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(71) Applicant *(for all designated States except US)*: **XENCOR, INC.** [US/US]; 111 West Lemon Avenue, Monrovia, CA 91016 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LAZAR, Gregory, Alan** [US/US]; 750 Arcadia Avenue, #6, Arcadia, CA 91007 (US). **DAHIYAT, Bassil, I.** [US/US]; 3829 Luna Court, Altadena, CA 91001 (US). **DANG, Wei** [CN/US]; 1111 Blanche Street, Apt. 312, Pasadena, CA 91106 (US). **KARKI, Sher, Bahadur** [NP/US]; 2833 Providence Way, Pomona, CA 91767 (US). **VAFA, Omid** [IR/US]; 724 Ocean View Avenue, Monrovia, CA 91016 (US).

(74) Agents: **SILVA, Robin, M.** et al.; Dorsey & Whitney LLP, 555 California Street, Suite 1000, San Francisco, CA 94104 (US).

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(54) **Title:** IgG IMMUNOGLOBULIN VARIANTS WITH OPTIMIZED EFFECTOR FUNCTION

(57) **Abstract:** The present application relates to optimized IgG immunoglobulin variants, engineering methods for their generation, and their application, particularly for therapeutic purposes.

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IgG IMMUNOGLOBULIN VARIANTS WITH OPTIMIZED EFFECTOR FUNCTION

[1] The present application claims benefit under 35 U.S.C. § 119(e) to U.S. Provisional Application Nos. 60/621,387, filed October 21, 2004; 60/629,068, filed November 18, 2004; 60/652,968, filed February 14, 2005, 60/659,004, filed March 3, 2005; and 60/723,294, filed October 3, 2005, each of which is incorporated herein by reference in its entirety.

FIELD

[2] The present application relates to optimized IgG immunoglobulin variants, engineering methods for their generation, and their application, particularly for therapeutic purposes.

BACKGROUND

[3] Antibodies are immunological proteins that bind a specific antigen. In most mammals, including humans and mice, antibodies are constructed from paired heavy and light polypeptide chains. Each chain is made up of individual immunoglobulin (Ig) domains, and thus the generic term immunoglobulin is used for such proteins. Each chain is made up of two distinct regions, referred to as the variable and constant regions. The light and heavy chain variable regions show significant sequence diversity between antibodies, and are responsible for binding the target antigen. The constant regions show less sequence diversity, and are responsible for binding a number of natural proteins to elicit important biochemical events. In humans there are five different classes of antibodies including IgA (which includes subclasses IgA1 and IgA2), IgD, IgE, IgG (which includes subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. The distinguishing features between these antibody classes are their constant regions, although subtler differences may exist in the V region. Figure 1 shows an IgG1 antibody, used here as an example to describe the general structural features of immunoglobulins. IgG antibodies are tetrameric proteins composed of two heavy chains and two light chains. The IgG heavy chain is composed of four immunoglobulin domains linked from N- to C-terminus in the order VH-CH1-CH2-CH3, referring to the heavy chain variable domain, heavy chain constant domain 1, heavy chain constant domain 2, and heavy chain constant domain 3 respectively (also referred to as VH-Cγ1-Cγ2-Cγ3, referring to the heavy chain variable domain, constant gamma 1 domain, constant gamma 2 domain, and constant gamma 3 domain respectively). The IgG light chain is composed of two immunoglobulin domains linked from N- to C-terminus in the order VL-CL, referring to the light chain variable domain and the light chain constant domain respectively.

[4] The variable region of an antibody contains the antigen binding determinants of the molecule, and thus determines the specificity of an antibody for its target antigen. The variable region is so named because it is the most distinct in sequence from other antibodies within the same class. The majority of sequence variability occurs in the complementarity determining regions (CDRs). There are 6 CDRs total, three each per heavy and light chain, designated VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3. The variable region outside of the CDRs is referred to as the framework (FR) region. Although not as diverse as the CDRs, sequence variability does occur in the FR region between different antibodies. Overall, this characteristic architecture of antibodies provides

a stable scaffold (the FR region) upon which substantial antigen binding diversity (the CDRs) can be explored by the immune system to obtain specificity for a broad array of antigens. A number of high-resolution structures are available for a variety of variable region fragments from different organisms, some unbound and some in complex with antigen. The sequence and structural features of antibody variable regions are well characterized (Morea *et al.*, 1997, *Biophys Chem* 68:9-16; Morea *et al.*, 2000, *Methods* 20:267-279), and the conserved features of antibodies have enabled the development of a wealth of antibody engineering techniques (Maynard *et al.*, 2000, *Annu Rev Biomed Eng* 2:339-376). For example, it is possible to graft the CDRs from one antibody, for example a murine antibody, onto the framework region of another antibody, for example a human antibody. This process, referred to in the art as "humanization", enables generation of less immunogenic antibody therapeutics from nonhuman antibodies. Fragments including the variable region can exist in the absence of other regions of the antibody, including for example the antigen binding fragment (Fab) including VH-Cγ1 and VH-CL, the variable fragment (Fv) including VH and VL, the single chain variable fragment (scFv) including VH and VL linked together in the same chain, as well as a variety of other variable region fragments (Little *et al.*, 2000, *Immunol Today* 21:364-370).

[5] The Fc region of an antibody interacts with a number of Fc receptors and ligands, imparting an array of important functional capabilities referred to as effector functions. For IgG the Fc region, as shown in Figure 1, comprises Ig domains Cγ2 and Cγ3 and the N-terminal hinge leading into Cγ2. An important family of Fc receptors for the IgG class are the Fc gamma receptors (FcγRs). These receptors mediate communication between antibodies and the cellular arm of the immune system (Raghavan *et al.*, 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ravetch *et al.*, 2001, *Annu Rev Immunol* 19:275-290). In humans this protein family includes FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIIb-NA1 and FcγRIIIb-NA2) (Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65). These receptors typically have an extracellular domain that mediates binding to Fc, a membrane spanning region, and an intracellular domain that may mediate some signaling event within the cell. These receptors are expressed in a variety of immune cells including monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, B cells, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, and γγ T cells. Formation of the Fc/FcγR complex recruits these effector cells to sites of bound antigen, typically resulting in signaling events within the cells and important subsequent immune responses such as release of inflammation mediators, B cell activation, endocytosis, phagocytosis, and cytotoxic attack. The ability to mediate cytotoxic and phagocytic effector functions is a potential mechanism by which antibodies destroy targeted cells. The cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell is referred to as antibody dependent cell-mediated cytotoxicity (ADCC) (Raghavan *et al.*, 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ghetie *et al.*, 2000, *Annu Rev Immunol* 18:739-766; Ravetch *et al.*, 2001, *Annu Rev Immunol* 19:275-290). The cell-mediated reaction wherein

nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell is referred to as antibody dependent cell-mediated phagocytosis (ADCP). A number of structures have been solved of the extracellular domains of human FcγRs, including FcγRIIa (pdb accession code 1H9V)(Sondermann *et al.*, 2001, *J Mol Biol* 309:737-749) (pdb accession code 1FCG)(Maxwell *et al.*, 1999, *Nat Struct Biol* 6:437-442), FcγRIIb (pdb accession code 2FCB)(Sondermann *et al.*, 1999, *Embo J* 18:1095-1103); and FcγRIIIb (pdb accession code 1E4J)(Sondermann *et al.*, 2000, *Nature* 406:267-273.). All FcγRs bind the same region on Fc, at the N-terminal end of the Cγ2 domain and the preceding hinge, shown in Figure 2. This interaction is well characterized structurally (Sondermann *et al.*, 2001, *J Mol Biol* 309:737-749), and several structures of the human Fc bound to the extracellular domain of human FcγRIIIb have been solved (pdb accession code 1E4K)(Sondermann *et al.*, 2000, *Nature* 406:267-273.) (pdb accession codes 1IIS and 1IIX)(Radaev *et al.*, 2001, *J Biol Chem* 276:16469-16477), as well as has the structure of the human IgE Fc/FcεRIα complex (pdb accession code 1F6A)(Garman *et al.*, 2000, *Nature* 406:259-266).

[6] The different IgG subclasses have different affinities for the FcγRs, with IgG1 and IgG3 typically binding substantially better to the receptors than IgG2 and IgG4 (Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65). All FcγRs bind the same region on IgG Fc, yet with different affinities: the high affinity binder FcγRI has a Kd for IgG1 of 10^{-8} M^{-1}, whereas the low affinity receptors FcγRII and FcγRIII generally bind at 10^{-6} and 10^{-5} respectively. The extracellular domains of FcγRIIIa and FcγRIIIb are 96% identical, however FcγRIIIb does not have a intracellular signaling domain. Furthermore, whereas FcγRI, FcγRIIa/c, and FcγRIIIa are positive regulators of immune complex-triggered activation, characterized by having an intracellular domain that has an immunoreceptor tyrosine-based activation motif (ITAM), FcγRIIb has an immunoreceptor tyrosine-based inhibition motif (ITIM) and is therefore inhibitory. Thus the former are referred to as activation receptors, and FcγRIIb is referred to as an inhibitory receptor. The receptors also differ in expression pattern and levels on different immune cells. Yet another level of complexity is the existence of a number of FcγR polymorphisms in the human proteome. A particularly relevant polymorphism with clinical significance is V158/F158 FcγRIIIa. Human IgG1 binds with greater affinity to the V158 allotype than to the F158 allotype. This difference in affinity, and presumably its effect on ADCC and/or ADCP, has been shown to be a significant determinant of the efficacy of the anti-CD20 antibody rituximab (Rituxan®, a registered trademark of IDEC Pharmaceuticals Corporation). Patients with the V158 allotype respond favorably to rituximab treatment; however, patients with the lower affinity F158 allotype respond poorly (Cartron *et al.*, 2002, *Blood* 99:754-758). Approximately 10-20% of humans are V158/V158 homozygous, 45% are V158/F158 heterozygous, and 35-45% of humans are F158/F158 homozygous (Lehrnbecher *et al.*, 1999, *Blood* 94:4220-4232; Cartron *et al.*, 2002, *Blood* 99:754-758). Thus 80-90% of humans are poor responders, that is they have at least one allele of the F158 FcγRIIIa.

[7] An overlapping but separate site on Fc, shown in Figure 1, serves as the interface for the complement protein C1q. In the same way that Fc/FcγR binding mediates ADCC, Fc/C1q binding

mediates complement dependent cytotoxicity (CDC). C1q forms a complex with the serine proteases C1r and C1s to form the C1 complex. C1q is capable of binding six antibodies, although binding to two IgGs is sufficient to activate the complement cascade. Similar to Fc interaction with FcγRs, different IgG subclasses have different affinity for C1q, with IgG1 and IgG3 typically binding substantially better to the FcγRs than IgG2 and IgG4 (Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65).

[8] A site on Fc between the Cγ2 and Cγ3 domains, shown in Figure 1, mediates interaction with the neonatal receptor FcRn, the binding of which recycles endocytosed antibody from the endosome back to the bloodstream (Raghavan *et al.*, 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ghetie *et al.*, 2000, *Annu Rev Immunol* 18:739-766). This process, coupled with preclusion of kidney filtration due to the large size of the full length molecule, results in favorable antibody serum half-lives ranging from one to three weeks. Binding of Fc to FcRn also plays a key role in antibody transport. The binding site for FcRn on Fc is also the site at which the bacterial proteins A and G bind. The tight binding by these proteins is typically exploited as a means to purify antibodies by employing protein A or protein G affinity chromatography during protein purification. Thus the fidelity of this region on Fc is important for both the clinical properties of antibodies and their purification. Structures of the rat Fc/FcRn complex have been disclosed (Martin *et al.*, 2001, *Mol Cell* 7:867-877). The complexes of Fc with proteins A and G have also been disclosed (Deisenhofer, 1981, *Biochemistry* 20:2361-2370; Sauer-Eriksson *et al.*, 1995, *Structure* 3:265-278; Tashiro *et al.*, 1995, *Curr Opin Struct Biol* 5:471-481).

[9] One feature of the Fc region is the conserved N-linked glycosylation that occurs at N297, shown in Figure 1. This carbohydrate, or oligosaccharide as it is sometimes referred, plays a critical structural and functional role for the antibody, and is one of the principle reasons that antibodies must be produced using mammalian expression systems. Umaña *et al.*, 1999, *Nat Biotechnol* 17:176-180; Davies *et al.*, 2001, *Biotechnol Bioeng* 74:288-294; Mimura *et al.*, 2001, *J Biol Chem* 276:45539-45547.; Radaev *et al.*, 2001, *J Biol Chem* 276:16478-16483; Shields *et al.*, 2001, *J Biol Chem* 276:6591-6604; Shields *et al.*, 2002, *J Biol Chem* 277:26733-26740; Simmons *et al.*, 2002, *J Immunol Methods* 263:133-147; Radaev *et al.*, 2001, *J Biol Chem* 276:16469-16477; and Krapp *et al.*, 2003, *J Mol Biol* 325:979-989).

[10] Antibodieshave been developed for therapeutic use. Representative publications related to such therapies include Chamow *et al.*, 1996, *Trends Biotechnol* 14:52-60; Ashkenazi *et al.*, 1997, *Curr Opin Immunol* 9:195-200, Cragg *et al.*, 1999, *Curr Opin Immunol* 11:541-547; Glennie *et al.*, 2000, *Immunol Today* 21:403-410, McLaughlin *et al.*, 1998, *J Clin Oncol* 16:2825-2833, and Cobleigh *et al.*, 1999, *J Clin Oncol* 17:2639-2648. Currently for anticancer therapy, any small improvement in mortality rate defines success. Certain IgG variants disclosed herein enhance the capacity of antibodies to destroy targeted cancer cells.

[11] Anti-tumor potency of antibodies is via enhancement of their ability to mediate cytotoxic effector functions such as ADCC, ADCP, and CDC. Examples include Clynes *et al.*, 1998, *Proc Natl Acad Sci U S A* 95:652-656; Clynes *et al.*, 2000, *Nat Med* 6:443-446, ; and Cartron *et al.*, 2002, *Blood* 99:754-758;.

[12] Human IgG1 is the most commonly used antibody for therapeutic purposes, and the majority of engineering studies have been constructed in this context. The different isotypes of the IgG class however, including IgG1, IgG2, IgG3, and IgG4, have unique physical, biological, and clinical properties. There is a need in the art to design IgG2, IgG3, and IgG4 variants. There is a futher need to design such variants to improve binding to an FcγR or enhance effector function as compared to native IgG polypeptides. The present application meets these and other needs.

SUMMARY

[13] The present application is directed to IgG2, IgG3, and IgG4 variants. Certain variants include isotopic amino acid modifications between IgG1, IgG2, IgG3, and IgG4. The variations can include isotopic modifications between in at least 2 domains, 3, domains, 3 domains, or 4 domains. Exchange domains can be CH1, CH2, hinge, and CH3 domains, CH1, CH2, and CH3 domains, or CH2 and CH3 domains.

[14] Alternatively, certain specific modifications can be made to IgG2, IgG3, and IgG4 variants that are not found in any other IgG subclass. In certain embodiments, the variations can occur within only the Fc region of the IgG subclass, or only within one or more specific domains.

[15] In additional aspects, IgG2, IgG3, and IgG4 variants that exhibit altered binding to an FcγR or enhances effector function as compared to native IgG polypeptides can be designed. For example, altered binding to an FcγR such as FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, or FcγRIIIa can be designed. Alternatively, one or more effector functions (e.g. ADCC, ADCP, and CDC) can be designed.

[16] In one aspect, the present application is directed to IgG2, IgG3, or IgG4 variants with one or more isotypic substitutions. In an embodiment, of such variants, the IgG2, IgG3, or IgG4 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAP-X(131)-S-X(133)-STS-X(137)-X(138)-
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS-X(192)-X(193)-GT-
X(196)-TY-X(199)-CNV-X(203)-HKPSNTKVDK-X(214)-VE-X(217)-K-X(219)-X(220)-X(221)-X(222)-
X(223)-X(224)-X(225)-CP-X(228)-CPAP-X(233)-X(234)-X(235)-X(236)-
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVS-X(268)-EDPEV-X(274)-F-X(276)-
WYVDGVEVHNAKTKPREEQ-X(296)-NST-X(300)-RVVSVLTV-X(309)-HQDWLNGKEYKCKVSNK-
X(327)-LP-X(330)-X(331)-IEKTISK-X(339)-KGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-
TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYS-
X(409)-LTVDKSRWQ-X(419)-GN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLS-X(445)-GK,
wherein
-X(131)- is selected from the group consisting of C and S;
-X(133)- is selected from the group consisting of R and K;
-X(137)- is selected from the group consisting of E and G;
-X(138)- is selected from the group consisting of S and G;
-X(192)- is selected from the group consisting of N and S;
-X(193)- is selected from the group consisting of F and L;

-X(196)- is selected from the group consisting of Q and K;

-X(199)- is selected from the group consisting of T and I;

-X(203)- is selected from the group consisting of D and N;

-X(214)- is selected from the group consisting of T, K and R;

-X(217)- is selected from the group consisting of R, P, L and S;

-X(219)- is selected from the group consisting of C, S, T and Y;

-X(220)- is selected from the group consisting of C, P and G;

-X(221)- is selected from the group consisting of no amino acid, D, L and the sequence LGD;

-X(222)- is selected from the group consisting of V, K, T and no amino acid;

-X(223)- is selected from the group consisting of no amino acid and T;

-X(224)- is selected from the group consisting of E, H and P;

-X(225)- is selected from the group consisting of no amino acid, T and P;

-X(228)- is selected from the group consisting of P, S, R, and the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR;

-X(233)- is selected from the group consisting of P and E;

-X(234)- is selected from the group consisting of V, L and F;

-X(235)- is selected from the group consisting of A and L;

-X(236)- is selected from the group consisting of no amino acid and G;

-X(268)- is selected from the group consisting of H and Q;

-X(274)- is selected from the group consisting of Q and K;

-X(276)- is selected from the group consisting of N and K;

-X(296)- is selected from the group consisting of F and Y;

-X(300)- is selected from the group consisting of F and Y;

-X(309)- is selected from the group consisting of V and L;

-X(327)- is selected from the group consisting of G and A;

-X(330)- is selected from the group consisting of A and S;

-X(331)- is selected from the group consisting of P and S;

-X(339)- is selected from the group consisting of T and A;

-X(355)- is selected from the group consisting of R and Q;

-X(356)- is selected from the group consisting of E and D;

-X(358)- is selected from the group consisting of M and L;

-X(384)- is selected from the group consisting of N and S;

-X(392)- is selected from the group consisting of K and N;

-X(397)- is selected from the group consisting of M and V;

-X(409)- is selected from the group consisting of K and R;

-X(419)- is selected from the group consisting of Q and E;

-X(422)- is selected from the group consisting of V and I;

-X(435)- is selected from the group consisting of H and R;

-X(436)- is selected from the group consisting of Y and F; and

-X(445)- is selected from the group consisting of P and L.

[17] Variants of the formula can have at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:12, SEQ ID NO:13, or SEQ ID NO: 4. In a further embodiment, at least two of the modifications can be in different domains, at least three modifications can be in different domains, or at least four modifications can be in different domains.

[18] In a further aspect, the present application is directed to an IgG2, IgG3, or IgG4 variant including at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:12, SEQ ID NO:13, or SEQ ID NO: 4. The modification can be at one or more positions selected from among positions 131, 133, 137, 138, 192, 193, 196, 199, 203, 214, 217, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 233, 234, 235, 236, 268, 274, 296, 300, 309, 327, 330, 335, 339, 356, 358, 384, 392, 397, 409, 419, 422, 435, 436 and 445. In further embodiments, at least two of the modifications can be in different domains, at least three modifications can be in different domains, or at least four modifications can be in different domains.

[19] In another aspect, the present application is directed to an IgG2 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAP-X(131)-S-X(133)-STS-X(137)-X(138)-
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS-X(192)-X(193)-
GTQTY-X(199)-CNV-X(203)-HKPSNTKVDK-X(214)-VE-X(217)-K-X(219)-C-X(221)-X(222)-X(223)-
X(224)-X(225)-CPPCPAP-X(233)-X(234)-X(235)-X(236)-
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV-X(274)-FNWYVDGVEVHNAKTKPREEQ-
X(296)-NST-X(300)-RVVSVLTV-X(309)-HQDWLNGKEYKCKVSNK-X(327)-LPAPIEKTISK-X(339)-
KGQPREPQVYTLPPSR-X(356)-E-X(358)-TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP-
X(397)-LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK,
wherein
X(131) is selected from the group consisting of C and S;
X(133) is selected from the group consisting of R and K;
X(137) is selected from the group consisting of E and G;
X(138) is selected from the group consisting of S and G;
X(192) is selected from the group consisting of N and S;
X(193) is selected from the group consisting of F and L;
X(199) is selected from the group consisting of T and I;
X(203) is selected from the group consisting of D and N;
X(214) is selected from the group consisting of T and K;
X(217) is selected from the group consisting of R and P;
X(219) is selected from the group consisting of C and S;
X(221) is selected from the group consisting of no amino acid and D;
X(222) is selected from the group consisting of V and K;
X(223) is selected from the group consisting of no amino acid and T;

X(224) is selected from the group consisting of E and H;
X(225) is selected from the group consisting of no amino acid and T;
X(233) is selected from the group consisting of P and E;
X(234) is selected from the group consisting of V and L;
X(235) is selected from the group consisting of A and L;
X(236) is selected from the group consisting of no amino acid and G;
X(274) is selected from the group consisting of Q and K;
X(296) is selected from the group consisting of F and Y;
X(300) is selected from the group consisting of F and Y;
X(309) is selected from the group consisting of V and L;
X(327) is selected from the group consisting of G and A;
X(339) is selected from the group consisting of T and A;
X(356) is selected from the group consisting of E and D;
X(358) is selected from the group consisting of M and L; and
X(397) is selected from the group consisting of M and V.

[20] In various embodiments, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:12. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains.

[21] In another aspect, the present application is directed to an IgG2 variant including two or more amino acid modifications as compared to SEQ ID NO:12. The modification can be selected from among C131S, R133K, E137G, S138G, N192S, F193L, T199I, D203N, T214K, R217P, C219S, insertion of 221D, V222K, insertion of 223T, E224H, insertion of 225T, P233E, V234L, A235L, insertion of 236G, Q274K, F296Y, F300Y, V309L, G327A, T339A, E356D, M358L, and M397V. In various embodiments, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:12. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains.

[22] In a further variation, the present application is directed to an IgG2 variant including an amino acid sequence having the formula:

-ASTKGPSVFPLAP-X(131)-S-X(133)-STS-X(137)-X(138)-
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS-X(192)-X(193)-GT-X(196)-TY-X(199)-CNV-X(203)-HKPSNTKVDK-X(214)-VE-X(217)-K-X(219)-X(220)-X(221)-X(222)-X(223)-X(224)-X(225)-C-X(227)-X(228)-C-X(230)-X(231)-X(232)-X(233)-X(234)-X(235)-X(236)-X(237)-X(238)-X(239)-X(240)-X(241)-L-X(243)-X(244)-X(245)-X(246)-X(247)-K-X(249)-TLMIS-X(255)-TP-X(258)-V-X(260)-C-X(262)-X(263)-X(264)-X(265)-X(266)-X(267)-X(268)-X(269)-X(270)-X(271)-X(272)-X(273)-X(274)-X(275)-X(276)-W-X(278)-V-X(280)-X(281)-X(282)-X(283)-X(284)-X(285)-X(286)-A-X(288)-T-X(290)-X(291)-X(292)-X(293)-X(294)-X(295)-X(296)-X(297)-X(298)-X(299)-X(300)-X(301)-X(302)-X(303)-X(304)-X(305)-LTV-X(309)-HQD-X(313)-LNG-X(317)-X(318)-Y-X(320)-C-X(322)-X(323)-X(324)-X(325)-X(326)-X(327)-X(328)-X(329)-X(330)-X(331)-X(332)-X(333)-X(334)-X(335)-X(336)-X(337)-K-X(339)-KGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-

TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYS-X(409)-LTVDKSRWQ-X(419)-GN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLS-X(445)-GK-, wherein

-X(131)- is selected from the group consisting of C and S;
-X(133)- is selected from the group consisting of R and K;
-X(137)- is selected from the group consisting of E and G;
-X(138)- is selected from the group consisting of S and G;
-X(192)- is selected from the group consisting of N and S;
-X(193)- is selected from the group consisting of F and L;
-X(196)- is selected from the group consisting of Q and K;
-X(199)- is selected from the group consisting of T and I;
-X(203)- is selected from the group consisting of D and N;
-X(214)- is selected from the group consisting of T, K and R;
-X(217)- is selected from the group consisting of R, P, L and S;
-X(219)- is selected from the group consisting of C, S, T and Y;
-X(220)- is selected from the group consisting of C, P and G;
-X(221)- is selected from the group consisting of no amino acid, D, K, L, Y and the sequence LGD;
-X(222)- is selected from the group consisting of V, K, T, no amino acid , E and Y;
-X(223)- is selected from the group consisting of no amino acid, T, E and K;
-X(224)- is selected from the group consisting of E, H, P and Y;
-X(225)- is selected from the group consisting of no amino acid, T, P, E, K and W;
-X(227)- is selected from the group consisting of P, E, G, K and Y;
-X(228)- is selected from the group consisting of P, S, R, E, G, K, Y, and the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR;
-X(230)- is selected from the group consisting of P, A, E, G and Y;
-X(231)- is selected from the group consisting of A, E, G, K, P and Y;
-X(232)- is selected from the group consisting of P, E, G, K and Y;
-X(233)- is selected from the group consisting of P, E, A, D, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(234)- is selected from the group consisting of V, L, F, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, W and Y;
-X(235)- is selected from the group consisting of A, L, D, F, G, H, I, K, M, N, P, Q, R, S, T, V, W , and Y;
-X(236)- is selected from the group consisting of no amino acid, G, A, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;
-X(237)- is selected from the group consisting of G, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;
-X(238)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(239)- is selected from the group consisting of S, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W and Y;

-X(240)- is selected from the group consisting of V, A, I, M and T;

-X(241)- is selected from the group consisting of F, D, E, L, R, S, W and Y;

-X(243)- is selected from the group consisting of F, E, H, L, Q, R, W, and Y;

-X(244)- is selected from the group consisting of P and H;

-X(245)- is selected from the group consisting of P and A;

-X(246)- is selected from the group consisting of , K, D, E, H and Y;

-X(247)- is selected from the group consisting of P, G and V;

-X(249)- is selected from the group consisting of D, H, Q and Y;

-X(255)- is selected from the group consisting of RE and Y;

-X(258)- is selected from the group consisting of E, H, S and Y;

-X(260)- is selected from the group consisting of T, D, E, H and Y;

-X(262)- is selected from the group consisting of V, A, E, F, I and T;

-X(263)- is selected from the group consisting of V, A, I, M and T;

-X(264)- is selected from the group consisting of V, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, and Y;

-X(265)- is selected from the group consisting of D, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(266)- is selected from the group consisting of V, A, I, M and T;

-X(267)- is selected from the group consisting of S, D, E, F, H, I, K, L, M, N, P, Q, R, V, W and Y;

-X(268)- is selected from the group consisting of H, Q, D, E, F, G, I, K, L, M, P, R, T, V and W;

-X(269)- is selected from the group consisting of E, F, G, H, I, K, L, M, N, P, R, S, T, V, W and Y;

-X(270)- is selected from the group consisting of D, F, G, H, I, L, M, P, Q, R, S, T, W and Y;

-X(271)- is selected from the group consisting of P, A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(272)- is selected from the group consisting of E, D, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;

-X(273)- is selected from the group consisting of V and I;

-X(274)- is selected from the group consisting of Q, K, D, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(275)- is selected from the group consisting of F, L and W;

-X(276)- is selected from the group consisting of N, K, D, E, F, G, H, I, L, M, P, R, S, T, V, W and Y;

-X(278)- is selected from the group consisting of Y, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V and W;

-X(280)- is selected from the group consisting of D, G, K, L, P and W;

-X(281)- is selected from the group consisting of G, D, E, K, N, P, Q and Y;

-X(282)- is selected from the group consisting of V, E, G, K, P and Y;

-X(283)- is selected from the group consisting of E, G, H, K, L, P, R and Y;

-X(284)- is selected from the group consisting of V, D, E, L, N, Q, T and Y;

-X(285)- is selected from the group consisting of H, D, E, K, Q, W and Y;

-X(286)- is selected from the group consisting of N, E, G, P and Y;

-X(288)- is selected from the group consisting of K, D, E and Y;

-X(290)- is selected from the group consisting of K, D, H, L, N and W;

-X(291)- is selected from the group consisting of P, D, E, G, H, I, Q and T;

-X(292)- is selected from the group consisting of R, D, E, T and Y;

-X(293)- is selected from the group consisting of E, F, G, H, I, L, M, N, P, R, S, T, V, W and Y;

-X(294)- is selected from the group consisting of E, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;

-X(295)- is selected from the group consisting of Q, D, E, F, G, H, I, M, N, P, R, S, T, V, W and Y;

-X(296)- is selected from the group consisting of F, Y, A, D, E, G, I, K, L, M, N, Q, R, S, T and V;

-X(297)- is selected from the group consisting of N, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(298)- is selected from the group consisting of S, E, F, H, I, K, M, Q, R, W and Y;

-X(299)- is selected from the group consisting of T, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W and Y;

-X(300)- is selected from the group consisting of F, Y, A, D, E, G, H, K, M, N, P, Q, R, S, T, V and W;

-X(301)- is selected from the group consisting of R, D, E, H and Y;

-X(302)- is selected from the group consisting of V and I;

-X(303)- is selected from the group consisting of V, D, E and Y;

-X(304)- is selected from the group consisting of S, D, H, L, N and T;

-X(305)- is selected from the group consisting of V, E, T and Y;

-X(309)- is selected from the group consisting of V and L;

-X(313)- is selected from the group consisting of W and F;

-X(317)- is selected from the group consisting of K, E and Q;

-X(318)- is selected from the group consisting of E, H, L, Q, R and Y;

-X(320)- is selected from the group consisting of K, D, F, G, H, I, L, N, P, S, T, V, W and Y;

-X(322)- is selected from the group consisting of K, D, F, G, H, I, P, S, T, V, W and Y;

-X(323)- is selected from the group consisting of V and I;

-X(324)- is selected from the group consisting of S, D, F, G, H, I, L, M, P, R, T, V, W and Y;

-X(325)- is selected from the group consisting of N, A, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(326)- is selected from the group consisting of K, I, L, P and T;

-X(327)- is selected from the group consisting of G, A, D, E, F, H, I, K, L, M, N, P, R, T, V, W and Y;

-X(328)- is selected from the group consisting of L, A, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;

-X(329)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(330)- is selected from the group consisting of A, S, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(331)- is selected from the group consisting of P, S, D, F, H, I, L, M, Q, R, T, V, W and Y;

-X(332)- is selected from the group consisting of I, A, D, E, F, H, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(333)- is selected from the group consisting of E, F, H, I, L, M, P, T and Y;

-X(334)- is selected from the group consisting of K, F, I, P and T;

-X(335)- is selected from the group consisting of T, D, F, G, H, I, L, M, N, P, R, S, V, W and Y;

-X(336)- is selected from the group consisting of I, E, K and Y;

-X(337)- is selected from the group consisting of S, E, H and N;
-X(339)- is selected from the group consisting of T and A;
-X(355)- is selected from the group consisting of R and Q;
-X(356)- is selected from the group consisting of E and D;
-X(358)- is selected from the group consisting of M and L;
-X(384)- is selected from the group consisting of N and S;
-X(392)- is selected from the group consisting of K and N;
-X(397)- is selected from the group consisting of M and V;
-X(409)- is selected from the group consisting of K and R;
-X(419)- is selected from the group consisting of Q and E;
-X(422)- is selected from the group consisting of V and I;
-X(435)- is selected from the group consisting of H and R;
-X(436)- is selected from the group consisting of Y and F; and
-X(445)- is selected from the group consisting of P and L.

[23] In certain variations, a first modification is selected from among C131S, R133K, E137G, S138G, N192S, F193L, Q196K, T199I, D203N, T214K, T214R, R217P, R217L, R217S, C219S, C219T, C219Y, C220P, C220G, insertion of 221D, insertion of 221L, insertion of 221LGD, V222K, V222T, deletion of V222, insertion of 223T, E224H, E224P, insertion of 225T, insertion of 225P, P228R, substitution of P228 with RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR, P228S, P233E, V234L, V234F, A235L, insertion of 236G, H268Q, Q274K, N276K, F296Y, F300Y, V309L, G327A, A330S, P331S, T339A, R355Q, E356D, M358L, N384S, K392N, M397V, K409R, Q419E, V422I, H435R, Y436F, and P445L. In a further variation, a second modification is selected from among 221K, 221Y, 222E, 222Y, 223E, 223K, 224Y, 225E, 225K, 225W, 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234W, 234Y, 235D, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P,

268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334I, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N.

[24] In another aspect, a first modification selected from among

[25] C131S, R133K, E137G, S138G, N192S, F193L, Q196K, T199I, D203N, T214K, T214R, R217P, R217L, R217S, C219S, C219T, C219Y, C220P, C220G, insertion of 221D, insertion of 221L, insertion of 221LGD, V222K, V222T, deletion of V222, insertion of 223T, E224H, E224P, insertion of 225T, insertion of 225P, P228R, substitution of P228 with

RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR, P228S, P233E, V234L, V234F, A235L, insertion of 236G, H268Q, Q274K, N276K, F296Y, F300Y, V309L, G327A, A330S, P331S, T339A, R355Q, E356D, M358L, N384S, K392N, M397V, K409R, Q419E, V422I, H435R, Y436F, and P445L. In a further variation, a second modification selected from among 221K, 221Y, 222E, 222Y, 223E, 223K, 224Y, 225E, 225K, 225W, 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234W, 234Y, 235D, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P,

300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334I, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, 337N.

[26] In another aspect, the present application is directed to an IgG2 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAP-X(131)-S-X(133)-STS-X(137)-X(138)-TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS-X(192)-X(193)-GT-X(196)-TY-X(199)-CNV-X(203)-HKPSNTKVDK-X(214)-VE-X(217)-K-X(219)-X(220)-X(221)-X(222)-X(223)-X(224)-X(225)-C-X(227)-X(228)-CPAP-X(233)-X(234)-X(235)-X(236)-X(237)-P-X(239)-X(240)-FLFPP-X(246)-PKDTLMIS-X(255)-TP-X(258)-V-X(260)-CVV-X(264)-DV-X(267)-X(268)-ED-X(271)-X(272)-V-X(274)-F-X(276)-W-X(278)-VD-X(281)-V-X(283)-X(284)-HNAKT-X(290)-PR-X(293)-E-X(295)-X(296)-NST-X(300)-RVV-X(304)-VLTV-X(309)-HQDWLNGKEYKCKV-X(324)-N-X(326)-X(327)-X(328)-P-X(330)-X(331)-X(332)-X(333)-X(334)-TISK-X(339)-KGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYS-X(409)-LTVDKSRWQ-X(419)-GN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLS-X(445)-GK; wherein

-X(131)- is selected from the group consisting of C and S;
-X(133)- is selected from the group consisting of R and K;
-X(137)- is selected from the group consisting of E and G;
-X(138)- is selected from the group consisting of S and G;
-X(192)- is selected from the group consisting of N and S;
-X(193)- is selected from the group consisting of F and L;
-X(196)- is selected from the group consisting of Q and K;
-X(199)- is selected from the group consisting of T and I;
-X(203)- is selected from the group consisting of D and N;
-X(214)- is selected from the group consisting of T, K and R;
-X(217)- is selected from the group consisting of R, P, L and S;

-X(219)- is selected from the group consisting of C, S, T and Y;
-X(220)- is selected from the group consisting of C, P and G;
-X(221)- is selected from the group consisting of no amino acid, D, K, L, and the sequence LGD;
-X(222)- is selected from the group consisting of V, K, T, and no amino acid;
-X(223)- is selected from the group consisting of no amino acid and T;
-X(224)- is selected from the group consisting of E, H and P;
-X(225)- is selected from the group consisting of no amino acid, T and P;
-X(227)- is selected from the group consisting of P and G;
-X(228)- is selected from the group consisting of P, S, R, and the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR;
-X(233)- is selected from the group consisting of P and E;
-X(234)- is selected from the group consisting of V, L, F, Y and I;
-X(235)- is selected from the group consisting of A, L, Y, I and D;
-X(236)- is selected from the group consisting of no amino acid, G, S and A;
-X(237)- is selected from the group consisting of G and D;
-X(239)- is selected from the group consisting of S, D, E, N, Q and T;
-X(240)- is selected from the group consisting of V, I and M;
-X(246)- is selected from the group consisting of K, H and Y;
-X(255)- is selected from the group consisting of R and Y;
-X(258)- is selected from the group consisting of E, H and Y;
-X(260)- is selected from the group consisting of T and H;
-X(264)- is selected from the group consisting of V, I, T and Y;
-X(267)- is selected from the group consisting of S, D and E;
-X(268)- is selected from the group consisting of H, Q, D and E;
-X(271)- is selected from the group consisting of P and G;
-X(272)- is selected from the group consisting of E, Y, H, R and I;
-X(274)- is selected from the group consisting of Q, K and E;
-X(276)- is selected from the group consisting of N and K;
-X(278)- is selected from the group consisting of Y and T;
-X(281)- is selected from the group consisting of G, D and E;
-X(283)- is selected from the group consisting of E, L and H;
-X(284)- is selected from the group consisting of V, E and D;
-X(290)- is selected from the group consisting of K and N;
-X(293)- is selected from the group consisting of E and R;
-X(295)- is selected from the group consisting of Q and E;
-X(296)- is selected from the group consisting of F and Y;
-X(300)- is selected from the group consisting of F and Y;
-X(304)- is selected from the group consisting of S and T;
-X(309)- is selected from the group consisting of V and L;
-X(324)- is selected from the group consisting of S, G and I;

-X(326)- is selected from the group consisting of K and T;
-X(327)- is selected from the group consisting of G, A and D;
-X(328)- is selected from the group consisting of L, A, F, I and T;
-X(330)- is selected from the group consisting of A, S, L, Y and I;
-X(331)- is selected from the group consisting of P and S;
-X(332)- is selected from the group consisting of I, D, E, N, Q and T;
-X(333)- is selected from the group consisting of E and Y;
-X(334)- is selected from the group consisting of K, F, I and T;
-X(339)- is selected from the group consisting of T and A;
-X(355)- is selected from the group consisting of R and Q;
-X(356)- is selected from the group consisting of E and D;
-X(358)- is selected from the group consisting of M and L;
-X(384)- is selected from the group consisting of N and S;
-X(392)- is selected from the group consisting of K and N;
-X(397)- is selected from the group consisting of M and V;
-X(409)- is selected from the group consisting of K and R;
-X(419)- is selected from the group consisting of Q and E;
-X(422)- is selected from the group consisting of V and I;
-X(435)- is selected from the group consisting of H and R;
-X(436)- is selected from the group consisting of Y and F; and
-X(445)- is selected from the group consisting of P and L.

[27] In certain variations, a first modification is selected from among C131S, R133K, E137G, S138G, N192S, F193L, Q196K, T199I, D203N, T214K, T214R, R217P, R217L, R217S, C219S, C219T, C219Y, C220P, C220G, the insertion of 221D, the insertion of 221LGD, the insertion of 221L, V222K, V222T, the deletion of V222, the insertion of 223T, E224H, E224P, the insertion of 225T, the insertion of 225P, P228R, the substitution of RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR for P228, P228S, P233E, V234L, V234F, A235L, the insertion of 236G, H268Q, Q274K, N276K, F296Y, F300Y, V309L, G327A, A330S, P331S, T339A, R355Q, E356D, M358L, N384S, K392N, M397V, K409R, Q419E, V422I, H435R, Y436F, and P445L. In further variations, a second modification is selected from among 221K, 227G, 234Y, 234I, 235Y, 235I, 235D, 236S, 236A, 237D, 239D, 239E, 239N, 239Q, 239T, 240I, 240M, 246H, 246Y, 255Y, 258H, 258Y, 260H, 264I, 264T, 264Y, 267D, 267E, 268D, 268E, 271G, 272Y, 272H, 272R, 272I, 274E, 278T, 281D, 281E, 283L, 283H, 284E, 284D, 290N, 293R, 295E, 304T, 324G, 324I, 326T, 327D, 328A, 328F, 328I, 328T, 330L, 330Y, 330I, 332D, 332E, 332N, 332Q, 332T, 333Y, 334F, 334I, and 334T.

[28] In another aspect, the present application is directed to an IgG2 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVEC-X(227)-X(228)-CPAP-X(233)-X(234)-

X(235)-X(236)--X(237)-X(238)-X(239)-X(240)-X(241)-L-X(243)-X(244)-X(245)-X(246)-X(247)-K-X(249)-TLMIS-X(255)-TP-X(258)-V-X(260)-C-X(262)-X(263)-X(264)-X(265)-X(266)-X(267)-X(268)-X(269)-X(270)-X(271)-X(272)-X(273)-X(274)-X(275)-X(276)-W-X(278)-V-X(280)-X(281)-X(282)-X(283)-X(284)-X(285)-X(286)-A-X(288)-T-X(290)-X(291)-X(292)-X(293)-X(294)-X(295)-X(296)-X(297)-X(298)-X(299)-X(300)-X(301)-X(302)-X(303)-X(304)-X(305)-LTV-X(309)-HQD-X(313)-LNG-X(317)-X(318)-Y-X(320)-C-X(322)-X(323)-X(324)-X(325)-X(326)-X(327)-X(328)-X(329)-X(330)-X(331)-X(332)-X(333)-X(334)-X(335)-X(336)-X(337)-K-X(339)-KGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYS-X(409)-LTVDKSRWQ-X(419)-GN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLS-X(445)-GK, wherein

-X(237)- is selected from the group consisting of G, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;
-X(238)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(239)- is selected from the group consisting of S, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W and Y;
-X(240)- is selected from the group consisting of V, A, I, M and T;
-X(241)- is selected from the group consisting of F, D, E, L, R, S, W and Y;
-X(243)- is selected from the group consisting of F, E, H, L, Q, RW, , and Y;
-X(244)- is selected from the group consisting of P and H;
-X(245)- is selected from the group consisting of P and A;
-X(246)- is selected from the group consisting of , K, D, E, H and Y;
-X(247)- is selected from the group consisting of P, G and V;
-X(249)- is selected from the group consisting of D, H, Q and Y;
-X(255)- is selected from the group consisting of RE and Y;
-X(258)- is selected from the group consisting of E, H, S and Y;
-X(260)- is selected from the group consisting of T, D, E, H and Y;
-X(262)- is selected from the group consisting of V, A, E, F, I and T;
-X(263)- is selected from the group consisting of V, A, I, M and T;
-X(264)- is selected from the group consisting of V, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, and Y;
-X(265)- is selected from the group consisting of D, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;
-X(266)- is selected from the group consisting of V, A, I, M and T;
-X(267)- is selected from the group consisting of S, D, E, F, H, I, K, L, M, N, P, Q, R, V, W and Y;
-X(268)- is selected from the group consisting of H, Q, D, E, F, G, I, K, L, M, P, R, T, V and W;
-X(269)- is selected from the group consisting of E, F, G, H, I, K, L, M, N, P, R, S, T, V, W and Y;
-X(270)- is selected from the group consisting of D, F, G, H, I, L, M, P, Q, R, S, T, W and Y;
-X(271)- is selected from the group consisting of P, A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(272)- is selected from the group consisting of E, D, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;

-X(273)- is selected from the group consisting of V and I;

-X(274)- is selected from the group consisting of Q, K, D, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(275)- is selected from the group consisting of FL and W;

-X(276)- is selected from the group consisting of N, K, D, E, F, G, H, I, L, M, P, R, S, T, V, W and Y;

-X(278)- is selected from the group consisting of Y, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V and W;

-X(280)- is selected from the group consisting of D, G, K, L, P and W;

-X(281)- is selected from the group consisting of G, D, E, K, N, P, Q and Y;

-X(282)- is selected from the group consisting of V, E, G, K, P and Y;

-X(283)- is selected from the group consisting of E, G, H, K, L, P, R and Y;

-X(284)- is selected from the group consisting of V, D, E, L, N, Q, T and Y;

-X(285)- is selected from the group consisting of H, D, E, K, Q, W and Y;

-X(286)- is selected from the group consisting of N, E, G, P and Y;

-X(288)- is selected from the group consisting of K, D, E and Y;

-X(290)- is selected from the group consisting of K, D, H, L, N and W;

-X(291)- is selected from the group consisting of P, D, E, G, H, I, Q and T;

-X(292)- is selected from the group consisting of R, D, E, T and Y;

-X(293)- is selected from the group consisting of E, F, G, H, I, L, M, N, P, R, S, T, V, W and Y;

-X(294)- is selected from the group consisting of E, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;

-X(295)- is selected from the group consisting of Q, D, E, F, G, H, I, M, N, P, R, S, T, V, W and Y;

-X(296)- is selected from the group consisting of F, Y, A, D, E, G, I, K, L, M, N, Q, R, S, T and V;

-X(297)- is selected from the group consisting of N, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(298)- is selected from the group consisting of S, E, F, H, I, K, M, Q, R, W and Y;

-X(299)- is selected from the group consisting of T, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W and Y;

-X(300)- is selected from the group consisting of F, Y, A, D, E, G, H, K, M, N, P, Q, R, S, T, V and W;

-X(301)- is selected from the group consisting of R, D, E, H and Y;

-X(302)- is selected from the group consisting of V and I;

-X(303)- is selected from the group consisting of V, D, E and Y;

-X(304)- is selected from the group consisting of S, D, H, L, N and T;

-X(305)- is selected from the group consisting of V, E, T and Y;

-X(309)- is selected from the group consisting of V and L;

-X(313)- is selected from the group consisting of W and F;

-X(317)- is selected from the group consisting of K, E and Q;

-X(318)- is selected from the group consisting of E, H, L, Q, R and Y;

-X(320)- is selected from the group consisting of K, D, F, G, H, I, L, N, P, S, T, V, W and Y;

-X(322)- is selected from the group consisting of K, D, F, G, H, I, P, S, T, V, W and Y;

-X(323)- is selected from the group consisting of V and I;

-X(324)- is selected from the group consisting of S, D, F, G, H, I, L, M, P, R, T, V, W and Y;

-X(325)- is selected from the group consisting of N, A, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(326)- is selected from the group consisting of K, I, L, P and T;

-X(327)- is selected from the group consisting of G, A, D, E, F, H, I, K, L, M, N, P, R, T, V, W and Y;

-X(328)- is selected from the group consisting of L, A, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;

-X(329)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(330)- is selected from the group consisting of A, S, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(331)- is selected from the group consisting of P, S, D, F, H, I, L, M, Q, R, T, V, W and Y;

-X(332)- is selected from the group consisting of I, A, D, E, F, H, , K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(333)- is selected from the group consisting of E, F, H, I, L, M, P, T and Y;

-X(334)- is selected from the group consisting of K, F, I, P and T;

-X(335)- is selected from the group consisting of T, D, F, G, H, I, L, M, N, P, R, S, V, W and Y;

-X(336)- is selected from the group consisting of I, E, K and Y;

-X(337)- is selected from the group consisting of S, E, H and N;

-X(339)- is selected from the group consisting of T and A;

-X(355)- is selected from the group consisting of R and Q;

-X(356)- is selected from the group consisting of E and D;

-X(358)- is selected from the group consisting of M and L;

-X(384)- is selected from the group consisting of N and S;

-X(392)- is selected from the group consisting of K and N;

-X(397)- is selected from the group consisting of M and V;

-X(409)- is selected from the group consisting of K and R;

-X(419)- is selected from the group consisting of Q and E;

-X(422)- is selected from the group consisting of V and I;

-X(435)- is selected from the group consisting of H and R;

-X(436)- is selected from the group consisting of Y and F; and

-X(445)- is selected from the group consisting of P and L.

[29] In certain variations, a first modification is selected from among P228R, substitution of P228 with RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR, P228S, P233E, V234L, V234F, A235L, insertion of 236G, H268Q, Q274K, N276K, F296Y, F300Y, V309L, G327A, A330S, P331S, T339A, R355Q, E356D, M358L, N384S, K392N, M397V, K409R, Q419E, V422I, H435R, Y436F, and P445L. In additional variations, a second modification is selected from among 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234W, 234Y, 235D, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I,

236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H,

330I, 330L, 330M, 330N, 330P, 330R, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334I, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N. In certain variations, X(227) is P and X(228) is P.

[30] In a further aspect, the present application is directed to an IgG2 variant amino acid sequence including at least two modifications as compared to SEQ ID. NO:2. In certain variations, a first modification is selected from among P228R, substitution of P228 with RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR, P228S, P233E, V234L, V234F, A235L, insertion of 236G, H268Q, Q274K, N276K, F296Y, F300Y, V309L, G327A, A330S, P331S, T339A, R355Q, E356D, M358L, N384S, K392N, M397V, K409R, Q419E, V422I, H435R, Y436F, and P445L. In further variations, a second modification is selected from among 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234W, 234Y, 235D, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H,

290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334I, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N.

[31] In a further aspect, the application is directed to an IgG2 variant including an amino acid sequence having the formula

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVEC-X(227)-X(228)-CPAP-X(233)-X(234)-X(235)-X(236)-X(237)-P-X(239)-X(240)-FLFPP-X(246)-PKDTLMIS-X(255)-TP-X(258)-V-X(260)-CVV-X(264)-DV-X(267)-X(268)-ED-X(271)-X(272)-V-X(274)-F-X(276)-W-X(278)-VD-X(281)-V-X(283)-X(284)-HNAKT-X(290)-PR-X(293)-E-X(295)-X(296)-NST-X(300)-RVV-X(304)-VLTV-X(309)-HQDWLNGKEYKCKV-X(324)-N-X(326)-X(327)-X(328)-P-X(330)-X(331)-X(332)-X(333)-X(334)-TISK-X(339)-KGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYS-X(409)-LTVDKSRWQ-X(419)-GN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLS-X(445)-GK

wherein

-X(237)- is selected from the group consisting of G and D;

-X(239)- is selected from the group consisting of S, D, E, N, Q and T;

-X(240)- is selected from the group consisting of V, I and M;

-X(246)- is selected from the group consisting of K, H and Y;

-X(255)- is selected from the group consisting of R and Y;
-X(258)- is selected from the group consisting of E, H and Y;
-X(260)- is selected from the group consisting of T and H;
-X(264)- is selected from the group consisting of V, I, T and Y;
-X(267)- is selected from the group consisting of S, D and E;
-X(268)- is selected from the group consisting of H, Q, D and E;
-X(271)- is selected from the group consisting of P and G;
-X(272)- is selected from the group consisting of E, Y, H, R and I;
-X(274)- is selected from the group consisting of Q, K and E;
-X(276)- is selected from the group consisting of N and K;
-X(278)- is selected from the group consisting of Y and T;
-X(281)- is selected from the group consisting of G, D and E;
-X(283)- is selected from the group consisting of E, L and H;
-X(284)- is selected from the group consisting of V, E and D;
-X(290)- is selected from the group consisting of K and N;
-X(293)- is selected from the group consisting of E and R;
-X(295)- is selected from the group consisting of Q and E;
-X(296)- is selected from the group consisting of F and Y;
-X(300)- is selected from the group consisting of F and Y;
-X(304)- is selected from the group consisting of S and T;
-X(309)- is selected from the group consisting of V and L;
-X(324)- is selected from the group consisting of S, G and I;
-X(326)- is selected from the group consisting of K and T;
-X(327)- is selected from the group consisting of G, A and D;
-X(328)- is selected from the group consisting of L, A, F, I and T;
-X(330)- is selected from the group consisting of A, S, L, Y and I;
-X(331)- is selected from the group consisting of P and S;
-X(332)- is selected from the group consisting of I, D, E, N, Q and T;
-X(333)- is selected from the group consisting of E and Y;
-X(334)- is selected from the group consisting of K, F, I and T;
-X(339)- is selected from the group consisting of T and A;
-X(355)- is selected from the group consisting of R and Q;
-X(356)- is selected from the group consisting of E and D;
-X(358)- is selected from the group consisting of M and L;
-X(384)- is selected from the group consisting of N and S;
-X(392)- is selected from the group consisting of K and N;
-X(397)- is selected from the group consisting of M and V;
-X(409)- is selected from the group consisting of K and R;
-X(419)- is selected from the group consisting of Q and E;
-X(422)- is selected from the group consisting of V and I;

-X(435)- is selected from the group consisting of H and R;
-X(436)- is selected from the group consisting of Y and F;
-X(445)- is selected from the group consisting of P and L;

[32] In certain variations, a first modification is selected from among P228R, the substitution of RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR for P228, P228S, P233E, V234L, V234F, A235L, the insertion of 236G, H268Q, K274Q, N276K, Y296F, Y300F, L309V, A327G, A330S, P331S, A339T, R355Q, D356E, L358M, N384S, K392N, V397M, K409R, Q419E, V422I, H435R, Y436F, and P445L. In additional variations, a second modification is selected from among 227G, 234Y, 234I, 235Y, 235I, 235D, 236S, 236A, 237D, 239D, 239E, 239N, 239Q, 239T, 240I, 240M, 246H, 246Y, 255Y, 258H, 258Y, 260H, 264I, 264T, 264Y, 267D, 267E, 268D, 268E, 271G, 272Y, 272H, 272R, 272I, 274E, 278T, 281D, 281E, 283L, 283H, 284E, 284D, 290N, 293R, 295E, 304T, 324G, 324I, 326T, 327D, 328A, 328F, 328I, 328T, 330L, 330Y, 330I, 332D, 332E, 332N, 332Q, 332T, 333Y, 334F, 334I, and 334T.

[33] In another aspect, the present application is directed to an IgG2 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC-X(221)-X(222)-X(223)-X(224)-X(225)-C-
X(227)-X(228)-C-X(230)-X(231)-X(232)-ELLGG-X(238)-X(239)-X(240)-X(241)-L-X(243)-X(244)-
X(245)-X(246)-X(247)-K-X(249)-TLMIS-X(255)-TP-X(258)-V-X(260)-C-X(262)-X(263)-X(264)-X(265)-
X(266)-X(267)-X(268)-X(269)-X(270)-X(271)-X(272)-X(273)-X(274)-X(275)-X(276)-W-X(278)-V-
X(280)-X(281)-X(282)-X(283)-X(284)-X(285)-X(286)-A-X(288)-T-X(290)-X(291)-X(292)-X(293)-
X(294)-X(295)-X(296)-X(297)-X(298)-X(299)-X(300)-X(301)-X(302)-X(303)-X(304)-X(305)-LTVVHQD-
X(313)-LNG-X(317)-X(318)-Y-X(320)-C-X(322)-X(323)-X(324)-X(325)-X(326)-X(327)-X(328)-X(329)-
X(330)-X(331)-X(332)-X(333)-X(334)-X(335)-X(336)-X(337)-
KTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK-, wherein
-X(221)- is selected from the group consisting of no amino acid, K and Y;
-X(222)- is selected from the group consisting of V, E and Y;
-X(223)- is selected from the group consisting of no amino acid, E and K;
-X(224)- is selected from the group consisting of E and Y;
-X(225)- is selected from the group consisting of no amino acid, E, K and W;
-X(227)- is selected from the group consisting of P, E, G, K and Y;
-X(228)- is selected from the group consisting of P, E, G, K and Y;
-X(230)- is selected from the group consisting of P, A, E, G and Y;
-X(231)- is selected from the group consisting of A, E, G, K, P and Y;
-X(232)- is selected from the group consisting of P, E, G, K and Y;
-X(233)- is selected from the group consisting of P, A, D, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(234)- is selected from the group consisting of V, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, W and Y;

-X(235)- is selected from the group consisting of A, D, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;

-X(236)- is selected from the group consisting of no amino acid, A, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(237)- is selected from the group consisting of G, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(238)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(239)- is selected from the group consisting of S, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W and Y;

-X(240)- is selected from the group consisting of V, A, I, M and T;

-X(241)- is selected from the group consisting of F, D, E, L, R, S, W and Y;

-X(243)- is selected from the group consisting of F, E, H, L, Q, R, W and Y;

-X(244)- is selected from the group consisting of P and H;

-X(245)- is selected from the group consisting of P and A;

-X(246)- is selected from the group consisting of K, D, E, H and Y;

-X(247)- is selected from the group consisting of P, G and V;

-X(249)- is selected from the group consisting of D, H, Q and Y;

-X(255)- is selected from the group consisting of R, E and Y;

-X(258)- is selected from the group consisting of E, H, S and Y;

-X(260)- is selected from the group consisting of T, D, E, H and Y;

-X(262)- is selected from the group consisting of V, A, E, F, I and T;

-X(263)- is selected from the group consisting of V, A, I, M and T;

-X(264)- is selected from the group consisting of V, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W and Y;

-X(265)- is selected from the group consisting of D, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(266)- is selected from the group consisting of V, A, I, M and T;

-X(267)- is selected from the group consisting of S, D, E, F, H, I, K, L, M, N, P, Q, R, V, W and Y;

-X(268)- is selected from the group consisting of H, D, E, F, G, I, K, L, M, P, R, T, V and W;

-X(269)- is selected from the group consisting of E, F, G, H, I, K, L, M, N, P, R, S, T, V, W and Y;

-X(270)- is selected from the group consisting of D, F, G, H, I, L, M, P, Q, R, S, T, W and Y;

-X(271)- is selected from the group consisting of P, A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(272)- is selected from the group consisting of E, D, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;

-X(273)- is selected from the group consisting of V and I;

-X(274)- is selected from the group consisting of Q, D, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(275)- is selected from the group consisting of F, L and W;

-X(276)- is selected from the group consisting of N, D, E, F, G, H, I, L, M, P, R, S, T, V, W and Y;

-X(278)- is selected from the group consisting of Y, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V and W;

-X(280)- is selected from the group consisting of D, G, K, L, P and W;

-X(281)- is selected from the group consisting of G, D, E, K, N, P, Q and Y;

-X(282)- is selected from the group consisting of V, E, G, K, P and Y;
-X(283)- is selected from the group consisting of E, G, H, K, L, P, R and Y;
-X(284)- is selected from the group consisting of V, D, E, L, N, Q, T and Y;
-X(285)- is selected from the group consisting of H, D, E, K, Q, W and Y;
-X(286)- is selected from the group consisting of N, E, G, P and Y;
-X(288)- is selected from the group consisting of K, D, E and Y;
-X(290)- is selected from the group consisting of K, D, H, L, N and W;
-X(291)- is selected from the group consisting of P, D, E, G, H, I, Q and T;
-X(292)- is selected from the group consisting of R, D, E, T and Y;
-X(293)- is selected from the group consisting of E, F, G, H, I, L, M, N, P, R, S, T, V, W and Y;
-X(294)- is selected from the group consisting of E, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;
-X(295)- is selected from the group consisting of Q, D, E, F, G, H, I, M, N, P, R, S, T, V, W and Y;
-X(296)- is selected from the group consisting of F, A, D, E, G, I, K, L, M, N, Q, R, S, T and V;
-X(297)- is selected from the group consisting of N, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;
-X(298)- is selected from the group consisting of S, E, F, H, I, K, M, Q, R, W and Y;
-X(299)- is selected from the group consisting of T, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W and Y;
-X(300)- is selected from the group consisting of F, A, D, E, G, H, K, M, N, P, Q, R, S, T, V and W;
-X(301)- is selected from the group consisting of R, D, E, H and Y;
-X(302)- is selected from the group consisting of V and I;
-X(303)- is selected from the group consisting of V, D, E and Y;
-X(304)- is selected from the group consisting of S, D, H, L, N and T;
-X(305)- is selected from the group consisting of V, E, T and Y;
-X(313)- is selected from the group consisting of W and F;
-X(317)- is selected from the group consisting of K, E and Q;
-X(318)- is selected from the group consisting of E, H, L, Q, R and Y;
-X(320)- is selected from the group consisting of K, D, F, G, H, I, L, N, P, S, T, V, W and Y;
-X(322)- is selected from the group consisting of K, D, F, G, H, I, P, S, T, V, W and Y;
-X(323)- is selected from the group consisting of V and I;
-X(324)- is selected from the group consisting of S, D, F, G, H, I, L, M, P, R, T, V, W and Y;
-X(325)- is selected from the group consisting of N, A, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;
-X(326)- is selected from the group consisting of K, I, L, P and T;
-X(327)- is selected from the group consisting of A, G, D, E, F, H, I, K, L, M, N, P, R, T, V, W and Y;
-X(328)- is selected from the group consisting of L, A, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;
-X(329)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(330)- is selected from the group consisting of A, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(331)- is selected from the group consisting of P, D, F, H, I, L, M, Q, R, T, V, W and Y;
-X(332)- is selected from the group consisting of I, A, D, E, F, H, K, L, M, N, P, Q, R, S, T, , V, W and Y;
-X(333)- is selected from the group consisting of E, F, H, I, L, M, P, T and Y;
-X(334)- is selected from the group consisting of K, F, I, P and T;
-X(335)- is selected from the group consisting of T, D, F, G, H, I, L, M, N, P, R, S, V, W and Y;
-X(336)- is selected from the group consisting of I, E, K and Y; and
-X(337)- is selected from the group consisting of S, E, H and N

[34] The variant differs from SEQ ID. NO:2 by at least one amino acid In a further aspect, X(327) is A.

[35] In another aspect, the present application is directed to an IgG2 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC-X(221)-VEC-X(227)-PCPAPELLGGP-X(239)-X(240)-FLFPP-X(246)-PKDTLMIS-X(255)-TP-X(258)-V-X(260)-CVV-X(264)-DV-X(267)-X(268)-ED-X(271)-X(272)-V-X(274)-FNW-X(278)-VD-X(281)-V-X(283)-X(284)-HNAKT-X(290)-PR-X(293)-E-X(295)-FNSTFRVV-X(304)-VLTVVHQDWLNGKEYKCKV-X(324)-N-X(326)-X(327)-X(328)-P-X(330)-P-X(332)-X(333)-X(334)-
TISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK, wherein

-X(221)- is selected from the group consisting of no amino acid and K;
-X(227)- is selected from the group consisting of P and G;
-X(237)- is selected from the group consisting of G and D;
-X(239)- is selected from the group consisting of S, D, E, N, Q and T;
-X(240)- is selected from the group consisting of V, I and M;
-X(246)- is selected from the group consisting of K, H and Y;
-X(255)- is selected from the group consisting of R and Y;
-X(258)- is selected from the group consisting of E, H and Y;
-X(260)- is selected from the group consisting of T and H;
-X(264)- is selected from the group consisting of V, I, T and Y;
-X(267)- is selected from the group consisting of S, D and E;
-X(268)- is selected from the group consisting of H, D and E;
-X(271)- is selected from the group consisting of P and G;
-X(272)- is selected from the group consisting of E, Y, H, R and I;
-X(274)- is selected from the group consisting of Q and E;
-X(278)- is selected from the group consisting of Y and T;
-X(281)- is selected from the group consisting of G, D and E;
-X(283)- is selected from the group consisting of E, L and H;

-X(284)- is selected from the group consisting of V, E and D;
-X(290)- is selected from the group consisting of K and N;
-X(293)- is selected from the group consisting of E and R;
-X(295)- is selected from the group consisting of Q and E;
-X(304)- is selected from the group consisting of S and T;
-X(324)- is selected from the group consisting of S, G and I;
-X(326)- is selected from the group consisting of K and T;
-X(327)- is selected from the group consisting of A, G and D;
-X(328)- is selected from the group consisting of L, A, F, I and T;
-X(330)- is selected from the group consisting of A, L, Y and I;
-X(332)- is selected from the group consisting of I, D, E, N, Q and T;
-X(333)- is selected from the group consisting of E and Y; and
-X(334)- is selected from the group consisting of K, F, I and T.

[36] In certain variations, at least one of the positions is different from the sequence of SEQ ID NO:5. In a further variation, X(327) is A.

