGAACAACCGCACCGTGCAGCATGGAGTGACTATTTCCTTGGGTGTGTGGGCCGCT GCTATACTTGTCGCCAGCCCTCAATTCATGTTTACCAAAAGGAAAGACAATGAGTGCCTCGGAGAT TACCCTGAGGTGTTGCAAGAAATGTGGCCTGTACTTCGAAATAGCGAAGTGAATATACTCGGCTTT GCTCTTCCTCTGCTCATCATGTCATTCTGTTATTTTCGAATAATCCAAACATTGTTCAGCTGTAAGAA CCGAAAGAAAGCCCGCGCCGTACGCCTGATTCTGCTCGTTGTGTTCGCCTTTTTTCTGTTTTGGACTC CTTACAACATAATGATATTCCTGGAGACTCTCAAATTCTATAACTTTTTTCCCTCCTGTGATATGAAA AGGGACCTTAGATTGGCTCTCAGTGTCACTGAAACAGTAGCCTTTAGCCATTGTTGTCTCAACCCTT TCATATATGCATTTGCAGGGGAAAAGTTCCGGCGGTATCTCGGACATTTGTATCGGAAGTGCTTGGC CGTGTTGTGTGGTCATCCTGTCCATACCGGATTCTCTCCTGAGAGTCAACGGAGCCGCCAAGATTCA ATCCTGTCCAGTTTCACTCACTATACTTCAGAGGGGGATGGCAGCCTTCTGCTC
Kynureinase #1 (SEQ ID NO: 100)
ATGGAGACCGACACTTTGTTGCTGTGGGTACTTTTGTTGTGGGTCCCAGGATCTACCGGGGATATGG AACCCTCTCCTCTTGAACTGCCAGTAGACGCCGTGCGCCGCATTGCAGCCGAGTTGAATTGCGATCC AACAGATGAACGCGTTGCCCTGAGGCTCGACGAAGAGGATAAATTGTCACATTTCAGGAACTGCTT TTACATTCCAAAGATGAGGGATCTTCCATCCATAGATCTTAGCCTCGTGTCCGAGGATGACGATGCC

ATATATTTTCTTGGGAACAGTCTTGGGTTGCAGCCAAAAATGGTACGGACATATCTCGAAGAGGAG CTGGACAAATGGGCTAAAATGGGTGCTTACGGCCACGACGTGGGAAAACGCCCCTGGATAGTTGGC GACGAATCTATCGTGAGTCTTATGAAAGATATAGTTGGAGCACATGAGAAAGAAATTGCACTGATG AATGCCCTTACTATCAATCTGCATCTCCTCTTGCTTTCATTCTTTAAGCCCACTCCTAAACGCCACAA AATACTTTTGGAAGCAAAAGCCTTTCCAAGCGACCACTACGCTATTGAGTCACAAATACAACTCCA TGGACTTGATGTGGAAAAGTCTATGCGGATGGTAAAACCACGCGAAGGCGAGGAGACCCTTCGAAT GGAGGACATACTTGAGGTCATCGAAGAAGAAGGAGATAGTATAGCAGTTATCCTTTTCAGCGGGCT GCACTTCTACACAGGTCAACTCTTTAACATTCCAGCTATTACTAAGGCAGGCCACGCTAAAGGATGC TTCGTGGGCTTTGACCTTGCACACGCAGTAGGAAACGTAGAGCTCCGCTTGCACGATTGGGGCGTT GATTTCGCCTGCTGGTGTTCATATAAGTATCTTAACTCAGGAGCTGGTGGGTTGGCAGGCGCATTCG TACACGAGAAACACGCTCATACCGTAAAGCCTGCACTGGTAGGGTGGTTCGGACACGATCTCTCTA CCCGCTTCAATATGGATAATAAACTCCAGCTTATACCTGGCGCCAATGGATTCAGGATCTCAAATCC TCCTATTTTGCTCGTTTGCAGTTTGCACGCATCTCTTGAGGTGTTCCAGCAGGCTACCATGACTGCAC TCCGCCGGAAGTCAATCCTTTTGACCGGATACTTGGAGTATATGCTGAAACATTATCACTCAAAAGA TAACACTGAGAATAAGGGCCCCATAGTAAACATTATCACTCCATCTCGGGCTGAAGAGCGCGGCTG CCAACTCACATTGACTTTTTCCATTCCCAAGAAGTCAGTGTTCAAAGAGTTGGAGAAACGGGGGGT TGTATGTGATAAGCGGGAGCCAGATGGAATCCGCGTTGCCCCAGTCCCCCTCTATAATTCTTTTCAC GATGTATACAAGTTTATTAGACTGCTGACAAGTATCTTGGACTCATCTGAGCGATCTtaG
Kynureinase #2 (SEQ ID NO: 101)
ATGGAACCCTCTCCTCTTGAACTGCCAGTAGACGCCGTGCGCCGCATTGCAGCCGAGTTGAATTGCG ATCCAACAGATGAACGCGTTGCCCTGAGGCTCGACGAAGAGGATAAATTGTCACATTTCAGGAACT GCTTTTACATTCCAAAGATGAGGGATCTTCCATCCATAGATCTTAGCCTCGTGTCCGAGGATGACGA TGCCATATATTTTCTTGGGAACAGTCTTGGGTTGCAGCCAAAAATGGTACGGACATATCTCGAAGA GGAGCTGGACAAATGGGCTAAAATGGGTGCTTACGGCCACGACGTGGGAAAACGCCCCTGGATAG TTGGCGACGAATCTATCGTGAGTCTTATGAAAGATATAGTTGGAGCACATGAGAAAGAAATTGCAC TGATGAATGCCCTTACTATCAATCTGCATCTCCTCTTGCTTTCATTCTTTAAGCCCACTCCTAAACGC CACAAAATACTTTTGGAAGCAAAAGCCTTTCCAAGCGACCACTACGCTATTGAGTCACAAATACAA CTCCATGGACTTGATGTGGAAAAGTCTATGCGGATGGTAAAACCACGCGAAGGCGAGGAGACCCTT CGAATGGAGGACATACTTGAGGTCATCGAAGAAGAAGGAGATAGTATAGCAGTTATCCTTTTCAGC GGGCTGCACTTCTACACAGGTCAACTCTTTAACATTCCAGCTATTACTAAGGCAGGCCACGCTAAAG GATGCTTCGTGGGCTTTGACCTTGCACACGCAGTAGGAAACGTAGAGCTCCGCTTGCACGATTGGG GCGTTGATTTCGCCTGCTGGTGTTCATATAAGTATCTTAACTCAGGAGCTGGTGGGTTGGCAGGCGC ATTCGTACACGAGAAACACGCTCATACCGTAAAGCCTGCACTGGTAGGGTGGTTCGGACACGATCT CTCTACCCGCTTCAATATGGATAATAAACTCCAGCTTATACCTGGCGCCAATGGATTCAGGATCTCA AATCCTCCTATTTTGCTCGTTTGCAGTTTGCACGCATCTCTTGAGGTGTTCCAGCAGGCTACCATGAC TGCACTCCGCCGGAAGTCAATCCTTTTGACCGGATACTTGGAGTATATGCTGAAACATTATCACTCA AAAGATAACACTGAGAATAAGGGCCCCATAGTAAACATTATCACTCCATCTCGGGCTGAAGAGCGC GGCTGCCAACTCACATTGACTTTTTCCATTCCCAAGAAGTCAGTGTTCAAAGAGTTGGAGAAACGG GGGGTTGTATGTGATAAGCGGGAGCCAGATGGAATCCGCGTTGCCCCAGTCCCCCTCTATAATTCTT TTCACGATGTATACAAGTTTATTAGACTGCTGACAAGTATCTTGGACTCATCTGAGCGATCTtaG
VEGF (SEQ ID NO: 102)
ATGAATTTCTTGCTGAGCTGGGTGCATTGGACACTCGCATTGTTGCTGTACTTGCACCATGCCAAGT GGTCCCAGGCTGCACCCACTACTGAGGGCGAGCAAAAGTCTCATGAGGTGATTAAATTTATGGACG TTTACCAACGATCATACTGTCGGCCAATCGAAACCCTCGTAGATATATTCCAGGAGTACCCAGACG AGATCGAATACATTTTCAAGCCCTCATGTGTCCCATTGATGCGATGTGCTGGGTGCTGTAACGACGA AGCACTTGAATGTGTCCCCACCTCCGAGAGTAACATCACAATGCAAATAATGAGAATCAAGCCCCA CCAATCCCAACATATCGGTGAAATGTCATTCCTTCAGCATTCCCGCTGCGAGTGCCGGCCTAAGAAG GACCGCACCAAACCAGAGAACCATTGTGAACCCTGTTCTGAGAGACGGAAGCACTTGTTCGTACAG GACCCTCAAACATGCAAGTGCAGCTGTAAGAATACCGACTCACGGTGTAAAGCTAGGCAACTGGAG CTTAATGAAAGGACCTGCCGATGCGATAAACCCAGGAGGtaa
GM-CSF (SEQ ID NO: 103)
ATGTGGTTGCAGAATTTGCTCTTCCTGGGGATTGTGGTCTACAGCCTCTCCGCACCTACCCGCTCTCC TATCACAGTTACAAGACCCTGGAAACATGTGGAGGCCATTAAAGAAGCATTGAATTTGTTGGACGA TATGCCCGTCACCCTGAATGAAGAAGTAGAAGTTGTTTCTAATGAGTTCAGCTTTAAAAAATTGACC TGTGTGCAGACACGGCTTAAAATTTTTGAACAGGGACTTAGAGGAAACTTTACTAAGCTGAAGGGG GCACTTAACATGACAGCTTCTTATTATCAGACCTATTGTCCTCCAACACCTGAAACCGACTGTGAAA

CACAGGTAACCACTTACGCCGATTTTATTGATTCTTTGAAAACATTCCTCACCGATATACCATTTGA GTGTAAGAAGCCAGGCCAAAAGtaG
Anti-PD1 (SEQ ID NO: 104)
ATGGAAACTGACACACTTCTTCTGTGGGTCTTGCTCCTGTGGGTCCCAGGCTCTACTGGTGACAGTC CTGATAGGCCATGGAACCCACCTACCTTTAGTCCAGCCTTGCTCGTCGTAACCGAAGGGGACAACG CTACATTCACCTGCTCTTTTAGCAATACTTCTGAGAGTTTTCATGTAGTCTGGCATCGGGAGAGTCC ATCCGGACAAACAGATACTTTGGCCGCTTTTCCAGAGGATAGGTCTCAACCTGGGCAAGACGCAAG GTTTCGAGTCACACAGCTTCCTAACGGGAGAGATTTTCACATGTCTGTAGTTCGGGCACGCCGAAAT GATTCTGGCACATATGTTTGCGGTGTGATCTCACTTGCTCCAAAGATTCAAATAAAGGAGAGCCTTC GCGCCGAGTTGCGGGTGACTGAGCGGGAGCCCAAGTCCTGCGACAAAACCCATACTTGTCCACCCT GTGGCGGCGGGTCATCCGGTGGCGGGTCTGGGGGCAACCAAGAGAGCCACAGGTATATACTCTTC CCCCCAGCAGAGAAGAAATGACAAAAAACCAAGTGTCCCTGACATGTCTGGTTAAAGGATTTTATC CCAGTGACATTGCTGTAGAATGGGAATCCAATGGTCAACCCGAGAATAACTACAAAACCACTCCTC CAGTATTGGACAGTGACGGTTCCTTCTTCCTCTATTCCAAACTTACAGTGGATAAATCCCGCTGGCA GCAAGGGAATGTATTCAGCTGTAGTGTCATGCACGAAGCTCTTCATAACCATTATACACAGAAATC TCTTTCCCTGAGCCCAGGTAAAtaG
Adenosine Deaminase (ADA) #1 (Mouse) (SEQ ID NO: 105)
ATGGAGACTGATACACTTTTGCTCTGGGTTTTGCTCTTGTGGGTACCAGGGTCTACTGGAGATGCAC AAACTCCTGCATTCAACAAGCCTAAGGTAGAGCTTCATGTCCATTTGGACGGAGCCATAAAACCTG AAACCATACTCTATTTCGGCAAGAAACGGGGTATAGCACTTCCCGCTGATACCGTGGAAGAGTTGA GAAATATCATTGGCATGGACAAACCTCTTAGCCTGCCTGGCTTTCTTGCAAAGTTCGACTACTATAT GCCAGTTATAGCAGGGTGTAGAGAAGCAATAAAGCGAATCGCCTATGAGTTCGTTGAGATGAAGGC TAAAGAAGGAGTTGTTTACGTGGAAGTCCGGTACTCACCTCATCTGCTTGCTAATAGCAAGGTGGA CCCAATGCCATGGAATCAAACTGAAGGTGATGTAACCCCTGACGATGTGGTCGATTTGGTCAATCA AGGTCTCCAAGAAGGCGAGCAGGCTTTCGGCATTAAGGTAAGAAGTATATTGTGCTGTATGCGACA TCAACCTTCATGGTCCCTGGAGGTCCTCGAATTGTGCAAAAAGTACAATCAAAAAACAGTGGTCGC AATGGATCTCGCTGGAGATGAGACCATAGAAGGTTCCTCTCTTTTCCCCGGTCATGTCGAAGCATAT GAAGGGGCTGTCAAAAATGGTATCCACCGCACCGTCCACGCAGGGGAAGTAGGGTCCCCAGAAGT AGTCAGGGAAGCCGTTGACATTTTGAAAACAGAAAGAGTCGGGCATGGCTACCATACAATAGAGG ACGAAGCCTTGTACAATCGACTTTTGAAAGAAAATATGCACTTCGAGGTCTGTCCCTGGAGTTCATA TCTCACCGGAGCATGGGACCCCAAAACAACCCACGCCGTCGTACGCTTCAAGAATGATAAGGCAAA CTACAGTTTGAATACAGATGATCCACTGATATTCAAGTCAACACTTGACACTGACTACCAGATGAC AAAAAAAGATATGGGTTTCACCGAAGAAGAGTTCAAGAGATTGAACATTAACGCAGCAAAAAGCT CCTTCCTGCCAGAGGAAGAGAAAAAAGAATTGCTTGAAAGGTTGTATCGAGAATACCAA
Adenosine Deaminase (ADA) #2 (Mouse) (SEQ ID NO: 106)
ATGGCACAAACTCCAGCTTTTAATAAGCCCAAAGTGGAACTTCATGTTCATCTGGATGGGGCAATT AAGCCCGAAACTATATTGTACTTTGGCAAAAAGAGGGGTATTGCCCTGCCAGCAGATACCGTTGAG GAGCTTCGCAACATCATTGGGATGGACAAGCCCCTCTCTCTGCCAGGTTTTCTCGCTAAATTCGATT ATTATATGCCTGTTATTGCTGGTTGCCGGGAGGCCATCAAGAGGATAGCCTACGAGTTTGTTGAGAT GAAGGCCAAAGAGGGCGTGGTGTACGTAGAGGTCAGATACAGCCCTCACCTGCTTGCCAACAGCA AGGTGGACCCAATGCCCTGGAACCAAACCGAGGGGGATGTCACTCCCGACGACGTTGTAGACCTCG TAAATCAGGGCCTTCAAGAGGGCGAGCAGGCATTTGGCATAAAAGTCCGGTCTATACTCTGCTGTA TGAGGCACCAACCCTCCTGGTCTTTGGAGGTACTTGAGTTGTGTAAGAAATACAATCAAAAGACTG TAGTCGCCATGGATCTTGCAGGCGATGAAACCATCGAGGGTAGCTCCTTGTTCCCTGGACATGTTGA AGCCTACGAGGGGGCCGTAAAAAATGGGATACACAGGACTGTCCACGCTGGTGAAGTCGGAAGCC CAGAGGTGGTAAGGGAGGCAGTTGACATACTCAAGACAGAGCGGGTTGGACACGGATACCACACA ATTGAGGACGAGGCCCTGTATAACCGCCTCCTCAAAGAGAACATGCATTTTGAGGTGTGTCCTTGGT CCAGCTACCTGACTGGTGCTTGGGACCCTAAAACAACTCACGCCGTGGTCCGGTTCAAGAACGATA AAGCCAATTACTCTTTGAATACCGACGACCCCCTCATATTCAAATCAACATTGGATACCGACTACCA AATGACCAAAAAGGATATGGGGTTTACTGAAGAGGAGTTCAAGAGGCTCAACATAAATGCCGCTA AATCCTCCTTTCTCCCCGAGGAAGAAAAAAAAGAACTCCTTGAGCGGCTGTATAGGGAGTATCAA
4-1BBL #1 (Mouse) (SEQ ID NO: 107)
ATGGAAACAGATACACTCTTGCTCTGGGTACTGCTTCTGTGGGTCCCCGGCTCTACTGGGGATGAAG ATGATGTAACTACTACAGAAGAACTCGCTCCCGCTCTTGTCCCCCCACCCAAGGGTACCTGCGCCGG TTGGATGGCTGGCATCCCAGGACATCCAGGTCACAACGGTACCCCCGGAAGAGATGGTCGGGATGG

AACTCCCGGCGAGAAGGGCGAAAAAGGGGATGCAGGGCTTCTGGGACCTAAAGGTGAAACAGGGG ACGTTGGAATGACTGGTGCAGAAGGGCCTCGCGGCTTTCCTGGCACCCCTGGGAGGAAAGGAGAGC CCGGAGAGCTCCAGAGAACTGAACCTCGGCCTGCACTCACTATAACTACTTCCCCTAATCTTGGGAC CCGCGAGAACAACGCCGATCAGGTTACACCTGTAAGCCATATCGGGTGCCCCAATACTACCCAGCA AGGGAGTCCCGTGTTCGCAAAGCTTTTGGCTAAAAACCAAGCATCCCTGTGTAACACTACTCTTAAT TGGCATTCACAAGACGGTGCTGGTAGCTCTTATCTTTCTCAGGGGCTGCGGTACGAAGAAGATAAG AAGGAATTGGTTGTGGATTCTCCAGGACTCTATTATGTCTTTCTCGAATTGAAGCTCAGTCCCACCT TCACAAACACTGGACACAAAGTCCAGGGCTGGGTAAGTCTGGTACTCCAAGCAAAGCCCCAGGTTG ACGATTTCGACAATTTGGCACTCACCGTAGAGCTTTTCCCATGCTCCATGGAAAATAAACTTGTTGA TCGGTCATGGTCACAGCTCTTGCTGCTTAAGGCAGGGCATCGCCTCTCAGTGGGTCTGAGAGCTTAT TTGCATGGTGCACAAGATGCTTACAGGGATTGGGAATTGTCCTACCCAAACACTACAAGTTTCGGG TTGTTCCTTGTCAAACCTGATAACCCATGGGAGtaG
4-1BBL #2 (Mouse) (SEQ ID NO: 108)
ATGGAAACTGATACACTCCTCCTGTGGGTCCTTCTTTTGTGGGTGCCCGGATCAACCGGCGATGGCT GGATGGCAGGCATCCCAGGACACCCAGGACACAACGGTACTCCAGGTCGAGACGGTCGGGATGGG ACTCCTGGGGAGAAAGGCGAGAAAGGGGACGCTGGTTTGCTCGGTCCTAAGGGGGAAACCGGGGA TGTAGGAATGACAGGGGCTGAAGGGCCTCGGGGATTTCCTGGGACACCAGGCAGGAAGGGTGAAC CAGGGGAGGCCCTCCAGCGCACCGAGCCACGGCCAGCTCTGACCATAACAACAAGTCCAAACCTG GGCACACGCGAAAACAATGCTGACCAGGTGACTCCTGTAAGTCACATCGGATGCCCTAACACTACA CAACAGGGCTCTCCTGTATTTGCAAAGCTTCTCGCAAAAAATCAAGCATCACTTTGTAATACAACCC TGAACTGGCATTCTCAGGACGGAGCAGGGTCCTCTTATTTGTCTCAAGGGCTCCGCTACGAAGAAG ATAAAAAGGAATTGGTTGTTGACAGTCCAGGTTTGTATTATGTGTTTTTGGAACTTAAGCTGTCACC AACCTTCACTAACACCGGCCACAAGGTCCAAGGCTGGGTTAGTCTTGTTTTGCAAGCCAAACCTCA AGTGGATGATTTTGACAATCTGGCTTTGACTGTTGAGCTTTTTCCATGCAGTATGGAGAATAAACTG GTTGATCGGTCATGGTCACAGCTCCTTCTGCTCAAGGCCGGACATAGGCTGAGTGTGGGACTTCGG GCCTACTTGCACGGCGCCCAGGACGCATACCGAGACTGGGAACTCAGCTACCCTAACACAACTTCT TTTGGGTTGTTCCTTGTCAAACCCGATAATCCTTGGGAAtaG
HPGE2 #1 (Mouse) (SEQ ID NO: 109)
ATGGAGACTGATACTTTGCTCCTGTGGGTTCTTCTCCTGTGGGTTCCTGGTTCCACAGGGGATATGC ATGTCAATGGCAAGGTAGCACTCGTGACTGGGGCTGCACAGGGTATCGGGAAAGCTTTTGCCGAGG CCCTGTTGCTGCATGGCGCCAAGGTCGCTTTGGTAGATTGGAACTTGGAGGCTGGAGTTAAATGCA AAGCTGCACTCGACGAACAATTTGAGCCTCAAAAAACCCTCTTTGTGCAGTGTGACGTTGCTGACC AAAAGCAACTCAGGGACACATTCAGGAAGGTCGTAGACCATTTCGGACGCCTCGATATACTCGTTA ATAATGCCGGGGTAAACAACGAAAAGAACTGGGAACAAACATTGCAAATCAACCTGGTAAGTGTC ATTAGCGGAACTTATCTGGGTCTTGATTATATGAGCAAGCAGAACGGGGGCGAGGGCGGGATCATT ATCAACATGTCAAGTCTTGCCGGATTGATGCCAGTTGCTCAGCAGCCTGTTTACTGTGCCAGCAAGC ACGGTATTATTGGGTTTACCCGGAGTGCCGCCATGGCCGCAAATCTTATGAAGAGTGGGGTAAGAC TGAATGTTATCTGCCCAGGTTTCGTAGATACCCCAATCCTGGAGAGCATCGAGAAGGAGGAAAATA TGGGACAATACATTGAATATAAAGATCAAATCAAGGCTATGATGAAGTTCTACGGGGTTCTGCATC CATCCACAATTGCCAACGGGCTCATTAATCTGATTGAGGACGACGCCTTGAACGGAGCTATAATGA AAATCACAGCTTCCAAAGGCATTCACTTCCAAGATTATGATATATCACCCTTGCTTGTCAAGGCTCC TCTGACAAGT
HPGE2 #2 (Mouse) (SEQ ID NO: 110)
ATGCATGTCAATGGCAAGGTAGCACTCGTGACTGGGGCTGCACAGGGTATCGGGAAAGCTTTTGCC GAGGCCCTGTTGCTGCATGGCGCCAAGGTCGCTTTGGTAGATTGGAACTTGGAGGCTGGAGTTAAA TGCAAAGCTGCACTCGACGAACAATTTGAGCCTCAAAAAACCCTCTTTGTGCAGTGTGACGTTGCTG ACCAAAAGCAACTCAGGGACACATTCAGGAAGGTCGTAGACCATTTCGGACGCCTCGATATACTCG TTAATAATGCCGGGGTAAACAACGAAAAGAACTGGGAACAAACATTGCAAATCAACCTGGTAAGT GTCATTAGCGGAACTTATCTGGGTCTTGATTATATGAGCAAGCAGAACGGGGGCGAGGGCGGGATC ATTATCAACATGTCAAGTCTTGCCGGATTGATGCCAGTTGCTCAGCAGCCTGTTTACTGTGCCAGCA AGCACGGTATTATTGGGTTTACCCGGAGTGCCGCCATGGCCGCAAATCTTATGAAGAGTGGGGTAA GACTGAATGTTATCTGCCCAGGTTTCGTAGATACCCCAATCCTGGAGAGCATCGAGAAGGAGGAAA ATATGGGACAATACATTGAATATAAAGATCAAATCAAGGCTATGATGAAGTTCTACGGGGTTCTGC ATCCATCCACAATTGCCAACGGGCTCATTAATCTGATTGAGGACGACGCCTTGAACGGAGCTATAA TGAAAATCACAGCTTCCAAAGGCATTCACTTCCAAGATTATGATATATCACCCTTGCTTGTCAAGGC TCCTCTGACAAGT

Additional Embodiments

Provided below are enumerated paragraphs describing specific embodiments:

1. An engineered cell comprising:

a) a promoter; and

5 b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide,

10 E1 comprises a polynucleotide sequence encoding a first effector molecule,

L comprises a linker polynucleotide sequence,

S2 comprises a polynucleotide sequence encoding a second signal peptide,

E2 comprises a polynucleotide sequence encoding a second effector molecule, and

wherein the promoter is operably linked to the expression cassette, the first signal 15 peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and

wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, 20 monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

2. The engineered cell of paragraph 1, wherein the promoter comprises an exogenous promoter polynucleotide sequence.

25 3. The engineered cell of paragraph 1, wherein the promoter comprises an endogenous promoter.

4. The engineered cell of any one of paragraphs 1-3, wherein the promoter is operably linked to the expression cassette such that the polynucleotides are capable of being transcribed as a single polynucleotide comprising the formula S1 – E1 – L – S2 – E2.

5. The engineered cell of paragraph 4, wherein the linker polynucleotide sequence is operably associated with the translation of the first effector molecule and the second effector molecule as separate polypeptides.

6. The engineered cell of paragraph 5, wherein the linker polynucleotide sequence encodes a 2A ribosome skipping tag.

7. The engineered cell of paragraph 6, wherein the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

8. The engineered cell of paragraph 5, wherein the linker polynucleotide sequence encodes a T2A ribosome skipping tag.

9. The engineered cell of paragraph 5, the linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES).

10. The engineered cell of any one of paragraphs 5-9, wherein the linker polynucleotide sequence encodes a cleavable polypeptide.

11. The engineered cell of paragraph 10, wherein the cleavable polypeptide comprises a Furin recognition polypeptide sequence.

12. The engineered cell of any one of paragraphs 5-9, wherein the linker polynucleotide sequence further encodes a Gly-Ser-Gly polypeptide sequence.

13. The engineered cell of any one of paragraphs 1-5, wherein the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus.

14. The engineered cell of any one of paragraphs 1-3, wherein the linker polynucleotide sequence encodes a second promoter,

wherein the promoter is operably linked to the expression cassette such that a first polynucleotide comprising the formula S1 – E1 is capable of being transcribed,

5 wherein the second promoter is operably linked to the expression cassette such that a second polynucleotide comprising the formula S2 – E2 is capable of being transcribed, and wherein the first and the second polynucleotide are separate polynucleotides.

15. The engineered cell of paragraph 14, wherein the promoter and the second promoter are identical.

10 16. The engineered cell of paragraph 14, wherein the promoter and the second promoter are different.

17. The engineered cell of any one of paragraphs 1-16, wherein the engineered cell is HLA-typed with reference to a subject in need of therapeutic treatment.

18. The engineered cell of any one of paragraphs 1-17, wherein the engineered cell is a 15 human cell.

19. The engineered cell of paragraph 18, wherein the human cell is an isolated cell from a subject.

20. The engineered cell of paragraph 19, wherein the isolated cell is isolated from a tissue consisting of the group of: bone marrow, adipose tissue, the umbilical cord, fetal liver, 20 muscle, and lung tissue.

21. The engineered cell of any one of paragraphs 1-20, wherein the engineered cell is a cultured cell.

22. The engineered cell of any one of paragraphs 1-21, wherein the engineered MSC comprises a cellular marker phenotype comprising the cellular markers CD105+, 25 CD73+,and CD90+.

23. The engineered cell of paragraph 22, wherein the cellular marker phenotype further comprises a phenotype lacking or substantially lacking one or more cellular markers selected from the group consisting of: CD45, CD34, CD14, CD11b, CD79α, CD19, HLA class II, and combinations thereof.

5 24. The engineered cell of any one of paragraphs 1-21, wherein the engineered MSC comprises a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD45-, CD34-, CD14-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD11b-, CD79α-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD19-, HLA class II-; or a cellular marker phenotype comprising CD73+, CD90+,
10 CD105+, and CD166+, CD11b-, CD14-, CD19-, CD34-, CD45-, and HLA-DR-.

25. The engineered cell of any one of paragraphs 22-24, wherein the cellular marker phenotype is determined or has been determined by flow-cytometry.

26. The engineered cell of any one of paragraphs 1-21, wherein the engineered cell comprises a T cell.

15 27. The engineered cell of any one of paragraphs 1-21, wherein the engineered cell comprises a NK cell.

28. The engineered cell of any one of paragraphs 1-21, wherein the engineered cell comprises a NKT cell.

29. The engineered cell of any of paragraphs 22-28, wherein the cellular marker phenotype
20 further comprises a cellular marker comprising a cognate receptor or a cognate receptor ligand for the first effector molecule, the second effector molecule, or the first and second effector molecules expressed in the engineered cells.

30. The engineered cell of paragraph 29, wherein the receptor is selected from the group consisting of: IL12RB1, IL12RB2, CCL7, and combinations thereof.

25 31. The engineered cell of any one of paragraphs 1-30, wherein the promoter and/or the second promoter comprises a constitutive promoter.

32. The engineered cell of paragraph 31, wherein the constitutive promoter is selected from the group consisting of: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb.

5 33. The engineered cell of any one of paragraphs 1-30, wherein the promoter comprises an SFFV promoter.

34. The engineered cell of any one of paragraphs 1-30, wherein the promoter and/or the second promoter comprises an inducible promoter.

35. The engineered cell of paragraph 34, wherein the inducible promoter is selected from the group consisting of: minP, NFkB response element, CREB response element, NFAT response element, SRF response element 1, SRF response element 2, AP1 response element, TCF-LEF response element promoter fusion, Hypoxia responsive element, SMAD binding element, STAT3 binding site, inducer molecule responsive promoters, and tandem repeats thereof.

15 36. The engineered cell of any one of paragraphs 1-35, wherein the first signal peptide or the second signal peptide comprises a native signal peptide native to the first effector molecule or the second effector molecule, respectively.

37. The engineered cell of any one of paragraphs 1-36, wherein the first signal peptide or the second signal peptide comprises a non-native signal peptide non-native to the first effector molecule or the second effector molecule, respectively.

38. The engineered cell of paragraph 37, wherein the non-native signal peptide is selected from the group consisting of: IL12, IL2, optimized IL2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, and IL21.

39. The engineered cell of any one of paragraphs 1-38, wherein the first signal peptide and the second signal peptide are identical.

40. The engineered cell of any one of paragraphs 1-39, wherein the polynucleotide sequence encoding the first signal peptide comprises a codon optimized polynucleotide sequence.

41. The engineered cell of any one of paragraphs 1-0, wherein the first secretion polypeptide is a human IL12 signal peptide.

5 42. The engineered cell of any one of paragraphs 1-0, wherein the polynucleotide sequence encoding the second signal peptide comprises a codon optimized polynucleotide sequence.

43. The engineered cell of any one of paragraphs 1-0, wherein the second secretion polypeptide is a human IL21 signal peptide.

10 44. The engineered cell of any one of paragraphs 1-0, wherein the first effector molecule is selected from a therapeutic class, wherein the therapeutic class is selected from the group consisting of: a cytokine, a chemokine, a growth factor, a co-activation molecule, a tumor microenvironment modifier a, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.

15 45. The engineered cell of any one of paragraphs 1-44, wherein the second effector molecule is selected from a therapeutic class, wherein the therapeutic class is selected from the group consisting of: a cytokine, a chemokine, a growth factor, a co-activation molecule, a tumor microenvironment modifier, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.

20 46. The engineered cell of paragraph 45, wherein the therapeutic class of the first effector molecule and the second effector molecule are different.

47. The engineered cell of any one of paragraphs 1-46, wherein the first effector molecule and/or the second effector molecule is a modified effector molecule.

48. The engineered cell of paragraph 47, wherein the first effector molecule and/or the
25 second effector molecule is modified to comprises a cell membrane tethering domain.

49. The engineered cell of paragraph 48, wherein the cell membrane tethering domain comprises a transmembrane-intracellular domain or a transmembrane domain.

50. The engineered cell of paragraph 48, wherein the cell membrane tethering domain comprises a cell surface receptor, or a cell membrane-bound portion thereof.

5 51. The engineered cell of paragraph 50, wherein the modified effector molecule is a fusion protein that comprises the cell surface receptor, or a cell membrane-bound portion thereof.

52. The engineered cell of any one of paragraphs 48-51, wherein the modified effector molecule further comprises a linker between the effector molecule and the cell membrane
10 tethering domain.

53. The engineered cell of any one of paragraphs 47-52, wherein when expressed the modified effector molecule is tethered to a cell membrane of the engineered cell.

54. The engineered cell of any one of paragraphs 44-53, wherein the cytokine is selected from the group consisting of: IL12, IL7, IL21, IL18, IL15, Type I interferons, and
15 Interferon-gamma.

55. The engineered cell of paragraph 54, wherein the IL12 cytokine is an IL12p70 fusion protein.

56. The engineered cell of any one of paragraphs 44-55, wherein the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, CXCL10-11
20 fusion, CCL19, CXCL9, and XCL1.

57. The engineered cell of any one of paragraphs 44-56, wherein the growth factor is selected from the group consisting of: Flt3L and GM-CSF.

58. The engineered cell of any one of paragraphs 44-57, wherein the co-activation molecule is selected from the group consisting of: 4-1BBL and CD40L.

59. The engineered cell of any one of paragraphs 34-41, wherein the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, TGFbeta inhibitors, immune checkpoint inhibitors, VEGF inhibitors, and HPGE2.

5 60. The engineered cell of paragraph 59, wherein the TGFbeta inhibitors are selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and combinations thereof.

61. The engineered cell of paragraph 59, wherein the immune checkpoint inhibitors comprise anti-PD-1 antibodies.

10 62. The engineered cell of paragraph 59, wherein the VEGF inhibitors comprise anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof.

63. The engineered cell of any one of paragraphs 1-59, wherein the first effector molecule and the second effector molecule are human-derived effector molecules.

64. The engineered cell of any one of paragraphs 1-63, wherein the first effector molecule
15 comprises IL12.

65. The engineered cell of any one of paragraphs 1-63, wherein the first effector molecule comprises an IL12p70 fusion protein.

66. The engineered cell of paragraph 15, wherein the IL12p70 fusion protein is a human IL12p70 fusion protein.

20 67. The engineered cell of any one of paragraphs 64-66, wherein the second effector molecule comprises CCL21a.

68. The engineered cell of paragraph 67, wherein the CCL21a is a human CCL21a.

69. The engineered cell of any one of paragraphs 64-66, wherein the second effector molecule comprises IL7.

25 70. The engineered cell of paragraph 69, wherein the IL7 is a human IL7.

71. The engineered cell of any one of paragraphs 64-66, wherein the second effector molecule comprises IL21.

72. The engineered cell of paragraph 71, wherein the IL21 is a human IL21.

73. The engineered cell of any one of paragraphs 1-72, wherein the expression cassette further comprises an E3 comprising a polynucleotide sequence encoding a third effector molecule.

74. The engineered cell of paragraph 73, wherein the third effector molecule comprises Flt3L.

75. The engineered cell of paragraph 73, wherein the third effector molecule comprises anti-PD1.

76. The engineered cell of paragraph 75, wherein the expression cassette further comprises an E4 comprising a polynucleotide sequence encoding a fourth effector molecule.

77. The engineered cell of paragraph 76, wherein the fourth effector molecule comprises adenosine deaminase.

78. The engineered cell of paragraph 73, wherein the third effector molecule comprises adenosine deaminase.

79. The engineered cell of paragraph 73, wherein the third effector molecule comprises CD40L.

80. The engineered cell of paragraph 73, wherein the third effector molecule comprises a CXCL10-CXCL11 fusion protein.

81. The engineered cell of paragraph 73, wherein the third effector molecule comprises XCL1.

82. The engineered cell of paragraph 64, wherein the second effector molecule comprises Flt3L.

83. The engineered cell of paragraph 64, wherein the second effector molecule comprises a CXCL10-CXCL11 fusion protein.

84. The engineered cell of paragraph 64, wherein the second effector molecule comprises anti-PD1.

5 85. The engineered cell of paragraph 64, wherein the second effector molecule comprises CD40L.

86. The engineered cell of any one of paragraphs 1-63, wherein the first effector molecule comprises interferon-beta and the second effector molecule comprises Flt3L.

87. The engineered cell of any one of paragraphs 1-86, wherein the polynucleotide sequence
10 encoding the first effector molecule comprises a codon optimized polynucleotide sequence.

88. The engineered cell of any one of paragraphs 1-87, wherein the polynucleotide sequence encoding the second effector molecule comprises a codon optimized polynucleotide sequence.

15 89. The engineered cell of any one of paragraphs 1-0, wherein the engineered cell comprises a polynucleotide sequence encoding the promoter and the expression cassette.

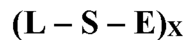
90. The engineered cell of paragraph 89, wherein the exogenous polynucleotide sequence comprises the sequence shown in SEQ ID NO: 144.

91. The engineered cell of any one of paragraphs 1-90, wherein the exogenous
20 polynucleotide sequence is integrated into the genome of the engineered cell.

92. The engineered cell of any one of paragraphs 1-91, wherein the exogenous polynucleotide sequence comprises one or more viral vector polynucleotide sequences.

93. The engineered cell of paragraph 92, wherein the one or more viral vector polynucleotide sequences comprise lentiviral, retroviral, retrotransposon, or adenoviral polynucleotide
25 sequences.

94. The engineered cell of any one of paragraphs 1-93, wherein the expression cassette further comprises following E2, an additional exogenous polynucleotide sequence comprising a formula, oriented from 5' to 3', comprising:



(L – S – E)X

5 wherein

S comprises a polynucleotide sequence encoding a signal peptide,

E comprises a polynucleotide sequence encoding an effector molecule,

L comprises a linker polynucleotide sequence,

X = 1 to 20

10 wherein the promoter is operably linked to the expression cassette, and wherein for each X the corresponding signal peptide is operably associated with the effector molecule.

95. An engineered cell comprising a construct, wherein the construct comprises:

a) an SFFV promoter; and

15 b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the
20 first signal peptide is a human IL12 signal peptide;

E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein;

L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly
25 polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus ;

S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide;

E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and

5 wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and

wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate 10 lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

96. The engineered cell of paragraph 17, wherein the construct comprises the polynucleotide 15 sequence shown in SEQ ID NO: 144.

97. The engineered cell of paragraph 17 or paragraph 18, wherein the engineered cell is HLA-typed with reference to a subject in need of therapeutic treatment.

98. The engineered cell of any one of paragraphs 17-0, wherein the engineered cell is a human cell.

20 99. The engineered cell of paragraph 0, wherein the human cell is an isolated cell from a subject.

100. The engineered cell of paragraph 0, wherein the isolated cell is isolated from a tissue consisting of the group of: bone marrow, adipose tissue, the umbilical cord, fetal liver, muscle, and lung tissue.

25 101. The engineered cell of any one of paragraphs 17-0, wherein the engineered cell is a cultured cell.

102. The engineered cell of any one of paragraphs 17-0, wherein the engineered MSC comprises a cellular marker phenotype comprising the cellular markers CD105+, CD73+, and CD90+.

103. The engineered cell of paragraph 0, wherein the cellular marker phenotype further comprises a phenotype lacking or substantially lacking one or more cellular markers selected from the group consisting of: CD45, CD34, CD14, CD11b, CD79α, CD19, HLA class II, and combinations thereof.

104. The engineered cell of any one of paragraphs 17-0, wherein the engineered MSC comprises a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD45-, CD34-, CD14-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD11b-, CD79α-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD19-, HLA class II-; or a cellular marker phenotype comprising CD73+, CD90+, CD105+, and CD166+, CD11b-, CD14-, CD19-, CD34-, CD45-, and HLA-DR-.

105. The engineered cell of any one of paragraphs 17-0, wherein the engineered cell comprises a T cell.

106. The engineered cell of paragraph 105, wherein the T cell is a CD8+ T cell, a CD4+ T cell, a cytotoxic T lymphocyte (CTL), a viral-specific T cell, a gamma-delta T cell, or a T regulatory cell.

107. The engineered cell of any one of paragraphs 17-0, wherein the engineered cell comprises a NK cell.

108. The engineered cell of any one of paragraphs 17-0, wherein the engineered cell comprises a NKT cell.

109. The engineered cell of any one of paragraphs 17-0, wherein the engineered cell comprises a monocyte cell.

110. The engineered cell of any one of paragraphs 17-0, wherein the engineered cell comprises a macrophage.

111. The engineered cell of any one of paragraphs 17-0, wherein the engineered cell comprises a TIL.

112. The engineered cell of any one of paragraphs 17-111, wherein the exogenous polynucleotide sequence is integrated into the genome of the engineered cell.

5 113. The engineered cell of any one of paragraphs 17-0, wherein the exogenous polynucleotide sequence comprises one or more viral vector polynucleotide sequences.

114. The engineered cell of paragraph 0, wherein the one or more viral vector polynucleotide sequences comprise lentiviral, retroviral, retrotransposon, or adenoviral polynucleotide sequences.

10 115. The engineered cell of paragraph 0, wherein the one or more viral vector polynucleotide sequences comprise lentiviral polynucleotide sequences.

116. The engineered cell of any one of paragraphs 1-115, wherein the cell secretes each effector molecule.

117. The engineered cell of paragraph 116, wherein the first effector molecule is secreted at a
15 ratio that is 10 fold higher relative to secretion of the second effector molecule.

118. The engineered cell of any one of paragraphs 1-117, wherein the cell further comprises an antigen recognizing receptor.

119. The engineered cell of paragraph 118, wherein the antigen recognizing receptor recognizes an antigen selected from the group consisting of: 5T4, ADAM9, ADGRE2, AFP, AXL, B7-H3, B7-H4, B7-H6, C4.4, CA6, Cadherin 3, Cadherin 6, CCR1, CCR4, CD117, CD123, CD131, CD133, CD138, CD142, CD166, CD25, CD244, CD30, CD300LF, CD33, CD352, CD37, CD38, CD44, CD56, CD66e, CD70, CD71, CD74, CD79b, CD80, CD93, CEA, CEACAM5, Claudin18.2, CLEC12A, cMet, CSPG4, CTLA, DLK1, DLL3, DR5, EGFR, EMB, ENPP3, EpCAM, EphA2, Ephrin A4, ETBR, FGFR2, FGFR3, FRalpha, FRb, FLT3, GAPT, GCC, GD2, GFRa4, gpA33, GPC3, gpNBM, GPRC5, HER2, IL-1RAP, IL-13R, IL-13Ra, IL-13Ra2, IL-8, IL-15, IL1RAP, Integrin aV, KIT, L1CAM, LAMP1, LAT2, Lewis Y, LeY, LILRA2, LILRB2, LIV-1, LRRC, LY6E, MCSP, Mesothelin, MLC1, MS4A3, MUC1, MUC16, MUC1C, MYADM, NaPi2B, Nectin 4, NKG2D, NOTCH3, NY ESO 1, Ovarin, P-cadherin, pan-Erb2, PIEZO1, PRAM1, PSCA, PSMA, PTK7, ROR1, S Aures, SCT, SLAMF7, SLC22A16, SLC17A9, SLITRK6, SPNS3, SSTR2, STEAP1, Survivin, TDGF1, TIM1, TROP2, VSTM1, and WT1

120. The engineered cell of paragraph 118 or paragraph 119, wherein the antigen recognizing receptor comprises an antigen-binding domain.

121. The engineered cell of paragraph120, wherein the antigen-binding domain comprises an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb).

122. The engineered cell of paragraph120, wherein the antigen-binding domain comprises a single chain variable fragment (scFv).

123. The engineered cell of paragraph 122, wherein the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

124. The engineered cell of paragraph 123, wherein the VH and VL are separated by a peptide linker.

125. The engineered cell of paragraph 124, wherein the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

126. The engineered cell of any one of paragraphs 118-125, the antigen recognizing receptor
5 is a chimeric antigen receptor (CAR) or T cell receptor (TCR).

127. The engineered cell of any one of paragraphs 118-125, the antigen recognizing receptor is a chimeric antigen receptor (CAR).

128. The engineered cell of paragraph 127, wherein the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are
10 selected from the group consisting of: a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB
15 intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain.

20 129. The engineered cell of paragraph 127 or paragraph 128, wherein the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of: a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane
25 domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

130. The engineered cell of any one of paragraphs 127-129, wherein the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain.

131. A population of cells, the population of cells comprising any of the engineered cells of any one of paragraphs 1-130.

5 132. The population of cells of paragraph 19, wherein the population of cells is enriched for the engineered cells.

133. The population of cells of paragraph 19 or paragraph 132, wherein the first effector molecule, the second effector molecule, or the first and second effector molecules expressed in the engineered cells promotes increased growth, viability, or growth and
10 viability relative to cells in the population that do not express the first effector molecule, the second effector molecule, or the first and second effector molecules.

134. The population of cells of paragraph 133, wherein the first effector molecule is IL12 or an IL12p70 fusion protein.

135. The population of cells of paragraph 134, wherein the population of cells enriched for the
15 engineered cells express IL12 receptor β1 or increased levels thereof, IL12 receptor β2 or increased levels thereof, or IL12 receptor β1 and IL12 receptor β2 or increased levels thereof.

136. The population of cells of any of paragraphs 133-135, wherein the second effector molecule is IL21.

20 137. The population of cells of any of paragraphs 133-135, wherein the second effector molecule is CCL21.

138. The population of cells of paragraph 137, wherein the population of cells enriched for the engineered cells express a CCL21 receptor or increased levels thereof.

139. The population of cells of paragraph 138, wherein the CCL21 receptor is CCR7.

140. A method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the engineered cells of any one of paragraphs 1-0 or the population of cells of any of paragraphs 19-139.

5 141. A method of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the engineered cells of any one of paragraphs 1-0 or the population of cells of any of paragraphs 19-139.

142. A method of treating a subject having cancer, the method comprising administering to a
10 subject having a tumor a therapeutically effective dose of any of the engineered cell of any one of paragraphs 1-0 or the population of cells of any of paragraphs 19-139.

143. A method of reducing tumor volume in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the engineered cells of any one of paragraphs 1-0 or the population of cells of any of paragraphs 19-139.

15 144. The method of any one of paragraphs 140-143, wherein the engineered cell is derived from the subject.

145. The method of any one of paragraphs 140-143, wherein the engineered cell is allogeneic with reference to the subject.

146. The method of any one of paragraphs 140-145, wherein the tumor is selected from the
20 group consisting of: an adenocarcinoma, an acute myeloid leukemia (AML), an acute lymphoblastic B-cell leukemia (BALL), an acute lymphoblastic T-cell leukemia (TALL), a B-cell prolymphocytic leukemia, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a chronic lymphocytic leukemia, a chronic myeloid leukemia (CML), a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung
25 tumor, a lymphoma, a melanoma, a mesothelioma, a myelodysplasia, an ovarian tumor, a pancreatic tumor, a plasma cell myeloma, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

147. The method of any one of paragraphs 140-145, wherein the tumor is an ovarian tumor.

148. The method of any one of paragraphs 140-147, wherein the tumor is a tumor located in a peritoneal space.

149. An engineered cell comprising:

5 a) a promoter; and

b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising:

$$(L - S - E)_X$$

wherein

10 S comprises a polynucleotide sequence encoding a signal peptide,

E comprises a polynucleotide sequence encoding an effector molecule,

L comprises a linker polynucleotide sequence,

X = 2 to 20,

wherein the promoter is operably linked to the expression cassette, wherein for the first

15 iteration of the (L – S – E) unit L is absent, and wherein for each X the corresponding signal peptide is operably associated with the effector molecule, and

wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte,

20 macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

150. A population of cells comprising one or more engineered cells, wherein the one or more engineered cells comprise:

25 a) a promoter; and

b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein

5 S1 comprises a polynucleotide sequence encoding a first signal peptide,

E1 comprises a polynucleotide sequence encoding a first effector molecule,

L comprises a linker polynucleotide sequence,

S2 comprises a polynucleotide sequence encoding a second signal peptide,

E2 comprises a polynucleotide sequence encoding a second effector molecule, and

10 wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and

wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate 15 lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

151. A population of cells comprising one or more engineered cells, wherein the one or more 20 engineered cells comprise:

a) a promoter; and

b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

25 wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide,

E1 comprises a polynucleotide sequence encoding a first effector molecule,

L comprises a linker polynucleotide sequence,

S2 comprises a polynucleotide sequence encoding a second signal peptide,

E2 comprises a polynucleotide sequence encoding a second effector molecule, and

5 wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and

wherein the first effector molecule, the second effector molecule, or the first and second effector molecules expressed in the engineered cells promotes increased 10 growth, viability, or growth and viability relative to cells in the population that do not express the first effector molecule, the second effector molecule, or the first and second effector molecules, and

wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate 15 lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

152. The population of cells of paragraph 151, wherein the one or more engineered cells 20 express a cognate receptor or cognate receptor ligand for the first effector molecule, the second effector molecule, or the first and second effector molecules expressed in the engineered cells.

153. The population of cells of paragraph 151 or paragraph 152, wherein the first effector molecule is IL12 or an IL12p70 fusion protein.

25 154. The population of cells of any of paragraphs 151-153, wherein the second effector molecule is IL21.

155. The population of cells of any of paragraphs 151-153, wherein the second effector molecule is CCL21.

156. A population of cells comprising one or more engineered cells, wherein the one or more engineered cells comprise a construct, wherein the construct comprises:

5 a) an SFFV promoter; and

b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein

10 S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide;

E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein;

L comprises a linker polynucleotide sequence, wherein the linker polynucleotide
15 sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus;

S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide;

20 E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and

wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and

25 wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil,

monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

157. A population of cells comprising one or more engineered cells, wherein the one or more engineered cells comprise a construct, wherein the construct comprises:

a) an SFFV promoter; and

b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide;

E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein;

L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus;

S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide;

E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and

wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and

wherein the first effector molecule, the second effector molecule, or the first and second effector molecules expressed in the engineered cells promotes increased

growth, viability, or growth and viability relative to cells in the population that do not express the first effector molecule, the second effector molecule, or the first and second effector molecules, and

wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

158. The population of cells of paragraph 0 or paragraph 0, wherein the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144.

159. A method of producing a population of cells enriched for one or more receptors or receptor ligands, comprising culturing one or more cells under conditions where the one or more cells are contacted with a first effector molecule, a second effector molecule, or a first and a second effector molecule, wherein the contacted cells express one or more cognate receptors or cognate receptor ligands for the first effector molecule, the second effector molecule, or the first and second effector molecules, and wherein the first effector molecule, the second effector molecule, or the first and the second effector molecules increase growth, viability, or growth and viability of the contacted cells relative to cells cultured in the absence of the first effector molecule, the second effector molecule, or the first and second effector molecules.

160. The method of paragraph 0, wherein the first effector molecule, the second effector molecule, or the first and second effector molecules are heterologously expressed in one or more cells, and the one or more cells are contacted with the first effector molecule, the second effector molecule, or the first and second effector molecules in an autocrine manner.

161. The method of paragraph 0, wherein the first effector molecule, the second effector molecule, or the first and second effector molecules are expressed in one or more

additional cells, and the one or more cells are contacted with the first effector molecule, the second effector molecule, or the first and second effector molecules in an paracrine manner.

162. The method of paragraph 0, wherein the one or more additional cells are a feeder cells.

5 163. The method of paragraph 0, wherein the one or more cells are cultured in media.

164. The method of paragraph 0, wherein the one or more cells are contacted with the first effector molecule, the second effector molecule, or the first and second effector molecules by addition of a soluble first effector molecule, a soluble second effector molecule, or a soluble first and second effector molecules to the media.

10 165. The method of paragraph 0 or paragraph 0, wherein the soluble first effector molecule and/or soluble second effector molecule is a recombinant effector molecule.

166. The method of paragraph 0, wherein the one or more cells are cultured under adherent conditions.

167. The method of paragraph 0, wherein the one or more cells are adhered onto a surface.

15 168. The method of paragraph 0, wherein the adhered cells are contacted with the first effector molecule, the second effector molecule, or the first and second effector molecules by exposing the one or more cells to first effector molecule, the second effector molecule, or the first and second effector molecules is immobilized on the surface.

169. The method of any one of paragraphs 0-168, wherein the first effector molecule is IL12
20 or an IL12p70 fusion protein.

170. The method of paragraph 169, wherein the population of cells is enriched for IL12 receptor β1 (IL12Rβ1), enriched for IL12 receptor β2 (IL12Rβ2), or enriched for IL12Rβ1 and IL12Rβ2.

171. The method of paragraph 0, wherein the population of MSCs comprises a cellular marker phenotype comprising the cellular markers CD105+, CD73+, CD90+, IL12Rβ1+, and IL12Rβ2+.

172. The method of paragraph 0, wherein the cellular marker phenotype further comprises a phenotype lacking or substantially lacking one or more cellular markers selected from the group consisting of: CD45, CD34, CD14, CD11b, CD79α, CD19, HLA class II, and combinations thereof.

173. The method of paragraph 0, wherein the population of cells comprises a cell selected from the group consisting of: natural killer (NK) cells, NKT cells, innate lymphoid cells, mast cells, eosinophils, basophils, monocytes, macrophages, neutrophils, and dendritic cells, T cells, CD8+ T cells, CD4+ T cells, gamma-delta T cells, and T regulatory cells, and B cells.

174. The method of paragraph 173, wherein the population of cells comprises a T cell, a NK cell, a NKT cell, a monocyte, a macrophage, or a myeloid derived cell.

175. The method of any one of paragraphs 0-174, wherein the second effector molecule is IL21.

176. The method of any one of paragraphs 0-174, wherein the second effector molecule is CCL21.

177. The method of paragraph 176, wherein the population of cells is enriched for CCR7.

178. The method of paragraph 0, wherein the population of MSCs comprises a cellular marker phenotype comprising the cellular markers CD105+, CD73+, CD90+, IL12Rβ1+, IL12Rβ2+, and CCR7+.

179. The method of paragraph 0, wherein the cellular marker phenotype further comprises a phenotype lacking or substantially lacking one or more cellular markers selected from the group consisting of: CD45, CD34, CD14, CD11b, CD79α, CD19, HLA class II, and combinations thereof.

180. A population of cells enriched for one or more receptors or receptor ligands produced by the method of any one of paragraphs 0-0.

181. An exogenous polynucleotide sequence comprising a promoter and an expression cassette described in a formula, oriented from 5' to 3', comprising

5 **S1 – E1 – L – S2 – E2**

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide,

E1 comprises a polynucleotide sequence encoding a first effector molecule,

L comprises a linker polynucleotide sequence,

10 S2 comprises a polynucleotide sequence encoding a second signal peptide,

E2 comprises a polynucleotide sequence encoding a second effector molecule, and

wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule.

15 182. The exogenous polynucleotide sequence of paragraph 181, wherein the promoter comprises an exogenous promoter polynucleotide sequence.

183. The exogenous polynucleotide sequence of paragraph 181, wherein the promoter comprises an endogenous promoter.

184. The exogenous polynucleotide sequence of any one of paragraphs 181-183, wherein the
20 promoter is operably linked to the expression cassette such that the polynucleotides are capable of being transcribed as a single polynucleotide comprising the formula S1 – E1 – L – S2 – E2.

185. The exogenous polynucleotide sequence of paragraph 184, wherein the linker polynucleotide sequence is operably associated with the translation of the first effector
25 molecule and the second effector molecule as separate polypeptides.

186. The exogenous polynucleotide sequence of paragraph 185, wherein the linker polynucleotide sequence encodes a 2A ribosome skipping tag.

187. The exogenous polynucleotide sequence of paragraph 186, wherein the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

5 188. The exogenous polynucleotide sequence of paragraph 185, wherein the linker polynucleotide sequence encodes a T2A ribosome skipping tag.

189. The exogenous polynucleotide sequence of paragraph 185, the linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES).

190. The exogenous polynucleotide sequence of any one of paragraphs 185-189, wherein the
10 linker polynucleotide sequence encodes a cleavable polypeptide.

191. The exogenous polynucleotide sequence of paragraph 181, wherein the cleavable polypeptide comprises a Furin recognition polypeptide sequence.

192. The exogenous polynucleotide sequence of any one of paragraphs 185-189, wherein the linker polynucleotide sequence further encodes a Gly-Ser-Gly polypeptide sequence.

15 193. The exogenous polynucleotide sequence of any one of paragraphs 181-185, wherein the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus.

194. The exogenous polynucleotide sequence of any one of paragraphs 181-183, wherein the
20 linker polynucleotide sequence encodes a second promoter,

wherein the promoter is operably linked to the expression cassette such that a first polynucleotide comprising the formula S1 – E1 is capable of being transcribed,

wherein the second promoter is operably linked to the expression cassette such that a second polynucleotide comprising the formula S2 – E2 is capable of being transcribed,
25 and wherein the first and the second polynucleotide are separate polynucleotides.

195. The exogenous polynucleotide sequence of paragraph 181, wherein the promoter and the second promoter are identical.

196. The exogenous polynucleotide sequence of paragraph 181, wherein the promoter and the second promoter are different.

5 197. The exogenous polynucleotide sequence of any one of paragraphs 181-196, wherein the promoter and/or the second promoter comprises a constitutive promoter.

198. The exogenous polynucleotide sequence of paragraph 197, wherein the constitutive promoter is selected from the group consisting of: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78,
10 hGRP94, hHSP70, hKINb, and hUBIb.

199. The exogenous polynucleotide sequence of any one of paragraphs 181-196, wherein the promoter comprises an SFFV promoter.

200. The exogenous polynucleotide sequence of any one of paragraphs 181-196, wherein the promoter and/or the second promoter comprises an inducible promoter.

15 201. The exogenous polynucleotide sequence of paragraph 200, wherein the inducible promoter is selected from the group consisting of: minP, NFkB response element, CREB response element, NFAT response element, SRF response element 1, SRF response element 2, AP1 response element, TCF-LEF response element promoter fusion, Hypoxia responsive element, SMAD binding element, STAT3 binding site, inducer molecule
20 responsive promoters, and tandem repeats thereof.

202. The exogenous polynucleotide sequence of any one of paragraphs 181-201, wherein the first signal peptide or the second signal peptide comprises a native signal peptide native to the first effector molecule or the second effector molecule, respectively.

203. The exogenous polynucleotide sequence of any one of paragraphs 181-202, wherein the
25 first signal peptide or the second signal peptide comprises a non-native signal peptide non-native to the first effector molecule or the second effector molecule, respectively.

204. The exogenous polynucleotide sequence of paragraph 203, wherein the non-native signal peptide is selected from the group consisting of: IL12, IL2, optimized IL2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, and IL21.

205. The exogenous polynucleotide sequence of any one of paragraphs 181-204, wherein the first signal peptide and the second signal peptide are identical.

206. The exogenous polynucleotide sequence of any one of paragraphs 181-205, wherein the polynucleotide sequence encoding the first signal peptide comprises a codon optimized polynucleotide sequence.

207. The exogenous polynucleotide sequence of any one of paragraphs 181-206, wherein the first secretion polypeptide is a human IL12 signal peptide.

208. The exogenous polynucleotide sequence of any one of paragraphs 181-206, wherein the polynucleotide sequence encoding the second signal peptide comprises a codon optimized polynucleotide sequence.

209. The exogenous polynucleotide sequence of any one of paragraphs 181-208, wherein the second secretion polypeptide is a human IL21 signal peptide.

210. The exogenous polynucleotide sequence of any one of paragraphs 181-208, wherein the first effector molecule is selected from a therapeutic class, wherein the therapeutic class is selected from the group consisting of: a cytokine, a chemokine, a growth factor, a co-activation molecule, a tumor microenvironment modifier a, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.

211. The exogenous polynucleotide sequence of any one of paragraphs 181-210, wherein the second effector molecule is selected from a therapeutic class, wherein the therapeutic class is selected from the group consisting of: a cytokine, a chemokine, a growth factor, a co-activation molecule, a tumor microenvironment modifier, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.

212. The exogenous polynucleotide sequence of paragraph 211, wherein the therapeutic class of the first effector molecule and the second effector molecule are different.

213. The exogenous polynucleotide sequence of any one of paragraphs 181-212, wherein the first effector molecule and/or the second effector molecule is a modified effector molecule.

214. The exogenous polynucleotide sequence of paragraph 213, wherein the first effector molecule and/or the second effector molecule is modified to comprises a cell membrane tethering domain.

215. The exogenous polynucleotide sequence of paragraph 214, wherein the cell membrane tethering domain comprises a transmembrane-intracellular domain or a transmembrane domain.

216. The exogenous polynucleotide sequence of paragraph 214, wherein the cell membrane tethering domain comprises a cell surface receptor, or a cell membrane-bound portion thereof.

217. The exogenous polynucleotide sequence of paragraph 216, wherein the modified effector molecule is a fusion protein that comprises the cell surface receptor, or a cell membrane-bound portion thereof.

218. The exogenous polynucleotide sequence of any one of paragraphs 214-217, wherein the modified effector molecule further comprises a linker between the effector molecule and the cell membrane tethering domain.

219. The exogenous polynucleotide sequence of any one of paragraphs 213-218, wherein when expressed in a cell, the modified effector molecule is tethered to a cell membrane of the cell.

220. The exogenous polynucleotide sequence of any one of paragraphs 210-219 wherein the cytokine is selected from the group consisting of: IL12, IL7, IL21, IL18, IL15, Type I interferons, and Interferon-gamma.

221. The exogenous polynucleotide sequence of paragraph 220, wherein the IL12 cytokine is an IL12p70 fusion protein.

222. The exogenous polynucleotide sequence of any one of paragraphs 210-221, wherein the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, CXCL10-11 fusion, CCL19, CXCL9, and XCL1.

223. The exogenous polynucleotide sequence of any one of paragraphs 210-222, wherein the growth factor is selected from the group consisting of: Flt3L and GM-CSF.

224. The exogenous polynucleotide sequence of any one of paragraphs 210-223, wherein the co-activation molecule is selected from the group consisting of: 4-1BBL and CD40L.

225. The exogenous polynucleotide sequence of any one of paragraphs 210-224, wherein the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, TGFbeta inhibitors, immune checkpoint inhibitors, VEGF inhibitors, and HPGE2.

226. The exogenous polynucleotide sequence of paragraph 225, wherein the TGFbeta inhibitors are selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and combinations thereof.

227. The exogenous polynucleotide sequence of paragraph 225, wherein the immune checkpoint inhibitors comprise anti-PD-1 antibodies.

228. The exogenous polynucleotide sequence of paragraph 225, wherein the VEGF inhibitors comprise anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof.

229. The exogenous polynucleotide sequence of any one of paragraphs 181-225, wherein the first effector molecule and the second effector molecule are human-derived effector molecules.

230. The exogenous polynucleotide sequence of any one of paragraphs 181-229, wherein the first effector molecule comprises IL12.

231. The exogenous polynucleotide sequence of any one of paragraphs 181-229, wherein the first effector molecule comprises an IL12p70 fusion protein.

232. The exogenous polynucleotide sequence of paragraph 231, wherein the IL12p70 fusion protein is a human IL12p70 fusion protein.

5 233. The exogenous polynucleotide sequence of any one of paragraphs 230-232, wherein the second effector molecule comprises CCL21a.

234. The exogenous polynucleotide sequence of paragraph 233, wherein the CCL21a is a human CCL21a.

235. The exogenous polynucleotide sequence of any one of paragraphs 230-232, wherein the
10 second effector molecule comprises IL7.

236. The exogenous polynucleotide sequence of paragraph 235, wherein the IL7 is a human IL7.

237. The exogenous polynucleotide sequence of any one of paragraphs 230-232, wherein the second effector molecule comprises IL21.

15 238. The exogenous polynucleotide sequence of paragraph 237, wherein the IL21 is a human IL21.

239. The exogenous polynucleotide sequence of any one of paragraphs 181-238, wherein the expression cassette further comprises an E3 comprising a polynucleotide sequence encoding a third effector molecule.

20 240. The exogenous polynucleotide sequence of paragraph 239, wherein the third effector molecule comprises Flt3L.

241. The exogenous polynucleotide sequence of paragraph 239, wherein the third effector molecule comprises anti-PD1.

242. The exogenous polynucleotide sequence of paragraph 241, wherein the expression cassette further comprises an E4 comprising a polynucleotide sequence encoding a fourth effector molecule.

243. The exogenous polynucleotide sequence of paragraph 242, wherein the fourth effector
5 molecule comprises adenosine deaminase.

244. The exogenous polynucleotide sequence of paragraph 239, wherein the third effector molecule comprises adenosine deaminase.

245. The exogenous polynucleotide sequence of paragraph 239, wherein the third effector molecule comprises CD40L.

10 246. The exogenous polynucleotide sequence of paragraph 239, wherein the third effector molecule comprises a CXCL10-CXCL11 fusion protein.

247. The exogenous polynucleotide sequence of paragraph 239, wherein the third effector molecule comprises XCL1.

248. The exogenous polynucleotide sequence of paragraph 230, wherein the second effector
15 molecule comprises Flt3L.

249. The exogenous polynucleotide sequence of paragraph 230, wherein the second effector molecule comprises a CXCL10-CXCL11 fusion protein.

250. The exogenous polynucleotide sequence of paragraph 230, wherein the second effector molecule comprises anti-PD1.

20 251. The exogenous polynucleotide sequence of paragraph 230, wherein the second effector molecule comprises CD40L.

252. The exogenous polynucleotide sequence of any one of paragraphs 181-229, wherein the first effector molecule comprises interferon-beta and the second effector molecule comprises Flt3L.

253. The exogenous polynucleotide sequence of any one of paragraphs 181-252, wherein the polynucleotide sequence encoding the first effector molecule comprises a codon optimized polynucleotide sequence.

254. The exogenous polynucleotide sequence of any one of paragraphs 181-253, wherein the polynucleotide sequence encoding the second effector molecule comprises a codon optimized polynucleotide sequence.

255. The exogenous polynucleotide sequence of any one of paragraphs 181-254, wherein the exogenous polynucleotide sequence comprises the polynucleotide sequence shown in SEQ ID NO: 144.

256. An exogenous polynucleotide sequence comprising an SFFV promoter and an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide;

E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein;

L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus;

S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide;

E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and

wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule.

257. The exogenous polynucleotide sequence of paragraph 256, wherein the polynucleotide sequence comprises the polynucleotide sequence shown in SEQ ID NO: 144.

258. An exogenous polynucleotide sequence comprising an SFFV promoter and an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide;

E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein;

L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus;

S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide;

E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21;

wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule;

wherein the promoter is operably linked to the expression cassette such that the polynucleotides are capable of being transcribed as a single polynucleotide comprising the formula S1 – E1 – L – S2 – E2; and

wherein the polynucleotide sequence comprises the polynucleotide sequence shown in SEQ ID NO: 144.

259. The exogenous polynucleotide sequence of any one of paragraphs 181-258, wherein the exogenous polynucleotide sequence is encoded by a nucleic acid selected from the group consisting of: a DNA, a cDNA, an RNA, an mRNA, and a naked plasmid.

260. An expression vector comprising the exogenous polynucleotide sequence of any one of paragraphs 181-259.

261. The expression vector of paragraph 260, wherein the expression vector is a viral vector.

262. The expression vector of paragraph 261, wherein the viral vector is a lentiviral vector.

263. A composition comprising the exogenous polynucleotide sequence of any one of paragraphs 181-259, and a pharmaceutically acceptable carrier.

264. An isolated cell comprising the exogenous polynucleotide sequence of any one of paragraphs 181-259, the expression vector of any one of paragraphs 260-262, or the composition of paragraph 263.

265. The isolated cell of paragraph 264, wherein the isolated cell is selected from the group consisting of: a T cell, a CD8+ T cell, a CD4+ T cell, a gamma-delta T cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a viral-specific T cell, a Natural Killer T (NKT) cell, a Natural Killer (NK) cell, a B cell, a tumor-infiltrating lymphocyte (TIL), an innate lymphoid cell, a mast cell, an eosinophil, a basophil, a neutrophil, a myeloid cell, a macrophage, a monocyte, a dendritic cell, an erythrocyte, a platelet cell, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, an MSC, an induced pluripotent stem cell (iPSC), and an iPSC-derived cell.

266. The isolated cell of paragraph 264, wherein the isolated cell is an MSC.

267. The isolated cell of any one of paragraphs 264-266, wherein the exogenous polynucleotide sequence is integrated into the genome of the cell.

268. The isolated cell of any one of paragraphs 264-267, wherein the exogenous polynucleotide sequence comprises one or more viral vector polynucleotide sequences.

269. The isolated cell of paragraph 268, wherein the one or more viral vector polynucleotide sequences comprise lentiviral, retroviral, retrotransposon, or adenoviral polynucleotide sequences.

270. The isolated cell of paragraph 268, wherein the one or more viral vector polynucleotide sequences comprise lentiviral polynucleotide sequences.

271. The isolated cell of any one of paragraphs 264-270, wherein the engineered cell is HLA-typed with reference to a subject in need of therapeutic treatment.

272. The isolated cell of any one of paragraphs 264-271, wherein the engineered cell is a human cell.

273. The isolated cell of paragraph 272, wherein the human cell is an isolated cell from a subject.

274. The isolated cell of paragraph 273, wherein the isolated cell is isolated from a tissue consisting of the group of: bone marrow, adipose tissue, the umbilical cord, fetal liver, muscle, and lung tissue.

275. The isolated cell of any one of paragraphs 264-272, wherein the cell is a cultured cell.

276. The isolated cell of any one of paragraphs 264-275, wherein the cell comprises a cellular marker phenotype comprising the cellular markers CD105+, CD73+,and CD90+.

277. The isolated cell of paragraph 276, wherein the cellular marker phenotype further comprises a phenotype lacking or substantially lacking one or more cellular markers selected from the group consisting of: CD45, CD34, CD14, CD11b, CD79α, CD19, HLA class II, and combinations thereof.

278. The isolated cell of any one of paragraphs 264-275, wherein the cell comprises a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD45-, CD34-, CD14-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD11b-, CD79α-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD19-, HLA class II-;
5 or a cellular marker phenotype comprising CD73+, CD90+, CD105+, and CD166+, CD11b-, CD14-, CD19-, CD34-, CD45-, and HLA-DR-.

279. The isolated cell of any one of paragraphs 264-278, wherein the cellular marker phenotype further comprises a cellular marker comprising a cognate receptor or a cognate receptor ligand for the first effector molecule, the second effector molecule, or the first
10 and second effector molecules expressed in the cell.

280. The isolated cell of paragraph 279, wherein the receptor is selected from the group consisting of: IL12RB1, IL12RB2, CCL7, and combinations thereof.

281. The isolated cell of any one of paragraphs 264-280, wherein the cell secretes each effector molecule.

15 282. The isolated cell of paragraph 281, wherein the first effector molecule is secreted at a ratio that is 10 fold higher relative to secretion of the second effector molecule.

283. The isolated cell of any one of paragraphs 264-282, wherein the cell further comprises an antigen recognizing receptor.

284. The isolated cell of paragraph 283, wherein the antigen recognizing receptor comprises
20 an antigen-binding domain.

285. The isolated cell of paragraph 284, wherein the antigen-binding domain comprises an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb).

286. The isolated cell of paragraph 284, wherein the antigen-binding domain comprises a
25 single chain variable fragment (scFv).

287. The isolated cell of paragraph 286, wherein the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

288. The isolated cell of paragraph 287, wherein the VH and VL are separated by a peptide linker.

5 289. The isolated cell of paragraph 288, wherein the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

290. The isolated cell of any one of paragraphs 283-289, the antigen recognizing receptor is a chimeric antigen receptor (CAR) or T cell receptor (TCR).

10 291. The isolated cell of any one of paragraphs 283-289, the antigen recognizing receptor is a chimeric antigen receptor (CAR).

292. The isolated cell of paragraph 291, wherein the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of: a CD3zeta-chain intracellular signaling domain, a CD97
15 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40
20 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain.

293. The isolated cell of paragraph 291 or paragraph 292, wherein the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of: a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

294. The isolated cell of any one of paragraphs 291-293, wherein the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain.

295. A virus comprising the exogenous polynucleotide sequence of any one of paragraphs 181-259 or the expression vector of any one of paragraphs 260-262.

296. The virus of paragraph 295, wherein the virus is selected from the group consisting of: a lentivirus, a retrovirus, a retrotransposon, and an adenovirus.

297. The virus of paragraph 295, wherein the virus is a lentivirus.

298. A method of reducing tumor volume in a subject, the method comprising delivering to a subject having a tumor a composition comprising cells engineered to produce multiple effector molecules that modulate tumor-mediated immunosuppressive mechanisms, in an effective amount to reduce the volume of the tumor, wherein the engineered cells comprise:

a) a promoter; and

b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5’ to 3’, comprising

S1 – E1 – L – S2 – E2

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide,

E1 comprises a polynucleotide sequence encoding a first effector molecule,

L comprises a linker polynucleotide sequence,

S2 comprises a polynucleotide sequence encoding a second signal peptide,

E2 comprises a polynucleotide sequence encoding a second effector molecule, and

wherein the promoter is operably linked to the expression cassette, the first signal
5 peptide is operably linked to the first effector molecule, and the second signal
peptide is operably linked to the second effector molecule, and

wherein the engineered cell is selected from the group consisting of: a mesenchymal
stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate
lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil,
10 monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell,
CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T
cell, T regulatory cell, and B cell.

299. A method of reducing tumor volume in a subject, the method comprising delivering to a
subject having a tumor a composition comprising cells engineered to produce IL12 and
15 IL21, in an effective amount to reduce the volume of the tumor, wherein the engineered
cells comprise a construct, wherein the construct comprises:

a) an SFFV promoter; and

b) an exogenous polynucleotide sequence comprising an expression cassette described in
a formula, oriented from 5’ to 3’, comprising

20 **S1 – E1 – L – S2 – E2**

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the
first signal peptide is a human IL12 signal peptide;

E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein
25 the first effector molecule is a human IL12p70 fusion protein;

L comprises a linker polynucleotide sequence, wherein the linker polynucleotide
sequence encodes Furin recognition polypeptide sequence, a Gly-Ser-Gly

polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus;

S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide;

5 E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and

wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and

10 wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T 15 cell, T regulatory cell, and B cell.

300. The method of paragraph 0, wherein the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144.

301. The method of any one of paragraphs 298-**Error! Reference source not found.**, wherein the method further comprises administering a checkpoint inhibitor.

20 302. The method of paragraph 0, wherein the checkpoint inhibitor is an anti-PD-1 antibody, anti-PD-1L antibody or an anti-CTLA-4 antibody.

303. The method of any one of paragraphs 298-302, wherein the method further comprises administering an anti-CD40 antibody.

304. The method of any one of paragraphs 298-303, wherein the tumor is selected from the group consisting of: an adenocarcinoma, an acute myeloid leukemia (AML), an acute lymphoblastic B-cell leukemia (BALL), an acute lymphoblastic T-cell leukemia (TALL), a B-cell prolymphocytic leukemia, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a chronic lymphocytic leukemia, a chronic myeloid leukemia (CML), a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a lymphoma, a melanoma, a mesothelioma, a myelodysplasia, an ovarian tumor, a pancreatic tumor, a plasma cell myeloma, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

305. The method of any one of paragraphs 298-303, wherein the tumor is an ovarian tumor.

306. The method of any one of paragraphs 298-303, wherein the tumor is a tumor located in a peritoneal space.

307. The method of any one of paragraphs 298-306, wherein the administering comprises systemic administration, intraperitoneal administration, or intratumoral administration

308. The method of any one of paragraphs 298-307, wherein the volume of the tumor is reduced by at least 25% relative to a control, optionally wherein the control is an unmodified cell.

309. The method of paragraph 307, wherein the volume of the tumor is reduced by at least 50% relative to a control, optionally wherein the control is an unmodified cell.

310. The method of paragraph 309, wherein the volume of the tumor is reduced by at least 75% relative to a control, optionally wherein the control is an unmodified cell.

311. A method of reducing tumor volume in a subject, the method comprising delivering to a subject having a tumor a composition capable of engineering an cell to produce multiple effector molecules that modulate tumor-mediated immunosuppressive mechanisms, in an effective amount to reduce the volume of the tumor, wherein each engineered cell comprises:

a) a promoter; and

b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein

5 S1 comprises a polynucleotide sequence encoding a first signal peptide,

E1 comprises a polynucleotide sequence encoding a first effector molecule,

L comprises a linker polynucleotide sequence,

S2 comprises a polynucleotide sequence encoding a second signal peptide,

E2 comprises a polynucleotide sequence encoding a second effector molecule, and

10 wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and

wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate 15 lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell..

312. A method of reducing tumor volume in a subject, the method comprising delivering to a 20 subject having a tumor a composition capable of engineering a cell to produce IL12 and IL21, in an effective amount to reduce the volume of the tumor, wherein the engineered cell comprises a construct, wherein the construct comprises:

a) an SFFV promoter; and

b) an exogenous polynucleotide sequence comprising an expression cassette described in 25 a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide;

E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein;

5 L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus;

S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein
10 the second signal peptide is a human IL21 signal peptide;

E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and

wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal
15 peptide is operably linked to the second effector molecule, and

wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell,
20 CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell..

313. The method of paragraph 0, wherein the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144.

314. The method of any one of paragraphs 311-**Error! Reference source not found.**, wherein
25 the composition comprises a delivery system selected from the group consisting of: a viral system, a transposon system, and a nuclease genomic editing system.

315. The method of paragraph 0, wherein the viral system is selected from the group consisting of: a lentivirus, a retrovirus, a retrotransposon, and an adenovirus.

316. The method of paragraph 0, wherein the nuclease genomic editing system is selected from the group consisting of: a zinc-finger system, a TALEN system, and a CRISPR system.