[37] In another aspect, the present application is directed to an IgG2 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC-X(221)-X(222)-X(223)-X(224)-X(225)-C-
X(227)-X(228)-C-X(230)-X(231)-X(232)-X(233)-X(234)-X(235)-X(236)-X(237)-X(238)-X(239)-X(240)-
X(241)-L-X(243)-X(244)-X(245)-X(246)-X(247)-K-X(249)-TLMIS-X(255)-TP-X(258)-V-X(260)-C-
X(262)-X(263)-X(264)-X(265)-X(266)-X(267)-X(268)-X(269)-X(270)-X(271)-X(272)-X(273)-X(274)-
X(275)-X(276)-W-X(278)-V-X(280)-X(281)-X(282)-X(283)-X(284)-X(285)-X(286)-A-X(288)-T-X(290)-
X(291)-X(292)-X(293)-X(294)-X(295)-X(296)-X(297)-X(298)-X(299)-X(300)-X(301)-X(302)-X(303)-
X(304)-X(305)-LTVVHQD-X(313)-LNG-X(317)-X(318)-Y-X(320)-C-X(322)-X(323)-X(324)-X(325)-
X(326)-X(327)-X(328)-X(329)-X(330)-X(331)-X(332)-X(333)-X(334)-X(335)-X(336)-X(337)-
KTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK-, wherein
-X(221)- is selected from the group consisting of no amino acid, K and Y;
-X(222)- is selected from the group consisting of V, E and Y;
-X(223)- is selected from the group consisting of no amino acid, E and K;
-X(224)- is selected from the group consisting of E and Y;
-X(225)- is selected from the group consisting of no amino acid, E, K and W;
-X(227)- is selected from the group consisting of P, E, G, K and Y;
-X(228)- is selected from the group consisting of P, E, G, K and Y;
-X(230)- is selected from the group consisting of P, A, E, G and Y;
-X(231)- is selected from the group consisting of A, E, G, K, P and Y;
-X(232)- is selected from the group consisting of P, E, G, K and Y;

-X(233)- is selected from the group consisting of P, A, D, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(234)- is selected from the group consisting of V, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, W and Y;

-X(235)- is selected from the group consisting of A, D, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;

-X(236)- is selected from the group consisting of no amino acid, A, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(237)- is selected from the group consisting of G, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(238)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(239)- is selected from the group consisting of S, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W and Y;

-X(240)- is selected from the group consisting of V, A, I, M and T;

-X(241)- is selected from the group consisting of F, D, E, L, R, S, W and Y;

-X(243)- is selected from the group consisting of F, E, H, L, Q, R, W and Y;

-X(244)- is selected from the group consisting of P and H;

-X(245)- is selected from the group consisting of P and A;

-X(246)- is selected from the group consisting of K, D, E, H and Y;

-X(247)- is selected from the group consisting of P, G and V;

-X(249)- is selected from the group consisting of D, H, Q and Y;

-X(255)- is selected from the group consisting of R, E and Y;

-X(258)- is selected from the group consisting of E, H, S and Y;

-X(260)- is selected from the group consisting of T, D, E, H and Y;

-X(262)- is selected from the group consisting of V, A, E, F, I and T;

-X(263)- is selected from the group consisting of V, A, I, M and T;

-X(264)- is selected from the group consisting of V, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W and Y;

-X(265)- is selected from the group consisting of D, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(266)- is selected from the group consisting of V, A, I, M and T;

-X(267)- is selected from the group consisting of S, D, E, F, H, I, K, L, M, N, P, Q, R, V, W and Y;

-X(268)- is selected from the group consisting of H, D, E, F, G, I, K, L, M, P, R, T, V and W;

-X(269)- is selected from the group consisting of E, F, G, H, I, K, L, M, N, P, R, S, T, V, W and Y;

-X(270)- is selected from the group consisting of D, F, G, H, I, L, M, P, Q, R, S, T, W and Y;

-X(271)- is selected from the group consisting of P, A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(272)- is selected from the group consisting of E, D, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;

-X(273)- is selected from the group consisting of V and I;

-X(274)- is selected from the group consisting of Q, D, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(275)- is selected from the group consisting of F, L and W;

-X(276)- is selected from the group consisting of N, D, E, F, G, H, I, L, M, P, R, S, T, V, W and Y;

-X(278)- is selected from the group consisting of Y, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V and W;
-X(280)- is selected from the group consisting of D, G, K, L, P and W;
-X(281)- is selected from the group consisting of G, D, E, K, N, P, Q and Y;
-X(282)- is selected from the group consisting of V, E, G, K, P and Y;
-X(283)- is selected from the group consisting of E, G, H, K, L, P, R and Y;
-X(284)- is selected from the group consisting of V, D, E, L, N, Q, T and Y;
-X(285)- is selected from the group consisting of H, D, E, K, Q, W and Y;
-X(286)- is selected from the group consisting of N, E, G, P and Y;
-X(288)- is selected from the group consisting of K, D, E and Y;
-X(290)- is selected from the group consisting of K, D, H, L, N and W;
-X(291)- is selected from the group consisting of P, D, E, G, H, I, Q and T;
-X(292)- is selected from the group consisting of R, D, E, T and Y;
-X(293)- is selected from the group consisting of E, F, G, H, I, L, M, N, P, R, S, T, V, W and Y;
-X(294)- is selected from the group consisting of E, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;
-X(295)- is selected from the group consisting of Q, D, E, F, G, H, I, M, N, P, R, S, T, V, W and Y;
-X(296)- is selected from the group consisting of F, A, D, E, G, I, K, L, M, N, Q, R, S, T and V;
-X(297)- is selected from the group consisting of N, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;
-X(298)- is selected from the group consisting of S, E, F, H, I, K, M, Q, R, W and Y;
-X(299)- is selected from the group consisting of T, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W and Y;
-X(300)- is selected from the group consisting of F, A, D, E, G, H, K, M, N, P, Q, R, S, T, V and W;
-X(301)- is selected from the group consisting of R, D, E, H and Y;
-X(302)- is selected from the group consisting of V and I;
-X(303)- is selected from the group consisting of V, D, E and Y;
-X(304)- is selected from the group consisting of S, D, H, L, N and T;
-X(305)- is selected from the group consisting of V, E, T and Y;
-X(313)- is selected from the group consisting of W and F;
-X(317)- is selected from the group consisting of K, E and Q;
-X(318)- is selected from the group consisting of E, H, L, Q, R and Y;
-X(320)- is selected from the group consisting of K, D, F, G, H, I, L, N, P, S, T, V, W and Y;
-X(322)- is selected from the group consisting of K, D, F, G, H, I, P, S, T, V, W and Y;
-X(323)- is selected from the group consisting of V and I;
-X(324)- is selected from the group consisting of S, D, F, G, H, I, L, M, P, R, T, V, W and Y;
-X(325)- is selected from the group consisting of N, A, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;
-X(326)- is selected from the group consisting of K, I, L, P and T;
-X(327)- is selected from the group consisting of G, D, E, F, H, I, K, L, M, N, P, R, T, V, W and Y;
-X(328)- is selected from the group consisting of L, A, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;

-X(329)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(330)- is selected from the group consisting of A, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(331)- is selected from the group consisting of P, D, F, H, I, L, M, Q, R, T, V, W and Y;

-X(332)- is selected from the group consisting of I, A, D, E, F, H, K, L, M, N, P, Q, R, S, T, , V, W and Y;

-X(333)- is selected from the group consisting of E, F, H, I, L, M, P, T and Y;

-X(334)- is selected from the group consisting of K, F, I, P and T;

-X(335)- is selected from the group consisting of T, D, F, G, H, I, L, M, N, P, R, S, V, W and Y;

-X(336)- is selected from the group consisting of I, E, K and Y; and

-X(337)- is selected from the group consisting of S, E, H and N.

[38] In certain variations, the variant differs from SEQ ID NO:12 by at least one amino acid.

[39] In a further aspect, the present application is directed to an IgG2 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC-X(221)-V-E-C-X(227)-PCPAPP-X(234)-
X(235)-X(236)-X(237)-P-X(239)-X(240)-FLFPP-X(246)-PKDTLMIS-X(255)-TP-X(258)-V-X(260)-CVV-
X(264)-DV-X(267)-X(268)-ED-X(271)-X(272)-V-X(274)-FNW-X(278)-VD-X(281)-V-X(283)-X(284)-
HNAKT-X(290)-PR-X(293)-E-X(295)-FNSTFRVV-X(304)-VLTVVHQDWLNGKEYKCKV-X(324)-N-
X(326)-X(327)-X(328)-P-X(330)-P-X(332)-X(333)-X(334)-
TISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK, wherein

-X(221)- is selected from the group consisting of no amino acid and K;

-X(227)- is selected from the group consisting of P and G;

-X(234)- is selected from the group consisting of V, Y and I;

-X(235)- is selected from the group consisting of A, Y, I and D;

-X(236)- is selected from the group consisting of no amino acid, S and A;

-X(237)- is selected from the group consisting of G and D;

-X(239)- is selected from the group consisting of S, D, E, N, Q and T;

-X(240)- is selected from the group consisting of V, I and M;

-X(246)- is selected from the group consisting of K, H and Y;

-X(255)- is selected from the group consisting of R and Y;

-X(258)- is selected from the group consisting of E, H and Y;

-X(260)- is selected from the group consisting of T and H;

-X(264)- is selected from the group consisting of V, I, T and Y;

-X(267)- is selected from the group consisting of S, D and E;

-X(268)- is selected from the group consisting of H, D and E;

-X(271)- is selected from the group consisting of P and G;

-X(272)- is selected from the group consisting of E, Y, H, R and I;
-X(274)- is selected from the group consisting of Q and E;
-X(278)- is selected from the group consisting of Y and T;
-X(281)- is selected from the group consisting of G, D and E;
-X(283)- is selected from the group consisting of E, L and H;
-X(284)- is selected from the group consisting of V, E and D;
-X(290)- is selected from the group consisting of K and N;
-X(293)- is selected from the group consisting of E and R;
-X(295)- is selected from the group consisting of Q and E;
-X(304)- is selected from the group consisting of S and T;
-X(324)- is selected from the group consisting of S, G and I;
-X(326)- is selected from the group consisting of K and T;
-X(327)- is selected from the group consisting of G and D;
-X(328)- is selected from the group consisting of L, A, F, I and T;
-X(330)- is selected from the group consisting of A, L, Y and I;
-X(332)- is selected from the group consisting of I, D, E, N, Q and T;
-X(333)- is selected from the group consisting of E and Y; and
-X(334)- is selected from the group consisting of K, F, I and T;

[40] In certain aspects, the variant differs from SEQ ID. NO:2 by at least one amino acid.

[41] In another aspect, the present application is directed to an IgG3 variant including two or more amino acid modifications as compared to SEQ ID NO:13. The modifications are selected from among C131S, R133K, G137E, G138S, S192N, L193F, Q196K, T199I, N203D, R214K R214T, L217P, L217R, L217S, T219S, T219C, T219Y, P220C P220G, L221D, L221-, deletion of the sequence LGD beginning at L221, T222K, T222V, deletion of T222, deletion of T223, H224E, H224P, deletion of T225, T225P, R228P, R228S, deletion of RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR beginning at 228, E233P, L234V, L234F, L235A, deletion of G236, H268Q, Q274K, K276N, Y296F, F300Y, L309V, A327G, A330S, P331S, T339A, R355Q, E356D, M358L, S384N, N392K, M397V, K409R, Q419E, I422V, R435H, F436Y, and P445L. In certain embodiments, at least two of the amino acid modifications are in different domains. In various embodiments, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:13. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains.

[42] In another embodiment, the an IgG3 variant includes an amino acid sequence having the formula:

ASTKGPSVFPLAP-X(131)-S-X(133)-
STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY-
X(199)-CNVNHKPSNTKVDK-X(214)-VE-X(217)-K-X(219)-X(220)-X(221)-GD-X(222)-THTCP-X(228)-
CPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEV-X(274)-F-X(276)-WYVDGVEVHNAKTKPREEQYNST-X(300)-

RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK-X(339)-KGQPREPQVYTLPPSR-X(356)-E-X(358)-TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYSKLTVDKSRWQQGN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLSPGK
wherein
X(131) is selected from the group consisting of C and S;
X(133) is selected from the group consisting of R and K;
X(199) is selected from the group consisting of T and I;
X(214) is selected from the group consisting of R and K;
X(217) is selected from the group consisting of L and P;
X(219) is selected from the group consisting of T and S;
X(220) is selected from the group consisting of P and C;
X(221) is selected from the group consisting of D L, and the sequence LGD;
X(222) is selected from the group consisting of T and K;
X(228) is selected from the group consisting of R, the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR and P;
X(274) is selected from the group consisting of Q and K;
X(276) is selected from the group consisting of K and N;
X(300) is selected from the group consisting of F and Y;
X(339) is selected from the group consisting of T and A;
X(356) is selected from the group consisting of E and D;
X(358) is selected from the group consisting of M and L;
X(384) is selected from the group consisting of S and N;
X(392) is selected from the group consisting of N and K;
X(397) is selected from the group consisting of M and V;
X(422) is selected from the group consisting of I and V;
X(435) is selected from the group consisting of R and H; and
X(436) is selected from the group consisting of F and Y.

[43] In certain variations, the formula has at least two amino acid modifications as compared to SEQ ID NO:13. In further variations, the two of modifications can in different domains. In various embodiments, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:13. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains.

[44] In another aspect, the present application is directed to an IgG3 variant including two or more amino acid modifications as compared to SEQ ID NO:13. The modifications can be selected from among C131S, R133K, G137E, G138S, S192N, L193F, Q196K, T199I, N203D, R214K R214T, L217P, L217R, L217S, T219S, T219C, T219Y, P220C P220G, L221D, the deletion of L221, deletion of GD, T222K, T222V, the deletion of T222, the deletion of T223, H224E, H224P, the deletion of T225, T225P, R228P, R228S, deletion of RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR beginning at R228, E233P,

L234V, L234F, L235A, G236-, H268Q, Q274K, K276N, Y296F, F300Y, L309V, A327G, A330S, P331S, T339A, R355Q, E356D, M358L, S384N, N392K, M397V, K409R, Q419E, I422V, R435H, F436Y, and P445L. In certain embodiments, at least two of the amino acid modifications are in different domains. In various embodiments, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:12. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains.

[45] In another aspect, the present application is directed to an IgG3 variant including an amino acid sequence having the formula:

-ASTKGPSVFPLAP-X(131)-S-X(133)-STS-X(137)-X(138)-
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS-X(192)-X(193)-GT-
X(196)-TY-X(199)-CNV-X(203)-HKPSNTKVDK-X(214)-VE-X(217)-K-X(219)-X(220)-X(221)-X(222)-
X(223)-X(224)-X(225)-C-X(227)-X(228)-C-X(230)-X(231)-X(232)-X(233)-X(234)-X(235)-X(236)-
X(237)-X(238)-X(239)-X(240)-X(241)-L-X(243)-X(244)-X(245)-X(246)-X(247)-K-X(249)-TLMIS-
X(255)-TP-X(258)-V-X(260)-C-X(262)-X(263)-X(264)-X(265)-X(266)-X(267)-X(268)-X(269)-X(270)-
X(271)-X(272)-X(273)-X(274)-X(275)-X(276)-W-X(278)-V-X(280)-X(281)-X(282)-X(283)-X(284)-
X(285)-X(286)-A-X(288)-T-X(290)-X(291)-X(292)-X(293)-X(294)-X(295)-X(296)-X(297)-X(298)-
X(299)-X(300)-X(301)-X(302)-X(303)-X(304)-X(305)-LTV-X(309)-HQD-X(313)-LNG-X(317)-X(318)-Y-
X(320)-C-X(322)-X(323)-X(324)-X(325)-X(326)-X(327)-X(328)-X(329)-X(330)-X(331)-X(332)-X(333)-
X(334)-X(335)-X(336)-X(337)-K-X(339)-KGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-
TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYS-
X(409)-LTVDKSRWQ-X(419)-GN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLS-X(445)-GK
wherein

-X(131)- is selected from the group consisting of C and S;
-X(133)- is selected from the group consisting of R and K;
-X(137)- is selected from the group consisting of E and G;
-X(138)- is selected from the group consisting of S and G;
-X(192)- is selected from the group consisting of N and S;
-X(193)- is selected from the group consisting of F and L;
-X(196)- is selected from the group consisting of Q and K;
-X(199)- is selected from the group consisting of T and I;
-X(203)- is selected from the group consisting of D and N;
-X(214)- is selected from the group consisting of T, K and R;
-X(217)- is selected from the group consisting of R, P, L and S;
-X(219)- is selected from the group consisting of C, S, T and Y;
-X(220)- is selected from the group consisting of C, P and G;
-X(221)- is selected from the group consisting of no amino acid, D, K, Y, L, and the sequence LGD;
-X(222)- is selected from the group consisting of V, K, T, no amino acid , E and Y;
-X(223)- is selected from the group consisting of no amino acid, T, E and K;

-X(224)- is selected from the group consisting of E, H, P and Y;

-X(225)- is selected from the group consisting of no amino acid, T, P, E, K and W;

-X(227)- is selected from the group consisting of P, E, G, K and Y;

-X(228)- is selected from the group consisting of P, S, E, G, K, Y, R, and the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR;

-X(230)- is selected from the group consisting of P, A, E, G and Y;

-X(231)- is selected from the group consisting of A, E, G, K, P and Y;

-X(232)- is selected from the group consisting of P, E, G, K and Y;

-X(233)- is selected from the group consisting of P, E, A, D, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(234)- is selected from the group consisting of V, L, F, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, W and Y;

-X(235)- is selected from the group consisting of A, L, D, F, G, H, I, K, M, N, P, Q, R, S, T, V, W , and Y;

-X(236)- is selected from the group consisting of no amino acid, G, A, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(237)- is selected from the group consisting of G, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(238)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(239)- is selected from the group consisting of S, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W and Y;

-X(240)- is selected from the group consisting of V, A, I, M and T;

-X(241)- is selected from the group consisting of F, D, E, L, R, S, W and Y;

-X(243)- is selected from the group consisting of F, E, H, L, Q, RW, , and Y;

-X(244)- is selected from the group consisting of P and H;

-X(245)- is selected from the group consisting of P and A;

-X(246)- is selected from the group consisting of , K, D, E, H and Y;

-X(247)- is selected from the group consisting of P, G and V;

-X(249)- is selected from the group consisting of D, H, Q and Y;

-X(255)- is selected from the group consisting of RE and Y;

-X(258)- is selected from the group consisting of E, H, S and Y;

-X(260)- is selected from the group consisting of T, D, E, H and Y;

-X(262)- is selected from the group consisting of V, A, E, F, I and T;

-X(263)- is selected from the group consisting of V, A, I, M and T;

-X(264)- is selected from the group consisting of V, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, and Y;

-X(265)- is selected from the group consisting of D, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(266)- is selected from the group consisting of V, A, I, M and T;

-X(267)- is selected from the group consisting of S, D, E, F, H, I, K, L, M, N, P, Q, R, V, W and Y;

-X(268)- is selected from the group consisting of H, Q, D, E, F, G, I, K, L, M, P, R, T, V and W;

-X(269)- is selected from the group consisting of E, F, G, H, I, K, L, M, N, P, R, S, T, V, W and Y;

-X(270)- is selected from the group consisting of D, F, G, H, I, L, M, P, Q, R, S, T, W and Y;

-X(271)- is selected from the group consisting of P, A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(272)- is selected from the group consisting of E, D, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;

-X(273)- is selected from the group consisting of V and I;

-X(274)- is selected from the group consisting of Q, K, D, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(275)- is selected from the group consisting of FL and W;

-X(276)- is selected from the group consisting of N, K, D, E, F, G, H, I, L, M, P, R, S, T, V, W and Y;

-X(278)- is selected from the group consisting of Y, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V and W;

-X(280)- is selected from the group consisting of D, G, K, L, P and W;

-X(281)- is selected from the group consisting of G, D, E, K, N, P, Q and Y;

-X(282)- is selected from the group consisting of V, E, G, K, P and Y;

-X(283)- is selected from the group consisting of E, G, H, K, L, P, R and Y;

-X(284)- is selected from the group consisting of V, D, E, L, N, Q, T and Y;

-X(285)- is selected from the group consisting of H, D, E, K, Q, W and Y;

-X(286)- is selected from the group consisting of N, E, G, P and Y;

-X(288)- is selected from the group consisting of K, D, E and Y;

-X(290)- is selected from the group consisting of K, D, H, L, N and W;

-X(291)- is selected from the group consisting of P, D, E, G, H, I, Q and T;

-X(292)- is selected from the group consisting of R, D, E, T and Y;

-X(293)- is selected from the group consisting of E, F, G, H, I, L, M, N, P, R, S, T, V, W and Y;

-X(294)- is selected from the group consisting of E, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;

-X(295)- is selected from the group consisting of Q, D, E, F, G, H, I, M, N, P, R, S, T, V, W and Y;

-X(296)- is selected from the group consisting of F, Y, A, D, E, G, I, K, L, M, N, Q, R, S, T and V;

-X(297)- is selected from the group consisting of N, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(298)- is selected from the group consisting of S, E, F, H, I, K, M, Q, R, W and Y;

-X(299)- is selected from the group consisting of T, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W and Y;

-X(300)- is selected from the group consisting of F, Y, A, D, E, G, H, K, M, N, P, Q, R, S, T, V and W;

-X(301)- is selected from the group consisting of R, D, E, H and Y;

-X(302)- is selected from the group consisting of V and I;

-X(303)- is selected from the group consisting of V, D, E and Y;

-X(304)- is selected from the group consisting of S, D, H, L, N and T;

-X(305)- is selected from the group consisting of V, E, T and Y;

-X(309)- is selected from the group consisting of V and L;

-X(313)- is selected from the group consisting of W and F;

-X(317)- is selected from the group consisting of K, E and Q;

-X(318)- is selected from the group consisting of E, H, L, Q, R and Y;

-X(320)- is selected from the group consisting of K, D, F, G, H, I, L, N, P, S, T, V, W and Y;

-X(322)- is selected from the group consisting of K, D, F, G, H, I, P, S, T, V, W and Y;

-X(323)- is selected from the group consisting of V and I;

-X(324)- is selected from the group consisting of S, D, F, G, H, I, L, M, P, R, T, V, W and Y;

-X(325)- is selected from the group consisting of N, A, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(326)- is selected from the group consisting of K, I, L, P and T;

-X(327)- is selected from the group consisting of G, A, D, E, F, H, I, K, L, M, N, P, R, T, V, W and Y;

-X(328)- is selected from the group consisting of L, A, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;

-X(329)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(330)- is selected from the group consisting of A, S, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(331)- is selected from the group consisting of P, S, D, F, H, I, L, M, Q, R, T, V, W and Y;

-X(332)- is selected from the group consisting of I, A, D, E, F, H, , K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(333)- is selected from the group consisting of E, F, H, I, L, M, P, T and Y;

-X(334)- is selected from the group consisting of K, F, I, P and T;

-X(335)- is selected from the group consisting of T, D, F, G, H, I, L, M, N, P, R, S, V, W and Y;

-X(336)- is selected from the group consisting of I, E, K and Y;

-X(337)- is selected from the group consisting of S, E, H and N;

-X(339)- is selected from the group consisting of T and A;

-X(355)- is selected from the group consisting of R and Q;

-X(356)- is selected from the group consisting of E and D;

-X(358)- is selected from the group consisting of M and L;

-X(384)- is selected from the group consisting of N and S;

-X(392)- is selected from the group consisting of K and N;

-X(397)- is selected from the group consisting of M and V;

-X(409)- is selected from the group consisting of K and R;

-X(419)- is selected from the group consisting of Q and E;

-X(422)- is selected from the group consisting of V and I;

-X(435)- is selected from the group consisting of H and R;

-X(436)- is selected from the group consisting of Y and F; and

-X(445)- is selected from the group consisting of P and L.

[46] In one variation, a first modification can be selected from among C131S, R133K, G137E, G138S, S192N, L193F, Q196K, T199I, N203D, R214K R214T, L217P, L217R, L217S, T219S, T219C, T219Y, P220C P220G, L221D, deletion of L221, deletion of the sequence LGD beginning at L221, T222K, T222V, deletion of T222, deletion of T223, H224E, H224P, deletion of T225, T225P, R228P, R228S, deletion of the sequence

RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR beginning at 228, E233P, L234V, L234F, L235A, deletion of G236, H268Q, Q274K, K276N, Y296F, F300Y, L309V, A327G, A330S, P331S, T339A, R355Q, E356D, M358L, S384N, N392K, M397V, K409R, Q419E, I422V, R435H, F436Y, and P445L. In a further variation, a second modification is selected from among 221K, 221Y, 222E, 222Y, 223E, 223K, 224Y, 225E, 225K, 225W, 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234W, 234Y, 235D, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M,

300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334I, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, 337N. In various embodiments, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:13. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains.

[47] In a further aspect, the present application is directed to an IgG3 variant amino acid sequence having at least two amino acid modifications as compared to SEQ ID NO:13, wherein a first modification is selected from among C131S, R133K, G137E, G138S, S192N, L193F, Q196K, T199I, N203D, R214K R214T, L217P, L217R, L217S, T219S, T219C, T219Y, P220C P220G, L221D, deletion of L221, deletion of the sequence LGD beginning at L221, T222K, T222V, deletion of T222, deletion of T223, H224E, H224P, deletion of T225, T225P, R228P, R228S, deletion of the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR beginning at 228, E233P, L234V, L234F, L235A, deletion of G236, H268Q, Q274K, K276N, Y296F, F300Y, L309V, A327G, A330S, P331S, T339A, R355Q, E356D, M358L, S384N, N392K, M397V, K409R, Q419E, I422V, R435H, F436Y, and P445L, and and a second modification is selected from among 221K, 221Y, 222E, 222Y, 223E, 223K, 224Y, 225E, 225K, 225W, 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234W, 234Y, 235D, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S,

258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334I, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N. In various embodiments, the formula has at least 1, 2, 3, 4, 5, 6,

7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:13. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains.

[48] In another aspect, the present application is directed to an IgG3 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAP-X(131)-S-X(133)-STS-X(137)-X(138)-
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS-X(192)-X(193)-GT-X(196)-TY-X(199)-CNV-X(203)-HKPSNTKVDK-X(214)-VE-X(217)-K-X(219)-X(220)-X(221)-X(222)-X(223)-X(224)-X(225)-C-X(227)-X(228)-CPAP-X(233)-X(234)-X(235)-X(236)-X(237)-P-X(239)-X(240)-FLFPP-X(246)-PKDTLMIS-X(255)-TP-X(258)-V-X(260)-CVV-X(264)-DV-X(267)-X(268)-ED-X(271)-X(272)-V-X(274)-F-X(276)-W-X(278)-VD-X(281)-V-X(283)-X(284)-HNAKT-X(290)-PR-X(293)-E-X(295)-X(296)-NST-X(300)-RVV-X(304)-VLTV-X(309)-HQDWLNGKEYKCKV-X(324)-N-X(326)-X(327)-X(328)-P-X(330)-X(331)-X(332)-X(333)-X(334)-TISK-X(339)-KGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYS-X(409)-LTVDKSRWQ-X(419)-GN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLS-X(445)-GK; wherein

-X(131)- is selected from the group consisting of C and S;
-X(133)- is selected from the group consisting of R and K;
-X(137)- is selected from the group consisting of E and G;
-X(138)- is selected from the group consisting of S and G;
-X(192)- is selected from the group consisting of N and S;
-X(193)- is selected from the group consisting of F and L;
-X(196)- is selected from the group consisting of Q and K;
-X(199)- is selected from the group consisting of T and I;
-X(203)- is selected from the group consisting of D and N;
-X(214)- is selected from the group consisting of T, K and R;
-X(217)- is selected from the group consisting of R, P, L and S;
-X(219)- is selected from the group consisting of C, S, T and Y;
-X(220)- is selected from the group consisting of C, P and G;
-X(221)- is selected from the group consisting of no amino acid, D, L, K, and the sequence LGD;
-X(222)- is selected from the group consisting of V, K, T, and no amino acid;
-X(223)- is selected from the group consisting of no amino acid and T;
-X(224)- is selected from the group consisting of E, H and P;
-X(225)- is selected from the group consisting of no amino acid, T and P;
-X(227)- is selected from the group consisting of P and G;
-X(228)- is selected from the group consisting of P, R, S, and the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR;
-X(233)- is selected from the group consisting of P and E;
-X(234)- is selected from the group consisting of V, , LF, Y and I;
-X(235)- is selected from the group consisting of A, L, Y, I and D;

-X(236)- is selected from the group consisting of no amino acid, G, S and A;
-X(237)- is selected from the group consisting of G and D;
-X(239)- is selected from the group consisting of S, D, E, N, Q and T;
-X(240)- is selected from the group consisting of V, I and M;
-X(246)- is selected from the group consisting of K, H and Y;
-X(255)- is selected from the group consisting of R and Y;
-X(258)- is selected from the group consisting of E, H and Y;
-X(260)- is selected from the group consisting of T and H;
-X(264)- is selected from the group consisting of V, I, T and Y;
-X(267)- is selected from the group consisting of S, D and E;
-X(268)- is selected from the group consisting of H, Q, D and E;
-X(271)- is selected from the group consisting of P and G;
-X(272)- is selected from the group consisting of E, Y, H, R and I;
-X(274)- is selected from the group consisting of Q, K and E;
-X(276)- is selected from the group consisting of N and K;
-X(278)- is selected from the group consisting of Y and T;
-X(281)- is selected from the group consisting of G, D and E;
-X(283)- is selected from the group consisting of E, L and H;
-X(284)- is selected from the group consisting of V, E and D;
-X(290)- is selected from the group consisting of K and N;
-X(293)- is selected from the group consisting of E and R;
-X(295)- is selected from the group consisting of Q and E;
-X(296)- is selected from the group consisting of F and Y;
-X(300)- is selected from the group consisting of F and Y;
-X(304)- is selected from the group consisting of S and T;
-X(309)- is selected from the group consisting of V and L;
-X(324)- is selected from the group consisting of S, G and I;
-X(326)- is selected from the group consisting of K and T;
-X(327)- is selected from the group consisting of G, A and D;
-X(328)- is selected from the group consisting of L, A, F, I and T;
-X(330)- is selected from the group consisting of A, S, L, Y and I;
-X(331)- is selected from the group consisting of P and S;
-X(332)- is selected from the group consisting of I, D, E, N, Q and T;
-X(333)- is selected from the group consisting of E and Y;
-X(334)- is selected from the group consisting of K, F, I and T;
-X(339)- is selected from the group consisting of T and A;
-X(355)- is selected from the group consisting of R and Q;
-X(356)- is selected from the group consisting of E and D;
-X(358)- is selected from the group consisting of M and L;
-X(384)- is selected from the group consisting of N and S;

-X(392)- is selected from the group consisting of K and N;
-X(397)- is selected from the group consisting of M and V;
-X(409)- is selected from the group consisting of K and R;
-X(419)- is selected from the group consisting of Q and E;
-X(422)- is selected from the group consisting of V and I;
-X(435)- is selected from the group consisting of H and R;
-X(436)- is selected from the group consisting of Y and F;
-X(445)- is selected from the group consisting of P and L.

[49] In certain variations, a first modification is selected from among C131S, R133K, G137E, G138S, S192N, L193F, Q196K, T199I, N203D, R214K R214T, L217P, L217R, L217S, T219S, T219C, T219Y, P220C P220G, L221D, deletion of L221, deletion of the sequence LGD beginning at L221, T222K, T222V, deletion of T222, deletion of T223, H224E, H224P, deletion of T225, T225P, R228P, R228S, deletion of R, deletion of the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR beginning at 228, E233P, L234V, L234F, L235A, deletion of G236, H268Q, Q274K, K276N, Y296F, F300Y, L309V, A327G, A330S, P331S, T339A, R355Q, E356D, M358L, S384N, N392K, M397V, K409R, Q419E, I422V, R435H, F436Y, and P445L. In a further variation, a second modification is selected from among 221K, 227G, 234Y, 234I, 235Y, 235I, 235D, 236S, 236A, 237D, 239D, 239E, 239N, 239Q, 239T, 240I, 240M, 246H, 246Y, 255Y, 258H, 258Y, 260H, 264I, 264T, 264Y, 267D, 267E, 268D, 268E, 271G, 272Y, 272H, 272R, 272I, 274E, 278T, 281D, 281E, 283L, 283H, 284E, 284D, 290N, 293R, 295E, 304T, 324G, 324I, 326T, 327D, 328A, 328F, 328I, 328T, 330L, 330Y, 330I, 332D, 332E, 332N, 332Q, 332T, 333Y, 334F, 334I, and 334T. In various embodiments, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more first and/or second amino acid modifications as compared to an amino acid sequence including SEQ ID NO:13. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains.

[50] In another aspect, the present application is directed to an IgG3 variant including an amino acid sequence having the formula:

C-X(227)-X(228)-C-X(230)-X(231)-X(232)-X(233)-X(234)-X(235)-X(236)-X(237)-X(238)-X(239)-X(240)-X(241)-L-X(243)-X(244)-X(245)-X(246)-X(247)-K-X(249)-TLMIS-X(255)-TP-X(258)-V-X(260)-C-X(262)-X(263)-X(264)-X(265)-X(266)-X(267)-X(268)-X(269)-X(270)-X(271)-X(272)-X(273)-X(274)-X(275)-X(276)-W-X(278)-V-X(280)-X(281)-X(282)-X(283)-X(284)-X(285)-X(286)-A-X(288)-T-X(290)-X(291)-X(292)-X(293)-X(294)-X(295)-X(296)-X(297)-X(298)-X(299)-X(300)-X(301)-X(302)-X(303)-X(304)-X(305)-LTV-X(309)-HQD-X(313)-LNG-X(317)-X(318)-Y-X(320)-C-X(322)-X(323)-X(324)-X(325)-X(326)-X(327)-X(328)-X(329)-X(330)-X(331)-X(332)-X(333)-X(334)-X(335)-X(336)-X(337)-K-X(339)-KGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYS-X(409)-LTVDKSRWQ-X(419)-GN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLS-X(445)-GK

wherein

-X(227)- is selected from the group consisting of P, E, G, K and Y;

-X(228)- is selected from the group consisting of P, S, E, G, K, Y, R, and the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR;

-X(230)- is selected from the group consisting of P, A, E, G and Y;

-X(231)- is selected from the group consisting of A, E, G, K, P and Y;

-X(232)- is selected from the group consisting of P, E, G, K and Y;

-X(233)- is selected from the group consisting of P, E, A, D, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(234)- is selected from the group consisting of V, L, F, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, W and Y;

-X(235)- is selected from the group consisting of A, L, D, F, G, H, I, K, M, N, P, Q, R, S, T, V, W , and Y;

-X(236)- is selected from the group consisting of no amino acid, G, A, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(237)- is selected from the group consisting of G, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(238)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(239)- is selected from the group consisting of S, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W and Y;

-X(240)- is selected from the group consisting of V, A, I, M and T;

-X(241)- is selected from the group consisting of F, D, E, L, R, S, W and Y;

-X(243)- is selected from the group consisting of F, E, H, L, Q, RW, , and Y;

-X(244)- is selected from the group consisting of P and H;

-X(245)- is selected from the group consisting of P and A;

-X(246)- is selected from the group consisting of , K, D, E, H and Y;

-X(247)- is selected from the group consisting of P, G and V;

-X(249)- is selected from the group consisting of D, H, Q and Y;

-X(255)- is selected from the group consisting of RE and Y;

-X(258)- is selected from the group consisting of E, H, S and Y;

-X(260)- is selected from the group consisting of T, D, E, H and Y;

-X(262)- is selected from the group consisting of V, A, E, F, I and T;

-X(263)- is selected from the group consisting of V, A, I, M and T;

-X(264)- is selected from the group consisting of V, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, and Y;

-X(265)- is selected from the group consisting of D, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(266)- is selected from the group consisting of V, A, I, M and T;

-X(267)- is selected from the group consisting of S, D, E, F, H, I, K, L, M, N, P, Q, R, V, W and Y;

-X(268)- is selected from the group consisting of H, Q, D, E, F, G, I, K, L, M, P, R, T, V and W;

-X(269)- is selected from the group consisting of E, F, G, H, I, K, L, M, N, P, R, S, T, V, W and Y;

-X(270)- is selected from the group consisting of D, F, G, H, I, L, M, P, Q, R, S, T, W and Y;

-X(271)- is selected from the group consisting of P, A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(272)- is selected from the group consisting of E, D, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;

-X(273)- is selected from the group consisting of V and I;

-X(274)- is selected from the group consisting of Q, K, D, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(275)- is selected from the group consisting of FL and W;

-X(276)- is selected from the group consisting of N, K, D, E, F, G, H, I, L, M, P, R, S, T, V, W and Y;

-X(278)- is selected from the group consisting of Y, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V and W;

-X(280)- is selected from the group consisting of D, G, K, L, P and W;

-X(281)- is selected from the group consisting of G, D, E, K, N, P, Q and Y;

-X(282)- is selected from the group consisting of V, E, G, K, P and Y;

-X(283)- is selected from the group consisting of E, G, H, K, L, P, R and Y;

-X(284)- is selected from the group consisting of V, D, E, L, N, Q, T and Y;

-X(285)- is selected from the group consisting of H, D, E, K, Q, W and Y;

-X(286)- is selected from the group consisting of N, E, G, P and Y;

-X(288)- is selected from the group consisting of K, D, E and Y;

-X(290)- is selected from the group consisting of K, D, H, L, N and W;

-X(291)- is selected from the group consisting of P, D, E, G, H, I, Q and T;

-X(292)- is selected from the group consisting of R, D, E, T and Y;

-X(293)- is selected from the group consisting of E, F, G, H, I, L, M, N, P, R, S, T, V, W and Y;

-X(294)- is selected from the group consisting of E, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;

-X(295)- is selected from the group consisting of Q, D, E, F, G, H, I, M, N, P, R, S, T, V, W and Y;

-X(296)- is selected from the group consisting of F, Y, A, D, E, G, I, K, L, M, N, Q, R, S, T and V;

-X(297)- is selected from the group consisting of N, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(298)- is selected from the group consisting of S, E, F, H, I, K, M, Q, R, W and Y;

-X(299)- is selected from the group consisting of T, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W and Y;

-X(300)- is selected from the group consisting of F, Y, A, D, E, G, H, K, M, N, P, Q, R, S, T, V and W;

-X(301)- is selected from the group consisting of R, D, E, H and Y;

-X(302)- is selected from the group consisting of V and I;

-X(303)- is selected from the group consisting of V, D, E and Y;

-X(304)- is selected from the group consisting of S, D, H, L, N and T;

-X(305)- is selected from the group consisting of V, E, T and Y;

-X(309)- is selected from the group consisting of V and L;

-X(313)- is selected from the group consisting of W and F;

-X(317)- is selected from the group consisting of K, E and Q;

-X(318)- is selected from the group consisting of E, H, L, Q, R and Y;

-X(320)- is selected from the group consisting of K, D, F, G, H, I, L, N, P, S, T, V, W and Y;

-X(322)- is selected from the group consisting of K, D, F, G, H, I, P, S, T, V, W and Y;

-X(323)- is selected from the group consisting of V and I;

-X(324)- is selected from the group consisting of S, D, F, G, H, I, L, M, P, R, T, V, W and Y;

-X(325)- is selected from the group consisting of N, A, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(326)- is selected from the group consisting of K, I, L, P and T;

-X(327)- is selected from the group consisting of G, A, D, E, F, H, I, K, L, M, N, P, R, T, V, W and Y;

-X(328)- is selected from the group consisting of L, A, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;

-X(329)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(330)- is selected from the group consisting of A, S, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(331)- is selected from the group consisting of P, S, D, F, H, I, L, M, Q, R, T, V, W and Y;

-X(332)- is selected from the group consisting of I, A, D, E, F, H, , K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(333)- is selected from the group consisting of E, F, H, I, L, M, P, T and Y;

-X(334)- is selected from the group consisting of K, F, I, P and T;

-X(335)- is selected from the group consisting of T, D, F, G, H, I, L, M, N, P, R, S, V, W and Y;

-X(336)- is selected from the group consisting of I, E, K and Y;

-X(337)- is selected from the group consisting of S, E, H and N;

-X(339)- is selected from the group consisting of T and A;

-X(355)- is selected from the group consisting of R and Q;

-X(356)- is selected from the group consisting of E and D;

-X(358)- is selected from the group consisting of M and L;

-X(384)- is selected from the group consisting of N and S;

-X(392)- is selected from the group consisting of K and N;

-X(397)- is selected from the group consisting of M and V;

-X(409)- is selected from the group consisting of K and R;

-X(419)- is selected from the group consisting of Q and E;

-X(422)- is selected from the group consisting of V and I;

-X(435)- is selected from the group consisting of H and R;

-X(436)- is selected from the group consisting of Y and F; and

-X(445)- is selected from the group consisting of P and L

[51] In various embodiments, a first modification is selected from among R228P, R228S, deletion of the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR beginning at 228, E233P, L234V, L234F, L235A, deletion of G236, H268Q, Q274K, K276N, Y296F, F300Y, L309V, A327G, A330S, P331S, T339A, R355Q, E356D, M358L, S384N, N392K, M397V, K409R, Q419E, I422V, R435H, F436Y, and P445L, and/or a second modification is selected from among 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R,

239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334I, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P,

335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N. In various embodiments, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:13. In additional embodiments, at least 2, 3, or 4 of the first and/or second modifications are in different domains. Alternatively, the substitutions can be selected from those beginning at position 230.

[52] In another aspect, the present application is directed to an IgG3 variant amino acid sequence including at least two modifications as compared to SEQ ID NO:13, wherein a first modification is selected from among R228P, R228S, deletion of the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR beginning at 228, E233P, L234V, L234F, L235A, deletion of G236, H268Q, Q274K, K276N, Y296F, F300Y, L309V, A327G, A330S, P331S, T339A, R355Q, E356D, M358L, S384N, N392K, M397V, K409R, Q419E, I422V, R435H, F436Y, and P445L. In a further variation, a second modification is selected from among 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234W, 234Y, 235D, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y,

293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334I, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N. In various embodiments, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:13. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains.

[53] In another aspect, the present application is directed to an IgG3 variant including an amino acid sequence having the formula

C-X(227)-X(228)-CPAP-X(233)-X(234)-X(235)-X(236)-X(237)-P-X(239)-X(240)-FLFPP-X(246)-PKDTLMIS-X(255)-TP-X(258)-V-X(260)-CVV-X(264)-DV-X(267)-X(268)-ED-X(271)-X(272)-V-X(274)-F-X(276)-W-X(278)-VD-X(281)-V-X(283)-X(284)-HNAKT-X(290)-PR-X(293)-E-X(295)-X(296)-NST-X(300)-RVV-X(304)-VLTV-X(309)-HQDWLNGKEYKCKV-X(324)-N-X(326)-X(327)-X(328)-P-X(330)-X(331)-X(332)-X(333)-X(334)-TISK-X(339)-KGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYS-X(409)-LTVDKSRWQ-X(419)-GN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLS-X(445)-GK-, wherein

-X(227)- is selected from the group consisting of P and G;

-X(228)- is selected from the group consisting of P, R, S, and the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR;

-X(233)- is selected from the group consisting of P and E;

-X(234)- is selected from the group consisting of V, , LF, Y and I;
-X(235)- is selected from the group consisting of A, L, Y, I and D;
-X(236)- is selected from the group consisting of no amino acid, G, S and A;
-X(237)- is selected from the group consisting of G and D;
-X(239)- is selected from the group consisting of S, D, E, N, Q and T;
-X(240)- is selected from the group consisting of V, I and M;
-X(246)- is selected from the group consisting of K, H and Y;
-X(255)- is selected from the group consisting of R and Y;
-X(258)- is selected from the group consisting of E, H and Y;
-X(260)- is selected from the group consisting of T and H;
-X(264)- is selected from the group consisting of V, I, T and Y;
-X(267)- is selected from the group consisting of S, D and E;
-X(268)- is selected from the group consisting of H, Q, D and E;
-X(271)- is selected from the group consisting of P and G;
-X(272)- is selected from the group consisting of E, Y, H, R and I;
-X(274)- is selected from the group consisting of Q, K and E;
-X(276)- is selected from the group consisting of N and K;
-X(278)- is selected from the group consisting of Y and T;
-X(281)- is selected from the group consisting of G, D and E;
-X(283)- is selected from the group consisting of E, L and H;
-X(284)- is selected from the group consisting of V, E and D;
-X(290)- is selected from the group consisting of K and N;
-X(293)- is selected from the group consisting of E and R;
-X(295)- is selected from the group consisting of Q and E;
-X(296)- is selected from the group consisting of F and Y;
-X(300)- is selected from the group consisting of F and Y;
-X(304)- is selected from the group consisting of S and T;
-X(309)- is selected from the group consisting of V and L;
-X(324)- is selected from the group consisting of S, G and I;
-X(326)- is selected from the group consisting of K and T;
-X(327)- is selected from the group consisting of G, A and D;
-X(328)- is selected from the group consisting of L, A, F, I and T;
-X(330)- is selected from the group consisting of A, S, L, Y and I;
-X(331)- is selected from the group consisting of P and S;
-X(332)- is selected from the group consisting of I, D, E, N, Q and T;
-X(333)- is selected from the group consisting of E and Y;
-X(334)- is selected from the group consisting of K, F, I and T;
-X(339)- is selected from the group consisting of T and A;
-X(355)- is selected from the group consisting of R and Q;
-X(356)- is selected from the group consisting of E and D;

-X(358)- is selected from the group consisting of M and L;
-X(384)- is selected from the group consisting of N and S;
-X(392)- is selected from the group consisting of K and N;
-X(397)- is selected from the group consisting of M and V;
-X(409)- is selected from the group consisting of K and R;
-X(419)- is selected from the group consisting of Q and E;
-X(422)- is selected from the group consisting of V and I;
-X(435)- is selected from the group consisting of H and R;
-X(436)- is selected from the group consisting of Y and F; and
-X(445)- is selected from the group consisting of P and L.

[54] In certain variations, a first modification is selected from among R228P, R228S, deletion of R, deletion of the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR beginning at 228, E233P, L234V, L234F, L235A, deletion of G236, H268Q, Q274K, K276N, Y296F, F300Y, L309V, A327G, A330S, P331S, T339A, R355Q, E356D, M358L, S384N, N392K, M397V, K409R, Q419E, I422V, R435H, F436Y, and P445L. In further variations, a second modification is selected from among 227G, 234Y, 234I, 235Y, 235I, 235D, 236S, 236A, 237D, 239D, 239E, 239N, 239Q, 239T, 240I, 240M, 246H, 246Y, 255Y, 258H, 258Y, 260H, 264I, 264T, 264Y, 267D, 267E, 268D, 268E, 271G, 272Y, 272H, 272R, 272I, 274E, 278T, 281D, 281E, 283L, 283H, 284E, 284D, 290N, 293R, 295E, 304T, 324G, 324I, 326T, 327D, 328A, 328F, 328I, 328T, 330L, 330Y, 330I, 332D, 332E, 332N, 332Q, 332T, 333Y, 334F, 334I, and 334T. In additional embodiments, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:13. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains. Alternatively, the modifications can be from position 230 until the C terminus.

[55] In another aspect, the present application is directed to an IgG3 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTP-X(221)-GD-X(222)-X(223)-X(224)-X(225)-C-
X(227)-X(228)-CPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRC-X(230)-X(231)-
X(232)-X(233)-X(234)-X(235)-X(236)-X(237)-X(238)-X(239)-X(240)-X(241)-L-X(243)-X(244)-X(245)-
X(246)-X(247)-K-X(249)-TLMIS-X(255)-TP-X(258)-V-X(260)-C-X(262)-X(263)-X(264)-X(265)-X(266)-
X(267)-X(268)-X(269)-X(270)-X(271)-X(272)-X(273)-X(274)-X(275)-X(276)-W-X(278)-V-X(280)-
X(281)-X(282)-X(283)-X(284)-X(285)-X(286)-A-X(288)-T-X(290)-X(291)-X(292)-X(293)-X(294)-
X(295)-X(296)-X(297)-X(298)-X(299)-X(300)-X(301)-X(302)-X(303)-X(304)-X(305)-LTVLHQD-X(313)-
LNG-X(317)-X(318)-Y-X(320)-C-X(322)-X(323)-X(324)-X(325)-X(326)-X(327)-X(328)-X(329)-X(330)-
X(331)-X(332)-X(333)-X(334)-X(335)-X(336)-X(337)-
KTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGS
FFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK, wherein
-X(221)- is selected from the group consisting of L, K and Y;

-X(222)- is selected from the group consisting of T, E and Y;
-X(223)- is selected from the group consisting of T, E and K;
-X(224)- is selected from the group consisting of H and Y;
-X(225)- is selected from the group consisting of T, E, K and W;
-X(227)- is selected from the group consisting of P, E, G, K and Y;
-X(228)- is selected from the group consisting of R, E, G, K and Y;
-X(230)- is selected from the group consisting of P, A, E, G and Y;
-X(231)- is selected from the group consisting of A, E, G, K, P and Y;
-X(232)- is selected from the group consisting of P, E, G, K and Y;
-X(233)- is selected from the group consisting of E, A, D, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(234)- is selected from the group consisting of L, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, W and Y;
-X(235)- is selected from the group consisting of L, D, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, and Y;
-X(236)- is selected from the group consisting of G, A, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;
-X(237)- is selected from the group consisting of G, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W , and Y;
-X(238)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(239)- is selected from the group consisting of S, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W and Y;
-X(240)- is selected from the group consisting of V, A, I, M and T;
-X(241)- is selected from the group consisting of F, D, E, L, R, S, W and Y;
-X(243)- is selected from the group consisting of F, E, H, L, Q, R, W and Y;
-X(244)- is selected from the group consisting of P and H;
-X(245)- is selected from the group consisting of P and A;
-X(246)- is selected from the group consisting of K, D, E, H and Y;
-X(247)- is selected from the group consisting of P, G and V;
-X(249)- is selected from the group consisting of D, H, Q and Y;
-X(255)- is selected from the group consisting of R, E and Y;
-X(258)- is selected from the group consisting of E, H, S and Y;
-X(260)- is selected from the group consisting of T, D, E, H and Y;
-X(262)- is selected from the group consisting of V, A, E, F, I and T;
-X(263)- is selected from the group consisting of V, A, I, M and T;
-X(264)- is selected from the group consisting of V, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W and Y;
-X(265)- is selected from the group consisting of D, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;
-X(266)- is selected from the group consisting of V, A, I, M and T;
-X(267)- is selected from the group consisting of S, D, E, F, H, I, K, L, M, N, P, Q, R, V, W and Y;
-X(268)- is selected from the group consisting of H, D, E, F, G, I, K, L, M, P, R, T, V and W;

-X(269)- is selected from the group consisting of E, F, G, H, I, K, L, M, N, P, R, S, T, V, W and Y;
-X(270)- is selected from the group consisting of D, F, G, H, I, L, M, P, Q, R, S, T, W and Y;
-X(271)- is selected from the group consisting of P, A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(272)- is selected from the group consisting of E, D, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;
-X(273)- is selected from the group consisting of V and I;
-X(274)- is selected from the group consisting of Q, D, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;
-X(275)- is selected from the group consisting of F, L and W;
-X(276)- is selected from the group consisting of K, D, E, F, G, H, I, L, M, P, R, S, T, V, W and Y;
-X(278)- is selected from the group consisting of Y, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V and W;
-X(280)- is selected from the group consisting of D, G, K, L, P and W;
-X(281)- is selected from the group consisting of G, D, E, K, N, P, Q and Y;
-X(282)- is selected from the group consisting of V, E, G, K, P and Y;
-X(283)- is selected from the group consisting of E, G, H, K, L, P, R and Y;
-X(284)- is selected from the group consisting of V, D, E, L, N, Q, T and Y;
-X(285)- is selected from the group consisting of H, D, E, K, Q, W and Y;
-X(286)- is selected from the group consisting of N, E, G, P and Y;
-X(288)- is selected from the group consisting of K, D, E and Y;
-X(290)- is selected from the group consisting of K, D, H, L, N and W;
-X(291)- is selected from the group consisting of P, D, E, G, H, I, Q and T;
-X(292)- is selected from the group consisting of R, D, E, T and Y;
-X(293)- is selected from the group consisting of E, F, G, H, I, L, M, N, P, R, S, T, V, W and Y;
-X(294)- is selected from the group consisting of E, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;
-X(295)- is selected from the group consisting of Q, D, E, F, G, H, I, M, N, P, R, S, T, V, W and Y;
-X(296)- is selected from the group consisting of Y, A, D, E, G, I, K, L, M, N, Q, R, S, T and V;
-X(297)- is selected from the group consisting of N, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;
-X(298)- is selected from the group consisting of S, E, F, H, I, K, M, Q, R, W and Y;
-X(299)- is selected from the group consisting of T, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W and Y;
-X(300)- is selected from the group consisting of F, A, D, E, G, H, K, M, N, P, Q, R, S, T, V and W;
-X(301)- is selected from the group consisting of R, D, E, H and Y;
-X(302)- is selected from the group consisting of V and I;
-X(303)- is selected from the group consisting of V, D, E and Y;
-X(304)- is selected from the group consisting of S, D, H, L, N and T;
-X(305)- is selected from the group consisting of V, E, T and Y;
-X(313)- is selected from the group consisting of W and F;
-X(317)- is selected from the group consisting of K, E and Q;
-X(318)- is selected from the group consisting of E, H, L, Q, R and Y;
-X(320)- is selected from the group consisting of K, D, F, G, H, I, L, N, P, S, T, V, W and Y;

-X(322)- is selected from the group consisting of K, D, F, G, H, I, P, S, T, V, W and Y;
-X(323)- is selected from the group consisting of V and I;
-X(324)- is selected from the group consisting of S, D, F, G, H, I, L, M, P, R, T, V, W and Y;
-X(325)- is selected from the group consisting of N, A, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;
-X(326)- is selected from the group consisting of K, I, L, P and T;
-X(327)- is selected from the group consisting of A, D, E, F, H, I, K, L, M, N, P, R, T, V, W and Y;
-X(328)- is selected from the group consisting of L, A, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;
-X(329)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(330)- is selected from the group consisting of A, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;
-X(331)- is selected from the group consisting of P, D, F, H, I, L, M, Q, R, T, V, W and Y;
-X(332)- is selected from the group consisting of I, A, D, E, F, H, K, L, M, N, P, Q, R, S, T, V, W and Y;
-X(333)- is selected from the group consisting of E, F, H, I, L, M, P, T and Y;
-X(334)- is selected from the group consisting of K, F, I, P and T;
-X(335)- is selected from the group consisting of T, D, F, G, H, I, L, M, N, P, R, S, V, W and Y;
-X(336)- is selected from the group consisting of I, E, K and Y; and
-X(337)- is selected from the group consisting of S, E, H and N.

[56] In certain variations, the variant differs from SEQ ID NO:13 by at least one amino acid.

[57] In anotother aspect, the present application is directed to an IgG3 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTP-X(221)-GDTTHTC-X(227)-
RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPE-X(234)-X(235)-X(236)-
X(237)-P-X(239)-X(240)-FLFPP-X(246)-PKDTLMIS-X(255)-TP-X(258)-V-X(260)-CVV-X(264)-DV-
X(267)-X(268)-ED-X(271)-X(272)-V-X(274)-FKW-X(278)-VD-X(281)-V-X(283)-X(284)-HNAKT-X(290)-
PR-X(293)-E-X(295)-YNSTFRVV-X(304)-VLTVLHQDWLNGKEYKCKV-X(324)-N-X(326)-X(327)-
X(328)-P-X(330)-P-X(332)-X(333)-X(334)-
TISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSD
GSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK-; wherein
-X(221)- is selected from the group consisting of L and K;
-X(227)- is selected from the group consisting of P and G;
-X(234)- is selected from the group consisting of L, Y and I;
-X(235)- is selected from the group consisting of L, Y, I and D;
-X(236)- is selected from the group consisting of G, S and A;
-X(237)- is selected from the group consisting of G and D;
-X(239)- is selected from the group consisting of S, D, E, N, Q and T;
-X(240)- is selected from the group consisting of V, I and M;

-X(246)- is selected from the group consisting of K, H and Y;
-X(255)- is selected from the group consisting of R and Y;
-X(258)- is selected from the group consisting of E, H and Y;
-X(260)- is selected from the group consisting of T and H;
-X(264)- is selected from the group consisting of V, I, T and Y;
-X(267)- is selected from the group consisting of S, D and E;
-X(268)- is selected from the group consisting of H, D and E;
-X(271)- is selected from the group consisting of P and G;
-X(272)- is selected from the group consisting of E, Y, H, R and I;
-X(274)- is selected from the group consisting of Q and E;
-X(278)- is selected from the group consisting of Y and T;
-X(281)- is selected from the group consisting of G, D and E;
-X(283)- is selected from the group consisting of E, L and H;
-X(284)- is selected from the group consisting of V, E and D;
-X(290)- is selected from the group consisting of K and N;
-X(293)- is selected from the group consisting of E and R;
-X(295)- is selected from the group consisting of Q and E;
-X(304)- is selected from the group consisting of S and T;
-X(324)- is selected from the group consisting of S, G and I;
-X(326)- is selected from the group consisting of K and T;
-X(327)- is selected from the group consisting of A and D;
-X(328)- is selected from the group consisting of L, A, F, I and T;
-X(330)- is selected from the group consisting of A, L, Y and I;
-X(332)- is selected from the group consisting of I, D, E, N, Q and T;
-X(333)- is selected from the group consisting of E and Y; and
-X(334)- is selected from the group consisting of K, F, I and T.

[58] In certain variations, the variant differs from SEQ ID NO:13 by at least one amino acid. In additional variations, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:13. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains.

[59] In another aspect, the present application is directed to an IgG4 variant including two or more amino acid modifications as compared to SEQ ID NO:14. The modifications can be selected from among C131S, R133K, E137G, S138G, S192N, L193F, K196Q, T199I, D203N, R214K, R214T, S217P, S217R, S217L, Y219S, Y219C, Y219T, G220C, G220P, -221D, -221L, insertion of the sequence LGD at -221, -222K, -222V, -222T, -223T, P224H, P224E, P225T, P225-, S228P, S228R, substitution of the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR at 228, E233P, F234L, F234V, L235A, G236-, Q268H, Q274K, N276K, F296Y, Y300F, L309V, G327A, S330A, S331P, A339T, Q355R, E356D, M358L, N384S, K392N, V397M, R409K, E419Q, V422I, H435R, Y436F, and L445P. In certain embodiments, at least two of the amino acid

modifications are in different domains. In various embodiments, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:14. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains.

[60] In another aspect, the present application is directed to an IgG4 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAP-X(131)-S-X(133)-STS-X(137)-X(138)-
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT-X(196)-TY-
X(199)-CNV-X(203)-HKPSNTKVDK-X(214)-VE-X(217)-K-X(219)-X(220)-X(221)-X(222)-X(223)-
X(224)-X(225)-CP-X(228)-CPAPE-X(234)-LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS-X(268)-
EDPEV-X(274)-FNWYVDGVEVHNAKTKPREEQ-X(296)-
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNK-X(327)-LP-X(330)-X(331)-
IEKTISKAKGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS-X(409)-LTVDKSRWQ-
X(419)-GNVFSCSVMHEALHNHYTQKSLSLS-X(445)-GK

wherein

X(131) is selected from the group consisting of C and S;
X(133) is selected from the group consisting of R and K;
X(137) is selected from the group consisting of E and G;
X(138) is selected from the group consisting of S and G;
X(196) is selected from the group consisting of K and Q;
X(199) is selected from the group consisting of T and I;
X(203) is selected from the group consisting of D and N;
X(214) is selected from the group consisting of R and K;
X(217) is selected from the group consisting of S and P;
X(219) is selected from the group consisting of Y and S;
X(220) is selected from the group consisting of G and C;
X(221) is selected from the group consisting of no amino acid and D;
X(222) is selected from the group consisting of no amino acid and K;
X(223) is selected from the group consisting of no amino acid and T;
X(224) is selected from the group consisting of P and H;
X(225) is selected from the group consisting of P and T;
X(228) is selected from the group consisting of S and P;
X(234) is selected from the group consisting of F and L;
X(268) is selected from the group consisting of Q and H;
X(274) is selected from the group consisting of Q and K;
X(296) is selected from the group consisting of F and Y;
X(327) is selected from the group consisting of G and A;
X(330) is selected from the group consisting of S and A;

X(331) is selected from the group consisting of S and P;
X(355) is selected from the group consisting of Q and R;
X(356) is selected from the group consisting of E and D;
X(358) is selected from the group consisting of M and L;
X(409) is selected from the group consisting of R and K;
X(419) is selected from the group consisting of E and Q; and
X(445) is selected from the group consisting of L and P.

[61] In certain embodiments, at least two of the amino acid modifications are in different domains. In various embodiments, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:14. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains.

[62] In another aspect, the present application is directed to an IgG4 variant including two or more amino acid modifications as compared to SEQ ID NO:14. In certain embodiments, the modifications selected from among C131S, R133K, E137G, S138G, K196Q, T199I, D203N, R214K, S217P, Y219S, G220C, 221D, -222K, -223T, P224H, P225T, S228P, F234L, Q268H, Q274K, F296Y, G327A, S330A, S331P, Q355R, E356D, M358L, R409K, E419Q, and L445P. In various embodiments, the formula has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid modifications as compared to an amino acid sequence including SEQ ID NO:14. In additional embodiments, at least 2, 3, or 4 of the modifications are in different domains.

[63] In another aspect, the present application is directed to an IgG4 variant including an amino acid sequence having the formula

-ASTKGPSVFPLAP-X(131)-S-X(133)-STS-X(137)-X(138)-
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS-X(192)-X(193)-GT-
X(196)-TY-X(199)-CNV-X(203)-HKPSNTKVDK-X(214)-VE-X(217)-K-X(219)-X(220)-X(221)-X(222)-
X(223)-X(224)-X(225)-C-X(227)-X(228)-C-X(230)-X(231)-X(232)-X(233)-X(234)-X(235)-X(236)-
X(237)-X(238)-X(239)-X(240)-X(241)-L-X(243)-X(244)-X(245)-X(246)-X(247)-K-X(249)-TLMIS-
X(255)-TP-X(258)-V-X(260)-C-X(262)-X(263)-X(264)-X(265)-X(266)-X(267)-X(268)-X(269)-X(270)-
X(271)-X(272)-X(273)-X(274)-X(275)-X(276)-W-X(278)-V-X(280)-X(281)-X(282)-X(283)-X(284)-
X(285)-X(286)-A-X(288)-T-X(290)-X(291)-X(292)-X(293)-X(294)-X(295)-X(296)-X(297)-X(298)-
X(299)-X(300)-X(301)-X(302)-X(303)-X(304)-X(305)-LTV-X(309)-HQD-X(313)-LNG-X(317)-X(318)-Y-
X(320)-C-X(322)-X(323)-X(324)-X(325)-X(326)-X(327)-X(328)-X(329)-X(330)-X(331)-X(332)-X(333)-
X(334)-X(335)-X(336)-X(337)-K-X(339)-KGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-
TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYS-
X(409)-LTVDKSRWQ-X(419)-GN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLS-X(445)-GK
wherein

-X(131)- is selected from the group consisting of C and S;
-X(133)- is selected from the group consisting of R and K;
-X(137)- is selected from the group consisting of E and G;

-X(138)- is selected from the group consisting of S and G;
-X(192)- is selected from the group consisting of N and S;
-X(193)- is selected from the group consisting of F and L;
-X(196)- is selected from the group consisting of Q and K;
-X(199)- is selected from the group consisting of T and I;
-X(203)- is selected from the group consisting of D and N;
-X(214)- is selected from the group consisting of T, K and R;
-X(217)- is selected from the group consisting of R, P, L and S;
-X(219)- is selected from the group consisting of C, S, T and Y;
-X(220)- is selected from the group consisting of C, P and G;
-X(221)- is selected from the group consisting of no amino acid, D, K, Y, L, and the sequence LGD;
-X(222)- is selected from the group consisting of V, K, T, no amino acid , E and Y;
-X(223)- is selected from the group consisting of no amino acid, T, E and K;
-X(224)- is selected from the group consisting of E, H, P and Y;
-X(225)- is selected from the group consisting of no amino acid, T, P, E, K and W;
-X(227)- is selected from the group consisting of P, E, G, K and Y;
-X(228)- is selected from the group consisting of P, S, E, G, K, Y, R, and the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR;
-X(230)- is selected from the group consisting of P, A, E, G and Y;
-X(231)- is selected from the group consisting of A, E, G, K, P and Y;
-X(232)- is selected from the group consisting of P, E, G, K and Y;
-X(233)- is selected from the group consisting of P, E, A, D, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(234)- is selected from the group consisting of V, L, F, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, W and Y;
-X(235)- is selected from the group consisting of A, L, D, F, G, H, I, K, M, N, P, Q, R, S, T, V, W , and Y;
-X(236)- is selected from the group consisting of no amino acid, G, A, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;
-X(237)- is selected from the group consisting of G, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;
-X(238)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(239)- is selected from the group consisting of S, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W and Y;
-X(240)- is selected from the group consisting of V, A, I, M and T;
-X(241)- is selected from the group consisting of F, D, E, L, R, S, W and Y;
-X(243)- is selected from the group consisting of F, E, H, L, Q, RW, , and Y;
-X(244)- is selected from the group consisting of P and H;
-X(245)- is selected from the group consisting of P and A;

-X(246)- is selected from the group consisting of , K, D, E, H and Y;
-X(247)- is selected from the group consisting of P, G and V;
-X(249)- is selected from the group consisting of D, H, Q and Y;
-X(255)- is selected from the group consisting of RE and Y;
-X(258)- is selected from the group consisting of E, H, S and Y;
-X(260)- is selected from the group consisting of T, D, E, H and Y;
-X(262)- is selected from the group consisting of V, A, E, F, I and T;
-X(263)- is selected from the group consisting of V, A, I, M and T;
-X(264)- is selected from the group consisting of V, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, and Y;
-X(265)- is selected from the group consisting of D, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;
-X(266)- is selected from the group consisting of V, A, I, M and T;
-X(267)- is selected from the group consisting of S, D, E, F, H, I, K, L, M, N, P, Q, R, V, W and Y;
-X(268)- is selected from the group consisting of H, Q, D, E, F, G, I, K, L, M, P, R, T, V and W;
-X(269)- is selected from the group consisting of E, F, G, H, I, K, L, M, N, P, R, S, T, V, W and Y;
-X(270)- is selected from the group consisting of D, F, G, H, I, L, M, P, Q, R, S, T, W and Y;
-X(271)- is selected from the group consisting of P, A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(272)- is selected from the group consisting of E, D, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;
-X(273)- is selected from the group consisting of V and I;
-X(274)- is selected from the group consisting of Q, K, D, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;
-X(275)- is selected from the group consisting of FL and W;
-X(276)- is selected from the group consisting of N, K, D, E, F, G, H, I, L, M, P, R, S, T, V, W and Y;
-X(278)- is selected from the group consisting of Y, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V and W;
-X(280)- is selected from the group consisting of D, G, K, L, P and W;
-X(281)- is selected from the group consisting of G, D, E, K, N, P, Q and Y;
-X(282)- is selected from the group consisting of V, E, G, K, P and Y;
-X(283)- is selected from the group consisting of E, G, H, K, L, P, R and Y;
-X(284)- is selected from the group consisting of V, D, E, L, N, Q, T and Y;
-X(285)- is selected from the group consisting of H, D, E, K, Q, W and Y;
-X(286)- is selected from the group consisting of N, E, G, P and Y;
-X(288)- is selected from the group consisting of K, D, E and Y;
-X(290)- is selected from the group consisting of K, D, H, L, N and W;
-X(291)- is selected from the group consisting of P, D, E, G, H, I, Q and T;
-X(292)- is selected from the group consisting of R, D, E, T and Y;
-X(293)- is selected from the group consisting of E, F, G, H, I, L, M, N, P, R, S, T, V, W and Y;
-X(294)- is selected from the group consisting of E, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;
-X(295)- is selected from the group consisting of Q, D, E, F, G, H, I, M, N, P, R, S, T, V, W and Y;
-X(296)- is selected from the group consisting of F, Y, A, D, E, G, I, K, L, M, N, Q, R, S, T and V;

-X(297)- is selected from the group consisting of N, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(298)- is selected from the group consisting of S, E, F, H, I, K, M, Q, R, W and Y;

-X(299)- is selected from the group consisting of T, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W and Y;

-X(300)- is selected from the group consisting of F, Y, A, D, E, G, H, K, M, N, P, Q, R, S, T, V and W;

-X(301)- is selected from the group consisting of R, D, E, H and Y;

-X(302)- is selected from the group consisting of V and I;

-X(303)- is selected from the group consisting of V, D, E and Y;

-X(304)- is selected from the group consisting of S, D, H, L, N and T;

-X(305)- is selected from the group consisting of V, E, T and Y;

-X(309)- is selected from the group consisting of V and L;

-X(313)- is selected from the group consisting of W and F;

-X(317)- is selected from the group consisting of K, E and Q;

-X(318)- is selected from the group consisting of E, H, L, Q, R and Y;

-X(320)- is selected from the group consisting of K, D, F, G, H, I, L, N, P, S, T, V, W and Y;

-X(322)- is selected from the group consisting of K, D, F, G, H, I, P, S, T, V, W and Y;

-X(323)- is selected from the group consisting of V and I;

-X(324)- is selected from the group consisting of S, D, F, G, H, I, L, M, P, R, T, V, W and Y;

-X(325)- is selected from the group consisting of N, A, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(326)- is selected from the group consisting of K, I, L, P and T;

-X(327)- is selected from the group consisting of G, A, D, E, F, H, I, K, L, M, N, P, R, T, V, W and Y;

-X(328)- is selected from the group consisting of L, A, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;

-X(329)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(330)- is selected from the group consisting of A, S, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(331)- is selected from the group consisting of P, S, D, F, H, I, L, M, Q, R, T, V, W and Y;

-X(332)- is selected from the group consisting of I, A, D, E, F, H, , K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(333)- is selected from the group consisting of E, F, H, I, L, M, P, T and Y;

-X(334)- is selected from the group consisting of K, F, I, P and T;

-X(335)- is selected from the group consisting of T, D, F, G, H, I, L, M, N, P, R, S, V, W and Y;

-X(336)- is selected from the group consisting of I, E, K and Y;

-X(337)- is selected from the group consisting of S, E, H and N;

-X(339)- is selected from the group consisting of T and A;

-X(355)- is selected from the group consisting of R and Q;

-X(356)- is selected from the group consisting of E and D;

-X(358)- is selected from the group consisting of M and L;

-X(384)- is selected from the group consisting of N and S;
-X(392)- is selected from the group consisting of K and N;
-X(397)- is selected from the group consisting of M and V;
-X(409)- is selected from the group consisting of K and R;
-X(419)- is selected from the group consisting of Q and E;
-X(422)- is selected from the group consisting of V and I;
-X(435)- is selected from the group consisting of H and R;
-X(436)- is selected from the group consisting of Y and F; and
-X(445)- is selected from the group consisting of P and L.

[64] In one variation, a first modification is selected from among C131S, R133K, E137G, S138G, S192N, L193F, K196Q, T199I, D203N, R214K, R214T, S217P, S217R, S217L, Y219S, Y219C, Y219T, G220C, G220P, -221D, -221L, insertion of the sequence LGD at -221, -222K, -222V, -222T, -223T, P224H, P224E, P225T, P225-, S228P, S228R, substitution of the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR at 228, E233P, F234L, F234V, L235A, G236-, Q268H, Q274K, N276K, F296Y, Y300F, L309V, G327A, S330A, S331P, A339T, Q355R, E356D, M358L, N384S, K392N, V397M, R409K, E419Q, V422I, H435R, Y436F, and L445P. In a further variation, a second modification is selected from among 221K, 221Y, 222E, 222Y, 223E, 223K, 224Y, 225E, 225K, 225W, 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234W, 234Y, 235D, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R,

274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334I, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N.

[65] In a further aspect, the present application is directed to an IgG4 variant amino acid sequence having at least two amino acid modifications as compared to SEQ ID NO:14. The IgG4 variant includes a first modification selected from among C131S, R133K, E137G, S138G, S192N, L193F, K196Q, T199I, D203N, R214K, R214T, S217P, S217R, S217L, Y219S, Y219C, Y219T, G220C, G220P, -221D, -221L, insertion of the sequence LGD at -221, -222K, -222V, -222T, -223T, P224H, P224E, P225T, P225-, S228P, S228R, substitution of the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR at 228, E233P, F234L, F234V, L235A, G236-, Q268H, Q274K, N276K, F296Y, Y300F, L309V, G327A, S330A, S331P, A339T, Q355R, E356D, M358L, N384S, K392N, V397M, R409K, E419Q, V422I, H435R, Y436F, and L445P. In a further variation, a second modification is selected from among 221K, 221Y, 222E, 222Y, 223E, 223K, 224Y, 225E, 225K, 225W, 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A,

230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234W, 234Y, 235D, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L,

325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334I, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N.

[66] In another aspect, the present application is directed to an IgG4 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAP-X(131)-S-X(133)-STS-X(137)-X(138)-TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS-X(192)-X(193)-GT-X(196)-TY-X(199)-CNV-X(203)-HKPSNTKVDK-X(214)-VE-X(217)-K-X(219)-X(220)-X(221)-X(222)-X(223)-X(224)-X(225)-C-X(227)-X(228)-CPAP-X(233)-X(234)-X(235)-X(236)-X(237)-P-X(239)-X(240)-FLFPP-X(246)-PKDTLMIS-X(255)-TP-X(258)-V-X(260)-CVV-X(264)-DV-X(267)-X(268)-ED-X(271)-X(272)-V-X(274)-F-X(276)-W-X(278)-VD-X(281)-V-X(283)-X(284)-HNAKT-X(290)-PR-X(293)-E-X(295)-X(296)-NST-X(300)-RVV-X(304)-VLTV-X(309)-HQDWLNGKEYKCKV-X(324)-N-X(326)-X(327)-X(328)-P-X(330)-X(331)-X(332)-X(333)-X(334)-TISK-X(339)-KGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYS-X(409)-LTVDKSRWQ-X(419)-GN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLS-X(445)-GK;

wherein

-X(131)- is selected from the group consisting of C and S;

-X(133)- is selected from the group consisting of R and K;

-X(137)- is selected from the group consisting of E and G;

-X(138)- is selected from the group consisting of S and G;

-X(192)- is selected from the group consisting of N and S;

-X(193)- is selected from the group consisting of F and L;

-X(196)- is selected from the group consisting of Q and K;

-X(199)- is selected from the group consisting of T and I;

-X(203)- is selected from the group consisting of D and N;

-X(214)- is selected from the group consisting of T, K and R;

-X(217)- is selected from the group consisting of R, P, L and S;

-X(219)- is selected from the group consisting of C, S, T and Y;

-X(220)- is selected from the group consisting of C, P and G;

-X(221)- is selected from the group consisting of no amino acid, D, L, K, and the sequence LGD;

-X(222)- is selected from the group consisting of V, K, T, and no amino acid;

-X(223)- is selected from the group consisting of no amino acid and T;

-X(224)- is selected from the group consisting of E, H and P;

-X(225)- is selected from the group consisting of no amino acid, T and P;

-X(227)- is selected from the group consisting of P and G;

-X(228)- is selected from the group consisting of P, R, S, and the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR;

-X(233)- is selected from the group consisting of P and E;

-X(234)- is selected from the group consisting of V, , LF, Y and I;

-X(235)- is selected from the group consisting of A, L, Y, I and D;

-X(236)- is selected from the group consisting of no amino acid, G, S and A;

-X(237)- is selected from the group consisting of G and D;

-X(239)- is selected from the group consisting of S, D, E, N, Q and T;

-X(240)- is selected from the group consisting of V, I and M;

-X(246)- is selected from the group consisting of K, H and Y;

-X(255)- is selected from the group consisting of R and Y;

-X(258)- is selected from the group consisting of E, H and Y;

-X(260)- is selected from the group consisting of T and H;

-X(264)- is selected from the group consisting of V, I, T and Y;

-X(267)- is selected from the group consisting of S, D and E;

-X(268)- is selected from the group consisting of H, Q, D and E;

-X(271)- is selected from the group consisting of P and G;

-X(272)- is selected from the group consisting of E, Y, H, R and I;

-X(274)- is selected from the group consisting of Q, K and E;

-X(276)- is selected from the group consisting of N and K;

-X(278)- is selected from the group consisting of Y and T;

-X(281)- is selected from the group consisting of G, D and E;

-X(283)- is selected from the group consisting of E, L and H;

-X(284)- is selected from the group consisting of V, E and D;

-X(290)- is selected from the group consisting of K and N;

-X(293)- is selected from the group consisting of E and R;

-X(295)- is selected from the group consisting of Q and E;

-X(296)- is selected from the group consisting of F and Y;

-X(300)- is selected from the group consisting of F and Y;

-X(304)- is selected from the group consisting of S and T;

-X(309)- is selected from the group consisting of V and L;

-X(324)- is selected from the group consisting of S, G and I;

-X(326)- is selected from the group consisting of K and T;

-X(327)- is selected from the group consisting of G, A and D;

-X(328)- is selected from the group consisting of L, A, F, I and T;

-X(330)- is selected from the group consisting of A, S, L, Y and I;

-X(331)- is selected from the group consisting of P and S;
-X(332)- is selected from the group consisting of I, D, E, N, Q and T;
-X(333)- is selected from the group consisting of E and Y;
-X(334)- is selected from the group consisting of K, F, I and T;
-X(339)- is selected from the group consisting of T and A;
-X(355)- is selected from the group consisting of R and Q;
-X(356)- is selected from the group consisting of E and D;
-X(358)- is selected from the group consisting of M and L;
-X(384)- is selected from the group consisting of N and S;
-X(392)- is selected from the group consisting of K and N;
-X(397)- is selected from the group consisting of M and V;
-X(409)- is selected from the group consisting of K and R;
-X(419)- is selected from the group consisting of Q and E;
-X(422)- is selected from the group consisting of V and I;
-X(435)- is selected from the group consisting of H and R;
-X(436)- is selected from the group consisting of Y and F;and
-X(445)- is selected from the group consisting of P and L.

[67] In one variation, a first modification is selected from among C131S, R133K, E137G, S138G, S192N, L193F, K196Q, T199I, D203N, R214K, R214T, S217P, S217R, S217L, Y219S, Y219C, Y219T, G220C, G220P, -221D, -221L, insertion of the sequence LGD at -221, -222K, -222V, -222T, -223T, P224H, P224E, P225T, P225-, S228P, S228R, substitution of the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR at 228, E233P, F234L, F234V, L235A, G236-, Q268H, Q274K, N276K, F296Y, Y300F, L309V, G327A, S330A, S331P, A339T, Q355R, E356D, M358L, N384S, K392N, V397M, R409K, E419Q, V422I, H435R, Y436F, and L445P. In a further variation, a second modification is selected from among 227G, 234Y, 234I, 235Y, 235I, 235D, 236S, 236A, 237D, 239D, 239E, 239N, 239Q, 239T, 240I, 240M, 246H, 246Y, 255Y, 258H, 258Y, 260H, 264I, 264T, 264Y, 267D, 267E, 268D, 268E, 271G, 272Y, 272H, 272R, 272I, 274E, 278T, 281D, 281E, 283L, 283H, 284E, 284D, 290N, 293R, 295E, 304T, 324G, 324I, 326T, 327D, 328A, 328F, 328I, 328T, 330L, 330Y, 330I, 332D, 332E, 332N, 332Q, 332T, 333Y, 334F, 334I, and 334T.

[68] In another aspect, the present application is directed to an IgG4 variant including an amino acid sequence having the formula:

-C-X(227)-X(228)-C-X(230)-X(231)-X(232)-X(233)-X(234)-X(235)-X(236)--X(237)-X(238)-X(239)-X(240)-X(241)-L-X(243)-X(244)-X(245)-X(246)-X(247)-K-X(249)-TLMIS-X(255)-TP-X(258)-V-X(260)-C-X(262)-X(263)-X(264)-X(265)-X(266)-X(267)-X(268)-X(269)-X(270)-X(271)-X(272)-X(273)-X(274)-X(275)-X(276)-W-X(278)-V-X(280)-X(281)-X(282)-X(283)-X(284)-X(285)-X(286)-A-X(288)-T-X(290)-X(291)-X(292)-X(293)-X(294)-X(295)-X(296)-X(297)-X(298)-X(299)-X(300)-X(301)-X(302)-X(303)-X(304)-X(305)-LTV-X(309)-HQD-X(313)-LNG-X(317)-X(318)-Y-X(320)-C-X(322)-X(323)-X(324)-X(325)-X(326)-X(327)-X(328)-X(329)-X(330)-X(331)-X(332)-X(333)-X(334)-X(335)-X(336)-X(337)-K-

X(339)-KGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYS-X(409)-LTVDKSRWQ-X(419)-GN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLS-X(445)-GK

wherein

-X(227)- is selected from the group consisting of P, E, G, K and Y;

-X(228)- is selected from the group consisting of P, S, E, G, K, Y, R, and the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR;

-X(230)- is selected from the group consisting of P, A, E, G and Y;

-X(231)- is selected from the group consisting of A, E, G, K, P and Y;

-X(232)- is selected from the group consisting of P, E, G, K and Y;

-X(233)- is selected from the group consisting of P, E, A, D, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(234)- is selected from the group consisting of V, L, F, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, W and Y;

-X(235)- is selected from the group consisting of A, L, D, F, G, H, I, K, M, N, P, Q, R, S, T, V, W , and Y;

-X(236)- is selected from the group consisting of no amino acid, G, A, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(237)- is selected from the group consisting of G, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(238)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(239)- is selected from the group consisting of S, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W and Y;

-X(240)- is selected from the group consisting of V, A, I, M and T;

-X(241)- is selected from the group consisting of F, D, E, L, R, S, W and Y;

-X(243)- is selected from the group consisting of F, E, H, L, Q, RW, , and Y;

-X(244)- is selected from the group consisting of P and H;

-X(245)- is selected from the group consisting of P and A;

-X(246)- is selected from the group consisting of , K, D, E, H and Y;

-X(247)- is selected from the group consisting of P, G and V;

-X(249)- is selected from the group consisting of D, H, Q and Y;

-X(255)- is selected from the group consisting of RE and Y;

-X(258)- is selected from the group consisting of E, H, S and Y;

-X(260)- is selected from the group consisting of T, D, E, H and Y;

-X(262)- is selected from the group consisting of V, A, E, F, I and T;

-X(263)- is selected from the group consisting of V, A, I, M and T;

-X(264)- is selected from the group consisting of V, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, and Y;

-X(265)- is selected from the group consisting of D, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(266)- is selected from the group consisting of V, A, I, M and T;
-X(267)- is selected from the group consisting of S, D, E, F, H, I, K, L, M, N, P, Q, R, V, W and Y;
-X(268)- is selected from the group consisting of H, Q, D, E, F, G, I, K, L, M, P, R, T, V and W;
-X(269)- is selected from the group consisting of E, F, G, H, I, K, L, M, N, P, R, S, T, V, W and Y;
-X(270)- is selected from the group consisting of D, F, G, H, I, L, M, P, Q, R, S, T, W and Y;
-X(271)- is selected from the group consisting of P, A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(272)- is selected from the group consisting of E, D, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;
-X(273)- is selected from the group consisting of V and I;
-X(274)- is selected from the group consisting of Q, K, D, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;
-X(275)- is selected from the group consisting of FL and W;
-X(276)- is selected from the group consisting of N, K, D, E, F, G, H, I, L, M, P, R, S, T, V, W and Y;
-X(278)- is selected from the group consisting of Y, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V and W;
-X(280)- is selected from the group consisting of D, G, K, L, P and W;
-X(281)- is selected from the group consisting of G, D, E, K, N, P, Q and Y;
-X(282)- is selected from the group consisting of V, E, G, K, P and Y;
-X(283)- is selected from the group consisting of E, G, H, K, L, P, R and Y;
-X(284)- is selected from the group consisting of V, D, E, L, N, Q, T and Y;
-X(285)- is selected from the group consisting of H, D, E, K, Q, W and Y;
-X(286)- is selected from the group consisting of N, E, G, P and Y;
-X(288)- is selected from the group consisting of K, D, E and Y;
-X(290)- is selected from the group consisting of K, D, H, L, N and W;
-X(291)- is selected from the group consisting of P, D, E, G, H, I, Q and T;
-X(292)- is selected from the group consisting of R, D, E, T and Y;
-X(293)- is selected from the group consisting of E, F, G, H, I, L, M, N, P, R, S, T, V, W and Y;
-X(294)- is selected from the group consisting of E, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;
-X(295)- is selected from the group consisting of Q, D, E, F, G, H, I, M, N, P, R, S, T, V, W and Y;
-X(296)- is selected from the group consisting of F, Y, A, D, E, G, I, K, L, M, N, Q, R, S, T and V;
-X(297)- is selected from the group consisting of N, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;
-X(298)- is selected from the group consisting of S, E, F, H, I, K, M, Q, R, W and Y;
-X(299)- is selected from the group consisting of T, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W and Y;
-X(300)- is selected from the group consisting of F, Y, A, D, E, G, H, K, M, N, P, Q, R, S, T, V and W;
-X(301)- is selected from the group consisting of R, D, E, H and Y;
-X(302)- is selected from the group consisting of V and I;
-X(303)- is selected from the group consisting of V, D, E and Y;
-X(304)- is selected from the group consisting of S, D, H, L, N and T;
-X(305)- is selected from the group consisting of V, E, T and Y;
-X(309)- is selected from the group consisting of V and L;

-X(313)- is selected from the group consisting of W and F;
-X(317)- is selected from the group consisting of K, E and Q;
-X(318)- is selected from the group consisting of E, H, L, Q, R and Y;
-X(320)- is selected from the group consisting of K, D, F, G, H, I, L, N, P, S, T, V, W and Y;
-X(322)- is selected from the group consisting of K, D, F, G, H, I, P, S, T, V, W and Y;
-X(323)- is selected from the group consisting of V and I;
-X(324)- is selected from the group consisting of S, D, F, G, H, I, L, M, P, R, T, V, W and Y;
-X(325)- is selected from the group consisting of N, A, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;
-X(326)- is selected from the group consisting of K, I, L, P and T;
-X(327)- is selected from the group consisting of G, A, D, E, F, H, I, K, L, M, N, P, R, T, V, W and Y;
-X(328)- is selected from the group consisting of L, A, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;
-X(329)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(330)- is selected from the group consisting of A, S, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;
-X(331)- is selected from the group consisting of P, S, D, F, H, I, L, M, Q, R, T, V, W and Y;
-X(332)- is selected from the group consisting of I, A, D, E, F, H, , K, L, M, N, P, Q, R, S, T, V, W and Y;
-X(333)- is selected from the group consisting of E, F, H, I, L, M, P, T and Y;
-X(334)- is selected from the group consisting of K, F, I, P and T;
-X(335)- is selected from the group consisting of T, D, F, G, H, I, L, M, N, P, R, S, V, W and Y;
-X(336)- is selected from the group consisting of I, E, K and Y;
-X(337)- is selected from the group consisting of S, E, H and N;
-X(339)- is selected from the group consisting of T and A;
-X(355)- is selected from the group consisting of R and Q;
-X(356)- is selected from the group consisting of E and D;
-X(358)- is selected from the group consisting of M and L;
-X(384)- is selected from the group consisting of N and S;
-X(392)- is selected from the group consisting of K and N;
-X(397)- is selected from the group consisting of M and V;
-X(409)- is selected from the group consisting of K and R;
-X(419)- is selected from the group consisting of Q and E;
-X(422)- is selected from the group consisting of V and I;
-X(435)- is selected from the group consisting of H and R;
-X(436)- is selected from the group consisting of Y and F; and
-X(445)- is selected from the group consisting of P and L.

[69] In one variation, a first modification is selected from among S228P, S228R, substitution of the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR at 228, E233P, F234L, F234V, L235A, G236-, Q268H, Q274K, N276K, F296Y, Y300F, L309V, G327A, S330A,

S331P, A339T, Q355R, E356D, M358L, N384S, K392N, V397M, R409K, E419Q, V422I, H435R, Y436F, and L445P. In a further variation, a modification is selected from among , 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234W, 234Y, 235D, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V,

320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334I, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N.

[70] In a further aspect, the present application is directed to an IgG4 variant amino acid sequence including at least two modifications as compared to SEQ ID NO:14. In certain variations, a first modification is selected from among Q268H, Q274K, N276K, F296Y, Y300F, L309V, G327A, S330A, S331P, A339T, Q355R, E356D, M358L, N384S, K392N, V397M, R409K, E419Q, V422I, H435R, Y436F, and L445P. In further variations, a second modification is selected from among 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F,

294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334I, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N.

[71] In another aspect, the present application is directed to an IgG4 variant including an amino acid sequence having the formula:

C-X(227)-X(228)-CPAP-X(233)-X(234)-X(235)-X(236)-X(237)-P-X(239)-X(240)-FLFPP-X(246)-PKDTLMIS-X(255)-TP-X(258)-V-X(260)-CVV-X(264)-DV-X(267)-X(268)-ED-X(271)-X(272)-V-X(274)-F-X(276)-W-X(278)-VD-X(281)-V-X(283)-X(284)-HNAKT-X(290)-PR-X(293)-E-X(295)-X(296)-NST-X(300)-RVV-X(304)-VLTV-X(309)-HQDWLNGKEYKCKV-X(324)-N-X(326)-X(327)-X(328)-P-X(330)-X(331)-X(332)-X(333)-X(334)-TISK-X(339)-KGQPREPQVYTLPPS-X(355)-X(356)-E-X(358)-TKNQVSLTCLVKGFYPSDIAVEWES-X(384)-GQPENNY-X(392)-TTPP-X(397)-LDSDGSFFLYS-X(409)-LTVDKSRWQ-X(419)-GN-X(422)-FSCSVMHEALHN-X(435)-X(436)-TQKSLSLS-X(445)-GK

wherein

-X(227)- is selected from the group consisting of P and G;

-X(228)- is selected from the group consisting of P, R, S, and the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR;

-X(233)- is selected from the group consisting of P and E;

-X(234)- is selected from the group consisting of V, , LF, Y and I;

-X(235)- is selected from the group consisting of A, L, Y, I and D;

-X(236)- is selected from the group consisting of no amino acid, G, S and A;

-X(237)- is selected from the group consisting of G and D;

-X(239)- is selected from the group consisting of S, D, E, N, Q and T;
-X(240)- is selected from the group consisting of V, I and M;
-X(246)- is selected from the group consisting of K, H and Y;
-X(255)- is selected from the group consisting of R and Y;
-X(258)- is selected from the group consisting of E, H and Y;
-X(260)- is selected from the group consisting of T and H;
-X(264)- is selected from the group consisting of V, I, T and Y;
-X(267)- is selected from the group consisting of S, D and E;
-X(268)- is selected from the group consisting of H, Q, D and E;
-X(271)- is selected from the group consisting of P and G;
-X(272)- is selected from the group consisting of E, Y, H, R and I;
-X(274)- is selected from the group consisting of Q, K and E;
-X(276)- is selected from the group consisting of N and K;
-X(278)- is selected from the group consisting of Y and T;
-X(281)- is selected from the group consisting of G, D and E;
-X(283)- is selected from the group consisting of E, L and H;
-X(284)- is selected from the group consisting of V, E and D;
-X(290)- is selected from the group consisting of K and N;
-X(293)- is selected from the group consisting of E and R;
-X(295)- is selected from the group consisting of Q and E;
-X(296)- is selected from the group consisting of F and Y;
-X(300)- is selected from the group consisting of F and Y;
-X(304)- is selected from the group consisting of S and T;
-X(309)- is selected from the group consisting of V and L;
-X(324)- is selected from the group consisting of S, G and I;
-X(326)- is selected from the group consisting of K and T;
-X(327)- is selected from the group consisting of G, A and D;
-X(328)- is selected from the group consisting of L, A, F, I and T;
-X(330)- is selected from the group consisting of A, S, L, Y and I;
-X(331)- is selected from the group consisting of P and S;
-X(332)- is selected from the group consisting of I, D, E, N, Q and T;
-X(333)- is selected from the group consisting of E and Y;
-X(334)- is selected from the group consisting of K, F, I and T;
-X(339)- is selected from the group consisting of T and A;
-X(355)- is selected from the group consisting of R and Q;
-X(356)- is selected from the group consisting of E and D;
-X(358)- is selected from the group consisting of M and L;
-X(384)- is selected from the group consisting of N and S;
-X(392)- is selected from the group consisting of K and N;
-X(397)- is selected from the group consisting of M and V;

-X(409)- is selected from the group consisting of K and R;
-X(419)- is selected from the group consisting of Q and E;
-X(422)- is selected from the group consisting of V and I;
-X(435)- is selected from the group consisting of H and R;
-X(436)- is selected from the group consisting of Y and F; and
-X(445)- is selected from the group consisting of P and L.

[72] In one variation, a first modification is selected from among S228P, S228R, substitution of the sequence RCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR at 228, E233P, F234L, F234V, L235A, G236-, Q268H, Q274K, N276K, F296Y, Y300F, L309V, G327A, S330A, S331P, A339T, Q355R, E356D, M358L, N384S, K392N, V397M, R409K, E419Q, V422I, H435R, Y436F, and L445P. In a further variation, a second modification is selected from among 227G, 234Y, 234I, 235Y, 235I, 235D, 236S, 236A, 237D, 239D, 239E, 239N, 239Q, 239T, 240I, 240M, 246H, 246Y, 255Y, 258H, 258Y, 260H, 264I, 264T, 264Y, 267D, 267E, 268D, 268E, 271G, 272Y, 272H, 272R, 272I, 274E, 278T, 281D, 281E, 283L, 283H, 284E, 284D, 290N, 293R, 295E, 304T, 324G, 324I, 326T, 327D, 328A, 328F, 328I, 328T, 330L, 330Y, 330I, 332D, 332E, 332N, 332Q, 332T, 333Y, 334F, 334I, and 334T. Alternatively, the modifications can be selected from among those beginning at position 230 until the C terminus.

[73] In another aspect, the present application is directed to an IgG4 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYG-X(221)-X(222)-X(223)-X(224)-X(225)-C-
X(227)-X(228)-C-X(230)-X(231)-X(232)-X(233)-X(234)-X(235)-X(236)-X(237)-X(238)-X(239)-X(240)-
X(241)-L-X(243)-X(244)-X(245)-X(246)-X(247)-K-X(249)-TLMIS-X(255)-TP-X(258)-V-X(260)-C-
X(262)-X(263)-X(264)-X(265)-X(266)-X(267)-X(268)-X(269)-X(270)-X(271)-X(272)-X(273)-X(274)-
X(275)-X(276)-W-X(278)-V-X(280)-X(281)-X(282)-X(283)-X(284)-X(285)-X(286)-A-X(288)-T-X(290)-
X(291)-X(292)-X(293)-X(294)-X(295)-X(296)-X(297)-X(298)-X(299)-X(300)-X(301)-X(302)-X(303)-
X(304)-X(305)-LTVLHQD-X(313)-LNG-X(317)-X(318)-Y-X(320)-C-X(322)-X(323)-X(324)-X(325)-
X(326)-X(327)-X(328)-X(329)-X(330)-X(331)-X(332)-X(333)-X(334)-X(335)-X(336)-X(337)-
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK-

wherein
-X(221)- is selected from the group consisting of no amino acid , K and Y;
-X(222)- is selected from the group consisting of no amino acid , E and Y;
-X(223)- is selected from the group consisting of no amino acid , E and K;
-X(224)- is selected from the group consisting of P and Y;
-X(225)- is selected from the group consisting of P, E, K and W;
-X(227)- is selected from the group consisting of P, E, G, K and Y;
-X(228)- is selected from the group consisting of S, E, G, K and Y;
-X(230)- is selected from the group consisting of P, A, E, G and Y;

-X(231)- is selected from the group consisting of A, E, G, K, P and Y;

-X(232)- is selected from the group consisting of P, E, G, K and Y;

-X(233)- is selected from the group consisting of E, A, D, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(234)- is selected from the group consisting of F, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, W and Y;

-X(235)- is selected from the group consisting of L, D, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;

-X(236)- is selected from the group consisting of G, A, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(237)- is selected from the group consisting of G, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(238)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(239)- is selected from the group consisting of S, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W and Y;

-X(240)- is selected from the group consisting of V, A, I, M and T;

-X(241)- is selected from the group consisting of F, D, E, L, R, S, W and Y;

-X(243)- is selected from the group consisting of F, E, H, L, Q, R, W and Y;

-X(244)- is selected from the group consisting of P and H;

-X(245)- is selected from the group consisting of P and A;

-X(246)- is selected from the group consisting of K, D, E, H and Y;

-X(247)- is selected from the group consisting of P, G and V;

-X(249)- is selected from the group consisting of D, H, Q and Y;

-X(255)- is selected from the group consisting of R, E and Y;

-X(258)- is selected from the group consisting of E, H, S and Y;

-X(260)- is selected from the group consisting of T, D, E, H and Y;

-X(262)- is selected from the group consisting of V, A, E, F, I and T;

-X(263)- is selected from the group consisting of V, A, I, M and T;

-X(264)- is selected from the group consisting of V, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W and Y;

-X(265)- is selected from the group consisting of D, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(266)- is selected from the group consisting of V, A, I, M and T;

-X(267)- is selected from the group consisting of S, D, E, F, H, I, K, L, M, N, P, Q, R, V, W and Y;

-X(268)- is selected from the group consisting of Q, D, E, F, G, I, K, L, M, P, R, T, V and W;

-X(269)- is selected from the group consisting of E, F, G, H, I, K, L, M, N, P, R, S, T, V, W and Y;

-X(270)- is selected from the group consisting of D, F, G, H, I, L, M, P, Q, R, S, T, W and Y;

-X(271)- is selected from the group consisting of P, A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(272)- is selected from the group consisting of E, D, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;

-X(273)- is selected from the group consisting of V and I;

-X(274)- is selected from the group consisting of Q, D, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(275)- is selected from the group consisting of F, L, W;

-X(276)- is selected from the group consisting of N, D, E, F, G, H, I, L, M, P, R, S, T, V, W and Y;

-X(278)- is selected from the group consisting of Y, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V and W;

-X(280)- is selected from the group consisting of D, G, K, L, P and W;

-X(281)- is selected from the group consisting of G, D, E, K, N, P, Q and Y;

-X(282)- is selected from the group consisting of V, E, G, K, P and Y;

-X(283)- is selected from the group consisting of E, G, H, K, L, P, R and Y;

-X(284)- is selected from the group consisting of V, D, E, L, N, Q, T and Y;

-X(285)- is selected from the group consisting of H, D, E, K, Q, W and Y;

-X(286)- is selected from the group consisting of N, E, G, P and Y;

-X(288)- is selected from the group consisting of K, D, E and Y;

-X(290)- is selected from the group consisting of K, D, H, L, N and W;

-X(291)- is selected from the group consisting of P, D, E, G, H, I, Q and T;

-X(292)- is selected from the group consisting of R, D, E, T and Y;

-X(293)- is selected from the group consisting of E, F, G, H, I, L, M, N, P, R, S, T, V, W and Y;

-X(294)- is selected from the group consisting of E, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;

-X(295)- is selected from the group consisting of Q, D, E, F, G, H, I, M, N, P, R, S, T, V, W and Y;

-X(296)- is selected from the group consisting of F, A, D, E, G, I, K, L, M, N, Q, R, S, T and V;

-X(297)- is selected from the group consisting of N, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(298)- is selected from the group consisting of S, E, F, H, I, K, M, Q, R, W and Y;

-X(299)- is selected from the group consisting of T, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W and Y;

-X(300)- is selected from the group consisting of Y, A, D, E, G, H, K, M, N, P, Q, R, S, T, V and W;

-X(301)- is selected from the group consisting of R, D, E, H and Y;

-X(302)- is selected from the group consisting of V and I;

-X(303)- is selected from the group consisting of V, D, E and Y;

-X(304)- is selected from the group consisting of S, D, H, L, N and T;

-X(305)- is selected from the group consisting of V, E, T and Y;

-X(313)- is selected from the group consisting of W and F;

-X(317)- is selected from the group consisting of K, E and Q;

-X(318)- is selected from the group consisting of E, H, L, Q, R and Y;

-X(320)- is selected from the group consisting of K, D, F, G, H, I, L, N, P, S, T, V, W and Y;

-X(322)- is selected from the group consisting of K, D, F, G, H, I, P, S, T, V, W and Y;

-X(323)- is selected from the group consisting of V and I;

-X(324)- is selected from the group consisting of S, D, F, G, H, I, L, M, P, R, T, V, W and Y;

-X(325)- is selected from the group consisting of N, A, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(326)- is selected from the group consisting of K, I, L, P and T;

-X(327)- is selected from the group consisting of G, D, E, F, H, I, K, L, M, N, P, R, T, V, W and Y;

-X(328)- is selected from the group consisting of L, A, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;

-X(329)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(330)- is selected from the group consisting of S, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(331)- is selected from the group consisting of S, D, F, H, I, L, M, Q, R, T, V, W and Y;

-X(332)- is selected from the group consisting of I, A, D, E, F, H, K, L, M, N, P, Q, R, S, T, V, W and Y;

-X(333)- is selected from the group consisting of E, F, H, I, L, M, P, T and Y;

-X(334)- is selected from the group consisting of K, F, I, P and T;

-X(335)- is selected from the group consisting of T, D, F, G, H, I, L, M, N, P, R, S, V, W and Y;

-X(336)- is selected from the group consisting of I, E, K and Y; and

-X(337)- is selected from the group consisting of S, E, H and N.

[74] In another aspect, the present application is directed to an IgG4 variant including an amino acid sequence having the formula:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYG-X(221)-PPC-X(227)-SCPAPE-X(234)-X(235)-
X(236)-X(237)-P-X(239)-X(240)-FLFPP-X(246)-PKDTLMIS-X(255)-TP-X(258)-V-X(260)-CVV-X(264)-
DV-X(267)-X(268)-ED-X(271)-X(272)-V-X(274)-FNW-X(278)-VD-X(281)-V-X(283)-X(284)-HNAKT-
X(290)-PR-X(293)-E-X(295)-FNSTYRVV-X(304)-VLTVLHQDWLNGKEYKCKV-X(324)-N-X(326)-
X(327)-X(328)-P-X(330)-S-X(332)-X(333)-X(334)-
TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK wherein

-X(221)- is selected from the group consisting of no amino acid and K;

-X(227)- is selected from the group consisting of P and G;

-X(234)- is selected from the group consisting of F, Y and I;

-X(235)- is selected from the group consisting of L, Y, I and D;

-X(236)- is selected from the group consisting of G, S and A;

-X(237)- is selected from the group consisting of G and D;

-X(239)- is selected from the group consisting of S, D, E, N, Q and T;

-X(240)- is selected from the group consisting of V, I and M;

-X(246)- is selected from the group consisting of K, H and Y;

-X(255)- is selected from the group consisting of R and Y;

-X(258)- is selected from the group consisting of E, H and Y;

-X(260)- is selected from the group consisting of T and H;

-X(264)- is selected from the group consisting of V, I, T and Y;

-X(267)- is selected from the group consisting of S, D and E;

-X(268)- is selected from the group consisting of Q, D and E;

-X(271)- is selected from the group consisting of P and G;

-X(272)- is selected from the group consisting of E, Y, H, R and I;

-X(274)- is selected from the group consisting of Q and E;

-X(278)- is selected from the group consisting of Y and T;
-X(281)- is selected from the group consisting of G, D and E;
-X(283)- is selected from the group consisting of E, L and H;
-X(284)- is selected from the group consisting of V, E and D;
-X(290)- is selected from the group consisting of K and N;
-X(293)- is selected from the group consisting of E and R;
-X(295)- is selected from the group consisting of Q and E;
-X(304)- is selected from the group consisting of S and T;
-X(324)- is selected from the group consisting of S, G and I;
-X(326)- is selected from the group consisting of K and T;
-X(327)- is selected from the group consisting of G and D;
-X(328)- is selected from the group consisting of L, A, F, I and T;
-X(330)- is selected from the group consisting of S, L, Y and I;
-X(332)- is selected from the group consisting of I, D, E, N, Q and T;
-X(333)- is selected from the group consisting of E and Y; and
-X(334)- is selected from the group consisting of K, F, I and T.

[75] In certain variations, the variant differs from SEQ ID NO:14 by at least one amino acid.

[76] Variations in which modifications are in 2, 3, or 4 different domains, the domains can be selected from among, for example, all IgG domains, only IgG heavy chain domains, and only hinge-CH2-CH3 domains. Alternatively, the domains can be limited to include only Fc region, or only CH2-CH3 domains.

[77] The IgG2, IgG3, or IgG4 variants can improves binding to one or more FcγR, or enhance effector function as compared to a polypeptide having the amino acid sequence of SEQ ID NO:12, SEQ ID NO:13, or SEQ ID NO:14. In certain variations, FcγR is selected from the group consisting of human FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, and FcγRIIIa. In other variations, the additionally reduces binding to human FcγRIIb. Exemplary effector function that is enhanced can be ADCC, ADCP, and CDC.

[78] The present application is also directed to sequence including the variants described herein identified by sequence identification number.

BRIEF DESCRIPTION OF THE DRAWINGS

[79] Figure 1. Antibody structure and function. Shown is a model of a full length human IgG1 antibody, modeled using a humanized Fab structure from pdb accession code 1CE1 (James *et al.*, 1999, *J Mol Biol* 289:293-301) and a human IgG1 Fc structure from pdb accession code 1DN2 (DeLano *et al.*, 2000, *Science* 287:1279-1283). The flexible hinge that links the Fab and Fc regions is not shown. IgG1 is a homodimer of heterodimers, made up of two light chains and two heavy chains. The Ig domains that comprise the antibody are labeled, and include VL and CL for the light chain, and V_H, CH1 (Cγ1), CH2 (Cγ2), and CH3 (Cγ3) for the heavy chain. The Fc region is labeled. Binding sites

for relevant proteins are labeled, including the antigen binding site in the variable region, and the binding sites for FcγRs, FcRn, C1q, and proteins A and G in the Fc region.

[80] Figure 2. The Fc/FcγRIIIb complex structure 1IIS. Fc is shown as a gray ribbon diagram, and FcγRIIIb is shown as a black ribbon. The N297 carbohydrate is shown as black sticks.

[81] Figure 3. Preferred embodiments of receptor binding profiles that include improvements to, reductions to, or no effect to the binding to various receptors, where such changes may be beneficial in certain contexts.

[82] Figure 4. Data for binding of IgG1 Fc variants to human FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, V158 FcγRIIIa, C1q, and FcRn. The table presents for each variant the variant number (Variant), the substitution(s) of the variant, the antibody context (Context), the fold affinity relative to WT (Fold) and the confidence (Conf) in the fold affinity for binding to each Fc ligand, and the IIIa:IIb specificity ratio (IIIa:IIb) (see below). Multiple data sets were acquired for many of the variants, and all data for a given variant are grouped together. The context of the antibody indicates which antibodies have been constructed with the particular Fc variant; a = alemtuzumab, t = trastuzumab, r = rituximab, c= cetuximab, and p = PRO70769. The data provided were acquired in the context of the first antibody listed, typically alemtuzumab, although in some cases trastuzumab. An asterix (*) indicates that the data for the given Fc ligand was acquired in the context of trastuzumab. A fold (Fold) above 1 indicates an enhancement in binding affinity, and a fold below 1 indicates a reduction in binding affinity relative to the parent antibody for the given Fc ligand. Confidence values (Conf) correspond to the log confidence levels, provided from the fits of the data to a sigmoidal dose response curve. As is known in the art, a lower Conf value indicates lower error and greater confidence in the Fold value. The lack of data for a given variant and Fc ligand indicates either that the fits to the data did not provide a meaningful value, or that the variant was not tested for that particular Fc ligand.

[83] Figure 5. Data for binding of IgG1 Fc variants to human V158 and F158 FcγRIIIa by AlphaScreen, binding to human V158 FcγRIIIa by SPR, and ADCC in the presence of human effector cells. The values are the fold-affinity (AlphaScreen and SPR) and fold-EC50 (ADCC) relative to WT. Context indicates the antibody variable region in which the data was acquired: a = alemtuzumab, t = trastuzumab, r = rituximab, c= cetuximab, and p = PRO70769.

[84] Figure 6. Non-naturally occurring modifications provided in Figure 4, listed according to EU position. Modifications in bolded grey indicate preferred modifications.

[85] Figure 7. Alignment of the human IgG immunoglobulin IgG1, IgG2, IgG3, and IgG4 amino acid sequences. Figure 7a provides the sequences of the CH1 domain and hinge region, and Figure 7b provides the sequences of the CH2 and CH3 domains. Positions are numbered according to the EU index, and differences between IgG1 and the other immunoglobulins IgG2, IgG3, and IgG4 are shown in grey. In Figure 7a the N-terminal end of the Fc region is indicated at EU position 226.

[86] Figure 8. Allotypes and isoallotypes of the human IgG1 constant chain showing the positions and the relevant amino acid substitutions (Gorman & Clark, 1990, Semin Immunol 2(6):457-66). For

comparison the amino acids found in the equivalent positions in human IgG2, IgG3 and IgG4 gamma chains are also shown.

[87] Figures 9a – 9b. Structure of the complex of human IgG1 Fc bound to human FcγRIIIb (pdb accession code 1E4K, Sondermann *et al.*, 2000, Nature 406:267-273), highlighting differences between IgG1 and IgG2 (Figure 9a), and between IgG1 and IgG4 (Figure 9b). IgG1 Fc is shown as grey ribbon, FcγRIIIb is shown as black ribbon, and IgG1 residues that differ in amino acid identity from IgG2 (Figure 9a) and IgG4 (Figure 9b) are shown as black sticks.

[88] Figures 10a and 10b. Competition AlphaScreen™ assay showing binding of IgG1, IgG2, and IgG4 isotypes to V158 FcγRIIIa (Figure 10a) and protein A (Figure 10b). The variable region of the antibodies is that of the anti-Her2 antibody trastuzumab. In the presence of competitor antibody, a characteristic inhibition curve is observed as a decrease in luminescence signal. These data were normalized to the maximum and minimum luminescence signal provided by the baselines at low and high concentrations of competitor antibody respectively. The curves represent the fits of the data to a one site competition model using nonlinear regression.

[89] Figures 11a – 11b. Competition AlphaScreen assay showing binding of WT and variant IgG1, IgG2, and IgG4 antibodies to human V158 FcγRIIIa (Figure 11a) and human FcγRI (Figure 11b). The variable region of the antibodies is that of the anti-Her2 antibody trastuzumab.

[90] Figure 12. SPR (Surface Plasmon Resonance) data showing binding of WT and variant IgG1, IgG2, and IgG4 antibodies to human V158 FcγRIIIa. The variable region of the antibodies is that of the anti-Her2 antibody trastuzumab.

[91] Figures 13a – 13b. IgG1 variants with isotypic and/or novel amino acid modifications. The amino acid sequences of the human immunoglobulin isotypes IgG1, IgG2, IgG3, and IgG4 are aligned according to Figure 7. Figure 13a provides the sequences of the CH1 domain and hinge regions, and Figure 13b provides the sequences of the CH2 and CH3 domains. The sequence of IgG1 is provided explicitly, and residues in the rows labeled "IgG2", "IgG3", and "IgG4" provide the amino acid identity at EU positions where they differ from IgG1; these modifications are isotypic modifications. Residues listed in the rows labeled "Novel" indicate novel modifications for human IgG1; these novel modifications are those indicated as preferred in Figure 6.

[92] Figures 14a – 14b. IgG2 variants with isotypic and/or non-naturally occurring modifications. The amino acid sequences of the human immunoglobulin isotypes IgG2, IgG1, IgG3, and IgG4 are aligned according to Figure 7. Figure 14a provides the sequences of the CH1 domain and hinge regions, and Figure 14b provides the sequences of the CH2 and CH3 domains. The sequence of IgG2 is provided explicitly, and residues in the rows labeled "IgG1", "IgG3", and "IgG4" provide the amino acid identity at EU positions where they differ from IgG2; these modifications are isotypic modifications. Residues listed in the rows labeled "Novel" indicate novel modifications for human IgG2; these novel modifications are those indicated as preferred in Figure 6.

[93] Figures 15a – 15b. IgG3 variants with isotypic and/or non-naturally occurring modifications. The amino acid sequences of the human immunoglobulin isotypes IgG3, IgG1, IgG2, and IgG4 are aligned according to Figure 7. Figure 15a provides the sequences of the CH1 domain and hinge regions, and Figure 15b provides the sequences of the CH2 and CH3 domains. The sequence of IgG3 is provided explicitly, and residues in the rows labeled "IgG1", "IgG2", and "IgG4" provide the amino acid identity at EU positions where they differ from IgG3; these modifications are isotypic modifications. Residues listed in the rows labeled "Novel" indicate novel modifications for human IgG3; these novel modifications are those indicated as preferred in Figure 6.

[94] Figures 16a – 16b. IgG4 variants with isotypic and/or non-naturally occurring modifications. The amino acid sequences of the human immunoglobulin isotypes IgG4, IgG1, IgG2, and IgG3 are aligned according to Figure 7. Figure 16a provides the sequences of the CH1 domain and hinge regions, and Figure 16b provides the sequences of the CH2 and CH3 domains. The sequence of IgG4 is provided explicitly, and residues in the rows labeled "IgG1", "IgG2", and "IgG3" provide the amino acid identity at EU positions where they differ from IgG4; these modifications are isotypic modifications. Residues listed in the rows labeled "Novel" indicate novel modifications for human IgG4; these novel modifications are those indicated as preferred in Figure 6.

[95] Figure 17. Anti-Her2 IgG2 Variants. Novel modifications and isotypic modifications are provided for each variant, all constructed in the context of the human IgG2 isotype. The variable region (VHVL), CH1 domain (CH1), hinge region (hinge), and Fc region (Fc) are described for each variant, and the full constant region is labeled (WT IgG2, IgG2 ELLGG, or IgG(1/2) ELLGG) accordingly.

[96] Figure 18. Competition AlphaScreen assay showing binding of WT and IgG variant antibodies to human V158 FcγRIIIa. The variable region of the antibodies is that of the anti-Her2 antibody trastuzumab.

[97] Figure 19. Anti-CD30 IgG(1/2) ELLGG Variants. Novel modifications and isotypic modifications are provided for each variant. All IgG variants comprise the variable region of the anti-CD30 antibody H3.69_V2_L3.71 AC10. The variants comprise the IgG(1/2) ELLGG constant region as described in Figure 18, and potentially one or more additional isotypic modifications and/or one or more novel modifications.

[98] Figures 20a – 20c. Competition AlphaScreen assay showing binding of WT and variant IgG antibodies to human V158 FcγRIIIa. IgG variants comprise the constant region of either IgG1 or IgG(1/2) ELLGG plus the indicated modifications. With the exception of I332E and S239D/I332E IgG1, all IgG variants comprise the variable region of the anti-CD30 antibody H3.69_V2_L3.71 AC10. Variants I332E IgG1 and S239D/I332E IgG1 comprise the variable region of the anti-CD30 antibody H3.69_L3.71 AC10.

[99] Figure 21. Data for binding of anti-CD30 IgG variants to human V158 FcγRIIIa as measured by the competition AlphaScreen. For each variant are provided the IC50 (M) and Fold IC50 relative to H3.69_V2_L3.71 AC10 IgG1.

[100] Figures 22a – 22d. Cell-based ADCC assay of WT and variant IgGs with the variable region of the anti-CD30 antibody H3.69_V2_L3.71 AC10 or H3.69_L3.71 AC10 (I33E and S239D/I332E IgG1). ADCC was measured by LDH activity using the Cytotoxicity Detection Kit (LDH, Roche Diagnostic Corporation, Indianapolis, IN) or the DELFIA® EuTDA-based cytotoxicity assay (Perkin Elmer, MA). For all assays, target cells were L540 Hodgkin's lymphoma cells and effector cells were human PBMCs. The figures show the dose-dependence of ADCC on antibody concentration for the indicated antibodies, normalized to the minimum and maximum fluorescence signal for each particular curve, provided by the baselines at low and high antibody concentrations respectively. The curves represent the fits of the data to a sigmoidal dose-response model using nonlinear regression.

[101] Figure 23. Anti-CD20 IgG(1/2) ELLGG Variants. Novel modifications and isotypic modifications are provided for each variant. All IgG variants comprise the variable region of the anti-CD20 antibody rituximab.The IgG variants comprise the IgG(1/2) ELLGG constant region and potentially one or more novel modifications.

[102] Figure 24. Cell-based ADCC assay of WT and variant IgGs with the variable region of the anti-CD20 antibody rituximab. ADCC was measured by LDH activity using the Cytotoxicity Detection Kit (LDH, Roche Diagnostic Corporation, Indianapolis, IN) according to the manufacturer's instructions, with WIL2-S lymphoma target cells and human PBMCs as effector cells.

[103] Figure 25. Anti-CD20 IgG(1/2) ELLGG Variants. Novel modifications and isotypic modifications are provided for each variant. All IgG variants comprise the variable region of the anti-CD20 antibody PRO70769. The variants comprise the IgG(1/2) ELLGG constant region and potentially one or more additional isotypic modifications and/or one or more novel modifications.

[104] Figures 26. Competition AlphaScreen assay showing binding of anti-CD20 IgG variant antibodies to human V158 FcγRIIIa. IgG variants comprise the constant region of either IgG1 or IgG(1/2) ELLGG plus the indicated modifications. All IgG variants comprise the variable region of the anti-CD20 antibody PRO70769.

[105] Figure 27. Cell-based ADCC assay of WT and variant IgGs with the variable region of the anti-CD20 antibody PRO70769. ADCC was measured using the DELFIA® EuTDA-based cytotoxicity assay with WIL2-S lymphoma target cells and human PBMCs as effector cells.

[106] Figure 28. Cell-based CDC assay of WT and variant IgGs with the variable region of the anti-CD20 antibody PRO70769. CDC assays were performed using Alamar Blue to monitor lysis of antibody -opsonized WIL2-S lymphoma cells by human serum complement (Quidel, San Diego, CA). The dose-dependence on antibody concentration of complement-mediated lysis is shown, normalized to the minimum and maximum fluorescence signal for each particular curve, provided by the baselines at low and high antibody concentrations respectively. The curves represent the fits of the data to a sigmoidal dose-response model using nonlinear regression.

[107] Figures 29a – 29h. Amino acid sequences of variable light (VL) and heavy (VH) chains used in the present invention.

[108] Figures 30a – 30g. Amino acid sequences of constant light and heavy chains used in the present invention. EU residues 233–236 are bolded in the IgG(1/2) (Figure 30f) and IgG(1/2) ELLGG (Figure 30g) sequences.

Figures 31a – 31d. Amino acid sequences of IgG variant antibodies of the present invention. Figures 31a and 31b provide the light and heavy chains respectively of an anti-CD20 antibody including the constant region IgG(1/2) ELLGG S239D/I332E/G327A. Figures 31c and 31d provide the light and heavy chains respectively of an anti-CD30 antibody including the constant region IgG(1/2) ELLGG S239D/I332E/G327A. EU residues 233–236, 239, 327, and 332 are bolded in the heavy chain sequences in Figures 31b and 31d.

DETAILED DESCRIPTION OF THE INVENTION

[109] In general, therapeutic antibodies have been based on IgG1 subclass, as these generally have the best binding profiles to Fc receptors of the four IgG subclasses. However, the present invention is directed to the use of several methods that result in compositions that confer good binding profiles and/or effector function on non-IgG1 subclasses. In general, there are two types of variations that allow the use of IgG2, IgG3 and IgG4 subclasses in place of IgG1, to achieve similar, and in some cases better binding profiles to Fc receptors and/or effector function. In one general embodiment, IgG subclass modifications are made within different domains of the constant region of the heavy chain (e.g. missing the variable heavy domain; CH1-hinge-CH2-CH3). These fall into two general classes. In the first case, IgG subclass modifications, either as individual amino acid modifications or as "domain swaps", are done. For example, some embodiments of the invention include one IgG subclass backbone with at least two domains exchanged with the same two domains of a different IgG subclass. For example, the invention provides IgG2 backbones with two different IgG1 domains. Alternatively, rather than swapping whole domains, individual amino acids are IgG subclass-modified. Thus, for example, variant IgG2 sequences contain amino acid modifications from the IgG1, IgG3 or IgG4 subclass, or combinations thereof. Similarly, variant IgG3 sequences contain amino acid modifications from the IgG1, IgG2 or IgG4 subclass, or combinations thereof. Variant IgG4 sequences contain amino acid modifications from the IgG1, IgG2 or IgG3 subclass, or combinations thereof. These changes are sometimes referred to herein as "IgG subclass modifications". In some embodiments, these changes may be within one domain (either one or more amino acid modifications), or in the case of a plurality of modifications, between two or more domains.

[110] A second category of variants are non-naturally occurring variants, sometimes referred to herein as "Fc variants" (it should be noted that there is positional overlap between these two groups; however, "Fc variants" do not include the IgG subclass modifications). These are amino acid modifications at particular positions that confer modified binding profiles (and/or effector function) as compared to the parent molecule but are not the specific amino acid changes seen in the different IgG subclasses.

[111] Also included within the invention are combinations of both approaches. Thus, for example, IgG2 variants are provided that have one or more isotypic modifications, in some cases from IgG1, and one or more Fc variants as well.

DEFINITIONS

[112] The present application is directed IgG2, IgG3, and IgG4 variants having amino acid modifications of IgG2, IgG3, and IgG4 sequences.

[113] In order that the application may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

[114] By "ADCC" or "antibody dependent cell-mediated cytotoxicity" as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell.

[115] By "ADCP" or antibody dependent cell-mediated phagocytosis as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell.

[116] By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence.

[117] By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with another amino acid. For example, the substitution E272Y refers to a variant polypeptide, in this case an Fc variant, in which the glutamic acid at position 272 is replaced with tyrosine.

[118] By "amino acid insertion" or "insertion" as used herein is meant the addition of an amino acid at a particular position in a parent polypeptide sequence. For example, -233E designates an insertion of glutamic acid at position 233.

[119] By "amino acid deletion" or "deletion" as used herein is meant the removal of an amino acid at a particular position in a parent polypeptide sequence. For example, E233- designates the deletion of glutamic acid at position 233.

[120] By "variant protein" or "protein variant", or "variant" as used herein is meant a protein that differs from that of a parent protein by virtue of at least one amino acid modification. Protein variant may refer to the protein itself, a composition comprising the protein, or the amino sequence that encodes it. Preferably, the protein variant has at least one amino acid modification compared to the parent protein, e.g. from about one to about ten amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. The protein variant sequence herein will preferably possess at least about 80% homology with a parent protein sequence, and most preferably at least about 90% homology, more preferably at least about 95% homology. Variant protein can refer to the variant protein itself, compositions comprising the protein variant, or the amino acid sequence that encodes it. Accordingly, by "antibody variant" or "variant antibody" as used herein

is meant an antibody that differs from a parent antibody by virtue of at least one amino acid modification, "IgG variant" or "variant IgG" as used herein is meant an antibody that differs from a parent IgG by virtue of at least one amino acid modification, and "immunoglobulin variant" or "variant immunoglobulin" as used herein is meant an immunoglobulin sequence that differs from that of a parent immunoglobulin sequence by virtue of at least one amino acid modification.

[121] By "Fab" or "Fab region" as used herein is meant the polypeptides that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody or antibody fragment.

[122] By "IgG subclass modification" as used herein is meant an amino acid modification that converts one amino acid of one IgG isotype to the corresponding amino acid in a different, aligned IgG isotype. For example, because IgG1 comprises a tyrosine and IgG2 a phenylalanine at EU position 296, a F296Y substitution in IgG2 is considered an IgG subclass modification.

[123] By "non-naturally occurring modification" as used herein is meant an amino acid modification that is not isotypic. For example, because none of the IgGs comprise a glutamic acid at position 332, the substitution I332E in IgG1, IgG2, IgG3, or IgG4 is considered a non-naturally occuring modification.

[124] By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids or any non-natural analogues that may be present at a specific, defined position.

[125] By "effector function" as used herein is meant a biochemical event that results from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include but are not limited to ADCC, ADCP, and CDC.

[126] By "effector cell" as used herein is meant a cell of the immune system that expresses one or more Fc receptors and mediates one or more effector functions. Effector cells include but are not limited to monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, B cells, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, and γδT cells, and may be from any organism including but not limited to humans, mice, rats, rabbits, and monkeys.

[127] By "IgG Fc ligand" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an IgG antibody to form an Fc / Fc ligand complex. Fc ligands include but are not limited to FcγRs, FcγRs, FcγRs, FcRn, C1q, C3, mannan binding lectin, mannose receptor, *staphylococcal* protein A, *streptococcal* protein G, and viral FcγR. Fc ligands also include Fc receptor homologs (FcRH), which are a family of Fc receptors that are homologous to the FcγRs (Davis *et al.*, 2002, *Immunological Reviews* 190:123-136). Fc ligands may include undiscovered molecules that bind Fc. Particular IgG Fc ligands are Fc gamma receptors.

[128] By "Fc gamma receptor" or "FcγR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene. In humans this family includes but is not limited to FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII

(CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIIb-NA1 and FcγRIIIb-NA2) (Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65), as well as any undiscovered human FcγRs or FcγR isoforms or allotypes. An FcγR may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. Mouse FcγRs include but are not limited to FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16), and FcγRIII-2 (CD16-2), as well as any undiscovered mouse FcγRs or FcγR isoforms or allotypes.

[129] By "parent polypeptide" as used herein is meant an unmodified polypeptide that is subsequently modified to generate a variant. The parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it. Accordingly, by "parent immunoglobulin" as used herein is meant an unmodified immunoglobulin polypeptide that is modified to generate a variant, and by "parent antibody" as used herein is meant an unmodified antibody that is modified to generate a variant antibody.

[130] By "position" as used herein is meant a location in the sequence of a protein. Positions may be numbered sequentially, or according to an established format, for example the EU index as in Kabat. For example, position 297 is a position in the human antibody IgG1.

[131] By "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides.

[132] By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297, also referred to as N297) is a residue in the human antibody IgG1.

[133] By "target antigen" as used herein is meant the molecule that is bound specifically by the variable region of a given antibody. A target antigen may be a protein, carbohydrate, lipid, or other chemical compound.

[134] By "target cell" as used herein is meant a cell that expresses a target antigen.

[135] By "variable region" as used herein is meant the region of an immunoglobulin that comprises one or more Ig domains substantially encoded by any of the Vκ, Vλ, and/or VH genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively.

[136] By "wild type or WT" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

Antibodies

[137] Accordingly, the present invention provides variant antibodies.

[138] Traditional antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. IgM has subclasses, including, but not limited to, IgM1 and IgM2. Thus, "isotype" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. The known human immunoglobulin isotypes are IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM1, IgM2, IgD, and IgE.

[139] The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. In the variable region, three loops are gathered for each of the V domains of the heavy chain and light chain to form an antigen-binding site. Each of the loops is referred to as a complementarity-determining region (hereinafter referred to as a "CDR"), in which the variation in the amino acid sequence is most significant.

[140] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Kabat et al. collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5th edition, NIH publication, No. 91-3242, E.A. Kabat et al.).

[141] In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-220 according to the EU index as in Kabat. "CH2" refers to positions 237-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat.

[142] Another type of Ig domain of the heavy chain is the hinge region. By "hinge" or "hinge region" or "antibody hinge region" or "immunoglobulin hinge region" herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for IgG the antibody hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the "lower hinge" generally referring to positions 226 or 230.

[143] Of particular interest in the present invention are the Fc regions. By "Fc" or "Fc region", as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain and in some cases, part of the hinge. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, as illustrated in Figure 1, Fc comprises immunoglobulin domains Cgamma2 and Cgamma3 (Cg2 and Cg3) and the lower hinge region between Cgamma1 (Cg1) and Cgamma2 (Cg2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. Fc may refer to this region in isolation, or this region in the context of an Fc polypeptide, as described below. By "Fc polypeptide" as used herein is meant a polypeptide that comprises all or part of an Fc region. Fc polypeptides include antibodies, Fc fusions, isolated Fcs, and Fc fragments.

[144] In some embodiments, the antibodies are full length. By "full length antibody" herein is meant the structure that constitutes the natural biological form of an antibody, including variable and constant regions, including one or more modifications as outlined herein.

[145] Alternatively, the antibodies can be a variety of structures, including, but not limited to, antibody fragments, monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), chimeric antibodies, humanized antibodies, antibody fusions (sometimes referred to as "antibody conjugates"), and fragments of each, respectively.

Antibody Fragments

[146] In one embodiment, the antibody is an antibody fragment. Of particular interest are antibodies that comprise Fc regions, Fc fusions, and the constant region of the heavy chain (CH1-hinge-CH2-CH3), again also including constant heavy region fusions.

[147] Specific antibody fragments include, but are not limited to, (i) the Fab fragment consisting of VL, VH, CL and CH1 domains, (ii) the Fd fragment consisting of the VH and CH1 domains, (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward et al., 1989, Nature 341:544-546) which consists of a single variable, (v) isolated CDR regions, (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al., 1988, Science 242:423-426, Huston et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883), (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies" or "triabodies", multivalent or multispecific fragments constructed by gene fusion (Tomlinson et. al., 2000, Methods Enzymol. 326:461-479; WO94/13804; Holliger et al., 1993, Proc. Natl. Acad. Sci. U.S.A. 90:6444-6448). The antibody fragments may be modified. For example, the molecules may be stabilized by the incorporation of disulphide bridges linking the VH and VL domains (Reiter et al., 1996, Nature Biotech. 14:1239-1245).