317. The method of any one of paragraphs 311-316, wherein the tumor is selected from the group consisting of: an adenocarcinoma, an acute myeloid leukemia (AML), an acute lymphoblastic B-cell leukemia (BALL), an acute lymphoblastic T-cell leukemia (TALL), a B-cell prolymphocytic leukemia, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a chronic lymphocytic leukemia, a chronic myeloid leukemia (CML), a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a lymphoma, a melanoma, a mesothelioma, a myelodysplasia, an ovarian tumor, a pancreatic tumor, a plasma cell myeloma, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

318. The method of any one of paragraphs 311-317, wherein the administering comprises systemic administration, intraperitoneal administration, or intratumoral administration.

EXAMPLES

Example 1

This Example describes the *in vitro* characterization of MSCs with individual and combination immunotherapy payloads. Direct anti-cancer effects of immunotherapy-expressing MSCs on cancer cells are first measured. Next, the effects of immunotherapy-expressing MSCs on co-cultures with primary immune cells (focusing on T cells) and cancer cells are measured. The immuno-stimulatory properties of immunotherapy-expressing MSCs are rank-ordered based on inflammatory biomarker panels in both mouse and human cell systems. Immunotherapy-expressing MSCs that significantly enhance cancer cell killing either on their own or together with T cells are identified, and the top candidates to advance to *in vivo* testing are selected.

Methods: Immunotherapy-expressing MSCs are engineered to express the effector molecules listed in Table 1 are evaluated for their functional effects using *in vitro* models relevant to cancer therapy. Human ovarian cancer cells (e.g., OVCAR8 and SKOV3) and

human immune cells isolated from circulating PBMCs are used to test the hMSCs expressing hITs. Mouse ovarian cancer cells (e.g., ID8) and mouse immune cells are used to test the mMSCs expressing mITs.

Checkpoint inhibitors. Cell-binding assays are used to verify the activity of the expressed antibodies. The targets of the antibodies, CTLA4 and PD1, both negatively regulate T cells, but they are upregulated at different stages of T-cell activation (Boutros C, et al. (2016) *Nat Rev Clin Oncol* 13(8):473-486; Valsecchi ME (2015) *New Engl J Med* 373(13):1270-1270). CTLA4 is briefly upregulated in the priming phase, whereas PD1 is consistently expressed in the effector phase of T cell activation (Pardoll DM (2012) *Nat Rev Cancer* 12(4):252-264; Legat A, et al. (2013) *Front Immunol* 4:455). Anti-CTLA4 antibody binds to CTLA4 on the T-cell surface, blocking CTLA4 from shutting down T-cell activation in the early stage, and the human anti-PD1 antibody binds to PD1, preventing tumor cells from inhibiting T-cell activity.

T cells are isolated from PBMC by negative selection using EASYSEP™ magnetic bead (STEMCELL Technologies). The isolated T cells are activated by Human T-Activator CD3/28 Dynabeads (Thermo Fisher) and expression of CTLA-4 and PD-1 is monitored over 5 days to select for optimal timing of expression for each surface marker. On the appropriate days, conditioned media from the MSCs expressing antibodies for CTLA-4 or PD-1, or control conditioned media from non-expressing MSCs, are applied to the activated T cells to validate direct cell-surface-receptor binding of these antibodies. Fluorochrome-labeled secondary detection antibodies together with flow cytometry should confirm binding.

Chemokines. CCL21 chemokine functionality is confirmed using cell migration assays and isolated naive T cells, which express chemokine receptor CCR7 that is responsive to CCL21 chemotaxis. Specifically, CCL21-expressing or control MSCs are added to one compartment of a trans-well and then cell migration is assessed by isolated naive T cells from the other compartment, followed by enumeration of numbers of migrated T cells (Justus CR, et al. (2014) *J Vis Exp* (88)).

Cytokines. The activity of IL2, IL12, and IL15 is measured. ELISA assays specific to IL2, IL12, and IL15 are used to detect levels of these cytokines in MSC supernatants. Functional bioactivity assays employ the CTLL-2 cell line to assess of IL2 or IL15-mediated proliferation, or the NKG cell line to assess IL12-mediated IFN-gamma production by MSC supernatants.

Multiplexed cytokine profiling assays using LUMINEX® technology may also be used to assess cytokine expression and effects on immune cells.

STING pathway. STING pathway activation is measured with the constitutive STING mutant payload. Using LUMINEX® beads, the secretion of Type I interferons (e.g. IFN-alpha2 and IFN-beta) with expression of the STING mutant are profiled in MSCs.

Direct effects of immunotherapy-expressing MSCs on ovarian cancer cells. Any direct effects of MSCs on ovarian cancer cell growth and viability are tested *in vitro*. For example, mMSC or hMSC candidates are co-cultured with the mouse ovarian cancer cell line (ID8) or human ovarian cancer cell lines (OVCAR8 and SKOV3) and cancer cell cytotoxicity is measured by the well-characterized lactate dehydrogenase (LDH) assay. After 24 hours of co-culture, the supernatants are collected and measured for LDH levels correlated to cellular death via an enzymatic reaction that is subsequently quantified by specific absorbance on a plate reader. Additionally, cancer cell numbers are assessed by counting live versus dead cells by Trypan Blue exclusion and live versus apoptotic/dead cells by flow cytometric measurement using Annexin-V and propidium iodide staining.

Effects of immunotherapy-expressing MSCs on T cell and ovarian cancer cell co-culture systems. Tests determine whether immunotherapy-expressing MSCs can stimulate immune cells, such as T cells, to have improved anti-cancer activity against ovarian cancer cells *in vitro*. Specifically, mMSC-mIT candidates are co-cultured with mouse splenocytes and the ID8 cancer cell line, or hMSC-hIT candidates are co-cultured with human PBMCs and the OVCAR8 or SKOV3 cell lines. The co-culture assays entail using PBMCs/splenocytes with the ovarian cancer cells, with or without the MSCs, and stimulation with anti-CD3/28 beads. To assess cancer cell death, 16 hour killing assays are performed using techniques such as LDH cytotoxicity measurements, combining dye-labeled ovarian cancer cells with non-labeled effector PBMCs/splenocytes at fixed ratios and assaying killing by flow cytometry (Jedema I, et al. (2004) *Blood* 103(7):2677-2682), and apoptosis readouts by flow cytometry using Annexin-V with propidium iodide. T cell activation/proliferation is specifically assay by CFSE cell division at 3-5 days and cytokine production of IFN-gamma at 1-3 days.

An alternative strategy to generate T cells expressing CTLA-4 and PD1 is to activate with phytohaemagglutinin (PHA) to express the cell surface receptors PD1 and CTLA4. On Day 3, ~99% of the activated T cells should express PD1 while ~15% of them should express CTLA4

(Pardoll DM (2012) *Nat Rev Cancer* 12(4):252-264; Legat A, et al. (2013) *Front Immunol* 4:455). On Day 10, the activated T cells should be in the effector phase, when CTLA4 expression is downregulated but PD1 expression is maintained. Direct cell-surface-receptor binding of these antibodies is evaluated. On Day 3 and Day 10 post-induction, MSCs with the respective checkpoint inhibitor antibody expression constructs are applied to the T cell cultures. Labeled detection antibodies are used together with flow cytometry to confirm binding. Commercial antibodies are used as controls.

Example 2

This Example describes the *in vivo* characterization of MSCs expressing immunotherapy payloads in a syngeneic ovarian cancer model. The anti-tumor efficacy of immunotherapy-expressing MSCs is characterized using syngeneic mouse models of ovarian cancer (mMSC-mIT with mouse immune system). Tumor homing of engineered MSCs and expression of individual and combinatorial immunotherapies in a syngeneic ovarian mouse model are measured. Ovarian tumor burden and mouse survival with engineered MSC treatments are also measured. This Example should demonstrate selective homing of engineered MSCs to the TME and localized production of immunotherapy factors in ovarian tumors versus other body sites. This Example should also demonstrate significant reductions in tumor burden and extension of mouse survival with immunotherapy-expressing engineered MSCs.

Methods: The mouse ID8 cell line originated from spontaneous transformation of mouse ovarian epithelial surface cells (MOSE), is used to create a syngeneic ovarian tumor model (Roby KF, et al. (2000) Carcinogenesis 21(4):585-591). Derivatives of the ID8 cell line are also used (*e.g.*, ID8-VEGF (ID8-Defb29/Vegf-a), ID8-P53DN, ID8-P53KO- PTEN KO, ID8-P53KO- BRCA2 KO, ID8-P53KO-BRCA1 KO, ID8-PD53KO-Nf1KO). The ID8 cell line is infected with a lentivirus expressing Renilla luciferase (rLuc) to allow for *in vivo* bioluminescence imaging that is orthogonal to MSCs expressing Firefly luciferase (ffLuc). Successful rLuc expression is confirmed in ID8 *in vitro* prior to establishing the syngeneic ovarian cancer model in mice. For the syngeneic model, $5x10^5$ ID8 cells are injected into the peritoneal cavity of C57BL/6 mice between 6 to 8 weeks old (36, 54). MSCs are engineered as in Example 1, along with an ffLuc-expressing plasmid.

mMSC-mIT candidates are introduced into the syngeneic mouse model starting on day 25 (after tumor cell injection) at a dose of 10^6 MSC per animal once per week for 5 weeks (Dembinski JL, et al. (2013) *Cytotherapy* 15(1):20-32). The ovarian tumor load and mMSC-mIT candidates are visualized over time through rLuc and ffLuc bioluminescence imaging, respectively, as well as histological analyses following terminal time points. Mice are euthanized when they develop signs of distress, such as body-weight loss, ruffled fur, poor body posture, distended abdomen, and jaundice. Survival curves for the mice are measured. Distal metastasis of tumor cells is quantified by bioluminescence imaging (BLI) and by necropsy at time of euthanasia. Immune system profiling and activity is measured at different time points as biomarkers of response to the therapy.

To assess for variability in the expected anti-tumor effects of the MSCs, the dose of ID8 cells used to establish the model is varied (e.g., increase the number of cells to $5x10^6$), the dose of MSCs used is changed, and the time when MSCs are delivered after tumor establishment is modulated.

Even though mMSCs have been shown to home to ovarian tumors in mouse models, it is possible that some payloads disrupt this homing activity. In these instances, expression of these payloads may be engineered to be inducible. This can be achieved, for example, with a phloretin-inducible system (Gitzinger M, et al. (2009) *Proc Natl Acad Sci U S A* 106(26):10638-10643). Alternatively, the Dimerizer system may be used to link a synthetic zinc-finger DNA-binding domain with a transactivator domain using a small molecule (Clackson T, et al. (1998) *Proc Natl Acad Sci U S A* 95(18):10437-10442). Alternatively or additionally, inducible payload expression constructs that are triggered in the tumor microenvironment based on signals such as low O_2 may be constructed.

Lentiviral ffLuc constructs may also be used to infect MSCs.

Example 3

This Example describes the *in vivo* characterization of the efficacy of MSCs expressing immunotherapy payloads in xenograft models of human ovarian cancer in mice with human immune cells. The activity of engineered MSCs in human ovarian cancer models in immunodeficient mice that are engrafted with human immune cells via CD34+ cell transplants (hMSC-hIT with humanized immune system) is tested. Homing of engineered MSCs and

expression of individual and combinatorial immunotherapies in human xenograft ovarian tumors in mice with human immune cells are measured. Ovarian tumor burden and mouse survival with engineered MSC treatments are also tested. This Example should demonstrate elevated homing of engineered MSCs and localized production of immunotherapy factors into human xenograft ovarian tumors versus other body sites in mice. This Example should also demonstrate significant reductions in tumor burden and extension of mouse survival with immunotherapy-expressing engineered MSCs correlating with changes in the immune system composition.

Methods. To enable translation of engineered MSCs into human clinical trials, hMSC-hIT constructs are tested in humanized mouse models of human cancers. The effects of the immunotherapy-expressing hMSCs in mice are modeled by using xenografts of human ovarian cancer cell lines in immuno-deficient mice (NSG) engrafted with $CD34^+$ hematopoietic stem cells (HSCs).

For human ovarian cancer cells, OVCAR8 and SKOV3 cell lines are used. Similar assays as described in Example 3 are used to investigate tumor load and mouse survival over time.

Two alternative approaches may also be used. (1) Human T cells can be infused into the mice. (2) Human PBMCs can be infused into the mice.

Expression Vector: pL+MCS

ACGCGTGTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTT
ACAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGAAGTAAGGTGGTACGATCGTGCCTTATTA
GGAAGGCAACAGACGGGTCTGACATGGATTGGACGAACCACTGAATTGCCGCATTGCAGAGATATTG
TATTTAAGTGCCTAGCTCGATACAATAAACGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCT
CTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGT
GTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCT
CTAGCAGTGGCGCCCGAACAGGGACCTGAAAGCGAAAGGGAAACCAGAGCTCTCTCGACGCAGGACT
CGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTTGA
CTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGAT
CGCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATG
GGCAAGCAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGA
CAAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAATAC
AGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGGAAGCTTTAGACAAG
ATAGAGGAAGAGCAAAACAAAAGTAAGACCACCGCACAGCAAGCGGCCACTGATCTTCAGACCTGGA
GGAGGAGATATGAGGGACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCAT
TAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAG
GAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCCTCAATGACGCTGACG
GTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGC
GCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGG
AAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAACTCATTTGCACCACT
GCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGGAACAGATTGGAATCACACGACCTGGATG
GAGTGGGACAGAGAAATTAACAATTACACAAGCTTAATACACTCCTTAATTGAAGAATCGCAAAACC

AGCAAGAAAAGAATGAACAAGAATTATTGGAATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAA
CATAACAAATTGGCTGTGGTATATAAAATTATTCATAATGATAGTAGGAGGCTTGGTAGGTTTAAGAA
TAGTTTTTGCTGTACTTTCTATAGTGAATAGAGTTAGGCAGGGATATTCACCATTATCGTTTCAGACCC
ACCTCCCAACCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACA
5 GAGACAGATCCATTCGATTAGTGAACGGATCTCGACGGTATCGGTTAACTTTTAAAAGAAAAGGGGG
GATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAACTAAAGA
ATTACAAAAACAAATTACAAAAATCAAAATTTTATCTCGACATGGTGGCGACCGGTAGCGCTAGCGG
ATCGATAAGCTTGATATCGCCTGCAGCCGAATTCCTTGACTTGGGATCCGCGTCAAGTGGAGCAAGGC
AGGTGGACAGTCCTGCAGGCATGCGTGACTGACTGAGGCCGCGACTCTAGTTTAAACTGCGTGACTGA
10 CTCTAGAAGATCCGGCAGTGCGGCCGCGTCGACAATCAACCTCTGGATTACAAAATTTGTGAAAGATT
GACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCAT
GCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGG
AGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGT
TGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCG
15 GAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGT
GGTGTTGTCGGGGAAATCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGG
GACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGC
TCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCC
GCCTGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAG
20 GGGGGACTGGAAGGGCTAATTCACTCCCAACGAAAATAAGATCTGCTTTTTGCTTGTACTGGGTCTCT
CTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAAT
AAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCC
TCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTAGTAGTTCATGTCATCTTATTATTCAGTATT
TATAACTTGCAAAGAAATGAATATCAGAGAGTGAGAGGAACTTGTTTATTGCAGCTTATAATGGTTAC
25 AAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTG
TCCAAACTCATCAATGTATCTTATCATGTCTGGCTCTAGCTATCCCGCCCCTAACTCCGCCCAGTTCCG
CCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTG
AGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGACTTTTGCAGAGACGGCCCAAATTCG
TAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCC
30 GGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTC
ACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGA
GAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGC
TGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGC
AGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGC
35 GTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAA
ACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCG
ACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCA
CGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTT
CAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATC
40 GCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTC
TTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCC
AGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTT
TTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCT
ACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAA
45 GGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAA
ACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCA
TCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAG
TGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCG
GAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGG
50 GAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGT
GGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATG
ATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGG
CCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGAT
GCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGC
55 TCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGG
AAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCA

CTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAA
GGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTT
CAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAA
AATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTAT
5 TATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATG
ACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGG
GAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCG
GCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGA
GAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCG
10 GGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGC
CAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCAAGCTG (SEQ ID NO: 111)

Example 4. 4T1 Triple Negative Breast Carcinoma

In the following experiments, MSCs were engineered to express one of the following
15 effector molecules, then administered, alone or in combinations, to an orthotopic breast cancer mouse model: IFNβ, IFNγ, IL12, IL15, IL36γ, IL7, TRAIL, cGAS, CCL21a, OX40L, CD40L, or HACv-PD1. In some examples, a checkpoint inhibitor (anti-CD40, anti-PD1, or anti-CTLA-4 antibody) was injected in combination with administration with the engineered MSCs.

20 **MSC Homing**

The following experiments demonstrate that murine MSCs home to tumors in an orthotopic mouse model of breast cancer. Luciferase-expressing 4T1 breast tumor cells ($5x10^5$) were orthotopically implanted into the dorsal fat pad of female BALB/cJ mice mice. After 5 days, mice were intraperitoneally injected with 1 million fluorescently-labeled (with XenoLight
25 DiR (Caliper Life Sciences)) murine BM-derived MSCs (BM-MSCs, therapeutic cells). At days 1 and 7 after MSC injection, fluorescence analysis was used to determine MSC localization using the Ami HT live animal imager (Spectral Instruments). On day 7, tumor localization and size was determined through the 4T1 cell's luciferase bioluminescence reporter using the Ami HT imager. As shown in **FIG. 3**, the injected MSCs co-localized to the site of the tumor,
30 indicating that these cells do in fact specifically home *in vivo* to sites of 4T1 breast tumors. The injected MSCs home to tumors within one day and persist for over 7 days. In contrast, injected MSCs do not home to the dorsum in the absence of tumor in normal mice. These results suggest that MSCs can be used as a delivery vehicle for anti-cancer molecules, proteins or compounds.

To determine whether engineered human MSCs can home toward mouse tumors,
35 different lines of engineered human MSC expressing either GFP, IL2 or CCL21a were injected into BALB/c mice with 4T1 tumors. Efficacy was determined by tumor volume from caliper

measurement every other day. FIGs. 11A-11B show that human MSCs do not home to mouse 4T1 tumors.

In Vivo **Efficacy**

5 The following experiments demonstrate the *in vivo* efficacy of MSCs expressing immunotherapy effectors (payloads) in the orthotopic model of breast cancer. 4T1-Neo-Fluc mouse breast tumor cells (Imanis Life Sciences, $5x10^5$ cells) were implanted orthotopically into the dorsal fat pad of female BALB/cJ mice (The Jackson Laboratory). Mice were then randomized into the treatment groups 5 days after tumor implantation. Mice received 10 intraperitoneal injection of either control MSC growth media or engineered MSCs ($2x10^6$ cells) expressing different immunotherapy effectors (payloads) once a week for two weeks. Each immunotherapy was expressed by a different MSC, and MSCs were combined (1:1 ratio) for combinatorial treatment. Tumor growth was monitored by caliper measurements every other day, and mouse weights were recorded twice weekly. Mice were euthanized 14 days after first 15 MSC treatment and tissues were collected for further analysis.

FIG. 4 shows that tumor growth was delayed in mice treated with engineered MSCs expressed combinatorial genes IL-12 and CCL21a compared to controls treated with media.

FIGs. 5A-5C show that engineered MSCs that express single immunotherapy effectors (e.g., IFN-β, IFN-γ, IL-12 or CCL21a) inhibited growth of syngeneic 4T1 mouse tumors 20 compared to media-treated mice. Surprisingly, a synergistic effect on tumor growth was observed when the immunotherapy effectors were combined, particularly the combination of IL-12 and CCL21a, and the combination of IFN-β, IFN-γ, IL-12 and CCL21a (**FIGs. 5A-5C**).

FIGs. 6A-6B show that engineered MSCs expressing OX40L, TRAIL, IL15, cGAS, or combinations thereof do not inhibit tumor growth.

25 **FIGs. 7A-7B** show that engineered MSCs expressing IL-12 and CCL21a inhibit tumor growth; however the addition of anti-CD40 antibody does not reduce tumor growth.

FIGs. 8A-8B show that engineered MSCs expressing OX40L, TRAIL, IL15, HACvPD-1, or combinations thereof do not inhibit tumor growth significantly in a subcutaneous breast cancer model.

FIGs. 9A-9B show that engineered MSCs expressing IL-12 and CCL21a inhibit tumor growth; however the combination of MSCs expressing CCL21a, IL-36 gamma and IL-7 does not reduce tumor growth. Some of the effector combinations tested, however, may cause toxicity.

5 **Dose Escalation**

A dose escalation study was performed. This experiment determined that engineered MSC cell expression GFP does not elicit toxicity **(FIGs. 10A-10B)**.

Effect on Large Tumors

10 This experiment tested whether engineered mouse MSCs expressing IL12 and CCL21a can reduce tumor burden from larger tumor (>800mm^3). Larger tumor are more difficult to treat than small tumor, and this experiment demonstrates this effector combination can reduce tumor expansion **(FIGs. 12A-12B)**.

15 **Checkpoint Inhibitors**

FIG. 13A shows that engineered MSCs expressing IL-12 and CCL21 are sufficient to inhibit tumor growth, although the addition of a checkpoint inhibitor (anti-PD-1 antibody or anti-CTLA-4 antibody) by injection did not increase efficacy in a subcutaneous tumor model.

20 **Example 5. CT26 Colorectal Carcinoma**

In the following experiments, MSCs were engineered to express one of the following effector molecules, then administered, alone or in combinations, to a colorectal carcinoma mouse model: IFNβ, IL12, IL15, IL36γ, IL7, CCL21a, HACv-PD1, or 41BB. In some examples, a checkpoint inhibitor (anti-CD40 or anti-CTLA-4 antibody) was injected in combination with 25 administration with the engineered MSCs.

FIG. 14 shows that engineered MSCs expressing IL-12 and CCL21a induced significant tumor growth delay.

FIG. 15 shows tumor growth kinetics in the CT26 mouse model to determine optimal time for dosing the engineered MSC cells.

30 ***In Vivo* Efficacy**

The following experiments demonstrate the *in vivo* efficacy of MSCs expressing immunotherapy effectors (payloads) in the subcutaneous mouse model of colon (colorectal) cancer. CT26-Neo-Fluc mouse colon cancer cells (Imanis Life Sciences, 5 x 10^5) were injected subcutaneously into the flanks of female BALB/cJ mice (The Jackson Laboratory). Seven days after tumor implantation, mice were then randomized into the following treatment groups: control MSC growth media, engineered MSCs (MSC-12+CCL21a), anti-CD40 antibody, anti-CTLA4 antibody (Bio X cell), MSC-12+CCL21a in combination with anti-CD40 antibody or MSC-12+CCL21a in combination with anti-CTLA4 antibody. Engineered MSCs (2x10^6 cells) were injected intraperitoneally (ip) once a week for two weeks (Day 0 and 7). Anti-CD40 antibodies were injected ip (100 μg) on Days 0 and 3. Anti-CTLA4 antibodies were injected ip (100 μg) on Days 0, 3 and 7. Tumor growth was monitored by caliper measurements every other day, and mouse weights were recorded twice weekly. Mice were euthanized 11 days after first MSC treatment and tumors were collected and weighed. The tumor weight of individual mice in each treatment group was measured and the results are shown in the bottom left of **FIG. 16B** (left graph). The average tumor volume of each treatment group was monitored over time (**FIG. 16B**, right graph). Treatment Groups 2 (IL-12+CCL21a+anti-CTLA4 antibody), 4 (IL-12+CCL21a) and 7 (IL-12+CCL21a+anti-CD40 antibody) inhibited the average growth of CT26 colon tumors compared to GFP-treated mice (**FIG. 16B**, right graph). Similar results were observed when the tumor volume of individual mice in each treatment group was measured over time (**FIG. 16A**). Therefore, combinatorial treatment with MSCs expressing immunotherapies inhibited the growth of colon cancer cells *in vivo*.

FIG. 18A shows that engineered MSCs expressing IL-12, CCL21a, and either IL15 or HACvPD-1 inhibit tumor growth significantly in a moue model colorectal cancer. **FIG. 18B** shows the tumor weight for individual mice in each treatment. **FIG. 18C** is a representative graph of the infiltrating immune population within the tumor microenvironment. **FIG. 18D** shows the percentage of regulatory T cells (Treg) in the total CD3 population. There was a significant decrease in the numbers of Tregs in the tumor microenvironment treated with engineered MSC-IL2 and CCL21a. **FIG. 18E** correlates the percentage of immune infiltration with tumor weight. Samples with increase in lymphocytes (CD3+) were found to correlate with low tumor weight, while samples with high myeloid (CD11b+) infiltration were correlated with higher tumor burden.

Long-Term Survival

Mice were dosed twice with different concentration of engineered MSC-IL12 and CCL21a therapy in combination with injected anti-CD40 antibody. After the second dose, tumor volume was monitored twice a week until tumor burden is greater than 1500 mm^3 and the mice were sacrificed. **FIG. 17A** shows the tumor volume of the individual group. **FIG. 17B**, left graph, tracks the mice weight and tumor volume from individual group over time. **FIG. 17B**, right graph, shows the survival plot of the different groups.

MSC Efficacy

FIG. 20A shows the tumor volume for individual mice in each treatment. **FIG. 20B** shows the tumor weight for individual mice in each treatment. Efficacy was determined by tumor volume from caliper measurement every other day.

Tumor Growth Kinetics

FIGs. 21A-21B show the kinetics of CT26-LUC (luciferase) tumor growth in the intraperitoneal space. A CT26 cell line was injected at day 0 and three (3) mice were harvested at day 7, day 10, day 14, and day 18 to determine the kinetics of tumor growth. The first row of **FIG. 21A** measures the mice body weight and ROI with an IVIS imager to monitor tumor burden. The second row monitors the tumor weight and the ROI of the tumor of individual mice in each group. The third row correlates the tumor weight with either whole body ROI or tumor ROI. **FIG. 21B** shows the immune profile of three (3) mice in the day 18 group to better understand the tumor microenvironment.

Tumor Infiltrate Statistics/Immune Percentage/Tumor Weight Subcutaneous Mouse Model

FIG. 22A includes data indicating that engineered MSCs expressing IL-12 and CCL21a inhibit tumor growth in an subcutaneous mouse model of colorectal cancer; however the combination of MSCs expressing CCL21a and IL-36 gamma or IL-7 does not reduce tumor growth. **FIGs. 23A-23B** include the tumor immune infiltrate statistics. Three mice were selected from PBS, Naïve MSC, and MSC-IL12+MSC-CCL21a (combo) group to run flow

cytometry to immune profile tumor microenvironment. **FIG. 23A** shows a significant increase in infiltrating CD3 and CD8 cytotoxic T population in the combo group compared to the group dosed with naïve MSC. **FIG. 23B** shows a significant reduction in granulocytic myeloid-derived suppressor cells (gMDSCs) and macrophage population in the combo group compared to group treated with Naïve MSC.

FIGs. 24A-24B include data relating to immune percentage and tumor weight, showing that samples with more CD3+ and CD8+ T cells (top left and center graph) correlate strongly with a decrease in tumor weight. These figures also show that samples with fewer CD11b myeloid cells, including macrophage, dendritic cells, and MDSC, display lower tumor burden (lower center and right graph of **FIG. 24A** and upper row of **FIG. 24B**).

Orthotopic Mouse Model

FIG. 26A shows that engineered MSCs expressing IL-12 and CCL21a, or CCL21a and IFN-β, inhibit tumor growth in an orthotopic mouse model of colorectal cancer; however the combination of MSCs expressing CCL21a and s41BBL does not reduce tumor growth. Each effector was expressed by a different MSC, and the MSCs were combined (at a 1:1 ratio) for combinatorial treatment. Each chart shows the effect of engineered MSCs expressing the indicated immunotherapies alone or in combination on the growth of 4T1 breast tumors in mice (n = 6-8). Each line of **FIG. 26A** represents an individual mouse. **FIG. 26B** shows the tumor weight for individual mice in each treatment. MSC-IL12 + MSC-CCL21a shows best efficacy compared to mice injected with naïve MSC. Treatment efficacy was also observed in the group treated with MSC-IFNb + MSC-CCL21a.

FIGs. 27A-27B are graphs that show immune profiles of each group treated with indicated engineered MSC. A consistent decrease in macrophage population was observed after treating with MSC-IL12 + MSC-CCL21a (**FIG. 27A**). A general trend of increased infiltration in CD3+ population and decreased infiltration in CD11b+ population was also observed when compared to group treated with MSC-IL12 + MSC-CCL21a against naïve MSC (**FIG. 27A** and **FIG. 27B**).

FIG. 28A-28B show the correlation of immune infiltration with tumor weight. Samples with low macrophage and dendritic cells have lower tumor burden (**FIG. 28B**, top center and top right). **FIG. 28C** shows the average tumor weight from each group. Statistical significance was

observed with both MSC-IL12 + MSC-CCL21a, or MSC-IFNb + MSC-CCL21a compared with naïve MSC.

FIG. 29 shows graphs combining the *in vivo* data from the colorectal cancer models above (**FIG. 22A** and **FIG. 26A**). The combined CT26 data from **FIG. 22A** and **FIG. 26A** capture three groups: tumor only (PBS), treated with naïve MSC, and treated with MSC-IL12 + MSC-CCL21a.

FIGs. 30A-30C also show combined data from **FIG. 22A** and **FIG. 26A**. The graphs show the average number of immune infiltration from the flow cytometry experiment data. Statistical significance was observed in CD8+T from **FIG. 30A**, demonstrating the ability of MSC-IL12 + MSC-CCL21a to repolarize tumor microenvironment and allow more cytotoxic T cell infiltration. Furthermore, there was a reduction in CD11b+ myeloid population infiltration in the groups that were treated by MSC-IL12 + MSC-CCL21a (**FIG. 30B**). The data collected using dendritic cells and the macrophage population was statistical significance.

IL12 and CCL21a Therapy in Intraperitoneal and Subcutaneous Mouse Models of Colorectal Cancer

FIGs. 25A-25B include data from MSC-IL-12+CCL21a therapy in intraperitoneal and subcutaneous colorectal cancer mouse models. Three different lots of a lentiviral transduced line was tested for MSC-IL12 and CCL21a (TLOO8-3/4, TL019-01/02, and TL022-01/02; each TL number represents one lot). **FIG. 25A** shows that all three lots of MSC-IL12 + MSC-CCL21a can reduce tumor burden in both subcutaneous and intraperitoneal model (first 5 graphs are from the SC model and last 3 are from the IP model). Tumors from all mice were collected on day 11. **FIG. 25B** shows the average tumor weight from each group.

Example 6. MSC Combination Cytokine Therapy Methods

The following methods were used in experiments, as indicated.

Methods:

MSC Culturing

Bone-marrow derived C57BL/6 and Balb/C murine MSCs (mMSCs) were purchased from Cyagen (Cat. No. MUBMX-01001 and MUCMX-01001, respectively). mMSC culturing

media was composed of : MEM Corning Cat # 10-022-CV (500ml) + MSC FBS Gibco Cat #12662-029 (final conc 10%) + L-Glut (200mM) Stem cell 07100 (Final conc 2mM) + PenStrep 100X VWR Cat # 97063-708 (Final conc 1X) + murine FGF Peprotech Cat# 450-33-100uG (Final conc-1:10,000 dilution). TrypLE Express was purchased (ThermoFisher - #12604021).
5 PBS did not contain magnesium, calcium, or phenol red.

mMSCs were passaged according to the protocol below:

1. mMSCs should be passaged at 70-90% confluency.
2. Aspirate media from dish/flask.
3. Rinse plate with PBS (e.g. 2 mL for 10cm dish, 3ml for 15cm dish).
10 4. Add TrypLE Express (e.g. 2 mL for 10cm dish, 3ml for 15cm dish)
5. Incubate for 3-4 minutes at 37degrees.
6. Knock plate on side to dislodge cells. Confirm by microscopy that most cells have been dislodged.
7. Wash cells off plate using media (e.g. 8mL for 10 cm dish).
15 8. Place cells in 15 conical and centrifuge 400Xg for 5 min.
9. Aspirate media.
10. Resuspend cells in appropriate media and plate cells into fresh plates/flasks. Note: 70% confluent cells can be
split 1:3. 90% confluent cells can be split 1:4. Alternatively, cells can be plated at
20 3000-5000 cells/cm2.

Bone-marrow derived human MSCs were purchased (RoosterBank-hBM-1M-XF, RoosterBio). Various hMSC culturing media were purchased: Xeno-free hMSC media - (RoosterBio - #KT-016); +FBS (serum-containing) hMSC media (Lonza - MSCGM media - #PT-
25 3001). TrypLE Express was purchased (ThermoFisher - #12604021). PBS did not contain magnesium, calcium, or phenol red.

hMSCs were passaged according to the exemplary protocol below:

1. hMSCs should be passaged at 70-90% confluency.
2. Aspirate media from dish/flask.
30 3. Rinse plate with PBS (e.g. 2 mL for 10cm dish).
4. Add TrypLE Express (e.g. 2 mL for 10cm dish)

5. Incubate for 3-4 minutes at 37degrees or 5 minutes RT.
6. Knock plate on side to dislodge cells. Confirm by microscopy that most cells have been dislodged.
7. Wash cells off plate using Lonza MSCGM media (e.g. 8mL for 10 cm dish).
5 8. Place cells in 15 conical and centrifuge 400Xg for 5 min.
9. Aspirate media.
10. Resuspend cells in Rooster xeno-free media and plate cells into fresh plates/flasks. Note: 70% confluent cells can be split 1:3. 90% confluent cells can be split 1:4. Alternatively, cells can be plated at 3000-5000 cells/cm2.

10

hMSCs were thawed according to the exemplary protocol below:
1. Pre-warm hMSC media to 37°.
2. Remove hMSC aliquot from liquid nitrogen.
3. Thaw by holding the tube 1/2 submerged in 37° bath for 60-90 seconds, until 2/3 of
15 the frozen sample has thawed.
4. Wipe the tube with 70% ethanol to sterilize tube.
5. Add 0.5 mL media to the cryotube, gently pipette 2-3 times, and then transfer cells into 9 mL media (10 mL total) in 15 mL conical tube.
6. Centrifuge 400Xg for 5 min.
20 7. Aspirate media, and then gently resuspend pellet in appropriate volume of Rooster xeno-free media. Plate cells at a concentration of 3000-5000 cells/cm2.

Lentiviral Production

Lentivirus was produced using: Lenti-X 293T packaging cell line (Clontech, Cat# 632180); LX293T Complete growth medium, without antibiotics; DMEM, hi-glucose; 1mM
25 Sodium Pyruvate; 10% FBS, heat-inactivated; Opti-Mem I Reduced Serum Media (Gibco/Thermo Fisher; Cat# 31985); FuGene HD (Promega, Cat#E2311); Envelope, Packaging, and Transfer Vector plasmids; VSV-G-pseudotyped envelope vector (pMD2.G); Packaging vector that contains Gag, Pol, Rev, and Tat that can be used with 2nd and 3rd generation transfer vectors (psMAX2). 293T(FT) cells from 90% confluent 10cm dishes were lifted and dispensed
30 at 1:3 dilution late in the afternoon the day before transfection and incubated cells as normal

overnight at 37°C, 5% CO2 (cells should be 60-85% confluent the next day at time of transfection).

A transfection reaction was prepped for each 10cm dish according to the protocol below:

1. Prep transfection reaction for each 10cm dish in a separate 1.7mL tube.
2. Add 900uL Opti-Mem I at RT.
3. Add 9ug vector backbone (containing gene of interest) per reaction.
4. Add 8ug packaging vector per reaction.
5. Add 1ug envelope vector per reaction (pMD2.G).
6. Mix thoroughly by quickly vortexing for 3 seconds.
7. Add 55uL Fugene HD per reaction.
8. Mix by quickly pipetting up and down 20-30 times.
9. Let sit at RT for 10 min (allowing DNA complexes to form).
10. Slowly add mixture in dropwise manner around the dish, then mix by gently rocking back-forth and up-down for 5-10 seconds (do not swirl).
11. Place dish into virus incubator.

Viral supernatants were harvested on days 2 and 3 using a serological pipette. Cellular debris was removed using a Millipore steriflip 0.45um filters. A Lenti-X Concentrator (Cat. Nos. 631231 & 631232) was used according to the protocol: 1) Combine 1 volume of Lenti-X Concentrator with 3 volumes of clarified supernatant. Mix by gentle inversion; 2) Incubate mixture on ice or at 4°C for 30 minutes to overnight; (3) Centrifuge sample at 1,500 x g for 45 minutes at 4°C; (4) Carefully remove and discard supernatant, taking care not to disturb the pellet; (5) Gently resuspend the pellet in 1/10 to 1/100th of the original volume using sterile PBS + 0.1% BSA.

Vectors

Cytokine expression cassettes were cloned into a pL17D, the vector map of which is shown in **Fig. 31** with salient features annotated; *e.g.*, a SFFV promoter; a FLAG and MYC epitope tag; LTRs, etc.

Lentiviral Transduction

Murine MSCs were seeded in 6-well plates and infected when cells were 50% confluent. Virus was added at the appropriate MOI and incubated for 3 hours to transduce cells. Following infection, fresh media was added to the cells.

5 Human MSCs were transduced following the exemplary protocol below:

1. 200,000 human MSCs were plated in each well of 6-well plate, in 2mL xeno-free human MSC media.
2. After 2 hours, the media was removed and replaced with 1mL of PBS.
3. Appropriate amount of virus was added to each well, as indicate by MOI below, and
10 cells were incubated with virus for 3 hours with occasional rocking, at 37 degrees and 5% CO2.
4. Virus was removed after 3 hours, plates were washed with media, and then the MSCs were cultured normally (as noted above) until cells reached senescence. Cells were counted at each passage, so that total cell numbers could be determined.

15

Example 7: MSC Combination Cytokine Therapy (CT26)

In the following example, balb/c mMSCs were engineered to express various cytokines using the lentiviral transduction method described in Example 6.

CT26 tumor cells ($5x10^4$ cells in 100μl) modified to constitutively express luciferase
20 enzyme (Cat no: CL043, Lot no: CL-IM147 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent balb/c (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. Mice were randomized into treatment groups and treated with intraperitoneally delivered mMSCs ($1x10^6$) expressing effector molecules as single agent or as a combination of mMSCs to
25 deliver a combination of agents. MSC-Flag-Myc and PBS were used as a negative control. Tumor burden was assessed at day 12 and 17. Bioluminescent signal (photons/second) was normalized for each individual mouse relative to the initial signal (pre-treatment). Reduction of BLI signal by more than 100 fold (0.01) was equivalent to a complete cure (no tumor was evident at the time of necropsy). As shown in **Fig. 32**, MSCs engineered to express different
30 effector molecules either alone or in combination demonstrated efficacy in reducing CT26 tumor burden in an IP tumor model as assessed by BLI levels.

Example 8: MSC Combination Cytokine Therapy (B16F10)

In the following example, C57BL/6 mMSCs were engineered to express various cytokines using the lentiviral transduction method described in Example 6.

B16F10 tumor cells ($5x10^4$ cells in 100μl) modified to constitutively express luciferase enzyme (B16F10-Fluc-Puro Cat#:CL052, lot#: CL-IM150 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent C57BL/6 (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. Mice were randomized into treatment groups and treated with intraperitoneally delivered mMSCs ($1x10^6$) expressing effector molecules as single agent or as a combination of mMSCs to deliver a combination of agents. MSC-Flag-Myc and PBS were used as a negative control. Tumor burden was assessed at day 12 and 17. Bioluminescent signal (photons/second) was normalized for each individual mouse relative to the initial signal (pre-treatment). Reduction of BLI signal by more than 100 fold (0.01) was equivalent to a complete cure (no tumor was evident at the time of necropsy). As shown in **Fig. 33**, MSCs engineered to express different effector molecules either alone or in combination demonstrated efficacy in reducing B16F10 tumor burden in an IP tumor model as assessed by BLI levels.

Example 9: Engineered Human MSC Cytokine Production

In the following example, bone-marrow derived hMSCs (derived from 3 human volunteer healthy donors) were engineered to express human IL12 (p70) and human CCL21a from a single lentiviral expression vector using the lentiviral transduction method described in Example 6. The lentiviral expression vector (schematic vector map of which is shown in **Fig. 34)** used a 2A ribosome skipping elements to express both cytokines from a single transcript.

As shown in **Fig. 35**, engineered hMSCs were able to produce both hIL12 **(Fig. 35A)** and hCCL21a **(Fig. 35B)**, as assessed by cytokine ELISA. Notably, protein secretion was correlated with the amount of viral particles (MOI) used during the transduction of MSCs.

Example 10: Engineered Human MSC Functional Assessment

In the following example, bone-marrow derived hMSCs were engineered to express human IL12 (p70) using the lentiviral transduction method described in Example 6. Engineered hMSCs were co-cultured into 0.4μm transwell inserts with human T-cells isolated from healthy blood donors (a schematic representation of the transwell assay is shown in **Fig. 36A**). To assess IL12 induced Th1 polarization on activated naïve T-cells, IFNγ production by T-cells was

measured by ELISA on the supernatant collected from the lower compartment (T-cells). As shown in **Fig. 36B**, IFNγ production was increased in a MOI dose-dependent manner by co-culturing CD3 T-cells with hMSCs expressing IL12p70.

Example 11: MSCs Home to Tumors in an IP Model

5 In the following example, balb/c MSCs ($2x10^6$ cells) expressing fLUC were injected IP into CT-26 IP tumor-bearing mice. Mice were euthanized and tissues were collected 24 hours after injection. As shown in **Fig. 37**, fLUC-MSCs were significantly enriched in the tumors as detected by bioluminescence imaging (images shown in **Fig. 37A**, quantification of images in **Fig. 37B**), quantitative real time PCR (**Fig. 37C**), and fluorescence microscopy against firefly 10 luciferase (**Fig. 37D**).

Additionally, C57Bl/6 mice were implanted with $5x10^4$ B16F10-fLUC cells IP. 7 days after tumor implantation, 1 $x10^6$ C57Bl/6 murine BM-MSCs engineered to express Nanoluc-EGFP were injected IP. Mice were euthanized at 24 hours post injection of MSCs and peritoneal organs (stomach, kidney, liver, colon, spleen, pancreas, omentum/tumor, ovaries and Fallopian 15 tubes) were imaged ex-vivo for nanoluc signaling (NanoGlo Substrate Kit ,Vendor: Promega, Catalog No.: N1110). As shown in **Fig. 37E**, murine MSC nanoluc signal was preferentially enriched in the tumor compared to the other organs in the peritoneal cavity in a B16F10 tumor model.

Example 12: IL12 Producing MSCs Reduce CT26 Tumor Burden in an IP Model

20 In the following example, balb/c mMSCs were engineered to express murine IL12p70 using the lentiviral transduction method described in Example 6.

CT26 tumor cells ($5x10^4$ cells in 100µl) modified to constitutively express luciferase enzyme (Cat no: CL043, Lot no: CL-IM147 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent balb/c (age 6-8 weeks). One week after tumor 25 implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. Mice were randomized into treatment groups and treated with intraperitoneally delivered mMSCs ($1x10^6$ cells) expressing IL12p70. MSC-Flag-Myc and PBS were used as a negative control. As shown in **Fig. 38**, IL12p70 expressing MSCs led to reduction in tumor burden as assessed by BLI (top panels and bottom left panel) and a complete elimination of detectable 30 intraperitoneal tumors by tumor weight (bottom right panel) in a CT26 model.

Example 13: IL12 Producing MSCs Reduce B16F10 Tumor Burden in an IP Model

In the following example, C57BL/6 mMSCs were engineered to express murine IL12p70 using the lentiviral transduction method described in Example 6.

B16F10 tumor cells ($5x10^4$ cells in 100μl) modified to constitutively express luciferase
5 enzyme (B16F10-Fluc-Puro Cat#:CL052, lot#: CL-IM150 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent C57BL/6 (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. Mice were randomized into treatment groups and treated with intraperitoneally delivered mMSCs $1x10^6$ expressing IL12p70. MSC-Flag-Myc and PBS were used as a negative control.
10 As shown in **Fig. 39**, IL12p70 expressing MSCs led to reduction in tumor burden as assessed by BLI (top panels and bottom left panel) and a complete elimination of detectable intraperitoneal tumors by tumor weight (bottom right panel) in a B16F10 model.

Example 14: MSCs Producing IL12 and CCL21a Reduce Tumor Burden and Prolong Survival in a CT26 IP Tumor Model

15 In the following example, balb/c mMSCs were engineered to express murine IL12 (p70) and murine CCL21a from a single lentiviral expression vector. The lentiviral expression vector used a 2A ribosome skipping elements to express both cytokines from a single transcript using the lentiviral transduction method described in Example 6.

CT26 tumor cells ($1x10^6$ cells) modified to constitutively express luciferase enzyme (Cat
20 no: CL043, Lot no: CL-IM147 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent balb/c mice (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. Mice were randomized into treatment groups and treated with intraperitoneally delivered mMSCs $1x10^6$ expressing IL12p70 and CCL21a by the same MSC ("MSC-IL-12p70_2A_CCL21a"). MSC-Flag-Myc and
25 PBS were used as a negative control. As shown in **Fig. 40**, IL12p70/CCL21a expressing MSCs led to reduction in tumor burden as assessed by BLI (top panels and bottom left panel) and a complete elimination of detectable intraperitoneal tumors by tumor weight (bottom right panel) in a CT26 model. **Fig. 40A** demonstrates the mean tumor burden as assessed by BLI for PBS treated (circle), MSC-Flag-Myc ("Naïve MSC" square), and IL12p70/CCL21a expressing MSCs
30 (triangle). **Fig. 40B** demonstrates the tumor burden in individual mice as assessed by BLI for PBS treated, MSC-Flag-Myc ("Naïve MSC"), and IL12p70/CCL21a expressing MSCs (left,

middle, and right panels, respectively). Notably, as shown in **Fig. 40C**, treatment with IL12p70/CCL21a expressing MSCs led to prolonged survival (100% survival greater than 90 days), while control treated mice all died or were euthanized by Day 20.

Example 15: MSCs Producing IL12 and IL21 Reduce Tumor Burden and Prolong Survival in a B16F10 IP Tumor Model

In the following example, C57BL/6 mMSCs were engineered to express murine IL12 (p70) or murine IL21 (*i.e.*, each MSC engineered to express only a single agent) using the lentiviral transduction method described in Example 6.

B16F10 tumor cells ($5x10^4$ cells in 100μl) modified to constitutively express luciferase enzyme (B16F10-Fluc-Puro Cat#:CL052, lot#: CL-IM150 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent C57BL/6 (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. Mice were randomized into treatment groups and treated with intraperitoneally delivered mMSCs ($1x10^6$ cells) expressing IL12p70 in combination with mMSCs ($1x10^6$ cells) expressing IL21, or mMSCs ($1x10^6$ cells) expressing IL12p70 alone. MSC-Flag-Myc and PBS were used as a negative control. As shown in **Fig. 41**, treatment with IL12p70 expressing MSCs led to prolonged survival relative to control treated mice but all mice still all died or were euthanized by Day 50. In contrast, treatment with IL12p70 expressing MSCs in combination with IL21 expressing MSCs led to prolonged survival relative to treatment with IL12p70 expressing MSCs (60% survival past 60 days). Thus, IL21 expression by MSCs enhanced the efficacy of IL12p70 expressing MSCs.

Example 16: Allogeneic MSCs Producing IL12 and CCL21a Reduce Tumor Burden and Prolong Survival in a CT26 IP Tumor Model

In the following example, balb/c mMSCs (syngeneic) and C57BL/6 mMSCs (allogeneic) were engineered to express murine IL12 (p70) and murine CCL21a from a single lentiviral expression vector. The lentiviral expression vector used a 2A ribosome skipping elements to express both cytokines from a single transcript using the lentiviral transduction method described in Example 6.

CT26 tumor cells ($1x10^6$ cells) modified to constitutively express luciferase enzyme (Cat no: CL043, Lot no: CL-IM147 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent balb/c mice (age 6-8 weeks). One week after tumor implantation, tumor

burden was measured by luciferase imaging (BLI) using an AMI imager. Mice were randomized into treatment groups and treated with intraperitoneally delivered mMSCs ($1x10^6$ cells) expressing IL12p70 and CCL21a by the same MSC ("MSC-IL12+CCL21"). Both balb/c control mMSCs (syngeneic) and C57BL/6 control mMSCs (allogeneic) were engineered to express
5 MSC-Flag-Myc ("Naïve"). PBS was also used as a negative control. As shown in **Fig. 1**, both syngeneic and allogeneic MSCs expressing IL12p70/CCL21a led to reduction in tumor burden as assessed by BLI in a CT26 model, while control treatments did not. Additionally, mice that were previously treated with mMSCs expressing IL12p70 and CCL21a in both syngeneic and allogeneic models and were determined to be tumor free for 90 days were subsequently
10 challenged with CT26 tumor cells ($0.5x10^6$ cells in 100μl PBS) implanted subcutaneously in the thigh, as schematized in **Fig. 2A**. As shown in **Fig. 2B**, tumor free mice rejected the tumor implant in contrast to naïve control mice where the tumor became established. Thus, treatment with MSCs expressing IL12p70/CCL21a led to prolonged tumor burden reduction as well as immunological memory.

15 **Example 17: MSCs Producing IL12 and CCL21a Demonstrate Enhanced Growth Relative to Unmodifed Cells**

In the following example, human MSCs from 3 different donors were engineered at different multiplicity of infections (MOIs) to express and secrete human IL-12 and human CCL21a from a single lentiviral expression vector. The lentiviral expression vector used a 2A
20 ribosome skipping elements to express both cytokines from a single transcript using the lentiviral transduction method described in Example 6.

As shown in **Fig. 42**, the genetically engineered MSCs (MOI=95000, 9500, or 950) exhibited enhanced cell expansion and growth compared to the non-genetically engineered human MSCs (MOI=0) in the three donors tested (**Fig. 42A**, Donor 1; **Fig. 42B**, Donor 2; **Fig.**
25 **42C**, Donor 3). Human MSCs genetically engineered with lentivirus to express GFP did not show a similar enhanced cell expansion or growth phenotype (data not shown).

Example 18: Selection of Promoter for Sustained Protein Expression in Human Bone-marrow MSCs (BM-MSCs)

In the following example, various promoters were tested for driving expression of a
30 reporter EGFP construct in human MSCs. Promoters tested were CMV, SFFV, EF1a, EF1a-LTR, EFS, MND, PGK, UbC (*see* Table 4). Cells were transduced using equivalent MOI

(multiplicity of infection) using the lentiviral transduction method described in Example. EGFP percentage and Median Fluorescence Intensity (MFI) were quantified over serial passages using flow cytometry.

As shown in **Fig. 43**, two independent human BM-MSC cell lines from 2 different donors (top and bottom row, respectively were engineered and percent GFP (left panels) and MFI (right panels) of engineered cells was assessed at day 25 post transduction. The SFFV promoter demonstrated GFP expression in both cell lines by both GFP percentage and MFI.

As shown in **Fig. 44**, EGFP MFI was tracked over time (day 7 to day 28 post-transduction) for either the two independent human BM-MSC cell lines individually (left panel) or with data from the two independent human BM-MSC cell lines combined (right panel). Protein expression was stable over time during more than 28 days. Additionally, in comparison to EF1a promoters, SFFV promoter consistently drove almost ten-fold more protein expression as quantified by MFI.

Example 19: Engineering Human MSCs to Produce IL12 and IL21

In the following example, human bone-marrow MSCs were stably transduced to express IL12p70 and IL21 from various constructs using the lentiviral transduction method described in Example 6. Cells were expanded for 3 to 4 passages post-transduction and $0.2x10^6$ cells were seeded in 6-well plates in 4mL of media. Conditioned media was collected after 24 hours and ELISAs were performed to determine the IL-12 and IL-21 concentrations produced.

Various constructs were tested with different combinations and/or arrangements of promoter – signal sequence 1 – cytokine 1- 2A linker – signal sequence 2 – cytokine 2. The combinations tested are described below in **Table 7**. Specific details of construct SB00880 are presented below in **Table 8**.

Table 7 – IL-12 and IL-21 Expression Constructs

Construct Name (SB#)	Promoter	Insert	Backbone	Codon Optimization
SB00743	SFFV_1	IL12ss-IL12	pL23d	None
SB00763	EFa1 (pEF6)	IL12ss-IL12-fT2A*-IL21ss-IL21	pL40g	None
SB00765	EFa1 (pEF6)	IL12ss-IL12-fT2A-IL12ss-IL21	pL40g	None
SB00766	EFa1 (pEF6)	IL12ss-IL12-fT2A-IL8ss-IL21	pL40g	None
SB00767	EF1a (pEF6)	IL12ss-IL12-fT2A-IL21	pL40g	None
SB00768	EFa1 (pEF6)	IL21ss-IL21-fT2A-IL12ss-IL12	pL40g	None
SB00769	EFa1 (pEF6)	IL12ss-IL21-fT2A-IL12ss-IL12	pL40g	None
SB00770	EFa1 (pEF6)	IL6ss-IL21-fT2A-IL12ss-IL12	pL40g	None

SB00771	EF1a (pEF6)	IL8ss-IL21-fT2A-IL12ss-IL12	pL40g	None
SB00772	EF1a (pEF6)	IL21ss-IL21-fT2A-IL12	pL40g	None
SB00773	EF1a (pEF6)	IL12ss-IL21-fT2A-IL12	pL40g	None
SB00774	EF1a (pEF6)	IL6ss-IL21-fT2A-IL12	pL40g	None
SB00775	EF1a (pEF6)	IL8ss-IL21-fT2A-IL12	pL40g	None
SB00772	EF1a (pEF6)	IL21ss-IL21-fT2A-IL12	pL40g	None
SB00620	SFFV_1	IL2ss-IL21	pL17d	None
SB00838	SFFV_1	IL12ss-IL12-fT2A-IL21ss-IL21	pL41g	None
SB00839	SFFV_1	IL12ss-IL12-fT2A-IL8ss-IL21	pL41g	None
SB00840	SFFV_1	IL12ss-IL12-fT2A-IL21	pL41g	None
SB00841	SFFV_1	IL21ss-IL21-fT2A-IL12ss-IL12	pL41g	None
SB00843	SFFV_1	IL21ss-IL21-fT2A-IL12	pL41g	None
SB00844	SFFV_1	IL8ss-IL21-fT2A-IL12	pL41g	None
SB00868	SFFV_1	IL12ss-IL12	pL41g	Yes
SB00870	EF1a (pEF6)	IL12ss-IL12-fT2A-IL21ss-IL21	pL40g	Yes
SB00872	EF1a (pEF6)	IL12ss-IL12-fT2A-IL21	pL40g	Yes
SB00869	EF1a (pEF6)	IL21ss-IL21-fT2A-IL12ss-IL12	pL40g	Yes
SB00871	EF1a (pEF6)	IL21ss-IL21-fT2A-IL12	pL40g	Yes
SB00879	SFFV_1	IL21ss-IL21-fT2A-IL12ss-IL12	pL41g	Yes
SB00880	SFFV_1	IL12ss-IL12-fT2A-IL21ss-IL21	pL41g	Yes
SB00881	SFFV_1	IL21ss-IL21-fT2A-IL12	pL41g	Yes
SB00882	SFFV_1	IL12ss-IL12-fT2A-IL21	pL41g	Yes
SB00862	SFFV_1	IL21ss-IL21	pL23d	None
SB00863	SFFV_1	IL2ss-IL21	pL41g	Yes
SB00968	SFFV_1	IL2ss-IL21-fT2A-IL12ss-IL12	pL41g	Yes
SB00969	SFFV_1	IL8ss-IL21-fT2A-IL12ss-IL12	pL41g	Yes
SB00970	SFFV_1	IL12ss-IL12-fT2A-IL2ss-IL21	pL41g	Yes
SB00971	SFFV_1	IL12ss-IL12-fT2A-IL8ss-IL21	pL41g	Yes
SB00862+SB00743	SFFV_1	Co-transduction IL12 + IL21	pL41g	None
SB00868+863	SFFV_1	Co-transduction IL12+IL21 (IL2ssIL21)	pL41g	Yes

* fT2A refers to Furin-T2A

Table 8 – SB00880 Expression Construct Sequences

SFFV promoter (SEQ ID NO: 17)
GTAACGCCATTTTGCAAGGCATGGAAAAATACCAAACCAAGAATAGAGAAGTTCAGATCAAGGGC GGGTACATGAAAATAGCTAACGTTGGGCCAAACAGGATATCTGCGGTGAGCAGTTTCGGCCCCGGC CCGGGGCCAAGAACAGATGGTCACCGCAGTTTCGGCCCCGGCCCGAGGCCAAGAACAGATGGTCCC CAGATATGGCCCAACCCTCAGCAGTTTCTTAAGACCCATCAGATGTTTCCAGGCTCCCCCAAGGACC TGAAATGACCCTGCGCCTTATTTGAATTAACCAATCAGCCTGCTTCTCGCTTCTGTTCGCGCGCTTCT GCTTCCCGAGCTCTATAAAAGAGCTCACAACCCCTCACTCGGCGCGCCAGTCCTCCGACAGACTGA GTCGCCCGGG
Human IL-12 signal sequence; codon optimized (nucleic acid) (SEQ ID NO: 32)
ATGTGCCATCAGCAACTCGTCATCTCCTGGTTCTCCCTTGTGTTCCTCGCTTCCCCTCTGGTCGCC
Human IL-12 signal sequence (amino acid) (SEQ ID NO: 112)
MCHQQLVISWFSLVFLASPLVA
Human IL-12 protein without signal sequence; codon optimized (nucleic acid) (SEQ ID NO: 136)
ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAGCTGGATTGGTACCCGGACGCCCCTGGAGAA ATGGTCGTGCTGACTTGCGATACGCCAGAAGAGGACGGCATAACCTGGACCCTGGATCAGAGCTCC GAGGTGCTCGGAAGCGGAAAGACCCTGACCATTCAAGTCAAGGAGTTCGGCGACGCGGGCCAGTA CACTTGCCACAAGGGTGGCGAAGTGCTGTCCCACTCCCTGCTGCTGCTGCACAAGAAAGAGGATGG AATCTGGTCCACTGACATCCTCAAGGACCAAAAAGAACCGAAGAACAAGACCTTCCTCCGCTGCGA AGCCAAGAACTACAGCGGTCGGTTCACCTGTTGGTGGCTGACGACAATCTCCACCGACCTGACTTTC

TCCGTGAAGTCGTCACGGGGATCAAGCGATCCTCAGGGCGTGACCTGTGGAGCCGCCACTCTGTCC
GCCGAGAGAGTCAGGGGAGACAACAAGGAATATGAGTACTCCGTGGAATGCCAGGAGGACAGCGC
CTGCCCTGCCGCGGAAGAGTCCCTGCCTATCGAGGTCATGGTCGATGCCGTGCATAAGCTGAAATA
CGAGAACTACACTTCCTCCTTCTTTATCCGCGACATCATCAAGCCTGACCCCCCCAAGAACTTGCAG
CTGAAGCCACTCAAGAACTCCCGCCAAGTGGAAGTGTCTTGGGAATATCCAGACACTTGGAGCACC
CCGCACTCATACTTCTCGCTCACTTTCTGTGTGCAAGTGCAGGGAAAGTCCAAACGGGAGAAGAAA
GACCGGGTGTTCACCGACAAAACCTCCGCCACTGTGATTTGTCGGAAGAACGCGTCAATCAGCGTC
CGGGCGCAGGATAGATACTACTCGTCCTCCTGGAGCGAATGGGCCAGCGTGCCTTGTTCCGGTGGC
GGATCAGGCGGAGGTTCAGGAGGAGGCTCCGGAGGAGGTTCCCGGAACCTCCCTGTGGCAACCCCC
GACCCTGGAATGTTCCCGTGCCTACACCACTCCCAAAACCTCCTGAGGGCTGTGTCGAACATGTTGC
AGAAGGCCCGCCAGACCCTTGAGTTCTACCCCTGCACCTCGGAAGAAATTGATCACGAGGACATCA
CCAAGGACAAGACCTCGACCGTGGAAGCCTGCCTGCCGCTGGAACTGACCAAGAACGAATCGTGTC
TGAACTCCCGCGAGACAAGCTTTATCACTAACGGCAGCTGCCTGGCGTCGAGAAAGACCTCATTCA
TGATGGCGCTCTGTCTTTCCTCGATCTACGAAGATCTGAAGATGTATCAGGTCGAGTTCAAGACCAT
GAACGCCAAGCTGCTCATGGACCCGAAGCGGCAGATCTTCCTGGACCAGAATATGCTCGCCGTGAT
TGATGAACTGATGCAGGCCCTGAATTTCAACTCCGAGACTGTGCCTCAAAAGTCCAGCCTGGAAGA
ACCGGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGTTGCACGCTTTCCGCATTCGAGCCGTG
ACCATTGACCGCGTGATGTCCTACCTGAACGCCAGT

Human IL-12 protein without signal sequence (amino acid) (SEQ ID NO: 137); p35 subunit in bold; p40 subunit in italics

*IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGG
EVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGV
TCGAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQL
KPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSS
WSEWASVPCS*GGGSGGGSGGGSGGGS**RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFY
PCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYED
LKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKL
CILLHAFRIRAVTIDRVMSYLNAS**

Human IL-12 protein with signal sequence (amino acid) (SEQ ID NO: 138) ; p35 subunit in bold; p40 subunit in italics

MCHQQLVISWFSLVFLASPLVA*IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGS
GKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCW
WLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKL
KYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTD
KTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS*GGGSGGGSGGGSGGGS**RNLPVATPDPGMFPCLHHS
QNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNG
SCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNF
NSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS**

Furin-T2A Linker (nucleic acid) (SEQ ID NO: 139)

AGACGGAAACGCGGAAGCGGAGAGGGCAGAGGCTCGCTGCTTACATGCGGGGACGTGGAAGAGAA
CCCCGGTCCG

Furin-T2A Linker (amino acid) (SEQ ID NO: 140)

RRKRGSGEGRGSLLTCGDVEENPGP

Human IL-21 signal sequence; codon optimized (nucleic acid) (SEQ ID NO: 55)

ATGGAACGCATTGTGATCTGCCTGATGGTCATCTTCCTGGGCACCTTAGTGCACAAGTCGAGCAGC

Human IL-21 signal sequence (amino acid) (SEQ ID NO: 135)

MERIVICLMVIFLGTLVHKSSS

Human IL-21 protein without signal sequence; codon optimized (nucleic acid) (SEQ ID NO: 141)

CAGGGACAGGACAGGCACATGATTAGAATGCGCCAGCTCATCGATATCGTGGACCAGTTGAAGAAC
TACGTGAACGACCTGGTGCCCGAGTTCCTGCCGGCCCCCGAAGATGTGGAAACCAATTGCGAATGG
TCGGCATTTTCCTGCTTTCAAAAGGCACAGCTCAAGTCCGCTAACACCGGGAACAACGAACGGATC
ATCAACGTGTCCATCAAAAAGCTGAAGCGGAAGCCTCCCTCCACCAACGCCGGACGGAGGCAGAA
GCATAGGCTGACTTGCCCGTCATGCGACTCCTACGAGAAGAAGCCGCCGAAGGAGTTCCTGGAGCG
GTTCAAGTCGCTCCTGCAAAAGATGATTCATCAGCACCTGTCCTCCCGGACTCATGGGTCTGAGGAT
TCA

Human IL-21 protein without signal sequence (amino acid) (SEQ ID NO: 142)
QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQLKSANTGNNERIINV SIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDS
Human IL-21 protein with signal sequence (amino acid) (SEQ ID NO: 143)
MERIVICLMVIFLGTLVHKSSSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFS CFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMI HQHLSSRTHGSEDS
SB00880 Cassette (SFFV-IL12ss-IL12-fT2A-IL21ss-IL21) (SEQ ID NO: 144)
GTAACGCCATTTTGCAAGGCATGGAAAAATACCAAACCAAGAATAGAGAAGTTCAGATCAAGGGC GGGTACATGAAAATAGCTAACGTTGGGCCAAACAGGATATCTGCGGTGAGCAGTTTCGGCCCCGGC CCGGGGCCAAGAACAGATGGTCACCGCAGTTTCGGCCCCGGCCCGAGGCCAAGAACAGATGGTCCC CAGATATGGCCCAACCCTCAGCAGTTTCTTAAGACCCATCAGATGTTTCCAGGCTCCCCCAAGGACC TGAAATGACCCTGCGCCTTATTTGAATTAACCAATCAGCCTGCTTCTCGCTTCTGTTCGCGCGCTTCT GCTTCCCGAGCTCTATAAAAGAGCTCACAACCCCTCACTCGGCGCGCCAGTCCTCCGACAGACTGA GTCGCCCGGGGGATCCGCGGAATTCGCCGCCACCATGTGCCATCAGCAACTCGTCATCTCCTGGTTC TCCCTTGTGTTCCTCGCTTCCCCTCTGGTCGCCATTTGGGAACTGAAGAAGGACGTCTACGTGGTCG AGCTGGATTGGTACCCGGACGCCCCTGGAGAAATGGTCGTGCTGACTTGCGATACGCCAGAAGAGG ACGGCATAACCTGGACCCTGGATCAGAGCTCCGAGGTGCTCGGAAGCGGAAAGACCCTGACCATTC AAGTCAAGGAGTTCGGCGACGCGGGCCAGTACACTTGCCACAAGGGTGGCGAAGTGCTGTCCCACT CCCTGCTGCTGCTGCACAAGAAAGAGGATGGAATCTGGTCCACTGACATCCTCAAGGACCAAAAAG AACCGAAGAACAAGACCTTCCTCCGCTGCGAAGCCAAGAACTACAGCGGTCGGTTCACCTGTTGGT GGCTGACGACAATCTCCACCGACCTGACTTTCTCCGTGAAGTCGTCACGGGGATCAAGCGATCCTCA GGGCGTGACCTGTGGAGCCGCCACTCTGTCCGCCGAGAGAGTCAGGGGAGACAACAAGGAATATG AGTACTCCGTGGAATGCCAGGAGGACAGCGCCTGCCCTGCCGCGGAAGAGTCCCTGCCTATCGAGG TCATGGTCGATGCCGTGCATAAGCTGAAATACGAGAACTACACTTCCTCCTTCTTTATCCGCGACAT CATCAAGCCTGACCCCCCCAAGAACTTGCAGCTGAAGCCACTCAAGAACTCCCGCCAAGTGGAAGT GTCTTGGGAATATCCAGACACTTGGAGCACCCCGCACTCATACTTCTCGCTCACTTTCTGTGTGCAA GTGCAGGGAAAGTCCAAACGGGAGAAGAAAGACCGGGTGTTCACCGACAAAACCTCCGCCACTGT GATTTGTCGGAAGAACGCGTCAATCAGCGTCCGGGCGCAGGATAGATACTACTCGTCCTCCTGGAG CGAATGGGCCAGCGTGCCTTGTTCCGGTGGCGGATCAGGCGGAGGTTCAGGAGGAGGCTCCGGAGG AGGTTCCCGGAACCTCCCTGTGGCAACCCCCGACCCTGGAATGTTCCCGTGCCTACACCACTCCCAA AACCTCCTGAGGGCTGTGTCGAACATGTTGCAGAAGGCCCGCCAGACCCTTGAGTTCTACCCCTGCA CCTCGGAAGAAATTGATCACGAGGACATCACCAAGGACAAGACCTCGACCGTGGAAGCCTGCCTGC CGCTGGAACTGACCAAGAACGAATCGTGTCTGAACTCCCGCGAGACAAGCTTTATCACTAACGGCA GCTGCCTGGCGTCGAGAAAGACCTCATTCATGATGGCGCTCTGTCTTTCCTCGATCTACGAAGATCT GAAGATGTATCAGGTCGAGTTCAAGACCATGAACGCCAAGCTGCTCATGGACCCGAAGCGGCAGAT CTTCCTGGACCAGAATATGCTCGCCGTGATTGATGAACTGATGCAGGCCCTGAATTTCAACTCCGAG ACTGTGCCTCAAAAGTCCAGCCTGGAAGAACCGGACTTCTACAAGACCAAGATCAAGCTGTGCATC CTGTTGCACGCTTTCCGCATTCGAGCCGTGACCATTGACCGCGTGATGTCCTACCTGAACGCCAGTA GACGGAAACGCGGAAGCGGAGAGGGCAGAGGCTCGCTGCTTACATGCGGGGACGTGGAAGAGAAC CCCGGTCCGATGGAACGCATTGTGATCTGCCTGATGGTCATCTTCCTGGGCACCTTAGTGCACAAGT CGAGCAGCCAGGGACAGGACAGGCACATGATTAGAATGCGCCAGCTCATCGATATCGTGGACCAGT TGAAGAACTACGTGAACGACCTGGTGCCCGAGTTCCTGCCGGCCCCCGAAGATGTGGAAACCAATT GCGAATGGTCGGCATTTTCCTGCTTTCAAAAGGCACAGCTCAAGTCCGCTAACACCGGGAACAACG AACGGATCATCAACGTGTCCATCAAAAAGCTGAAGCGGAAGCCTCCCTCCACCAACGCCGGACGGA GGCAGAAGCATAGGCTGACTTGCCCGTCATGCGACTCCTACGAGAAGAAGCCGCCGAAGGAGTTCC TGGAGCGGTTCAAGTCGCTCCTGCAAAAGATGATTCATCAGCACCTGTCCTCCCGGACTCATGGGTC TGAGGATTCATGA
SB00880 Full Vector (PL41g + SB00880 Cassette) (SEQ ID NO: 145)
TGACTCCTGCGCAGTCCAAAAAAAAAGGCTCCAAAAGGAGCCTTTAATTGTATCGGTGGGCCCTTA GAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTT TGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCC TGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAA TAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGCTTAT GCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAAC CAAACCGTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACA

ATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACC
TGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCAT
GCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTT
AGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTG
GCGCATCGGGCTTCCCATACAATCGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCA
TTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGAGCAAGACGTTTCCCGT
TGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTCATGATGA
TATATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACACAACGTGGTTTAAACAAATAGTC
AAAAGCCTCCGGCGACTAGTCGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAA
CTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACG
TATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAA
CTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGG
TAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATC
TACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGC
GGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCA
AAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCG
TGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGGGTCTCTCTGGTTAGACCAGA
TTTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTG
AGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTT
TAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACCTGAAAGCGAAAGGGAAACCA
GAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCG
ACTGCAGAGTACGCCAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGT
CAGTATTAAGCGGGGGAAAATAGCGGCCGCCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTA
CAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAACTAAAGAATTACAAAAAC
AAATTACAAAAATTCAAATTTTCGGGGGATCCGTAACGCCATTTTGCAAGGCATGGAAAAATACCA
AACCAAGAATAGAGAAGTTCAGATCAAGGGCGGGTACATGAAAATAGCTAACGTTGGGCCAAACA
GGATATCTGCGGTGAGCAGTTTCGGCCCCGGCCCGGGGCCAAGAACAGATGGTCACCGCAGTTTCG
GCCCCGGCCCGAGGCCAAGAACAGATGGTCCCCAGATATGGCCCAACCCTCAGCAGTTTCTTAAGA
CCCATCAGATGTTTCCAGGCTCCCCCAAGGACCTGAAATGACCCTGCGCCTTATTTGAATTAACCAA
TCAGCCTGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTTCCCGAGCTCTATAAAAGAGCTCACAACCCC
TCACTCGGCGCGCCAGTCCTCCGACAGACTGAGTCGCCCGGGGGATCCGCGGAATTCGCCGCCACC
ATGTGCCATCAGCAACTCGTCATCTCCTGGTTCTCCCTTGTGTTCCTCGCTTCCCCTCTGGTCGCCAT
TTGGGAACTGAAGAAGGACGTCTACGTGGTCGAGCTGGATTGGTACCCGGACGCCCCTGGAGAAAT
GGTCGTGCTGACTTGCGATACGCCAGAAGAGGACGGCATAACCTGGACCCTGGATCAGAGCTCCGA
GGTGCTCGGAAGCGGAAAGACCCTGACCATTCAAGTCAAGGAGTTCGGCGACGCGGGCCAGTACA
CTTGCCACAAGGGTGGCGAAGTGCTGTCCCACTCCCTGCTGCTGCTGCACAAGAAAGAGGATGGAA
TCTGGTCCACTGACATCCTCAAGGACCAAAAAGAACCGAAGAACAAGACCTTCCTCCGCTGCGAAG
CCAAGAACTACAGCGGTCGGTTCACCTGTTGGTGGCTGACGACAATCTCCACCGACCTGACTTTCTC
CGTGAAGTCGTCACGGGGATCAAGCGATCCTCAGGGCGTGACCTGTGGAGCCGCCACTCTGTCCGC
CGAGAGAGTCAGGGGAGACAACAAGGAATATGAGTACTCCGTGGAATGCCAGGAGGACAGCGCCT
GCCCTGCCGCGGAAGAGTCCCTGCCTATCGAGGTCATGGTCGATGCCGTGCATAAGCTGAAATACG
AGAACTACACTTCCTCCTTCTTTATCCGCGACATCATCAAGCCTGACCCCCCCAAGAACTTGCAGCT
GAAGCCACTCAAGAACTCCCGCCAAGTGGAAGTGTCTTGGGAATATCCAGACACTTGGAGCACCCC
GCACTCATACTTCTCGCTCACTTTCTGTGTGCAAGTGCAGGGAAAGTCCAAACGGGAGAAGAAAGA
CCGGGTGTTCACCGACAAAACCTCCGCCACTGTGATTTGTCGGAAGAACGCGTCAATCAGCGTCCG
GGCGCAGGATAGATACTACTCGTCCTCCTGGAGCGAATGGGCCAGCGTGCCTTGTTCCGGTGGCGG
ATCAGGCGGAGGTTCAGGAGGAGGCTCCGGAGGAGGTTCCCGGAACCTCCCTGTGGCAACCCCCGA
CCCTGGAATGTTCCCGTGCCTACACCACTCCCAAAACCTCCTGAGGGCTGTGTCGAACATGTTGCAG
AAGGCCCGCCAGACCCTTGAGTTCTACCCCTGCACCTCGGAAGAAATTGATCACGAGGACATCACC
AAGGACAAGACCTCGACCGTGGAAGCCTGCCTGCCGCTGGAACTGACCAAGAACGAATCGTGTCTG
AACTCCCGCGAGACAAGCTTTATCACTAACGGCAGCTGCCTGGCGTCGAGAAAGACCTCATTCATG
ATGGCGCTCTGTCTTTCCTCGATCTACGAAGATCTGAAGATGTATCAGGTCGAGTTCAAGACCATGA
ACGCCAAGCTGCTCATGGACCCGAAGCGGCAGATCTTCCTGGACCAGAATATGCTCGCCGTGATTG
ATGAACTGATGCAGGCCCTGAATTTCAACTCCGAGACTGTGCCTCAAAAGTCCAGCCTGGAAGAAC
CGGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGTTGCACGCTTTCCGCATTCGAGCCGTGAC
CATTGACCGCGTGATGTCCTACCTGAACGCCAGTAGACGGAAACGCGGAAGCGGAGAGGGCAGAG

GCTCGCTGCTTACATGCGGGGACGTGGAAGAGAACCCCGGTCCGATGGAACGCATTGTGATCTGCC
TGATGGTCATCTTCCTGGGCACCTTAGTGCACAAGTCGAGCAGCCAGGGACAGGACAGGCACATGA
TTAGAATGCGCCAGCTCATCGATATCGTGGACCAGTTGAAGAACTACGTGAACGACCTGGTGCCCG
AGTTCCTGCCGGCCCCCGAAGATGTGGAAACCAATTGCGAATGGTCGGCATTTTCCTGCTTTCAAAA
GGCACAGCTCAAGTCCGCTAACACCGGGAACAACGAACGGATCATCAACGTGTCCATCAAAAAGCT
GAAGCGGAAGCCTCCCTCCACCAACGCCGGACGGAGGCAGAAGCATAGGCTGACTTGCCCGTCATG
CGACTCCTACGAGAAGAAGCCGCCGAAGGAGTTCCTGGAGCGGTTCAAGTCGCTCCTGCAAAAGAT
GATTCATCAGCACCTGTCCTCCCGGACTCATGGGTCTGAGGATTCATGAGGTTAGTCGACAATCAAC
CTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGT
GGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTG
TATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGT
GCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGG
GACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGA
CAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCTTTCCTTG
GCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCA
ATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGC
CCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCTTAGTACTGGTACCTTTAAGACCAATG
ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATT
CACTCCCAACGAAGACAAGATTCCGGAATTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTA
AACCGGTGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCT
CTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTG
TGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAA
TCTCTAGCATCTAGAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCC
CAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGC
CCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACT
AATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGA
GGCTTTTTTGGAGGCCTAGGCTAGAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGC
TTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAAC
TTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCAT
TTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGCTAGCCG
GGCTTTTTTTTCTTAGGCCTTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGC
GGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAG
GAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCG
TTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGA
AACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTC
CGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAG
CTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCC
CCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACG
ACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTA
CAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCT
GCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGG
TAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCC
TTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATG
AGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAA
GTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGAT
CTGTCTATTTCGTTCATCCATAGTTGCC

Secretion of IL-12p70 and IL-21 by engineered MSCs are shown in **Fig. 45** and **Fig. 46**, respectively, as assessed by ELISA. SB00880 demonstrated expression of both cytokines by engineered MSCs at higher levels than the majority of constructs tested. Additionally, the ratio of IL-12 to IL-21 was determined, as assessed by ELISA and shown in **Fig. 47**. MSCs

engineered using SB00880 demonstrated a 10 fold higher ratio of IL-12p70 relative to IL-21. Notably, a ratio of 10:1 has demonstrated pre-clinical efficacy (data not shown).

Functional assays demonstrating expression of IL-12p70 by engineered MSCs were performed. HEK-293T cells with a STAT4-SEAP reporter, which reports IL12p70 binding to its receptor and signaling through the JAK-STAT4 pathway, were used to determine potency and activity of IL12p70 produced by engineered hMSCs. Engineered MSCs were cultured for 24 hours and media was collected and incubated with HEK-293T STAT4-SEAP reporter cells. SEAP production was determined with spectrophotometer. As shown in **Fig. 48**, all constructs that encode IL-12 demonstrated reporter activity indicating functional IL12p70 signaling.