Chimeric and Humanized Antibodies

[148] In some embodiments, the scaffold components can be a mixture from different species. As such, if the antibody is an antibody, such antibody may be a chimeric antibody and/or a humanized antibody. In general, both "chimeric antibodies" and "humanized antibodies" refer to antibodies that combine regions from more than one species. For example, "chimeric antibodies" traditionally comprise variable region(s) from a mouse (or rat, in some cases) and the constant region(s) from a human. "Humanized antibodies" generally refer to non-human antibodies that have had the variable-domain framework regions swapped for sequences found in human antibodies. Generally, in a humanized antibody, the entire antibody, except the CDRs, is encoded by a polynucleotide of human origin or is identical to such an antibody except within its CDRs. The CDRs, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, e.g., WO 92/11018, Jones, 1986, Nature 321:522-525, Verhoeyen et al., 1988, Science 239:1534-1536. "Backmutation" of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (US 5530101; US 5585089; US 5693761; US 5693762; US 6180370; US 5859205; US 5821337; US 6054297; US 6407213). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, and thus will typically comprise a human Fc region. Humanized antibodies can also be generated using mice with a genetically engineered immune system. Roque et al., 2004, Biotechnol. Prog. 20:639-654. A variety of techniques and methods for humanizing and reshaping non-human antibodies are well known in the art (See Tsurushita & Vasquez, 2004, Humanization of Monoclonal Antibodies, Molecular Biology of B Cells, 533-545, Elsevier Science (USA), and references cited therein). Humanization methods include but are not limited to methods described in Jones *et al.*, 1986, *Nature* 321:522-525; Riechmann *et al.*,1988; *Nature* 332:323-329; Verhoeyen *et al.*, 1988, *Science*, 239:1534-1536; *Queen et al.*, 1989, *Proc Natl Acad Sci*, USA 86:10029-33; He et al., 1998, J. Immunol. 160: 1029-1035; Carter *et al.*, 1992, *Proc Natl Acad Sci USA 89:4285-9*, Presta et al., 1997, Cancer Res.57(20):4593-9; Gorman et al., 1991, Proc. Natl. Acad. Sci. USA 88:4181-4185; O'Connor *et al.*, 1998, *Protein Eng* 11:321-8. Humanization or other methods of reducing the immunogenicity of nonhuman antibody variable regions may include resurfacing methods, as described for example in Roguska et al., 1994, Proc. Natl. Acad. Sci. USA 91:969-973. In one embodiment, the parent antibody has been affinity matured, as is known in the art. Structure-based methods may be employed for humanization and affinity maturation, for example as described in USSN 11/004,590. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu et al., 1999, J. Mol. Biol. 294:151-162; Baca et al., 1997, J. Biol. Chem. 272(16):10678-10684; Rosok et al., 1996, J. Biol. Chem. 271(37): 22611-22618; Rader et al., 1998, Proc. Natl. Acad. Sci. USA 95: 8910-8915; Krauss et al., 2003, Protein Engineering 16(10):753-759. Other humanization methods may involve the grafting of only parts of the CDRs, including but not

limited to methods described in USSN 09/810,502; Tan et al., 2002, J. Immunol. 169:1119-1125; De Pascalis et al., 2002, J. Immunol. 169:3076-3084.

Bispecific Antibodies

[149] In one embodiment, the antibodies of the invention multispecific antibody, and notably a bispecific antibody, also sometimes referred to as "diabodies". These are antibodies that bind to two (or more) different antigens. Diabodies can be manufactured in a variety of ways known in the art (Holliger and Winter, 1993, Current Opinion Biotechnol. 4:446-449), e.g., prepared chemically or from hybrid hybridomas.

Minibodies

[150] In one embodiment, the antibody is a minibody. Minibodies are minimized antibody-like proteins comprising a scFv joined to a CH3 domain. Hu et al., 1996, Cancer Res. 56:3055-3061. In some cases, the scFv can be joined to the Fc region, and may include some or all of the hinge region.

Human Antibodies

[151] In one embodiment, the antibody is a fully human antibody with at least one modification as outlined herein. "Fully human antibody " or "complete human antibody" refers to a human antibody having the gene sequence of an antibody derived from a human chromosome with the modifications outlined herein.

Antibody Fusions

[152] In one embodiment, the antibodies of the invention are antibody fusion proteins (sometimes referred to herein as an "antibody conjugate"). One type of antibody fusions are Fc fusions, which join the Fc region with a conjugate partner. By "Fc fusion" as used herein is meant a protein wherein one or more polypeptides is operably linked to an Fc region. Fc fusion is herein meant to be synonymous with the terms "immunoadhesin", "Ig fusion", "Ig chimera", and "receptor globulin" (sometimes with dashes) as used in the prior art (Chamow et al., 1996, Trends Biotechnol 14:52-60; Ashkenazi et al., 1997, Curr Opin Immunol 9:195-200). An Fc fusion combines the Fc region of an immunoglobulin with a fusion partner, which in general can be any protein or small molecule. Virtually any protein or small molecule may be linked to Fc to generate an Fc fusion. Protein fusion partners may include, but are not limited to, the variable region of any antibody, the target-binding region of a receptor, an adhesion molecule, a ligand, an enzyme, a cytokine, a chemokine, or some other protein or protein domain. Small molecule fusion partners may include any therapeutic agent that directs the Fc fusion to a therapeutic target. Such targets may be any molecule, preferably an extracellular receptor, that is implicated in disease.

[153] In addition to Fc fusions, antibody fusions include the fusion of the constant region of the heavy chain with one or more fusion partners (again including the variable region of any antibody), while other antibody fusions are substantially or completely full length antibodies with fusion partners. In one embodiment, a role of the fusion partner is to mediate target binding, and thus it is functionally analogous to the variable regions of an antibody (and in fact can be). Virtually any protein or small

molecule may be linked to Fc to generate an Fc fusion (or antibody fusion). Protein fusion partners may include, but are not limited to, the target-binding region of a receptor, an adhesion molecule, a ligand, an enzyme, a cytokine, a chemokine, or some other protein or protein domain. Small molecule fusion partners may include any therapeutic agent that directs the Fc fusion to a therapeutic target. Such targets may be any molecule, preferably an extracellular receptor, that is implicated in disease.

[154] The conjugate partner can be proteinaceous or non-proteinaceous; the latter generally being generated using functional groups on the antibody and on the conjugate partner. For example linkers are known in the art; for example, homo-or hetero-bifunctional linkers as are well known (see, 1994 Pierce Chemical Company catalog, technical section on cross-linkers, pages 155-200, incorporated herein by reference).

[155] Suitable conjugates include, but are not limited to, labels as described below, drugs and cytotoxic agents including, but not limited to, cytotoxic drugs (e.g., chemotherapeutic agents) or toxins or active fragments of such toxins. Suitable toxins and their corresponding fragments include diptheria A chain, exotoxin A chain, ricin A chain, abrin A chain, curcin, crotin, phenomycin, enomycin and the like. Cytotoxic agents also include radiochemicals made by conjugating radioisotopes to antibodies, or binding of a radionuclide to a chelating agent that has been covalently attached to the antibody. Additional embodiments utilize calicheamicin, auristatins, geldanamycin, maytansine, and duocarmycins and analogs; for the latter, see U.S. 2003/0050331, hereby incorporated by reference in its entirety.

Covalent modifications of Antibodies

[156] Covalent modifications of antibodies are included within the scope of this invention, and are generally, but not always, done post-translationally. For example, several types of covalent modifications of the antibody are introduced into the molecule by reacting specific amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

[157] Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

[158] Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

[159] Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride;

trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

[160] Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

[161] The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using 125I or 131I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

[162] Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'—N=C=N--R'), where R and R' are optionally different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

[163] Derivatization with bifunctional agents is useful for crosslinking antibodies to a water-insoluble support matrix or surface for use in a variety of methods, in addition to methods described below. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis (succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

[164] Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

[165] Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, pp. 79-86 [1983]), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Glycosylation

[166] Another type of covalent modification is glycosylation. In another embodiment, the IgG variants disclosed herein can be modified to include one or more engineered glycoforms. By "engineered glycoform" as used herein is meant a carbohydrate composition that is covalently attached to an IgG, wherein said carbohydrate composition differs chemically from that of a parent IgG. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. Engineered glycoforms may be generated by a variety of methods known in the art (Umaña et al., 1999, Nat Biotechnol 17:176-180; Davies et al., 2001, Biotechnol Bioeng 74:288-294; Shields et al., 2002, J Biol Chem 277:26733-26740; Shinkawa et al., 2003, J Biol Chem 278:3466-3473); (US 6,602,684; USSN 10/277,370; USSN 10/113,929; PCT WO 00/61739A1; PCT WO 01/29246A1; PCT WO 02/31140A1; PCT WO 02/30954A1); (Potelligent™ technology [Biowa, Inc., Princeton, NJ]; GlycoMAb™ glycosylation engineering technology [GLYCART biotechnology AG, Zürich, Switzerland]). Many of these techniques are based on controlling the level of fucosylated and/or bisecting oligosaccharides that are covalently attached to the Fc region, for example by expressing an IgG in various organisms or cell lines, engineered or otherwise (for example Lec-13 CHO cells or rat hybridoma YB2/0 cells), by regulating enzymes involved in the glycosylation pathway (for example FUT8 [α1,6-fucosyltranserase] and/or β1-4- N-acetylglucosaminyltransferase III [GnTIII]), or by modifying carbohydrate(s) after the IgG has been expressed. Engineered glycoform typically refers to the different carbohydrate or oligosaccharide; thus an IgG variant, for example an antibody or Fc fusion, can include an engineered glycoform. Alternatively, engineered glycoform may refer to the IgG variant that comprises the different carbohydrate or oligosaccharide. As is known in the art, glycosylation patterns can depend on both the sequence of the protein (e.g., the presence or absence of particular glycosylation amino acid residues, discussed below), or the host cell or organism in which the protein is produced. Particular expression systems are discussed below.

[167] Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[168] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the starting sequence (for O-linked glycosylation sites). For ease, the antibody amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

[169] Another means of increasing the number of carbohydrate moieties on the antibody is by chemical or enzymatic coupling of glycosides to the protein. These procedures are advantageous in that they do not require production of the protein in a host cell that has glycosylation capabilities for N- and O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston, 1981, CRC Crit. Rev. Biochem., pp. 259-306.

[170] Removal of carbohydrate moieties present on the starting antibody may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the protein to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin et al., 1987, Arch. Biochem. Biophys. 259:52 and by Edge et al., 1981, Anal. Biochem. 118:131. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., 1987, Meth. Enzymol. 138:350. Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin et al., 1982, J. Biol. Chem. 257:3105. Tunicamycin blocks the formation of protein-N-glycoside linkages.

[171] Another type of covalent modification of the antibody comprises linking the antibody to various nonproteinaceous polymers, including, but not limited to, various polyols such as polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the manner set forth in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337. In addition, as is known in the art, amino acid substitutions may be made in various positions within the antibody to facilitate the addition of polymers such as PEG. See for example, U.S. Publication No. 2005/0114037, incorporated herein by reference in its entirety.

Labeled Antibodies

[172] In some embodiments, the covalent modification of the antibodies of the invention comprises the addition of one or more labels. In some cases, these are considered antibody fusions.

[173] The term "labelling group" means any detectable label. In some embodiments, the labelling group is coupled to the antibody via spacer arms of various lengths to reduce potential steric hindrance. Various methods for labelling proteins are known in the art and may be used in performing the present invention.

[174] In general, labels fall into a variety of classes, depending on the assay in which they are to be detected: a) isotopic labels, which may be radioactive or heavy isotopes; b) magnetic labels (e.g., magnetic particles); c) redox active moieties; d) optical dyes; enzymatic groups (e.g. horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase); e) biotinylated groups; and f)

predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags, etc.). In some embodiments, the labelling group is coupled to the antibody via spacer arms of various lengths to reduce potential steric hindrance. Various methods for labelling proteins are known in the art and may be used in performing the present invention.

[175] Specific labels include optical dyes, including, but not limited to, chromophores, phosphors and fluorophores, with the latter being specific in many instances. Fluorophores can be either "small molecule" fluores, or proteinaceous fluores.

[176] By "fluorescent label" is meant any molecule that may be detected via its inherent fluorescent properties. Suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade BlueJ, Texas Red, IAEDANS, EDANS, BODIPY FL, LC Red 640, Cy 5, Cy 5.5, LC Red 705, Oregon green, the Alexa-Fluor dyes (Alexa Fluor 350, Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 546, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 660, Alexa Fluor 680), Cascade Blue, Cascade Yellow and R-phycoerythrin (PE) (Molecular Probes, Eugene, OR), FITC, Rhodamine, and Texas Red (Pierce, Rockford, IL), Cy5, Cy5.5, Cy7 (Amersham Life Science, Pittsburgh, PA). Suitable optical dyes, including fluorophores, are described in Molecular Probes Handbook by Richard P. Haugland, hereby expressly incorporated by reference.

[177] Suitable proteinaceous fluorescent labels also include, but are not limited to, green fluorescent protein, including a Renilla, Ptilosarcus, or Aequorea species of GFP (Chalfie et al., 1994, Science 263:802-805), EGFP (Clontech Laboratories, Inc., Genbank Accession Number U55762), blue fluorescent protein (BFP, Quantum Biotechnologies, Inc. 1801 de Maisonneuve Blvd. West, 8th Floor, Montreal, Quebec, Canada H3H 1J9; Stauber, 1998, Biotechniques 24:462-471; Heim et al., 1996, Curr. Biol. 6:178-182), enhanced yellow fluorescent protein (EYFP, Clontech Laboratories, Inc.), luciferase (Ichiki et al., 1993, J. Immunol. 150:5408-5417), β galactosidase (Nolan et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:2603-2607) and Renilla (WO92/15673, WO95/07463, WO98/14605, WO98/26277, WO99/49019, U.S. Patent Nos. 5292658, 5418155, 5683888, 5741668, 5777079, 5804387, 5874304, 5876995, 5925558). All of the above-cited references are expressly incorporated herein by reference.

IgG Variants

[178] In one embodiment, the invention provides variant IgG proteins. At a minimum, IgG variants comprise an antibody fragment comprising the CH2-CH3 region of the heavy chain. In addition, suitable IgG variants comprise Fc domains (e.g. including the lower hinge region), as well as IgG variants comprising the constant region of the heavy chain (CH1-hinge-CH2-CH3) also being useful in the present invention, all of which can be fused to fusion partners.

[179] An IgG variant includes one or more amino acid modifications relative to a parent IgG polypeptide, in some cases relative to the wild type IgG. The IgG variant can have one or more optimized properties. An IgG variant differs in amino acid sequence from its parent IgG by virtue of at

least one amino acid modification. Thus IgG variants have at least one amino acid modification compared to the parent. Alternatively, the IgG variants may have more than one amino acid modification as compared to the parent, for example from about one to fifty amino acid modifications, preferably from about one to ten amino acid modifications, and most preferably from about one to about five amino acid modifications compared to the parent.

[180] Thus the sequences of the IgG variants and those of the parent Fc polypeptide are substantially homologous. For example, the variant IgG variant sequences herein will possess about 80% homology with the parent IgG variant sequence, preferably at least about 90% homology, and most preferably at least about 95% homology. Modifications may be made genetically using molecular biology, or may be made enzymatically or chemically.

[181] Virtually any antigen may be targeted by the IgG variants, including but not limited to proteins, subunits, domains, motifs, and/or epitopes belonging to the following list of target antigens: 17-IA, 4-1BB, 4Dc, 6-keto-PGF1a, 8-iso-PGF2a, 8-oxo-dG, A1 Adenosine Receptor, A33, ACE, ACE-2, Activin, Activin A, Activin AB, Activin B, Activin C, Activin RIA, Activin RIA ALK-2, Activin RIB ALK-4, Activin RIIA, Activin RIIB, ADAM, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADAM8, ADAM9, ADAMTS, ADAMTS4, ADAMTS5, Addressins, aFGF, ALCAM, ALK, ALK-1, ALK-7, alpha-1-antitrypsin, alpha-V/beta-1 antagonist, ANG, Ang, APAF-1, APE, APJ, APP, APRIL, AR, ARC, ART, Artemin, anti-Id, ASPARTIC, Atrial natriuretic factor, av/b3 integrin, Axl, b2M, B7-1, B7-2, B7-H, B-lymphocyte Stimulator (BlyS), BACE, BACE-1, Bad, BAFF, BAFF-R, Bag-1, BAK, Bax, BCA-1, BCAM, Bcl, BCMA, BDNF, b-ECGF, bFGF, BID, Bik, BIM, BLC, BL-CAM, BLK, BMP, BMP-2 BMP-2a, BMP-3 Osteogenin, BMP-4 BMP-2b, BMP-5, BMP-6 Vgr-1, BMP-7 (OP-1), BMP-8 (BMP-8a, OP-2), BMPR, BMPR-IA (ALK-3), BMPR-IB (ALK-6), BRK-2, RPK-1, BMPR-II (BRK-3), BMPs, b-NGF, BOK, Bombesin, Bone-derived neurotrophic factor, BPDE, BPDE-DNA, BTC, complement factor 3 (C3), C3a, C4, C5, C5a, C10, CA125, CAD-8, Calcitonin, cAMP, carcinoembryonic antigen (CEA), carcinoma-associated antigen, Cathepsin A, Cathepsin B, Cathepsin C/DPPI, Cathepsin D, Cathepsin E, Cathepsin H, Cathepsin L, Cathepsin O, Cathepsin S, Cathepsin V, Cathepsin X/Z/P, CBL, CCI, CCK2, CCL, CCL1, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/10, CCR, CCR1, CCR10, CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CD1, CD2, CD3, CD3E, CD4, CD5, CD6, CD7, CD8, CD10, CD11a, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD25, CD27L, CD28, CD29, CD30, CD30L, CD32, CD33 (p67 proteins), CD34, CD38, CD40, CD40L, CD44, CD45, CD46, CD49a, CD52, CD54, CD55, CD56, CD61, CD64, CD66e, CD74, CD80 (B7-1), CD89, CD95, CD123, CD137, CD138, CD140a, CD146, CD147, CD148, CD152, CD164, CEACAM5, CFTR, cGMP, CINC, *Clostridium botulinum* toxin, *Clostridium perfringens* toxin, CKb8-1, CLC, CMV, CMV UL, CNTF, CNTN-1, COX, C-Ret, CRG-2, CT-1, CTACK, CTGF, CTLA-4, CX3CL1, CX3CR1, CXCL, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCR, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, cytokeratin tumor-associated antigen, DAN, DCC, DcR3, DC-SIGN, Decay accelerating factor, des(1-

3)-IGF-I (brain IGF-1), Dhh, digoxin, DNAM-1, Dnase, Dpp, DPPIV/CD26, Dtk, ECAD, EDA, EDA-A1, EDA-A2, EDAR, EGF, EGFR (ErbB-1), EMA, EMMPRIN, ENA, endothelin receptor, Enkephalinase, eNOS, Eot, eotaxin1, EpCAM, Ephrin B2/ EphB4, EPO, ERCC, E-selectin, ET-1, Factor IIa, Factor VII, Factor VIIIc, Factor IX, fibroblast activation protein (FAP), Fas, FcR1, FEN-1, Ferritin, FGF, FGF-19, FGF-2, FGF3, FGF-8, FGFR, FGFR-3, Fibrin, FL, FLIP, Flt-3, Flt-4, Follicle stimulating hormone, Fractalkine, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, G250, Gas 6, GCP-2, GCSF, GD2, GD3, GDF, GDF-1, GDF-3 (Vgr-2), GDF-5 (BMP-14, CDMP-1), GDF-6 (BMP-13, CDMP-2), GDF-7 (BMP-12, CDMP-3), GDF-8 (Myostatin), GDF-9, GDF-15 (MIC-1), GDNF, GDNF, GFAP, GFRa-1, GFR-alpha1, GFR-alpha2, GFR-alpha3, GITR, Glucagon, Glut 4, glycoprotein IIb/IIIa (GP IIb/IIIa), GM-CSF, gp130, gp72, GRO, Growth hormone releasing factor, Hapten (NP-cap or NIP-cap), HB-EGF, HCC, HCMV gB envelope glycoprotein, HCMV) gH envelope glycoprotein, HCMV UL, Hemopoietic growth factor (HGF), Hep B gp120, heparanase, Her2, Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), herpes simplex virus (HSV) gB glycoprotein, HSV gD glycoprotein, HGFA, High molecular weight melanoma-associated antigen (HMW-MAA), HIV gp120, HIV IIIB gp 120 V3 loop, HLA, HLA-DR, HM1.24, HMFG PEM, HRG, Hrk, human cardiac myosin, human cytomegalovirus (HCMV), human growth hormone (HGH), HVEM, I-309, IAP, ICAM, ICAM-1, ICAM-3, ICE, ICOS, IFNg, Ig, IgA receptor, IgE, IGF, IGF binding proteins, IGF-1R, IGFBP, IGF-I, IGF-II, IL, IL-1, IL-1R, IL-2, IL-2R, IL-4, IL-4R, IL-5, IL-5R, IL-6, IL-6R, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-18, IL-18R, IL-23, interferon (INF)-alpha, INF-beta, INF-gamma, Inhibin, iNOS, Insulin A-chain, Insulin B-chain, Insulin-like growth factor 1, integrin alpha2, integrin alpha3, integrin alpha4, integrin alpha4/beta1, integrin alpha4/beta7, integrin alpha5 (alphaV), integrin alpha5/beta1, integrin alpha5/beta3, integrin alpha6, integrin beta1, integrin beta2, interferon gamma, IP-10, I-TAC, JE, Kallikrein 2, Kallikrein 5, Kallikrein 6, , Kallikrein 11, Kallikrein 12, Kallikrein 14, Kallikrein 15, Kallikrein L1, Kallikrein L2, Kallikrein L3, Kallikrein L4, KC, KDR, Keratinocyte Growth Factor (KGF), laminin 5, LAMP, LAP, LAP (TGF- 1), Latent TGF-1, Latent TGF-1 bp1, LBP, LDGF, LECT2, Lefty, Lewis-Y antigen, Lewis-Y related antigen, LFA-1, LFA-3, Lfo, LIF, LIGHT, lipoproteins, LIX, LKN, Lptn, L-Selectin, LT-a, LT-b, LTB4, LTBP-1, Lung surfactant, Luteinizing hormone, Lymphotoxin Beta Receptor, Mac-1, MAdCAM, MAG, MAP2, MARC, MCAM, MCAM, MCK-2, MCP, M-CSF, MDC, Mer, METALLOPROTEASES, MGDF receptor, MGMT, MHC (HLA-DR), MIF, MIG, MIP, MIP-1-alpha, MK, MMAC1, MMP, MMP-1, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-2, MMP-24, MMP-3, MMP-7, MMP-8, MMP-9, MPIF, Mpo, MSK, MSP, mucin (Muc1), MUC18, Muellerian-inhibitin substance, Mug, MuSK, NAIP, NAP, NCAD, N-Cadherin, NCA 90, NCAM, NCAM, Neprilysin, Neurotrophin-3,-4, or -6, Neurturin, Neuronal growth factor (NGF), NGFR, NGF-beta, nNOS, NO, NOS, Npn, NRG-3, NT, NTN, OB, OGG1, OPG, OPN, OSM, OX40L, OX40R, p150, p95, PADPr, Parathyroid hormone, PARC, PARP, PBR, PBSF, PCAD, P-Cadherin, PCNA, PDGF, PDGF, PDK-1, PECAM, PEM, PF4, PGE, PGF, PGI2, PGJ2, PIN, PLA2, placental alkaline phosphatase (PLAP), PlGF, PLP, PP14, Proinsulin, Prorelaxin, Protein C, PS, PSA, PSCA, prostate specific membrane antigen (PSMA), PTEN, PTHrp, Ptk, PTN, R51, RANK, RANKL, RANTES, RANTES, Relaxin A-chain, Relaxin B-chain, renin, respiratory syncytial virus (RSV) F, RSV Fgp, Ret, Rheumatoid factors, RLIP76, RPA2, RSK, S100, SCF/KL, SDF-1, SERINE, Serum albumin, sFRP-3, Shh, SIGIRR, SK-1,

SLAM, SLPI, SMAC, SMDF, SMOH, SOD, SPARC, Stat, STEAP, STEAP-II, TACE, TACI, TAG-72 (tumor-associated glycoprotein-72), TARC, TCA-3, T-cell receptors (e.g., T-cell receptor alpha/beta), TdT, TECK, TEM1, TEM5, TEM7, TEM8, TERT, testicular PLAP-like alkaline phosphatase, TfR, TGF, TGF-alpha, TGF-beta, TGF-beta Pan Specific, TGF-beta RI (ALK-5), TGF-beta RII, TGF-beta RIIb, TGF-beta RIII, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5, Thrombin, Thymus Ck-1, Thyroid stimulating hormone, Tie, TIMP, TIQ, Tissue Factor, TMEFF2, Tmpo, TMPRSS2, TNF, TNF-alpha, TNF-alpha beta, TNF-beta2, TNFc, TNF-RI, TNF-RII, TNFRSF10A (TRAIL R1 Apo-2, DR4), TNFRSF10B (TRAIL R2 DR5, KILLER, TRICK-2A, TRICK-B), TNFRSF10C (TRAIL R3 DcR1, LIT, TRID), TNFRSF10D (TRAIL R4 DcR2, TRUNDD), TNFRSF11A (RANK ODF R, TRANCE R), TNFRSF11B (OPG OCIF, TR1), TNFRSF12 (TWEAK R FN14), TNFRSF13B (TACI), TNFRSF13C (BAFF R), TNFRSF14 (HVEM ATAR, HveA, LIGHT R, TR2), TNFRSF16 (NGFR p75NTR), TNFRSF17 (BCMA), TNFRSF18 (GITR AITR), TNFRSF19 (TROY TAJ, TRADE), TNFRSF19L (RELT), TNFRSF1A (TNF RI CD120a, p55-60), TNFRSF1B (TNF RII CD120b, p75-80), TNFRSF26 (TNFRH3), TNFRSF3 (LTbR TNF RIII, TNFC R), TNFRSF4 (OX40 ACT35, TXGP1 R), TNFRSF5 (CD40 p50), TNFRSF6 (Fas Apo-1, APT1, CD95), TNFRSF6B (DcR3 M68, TR6), TNFRSF7 (CD27), TNFRSF8 (CD30), TNFRSF9 (4-1BB CD137, ILA), TNFRSF21 (DR6), TNFRSF22 (DcTRAIL R2 TNFRH2), TNFRST23 (DcTRAIL R1 TNFRH1), TNFRSF25 (DR3 Apo-3, LARD, TR-3, TRAMP, WSL-1), TNFSF10 (TRAIL Apo-2 Ligand, TL2), TNFSF11 (TRANCE/RANK Ligand ODF, OPG Ligand), TNFSF12 (TWEAK Apo-3 Ligand, DR3 Ligand), TNFSF13 (APRIL TALL2), TNFSF13B (BAFF BLYS, TALL1, THANK, TNFSF20), TNFSF14 (LIGHT HVEM Ligand, LTg), TNFSF15 (TL1A/VEGI), TNFSF18 (GITR Ligand AITR Ligand, TL6), TNFSF1A (TNF-a Conectin, DIF, TNFSF2), TNFSF1B (TNF-b LTa, TNFSF1), TNFSF3 (LTb TNFC, p33), TNFSF4 (OX40 Ligand gp34, TXGP1), TNFSF5 (CD40 Ligand CD154, gp39, HIGM1, IMD3, TRAP), TNFSF6 (Fas Ligand Apo-1 Ligand, APT1 Ligand), TNFSF7 (CD27 Ligand CD70), TNFSF8 (CD30 Ligand CD153), TNFSF9 (4-1BB Ligand CD137 Ligand), TP-1, t-PA, Tpo, TRAIL, TRAIL R, TRAIL-R1, TRAIL-R2, TRANCE, transferring receptor, TRF, Trk, TROP-2, TSG, TSLP, tumor-associated antigen CA 125, tumor-associated antigen expressing Lewis Y related carbohydrate, TWEAK, TXB2, Ung, uPAR, uPAR-1, Urokinase, VCAM, VCAM-1, VECAD, VE-Cadherin, VE-cadherin-2, VEFGR-1 (flt-1), VEGF, VEGFR, VEGFR-3 (flt-4), VEGI, VIM, Viral antigens, VLA, VLA-1, VLA-4, VNR integrin, von Willebrands factor, WIF-1, WNT1, WNT2, WNT2B/13, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, WNT16, XCL1, XCL2, XCR1, XCR1, XEDAR, XIAP, XPD, and receptors for hormones and growth factors.

Optimized IgG Variant Properties

[182] The present application also provides IgG variants that are optimized for a variety of therapeutically relevant properties. An IgG variant that is engineered or predicted to display one or more optimized properties is herein referred to as an "optimized IgG variant". Properties that may be optimized include but are not limited to enhanced or reduced affinity for an FcγR. In a preferred embodiment, the IgG variants are optimized to possess enhanced affinity for a human activating FcγR, preferably FcγRI, FcγRIIa, FcγRIIc, FcγRIIIa, and FcγRIIIb, most preferably FcγRIIIa. In an

alternate embodiment, the IgG variants are optimized to possess reduced affinity for the human inhibitory receptor FcγRIIb. These embodiments are anticipated to provide IgG polypeptides with enhanced therapeutic properties in humans, for example enhanced effector function and greater anti-cancer potency. In an alternate embodiment, the IgG variants are optimized to have reduced or ablated affinity for a human FcγR, including but not limited to FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, and FcγRIIIb. These embodiments are anticipated to provide IgG polypeptides with enhanced therapeutic properties in humans, for example reduced effector function and reduced toxicity. In other embodiments, IgG variants provide enhanced affinity for one or more FcγRs, yet reduced affinity for one or more other FcγRs. For example, an IgG variant may have enhanced binding to FcγRIIIa, yet reduced binding to FcγRIIb. Alternately, an IgG variant may have enhanced binding to FcγRIIa and FcγRI, yet reduced binding to FcγRIIb. In yet another embodiment, an IgG variant may have enhanced affinity for FcγRIIb, yet reduced affinity to one or more activating FcγRs.

[183] Preferred embodiments comprise optimization of binding to a human FcγR, however in alternate embodiments the IgG variants possess enhanced or reduced affinity for FcγRs from nonhuman organisms, including but not limited to rodents and non-human primates. IgG variants that are optimized for binding to a nonhuman FcγR may find use in experimentation. For example, mouse models are available for a variety of diseases that enable testing of properties such as efficacy, toxicity, and pharmacokinetics for a given drug candidate. As is known in the art, cancer cells can be grafted or injected into mice to mimic a human cancer, a process referred to as xenografting. Testing of IgG variants that comprise IgG variants that are optimized for one or more mouse FcγRs, may provide valuable information with regard to the efficacy of the protein, its mechanism of action, and the like. The IgG variants may also be optimized for enhanced functionality and/or solution properties in aglycosylated form. In a preferred embodiment, the aglycosylated IgG variants bind an Fc ligand with greater affinity than the aglycosylated form of the parent IgG variant. The Fc ligands include but are not limited to FcγRs, C1q, FcRn, and proteins A and G, and may be from any source including but not limited to human, mouse, rat, rabbit, or monkey, preferably human. In an alternately preferred embodiment, the IgG variants are optimized to be more stable and/or more soluble than the aglycosylated form of the parent IgG variant.

[184] IgG variants can include modifications that modulate interaction with Fc ligands other than FcγRs, including but not limited to complement proteins, FcRn, and Fc receptor homologs (FcRHs). FcRHs include but are not limited to FcRH1, FcRH2, FcRH3, FcRH4, FcRH5, and FcRH6 (Davis et al., 2002, Immunol. Reviews 190:123-136).

[185] Preferably, the Fc ligand specificity of the IgG variant will determine its therapeutic utility. The utility of a given IgG variant for therapeutic purposes will depend on the epitope or form of the Target antigen and the disease or indication being treated. For some targets and indications, enhanced FcγR-mediated effector functions may be preferable. This may be particularly favorable for anti-cancer IgG variants. Thus IgG variants may be used that comprise IgG variants that provide enhanced affinity for activating FcγRs and/or reduced affinity for inhibitory FcγRs. For some targets and indications, it

may be further beneficial to utilize IgG variants that provide differential selectivity for different activating FcγRs; for example, in some cases enhanced binding to FcγRIIa and FcγRIIIa may be desired, but not FcγRI, whereas in other cases, enhanced binding only to FcγRIIa may be preferred. For certain targets and indications, it may be preferable to utilize IgG variants that enhance both FcγR-mediated and complement-mediated effector functions, whereas for other cases it may be advantageous to utilize IgG variants that enhance either FcγR-mediated or complement-mediated effector functions. For some targets or cancer indications, it may be advantageous to reduce or ablate one or more effector functions, for example by knocking out binding to C1q, one or more FcγR's, FcRn, or one or more other Fc ligands. For other targets and indications, it may be preferable to utilize IgG variants that provide enhanced binding to the inhibitory FcγRIIb, yet WT level, reduced, or ablated binding to activating FcγRs. This may be particularly useful, for example, when the goal of an IgG variant is to inhibit inflammation or auto-immune disease, or modulate the immune system in some way.

[186] Clearly an important parameter that determines the most beneficial selectivity of a given IgG variant to treat a given disease is the context of the IgG variant, that is what type of IgG variant is being used. Thus the Fc ligand selectivity or specificity of a given IgG variant will provide different properties depending on whether it composes an antibody, Fc fusion, or an IgG variants with a coupled fusion or conjugate partner. For example, toxin, radionucleotide, or other conjugates may be less toxic to normal cells if the IgG variant that comprises them has reduced or ablated binding to one or more Fc ligands. As another example, in order to inhibit inflammation or auto-immune disease, it may be preferable to utilize an IgG variant with enhanced affinity for activating FcγRs, such as to bind these FcγRs and prevent their activation. Conversely, an IgG variant that comprises two or more Fc regions with enhanced FcγRIIb affinity may co-engage this receptor on the surface of immune cells, thereby inhibiting proliferation of these cells. Whereas in some cases an IgG variants may engage its target antigen on one cell type yet engage FcγRs on separate cells from the target antigen, in other cases it may be advantageous to engage FcγRs on the surface of the same cells as the target antigen. For example, if an antibody targets an antigen on a cell that also expresses one or more FcγRs, it may be beneficial to utilize an IgG variant that enhances or reduces binding to the FcγRs on the surface of that cell. This may be the case, for example when the IgG variant is being used as an anti-cancer agent, and co-engagement of target antigen and FcγR on the surface of the same cell promote signaling events within the cell that result in growth inhibition, apoptosis, or other anti-proliferative effect. Alternatively, antigen and FcγR co-engagement on the same cell may be advantageous when the IgG variant is being used to modulate the immune system in some way, wherein co-engagement of target antigen and FcγR provides some proliferative or anti-proliferative effect. Likewise, IgG variants that comprise two or more Fc regions may benefit from IgG variants that modulate FcγR selectivity or specificity to co-engage FcγRs on the surface of the same cell.

[187] The Fc ligand specificity of the IgG variants can be modulated to create different effector function profiles that may be suited for particular target antigens, indications, or patient populations.

Figure 3 describes several preferred embodiments of receptor binding profiles that include improvements to, reductions to or no effect to the binding to various receptors, where such changes may be beneficial in certain contexts. The receptor binding profiles in the table could be varied by degree of increase or decrease to the specified receptors. Additionally, the binding changes specified could be in the context of additional binding changes to other receptors such as C1q or FcRn, for example by combining with ablation of binding to C1q to shut off complement activation, or by combining with enhanced binding to C1q to increase complement activation. Other embodiments with other receptor binding profiles are possible, the listed receptor binding profiles are exemplary.

[188] The presence of different polymorphic forms of FcγRs provides yet another parameter that impacts the therapeutic utility of the IgG variants . Whereas the specificity and selectivity of a given IgG variant for the different classes of FcγRs signficantly affects the capacity of an IgG variant to target a given antigen for treatment of a given disease, the specificity or selectivity of an IgG variant for different polymorphic forms of these receptors may in part determine which research or pre-clinical experiments may be appropriate for testing, and ultimately which patient populations may or may not respond to treatment. Thus the specificity or selectivity of IgG variants to Fc ligand polymorphisms, including but not limited to FcγR, C1q, FcRn, and FcRH polymorphisms, may be used to guide the selection of valid research and pre-clinical experiments, clinical trial design, patient selection, dosing dependence, and/or other aspects concerning clinical trials.

[189] Modification may be made to improve the IgG stability, solubility, function, or clinical use. In a preferred embodiment, the IgG variants can include modifications to reduce immunogenicity in humans. In a most preferred embodiment, the immunogenicity of an IgG variant is reduced using a method described in USSN 11/004,590, filed December 3, 2004, entitled "Methods of Generating Variant Proteins with Increased Host String Content and Compositions Thereof". In alternate embodiments, the IgG variants are humanized (Clark, 2000, *Immunol Today* 21:397-402).

[190] The IgG variants can include modifications that reduce immunogenicity. Modifications to reduce immunogenicity can include modifications that reduce binding of processed peptides derived from the parent sequence to MHC proteins. For example, amino acid modifications would be engineered such that there are no or a minimal number of immune epitopes that are predicted to bind, with high affinity, to any prevalent MHC alleles. Several methods of identifying MHC-binding epitopes in protein sequences are known in the art and may be used to score epitopes in an IgG variant . See for example WO 98/52976; WO 02/079232; WO 00/3317; USSN 09/903,378; USSN 10/039,170; USSN 60/222,697; USSN 10/754,296; PCT WO 01/21823; and PCT WO 02/00165; Mallios, 1999, *Bioinformatics* 15: 432-439; Mallios, 2001, *Bioinformatics* 17: 942-948; Sturniolo *et al.*, 1999, *Nature Biotech.* 17: 555-561; WO 98/59244; WO 02/069232; WO 02/77187; Marshall *et al.*, 1995, *J. Immunol.* 154: 5927-5933; and Hammer *et al.*, 1994, *J. Exp. Med.* 180: 2353-2358. Sequence-based information can be used to determine a binding score for a given peptide – MHC interaction (see for example Mallios, 1999, *Bioinformatics* 15: 432-439; Mallios, 2001, *Bioinformatics* 17: p942-948; Sturniolo *et. al.*, 1999, *Nature Biotech.* 17: 555-561).

Fusion Partners

[191] The IgG variants can be linked to one or more fusion partners. In one alternate embodiment, the IgG variant is conjugated or operably linked to another therapeutic compound. The therapeutic compound may be a cytotoxic agent, a chemotherapeutic agent, a toxin, a radioisotope, a cytokine, or other therapeutically active agent. The IgG may be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol.

Engineering IgG Variants

[192] The IgG variants can be based on human IgG sequences, and thus human IgG sequences are used as the "base" sequences against which other sequences are compared, including but not limited to sequences from other organisms, for example rodent and primate sequences. IgG variants may also comprise sequences from other immunoglobulin classes such as IgA, IgE, IgGD, IgGM, and the like. It is contemplated that, although the IgG variants are engineered in the context of one parent IgG, the variants may be engineered in or "transferred" to the context of another, second parent IgG. This is done by determining the "equivalent" or "corresponding" residues and substitutions between the first and second IgG, typically based on sequence or structural homology between the sequences of the two IgGs. In order to establish homology, the amino acid sequence of a first IgG outlined herein is directly compared to the sequence of a second IgG. After aligning the sequences, using one or more of the homology alignment programs known in the art (for example using conserved residues as between species), allowing for necessary insertions and deletions in order to maintain alignment (i.e., avoiding the elimination of conserved residues through arbitrary deletion and insertion), the residues equivalent to particular amino acids in the primary sequence of the first IgG variant are defined. Alignment of conserved residues preferably should conserve 100% of such residues. However, alignment of greater than 75% or as little as 50% of conserved residues is also adequate to define equivalent residues. Equivalent residues may also be defined by determining structural homology between a first and second IgG that is at the level of tertiary structure for IgGs whose structures have been determined. In this case, equivalent residues are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the parent or precursor (N on N, CA on CA, C on C and O on O) are within 0.13 nm and preferably 0.1 nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the proteins. Regardless of how equivalent or corresponding residues are determined, and regardless of the identity of the parent IgG in which the IgGs are made, what is meant to be conveyed is that the IgG variants discovered by can be engineered into any second parent IgG that has significant sequence or structural homology with the IgG variant. Thus for example, if a variant antibody is generated wherein the parent antibody is human IgG1, by using the methods described above or other methods for determining equivalent residues, the variant antibody may be engineered in another IgG1 parent antibody that binds a different antigen, a human IgG2 parent antibody, a human IgA parent antibody,

a mouse IgG2a or IgG2b parent antibody, and the like. Again, as described above, the context of the parent IgG variant does not affect the ability to transfer the IgG variants to other parent IgGs.

[193] Methods for engineering, producing, and screening IgG variants are provided. The described methods are not meant to constrain to any particular application or theory of operation. Rather, the provided methods are meant to illustrate generally that one or more IgG variants may be engineered, produced, and screened experimentally to obtain IgG variants with optimized effector function. A variety of methods are described for designing, producing, and testing antibody and protein variants in USSN 10/754,296, and USSN 10/672,280, which are herein expressly incorporated by reference.

[194] A variety of protein engineering methods may be used to design IgG variants with optimized effector function. In one embodiment, a structure-based engineering method may be used, wherein available structural information is used to guide substitutions. In a preferred embodiment, a computational screening method may be used, wherein substitutions are designed based on their energetic fitness in computational calculations. See for example USSN 10/754,296 and USSN 10/672,280, and references cited therein.

[195] An alignment of sequences may be used to guide substitutions at the identified positions. One skilled in the art will appreciate that the use of sequence information may curb the introduction of substitutions that are potentially deleterious to protein structure. The source of the sequences may vary widely, and include one or more of the known databases, including but not limited to the Kabat database (Northwestern University); Johnson & Wu, 2001, *Nucleic Acids Res.* 29:205-206; Johnson & Wu, 2000, *Nucleic Acids Res.* 28:214-218), the IMGT database (IMGT, the international ImMunoGeneTics information system®; ; Lefranc *et al.*, 1999, *Nucleic Acids Res.* 27:209-212; Ruiz *et al.*, 2000 *Nucleic Acids Res.* 28:219-221; Lefranc *et al.*, 2001, *Nucleic Acids Res.* 29:207-209; Lefranc *et al.*, 2003, *Nucleic Acids Res.* 31:307-310), and VBASE. Antibody sequence information can be obtained, compiled, and/or generated from sequence alignments of germline sequences or sequences of naturally occurring antibodies from any organism, including but not limited to mammals. One skilled in the art will appreciate that the use of sequences that are human or substantially human may further have the advantage of being less immunogenic when administered to a human. Other databases which are more general nucleic acid or protein databases, i.e. not particular to antibodies, include but are not limited to SwissProt, GenBank Entrez, and EMBL Nucleotide Sequence Database. Aligned sequences can include VH, VL, CH, and/or CL sequences. There are numerous sequence-based alignment programs and methods known in the art, and all of these find use in for generation of sequence alignments.

[196] Alternatively, random or semi-random mutagenesis methods may be used to make amino acid modifications at the desired positions. In these cases positions are chosen randomly, or amino acid changes are made using simplistic rules. For example all residues may be mutated to alanine, referred to as alanine scanning. Such methods may be coupled with more sophisticated engineering approaches that employ selection methods to screen higher levels of sequence diversity. As is well known in the art, there are a variety of selection technologies that may be used for such approaches,

including, for example, display technologies such as phage display, ribosome display, cell surface display, and the like, as described below.

[197] Methods for production and screening of IgG variants are well known in the art. General methods for antibody molecular biology, expression, purification, and screening are described in Antibody Engineering, edited by Duebel & Kontermann, Springer-Verlag, Heidelberg, 2001; and Hayhurst & Georgiou, 2001, *Curr Opin Chem Biol* 5:683-689; Maynard & Georgiou, 2000, *Annu Rev Biomed Eng* 2:339-76. Also see the methods described in USSN 10/754,296, filed on March 3, 2003, USSN 10/672,280, filed September 29, 2003, and USSN 10/822,231, filed March 26, 2004.

Making IgG Variants

[198] The IgG variants can be made by any method known in the art. In one embodiment , the IgG variant sequences are used to create nucleic acids that encode the member sequences, and that may then be cloned into host cells, expressed and assayed, if desired. These practices are carried out using well-known procedures, and a variety of methods that may find use in are described in Molecular Cloning - A Laboratory Manual, 3rd Ed. (Maniatis, Cold Spring Harbor Laboratory Press, New York, 2001), and Current Protocols in Molecular Biology (John Wiley & Sons). The nucleic acids that encode the IgG variants may be incorporated into an expression vector in order to express the protein. Expression vectors typically include a protein operably linked, that is placed in a functional relationship, with control or regulatory sequences, selectable markers, any fusion partners, and/or additional elements. The IgG variants may be produced by culturing a host cell transformed with nucleic acid, preferably an expression vector, containing nucleic acid encoding the IgG variants, under the appropriate conditions to induce or cause expression of the protein. A wide variety of appropriate host cells may be used, including but not limited to mammalian cells, bacteria, insect cells, and yeast. For example, a variety of cell lines that may find use in are described in the ATCC cell line catalog, available from the American Type Culture Collection. The methods of introducing exogenous nucleic acid into host cells are well known in the art, and will vary with the host cell used.

[199] In a preferred embodiment, IgG variants are purified or isolated after expression. Antibodies may be isolated or purified in a variety of ways known to those skilled in the art. Standard purification methods include chromatographic techniques, electrophoretic, immunological, precipitation, dialysis, filtration, concentration, and chromatofocusing techniques. As is well known in the art, a variety of natural proteins bind antibodies, for example bacterial proteins A, G, and L, and these proteins may find use in for purification. Purification can often be enabled by a particular fusion partner. For example, proteins may be purified using glutathione resin if a GST fusion is employed, Ni^{+2} affinity chromatography if a His-tag is employed, or immobilized anti-flag antibody if a flag-tag is used. For general guidance in suitable purification techniques, see Antibody Purification: Principles and Practice, 3rd Ed., Scopes, Springer-Verlag, NY, 1994.

Screening IgG Variants

[200] IgG variants may be screened using a variety of methods, including but not limited to those that use *in vitro* assays, *in vivo* and cell-based assays, and selection technologies. Automation and

high-throughput screening technologies may be utilized in the screening procedures. Screening may employ the use of a fusion partner or label, for example an immune label, isotopic label, or small molecule label such as a fluorescent or colorimetric dye.

[201] In a preferred embodiment, the functional and/or biophysical properties of IgG variants are screened in an *in vitro* assay. In a preferred embodiment, the protein is screened for functionality, for example its ability to catalyze a reaction or its binding affinity to its target. Binding assays can be carried out using a variety of methods known in the art, including but not limited to FRET (Fluorescence Resonance Energy Transfer) and BRET (Bioluminescence Resonance Energy Transfer) -based assays; AlphaScreen™ (Amplified Luminescent Proximity Homogeneous Assay), Scintillation Proximity Assay, ELISA (Enzyme-Linked Immunosorbent Assay), SPR (Surface Plasmon Resonance, also known as BIACORE®), isothermal titration calorimetry, differential scanning calorimetry, gel electrophoresis, and chromatography including gel filtration. These and other methods may take advantage of some fusion partner or label. Assays may employ a variety of detection methods including but not limited to chromogenic, fluorescent, luminescent, or isotopic labels. The biophysical properties of proteins, for example stability and solubility, may be screened using a variety of methods known in the art. Protein stability may be determined by measuring the thermodynamic equilibrium between folded and unfolded states. For example, IgG variants may be unfolded using chemical denaturant, heat, or pH, and this transition may be monitored using methods including but not limited to circular dichroism spectroscopy, fluorescence spectroscopy, absorbance spectroscopy, NMR spectroscopy, calorimetry, and proteolysis. As will be appreciated by those skilled in the art, the kinetic parameters of the folding and unfolding transitions may also be monitored using these and other techniques. The solubility and overall structural integrity of a IgG variant may be quantitatively or qualitatively determined using a wide range of methods that are known in the art. Methods which may find use in for characterizing the biophysical properties of IgG variants include gel electrophoresis, chromatography such as size exclusion chromatography and reversed-phase high performance liquid chromatography, mass spectrometry, ultraviolet absorbance spectroscopy, fluorescence spectroscopy, circular dichroism spectroscopy, isothermal titration calorimetry, differential scanning calorimetry, analytical ultra-centrifugation, dynamic light scattering, proteolysis, and cross-linking, turbidity measurement, filter retardation assays, immunological assays, fluorescent dye binding assays, protein-staining assays, microscopy, and detection of aggregates via ELISA or other binding assay. Structural analysis employing X-ray crystallographic techniques and NMR spectroscopy may also find use.

[202] As is known in the art, a subset of screening methods are those that select for favorable members of a library. The methods are herein referred to as "selection methods", and these methods find use in for screening IgG variants. When protein libraries are screened using a selection method, only those members of a library that are favorable, that is which meet some selection criteria, are propagated, isolated, and/or observed. As will be appreciated, because only the most fit variants are observed, such methods enable the screening of libraries that are larger than those screenable by methods that assay the fitness of library members individually. Selection is enabled by any method,

technique, or fusion partner that links, covalently or noncovalently, the phenotype of a protein with its genotype, that is the function of a protein with the nucleic acid that encodes it. For example the use of phage display as a selection method is enabled by the fusion of library members to the gene III protein. In this way, selection or isolation of IgG variants that meet some criteria, for example binding affinity to the protein's target, also selects for or isolates the nucleic acid that encodes it. Once isolated, the gene or genes encoding Fc variants may then be amplified. This process of isolation and amplification, referred to as panning, may be repeated, allowing favorable IgG variants in the library to be enriched. Nucleic acid sequencing of the attached nucleic acid ultimately allows for gene identification.

[203] A variety of selection methods are known in the art that may find use in for screening protein libraries. These include but are not limited to phage display (Phage display of peptides and proteins: a laboratory manual, Kay *et al.*, 1996, Academic Press, San Diego, CA, 1996; Lowman *et al.*, 1991, *Biochemistry* 30:10832-10838; Smith, 1985, *Science* 228:1315-1317) and its derivatives such as selective phage infection (Malmborg *et al.*, 1997, J Mol Biol 273:544-551), selectively infective phage (Krebber *et al.*, 1997, *J Mol Biol* 268:619-630), and delayed infectivity panning (Benhar *et al.*, 2000, *J Mol Biol* 301:893-904), cell surface display (Witrrup, 2001, *Curr Opin Biotechnol*, 12:395-399) such as display on bacteria (Georgiou *et al.*, 1997, *Nat Biotechnol* 15:29-34; Georgiou *et al.*, 1993, *Trends Biotechnol* 11:6-10; Lee *et al.*, 2000, *Nat Biotechnol* 18:645-648; Jun *et al.*, 1998, *Nat Biotechnol* 16:576-80), yeast (Boder & Wittrup, 2000, *Methods Enzymol* 328:430-44; Boder & Wittrup, 1997, *Nat Biotechnol* 15:553-557), and mammalian cells (Whitehorn *et al.*, 1995, *Bio/technology* 13:1215-1219), as well as *in vitro* display technologies (Amstutz *et al.*, 2001, *Curr Opin Biotechnol* 12:400-405) such as polysome display (Mattheakis *et al.*, 1994, *Proc Natl Acad Sci USA* 91:9022-9026), ribosome display (Hanes *et al.*, 1997, *Proc Natl Acad Sci USA* 94:4937-4942), mRNA display (Roberts & Szostak, 1997, *Proc Natl Acad Sci USA 94*:12297-12302; Nemoto *et al.*, 1997, *FEBS Lett* 414:405-408), and ribosome-inactivation display system (Zhou *et al.*, 2002, *J Am Chem Soc* 124, 538-543).

[204] Other selection methods that may find use in include methods that do not rely on display, such as *in vivo* methods including but not limited to periplasmic expression and cytometric screening (Chen *et al.*, 2001, *Nat Biotechnol* 19:537-542), the protein fragment complementation assay (Johnsson & Varshavsky, 1994, *Proc Natl Acad Sci USA* 91:10340-10344; Pelletier *et al.*, 1998, *Proc Natl Acad Sci USA* 95:12141-12146), and the yeast two hybrid screen (Fields & Song, 1989, *Nature* 340:245-246) used in selection mode (Visintin *et al.*, 1999, *Proc Natl Acad Sci USA* 96:11723-11728). In an alternate embodiment, selection is enabled by a fusion partner that binds to a specific sequence on the expression vector, thus linking covalently or noncovalently the fusion partner and associated Fc variant library member with the nucleic acid that encodes them. For example, USSN 09/642,574; USSN 10/080,376; USSN 09/792,630; USSN 10/023,208; USSN 09/792,626; USSN 10/082,671; USSN 09/953,351; USSN 10/097,100; USSN 60/366,658; PCT WO 00/22906; PCT WO 01/49058; PCT WO 02/04852; PCT WO 02/04853; PCT WO 02/08023; PCT WO 01/28702; and PCT WO 02/07466 describe such a fusion partner and technique that may find use in . In an alternative

embodiment, *in vivo* selection can occur if expression of the protein imparts some growth, reproduction, or survival advantage to the cell.

[205] A subset of selection methods referred to as "directed evolution" methods are those that include the mating or breading of favorable sequences during selection, sometimes with the incorporation of new mutations. As will be appreciated by those skilled in the art, directed evolution methods can facilitate identification of the most favorable sequences in a library, and can increase the diversity of sequences that are screened. A variety of directed evolution methods are known in the art that may find use in for screening IgG variants, including but not limited to DNA shuffling (PCT WO 00/42561 A3; PCT WO 01/70947 A3), exon shuffling (US 6,365,377; Kolkman & Stemmer, 2001, *Nat Biotechnol* 19:423-428), family shuffling (Crameri *et al.*, 1998, *Nature* 391:288-291; US 6,376,246), RACHITT™ (Coco *et al.*, 2001, *Nat Biotechnol* 19:354- 359; PCT WO 02/06469), STEP and random priming of *in vitro* recombination (Zhao *et al.*, 1998, *Nat Biotechnol* 16:258-261; Shao *et al.*, 1998, *Nucleic Acids Res* 26:681-683), exonuclease mediated gene assembly (US 6,352,842; US 6,361,974), Gene Site Saturation Mutagenesis™ (US 6,358,709), Gene Reassembly™ (US 6,358,709), SCRATCHY (Lutz et al., 2001, *Proc Natl Acad Sci USA* 98:11248-11253), DNA fragmentation methods (Kikuchi *et al.*, *Gene* 236:159-167), single-stranded DNA shuffling (Kikuchi *et al.*, 2000, *Gene* 243:133-137), and AMEsystem™ directed evolution protein engineering technology (Applied Molecular Evolution) (US 5,824,514; US 5,817,483; US 5,814,476; US 5,763,192; US 5,723,323).

[206] In a preferred embodiment, IgG variants are screened using one or more cell-based or *in vivo* assays. For such assays, purified or unpurified proteins are typically added exogenously such that cells are exposed to individual variants or pools of variants belonging to a library. These assays are typically, but not always, based on the function of the IgG; that is, the ability of the IgG to bind to its target and mediate some biochemical event, for example effector function, ligand/receptor binding inhibition, apoptosis, and the like. Such assays often involve monitoring the response of cells to the IgG, for example cell survival, cell death, change in cellular morphology, or transcriptional activation such as cellular expression of a natural gene or reporter gene. For example, such assays may measure the ability of IgG variants to elicit ADCC, ADCP, or CDC. For some assays additional cells or components, that is in addition to the target cells, may need to be added, for example example serum complement, or effector cells such as peripheral blood monocytes (PBMCs), NK cells, macrophages, and the like. Such additional cells may be from any organism, preferably humans, mice, rat, rabbit, and monkey. Antibodies may cause apoptosis of certain cell lines expressing the target, or they may mediate attack on target cells by immune cells which have been added to the assay. Methods for monitoring cell death or viability are known in the art, and include the use of dyes, immunochemical, cytochemical, and radioactive reagents. For example, caspase staining assays may enable apoptosis to be measured, and uptake or release of radioactive substrates or fluorescent dyes such as alamar blue may enable cell growth or activation to be monitored. In a preferred embodiment, the DELFIA® EuTDA-based cytotoxicity assay (Perkin Elmer, MA) is used. Alternatively, dead or damaged target cells may be monitoried by measuring the release of one or

more natural intracellular proteins, for example lactate dehydrogenase. Transcriptional activation may also serve as a method for assaying function in cell-based assays. In this case, response may be monitored by assaying for natural genes or proteins which may be upregulated, for example the release of certain interleukins may be measured, or alternatively readout may be via a reporter construct. Cell-based assays may also involve the measure of morphological changes of cells as a response to the presence of a protein. Cell types for such assays may be prokaryotic or eukaryotic, and a variety of cell lines that are known in the art may be employed. Alternatively, cell-based screens are performed using cells that have been transformed or transfected with nucleic acids encoding the variants. That is, IgG variants are not added exogenously to the cells. For example, in one embodiment, the cell-based screen utilizes cell surface display. A fusion partner can be employed that enables display of IgG variants on the surface of cells (Witrrup, 2001, *Curr Opin Biotechnol*, 12:395-399).

[207] In a preferred embodiment, the immunogenicity of the IgG variants is determined experimentally using one or more cell-based assays. Several methods can be used for experimental confirmation of epitopes. In a preferred embodiment, *ex vivo* T-cell activation assays are used to experimentally quantitate immunogenicity. In this method, antigen presenting cells and naïve T cells from matched donors are challenged with a peptide or whole protein of interest one or more times. Then, T cell activation can be detected using a number of methods, for example by monitoring production of cytokines or measuring uptake of tritiated thymidine. In the most preferred embodiment, interferon gamma production is monitored using Elispot assays (Schmittel *et. al.*, 2000, *J. Immunol. Meth.*, 24: 17-24).

[208] The biological properties of the IgG variants may be characterized in cell, tissue, and whole organism experiments. As is known in the art, drugs are often tested in animals, including but not limited to mice, rats, rabbits, dogs, cats, pigs, and monkeys, in order to measure a drug's efficacy for treatment against a disease or disease model, or to measure a drug's pharmacokinetics, toxicity, and other properties. The animals may be referred to as disease models. Therapeutics are often tested in mice, including but not limited to nude mice, SCID mice, xenograft mice, and transgenic mice (including knockins and knockouts). Such experimentation may provide meaningful data for determination of the potential of the protein to be used as a therapeutic. Any organism, preferably mammals, may be used for testing. For example because of their genetic similarity to humans, monkeys can be suitable therapeutic models, and thus may be used to test the efficacy, toxicity, pharmacokinetics, or other property of the IgGs . Tests of the in humans are ultimately required for approval as drugs, and thus of course these experiments are contemplated. Thus the IgGs may be tested in humans to determine their therapeutic efficacy, toxicity, immunogenicity, pharmacokinetics, and/or other clinical properties.

Methods of Using IgG Variants

[209] The IgG variants may find use in a wide range of products. In one embodiment the IgG variant is a therapeutic, a diagnostic, or a research reagent, preferably a therapeutic. The IgG variant may find use in an antibody composition that is monoclonal or polyclonal. In a preferred embodiment,

the IgG variants are used to kill target cells that bear the target antigen, for example cancer cells. In an alternate embodiment, the IgG variants are used to block, antagonize, or agonize the target antigen, for example for antagonizing a cytokine or cytokine receptor. In an alternately preferred embodiment, the IgG variants are used to block, antagonize, or agonize the target antigen and kill the target cells that bear the target antigen.

[210] The IgG variants may be used for various therapeutic purposes. In a preferred embodiment, an antibody comprising the IgG variant is administered to a patient to treat an antibody-related disorder. A "patient" for the purposes includes both humans and other animals, preferably mammals and most preferably humans. By "antibody related disorder" or "antibody responsive disorder" or "condition" or "disease" herein are meant a disorder that may be ameliorated by the administration of a pharmaceutical composition comprising an IgG variant . Antibody related disorders include but are not limited to autoimmune diseases, immunological diseases, infectious diseases, inflammatory diseases, neurological diseases, and oncological and neoplastic diseases including cancer. By "cancer" and "cancerous" herein refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to carcinoma, lymphoma, blastoma, sarcoma (including liposarcoma), neuroendocrine tumors, mesothelioma, schwanoma, meningioma, adenocarcinoma, melanoma, and leukemia and lymphoid malignancies.

[211] In one embodiment, an IgG variant is the only therapeutically active agent administered to a patient. Alternatively, the IgG variant is administered in combination with one or more other therapeutic agents, including but not limited to cytotoxic agents, chemotherapeutic agents, cytokines, growth inhibitory agents, anti-hormonal agents, kinase inhibitors, anti-angiogenic agents, cardioprotectants, or other therapeutic agents. The IgG vararíants may be administered concomitantly with one or more other therapeutic regimens. For example, an IgG variant may be administered to the patient along with chemotherapy, radiation therapy, or both chemotherapy and radiation therapy. In one embodiment, the IgG variant may be administered in conjunction with one or more antibodies, which may or may not be an IgG variant. In accordance with another embodiment , the IgG variant and one or more other anti-cancer therapies are employed to treat cancer cells *ex vivo*. It is contemplated that such *ex vivo* treatment may be useful in bone marrow transplantation and particularly, autologous bone marrow transplantation. It is of course contemplated that the IgG variants can be employed in combination with still other therapeutic techniques such as surgery.

[212] A variety of other therapeutic agents may find use for administration with the IgG variants . In one embodiment, the IgG is administered with an anti-angiogenic agent. By "anti-angiogenic agent" as used herein is meant a compound that blocks, or interferes to some degree, the development of blood vessels. The anti-angiogenic factor may, for instance, be a small molecule or a protein, for example an antibody, Fc fusion, or cytokine, that binds to a growth factor or growth factor receptor involved in promoting angiogenesis. The preferred anti-angiogenic factor herein is an antibody that binds to Vascular Endothelial Growth Factor (VEGF). In an alternate embodiment, the IgG is administered with a therapeutic agent that induces or enhances adaptive immune response, for

example an antibody that targets CTLA-4. In an alternate embodiment, the IgG is administered with a tyrosine kinase inhibitor. By "tyrosine kinase inhibitor" as used herein is meant a molecule that inhibits to some extent tyrosine kinase activity of a tyrosine kinase. In an alternate embodiment, the IgG variants are administered with a cytokine. By "cytokine" as used herein is meant a generic term for proteins released by one cell population that act on another cell as intercellular mediators.

[213] Pharmaceutical compositions are contemplated wherein an IgG variant and one or more therapeutically active agents are formulated. Formulations of the IgG variants are prepared for storage by mixing the IgG having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed.,1980), in the form of lyophilized formulations or aqueous solutions. The formulations to be used for *in vivo* administration are preferably sterile. This is readily accomplished by filtration through sterile filtration membranes or other methods. The IgG variants and other therapeutically active agents disclosed herein may also be formulated as immunoliposomes, and/or entrapped in microcapsules

[214] The concentration of the therapeutically active IgG variant in the formulation may vary from about 0.1 to 100 weight %. In a preferred embodiment, the concentration of the IgG is in the range of 0.003 to 1.0 molar. In order to treat a patient, a therapeutically effective dose of the IgG variant may be administered. By "therapeutically effective dose" herein is meant a dose that produces the effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. Dosages may range from 0.01 to 100 mg/kg of body weight or greater, for example 0.1, 1, 10, or 50 mg/kg of body weight, with 1 to 10mg/kg being preferred. As is known in the art, adjustments for protein degradation, systemic versus localized delivery, and rate of new protease synthesis, as well as the age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

[215] Administration of the pharmaceutical composition comprising an IgG variant , preferably in the form of a sterile aqueous solution, may be done in a variety of ways, including, but not limited to, orally, subcutaneously, intravenously, intranasally, intraotically, transdermally, topically (e.g., gels, salves, lotions, creams, etc.), intraperitoneally, intramuscularly, intrapulmonary (e.g., AERx® inhalable technology commercially available from Aradigm, or Inhance™ pulmonary delivery system commercially available from Inhale Therapeutics), vaginally, parenterally, rectally, or intraocularly.

EXAMPLES

[216] Examples are provided below to illustrate the present invention. These examples are not meant to constrain the present invention to any particular application or theory of operation. For all positions discussed in the present invention, numbering is according to the EU index as in Kabat (Kabat *et al.*, 1991, *Sequences of Proteins of Immunological Interest,* 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda). Those skilled in the art of antibodies will appreciate that this convention consists of nonsequential numbering in specific regions of an

immunoglobulin sequence, enabling a normalized reference to conserved positions in immunoglobulin families. Accordingly, the positions of any given immunoglobulin as defined by the EU index will not necessarily correspond to its sequential sequence.

Example 1. Non-naturally occurring Modifications

[217] Novel Fc variants have been successfully engineered, primarily in the context of the IgG1 isotype, with selectively enhanced binding to FcγRs, and these variants have been shown to provide enhanced potency and efficacy in cell-based effector function assays (USSN 10/672,280, USSN 10/822,231, USSN 60/627,774, USSN 60/642,477, and USSN 60/723,294, entitled "Optimized Fc Variants", filed 10/3/2005, all expressly incorporated by reference). Figures 4 and 5 summarize these variants and the data detailing their properties with respect to Fc ligand affinity and effector function. Figure 6 summarizes the amino acid modifications that compose this set of variants.

[218] The variants described in Figures 4 - 6 provide a variety of unique biological and clinical properties. A number of variants provide substantial enhancements in FcγR affinity, in particular to one or both isoforms (V158 and F158) of the activating receptor FcγRIIIa. For example substitutions at positions 239, 268, and 332 provide substantial improvements in FcγR binding and effector function. A number of variants have been obtained with altered specificities for the various Fc ligands. The selective affinity of a variant for the different FcγRs may be an important factor in determining the optimal therapeutic IgG. For example, the affinity of a variant for FcγRI, the relative affinity for FcγRIII versus FcγRIIb, and/or the relative affinity for FcγRIIa versus FcγRIIb may be important determinants of the capacity of an antibody or Fc fusion to mediate ADCC or ADCP, or elicit long-term immunity. For example, the balance between FcγRIIa and FcγRIIb establishes a threshold of DC activation and enables immune complexes to mediate opposing effects on debdritic cell (DC) maturation and function (Boruchov et al., 2005, *J Clin Invest*, Sep 15, 1-10). Thus variants that selectively ligate FcγRIIa or FcγRIIb may affect DC processing, T cell priming and activation, antigen immunization, and/or efficacy against cancer (Dhodapkar & Dhodapkar, 2005, *Proc Natl Acad Sci USA*, 102, 6243–6244). Such variants may be employed as novel strategies for targeting antigens to the activating or inhibitory FcγRs on human DCs to generate either antigen-specific immunity or tolerance. Some variants provide selective enhancement in binding affinity to different Fc ligands, whereas other provide selective reduction in binding affinity to different Fc ligands. By "selective enhancement" as used herein is meant an improvement in or a greater improvement in binding affinity of a variant to one or more Fc ligands relative to one or more other Fc ligands. For example, for a given variant, the Fold WT for binding to, say FcγRIIa, may be greater than the Fold WT for binding to, say FcγRIIb. By "selective reduction" as used herein is meant a reduction in or a greater reduction in binding affinity of a variant to one or more Fc ligands relative to one or more other Fc ligands. For example, for a given variant, the Fold WT for binding to, say FcγRI, may be lower than the Fold WT for binding to, say FcγRIIb. As an example of such selectivity, G236S provides a selective enhancement to FcγRII's (IIa, IIb, and IIc) relative to FcγRI and FcγRIIIa, with a somewhat greater enhancement to FcγRIIa relative to FcγRIIb and FcγRIIc. G236A, however, is highly selectively enhanced for FcγRIIa, not only with

respect to FcγRI and FcγRIIIa, but also over FcγRIIb and FcγRIIc. Selective enhancements and reductions are observed for a number of Fc variants, including but not limited to variants comprising substitutions at EU positions 234, 235, 236, 267, 268, 292, 293, 295, 300, 324, 327, 328, 330, and 335. In particular, receptor selectivity may be provided by variants comprising one or more substitutions selected from the group consisting of 236S, 236A, 267D, 267E, 268D, 268E, 293R, 324I, 327D, 272R, 328A, 328F, 271G, 235Y, 327D, 328A, 328F, 324G, 330Y, 330L, and 330I. Figure 6 highlights preferred non-naturally occurring modifications that provide optimized Fc ligand binding and/or effector function properties. Alternately preferred non-naturally occurring modifications include 234Y, 234I, 235Y, 235I, 235D, 236S, 237D, 239D, 239E, 239N, 239Q, 239T, 240M, 246H, 246Y, 255Y, 258Y, 264I, 264T, 264Y, 267D, 267E, 271G, 272Y, 272H, 272R, 272I, 274E, 278T, 283L, 283H, 293R, 324G, 324I, 326T, 327D, 328A, 328F, 328T, 330L, 330Y, 330I, 332D, 332E, 332N, 332Q, 332T, 333Y, 334F, and 334T. Most preferred non-naturally occurring modifications include 234Y, 234I, 235Y, 235I, 235D, 236S, 237D, 239D, 239E, 239N, 239Q, 239T, 264I, 264T, 264Y, 267D, 267E, 324G, 324I, 327D, 328A, 328F, 328T, 330L, 330Y, 330I, 332D, 332E, 332N, 332Q, and 332T.

Example 2. IgG Variants with Non-naturally occurring Modifications

[219] The present invention provides immunoglobulins wherein the aforedescribed novel variants are utilized in the context of alternate IgG isotypes. Figure 7 shows the sequences of the four IgG isotypes IgG1, IgG2, IgG3, and IgG4, with differences from IgG1 highlighted. Thus Figure 7 provides the isotypic differences between the four IgGs. For completeness, it is noted that in addition to isotypic differences, a number of immunoglobulin polmorphisms (referred to as Gm polymorphisms) or allotypes exist in the human population. Gm polymorphism is determined by the IGHG1, IGHG2 and IGHG3 genes which have alleles encoding allotypic antigenic determinants referred to as G1m, G2m, and G3m allotypes for markers of the human IgG1, IgG2 and IgG3 molecules (no Gm allotypes have been found on the gamma 4 chain) (Clark, 1997, IgG effector mechanisms, Chem Immunol. 65:88-110; Gorman & Clark, 1990, Semin Immunol 2(6):457-66). Allelic forms of human immunoglobulins have been well-characterized (WHO Review of the notation for the allotypic and related markers of human immunoglobulins. J Immunogen 1976, 3:357-362; WHO Review of the notation for the allotypic and related markers of human immunoglobulins. 1976, Eur. J. Immunol. 6, 599-601; Loghem E van, 1986, Allotypic markers, Monogr Allergy 19: 40-51). At present, 18 Gm allotypes are known: G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b5, b0, b3, b4, s, t, g1, c5, u, v, g5) (Lefranc, et al., The human IgG subclasses: molecular analysis of structure, function and regulation. Pergamon, Oxford, pp. 43-78 (1990); Lefranc, G. et al., 1979, Hum. Genet.: 50, 199-211). Additionally, other polymorphisms have been characterized (Kim et al., 2001, J. Mol. Evol. 54:1-9). As an example, Figure 8 shows the allotypes and isoallotypes of the gamma1 chain of human IgG1 showing the positions and the relevant amino acid substitutions.

[220] The different IgG isotypes offer a variety of unique physical, biological, and therapeutic properties. For example there are significant differences in stability, solubility, FcγR-mediated effector functions, complement-mediated effector functions, in vivo pharmacokinetics, and oligomerization

state among the isotypes IgG1, IgG2, IgG3, and IgG4. These differences must be due to one or more of the isotypic differences between the IgGs shown in Figure 7. For example, because the binding site for FcγRs resides on the Fc region, it is likely that the IgG differences in Fc, and even more likely the lower hinge and the CH2 domain, are responsible for the differences in their FcγR-mediated effector functions. Figures 9a and 9b highlight the differences between the Fc region of IgG1 and those of IgG2 and IgG4 respectively, mapped in the context of the IgG1 Fc/FcγRIIIb complex (pdb accession code 1E4K)(Sondermann *et al.*, 2000, Nature 406:267-273).

[221] In order to explore the properties of the different IgG isotypes, a matched set of IgG1, IgG2, and IgG4 antibodies were constructed with the variable region of the anti-Her2/neu antibody trastuzumab (Herceptin®, a registered trademark of Genentech, currently approved for treatment of breast cancer). The genes for the variable regions of trastuzumab were constructed using recursive PCR, and subcloned into the mammalian expression vector pcDNA3.1Zeo (Invitrogen) comprising the full length light kappa (Cκ) and heavy chain IgG1 constant regions. DNA was sequenced to confirm the fidelity of the sequences. Plasmids containing heavy chain gene (VH-Cγ1-Cγ2-Cγ3) (wild-type or variants) were co-transfected with plasmid containing light chain gene (VL-Cκ) into 293T cells. Media were harvested 5 days after transfection, and antibodies were purified from the supernatant using protein A affinity chromatography (Pierce). Antibody concentrations were determined by bicinchoninic acid (BCA) assay (Pierce).

[222] In order to screen for FcγR binding, the extracellular region of human V158 FcγRIIIa was expressed and purified. The extracellular region of this receptor was obtained by PCR from a clone obtained from the Mammalian Gene Collection (MGC:22630). The receptor was fused at the C-terminus with a 6x His-tag and a GST-tag, and subcloned into pcDNA3.1zeo. Vector containing receptor was transfected into 293T cells, media were harvested, and receptors were purified using Nickel affinity chromatography. Receptor concentrations were determined by bicinchoninic acid (BCA) assay (Pierce). Binding affinity to human FcγRIIIa by the antibodies was measured using a quantitative and extremely sensitive method, AlphaScreen™ assay. The AlphaScreen is a bead-based luminescent proximity assay. Laser excitation of a donor bead excites oxygen, which if sufficiently close to the acceptor bead will generate a cascade of chemiluminescent events, ultimately leading to fluorescence emission at 520-620 nm. The AlphaScreen was applied as a competition assay for screening the antibodies. Commercial IgG was biotinylated by standard methods for attachment to streptavidin donor beads, and tagged human FcγRIIIa (V158 isoform) was bound to glutathione chelate acceptor beads. In the absence of competing antibody, antibody and FcγR interact and produce a signal at 520-620 nm. Addition of untagged antibody competes with the Fc/FcγR interaction, reducing fluorescence quantitatively to enable determination of relative binding affinities.

[223] Figure 10a presents the competition AlphaScreen binding data for binding of trastuzumab IgGs to human V158 FcγRIIIa. The binding data were normalized to the maximum and minimum luminescence signal provided by the baselines at low and high concentrations of competitor antibody respectively. The data were fit to a one site competition model using nonlinear regression, and these

fits are represented by the curves in the figure. The results show that the FcγR-mediated effector functions are substantially greater for IgG1 than for IgG2 and IgG4, consistent with prior studies (Michaelsen et al., 1992, Molecular Immunology, 29(3): 319-326). Figure 10b presents competitition AlphaScreen data for binding of the IgGs to protein A, carried out using commercial protein A-conjugated acceptor beads. The data show that all of the variants bind comparably to protein A, indicating that the FcγR-affinity differences are not due to differences in stability, solubility, or other properties between the IgG isotypes.

[224] Non-naturally occurring modifications were constructed in the context of all three antibody isotypes. The substitutions S239D and I332E were introduced into the heavy chains of the trastuzumab IgG1, IgG2, and IgG4 antibodies using quick-change mutagenesis techniques (Stratagene), and antibodies were expressed and purified as described above. Competition AlphaScreen data were acquired as described above for binding to human V158 FcγRIIIa, as well as human FcγRI, which was constructed using recursive PCR and expressed and purified as described above. Figures 11a and 11b show the data for binding of the IgG variants to these receptors. The results show that the novel modifications S239D/I332E provide enhanced receptor binding to all three isotypes, despite the poor FcγR affinity of IgG2 and IgG4 relative to IgG1.

[225] Surface Plasmon Resonance (SPR) (Biacore, Uppsala, Sweden) was carried out to further investigate the FcγRIIIa affinity of the IgG variants. Protein A (Pierce) was covalently coupled to a CM5 sensor chip using NHS/EDC chemistry. WT or variant trastuzumab antibody was bound to the protein A CM5 chip, and FcγRIIIa-His-GST analyte, in serial dilutions was injected (association phase) and washed (dissociation phase). Response in resonance units (RU) was acquired, and data were normalized for baseline response, obtained from a cycle with antibody and buffer alone. Figure 12 provides the kinetic traces for the binding of WT IgG1, WT IgG2, WT IgG4, S239D/I332E IgG2, and S239D/I332E IgG4 antibodies to human V158 FcγRIIIa. The relative amplitudes of the binding traces reflect the relative FcγR affinities of the variants. The data corroborate the AlphaScreen data, indicating further that the novel modifications provide significant FcγR binding enhancements to IgG2 and IgG4.

Example 3. IgGs Variants with Novel and Isotypic Amino Acid Modifications

[226] The present invention provides immunoglobulins wherein the aforedescribed novel variants are coupled with isotypic modifications to provide IgG variants with optimized properties. Figures 13 – 16 describe a set of novel and isotypic amino acid modifications for each isotype IgG1 (Figure 13), IgG2 (Figure 14), IgG3 (Figure 15), and IgG4 (Figure 16). The sequence of the parent IgG is provided explicitly, and novel and isotypic residues are provided at appropriate EU positions according to Figure 6. As an example in Figure 14, IgG2 is the parent immunoglobulin and comprises a deletion at EU position 236. IgG1, IgG2, and IgG3 all comprise glycines at position 236, and serine and alanine are two preferred novel substitutions at position 236. Thus Figure 14 describes in the parent immunoglobulin IgG2 the isotypic modifications -236G and the novel modifications -236S and -236A. According to Figure 14 and 6, the full set of novel modifications in the parent IgG2 at position 236

include -236A, -236D, -236E, -236F, -236H, -236I, -236K, -236L, -236M, -236N, -236P, -236Q, -236R, -236S, -236T, -236V, -236W, and -236Y.

[227] A set of IgG2 trastuzumab variants were constructed comprising novel and isotypic modifications using the information provided in Figure 14. Figure 17 provides this set of IgG variants. For simplicity, constant regions are labeled for easy reference. P233E/V234L/A235L/-236G IgG2, referred to as IgG2 ELLGG, is an IgG2 variant described previously (Chappel et al., 1991, Proc. Natl. Acad. Sci. USA 88(20):9036-9040; Chappel et al., 1993, Journal of Biological Chemistry 268:25124-25131). γ1(118-225) / P233E/V234L/A235L/-236G IgG2, referred to as IgG(1/2) ELLGG, is a novel IgG2 variant comprising the P233E/V234L/A235L/-236G modifications of IgG2 ELLGG and the full set of IgG2 to IgG1 isotypic modifications in the CH1 domain and hinge region (γ1(118-225)). These variants were constructed, expressed, and purified as described previously. Figure 18 shows competition AlphaScreen data for binding of the IgG2 trastuzumab variants to human V158 FcγRIIIa, carried out as described. The results show the favorable FcγR binding properties of the IgG2 ELLGG and IgG(1/2) ELLGG variants. Furthermore, the results show that a number of novel and isotypic modifications significantly improve the FcγR binding affinity of the IgG2 isotype.

[228] A series of isotypic and novel modifications were made and tested in the context of IgG(1/2) ELLGG to further explore the properties of this IgG variant. These variants are provided in Figure 19. The variable region of these IgG variants is that of H3.69_V2_L3.69 AC10, which is an anti-CD30 antibody with reduced immunogenicity. H3.69_V2_L3.69 AC10 is a variant of H3.69_L3.71 AC10 described in USSN 11/004,590 (herein expressly incorporated by reference) with a mutation I2V in the H3.69 VH region. The set of variants in Figure 19 comprise novel and isotypic modifications in the context of IgG(1/2) ELLGG. These variants were constructed, expressed, and purified as described previously. Figure 20 shows competition AlphaScreen data for binding of the the anti-CD30 IgG2 variants to human V158 FcγRIIIa, carried out as described. The fits to the data provide the inhibitory concentration 50% (IC50) (i.e. the concentration required for 50% inhibition) for each antibody, thus enabling the relative binding affinities of Fc variants to be quantitatively determined. By dividing the IC50 for each variant by that of H3.69_V2_L3.71 AC10 IgG1, the fold- enhancement or reduction in receptor binding (Fold V158 FcγRIIIa) are obtained. These values are provided in Figure 21. The results further show that the Fc ligand binding properties of the IgG isotypes can be significantly improved via engineering of novel and isotypic amino acid modificaitons.

[229] Cell-based ADCC assays were carried out on the anti-CD30 IgG variants to investigate their effector function properties. ADCC was measured using either the DELFIA® EuTDA-based cytotoxicity assay (Perkin Elmer) or LDH Cytotoxicity Detection Kit (Roche Diagnostic Corporation, Indianapolis, IN). Human PBMCs were purified from leukopacks using a ficoll gradient. For europium-based detection, target cells were first loaded with BATDA at $1x10^6$ cells/ml and washed 4 times. For both europium- and LDH-based detection, CD30+ L540 Hodgkin's lymphoma target cells were seeded into 96-well plates at 10,000 cells/well, and opsonized using Fc variant or WT antibodies at the indicated final concentration. Triton X100 and PBMCs alone were typically run as controls.

Effector cells were added at 25:1 PBMCs:target cells, and the plate was incubated at 37°C for 4 hrs. Cells were incubated with either Eu3+ solution or LDH reaction mixture, and relative fluorescence units were measured. Data were normalized to maximal (triton) and minimal (PBMCs alone) lysis, and fit to a sigmoidal dose-response model using nonlinear regression. Figure 22a – 22d provide these data. The results show that the optimized FcγR binding properties of the IgG variants result in improved effector function.

[230] A set of IgG variants comprising novel and isotypic modifications were made and tested in the context of two antibodies that target the B-cell antigen CD20. Figure 23 provides a set of IgG variants comprising the variable region of C2B8, an anti-CD20 antibody currently marketed as the biotherapeutic rituximab (US 5,736,137). These variants were constructed, expressed, and purified as described previously. Figure 24 shows cell-based ADCC data for select rituximab IgG2 variants against CD20+ WIL2-S lymphoma target cells. Figure 25 provides a set of IgG variants comprising the variable region of the anti-CD20 antibody PRO70769 (PCT/US2003/040426). These variants were constructed, expressed, and purified as described previously. Figure 26 shows competition AlphaScreen data for binding of these anti-CD20 IgG variants to human V158 FcγRIIIa, and Figure 27 provides a cell-based ADCC for one of the PRO70769 IgG variants against WIL2-S cells. The results are consistent with the aforedescribed results, indicating that the IgG variants are the invention are broadly applicable for improving clinically relevant antibodies.

[231] To explore the effect of the novel and isotypic modifications on complement activity, a cell-based CDC assay was performed. Target WIL2-S lymphoma cells were washed 3x in 10% FBS medium by centrifugation and resuspension, and seeded at 50,000 cells/well. Anti-CD20 antibodies was added at the indicated final concentrations. Human serum complement (Quidel, San Diego, CA) was diluted 50% with medium and added to antibody-opsonized target cells. Final complement concentration was approximately $1/6^{th}$ original stock. Plates were incubated for 2 hrs at 37 °C, Alamar Blue was added, and cells were cultured for two days. Fluorescence was measured, and data were normalized to the maximum and minimum signal and fit to a sigmoidal dose-response curve. Figure 28 shows these data. The results indicate that the novel and isotypic modifications of the invention can be further employed to modulate IgG CDC activity.

[232] Figure 29 provides the amino acid sequences of the variable region VL and VH domains utilized in the present invention, including the anti-CD20, anti-Her2, and anti-CD30 antibodies. These sequences are not meant to constrain the present invention to these variable regions. The present invention contemplates application of the described IgG variants to other antibodies that target CD20, Her2, and CD30. Particularly preferred are anti-CD20 antibodies that bind to an identical or overlapping CD20 epitope as C2B8, anti-CD20 antibodies that bind to an identical or overlapping CD20 epitope as PRO70769, anti-Her2 antibodies that bind to an identical or overlapping Her2 epitope as trastuzumab, and anti-CD30 antibodies that bind to an identical or overlapping CD30 epitope as H3.69_V2_L3.71 AC10. The present invention of course contemplates application of the described IgG variants to antibodies that target other antigens besides CD20, Her2, and CD30.

[233] Figure 30 provides the constant region amino acid sequences described in the present invention. These include the constant light chain kappa region, the four IgG isotypes IgG1, IgG2, IgG3, and IgG4, the IgG2 ELLGG constant region, and the IgG(1/2) ELLGG constant region. These sequences are not meant to constrain the present invention to these constant regions. For example, although the kappa constant chain (Cκ) was used in the present study, the lambda constant chain (Cλ) may be employed.

[234] Figures 31a and 31b provide the amino acid sequences of the full length light and heavy chains of one of the anti-CD20 IgG variants described in the present invention. Figures 31c and 31d provide the amino acid sequences of the full length light and heavy chains of one of the anti-CD30 IgG variant described in the present invention.

[235] All references are herein expressly incorporated by reference.

[236] Whereas particular embodiments of the invention have been described above for purposes of illustration, it will be appreciated by those skilled in the art that numerous variations of the details may be made without departing from the invention as described in the appended claims.

We claim:

1. An IgG2 variant comprising an amino acid sequence having the formula:
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC-X(221)-X(222)-X(223)-X(224)-X(225)-C-
X(227)-X(228)-C-X(230)-X(231)-X(232)-ELLGG-X(238)-X(239)-X(240)-X(241)-L-X(243)-X(244)-
X(245)-X(246)-X(247)-K-X(249)-TLMIS-X(255)-TP-X(258)-V-X(260)-C-X(262)-X(263)-X(264)-X(265)-
X(266)-X(267)-X(268)-X(269)-X(270)-X(271)-X(272)-X(273)-X(274)-X(275)-X(276)-W-X(278)-V-
X(280)-X(281)-X(282)-X(283)-X(284)-X(285)-X(286)-A-X(288)-T-X(290)-X(291)-X(292)-X(293)-
X(294)-X(295)-X(296)-X(297)-X(298)-X(299)-X(300)-X(301)-X(302)-X(303)-X(304)-X(305)-LTVVHQD-
X(313)-LNG-X(317)-X(318)-Y-X(320)-C-X(322)-X(323)-X(324)-X(325)-X(326)-X(327)-X(328)-X(329)-
X(330)-X(331)-X(332)-X(333)-X(334)-X(335)-X(336)-X(337)-
KTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK-, wherein
-X(221)- is selected from the group consisting of no amino acid, K and Y;
-X(222)- is selected from the group consisting of V, E and Y;
-X(223)- is selected from the group consisting of no amino acid, E and K;
-X(224)- is selected from the group consisting of E and Y;
-X(225)- is selected from the group consisting of no amino acid, E, K and W;
-X(227)- is selected from the group consisting of P, E, G, K and Y;
-X(228)- is selected from the group consisting of P, E, G, K and Y;
-X(230)- is selected from the group consisting of P, A, E, G and Y;
-X(231)- is selected from the group consisting of A, E, G, K, P and Y;
-X(232)- is selected from the group consisting of P, E, G, K and Y;
-X(233)- is selected from the group consisting of P, A, D, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(234)- is selected from the group consisting of V, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, W and Y;
-X(235)- is selected from the group consisting of A, D, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;
-X(236)- is selected from the group consisting of no amino acid, A, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;
-X(237)- is selected from the group consisting of G, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y;
-X(238)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(239)- is selected from the group consisting of S, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W and Y;
-X(240)- is selected from the group consisting of V, A, I, M and T;
-X(241)- is selected from the group consisting of F, D, E, L, R, S, W and Y;
-X(243)- is selected from the group consisting of F, E, H, L, Q, R, W and Y;
-X(244)- is selected from the group consisting of P and H;

-X(245)- is selected from the group consisting of P and A;
-X(246)- is selected from the group consisting of K, D, E, H and Y;
-X(247)- is selected from the group consisting of P, G and V;
-X(249)- is selected from the group consisting of D, H, Q and Y;
-X(255)- is selected from the group consisting of R, E and Y;
-X(258)- is selected from the group consisting of E, H, S and Y;
-X(260)- is selected from the group consisting of T, D, E, H and Y;
-X(262)- is selected from the group consisting of V, A, E, F, I and T;
-X(263)- is selected from the group consisting of V, A, I, M and T;
-X(264)- is selected from the group consisting of V, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W and Y;
-X(265)- is selected from the group consisting of D, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;
-X(266)- is selected from the group consisting of V, A, I, M and T;
-X(267)- is selected from the group consisting of S, D, E, F, H, I, K, L, M, N, P, Q, R, V, W and Y;
-X(268)- is selected from the group consisting of H, D, E, F, G, I, K, L, M, P, R, T, V and W;
-X(269)- is selected from the group consisting of E, F, G, H, I, K, L, M, N, P, R, S, T, V, W and Y;
-X(270)- is selected from the group consisting of D, F, G, H, I, L, M, P, Q, R, S, T, W and Y;
-X(271)- is selected from the group consisting of P, A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;
-X(272)- is selected from the group consisting of E, D, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;
-X(273)- is selected from the group consisting of V and I;
-X(274)- is selected from the group consisting of Q, D, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;
-X(275)- is selected from the group consisting of F, L and W;
-X(276)- is selected from the group consisting of N, D, E, F, G, H, I, L, M, P, R, S, T, V, W and Y;
-X(278)- is selected from the group consisting of Y, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V and W;
-X(280)- is selected from the group consisting of D, G, K, L, P and W;
-X(281)- is selected from the group consisting of G, D, E, K, N, P, Q and Y;
-X(282)- is selected from the group consisting of V, E, G, K, P and Y;
-X(283)- is selected from the group consisting of E, G, H, K, L, P, R and Y;
-X(284)- is selected from the group consisting of V, D, E, L, N, Q, T and Y;
-X(285)- is selected from the group consisting of H, D, E, K, Q, W and Y;
-X(286)- is selected from the group consisting of N, E, G, P and Y;
-X(288)- is selected from the group consisting of K, D, E and Y;
-X(290)- is selected from the group consisting of K, D, H, L, N and W;
-X(291)- is selected from the group consisting of P, D, E, G, H, I, Q and T;
-X(292)- is selected from the group consisting of R, D, E, T and Y;
-X(293)- is selected from the group consisting of E, F, G, H, I, L, M, N, P, R, S, T, V, W and Y;
-X(294)- is selected from the group consisting of E, F, G, H, I, K, L, M, P, R, S, T, V, W and Y;
-X(295)- is selected from the group consisting of Q, D, E, F, G, H, I, M, N, P, R, S, T, V, W and Y;
-X(296)- is selected from the group consisting of F, A, D, E, G, I, K, L, M, N, Q, R, S, T and V;

-X(297)- is selected from the group consisting of N, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(298)- is selected from the group consisting of S, E, F, H, I, K, M, Q, R, W and Y;

-X(299)- is selected from the group consisting of T, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W and Y;

-X(300)- is selected from the group consisting of F, A, D, E, G, H, K, M, N, P, Q, R, S, T, V and W;

-X(301)- is selected from the group consisting of R, D, E, H and Y;

-X(302)- is selected from the group consisting of V and I;

-X(303)- is selected from the group consisting of V, D, E and Y;

-X(304)- is selected from the group consisting of S, D, H, L, N and T;

-X(305)- is selected from the group consisting of V, E, T and Y;

-X(313)- is selected from the group consisting of W and F;

-X(317)- is selected from the group consisting of K, E and Q;

-X(318)- is selected from the group consisting of E, H, L, Q, R and Y;

-X(320)- is selected from the group consisting of K, D, F, G, H, I, L, N, P, S, T, V, W and Y;

-X(322)- is selected from the group consisting of K, D, F, G, H, I, P, S, T, V, W and Y;

-X(323)- is selected from the group consisting of V and I;

-X(324)- is selected from the group consisting of S, D, F, G, H, I, L, M, P, R, T, V, W and Y;

-X(325)- is selected from the group consisting of N, A, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W and Y;

-X(326)- is selected from the group consisting of K, I, L, P and T;

-X(327)- is selected from the group consisting of A, G, D, E, F, H, I, K, L, M, N, P, R, T, V, W and Y;

-X(328)- is selected from the group consisting of L, A, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W and Y;

-X(329)- is selected from the group consisting of P, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W and Y;

-X(330)- is selected from the group consisting of A, E, F, G, H, I, L, M, N, P, R, T, V, W and Y;

-X(331)- is selected from the group consisting of P, D, F, H, I, L, M, Q, R, T, V, W and Y;

-X(332)- is selected from the group consisting of I, A, D, E, F, H, K, L, M, N, P, Q, R, S, T, , V, W and Y;

-X(333)- is selected from the group consisting of E, F, H, I, L, M, P, T and Y;

-X(334)- is selected from the group consisting of K, F, I, P and T;

-X(335)- is selected from the group consisting of T, D, F, G, H, I, L, M, N, P, R, S, V, W and Y;

-X(336)- is selected from the group consisting of I, E, K and Y;

-X(337)- is selected from the group consisting of S, E, H and N, and

wherein the variant differs from SEQ ID NO:16 by at least one amino acid.

2. The IgG2 variant according to claim 1, wherein the X(327) is A.

3. An IgG2 variant comprising an amino acid sequence having the formula:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC-X(221)-V-E-C-X(227)-PCPAPELLGGP-

X(239)-X(240)-FLFPP-X(246)-PKDTLMIS-X(255)-TP-X(258)-V-X(260)-CVV-X(264)-DV-X(267)-X(268)-ED-X(271)-X(272)-V-X(274)-FNW-X(278)-VD-X(281)-V-X(283)-X(284)-HNAKT-X(290)-PR-X(293)-E-X(295)-FNSTFRVV-X(304)-VLTVVHQDWLNGKEYKCKV-X(324)-N-X(326)-X(327)-X(328)-P-X(330)-P-X(332)-X(333)-X(334)-
TISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

wherein

-X(221)- is selected from the group consisting of no amino acid and K;

-X(227)- is selected from the group consisting of P and G;

-X(237)- is selected from the group consisting of G and D;

-X(239)- is selected from the group consisting of S, D, E, N, Q and T;

-X(240)- is selected from the group consisting of V, I and M;

-X(246)- is selected from the group consisting of K, H and Y;

-X(255)- is selected from the group consisting of R and Y;

-X(258)- is selected from the group consisting of E, H and Y;

-X(260)- is selected from the group consisting of T and H;

-X(264)- is selected from the group consisting of V, I, T and Y;

-X(267)- is selected from the group consisting of S, D and E;

-X(268)- is selected from the group consisting of H, D and E;

-X(271)- is selected from the group consisting of P and G;

-X(272)- is selected from the group consisting of E, Y, H, R and I;

-X(274)- is selected from the group consisting of Q and E;

-X(278)- is selected from the group consisting of Y and T;

-X(281)- is selected from the group consisting of G, D and E;

-X(283)- is selected from the group consisting of E, L and H;

-X(284)- is selected from the group consisting of V, E and D;

-X(290)- is selected from the group consisting of K and N;

-X(293)- is selected from the group consisting of E and R;

-X(295)- is selected from the group consisting of Q and E;

-X(304)- is selected from the group consisting of S and T;

-X(324)- is selected from the group consisting of S, G and I;

-X(326)- is selected from the group consisting of K and T;

-X(327)- is selected from the group consisting of A, G and D;

-X(328)- is selected from the group consisting of L, A, F, I and T;

-X(330)- is selected from the group consisting of A, L, Y and I;

-X(332)- is selected from the group consisting of I, D, E, N, Q and T;

-X(333)- is selected from the group consisting of E and Y;

-X(334)- is selected from the group consisting of K, F, I and T;

and wherein at least one of the positions is different from the sequence of SEQ ID NO:16.

4. The IgG2 variant according to claim 3, wherein the X(327) is A.

Figure 1

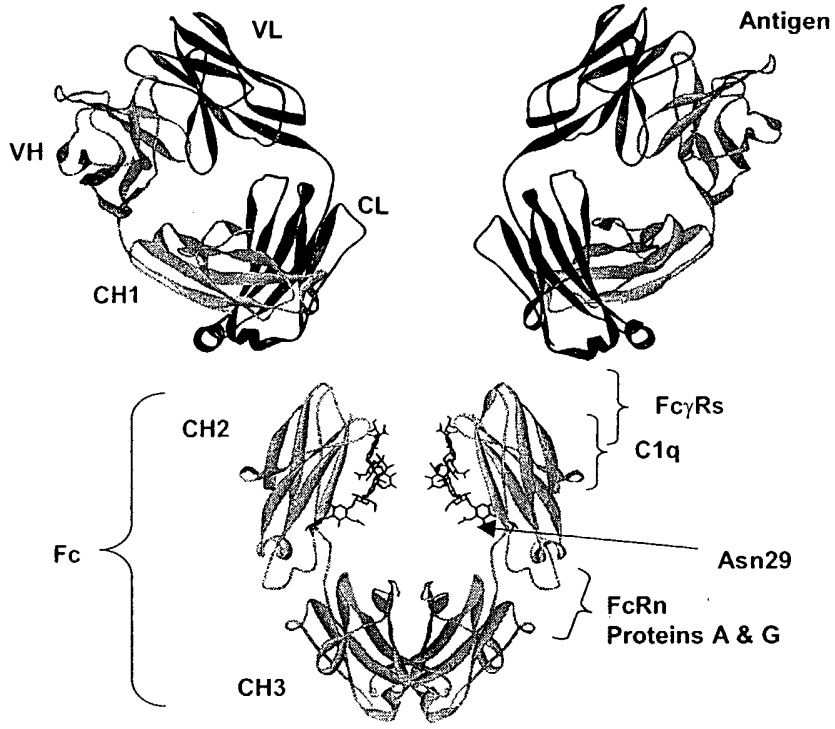

Figure 2

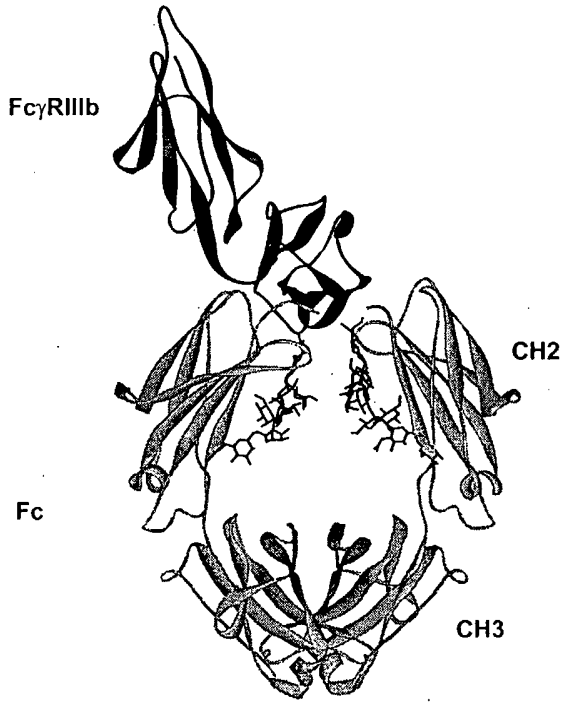

Figure 3

Affinity Enhancement	Affinity Reduction	Cell Activity	Therapeutic Activity
FcγRI only	-	Enhanced dendritic cell activity and uptake, and subsequent presentation of antigens Enhanced monocyte and macrophage response to antibody	Enhanced cell-based immune response against target
FcγRIIIa	-	Enhanced ADCC and phagocytosis of broad range of cell types	Increased target cell lysis
FcγRIIIa	FcγRIIb	Enhanced ADCC and phagocytosis of broad range of cell types	Increased target cell lysis
FcγRIIb FcγRIIc	-	Reduced activity of all FcγR bearing cell types except NK cells Possible activation of NK cells via FcγRIIc receptor signaling	Enhancement of target cell lysis selective for NK cell accessible target cells
FcγRIIb FcγRIIIa	-	Possible NK cell specific activation and enhancement of NK cell mediated ADCC	Enhanced target cell lysis selective for NK cell accessible target cells
FcγRIIIb	-	Neutrophil mediated phagocytosis enhancement	Enhanced target cell destruction for neutrophil accessible cells
FcαR	-	Neutrophil mediated phagocytosis enhancement	Enhanced target cell destruction for neutrophil accessible cells
FcγRI FcγRIIa FcγRIIIa	FcγRIIb	Enhanced dendritic cell activity and uptake, and subsequent presentation of antigens to T cells Enhanced monocyte and macrophage response to antibody	Enhanced cell-based immune response against target
FcγRIIb	FcγRI FcγRIIa FcγRIIIa	Reduced activity of monocytes, macrophages, neutrophils, NK, dendritic and other gamma receptor bearing cells	Eliminated or reduced cell-mediated cytotoxicity against target bearing cells

Figure 4

Variant	Substitution(s)	Context	FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		IIIa:IIb
			Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	
1	V264A	a	0.67	0.06	0.32	0.69	1.03	0.40	0.91	0.57	0.14	0.25	2.16	0.23	1.12	0.18	0.13
											0.53						
2	V264L	a	0.76	0.07	0.43	0.67	1.17	0.33	0.90	0.56	0.22	0.31	1.44	0.21	0.81	0.17	0.19
											0.56						
3	V264I	atr	1.35	0.05	0.70	0.57	1.28	0.33	1.05	0.51	0.84	0.22	8.58	0.56	1.32	0.18	0.66
											1.43						
4	F241W	a	1.59	0.13	0.68	0.62	1.10	0.34	0.85	0.57	0.32	0.23	3.68	0.21	1.70	0.25	0.29
											0.29						
5	F241L	a	0.84	0.25	0.77	0.57	1.20	0.34	1.12	0.50	0.24	0.27	3.24	0.30	1.99	0.48	0.20
											0.26						
6	F243W	a	1.61	0.23	1.71	0.58	1.44	0.34	1.11	0.45	1.12	0.18	2.88	0.24	4.63	0.26	0.78
											0.51						
7	F243L	a	0.51	0.07	0.31	0.63	1.00	0.44	0.72	0.72	0.43	0.26	2.47	0.21	0.64	0.12	0.43
											0.51						
8	F241L/F243L/V262I/V264I	a	0.80	0.21	0.10	0.36		24.05	8.39	6.19	0.06	0.66					
			0.51	0.10			0.49	0.57			0.05	0.48	1.24	0.20	0.44	0.19	0.10
											0.09						
9	F241W/F243W	a	1.13	0.07	0.81	0.59	1.39	0.38	0.96	0.54	0.28	0.21	1.88	0.27	1.12	0.12	0.20
											0.07						
10	F241W/F243W/V262A/V264A	a	0.50	0.12	0.28	0.58	1.33	0.32	1.06	0.53	0.19	0.23	2.13	0.19	1.91	0.15	0.15
											0.04						
11	F241L/V262I	a	1.30	0.10	0.27	0.59	0.75	0.36			0.17	0.25	5.29	0.63	1.37	0.17	0.23
											0.06						
12	F243L/V264I	a	1.02	0.12	0.34	0.68	0.69	0.38			0.72	0.21	3.94	0.30	0.71	0.34	1.04
											1.23						
13	F243L/V262I/V264W	a	0.32	0.26	0.12	0.31		24.05	15.82	5.78	0.03	0.51					
			0.30	0.18	0.24	195	0.91	0.44	1.05	28.03	0.55	0.24	3.72	0.62	1.35	0.42	0.60
											0.02						
14	F241Y/F243Y/V262T/V264T	a	0.58	0.19	0.18	0.32		24.04	30.49	5.72	0.06	0.42					
			0.66	0.23	0.25	1.97	1.17	0.35	1.01	9.71	0.47	0.19	1.19	0.36	1.89	0.57	0.40
											0.05						
15	F241E/F243R/V262E/V264R	a	0.05	0.39	0.04	0.61		24.05	10.73	5.99							
			0.06	0.11			0.69	0.52	0.67	1.52	0.48	0.24	1.51	0.18	0.28	0.16	0.70
											0.05						
16	F241E/F243Q/V262T/V264E	a	0.02	0.31	0.10	0.99		24.07	5.62	6.06							
			0.01	0.27			0.36	0.67			0.07	0.30	1.34	0.24	0.24	0.23	0.19
											0.07						
17	F241R/F243Q/V262T/V264R	a	0.01	0.63	0.07	0.51		24.06	17.05	5.77		54.64					
			0.16	0.11	0.21	521	1.00	0.35	0.71	0.80	0.80	0.19	1.58	0.22	0.45	0.27	0.79
											0.02						
18	F241E/F243Y/V262T/V264R	a	0.17	0.24	0.08	0.30		24.05	17.48	5.79	0.02	1.23					
			0.12	0.18	0.18	63.41	0.94	0.38	0.83	1.39	0.51	0.19	1.24	0.19	0.54	0.29	0.55
											0.05						
19	L328M	e	2.07	5407	0.47	0.35		24.04	0.19	9.68	0.05	0.28					
			1.33	0.07	0.85	0.56	1.90	0.32	1.29	0.46	0.62	0.22	5.40	0.57	0.84	0.21	0.32
											0.21						
20	L328E	e	2.17	0.17	0.07	0.34		24.04			0.07	0.34					
			1.23	0.10	0.23	124	1.90	0.33	1.07	0.54	0.72	0.21	2.55	0.36	0.73	0.31	0.38
											0.12						
21	L328F	e	1.38	0.17	0.13	0.30		24.05	71.81	5.62	0.00	4.94					
			0.99	0.18	0.25	2.89	5.14	0.33	4.13	0.45			2.48	0.35	2.06	0.46	
											0.24						
22	I332E	atrp	3.97	0.19	1.09	0.60	1.92	0.33	1.27	0.49	3.57	0.19	4.28	0.56	1.32	0.44	1.86
			2.08	0.13	1.26	0.21	2.37	0.37	2.64	0.34	1.22	2.82			0.78	0.41	0.52
							3.93				6.72						1.71
23	L328M/I332E	e	0.30	0.19	0.28	0.35	0.90	0.12	0.82	0.12	0.21	0.15	0.05	0.64			0.24
											2.6						
24	P244H	e	1.05	0.34	0.53	0.67	1.02	0.42	0.79	0.68	0.64	0.23	1.42	0.26	0.46	0.14	0.62
											0.83						
25	P245A	a	0.84	0.11	0.46	0.65	0.70	0.52			0.45	0.22	2.00	0.26	0.44	0.27	0.64
											0.25						
26	P247V	a	1.06	0.12	0.99	0.58	1.34	0.48	0.88	0.54	0.62	0.20	1.45	0.31	0.43	0.24	0.46
											0.53						
27	W313F	a	0.82	0.11	0.38	0.65	0.64	0.39			0.24	0.20	1.36	0.19	0.47	0.25	0.38
											0.88						
28	P244H/P245A/P247V	a	0.98	0.09	0.68	0.57	0.73	0.32			0.52	0.23	4.95	0.54	0.52	0.25	0.71
											0.93						
29	P247G	e	1.57	0.20	0.35	0.70	0.69	0.40	0.83	8.15	0.28	0.22	1.72	0.24	0.68	0.45	0.41
											0.54						
30	V264I/I332E	atrc	1.65	0.18	0.79	0.54	2.13	0.36	1.41	0.49	4.80	0.19	4.07	0.38	1.70	0.61	2.25
			4.58	0.20	0.89	0.23	5.08	0.40	2.80	0.31	3.00	2.82			0.89	0.35	0.59
							1.57				12.5						7.96

Figure 4 (continued)

Variant	Substitution(s)	Context	FcγRI Fold	FcγRI Conf	FcγRIIa Fold	FcγRIIa Conf	FcγRIIb Fold	FcγRIIb Conf	FcγRIIc Fold	FcγRIIc Conf	FcγRIIIa Fold	FcγRIIIa Conf	C1q Fold	C1q Conf	FcRn Fold	FcRn Conf	IIIa:IIb
31	F241E/F243R/V262E/ V264R/I332E	a									0.19						
32	F241E/F243Q/V262T/ V264E/I332E	a															
33	F241R/F243Q/V262T/ V264R/I332E	a															
34	F241E/F243Y/V262T/ V264R/I332E	a									0.1						
35	S298A	a									2.21						
36	S298A/I332E	a									21.7						
37	S298A/E333A/K334A	a t r p									2.56						
41	S239E/I332E	a	3.64	0.21	3.22	0.13	8.73	0.11	8.29	0.17	88.98	0.33	0.76	0.29	1.34	0.31	10.19
							3.49				5.8						1.66
42	S239Q/I332E	a	2.08	0.23	1.91	0.14	3.68	0.13	3.64	0.18	13.85	0.42	1.67	0.20	1.33	0.37	3.77
							4.68				6.6						1.41
43	S239E	a t	2.17	0.23	1.38	0.15	2.18	0.11	3.01	0.23	51.22	0.47	0.95	0.21	1.47	0.85	23.47
											10.2						
44	D265G	a	0.17	0.22	1.97	0.10	4.86	0.09	3.19	0.15	4.37	0.38	0.72	0.28	1.74	0.52	0.90
											<0.02						
45	D265N	a	0.02	0.31	0.95	0.14	0.90	0.14	0.58	0.18		1.30	0.55	0.31	0.48	0.28	
											<0.02						
46	S239E/D265G	a	0.11	0.42	0.75	0.12	0.93	0.11	0.95	0.22			1.15	0.22	0.50	0.34	
											<0.02						
47	S239E/D265N	a	0.12	0.26	0.61	0.12	1.39	0.09	1.11	0.15	1.51	0.38	0.63	0.25	0.52	0.35	1.08
											0.02						
48	S239E/D265Q	a	0.65	0.23	0.53	0.15	0.99	0.12	1.00	0.18			1.74	0.19	0.37	0.55	
											0.05						
49	Y296E	a	1.56	0.22	1.09	0.13	1.28	0.12	1.66	0.19	1.04	0.49	1.85	0.18	1.23	0.60	0.81
							1.11				0.73						0.66
50	Y296Q	a	0.81	0.23	1.18	0.19	2.49	0.15	2.45	0.19	2.13	0.37	1.18	0.22	0.31	0.50	0.85
							0.43				0.52						1.21
51	S298T	a	1.33	0.23	1.15	0.15	1.44	0.10	1.63	0.19	2.04	0.35	1.90	0.19	2.13	0.76	1.41
							<0.02				0.94						
52	S298N	a	0.59	0.24	0.80	0.14	1.27	0.13	0.91	0.17			1.28	0.21	0.94	0.31	
							<0.02				0.41						
53	T299I	a	0.13	0.22	0.90	0.13	1.26	0.11	0.57	0.17			0.80	0.24	0.34	0.36	
											<0.02						
54	A327S	a	0.39	0.26	0.64	0.12	0.89	0.10	1.15	0.24		3.86	0.47	0.25	0.13	0.44	
							0.39				0.23						0.59
55	A327N	a	1.25	0.32	0.77	0.16	1.07	0.10	1.31	0.17	0.61	0.40	0.66	0.32	0.88	0.38	0.57
							1.15				0.19						0.17
56	S267Q/A327S	a	0.44	0.22	0.52	0.11	1.14	0.16	1.08	0.18			0.88	0.22	0.71	0.51	
											0.03						
57	S267L/A327S	a	0.35	0.23	0.64	0.14	1.23	0.13	0.97	0.19	0.27	0.35	1.93	0.20	1.05	0.35	0.22
											<0.02						
58	A327L	a	0.53	0.28	0.55	0.21	1.21	0.19	0.97	0.29			0.89	0.25	1.55	0.52	
											0.05						
59	P329F	a	0.33	0.25	0.93	0.16	1.86	0.09	1.91	0.17	0.96	0.33	2.29	0.18	0.61	0.29	0.51
											<0.02						
60	A330L	a p	1.37	0.23	1.27	0.13	1.23	0.10	1.20	0.18	1.08	0.41	0.83	0.24	0.96	0.47	0.87
							0.38				0.73						1.92
61	A330Y	a p	1.18	0.23	1.08	0.15	1.62	0.09	1.11	0.20	1.04	0.47	2.00	0.24	0.94	0.41	0.64
							0.75				1.64						2.19
62	I332D	a	2.70	0.26	5.41	0.11	3.76	0.11	3.69	0.17	9.03	0.36	2.73	0.20	0.42	0.36	2.40
			3.95	0.10	8.79	0.23	8.32	0.39	7.11	0.29	2.85	2.82			1.75	0.39	0.34
							3.34				17.8						5.33
63	N297S	a	0.03	0.26	0.68	0.12	0.65	0.11	0.68	0.23			1.77	0.23	0.38	0.55	
											<0.02						
64	N297D	a	0.22	0.23	0.62	0.19	1.30	0.09	1.07	0.16	1.00	0.36	1.01	0.22	0.63	0.32	0.77
65	N297S/I332E	a	0.18	0.24	0.62	0.24	0.89	0.13	0.69	0.20	<0.02		0.89	0.19	0.19	0.52	
											<0.02						
66	N297D/I332E	a	1.41	0.23	0.60	0.23	0.94	0.20	0.80	0.29			0.97	0.23	0.96	0.82	
							<0.02				0.08						
67	N297E/I332E	a	0.45	0.24	0.54	0.15	0.96	0.17	0.76	0.24			0.65	0.22	0.58	0.31	
											<0.02						
68	D265Y/N297D/I332E	a	0.09	0.24	0.65	0.14	1.16	0.11	0.83	0.16	0.31	0.38	2.78	0.21	0.25	0.33	0.27
											<0.02						
69	D265Y/N297D/T299L/I332E	a	0.33	0.22	1.63	0.13	1.99	0.09	1.30	0.15	3.15	0.40	1.33	0.21	0.63	0.57	1.58
											<0.02						
70	D265F/N297E/I332E	a	0.01	0.29	0.79	0.13	0.65	0.13	0.87	0.20			1.56	0.18	0.31	0.47	
			0.37	0.36	0.80	0.26	1.68	0.32	2.64	0.29	5.65	0.71	1.00	0.46	1.38	0.75	3.37
											<0.02						
71	L328M/I332E	a	2.79	0.11	1.44	0.17	7.61	0.24	6.59	0.25	11.11	0.70	1.14	0.47	2.21	0.55	1.46
			3.95	0.13	1.22	0.23	11.60	0.27	7.87	0.94	12.61	0.12	2.62	0.31	0.82	0.24	1.09
											7.03						

Figure 4 (continued)

Variant	Substitution(s)	Context	FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		IIIa:IIb
			Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	
72	L328Q/I332E	a	2.87	0.12	1.76	0.13	11.30	0.29	6.52	0.23	1.93	0.69	0.91	0.48	4.29	0.65	0.17
			2.15	0.14	1.05	0.16	15.13	0.29	7.38	0.93	1.69	0.22	1.90	0.31	0.88	0.33	0.11
											1.54						
73	I332N	a	1.64	0.20	2.96	0.14	1.43	0.32	1.82	0.26	1.70	0.71	0.98	0.52	3.20	0.54	1.19
			0.82	0.12	1.52	0.19	1.26	0.57		46.97	0.33	0.11	2.35	0.33	0.71	0.33	0.26
											0.39						
74	I332Q	a	1.49	0.33	2.70	0.16	1.10	0.28	1.10	0.29	0.67	0.70	1.10	0.49	1.07	0.51	0.61
			1.29	0.15	2.08	0.19	1.46	0.29	1.11	1.34	0.65	0.14	1.82	0.36	1.20	0.32	0.45
											0.37						
75	V264T	a	1.36	0.21	2.82	0.15	2.94	0.20	2.86	0.24	1.30	0.69	1.08	0.49	1.00	0.47	0.44
			1.25	0.15	2.50	0.16	4.84	0.21	6.12	1.16	1.73	0.10	1.42	0.33	1.66	0.35	0.36
											2.73						
76	V264F	a	0.35	0.17	0.14	0.43	0.96	0.56	0.47	0.82	0.16	0.79	1.16	0.50	0.82	0.51	0.17
			0.43	0.22	0.05	0.23	0.22	0.46			0.06	0.13	1.87	0.29	1.07	0.36	0.29
											0.16						
77	V240I	a	0.95	0.12	1.19	0.16	1.02	0.28	1.06	0.25	1.37	0.69			1.21	0.51	1.34
			1.17	0.14	0.38	22.08	1.04	0.22	0.86	0.91	0.87	0.10	2.02	0.33	1.28	0.34	0.84
											3.25						
78	V263I	a	1.39	0.31	0.61	0.21	0.68	0.60	0.94	0.42	0.15	0.71	1.11	0.49	2.71	0.73	0.22
											0.1						
79	V266I	a	1.64	0.12	1.63	0.24	3.66	0.36	3.85	1.17	1.41	0.15	1.25	0.31	1.17	0.32	0.39
											1.86						
80	T299A	a	0.01	0.18	0.10	0.25	0.56	0.48	72.84	6.18	0.06	0.37	2.31	0.32	0.82	0.33	0.11
											0.03						
81	T299S	a	0.80	0.19	0.16	0.24	2.01	0.81			0.19	0.20	1.52	0.32	0.86	0.31	0.09
											0.15						
82	T299V	a	0.02	0.20	0.14	0.20	0.21	0.50	19.44	7.11	0.21	0.14	1.92	0.41	0.35	0.31	1.03
											<0.02						
83	N325Q	a	0.65	0.17	0.07	0.23	0.26	0.42	62.17	9.35	0.04	0.57	1.92	0.34	0.69	0.34	0.16
											<0.02						
84	N325L	a	0.42	0.25	0.04	0.42	1.46	0.39		18.73	0.03	0.93	2.18	0.28	0.91	0.33	0.02
											<0.02						
85	N325I	a	0.35	0.12	0.05	0.31	0.86	0.26	0.97	1.09	0.09	0.13	2.05	0.33	0.89	0.33	0.11
											<0.02						
86	S239D	a/p	4.40	0.25	1.74	0.57	6.21	0.29	5.13	0.45	6.29	0.20	3.54	0.42	1.73	0.48	1.01
			2.63	0.13	2.29	0.13	11.42	0.17	3.83	0.80	23.17	0.10	0.96	0.46	0.80	0.31	2.03
			1.86	0.13	2.17	0.22	9.04	0.37	10.98	0.28	2.55	2.82			1.07	0.41	0.28
							4.47*				11.6						2.60
87	S239N	a									<0.02						
88	S239F	a	0.28	0.17	0.02	1.14	0.33	0.68		51.36	0.10	0.24	0.95	0.43	0.85	0.34	0.30
							<0.02				0.22						
89	S239D/I332D	a	2.33	0.11	2.68	0.16	41.43	0.18	18.96	0.78	25.53	0.11	1.53	0.29	1.12	0.33	0.62
											14.1						
90	S239D/I332E	a/t/r/c/p	3.89	0.12	11.57	0.15	128	0.26	145	0.99	192	0.10	1.88	0.35	1.01	0.36	1.50
			9.42	0.13	15.46	0.19	70.44	0.37	84.57	0.29	27.74	2.82			1.22	0.36	0.39
							19.71*				56.1						2.85
91	S239D/I332N	a	1.97	0.12	4.95	0.16	14.13	0.20	8.14	0.82	10.79	0.12	1.16	0.35	0.76	0.31	0.76
											7.19						
92	S239D/I332Q	a	1.81	0.21	3.05	0.33	15.24	0.19	9.75	0.91	9.41	0.09	1.28	0.32	0.64	0.35	0.62
											9.28						
93	S239E/I332D	a	4.52	0.18	1.72	0.15	10.87	0.29	30.32	1.31	21.77	0.10	2.42	0.31	1.00	0.37	2.00
											9.33						
94	S239E/I332N	a	1.62	0.12	1.81	0.16	4.55	0.23	6.01	1.16	16.86	0.13	2.00	0.32	0.88	0.33	3.71
											11.9						
95	S239E/I332Q	a	1.66	0.19	0.93	0.11	2.52	0.34	1.41	0.97	3.21	0.10	1.78	0.29	0.64	0.31	1.28
											3.8						
96	S239N/I332D	a	2.01	0.21	2.40	0.16	11.11	0.32	8.21	0.91	9.69	0.12	2.09	0.27	0.73	0.34	0.89
											3.06						
97	S239N/I332E	a	1.93	0.12	1.64	0.16	29.42	0.24	8.44	0.81	19.80	0.12	1.36	0.29	0.73	0.33	0.67
											14.2						
98	S239N/I332N	a	0.55	0.18	0.67	0.21	1.88	0.46		13.55	0.55	0.15	1.08	0.33	0.84	0.35	0.29
											0.43						
99	S239N/I332Q	a	0.73	0.13	0.72	0.17	2.55	0.35	11.03	2.16	0.59	0.11	1.86	0.29	0.73	0.33	0.23
											0.56						
100	S239Q/I332D	a	1.40	0.13	1.08	0.14	5.76	0.36	5.26	1.05	2.33	0.11	1.89	0.29	0.74	0.35	0.40
											5.05						
101	S239Q/I332N	a	0.52	0.25	0.80	0.15	1.55	0.50		10.71	0.32	0.09	2.68	0.30	1.11	0.35	0.21
											0.39						
102	S239Q/I332Q	a	0.86	0.22	0.69	0.12	1.51	0.42			0.42	0.21	1.41	0.21	1.04	0.14	0.28
											0.59						

Figure 4 (continued)

			FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
Variant	Substitution(s)	Context	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
103	K326E	ap	3.17	0.17	1.55	0.13	4.15	0.47			8.07	0.34	0.96	0.18			1.95
			3.41	0.11	0.79	0.24	4.99	0.41	3.68	0.31	0.25	2.82			0.82	0.38	0.05
104	Y296D	a	1.28	0.18	0.88	0.10	1.28	0.55			3.85 0.86 0.62	0.21	0.83	0.21	0.50	0.09	0.67
105	Y296N	a	0.84	0.23	0.68	0.13	1.30	0.41			0.21	0.21	0.67	0.20	0.26	0.14	0.16
106	F241Y/F243Y/V262T/ V264T/N297D/I332E	a	0.38	0.25	0.11	0.23					0.29 0.25 0.15	0.23	1.35	0.22	0.25	0.09	
107	A330Y/I332E	a	4.49	0.18	4.81	0.13	10.81	0.36			68.79	0.20	0.74	0.18	1.53	0.09	6.37
108	V264I/A330Y/I332E	a	3.89	0.19	1.17	0.07	4.4 4.18	0.35			12 22.76	0.20	0.61	0.17	0.85	0.08	2.73 5.44
109	A330L/I332E	a	4.94	0.17	1.28	0.09	3.54 3.15 2.03	0.35			12 55.42 10.3	0.20	0.72	0.18	1.50	0.20	3.39 17.59 5.09
110	V264I/A330L/I332E	a	4.31	0.22	0.66	0.08	2.73	0.34			24.96	0.20	1.12	0.21	1.13	0.17	9.13
111	L234D	a	0.36	0.30	0.40	0.13	1.79 4.95	0.35			11.2 3.89	0.21	0.96	0.21	1.54	0.16	6.23 0.79
112	L234E	a	0.42	0.29	0.24	0.08	4.78	0.36			0.21 1.86	0.21	1.19	0.21	1.25	0.11	0.39
113	L234N	a	0.10	0.32	0.19	0.11	2.21 2.05	0.41			1.34 0.49	0.22	1.18	0.33	1.06	0.13	0.61 0.24
114	L234Q	a	0.28	0.27	0.28	0.09	1.39 3.53	0.38			0.56 0.52	0.20	0.94	0.18	0.97	0.11	0.40 0.15
115	L234T	a	0.49	0.26	0.20	0.10	1.79	0.43			0.37 0.26	0.22	0.56	0.21	0.99	0.08	0.14
116	L234H	a	0.11	0.34	0.29	0.08	1.56	0.49			0.35 0.27 0.33	0.21	0.65	0.19	1.48	0.08	0.18
117	L234Y	ap	1.45	0.24	0.51	0.09	1.93	0.39			0.80	0.21	0.99	0.22	1.90	0.21	0.41
118	L234I	a	1.20	0.27	0.78	0.08	1.08 2.57	0.40			1.42 1.30	0.21	1.28	0.28	1.26	0.12	1.31 0.50
119	L234V	a	1.66	0.26	0.78	0.08	1.14 3.94	0.35			1.55 1.61	0.22	0.64	0.18	1.45	0.13	1.36 0.41
120	L234F	a	0.74	0.26	0.47	0.07	2.36	0.37			0.38 0.37 0.3	0.21	0.72	0.21	1.46	0.13	0.15
121	L235D	ap			0.76	0.09	5.48	0.37			1.61	0.20	1.05	0.17	0.90	0.15	0.29
122	L235S	a			0.27	0.08	3.63 2.99	0.37			1.66 0.95	0.21	0.66	0.21	1.51	0.09	0.46 0.32
123	L235N	a	0.06	0.37	0.21	0.15	1.59	0.46			1.25 0.37	0.22	0.70	0.20	1.32	0.09	0.23
124	L235Q	a	0.09	0.28	0.30	0.10	1.40	0.44			0.4 1.02	0.21	0.85	0.22	1.67	0.14	0.73
125	L235T	a	0.13	0.26	0.53	0.11	3.55	0.34			0.51 2.15	0.21	1.06	0.23	1.65	0.38	0.60
126	L235H	a	0.06	0.37	0.51	0.09	1.77	0.37			0.52 0.30	0.23	0.54	0.19	0.96	0.14	0.17
127	L235Y	ap	0.24 0.02	0.31 0.55	3.32 0.70	0.08 0.20	4.44 1.39	0.35 0.64	1.15	0.38	0.41 1.74 0.05	0.23 2.82	0.86	0.22	1.02 0.78	0.10 0.39	0.39 0.04
128	L235I	a	0.16	0.26	0.67	0.10	10.15 1.24	0.55			1.19 1.47	0.26	0.68	0.21	0.81	0.10	0.12 1.18
129	L235V	a	0.16 0.11	0.31 0.21	0.43 0.23	0.10 0.22	0.94 0.76	0.52	0.57	1.26	1.1 0.58 0.05	0.22 2.82	0.94	0.26	1.31 0.86	0.17 0.45	1.17 0.07
130	L235F	a			1.25	0.17	1.62	0.45			0.48 0.56	0.22	0.71	0.21	0.80	0.11	0.35
131	S239T	a	0.88	0.17	0.91	0.13	3.53 1.47	0.52			0.73 0.90	0.21	0.84	0.25	1.12	0.10	0.21 0.61
132	S239H	a	0.08	0.10	0.11	0.38	0.20	0.65	0.23	1.24	1.34	2.04	0.88	0.24	0.72	0.11	
133	S239Y	a	0.19	0.12	0.12	0.34	0.69	0.48	1.88	0.42	0.2 0.21		1.70	0.23	1.08	0.13	
134	V240A	a	0.80	0.10	0.62	0.28	1.45	0.39	1.02	0.44	1.49	0.20	0.91	0.22	0.93	0.11	1.03
135	V240T	a	0.92	0.12	0.67	0.30	0.14 1.20	0.46	0.13	0.97	0.7 1.16	0.16	0.78	0.23	0.46	0.69	5.00 0.96
136	V240M	a	1.60	0.07	0.67	0.30	0.94	0.55	0.68	0.68	0.86	0.22	0.74	0.21	0.93	0.09	0.92
137	V263A	a	1.05	0.14	0.45	0.31	1.38 0.04	1.64			2.06 0.54	0.26	1.35	0.25	0.91	0.09	1.49 12.93
138	V263T	a	1.00	0.08	1.57	0.27	1.20	0.39	1.02	0.67	2.67 0.43	0.32	0.59	0.20	0.93	0.15	2.23

Figure 4 (continued)