Functional assays demonstrating expression of IL-21 by engineered MSCs were performed. NK-92 human natural killer cells were used to determine function of IL-21 produced by engineered hMSCs. Engineered hMSCs were cultured for 24 hours and conditioned media was collected and used to treat NK-92 cells that were deprived from IL-2. Intracellular phospho-flow was performed to quantify phospho-STAT1 and phospho-STAT3 activation as a readout for IL-21 activity. As shown in **Fig. 49**, all constructs that encode IL-21 demonstrated STAT1 (left panel) and STAT3 (right panel) phosphorylation indicating functional IL-21 signaling.

Functional assays for IL-21 was also performed using a IL21R-U2OS IL21R/IL2RG dimerization reporter (PathHunter® U2OS IL21R/IL2RG Dimerization Cell Line, DiscoverX Cat. No: 93-1035C3). Reporter cells were incubated with conditioned media from engineered human MSCs or the appropriate positive (recombinant cytokine) or negative controls. As shown in **Fig. 50**, all constructs that encode IL-21 demonstrated dimerization.

Example 20: Engineered MSC Efficacy in CT26 Tumor Model

In the following example, balb/c mMSCs were engineered to express each of the various murine immune effectors shown in **Fig. 51A** using the lentiviral transduction method described in Example 6. Each MSC was engineered to express only a single agent. CT26 tumor cells ($5x10^4$ cells in 100μl) modified to constitutively express luciferase enzyme (Cat no: CL043, Lot no: CL-IM147 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent balb/c female mice (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. Mice were randomized into treatment groups and treated with intraperitoneally delivered engineered mMSCs ($1x10^6$ cells). MSC-Flag-Myc and PBS were used as a negative control.

As shown in **Fig. 51A**, significant reductions in tumor burden were achieved with select effector-producing engineered-MSCs and select effector-producing engineered-MSCs in a CT26 syngeneic tumor model. Tumor burden fold change was calculated for each individual mouse by normalization of post-treatment BLI (day 10) vs pre-treatment BLI. All the cases where tumor 5 burden fold change was lower than 1 (dotted line) represent tumor burden reduction. The top MSC-effectors that achieved significant reduction in tumor burden were: IL12, IL15, IL12+anti-PD1(microbody), IL12+IL21, IL12+CCL21a, IL12+CXCL10, IL12+CXCL11, IL21+CXCL11, IL21+CCL21a, IL15+CXCL10, GM-CSF+IL12, IL12+IL21+CCL21a.

Example 21: Engineered MSC Efficacy in B16F10 Tumor Model

10 In the following example, C57BL/6 mMSCs were engineered to express each of the various murine immune effectors shown in **Fig. 51B** using the lentiviral transduction method described in Example 6. Each MSC was engineered to express only a single agent. B16F10 tumor cells ($5x10^4$ cells in 100μl) modified to constitutively express luciferase enzyme (B16F10-Fluc-Puro Cat#:CL052, lot#: CL-IM150 Imanis Life Sciences) were injected into the peritoneal 15 space of immunocompetent C57BL/6 female mice (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. Mice were randomized into treatment groups and treated with intraperitoneally delivered mMSCs $1x10^6$ expressing immune-modulatory cytokines or chemokines such as IL12p70. MSC-Flag-Myc and PBS were used as a negative control.

20 As shown in **Fig. 51B**, significant reductions in tumor burden were achieved with select effector-producing engineered-MSCs and select effector-producing engineered-MSCs in a CT26 syngeneic tumor model. Selected effectors or combinations were achieving significant reduction in tumor burden: IL12, IL12+CD40L, IL12+CXCL10, IL12+IL21, IL12+IL21+Flt3L, IL12+IL21+CXCL10, IL12+CCL21a+Flt3L.

25 **Example 22: IL12 Producing MSCs Reduce CT26 Tumor Burden in an IP Model**

In the following example, balb/c mMSCs were engineered to express murine IL12p70 or murine IL21 (*i.e.*, each MSC engineered to express only a single agent) using the lentiviral transduction method described in Example 6. CT26 tumor cells ($5x10^4$ cells in 100μl) modified to constitutively express luciferase enzyme (Cat no: CL043, Lot no: CL-IM147 Imanis Life 30 Sciences) were injected into the peritoneal space of immunocompetent balb/c (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI)

using an AMI imager. In addition, tumor weights were determined at the time of termination (day 17 post tumor implant). Mice were randomized into treatment groups and treated with intraperitoneally delivered mMSCs (1x10^6 cells). MSC-Flag-Myc and PBS were used as a negative control. Experimental cohorts included: murine IL12-expressing murine MSCs, murine IL21-expressing murine MSCs, and combination treatment of murine IL12-expressing murine MSCs and murine IL21-expressing murine MSCs (1x10^6 cells delivered for each in the combination).

As shown in **Fig. 52A** and **Fig. 52B**, the groups receiving IL12p70-expressing MSCs, IL21-expressing MSCs, and the combination of IL12p70 and IL21-expressing MSCs led to reduction in tumor burdens as assessed by BLI (**Fig. 52A** left panel) and by tumor weight (**Fig. 52A** right panel) in a CT26 model, including a significant reduction in the combination treatment, relative to the controls. **Fig. 52B** demonstrates the BLI luciferase measurements of individual mice (results summarized in **Fig. 52A** left panel).

The above experiment was repeated with the modification of delivering a lower dose of engineered mMSCs (1x10^5 cells). As shown in **Fig. 53A**, the groups receiving IL12p70-expressing MSCs, and the combination of IL12p70 and IL21-expressing MSCs led to reduction in tumor burdens as assessed by BLI (**Fig. 53A**; individual BLI measurements of mice - left panel; summary of BLI measurments – right panel) in a CT26 model, including a significant reduction in the combination treatment, relative to the controls. Additionally, the combination treatment demonstrated increased efficacy relative to groups receiving IL12p70-expressing MSCs alone. As shown in **Fig. 53B**, treatment with 1x10^5 IL12p70 expressing MSCs in combination with 1x10^5 IL21 expressing MSCs led to tumor-free survival up to 40 days in all mice treated (n=8; median survival not reached). In constrast, treatment with 1x10^5 IL12p70 expressing MSCs alone only resulted in a 25% survival rate by day 40 (n=8; median survival 19 days). Control groups treated with PBS for FLAG-MSCs resulted in a 0% survival rate by day 40 (n=8 each; median survival 12 days each). Thus, IL21 expression by MSCs enhanced the efficacy of IL12p70 expressing MSCs.

Example 23: IL12 Producing MSCs Reduce B16F10 Tumor Burden in an IP Model

In the following example, C57BL/6 mMSCs were engineered to express murine IL12p70 or murine IL21 (*i.e.*, each MSC engineered to express only a single agent) using the lentiviral transduction method described in Example 6. B16F10 tumor cells (5x10^4 cells in 100μl)

modified to constitutively express luciferase enzyme (B16F10-Fluc-Puro Cat#:CL052, lot#: CL-IM150 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent C57BL/6 (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. In addition, tumor weights were determined at the time of termination (day 17 post tumor implant). Mice were randomized into treatment groups and treated with intraperitoneally delivered mMSCs ($1x10^6$ cells). MSC-Flag-Myc and PBS were used as a negative control. Experimental cohorts included: murine IL12-expressing murine MSCs, murine IL21-expressing murine MSCs, and combination treatment of murine IL12-expressing murine MSCs and murine IL21-expressing murine MSCs ($1x10^6$ cells delivered for each in the combination).

As shown in **Fig. 54** and **Fig. 55**, the groups receiving IL12p70-expressing MSCs, and the combination of IL12p70 and IL21-expressing MSCs led to reduction in tumor burdens as assessed by BLI (**Fig. 54** left panel) and by tumor weight (**Fig. 54** right panel) in a B16F10 model, including a significant reduction in the combination treatment, relative to the controls. Notably, IL21-expressing MSCs alone did not demonstrate a significant reduction in tumor burden or tumor weight. **Fig. 55** demonstrates the BLI luciferase measurements of individual mice for the control FLAG-expressing MSCs and the combination of IL12-expressing MSCs and IL21-expressing MSCs (results summarized in **Fig. 54** left panel).

Example 24: MSCs Producing IL12 and IL21 Prolong Tumor-Free Survival in a B16F10 IP Tumor Model and Survive Tumor Rechallenge

In the following example, C57BL/6 mMSCs were engineered to express murine IL12 (p70) or murine IL21 (*i.e.*, each MSC engineered to express only a single agent) using the lentiviral transduction method described in Example 6. B16F10 tumor cells ($5x10^4$ cells in 100μl) modified to constitutively express luciferase enzyme (B16F10-Fluc-Puro Cat#:CL052, lot#: CL-IM150 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent C57BL/6 (age 6-8 weeks). Mice were randomized into treatment groups and treated with intraperitoneally delivered mMSCs ($1x10^6$ cells). MSC-Flag-Myc and PBS were used as a negative control. Experimental cohorts included: murine IL12-expressing murine MSCs, murine IL21-expressing murine MSCs, and combination treatment of murine IL12-expressing murine MSCs and murine IL21-expressing murine MSCs ($1x10^6$ cells delivered for each in the combination).

As shown in **Fig. 56**, treatment with IL12p70 expressing MSCs led to prolonged survival (median survival 27 days post-treatment) relative to control treated mice (median survival of 8 days post-treatment for both PBS treated and FLAG-expressing MSCs). Treatment with IL12p70 expressing MSCs in combination with IL21 expressing MSCs led to prolonged survival (54.5% survival; median survival not reached) relative to treatment with IL12p70 expressing MSCs alone. Thus, IL21 expression by MSCs enhanced the efficacy of IL12p70 expressing MSCs.

Additionally, mice that were tumor-free for more than 90 days were subsequently re-challenged with B16-F10 tumor cells implanted in the flank ($1x10^6$ cells). Naïve un-treated mice were implanted at the same time as controls. Sub-cutaneous tumor burden was measured by caliper. As shown in **Fig. 57C**, all mice (n=4) that previously received the combination treatment of IL12-expressing MSCs and IL21-expressing MSCs survived rejected the newly implanted tumor, indicating that the treatment resulted in achievement of anti-tumor immune memory. Mice that previously received the treatment of IL12-expressing MSCs alone had a 50% tumor-rejection rate (2 out of 4 mice; **Fig. 57B**). In contrast, tumor were established in 60% of naïve mice (3 out of 5 mice; **Fig. 57A**).

Example 25: MSCs Producing IL12 in Combination with Immune Checkpoint Therapy Prolong Survival in a B16F10 IP Tumor Model

In the following example, C57BL/6 mMSCs were engineered to express murine IL12 (p70) using the lentiviral transduction method described in Example 6. B16F10 tumor cells ($5x10^4$ cells in 100μl) modified to constitutively express luciferase enzyme (B16F10-Fluc-Puro Cat#:CL052, lot#: CL-IM150 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent C57BL/6 (age 6-8 weeks). Mice were randomized into treatment groups and treated with IP administration of anti-PD1 antibody (clone RMP1-14) at a dose of 200mg/kg alone or in combination with low dose (1e5) of IL12-expressing murine MSCs.

As shown in **Fig. 56**, treatment with anti-PD1 alone resulted in a 12.5% survival rate and median survival of 23 days (Fig. 56 "Anti-PD1"; 1 out of 8 mice had long term tumor-free survival). In contrast, the combined treatment of anti-PD1 with IL12p70 expressing MSCs resulted in a 50% survival rate (Fig. 56 "MSC-IL12 (p70) + Anti-PD1"; 4 out of 8 mice had long term tumor-free survival; median survival not yet established). Thus, IL12 expression by MSCs enhanced the efficacy of anti-PD1 immune checkpoint therapy and convert a checkpoint refractory or resistant model (B16F10) into responsive..

Example 26: MSCs Producing Both IL12 and IL21 Reduce Tumor Burden in a CT26 IP Tumor Model

In the following example, balb/c mMSCs were engineered to express murine IL12 (p70) and murine IL21 from a single lentiviral expression vector. The lentiviral expression vector used a 2A ribosome skipping elements to express both cytokines from a single transcript using the lentiviral transduction method described in Example 6. CT26 tumor cells ($1x10^5$ cells in 100μl) modified to constitutively express luciferase enzyme (Cat no: CL043, Lot no: CL-IM147 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent female balb/c mice (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. Mice were randomized into treatment groups and treated IP with different amounts of mMSCs ranging from $1x10^4$ to $1x10^6$ cells. MSC-Flag-Myc ($1x10^6$ cells) and PBS were used as a negative control.

As shown in **Fig. 58A-C**, anti-tumor activity was observed in a dose-dependent manner of MSCs expressing both IL12 and IL21, as assessed by BLI (**Fig. 58A** normalized day 17 vs day 7; **Fig. 58B** and **Fig. 58C** BLI over time for individual mice). No efficacy was observed in control FLAG or PBS mice (**Fig. 58A** and **Fig. 58B**). In constract, minimal efficacy was observed at a dose of $1x10^4$, with efficacy increasing at each increased dose (**Fig. 58A** and **Fig. 58C**). As shown in **Fig. 58D**, long term tumor-free survival up to 60 days post tumor implant was observed in a dose-dependent manner, with mice treated with $1x10^6$ to $1x10^5$ having siginificantly extended tumor-free survival (Median survial post-implant: PBS/FLAG – 19 days; $1x10^6$ to $1x10^5$ – not reached; $3x10^4$ – 53 days; $1x10^4$ – 18-19 days).

Example 27: MSCs Producing Both IL12 and IL21 Reduce Tumor Burden in a B16F10 IP Tumor Model

In the following example, C57BL/6 mMSCs were engineered to express murine IL12 (p70) and murine IL21 from a single lentiviral expression vector. The lentiviral expression vector used a 2A ribosome skipping elements to express both cytokines from a single transcript using the lentiviral transduction method described in Example 6. B16F10 tumor cells ($5x10^4$ cells in 100μl) modified to constitutively express luciferase enzyme (B16F10-Fluc-Puro Cat#:CL052, lot#: CL-IM150 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent C57BL/6 (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. Mice were randomized into treatment groups and treated with different amounts of mMSCs ranging from $1x10^5$ to $1x10^7$

cells). MSC-Flag-Myc ($3x10^6$ cells) and PBS were used as a negative control. Some groups were treated with multiple doses separated by 5 days (treatment on day 7, 12 and 17 post tumor-implant).

As shown in **Fig. 59A-D**, anti-tumor activity was observed in a dose-dependent manner of MSCs expressing both IL12 and IL21, as assessed by BLI (**Fig. 59A** normalized day 17 vs day 7; **Fig. 59B-D** BLI over time for individual mice). No efficacy was observed in control FLAG or PBS mice (**Fig. 59A** and **Fig. 59B**). No efficacy was also observed at doses of $1x10^5$ or $3x10^5$ cells (**Fig. 59A** and **Fig. 59C**). In contrast, minimal efficacy was observed at a dose of $1x10^6$, with efficacy increasing at each increased dose (**Fig. 59A** and **Fig. 59C**). Efficacy was also observed following multiple administrations of higher doses (**Fig. 59D**). As shown in **Fig. 59E**, long term tumor-free survival was observed in a dose-dependent manner, and also observed following multiple administrations of higher doses (Median survial post-implant: PBS – 20 days; FLAG (x3) – 27 days; $1x10^7$ – 31.5 days; $3x10^6$ – 36 days; $3x10^6$ (x3) – 39 days; $1x10^6$ – 33 days; $1x10^6$ (x3) – 39 days; $3x10^5$ – 27 days; $3x10^5$ (x3) – 27 days [curve overlaps with $3x10^5$ treatment]; $1x10^5$ – 24 days).

Example 28: MSCs Producing Both IL12 and IL21 Reduce Tumor Burden in a MC-38 IP Tumor Model

In the following example, C57BL/6 mMSCs were engineered to express murine IL12 (p70) and murine IL21 from a single lentiviral expression vector. The lentiviral expression vector used a 2A ribosome skipping elements to express both cytokines from a single transcript using the lentiviral transduction method described in Example 6. MC-38 tumor cells were transduced with fLUC-EGFP construct and sorted based on EGFP fluorescence, then $5x10^5$ cells were injected into the peritoneal space of immunocompetent C57BL/6 (age 6-8 weeks). Nine days after tumor implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. Mice were randomized into treatment groups and treated with different amounts of mMSCs ranging from $3x10^4$ to $1x10^6$ cells. MSC-Flag-Myc and PBS were used as a negative control.

As shown in **Fig. 60A** and **Fig. 60B**, anti-tumor activity was observed in a dose-dependent manner of MSCs expressing both IL12 and IL21, as assessed by BLI (**Fig. 60A** normalized day 18 vs day 9; **Fig. 60B** BLI over time for individual mice). No efficacy was observed in control FLAG or PBS mice (**Fig. 60A** and **Fig. 60B**). No efficacy was also observed

at doses of $1x10^5$ or $3x10^4$ cells (**Fig. 60A** and **Fig. 60B**). In contrast, minimal efficacy was observed at a dose of $3x10^5$, with efficacy increasing at an increased dose of $1x10^6$ cells (**Fig. 60A** and **Fig. 60B**). As shown in **Fig. 60C**, long term tumor-free survival was observed in a dose-dependent manner, with all mice treated with $1x10^6$ cells surviving past at least day 30 (Median survial post-implant: PBS – 21 days; FLAG – 29 days; $1x10^6$ – not reached; $3x10^5$ – 28 days; $1x10^5$ – 21 days; $3x10^4$ – 21 days [PBS, $1x10^5$, and $3x10^4$ overlap). Accordingly, mMSCs engineered to express murine IL12 (p70) and murine IL21 demonstrated efficacy in a MC-38 tumor model.

Example 29: Human MSCs Home to Tumors in an IP Model

In the following example, NSG mice were implanted with OVCAR8-fLUC cells IP. 14-21 days after tumor implantation, $1x10^6$ human BM-MSCs engineered to express Nanoluc-EGFP were delivered IP. Mice were euthanized at 24 hours post injection of MSCs and peritoneal organs (stomach, kidney, liver, colon, spleen, pancreas, omentum/tumor, ovaries and Fallopian tubes) were imaged ex-vivo for NanoLuc signaling (NanoGlo Substrate Kit ,Vendor: Promega, Catalog No.: N1110). Human MSCs were imaged by EGFP fluorescence in tumor sections collected at 24 hours as well as 22 days post injection.

As shown in **Fig. 61A** and **Fig. 61B**, human MSC NanoLuc signal was preferentially enriched in the tumor compared to the other organs in the peritoneal cavity (**Fig. 61A** summarized luciferase quantification; **Fig. 61B** representative images of luciferase signal). Additionally, persistence of MSCs was lower than 22 days, with no cells being detected at the latest time point (**Fig. 61B** right most panel).

Example 30: Biodistribution and PK of effector cytokines

In the following example, biodistribution and PK of effector cytokines produced by engineered MSCs was assessed.

In a first experiment, NSG mice were implanted with $5x10^6$ OVCAR8-fLUC tumor cells IP. 21-27 days after tumor implantation, mice were randomized based on tumor burden measured by BLI and treated with $1x10^6$ hMSCs engineered to express human IL12 (p70) and human IL21 from a single lentiviral expression vector. The lentiviral expression vector used a 2A ribosome skipping elements to express both cytokines from a single transcript using the lentiviral transduction method described in Example 6. Mice were euthanized at 16-24 hours or 3, 4 and 7 days post MSC treatment and peritoneal fluid was collected via IP lavage by injecting

1mL of PBS into the peritoneal space and collecting it. Serum was separated from whole blood after intracardiac puncture. ELISA (R&D systems) was used to determine the protein amount in each compartment (peritoneal fluid vs serum) for each time point and treatment type.

As shown in **Fig. 62A**, transient production of both human IL12 (left panel) and human IL21 (right panel) was observed in both the peritoneal fluid (left column for each respective time point) and serum (right column for each respective time point). At least a 10 fold increased protein abundance was observed in the peritoneal space (local) compared to systemic (serum), demonstrating localized delivery of cytokines by engineered MSCs.

In another experiment, balb/c mMSCs were engineered to express murine IL12p70 or murine IL21 (*i.e.*, each MSC engineered to express only a single agent) using the lentiviral transduction method described in Example 6. CT26-fLUC tumor cells (1x10^5 cells in 100μl) were injected into the peritoneal space of immunocompetent balb/c (age 6-8 weeks). Murine IL12-expressing murine MSCs and murine IL21-expressing murine MSCs (1x10^6 cells delivered for each in the combination) were delivered IP. Mice were euthanized at 24 or 72 hours post MSC treatment and peritoneal fluid was collected via IP lavage by injecting 1mL of PBS into the peritoneal space and collecting it. Serum was separated from whole blood after intracardiac puncture. Luminex (Millipore) was used to determine the protein amount in each compartment (peritoneal fluid vs serum) for each time point and treatment type.

As shown in **Fig. 62B**, transient production of both murine IL12 (left panel) and murine IL21 (right panel) was observed in both the peritoneal fluid (left column for each respective time point) and serum (right column for each respective time point). At least a 10 fold increased protein abundance was observed in the peritoneal space (local) compared to systemic (serum), demonstrating localized delivery of cytokines by engineered MSCs.

Example 31: Comparison of MSC Treatment and Recombinant Cytokine Treatment in a CT26 IP Tumor Model

In the following example, balb/c mMSCs were engineered to express murine IL12 (p70) and murine IL21 from a single lentiviral expression vector. Balb/c mMSCs were also engineered to express either murine IL12 (p70) or murine IL21. The lentiviral expression vector used a 2A ribosome skipping elements to express both cytokines from a single transcript using the lentiviral transduction method described in Example 6. CT26 tumor cells (1x10^5 cells in 100μl) modified to constitutively express luciferase enzyme (Cat no: CL043, Lot no: CL-IM147 Imanis Life

Sciences) were injected into the peritoneal space of immunocompetent female balb/c mice (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. For MSC treated mice, mice were randomized into treatment groups and treated with intraperitoneally delivered mMSCs ($1x10^6$ cells), receiving murine IL12-expressing murine MSCs, murine IL21-expressing murine MSCs, or murine IL12 and IL21-expressing murine MSCs, with MSC-Flag-Myc and PBS were used as a negative control. Additionally, treatment groups also included mice receiving a bolus dose of the respective recombinant cytokines at a dose of 4-times the amount produced by MSCs in vitro (measured by ELISA - recombinant IL12: 5ug/mouse; Recombinant IL21: 0.4ug/mouse). Tumor burden was measured by fLUC BLI across time points and mice were euthanized when reaching endpoint criteria due to tumor burden. Kaplan Meier survival curves were determined to calculate tumor-free survival.

As shown in **Fig. 63A-C**, mice treated with MSCs engineered to produce cytokines outperformed recombinant cytokine therapy in terms of prolonged tumor-free survival, in all cases (**Fig. 63A** – MSC-IL12 vs rIL12; **Fig. 63B** – MSC-IL21 vs rIL21; **Fig. 63C** – MSC-IL12/IL21 vs rIL12+rIL21). Additionally, as shown in **Fig. 63D-E**, mice treated with MSCs engineered to produce cytokines outperformed recombinant cytokine therapy as assessed by tumor burden BLI), in all cases (**Fig. 63D** bottom row – MSC-IL12 vs rIL12; **Fig. 63E** top row – MSC-IL21 vs rIL21; **Fig. 63E** bottom row – MSC-IL12/IL21 vs rIL12+rIL21).

Example 32: Comparison of MSC Treatment and Recombinant Cytokine Treatment in a B16F10 IP Tumor Model

In the following example, C57BL/6 mMSCs were engineered to express murine IL12 (p70) and murine IL21 from a single lentiviral expression vector. C57BL/6 mMSCs were also engineered to express either murine IL12 (p70) or murine IL21. The lentiviral expression vector used a 2A ribosome skipping elements to express both cytokines from a single transcript using the lentiviral transduction method described in Example 6. B16F10 tumor cells ($1x10^5$ cells in 100μl) modified to constitutively express luciferase enzyme (Cat no: CL043, Lot no: CL-IM147 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent female balb/c mice (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI) using an AMI imager. For MSC treated mice, mice were randomized into treatment groups and treated with intraperitoneally delivered mMSCs ($3x10^6$ cells)

engineered to express both IL12 and IL21-expressing murine MSCs, with MSC-Flag-Myc and PBS were used as a negative control. Additionally, treatment groups also included mice receiving a bolus dose of the respective recombinant cytokines at a dose of 4-times the amount produced by MSCs in vitro (measured by ELISA - recombinant IL12: 3ug/mouse; Recombinant
5 IL21: 0.03ug/mouse). Tumor burden was measured by tumor weight at day 7 post treatment and mice were euthanized when reaching endpoint criteria due to tumor burden. Kaplan Meier survival curves were determined to calculate tumor-free survival.

As shown in **Fig. 64A**, mice treated with MSCs engineered to produce both IL12 and IL21 outperformed recombinant cytokine therapy as assessed by tumor weight. Additionally, as
10 shown in **Fig. 64B**, mice treated with MSCs engineered to produce both IL12 and IL21 outperformed recombinant cytokine therapy as assessed by tumor-free prolonged survival.

Example 33: Immune Profile Following Treatment with MSCs Producing Both IL12 and IL21 in a CT26 IP Tumor Model

In the following example, balb/c mMSCs were engineered to express murine IL12p70 or
15 murine IL21 (*i.e.*, each MSC engineered to express only a single agent) using the lentiviral transduction method described in Example 6. CT26 tumor cells ($1x10^5$ cells) modified to constitutively express luciferase enzyme (Cat no: CL043, Lot no: CL-IM147 Imanis Life Sciences) were injected into the peritoneal space of immunocompetent balb/c (age 6-8 weeks). One week after tumor implantation, tumor burden was measured by luciferase imaging (BLI)
20 using an AMI imager. Mice were randomized into treatment groups and treated with a intraperitoneally delivered combination treatment of murine IL12-expressing murine MSCs and murine IL21-expressing murine MSCs ($1x10^6$ cells delivered for each in the combination), or MSC-Flag-Myc and PBS as a negative controls. Mice were euthanized and organs collected at 72 hours after treatment. Multicolor flow cytometry was used to characterize immune infiltrates
25 in response to treatment.

As shown in **Fig. 65A** and **Fig. 65B**, T-cell subsets and activation markers (CD3, CD4, CD8, CD8/CD38+, CD8/IFNg+, CD8/Gzmb+, NK/Gzmb+ and ratio CD8:Tregs-FoxP3) were significantly increased in the peritoneal fluid after treatment with MSCs-IL12 +MSCs IL21. Additionally, as shown in **Fig. 65C**, antigen-presenting cells such as dendritic cells
30 (CD11c/MHC-II hi, CD86+, CD103+, CD11b+) were also significantly increased in peritoneal tumor-draining lymph nodes after treatment with MSC-IL12 + MSC-IL21. Accordingly,

combination treatment of murine IL12-expressing murine MSCs and murine IL21-expressing murine MSCs demonstrated an activated immune profile.

Example 34: Optimization of Signal Peptide Sequences

In the following example, effector molecules are modified to replace their native signal peptide sequence with an exogenous signal peptide sequence (*see* Table 5 for exemplary signal peptide sequences that are tested). Modified effector molecules are tested for functional improvements such as improved expression and maintained secretion, such as in particular environments (*e.g.*, tumor microenvironments). Functional performance for the modified effector molecules is also tested in tumor models (*e.g.*, improved ability to clear tumors, improved ability to clear tumors in different environments, or improved ability to clear different types of tumors).

Example 35: Enrichment of Engineered MSCs.

In the following example, MSCs are engineered to express effector molecules within a population of cells that include unmodified cells, such as unmodified MSCs. The engineered MSCs are enriched within the population by contacting the engineered MSCs with a growth factor (such as the effector molecules described in Table 1) such that those engineered MSCs that are enriched are a sub-population of engineered MSCs that express a receptor or receptor ligand for the growth factor. The sub-population of engineered MSCs of interest are contacted with the growth factor in various manners:

1. In an autocrine manner by genetically engineering the MSCs themselves to express the factors.
2. In a paracrine manner by genetically engineering feeder or support cells to express the factors and supply those factors to the MSCs, or by using conditioned media containing the factors from the feeder or support cells (such as 293Ts) engineered to express these factors.
3. In an endocrine manner, by injecting recombinant protein or nucleic acid versions of these factors into patients following MSC transplantation.
4. Via addition of soluble recombinant protein versions of these factors to the MSC culture conditions.
5. Via coating of the tissue culture plate/flask surfaces used for MSC propagation with recombinant versions of these factors.

References:

1. Kidd S, et al. (2009) Stem Cells 27(10):2614-2623.
2. Dembinski JL, et al. (2013) Cytotherapy 15(1):20-32.
3. Siegel RL, et al. (2016) CA Cancer J Clin 66(1):7-30.
4. Dizon D MJ (2010) Gynecol Oncol 116(3).
5. Woo SR, et al. (2015) Trends Immunol 36(4):250-256.
6. Hamanishi J, et al. (2016) Int Immunol 28(7):339-348.
7. Li S, et al. (2012) Oncolytic Virother 1:1-21.
8. Koneru M, et al. (2015) J Transl Med 13:102.
9. Cruz CR, et al. (2010) Cytotherapy 12(6):743-749.
10. Li YQ, et al. (2013) PLoS One 8(10):e76379.
11. Wiedemann GM, et al. (2016) Oncoimmunology 5(9):e1175794.
12. Squillaro T, et al. (2016) Cell Transplant 25(5):829-848.
13. Studeny M, et al. (2004) J Natl Cancer Inst 96(21):1593-1603.
14. Ling X, et al. (2010) Cancer Microenviron 3(1):83-95.
15. Schukur L, et al. (2015) Sci Transl Med 7(318):318ra201.
16. Howlader N NA, Krapcho M, Garshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ and Cronin KA. (2015).
17. Lengyel E (2010) Am J Pathol 177(3):1053-1064.
18. McGuire WP, et al. (1996) The New England journal of medicine 334(1):1-6.
19. McGuire WP, et al. (1989) Annals of internal medicine 111(4):273-279.
20. Adams SF & Benencia F (2015) Future Oncology 11(9):1293-1296.
21. Maude SL, et al. (2014) N Engl J Med 371(16):1507-1517.
22. Bargou R, et al. (2008) Science 321(5891):974-977.
23. Kershaw MH, et al. (2014) Clin Trans Immunol 3:e16.
24. Gilham DE, et al. (2012) Trends Mol Med 18(7):377-384.
25. Klinger M, et al. (2012) Blood 119(26):6226-6233.
26. Fu J, et al. (2015) Sci Transl Med 7(283):283ra252.
27. Moynihan KD, et al. (2016) Nat Med 22(12):1402-1410.
28. Mohammadi M, et al. (2016) Cancer Gene Ther 23(9):285-286.
29. Wang D, et al. (2013) Cell Transplant 22(12):2267-2277.
30. Nowakowski A, et al. (2016) Stem Cells Int 2016:4956063.
31. Sun Z, et al. (2014) J Hematol Oncol 7:14.
32. Ando M, et al. (2015) Stem Cell Reports 5(4):597-608.
33. Zhao Q, et al. (2015) Proc Natl Acad Sci U S A 112(2):530-535.
34. Xie C, et al. (2013) Br J Cancer 109(5):1198-1205.
35. Parker BS, et al. (2016) Nat Rev Cancer 16(3):131-144.
36. Roby KF, et al. (2000) Carcinogenesis 21(4):585-591.

37. Sharma AD, et al. (2015) J Vis Exp (95):e52242.
38. Waterman RS, et al. (2012) PLoS One 7(9):e45590.
39. Dubinett SM, et al. (2010) Cancer J 16(4):325-335.
40. Tang ED & Wang CY (2015) PLoS One 10(3):e0120090.
41. Cieri N, et al. (2013) Blood 121(4):573-584.
42. Fitzgerald KA, et al. (2003) Nat Immunol 4(5):491-496.
43. Wong AS, et al. (2016) Proc Natl Acad Sci U S A 113(9):2544-2549.
44. Wong AS, et al. (2015) Nat Biotechnol 33(9):952-961.
45. Nissim L, et al. (2014) Mol Cell 54(4):698-710.
46. Deng P, et al. (2016) Neural Regen Res 11(5):702-705.
47. Beegle JR, et al. (2016) Mol Ther Methods Clin Dev 3:16053.
48. Boutros C, et al. (2016) Nat Rev Clin Oncol 13(8):473-486.
49. Valsecchi ME (2015) New Engl J Med 373(13):1270-1270.
50. Pardoll DM (2012) Nat Rev Cancer 12(4):252-264.
51. Legat A, et al. (2013) Front Immunol 4:455.
52. Justus CR, et al. (2014) J Vis Exp (88).
53. Jedema I, et al. (2004) Blood 103(7):2677-2682.
54. Peng D, et al. (2015) Nature 527(7577):249-253.
55. Gitzinger M, et al. (2009) Proc Natl Acad Sci U S A 106(26):10638-10643.
56. Clackson T, et al. (1998) Proc Natl Acad Sci U S A 95(18):10437-10442.
57. Siuti P, et al. (2013) Nature Biotechnology 31(5):448-452.
58. Farzadfard F & Lu TK (2014) Science 346(6211):1256272.
59. Perli SD, et al. (2016) Science 353(6304).
60. Roquet N, et al. (2016) Science 353(6297):aad8559.
61. Wong ASL, et al. (2016) Proceedings of the National Academy of Sciences.
62. Gardner TS, et al. (2000) Nature 403(6767):339-342.
63. Deans TL, et al. (2007) Cell 130(2):363-372.
64. Warren L, et al. (2010) Cell Stem Cell 7(5):618-630.
65. Yang BX, et al. (2015) Cell 163(1):230-245.
66. Kumar RM, et al. (2014) Nature 516(7529):56-61.
67. Zhang J, et al. (2016) Cell Stem Cell 19(1):66-80.
68. Cahan P, et al. (2014) Cell 158(4):903-915.
69. Doulatov S, et al. (2013) Cell Stem Cell 13(4):459-470.
70. Kim K, et al. (2011) Nat Biotechnol 29(12):1117-1119.
71. Chavez A, et al. (2016) Nat Methods 13(7):563-567.
72. Slomovic S & Collins JJ (2015) Nat Methods 12(11):1085-1090.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, *i.e.*, to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

CLAIMS

What is claimed is:

1. An engineered cell comprising:

a) a promoter; and

b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide,

E1 comprises a polynucleotide sequence encoding a first effector molecule,

L comprises a linker polynucleotide sequence,

S2 comprises a polynucleotide sequence encoding a second signal peptide,

E2 comprises a polynucleotide sequence encoding a second effector molecule, and

wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule,

and wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

2. The engineered cell of claim 1, wherein the promoter is operably linked to the expression cassette such that the polynucleotides are capable of being transcribed as a single polynucleotide comprising the formula S1 – E1 – L – S2 – E2.

3. The engineered cell of claim 1 or claim 2, wherein the linker polynucleotide sequence is operably associated with the translation of the first effector molecule and the second effector molecule as separate polypeptides.

4. The engineered cell of claim 3, wherein the linker polynucleotide sequence encodes a 2A ribosome skipping tag or encodes an Internal Ribosome Entry Site (IRES), optionally wherein when the linker polynucleotide sequence encodes a 2A ribosome skipping tag, and the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

5. The engineered cell of claim 1, wherein the linker polynucleotide sequence encodes a second promoter,

   wherein the promoter is operably linked to the expression cassette such that a first polynucleotide comprising the formula S1 – E1 is capable of being transcribed,

   wherein the second promoter is operably linked to the expression cassette such that a second polynucleotide comprising the formula S2 – E2 is capable of being transcribed, and wherein the first and the second polynucleotide are separate polynucleotides.

6. The engineered cell of any one of claims 1-5, wherein the engineered cell is a human cell, optionally wherein the human cell is isolated from a tissue selected from the group consisting of: bone marrow, adipose tissue, the umbilical cord, fetal liver, muscle, and lung tissue.

7. The engineered cell of any one of claims 1-6, wherein the engineered cell is an MSC, and wherein MSC comprises a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD45-, CD34-, CD14-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD11b-, CD79α-; a cellular marker phenotype comprising CD105+, CD73+, CD90+, CD19-, HLA class II-; or a cellular marker phenotype comprising CD73+, CD90+, CD105+, and CD166+, CD11b-, CD14-, CD19-, CD34-, CD45-, and HLA-DR-.

8. The engineered cell of any one of claims 1-7, wherein the promoter and/or the second promoter comprises a constitutive promoter, optionally wherein the constitutive promoter is selected from the group consisting of: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb .

9. The engineered cell of any one of claims 1-7, wherein the promoter and/or the second promoter comprises an inducible promoter, optionally wherein the inducible promoter is selected from the group consisting of: minP, NFkB response element, CREB response element, NFAT response element, SRF response element 1, SRF response element 2, AP1 response element, TCF-LEF response element promoter fusion, Hypoxia responsive element, SMAD binding element, STAT3 binding site, inducer molecule responsive promoters, and tandem repeats thereof.

10. The engineered cell of any one of claims 1-9, wherein the first signal peptide or the second signal peptide comprises a native signal peptide native to the first effector molecule or the second effector molecule, respectively.

11. The engineered cell of any one of claims 1-9, wherein the first signal peptide or the second signal peptide comprises a non-native signal peptide non-native to the first effector molecule or the second effector molecule, respectively, optionally wherein the non-native signal peptide is selected from the group consisting of: IL12, IL2, optimized IL2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, and IL21.

12. The engineered cell of any one of claims 1-11, wherein the first signal peptide and the second signal peptide are identical.

13. The engineered cell of any one of claims 1-12, wherein each effector molecule is independently selected from a therapeutic class, wherein the therapeutic class is selected from the group consisting of: a cytokine, a chemokine, a growth factor, a co-activation molecule, a tumor microenvironment modifier a, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme, optionally wherein the therapeutic class of the first effector molecule and the second effector molecule are different, optionally wherein the first effector molecule and/or the second effector molecule is a modified effector molecule that when expressed is tethered to a cell membrane of the engineered MSC.

14. The engineered cell of claim 13, wherein:

i) the cytokine is selected from the group consisting of: IL12, an IL12p70 fusion protein, IL7, IL21, IL18, IL15, Type I interferons, and Interferon-gamma;

ii) the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1;

iii) the growth factor is selected from the group consisting of: Flt3L and GM-CSF;

iv) the co-activation molecule is selected from the group consisting of: 4-1BBL and CD40L; and

v) the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, TGFbeta inhibitors, immune checkpoint inhibitors, VEGF inhibitors, and HPGE2,
optionally wherein the TGFbeta inhibitors are selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and combinations thereof,
optionally wherein the immune checkpoint inhibitors comprise anti-PD-1 antibodies, and
optionally wherein the VEGF inhibitors comprise anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof.

15. The engineered cell of any one of claims 1-14, wherein the first effector molecule comprises an IL12p70 fusion protein and the second effector molecule comprises CCL21a, IL7, IL15, IL21, Flt3L, an anti-PD1 antibody, CD40L, or a CXCL10-CXCL11 fusion protein.

16. The engineered cell of any one of claims 1-15, wherein the expression cassette further comprises following E2, an additional exogenous polynucleotide sequence comprising a formula, oriented from 5' to 3', comprising:

(L – S – E)$_X$

wherein

S comprises a polynucleotide sequence encoding a signal peptide,

E comprises a polynucleotide sequence encoding an effector molecule,

L comprises a linker polynucleotide sequence,

X = 1 to 20

wherein the promoter is operably linked to the expression cassette, and wherein for each X the corresponding signal peptide is operably associated with the effector molecule,
optionally wherein one or more of the additional effector molecules comprises IL15, Flt3L, an anti-PD1 antibody, adenosine deaminase, CD40L, a CXCL10-CXCL11 fusion protein, and/or XCL1.

17. The engineered cell of any one of claims 1-16, wherein the construct comprises:

a) an SFFV promoter; and

b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide;

E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein;

L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes a Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus;

S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide;

E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and

wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule,
optionally wherein the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144.

18. The engineered cell of any one of claims 1-17, wherein the exogenous polynucleotide sequence comprises one or more viral vector polynucleotide sequences, optionally wherein the one or more viral vector polynucleotide sequences comprise lentiviral, retroviral, retrotransposon, or adenoviral polynucleotide sequences.

19. A population of cells, wherein the population of cells comprises one or more engineered cells of any one of claims 1-18.

20. A pharmaceutical composition, wherein the pharmaceutical composition comprises the engineered cells of any one of claims 1-18 or the population of cells of claim 19.

21. A method of reducing tumor volume in a subject, the method comprising delivering to a subject having a tumor a composition comprising any of the engineered cells of any one of claims 1-18, the population of cells of claim 19, or the pharmaceutical composition of claim 20, optionally wherein the engineered cell is allogeneic with reference to the subject.

22. A method of inducing an immune response in a subject, the method comprising administering a therapeutically effective dose of any of the engineered cells of any one of claims 1-18, the population of cells of claim 19, or the pharmaceutical composition of claim 20, optionally wherein the engineered cell is allogeneic with reference to the subject.

23. An exogenous polynucleotide sequence comprising a promoter and an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide,

E1 comprises a polynucleotide sequence encoding a first effector molecule,

L comprises a linker polynucleotide sequence,

S2 comprises a polynucleotide sequence encoding a second signal peptide,

E2 comprises a polynucleotide sequence encoding a second effector molecule, and

wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule.

24. The exogenous polynucleotide of claim 23, wherein the exogenous polynucleotide comprises a sequence comprising an SFFV promoter and an expression cassette described in a formula, oriented from 5' to 3', comprising

S1 – E1 – L – S2 – E2

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide, wherein the first signal peptide is a human IL12 signal peptide;

E1 comprises a polynucleotide sequence encoding a first effector molecule, wherein the first effector molecule is a human IL12p70 fusion protein;

L comprises a linker polynucleotide sequence, wherein the linker polynucleotide sequence encodes Furin recognition polypeptide sequence, a Gly-Ser-Gly polypeptide sequence, and a T2A ribosome skipping tag in a Furin:Gly-Ser-Gly:T2A orientation from N-terminus to C-terminus;

S2 comprises a polynucleotide sequence encoding a second signal peptide, wherein the second signal peptide is a human IL21 signal peptide;

E2 comprises a polynucleotide sequence encoding a second effector molecule, wherein the second effector molecule is human IL21; and

wherein the SFFV promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, optionally wherein the polynucleotide sequence comprises the polynucleotide sequence shown in SEQ ID NO: 144.

25. A method of inducing an immune response in a subject, the method comprising delivering to a subject a composition comprising cells engineered to produce multiple effector molecules that modulate tumor-mediated immunosuppressive mechanisms, in an effective amount to induce an immune response, wherein each engineered cell comprises:

a) a promoter; and

b) an exogenous polynucleotide sequence comprising an expression cassette described in a formula, oriented from 5’ to 3’, comprising

S1 – E1 – L – S2 – E2

wherein

S1 comprises a polynucleotide sequence encoding a first signal peptide,

E1 comprises a polynucleotide sequence encoding a first effector molecule,

L comprises a linker polynucleotide sequence,

S2 comprises a polynucleotide sequence encoding a second signal peptide,

E2 comprises a polynucleotide sequence encoding a second effector molecule, and

wherein the promoter is operably linked to the expression cassette, the first signal peptide is operably linked to the first effector molecule, and the second signal peptide is operably linked to the second effector molecule, and

wherein the engineered cell is selected from the group consisting of: a mesenchymal stem cell (MSC), stem cell, immune cell, natural killer (NK) cell, NKT cell, innate lymphoid cell, tumor-infiltrating lymphocyte (TIL), mast cell, eosinophil, basophil, monocyte, macrophage, neutrophil, myeloid cell, dendritic cell, T cell, CD8+ T cell, CD4+ T cell, cytotoxic T lymphocyte (CTL), viral-specific T cell, gamma-delta T cell, T regulatory cell, and B cell.

26. The method of claim 25, wherein the construct comprises the polynucleotide sequence shown in SEQ ID NO: 144.

27. A method of inducing an immune response in a subject, the method comprising delivering to a subject a composition capable of engineering an cell to produce multiple effector molecules that modulate tumor-mediated immunosuppressive mechanisms, in an effective amount to induce an immune response, wherein the composition comprises the exogenous polynucleotide of claim 23 or claim 24.

28. The method of claim 27, wherein the composition comprises a delivery system selected from the group consisting of: a viral system, a transposon system, and a nuclease genomic editing system,
optionally wherein the viral system is selected from the group consisting of: a lentivirus, a retrovirus, a retrotransposon, and an adenovirus, and
optionally wherein the nuclease genomic editing system is selected from the group consisting of: a zinc-finger system, a TALEN system, and a CRISPR system.

29. The method of any one of claims 25-28, wherein the method further comprises administering a checkpoint inhibitor and/or an anti-CD40 antibody, optionally wherein the checkpoint inhibitor is an anti-PD-1 antibody, an anti-PD-1L antibody, or an anti-CTLA-4 antibody.

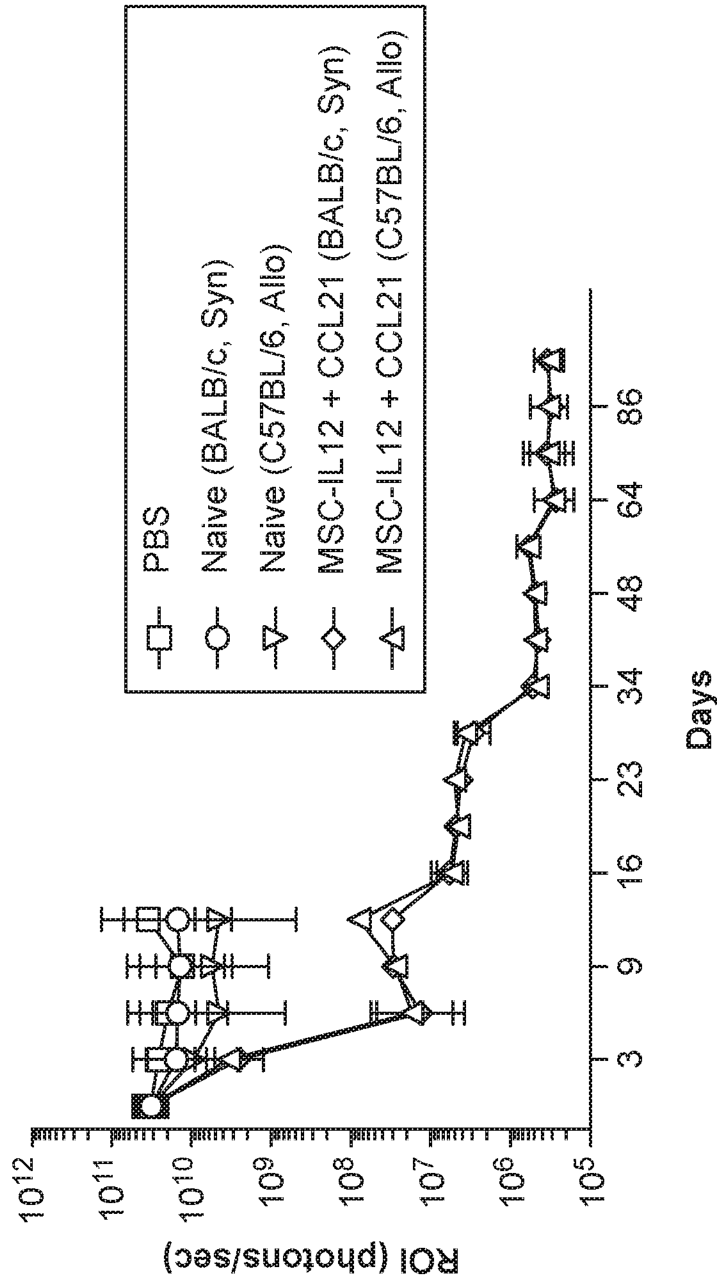


FIG. 1

FIG. 2A

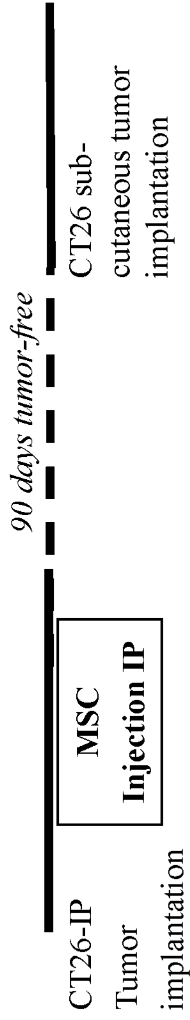
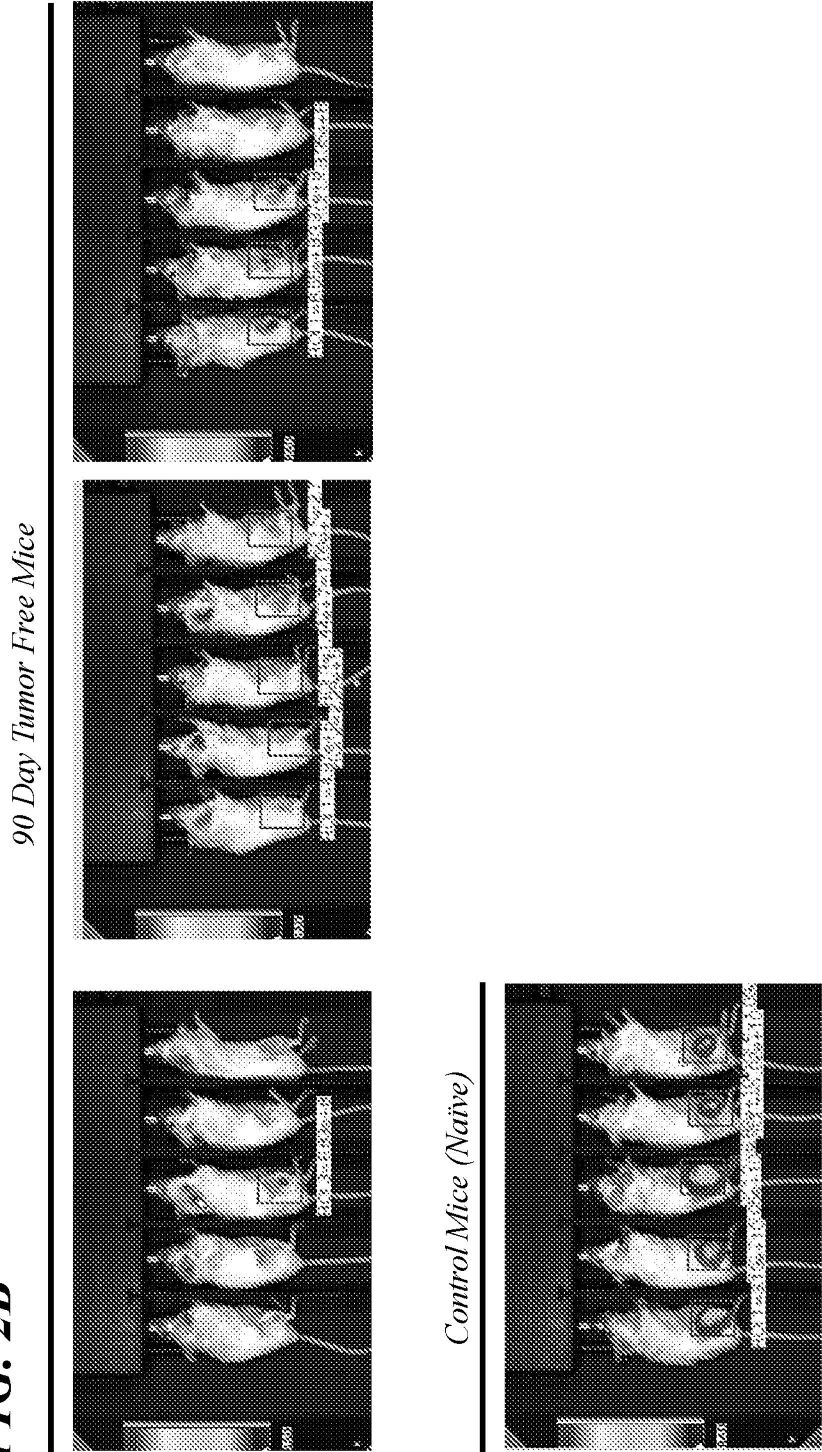


FIG. 2B



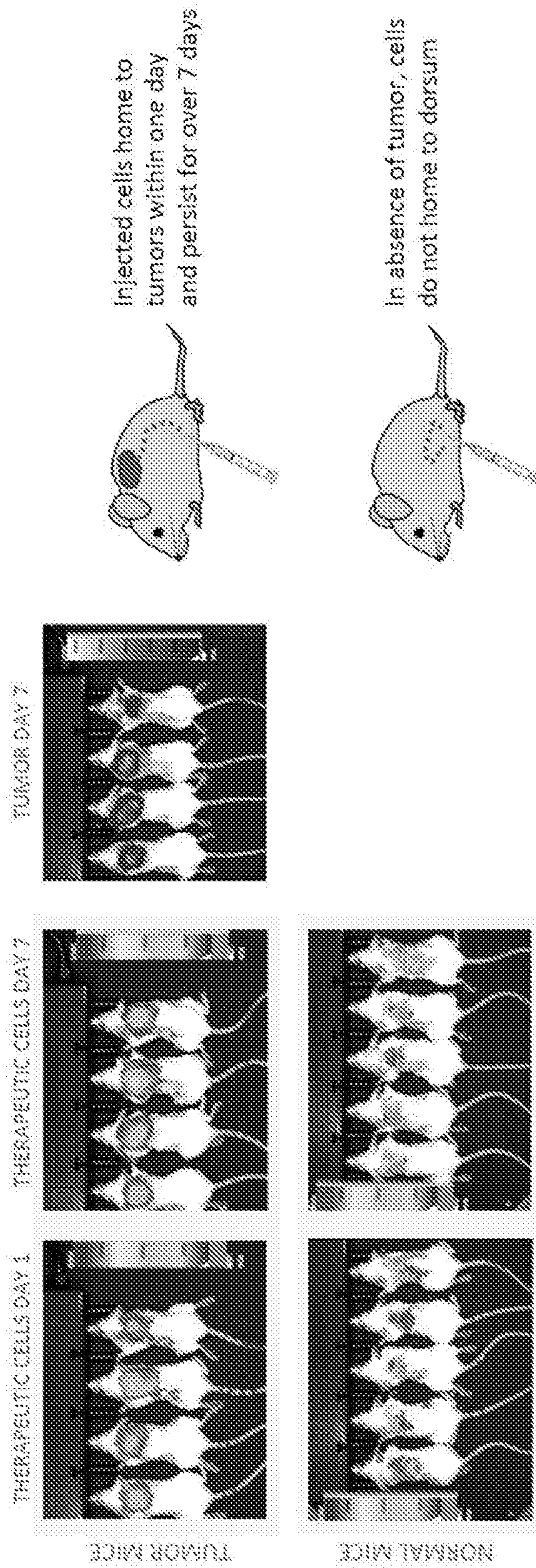
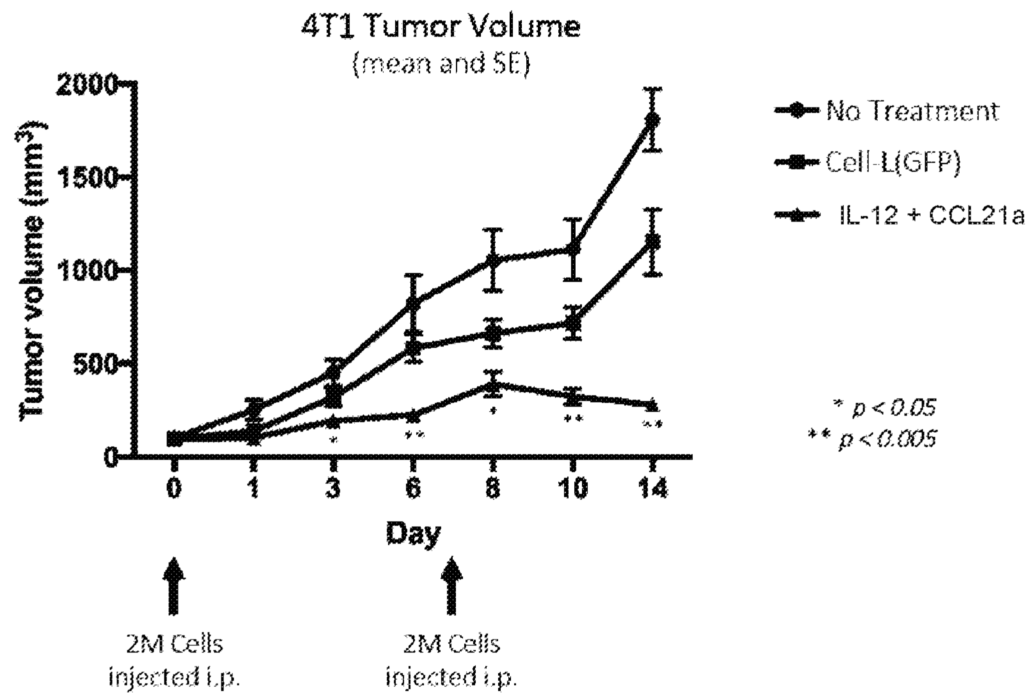