Variant	Substitution(s)	Context	FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		IIIa:IIb
			Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	
139	V263M	a	0.69	0.07	0.41	0.26	1.69	0.41	1.65	0.38		3.66	1.32	0.26	0.58	0.19	
											0.05						
140	V264M	a	0.64	0.08	0.30	0.29	0.83	0.44	0.94	0.87	0.21	0.41	0.73	0.21	0.63	0.11	0.25
											0.26						
141	V264Y	a	0.51	0.08	0.32	0.29	0.57	0.44	0.81	0.59	0.30	0.29	1.19	0.22	0.75	0.12	0.52
							0.27				1.02						3.78
142	V266A	a	0.61	0.12	0.30	0.28	0.76	0.64	0.06	1.67	0.61	0.25	0.95	0.22	0.71	0.12	0.80
											<0.02						
143	V266T	a	0.28	0.12	0.10	0.32	1.16	0.48	0.18	1.05			1.21	0.24	0.53	0.14	
											0.45						
144	V266M	a	1.32	0.09	0.43	0.29	16.45	0.45	3.32	0.45		7.83	0.83	0.21	0.71	0.11	
			1.11	0.15	0.19	0.23	2.63	0.40	2.57	0.30	0.01	2.84			0.86	0.45	0.01
											0.62						
145	E269H	a	0.16	0.13	0.18	0.31	0.07	1.25	0.28	0.92		5.90	1.11	0.25	0.72	0.12	
											<0.02						
146	E269Y	a	0.51	0.07	0.48	0.30	0.79	0.54	0.37	0.94	0.27	0.25	0.79	0.29	0.76	0.11	0.34
											0.12						
147	E269F	a	0.21	0.07	0.20	0.30	0.48	0.56	0.51	1.33			0.94	0.26	0.74	0.15	
											0.16						
148	E269R	a	0.07	0.11	0.07	0.28	0.13	1.73	0.06	2.21		1.97	1.15	0.26	0.72	0.15	
											0.05						
149	Y296S	a	0.63	0.08	0.71	0.28	0.51	0.49	0.64	1.31			1.21	0.26	0.67	0.13	
											0.12						
150	Y296T	a	0.72	0.09	0.71	0.25	0.97	0.39	0.58	0.80		3.07	1.07	0.26	0.60	0.15	
											<0.02						
151	Y296L	a	0.81	0.10	0.41	0.29	1.15	0.43	0.17	1.20	1.24	0.19	0.53	0.20	0.62	0.12	1.08
											0.22						
152	Y296I	a	0.87	0.11	0.43	0.27	0.52	0.85	0.20	1.02	0.35	0.24	1.51	0.26	0.56	0.11	0.67
											0.09						
153	S298H	a	0.58	0.10	0.23	0.31	0.09	1.22	0.18	0.86			1.22	0.25	0.60	0.10	
											0.27						
154	T299H	a	0.03	0.09	0.16	0.29	0.44	0.48	0.33	1.23		1.02	1.65	0.22	0.57	0.15	
											<0.02						
155	A330V	ap	1.12	0.09	0.41	0.28	0.37	0.58	0.79	1.13	0.55	0.20	0.81	0.26	0.75	0.16	1.47
											0.43						
156	A330I	ap	1.14	0.09	0.35	0.28	0.21	0.68	0.27	0.74	0.40	0.19	1.33	0.28	0.61	0.19	1.90
							0.02				1.71						85.50
157	A330F	a	1.65	0.12	0.89	0.29	1.19	0.47	0.32	0.87	2.02	0.19	1.59	0.28	0.98	0.12	1.70
											0.6						
158	A330R	a	0.45	0.09	1.42	0.36	1.01	0.53	0.90	0.45	0.45	0.22	0.74	0.22	0.58	0.14	0.45
			0.46	0.14	1.16	0.22	1.05	0.39	1.63	0.35	0.07	2.82			0.95	0.42	0.07
											<0.02						
159	A330H	ap	1.09	0.12	1.16	0.33	2.09	0.41	1.62	0.48	1.41	0.16	0.81	0.20	0.91	0.14	0.67
											0.52						
160	N325D	a	1.20	0.11	0.14	0.34	0.38	0.99					0.79	0.24	0.68	0.11	
			0.87	0.12	0.14	3.34	1.62	0.44	0.63	0.54	0.02	2.82			0.78	0.39	0.01
											0.41						
161	N325E	a	1.34	0.10	0.09	0.38	0.05	1.35	0.03	2.06		7.72	0.86	0.23	0.55	0.11	
											<0.02						
162	N325A	a	0.31	0.06					0.05	3.84			1.27	0.14	0.74	0.08	
											0.11						
163	N325T	a	0.83	0.05	0.41	0.37			0.13	3.68	0.93	0.18	1.35	0.16	1.23	0.08	
											1.1						
164	N325V	a	0.61	0.08	0.42	0.36			0.16	3.68	0.66	0.17	1.24	0.15	1.29	0.08	
											0.48						
165	N325H	a	0.52	0.08	0.25	2.55			0.13	3.68	0.64	0.22	1.28	0.15	0.03	1.97	
											0.73						
166	L328D/I332E	a	3.56	0.05	1.64	0.29			1.38	3.69	2.01	0.37	1.97	0.20	1.54	0.10	
											1.34						
167	L328E/I332E	a	2.02	0.15	0.94	0.34			1.39	3.66	0.95	0.37	2.59	0.20	1.44	0.11	
											0.2						
168	L328N/I332E	a	2.69	0.05	0.32	0.33			2.29	3.69	10.24	0.24	1.12	0.16	1.18	0.14	
											<0.02						
169	L328Q/I332E	a									0.7						
170	L328V/I332E	a	3.12	0.07	0.91	0.32			8.43	3.69	17.20	0.23	1.34	0.15	0.93	0.10	
											2.06						
171	L328T/I332E	a	2.70	0.07	1.46	0.35			14.23	3.66	19.76	10.55	2.36	0.39	0.99	0.15	
											1.1						
172	L328H/I332E	a	1.26	0.08	0.32	0.36			0.44	3.67	1.63	0.20	1.39	0.15	1.16	0.09	
											<0.02						
173	L328I/I332E	a									3.49						
174	L328A	a	0.86	0.05	10.48	0.34			0.49	3.67	3.81	0.30	1.23	0.19	1.32	0.09	
											0.2						

Figure 4 (continued)

			FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
Variant	Substitution(s)	Context	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
175	I332T	a	1.58	0.05	2.82	0.30			0.74	3.66	3.18	0.38	1.15	0.22	1.02	0.09	
											0.72						
176	I332H	a	1.19	0.06	2.12	0.34			0.73	3.71	1.05	0.14	2.13	0.14	1.24	0.17	
											0.46						
177	I332Y	a	1.88	0.07	4.18	0.29			0.63	3.68	2.14	0.16	1.53	0.21	1.43	0.17	
											0.76						
178	I332A	a	1.60	0.10	3.01	0.31			0.48	3.67	1.97	0.20	1.57	0.17	0.96	0.13	
											0.89						
179	S239E/V264I/I332E	a	4.75	0.06	1.73	0.40			2.58	3.66	142	1.57	1.45	0.18	1.07	0.24	
											15.5						
180	S239Q/V264I/I332E	a	2.44	0.07	0.50	0.41			0.45	3.66	21.84	0.24	1.07	0.16	0.97	0.16	
											2.14						
181	S239E/V264I/A330Y/I332E	a	7.52	0.12	1.80	0.32			4.69	3.66	318	0.27	2.05	2309	2.36	0.85	
											8.53						
182	S239E/V264I/S298A/A330Y/I332E	a	6.80	0.11	0.32	0.35			0.73	3.67	862	0.24	1.98	0.23	1.22	0.10	
183	S239D/N297D/I332E	a	2.82	0.09					0.06	3.67	0.82	0.37	3.14	0.29	0.76	0.07	
											0.28						
184	S239E/N297D/I332E	a	2.27	0.06							0.28	0.18	2.48	0.25	0.76	0.20	
											0.06						
185	N297D/I332E/S239D/D265V	a									0.03						
186	S239D/D265I/N297D/I332E	a	0.11	0.11					0.31	4.45	0.44	0.13	7.68	0.24	0.45	0.15	
											0.01						
187	S239D/D265L/N297D/I332E	a	0.10	0.09					0.05	3.74	0.10	0.24	2.78	0.20	0.47	0.09	
											<0.02						
188	S239D/D265F/N297D/I332E	a	0.20	0.09					0.17	3.67	0.54	0.35	1.47	0.13	0.62	0.08	
											<0.02						
189	S239D/D265Y/N297D/I332E	a	0.19	0.09					0.08	3.69	0.39	0.14	2.96	0.24	0.52	0.13	
											0.02						
190	S239D/D265H/N297D/I332E	a	0.09	0.09					0.12	3.68	0.51	0.23	2.16	0.23	1.26	0.24	
											0.04						
191	S239D/D265T/N297D/I332E	a	0.35	0.07	0.24	0.85			0.11	3.70	0.51	0.42	8.28	0.25	0.59	0.07	
											<0.02						
192	V264E/N297D/I332E	a	0.90	0.08	0.26	0.42			0.04	3.69	0.26	0.47	2.41	0.25	0.96	0.07	
193	N297D/I332E/Y296D	a									0.05						
194	N297D/I332E/Y296E	a									<0.02						
195	N297D/I332E/Y296N	a									0.04						
196	N297D/I332E/Y296Q	a									<0.02						
197	N297D/I332E/Y296H	a									<0.02						
198	N297D/I332E/Y296T	a									<0.02						
199	N297D/I332E/T299V	a									<0.02						
200	N297D/I332E/T299I	a									<0.02						
201	N297D/I332E/T299L	a									<0.02						
202	N297D/I332E/T299F	a									<0.02						
203	N297D/I332E/T299H	a									<0.02						
204	N297D/I332E/T299E	a									<0.02						
205	N297D/I332E/A330Y	a									0.43						
206	N297D/I332E/S298A/A330Y	a									0.16						
207	S239D/I332E/A330Y	ap									130						
208	S239N/I332E/A330Y	a									14.2						
209	S239D/A330L/I332E	atrcp	5.54	0.21	2.52	0.33		25.98	31.11	5.64		6.50					
							7.5				139						18.48
210	S239N/I332E/A330L	a									13						
211	I332E/V264I/S298A	t									16.5						
212	I332E/S239D/S298A	tp					6.16				295						47.92
213	I332E/S239N/S298A	t					5.15				32.1						6.24
214	S239D/I332E/V264I	t					14.39				36.6						2.54
215	S239D/I332E/V264I/S298A	t															
216	S239D/I332E/V264I/A330L	t															
217	L328N	a									0.59						
218	L328H	a									<0.02						
219	S239D/I332E/A330I	ap									59.1						
220	N297D/I332E/S239D/A330L	a															
221	P230A	t	1.28	0.13	0.99	0.18	1.13	39.94			0.55	0.20	2.53	0.25	0.77	0.19	0.49
											1.09						
222	E233D	t	0.85	0.15	0.81	0.16	1.05	1.12			0.64	0.18	2.66	0.36	0.76	0.23	0.61
											0.85						
223	P230A/E233D	t	2.03	0.22	0.76	0.13					0.54	0.17	2.04	0.27	0.84	0.18	
											0.92						
224	P230A/E233D/I332E	t	4.92	0.16	0.97	0.16	2.01	0.90	1.36	75.92	7.81	0.14	3.64	0.35	0.78	0.16	3.88
											1.87						

Figure 4 (continued)

			FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
Variant	Substitution(s)	Context	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
225	S267T	t	0.93	0.14	0.15	0.32	1.06	1.26	1.01	450	0.86	0.18	3.09	0.28	0.75	0.19	0.81
226	S267H	t	0.34	0.15	0.16	0.23	0.93	14.97			0.34	0.23	3.26	0.28	0.57	0.23	0.36
227	S267D	tp	1.41	0.13	0.75	0.09	5.02	0.89	4.78	75.92	1.45	0.11	2.88	0.18	1.00	0.20	0.29
228	S267N	t	0.72	0.20	0.08	0.71					0.27	0.19	3.18	0.34	0.85	0.16	
229	E269T	t	0.32	0.16	0.20	0.31					0.14	0.60	1.17	0.20	0.92	0.18	
230	E269L	t	0.28	0.15	0.16	0.24					<0.02		1.83	0.29	0.75	0.21	
231	E269N	t	0.19	0.15	0.12	0.38					<0.02		1.36	0.27	0.65	0.22	
232	D270Q	t	0.51	0.22	0.42	0.19					<0.02						
233	D270T	t	0.33	0.22	0.16	0.23					0.30	0.19	2.41	0.24	0.76	0.18	
234	D270H	t	0.33	0.18	0.24	0.17					<0.02						
235	E272S	t	1.04	0.20	0.53	0.25	1.15	4.37	1.07	6.40	<0.02		1.45	0.28	0.85	0.19	
236	E272K	t	0.79	0.19	0.55	0.10	1.85	0.30	1.33	0.35	0.39	0.20	1.28	0.31	0.90	0.23	0.34
237	E272I	tp	0.83	0.16	0.72	0.12	1.04	0.40	1.11	2.20	3.41	0.19	1.09	0.30	0.96	0.18	1.84
238	E272Y	t	0.58	0.16	0.80	0.09					4.96	0.16	1.48	0.31	1.05	0.20	4.79
239	V273I	t	0.98	0.17	0.55	0.09	2.20	0.31	1.62	0.34	0.78	0.17	1.68	0.32	2.08	0.26	
240	K274T	t	1.01	0.24	0.82	0.08			1.10	13.52	8.7						
241	K274E	tp	1.46	0.13	0.86	0.11	1.17	0.29	1.07	0.50	0.60	0.17	1.11	0.37	0.95	0.28	0.27
242	K274R	tp	0.95	0.16	0.77	0.11			0.85	0.56	0.79						
243	K274L	t	1.17	0.15	0.91	0.11	1.71	0.30	1.41	0.37	0.90	0.19	1.55	0.33	1.08	0.42	
244	K274Y	tp	1.02	0.16	0.79	0.11	1.09	0.37	1.16	10.90	1.41						
245	F275W	t	1.16	0.17	0.57	0.10	1.15	0.37	1.09	0.48	1.00	0.12	2.73	0.37	1.05	0.27	0.86
246	N276S	tp	0.84	0.15	0.62	0.09	0.97	0.46	1.24	25.25	6.11						
247	N276E	t	2.07	0.15			1.29	2.40			0.71	0.17	2.22	0.35	0.65	0.23	
248	N276R	t	0.84	0.22	0.64	0.11	0.87	0.50	1.26	21.80	1.41						
249	N276L	tp	0.65	0.29	0.66	0.10	0.94	0.41	1.10	10.72	4.35	0.15	1.15	0.30	0.51	0.24	2.54
250	N276Y	t	1.23	0.16	1.18	0.09	3.02	0.29	2.97	0.31	1.09						
251	Y278T	t	0.73	0.17	0.35	0.10	0.71	0.55	1.08	2.80	0.93	0.13	1.42	0.33	0.63	0.25	0.85
252	Y278E	tp	2.11	0.15	0.59	0.14	1.09	0.35	1.04	0.76	1.06						
253	Y278K	t	0.63	0.24	0.45	0.14	1.12	4.55	1.02	5.44	0.51	0.19	1.43	0.35	1.19	0.31	0.59
254	Y278W	t	0.65	0.15	0.45	0.10	0.83	0.93	1.06	15.47	0.66						
255	E283R	t	0.67	0.14	0.62	0.08	0.91	0.70	1.11	3.27	0.52	0.22	1.37	0.36	1.21	0.43	0.56
256	V302I	t	0.75	0.20	0.66	0.11	1.20	0.41	1.08	0.44	1.07						
257	E318R	t	0.71	0.35	0.57	0.13	1.14	6.22			3.47	0.10	1.34	0.31	0.74	0.18	1.15
258	K320T	tp	1.37	0.41	1.10	0.16	1.23	0.29	0.91	0.33	0.56						
259	K320D	t	2.29	0.14	0.79	0.19	1.37	0.29	1.06	0.35	0.54	0.13	1.43	0.33	0.61	0.30	0.49
260	K320I	tp	1.87	0.13	0.99	0.15	1.65	0.27	1.21	0.37	0.9						
261	K322T	tp	1.64	0.16	0.56	0.16	1.14	0.32	1.08	1.26	0.37	0.17	1.18	0.30	0.78	0.20	0.34
262	K322H	tp	1.20	0.17	0.66	0.14	0.92	0.52	1.20	3.35	0.31	0.16	1.40	0.35	0.74	0.22	0.38
263	V323I	t	0.90	0.13	0.74	0.13	1.64	0.33	1.29	0.35	0.41						
264	S324T	tp	2.07	0.28	1.29	0.09	1.15	0.08	1.15	0.06	0.49	0.14	1.36	0.32	1.86	0.18	0.54
			1.03	0.23	0.89	0.09	1.00	0.42	1.13	1.80	0.67						
265	S324D	tp	3.36	0.25	1.71	0.11	1.25	0.08	1.31	0.07	0.81	0.15	1.07	0.24	1.12	0.26	1.12
			0.94	0.20	1.03	0.10	0.93	0.67			1.54	0.35	1.71	0.08			1.23
266	S324R	t	2.67	0.27	1.18	0.15	1.39	0.10	2.52	0.33	0.75	0.18	1.46	0.33	1.27	0.44	0.80
			0.64	0.16	0.73	0.17					1.07						
											1.86	0.38	1.23	0.08			1.34
											0.56	0.17	2.06	0.19	0.61	0.37	
											0.71						

Figure 4 (continued)

			FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
Variant	Substitution(s)	Context	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
267	S324I	tp	9.07	0.25	1.98	0.15	6.71	0.34	7.80	0.30	1.88	0.30	0.69	0.12			0.28
			1.43	0.17	0.69	0.10					0.54	0.16	2.02	0.21	0.90	0.20	
268	S324V	tp	2.47	0.24	1.96	0.08	1.88	0.09	1.94	0.07	1.15 2.25	0.36	0.90	0.10			1.19
			2.12	0.16	1.32	0.23	1.01	0.98	1.03	76.44	1.06	0.19	1.66	0.19	0.90	0.19	1.05
269	S324L	tp	2.01	0.26	1.95	0.07	1.22	0.08	1.24	0.06	1.17 2.28	0.38	0.62	0.08			1.86
			0.93	0.14	0.96	0.13	0.93	1.00	0.90	79.37	0.74	0.14	1.71	0.19	0.82	0.22	0.79
270	S324Y	t	1.89	0.25	1.53	0.06	1.48	0.07	1.40	0.06	<0.02 1.86	0.44	1.83	0.10			1.26
			1.51	0.13	0.86	0.09	0.97	1.05			0.89	0.14	2.25	0.23	0.76	0.21	0.91
271	K326L	tp	2.19	0.26	1.50	0.09	1.40	0.08	1.37	0.04	0.98 3.20	0.28	1.13	0.08			2.29
			2.66	0.19	0.70	0.18	1.55	0.91	1.79	75.92	1.54	0.17	1.64	0.20	0.70	0.19	1.00
272	K326I	t	2.79	0.25	1.62	0.09	1.39	0.07	1.41	0.05	3.12	0.31	1.06	0.09			2.24
			2.10	0.17	0.67	0.17	1.79	0.92	1.69	75.92	1.68	0.15	2.49	0.21	0.65	0.20	0.94
273	K326T	tp	4.10	0.26	2.07	0.12	1.95	0.07	2.05	0.06	1.43 2.75	0.36	2.25	0.11			1.41
			2.92	0.19	1.26	0.13	1.33	0.91	1.06	75.92	1.79	0.14	2.65	0.21	0.68	0.22	1.34
274	A327D	t	15.80	0.25	2.09	0.19	7.30	0.36	11.30	0.39	1.88 3.34	0.24	0.62	0.17			0.46
			2.55	0.13	0.73	0.16	1.62	0.89	1.11	75.92	0.51	0.14	3.00	0.21	1.01	0.18	0.32
275	A327T	t	1.13	0.25	0.82	0.15	1.06	0.08	1.15	0.06	<0.02 0.65	0.27	2.64	0.08			0.62
			0.19	0.19	0.13	1.18							2.63	0.29	0.67	0.17	
276	A330S	tp	4.00	0.25	1.58	0.13	1.91	0.09	1.84	0.11	<0.02 1.56	0.36	1.88	0.07			0.81
			2.38	0.19	0.80	0.21	0.97	1.11			0.67	0.18	1.64	0.24	0.91	0.21	0.69
277	A330W	t	3.94	0.25	1.33	0.07	1.45	0.08	1.49	0.06	1.37	0.38	1.96	0.08			0.94
			2.14	0.16	0.37	0.17	1.00	8.31			0.76	0.19	2.44	0.23	1.02	0.19	0.76
278	A330M	t	2.30	0.25	1.36	0.09	1.26	0.07	1.09	0.05	1.79	0.38	1.91	0.08			1.42
			2.01	0.15	0.53	0.14	1.15	17.59			1.00	0.15	2.31	0.22	0.77	0.24	0.87
279	P331V	t	2.52	0.24	1.01	0.06	1.30	0.07	1.36	0.05	1.19	0.29	2.12	0.08			0.92
			1.43	0.13	0.34	0.18	0.88	1.06	0.93	76.26	0.26	0.22	3.49	0.18	0.83	0.19	0.29
280	P331H	t	2.28	0.25	1.40	0.09	1.27	0.08	1.34	0.05	1.12	0.29	2.24	0.07			0.88
			2.09	0.46	0.42	0.18	1.24	0.93	0.95	76.55	0.35	0.18	2.80	0.25	0.90	0.22	0.36
281	E333T	t	2.46	0.26	1.43	0.11	1.27	0.08	1.22	0.05	1.77	0.34	1.68	0.75			1.39
			1.21	0.16	0.68	0.12	1.05	1.34	0.98	454	0.72	0.15	3.17	0.21	0.85	0.18	0.68
282	E333H	t	2.91	0.25	1.23	0.09	1.24	0.11	1.30	0.06	0.78 1.77	0.34	2.21	0.12			1.43
			0.40	0.06	0.59	0.33	1.19	0.46	0.73	0.32	0.46	0.17	1.06	0.73	0.84	0.29	0.39
283	E333I	t	2.60	0.26	1.53	0.11	1.78	0.09	2.10	0.07	0.75 1.94	0.34	2.55	0.07			1.09
			0.34	0.13	0.58	0.40	1.72	0.30	0.93	0.23	0.38	0.11	1.52	0.60	0.84	0.26	0.22
284	E333Y	t	7.61	0.25	2.51	0.10	10.60	0.17	11.60	0.21	8.70	0.23	1.10	0.14			0.82
			0.45	0.11	0.64	0.32	1.93	0.31	0.88	0.29	0.90	0.15	0.81	0.94	0.83	0.27	0.47
285	K334I	t	3.99	0.25	1.82	0.07	1.67	0.05	1.60	0.05	10.32	0.28	1.84	0.08			6.18
			0.97	0.15	0.85	0.36	1.89	0.33	1.00	0.28	2.47	0.11	1.16	0.72	0.64	0.27	1.31
286	K334T	tp	3.63	0.26	2.15	0.07	0.93	0.38	0.96	0.06	5.35	0.36	1.03	0.10			5.75
			1.24	0.25	0.98	0.31	2.28	0.30	1.39	0.23	1.69	0.13	1.06	0.80	0.82	0.29	0.74
287	K334F	t	4.03	0.25	1.57	0.10	1.26	0.07	1.15	0.05	5.47	0.42	2.70	0.08			4.33
			1.54	0.11	0.75	0.29	1.77	0.32	0.92	0.23	1.46	0.12	1.01	1.00	0.77	0.26	0.83
288	T335D	tp	3.52	0.28	1.68	0.13	1.21	0.07	1.13	0.07	3.03	0.33	1.93	0.09			2.51
			1.37	0.07	0.82	0.26	1.37	0.30	0.66	0.32	0.96	0.14	1.25	0.67	0.99	0.24	0.70
289	T335R	t	2.72	0.26	1.28	0.09	1.23	0.10	1.22	0.07	2.79 1.47	0.33	2.06	0.15			1.19
			0.38	0.07	0.66	0.26	1.23	0.31	0.66	0.30	0.38	0.12	1.00	0.70	0.74	0.26	0.31
290	T335Y	tp	2.72	0.28	1.48	0.09	1.23	0.06	1.19	0.05	2.58 2.29	0.37	3.22	0.11			1.86
			0.46	0.10	0.70	0.37	0.81	0.62	0.62	0.41	0.52	0.12	1.31	0.69	0.86	0.26	0.64
291	L234I/L235D	t	0.81	0.32	2.20	0.09	0.96	0.10	0.89	0.06	1.56 3.68	0.37	0.60	0.12			3.82
			0.04	0.15	0.25	2.73	1.14	0.34	0.52	0.25	0.57	0.12	2.07	0.60	1.03	0.25	0.50
292	V240I/V266I	t	3.99	0.24	1.86	0.09	1.99	0.11	1.90	0.06	0.07 3.42	0.35	3.08	0.07			1.71
			0.60	0.12	0.46	0.32	1.91	0.37	1.05	0.27	0.52	0.14	1.16	0.73	0.81	0.25	0.27
293	S239D/A330Y/I332E/L234I	t									1.72 22.4						
294	L235D/S239D/A330Y/I332E	t	5.43	0.28	1.62	0.06	1.59	0.06	1.35	0.06	65.84	0.29	1.16	0.09			41.38
			0.15	0.12	0.34	0.35	7.07	0.30	3.66	0.26	80.04	0.11	1.11	0.83	0.78	0.28	11.33
295	S239D/V240I/A330Y/I332E	t	2.64	0.13	2.78	0.25	21.68	0.29	13.70	0.22	7.04 115	0.13	0.99	0.90	0.71	0.27	5.33
											28						

Figure 4 (continued)

			FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
Variant	Substitution(s)	Context	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
296	S239D/V264T/A330Y/I332E	t	1.64	0.10	4.85	0.26	22.14	0.30	14.88	0.23	59.09	0.11	0.89	1.07	0.98	0.25	2.67
											17.7						
297	S239D/A330Y/I332E/V266I	t															
298	S239D/K326E/A330Y/I332E	tp	3.21	0.08	3.00	0.26	48.74	0.30	27.56	0.23	298	0.12	1.20	0.66	0.39	0.27	6.12
											64.1						
299	S239D/K326T/A330Y/I332E	t	1.83	0.08	8.89	0.22	41.48	0.29	22.59	0.22	185	0.10	1.27	0.60	0.40	0.28	4.47
											59						
300	S239D/N297D/A330Y/I332E	t	0.10	0.13			0.23	1.70	0.85	11.57	0.08	0.33	1.27	0.62	0.19	0.81	0.32
											<0.02						
301	S239D/F241S/F243H/V262T/V264T/N297D/A330Y/I332E	t	0.08	0.11			0.90	0.87	0.84	9.38	0.03	0.41	1.65	0.59	0.23	0.64	0.03
											<0.02						
302	L235D/S239D/N297D/I332E	t	0.02	0.20							0.08	0.48	1.10	0.78	0.19	0.59	
303	S239D/N297D/K326E/I332E	t	0.23	0.10			0.81	0.68	0.57	0.34	0.36	0.13	1.33	0.66	0.19	0.63	0.45
321	P232E	a	2.97	0.14	0.80	0.14	1.59	0.21	0.99	0.23	1.51	0.36	1.18	0.16	0.76	0.23	0.95
			0.18	0.19	0.06	0.38	0.27	0.20	0.30	0.16	0.32	0.16	0.08	0.59			1.19
322	P232K	a	0.70	0.14	0.87	0.16	0.85	0.17	0.61	0.24	0.77	0.39	0.78	0.18	1.56	0.30	0.91
			0.07	0.25	0.05	0.45	0.27	0.17	0.33	0.20	0.20	0.15	0.07	0.60			0.72
323	P232Y	a	1.91	0.21	1.51	0.12	1.49	0.19	0.96	0.28	0.99	0.34	0.87	0.18	0.68	0.26	0.66
			0.15	0.16	0.22	0.32	0.54	0.14	0.74	0.19	0.21	0.13	0.16	0.61			0.40
324	P232G	a	1.31	0.18	0.49	0.13	0.69	0.18	0.42	0.23	0.51	0.31	0.84	0.15	0.70	0.26	0.74
			0.10	0.19	0.05	0.41	0.11	0.30	0.01	1.67	0.04	0.22	0.14	0.60			0.36
325	S239Q	a	0.98	0.15	1.05	0.12	1.11	0.25	0.59	0.24	1.59	0.31	0.81	0.15	0.57	0.22	1.44
			0.07	0.21	0.09	0.34	0.68	0.16	0.91	0.21	0.92	0.11	0.17	0.59			1.35
326	S239K	a	0.18	0.12	0.94	0.11	0.76	0.22	0.43	0.23	1.20	0.36	0.98	0.15	0.86	0.27	1.58
			0.02	0.16	0.14	0.29	0.78	0.17	0.94	0.17	0.88	0.14	0.49	0.86			1.12
327	S239R	a	0.05	0.14	0.41	0.14	0.51	0.16	0.19	0.37	0.11	0.28	1.29	0.18	1.04	0.20	0.22
			0.01	0.15			0.08	0.63	0.00	2.30			0.12	0.58			
328	S239V	a	0.08	0.19	0.36	0.26	2.49	0.16	1.98	0.15	1.10	0.14	0.36	0.65			0.44
			1.28	0.19	0.29	0.22	1.92	0.49	1.23	0.32	0.05	2.82			1.17	0.36	0.02
329	S239L	ap	0.99	0.16	3.98	0.13	6.06	0.22	3.69	0.22	4.49	0.25	6.30	0.19	4.60	0.25	0.74
			0.00	0.20			0.10	0.35	0.14	0.27			0.06	0.58			
330	S239I	a	0.06	0.18	0.57	0.17	1.47	0.19	0.87	0.26	0.13	0.25	1.28	0.16	0.71	0.29	0.09
			0.14	0.15	0.10	0.35	0.16	0.24	0.07	0.62	0.05	0.14	0.11	0.60			0.34
331	S239M	a	0.90	0.12	0.81	0.16	1.02	0.17	0.91	0.33	0.64	0.29	1.36	0.16	1.03	0.26	0.63
			0.05	0.16	0.05	25.50	0.01	2.15	0.02	5.02	0.02	0.17	0.20	0.61			0.26
332	S239W	a	0.43	0.13	0.52	0.16	0.49	0.17	0.38	0.23	0.45	0.28	1.53	0.15	0.57	0.27	0.92
			0.04	0.16	0.04	13.71	0.11	0.40	0.02	1.84	0.02	0.22	0.15	0.59			0.16
333	S239P	a	0.27	0.16	0.63	0.15	0.91	0.18	0.73	0.23	0.19	0.30	0.96	0.15	0.30	0.30	0.21
			0.15	0.15	0.16	0.30	0.82	0.15	0.55	0.30	0.04	0.19	0.13	0.58			0.05
334	S239G	a	1.20	0.14	0.97	0.14	1.80	0.14	1.31	0.23	0.47	0.30	0.91	0.16	1.16	0.38	0.26
			0.08	0.16	0.05	0.33	0.05	0.50	0.09	0.58	0.03	0.18	0.30	0.81			0.69
335	F241D	a	1.76	0.18	1.37	0.12	1.36	0.14	0.86	0.21	0.60	0.29	0.78	0.16	0.69	0.26	0.45
			0.15	0.16	0.08	0.33	0.09	0.50	0.11	0.63	0.03	0.13	0.13	0.60			0.34
336	F241E	a	0.96	0.15	1.90	0.11	1.18	0.17	1.02	0.23	0.57	0.33	1.10	0.16	1.09	0.19	0.48
			0.14	0.16	0.16	0.28	0.33	0.17	0.29	0.13	0.09	0.15	0.07	0.63			0.29
337	F241Y	a	1.33	0.15	1.15	0.13	2.04	0.17	1.51	0.22	1.04	0.28	1.23	0.23	0.74	0.27	0.51
			0.15	0.17	0.05	152	0.68	0.16	0.55	0.17	0.27	0.13	0.06	0.58			0.40
338	S267E	ap	4.43	0.16	4.76	0.18	337	0.42	248	0.39	1.30	0.33	21.33	0.17	0.20	0.28	0.00
			2.39	0.21	5.92	0.13	97.52	0.21	438	0.49	1.18	0.39	30.15	0.14	1.09	0.19	0.01
			3.50	0.16	11.50	0.20	335	0.37	1321	0.28	0.65	2.82			2.24	0.39	0.00
339	S267Q	ap	1.01	0.12	0.67	0.21	2.03	0.17	1.50	0.23	0.74	0.40	1.14	0.17	0.65	0.27	0.36
			0.02	0.14			0.14	0.24	0.05	0.84		3.25	0.19	0.61			
340	S267K	a	0.32	0.19	1.65	0.14	1.25	0.23	0.69	0.25	0.30	0.24	0.96	0.18	1.70	0.35	0.24
			0.02	0.16			0.01	1.29	0.02	79.64			0.14	0.60			
341	S267R	a	0.15	0.15	0.45	0.19	0.36	0.17	0.17	0.24	0.16	0.24	1.03	0.15	0.75	0.30	0.44
			0.12	0.18	0.05	283	1.18	0.19	1.38	0.22			0.14	0.60			
342	S267V	a	1.21	0.16	0.81	0.13	3.26	0.16	2.35	0.22	0.17	0.25	1.38	0.16	0.50	0.33	0.05
			0.09	0.14			0.35	0.33	0.24	0.45	0.01	0.72	0.15	0.62			0.04
343	S267L	a	0.82	0.15	0.90	0.12	1.29	0.18	1.22	0.22	0.16	0.25	0.90	0.15	0.96	0.27	0.13
			0.10	0.12			0.92	0.17	1.18	0.14	0.02	5.05	0.39	0.84			0.02
344	S267I	a	0.76	0.15	0.55	0.13	2.81	0.18	2.07	0.22	0.13	0.28	1.23	0.16	0.63	0.29	0.04
			0.03	0.13			0.04	0.63	0.03	1.06		6.12	0.12	0.60			
345	S267F	a	0.40	0.17	0.54	0.13	0.71	0.16	0.61	0.25	0.22	0.24	0.87	0.23	0.42	0.34	0.20
			0.18	0.16			0.51	0.17	0.66	0.19	0.02	0.23	0.09	0.65			0.04
346	S267M	a	2.16	0.12	0.91	0.13	2.46	0.23	1.73	0.28	0.44	0.26	1.11	0.18	0.48	0.33	0.18
			0.03	0.14			0.07	0.44	0.09	0.74			0.07	0.63			
347	S267Y	a	0.37	0.13	1.28	0.20	0.95	0.23	0.60	0.30	0.35	0.28	1.10	0.18	0.93	0.33	0.37
			0.05	0.15			0.12	0.33	0.01	1.75			0.10	0.61			
348	S267W	a	0.48	0.16	0.48	0.28	1.09	0.17	0.38	0.27	0.18	0.24	1.36	0.16	0.36	0.33	0.17
			0.02	0.16			0.00	1.59	0.00	5.22	0.02	0.18	0.12	0.62			7.37
349	S267P	a	0.27	0.14	0.58	0.14	0.97	0.15	0.50	0.24	0.49	2.27	1.42	0.16	0.35	0.35	0.50
			0.51	0.16	0.79	0.29	2.99	0.16	3.81	0.15	0.56	0.14	0.11	0.62			0.19

Figure 4 (continued)

			FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
Variant	Substitution(s)	Context	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
350	H268D	a	3.92	0.13	7.02	0.12	12.81	0.17	9.43	0.22	4.97	0.29	1.89	0.17	0.43	0.48	0.39
			0.29	0.13	0.71	0.23	4.42	0.14	5.80	0.20	0.80	0.13	0.29	0.60			0.18
351	H268E	ap	3.25	0.14	5.03	0.14	7.91	0.19	6.42	0.25	4.42	0.27	1.40	0.15	1.71	0.39	0.56
352	H268Q	a	2.13	0.43	1.26	0.12	1.70	0.15	2.05	0.14	1.38	0.49	0.79	0.42	0.98	0.22	0.81
353	H268K	a	0.87	0.40	0.58	0.13	0.38	0.17	0.37	0.11	0.47	0.49	0.50	0.32	0.54	0.25	1.25
354	H268R	a	0.80	0.43	0.55	0.13	0.51	0.12	0.32	0.17	0.41	0.54	0.64	0.39	0.67	0.21	0.79
355	H268T	a	2.20	0.45	0.78	0.09	0.57	0.14	0.39	0.15	0.57	0.68	0.49	1314	0.68	0.29	1.00
356	H268V	a	2.61	0.44	1.03	0.10	0.85	0.15	0.51	0.14	0.58	0.49	0.38	0.49	0.63	0.26	0.88
357	H268L	a	1.33	0.40	0.46	0.13	0.45	0.13	0.31	0.11	0.65	0.49	1.12	0.39	0.39	0.26	1.46
358	H268I	a	3.69	0.43	0.49	0.08	0.61	0.12	0.37	0.13	0.53	0.48	0.52	0.34	0.93	0.23	0.86
			0.43	0.23			1.04	0.85	0.72	15.94	0.07	2.82			0.54	0.42	0.07
359	H268F	a	2.70	0.41	2.13	0.09	0.98	0.12	0.86	0.12	1.44	0.49	2.12	0.42	0.40	0.25	1.48
360	H268M	a	1.15	0.13	0.50	0.24	1.08	0.46	0.73	0.36	0.43	2.82			1.88	0.40	0.40
361	H268W	a	1.41	0.43	0.88	0.11	2.63	0.35	1.29	0.14	0.56	0.66	1.41	0.40	0.35	0.23	0.21
362	H268P	a	1.78	0.40	0.35	0.14	0.91	0.14	0.71	0.12	0.57	0.49	0.67	0.41	0.68	0.16	0.63
363	H268G	ap	2.41	0.42	0.99	0.14	1.38	0.13	1.58	0.08	0.84	0.50	1.11	0.40	0.38	0.19	0.61
364	S298D	a	2.96	0.40	0.20	0.21	0.30	0.12	0.15	0.16	0.66	0.50	1.06	0.42	0.55	0.18	2.24
365	S298E	a	3.36	0.39	0.25	0.19	0.26	0.14	0.17	0.27	0.19	0.50	0.97	0.43	0.31	0.18	0.74
			1.90	0.21	0.09	3.80	0.58	0.44	0.63	0.53	0.04	2.83			1.54	0.37	0.06
366	S298Q	a	1.82	0.40	0.49	0.08	0.87	0.09	0.78	0.10	0.59	0.49	0.66	0.48	0.45	0.19	0.68
367	S298K	a	0.33	0.39	0.21	0.16	0.39	0.15	0.29	0.16	0.14	0.48	0.78	0.28	0.54	0.17	0.36
368	S298R	a	0.86	0.40	0.30	0.12	0.17	0.24	0.13	0.26	0.24	0.48	0.92	0.41	0.48	0.16	1.38
369	S298I	a	3.25	0.40	0.44	0.22	0.69	0.16	0.56	0.16	0.94	0.49	1.54		0.13	0.23	1.36
370	S298F	a	3.35	0.39	1.05	0.15	2.57	0.10	1.48	0.13	1.50	0.48	1.20	0.28	0.25	0.16	0.58
			2.42	0.10	0.16	0.29	0.93	0.42	0.55	0.72	0.08	2.82			1.15	0.38	0.09
371	S298M	a	3.34	0.42	1.76	0.13	2.53	0.12	1.73	0.11	1.24	0.49	1.40	0.62	0.60	0.21	0.49
372	S298Y	a	2.51	0.41	0.54	0.15	0.68	0.11	0.49	0.19	0.98	0.50	0.91	0.49	0.50	0.19	1.43
373	S298W	a	2.46	0.40	0.31	0.16	0.32	0.21	0.22	0.19	0.32	0.48	0.80	0.46	0.23	0.25	0.98
374	T299D	a	0.12	0.41	0.26	0.16	0.26	0.19	0.22	0.12	0.18	0.50	1.50	1.25	0.44	0.34	0.67
375	T299E	a	0.24	0.40	0.39	0.08	0.37	0.14	0.28	0.15	0.28	0.48	1.07	0.29	0.47	0.18	0.76
376	T299N	a	0.22	0.42	0.33	0.21	0.36	0.15	0.27	0.18	0.21	0.48	1.90	0.39	0.61	0.26	0.60
377	T299Q	a	0.15	0.40	0.19	0.39	0.11	0.22	0.16	0.22	0.17	0.48	1.16	0.39	0.25	0.27	1.50
378	T299K	a	0.04	0.54	0.58	0.29	0.30	0.18	0.27	0.16	0.10	0.49	0.50	0.31	0.49	0.15	0.35
379	T299R	a	0.02	0.99	0.23	0.41	0.20	0.36	0.08	0.42	0.00	24.61	0.61	0.41	0.44	0.21	0.00
380	T299L	a	0.26	0.44	0.18	0.30	0.58	0.22	0.43	0.09	0.06	0.59	0.60	12.53	0.34	0.19	0.11
381	T299F	a	0.61	0.43	1.77	0.26	0.95	0.17	0.33	0.20	0.12	0.49	1.31	0.47	1.86	0.20	0.12
382	T299H	a	0.17	0.44	0.21	0.34	0.85	0.13	0.46	0.12	0.06	0.66	0.85	0.33	0.36	0.23	0.08
383	T299Y	a	0.03	0.33	0.77	0.26	0.86	0.27	0.62	0.21	0.11	0.20	1.08	0.38	3.85	0.13	0.13
384	T299W	a	0.09	0.32	0.31	0.20	0.85	0.18	0.59	0.12	0.20	0.13	1.46	0.33	1.09	0.10	0.24
385	T299P	a	0.02	0.36	0.20	0.16	0.34	0.17	0.26	0.17	0.10	0.23	2.60	0.36	0.72	0.11	0.29
386	T299G	a	0.02	0.45	0.15	0.29	0.24	0.21	0.09	0.23	0.03	0.61	1.94	0.52			0.12
387	Y300D	ap	1.58	0.32	0.67	0.08	1.68	0.15	1.37	0.16	1.19	0.15	3.22	0.46	0.97	0.10	0.71
388	Y300E	a	0.12	0.37	0.10	0.33	0.22	0.17	0.11	0.20	0.06	0.24	2.51	0.37	0.82	0.12	0.26
			1.53	0.17	1.00	0.23	3.51	0.40	2.95	0.30	0.14	2.82			1.27	0.41	0.04
389	Y300N	a	1.87	0.44	0.98	0.08	11.42	2509	0.56	0.12	0.63	0.14	0.88	0.38	1.14	0.10	0.06
390	Y300Q	a	0.86	0.31	1.02	0.07	1.71	0.15	1.88	0.12	1.22	0.14	2.29	0.42	1.11	0.12	0.71
391	Y300K	a	0.42	0.29	0.48	0.11	0.54	0.17	0.50	0.13	0.58	0.13	1.14	0.34	1.01	0.18	1.08
392	Y300R	a	0.41	0.38	0.35	0.11	0.57	0.16	0.57	0.14	0.41	0.14	2.12	0.32	0.86	0.13	0.72
393	Y300S	a	0.72	0.31	0.73	0.10	0.48	0.15	0.46	0.14	0.59	0.15	2.79	0.41	0.80	0.14	1.25
394	Y300T	ap	1.16	0.27	0.87	0.11	1.31	0.16	1.18	0.13	2.60	0.15	17.00	0.51			2.14
395	Y300H	a	1.23	0.31	1.23	0.08	3.85	0.13	3.86	0.12	1.31	0.14	3.90	0.45	0.81	0.14	0.34
			1.64	0.13	0.78	0.25	1.47	0.46	0.92	0.36	0.06	2.82			0.80	0.38	0.04
396	Y300A	a	1.16	0.32	0.63	0.08	0.74	0.12	0.55	0.12	0.75	0.13	1.64	0.26	1.04	0.14	1.01
397	Y300V	a	0.80	0.38	1.19	0.10	0.83	0.12	1.07	0.12	0.84	0.14	1.72	0.42	1.09	0.19	1.02
398	Y300M	a	1.32	0.30	1.22	0.11	0.89	0.14	0.84	0.13	1.00	0.19	1.85	0.48	1.10	0.14	1.13
399	Y300W	a	0.68	0.31	0.82	0.10	1.10	0.13	0.99	0.15	1.01	0.14	1.55	0.36	0.96	0.15	0.93
400	Y300P	a	0.11	0.28	0.54	0.13	0.61	0.17	0.47	0.13	0.90	0.12	1.93	0.30	0.61	0.14	1.48
401	Y300G	a	0.83	0.33	0.62	0.16	0.67	0.12	0.64	0.12	0.80	0.13	2.96	0.41	1.07	0.12	1.20
402	A330E	ap	3.27	0.30	0.58	0.08	0.59	0.15	0.51	0.20	1.92	0.18	2.14	0.53			3.25
			2.18	0.14	0.26	0.25	1.35	0.55	0.55	0.71	0.11	2.82			0.86	0.39	0.08
403	A330N	ap	1.44	0.34	0.39	0.11	0.47	0.19	0.22	0.21	0.58	0.17	2.03	0.37	0.80	0.13	1.23
404	A330T	a	0.94	0.32	0.74	0.08	0.71	0.14	0.53	0.15	0.91	0.15	1.06	0.31	0.96	0.12	1.27
405	A330P	a	0.33	0.35	0.40	0.35	1.26	0.23	0.79	0.23	0.12	0.17	0.72	0.31	5.48	0.15	0.09
			0.38	0.21	0.13	22.81	1.86	0.63	1.44	0.31	0.00	2.84			1.37	0.35	0.00
406	A330G	ap	0.91	0.31	1.27	0.11	1.14	0.16	1.34	0.12	1.32	0.19	1.54	0.37	1.01	0.15	1.16
407	I332K	a	0.15	0.29	0.47	0.11	0.59	0.13	0.60	0.15	0.40	0.13	1.52	0.34	0.90	0.17	0.68
408	I332R	a	0.14	0.33	0.39	0.07	0.58	0.16	0.37	0.17	0.27	0.14	1.25	0.33	0.79	0.13	0.47
409	I332S	a	1.63	0.35	1.83	0.12	1.11	0.20	1.00	0.13	1.05	0.13	2.45	0.41	0.98	0.15	0.94
410	I332V	a	1.91	0.37	1.18	0.11	0.78	0.20	0.51	0.21	1.05	0.21	5.94	0.50	59.27	0.70	1.35
411	I332L	a	1.76	0.31	0.86	0.07	0.99	0.18	0.76	0.17	0.97	0.14	3.98	0.56	0.73	0.19	0.98
412	I332F	a	1.56	0.32	1.66	0.09	1.55	0.16	1.26	0.16	1.13	0.14	3.32	0.34	0.78	0.18	0.73
413	I332M	a															
414	I332W	a															
415	I332P	a								20.57							

Figure 4 (continued)

			FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
Variant	Substitution(s)	Context	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
417	L234K	a	0.43	0.34	0.65	0.50	1.42	0.32	1.09	0.12	0.53	0.75	2.02	0.07	0.62	0.14	0.38
418	L234R	a	0.38	0.36	0.87	0.57	1.49	0.37	1.52	0.19	0.73	0.59	1.72	0.10	1.19	0.12	0.49
419	L234S	a	0.49	0.20	1.01	0.41	1.40	0.26	1.30	0.14	0.69	0.32	1.10	9.38	0.93	0.10	0.49
420	L234A	a	0.44	0.09	0.80	0.30	0.85	0.19	0.62	0.09	0.35	0.24	0.88	0.04	0.58	0.10	0.41
421	L234M	a	0.64	0.08	0.89	0.24	0.90	0.17	0.65	0.10	0.49	0.16	0.88	0.26	0.55	0.10	0.54
422	L234W	a															
423	L234P	a															
424	L234G	a	0.70	0.47	3.26	0.53	3.62	0.34	3.48	0.26	2.17	0.61	1.91	0.08	2.54	0.11	0.60
			0.08	0.20			0.62	0.68	0.69	26.67	0.01	2.83			1.75	0.37	0.02
425	L235E	a	0.34	0.28	0.63	0.31	0.83	0.19	0.80	0.08	1.06	0.18	0.93	0.04	0.78	0.14	1.28
426	L235K	a	0.42	0.64	0.56	0.55	1.28	0.35	1.34	0.13	0.63	0.71	1.55	0.13	0.96	0.17	0.49
427	L235R	a	0.35	0.69	0.71	0.52	1.93	0.29	1.15	0.11	0.62	0.68	1.73	0.07	0.53	0.17	0.32
428	L235A	a	0.34	0.22	0.62	0.35	0.84	0.21	0.78	0.10	0.41	0.30	1.00	0.04	0.97	0.15	0.49
429	L235M	a	0.38	0.09	0.79	0.26	0.89	0.19	0.64	0.09	0.46	0.23	1.05	0.05	0.69	0.11	0.51
430	L235W	a	0.11	0.34	0.90	0.19	0.77	0.17	0.50	0.08	0.32	0.24	0.83	0.04	0.46	0.11	0.42
431	L235P	a	0.13	0.62	1.16	0.27	1.16	0.18	0.89	0.10	0.78	0.23	1.31	0.05	0.86	0.12	0.67
432	L235G	a		579	0.99	0.31	1.02	0.19	0.74	0.11	0.43	0.34	1.12	0.06	0.68	0.11	0.42
433	V264D	a	0.56	0.20	0.19	0.22	0.24	0.49	0.54	0.38	0.93	0.69	0.54	0.67	7.67	0.51	3.92
434	V264E	a	0.67	0.06	0.37	0.44	0.74	0.26	0.94	0.09	0.44	0.67	0.60	0.07	0.93	0.17	0.60
435	V264N	a	0.93	0.15	1.04	0.17	1.63	0.28	1.60	0.25	0.34	0.69			3.39	0.56	0.21
436	V264Q	a	0.62	0.08	0.48	0.25	0.68	0.20	0.61	0.09	0.38	0.20	0.73	0.13	0.72	0.14	0.55
437	V264K	a	0.48	0.13	0.46	0.39	0.77	0.24	0.67	0.09	0.36	0.45	0.97	0.05	0.50	0.11	0.47
438	V264R	a	0.31	1.12	1.21	0.66	2.37	0.30	2.01	0.13	7.42	11.18	3.05	0.04	0.67	0.10	3.13
439	V264S	a	1.15	0.23	0.69	0.17	1.37	0.51	0.81	0.37	0.35	0.69	0.83	0.72	5.13	0.52	0.26
440	V264H	a	0.34	0.09	0.27	0.25	0.30	0.20	0.22	0.13	0.16	0.22	0.58	0.05	0.55	0.18	0.52
441	V264W	a	0.59	0.06	0.81	0.27	0.81	0.18	0.67	0.09	0.39	0.27	0.86	0.03	0.87	0.15	0.48
442	V264P	a															
443	V264G	a	0.41	0.19	0.26	0.19	1.11	0.26	1.14	0.33	0.21	0.70	1.13	0.53	0.93	0.57	0.19
444	D265Q	a	0.33	0.23	0.94	0.30	0.89	0.19	0.71	0.11	0.51	0.45	1.04	0.13	0.76	0.14	0.57
445	D265K	a	0.20	1.32	1.72	0.42	2.15	0.23	1.46	0.13	1.00	1.01	2.12	0.05	0.87	0.19	0.47
446	D265R	a	0.71	1.23	0.77	0.79	2.55	0.45	2.18	0.14	1.50	1.54	2.98	0.04	1.16	0.13	0.59
447	D265S	a	0.29	0.47	0.50	0.44	1.07	0.27	0.89	0.10	0.49	0.65	1.47	0.07	0.53	0.11	0.46
448	D265T	a	0.41	0.39	0.64	0.46	1.41	0.26	1.04	0.12	0.61	0.67	1.66	0.07	0.74	0.13	0.43
449	D265H	a	0.33	0.23	0.48	0.53	0.86	0.23	0.74	0.09	0.40	0.52	1.24	0.04	0.72	0.10	0.47
450	D265V	a	0.11	0.76	0.72	0.38	0.93	0.22	0.92	0.11	0.47	0.60	1.39	0.06	0.44	0.11	0.50
451	D265L	a	0.14	0.55	0.86	0.34	0.90	0.19	0.78	0.10	0.52	0.52	0.93	0.04	0.55	0.11	0.59
452	D265I	a	0.24	1.08	1.70	0.36	1.62	0.20	1.44	0.11	0.90	0.65	1.60	0.06	0.49	0.12	0.55
453	D265F	a	0.12	0.67	1.00	0.32	0.98	0.19	0.88	0.11	0.67	0.75	0.97	0.04	0.55	0.09	0.68
454	D265M	a	0.49	0.28	0.56	0.34	0.80	0.18	0.74	0.09	0.59	0.32	0.63	0.08	0.61	0.13	0.74
455	D265Y	a	0.01	1.21		13.21	0.58	0.40	0.30	1.04	0.02	1.09			0.43	0.46	0.03
456	D265W	a	0.52	0.43	0.72	0.34	0.99	0.17	0.98	0.09	0.84	0.42	1.02	0.09	0.67	0.13	0.85
457	D265P	a	0.74	0.43	1.13	0.41	1.94	0.20	1.56	0.10	1.19	0.56	1.92	0.10	1.18	0.12	0.61
458	K326P	a															
459	A327E	a	0.99	0.11	0.71	0.17	0.54	0.12	0.45	0.07	0.64	0.20	0.59	0.08	0.91	0.15	1.18
460	A327K	a	0.93	0.17	1.19	0.29	1.25	0.15	0.96	0.09	1.01	0.46	1.21	0.10	1.01	0.14	0.81
461	A327R	ap	0.91	0.17	1.20	0.27	1.36	0.15	0.95	0.10	0.98	0.51	1.29	0.10	0.80	0.12	0.72
462	A327H	ap	1.36	0.14	0.52	0.47	1.01	0.23	0.97	0.13	0.75	0.54	1.39	0.11	1.29	0.15	0.74
463	A327V	a															
464	A327I	a	0.65	0.07	0.26	0.26	0.44	0.21	0.33	0.11	0.27	0.25	0.56	0.09			0.60
465	A327F	a	0.69	0.07	0.45	0.27	0.64	0.17	0.51	0.09	0.37	0.27	0.60	0.08	0.71	0.20	0.58
466	A327M	a	0.82	0.07	0.64	0.26	0.78	0.16	0.67	0.09	0.51	0.24	0.75	0.08	1.08	0.14	0.66
467	A327Y	a	1.04	0.09	0.70	0.31	0.86	0.18	0.76	0.10	0.61	0.30	0.86	0.08	1.23	0.13	0.71
468	A327W	a	0.76	0.09	0.66	0.25	0.70	0.14	0.59	0.08	0.47	0.28	0.68	0.08	0.90	0.12	0.67
469	A327P	a	1.06	0.09	0.78	0.23	0.76	0.13	0.63	0.08	0.47	0.22	0.80	0.07	0.86	0.14	0.61
470	L328D	a	0.95	0.15	0.64	0.19	0.54	0.12	0.51	0.09	0.51	0.31	0.43	0.09	0.89	0.15	0.95
471	L328Q	ap	1.15	0.07	0.70	0.22	0.86	0.14	0.77	0.07	0.67	0.22	0.79	0.10	0.89	0.16	0.78
472	L328K	a	0.77	0.22	0.51	0.38	0.99	0.20	0.91	0.09	0.87	0.32	1.13	0.09	0.95	0.19	0.88
473	L328R	a	0.07	0.27	0.10	0.82	0.88	0.58	0.37	1.05	0.11	0.93	1.21	0.52	1.82	0.46	0.12
474	L328S	a	0.96	0.08	1.14	0.17	0.90	0.13	0.70	0.07	0.59	0.19	0.76	0.08	0.66	0.20	0.65
475	L328T	a	0.60	0.07	0.62	0.23	0.61	0.12	0.44	0.07	0.46	0.24	0.59	0.08	0.74	0.14	0.76
476	L328V	a	0.70	0.06	0.54	0.18	0.60	0.13	0.43	0.08	0.49	0.22	0.57	0.09	0.52	0.12	0.82
477	L328I	a	0.79	0.08	0.69	0.19	0.67	0.12	0.55	0.07	0.58	0.20	0.60	0.08	0.77	0.14	0.86
478	L328Y	a	1.05	0.09	0.88	0.19	0.75	0.12	0.63	0.08	0.47	0.30	0.71	0.07	0.92	0.13	0.63
479	L328W	a	1.01	0.06	1.16	0.19	0.90	0.13	0.82	0.09	0.73	0.30	0.63	0.09	1.35	0.13	0.81
480	L328F	a	0.72	0.07	0.38	0.29	0.61	0.17	0.52	0.08	0.42	0.33	0.56	0.09	0.76	0.19	0.68
481	L328G	a	1.18	0.10	0.71	0.34	0.90	0.16	0.93	0.08	0.64	0.41	0.80	0.08	1.16	0.24	0.70
482	P329D	a	0.72	0.12	0.55	0.32	0.82	0.17	0.80	0.09	0.62	0.29	0.80	0.09	0.79	0.26	0.76
483	P329E	a															
484	P329N	a	0.76	0.08	0.65	0.54	0.98	0.15	0.86	0.08	0.97	0.21	0.97	0.08	1.00	0.13	0.99
485	P329Q	a	0.72	0.15	0.70	0.33	0.84	0.17	0.81	0.09	0.68	0.41	0.91	0.08			0.80
486	P329K	a	0.20	2.88			0.16	0.47	0.57	0.42	0.02	0.90	1.17	0.53	0.72	0.12	0.10
487	P329R	a	1.83	0.18	2.06	0.33	2.38	0.17	2.03	0.10	1.52	0.43	2.23	0.07	1.64	0.16	0.64
488	P329S	a	0.60	0.13	0.77	0.25	0.76	0.14	0.75	0.52	0.62	0.36	0.87	0.07	0.77	0.13	0.82

Figure 4 (continued)

Variant	Substitution(s)	Context	FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
			Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
489	P329T	a	0.34	0.11	0.49	0.21	0.47	0.14	0.33	0.10	0.41	0.32	0.52	0.09	0.70	0.13	0.88
490	P329H	a	0.09	0.21	0.24	0.57	0.24	0.15	0.30	0.23	0.03	0.25	1.05	0.12	0.64	0.41	0.12
491	P329V	a	0.51	0.19	0.33	0.49	0.31	0.21	0.28	0.26	0.04	0.27	1.62	0.12	0.64	0.39	0.12
492	P329L	a	0.17	0.21	0.24	0.58	0.22	0.13	0.22	0.17	0.04	0.19	2.78	0.16	0.56	0.42	0.18
493	P329I	a	0.40	0.15	0.31	0.54	0.27	0.21	0.27	0.21	0.03	0.18	2.57	0.14	0.48	0.36	0.12
494	P329M	a	0.21	0.16	0.23	0.57	0.21	0.21	0.16	0.18	0.03	0.21	4.36	0.15	0.62	0.43	0.13
495	P329Y	a															
496	P329W	a	0.54	0.55			0.09	1.39	0.61	0.76	0.02	1.47	1.04	0.49	3.16	0.72	0.25
497	P329G	a	1.03	0.14	0.30	0.47	0.25	0.16	0.29	0.14	0.06	0.19	4.72	0.13	0.45	0.40	0.23
498	P331D	ap	3.14	0.18	0.92	0.49	1.18	0.12	1.23	0.19	0.74	0.18	5.89	0.12	0.79	0.40	0.63
			1.65	0.12	0.32	0.19	1.00	0.50	0.86	4.39	0.05	2.82			0.80	0.42	0.05
499	P331Q	a	1.71	0.14	0.60	0.40	0.63	0.13	0.77	0.17	0.64	0.12	2.46	0.12	0.60	0.46	1.01
500	P331R	a	2.29	0.19	0.39	0.42	0.54	0.22	0.32	0.22	0.29	0.09	0.96	0.15	0.89	0.37	0.54
501	P331T	a	1.67	0.13	0.53	0.41	0.71	0.17	0.88	0.23	0.57	0.10	3.25	0.14	0.57	0.37	0.81
502	P331L	ap	1.95	0.33	0.53	0.45	0.92	0.12	0.99	0.24	0.50	0.12	6.07	0.15	0.54	0.40	0.54
			0.64	0.16	0.68	0.14	1.14	0.11	1.37	0.36	0.35	0.36	5.28	0.12	1.77	0.13	0.31
503	P331I	a	1.63	0.15	0.41	0.41	0.71	0.18	0.78	0.19	0.22	0.19	3.81	0.16	0.56	0.41	0.32
504	P331F	a	1.02	0.13	0.51	0.18	0.67	0.41	0.97	0.26	0.29	0.69	0.96	0.48	3.91	0.90	0.44
505	P331M	a	1.74	0.12	0.60	0.47	1.13	0.18	0.94	0.16	0.77	0.12	2.50	0.12	0.53	0.37	0.68
506	P331Y	a	1.20	0.13	1.24	0.16	0.47	0.47	0.82	0.35	0.69	0.69	0.95	0.55	6.06	0.75	1.47
507	P331W	a	1.95	0.13	0.79	0.41	1.46	0.14	1.32	0.18	0.76	0.12	2.41	0.13	0.87	0.46	0.52
508	E333L	a	1.03	0.14	0.56	0.44	0.93	0.15	0.94	0.23	0.90	0.10	3.06	0.16	1.03	0.49	0.97
509	E333F	ap	2.22	0.14	0.69	0.45	0.93	0.18	1.55	0.30	1.69	0.15	6.96	0.16	0.82	0.42	1.82
			0.91	0.17	0.86	0.14	1.58	0.18	1.66	0.39	1.22	0.40	5.83	0.20	1.96	0.15	0.77
510	E333M	a	1.17	0.18	0.86	0.15	1.20	1.87	0.21	0.76	0.74	0.70	0.96	0.53	5.46	0.78	0.62
511	E333P	a															
512	K334P	a															
513	K222E	a	1.77	0.13	1.26	0.41	1.04	0.16	1.22	0.20	1.51	0.14	1.23	0.15	0.64	0.38	1.44
514	K222Y	a	0.77	0.14	0.48	0.51	0.50	0.15	0.34	0.26	0.34	0.10	1.06	0.12	0.66	0.41	0.68
			1.92	0.22	1.28	0.28	1.69	0.42	1.33	0.44	0.11	2.86			1.31	0.39	0.07
515	F243E	a	0.93	0.13	0.18	0.33	1.24	0.49	0.63	0.52	0.14	2.84			1.14	0.40	0.11
516	D270R	a	0.60	0.32	0.37	0.56	0.94	0.12	0.36	0.19	0.14	0.13	0.47	0.16	0.82	0.43	0.14
517	D270S	a	0.81	0.16	0.69	0.44	1.80	0.14	0.61	0.18	0.19	0.13	0.81	0.19	1.03	0.49	0.10
518	D270L	a	1.03	0.18	0.59	0.47	0.35	0.19	0.31	0.16	0.40	0.11	0.72	0.12	0.54	0.43	1.15
519	D270I	a	0.58	0.15	0.15	0.30	0.76	0.60			0.02	2.83			1.23	0.38	0.03
520	D270F	a	0.66	0.18	0.64	0.40	0.39	0.17	0.21	0.15	0.19	0.14	0.70	0.13	0.70	0.40	0.49
521	D270M	a	0.74	0.19	0.43	0.49	0.21	0.18	0.37	0.19	0.36	0.09	1.11	0.12	0.99	0.44	1.72
522	D270Y	a	0.51	0.22	0.54	0.48	0.34	0.14	0.37	0.16	0.28	0.11	0.62	0.18	0.93	0.37	0.83
523	D270W	a	0.84	0.20	0.49	0.44	0.25	0.20	0.38	0.21	0.17	0.10	0.62	0.21	0.92	0.43	0.68
524	D270P	a	0.37	0.21	0.32	0.60	0.25	0.19	0.32	0.22	0.04	0.23	0.91	0.27	0.55	0.34	0.15
525	D270G	a	0.52	0.24	0.42	0.50	0.38	0.12	0.22	0.23	0.08	0.18	0.63	0.30	0.97	0.46	0.21
526	P271D	ap	1.48	0.15	0.77	0.43	1.20	0.13	1.02	0.14	1.12	0.13	1.80	0.14	0.52	0.42	0.93
527	P271E	a	1.87	0.14	0.65	0.46	0.95	0.15	0.72	0.17	0.75	0.17	0.72	0.14	0.41	0.50	0.79
528	P271N	a	1.61	0.13	0.78	0.41	0.98	0.11	0.79	0.12	0.63	0.14	1.17	0.13	0.54	0.41	0.64
529	P271Q	a	0.55	0.24	0.71	0.09	1.07	0.79	0.66	0.30	0.60	0.23	0.86	0.18	0.56	0.12	0.56
530	P271K	a	0.63	0.27	0.67	0.09	0.90	0.87	0.36	0.29	0.71	0.17	0.71	0.20	0.87	0.16	0.79
531	P271R	a	0.60	0.30	0.67	0.08	1.68	1.14	0.28	0.31	0.51	0.19	0.52	0.20	0.78	0.14	0.31
532	P271S	a	0.55	0.24	0.73	0.08	1.71	1.10	0.61	0.27	0.70	0.21	0.71	0.16	1.14	0.14	0.41
533	P271T	a	0.77	0.22	0.97	0.07	2.73	0.90	0.83	0.30	0.53	0.19	0.95	0.17	3.88	0.12	0.19
534	P271H	a	0.56	0.30	0.63	0.13	0.37	0.66	0.45	0.31	0.67	0.20	0.88	0.18	0.74	0.13	1.80
535	P271A	ap	0.85	0.24	0.70	0.07	1.05	0.87	0.93	0.27	0.73	0.23	1.02	0.17	1.01	0.21	0.70
536	P271V	a	0.33	0.30	0.43	0.14	1.73	0.95	0.15	0.33	0.17	0.18	0.50	0.18	0.54	0.16	0.10
			1.52	0.15	0.35	0.27	0.84	0.43	0.75	0.42	0.06	2.82			1.06	0.38	0.07
537	P271L	a	1.77	0.27	0.73	0.09	1.09	0.79	1.17	0.30	0.46	0.22	1.56	0.16	0.43	0.23	0.42
538	P271I	ap	0.99	0.22	2.13	0.08	1.42	0.71	1.09	0.27	1.28	0.19	1.51	0.16	1.99	0.20	0.90
539	P271F	a	0.64	0.25	0.54	0.12	1.66	0.92	0.45	0.29	0.44	0.20	0.89	0.19	0.68	0.12	0.26
540	P271M	a	0.91	0.30	0.62	0.08	2.37	1.38	0.67	0.28	0.52	0.20	0.91	0.20	0.54	0.14	0.22
541	P271Y	a	0.71	0.31	0.70	0.12	3.65	1.10	0.21	0.36	0.37	0.21	0.64	0.17	1.48	0.14	0.10
542	P271W	a	0.60	0.24	0.63	0.47	0.52	0.73	0.25	0.32	0.72	0.22	1.06	0.18	0.98	0.15	1.37
543	P271G	a	0.88	0.21	2.33	0.04	1.79	0.86	5.03	0.27	1.09	0.27	0.96	0.17	0.57	0.22	0.61
			2.07	0.14	1.50	0.25	4.50	0.37	4.02	0.34	0.07	2.82			0.79	0.37	0.02
544	D280K	a	1.99	0.32	0.68	0.08	1.54	0.83	0.51	0.32	0.59	0.17	0.68	0.20	0.38	0.17	0.38
545	D280L	a	0.62	0.25	0.84	0.06	1.33	0.78	0.39	0.29	0.65	0.29	0.86	0.19	0.45	0.16	0.49
546	D280W	a	0.47	0.26	0.83	0.07	1.30	0.78	0.33	0.30	0.57	0.24	0.76	0.18	0.38	0.16	0.44
547	D280P	a	0.81	0.12			0.91	0.34			0.28	0.17					0.31
548	D280G	a	1.75	0.15	0.92	0.25	2.12	0.63	1.71	0.31	0.15	2.82			0.98	0.38	0.07
549	K290D	a	0.91	0.31	1.42	0.07	1.36	1.07	1.87	0.28	1.81	0.23	2.20	0.20	0.33	0.15	1.33
550	K290N	a	0.85	0.30	1.26	0.07	0.76	0.90	1.31	0.28	1.48	0.21	1.97	0.17	0.39	0.14	1.93
551	K290H	a	1.14	0.34	1.13	0.06	1.66	0.86	0.83	0.30	1.50	0.23	1.88	0.19	0.41	0.16	0.90
552	K290L	a	1.07	0.10			0.86	0.29			0.30	0.18					0.35
553	K290W	a	0.76	0.19	1.00	0.15	0.38	0.66	1.44	0.29	1.37	0.28	1.53	0.19	0.68	0.13	3.63
554	E293N	a	1.24	0.25	0.83	0.08	1.43	0.91	0.62	0.30	0.67	0.18	1.06	0.21	0.71	0.24	0.47