FIG. 3



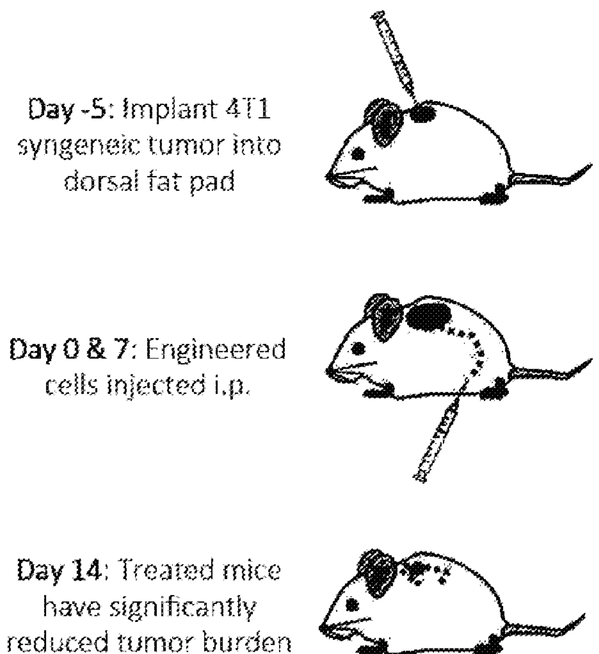


FIG. 4

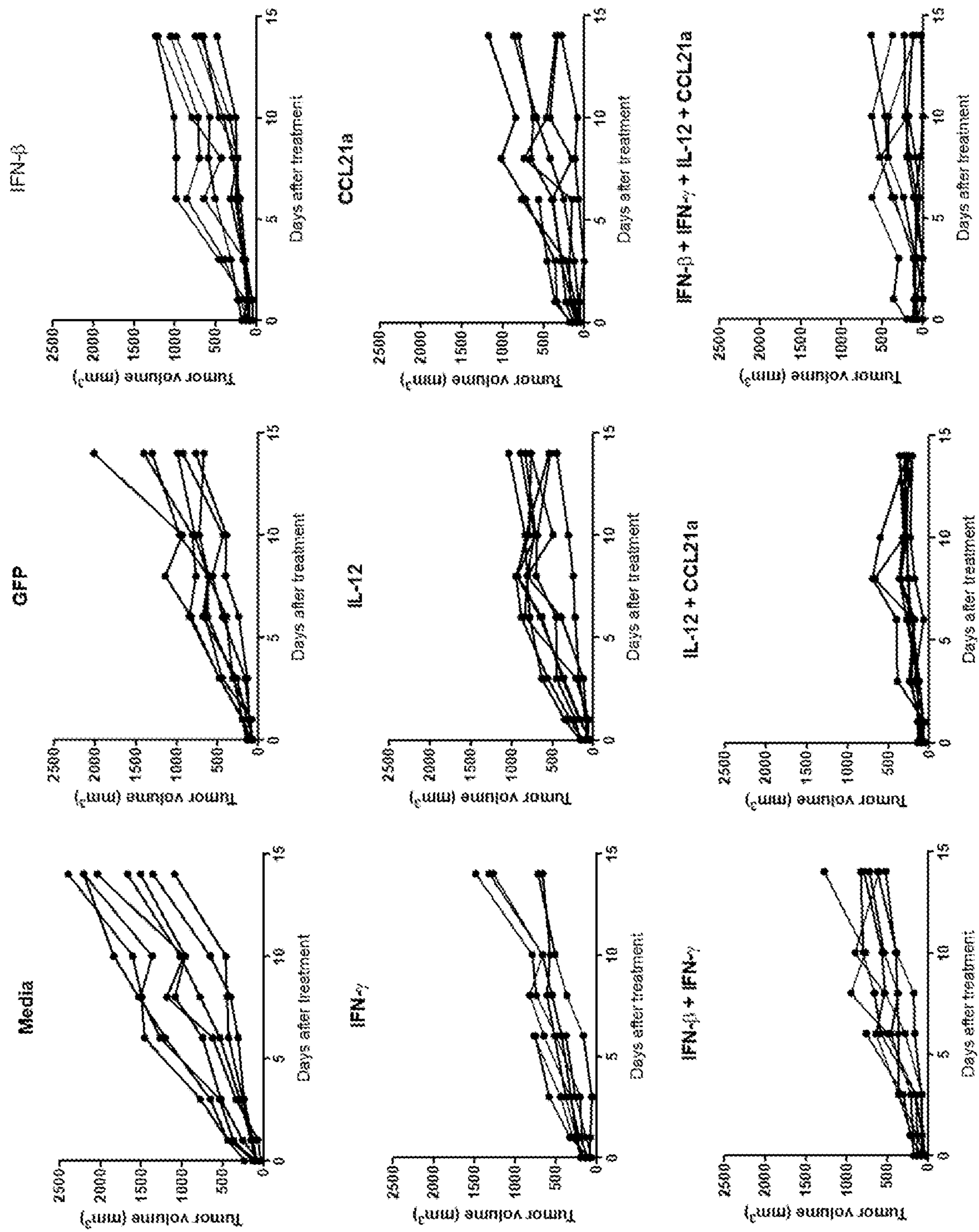


FIG. 5A

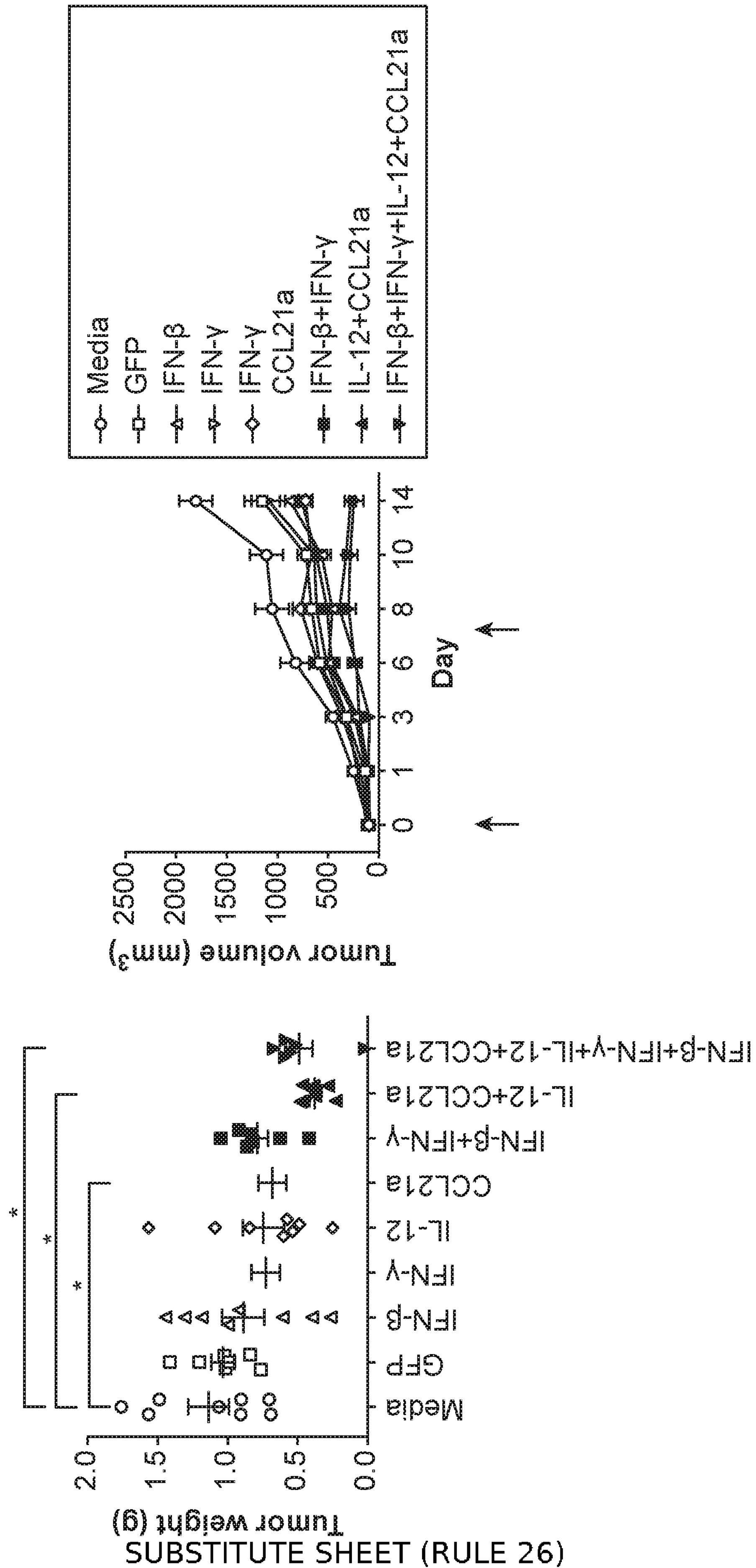


FIG. 5B

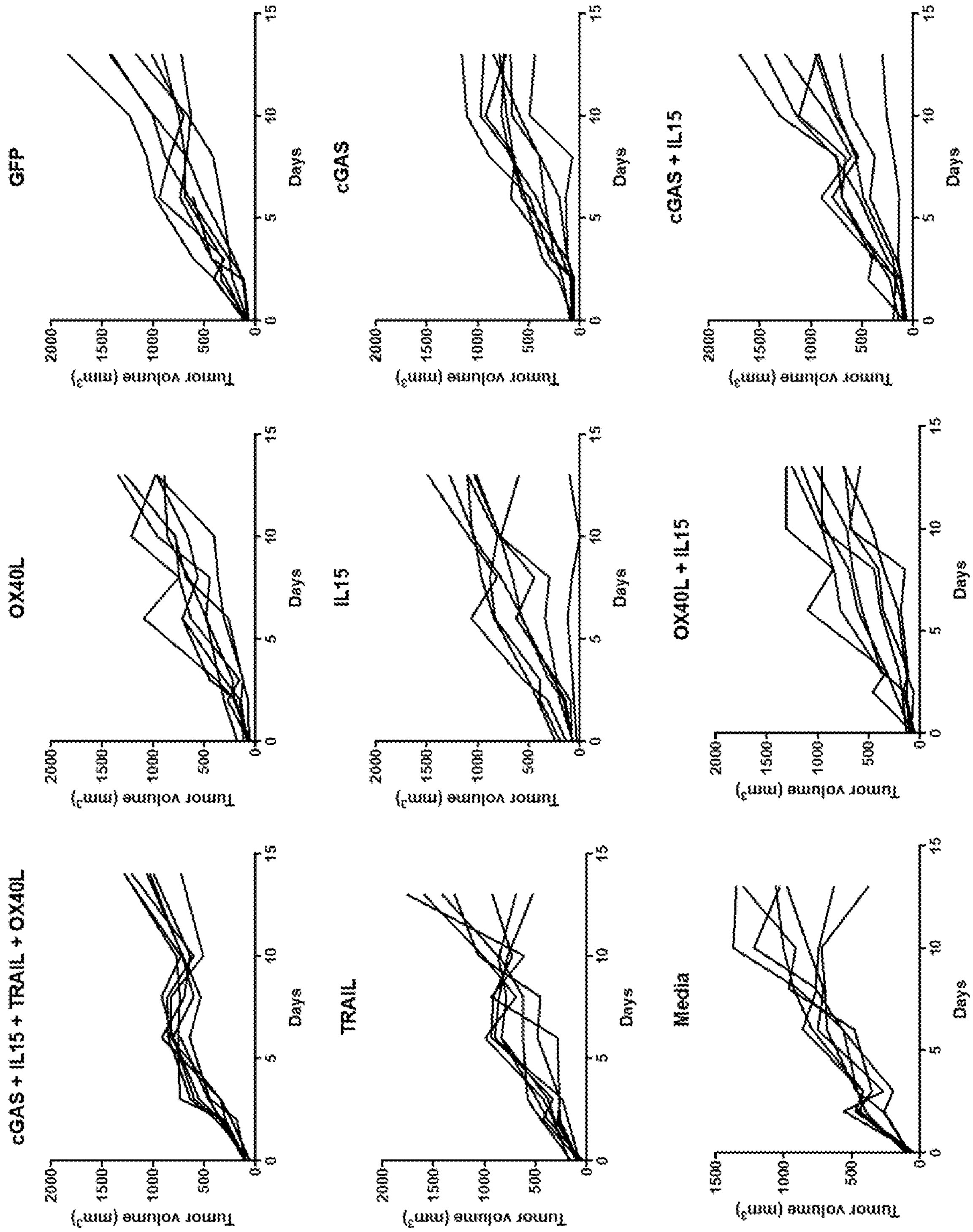


FIG. 6A

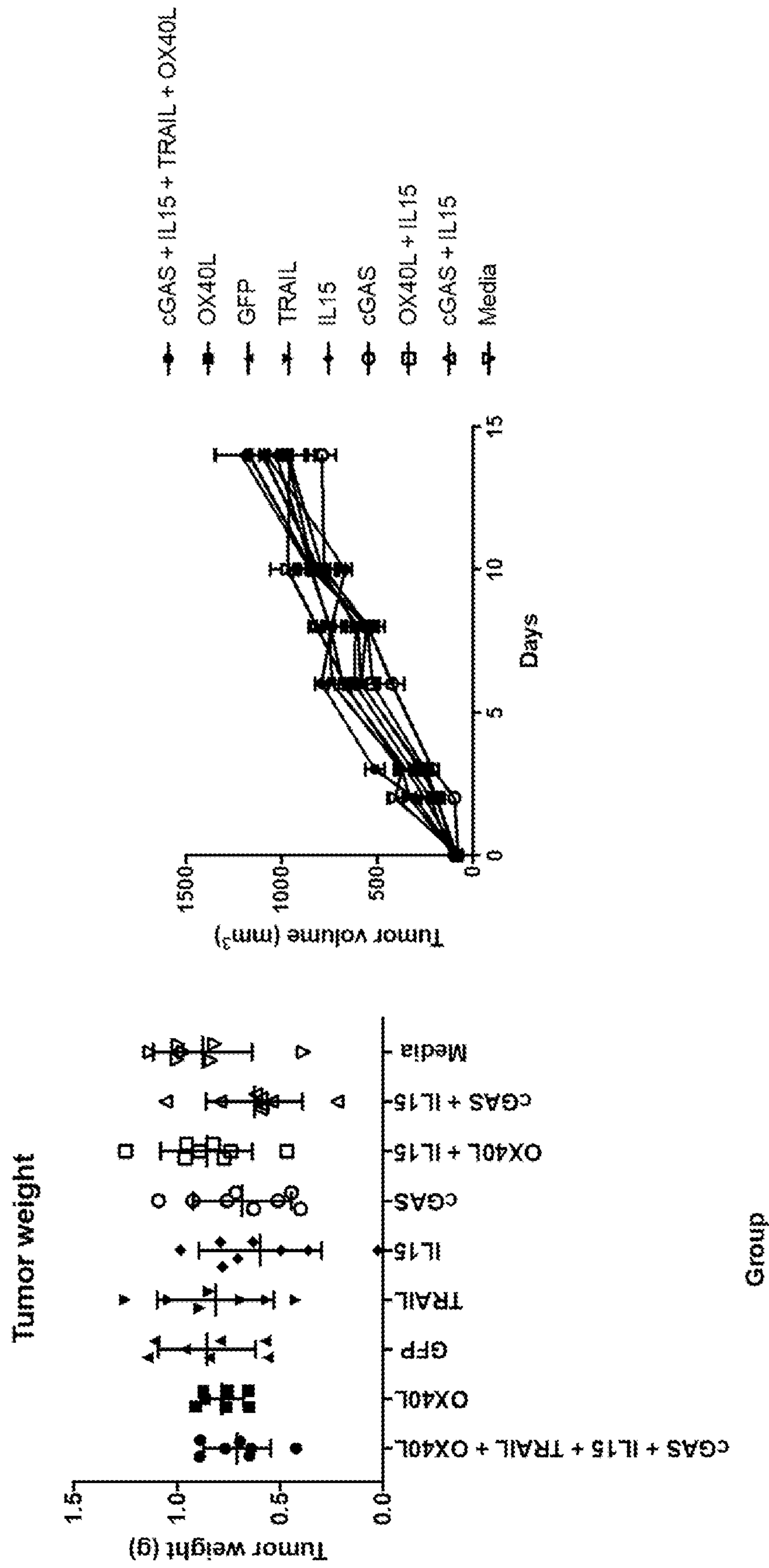


FIG. 6B

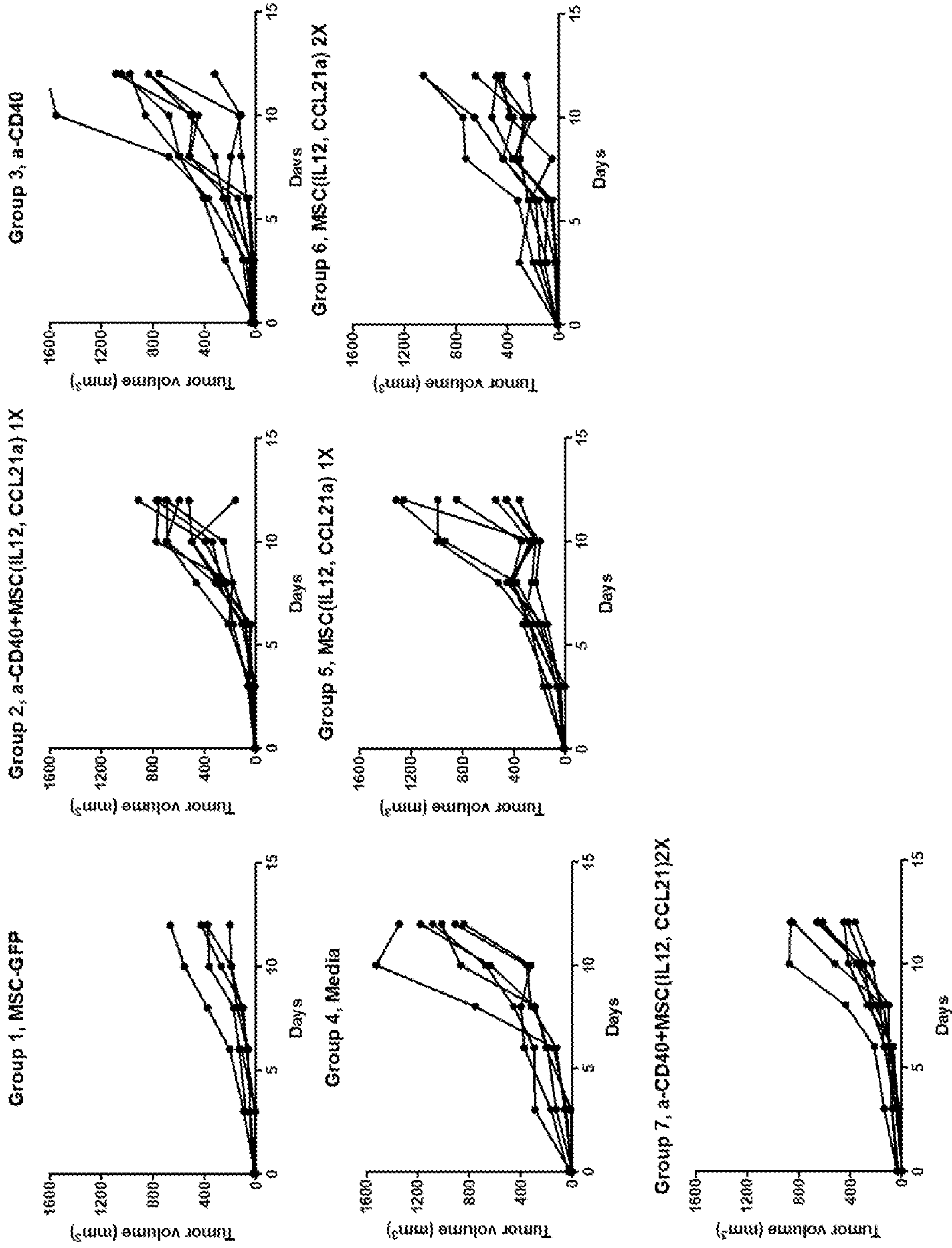


FIG. 7A

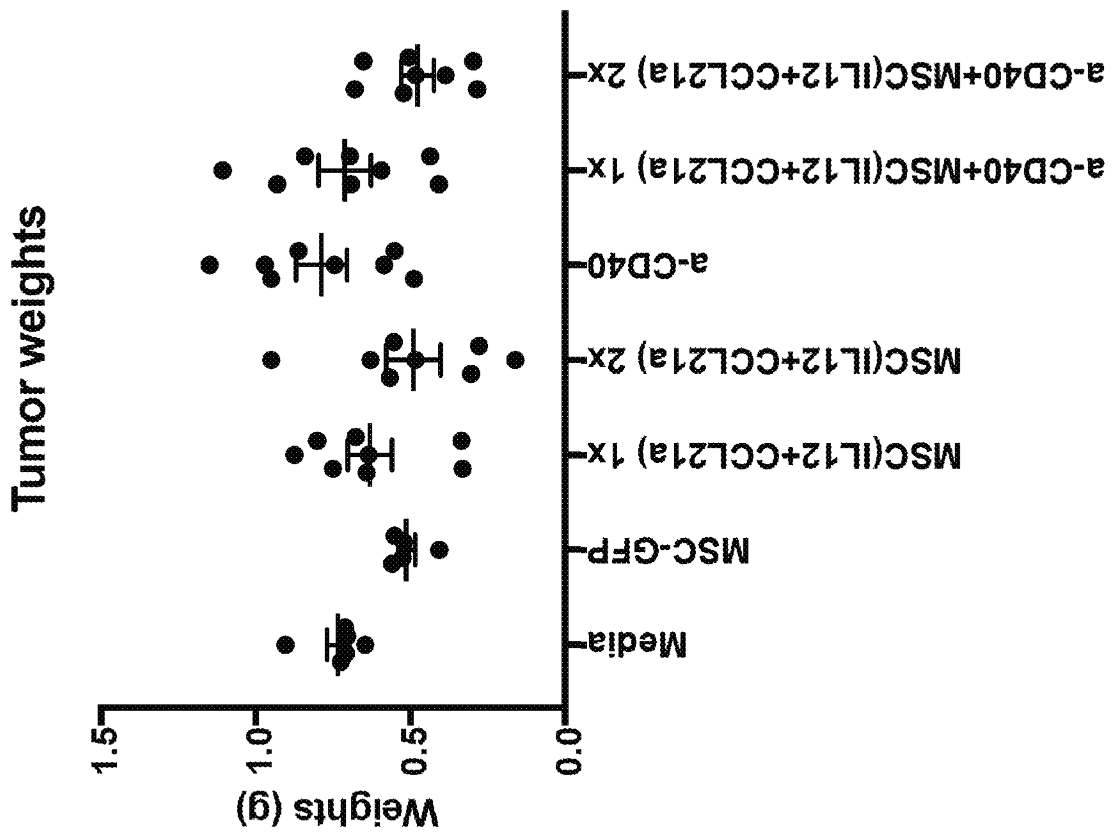


FIG. 7B

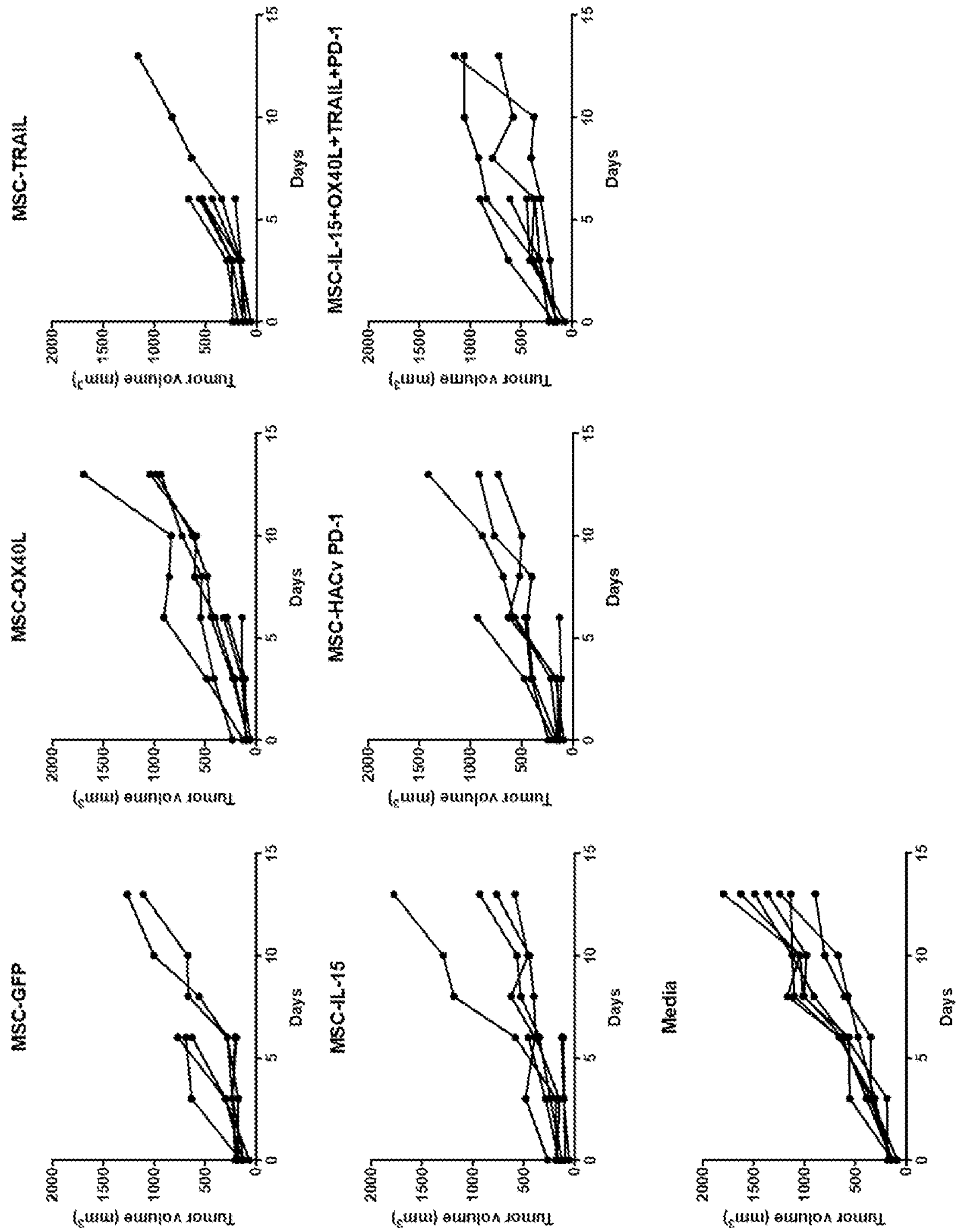


FIG. 8A

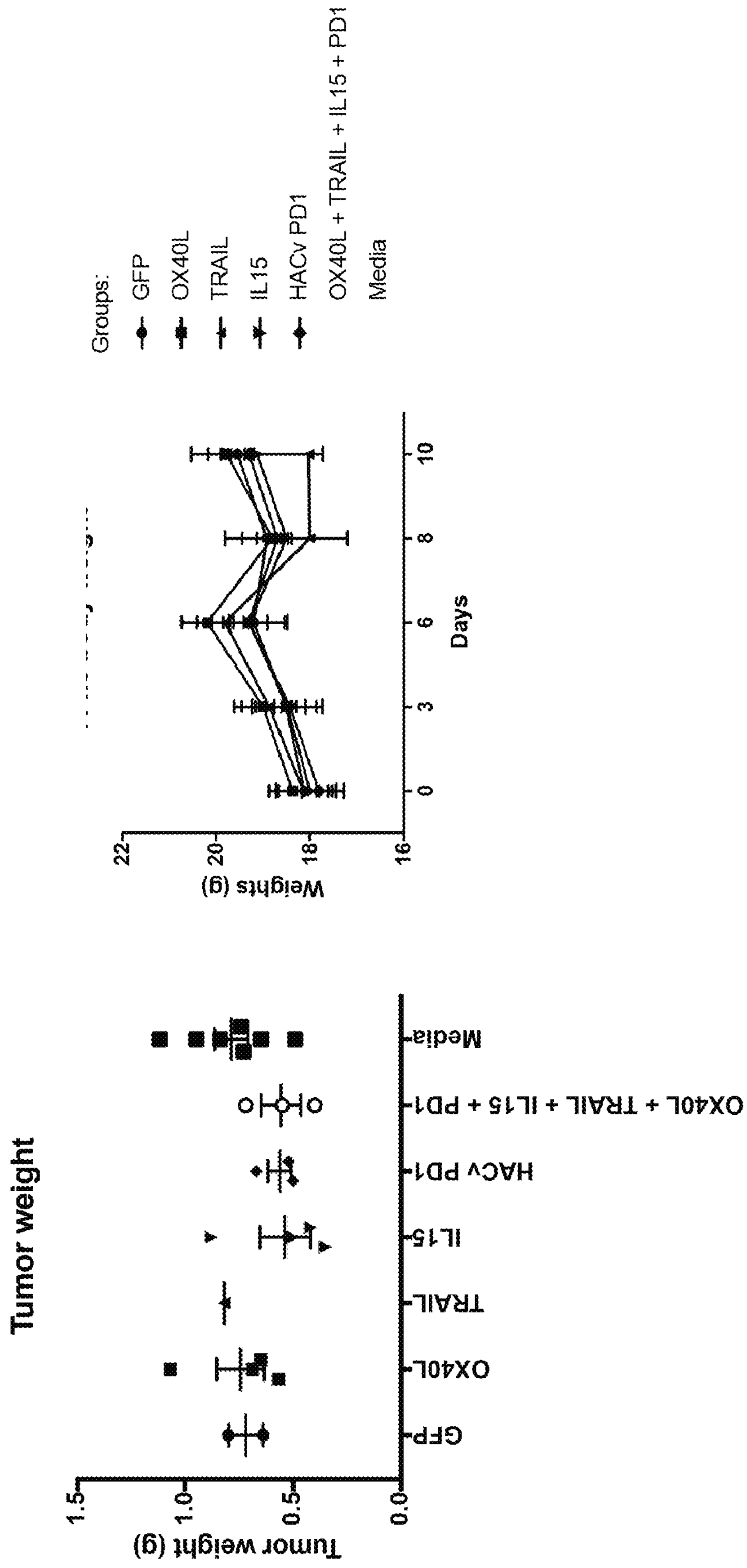


FIG. 8B

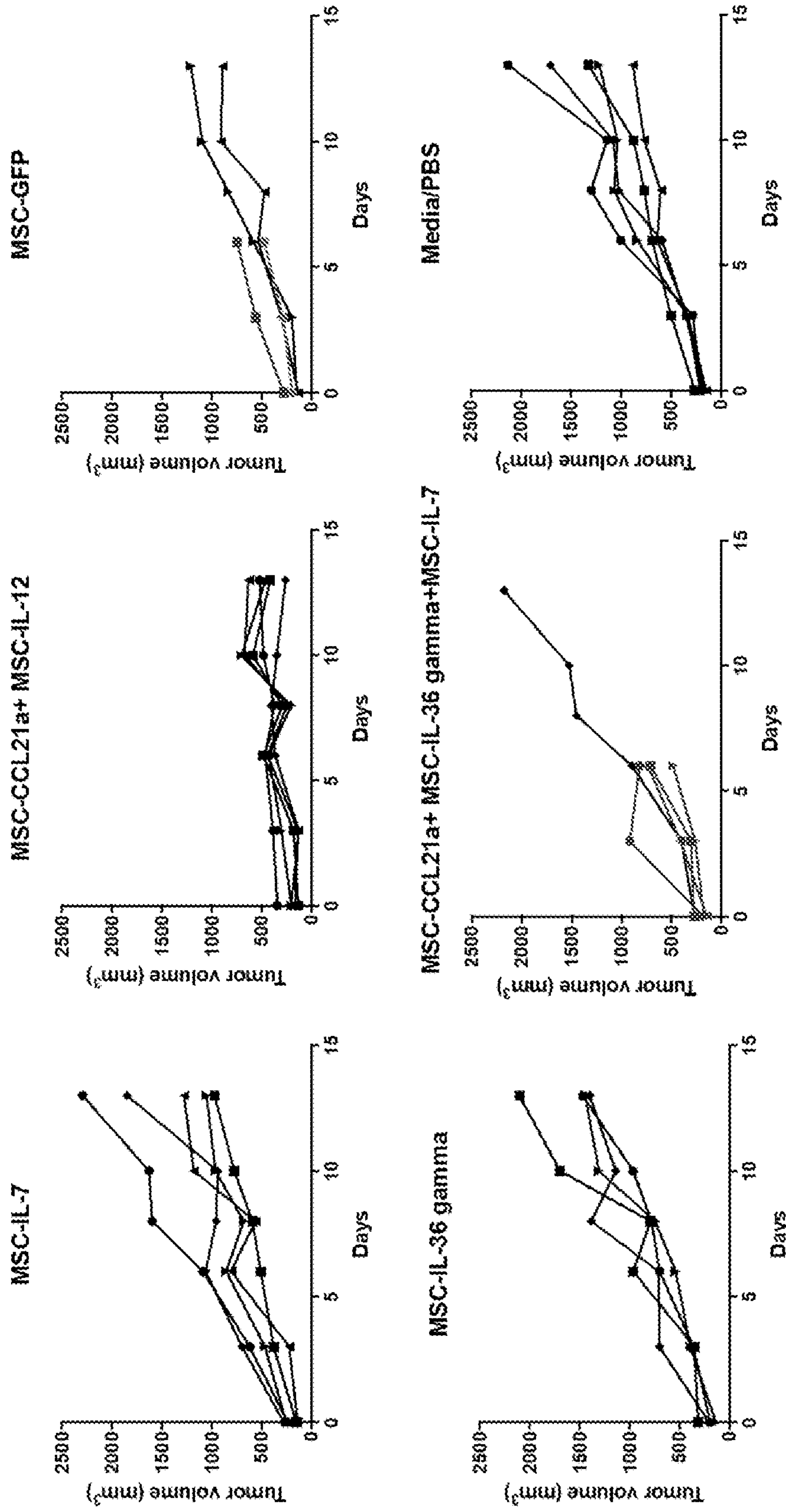


FIG. 9A

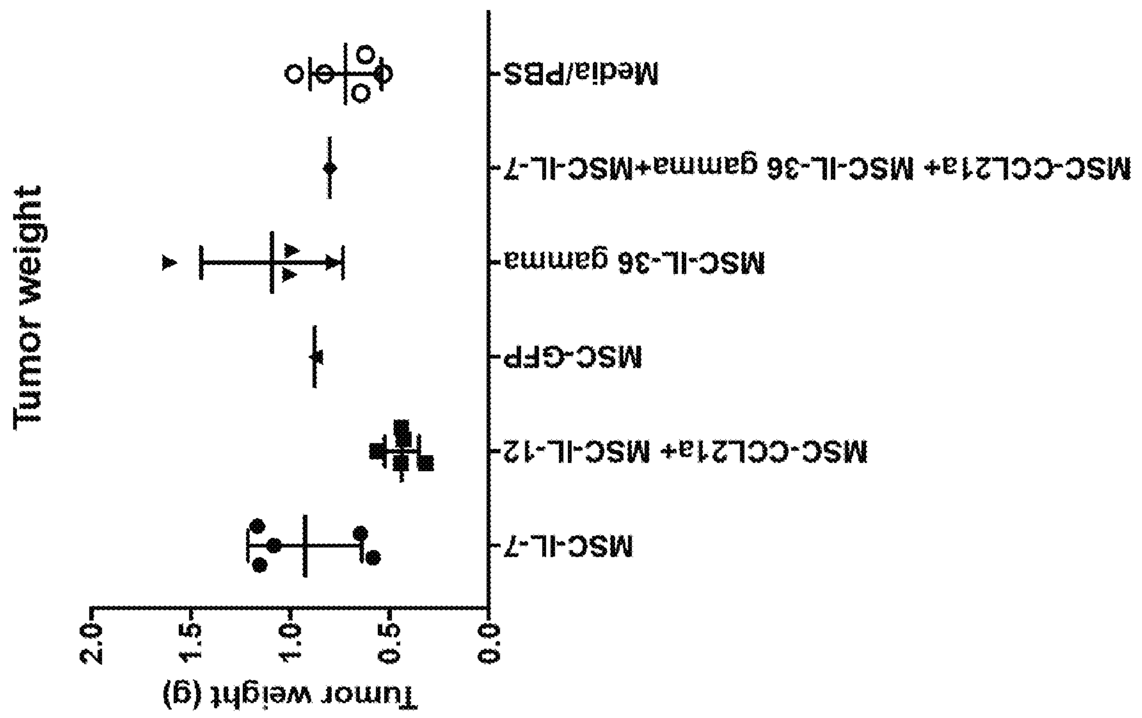


FIG. 9B

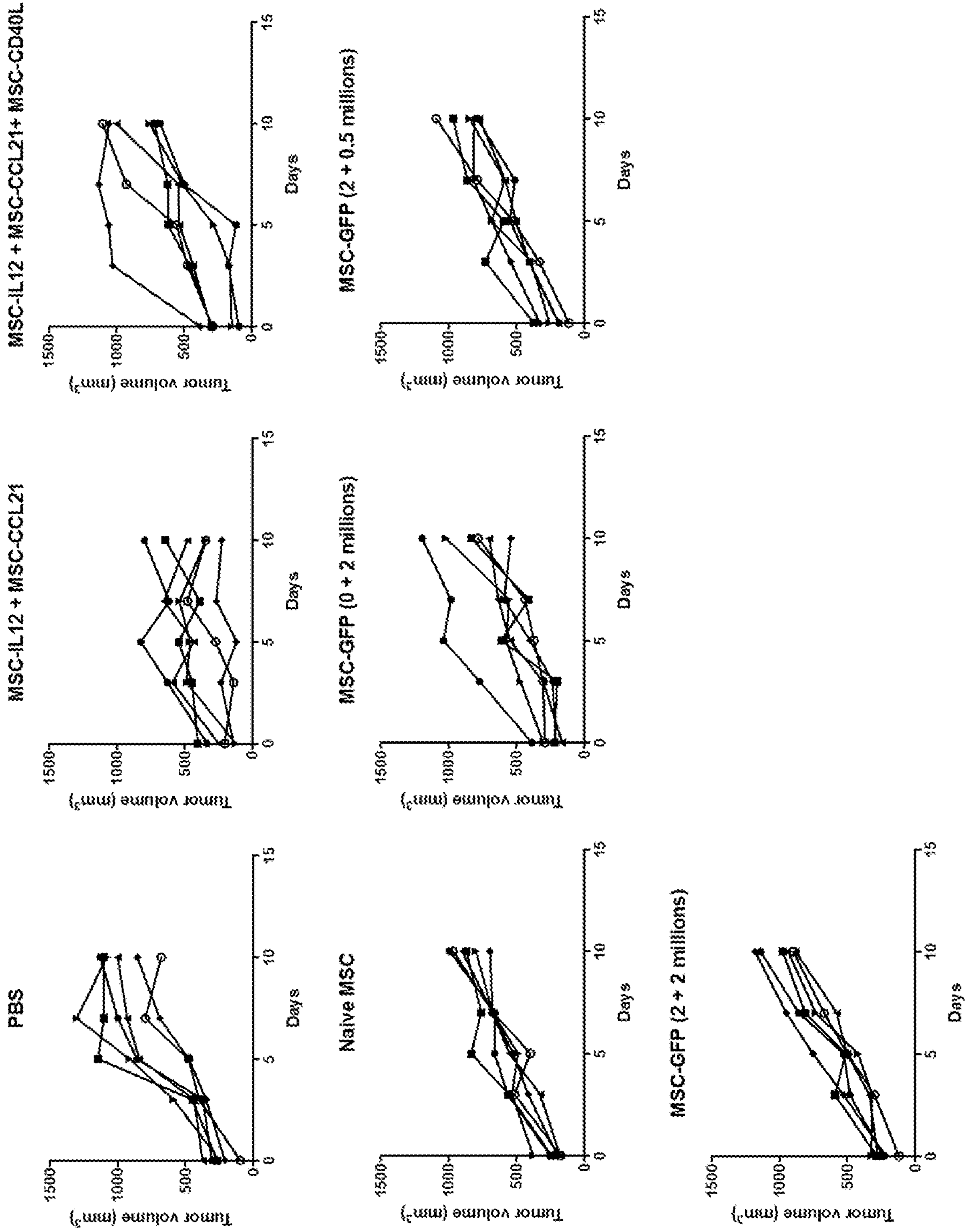


FIG. 10A

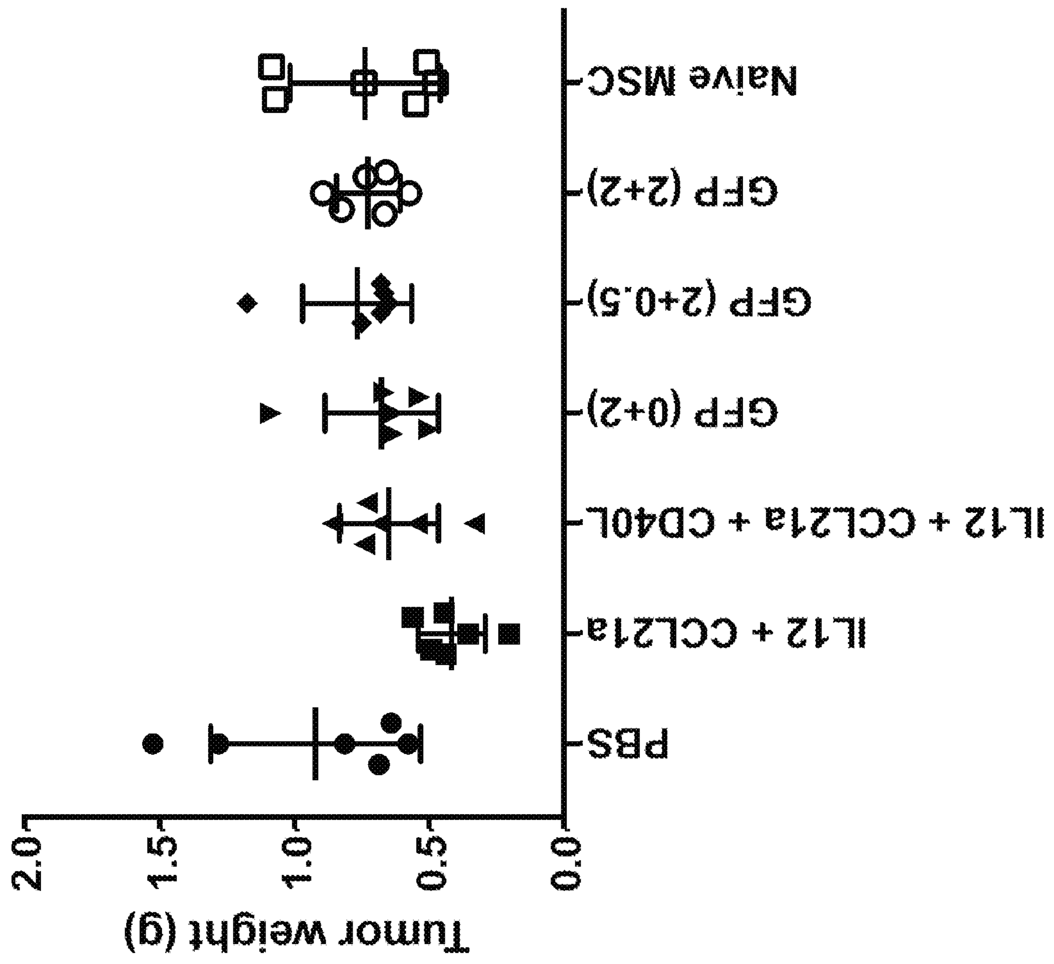


FIG. 10B

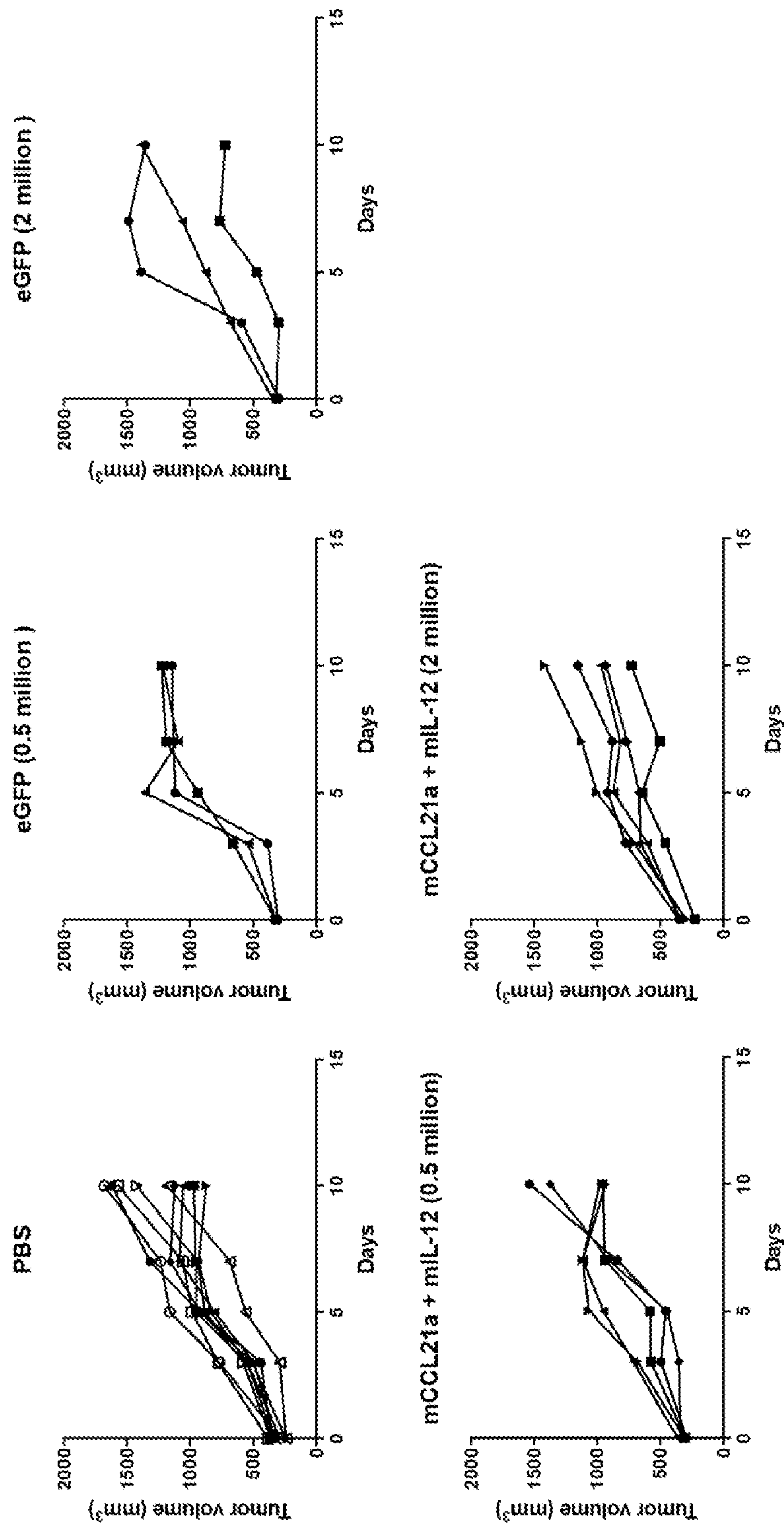


FIG. 11A

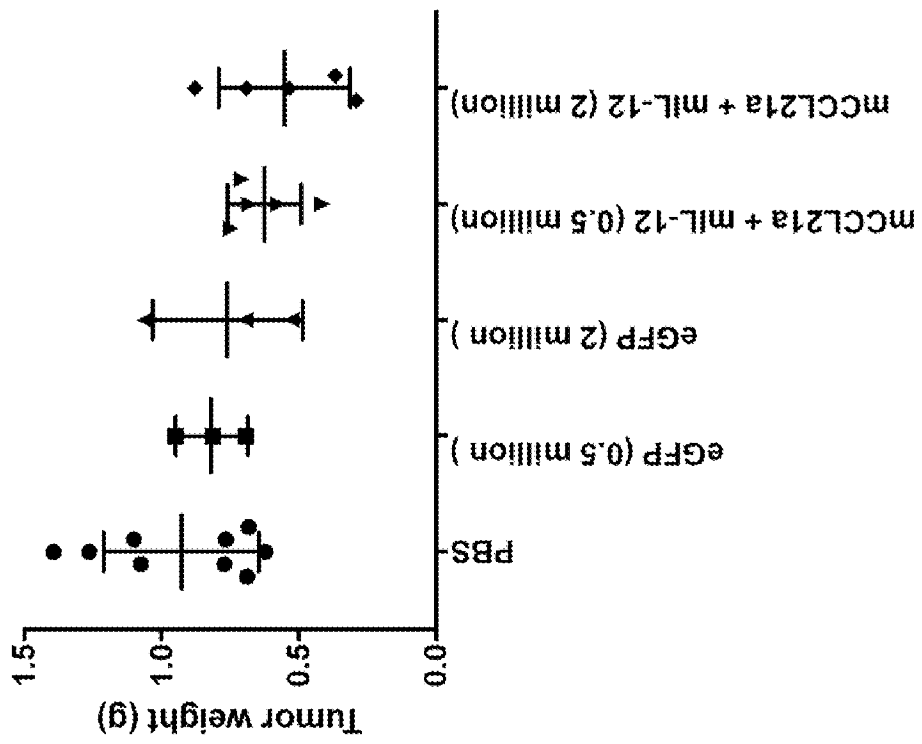


FIG. 11B

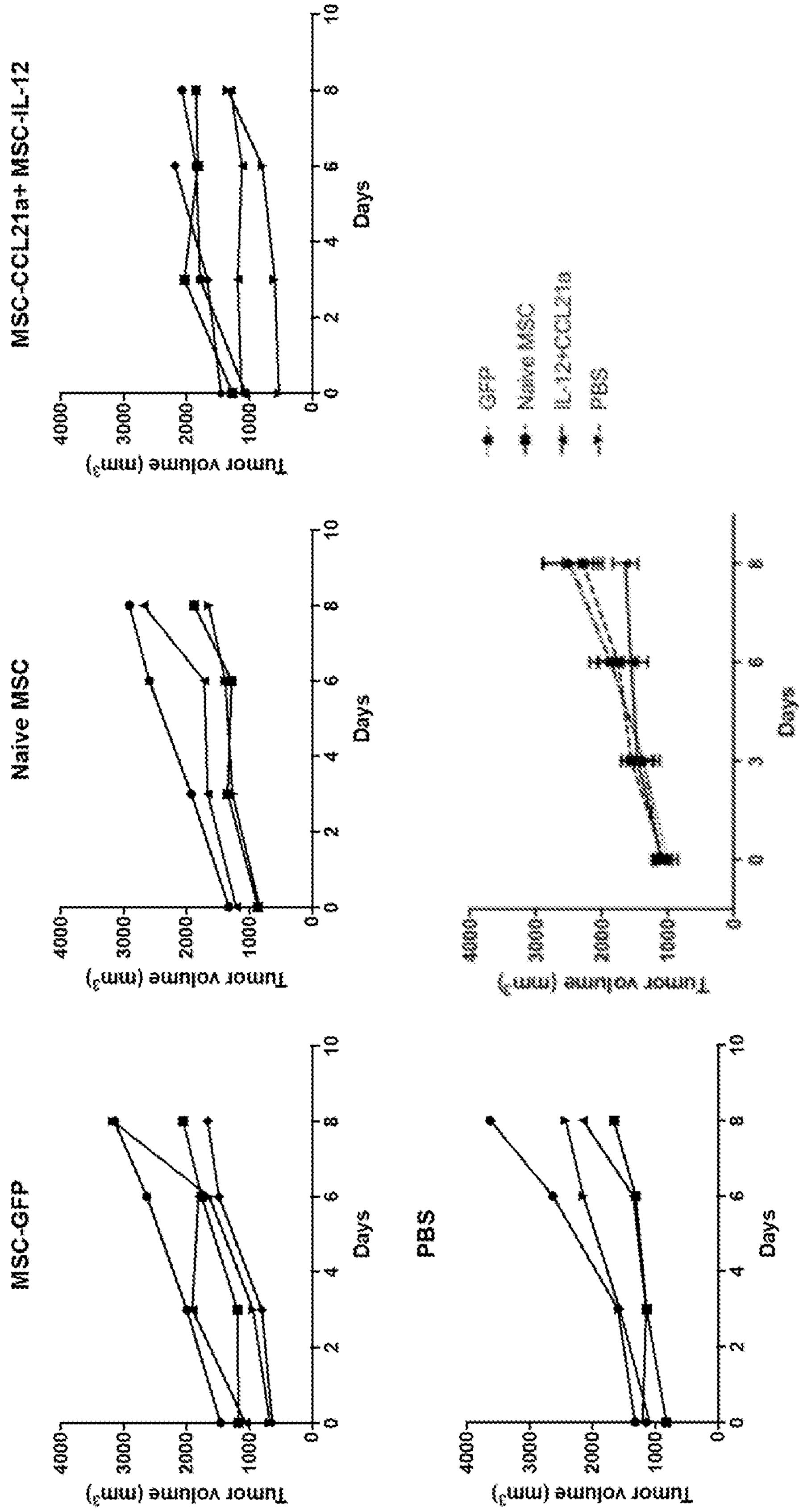


FIG. 12

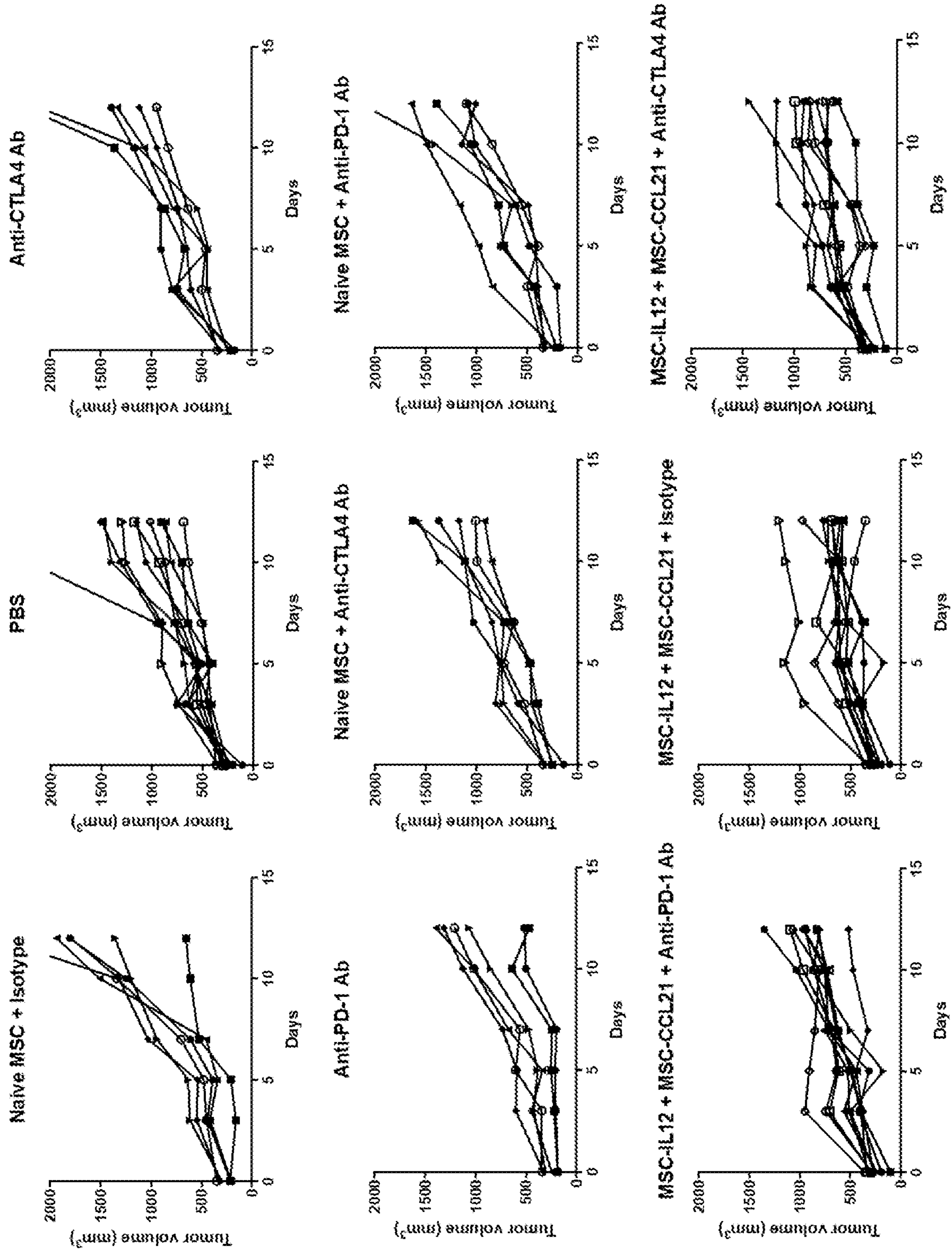


FIG. 13A

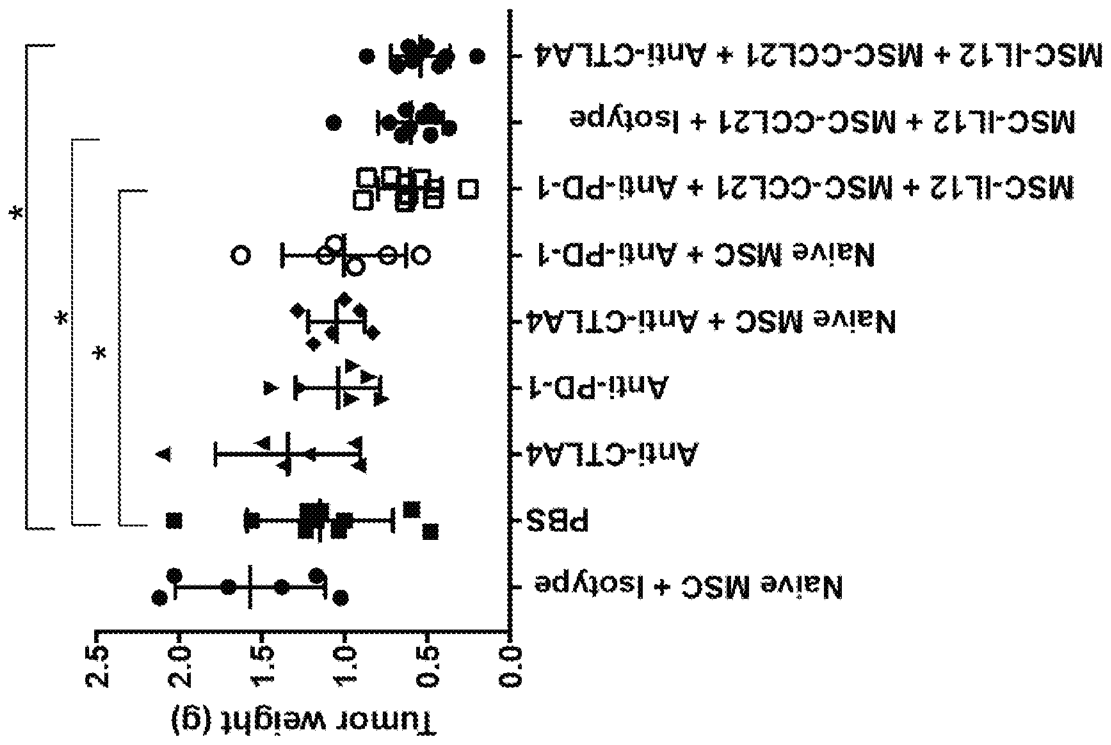
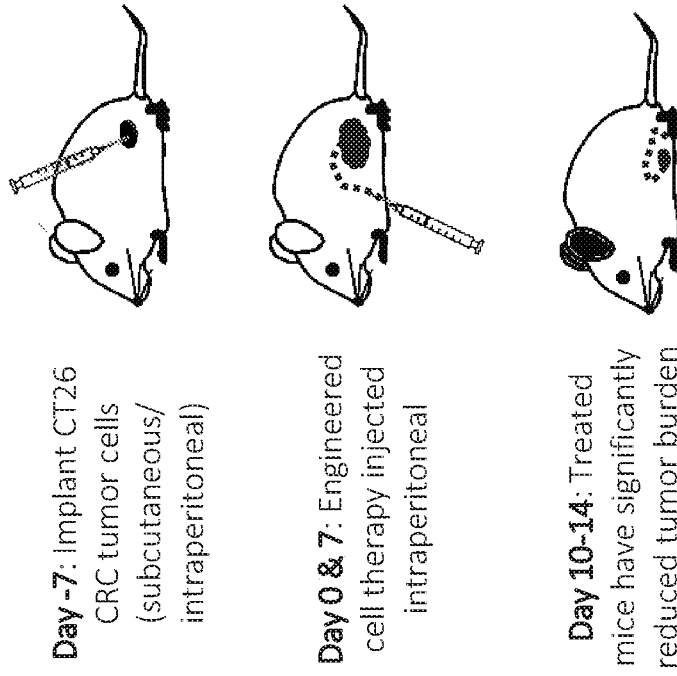