Figure 4 (continued)

			FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
Variant	Substitution(s)	Context	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
555	E293R	ap	172	0.64	0.66	0.20	1.75	0.82	0.40	0.46	68.81	2.94	3.16	0.48	0.67	0.15	39.28
			18.03	0.43	0.93	0.16	2.22	0.28	0.88	0.31	2.90	1.26	1.77	0.36	0.38	0.31	1.31
556	E293S	a	2.69	0.25	0.91	0.17	1.61	0.76	0.57	0.34	0.86	0.22	1.01	0.21	0.53	0.20	0.53
557	E293T	a	1.50	0.38	0.73	0.09	2.11	0.91	0.55	0.27	0.69	0.21	0.91	0.24	0.58	0.15	0.33
558	E293H	a	2.26	0.21	0.59	0.09	1.85	1.06	0.29	0.26	0.61	0.18	1.05	0.28	0.48	0.13	0.33
559	E293V	a	2.23	0.30	0.77	0.13	4.03	0.97	0.55	0.28	0.90	0.19	1.08	0.22	0.63	0.14	0.22
560	E293L	a	1.66	0.28	0.85	0.14	2.79	0.83	0.56	0.30	0.51	0.18	1.02	0.26	0.77	0.15	0.18
561	E293I	a	1.28	0.22	0.79	0.16	0.47	0.68	0.33	0.29	0.61	0.19	0.77	0.23	0.60	0.13	1.29
562	E293F	a	0.74	0.13	0.51	0.09	1.27	0.95	0.31	0.31	0.23	0.25	0.64	0.12	0.61	0.20	0.18
563	E293M	a	1.30	0.21	1.06	0.09	0.60	0.95	1.01	0.29	0.39	0.26	0.53	0.14	0.90	0.21	0.65
564	E293Y	a	0.84	0.14	0.59	0.13	0.91	1.15	0.28	0.33	0.24	0.24	0.61	0.13	0.66	0.20	0.26
565	E293W	a	0.92	0.21	0.68	0.12	4.93	1.73	0.21	0.56	0.24	0.23	0.45	0.14	0.65	0.23	0.05
566	E293P	a	0.34	0.28	0.26	0.14	9.25	1.72	0.10	0.31	0.10	0.23	0.66	0.14	0.44	0.17	0.01
567	E293G	a	1.25	0.26	0.68	0.09	1.00	1.38	0.24	0.31	0.31	0.25	0.77	0.13	0.59	0.18	0.31
568	E294K	a	0.70	0.29	0.57	0.13	1.44	1.19	0.45	0.34	1.02	0.25	0.50	0.14	0.74	0.23	0.71
			0.68	0.19	0.36	0.27	1.57	0.64	0.61	0.81	0.07	2.82			1.16	0.40	0.04
569	E294R	a	0.71	0.10	0.60	0.14	0.98	0.98	0.65	0.22	0.86	0.25	0.58	0.13	0.92	0.28	0.89
570	E294S	a	0.87	0.11			0.84	0.72			0.30	0.18					0.36
571	E294T	a	1.28	0.11	1.37	0.10	0.68	0.98	1.16	0.26	0.45	0.25	1.07	0.15	0.71	0.33	0.66
572	E294H	a	1.50	0.27	1.11	0.13	0.58	0.92	0.55	0.22	0.45	0.27	0.81	0.12	0.76	0.20	0.77
573	E294V	a	0.86	0.21	0.74	0.10	0.39	1.28	0.87	0.22	0.43	0.49	0.87	0.13	0.70	0.17	1.11
574	E294L	a	0.43	0.26	0.35	0.15	0.42	1.14	0.45	0.24	0.35	0.58	0.93	0.12	0.45	0.19	0.83
			1.23	0.26	0.70	0.32	1.30	0.20	1.26	0.30	1.65	0.16			0.98	0.47	1.26
575	E294I	a	1.70	0.25	0.95	0.14	3.65	1.09	0.83	0.26	0.71	0.23	1.05	0.13	1.09	0.22	0.19
576	E294F	a	1.08	0.35	0.89	0.16	0.56	0.95	0.58	0.22	0.43	0.31	0.72	0.16	0.90	0.18	0.78
577	E294M	a	0.96	0.15	0.84	0.09	1.70	1.26	0.65	0.27	0.67	0.28	1.17	0.14	0.78	0.21	0.39
578	E294Y	a	0.70	0.12			0.68	0.49			1.32	0.17					1.95
579	E294W	a	0.74	0.18	0.93	0.10	0.99	1.08	0.77	0.22	1.70	0.38	1.37	0.15	0.80	0.20	1.73
580	E294P	a	1.00	0.20	1.09	0.12	1.34	0.97	0.50	0.23	0.67	0.24	0.50	0.12	0.77	0.26	0.50
581	E294G	a	1.55	0.20	1.18	0.09	0.55	0.97	1.45	0.21	0.61	0.25	0.94	0.14	1.25	0.27	1.10
582	Q295D	a	1.73	0.24	0.57	0.10	3.31	1.54	0.67	0.30	0.54	0.26	2.41	0.27	0.55	0.17	0.16
583	Q295E	a	4.42	0.12	1.49	0.14	1.91	1.40	0.88	0.22	1.03	0.24	0.84	0.17	0.96	0.23	0.54
			1.36	0.26	1.37	0.26	0.80	0.27	0.72	0.20	0.89	0.15			0.81	0.49	1.10
584	Q295N	a	2.09	0.18	0.81	0.12	5.76	1.58	0.50	0.34	0.45	0.25	0.69	0.15	0.93	0.17	0.08
585	Q295R	a	1.30	0.19	0.78	0.11	0.51	0.87	0.45	0.30	0.35	0.28	1.43	0.22	0.67	0.16	0.69
586	Q295S	a	1.37	0.12	0.74	0.13	0.95	1.07	0.77	0.31	0.55	0.24	1.66	0.13	0.72	0.23	0.57
587	Q295T	a	1.46	0.12	1.84	0.11	1.09	1.04	1.54	0.23	1.36	0.24	1.48	0.21	3.82	0.20	1.25
588	Q295H	a	1.14	0.20	1.01	0.11	4.39	1.62	0.79	0.31	0.43	0.25	1.39	0.20	0.76	0.32	0.10
589	Q295V	a	2.12	0.19	1.63	0.10	0.99	1.15	1.56	0.24	0.61	0.25	1.35	0.18	1.19	0.20	0.62
590	Q295I	a	1.42	0.17	1.99	0.12	1.94	1.00	1.04	0.21	0.53	0.27	1.28	0.15	0.68	0.37	0.27
591	Q295F	a	1.38	0.27	0.87	0.13	1.79	1.17	0.28	0.28	0.33	0.26	1.73	0.12	0.83	0.24	0.19
592	Q295M	a	2.88	0.26	1.69	0.11	1.50	1.00	0.66	0.40	0.81	0.27	2.93	0.17	0.70	0.25	0.54
593	Q295Y	a	0.94	0.22	0.61	0.11	0.18	0.86	0.56	0.22	0.27	0.29	2.30	0.14	0.62	0.34	1.47
594	Q295W	a	0.12	0.24	0.43	0.48	0.38	0.42	0.50	0.33	0.27	0.24	3.15	0.16	0.32	0.38	0.71
595	Q295P	a	0.38	0.30	0.94	0.28	0.57	0.37	0.71	0.20	0.48	0.32	3.14	0.14	0.49	0.36	0.83
596	Q295G	a	0.19	0.23	0.62	0.35	0.35	0.51	0.47	0.25	0.54	0.25	4.08	0.12	0.35	0.37	1.54
597	Y296K	a	0.21	0.26	0.40	0.36	0.33	0.53	0.54	0.30	0.35	0.16	2.67	0.14	0.58	0.34	1.07
598	Y296R	a	0.26	0.41	0.58	0.28	0.41	0.48	0.74	0.20	0.54	0.15	2.51	0.13	0.49	0.34	1.33
599	Y296A	a	0.56	0.35	0.44	0.27	0.48	0.39	0.68	0.33	1.06	0.23	4.09	0.14	0.42	0.40	2.20
600	Y296V	a	0.41	0.29	1.08	0.51	0.58	0.31	0.88	0.14	0.72	0.26	6.49	0.13	0.48	0.41	1.25
601	Y296M	a	0.21	0.33	0.94	0.36	0.64	0.34	0.90	0.17	0.65	0.28	3.02	0.13	0.53	0.36	1.01
602	Y296G	a	0.63	0.82	1.65	0.42	0.58	0.32	1.01	0.21	0.33	0.27	5.51	0.19	0.59	0.38	0.56
603	S324H	ap	0.99	0.33	0.84	0.25	0.74	0.29	0.57	0.17	0.73	0.36	3.54	0.13	0.57	0.34	0.98
604	S324F	a	1.17	0.40	0.94	0.29	0.65	0.33	0.74	0.23	0.92	0.30	4.10	0.15	0.50	0.33	1.43
605	S324M	a	0.76	0.80	1.13	0.28	0.88	0.23	1.07	0.12	1.46	0.24	2.02	0.13	0.61	0.33	1.66
606	S324W	a															
607	S324P	a	0.28	0.30	0.42	0.29	1.23	0.26	1.01	0.15	1.38	0.29	1.68	0.14	0.63	0.34	1.12
608	S324G	a	1.61	0.33	2.07	0.27	1.92	0.27	1.89	0.34	6.18	0.22	3.22	0.16	1.70	0.36	3.22
			0.60	0.15	3.06	0.11	2.56	0.18	2.18	0.30	3.92	0.30	2.14	0.13	2.74	0.18	1.53
			1.46	0.20	1.55	0.23	1.07	0.20	0.89	0.27	1.88	0.10			1.25	0.50	1.77
609	P230E	a	2.07	0.52	1.25	0.31	2.34	0.45	1.47	0.15	1.40	0.24	2.70	0.13	1.01	0.34	0.60
610	P230Y	a	0.44	0.52	0.40	0.30	0.85	0.34	1.22	0.20	0.56	0.33	2.00	0.12	0.65	0.33	0.66
611	P230G	a	1.04	0.45	0.99	0.38	1.13	0.36	0.75	0.21	0.48	0.22	1.27	0.16	1.16	0.34	0.42
612	A231E	a															
613	A231K	a	0.50	0.36	0.62	0.34	0.41	0.61	0.58	0.24	0.58	0.49	1.40	0.16	0.52	0.33	1.39
614	A231Y	a															
615	A231P	a	1.17	0.15			0.81	0.43			0.31	0.17					0.38
616	A231G	a															
617	E233N	a	0.16	0.28	0.50	0.44	0.34	0.79	0.46	0.22	0.48	0.17	1.28	0.15	0.74	0.33	1.42
618	E233Q	a	0.19	0.22	0.52	0.42	0.27	0.62	0.39	0.25	0.51	0.17	1.17	0.16	0.61	0.34	1.88
619	E233K	a															
620	E233R	a	0.14	0.26	0.75	0.27	0.36	0.68	0.60	0.19	0.35	0.16	1.13	0.18	1.05	0.33	0.97
621	E233S	a	0.17	0.18	0.62	0.35	0.31	0.59	0.37	0.35	0.36	0.19	1.18	0.16	1.10	0.33	1.14

Figure 4 (continued)

			FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
Variant	Substitution(s)	Context	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
622	E233T	a	0.15	0.35	1.28	0.39	0.78	0.40	0.83	0.13	0.42	0.19	0.99	0.17	1.19	0.35	0.54
623	E233H	a	0.17	0.32	0.74	0.27	0.58	0.40	0.78	0.14	0.32	0.27	1.05	0.15	0.99	0.33	0.55
624	E233A	a	0.10	0.31	1.23	0.33	0.60	0.44	0.71	0.20	0.46	0.21	1.02	0.13	0.93	0.38	0.75
625	E233V	a	0.25	0.48	0.71	0.31	0.37	0.61	0.61	0.17	0.50	0.21	0.70	0.15	0.91	0.33	1.34
626	E233L	a	0.53	0.40	0.55	0.29	0.26	0.53	0.54	0.27	0.52	0.19	2.24	0.17	0.60	0.34	1.99
627	E233I	ap	0.30	0.28	1.09	0.29	1.69	0.27	1.80	0.14	0.88	0.27	2.30	0.14	0.95	0.34	0.52
628	E233F	a	0.23	0.34	0.64	0.31	0.73	0.38	0.84	0.27	0.58	0.20	1.27	0.15	0.90	0.35	0.79
629	E233M	a	0.29	0.27	0.67	0.27	0.49	0.48	0.85	0.15	0.70	0.18	1.56	0.14	1.13	0.43	1.42
630	E233Y	a	0.31	0.14	0.96	0.10	0.97	0.16	0.55	0.14	0.37	0.09	0.70	410	1.86	0.27	0.38
631	E233W	a	0.28	0.17	0.86	0.12	0.82	0.14	0.70	0.15	0.35	0.13	0.91	0.34	1.64	0.24	0.43
632	E233G	a	0.36	0.14	1.21	0.11	0.94	0.22	0.74	0.13	1.21	0.10	1.03	0.29	2.09	0.25	1.29
633	E272D	a	1.26	0.24	1.25	0.10	1.12	0.13	1.41	0.12	1.49	0.16	1.48	0.18	1.37	0.22	1.33
634	E272R	ap	66.62	0.43	0.84	0.11	0.80	0.17	1.91	0.27	41.60	2.06		26.26	1.83	0.22	51.94
			98.41	0.98	0.94	0.21	1.26	0.31	0.47	0.30	0.67	0.35		2.58	1.62	0.25	0.53
635	E272T	a	3.32	0.22	1.24	0.11	1.74	0.31	1.34	0.18	3.04	0.25	0.69	0.23	2.46	0.21	1.75
			0.96	0.23	0.44	0.26	0.75	0.23	0.74	0.17	0.31	0.17			0.79	0.48	0.41
636	E272H	ap	7.21	0.20	0.84	0.09	1.92	0.10	0.83	0.13	28.57	0.15	0.55	0.20	1.45	0.27	14.86
			4.09	0.51	0.88	0.15	1.70	0.15	1.18	0.26	9.83	0.37	1.42	0.25	0.90	0.23	5.77
			1.02	0.26	0.49	0.29	0.43	0.39	0.26	0.47	0.35	0.10			0.92	0.43	0.80
637	E272V	a	2.80	0.28	0.96	0.10	0.72	0.13	0.50	0.17	1.19	0.23	0.67	0.20	1.77	0.25	1.64
638	E272L	a	2.07	0.22	1.10	0.10	0.83	0.13	0.62	0.13	1.07	0.19	0.75	0.21	1.81	0.21	1.29
639	E272F	a															
640	E272M	a															
641	E272W	a	1.61	0.32	1.06	0.11	1.09	0.12	0.68	0.15	2.59	0.15	0.62	0.23	1.82	0.23	2.38
642	E272P	a	5.27	0.21	1.14	0.14	2.99	0.14	3.36	0.22	0.67	0.11	0.55	0.30	2.17	0.21	0.23
			1.73	0.23	0.37	0.30	3.46	0.17	4.31	0.13	0.32	0.11			0.67	0.62	0.09
643	E272G	a	1.25	0.11			0.46	0.45			0.24	0.18					0.52
644	K274D	a															
645	K274N	a	1.25	0.17	1.37	0.10	1.17	0.13	1.42	0.13	1.18	0.09	1.55	0.21	1.26	0.25	1.01
647	K274H	a	1.02	0.22			1.21	0.30			0.27	0.24					0.22
648	K274V	a															
649	K274I	a	1.83	0.16	1.42	0.08	1.51	0.14	2.20	0.13	1.47	0.16	2.03	0.19	1.08	0.28	0.98
650	K274F	a	1.98	0.15	1.22	0.09	1.04	0.14	1.89	0.16	1.25	0.09	3.52	0.18	1.02	0.22	1.20
651	K274M	a	1.42	0.14	1.65	0.09	1.28	0.10	1.18	0.12	1.42	0.08	1.89	0.21	1.01	0.22	1.11
652	K274W	a															
653	K274F	a															
654	K274G	a	1.57	0.14	1.70	0.11	1.06	0.11	0.92	0.14	1.41	0.08	1.20	0.25	1.04	0.22	1.36
655	N276D	ap	1.54	0.13	1.34	0.13	1.21	0.11	1.04	0.11	0.88	0.05	1.10	0.26	1.18	0.21	0.72
656	N276T	a															
657	N276H	a	2.78	0.18	0.98	0.09	1.10	0.10	0.72	0.14	0.71	0.15	0.79	2.83	1.19	0.21	0.64
658	N276V	a	1.25	0.15	1.06	0.12	1.03	0.13	1.12	0.15	1.04	0.07	0.91	0.21	1.27	0.24	1.01
659	N276I	a															
660	N276F	a	1.34	0.30	1.16	0.10	1.30	0.15	1.57	0.14	1.53	0.15	2.20	0.17	1.18	0.21	1.17
661	N276M	a	1.20	0.26			0.99	0.36			0.26	0.24					0.26
662	N276W	a	2.53	0.26	1.24	0.11	1.32	0.16	2.06	0.14	1.21	0.09	1.57	0.26	2.00	0.21	0.92
663	N276P	a															
664	N276G	a															
665	Y278D	a	2.17	0.12	1.25	0.09	1.38	0.10	1.19	0.14	1.25	0.12	2.00	0.17	1.79	0.22	0.91
666	Y278N	a	1.54	0.18	0.88	0.10	0.78	0.12	0.66	0.12	0.82	0.08	0.74	9.38	1.33	0.22	1.05
667	Y278Q	ap															
668	Y278R	ap	1.98	0.25	0.92	0.12	0.62	0.11	0.58	0.15	0.54	0.10	0.82	0.25	1.23	0.22	0.87
669	Y278S	a	2.19	0.18	0.97	0.10	1.10	0.13	0.99	0.12	0.79	0.07	2.24	0.18	1.23	0.22	0.72
670	Y278H	ap															
671	Y278V	a	1.64	0.15	1.37	0.09	1.82	0.11	1.73	0.14	0.97	0.10	2.21	0.18	1.27	0.23	0.53
672	Y278L	a	2.57	0.20	1.05	0.13	1.32	0.12	0.94	0.12	2.92	0.09	0.44	0.21	1.74	0.21	2.21
673	Y278I	a	1.25	0.34	1.09	0.10	0.94	0.13	1.03	0.11	0.97	0.12	1.18	0.19	1.52	0.22	1.04
674	Y278M	a	1.49	0.16	0.94	0.09	0.71	0.10	1.02	0.15	0.55	0.15	1.22	0.20	1.28	0.21	0.77
675	Y278P	a															
676	Y278G	a															
677	K320N	a	2.63	0.13	1.33	0.10	1.45	0.11	1.42	0.12	1.64	0.11	1.89	0.18	2.01	0.22	1.13
678	K320S	a															
679	K320H	a	0.80	0.18	1.12	0.14	1.32	0.13	2.45	0.31	1.21	0.35	2.24	0.12	1.13	0.21	0.92
680	K320V	a	0.66	0.15	1.18	0.12	1.13	0.11	1.83	0.30	1.83	0.37	2.45	0.13	0.83	0.18	1.61
681	K320L	a	0.97	0.13	0.95	0.11	1.20	0.14	1.19	0.36	1.84	0.38	4.14	0.10	1.07	0.16	1.53
682	K320F	a	0.79	0.15	1.18	0.11	1.16	0.15	1.18	0.32	1.23	0.37	1.64	0.13	0.76	0.21	1.06
683	K320Y	ap	0.87	0.14	0.95	0.11	1.00	0.20	1.01	0.32	1.11	0.37	1.88	0.14	0.80	0.20	1.11
684	K320W	a	0.98	0.18	1.19	0.13	1.10	0.15	1.39	0.30	1.31	0.33	1.70	0.29	1.11	0.19	1.20
685	K320P	a															
686	K320G	a	0.73	0.20	1.11	0.12	1.06	0.14	1.19	0.37	1.21	0.36	1.53	0.12	0.69	0.22	1.13
687	K322D	a	1.11	0.15	0.80	0.12	0.78	0.10	1.43	0.31	0.80	0.36	1.69	0.11	0.48	0.25	1.03
688	K322S	a	0.93	0.43	0.94	0.11	0.97	0.11	1.99	0.31	1.14	0.36	1.86	0.11	0.87	0.20	1.17
689	K322V	a	0.69	0.14	1.07	0.11	1.25	0.14	2.00	0.31	1.16	0.39	1.51	0.11	0.72	0.18	0.92
690	K322I	a	0.69	0.28	0.90	0.11	1.33	0.15	1.54	0.32	0.92	0.36	1.61	0.11	0.75	0.24	0.69

Figure 4 (continued)

			FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
Variant	Substitution(s)	Context	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
691	K322F	a	0.79	0.15	0.95	0.11	1.33	0.13	1.30	0.29	0.86	0.38	1.15	0.15	0.75	0.19	0.65
692	K322Y	ap	0.77	0.14	0.95	0.11	1.11	0.17	1.18	0.37	1.03	0.34	2.10	0.11	0.87	0.22	0.93
693	K322W	a	0.65	0.17	0.87	0.13	0.73	0.18	0.77	0.35	0.95	0.32	1.48	0.11	0.66	0.18	1.31
694	K322P	a															
695	K322G	a	0.86	0.18	0.64	0.14	0.73	0.11	0.69	0.40	0.57	0.39	1.86	0.11	0.62	0.18	0.78
696	N325K	a	0.06	0.14	0.59	0.14	0.76	0.13	0.43	0.45	0.67	0.29	1.09	0.15	0.77	0.13	0.88
697	N325R	a	2.02	0.36	0.47	0.15	0.81	0.16	0.58	0.38	0.43	0.35	1.15	0.42	0.72	0.22	0.53
698	N325S	a	0.43	0.17	0.60	0.15	1.05	0.12	1.21	0.30	0.84	0.33	0.49	0.14	0.79	0.16	0.80
699	N325F	a	0.32	0.17	0.71	0.13	2.87	0.17	3.26	0.27	0.62	0.27	0.60	0.13	1.22	0.21	0.21
700	N325M	a	0.39	0.15	0.82	0.11	1.54	0.14	1.92	0.29	1.03	0.28	1.61	0.13	1.43	0.19	0.67
701	N325Y	a	0.27	0.14	0.54	0.13	0.73	0.23	0.65	0.35	0.57	0.30	1.72	0.13	0.91	0.24	0.77
702	N325W	a	0.23	0.19	0.45	0.17	0.57	0.25	0.40	0.49	0.51	0.30	1.35	0.12	0.86	0.23	0.89
703	N325P	a	0.06	0.21			0.37	2.05			0.03	0.53					0.07
704	N325G	a	0.18	0.21	0.46	0.16	0.76	0.16	0.70	0.34	0.75	0.27	0.69	0.66	0.53	0.15	0.99
705	P227E	a	1.47	0.23	1.55	0.32	1.55	0.19	1.67	0.12	2.10	0.10			1.49	0.49	1.36
706	P227K	a	0.44	0.37	0.64	0.17	0.84	0.27	3.04	0.28	0.38	0.24	0.92	0.46			0.46
			0.97	0.10	1.00	0.42	0.79	0.53	0.94	0.56	0.26	0.25	2.21	0.28	1.45	0.39	0.33
			0.92	0.20	0.85	0.29	0.89	0.22	0.77	0.22	1.03	0.13			1.16	0.51	1.16
707	P227Y	a	1.06	0.27	0.89	0.33	0.79	0.21	0.73	0.27	0.44	0.15			0.80	0.49	0.56
708	P227G	a	0.70	0.38	1.39	0.16	1.93	0.22	6.56	0.26	15.77	0.20	0.59	0.37			8.18
			1.67	0.15	0.91	0.37	1.98	0.24	3.47	0.32	12.61	0.37	1.16	0.32	1.67	0.40	6.36
709	P228E	a															
710	P228K	a	0.78	0.37	1.42	0.17	0.72	0.38	1.23	0.33	1.86	0.18	0.73	0.36			2.58
			1.91	0.28	1.31	0.58	1.14	0.25	1.13	0.54	1.28	0.25	1.62	0.29	1.55	0.39	1.12
			0.48	0.29	2.65	0.26	1.67	0.20	1.94	0.18	1.73	0.14			1.29	0.53	1.04
711	P228Y	a	0.62	0.39	1.10	0.17	0.60	0.31	2.04	0.29	0.71	0.21	1.11	0.34			1.18
			1.15	0.11	1.68	0.64	1.59	0.29	1.61	0.43	0.75	0.19	2.25	0.31	1.45	0.35	0.47
712	P228G	a	1.69	0.49	0.70	0.17	0.66	0.25	1.14	0.30	0.98	0.45	0.99	0.41			1.49
			1.00	0.19	2.60	0.68	0.96	0.24	0.54	0.54	0.98	0.18	1.89	0.42	1.77	0.37	1.01
713	G236D	a	0.42	0.57	1.31	0.16	3.39	0.21	7.15	0.54	0.19	9.45	1.08	0.38			0.05
			0.15	0.16	5.01	0.70	3.77	0.19	4.74	0.34	0.11	0.23	1.64	0.32	1.53	0.37	0.03
714	G236E	a	0.06	0.42	4.88	0.20	1.53	0.22	3.39	0.30	1.41	0.19	0.48	0.39			0.92
			0.22	0.10	3.04	0.39	1.68	0.22	2.15	0.29	1.05	0.19	1.58	0.25	1.46	0.39	0.62
			0.07	0.34	1.65	0.24	0.98	0.18	0.67	0.21	0.47	0.13			0.56	0.52	0.48
715	G236N	a	0.07	0.34	0.26	0.29	0.69	0.21	0.79	0.26	0.07	0.44			1.89	0.47	0.10
716	G236Q	a	0.03	0.44	0.82	0.14	0.49	0.72	1.28	0.34	0.21	0.39	0.99	0.35			0.43
			0.12	0.15	0.60	0.34	0.89	7.57	0.26	0.53	0.17	0.20	1.89	0.33	1.46	0.40	0.18
717	G236K	a	0.01	0.40	0.27	11.52	45.05	8.94	1.38	0.29	0.48	0.28	1.15	0.35			0.01
			0.11	0.29	3.21	4.73	0.90	3.50	0.55	0.69	0.46	0.25	2.18	0.37	1.36	0.35	0.51
			0.02	0.40					0.06	0.70	0.01	1.89			0.73	0.46	
718	G236R	a	0.01	0.38	0.29	0.23	0.63	0.33	0.62	0.69	0.20	0.72	1.04	0.34			0.32
			0.10	0.09	0.31	0.56	0.98	16.50	0.19	0.55	0.10	0.27	2.03	0.35	1.99	0.36	0.10
719	G236S	a	0.12	0.38	28.92	0.14	2.23	0.29	7.98	0.26	6.10	0.21	0.68	0.36			2.73
			0.55	0.12	22.71	0.37	2.77	0.21	3.68	0.45	5.77	0.26	1.82	0.29	1.77	0.38	2.08
720	G236T	a	0.02	0.42	1.58	0.16	0.36	0.55	1.35	0.28	0.21	0.91	0.99	0.33			0.59
			0.11	0.13	1.89	0.39	0.95	0.32	0.48	0.57	0.11	0.22	3.25	0.34	1.56	0.35	0.12
			0.06	0.36	0.56	0.26	0.34	0.47	0.15	0.35	0.04	0.85			0.43	0.58	0.10
721	G236H	a	0.05	0.47	0.69	0.20	0.36	1.36	0.46	0.39			0.87	0.36			
			0.19	0.16	2.02	0.51	0.87	15.81	0.28	0.97	0.07	0.33	2.32	0.47	1.48	0.37	0.07
			0.03	0.40	0.37	0.31	0.31	0.49	0.21	0.45	0.08	0.13			0.52	0.51	0.26
722	G236A	a	0.37	0.43	45.05	0.19	1.20	0.22	1.58	0.32	0.73	0.22	1.05	0.36			0.61
			0.48	0.16	44.99	0.28	1.05	0.24	1.45	0.50	0.65	0.18	1.64	0.27	1.75	0.35	0.62
723	G236V	a	0.03	0.52	2.06	0.15	0.23	0.88	0.96	0.36	0.24	0.37	0.99	0.37			1.02
			0.14	0.15	1.52	0.36	0.82	0.46	0.47	0.38	0.27	0.19	1.59	0.28	1.35	0.39	0.33
724	G236L	a	0.04	0.28			1.07	0.48			0.18	0.25					0.17
725	G236I	a	0.02	0.40	1.95	0.15	0.11	2.97	1.10	0.38			1.04	0.34			
			0.11	0.18	1.33	0.30	0.75	2.41	0.12	0.69	0.02	0.87	1.96	0.26	1.33	0.37	0.02
726	G236F	a	0.06	0.41	0.43	0.17	0.55	0.43	0.88	0.34	0.18	0.64	1.19	0.34			0.33
			0.21	0.23	0.44	0.54	0.92	46.70	0.08	1.29	0.05	0.76	2.56	0.48	1.75	0.36	0.06
727	G236M	a	0.03	0.38	0.29	0.19	0.43	0.37	1.22	0.42	0.21	0.44	0.67	0.34			0.49
			0.14	0.12	0.34	0.44	0.79	0.83	0.40	0.67	0.19	0.20	1.40	0.55	1.60	0.35	0.24
728	G236Y	a	0.04	0.39	0.83	0.16	0.44	1.15	0.91	0.44	0.22	1.31	0.77	0.36			0.50
			0.17	0.13	0.78	0.58	0.85	1.07	0.44	1.38	0.14	0.26	1.34	0.33	1.52	0.35	0.16
			0.10	0.38	0.37	0.31	0.41	1.22	0.00	2.84	0.04	0.88			0.56	0.52	0.09
729	G236W	a	0.32	0.39	1.71	0.18	0.52	0.55	0.67	0.30	0.33	0.33	0.98	0.34			0.63
			0.78	0.17	2.13	0.40	0.70	0.51	0.29	0.69	0.29	0.20	1.40	0.26	1.52	0.36	0.41
			0.68	0.27	1.23	0.27	0.83	0.21	1.56	0.15	0.34	0.13			0.56	0.51	0.40
730	G236P	a	0.04	0.48	0.25	0.30	0.27	1.53	0.42	0.41	0.19	28.37	1.25	0.35			0.70
			0.15	0.16		7.93	0.90	10.17	0.15	0.68	0.10	0.23	1.38	0.86	1.41	0.37	0.11
			0.04	0.47	0.11	0.72	0.24	0.76	0.10	1.03	0.07	0.21			0.66	0.52	0.30
731	G237D	a	0.18	0.55	0.21	2.59	54.08	9.16	2.51	0.27			1.50	0.37			
			0.10	0.25		8.65	1.00	0.27	1.13	0.52	0.13	0.19	2.86	0.27	1.84	0.36	0.13
			0.08	0.33			1.04	0.19	0.74	0.17					0.80	0.50	

Figure 4 (continued)

Variant	Substitution(s)	Context	FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		IIIa:IIb
			Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	
732	G237E	a	0.01	0.48	0.26	9.34	0.88	0.24	1.03	0.32	0.21	12.54	0.89	0.36			0.24
			0.09	0.13	0.70	0.95	0.88	0.45	0.42	0.48	0.02	0.60	1.16	0.24	1.36	0.42	0.02
733	G237N	a	0.02	0.40	0.27	13.28	0.65	0.38	2.79	0.26	0.18	5.37	0.71	0.36			0.27
			0.08	0.24	0.10	0.62	0.98	0.26	0.73	0.43	0.02	1.05	1.29	0.24	1.39	0.36	0.02
734	G237Q	a	0.01	0.39	0.26	14.91	0.85	8.46	1.20	0.30			0.84	0.34			
			0.07	0.32	0.46	4.47	0.78	17.18	1.80	5.62	0.00	3.01	1.33	0.31	1.39	0.37	0.00
735	G237K	a	0.01	0.39	0.22	0.34	0.32	0.81	1.29	0.38	0.17	0.73	0.81	0.35			0.54
			0.09	0.18	0.32	0.63	0.74	0.73	0.32	0.56	0.10	0.28	1.52	0.29	1.47	0.36	0.13
			0.02	0.56			0.46	0.27	0.11	0.85	0.07	2.82			1.28	0.48	0.15
736	G237R	a	0.01	0.38	0.21	0.32	0.48	0.83			0.21	0.66	0.73	0.35			0.43
			0.08	0.20		41.28			0.39	1.04	0.16	0.28	1.01	0.34	1.36	0.36	
737	G237S	a	0.03	0.42	0.17	1.31	0.75	0.34	1.94	0.26			1.04	0.33			
			0.12	0.15		6.61	1.02	0.24	0.78	0.53	0.07	0.49	1.86	0.34	1.29	0.36	0.06
738	G237T	a	0.05	0.54			0.50	0.68	0.77	0.29			0.69	0.35			
			0.13	0.18		5.66			0.21	0.72	0.09	0.21	1.08	0.35	1.32	0.38	
739	G237H	a	0.06	0.25	0.24	0.38	0.59	0.29	1.76	1.61	0.09	0.24	0.97	0.36	1.48	0.36	0.15
740	G237V	a	0.03	0.26			1.07	0.83	1.81	0.40	0.06	0.41	2.03	0.24	1.00	0.19	0.06
741	G237L	a	0.01	0.35			0.54	0.81	1.19	0.46	0.03	0.56	1.88	0.29	1.21	0.25	0.06
742	G237I	a					0.29	0.84	0.43	0.56	0.35	0.21	1.51	0.28	0.60	0.70	1.18
			0.02	0.22							0.40	0.15			1.01	0.27	
743	G237F	a	0.07	0.36			0.41	0.73	1.08	0.45	0.04	0.56	1.65	0.29	0.81	0.28	0.11
744	G237M	a	0.04	0.32			0.30	0.58	1.06	0.49	0.06	0.65	1.80	0.27	0.94	0.25	0.18
			0.07	0.37	0.14	0.35	0.57	0.26	0.66	0.27	0.10	0.15			1.74	0.45	0.17
745	G237Y	a	0.21	0.44			0.37	0.58	0.67	0.71	0.08	0.22	2.06	0.32	1.00	0.21	0.21
746	G237W	a	0.04	1.92			2.62	0.83	1.92	0.38			0.85	0.24	1.20	0.21	
747	G237P	a	0.02	0.18			0.52	0.90	0.52	0.51	0.01	0.69	1.17	0.25	1.21	0.21	0.02
748	P238D	a	0.26	0.32			13.02	1.16	5.89	0.39	0.72	0.25	1.03	0.24	0.63	0.89	0.06
			0.07	0.24							0.58	0.13			1.03	0.21	
749	P238E	a	0.95	0.29			1.18	0.86	1.46	0.41		3.53	1.20	0.25	3.71	1.18	
			0.30	0.14							0.04	0.32			0.68	0.28	
			0.19	0.34			0.42	0.33	0.36	0.19	0.05	0.61			1.43	0.62	0.12
750	P238N	a	0.21	0.31			0.53	0.91	1.11	0.49			0.99	0.22	1.15	0.99	
			0.09	0.16							0.01	1.23			1.01	0.28	
751	P238Q	a															
752	P238K	a		1.05			0.24	0.59	0.20	0.85			1.00	0.25	5.01	0.83	
			0.04	8.30							0.00	3.26			0.77	0.29	
753	P238R	a	0.17	0.32			0.35	0.58	0.54	0.67	0.62	0.20	1.18	0.24	0.83	0.66	1.78
			0.25	0.21	0.31	3.96					0.50	0.16			0.92	0.26	
754	P238S	a	0.29	0.31			0.31	0.55	0.75	0.77		3.07	0.97	0.27	0.42	0.60	
			0.44	0.12							0.08	0.17			0.93	0.22	
			0.57	0.26	0.15	0.38	0.63	0.40	1.08	0.22	0.23	0.13			1.12	0.51	0.37
755	P238T	a	0.21	0.35			0.60	0.66	0.78	0.54		3.34	1.51	0.30	0.91	0.61	
			0.11	0.22	0.26	277					0.02	0.64			1.12	0.19	
756	P238H	a	0.44	0.35			1.89	0.85	1.35	0.41	1.51	0.18	1.32	0.24	0.90	0.53	0.80
			0.26	0.09	0.32	2638					1.12	0.13			1.46	0.22	
757	P238V	a	1.09	0.28			2.24	0.91	1.99	0.38		2.80	1.55	0.28	1.43	0.57	
			1.22	0.18	0.38	0.37					0.06	0.43			1.09	0.19	
758	P238L	a	1.78	0.31			2.54	0.95	3.64	0.41	0.79	0.22	2.34	0.29	1.71	0.54	0.31
			1.17	0.14	0.31	12.20					0.69	0.15			1.40	0.30	
759	P238I	a	1.32	0.29			0.40	0.82	0.38	0.73			1.79	0.29	1.06	0.52	
			0.62	0.21							0.02	0.77			1.44	0.26	
760	P238F	a	1.70	0.31					3.72	0.38		2.56	2.32	0.32	2.41	0.55	
			2.60	0.12							0.02	0.84			1.09	0.26	
761	P238M	a	1.38	0.27			0.44	0.66	1.27	0.44		2.89	3.23	0.35	2.33	1.22	
			1.39	0.09							0.03	0.55			0.91	0.24	
762	P238Y	a	0.66	0.26			0.43	0.62	0.99	0.56		6.35	1.16	0.29	0.80	0.54	
			3.05	0.20											0.92	0.21	
763	P238W	a	1.27	0.27			0.73	0.69	1.12	0.53		2.95	1.92	0.31	0.10	0.54	
			0.73	0.21											1.30	0.20	
764	P238G	a	0.33	0.35			1.30	0.86	0.84	0.41	0.39	0.21	1.41	0.26	0.12	0.53	0.30
			0.11	0.16	0.34	0.27					0.36	0.20			1.53	0.22	
765	E269K	a	0.14	0.38			1.00	0.79	0.95	0.44			1.46	0.26	0.13	0.53	
			0.08	0.17							0.14	0.12			1.23	0.20	
766	E269S	a	0.31	0.30			0.74	0.77	1.19	0.44	0.30	0.20	0.93	0.28	0.13	0.55	0.41
			0.21	0.15	0.29	8.99					0.21	0.20			1.31	0.33	
767	E269V	a	0.30	0.29			0.35	0.80	0.32	0.68		4.69	1.48	0.28	0.13	0.52	
			0.24	0.17	0.26	34.19					0.19	0.54			1.38	0.28	
768	E269I	a	0.35	0.29			0.29	0.63	0.40	0.42			2.10	0.40	0.13	0.52	
			0.41	0.22	0.28	1372					0.12	0.24			1.26	0.30	
769	E269M	a	0.38	0.25			0.37	0.58	0.66	0.58		14.73	2.66	0.34	0.14	0.52	
			0.51	0.14							0.12	0.16			1.11	0.24	
770	E269W	a	0.20	0.29			0.34	0.55	0.92	0.62		1.83	1.43	0.32	0.08	0.52	
			0.37	0.19							0.06	0.38			1.19	0.22	

Figure 4 (continued)

			FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
Variant	Substitution(s)	Context	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
771	E269P	a	0.36	0.16	0.13	0.41	0.73	0.25	1.33	0.66	0.02	0.70	0.00	12605	1.23	0.21	0.02
772	E269G	a			0.58	0.54	0.51	0.28	1.11	0.66	0.03	0.63	0.00	7.64	1.34	0.18	0.05
			0.39	0.29	0.23	0.37	0.58	0.28	0.44	0.51	0.23	0.16			1.18	0.50	0.39
773	H285D	a	1.81	0.16	1.57	0.54	1.18	0.33	2.56	0.72	1.22	0.64	0.00	7.64	2.40	0.27	1.03
774	H285E	a	1.67	0.25	1.39	0.32	0.98	0.29	1.53	0.35	1.46	0.19			1.93	0.50	1.50
775	H285Q	a	2.42	0.16	1.68	0.55	0.66	0.45	1.45	0.67	0.33	0.60	0.00	7.64	1.25	0.27	0.50
			1.28	0.22	1.41	0.29	1.81	0.22	2.15	0.17	1.59	0.16			1.38	0.49	0.88
776	H285K	a	1.80	0.20	2.04	0.50	1.37	0.37	2.89	0.80	2.46	0.66	0.00	7.64	1.76	0.30	1.79
777	H285Y	ap	2.68	0.31	1.95	0.56	0.95	0.49	4.24	1.43	1.77	0.97			1.25	0.24	1.87
778	H285W	a	1.32	0.14	0.84	0.41	1.31	0.23	2.37	0.66	0.69	0.48	0.00	7.65	1.33	0.17	0.53
779	N286E	a		3.40	0.07	0.40	0.38	0.25	0.37	0.94	0.01	1.71	0.00	7.65	2.12	0.18	0.03
780	N286Y	a		2.92	0.07	0.44	0.42	0.22	1.16	0.66	0.02	0.62	0.00	7.66	1.45	0.17	0.05
781	N286P	a	2.62	0.15	1.80	0.40	2.35	0.23	5.43	0.62	1.18	0.51	0.17	8.96	2.31	0.18	0.50
			1.16	0.28	1.05	0.32	2.50	0.21	3.86	0.13	0.71	0.11			2.90	0.49	0.29
782	N286G	a															
783	K288D	a	2.19	0.10			1.85	0.21			1.78	0.18					0.96
784	K288E	a	2.76	0.14	1.72	0.41	3.27	0.26	8.05	0.63	2.77	0.52	0.00	7.64	0.89	0.19	0.85
785	K288Y	a	2.17	0.13	1.80	0.53	1.14	0.27	2.71	0.64	0.69	0.54	0.00	7.64	1.10	0.19	0.61
786	R292D	a	2.38	0.17	5.10	0.39	1.12	0.25	2.81	0.68	0.51	0.50	0.00	7.64	1.67	0.22	0.46
787	R292E	a	1.56	0.13	1.00	0.44	0.73	0.41	4.46	4.23	0.34	0.50	0.00	7.64	1.45	0.22	0.47
788	R292T	a	1.30	0.15	1.11	0.40	0.95	0.24	1.11	0.75	0.41	0.51	0.25	8.57	0.85	0.18	0.43
789	R292Y	a	1.59	0.13	0.35	0.40	0.86	0.27	2.24	0.60	0.40	0.50	0.00	7.65	1.36	0.16	0.47
790	T335N	a	1.89	0.12	1.21	0.40	1.27	0.22	2.14	0.64	0.85	0.51	0.00	7.65	1.23	0.16	0.67
791	T335S	a	2.02	0.14	6.31	0.38	1.27	0.22	2.70	0.68	1.43	0.58			1.42	0.16	1.12
792	T335H	a	2.26	0.19	1.31	0.45	1.07	0.24	1.67	0.67	1.25	0.61	0.00	7.65	1.16	0.21	1.17
793	T335V	a	2.33	0.13	1.80	0.53	0.97	0.27	2.53	0.72	1.41	0.63	0.00	7.64	1.25	0.17	1.46
794	T335L	a	2.48	0.18	1.96	0.51	2.62	0.32	14.39	0.70	2.22	0.50	0.00	2101	1.50	0.26	0.85
795	T335I	a	2.25	0.15	2.47	0.50	1.16	0.36	1.76	1.04	1.51	0.70	0.00	7.64	1.41	0.22	1.30
796	T335F	a	1.21	0.11	0.71	0.41	1.21	0.24	1.70	0.76	0.49	0.54	0.25	9.47	1.19	0.17	0.40
797	T335M	a	2.06	0.12	0.60	0.41	1.13	0.26	1.98	0.64	0.52	0.51	0.00	7.65	0.79	0.19	0.46
			1.31	0.24	1.14	0.26	1.13	0.24	1.46	0.13	1.18	0.10			1.35	0.48	1.04
798	T335W	a	2.11	0.13	0.68	0.40	1.16	0.22	2.08	0.66	0.59	0.51			1.35	0.17	0.51
799	T335P	a	2.80	0.12	2.42	0.46	0.93	0.26	1.75	0.65	1.35	0.60			1.45	0.17	1.45
800	T335G	a	1.99	0.16	0.93	0.46	0.77	0.25	1.47	0.66	0.32	0.51			0.90	0.24	0.42
801	D221K	a	4.53	0.19	4.32	0.39	3.50	0.28	17.75	0.61	65.60	0.48	0.00	7.64	5.66	0.18	18.72
			0.97	0.25	1.01	0.30	0.68	0.40	1.04	0.17	2.82	0.09			1.84	0.46	4.17
802	D221Y	a	2.43	0.19	2.62	0.44	2.41	0.28	10.01	0.67	9.56	0.48			2.74	0.21	0.23
803	T223E	a	2.12	0.14	2.37	0.42	1.74	0.23	3.70	0.69	0.75	0.49	0.00	7.64	2.04	0.22	0.43
804	T223K	a	1.61	0.17	2.18	0.29	1.81	0.25		11.88	9.37	0.36	1.31	0.30	2.97	0.36	5.19
			1.15	0.27	0.55	0.27	0.60	0.23	0.41	0.26	0.67	0.12			0.78	0.60	1.11
805	H224E	a	1.91	0.13	3.67	0.31	2.18	0.25		11.88	14.28	0.36	2.80	0.32	5.11	0.38	6.54
			0.54	0.09	0.27	0.21	0.21	0.36	0.66	0.71	0.58	1.31			0.43	0.23	2.73
806	H224Y	a	1.74	0.20	1.17	0.30	1.29	0.31		11.88	0.86	0.37	2.01	0.31	1.68	0.44	0.66
807	T225E	a	2.28	0.16	2.75	0.30	2.38	0.25	97.09	11.88	9.84	0.36	1.96	0.31	4.89	0.47	4.14
			0.46	0.14	0.35	0.19	0.44	0.44	1.21	0.66	0.76	1.31			0.41	0.16	1.71
808	T225K	a	0.30	0.17	0.37	0.32	0.45	0.34	95.70	11.88	0.22	0.44	1.12	0.28	5.68	0.68	0.48
			0.53	0.27	0.31	0.23	0.45	0.26	1.01	0.65	0.68	1.31			0.50	0.20	1.51
809	T225W	a	2.30	0.29	1.04	0.42	1.44	0.24	93.33	11.88	0.59	0.37	1.92	0.29	6.89	0.75	0.41
810	K246D	a	1.78	0.13	0.92	0.30	1.36	0.24	67.73	11.90	0.56	0.39	1.22	0.33	0.93	0.41	0.41
811	K246E	a	1.58	0.15	1.25	0.28	0.98	0.32	35.41	11.89	2.86	0.40	1.86	0.33	0.51	0.38	2.91
812	K246H	a	1.67	0.15	1.66	0.29	1.24	0.26		11.88	14.37	0.36	1.49	0.29	4.02	0.36	11.56
			0.35	0.17	0.26	0.21	0.16	0.76	0.24	0.83	0.36	1.31			0.38	0.14	2.22
813	K246Y	a	1.53	0.13	1.16	0.28	1.18	0.28	49.45	11.89	5.25	0.36	1.87	0.32	2.47	0.40	4.46
814	D249Q	a	0.30	0.20	0.22	0.33	0.61	0.82	74.56	11.89	0.36	0.36	2.13	0.33	2.06	0.37	0.59
			0.46	0.17	0.52	0.22	0.46	0.32	1.07	0.74	1.06	1.31			0.49	0.15	2.30
815	D249H	a	0.39	0.14	0.37	0.23	0.28	0.46	0.64	0.78	0.86	1.32			0.41	0.19	3.04
816	D249Y	a	1.80	0.14	1.87	0.29	1.13	0.28		11.88	3.92	0.37	2.09	0.30	2.57	0.45	3.47
			0.47	0.17	0.37	0.39	0.55	0.34	0.79	1.42	749	42.72			0.41	0.22	
817	R255E	a	1.80	0.13	1.38	0.31	0.95	0.28	89.36	11.89	0.72	0.36	1.50	0.30	1.16	0.41	0.76
818	R255Y	a	3.21	0.28	5.87	0.31	2.30	0.23		11.88	34.11	0.39	2.07	0.34	12.88	0.52	14.80
			0.38	0.23	0.35	0.33	0.33	0.46	0.79	0.75	0.43	1.32			0.36	0.21	1.33
819	E258S	a	2.79	0.30	1.13	0.34	1.39	0.27	40.92	11.92	0.65	0.56	1.36	0.32	0.54	0.52	0.47
820	E258H	a	2.62	0.23	2.92	0.40	1.77	0.29	48.21	11.89	11.08	0.58	1.69	0.28	0.89	0.39	6.25
821	E258Y	a	2.62	0.18	1.28	0.36	2.12	0.27		11.88	10.52	0.43	1.66	0.29	1.07	0.49	4.96
			0.56	0.27	0.30	0.24	0.39	0.30	0.95	0.66	0.36	1.31			0.31	0.28	0.91
822	T260D	a	1.79	0.16	0.58	0.32	0.65	0.29			0.31	0.37	4.06	0.32	1.71	0.53	0.47
823	T260E	a	2.21	0.14	1.00	0.31	1.21	0.44	94.92	11.88	1.70	0.41	2.89	0.34	5.49	0.61	1.41
824	T260H	a	2.03	0.20	1.85	0.34	1.70	0.29		11.88	11.25	0.43	1.63	0.28	7.30	0.53	6.61
			0.52	0.16	0.27	0.19	0.16	0.93	0.50	0.77	0.53	1.32			0.50	0.16	3.40
825	T260Y	a	1.59	0.16	0.84	0.37	1.03	0.27		11.88	0.48	0.38	1.62	0.27	7.51	0.93	0.47
			0.38	0.32	0.59	0.29	0.51	0.26	1.08	0.65	1.40	1.31			0.69	0.18	2.73
826	V262E	a	1.03	0.34	0.16	0.43	0.92	0.24	36.47	11.91			2.85	0.29	9.27	0.52	
827	V262F	a	1.04	0.15	0.43	0.28	1.23	0.26					5.20	0.39	0.50	0.38	

Figure 4 (continued)

Variant	Substitution(s)	Context	FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
			Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
828	F275L	a	1.85	0.15	0.83	0.31	1.43	0.31	97.71	11.88	1.17	0.38	3.58	0.37	0.89	0.35	0.82
829	G281D	ap	2.19	0.17	1.81	0.29	2.15	0.23		11.88	4.55	0.38	6.23	0.38	1.33	0.36	2.11
830	G281K	a	1.25	0.15	1.01	0.26	1.20	0.29		11.88	0.79	0.39	1.92	0.30	0.95	0.35	0.66
831	G281Y	a	1.89	0.14	2.08	0.52	2.47	0.34		11.88	0.89	0.39	2.87	0.35	1.46	0.35	0.36
			0.42	0.18	0.80	0.21	0.73	0.27	1.17	0.64	1.31	1.31			0.69	0.14	1.79
832	G281P	a															
833	V282E	a	1.89	0.23	1.10	0.33	1.43	0.28	85.43	11.88	0.49	0.36	5.37	0.30	1.91	0.40	0.34
834	V282K	a	1.50	0.23	0.58	0.31	0.65	0.28	82.37	11.89	0.54	0.39	2.23	0.37	1.44	0.44	0.83
			0.40	0.20	0.85	0.19	1.26	0.23	1.76	0.64	2.97	1.31			1.11	0.17	2.37
835	V282Y	a	2.34	0.26	1.43	0.29	1.41	0.24		11.88	1.10	0.37	2.38	0.36	1.92	0.37	0.78
			0.37	0.16	0.53	0.22	0.49	0.40	0.86	0.72	0.89	1.32			0.65	0.18	1.83
836	V282P	a															
837	V282G	ap	1.41	0.19	1.58	0.18	1.12	0.39	1.08	0.70	0.98	0.26	8.93	0.45	1.16	0.18	0.87
838	E283K	a	2.47	0.14	1.20	0.16	2.81	0.28	3.63	0.64	3.26	0.22	1.25	0.35	2.74	0.94	1.16
839	E283H	a	4.36	0.41	3.56	0.24	3.85	0.36	2.13	0.71	27.06	0.35	3.62	0.40	6.25	1.29	7.04
			0.78	0.16	0.50	0.41	0.32	0.39	0.66	1.13		28.71			1.18	0.16	
840	E283L	a	1.44	0.15	4.67	0.12	6.05	0.28	9.30	0.65	22.32	0.26	3.95	0.40	9.25	0.69	3.69
			0.42	0.24	0.34	0.34	0.67	0.46	1.06	0.79	0.52	1.32			0.82	0.20	0.78
841	E283Y	a	1.68	0.16	1.19	0.15	1.51	0.30	0.85	0.71	1.43	0.23	2.35	0.49	1.42	0.68	0.94
842	E283P	a	1.56	0.18	0.95	0.23	2.51	0.32	1.14	0.71	1.65	0.26	3.62	0.50	1.33	0.41	0.66
843	E283G	a	0.47	0.11	0.33	0.24	0.45	0.27	0.89	0.68	0.43	1.31			0.77	0.20	0.97
844	V284E	a	1.45	0.17	5.92	0.13	4.86	0.30	2.89	0.66	18.48	0.28	2.72	0.57	25.76	0.15	3.80
			0.59	0.10	0.41	0.22	0.57	0.61	0.97	0.67	0.89	1.32			1.11	0.14	1.55
845	V284N	a	0.45	0.29	0.43	0.27	0.60	0.28	1.13	0.66	0.73	1.32			0.77	0.17	1.21
846	V284T	ap	0.25	0.18	0.41	0.17	1.26	0.47	1.08	0.67	1.16	0.23	4.49	0.57	1.15	0.16	0.92
			0.37	0.11	0.45	0.19	0.53	0.34	0.95	0.68	0.78	1.31			0.79	0.16	1.47
847	V284L	a	1.56	0.16	1.90	0.16	3.13	0.32	1.32	0.70	2.71	0.23	2.56	0.48	1.51	0.20	0.87
848	V284Y	a															
849	P291D	a	1.11	0.18	1.19	0.14	1.07	0.32	1.89	0.65	0.72	0.22	1.06	0.37	0.89	0.86	0.67
850	P291E	a	1.82	0.28	1.00	0.30	1.24	0.44	0.68	0.98	0.92	0.28	1.08	0.44	0.76	0.94	0.74
851	P291Q	a	1.06	0.17	1.40	0.16	1.81	0.32	1.33	0.65	0.80	0.31	1.26	0.40	1.24	0.51	0.44
852	P291T	a	0.55	0.18	0.49	0.14	1.00	0.34	0.67	0.73	0.53	0.25	1.44	0.40	0.94	0.44	0.53
853	P291H	a	1.85	0.18	2.66	0.23	1.47	0.29	1.35	0.73	2.40	0.28	0.75	0.39	2.56	0.57	1.63
854	P291I	a	1.24	0.25	1.20	0.13	1.19	0.34	2.15	0.67	0.52	0.25	1.30	0.41	1.15	0.17	0.44
855	P291G	a	0.08	0.29	0.35	0.22	1.26	0.37	0.68	0.75	0.31	0.24	2.19	0.41	0.81	0.18	0.24
			0.46	0.17	0.64	0.20	0.98	0.26	1.66	0.65	1.14	1.31			0.78	0.14	1.17
856	N297Q	a															
857	N297K	a															
858	N297R	a	0.01	0.20	0.01	0.80	0.01	2.77	0.06	1.66	0.01	3.89			0.45	0.17	1.03
859	N297T	a															
860	N297H	a															
861	N297V	a															
862	N297L	a															
863	N297I	a															
864	N297F	a															
865	N297M	a															
866	N297Y	a															
867	N297W	a															
868	N297P	a															
869	N297G	a															
870	R301D	a	0.87	0.16	0.11	0.60	0.06	2.49	0.04	2.89	0.03	1.45	1.58	0.43	0.50	0.24	0.47
871	R301E	a	0.62	0.23	0.36	0.15	0.84	0.43	0.44	0.73	0.69	0.24	2.71	0.49	2.92	0.99	0.82
872	R301H	a	1.65	0.25	0.37	0.24	0.97	0.64	0.74	0.93	0.41	0.30	1.58	0.48	0.76	0.98	0.42
873	R301Y	a	0.72	0.15	0.64	0.17	1.27	0.35	0.95	0.68	0.17	0.31	1.49	0.44	0.78	0.51	0.13
874	V303D	a	0.69	0.18	0.67	0.15	0.55	0.64	0.53	0.75	0.29	0.30	1.45	0.45	0.91	0.33	0.52
875	V303E	a	2.29	0.23	1.02	0.19	1.41	0.28	1.31	0.68	1.43	0.25	1.43	0.40	1.63	0.35	1.02
876	V303Y	a	0.78	0.18	2.56	0.21	1.43	0.33	1.26	0.73	0.44	0.25	1.02	0.42	1.15	0.16	0.31
877	S304D	a	1.12	0.19	0.53	0.29	1.17	0.57			0.14	0.28	2.17	0.52	0.61	0.20	0.12
878	S304N	a	0.95	0.18	0.69	0.19	0.81	0.42	0.90	0.90	1.08	0.25	1.32	0.41	1.19	0.19	1.34
879	S304T	a	1.00	0.15	2.86	0.18	1.61	0.31	0.73	0.80	6.13	0.21	3.30	0.48	2.86	1.08	3.80
880	S304H	a	1.11	0.29	0.32	0.31	0.79	0.97	1.65	15.76	0.18	0.28	1.59	0.50	0.38	0.97	0.23
881	S304L	a															
882	V305E	a	0.89	0.16	0.90	0.16	1.45	0.31	1.14	0.76	0.65	0.24	1.99	0.49	0.67	0.51	0.45
883	V305T	a	1.17	0.22	0.91	0.16	1.23	0.33	0.07	1.74	0.68	0.25					0.55
884	V305Y	a	1.59	0.22	0.98	0.24	0.95	0.31	0.22	1.23	0.66	0.30	1.12	0.49	0.74	0.30	0.70
885	K317E	a	0.85	0.18	0.79	0.22	0.48	0.55	0.29	0.95	0.39	0.72			0.45	0.56	0.81
886	K317Q	a	1.22	0.10			0.98	0.42			1.25	0.17					1.27
887	E318Q	a	0.62	0.14	0.80	0.18	0.35	0.37	0.72	0.33	0.40	0.70	0.99	0.55	0.56	0.66	1.17
888	E318H	a	0.49	0.15	0.46	0.35	0.38	0.45			0.26	1.71			0.61	0.21	0.69
889	E318L	a	1.05	0.37	0.67	0.23	0.18	1.28	0.87	0.45	0.38	0.71			3.61	1.60	2.12
890	E318Y	a	0.67	0.16	0.63	0.14	0.22	0.83	0.51	0.80	0.25	0.69			6.14	1.63	1.13
891	I336E	a	0.36	0.27	0.08	0.77	0.05	0.95	0.17	1.59	0.06	0.82	1.07	0.52	8.11	1.03	1.10
			0.70	0.44	0.38	0.29	0.34	0.59	1.02	0.86	1.79	1.33			0.64	0.21	5.20

Figure 4 (continued)

			FcγRI		FcγRIIa		FcγRIIb		FcγRIIc		FcγRIIIa		C1q		FcRn		
Variant	Substitution(s)	Context	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	Fold	Conf	IIIa:IIb
892	I336K	a	1.11	0.21	0.94	0.20	1.05	1.26	0.47	1.27	0.73	0.71	1.13	0.40	12.12	0.94	0.69
893	I336Y	a	0.45	0.15	0.28	0.19	0.59	0.50	0.64	0.68	0.21	0.71			0.49	0.50	0.36
894	S337E	a	0.71	0.17	1.15	0.18	1.13	0.27	0.95	0.24	0.74	0.69			0.39	0.47	0.65
895	S337N	a	0.77	0.20	1.45	0.24	1.50	0.37	0.93	0.50	0.58	0.75			0.52	0.48	0.39
896	S337H	a	0.66	0.14	0.78	0.14	0.64	0.29	0.85	0.25	0.35	0.69			0.56	0.51	0.55
912	S239D/E272Y/I332E	tr	1.98	0.18	3.33	0.30	12.09	0.30	9.89	0.23	43.67	0.12	0.88	0.96	1.31	0.27	3.61
913	S239D/E272S/I332E	tr	1.23	0.09	3.11	0.24	14.47	0.29	12.02	0.23	18.57	0.11	0.87	1.07	1.49	0.25	1.28
914	S239D/E272K/I332E	tr															
915	S239D/E272I/I332E	tr	3.23	0.07	7.99	0.24	22.11	0.30	18.91	0.23	64.80	0.10	1.07	0.75	1.81	0.23	2.93
916	S239D/E272Y/A330L/I332E	tr	1.83	0.12	0.89	0.28	2.92	0.34	3.04	0.23	46.36	0.12			1.00	0.25	15.90
917	S239D/E272S/A330L/I332E	tr															
918	S239D/E272K/A330L/I332E	tr															
919	S239D/E272I/A330L/I332E	tr	1.83	0.09	2.34	0.30	11.04	0.32	9.15	0.23	70.29	0.10	0.88	0.75	0.98	0.27	6.37
920	S239D/K274E/I332E	tr	3.20	0.09	3.66	0.31	26.79	0.34	14.88	0.23	86.88	0.10	1.23	0.70	0.60	0.27	3.24
921	S239D/Y278T/I332E	tr	0.04	0.15	0.26	16.94	1.36	1.57	0.75	0.38	0.60	0.14	1.12	0.74	0.21	0.51	0.44
922	S239D/K326T/I332E	trp	5.76	0.13	20.99	0.23	122	0.31	78.15	0.22	332	0.11	0.70	1.34	2.22	0.26	2.71
923	S239D/K326E/I332E	trp	2.87	0.18	4.08	0.29	59.31	0.29	35.60	0.22	184	0.11	0.89	1.20	0.64	0.27	3.10
924	S239D/K274E/A330L/I332E	tr	1.94	0.10	1.26	0.29	10.27	0.29	7.71	0.22	84.23	0.10	0.92	2.08	0.72	0.30	8.20
925	S239D/Y278T/A330L/I332E	tr															
926	S239D/K326E/A330L/I332E	tr															
927	S267E	r															
928	S239D/S267E/I332E	r															
929	S239D/S267E/A330L/I332E	r															
930	Y278W	r															
931	E283R/V302I/Y278W/E283R	r															
934	Y278W/V302I	r															
935	Y278W/E283R/V302I	r															
1145	S239D/I332E/G236S	t															
1146	S239D/I332E/G236A	t															
1147	S239D/I332E/K246H	t															
1148	S239D/I332E/R255Y	t															
1149	S239D/I332E/S267E	t															
1150	S239D/I332E/E272R	t															
1151	S239D/I332E/E272H	t															
1152	I332E/G281D	t															
1153	S239D/I332E/E283H	t															
1154	S239D/I332E/E283L	t															
1155	I332E/V284E	t															
1156	S239D/I332E/V284E	t															
1157	S267E/S324I	t															
1158	S267E/A327D	t															
1159	S324I/A327D	t															
1160	S267E/P331D	t															
1161	S267E/V282G	t															
1162	G281D/V282G	t															
1163	V282G/P331D	t															
1164	G281E	t															
1165	G281N	t															
1166	G281Q	t															
1167	V284D	t															
1168	V284Q	t															
1169	S298A/K326E	t															
1170	S298A/K334L	t															
1171	S298A/K326E/K334L	t															
1608	S239D/S298A/K326E/I332E	tp															
1609	S239D/S298A/K326T/I332E	t															
1877	I332E/H268E	p															
1878	I332E/H268D	p															
1879	S239D/H268E	p															
1880	S239D/H268D	p															
1881	S239D/I332E/H268E	p															
1882	S239D/I332E/H268D	p															
1883	S239D/I332E/A327D	p															
1884	S239D/I332E/V284D	p															
1885	S239D/I332E/V284E	p															