FIG. 13B



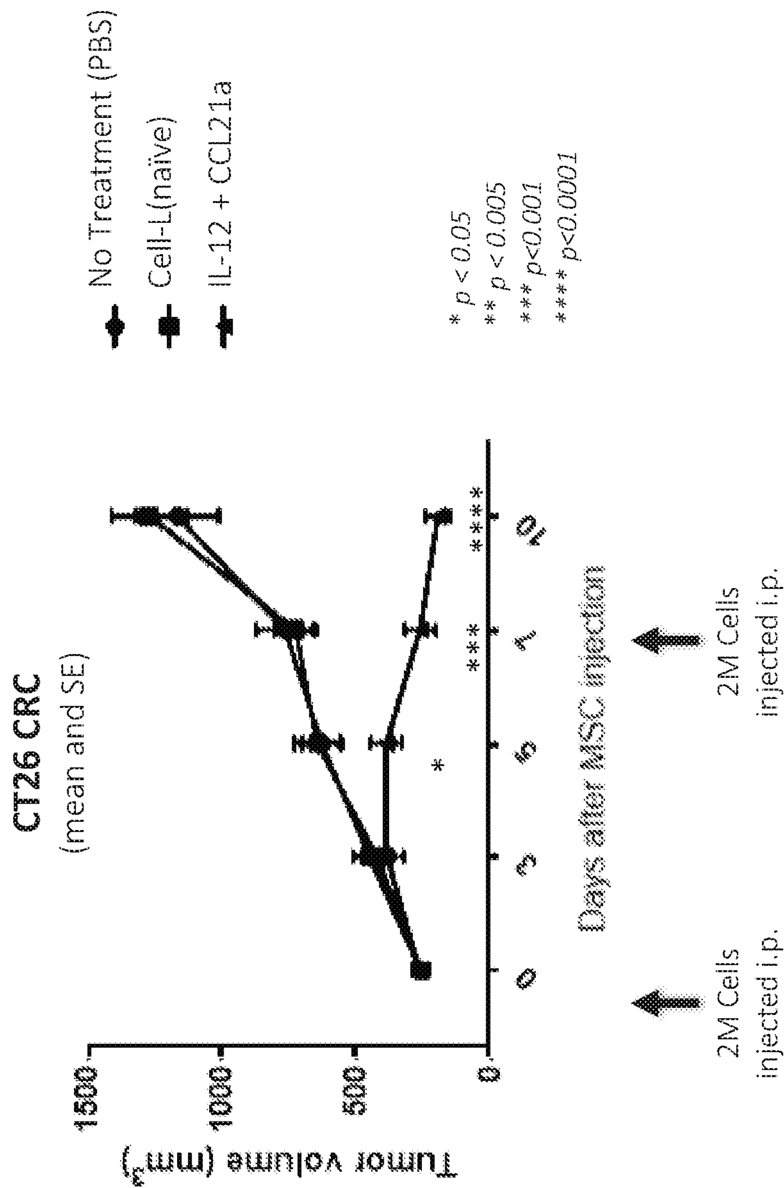


FIG. 14

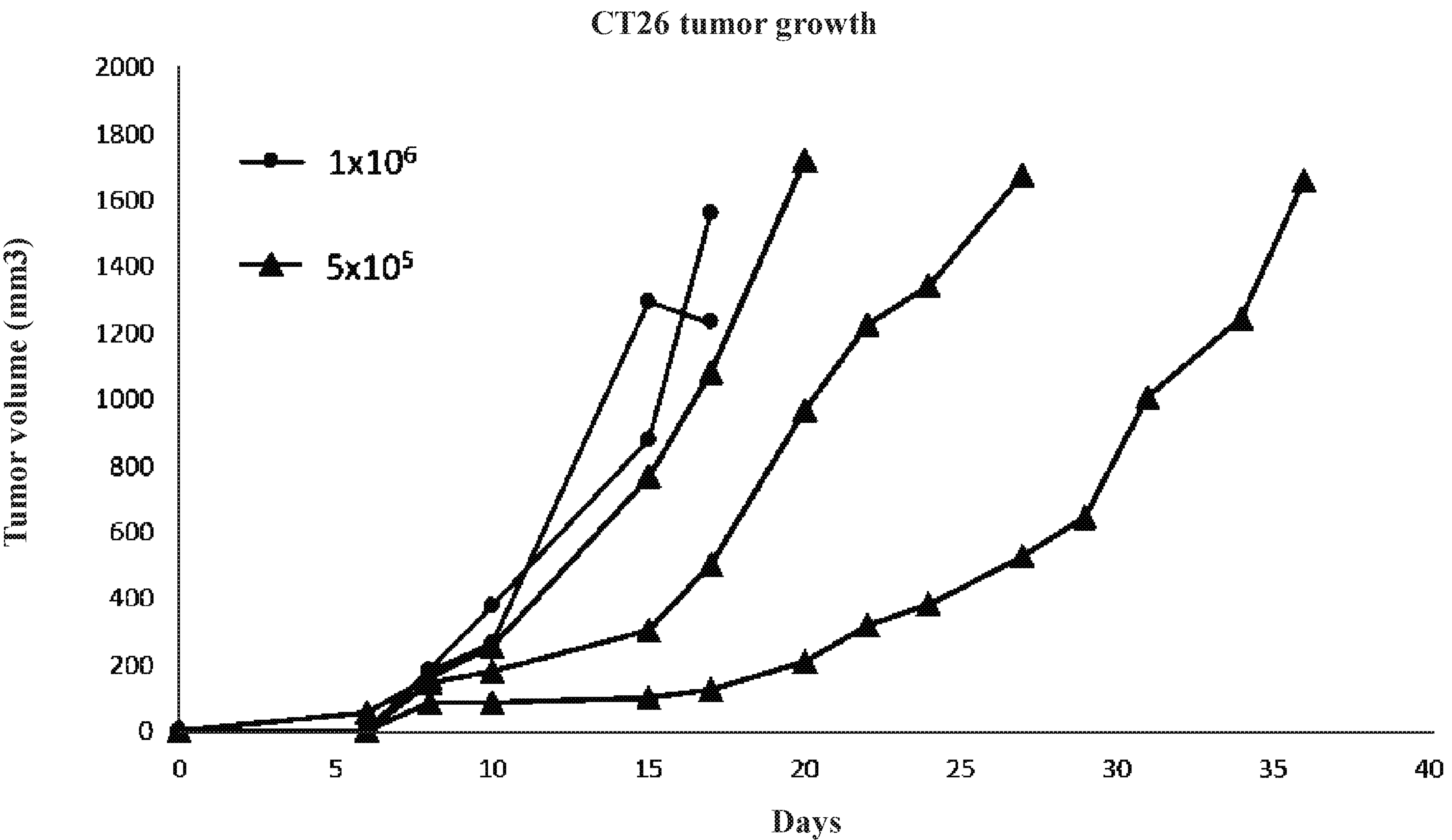


FIG. 15

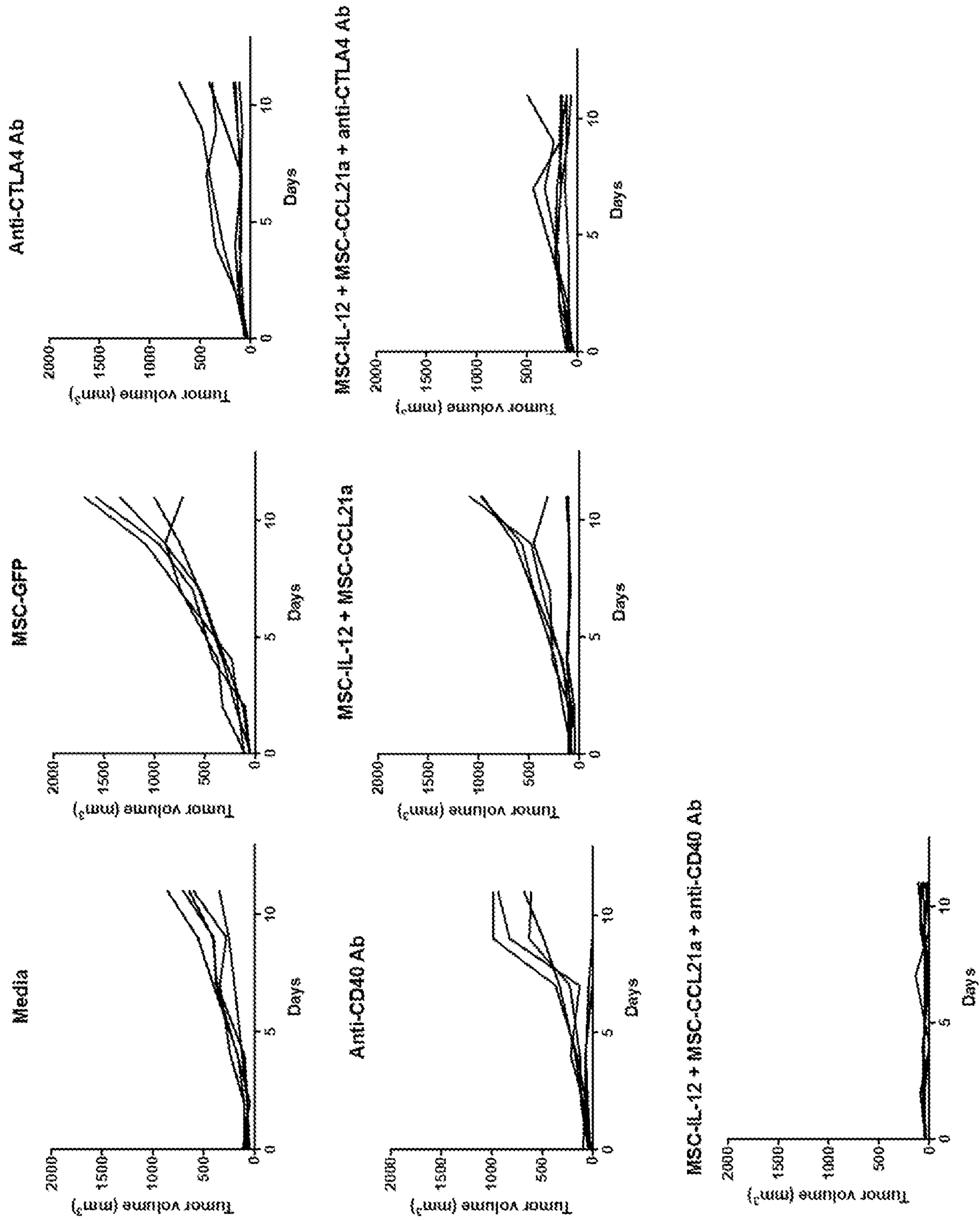


FIG. 16A

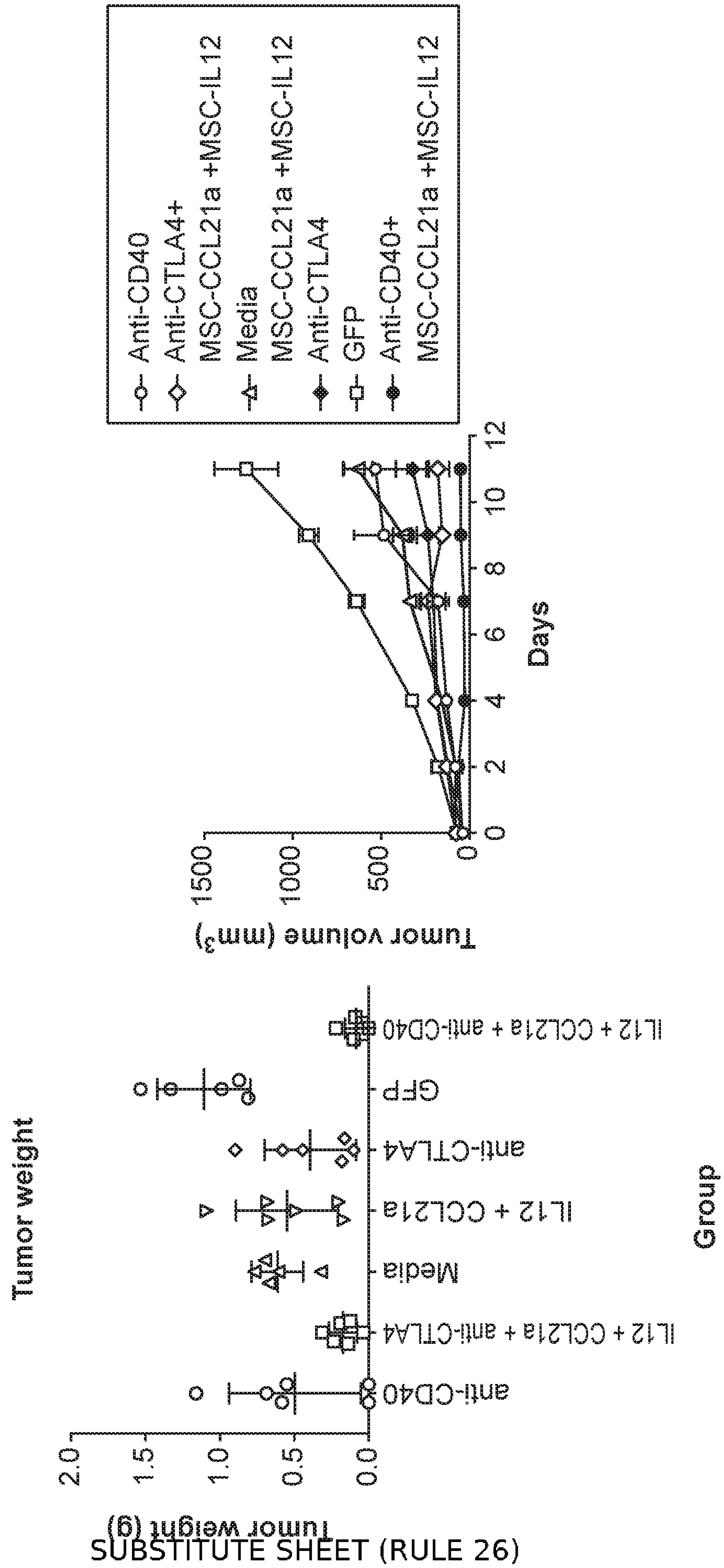


FIG. 16B

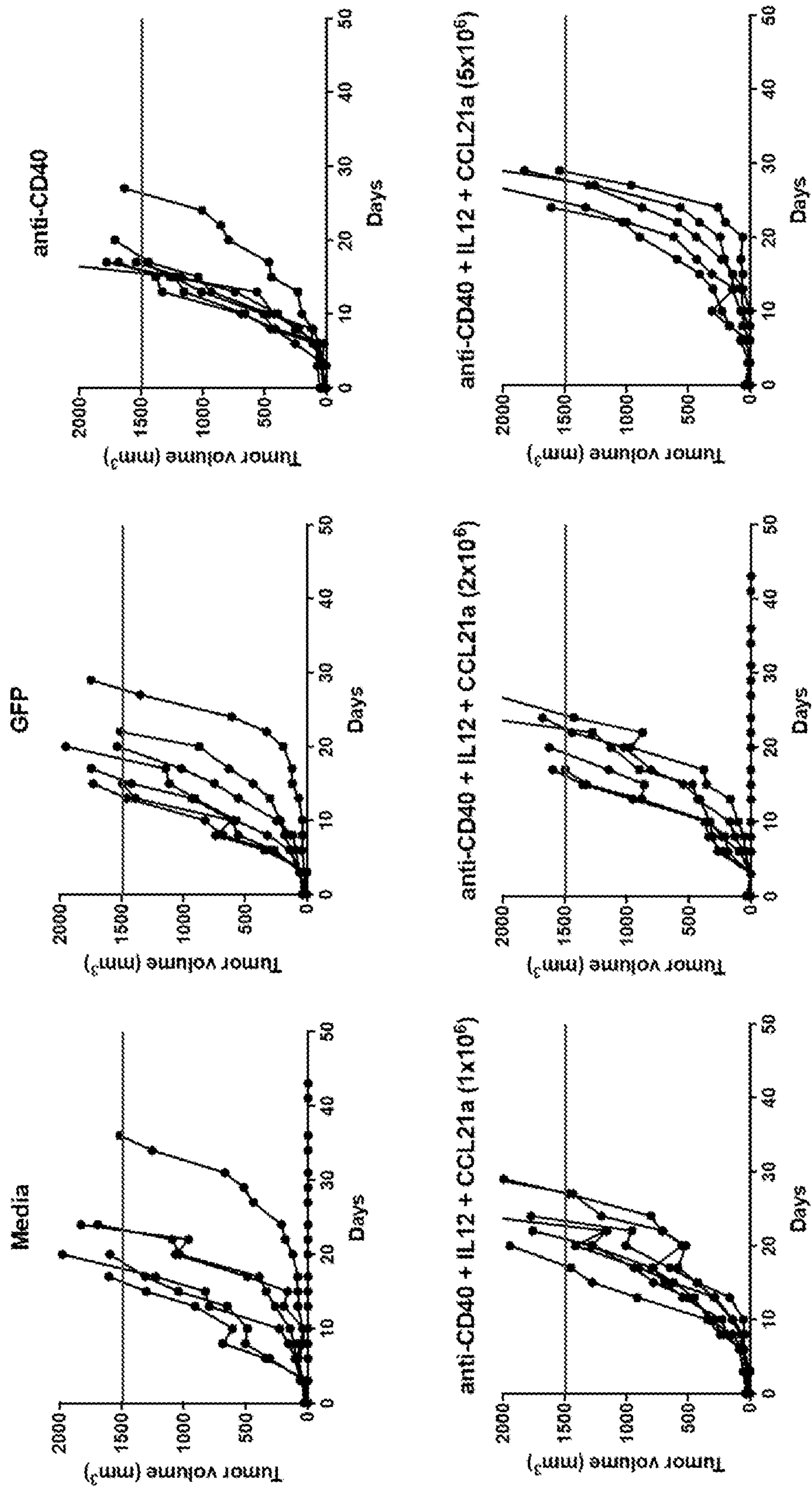


FIG. 17A

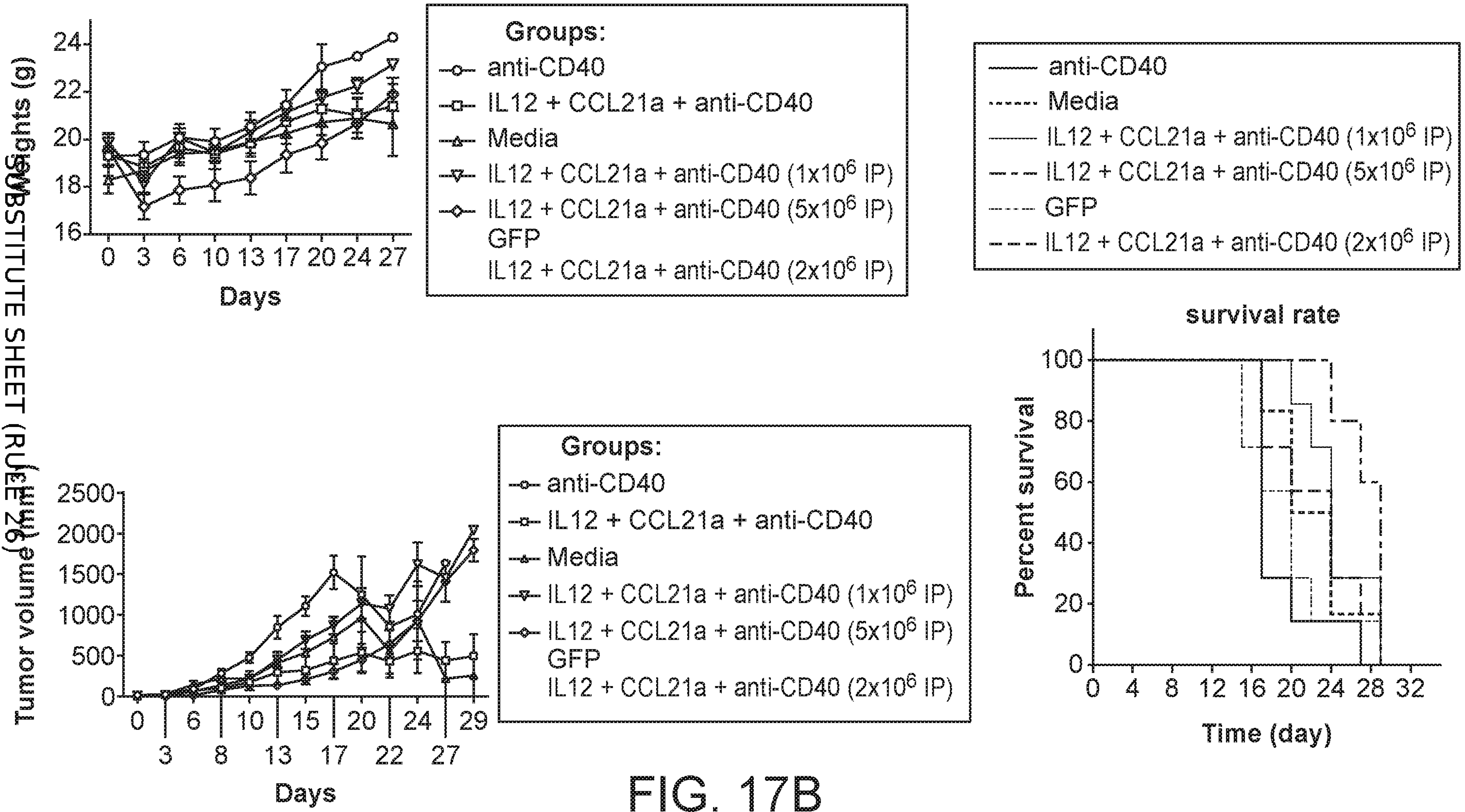


FIG. 17B

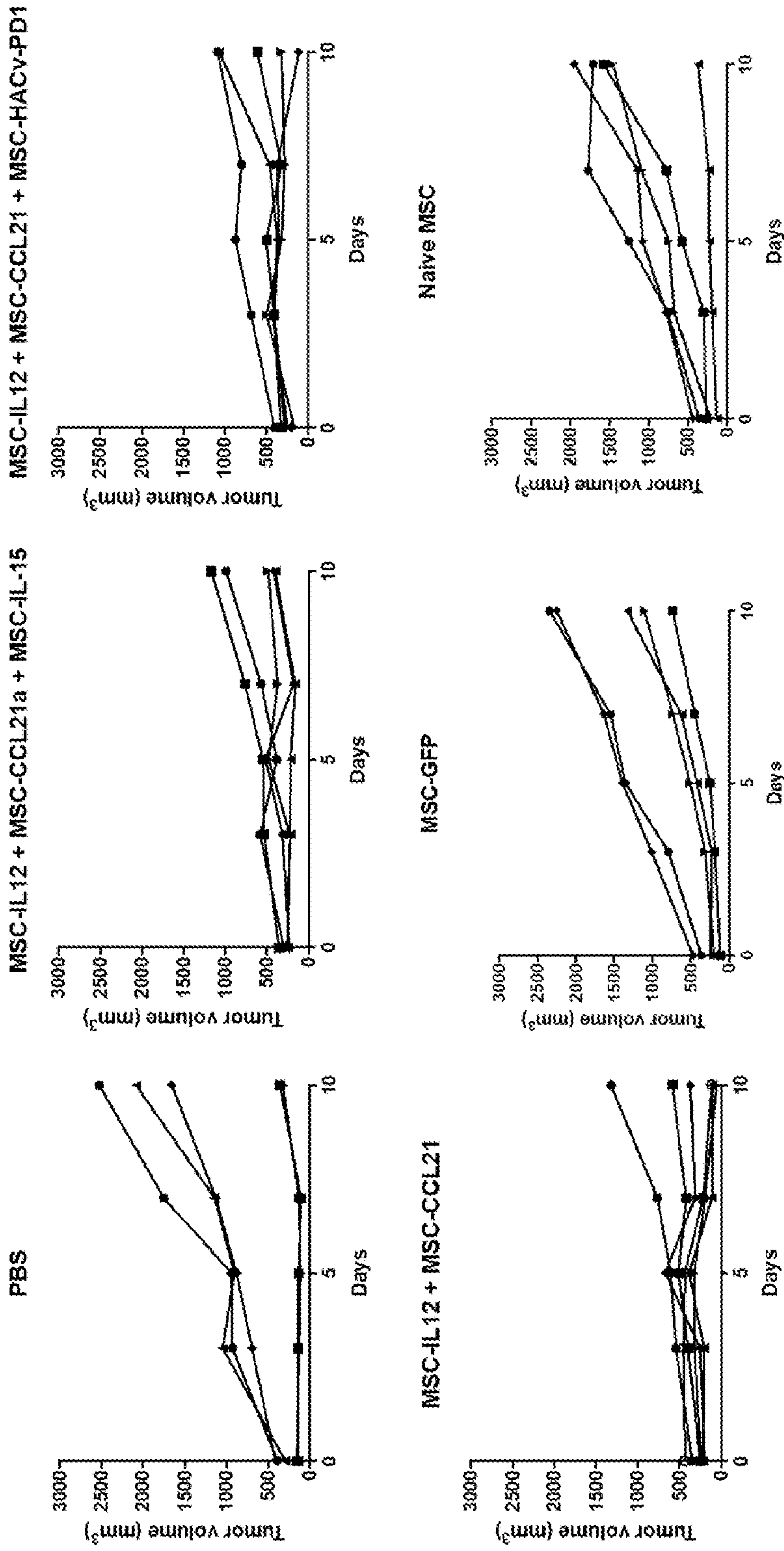


FIG. 18A

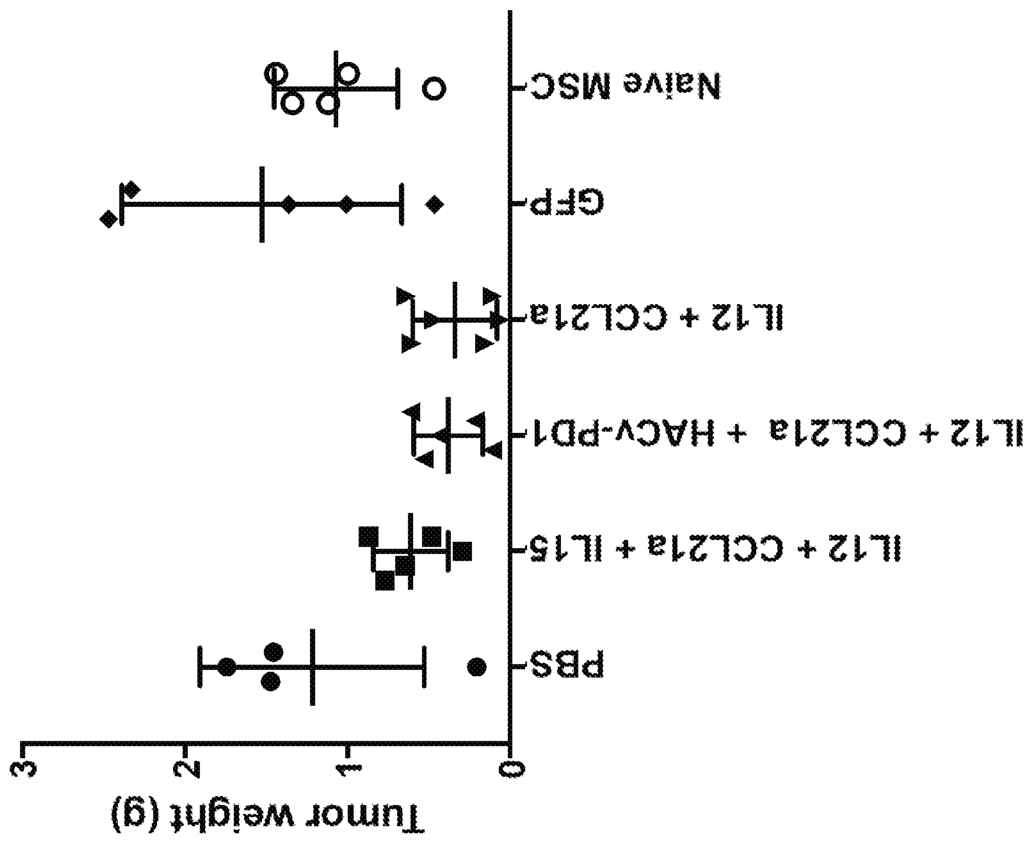


FIG. 18B

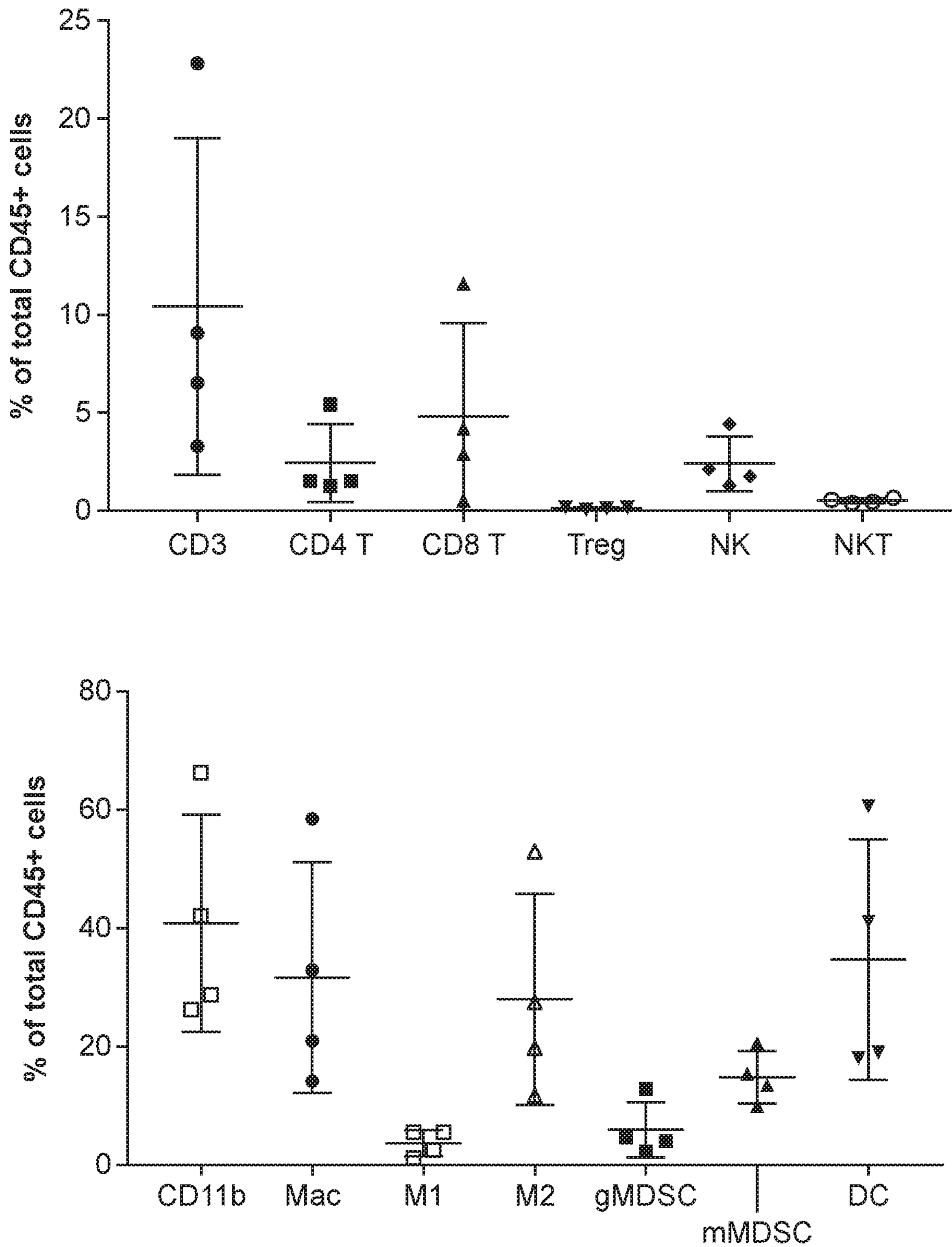


FIG. 18C

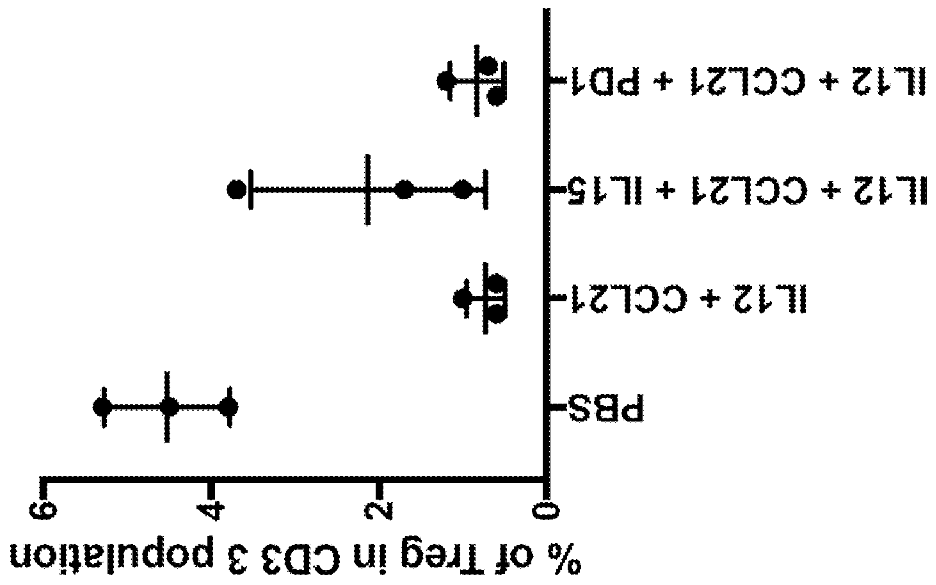


FIG. 18D

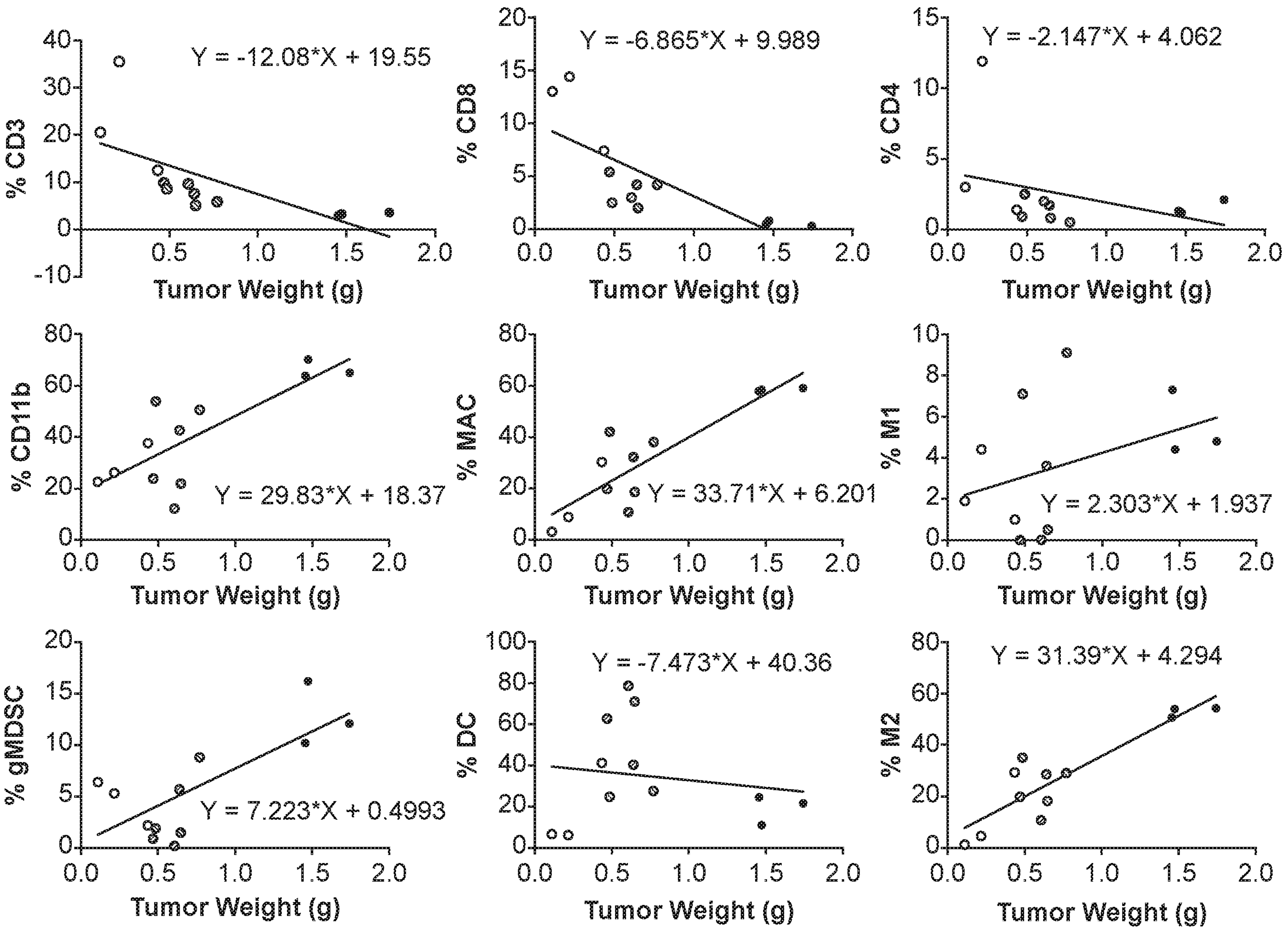


FIG. 18E

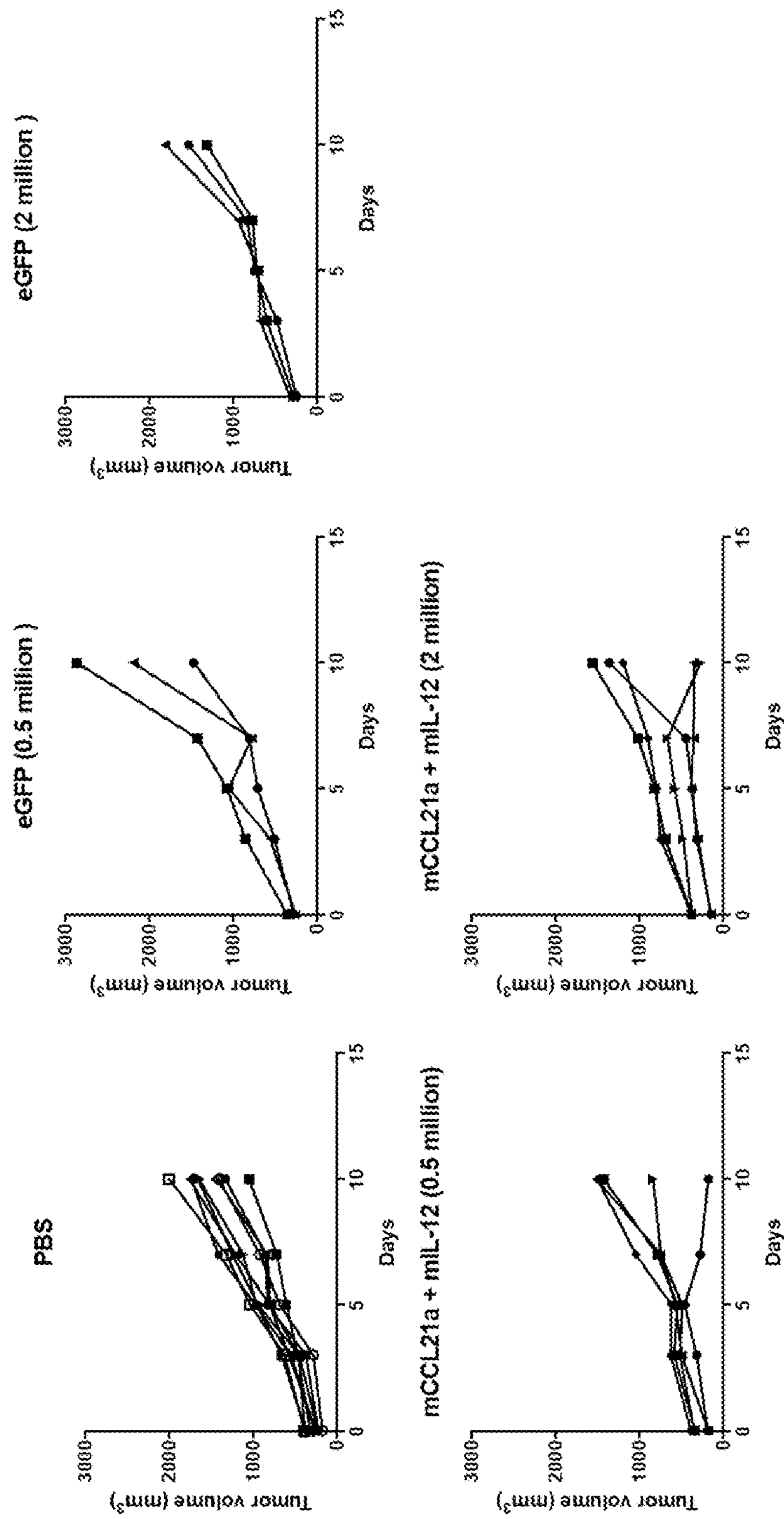


FIG. 19

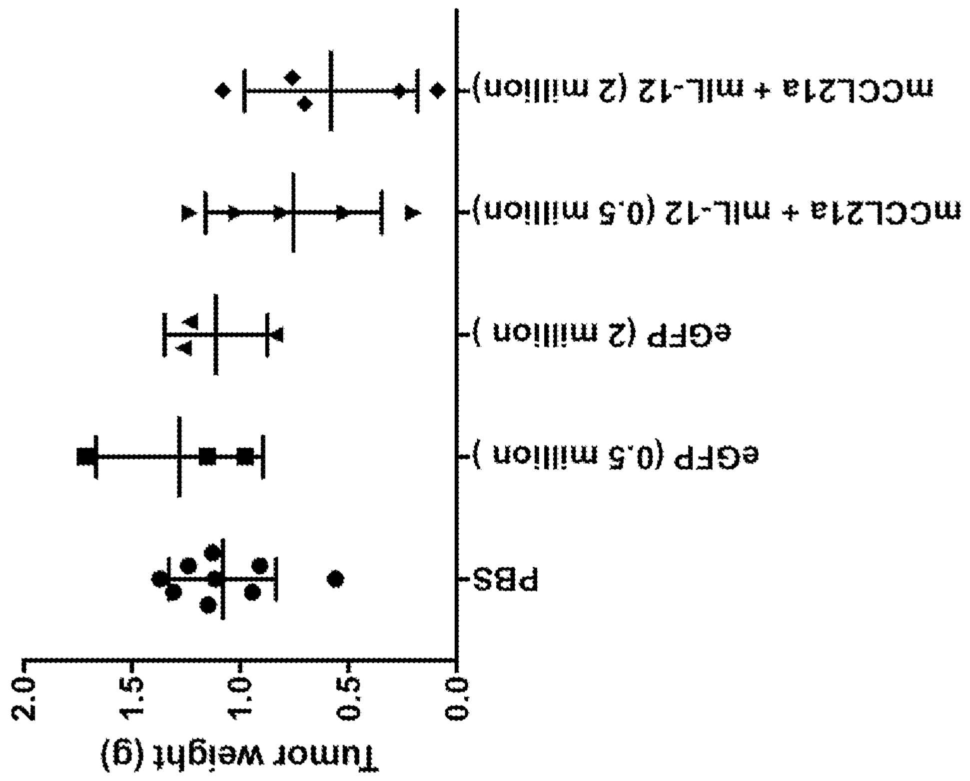


FIG. 20

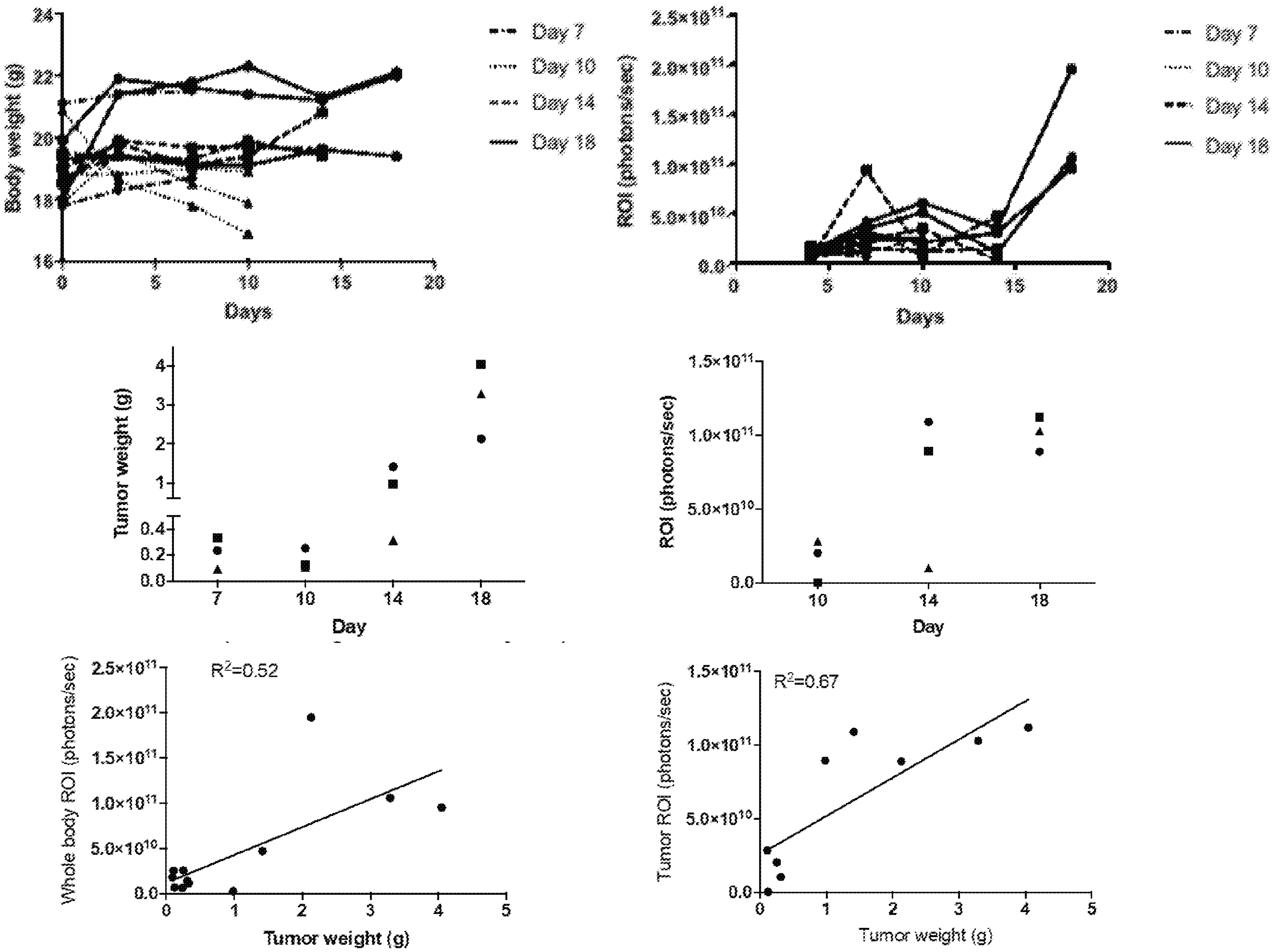


FIG. 21A

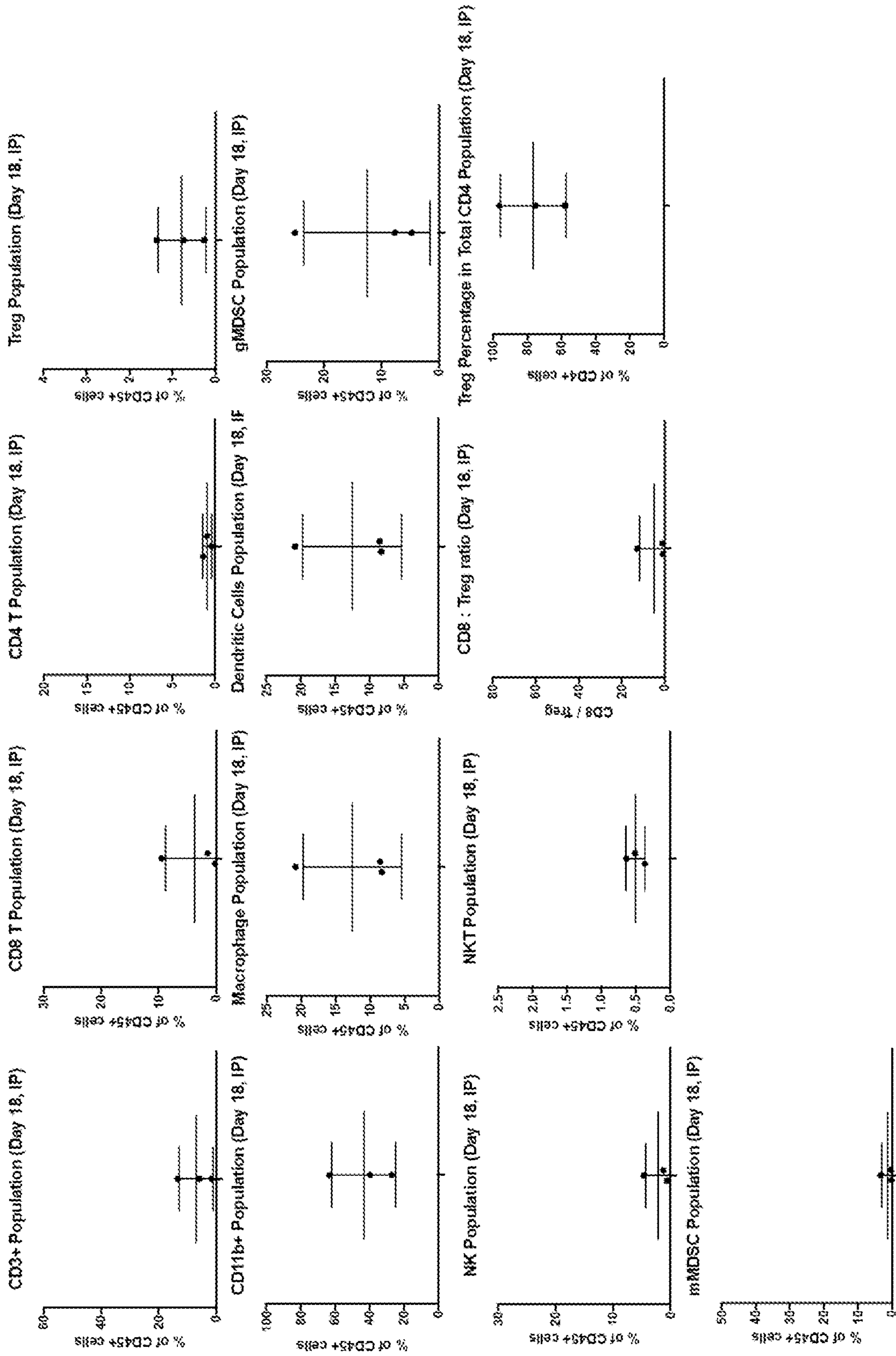


FIG. 21B

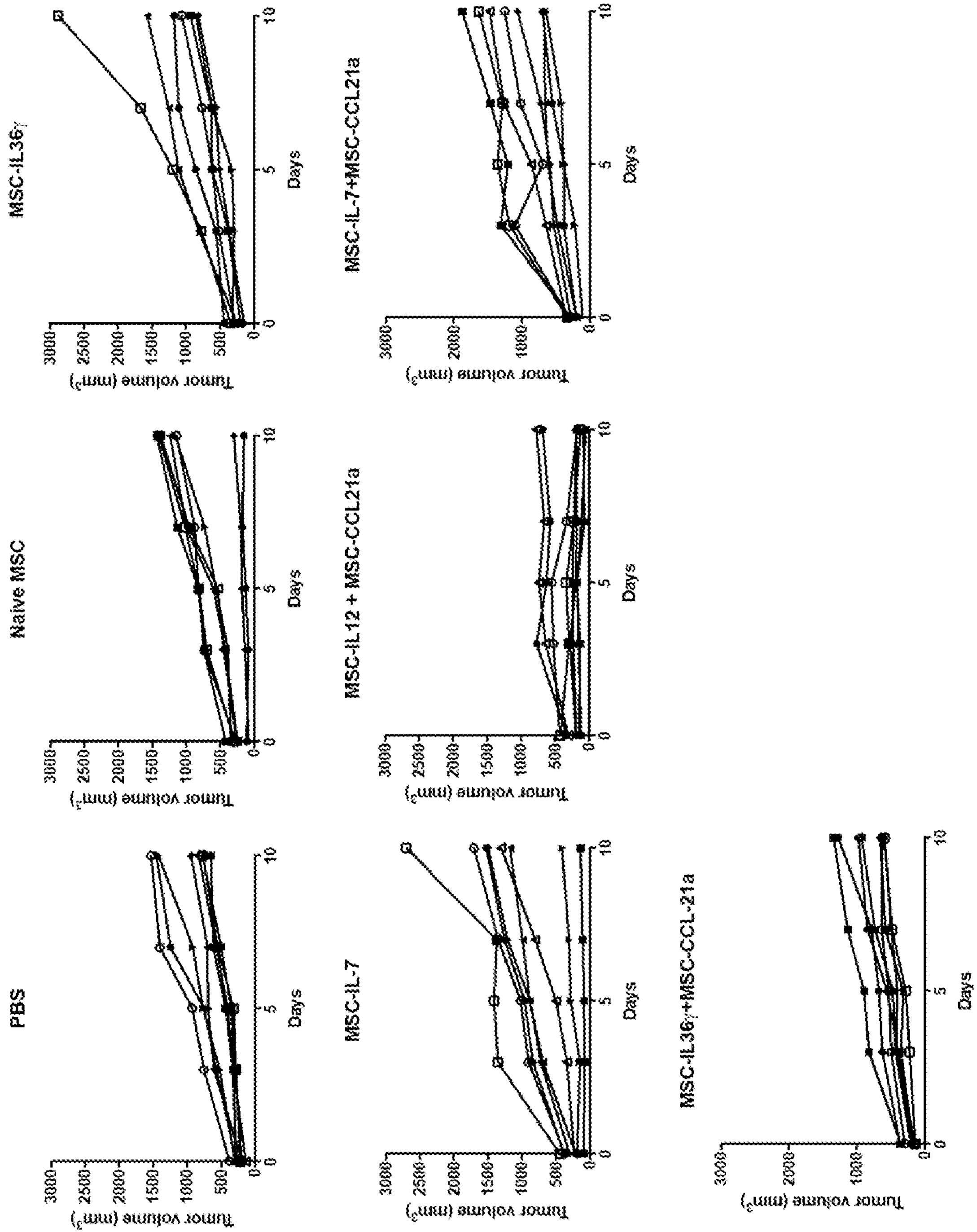


FIG. 22A

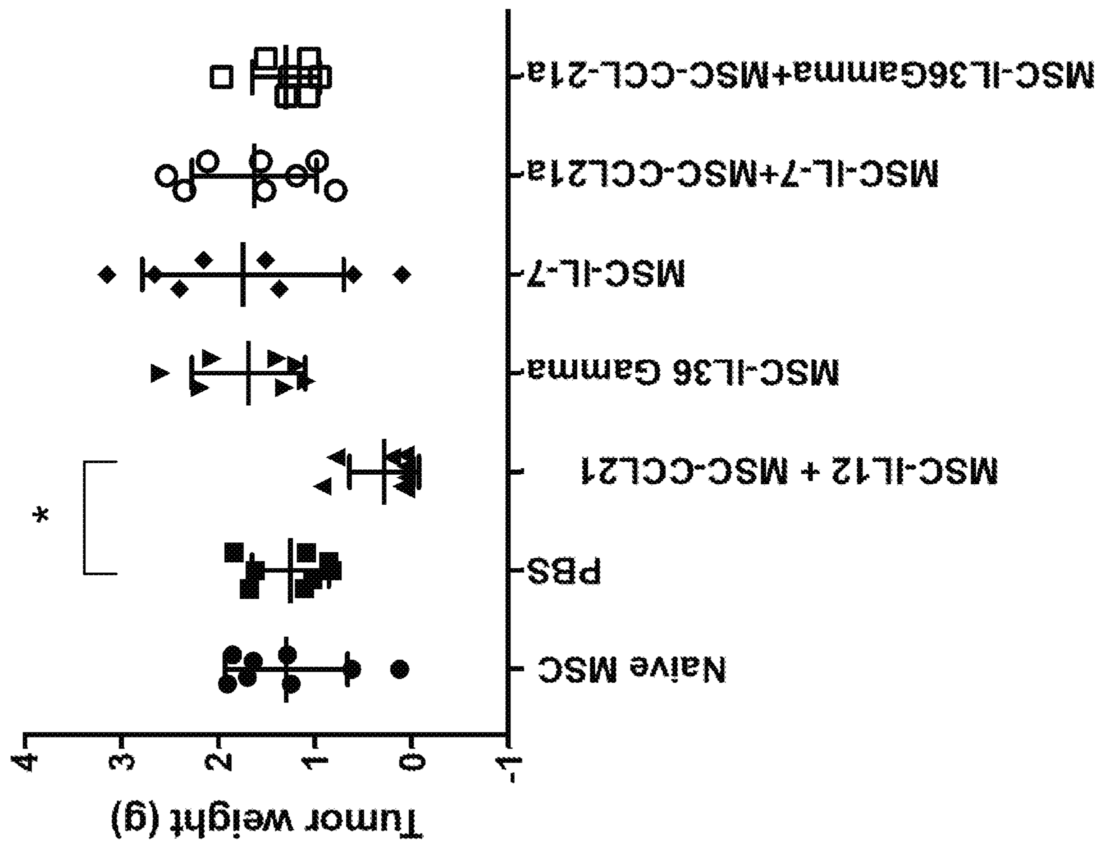


FIG. 22B

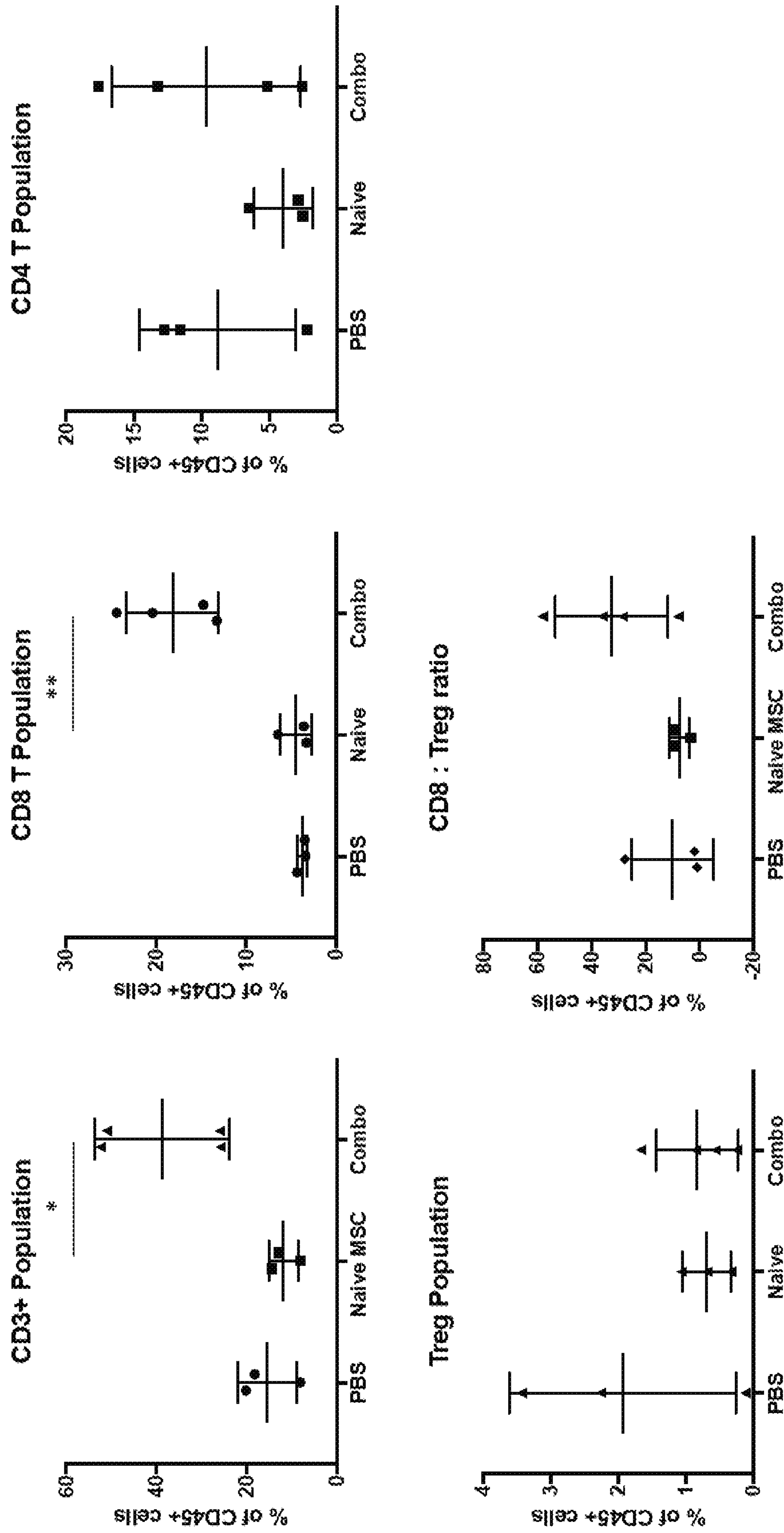


FIG. 23A

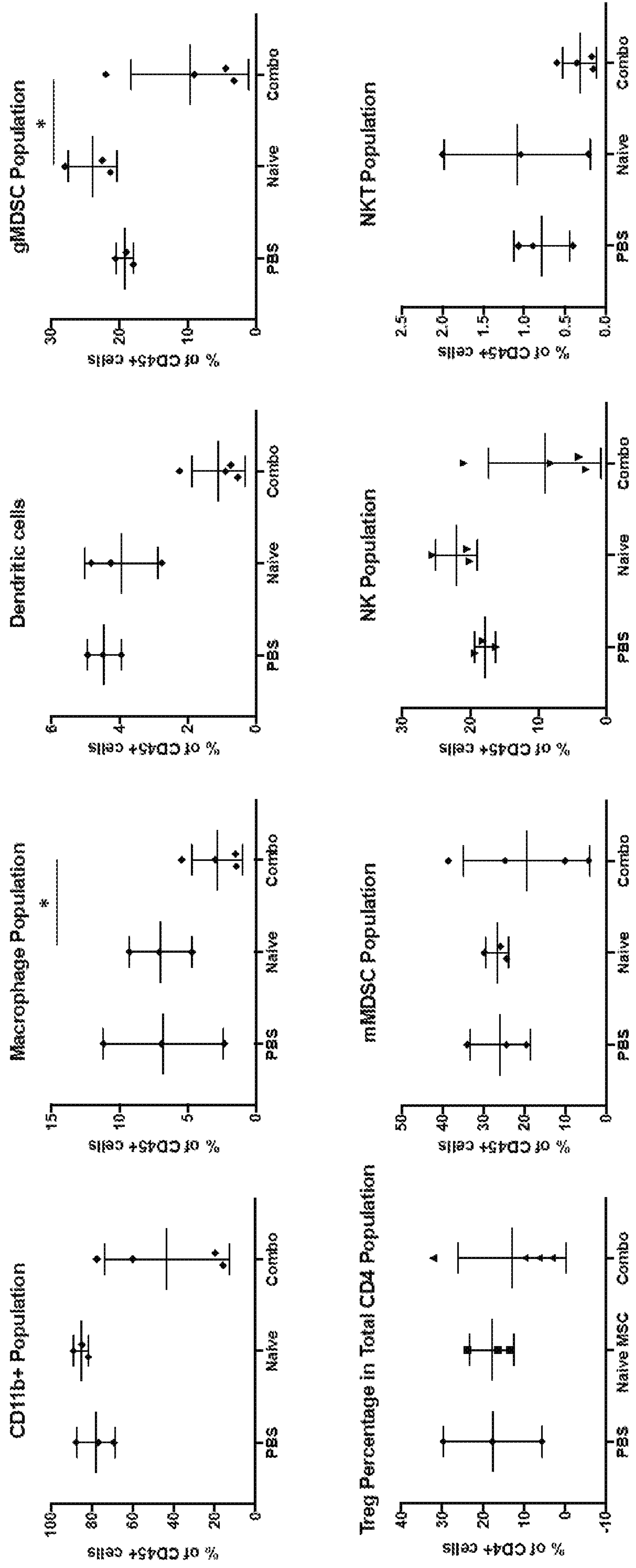


FIG. 23B

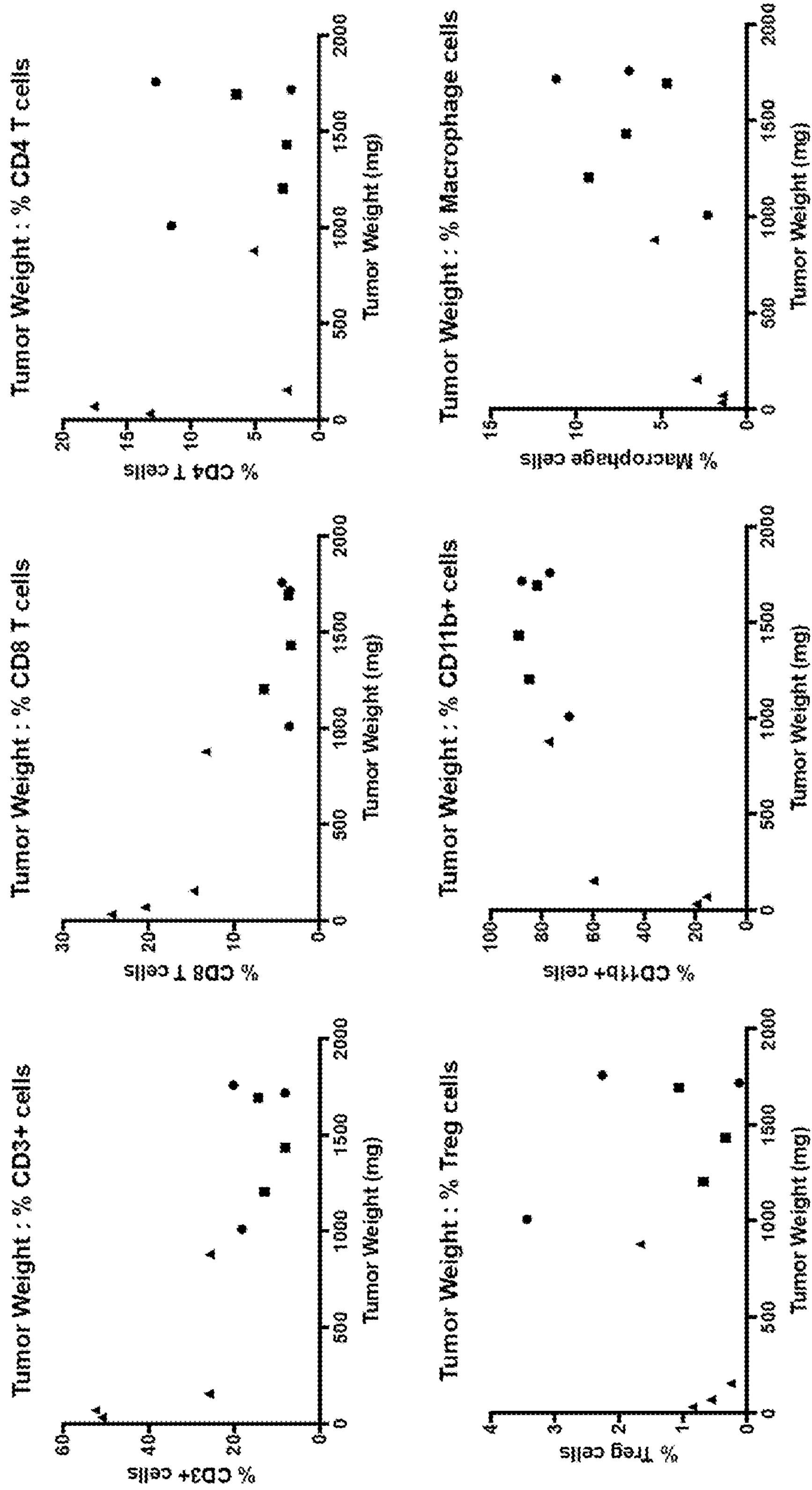


FIG. 24A

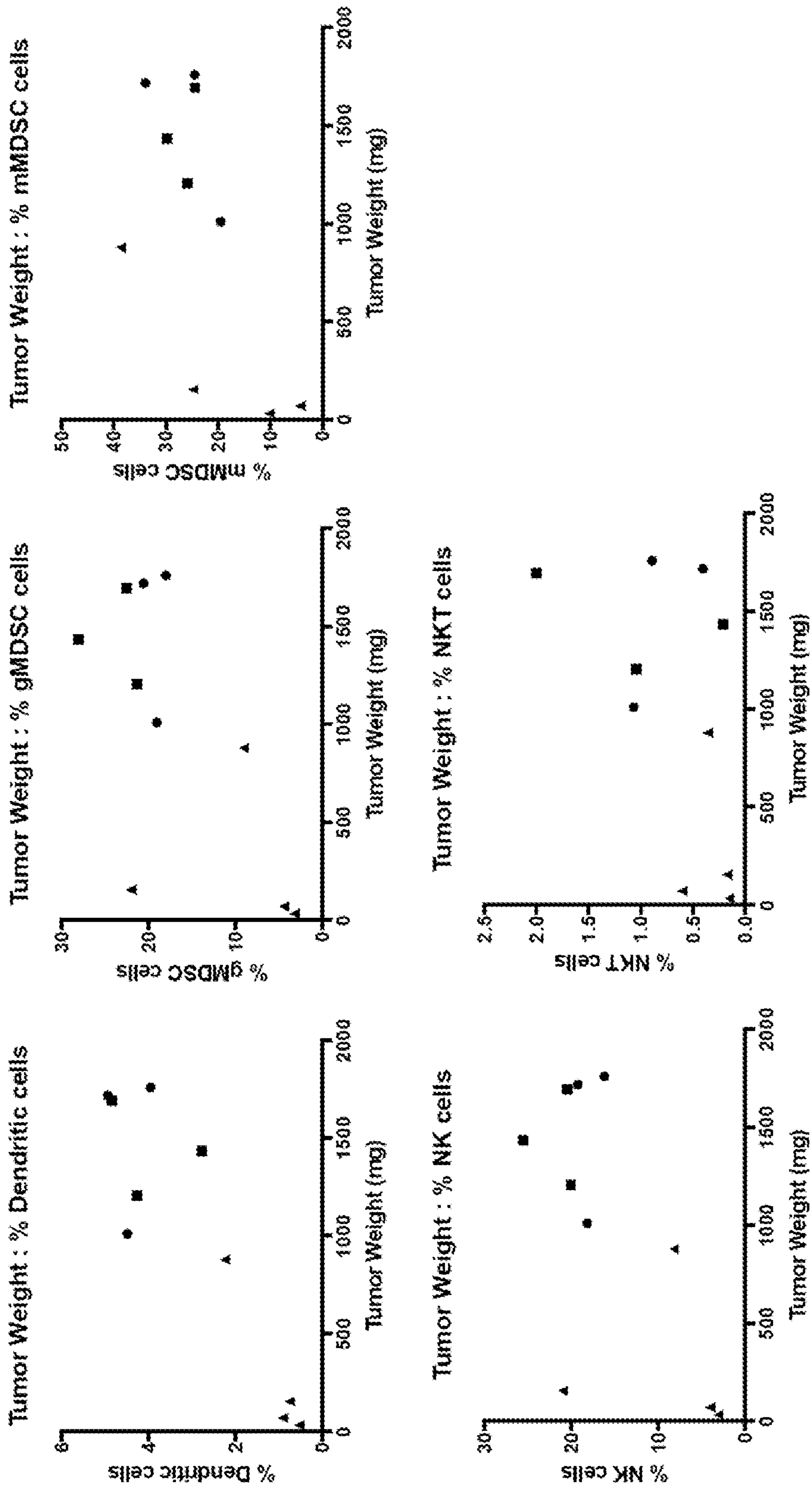


FIG. 24B

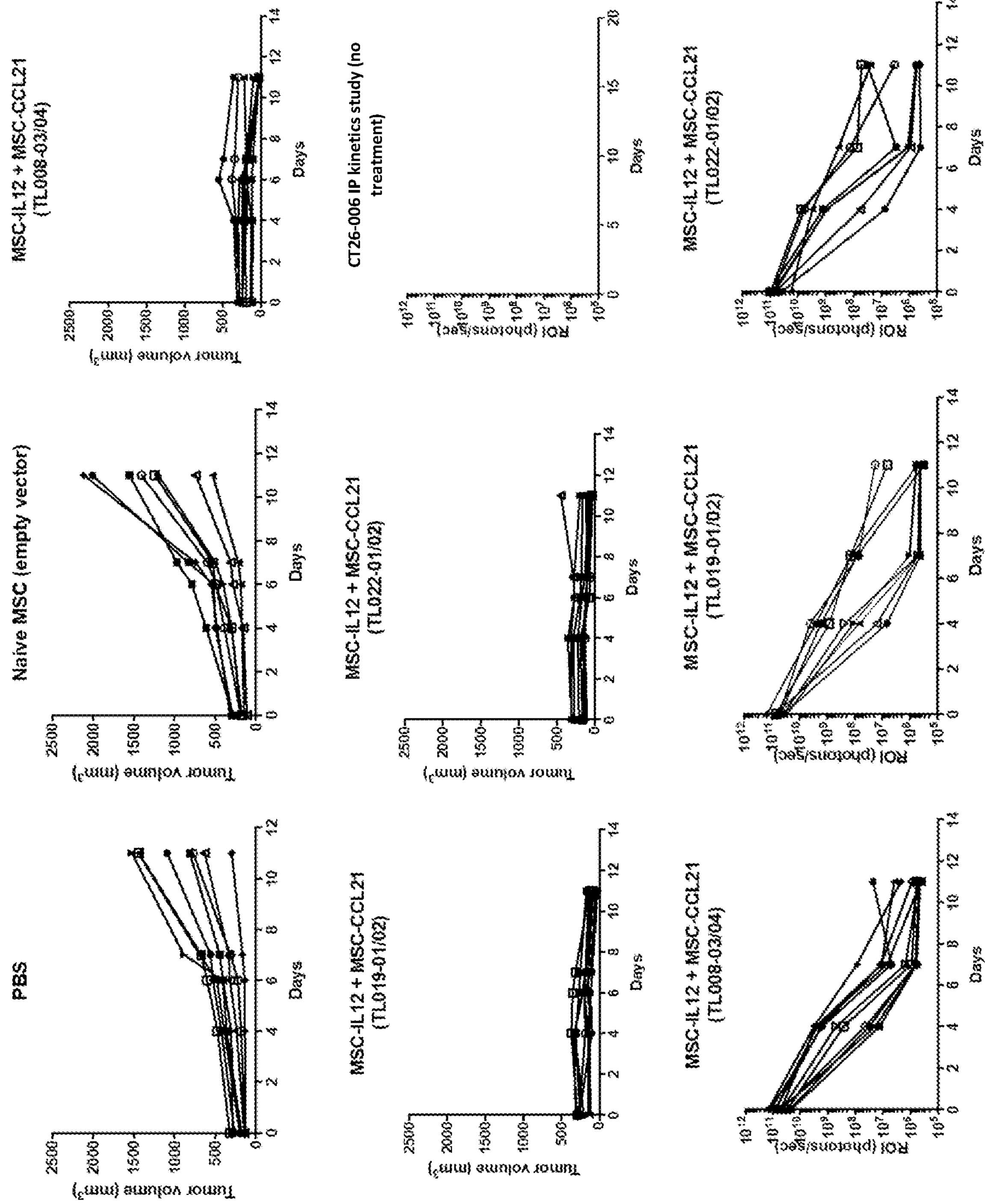


FIG. 25A

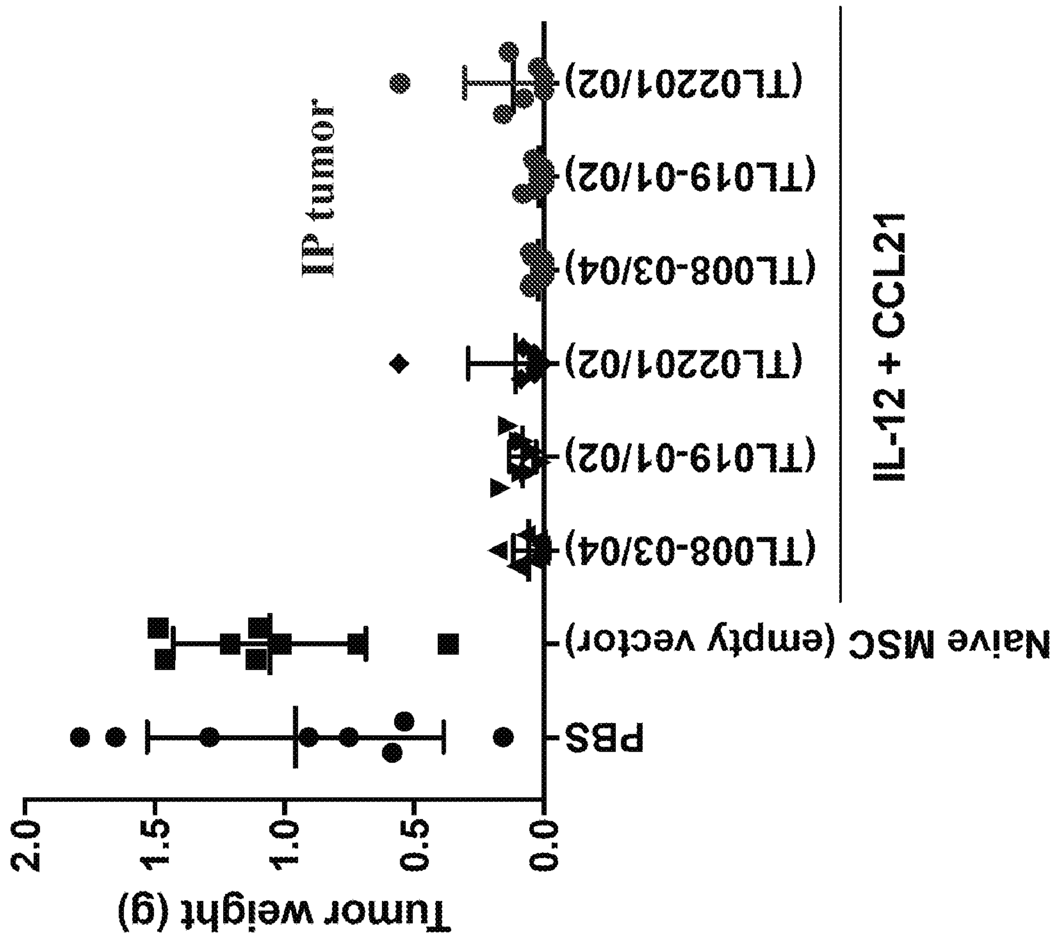


FIG. 25B

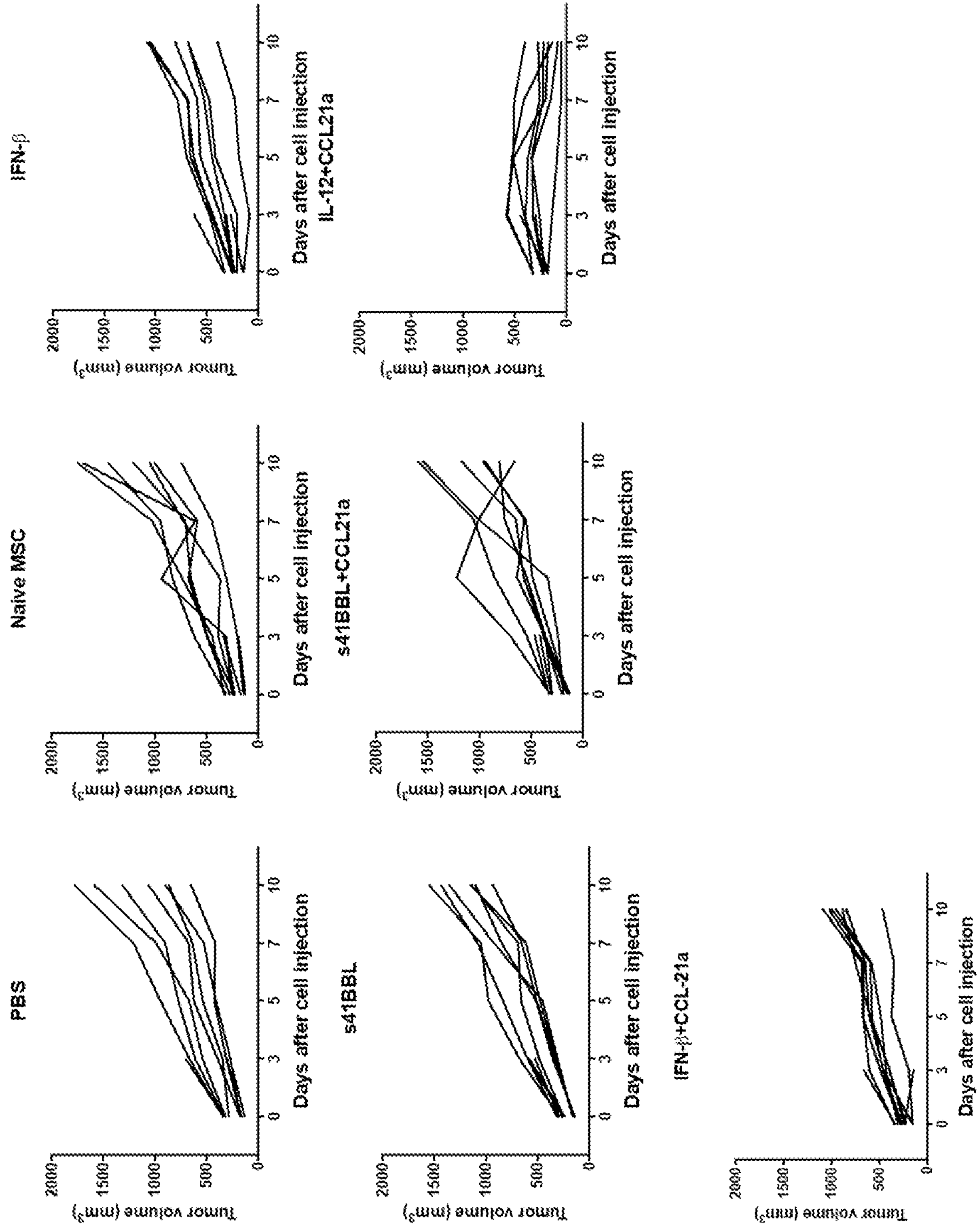


FIG. 26A

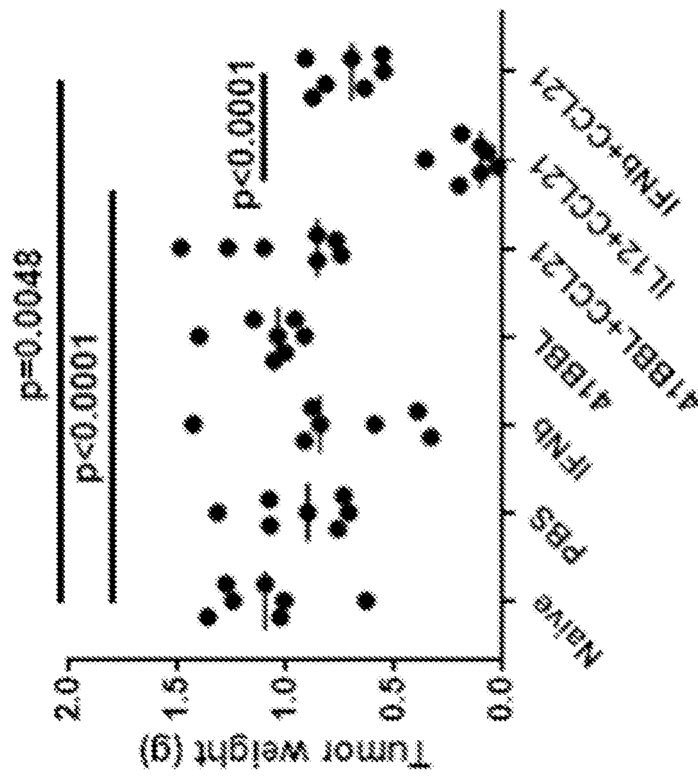


FIG. 26B

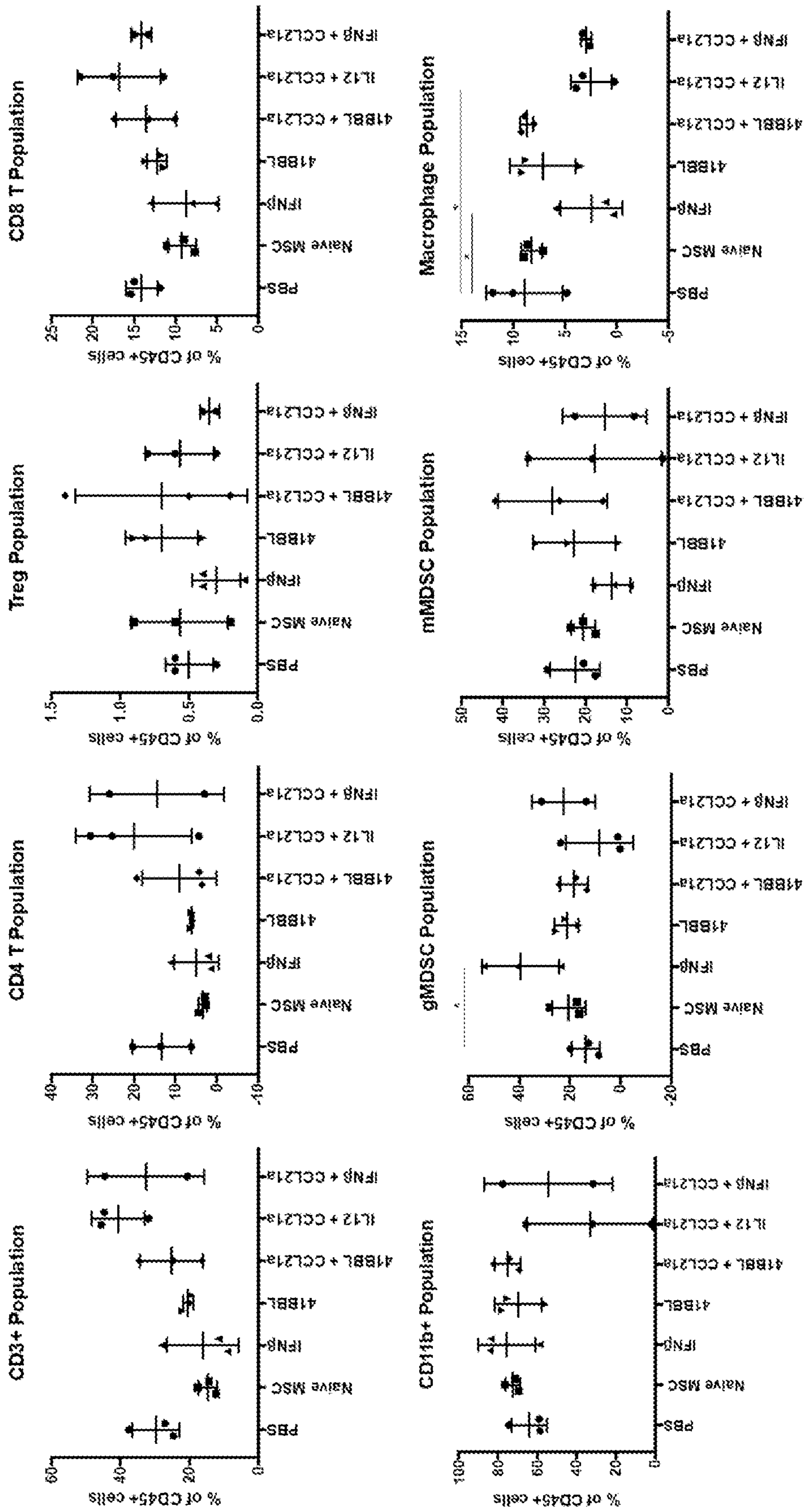


FIG. 27A

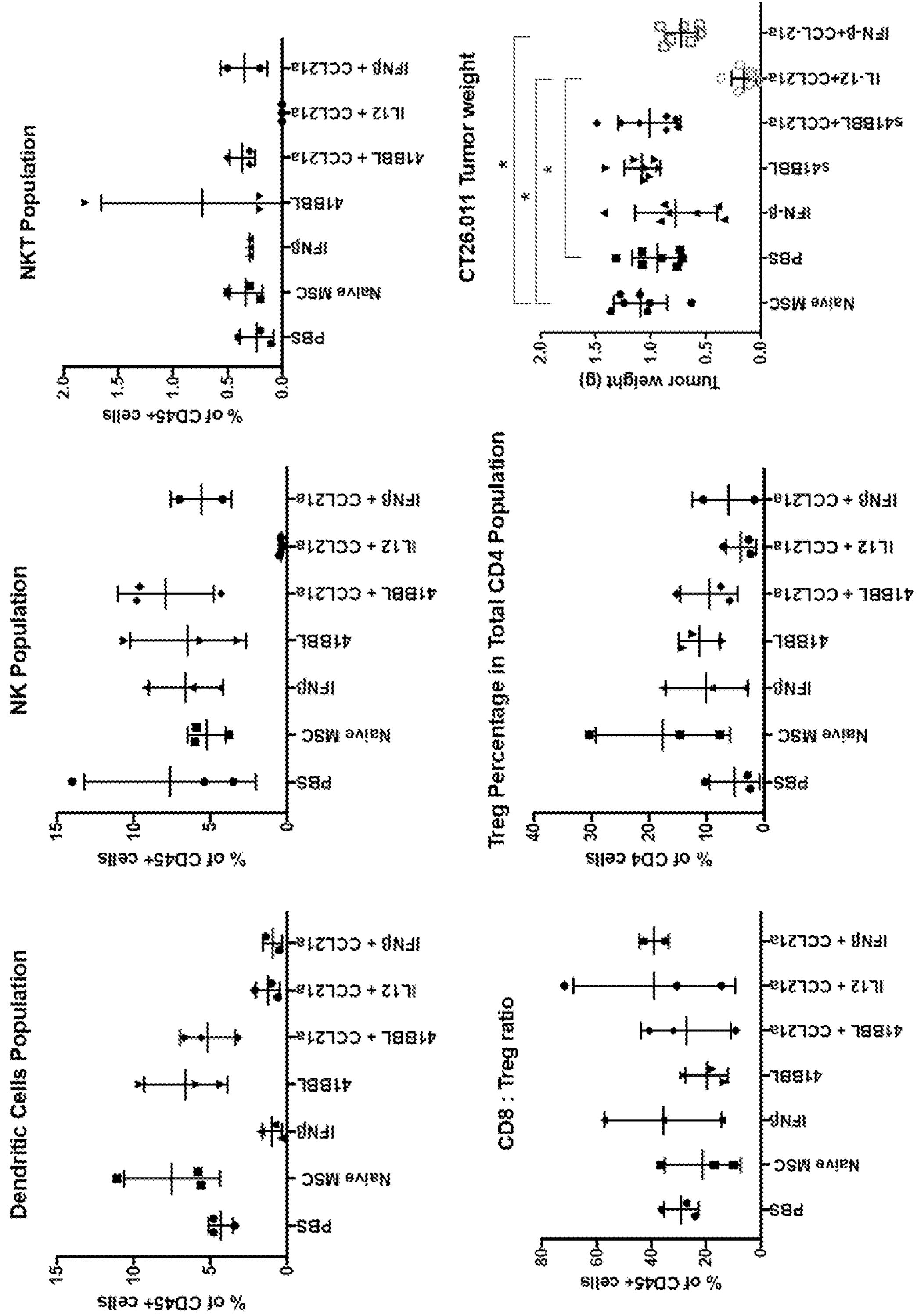


FIG. 27B

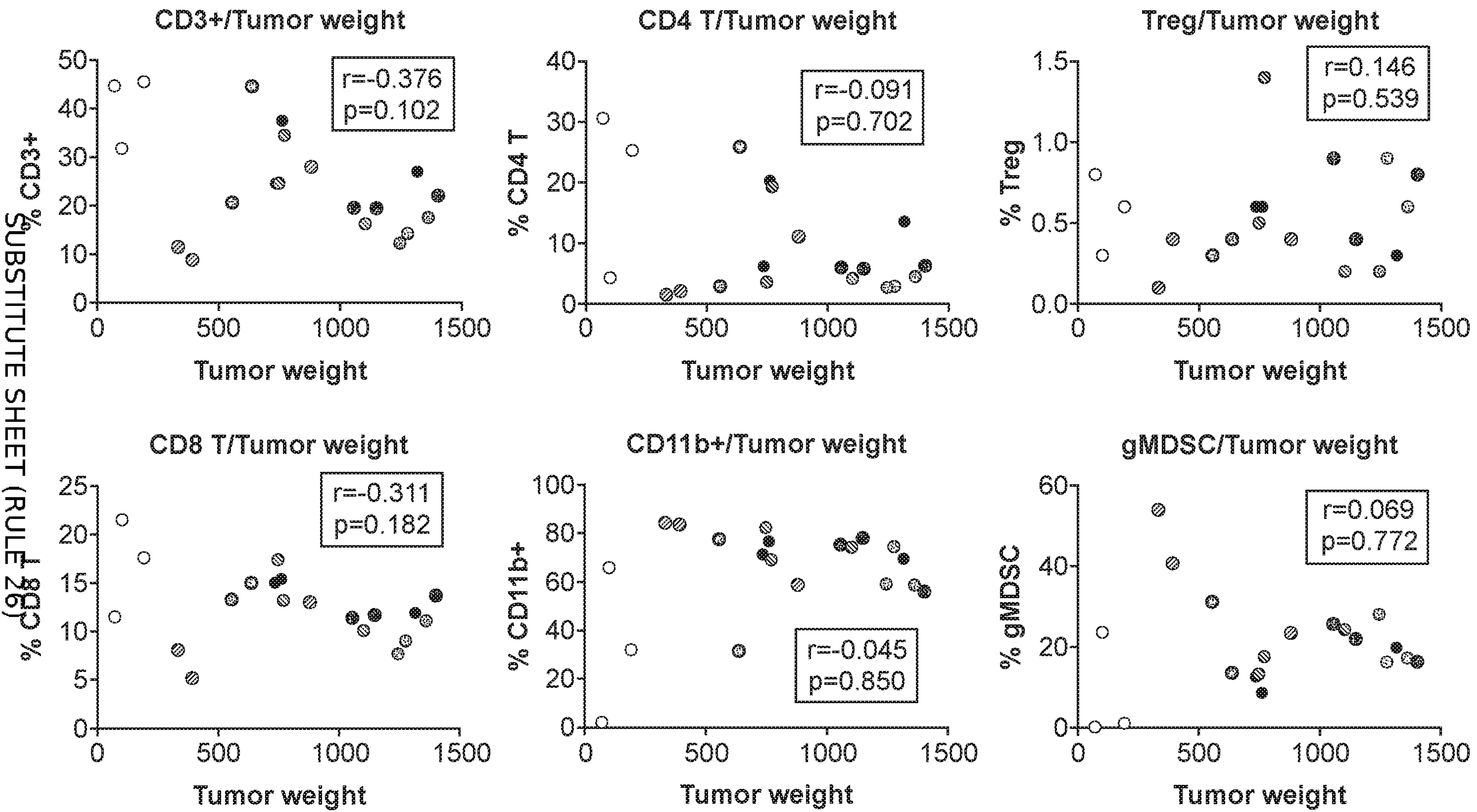


FIG. 28A

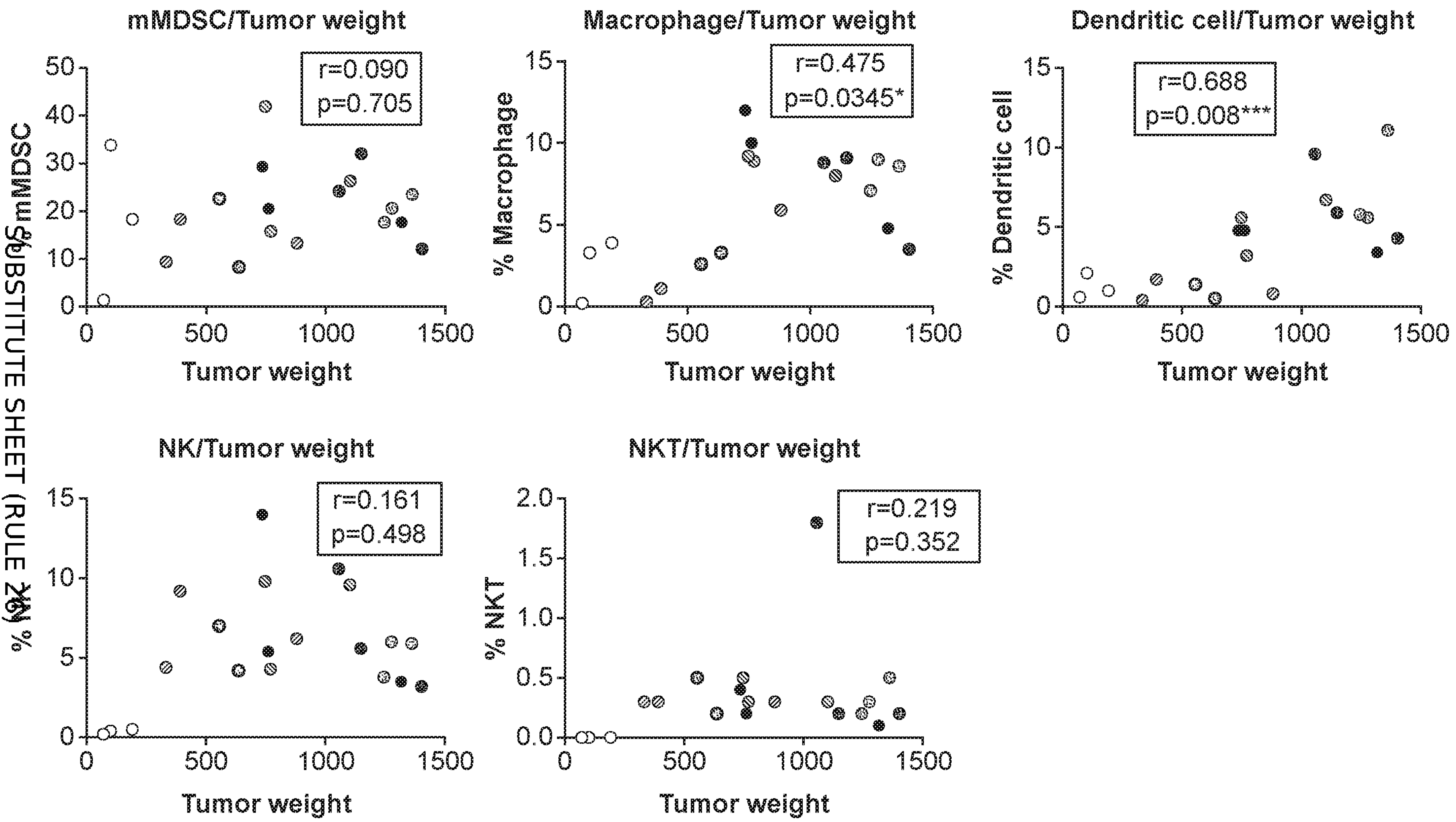


FIG. 28B

FIG. 29

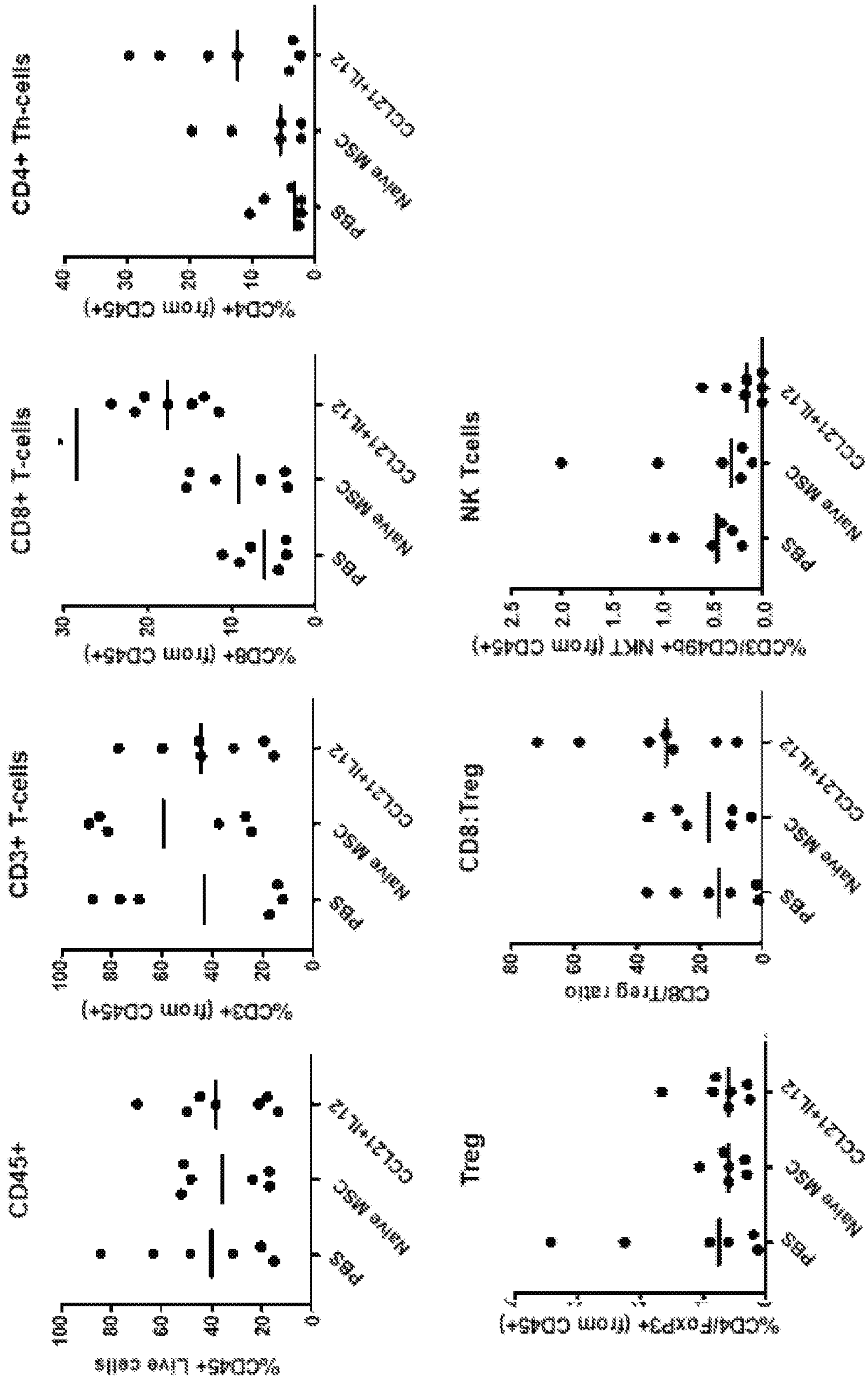


FIG. 30A

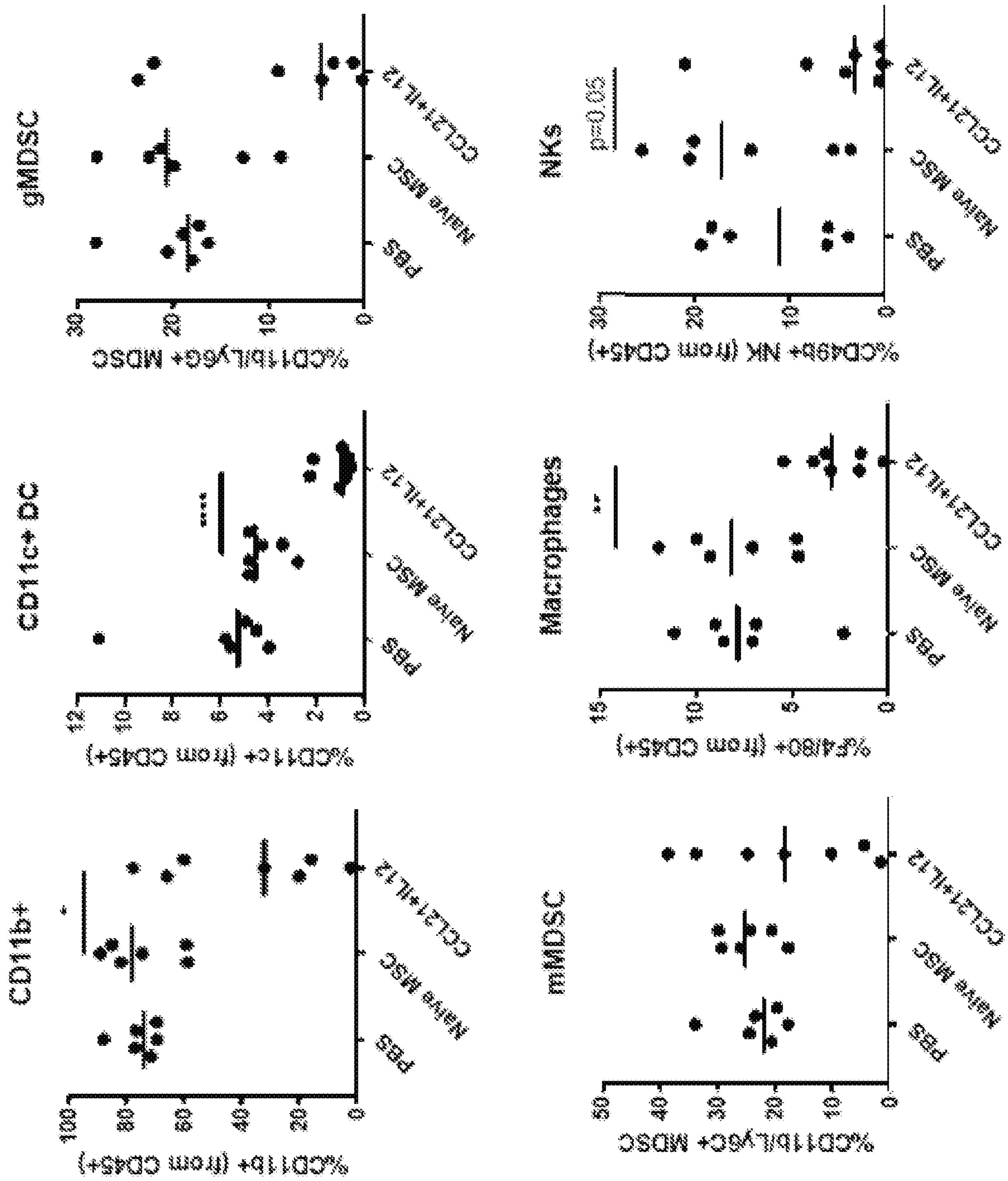


FIG. 30B

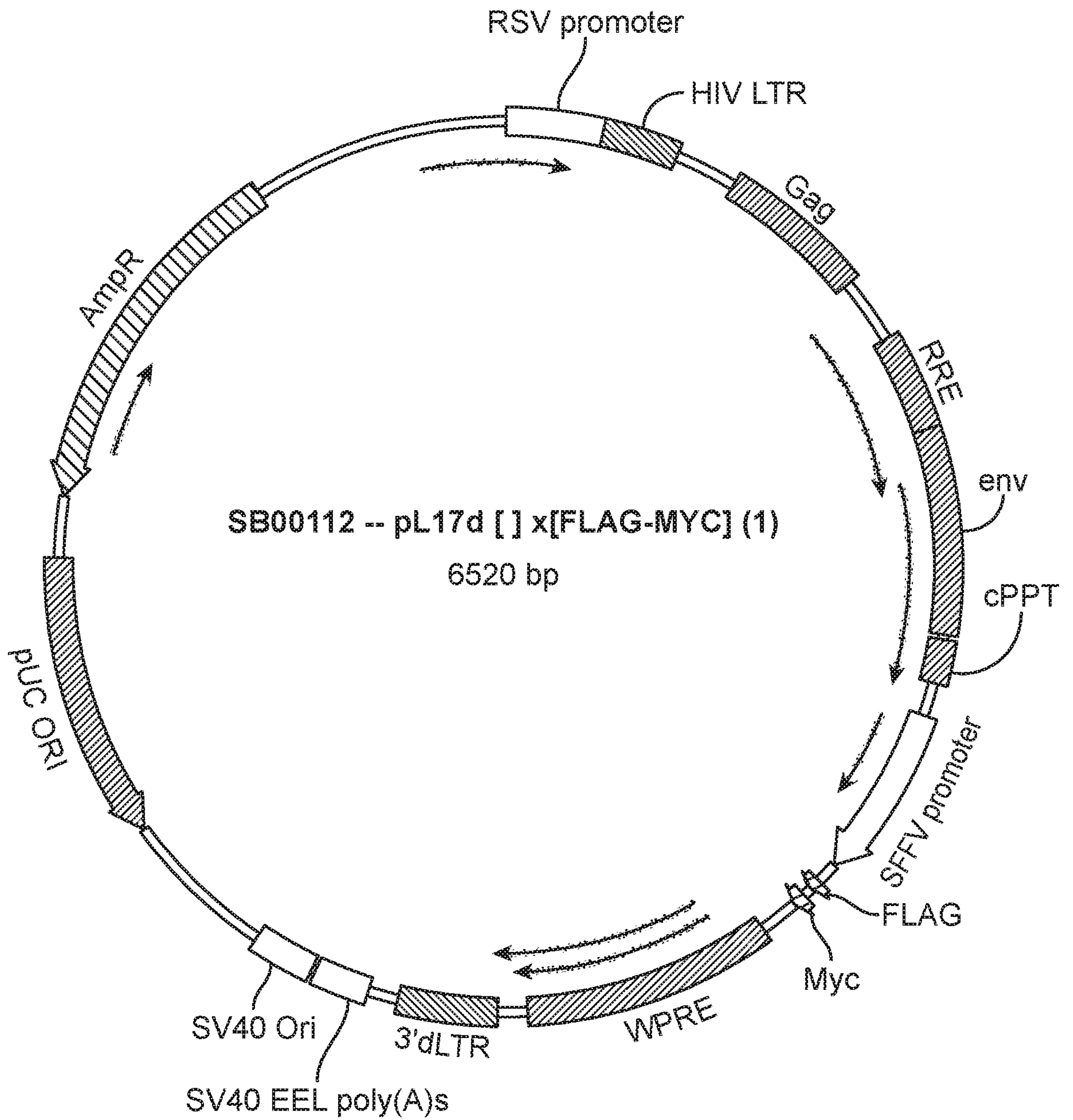


FIG. 31

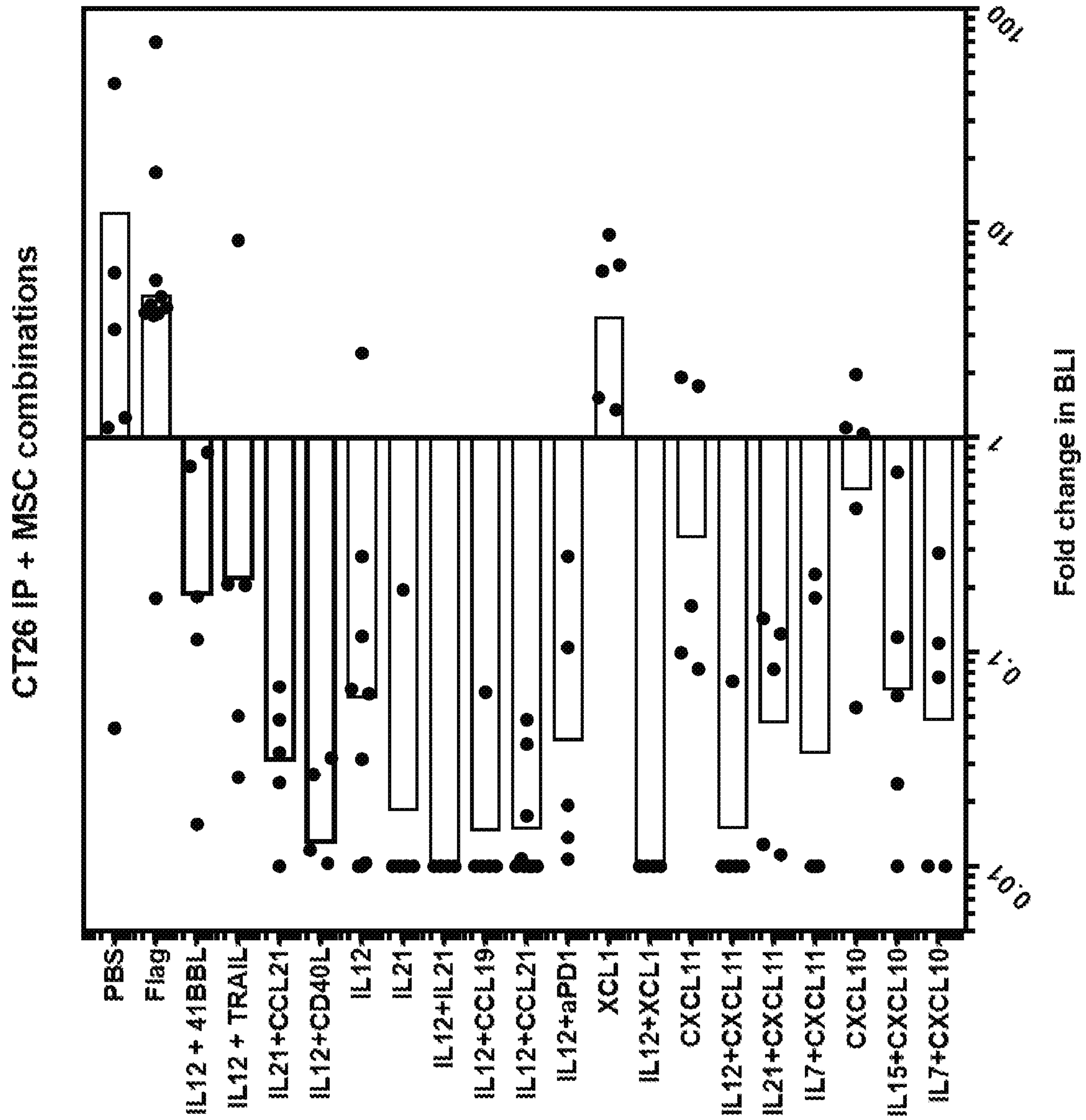


FIG. 32

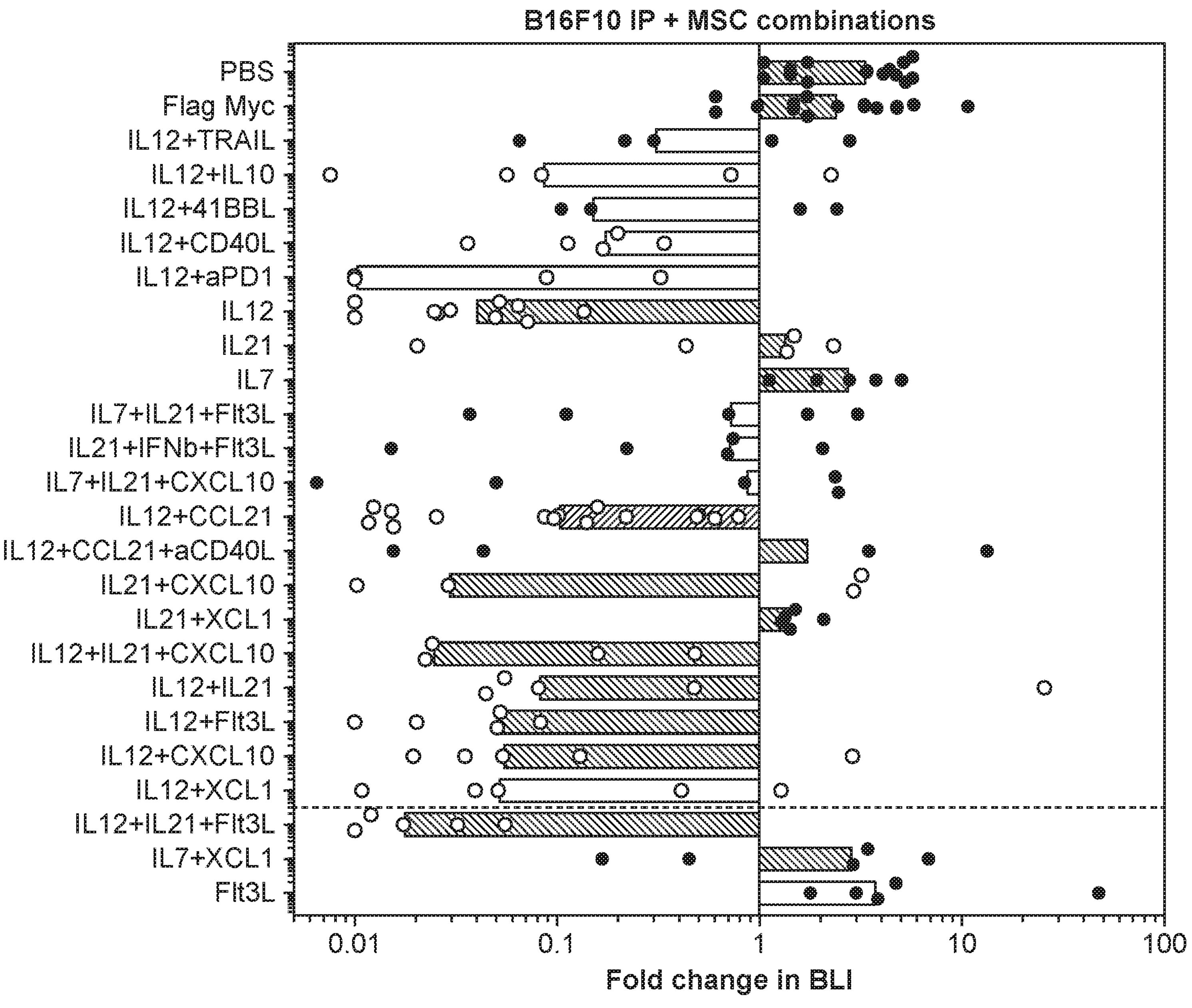


FIG. 33

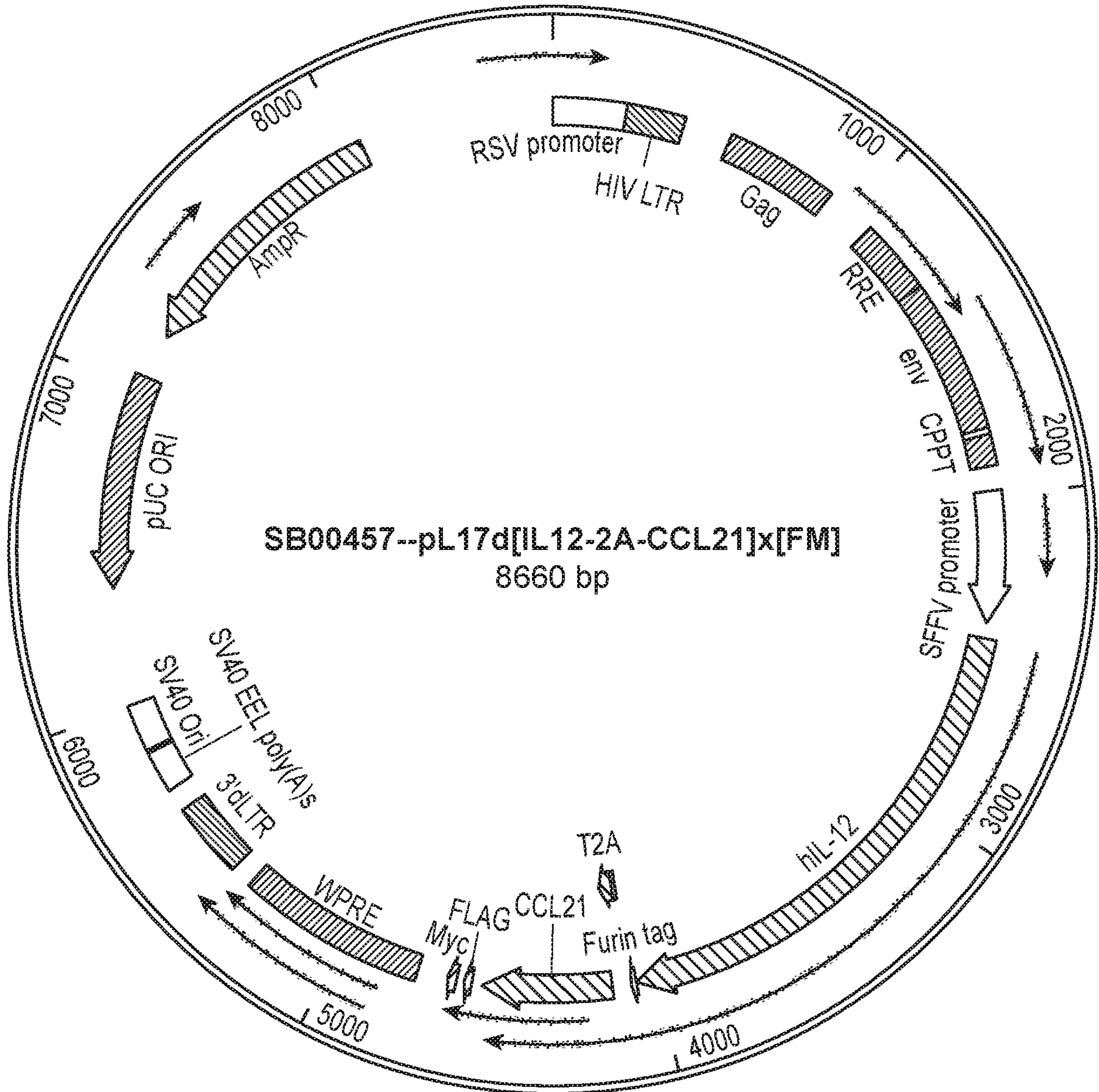


FIG. 34

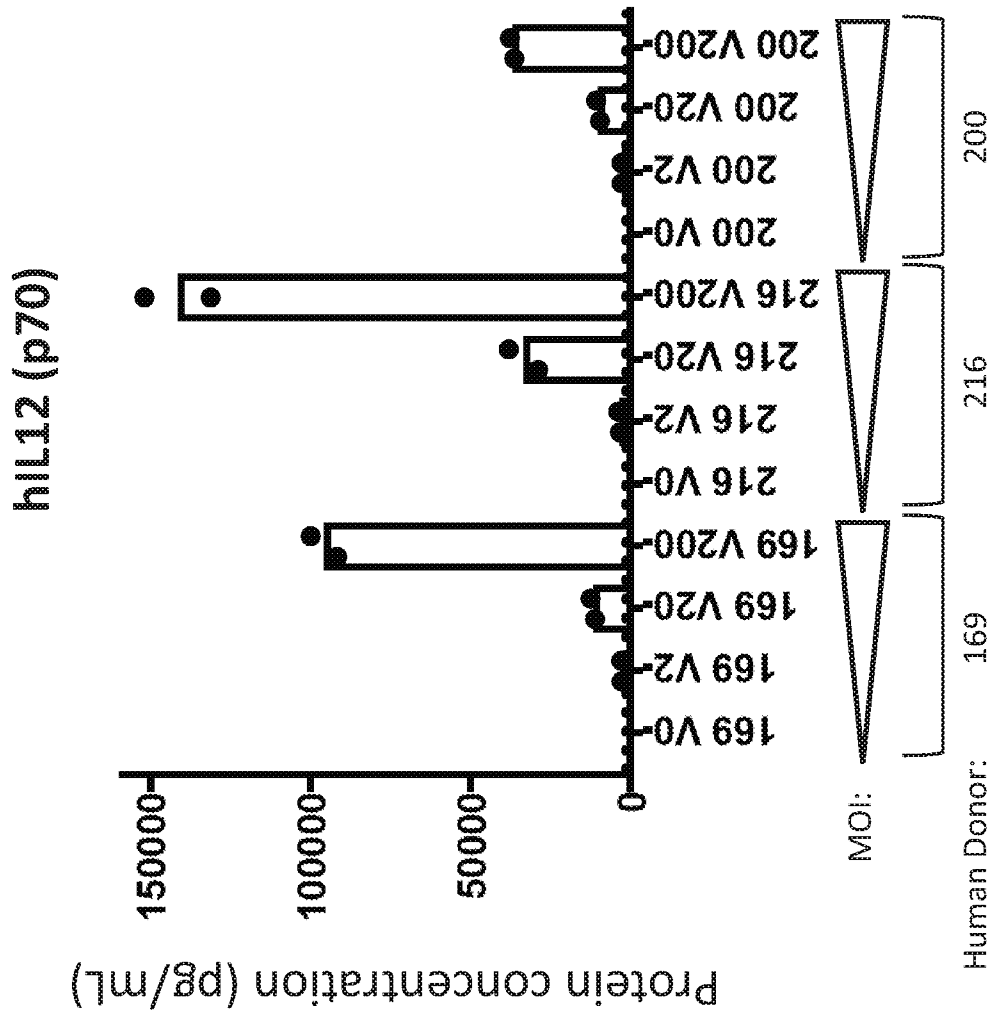


FIG. 35A

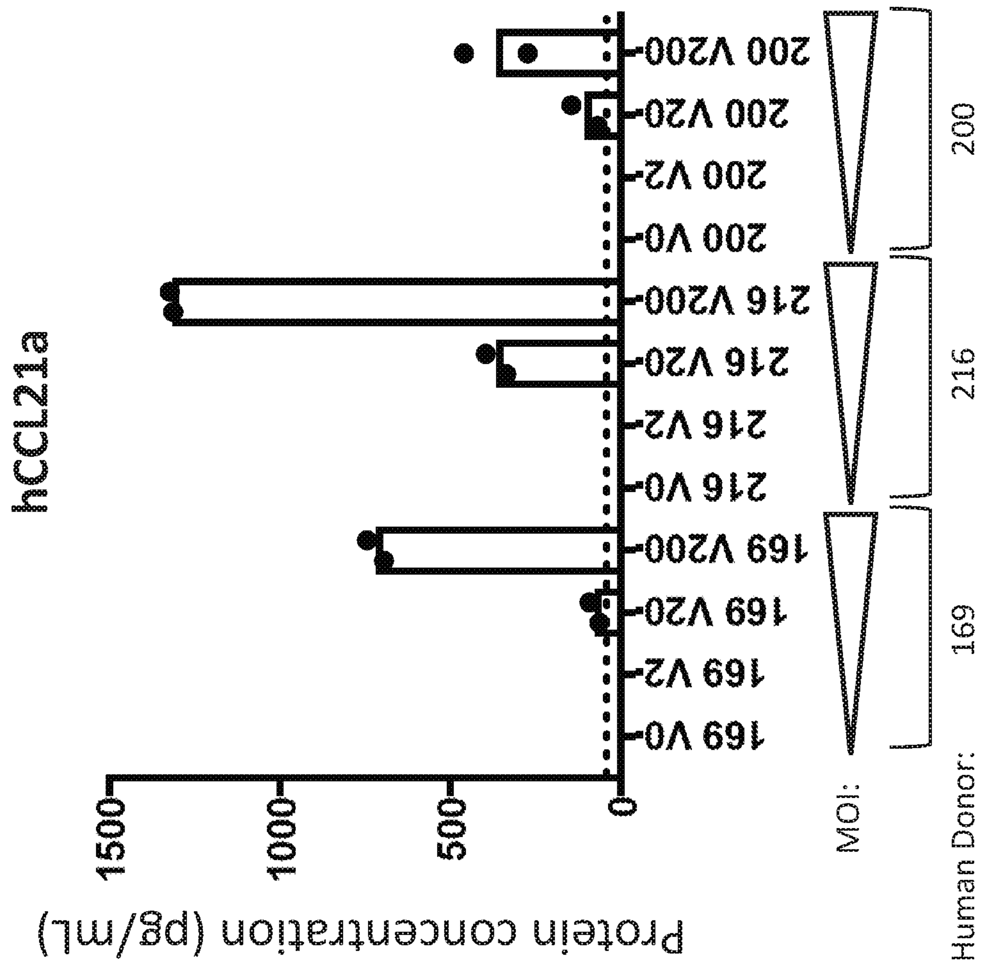


FIG. 35B

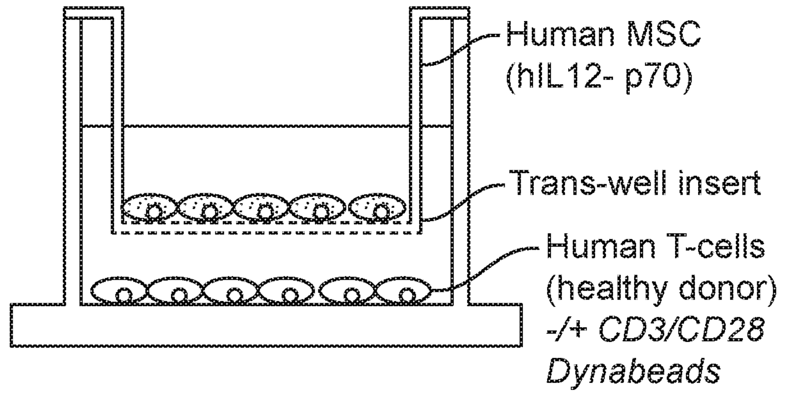


FIG. 36A

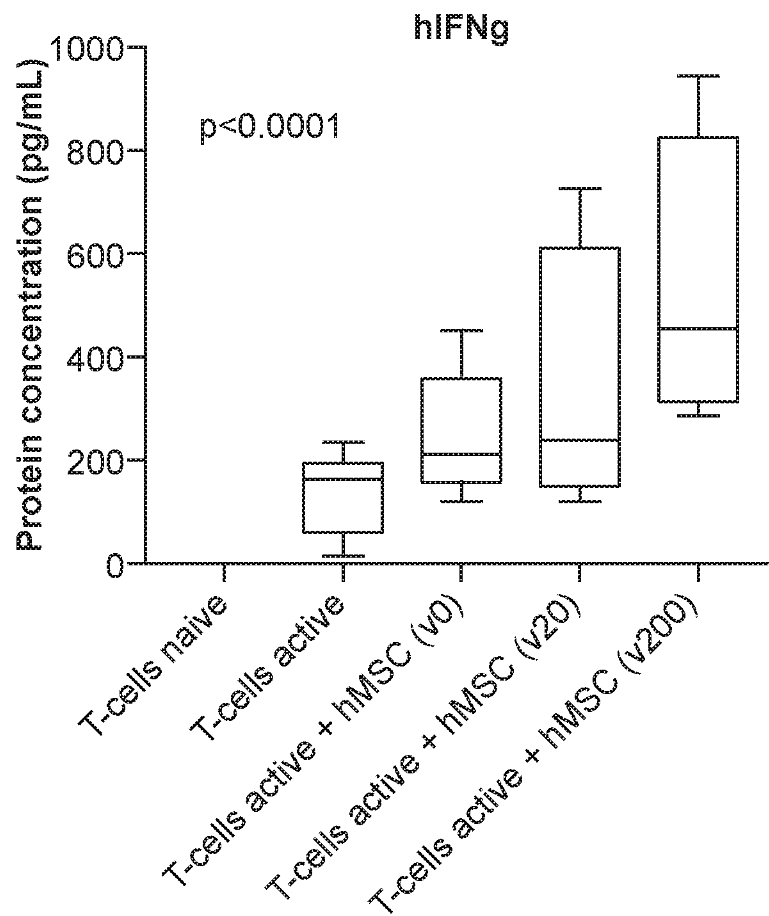


FIG. 36B

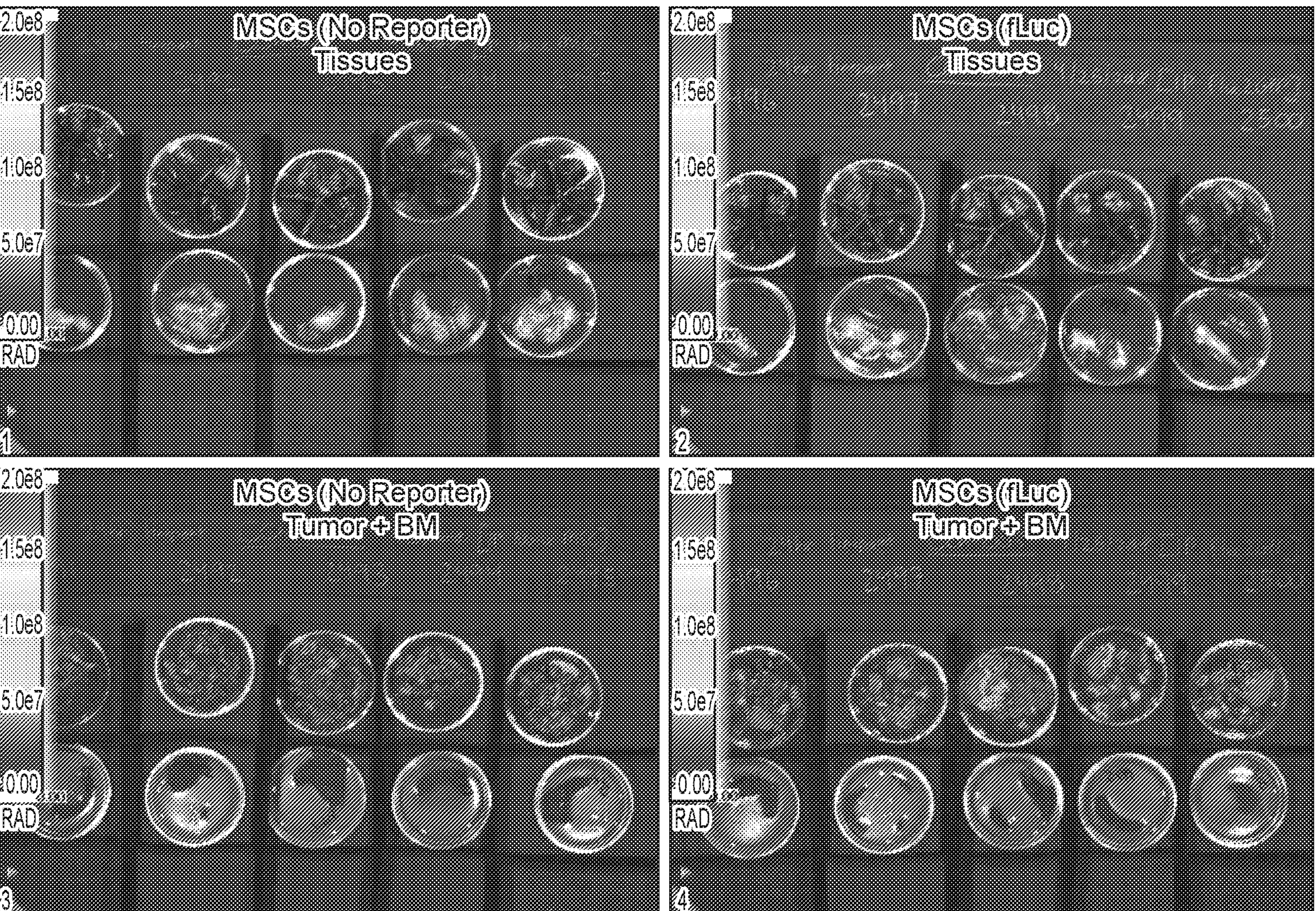


FIG. 37A

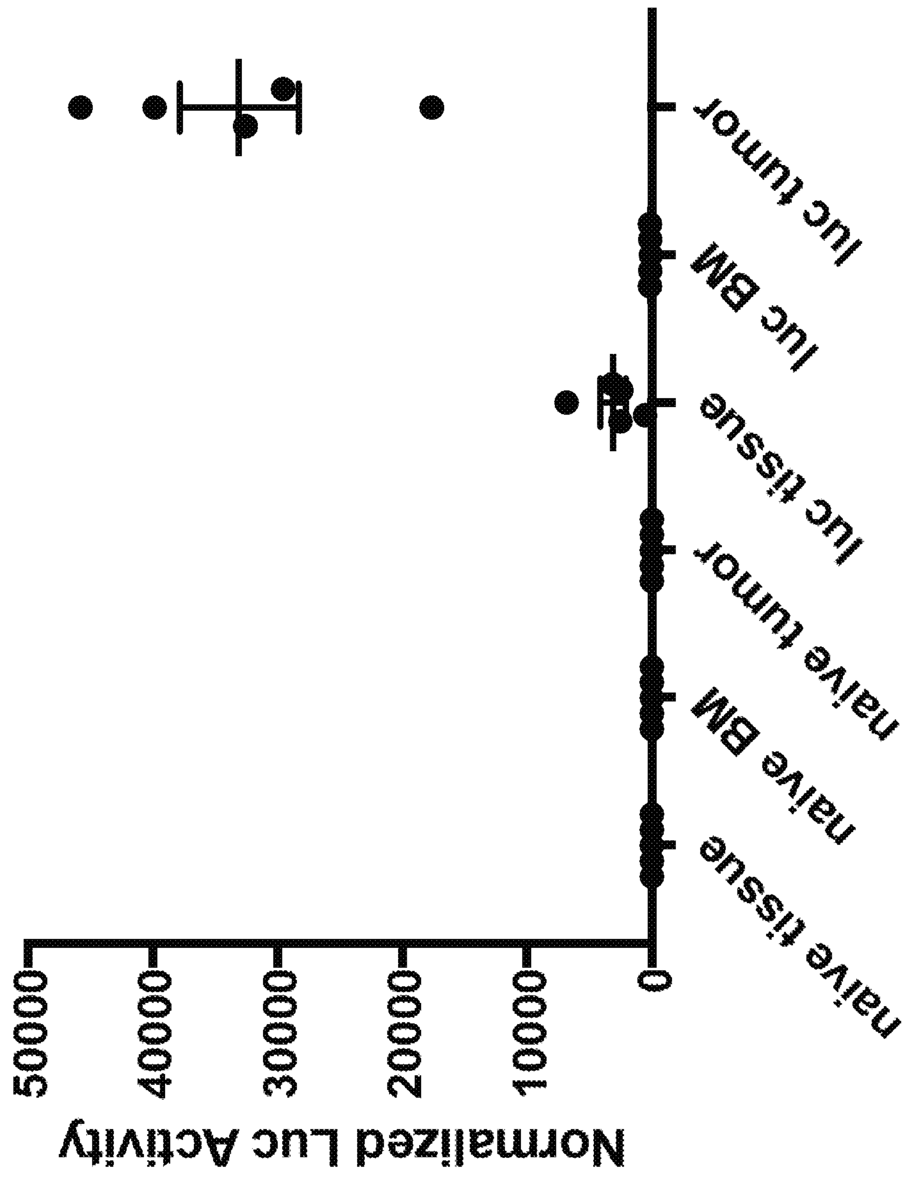


FIG. 37B

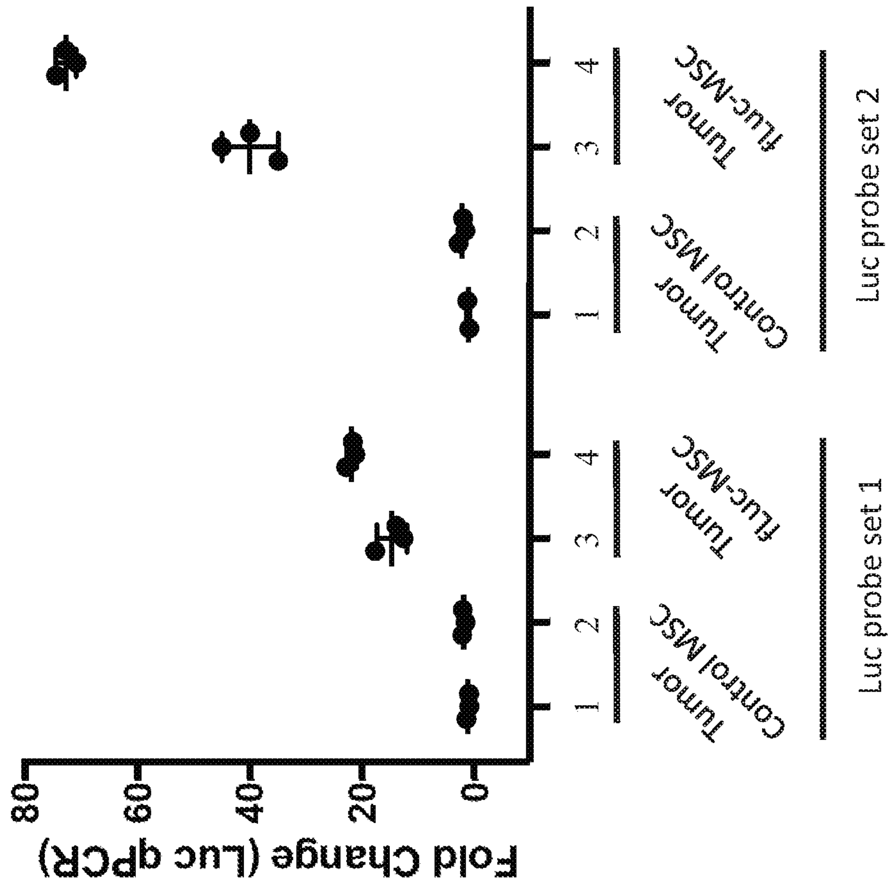
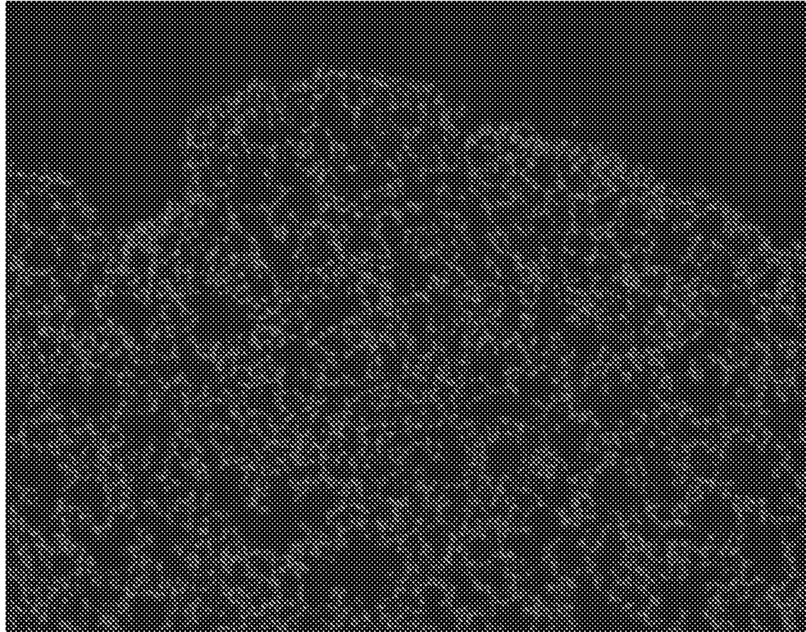


FIG. 37C

Negative control



Anti-firefly luciferase (MSCs)

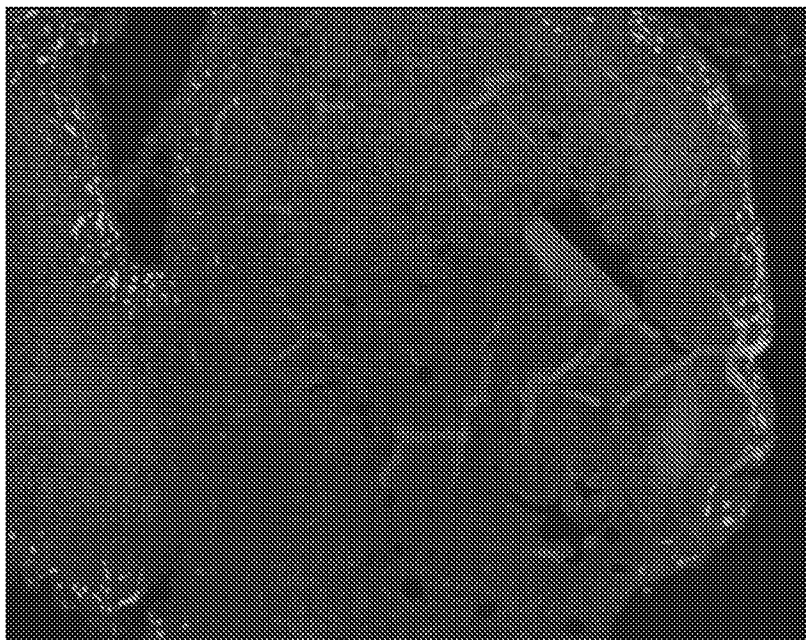


FIG. 37D

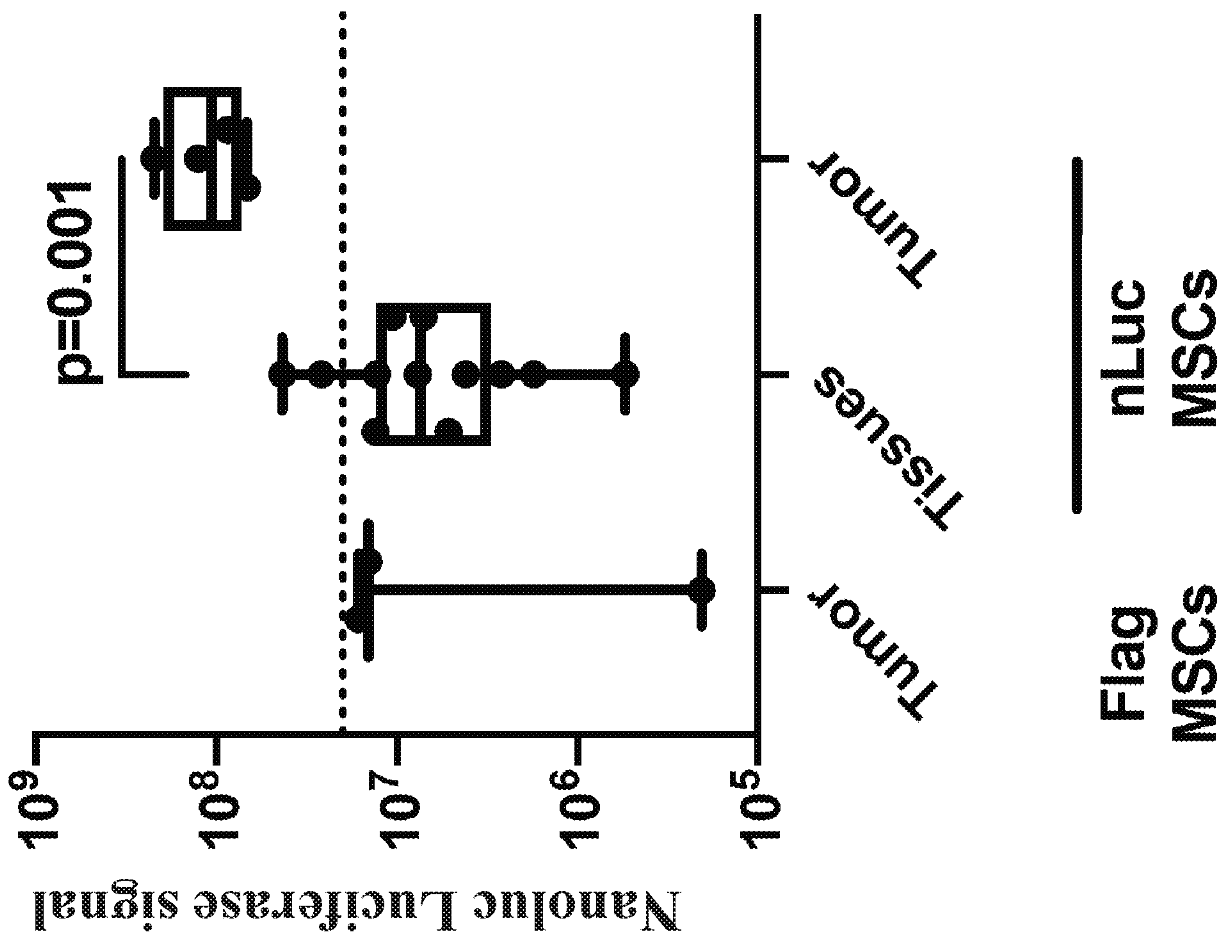
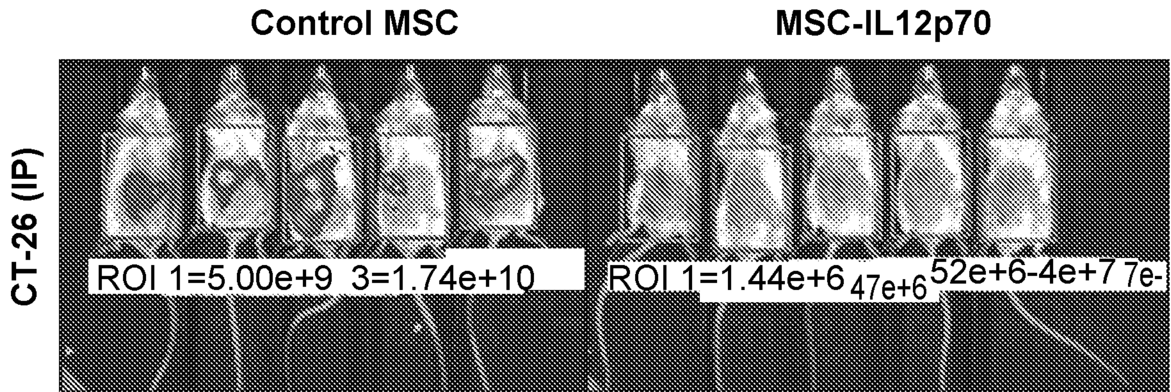
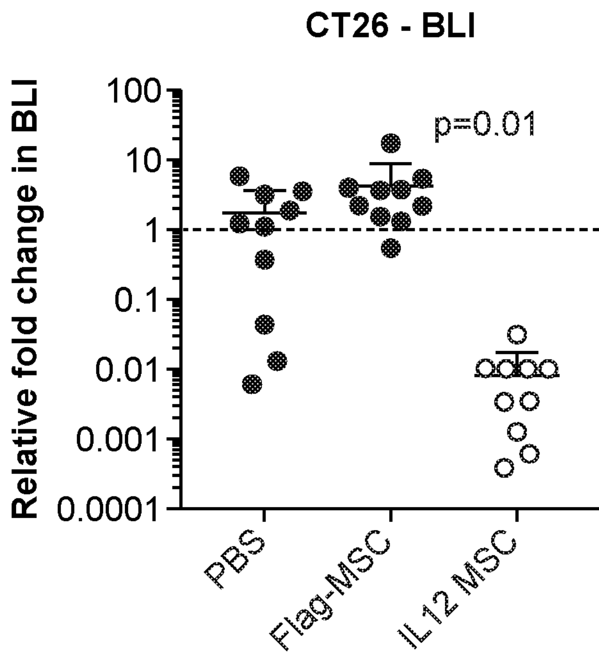


FIG. 37E





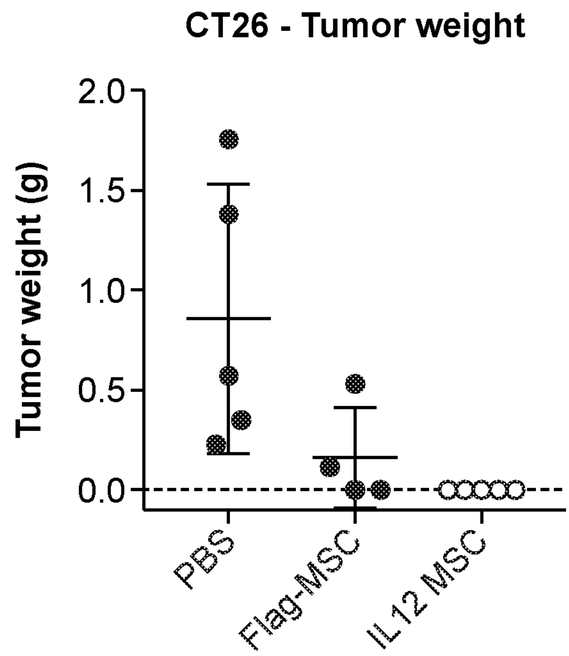


FIG. 38

Control MSC

MSC-IL12p70

B16F10 (IP)

ROI 4=5.16e+9
ROI 2=6.33e+10
1=1.22e+10
ROI 5=2.49e+10
ROI 3=1.76e+10

ROI 4=7.54e+7
ROI 2=3.32e+7
ROI 1=8.67e+8
ROI 5=1.32e+8
ROI 3=1.18e+8

B16F10 - BLI

Relative fold change in BLI
100
10
1
0.1
0.01
0.001
p=0.0017
PBS
Flag-MSC
IL12 MSC

B16F10 - tumor weights

Tumor weight (g)
2.0
1.5
1.0
0.5
0.0
p=0.01
PBS
Flag-MSC
IL12 MSC

FIG. 39

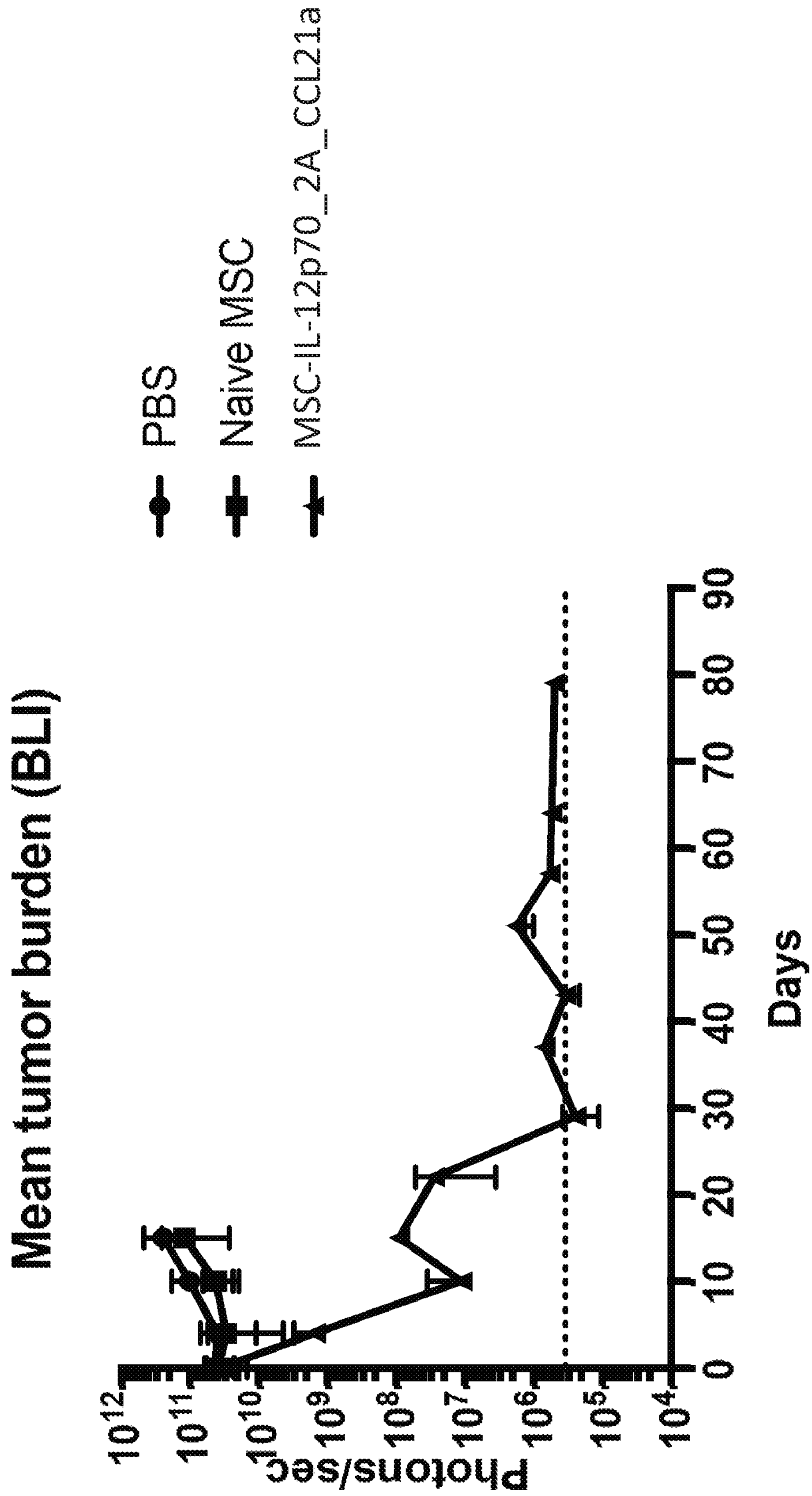


FIG. 40A

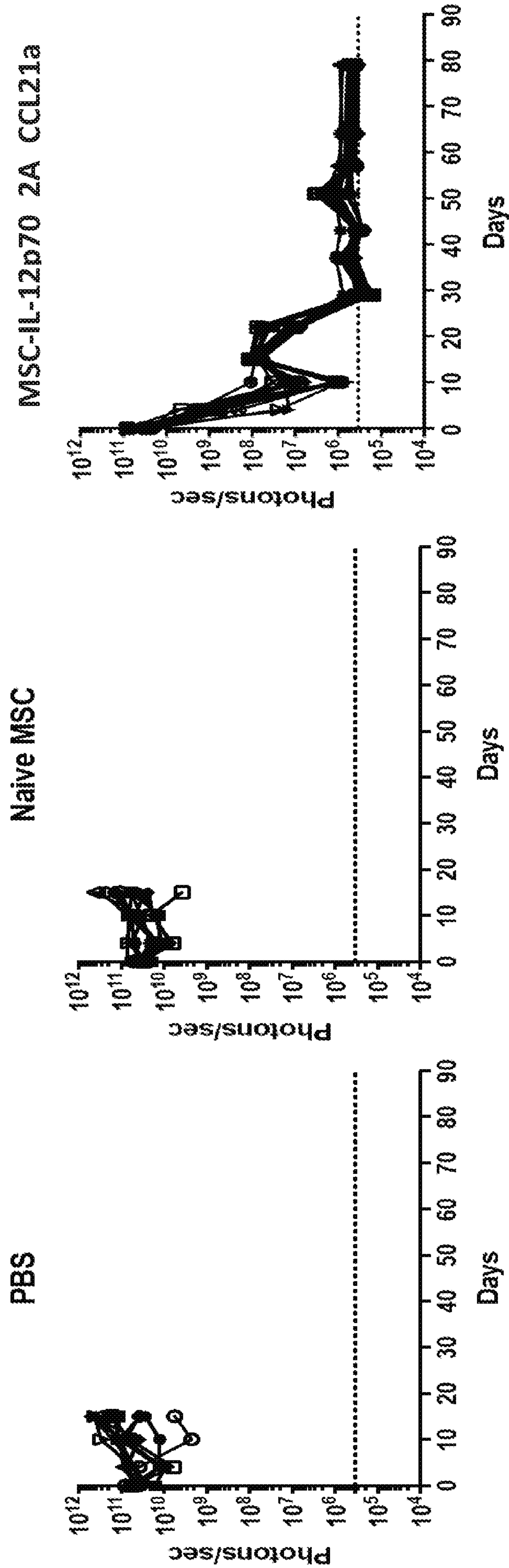


FIG. 40B

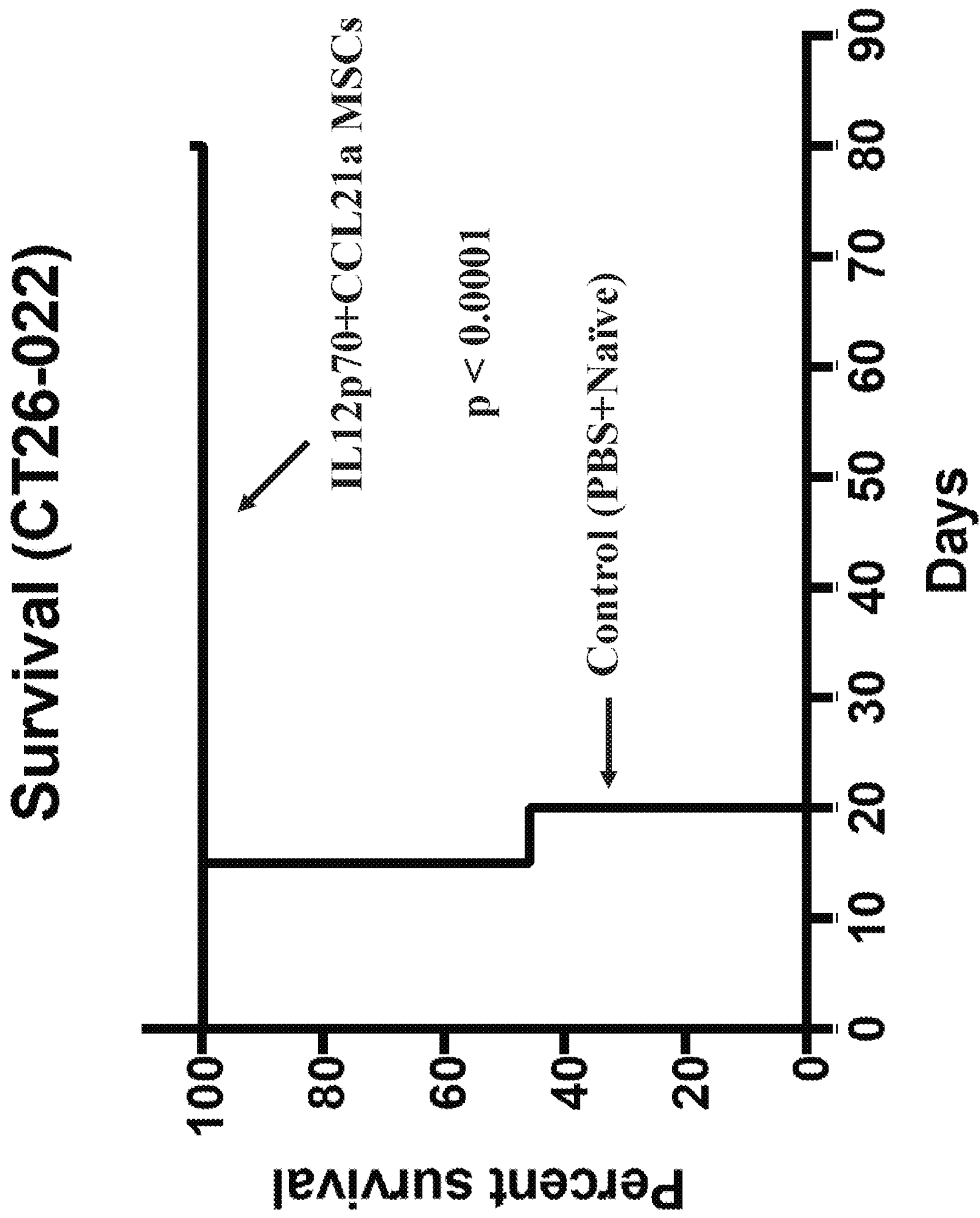


FIG. 40C

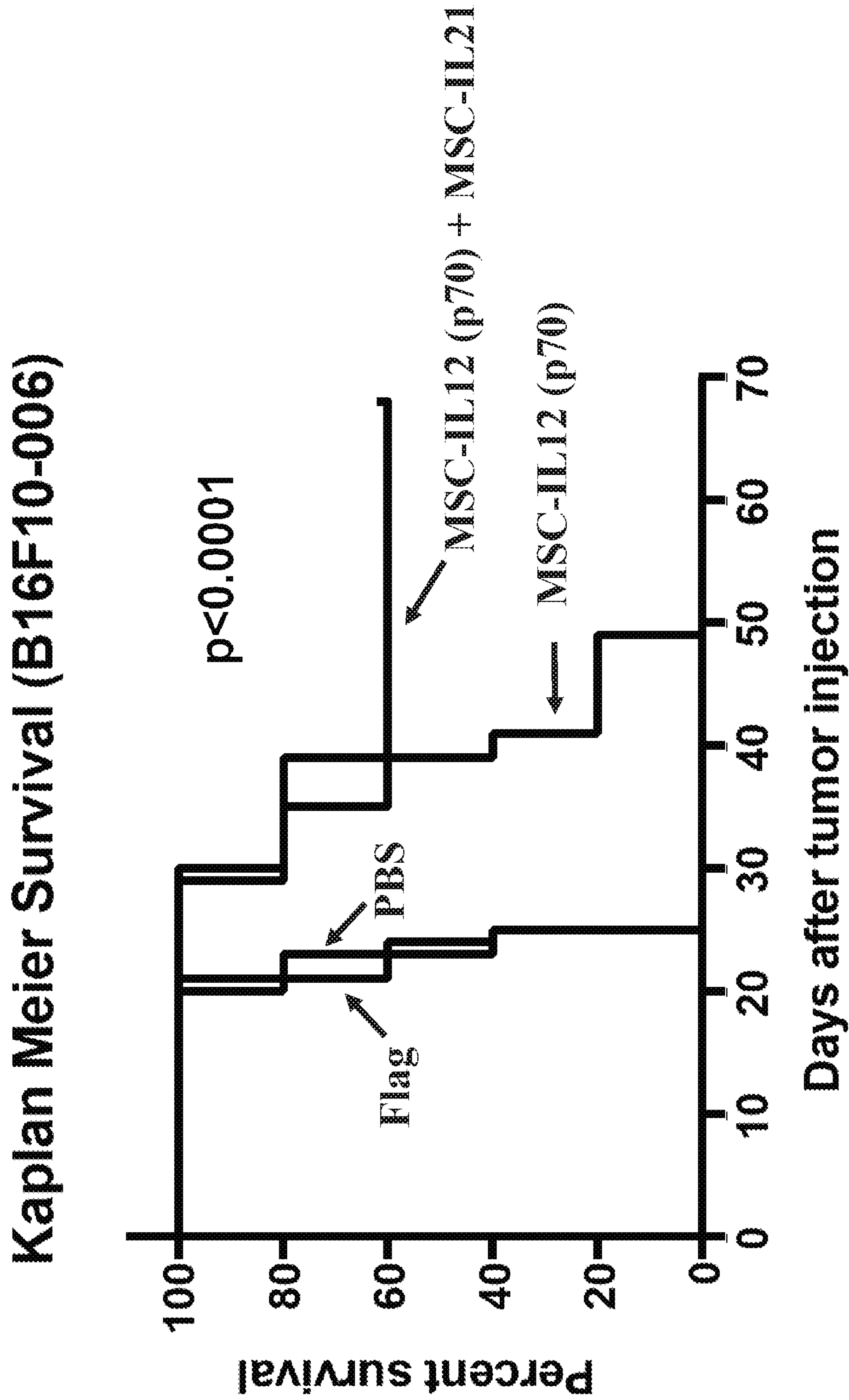


FIG. 41

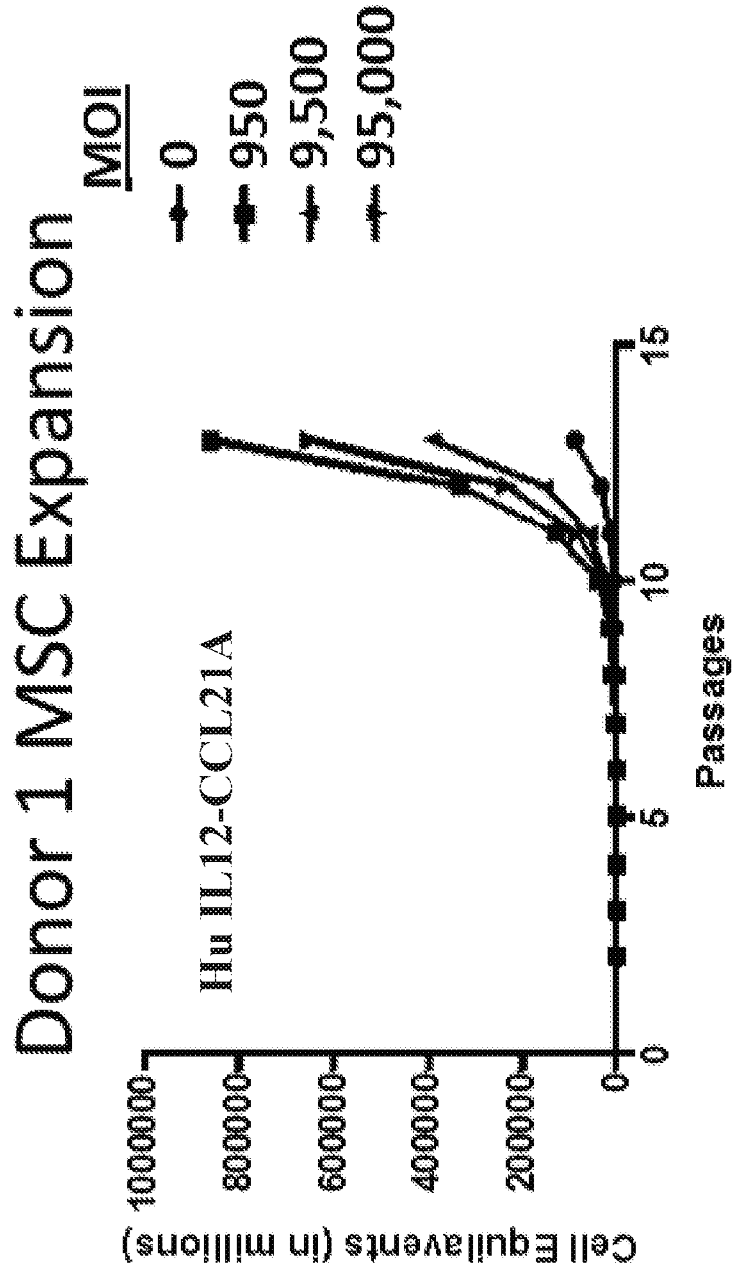


FIG. 42A

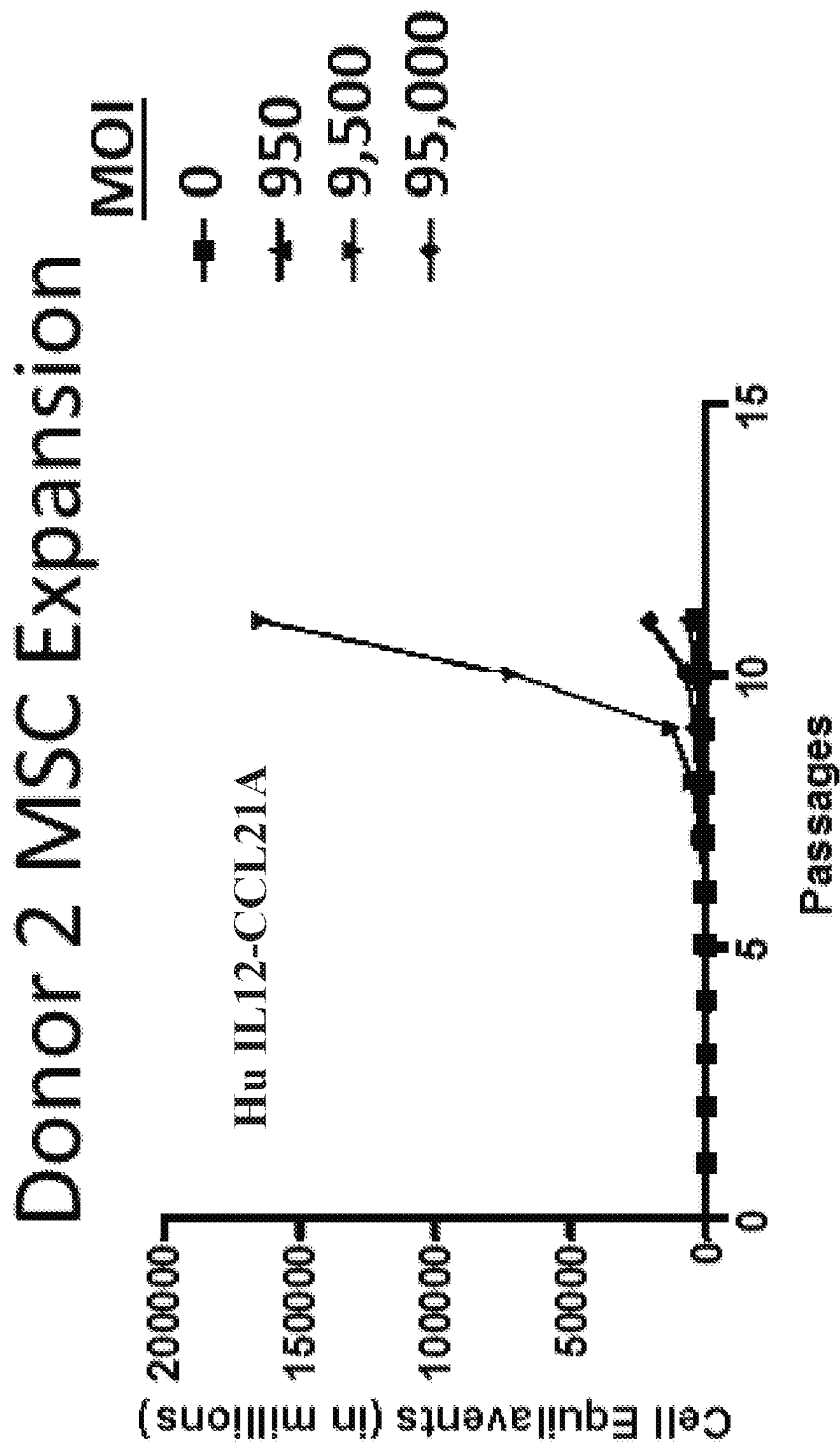


FIG. 42B

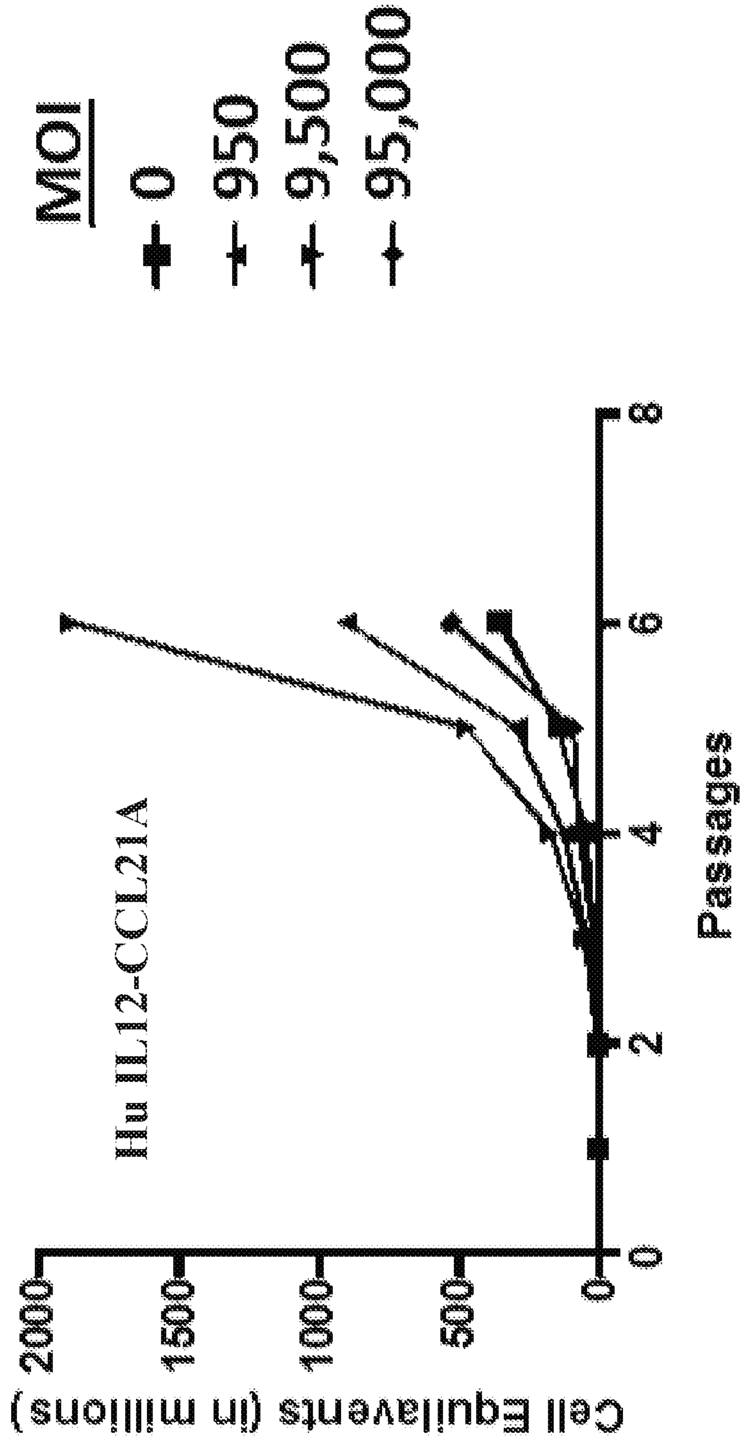


FIG. 42C

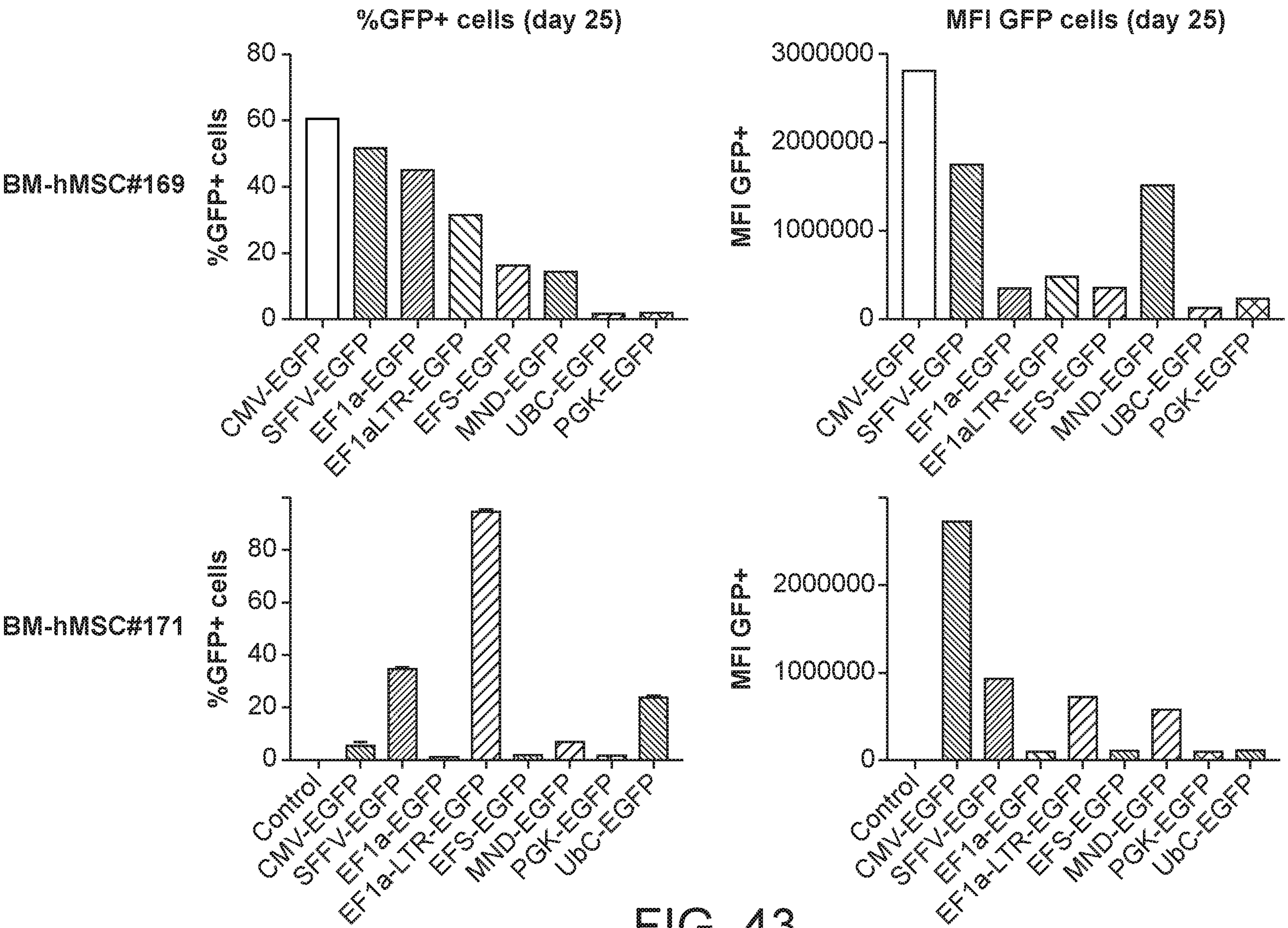


FIG. 43

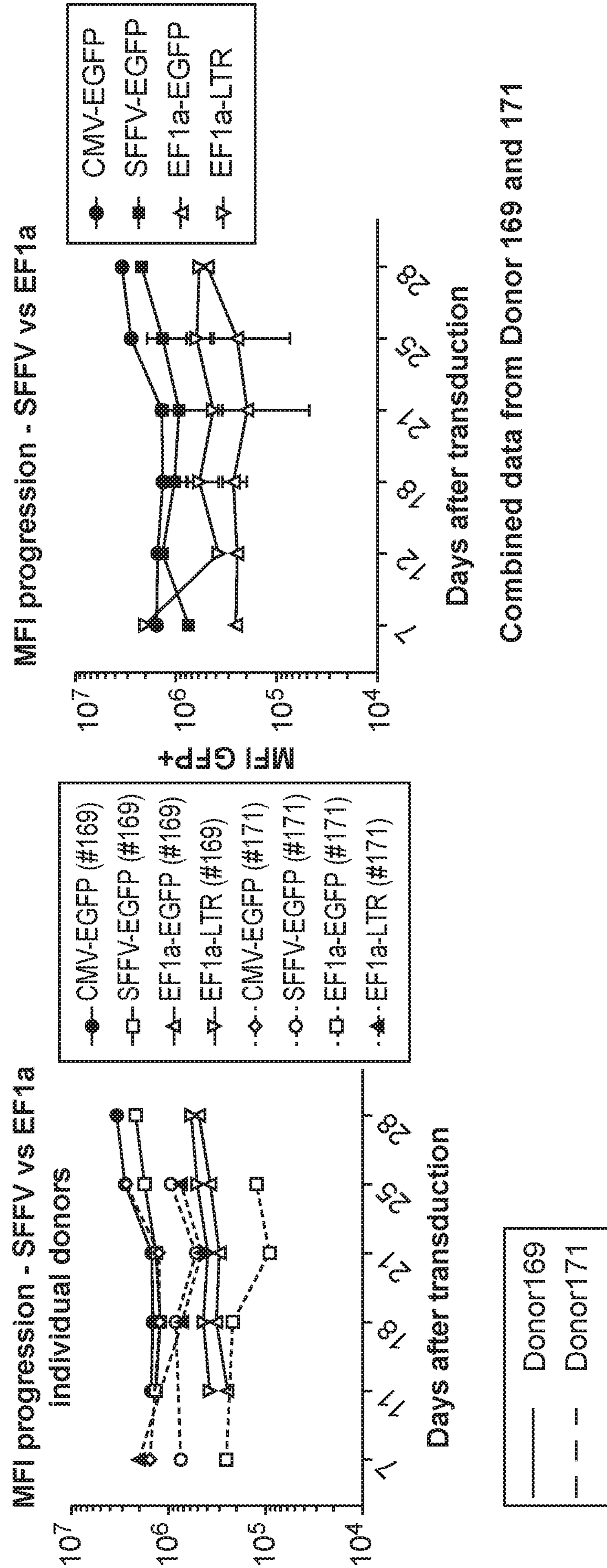


FIG. 44

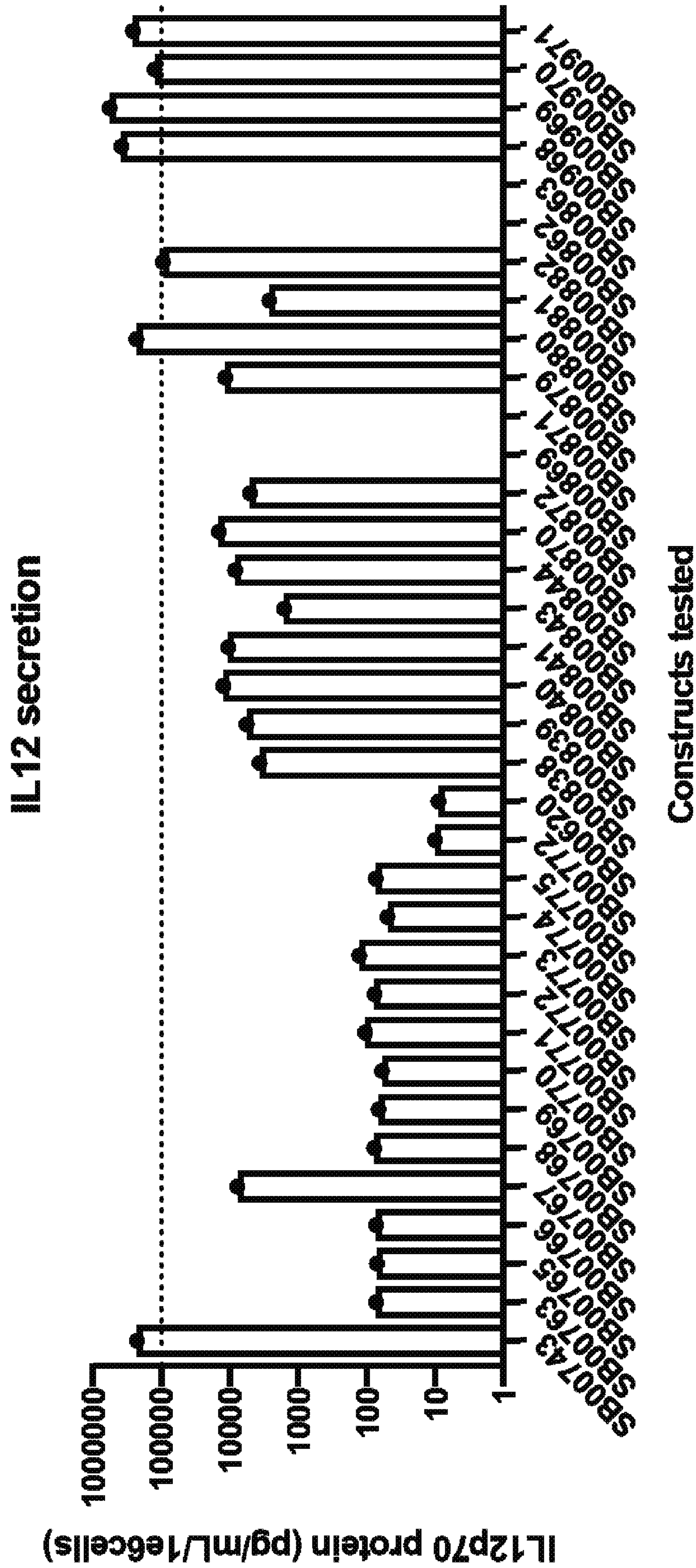


FIG. 45

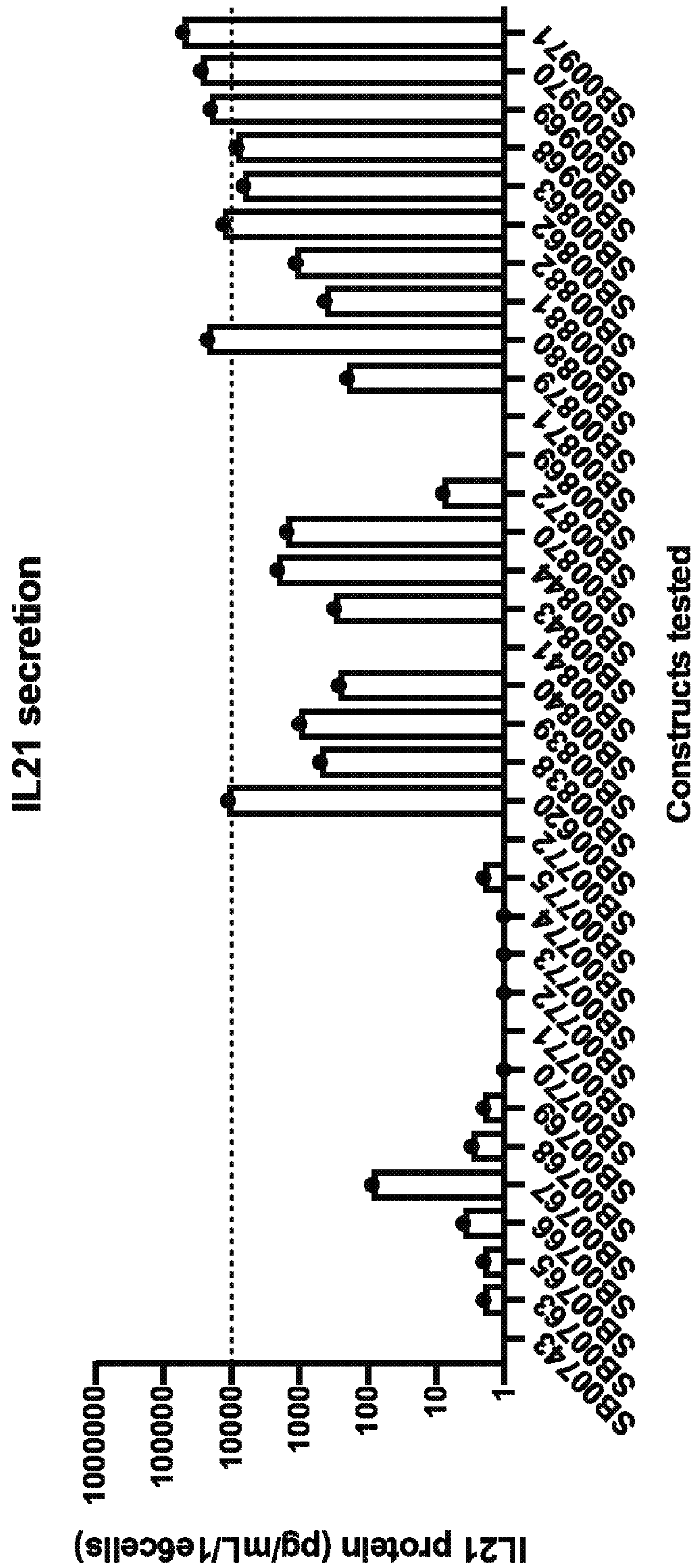


FIG. 46

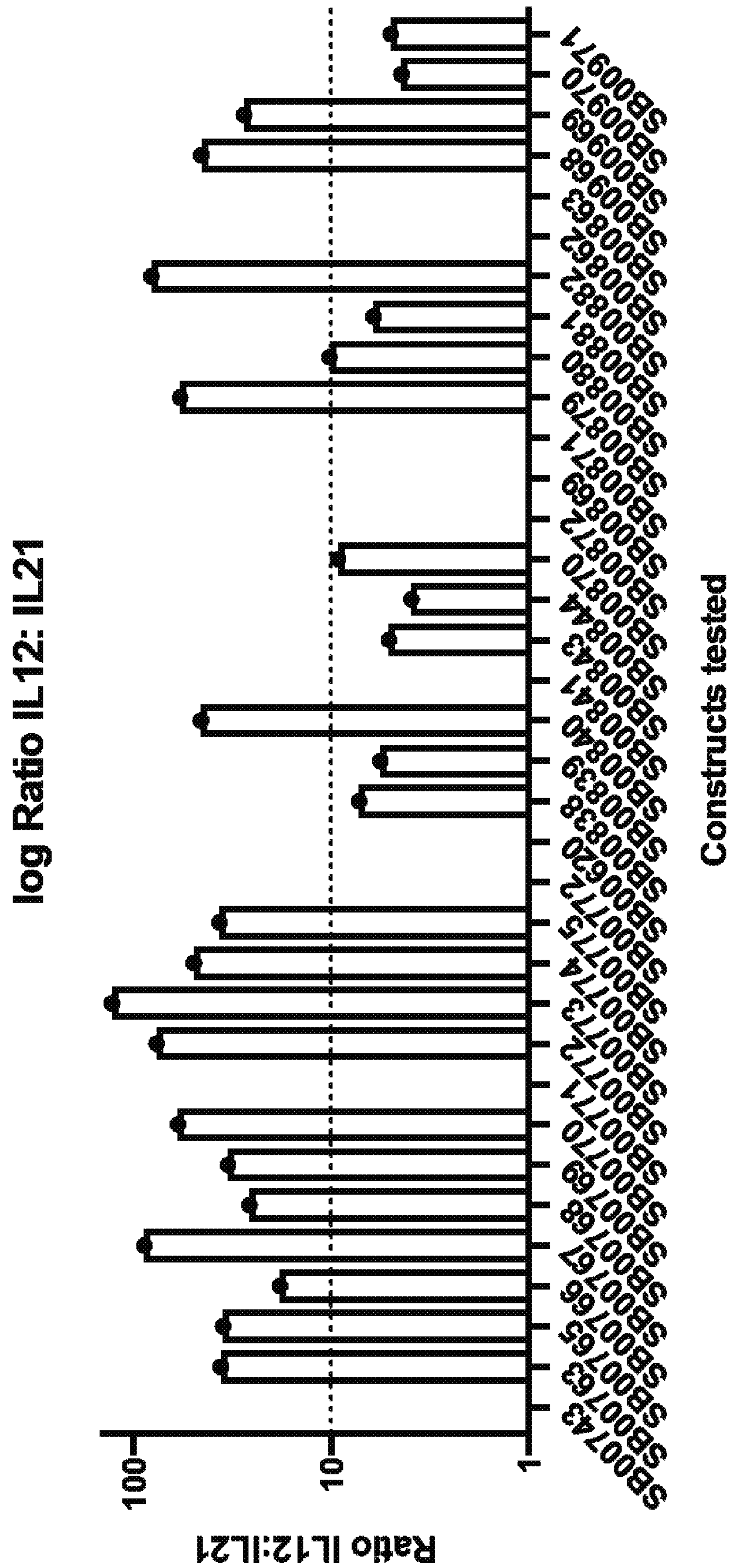


FIG. 47

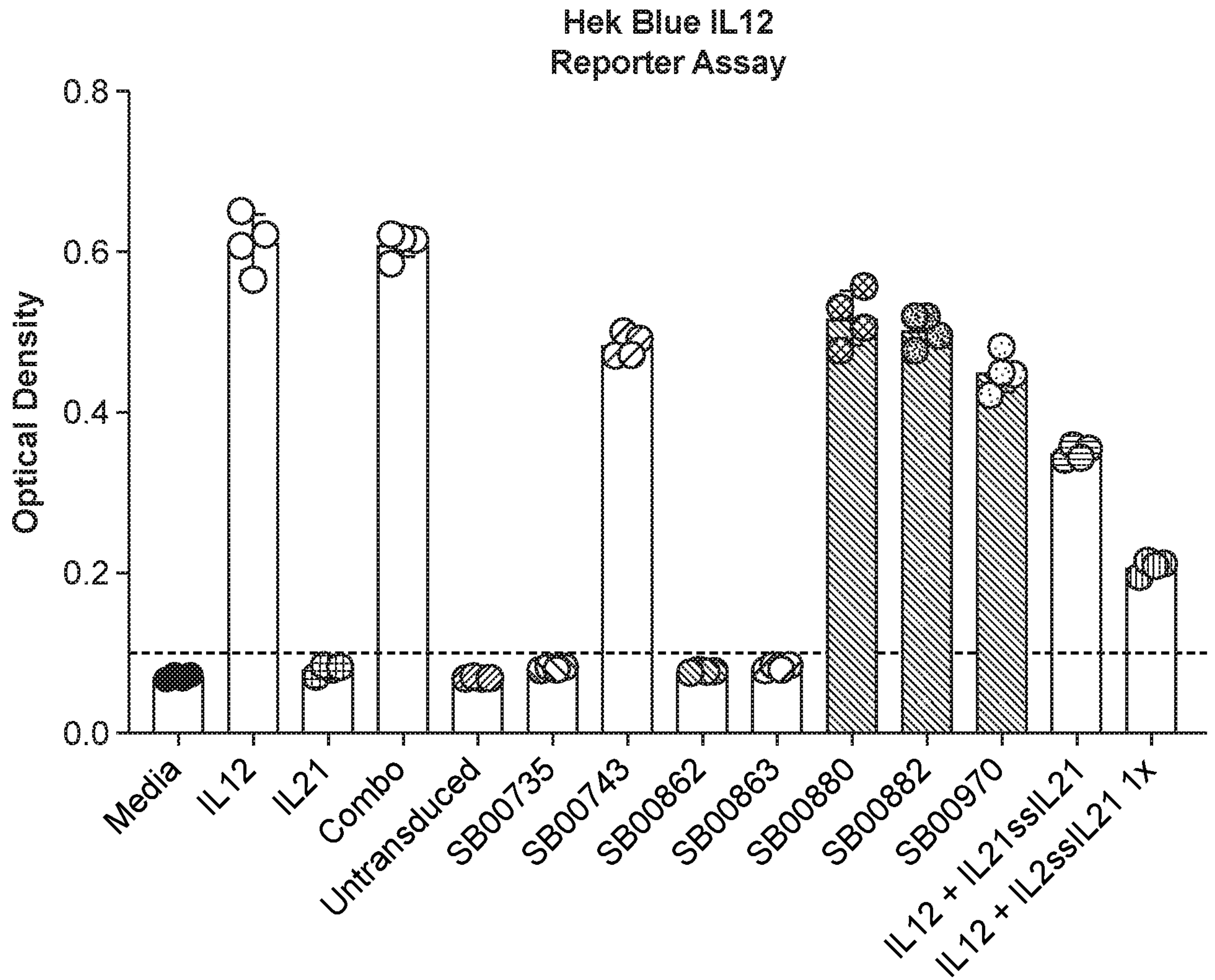


FIG. 48

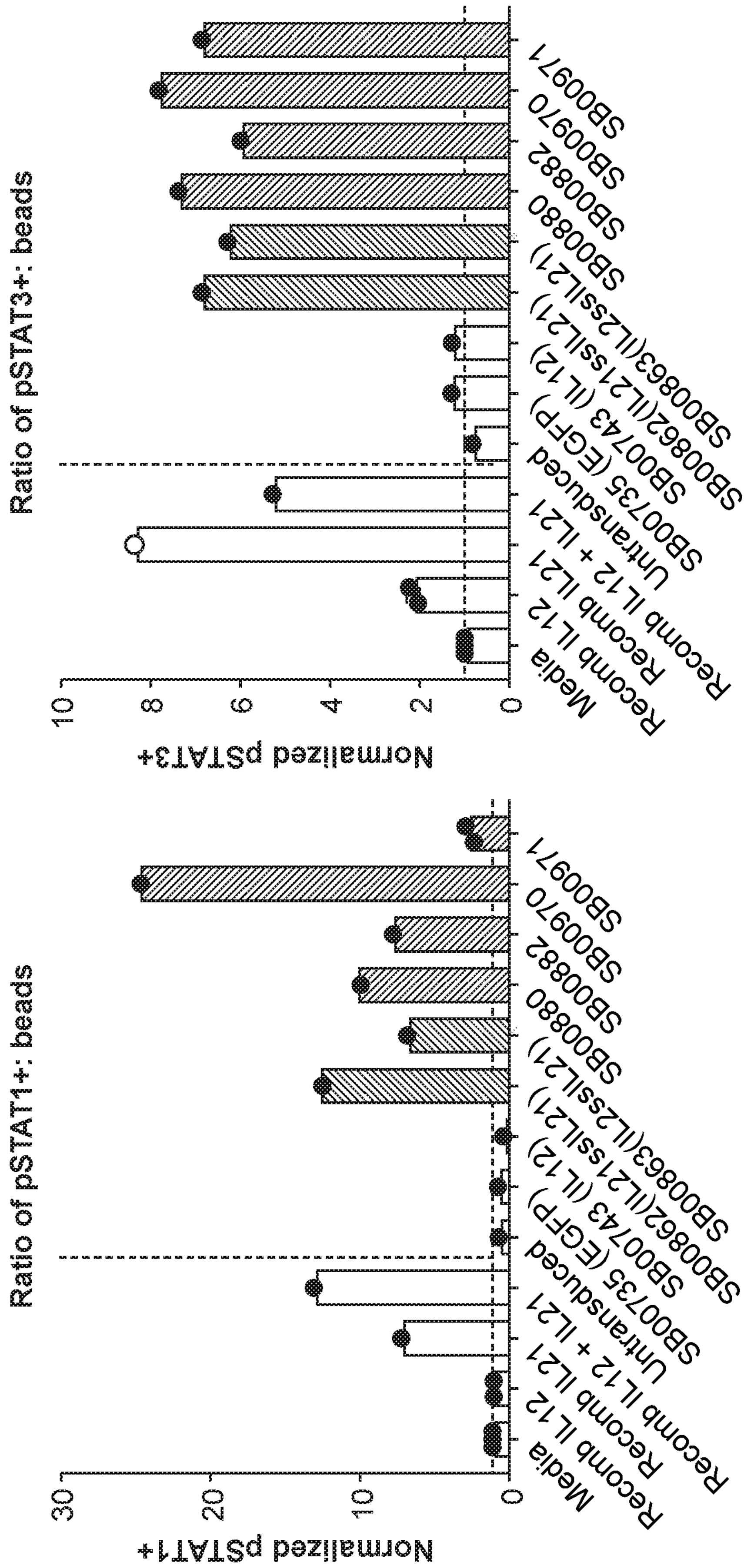
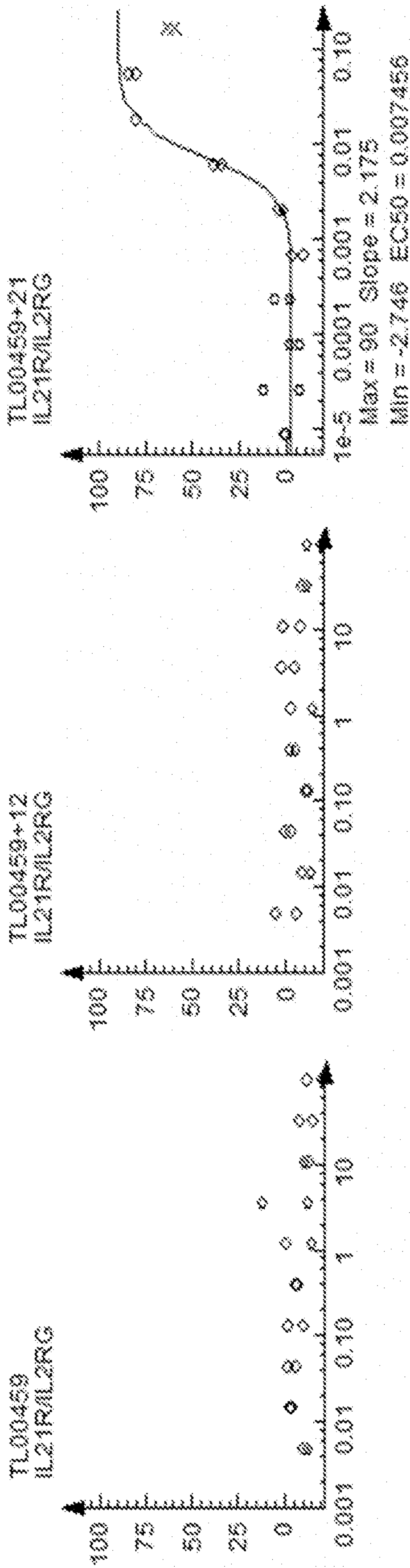


FIG. 49



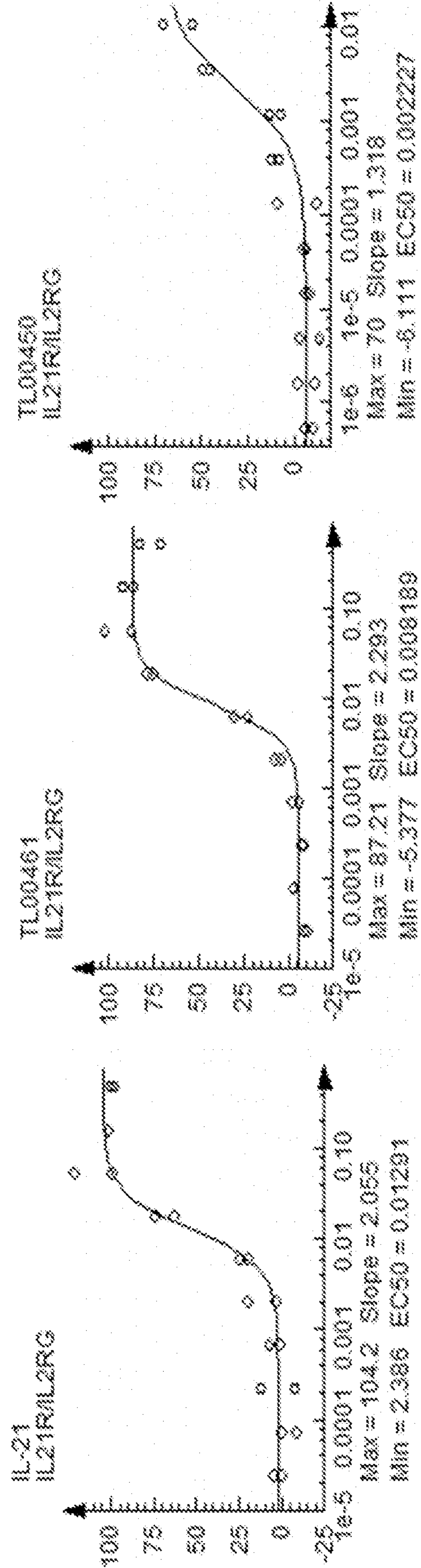


FIG. 50

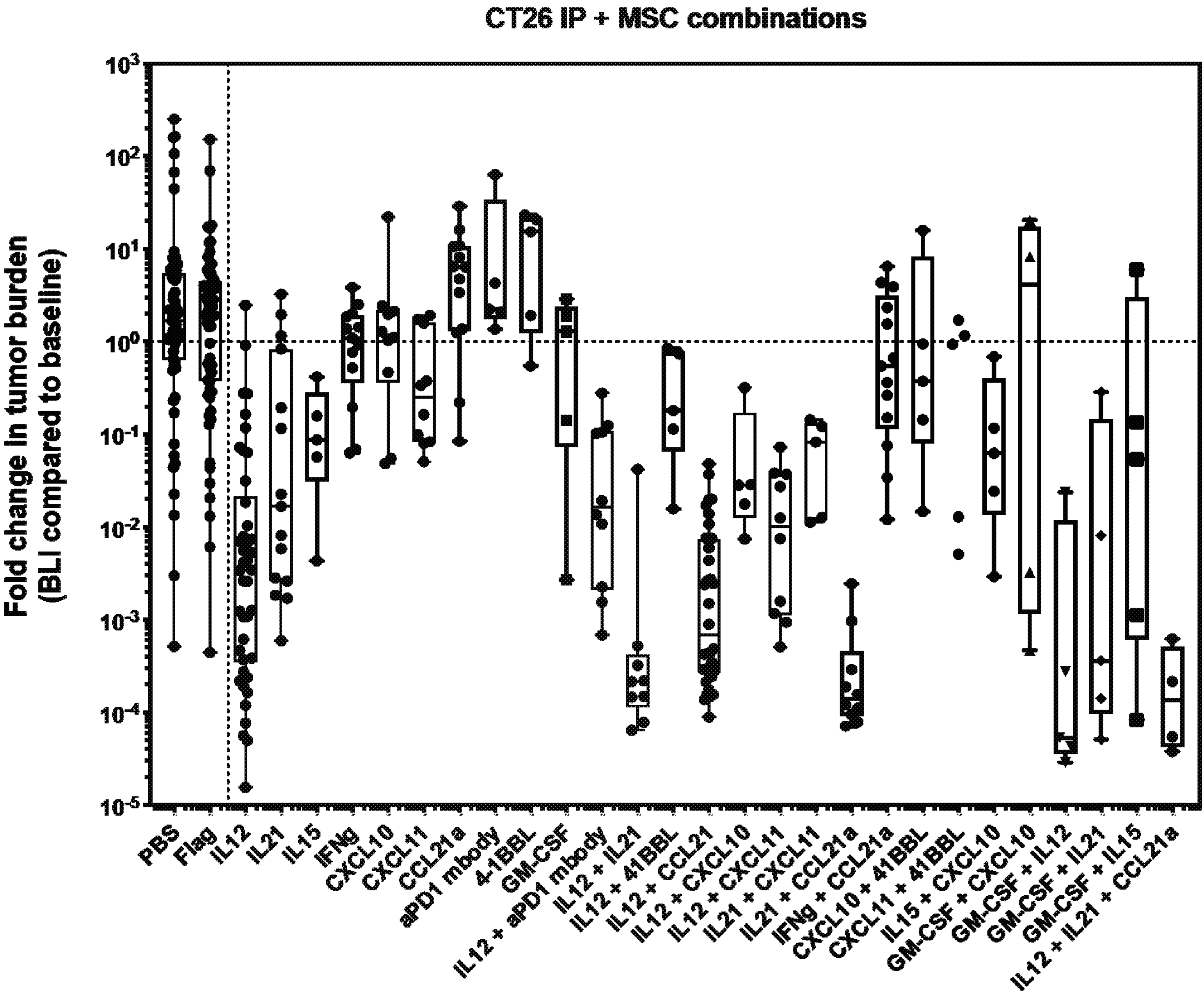


FIG. 51A

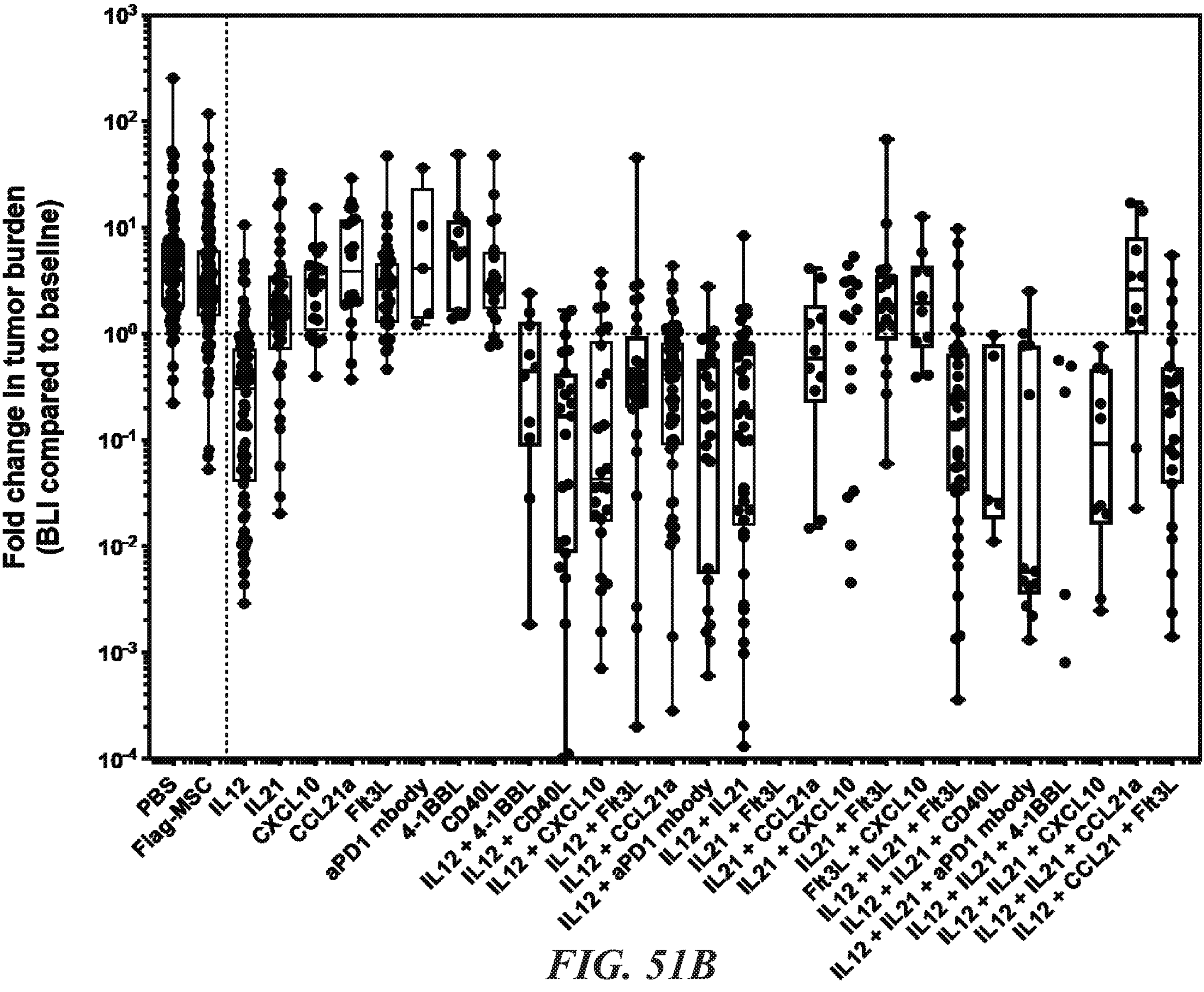


FIG. 51B

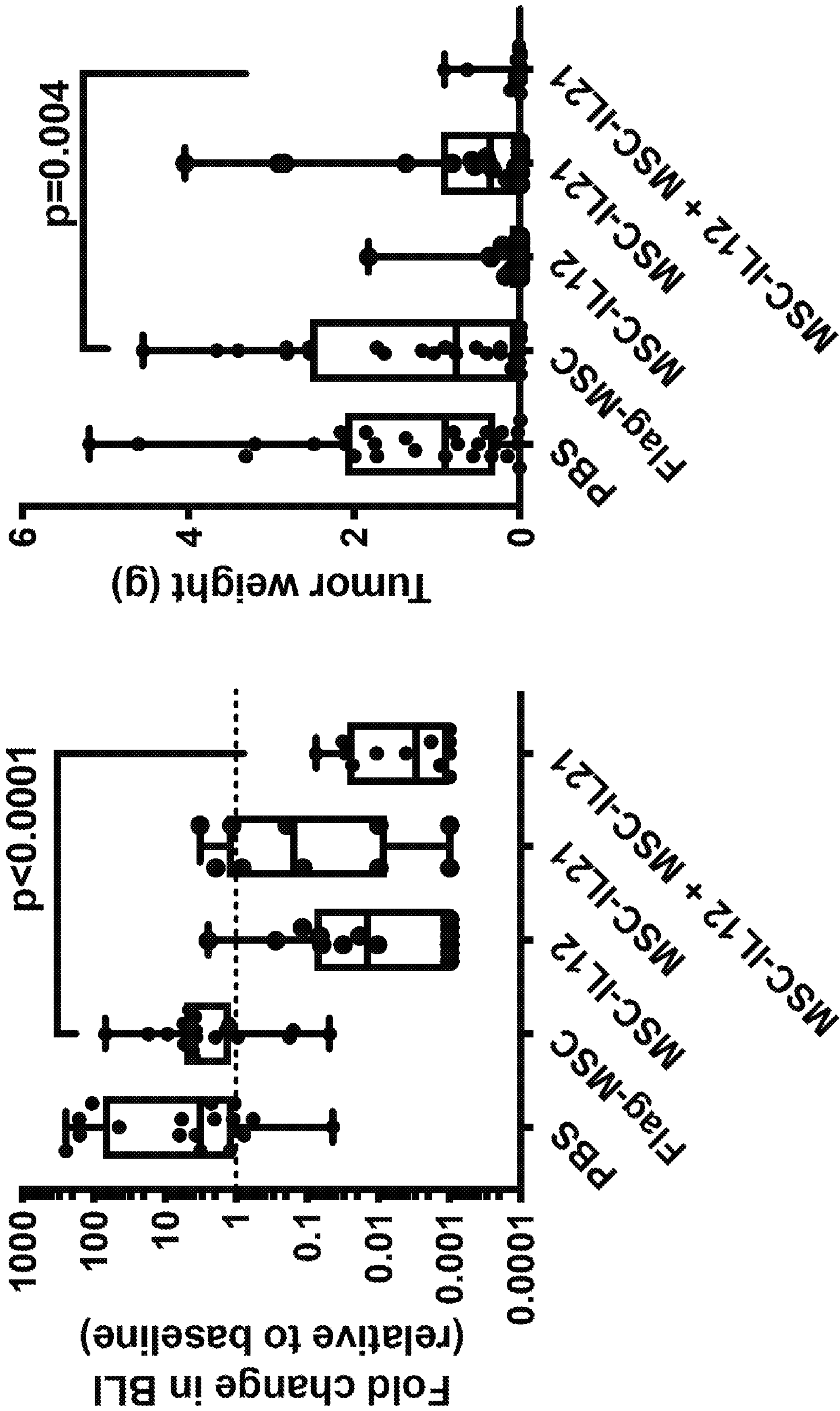


FIG. 52A

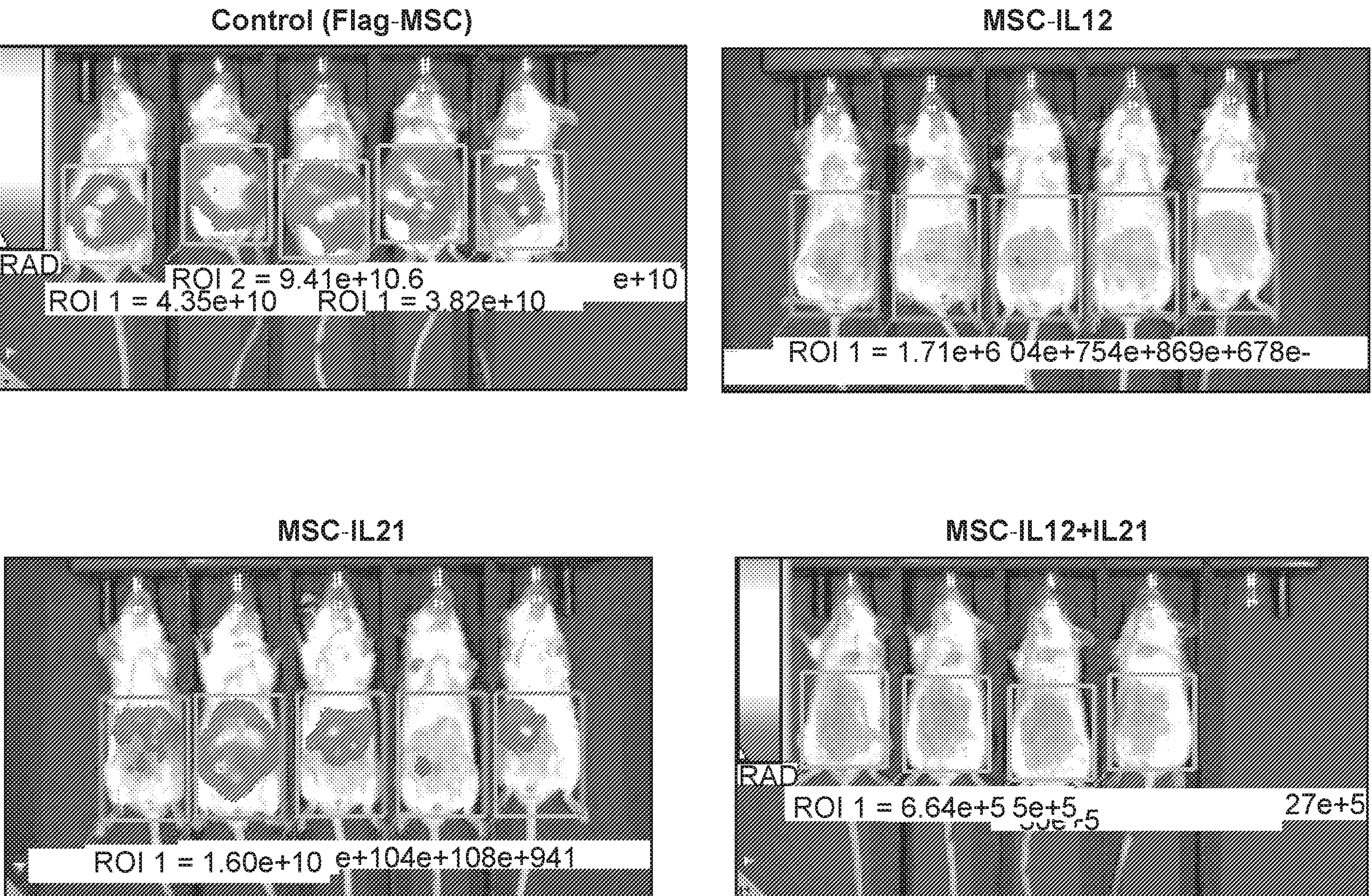


FIG. 52B

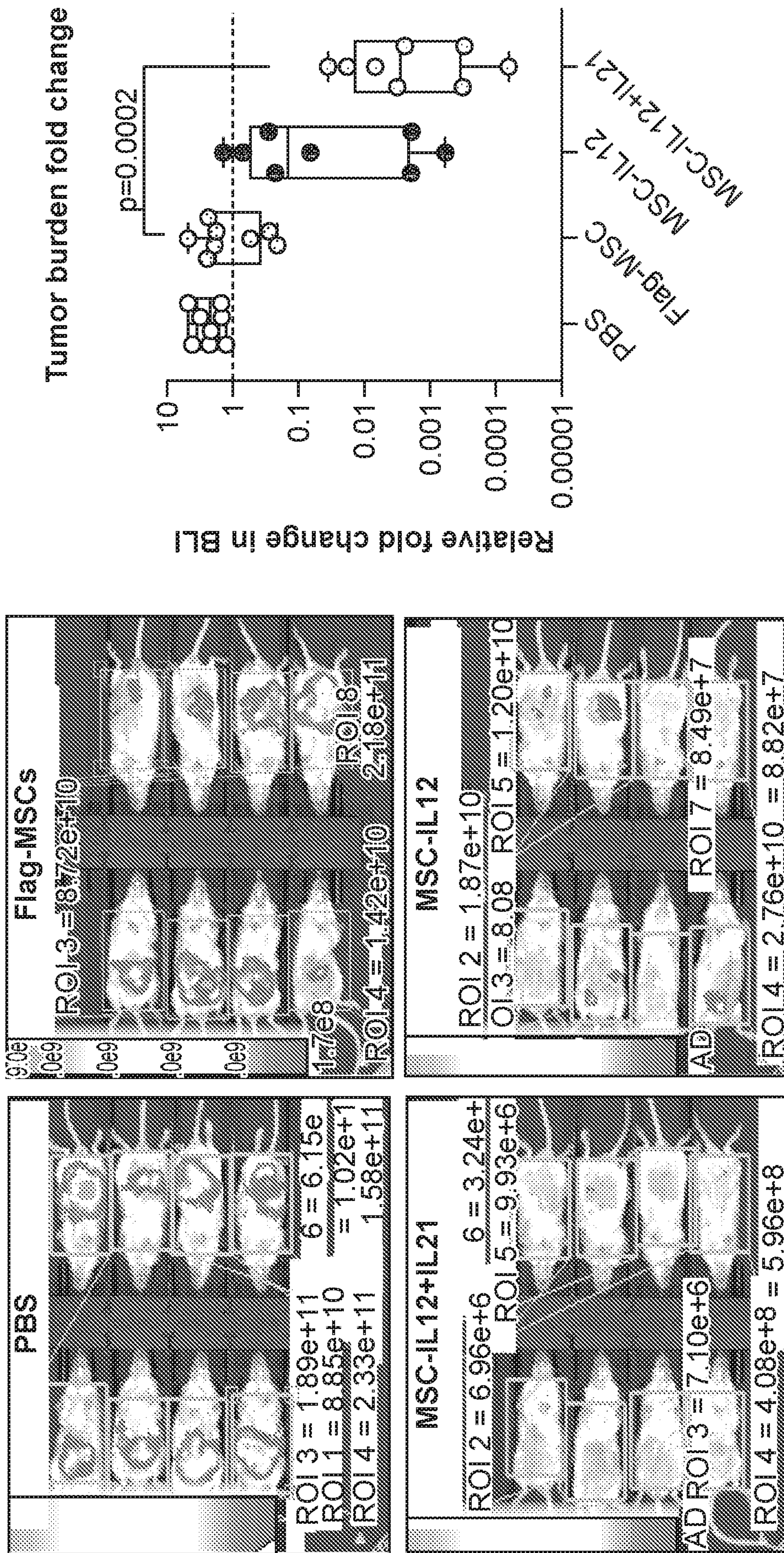


FIG. 53A

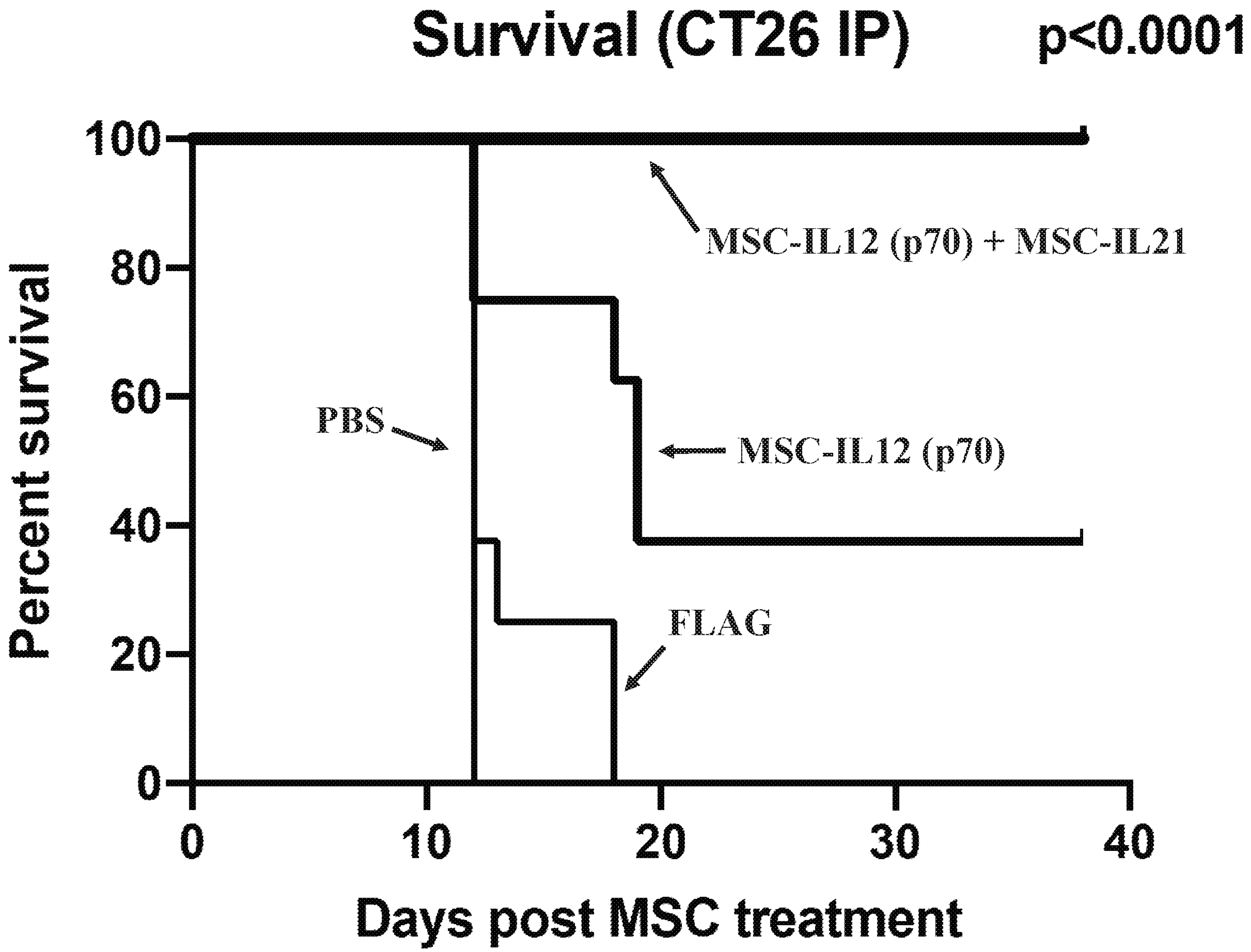


FIG. 53B

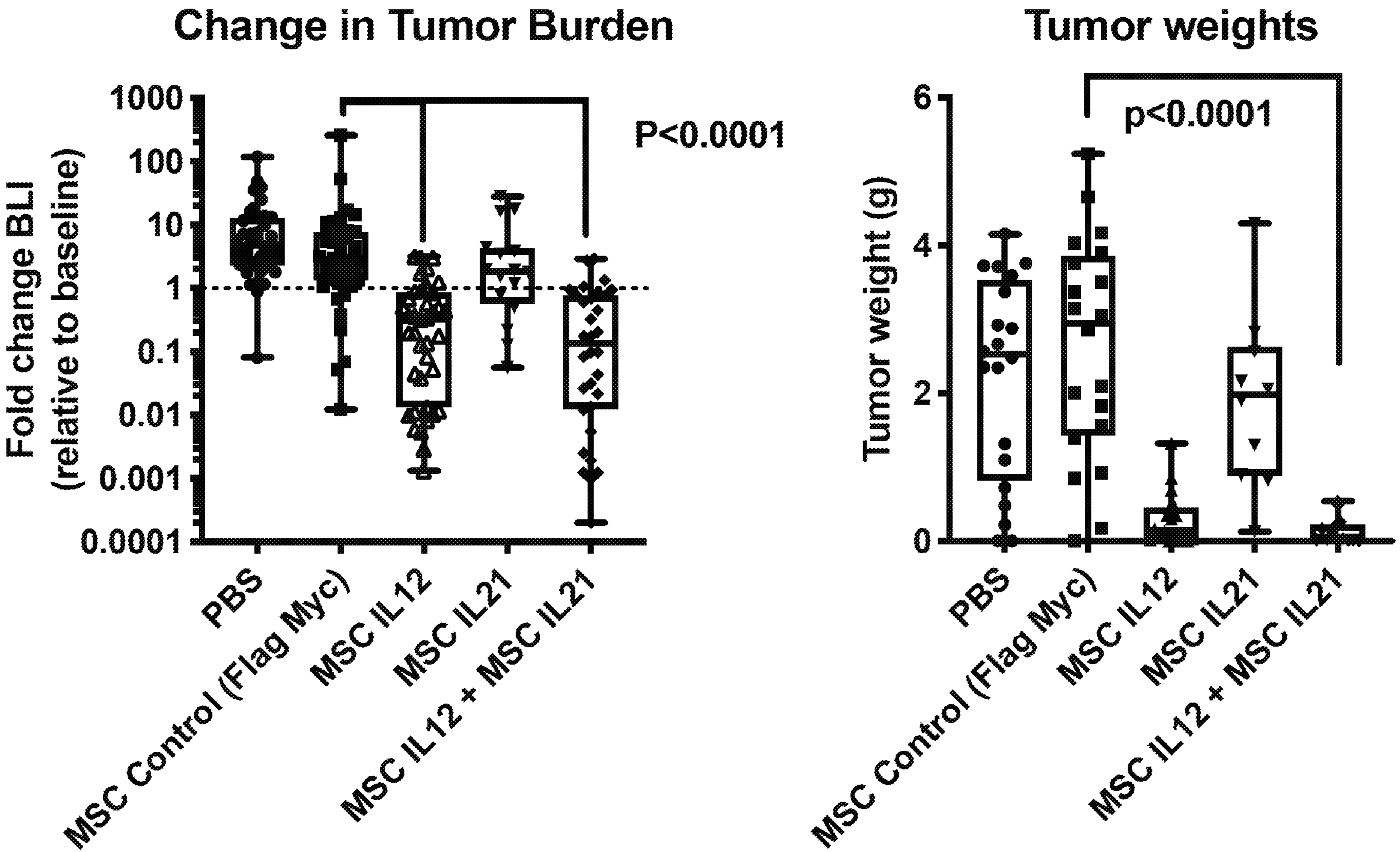


FIG. 54

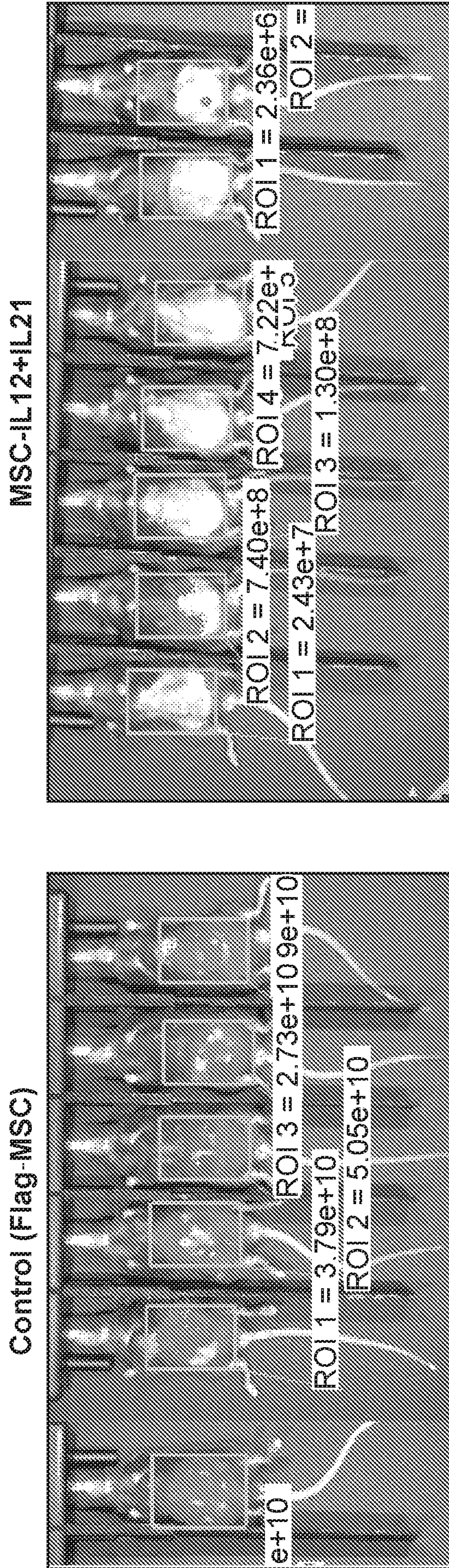


FIG. 55

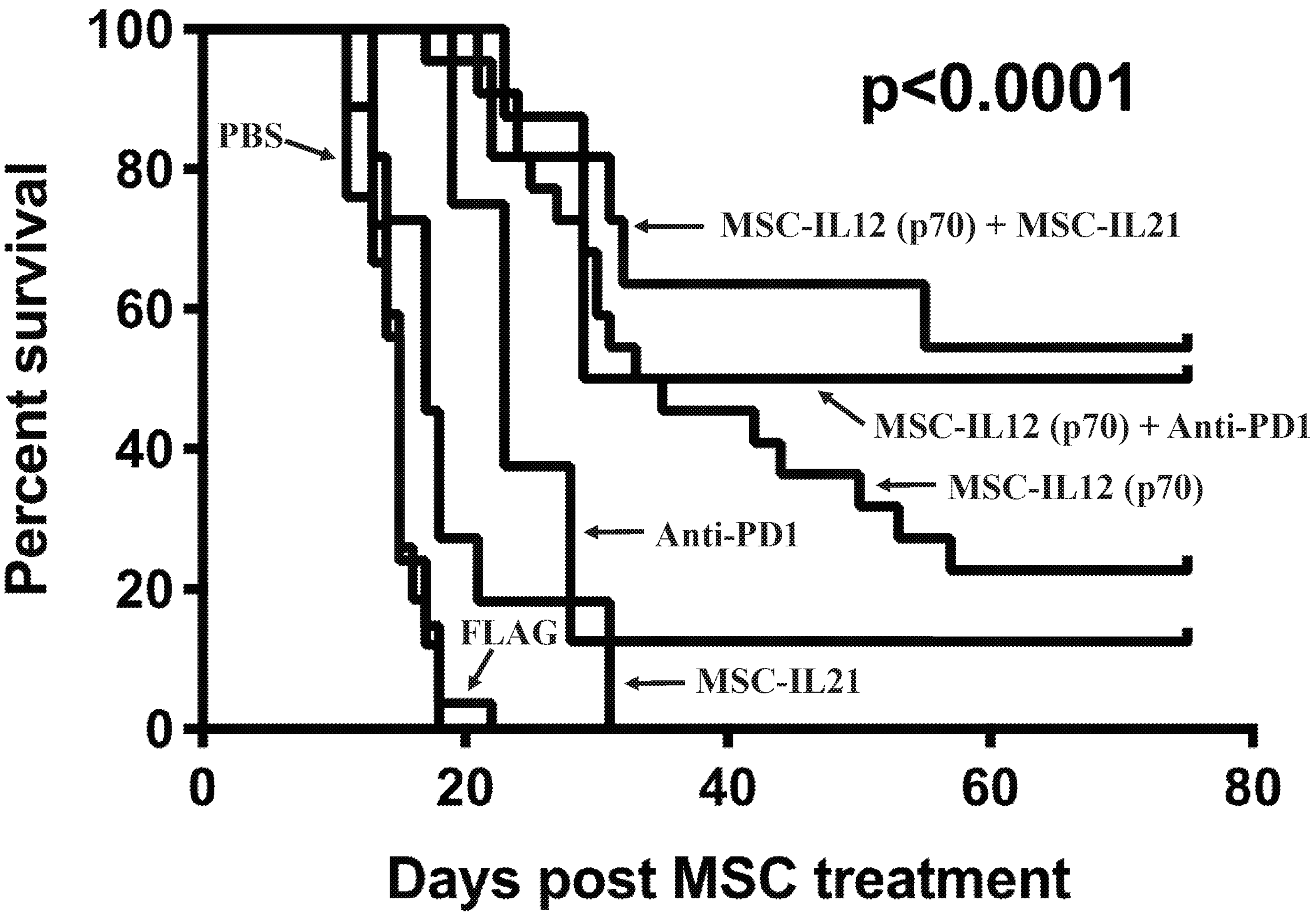


FIG. 56

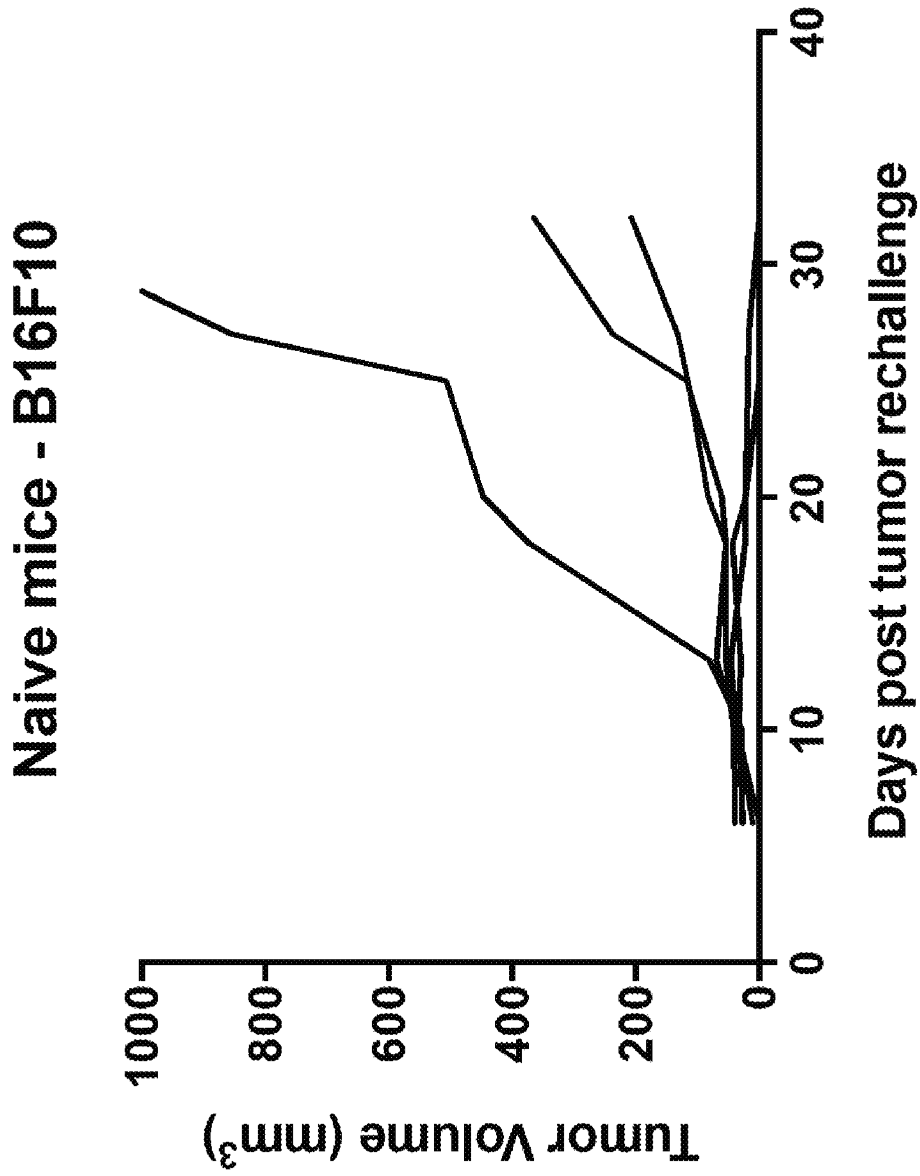


FIG. 57A

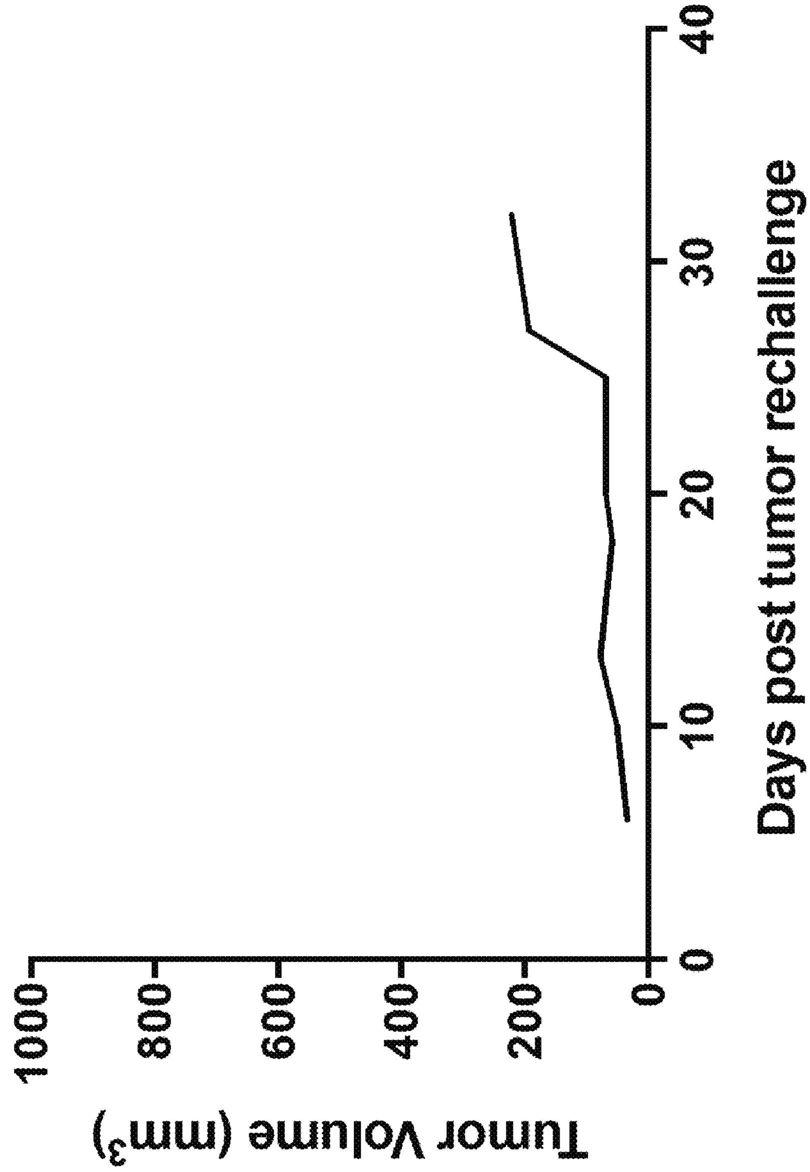


FIG. 57B

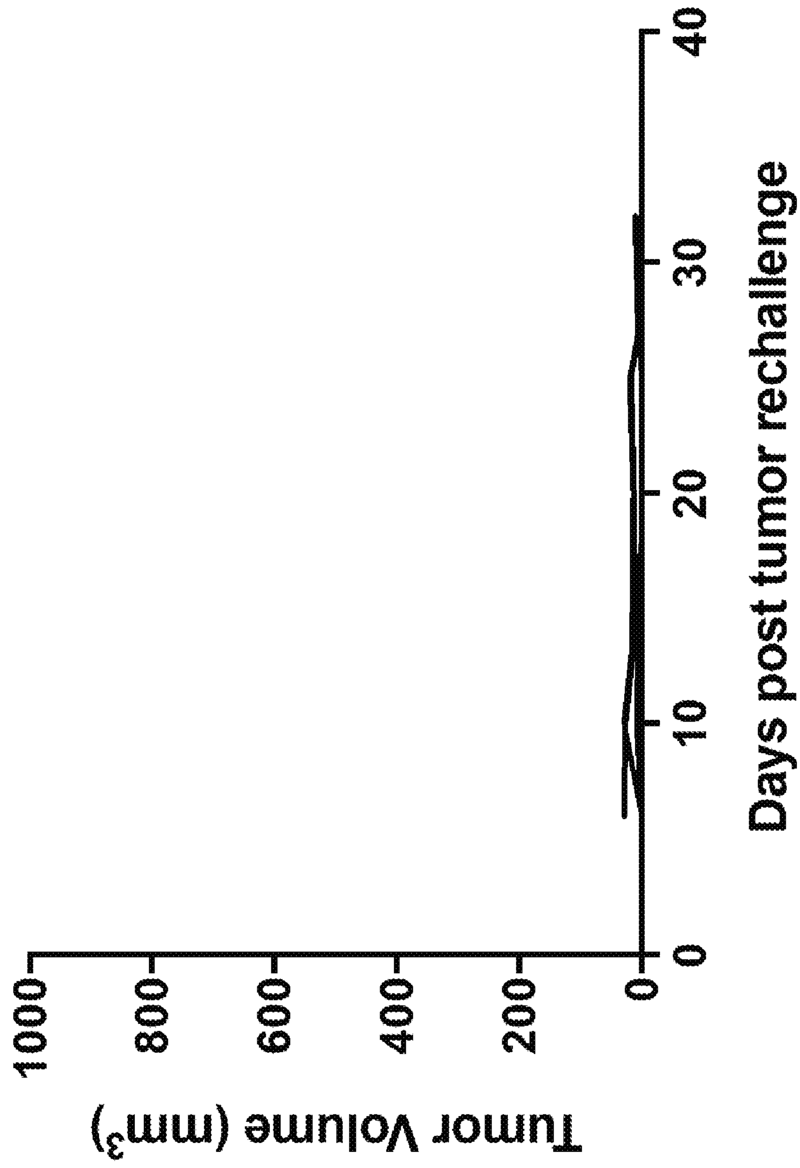


FIG. 57C

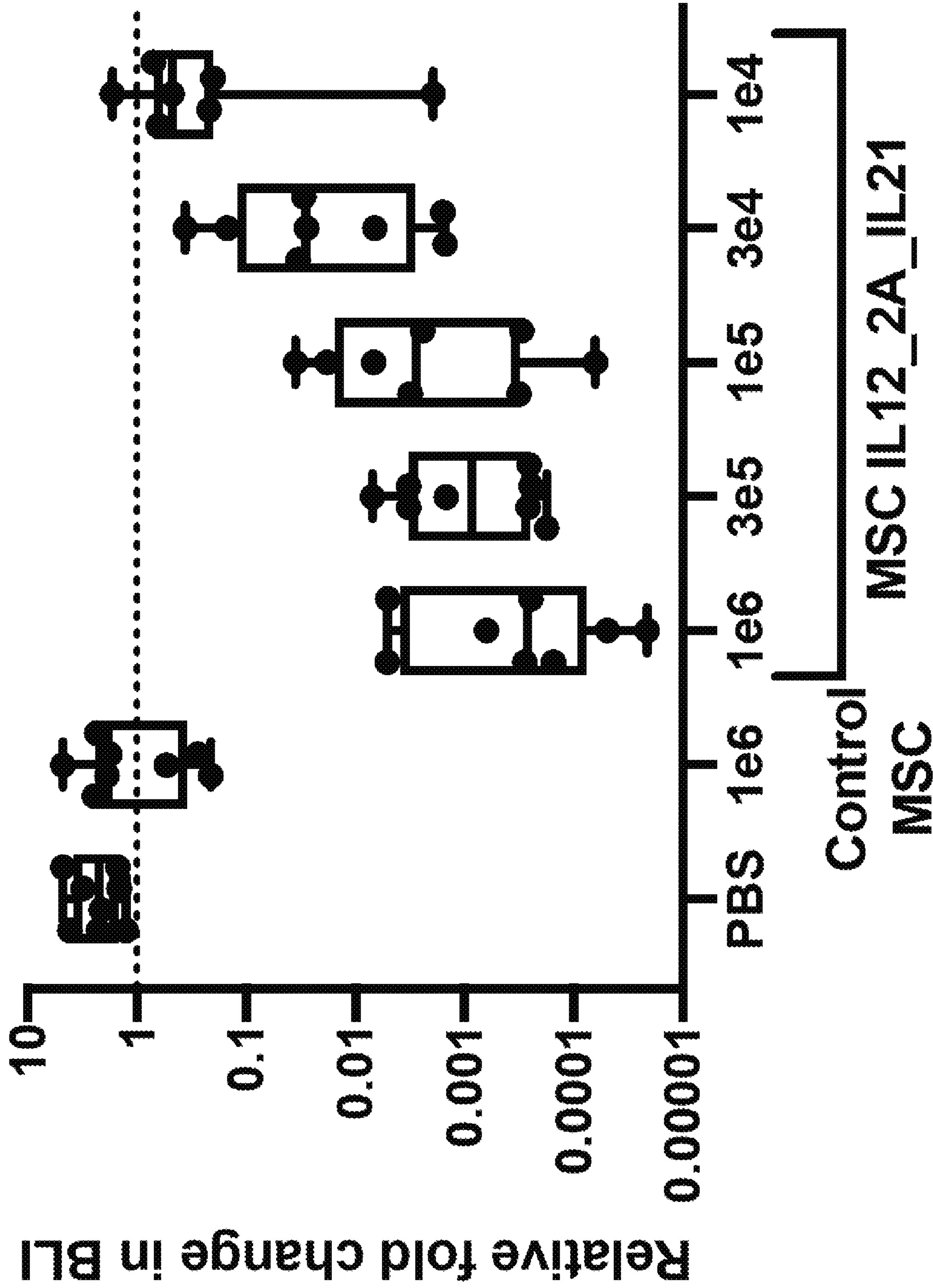


FIG. 58A

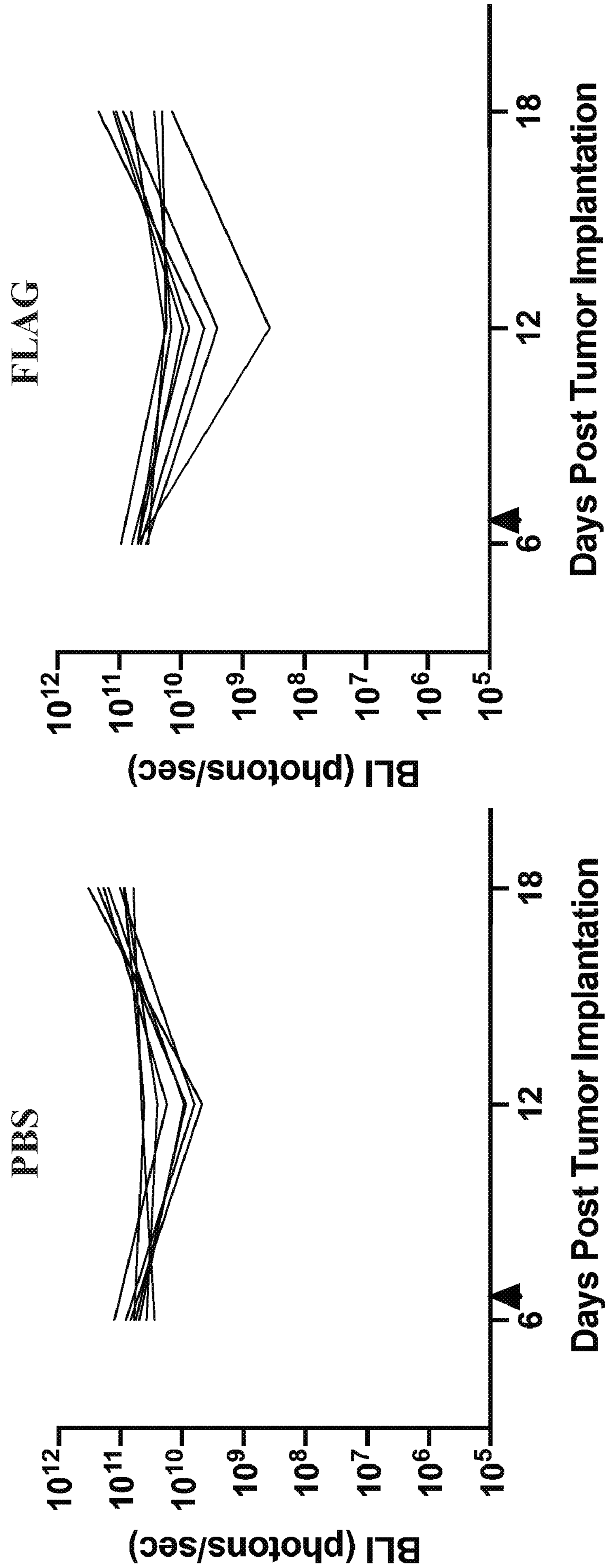


FIG. 58B

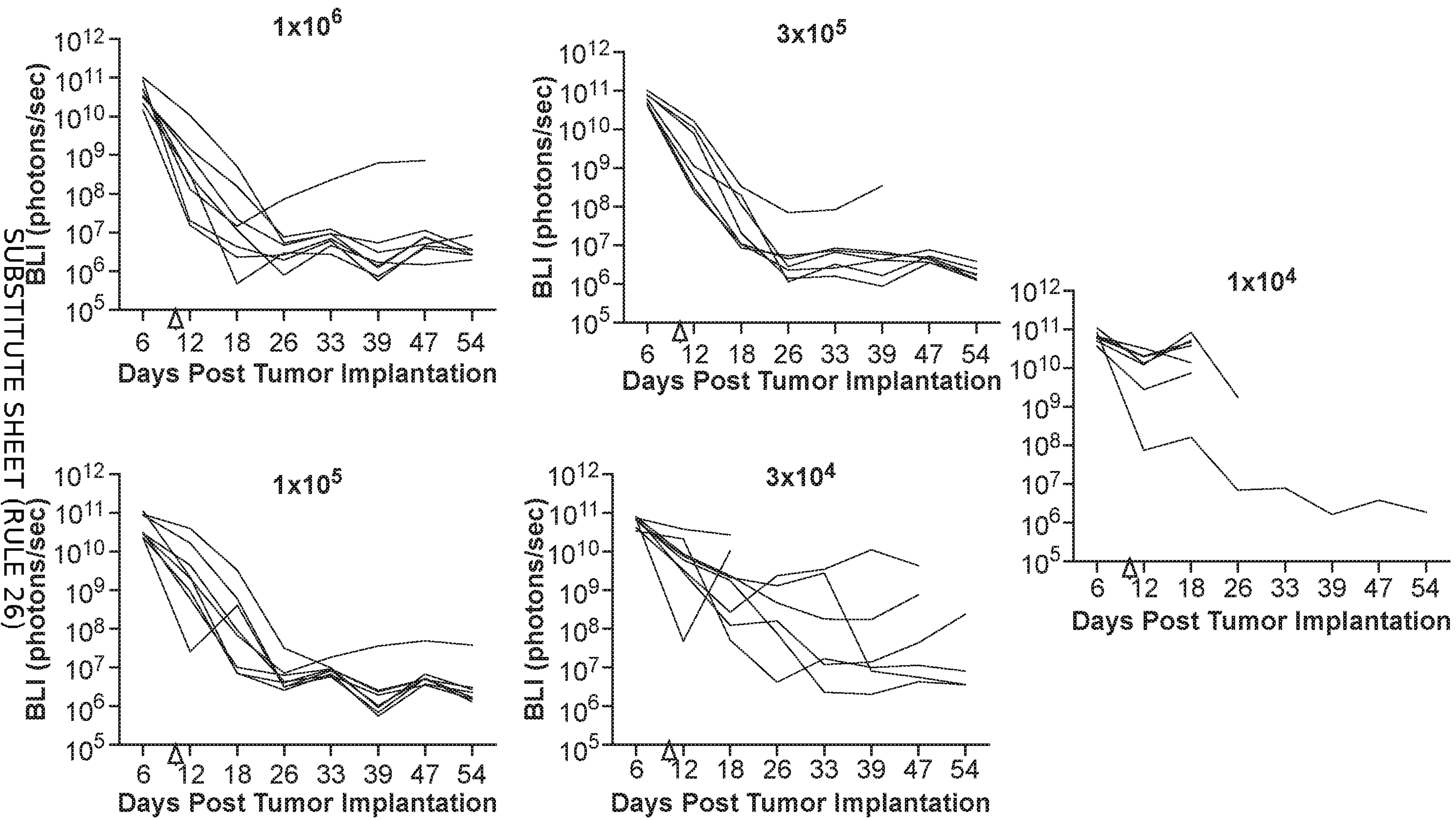


FIG. 58C

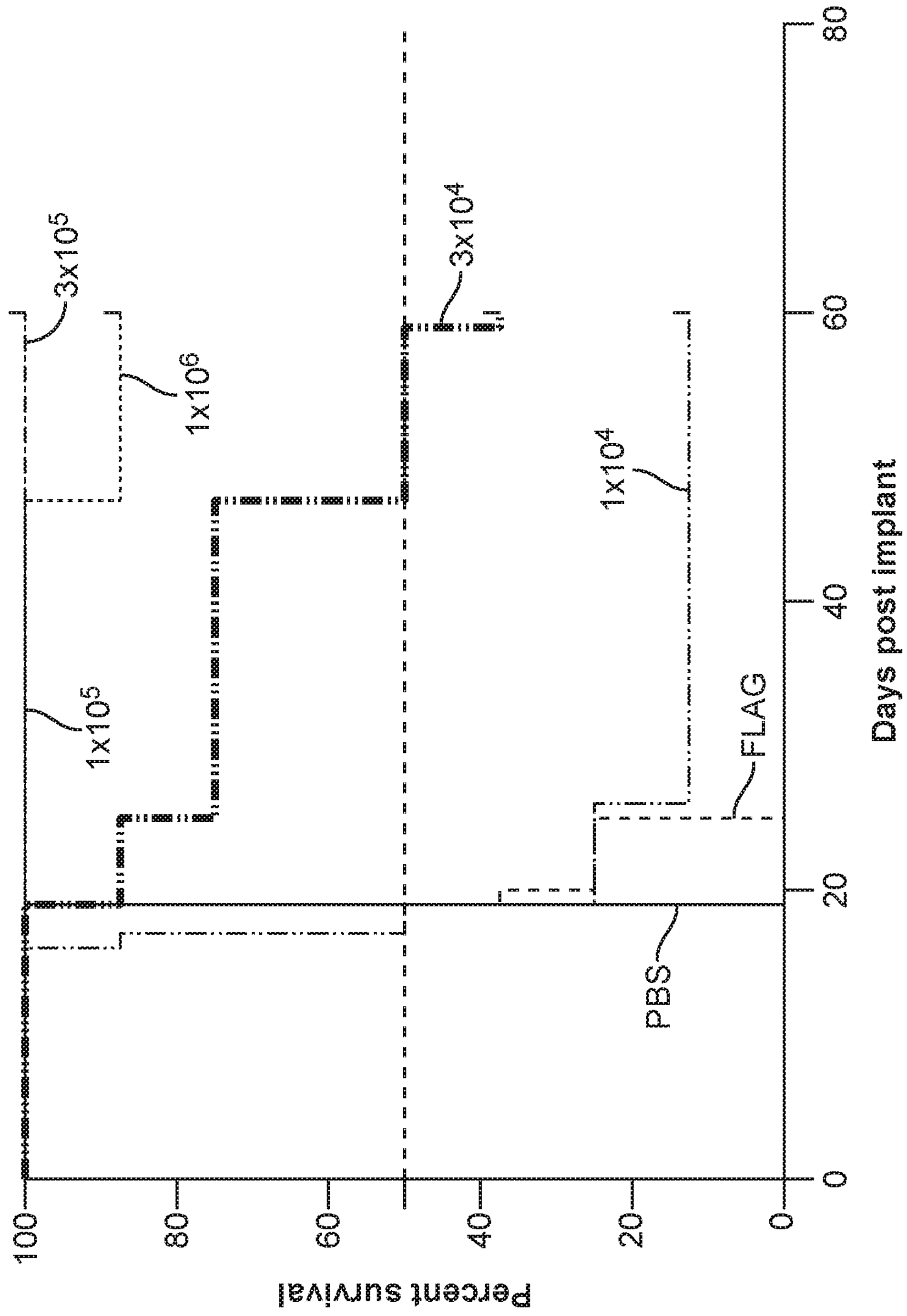


FIG. 58D

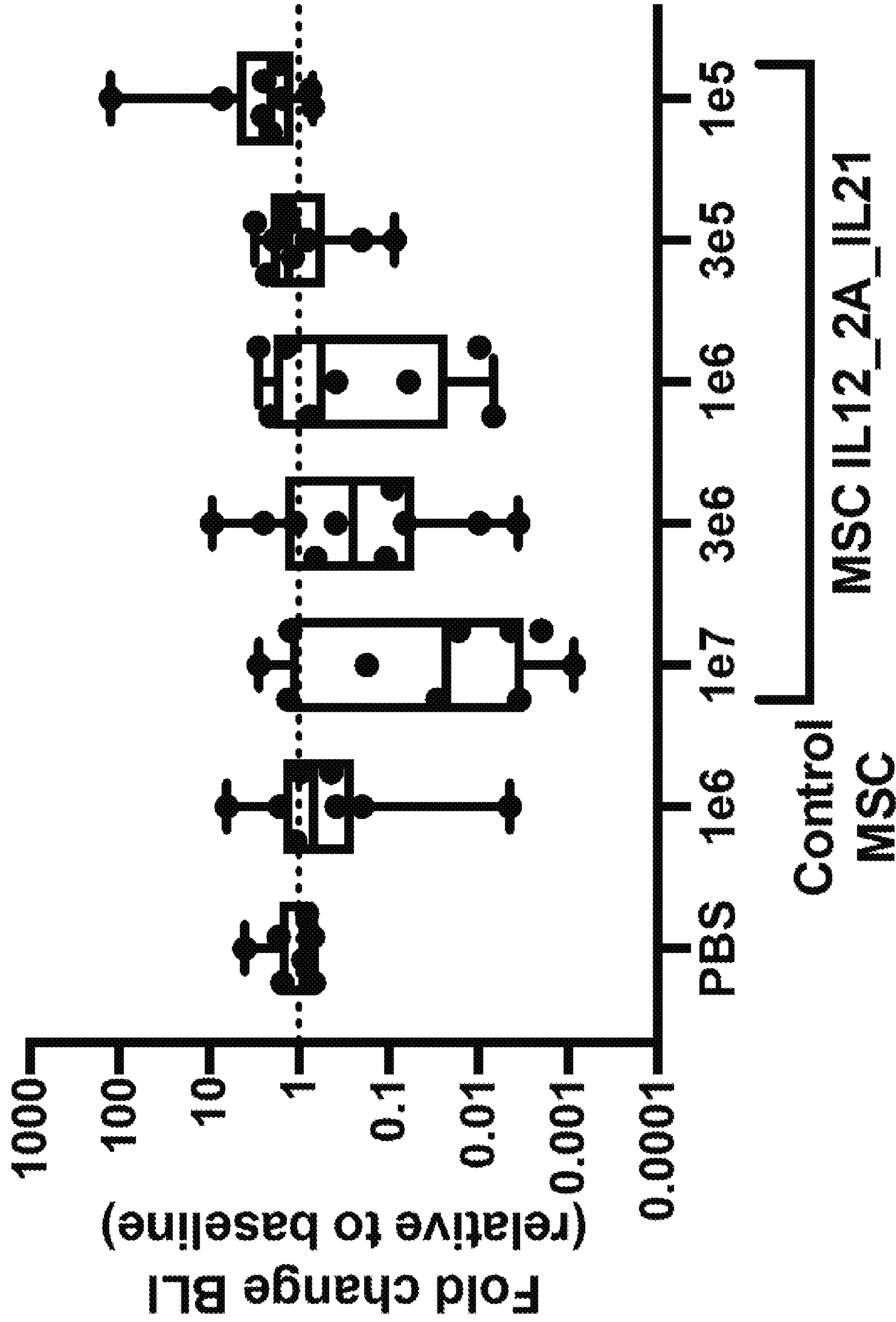


FIG. 59A

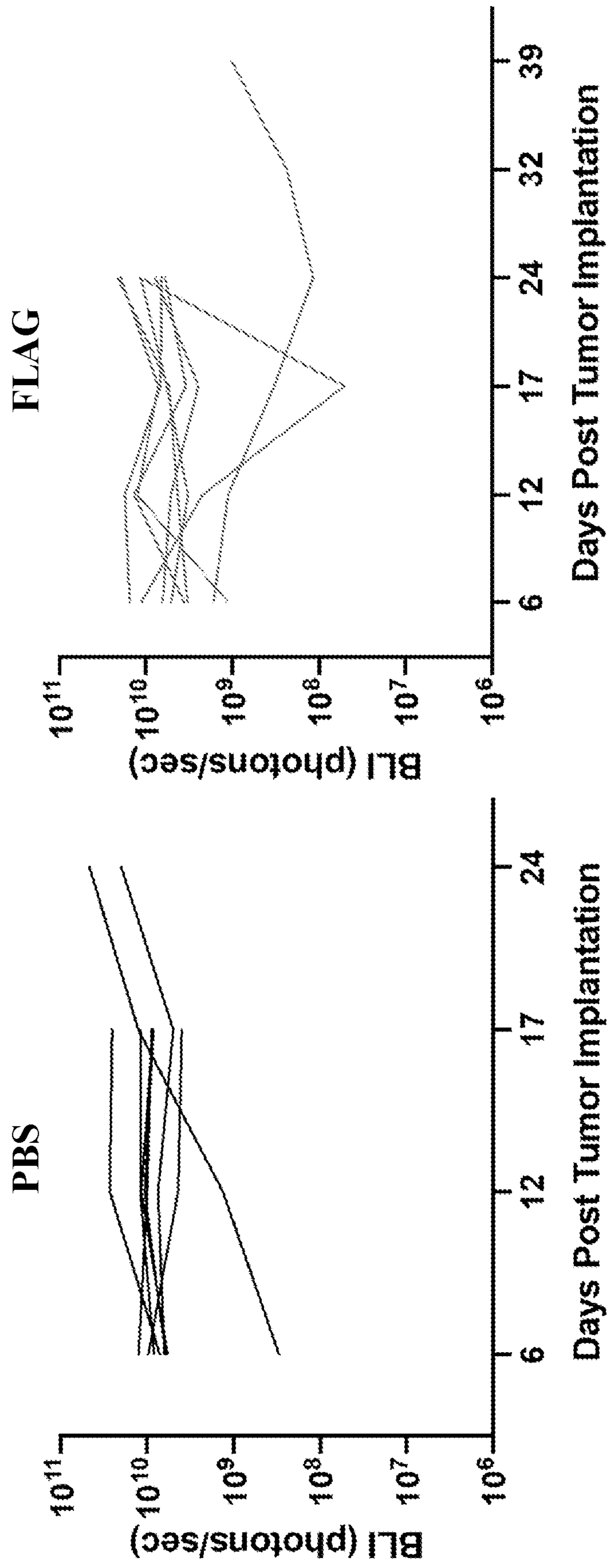


FIG. 59B

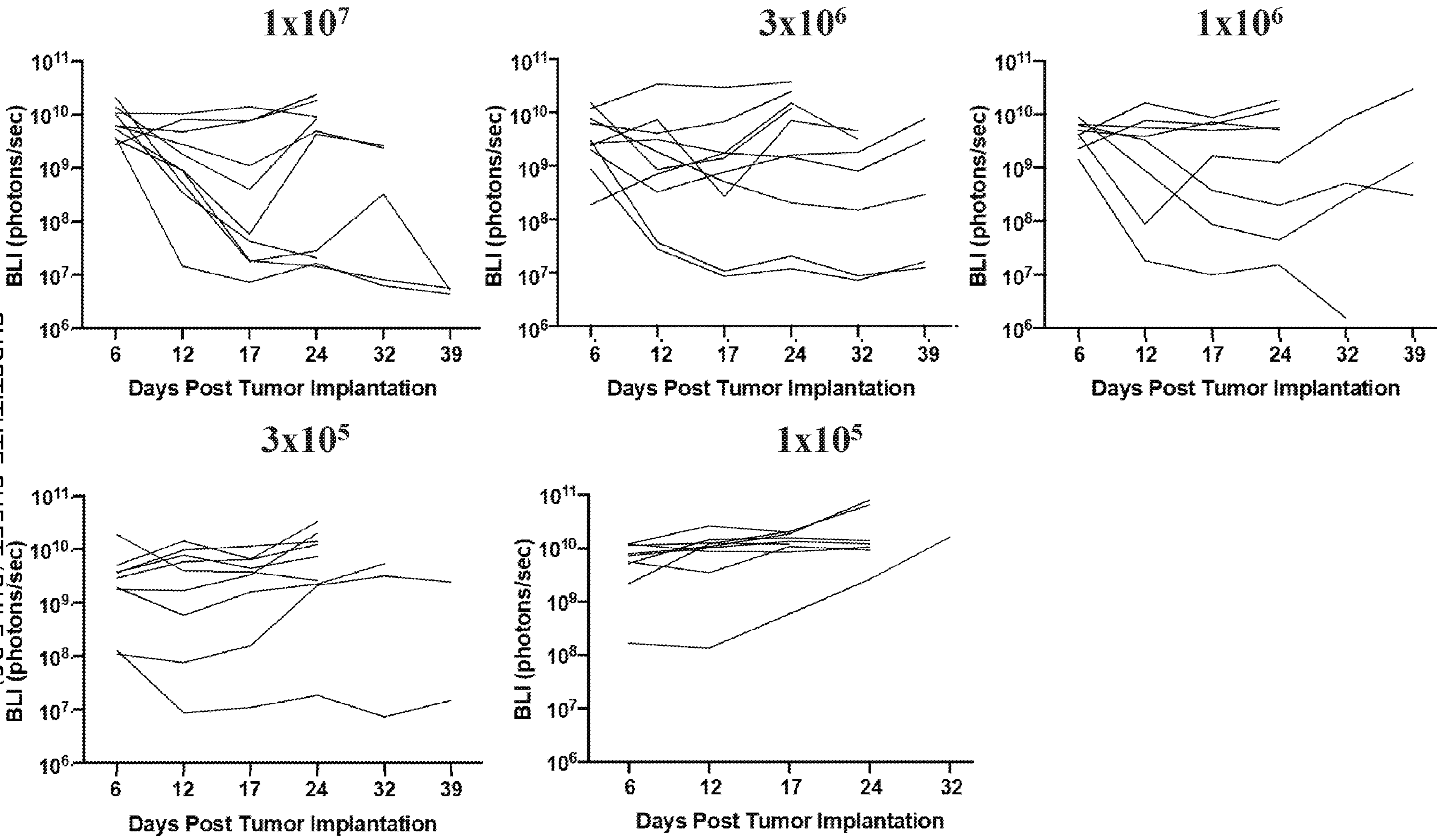


FIG. 59C

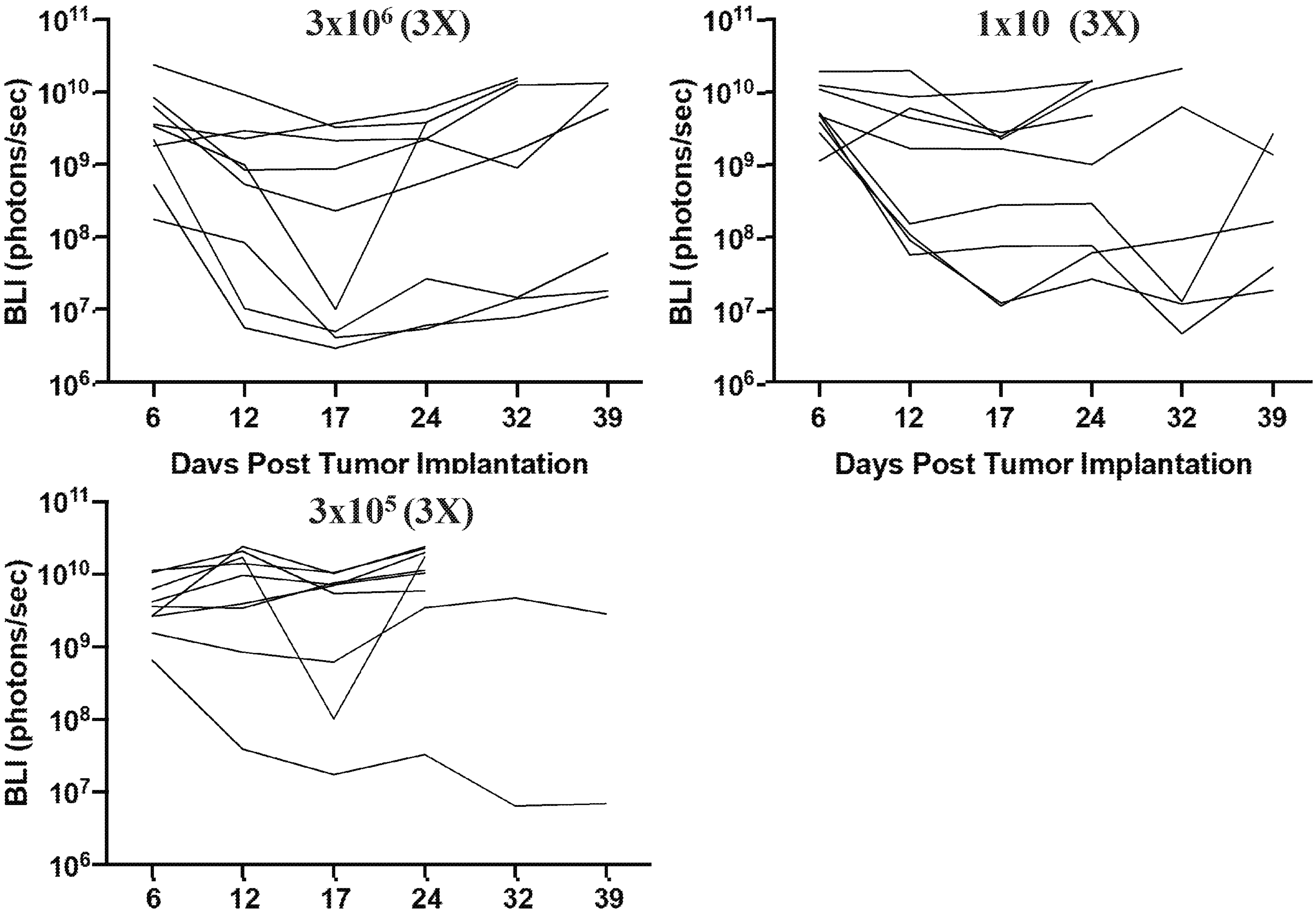


FIG. 59D

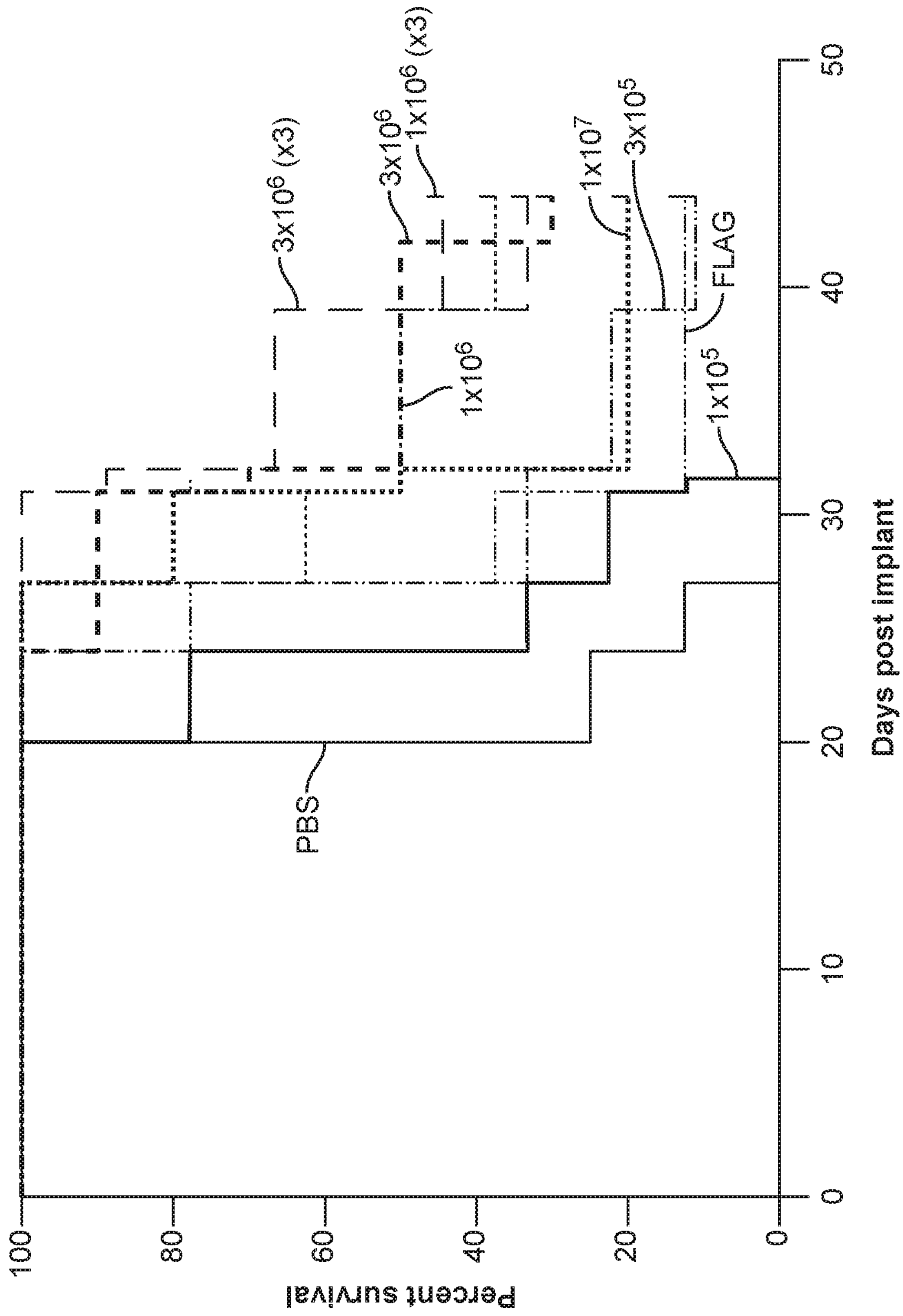


FIG. 59E

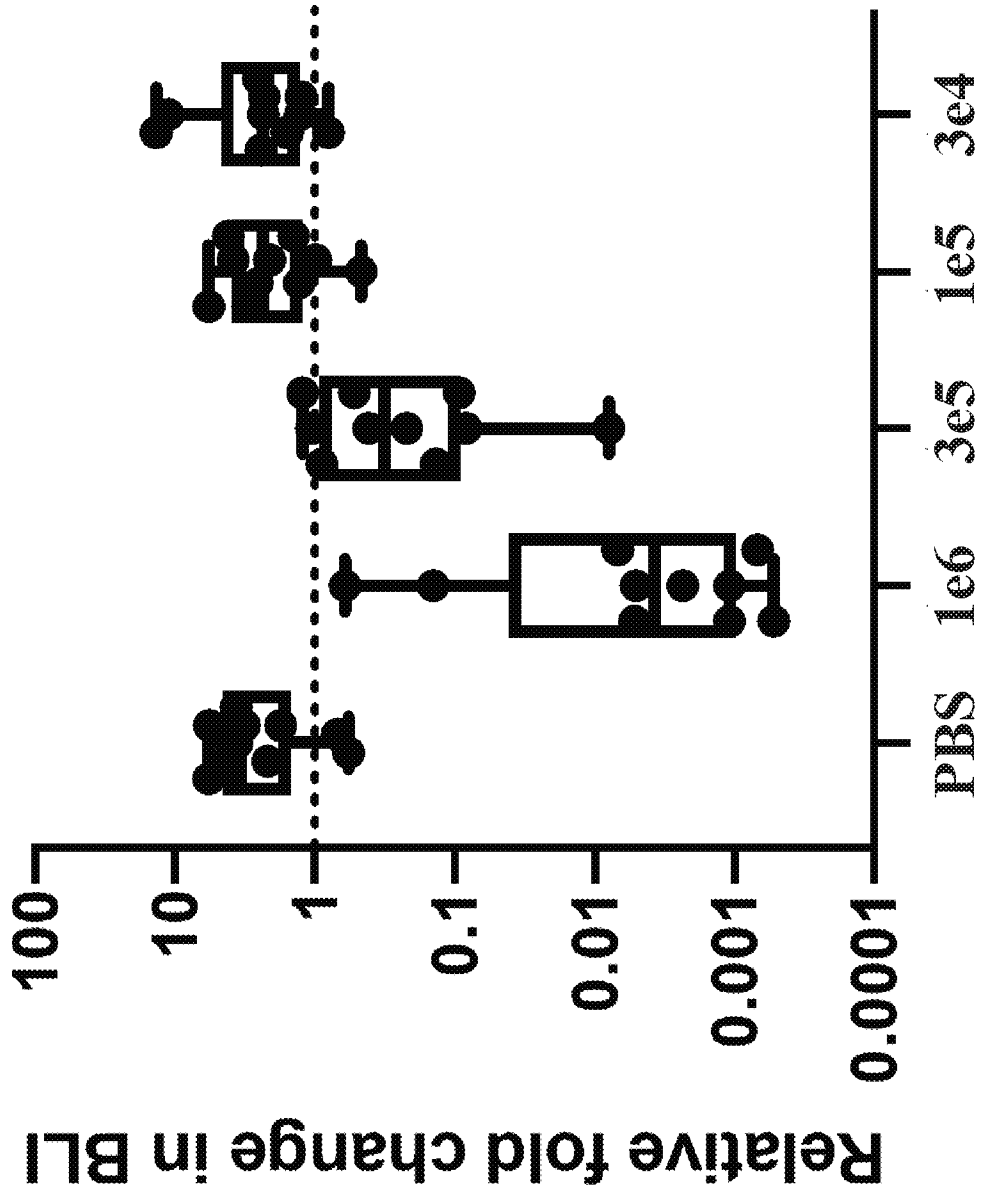


FIG. 60A

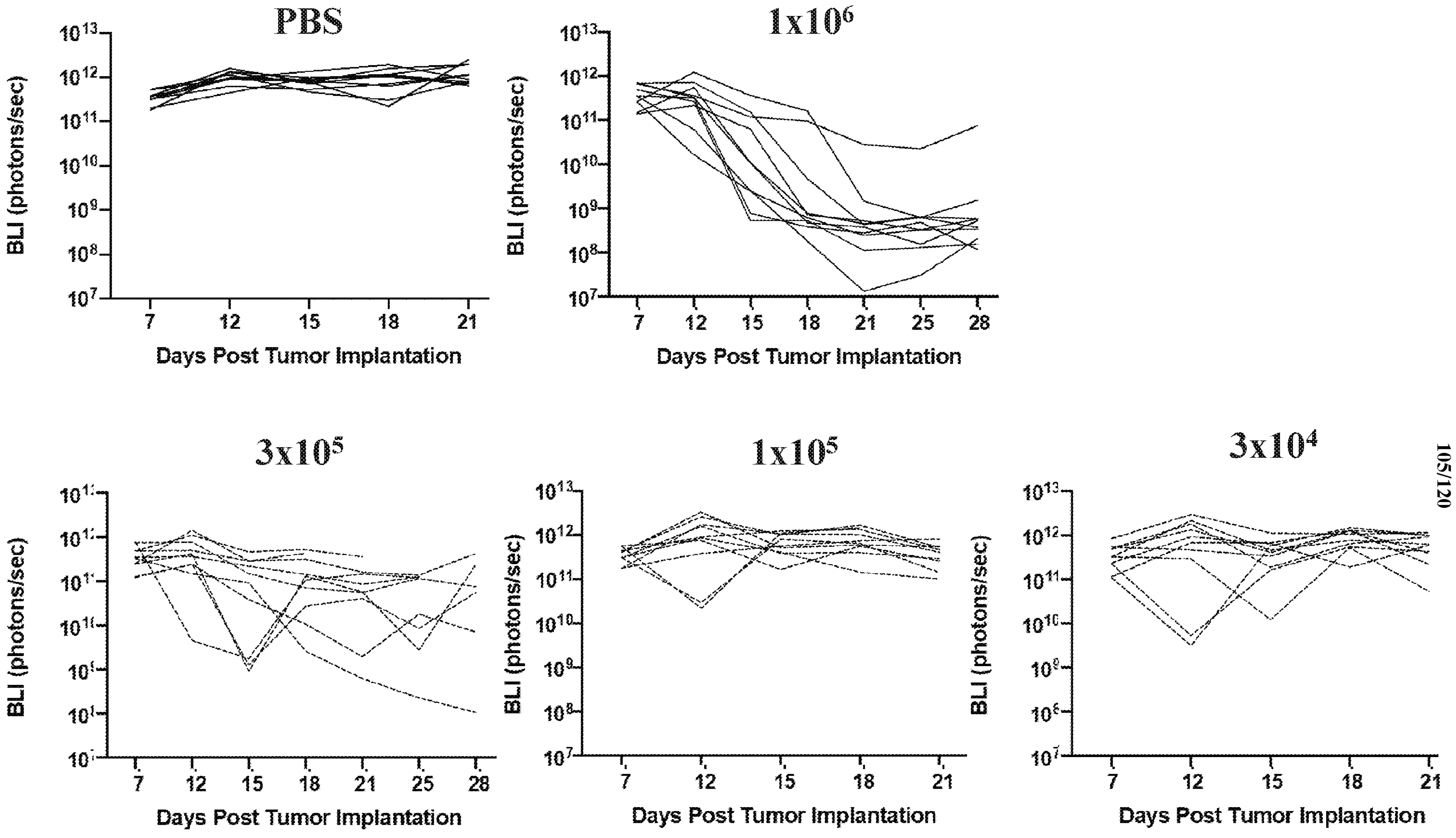


FIG. 60B

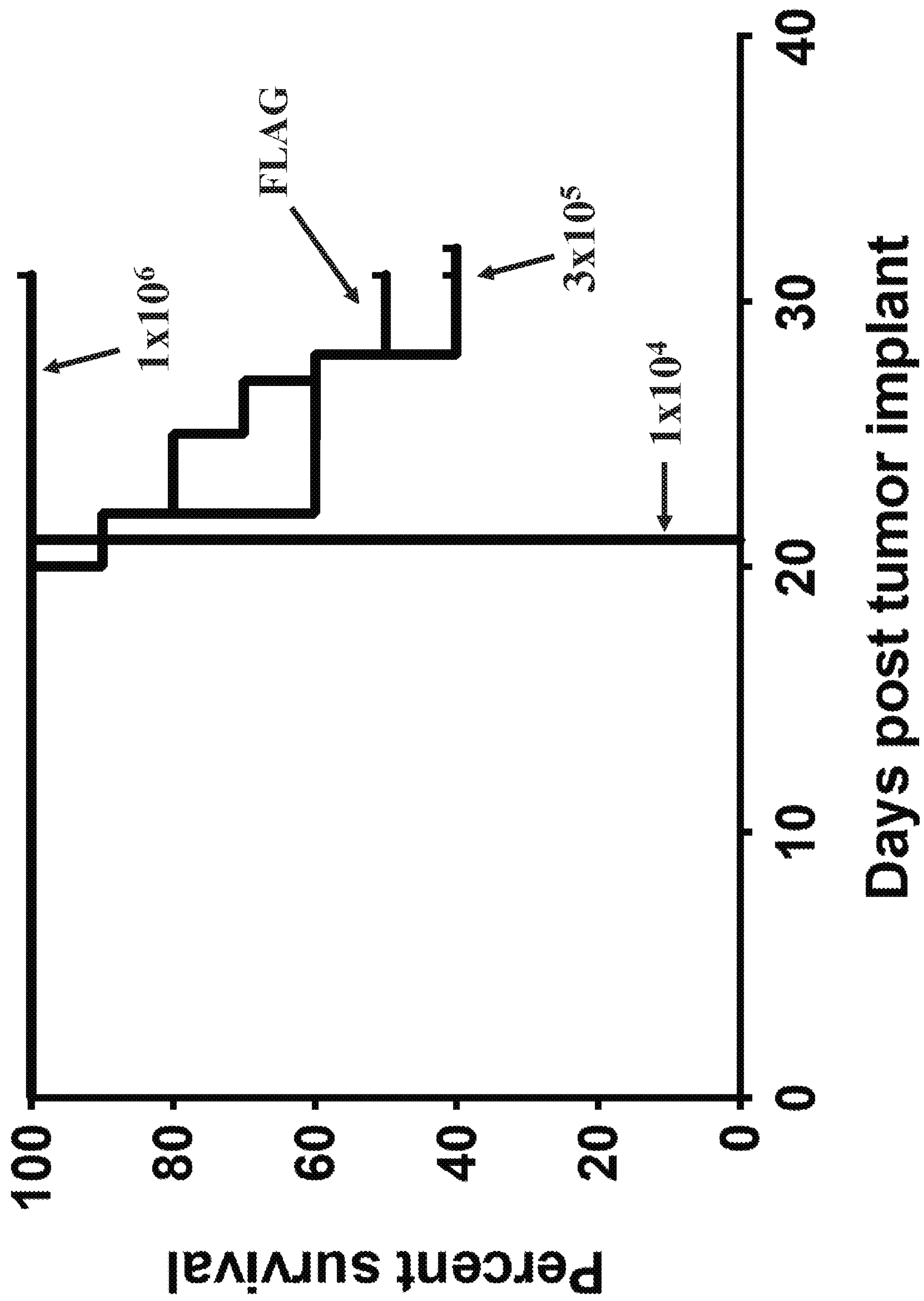


FIG. 60C

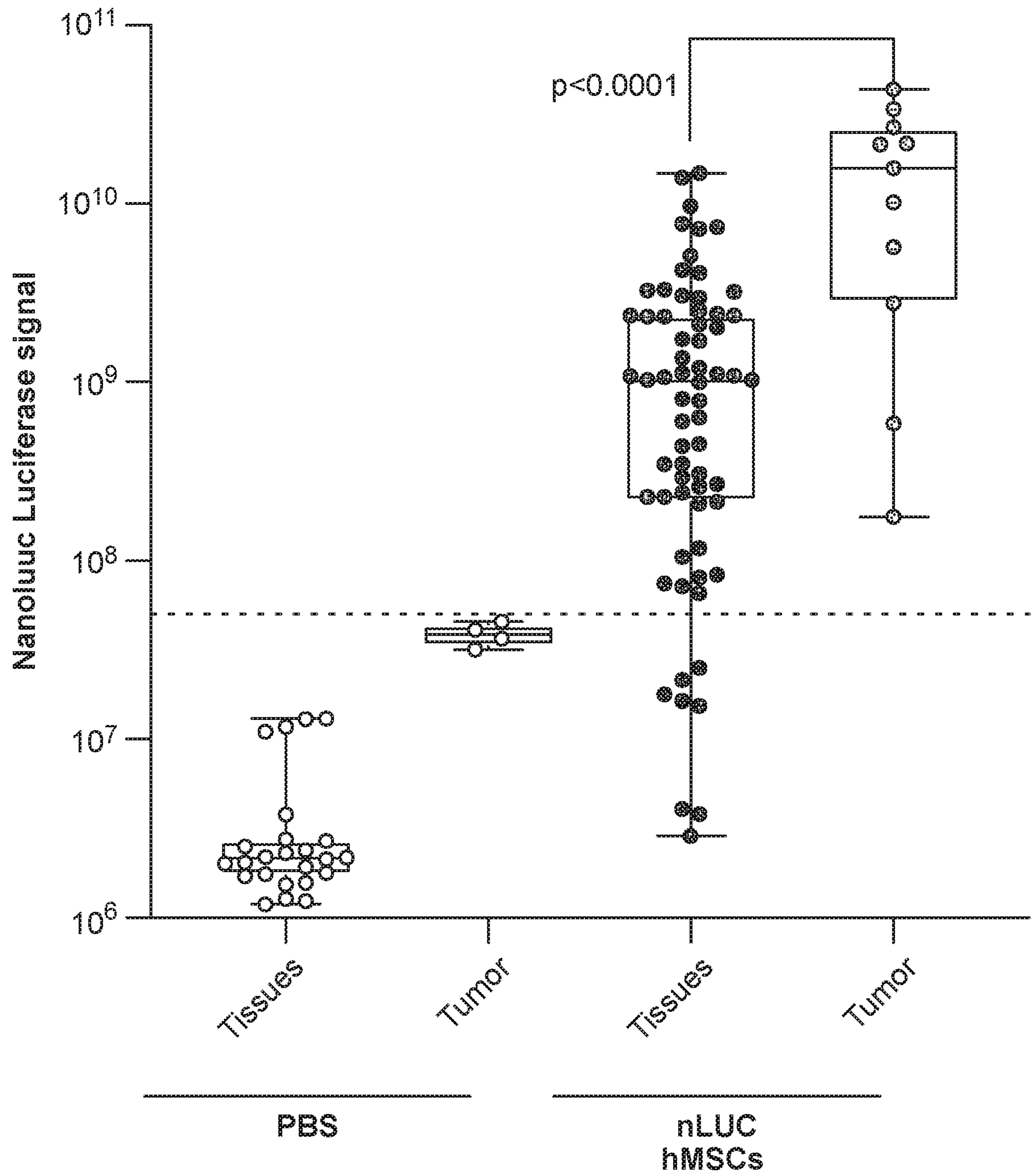


FIG. 61A

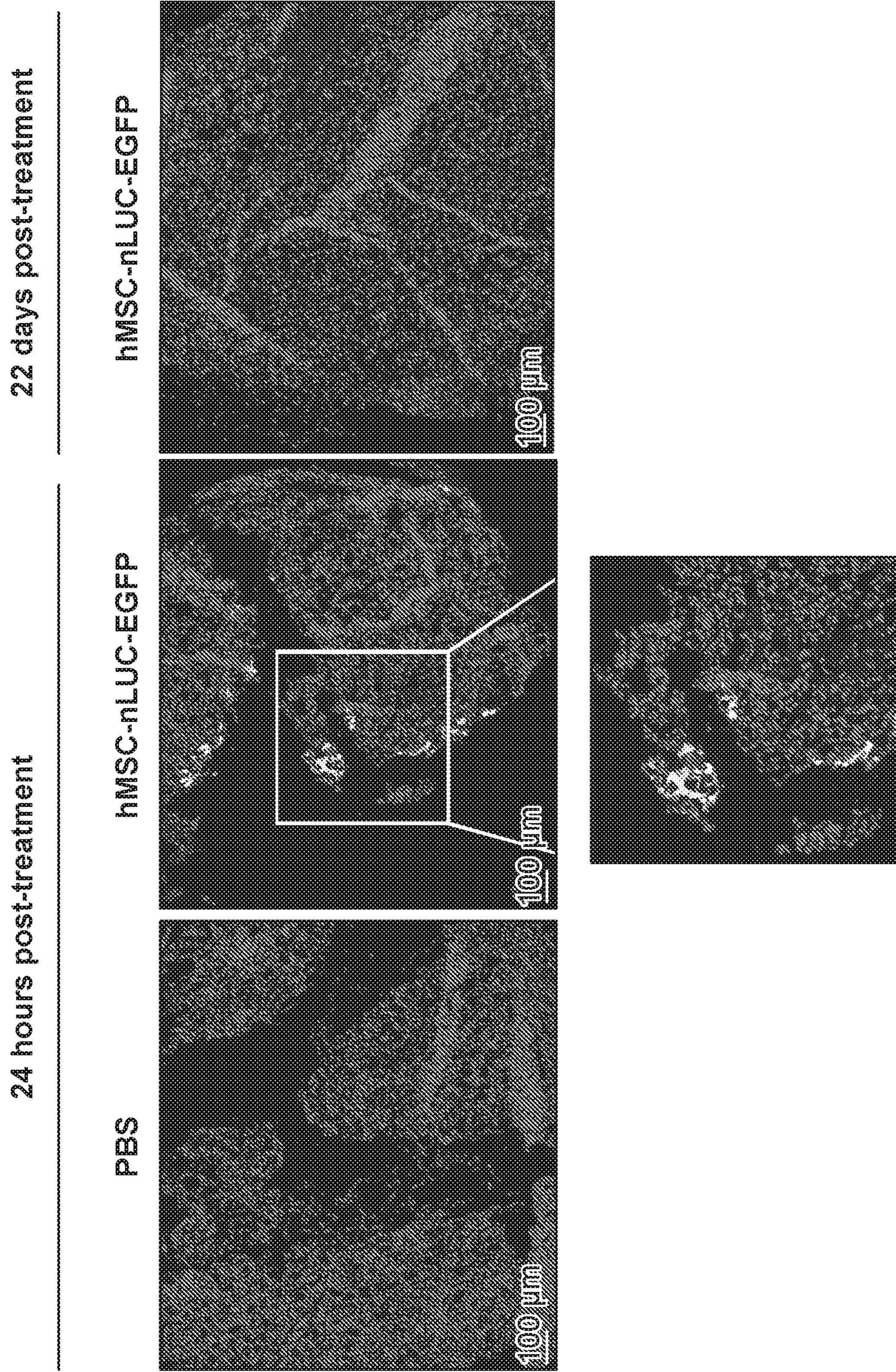


FIG. 61B

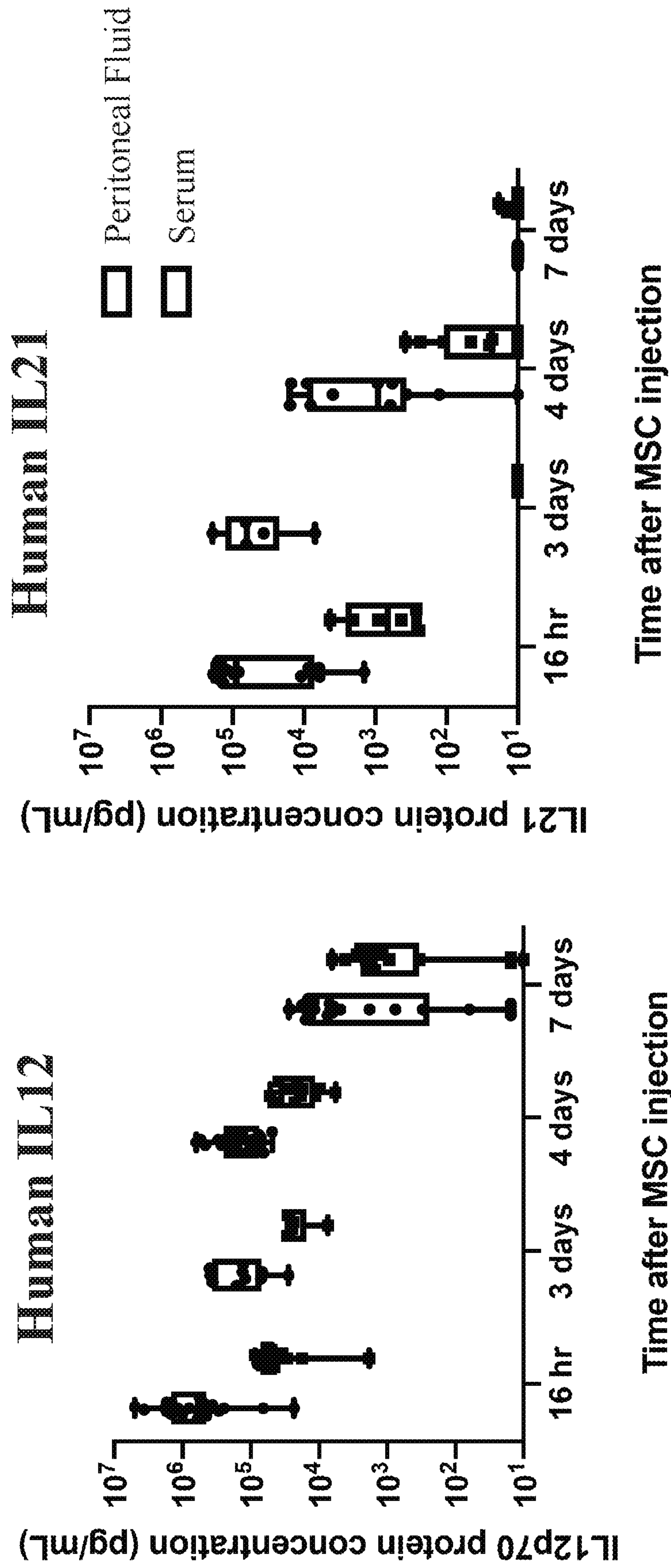
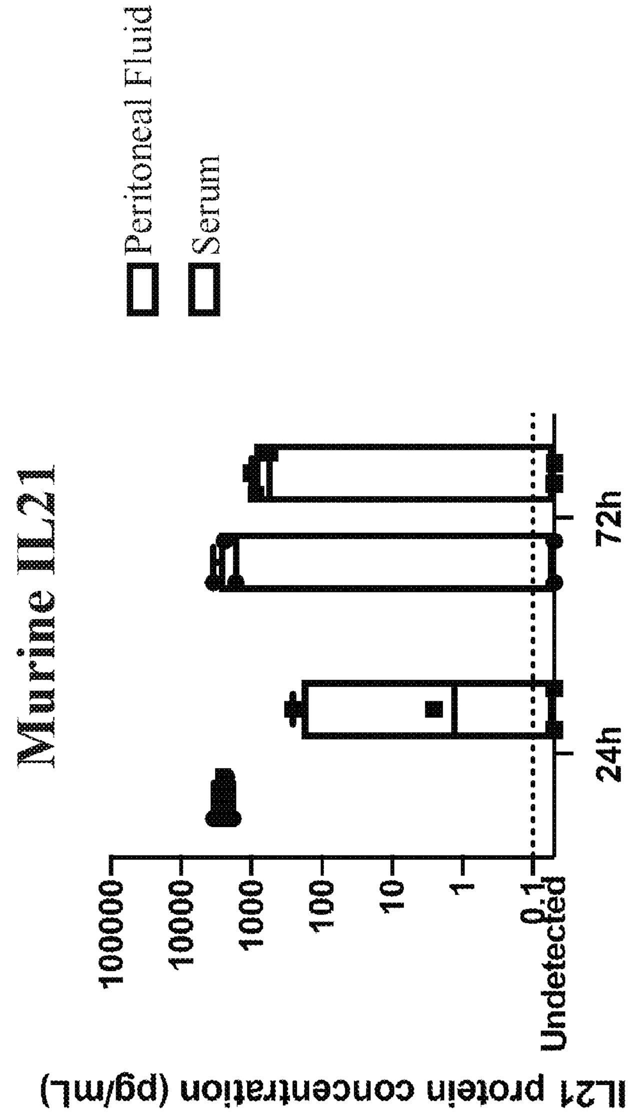


FIG. 62A



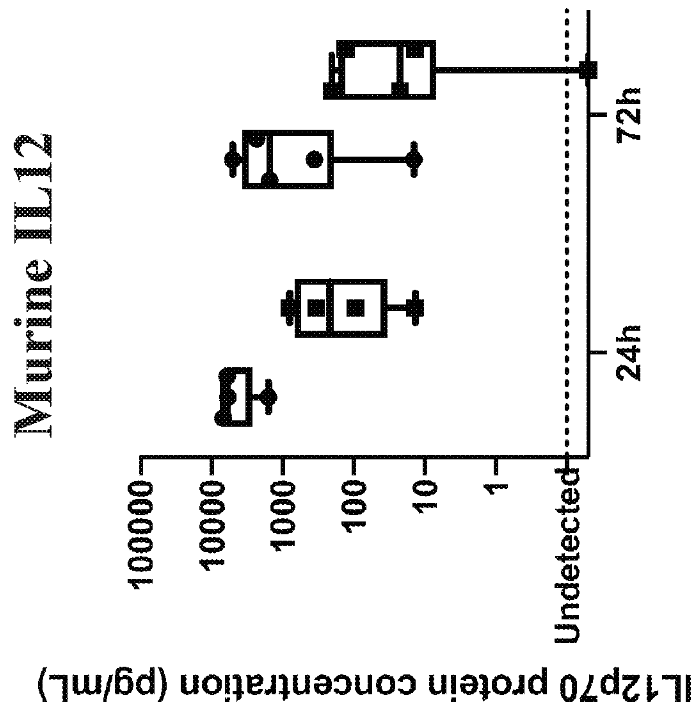


FIG. 62B

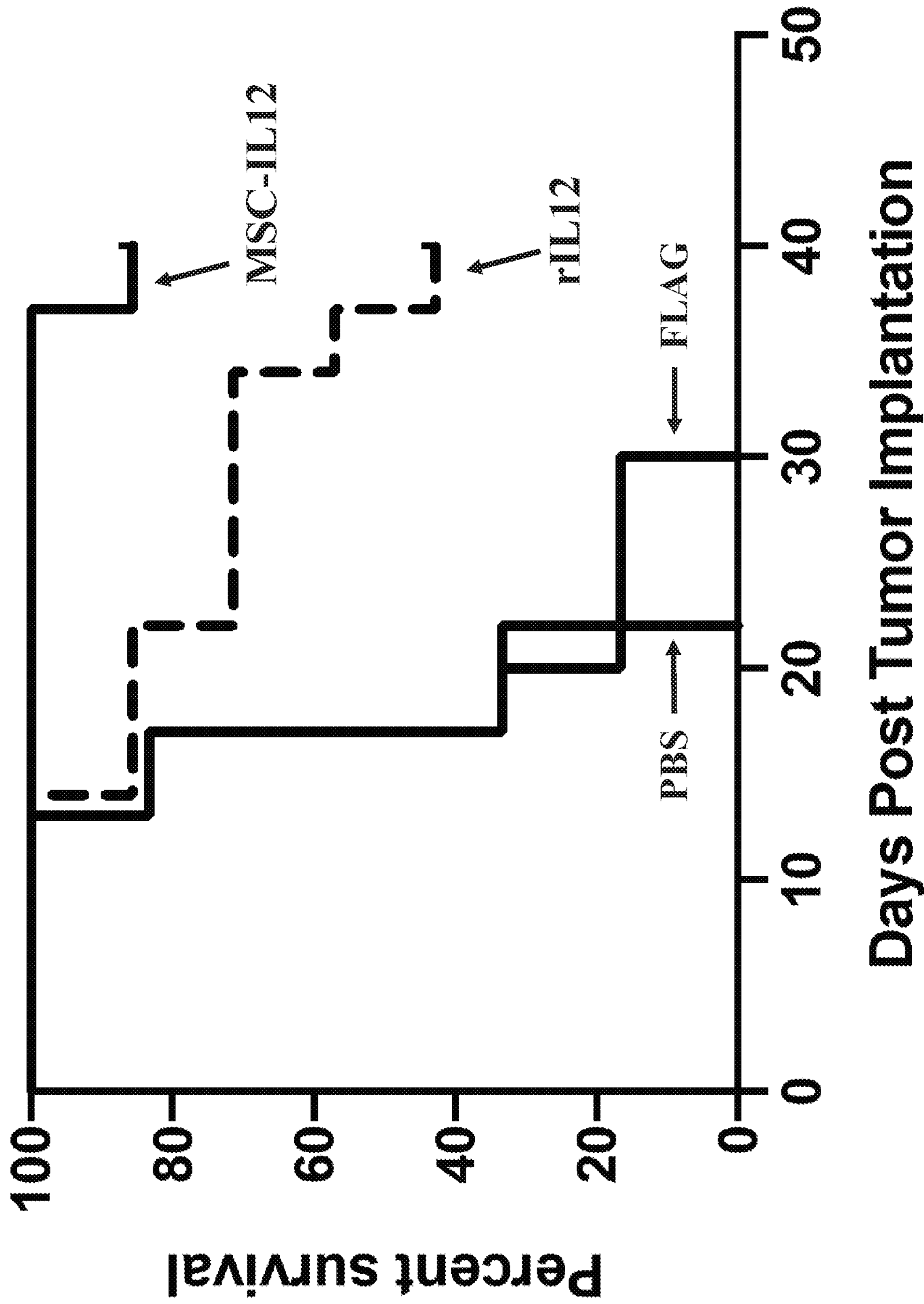


FIG. 63A

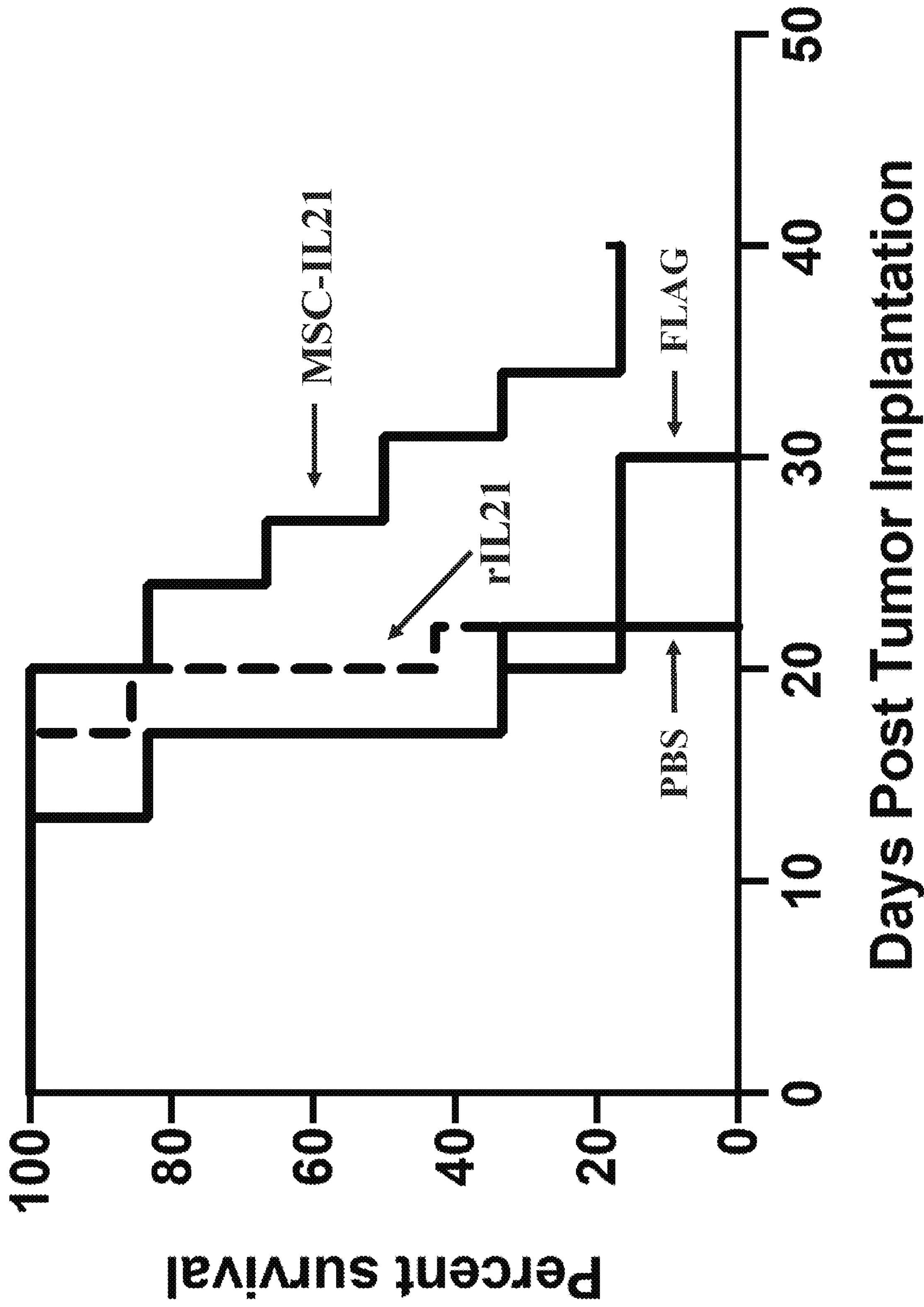


FIG. 63B

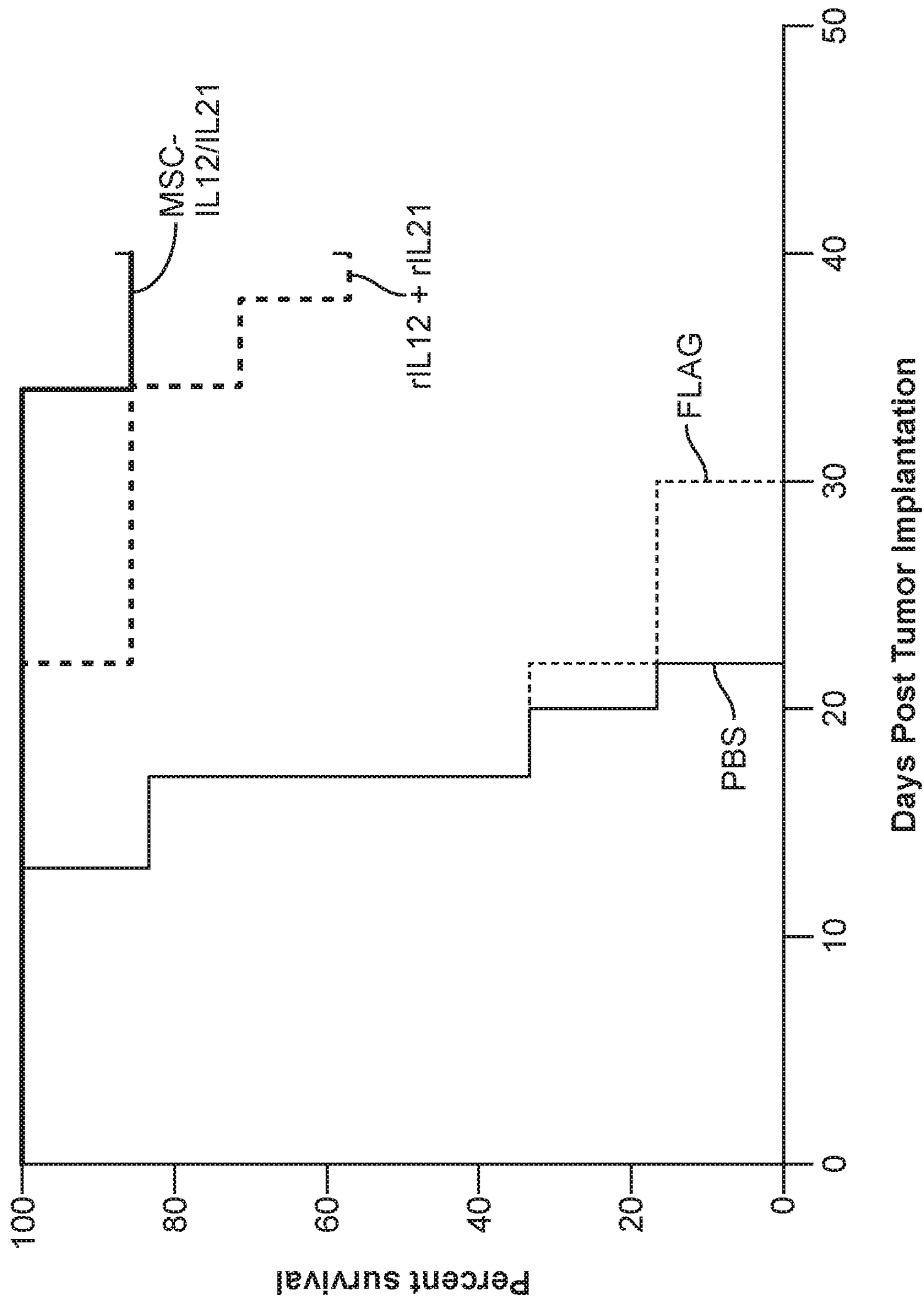
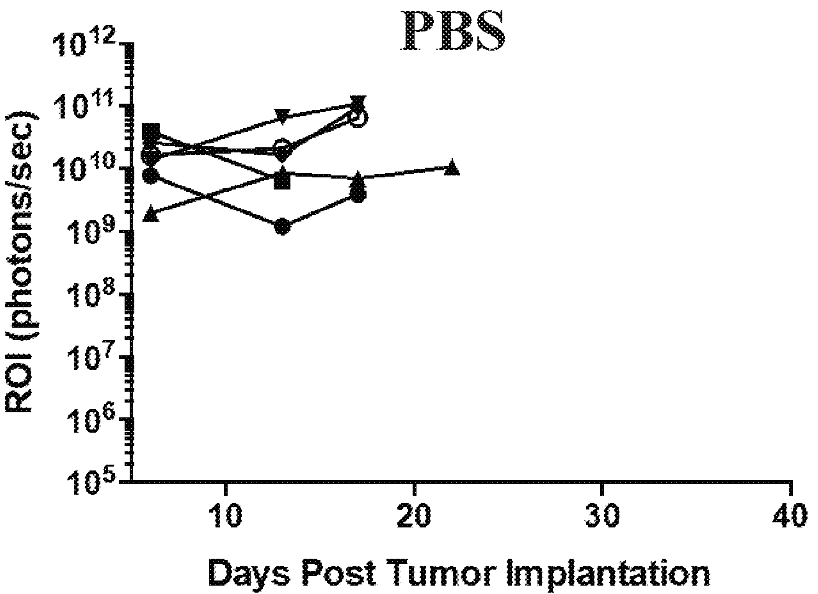
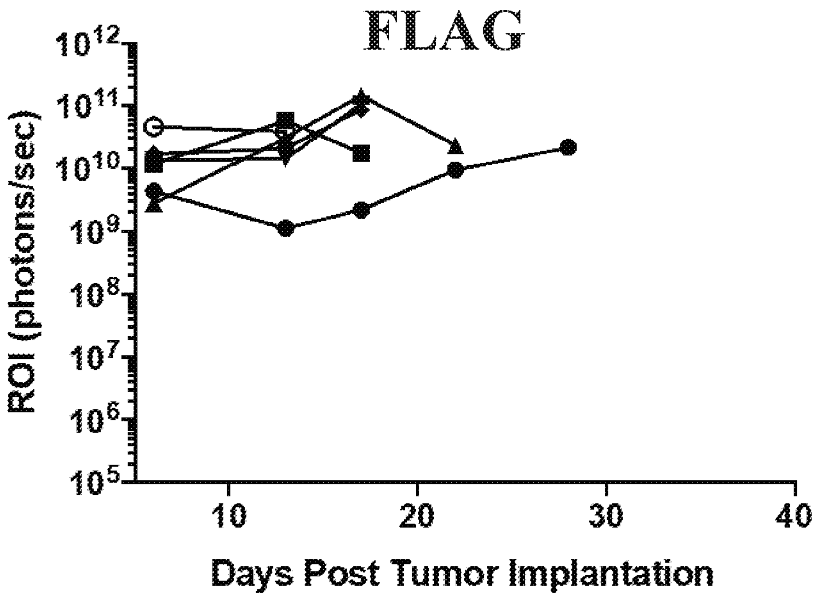
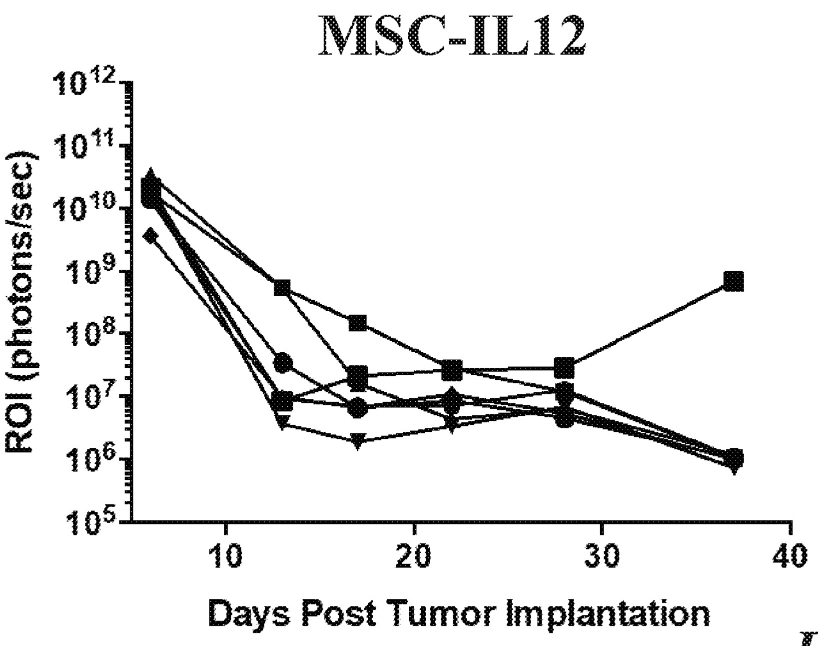


FIG. 63C







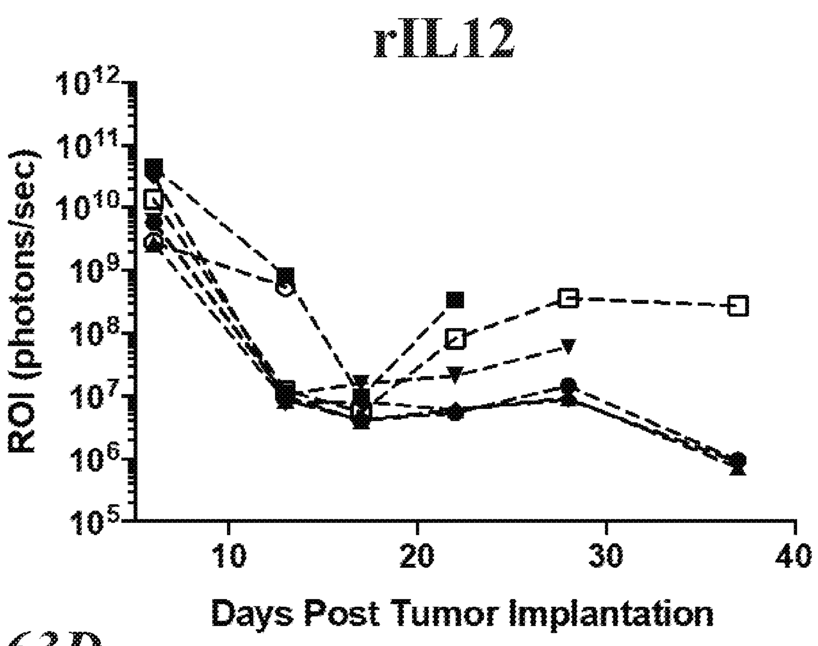
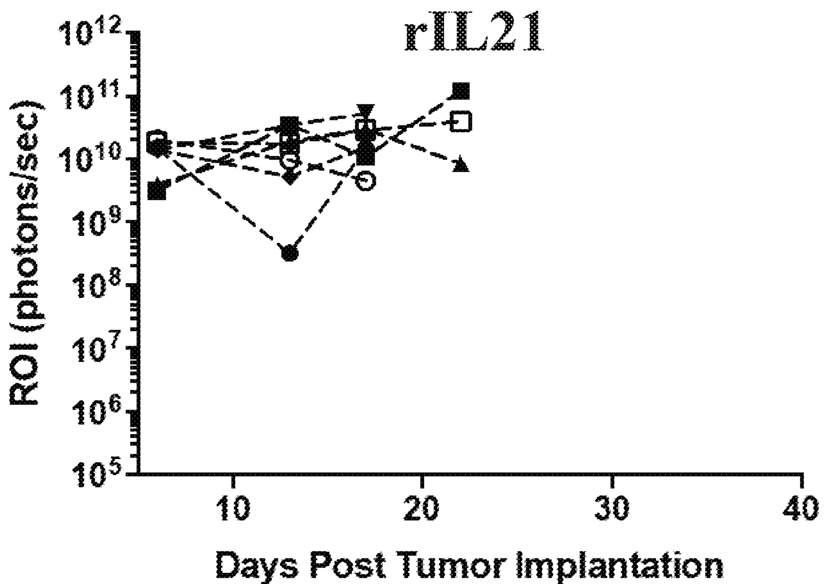
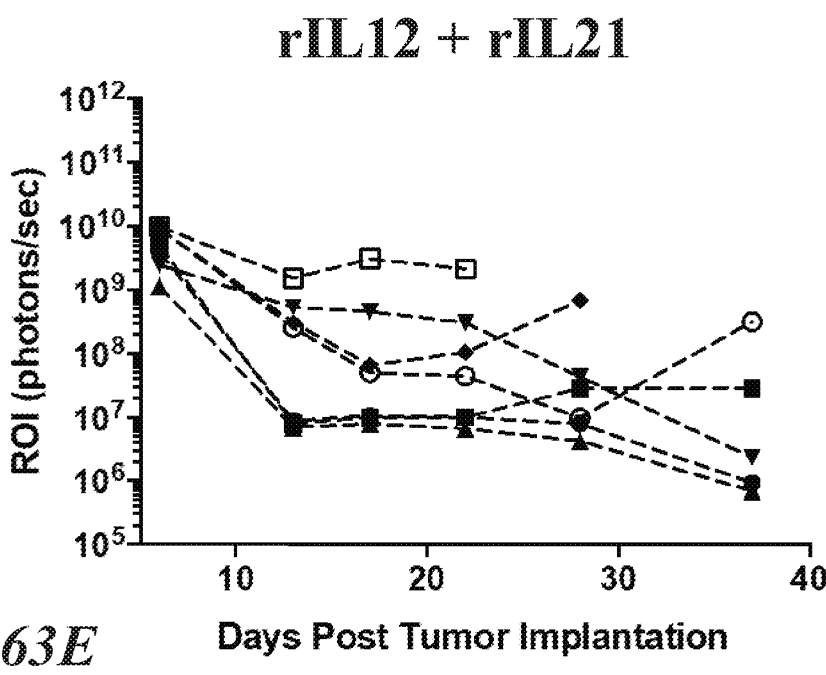
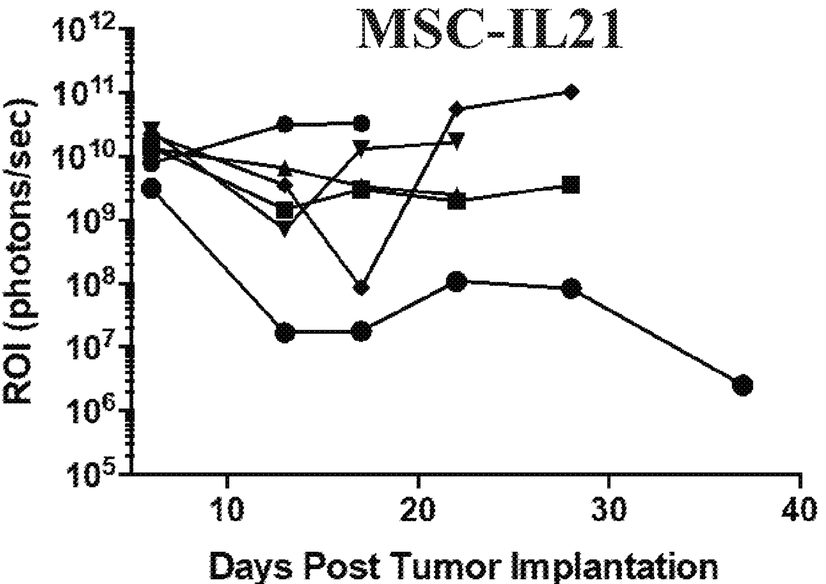


FIG. 63D







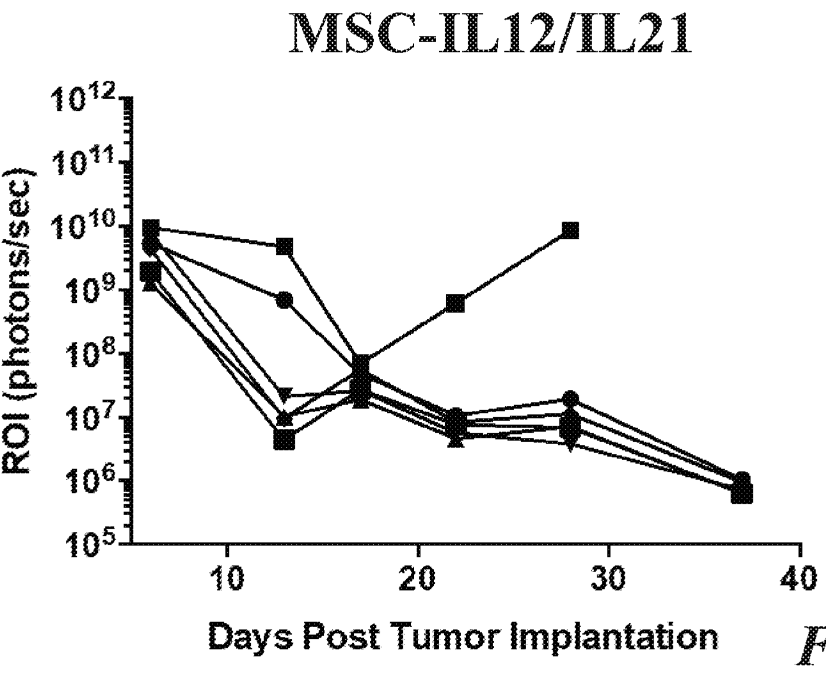


FIG. 63E

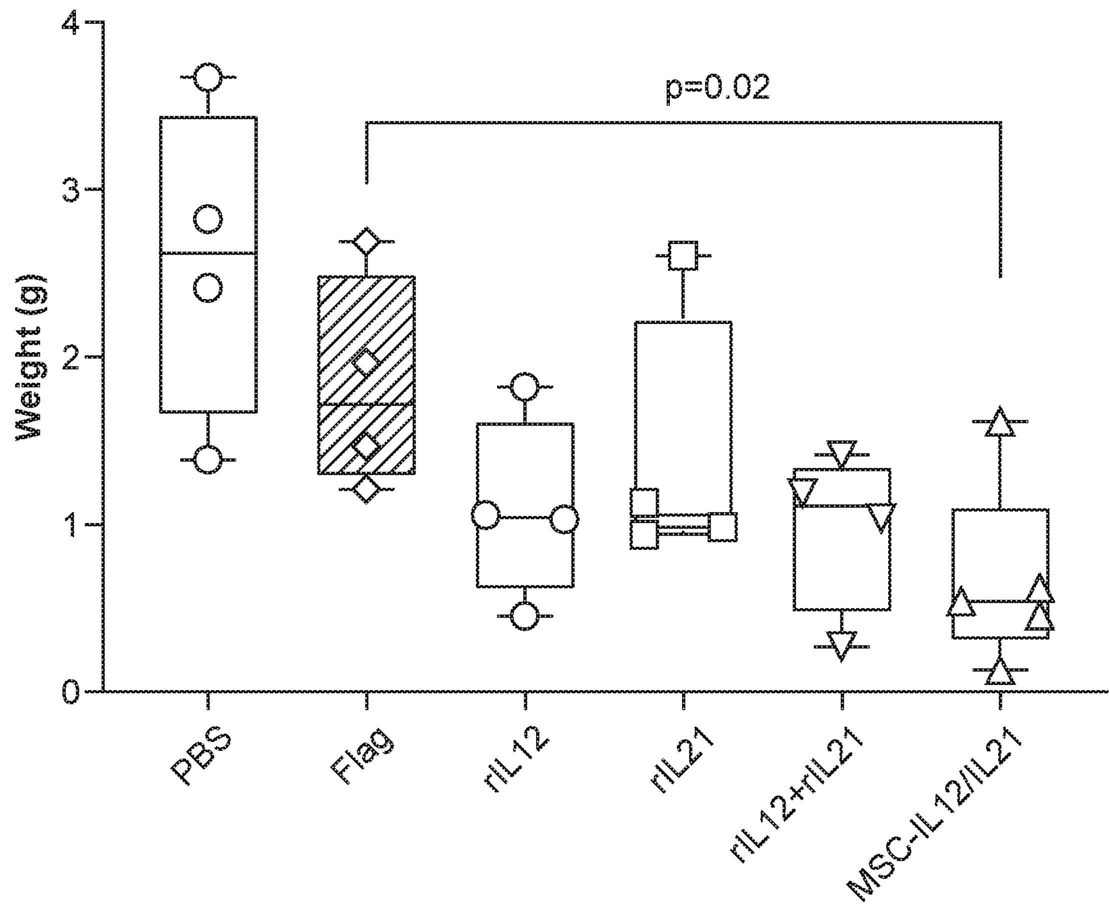


FIG. 64A

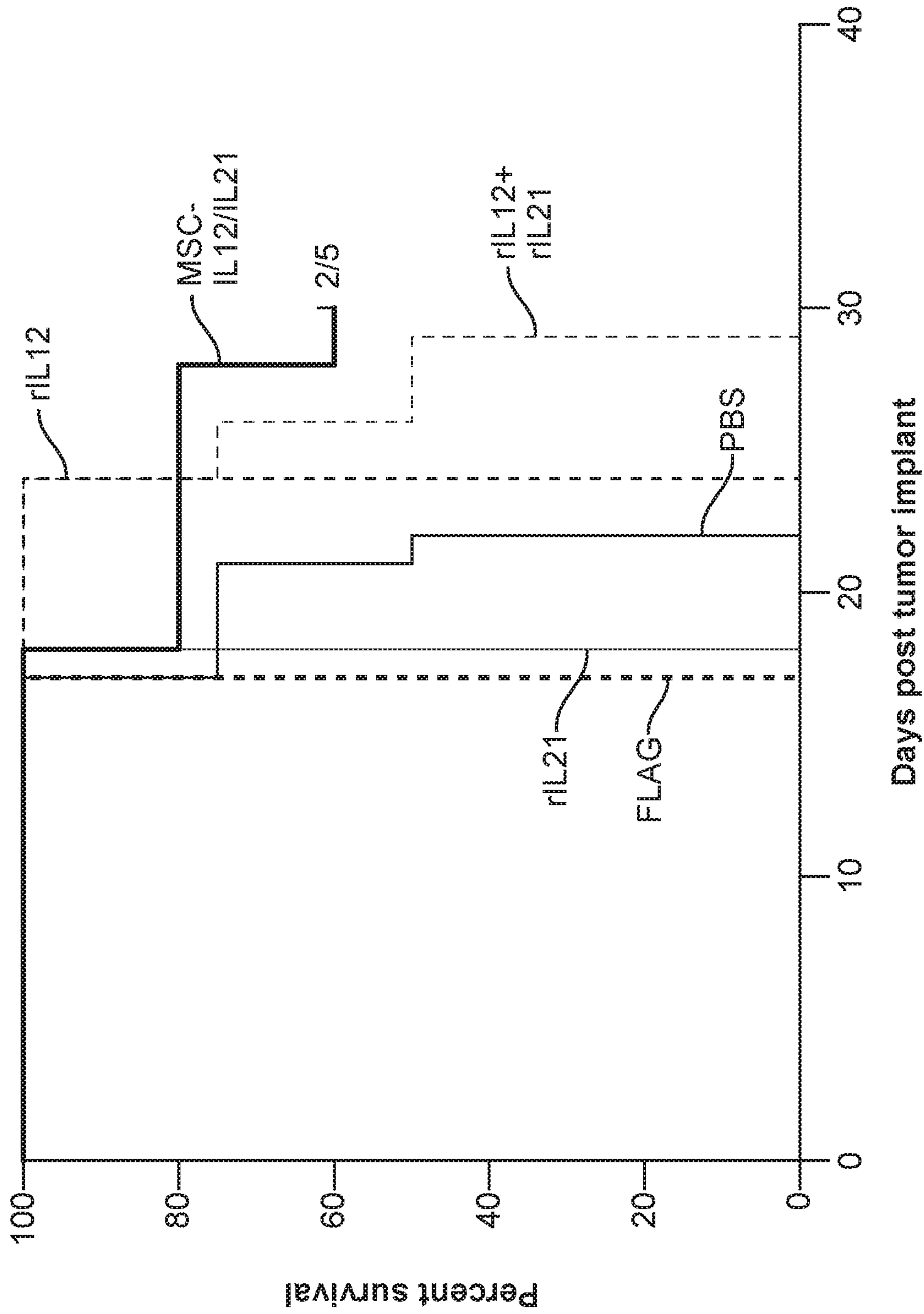


FIG. 64B

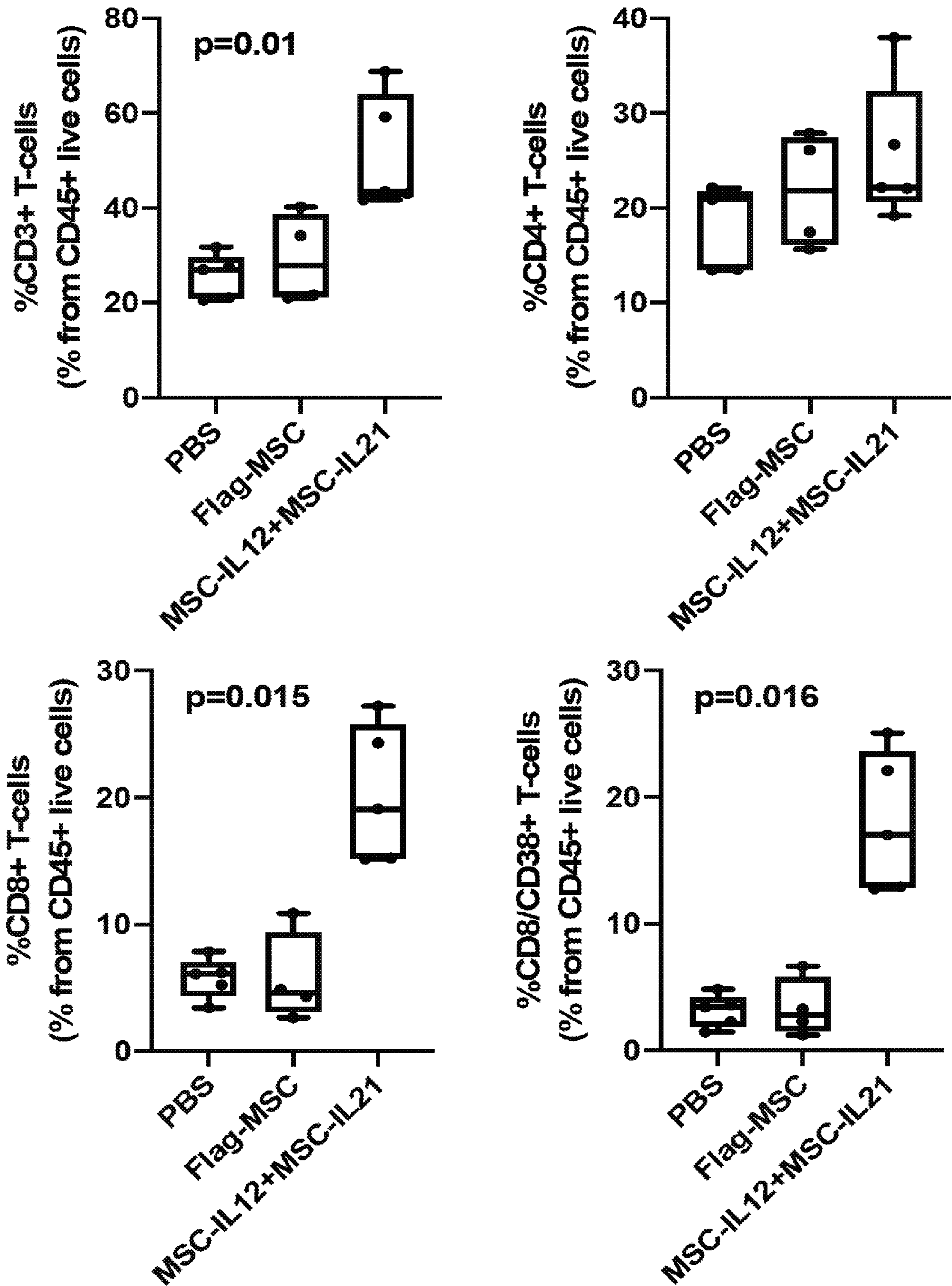


FIG. 65A

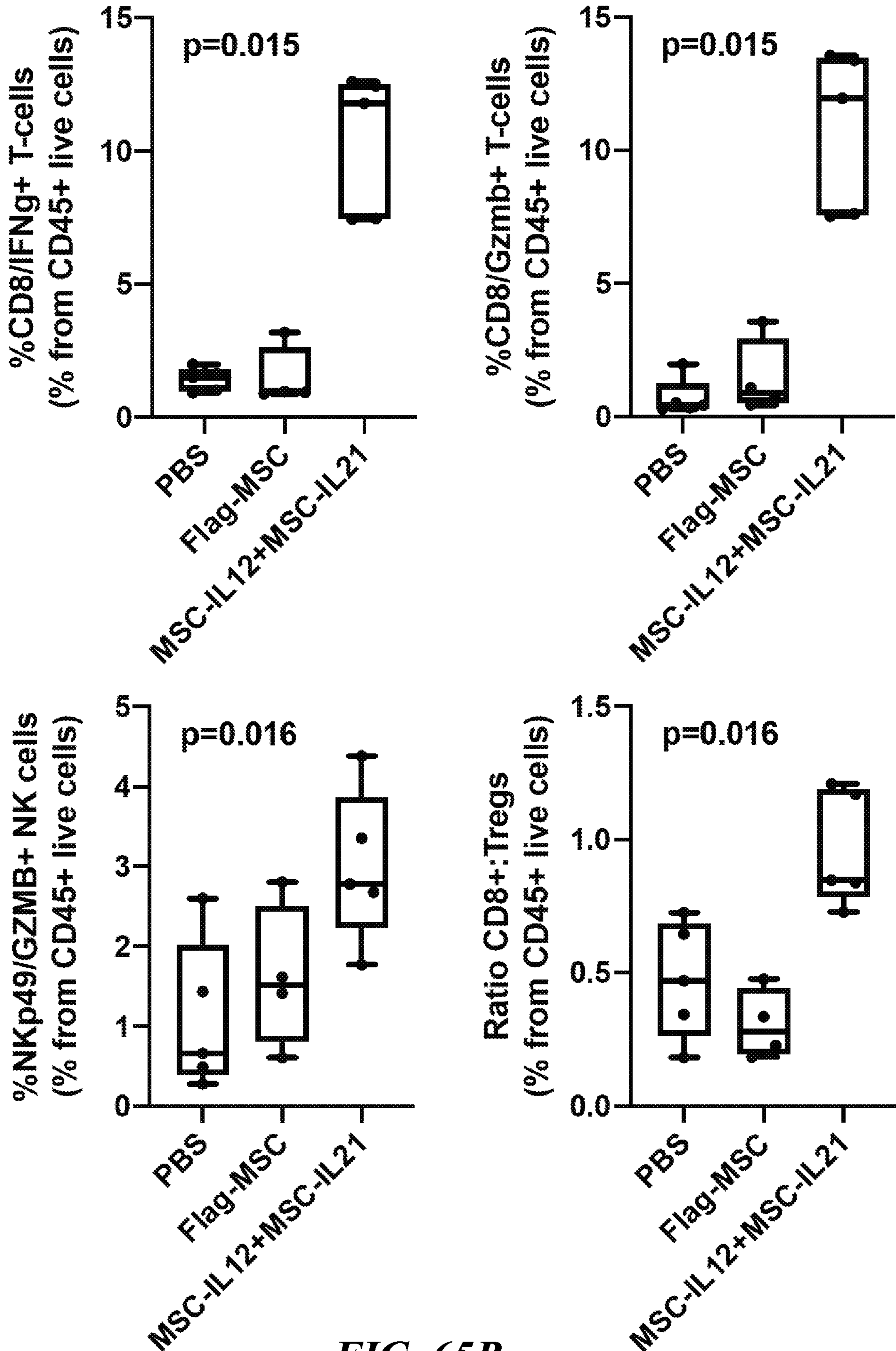


FIG. 65B

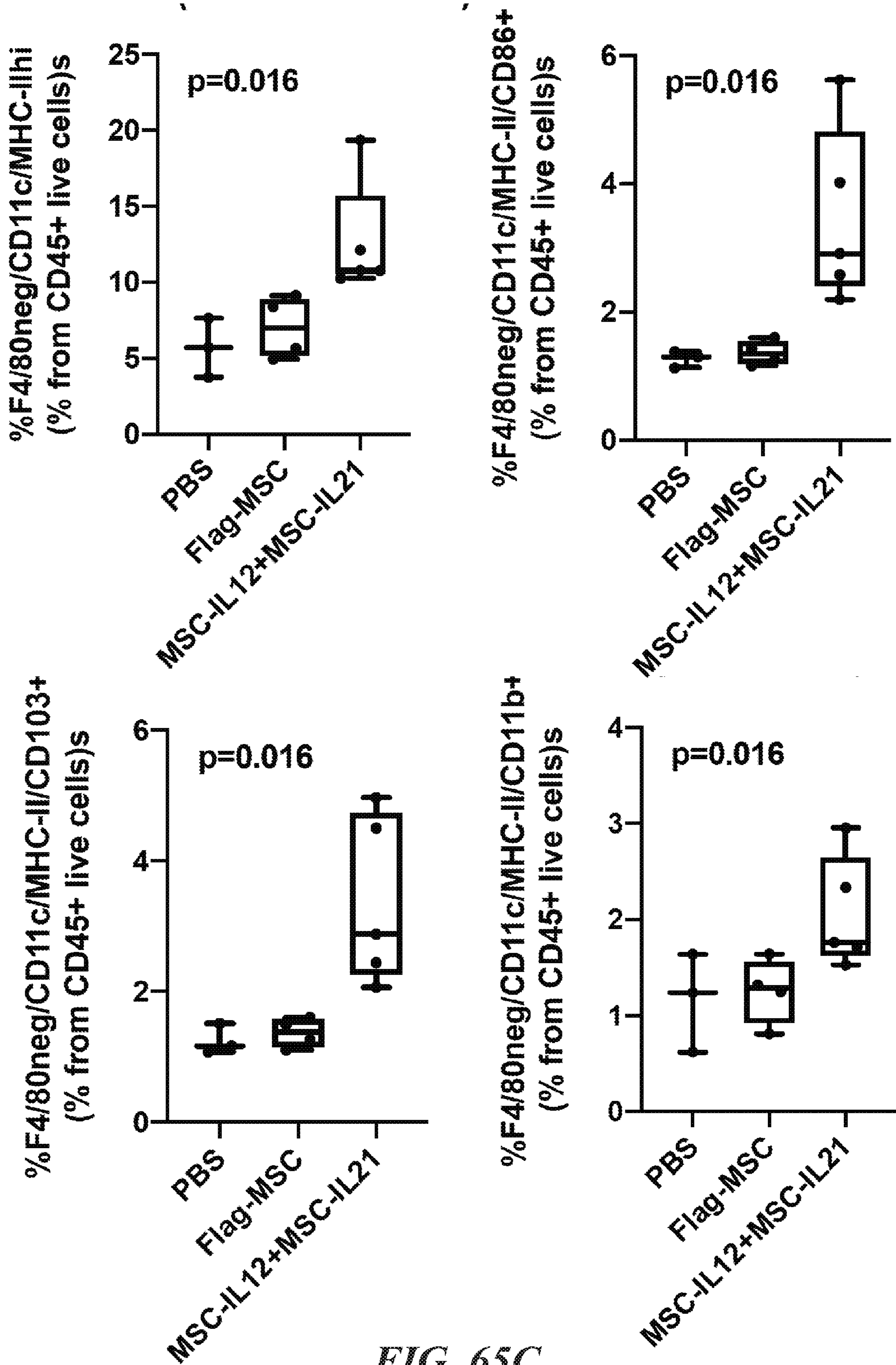


FIG. 65C