Figure 5

Variant	Context	AlphaScreen		SPR	ADCC
		V158 FcγRIIIa	F158 FcγRIIIa	V158 FcγRIIIa	
L235D	p	1.68	1.33		
G236S	t	2.78		1.34	0.37
G236S	a	6.22		6.69	
G236S	p	0.22	0.21		
G236A	a	0.31			
G236A	p	0.36	0.45		
S239E	t	29.99		4.17	7.60
S239E	a	2.64		3.28	
S239D	t	16.90		3.50	6.10
S239D	a	36.56		16.61	
S239D	p	32.84			
K246H	t	17.91		2.67	2.00
K246H	a	13.58		22.36	
K246H	p	0.75	0.71		
K246Y	t	17.44		2.39	1.36
K246Y	a	4.32		7.07	
R255Y	t	21.14		2.75	1.60
R255Y	a	0.92		1.41	
E258H	t	1.18		0.77	0.76
E258H	a	2.35		5.50	
E258Y	t	2.82		1.69	0.92
E258Y	a	0.64		1.77	
T260H	t	35.32		2.82	
T260H	a	1.00		1.86	
V264I	p	1.19	1.03		
S267E	a	9.33		2.62	
S267E	p				
S267D	p				
H268D	t	45.27		4.76	4.59
H268D	a	10.55		5.66	
H268D	a	3.31			
H268D	p	3.90	3.19		
H268E	a	4.91			
H268E	p	2.58	2.09		
E272I	t	5.86		1.63	1.38
E272I	t	3.24		1.99	
E272I	p	1.18	2.04		
E272R	a			1.38	
E272H	t	1.02		0.66	1.28
E272H	a	187.10		583.68	
E272H	p	0.85	2.35		
E272P	t	0.01		0.52	0.39
E272P	a	1.46		1.41	
G281D	p	1.14	1.55		
V282G	p	0.90	2.28		
E283H	t	0.99		0.71	1.40
E283H	a			2.31	
E283L	t	19.88		3.68	5.20
E283L	a	1.36		2.56	
V284E	t	2.82		1.26	0.84
V284E	a			1.51	
V284E	a	0.71			
E293R	t	1.15		0.94	0.47
E293R	p	1.01	1.97		
S298D	t	3.48		1.49	0.58
S304T	t	6.33		1.65	1.02
S304T	a			12.85	
S324G	t	3.04		1.76	3.23
S324G	a	13.62		14.17	
S324I	t	5.26		1.46	2.21
S324I	p	0.58	0.43		
K326E	t	6.12		2.12	2.87
K326E	a	1.86		3.13	
A327D	t	2.44		1.31	1.04
A327D	a	1.11			
A330L	p	0.57	0.75		
A330Y	p	1.35	1.29		
A330I	p	0.31	0.83		
I332E	p	10.52			3.55
I332E	p	15.65	14.10		
I332E	p	18.00	13.50		
I332E	p	13.90	11.20		
I332E	p	13.90	12.87		
I332D	t	19.00		2.57	5.00
I332D	a	21.65		11.16	
E333Y	t	8.24		1.94	2.23
K334I	t	15.24		7.10	1.20
K334T	t	15.73		6.79	3.14
K334F	t	10.46		5.82	1.92
D221K/I332E	p	16.57	15.65		
H224E/I332E	p	13.52	10.64		
P227G/I332E	p	13.52	10.15		
L234D/I332E	p	1.86	2.20		
L234E/I332E	p	3.48	5.24		
L234Y/I332E	p	16.01	12.24		

Variant	Context	AlphaScreen		SPR	ADCC
		V158 FcγRIIIa	F158 FcγRIIIa	V158 FcγRIIIa	
L234I/I332E	p	18.98	12.88		
L234G/I332E	p	1.17	1.08		
L235I/I332E	p	150.49	65.22		
L235S/I332E	p	13.39	7.95		
L235D/I332E	p	16.46	8.41		
L235E/I332E	p	15.74	10.11		
G236S/I332E	p	3.34	5.76		
G236A/I332E	p	12.26	18.32		
G236S/I332D	p	1.11	1.53		
G236A/I332D	p	1.81	3.94		
S239D/I332E	p	181.35			252.52
S239D/I332E	a	248.56			
S239D/I332E	p	197.31	146.91		
S239D/I332E	p	475.95	271.59		
S239D/I332E	p	146.04	92.08		
S239D/I332E	p	327.63	229.27		
S239D/H268E	p				
S239D/H268D	p	135.14			8.68
K246H/I332E	p	9.78	8.73		
R255Y/I332E	p	20.42	16.44		
E258H/I332E	p	391.08	101.46		
T260H/I332E	p	16.25	10.16		
V264I/I332E	a	2.66			
V264I/I332E	p	20.95	16.08		
S267E/I332E	p	4.66	4.19		
S267D/I332E	p	21.77	14.11		
S267D/I332E	p	26.95	22.93		
H268E/I332D	p	25.90	29.12		
H268D/I332D	p	25.68	28.26		
H268E/I332E	p	58.99			32.36
H268D/I332E	p				
E272R/I332E	p	4.79	4.66		
E272H/I332E	p				
E283H/I332E	p	11.55	10.07		
V284E/I332E	p	24.30	14.63		
E293R/I332E	p	7.44	7.42		
Q295E/I332E	p	9.72	6.91		
S304T/I332E	p	2.23	1.97		
S324I/I332E	p	8.17	3.53		
S324G/I332E	p	0.75	1.72		
S324I/I332D	p	3.41	4.98		
S324G/I332D	p				
A327D/I332E	p	0.13	0.36		
L328A/I332E	p	0.99	0.78		
L328T/I332E	p	17.48	18.25		
L328V/I332E	p	6.83	9.17		
L328I/I332E	p	25.70	19.59		
L328F/I332E	p	14.45	10.24		
L328Y/I332E	p	2.96	2.22		
L328M/I332E	p	2.67	1.96		
L328D/I332E	p	3.29	3.29		
L328E/I332E	p				
L328N/I332E	p				
L328Q/I332E	p	0.71	0.58		
L328A/I332D	p	0.29	0.38		
L328T/I332D	p				
L328V/I332D	p	1.59	1.01		
L328I/I332D	p	3.04	1.24		
L328F/I332D	p	0.48	2.02		
L328Y/I332D	p	0.75	1.15		
L328M/I332D	p	2.28	2.20		
L328D/I332D	p				
L328E/I332D	p				
L328N/I332D	p				
L328Q/I332D	p	1.54	1.81		
A330L/I332E	a	8.82			
A330L/I332E	p	2.13	1.47		
A330Y/I332E	a	3.92			
A330Y/I332E	p	16.09	16.48		
A330I/I332E	p				
T335D/I332E	p	17.92	19.78		
S239D/I332E/H268E	p	461.36	307.87		
S239D/I332E/H268D	p	590.49	377.89		
S239D/I332E/V284E	p				
S239D/I332E/A327D	p				
S239D/I332E/A330Y	p	309.25	293.30		
S239D/I332E/A330L	p	220.83	178.24		
S239D/I332E/A330I	p	115.65	123.50		
I332D/A330Y	p				
H268E/A330Y	p				
H268D/A330Y	p				
S239D/A330Y	p				
I332E/A330Y/H268E	p				
S239D/H268E/A330Y	p				
S239D/I332E/H268E/A330Y	p				

Figure 6

WT IgG1	EU	Amino Acid Modification(s)
D	221	K Y
K	222	E Y
T	223	E K
H	224	E Y
T	225	E K W
P	227	E G K Y
P	228	E G K Y
P	230	A E G Y
A	231	E G K P Y
P	232	E G K Y
E	233	A D F G H I K L M N Q R S T V W Y
L	234	A D E F G H K M N P Q R S T V W Y
L	235	A D E F G H I K M N P Q R S T V W Y
G	236	A D E F H I K L M N P Q R S T V W Y
G	237	D E F H I K L M N P Q R S T V W Y
P	238	D E F G H I K L M N Q R S T V W Y
S	239	D E F G H I K L M N P Q R T V W Y
V	240	A T M T
F	241	D E L R S W Y
F	243	E H L Q R W Y
P	244	H
P	245	A
K	246	D E H Y
P	247	G V
D	249	H Q Y
R	255	E Y
E	258	H S Y
T	260	D E H Y
V	262	A E F I T
V	263	A I M T
V	264	A D E F G H K L M N P Q R S T W Y
D	265	F G H I K L M N P Q R S T V W Y
V	266	A I M T
S	267	D E F H I K L M N P Q R T V W Y
H	268	D E F G I K L M P Q R T V W
E	269	F G H I K L M N P R S T V W Y
D	270	F G H I L M P Q R S T W Y
P	271	A D E F G H I K L M N Q R S T V W Y
E	272	D F G H K L M P R S T V W Y
V	273	I
K	274	D E F G H I L M N P R T V W Y
F	275	L W
N	276	D E F G H I L M P R S T V W Y
Y	278	D E G H I K L M N P Q R S T V W

WT IgG1	EU	Amino Acid Modification(s)
D	280	G K L P W
G	281	D E K N P Q Y
V	282	E G K P Y
E	283	G H K L P R Y
V	284	D E L N Q T Y
H	285	D E K Q W Y
N	286	E G P Y
K	288	D E Y
K	290	D H L N W
P	291	D E G H I Q T
R	292	D E T Y
E	293	F G H I L M N P R S T V W Y
E	294	F G H I K L M P R S T V W Y
Q	295	D E F G H I M N P R S T V W Y
Y	296	A D E G H I K L M N Q R S T V
N	297	D E F G H I K L M P Q R S T V W Y
S	298	A D E F H I K M N Q R T W Y
T	299	A D E F G H I K L M N P Q R S V W Y
Y	300	A D E G H K M N P Q R S T V W
R	301	D E H Y
V	302	I
V	303	D E Y
S	304	D H L N T
V	305	E T Y
W	313	F
K	317	E Q
E	318	H L Q R Y
K	320	D F G H I L N P S T V W Y
K	322	D F G H I P S T V W Y
V	323	I
S	324	D F G H L M P R T V W Y
N	325	A D E F G H I K L M P Q R S T V W Y
K	326	E I L P T
A	327	D E F H I K L M N P R S T V W Y
L	328	A D E F G H K M N P Q R S T V W Y
P	329	D E F G H I K L M N Q R S T V W Y
A	330	E F G H L M N P R S T V W Y
P	331	D F H I L M Q R T V W Y
I	332	A D E F H K L M N P Q R S T V W Y
E	333	A F H I L M P T Y
K	334	A E L P T
T	335	D F G H I L M N P R S V W Y
I	336	E K Y
S	337	E H N

Figure 7a

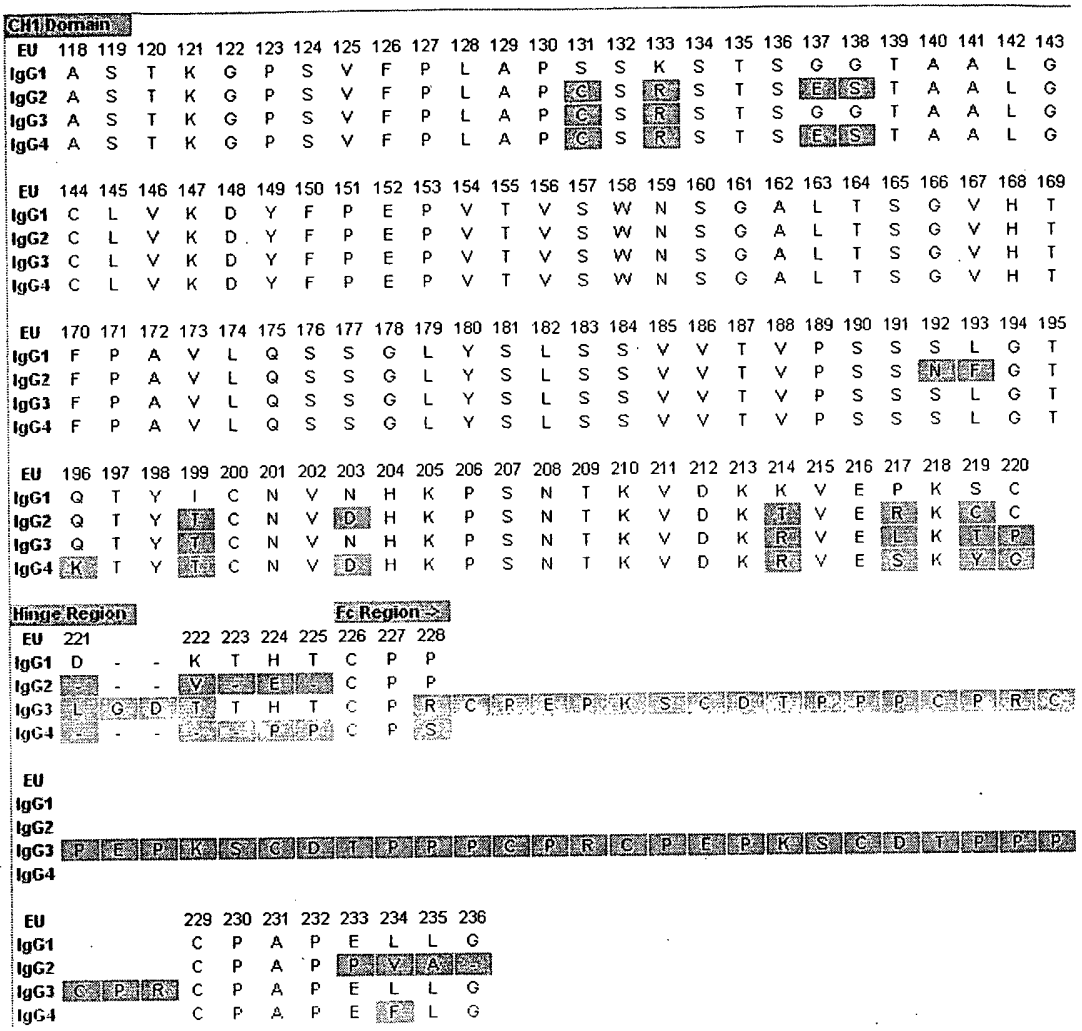
CH1 Domain
EU 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143
IgG1 A S T K G P S V F P L A P S S K S T S G G T A A L G
IgG2 A S T K G P S V F P L A P C S R S T S E S T A A L G
IgG3 A S T K G P S V F P L A P C S R S T S G G T A A L G
IgG4 A S T K G P S V F P L A P C S R S T S E S T A A L G
EU 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169
IgG1 C L V K D Y F P E P V T V S W N S G A L T S G V H T
IgG2 C L V K D Y F P E P V T V S W N S G A L T S G V H T
IgG3 C L V K D Y F P E P V T V S W N S G A L T S G V H T
IgG4 C L V K D Y F P E P V T V S W N S G A L T S G V H T
EU 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
IgG1 F P A V L Q S S G L Y S L S S V V T V P S S S L G T
IgG2 F P A V L Q S S G L Y S L S S V V T V P S S N F G T
IgG3 F P A V L Q S S G L Y S L S S V V T V P S S S L G T
IgG4 F P A V L Q S S G L Y S L S S V V T V P S S S L G T
EU 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220
IgG1 Q T Y I C N V N H K P S N T K V D K K V E P K S C
IgG2 Q T Y T C N V D H K P S N T K V D K T V E R K C C
IgG3 Q T Y T C N V N H K P S N T K V D K R V E L K T P
IgG4 K T Y T C N V D H K P S N T K V D K R V E S K Y G
Hinge Region
Fc Region >
EU 221 222 223 224 225 226 227 228
IgG1 D - - K T H T C P P
IgG2 - - - V - E - C P P
IgG3 L G D T T H T C P R C P E P K S C D T P P P C P R C
IgG4 - - - S - P P C P S
EU
IgG1
IgG2
IgG3 P E P K S C D T P P P C P R C P E P K S C D T P P P
IgG4
EU 229 230 231 232 233 234 235 236
IgG1 C P A P E L L G
IgG2 C P A P P V A P
IgG3 C P R C P A P E L L G
IgG4 C P A P E F L G

Figure 7b

CH2 Domain

EU	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263
IgG1	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E	V	T	C	V	V
IgG2	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E	V	T	C	V	V
IgG3	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E	V	T	C	V	V
IgG4	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E	V	T	C	V	V

EU	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290
IgG1	V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y	V	D	G	V	E	V	H	N	A	K	T	K
IgG2	V	D	V	S	H	E	D	P	E	V	Q	F	N	W	Y	V	D	G	V	E	V	H	N	A	K	T	K
IgG3	V	D	V	S	H	E	D	P	E	V	Q	F	K	W	Y	V	D	G	V	E	V	H	N	A	K	T	K
IgG4	V	D	V	S	Q	E	D	P	E	V	Q	F	N	W	Y	V	D	G	V	E	V	H	N	A	K	T	K

EU	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317
IgG1	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K
IgG2	P	R	E	E	Q	F	N	S	T	F	R	V	V	S	V	L	T	V	V	H	Q	D	W	L	N	G	K
IgG3	P	R	E	E	Q	Y	N	S	T	F	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K
IgG4	P	R	E	E	Q	F	N	S	T	Y	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K

EU	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340
IgG1	E	Y	K	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K
IgG2	E	Y	K	C	K	V	S	N	K	G	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG3	E	Y	K	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG4	E	Y	K	C	K	V	S	N	K	G	L	P	S	S	I	E	K	T	I	S	K	A	K

CH3 Domain

EU	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367
IgG1	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N	Q	V	S	L	T	C
IgG2	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C
IgG3	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C
IgG4	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	Q	E	E	M	T	K	N	Q	V	S	L	T	C

EU	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394
IgG1	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T
IgG2	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T
IgG3	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	S	G	Q	P	E	N	N	Y	N	T	T
IgG4	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T

EU	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421
IgG1	P	P	V	L	D	S	D	G	S	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N
IgG2	P	P	M	L	D	S	D	G	S	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N
IgG3	P	P	M	L	D	S	D	G	S	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N
IgG4	P	P	V	L	D	S	D	G	S	F	F	L	Y	S	R	L	T	V	D	K	S	R	W	Q	E	G	N

EU	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447
IgG1	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	K
IgG2	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	K
IgG3	S	F	S	C	S	V	M	H	E	A	L	H	N	R	F	T	Q	K	S	L	S	L	S	P	G	K
IgG4	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	L	G	K

Figure 8

Name	Position	IgG1	IgG2	IgG3	IgG4
Allotypes					
G1m(1)	356	D	E	E	E
	358	L	M	M	M
G1m(2)	431	G	A	A	A
G1m(3)	214	R	T	R	R
G1m(17)	214	K	T	R	R
Isoallotypes					
nG1m(1)	356	E	E	E	E
	358	M	M	M	M
nG1m(2)	431	A	A	A	A
nG1m(17)	214	R	T	R	R

Figure 9a

Figure 9b

Figure 10a

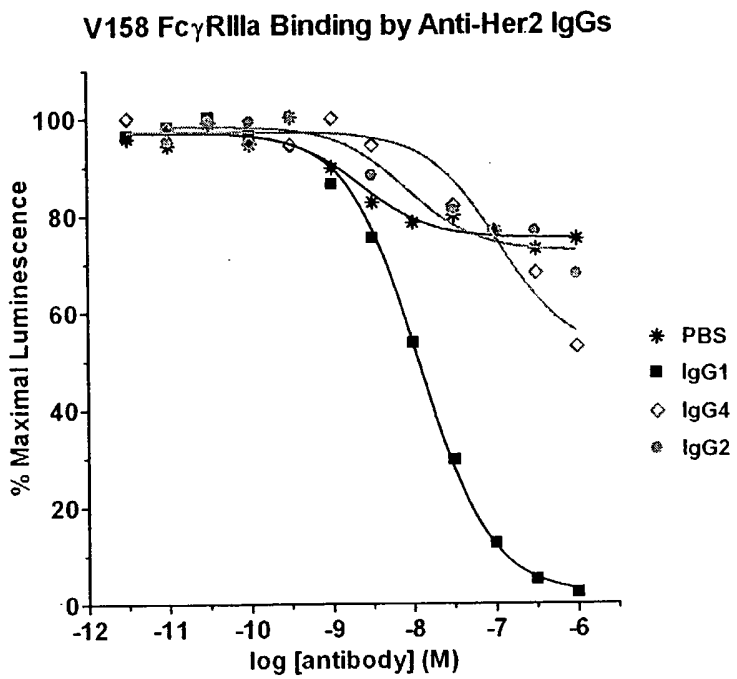

Figure 10b

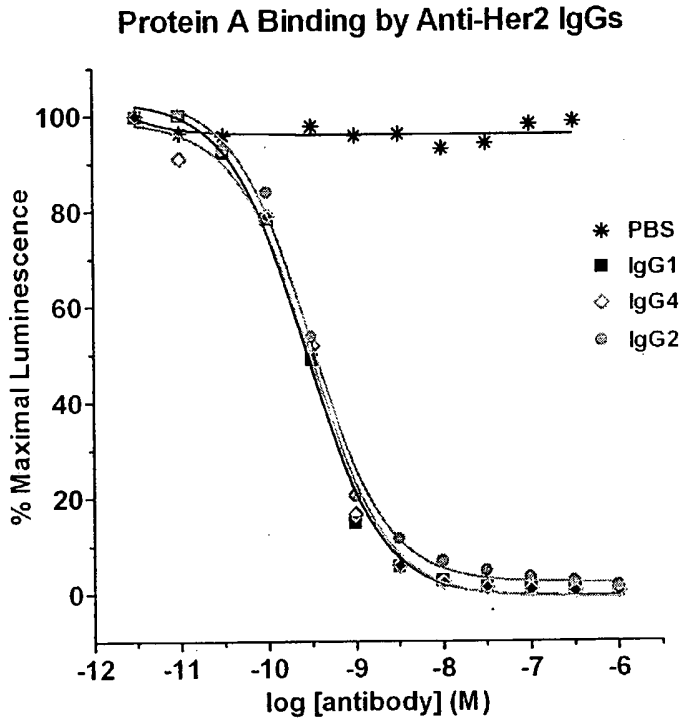

Figure 11a

V158 FcγRIIIa Binding by Anti-Her2 IgG Variants

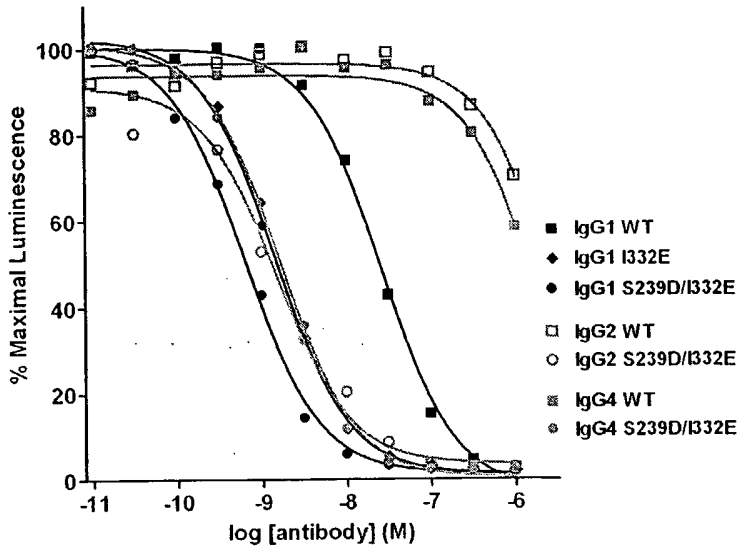

Figure 11b

FcγRI Binding by Anti-Her2 IgG Variants

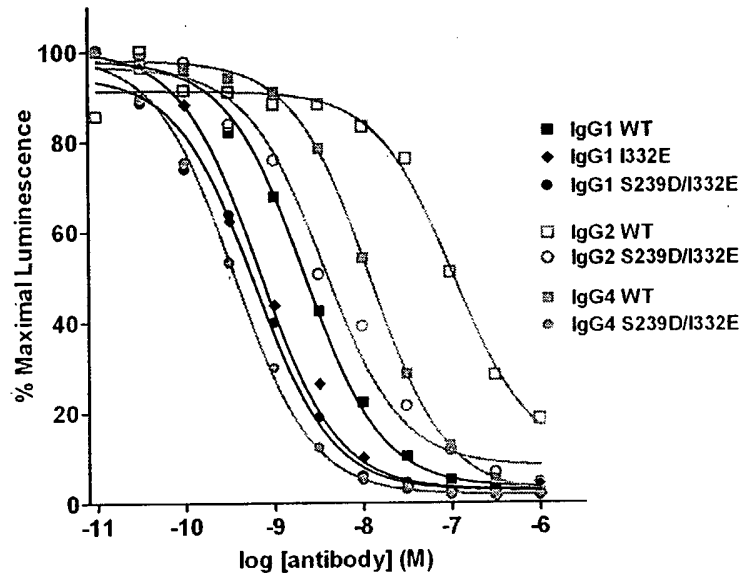

Figure 12.

V158 FcγRIIIa Binding by Anti-Her2 IgG Variants

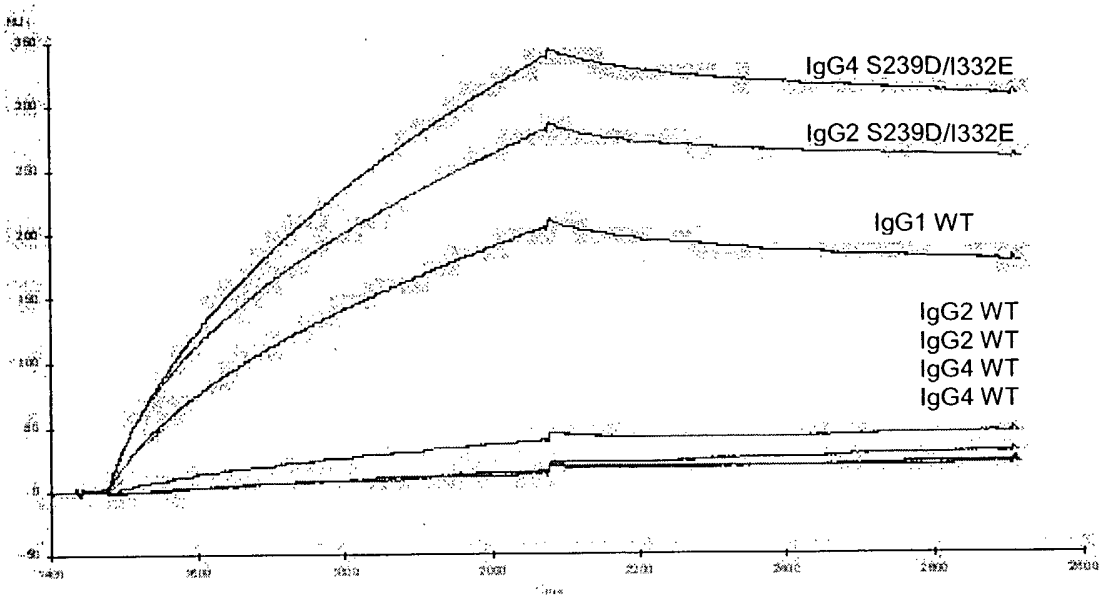

Figure 13a

CH1 Domain

EU	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143
IgG1	A	S	T	K	G	P	S	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L	G
IgG2														C		R				E	S					
IgG3														C		R										
IgG4														C		R				E	S					

EU	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169
IgG1	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G	A	L	T	S	G	V	H	T
IgG2																										
IgG3																										
IgG4																										

EU	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195
IgG1	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T
IgG2																							N	F		
IgG3																										
IgG4																										

EU	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220
IgG1	Q	T	Y	I	C	N	V	N	H	K	P	S	N	T	K	V	D	K	K	V	E	P	K	S	C
IgG2				T				D											T			R		C	
IgG3				T															R			L		T	P
IgG4	K			T				D											R			S		Y	G

Hinge Region → Fc Region →

EU	221			222	223	224	225	226	227	228	229	230	231	232	233	234	235	236
IgG1	D	-	-	K	T	H	T	C	P	P	C	P	A	P	E	L	L	G
IgG2	-			V	-	E	-								P	V	A	-
IgG3	L	G	D	T						R								
IgG4	-			-	-	P	P			S						F		
Novel	K								G							Y	Y	S
Novel																I	I	A
Novel																	D	

Figure 13b

CH2 Domain

EU	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263
IgG1	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E	V	T	C	V	V
IgG2																											
IgG3																											
IgG4																											
Novel	D		D	I						H									Y			H		H			
Novel			E	M						Y												Y					
Novel			N																								
Novel			Q																								
Novel			T																								

EU	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290
IgG1	V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y	V	D	G	V	E	V	H	N	A	K	T	K
IgG2											Q																
IgG3											Q		K														
IgG4					Q						Q																
Novel	I			D	D			G	Y		E				T			D		L	E					N
Novel	T			E	E				H									E		H	D					
Novel	Y								R																		
Novel									I																		

EU	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317
IgG1	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K
IgG2						F				F									V								
IgG3										F																	
IgG4						F																					
Novel			R		E									T													

EU	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340
IgG1	E	Y	K	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K
IgG2										G												T	
IgG3																						T	
IgG4										G			S	S									
Novel							G		T	D	A		L		D	Y	F						
Novel							I				F		Y		E		I						
Novel											I		I		N		T						
Novel											T				Q								
Novel															T								

CH3 Domain

EU	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367
IgG1	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N	Q	V	S	L	T	C
IgG2																E		M									
IgG3																E		M									
IgG4															Q	E		M									

EU	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394
IgG1	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T
IgG2																											
IgG3																	S								N		
IgG4																											

EU	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421
IgG1	P	P	V	L	D	S	D	G	S	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N
IgG2			M																								
IgG3			M																								
IgG4															R										E		

EU	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447
IgG1	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	K
IgG2																										
IgG3	I													R	F											
IgG4																								L		

Figure 14a

CH1 Domain

EU	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143
IgG2	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S	T	A	A	L	G
IgG1														S		K				G	G					
IgG3																				G	G					
IgG4																										

EU	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169
IgG2	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G	A	L	T	S	G	V	H	T
IgG1																										
IgG3																										
IgG4																										

EU	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195
IgG2	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S	S	V	V	T	V	P	S	S	N	F	G	T
IgG1																							S	L		
IgG3																							S	L		
IgG4																							S	L		

EU	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220
IgG2	Q	T	Y	T	C	N	V	D	H	K	P	S	N	T	K	V	D	K	T	V	E	R	K	C	C
IgG1				I				N											K			P		S	
IgG3								N											R			L		T	P
IgG4	K																		R			S		Y	G

Hinge Region | Fc Region ->

EU	221			222	223	224	225	226	227	228	229	230	231	232	233	234	235	236
IgG2	-	-	-	V	-	E	-	C	P	P	C	P	A	P	P	V	A	-
IgG1	D			K	T	H	T								E	L	L	G
IgG3	L	G	D	T	T	H	T			R					E	L	L	G
IgG4				-		P	P			S					E	F	L	G
Novel	K								G							Y	Y	S
Novel																I	I	A
Novel																	D	

Figure 14b

CH2 Domain

EU	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263
IgG2	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E	V	T	C	V	V
IgG1																											
IgG3																											
IgG4																											
Novel	D		D	I						H									Y			H		H			
Novel			E	M						Y												Y					
Novel			N																								
Novel			Q																								
Novel			T																								

EU	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290
IgG2	V	D	V	S	H	E	D	P	E	V	Q	F	N	W	Y	V	D	G	V	E	V	H	N	A	K	T	K
IgG1											K																
IgG3													K														
IgG4					Q																						
Novel	I			D	D			G	Y		E				T			D		L	E					N
Novel	T			E	E				H									E		H	D						
Novel	Y								R																		
Novel									I																		

EU	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317
IgG2	P	R	E	E	Q	F	N	S	T	F	R	V	V	S	V	L	T	V	V	H	Q	D	W	L	N	G	K
IgG1						Y				Y									L								
IgG3						Y													L								
IgG4										Y									L								
Novel			R		E									T													

EU	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340
IgG2	E	Y	K	C	K	V	S	N	K	G	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG1										A												A	
IgG3										A													
IgG4													S	S								A	
Novel							G		T	D	A		L		D	Y	F						
Novel							I				F		Y		E		I						
Novel											I		I		N		T						
Novel											T				Q								
Novel															T								

CH3 Domain

EU	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367
IgG2	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C
IgG1																D		L									
IgG3																											
IgG4															Q												

EU	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394
IgG2	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T
IgG1																											
IgG3																	S								N		
IgG4																											

EU	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421
IgG2	P	P	M	L	D	S	D	G	S	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N
IgG1			V																								
IgG3																											
IgG4			V												R										E		

EU	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447
IgG2	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	K
IgG1																										
IgG3	I													R	F									L		
IgG4																										

Figure 15a

CH1 Domain

EU	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143
IgG3	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	G	G	T	A	A	L	G
IgG1														S		K										
IgG2																				E	S					
IgG4																				E	S					

EU	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169
IgG3	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G	A	L	T	S	G	V	H	T
IgG1																										
IgG2																										
IgG4																										

EU	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195
IgG3	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T
IgG1																										
IgG2																							N	F		
IgG4																										

EU	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220
IgG3	Q	T	Y	T	C	N	V	N	H	K	P	S	N	T	K	V	D	K	R	V	E	L	K	T	P
IgG1				I				D											K			P		S	C
IgG2								D											T			R		C	C
IgG4	K							D														S		Y	G

Hinge Region **Fc Region**

EU	221			222	223	224	225	226	227	228																
IgG3	L	G	D	T	T	H	T	C	P	R	C	P	E	P	K	S	C	D	T	P	P	P	C	P	R	C
IgG1	D	-	-	K						P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IgG2	-	-	-	V	-	E	-			P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IgG4	-	-	-	-	-	P	P			S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Novel	K								G																	

EU																										
IgG3	P	E	P	K	S	C	D	T	P	P	P	C	P	R	C	P	E	P	K	S	C	D	T	P	P	P
IgG1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IgG2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IgG4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

EU				229	230	231	232	233	234	235	236
IgG3	C	P	R	C	P	A	P	E	L	L	G
IgG1	-	-	-								
IgG2	-	-	-					P	V	A	-
IgG4	-	-	-						F		
Novel									Y	Y	S
Novel									I	I	A
Novel										D	

Figure 15b

CH2 Domain

EU	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263
IgG3	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E	V	T	C	V	V
IgG1																											
IgG2																											
IgG4																											
Novel	D		D	I						H									Y			H		H			
Novel			E	M						Y												Y					
Novel			N																								
Novel			Q																								
Novel			T																								

EU	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290
IgG3	V	D	V	S	H	E	D	P	E	V	Q	F	K	W	Y	V	D	G	V	E	V	H	N	A	K	T	K
IgG1											K		N														
IgG2													N														
IgG4					Q								N														
Novel	I			D	D			G	Y		E				T			D		L	E					N
Novel	T			E	E				H									E		H	D						
Novel	Y								R																		
Novel									I																		

EU	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317
IgG3	P	R	E	E	Q	Y	N	S	T	F	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K
IgG1										Y																	
IgG2						F													V								
IgG4						F				Y																	
Novel			R		E									T													

EU	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340
IgG3	E	Y	K	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG1																						A	
IgG2										G													
IgG4										G			S	S								A	
Novel							G		T	D	A		L		D	Y	F						
Novel							I				F		Y		E		I						
Novel											I		I		N		T						
Novel											T				Q								
Novel															T								

CH3 Domain

EU	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367
IgG3	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q	V	S	L	T	C
IgG1																D		L									
IgG2																											
IgG4															Q												

EU	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394
IgG3	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	S	G	Q	P	E	N	N	Y	N	T	T
IgG1																	N								K		
IgG2																	N								K		
IgG4																	N								K		

EU	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421
IgG3	P	P	M	L	D	S	D	G	S	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N
IgG1			V																								
IgG2																											
IgG4			V												R										E		

EU	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447
IgG3	I	F	S	C	S	V	M	H	E	A	L	H	N	R	F	T	Q	K	S	L	S	L	S	P	G	K
IgG1	V													H	Y											
IgG2	V													H	Y											
IgG4	V													H	Y									L		

Figure 16a

CH1 Domain

EU	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143
IgG4	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S	T	A	A	L	G
IgG1														S		K				G	G					
IgG2																										
IgG3																				G	G					

EU	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169
IgG4	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G	A	L	T	S	G	V	H	T
IgG1																										
IgG2																										
IgG3																										

EU	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195
IgG4	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T
IgG1																										
IgG2																							N	F		
IgG3																										

EU	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220
IgG4	K	T	Y	T	C	N	V	D	H	K	P	S	N	T	K	V	D	K	R	V	E	S	K	Y	G
IgG1	Q			I				N											K			P		S	C
IgG2	Q																		T			R		C	C
IgG3	Q							N														L		T	P

Hinge Region — Fc Region

EU	221			222	223	224	225	226	227	228	229	230	231	232	233	234	235	236
IgG4	-	-	-	-	-	P	P	C	P	S	C	P	A	P	E	F	L	G
IgG1	D			K	T	H	T			P						L		
IgG2				V		E	-			P					P	V	A	-
IgG3	L	G	D	T	T	H	T			R						L		
Novel	K								G							Y	Y	S
Novel																I	I	A
Novel																	D	

Figure 16b

CH2 Domain

EU	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263
IgG4	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E	V	T	C	V	V
IgG1																											
IgG2																											
IgG3																											
Novel	D		D	I						H									Y			H		H			
Novel			E	M						Y												Y					
Novel			N																								
Novel			Q																								
Novel			T																								

EU	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290
IgG4	V	D	V	S	Q	E	D	P	E	V	Q	F	N	W	Y	V	D	G	V	E	V	H	N	A	K	T	K
IgG1					H						K																
IgG2					H																						
IgG3					H								K														
Novel	I			D	D			G	Y		E				T			D		L	E					N
Novel	T			E	E				H									E		H	D						
Novel	Y								R																		
Novel									I																		

EU	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317
IgG4	P	R	E	E	Q	F	N	S	T	Y	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K
IgG1						Y																					
IgG2										F								V	V								
IgG3						Y				F																	
Novel			R		E									T													

EU	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340
IgG4	E	Y	K	C	K	V	S	N	K	G	L	P	S	S	I	E	K	T	I	S	K	A	K
IgG1										A			A	P									
IgG2													A	P								T	
IgG3										A			A	P								T	
Novel							G		T	D	A		L		D	Y	F						
Novel							I				F		Y		E		I						
Novel											I		I		N		T						
Novel											T				Q								
Novel															T								

CH3 Domain

EU	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367
IgG4	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	Q	E	E	M	T	K	N	Q	V	S	L	T	C
IgG1															R	D		L									
IgG2															R												
IgG3															R												

EU	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394
IgG4	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T
IgG1																											
IgG2																											
IgG3																	S								N		

EU	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421
IgG4	P	P	V	L	D	S	D	G	S	F	F	L	Y	S	R	L	T	V	D	K	S	R	W	Q	E	G	N
IgG1															K										Q		
IgG2			M												K										Q		
IgG3			M												K										Q		

EU	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447
IgG4	V	F	S	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	L	G	K
IgG1																								P		
IgG2																								P		
IgG3	I													R	F									P		

Figure 17

Anti-Her2 IgG2 Variants

Novel Modification(s)	Isotypic Modification(s)	VHVL	CH1	hinge	Fc	Constant Region
		trastuzumab	γ2	γ2	γ2	WT IgG2
		trastuzumab	γ2	γ2	γ2 ELLGG	IgG2 ELLGG
I332E	P233E/V234L/A235L/-236G	trastuzumab	γ2	γ2	γ2 ELLGG	IgG2 ELLGG
S239D/I332E	P233E/V234L/A235L/-236G	trastuzumab	γ2	γ2	γ2 ELLGG	IgG2 ELLGG
S239D/I332E/A330L	P233E/V234L/A235L/-236G	trastuzumab	γ2	γ2	γ2 ELLGG	IgG2 ELLGG
	G327A/P233E/V234L/A235L/-236G	trastuzumab	γ2	γ2	γ2 ELLGG	IgG2 ELLGG
S239D/I332E	G327A/P233E/V234L/A235L/-236G	trastuzumab	γ2	γ2	γ2 ELLGG	IgG2 ELLGG
	F296Y/P233E/V234L/A235L/-236G	trastuzumab	γ2	γ2	γ2 ELLGG	IgG2 ELLGG
	F300Y/P233E/V234L/A235L/-236G	trastuzumab	γ2	γ2	γ2 ELLGG	IgG2 ELLGG
	Q274K/P233E/V234L/A235L/-236G	trastuzumab	γ2	γ2	γ2 ELLGG	IgG2 ELLGG
	V309L/P233E/V234L/A235L/-236G	trastuzumab	γ2	γ2	γ2 ELLGG	IgG2 ELLGG
	T339A/P233E/V234L/A235L/-236G	trastuzumab	γ2	γ2	γ2 ELLGG	IgG2 ELLGG
	-221D/V222K/-223T/E224H/-225T/ P233E/V234L/A235L/-236G	trastuzumab	γ2	γ1	γ2 ELLGG	IgG2 ELLGG
	γ1(118-225)* / P233E/V234L/A235L/-236G	trastuzumab	γ1	γ1	γ2 ELLGG	IgG(1/2) ELLGG

* γ1(118-225) = C131S/R133K/E137G/S138G/N192S/F193L/T199I/D203N/T214K/R217P/C219S/ -221D/V222K/-223T/E224H/-225T

Figure 18

V158 FcγRIIIa Binding by Anti-Her2 IgG Variants

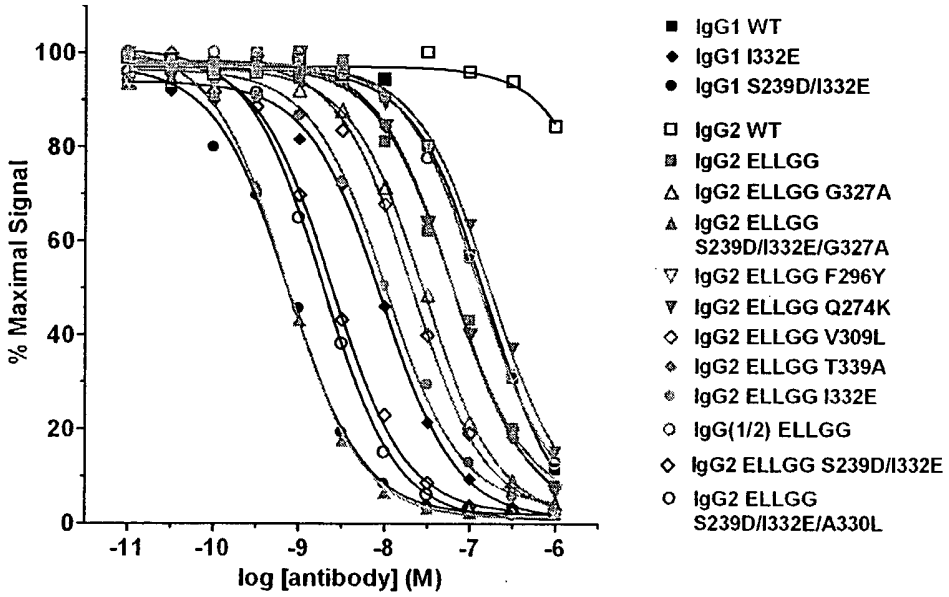

Figure 19

Anti-CD30 IgG(1/2) ELLGG Variants

Novel Modification(s)	Isotypic Modification(s)	Isotypic Modification(s)
	(All are IgG(1/2) ELLGG)	
	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/K246H	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/S267E	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/H268D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/H268E	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/S298A	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/S324G	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/K326T	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/A330Y	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/K334T	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/H268D/S324G	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/K326E/A330Y	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/K246H/T260H	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/S324I	γ1(118-225) / P233E/V234L/A235L/-236G	
	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/K246H	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/K246H/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/S267E	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/S267E/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/H268D	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/H268D/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/H268E	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/H268E/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/S298A	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/S298A/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/S324G	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/S324G/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/K326T	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/K326T/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/A330Y	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/A330Y/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/K334T	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/K334T/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/H268D/S324G	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/H268D/S324G/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/K326E/A330Y	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/K326E/A330Y/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/K246H/T260H	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/K246H/T260H/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/S324I	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/S324I/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/V284D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/V284E	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/M428L	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/T250Q/M428L	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/V284D	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/V284D/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/V284E	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/V284E/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	

Figure 20a

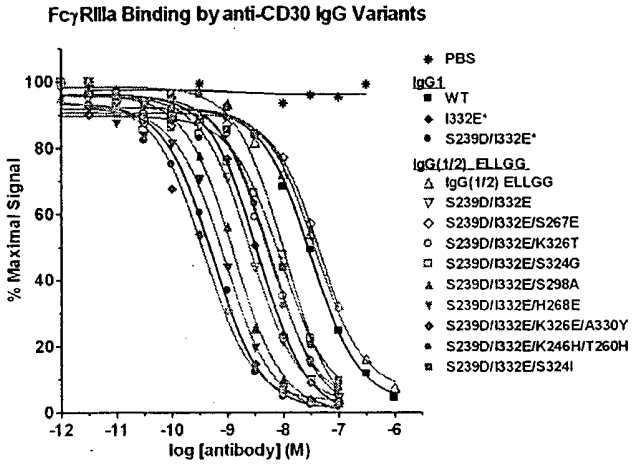

Figure 20b

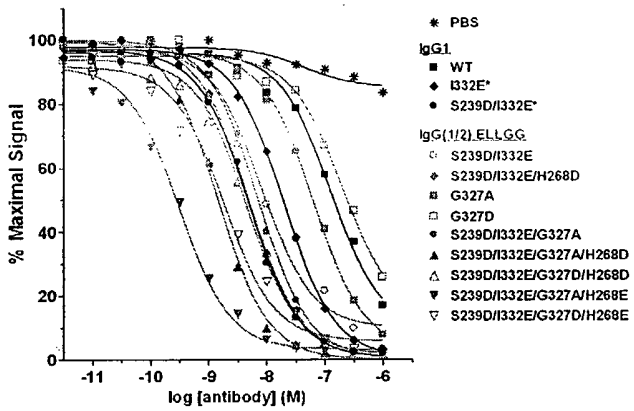

Figure 20c

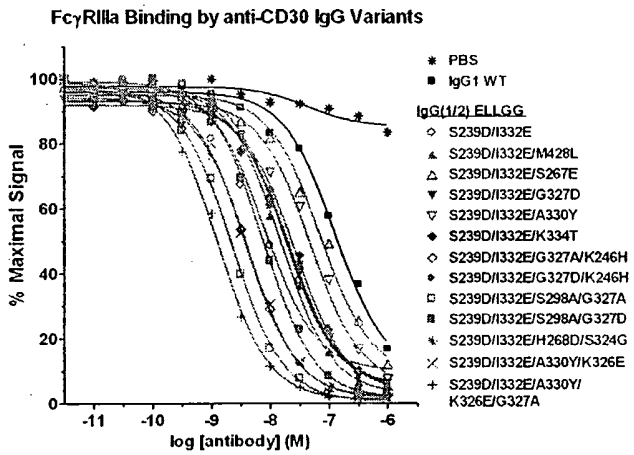

Figure 21

Amino Acid Modification	Variable Region	Constant Region	AlphaScreen IC50 (M)	AlphaScreen Fold V158 FcγRIIIa
None (WT IgG1)	H3.69_V2_L3.71 AC10	IgG1	1.3E-07	1.0
I332E	H3.69_L3.71 AC10	IgG1	1.9E-08	6.8
I332E	H3.69_L3.71 AC10	IgG1	3.56E-09	8.7
S239D/I332E	H3.69_L3.71 AC10	IgG1	5.1E-09	25.4
S239D/I332E	H3.69_L3.71 AC10	IgG1	5.31E-10	58.6
None (IgG(1/2) ELLGG)	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	3.8E-08	0.8
S239/I332E	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	8.1E-09	16.0
S239/I332E/K334T	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	2.8E-08	4.7
S239D/I332E/H268E	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	8.82E-10	35.3
S239/I332E/H268D	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	4.7E-09	27.4
S239D/I332E/A330Y	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	5.2E-08	2.5
S239/I332E/S267E	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	7.2E-08	1.8
S239/I332E/M428L	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	1.5E-08	8.4
S239D/I332E/K326T	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	5.26E-09	5.9
S239D/I332E/S324G	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	8.93E-09	3.5
S239D/I332E/S298A	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	1.28E-09	24.4
S239/I332E/H268D/S324G	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	2.1E-08	6.1
S239D/I332E/A330Y/K326E	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	4.0E-09	32.8
S239D/I332E/K246H/T260H	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	5.79E-09	5.4
S239D/I332E/K246H/S324I	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	1.07E-08	2.9
G327A	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	6.4E-08	2.0
G327D	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	1.9E-07	0.7
S239/I332E/G327A	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	6.9E-09	18.7
S239/I332E/G327D	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	1.7E-08	7.7
S239/I332E/G327A/H268E	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	3.9E-10	330.1
S239/I332E/G327D/H268E	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	2.1E-09	61.7
S239/I332E/G327A/H268D	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	1.4E-09	90.8
S239/I332E/G327D/H268D	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	4.9E-09	26.7
S239/I332E/S298A/G327A	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	2.4E-09	55.2
S239/I332E/S298A/G327D	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	7.6E-09	17.0
S239/I332E/G327A/K246H	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	4.4E-09	29.4
S239/I332E/G327D/K246H	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	2.2E-08	5.9
S239/I332E/A330Y/K326E/G327A	H3.69_V2_L3.71 AC10	IgG(1/2) ELLGG	1.3E-09	102.9

Figure 22a

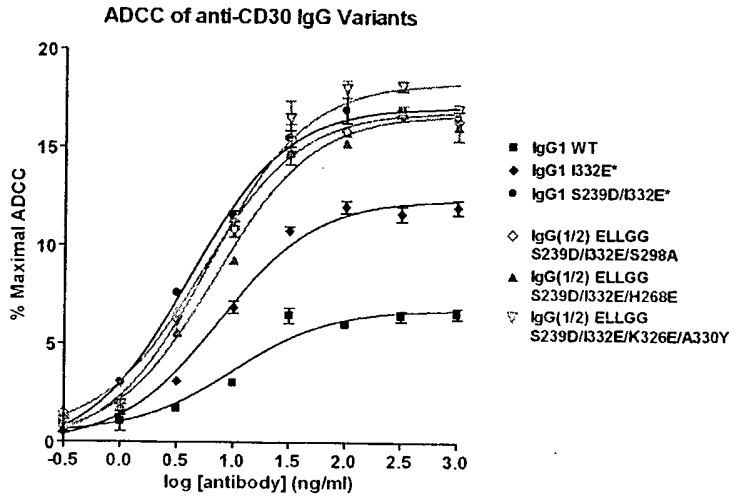

Figure 22b

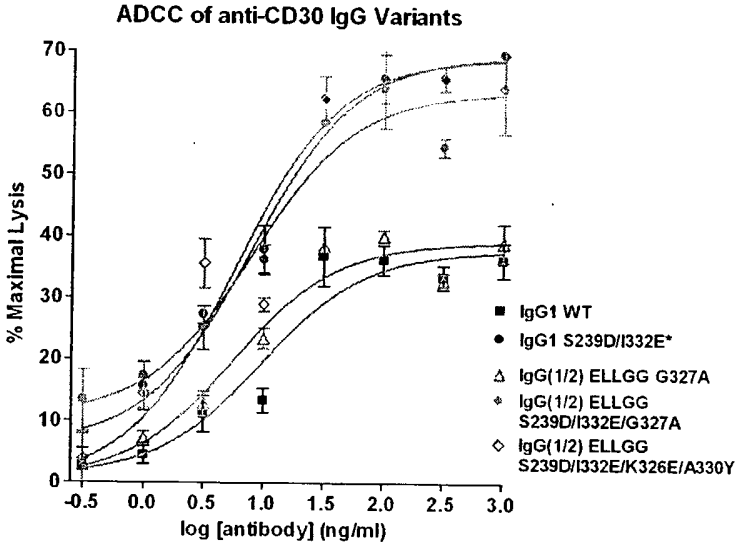

Figure 22c

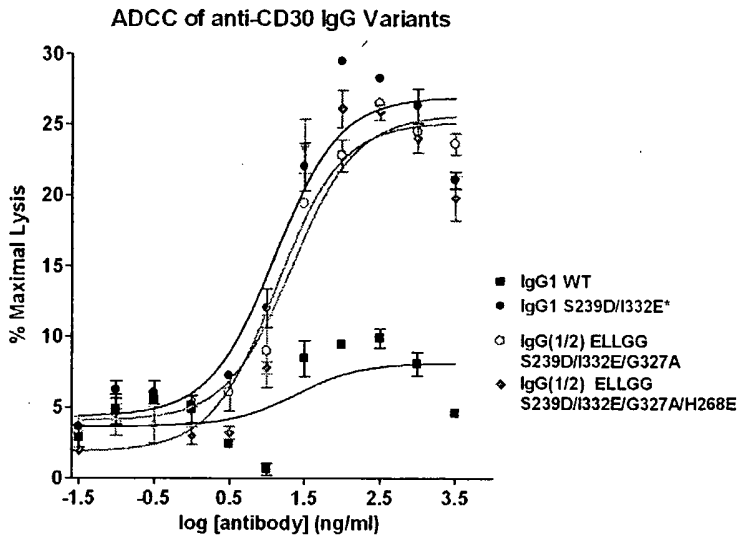

Figure 22d

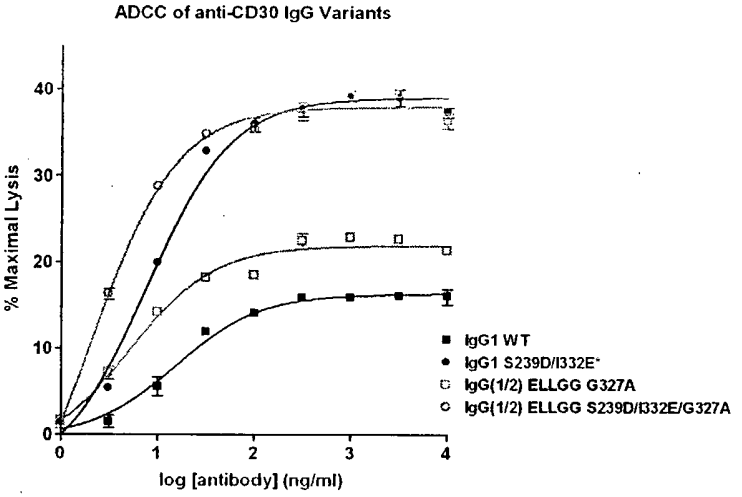

Figure 23

Anti-CD20 IgG(1/2) ELLGG Variants

Novel Modification(s)	Isotypic Modification(s)
	(All are IgG(1/2) ELLGG)
	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/K246H	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/S267E	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/H268D	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/H268E	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/S298A	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/S324G	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/K326T	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/G327D	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/A330Y	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/K334T	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/H268D/S324G	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/K326E/A330Y	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/K246H/T260H	γ1(118-225) / P233E/V234L/A235L/-236G
S239D/I332E/S324I	γ1(118-225) / P233E/V234L/A235L/-236G

Figure 24

ADCC of Anti-CD20 IgG Variants

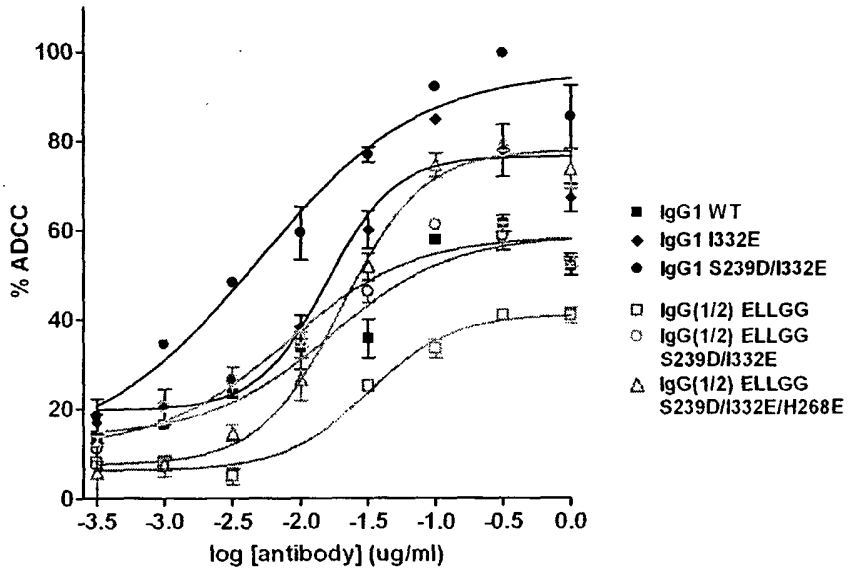

Figure 25

Anti-CD20 IgG(1/2) ELLGG Variants

Novel Modification(s)	Isotypic Modification(s)	Isotypic Modification(s)
	(All are IgG(1/2) ELLGG)	
	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/H268D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/H268E	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/V284D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/V284E	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/H268E/G327D	γ1(118-225) / P233E/V234L/A235L/-236G	
S239D/I332E/H268E	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/A330Y	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
I332E/A330Y/H268E	γ1(118-225) / P233E/V234L/A235L/-236G	G327A
S239D/I332E/H268E/A330Y	γ1(118-225) / P233E/V234L/A235L/-236G	G327A

Figure 26

V158 FcγRIIIa Binding by Anti-CD20 IgG Variants

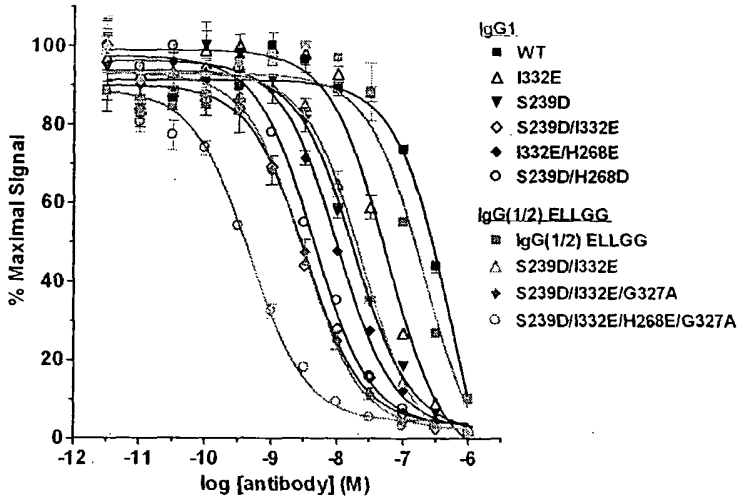

Figure 27

ADCC of Anti-CD20 IgG Variants

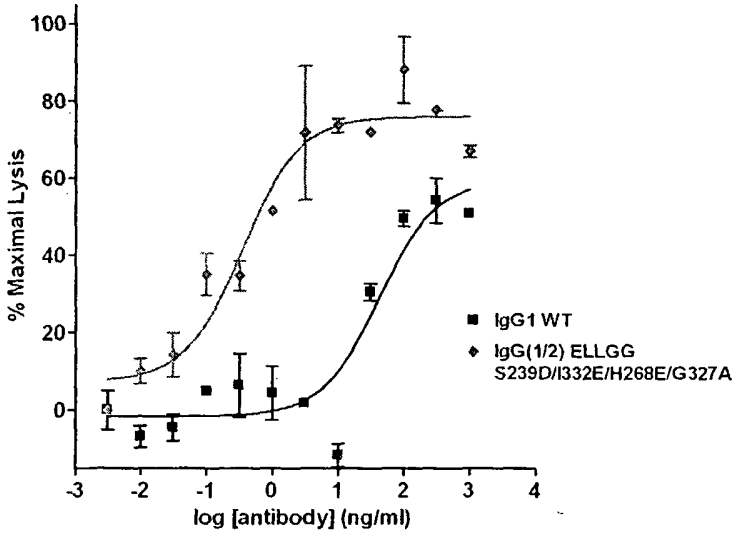

Figure 28

CDC of Anti-CD20 IgG Variants

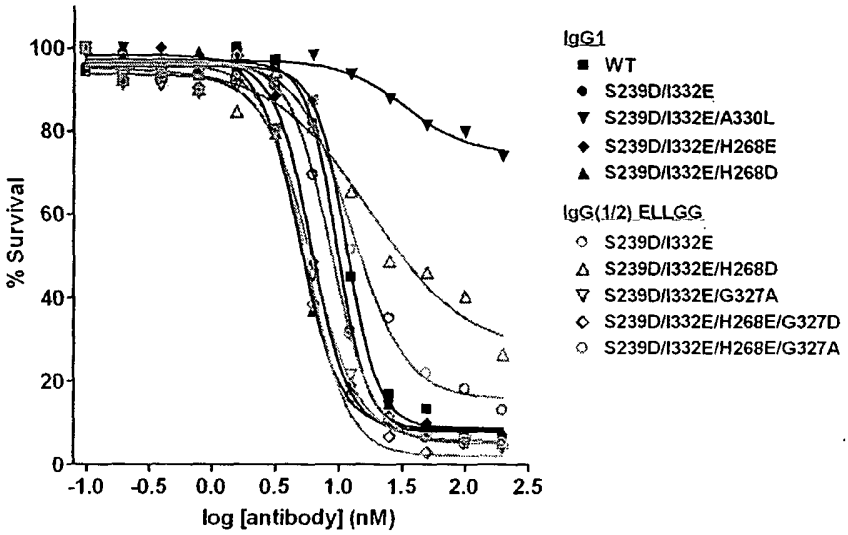

Figure 29a (SEQ ID NO:1)

Anti-CD20 rituximab variable light chain (VL)

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGSG
TSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIK

Figure 29b (SEQ ID NO:2)

Anti-CD20 rituximab variable heavy chain (VH)

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFK
GKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSA

Figure 29c (SEQ ID NO:3)

Anti-CD20 PRO70769 variable light chain (VL)

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIK

Figure 29d (SEQ ID NO:4)

Anti-CD20 PRO70769 variable heavy chain (VH)

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFK
GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSS

Figure 29e (SEQ ID NO:5)

Anti-Her2 trastuzumab variable light chain (VL)

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRS
GTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK

Figure 29f (SEQ ID NO:6)

Anti-Her2 trastuzumab heavy chain (VH)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKG
RFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS

Figure 29g (SEQ ID NO:7)

Anti-CD30 L3.71 AC10 variable light chain (VL)

EIVLTQSPDSLAVSLGERATINCKASQSVDFDGDSYLNWYQQKPGQPPKVLIYAASTLQSGVPSRFS
GSGSGTDFTLTINSLEAEDAATYYCQQSNEDPWTFGGGTKVEIK

Figure 29h (SEQ ID NO:8)

Anti-CD30 H3.69_V2 AC10 variable heavy chain (VH)

QVQLVQSGAEVKKPGASVKVSCKVSGYTFTDYYITWVRQAPGQALEWMGWIYPGSGNTKYSQKFQ
GRFVFSVDTSASTAYLQISSLKAEDTAVYYCANYGNYWFAYWGQGTLVTVSS

Figure 30a (SEQ ID NO:9)

Kappa constant light chain (Cκ)
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 30b (SEQ ID NO:11)

IgG1 constant heavy chain (CH1-hinge-CH2-CH3)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 30c (SEQ ID NO:12)

IgG2 constant heavy chain (CH1-hinge-CH2-CH3)
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYK
CKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 30d (SEQ ID NO:13)

IgG3 constant heavy chain (CH1-hinge-CH2-CH3)
ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKS
CDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
TKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSF
FLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK

Figure 30e (SEQ ID NO:14)

IgG4 constant heavy chain (CH1-hinge-CH2-CH3)
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Figure 30f (SEQ ID NO:15)

IgG(1/2) constant heavy chain (CH1-hinge-CH2-CH3)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP**PVA**GPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKE
YKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 30g (SEQ ID NO:16)

IgG(1/2) ELLGG constant heavy chain (CH1-hinge-CH2-CH3)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP**ELLG**GPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGK
EYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 31a (SEQ ID NO:17)

Anti-CD20 light chain (VL-CL)

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGSG
TSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS
SPVTKSFNRGEC

Figure 31b (SEQ ID NO:18)

Anti-CD20 heavy chain (VH-CH1-hinge-CH2-CH3)

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFK
GKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP**ELLG**GP**D**VFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNK**A**
LPAP**E**EKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 31c (SEQ ID NO:19)

Anti-CD30 light chain (VL-CL)

EIVLTQSPDSLAVSLGERATINCKASQSVDFDGDSYLNWYQQKPGQPPKVLIYAASTLQSGVPSRFS
GSGSGTDFTLTINSLEAEDAATYYCQQSNEDPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT
HQGLSSPVTKSFNRGEC

Figure 31d (SEQ ID NO:20)

Anti-CD30 heavy chain (VH-CH1-hinge-CH2-CH3)

QVQLVQSGAEVKKPGASVKVSCKVSGYTFTDYYITWVRQAPGQALEWMGWIYPGSGNTKYSQKFQ
GRFVFSVDTSASTAYLQISSLKAEDTAVYYCANYGNYWFAYWGQGTLVTVSSASTKGPSVFPLAPSS
KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI
CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP**ELLG**GP**D**VFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNK**AL**PAP
EEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPML
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK