

MUTATIONS IN FELINE ANTIBODY CONSTANT REGIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of United States Provisional Patent Application 63/127313, filed December 18, 2020, which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates generally to feline antibody variants and uses thereof. Specifically, the invention relates to one or more mutations in the Fc constant region of feline antibody for improving various characteristics.

BACKGROUND OF THE INVENTION

[0003] Feline IgG monoclonal antibodies (mAbs) are being developed as effective therapeutics in veterinary medicine. Several years ago, feline IgG subclasses were identified and characterized (Strietzel *et al.*, 2014, *Vet Immunol Immunopathol.*, vol. 158(3-4), pages 214-223). However, not much work has been done on extending the half-life of feline IgGs.

[0004] Through a recycling mechanism, the neonatal Fc receptor (FcRn) prolongs the half-life of an IgG in a pH-dependent interaction with its fragment crystallizable (Fc) region. Specifically, the Fc region spanning the interface of CH2 and CH3 domains interacts with the FcRn on the surface of cells to regulate IgG homeostasis. This interaction is favored by an acidic interaction after IgG pinocytosis and thus IgG is protected from degradation. The endocytosed IgG is then recycled back to the cell surface and released into the blood stream at an alkaline pH thereby maintaining sufficient serum IgG for proper function. Accordingly, the pharmacokinetic profile of IgGs depend on to the structural and functional properties of their Fc regions.

[0005] Three feline IgG subclasses bind feline FcRn and have been compared to human IgG analogues. Half-life of feline IgG remains to be fully studied because, without any experimental support, one cannot expect or predict whether or not they will align closely with human IgGs.

[0006] Extended half-life of IgG could allow less frequent dosing and/or lower dose of the antibody drug, which in turn reduces veterinary visits, improves patient compliance, and lowers the concentration-dependent cytotoxicity/adverse events.

[0007] Accordingly, there exists a need to identify mutations in the Fc constant regions to
5 improve half-life.

SUMMARY OF THE INVENTION

[0008] The invention relates to mutant feline IgGs that provide higher FcRn affinity, relative to wild-type feline IgGs. Specifically, the inventors of the instant application have found that substituting one or more amino acid residues surprisingly and unexpectedly enhanced the
10 affinity to FcRn.

[0009] In one aspect, the invention provides a modified IgG comprising: a feline IgG constant domain comprising at least one amino acid substitution relative to a wild-type feline IgG constant domain, wherein said substitution is at amino acid residue 247, 249, 250, 252, 254, 256, 285, 309, 311, 312, 314, 378, 399, 401, 402, 403, 404, 428, 430, 431, 432, 434, 436, or
15 437, numbered according to the Eu index as in Kabat.

[00010] In some embodiments, the constant domain comprises one or more of substitutions P247I, P247L, P247V, D249A, D249E, D249S, T250E, T250I, T250Q, T250S, T250V, S252A, S252C, S252D, S252E, S252F, S252G, S252H, S252I, S252K, S252L, S252N, S252P, S252Q, S252R, S252T, S252V, S252Y, S252M, S252W, S254A, S254D, S254E, S254F, S254G, S254H, S254K, S254L, S254M, S254C, S254I, S254N, S254P, S254Q, S254R, S254T, S254V, S254W, S254Y, T256A, T256C, T256D, T256E, T256F, T256G, T256H, T256I, T256K, T256L, T256M, T256N, T256P, T256Q, T256R, T256V, T256W, T256Y, T256S, Y285A, L309G, L309I, Q311F, Q311H, Q311I, Q311K, Q311L, Q311M, Q311R, Q311W, Q311Y, D312A, D312H, D312K, D312R, D312P, L314K, 316Q, A378C, A378D, A378E, A378F, A378G, A378H, A378I, A378K, A378L, A378M, A378N, A378P, A378Q, A378R, A378S, A378T, A378V, A378W, A378Y, D399M, D399T, D399V, D401R, G402R, T403R, Y404Q, S428A, S428C, S428D, S428E, S428F, S428G, S428H, S428I, S428K, S428L, S428N, S428P, S428R, S428T, S428V, S428W, S428Y, S428M, E430I, E430Q, A431Q, A431R, A431V, A431K, L432S, S434A, S434C, S434D, S434E, S434F, S434G, S434H, S434I, S434K, S434L, S434M, S434N, S434P, S434Q, S434R, S434T, S434V, S434W, S434Y, H436G, H436K, H436M, H436R, H436Y, T437A, and T437R.

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[00011] In another aspect, the invention provides a polypeptide comprising: a feline IgG constant domain comprising at least one amino acid substitution relative to a wild-type feline IgG constant domain, wherein said substitution is at amino acid residue 247, 249, 250, 252, 254, 256, 285, 309, 311, 312, 314, 378, 399, 401, 402, 403, 404, 428, 430, 431, 432, 434, 436, or 437, numbered according to the Eu index as in Kabat.

[00012] In yet another aspect, the invention provides an antibody comprising: a feline IgG constant domain comprising at least one amino acid substitution relative to a wild-type feline IgG constant domain, wherein said substitution is at amino acid residue 247, 249, 250, 252, 254, 256, 285, 309, 311, 312, 314, 378, 399, 401, 402, 403, 404, 428, 430, 431, 432, 434, 436, or 437, numbered according to the Eu index as in Kabat.

[00013] In a further aspect, the invention provides a method for producing or manufacturing an antibody or a molecule, the method comprising: providing a vector or a host cell having an antibody comprising a feline IgG constant domain, said feline IgG constant domain comprising one or more amino acid substitutions relative to a wild-type feline IgG constant domain, wherein said one or more substitutions are at amino acid residues 247, 249, 250, 252, 254, 256, 285, 309, 311, 312, 314, 378, 399, 401, 402, 403, 404, 428, 430, 431, 432, 434, 436, 437, or a combination thereof.

[00014] In another aspect, the invention provides a fusion molecule comprising: a feline IgG constant domain comprising at least one amino acid substitution relative to a wild-type feline IgG constant domain, wherein said substitution is at amino acid residue 247, 249, 250, 252, 254, 256, 285, 309, 311, 312, 314, 378, 399, 401, 402, 403, 404, 428, 430, 431, 432, 434, 436, or 437, numbered according to the Eu index as in Kabat.

[00015] In another aspect, the invention provides a method for increasing an antibody serum half-life in a cat, the method comprising: administering said cat a therapeutically effective amount of an antibody comprising a feline IgG constant domain, said feline IgG constant domain comprising at least one amino acid substitution relative to a wild-type feline IgG constant domain, wherein said substitution is at amino acid residue 252, 311, or 428, numbered according to the EU index as in Kabat. In one exemplary embodiment, the feline IgG constant domain comprises one or more of mutations S252H, S252Y, Q311W, S428L, S428M, and S428Y. In another exemplary embodiment, the feline IgG constant domain comprises one or more mutations selected from a group: (1) S428L; (2) S252H and S428M; (3) S252Y and S428M; (4) S428M and Q311W; or (5) S428Y and Q311W.

[00016] Other features and advantages of the present invention will become apparent from the following detailed description examples and figures. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within
5 the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[00017] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the
10 Office upon request and payment of the necessary fee.

[00018] FIG. 1 illustrates domain structure of IgG.

[00019] FIG. 2 shows the alignment of the amino acid sequences of wild-type (WT) human IgG1, WT feline IgG1a, WT feline IgG1b, WT feline IgG2 and mutant feline IgG2 having hinge mutation. The amino acid residues are numbered according to the Eu index as in Kabat.
15 The CH1, hinge, CH2, and CH3 amino acid residues are in red, violet, blue, and green, respectively.

[00020] FIG. 3 shows feline Fc IgG1a WT nucleotide sequence.

[00021] FIG. 4 shows that feline IgG point mutations increase the half-life in domestic cats.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

20 [00022] SEQ ID NO.: 1 is the amino acid sequence of feline IgG1a wildtype constant region.

[00023] SEQ ID NO.: 2 is the nucleic acid sequence of feline Fc IgG1a wildtype constant region.

[00024] SEQ ID NO.: 3 is the amino acid sequence of feline IgG1b wildtype constant region.

[00025] SEQ ID NO.: 4 is the amino acid sequence of feline IgG2 wildtype constant region.

[00026] SEQ ID NO.: 5 is the amino acid sequence of feline IgG2_Hinge mutant constant
25 region.

[00027] SEQ ID NO.: 6 is the amino acid sequence of human IgG1 constant region.

[00028] SEQ ID NO.: 7 is the nucleic acid sequence of feline IgG1b wildtype constant region.

[00029] SEQ ID NO.: 8 is the nucleic acid sequence of feline IgG2 wildtype constant region.

[00030] SEQ ID NO.: 9 is the nucleic acid sequence of anti-IL31 antibody (ZTS-5864) Heavy Chain Variable Region.

[00031] SEQ ID NO.: 10 is the amino acid sequence of anti-IL31 antibody (ZTS-5864) Heavy Chain Variable Region.

- 5 [00032] SEQ ID NO.: 11 is the amino acid sequence of anti-IL31 antibody (ZTS-5864) Heavy Chain Variable Region CDR1.

[00033] SEQ ID NO.: 12 is the amino acid sequence of anti-IL31 antibody (ZTS-5864) Heavy Chain Variable Region CDR2.

- 10 [00034] SEQ ID NO.: 13 is the amino acid sequence of anti-IL31 antibody (ZTS-5864) Heavy Chain Variable Region CDR3.

[00035] SEQ ID NO.: 14 is the nucleic acid sequence of anti-IL31 antibody (ZTS-5864) Light Chain Variable Region.

[00036] SEQ ID NO.: 15 is the amino acid sequence of anti-IL31 antibody (ZTS-5864) Light Chain Variable Region.

- 15 [00037] SEQ ID NO.: 16 is the amino acid sequence of anti-IL31 antibody (ZTS-5864) Light Chain Variable Region CDR1.

[00038] SEQ ID NO.: 17 is the amino acid sequence of anti-IL31 antibody (ZTS-5864) Light Chain Variable Region CDR2.

- 20 [00039] SEQ ID NO.: 18 is the amino acid sequence of anti-IL31 antibody (ZTS-5864) Light Chain Variable Region CDR3.

DETAILED DESCRIPTION OF THE INVENTION

- 25 [00040] The present subject matter may be understood more readily by reference to the following detailed description which forms a part of this disclosure. It is to be understood that this invention is not limited to the specific products, methods, conditions or parameters described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed invention.

[00041] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of

ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[00042] As employed above and throughout the disclosure, the following terms and abbreviations, unless otherwise indicated, shall be understood to have the following meanings.

5 Definitions

[00043] In the present disclosure the singular forms "a," "an," and "the" include the plural reference, and reference to a particular numerical value includes at least that particular value, unless the context clearly indicates otherwise. Thus, for example, a reference to "a molecule" or "a compound" is a reference to one or more of such molecules or compounds and equivalents
10 thereof known to those skilled in the art, and so forth. The term "plurality", as used herein, means more than one. When a range of values is expressed, another embodiment includes from the one particular and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it is understood that the particular value forms another embodiment. All ranges are inclusive and combinable.

15 [00044] In the specification and claims, the numbering of the amino acid residues in an immunoglobulin heavy chain is that of the Eu index as in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). The "Eu index as in Kabat" refers to the residue numbering of the IgG antibody and is reflected herein in FIG. 2.

20 [00045] The term "isolated" when used in relation to a nucleic acid is a nucleic acid that is identified and separated from at least one contaminant nucleic acid with which it is ordinarily associated in its natural source. Isolated nucleic acid is in a form or setting different from that in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. An isolated nucleic acid molecule includes
25 a nucleic acid molecule contained in cells that ordinarily express the polypeptide encoded therein where, for example, the nucleic acid molecule is in a plasmid or a chromosomal location different from that of natural cells. The isolated nucleic acid may be present in single-stranded or double-stranded form. When an isolated nucleic acid molecule is to be utilized to express a protein, the oligonucleotide or polynucleotide will contain at a minimum the sense or coding
30 strand, but may contain both the sense and anti-sense strands (i.e., may be double-stranded).

[00046] A nucleic acid molecule is "operably linked" or "operably attached" when it is placed into a functional relationship with another nucleic acid molecule. For example, a promoter or

enhancer is operably linked to a coding sequence of nucleic acid if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence of nucleic acid if it is positioned so as to facilitate translation. A nucleic acid molecule encoding a variant Fc region is operably linked to a nucleic acid molecule encoding a heterologous protein (i.e., a protein or functional fragment thereof which does not, as it exists in nature, comprise an Fc region) if it is positioned such that the expressed fusion protein comprises the heterologous protein or functional fragment thereof adjoined either upstream or downstream to the variant Fc region polypeptide; the heterologous protein may be immediately adjacent to the variant Fc region polypeptide or may be separated therefrom by a linker sequence of any length and composition. Likewise, a polypeptide (used synonymously herein with "protein") molecule is "operably linked" or "operably attached" when it is placed into a functional relationship with another polypeptide.

[00047] As used herein the term "functional fragment" when in reference to a polypeptide or protein (e.g., a variant Fc region, or a monoclonal antibody) refers to fragments of that protein which retain at least one function of the full-length polypeptide. The fragments may range in size from six amino acids to the entire amino acid sequence of the full-length polypeptide minus one amino acid. A functional fragment of a variant Fc region polypeptide of the present invention retains at least one "amino acid substitution" as herein defined. A functional fragment of a variant Fc region polypeptide retains at least one function known in the art to be associated with the Fc region (e.g., ADCC, CDC, Fc receptor binding, C1q binding, down regulation of cell surface receptors or may, e.g., increase the in vivo or in vitro half-life of a polypeptide to which it is operably attached).

[00048] The term "purified" or "purify" refers to the substantial removal of at least one contaminant from a sample. For example, an antigen-specific antibody may be purified by complete or substantial removal (at least 90%, 91%, 92%, 93%, 94%, 95%, or more preferably at least 96%, 97%, 98% or 99%) of at least one contaminating non-immunoglobulin protein; it may also be purified by the removal of immunoglobulin protein that does not bind to the same antigen. The removal of non-immunoglobulin proteins and/or the removal of immunoglobulins that do not bind a particular antigen results in an increase in the percent of antigen-specific immunoglobulins in the sample. In another example, a polypeptide (e.g., an immunoglobulin) expressed in bacterial host cells is purified by the complete or substantial removal of host cell proteins; the percent of the polypeptide is thereby increased in the sample.

[00049] The term "native" as it refers to a polypeptide (e.g., Fc region) is used herein to indicate that the polypeptide has an amino acid sequence consisting of the amino acid sequence of the polypeptide as it commonly occurs in nature or a naturally occurring polymorphism thereof. A native polypeptide (e.g., native Fc region) may be produced by recombinant means or may be
5 isolated from a naturally occurring source.

[00050] The term "expression vector" as used herein refers to a recombinant DNA molecule containing a desired coding sequence and appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host organism.

[00051] As used herein, the term "host cell" refers to any eukaryotic or prokaryotic cell (e.g.,
10 bacterial cells such as E. coli, CHO cells, yeast cells, mammalian cells, avian cells, amphibian cells, plant cells, fish cells, and insect cells), whether located in vitro or in situ, or in vivo

[00052] As used herein, the term "Fc region" refers to a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a variant Fc region. Although the generally accepted boundaries of the Fc region of an immunoglobulin heavy
15 chain might vary, the feline IgG heavy chain Fc region is usually defined to stretch, for example, from an amino acid residue at position 231, to the carboxyl-terminus thereof. In some embodiments, variants comprise only portions of the Fc region and can include or not include the carboxy-terminus. The Fc region of an immunoglobulin generally comprises two constant
20 domains, CH2 and CH3. In some embodiments, variants having one or more of the constant domains are contemplated. In other embodiments, variants without such constant domains (or with only portions of such constant domains) are contemplated.

[00053] The "CH2 domain" of a feline IgG Fc region usually extends, for example, from about amino acid 231 to about amino acid 340 (see FIG. 2). The CH2 domain is unique in that it is not closely paired with another domain. Two N-linked branched carbohydrate chains are
25 interposed between the two CH2 domains of an intact native IgG molecule.

[00054] The "CH3 domain" of a feline IgG Fc region generally is the stretch of residues C-terminal to a CH2 domain in an Fc region extending, for example, from about amino acid residue 341 to about amino acid residue 447 (see FIG. 2).

[00055] A "functional Fc region" possesses an "effector function" of a native sequence Fc region. At least one effector function of a polypeptide comprising a variant Fc region of the
30 present invention may be enhanced or diminished with respect to a polypeptide comprising a native Fc region or the parent Fc region of the variant. Examples of effector functions include,

but are not limited to: Clq binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc. Such effector functions may require the Fc region to be operably linked to a binding domain (e.g., an antibody variable domain) and can be assessed using various assays (e.g., Fc binding assay, ADCC assays, CDC assays, target cell depletion from whole or fractionated blood samples, etc.).

[00056] A "native sequence Fc region" or "wild type Fc region" refers to an amino acid sequence that is identical to the amino acid sequence of an Fc region commonly found in nature. Exemplary native sequence feline Fc regions are shown in FIG. 2 and include a native sequence of feline IgG1a Fc region.

[00057] A "variant Fc region" comprises an amino acid sequence that differs from that of a native sequence Fc region (or fragment thereof) by virtue of at least one "amino acid substitution" as defined herein. In preferred embodiments, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or in the Fc region of a parent polypeptide, preferably 1, 2, 3, 4 or 5 amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. In an alternative embodiment, a variant Fc region may be generated according to the methods herein disclosed and this variant Fc region can be fused to a heterologous polypeptide of choice, such as an antibody variable domain or a non-antibody polypeptide, e.g., binding domain of a receptor or ligand.

[00058] As used herein, the term "derivative" in the context of polypeptides refers to a polypeptide that comprises an amino acid sequence which has been altered by introduction of an amino acid residue substitution. The term "derivative" as used herein also refers to a polypeptide which has been modified by the covalent attachment of any type of molecule to the polypeptide. For example, but not by way of limitation, an antibody may be modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative polypeptide may be produced by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative polypeptide possesses a similar or identical function as the polypeptide from which it was derived. It is understood that a polypeptide comprising a variant Fc region of the present invention may be a derivative as defined herein, preferably the derivatization occurs within the Fc region.

[00059] "Substantially of feline origin" as used herein in reference to a polypeptide (e.g., an Fc region or a monoclonal antibody), indicates the polypeptide has an amino acid sequence at least 80%, at least 85%, more preferably at least 90%, 91%, 92%, 93%, 94% or even more preferably at least 95%, 95%, 97%, 98% or 99% homologous to that of a native feline amino polypeptide.

5 [00060] The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to an Fc region (e.g., the Fc region of an antibody). The preferred FcR is a native sequence FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the Fc gamma RI, Fc gamma RII, Fc gamma RIII subclasses, including allelic variants and alternatively spliced forms of these receptors. Another preferred FcR includes the
10 neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.

[00061] The phrase "antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells (e.g., nonspecific) that express
15 FcRs (e.g., Natural Killer ("NK") cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cells. The primary cells for mediating ADCC, NK cells, express Fc gamma RIII only, whereas monocytes express Fc gamma RI, Fc gamma RII and Fc gamma RIII.

[00062] As used herein, the phrase "effector cells" refers to leukocytes (preferably feline) which
20 express one or more FcRs and perform effector functions. Preferably, the cells express at least Fc gamma RIII and perform ADCC effector function. Examples of leukocytes which mediate ADCC include PBMC, NK cells, monocytes, cytotoxic T cells and neutrophils. The effector cells may be isolated from a native source (e.g., from blood or PBMCs).

[00063] A variant polypeptide with "altered" FcRn binding affinity is one which has either
25 enhanced (i.e., increased, greater or higher) or diminished (i.e., reduced, decreased or lesser) FcRn binding affinity compared to the variant's parent polypeptide or to a polypeptide comprising a native Fc region when measured at pH 6.0. A variant polypeptide which displays increased binding or increased binding affinity to an FcRn binds FcRn with greater affinity than the parent polypeptide. A variant polypeptide which displays decreased binding or
30 decreased binding affinity to an FcRn, binds FcRn with lower affinity than its parent polypeptide. Such variants which display decreased binding to an FcRn may possess little or no appreciable binding to an FcRn, e.g., 0-20% binding to the FcRn compared to a parent

polypeptide. A variant polypeptide which binds an FcRn with "enhanced affinity" as compared to its parent polypeptide, is one which binds FcRn with higher binding affinity than the parent polypeptide, when the amounts of variant polypeptide and parent polypeptide in a binding assay are essentially the same, and all other conditions are identical. For example, a variant polypeptide with enhanced FcRn binding affinity may display from about 1.10 fold to about 100 fold (more typically from about 1.2 fold to about 50 fold) increase in FcRn binding affinity compared to the parent polypeptide, where FcRn binding affinity is determined, for example, in an ELISA assay or other method available to one of ordinary skill in the art.

[00064] As used herein, an "amino acid substitution" refers to the replacement of at least one existing amino acid residue in a given amino acid sequence with another different "replacement" amino acid residue. The replacement residue or residues may be "naturally occurring amino acid residues" (i.e., encoded by the genetic code) and selected from: alanine (Ala); arginine (Arg); asparagine (Asn); aspartic acid (Asp); cysteine (Cys); glutamine (Gln); glutamic acid (Glu); glycine (Gly); histidine (His); isoleucine (Ile); leucine (Leu); lysine (Lys); methionine (Met); phenylalanine (Phe); proline (Pro); serine (Ser); threonine (Thr); tryptophan (Trp); tyrosine (Tyr); and valine (Val). Substitution with one or more non-naturally occurring amino acid residues is also encompassed by the definition of an amino acid substitution herein. A "non-naturally occurring amino acid residue" refers to a residue, other than those naturally occurring amino acid residues listed above, which is able to covalently bind adjacent amino acid residues (s) in a polypeptide chain. Examples of non-naturally occurring amino acid residues include norleucine, ornithine, norvaline, homoserine and other amino acid residue analogues such as those described in Ellman et al. Meth. Enzym. 202: 301-336 (1991).

[00065] The term "assay signal" refers to the output from any method of detecting protein-protein interactions, including but not limited to, absorbance measurements from colorimetric assays, fluorescent intensity, or disintegrations per minute. Assay formats could include ELISA, facs, or other methods. A change in the "assay signal" may reflect a change in cell viability and/or a change in the kinetic off-rate, the kinetic on-rate, or both. A "higher assay signal" refers to the measured output number being larger than another number (e.g., a variant may have a higher (larger) measured number in an ELISA assay as compared to the parent polypeptide). A "lower" assay signal refers to the measured output number being smaller than another number (e.g., a variant may have a lower (smaller) measured number in an ELISA assay as compared to the parent polypeptide).

[00066] The term "binding affinity" refers to the equilibrium dissociation constant (expressed in units of concentration) associated with each Fc receptor-Fc binding interaction. The binding affinity is directly related to the ratio of the kinetic off-rate (generally reported in units of inverse time, e.g., seconds⁻¹) divided by the kinetic on-rate (generally reported in units of concentration per unit time, e.g., molar/second). In general it is not possible to unequivocally state whether changes in equilibrium dissociation constants (K_D or K_D) are due to differences in on-rates, off-rates or both unless each of these parameters are experimentally determined (e.g., by BIACORE or SAPIDYNE measurements).

[00067] As used herein, the term "hinge region" refers to the stretch of amino acids, for example, in feline IgG1a (e.g. stretching from position 216 to position 230 of feline IgG1a). Hinge regions of other IgG isotypes may be aligned with the IgG sequence by placing the first and last cysteine residues forming inter-heavy chain disulfide (S—S) bonds in the same positions.

[00068] "Clq" is a polypeptide that includes a binding site for the Fc region of an immunoglobulin. Clq together with two serine proteases, Clr and Cls, forms the complex Cl, the first component of the CDC pathway.

[00069] As used herein, the term "antibody" is used interchangeably with "immunoglobulin" or "Ig." is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity or functional activity. Single chain antibodies, and chimeric, feline, or felinized antibodies, as well as chimeric or CDR-grafted single chain antibodies, and the like, comprising portions derived from different species, are also encompassed by the present invention and the term "antibody". The various portions of these antibodies can be joined together chemically by conventional techniques, synthetically, or can be prepared as a contiguous protein using genetic engineering techniques. For example, nucleic acids encoding a chimeric or felinized chain can be expressed to produce a contiguous protein. See, e.g., U.S. Pat. No. 4,816,567; U.S. Pat. No. 4,816,397; WO 86/01533; U.S. Pat. No. 5,225,539; and U.S. Pat. Nos. 5,585,089 and 5,698,762. See also, Newman, R. et al. BioTechnology, 10: 1455-1460, 1993, regarding primatized antibody, and Ladner et al., U.S. Pat. No. 4,946,778 and Bird, R. E. et al., Science, 242:423-426, 1988, regarding single chain antibodies. It is understood that all forms of the antibodies comprising an Fc region (or portion thereof) are encompassed herein within the term "antibody." Furthermore, the antibody may be labeled with a detectable label, immobilized on

a solid phase and/or conjugated with a heterologous compound (e.g., an enzyme or toxin) according to methods known in the art.

[00070] As used herein, the term "antibody fragments" refers to a portion of an intact antibody. Examples of antibody fragments include, but are not limited to, linear antibodies; single-chain
5 antibody molecules; Fc or Fc' peptides, Fab and Fab fragments, and multispecific antibodies formed from antibody fragments. The antibody fragments preferably retain at least part of the hinge and optionally the CH1 region of an IgG heavy chain. In other preferred embodiments, the antibody fragments comprise at least a portion of the CH2 region or the entire CH2 region.

[00071] As used herein, the term "functional fragment", when used in reference to a monoclonal
10 antibody, is intended to refer to a portion of the monoclonal antibody that still retains a functional activity. A functional activity can be, for example, antigen binding activity or specificity, receptor binding activity or specificity, effector function activity and the like. Monoclonal antibody functional fragments include, for example, individual heavy or light chains and fragments thereof, such as VL, VH and Fd; monovalent fragments, such as Fv, Fab,
15 and Fab'; bivalent fragments such as F(ab')₂; single chain Fv (scFv); and Fc fragments. Such terms are described in, for example, Harlowe and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York (1989); *Molec. Biology and Biotechnology: A Comprehensive Desk Reference* (Myers, R. A. (ed.), New York: VCH Publisher, Inc.); Huston et al., *Cell Biophysics*, 22:189-224 (1993); Pluckthun and Skerra, *Meth. Enzymol.*, 178:497-
20 515 (1989) and in Day, E. D., *Advanced Immunochimistry*, Second Ed., Wiley-Liss, Inc., New York, N.Y. (1990). The term functional fragment is intended to include, for example, fragments produced by protease digestion or reduction of a monoclonal antibody and by recombinant DNA methods known to those skilled in the art.

[00072] As used herein, the term "fragment" refers to a polypeptide comprising an amino acid
25 sequence of at least 5, 15, 20, 25, 40, 50, 70, 90, 100 or more contiguous amino acid residues of the amino acid sequence of another polypeptide. In a preferred embodiment, a fragment of a polypeptide retains at least one function of the full-length polypeptide.

[00073] As used herein, the term "chimeric antibody" includes monovalent, divalent or
30 polyvalent immunoglobulins. A monovalent chimeric antibody is a dimer formed by a chimeric heavy chain associated through disulfide bridges with a chimeric light chain. A divalent chimeric antibody is a tetramer formed by two heavy chain-light chain dimers associated through at least one disulfide bridge. A chimeric heavy chain of an antibody for use

in feline comprises an antigen-binding region derived from the heavy chain of a non-feline antibody, which is linked to at least a portion of a feline heavy chain constant region, such as CH1 or CH2. A chimeric light chain of an antibody for use in feline comprises an antigen binding region derived from the light chain of a non-feline antibody, linked to at least a portion of a feline light chain constant region (CL). Antibodies, fragments or derivatives having chimeric heavy chains and light chains of the same or different variable region binding specificity, can also be prepared by appropriate association of the individual polypeptide chains, according to known method steps. With this approach, hosts expressing chimeric heavy chains are separately cultured from hosts expressing chimeric light chains, and the immunoglobulin chains are separately recovered and then associated. Alternatively, the hosts can be co-cultured and the chains allowed to associate spontaneously in the culture medium, followed by recovery of the assembled immunoglobulin or fragment or both the heavy and light chains can be expressed in the same host cell. Methods for producing chimeric antibodies are well known in the art (see, e.g., U.S. Pat. Nos. 6,284,471; 5,807,715; 4,816,567; and 4,816,397).

[00074] As used herein, "felinized" forms of non-feline (e.g., murine) antibodies (i.e., felinized antibodies) are antibodies that contain minimal sequence, or no sequence, derived from non-feline immunoglobulin. For the most part, felinized antibodies are feline immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-feline species (donor antibody) such as mouse, rat, rabbit, human or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the feline immunoglobulin are replaced by corresponding non-feline residues. Furthermore, felinized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are generally made to further refine antibody performance. In general, the felinized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops (CDRs) correspond to those of a non-feline immunoglobulin and all or substantially all of the FR residues are those of a feline immunoglobulin sequence. The felinized antibody may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a feline immunoglobulin.

[00075] As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding domain of a heterologous "adhesin" protein (e.g., a receptor, ligand or enzyme) with an immunoglobulin constant domain. Structurally, immunoadhesins comprise a

fusion of the adhesin amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site (antigen combining site) of an antibody (i.e., is "heterologous") with an immunoglobulin constant domain sequence.

[00076] As used herein, the term "ligand binding domain" refers to any native receptor or any region or derivative thereof retaining at least a qualitative ligand binding ability of a corresponding native receptor. In certain embodiments, the receptor is from a cell-surface polypeptide having an extracellular domain that is homologous to a member of the immunoglobulin supergene family. Other receptors, which are not members of the immunoglobulin supergene family but are nonetheless specifically covered by this definition, are receptors for cytokines, and in particular receptors with tyrosine kinase activity (receptor tyrosine kinases), members of the hematopoietin and nerve growth factor receptor superfamilies, and cell adhesion molecules (e.g., E-, L-, and P-selectins).

[00077] As used herein, the term "receptor binding domain" refers to any native ligand for a receptor, including, e.g., cell adhesion molecules, or any region or derivative of such native ligand retaining at least a qualitative receptor binding ability of a corresponding native ligand.

[00078] As used herein, an "isolated" polypeptide is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In certain embodiments, the isolated polypeptide is purified (1) to greater than 95% by weight of polypeptides as determined by the Lowry method, and preferably, more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-page under reducing or nonreducing conditions using Coomassie blue or silver stain. Isolated polypeptide includes the polypeptide in situ within recombinant cells since at least one component of the polypeptide's natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by a least one purification step.

[00079] As used herein, the term "disorder" and "disease" are used interchangeably to refer to any condition that would benefit from treatment with a variant polypeptide (a polypeptide comprising a variant Fc region of the invention), including chronic and acute disorders or diseases (e.g., pathological conditions that predispose a patient to a particular disorder).

[00080] As used herein, the term "receptor" refers to a polypeptide capable of binding at least one ligand. The preferred receptor is a cell-surface or soluble receptor having an extracellular ligand-binding domain and, optionally, other domains (e.g., transmembrane domain, intracellular domain and/or membrane anchor). A receptor to be evaluated in an assay described herein may be an intact receptor or a fragment or derivative thereof (e.g. a fusion protein comprising the binding domain of the receptor fused to one or more heterologous polypeptides). Moreover, the receptor to be evaluated for its binding properties may be present in a cell or isolated and optionally coated on an assay plate or some other solid phase or labeled directly and used as a probe.

10 Feline Wildtype IgG

[00081] Feline IgGs are well known in the art and fully described, for example, in Strietzel *et al.*, 2014, *Vet Immunol Immunopathol.*, vol. 158(3-4), pages 214-223. In one embodiment, feline IgG is IgG1_a. In another embodiment, feline IgG is IgG1_b. In yet another embodiment, feline IgG is IgG2. In a particular embodiment, feline IgG is IgG1_a.

15 [00082] The amino acid and nucleic acid sequences of IgG1_a, IgG1_b, and IgG2 are also well known in the art.

[00083] In one example, IgG of the invention comprises a constant domain, for example, CH1, CH2, or CH3 domains, or a combination thereof. In another example, the constant domain of the invention comprises Fc region, including, for example, CH2 or CH3 domains or a combination thereof.

20 [00084] In a particular example, the wild-type constant domain comprises the amino acid sequence set forth in SEQ ID NO.: 1, 3, or 4. In some embodiments, the wild-type IgG constant domain is a homologue, a variant, an isomer, or a functional fragment of SEQ ID NO.: 1, 3, or 4, but without any mutation. Each possibility represents a separate embodiment of the present invention.

25 [00085] IgGs constant domains also include polypeptides with amino acid sequences substantially similar to the amino acid sequence of the heavy and/or light chain. Substantially the same amino acid sequence is defined herein as a sequence with at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity to a compared amino acid sequence, as determined by the FASTA search method in accordance with Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444-2448 (1988).

[00086] The present invention also includes nucleic acid molecules that encode IgGs or portion thereof, described herein. In one embodiment, the nucleic acids may encode an antibody heavy chain comprising, for example, CH1, CH2, CH3 regions, or a combination thereof. In another embodiment, the nucleic acids may encode an antibody heavy chain comprising, for example, any one of the VH regions or a portion thereof, or any one of the VH CDRs, including any variants thereof. The invention also includes nucleic acid molecules that encode an antibody light chain comprising, for example, any one of the CL regions or a portion thereof, any one of the VL regions or a portion thereof or any one of the VL CDRs, including any variants thereof. In certain embodiments, the nucleic acid encodes both a heavy and light chain, or portions thereof.

[00087] The amino acid sequence of the wild-type constant domain set forth in SEQ ID NO.: 1, 3, or 4 is encoded by a nucleic acid sequence comprising the sequence set forth in SEQ ID NO.: 2, 7, or 8, respectively.

Modified Feline IgG

[00088] The inventors of the instant application have found that substituting one or more amino acid residues surprisingly and unexpectedly enhanced the affinity to FcRn. The amino acid position number, as used herein, refers to a position numbered according to the Eu index as in Kabat (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)).

[00089] Accordingly, in one embodiment, the invention provides a modified IgG comprising: a feline IgG constant domain comprising at least one amino acid substitution relative to a wild-type feline IgG constant domain, wherein said substitution is at amino acid residue 247, 249, 250, 252, 254, 256, 285, 309, 311, 312, 314, 378, 399, 401, 402, 403, 404, 428, 430, 431, 432, 434, 436, or 437, numbered according to the Eu index as in Kabat.

[00090] In some embodiments, the constant domain comprises one or more of substitutions P247I, P247L, P247V, D249A, D249E, D249S, T250E, T250I, T250Q, T250S, T250V, S252A, S252C, S252D, S252E, S252F, S252G, S252H, S252I, S252K, S252L, S252N, S252P, S252Q, S252R, S252T, S252V, S252Y, S252M, S252W, S254A, S254D, S254E, S254F, S254G, S254H, S254K, S254L, S254M, S254C, S254I, S254N, S254P, S254Q, S254R, S254T, S254V, S254W, S254Y, T256A, T256C, T256D, T256E, T256F, T256G, T256H, T256I, T256K, T256L, T256M, T256N, T256P, T256Q, T256R, T256V, T256W, T256Y, T256S, Y285A, L309G, L309I, Q311F, Q311H, Q311I, Q311K, Q311L, Q311M, Q311R,

Q311W, Q311Y, D312A, D312H, D312K, D312R, D312P, L314K, 316Q, A378C, A378D, A378E, A378F, A378G, A378H, A378I, A378K, A378L, A378M, A378N, A378P, A378Q, A378R, A378S, A378T, A378V, A378W, A378Y, D399M, D399T, D399V, D401R, G402R, T403R, Y404Q, S428A, S428C, S428D, S428E, S428F, S428G, S428H, S428I, S428K, S428L, S428N, S428P, S428R, S428T, S428V, S428W, S428Y, S428M, E430I, E430Q, A431Q, A431R, A431V, A431K, L432S, S434A, S434C, S434D, S434E, S434F, S434G, S434H, S434I, S434K, S434L, S434M, S434N, S434P, S434Q, S434R, S434T, S434V, S434W, S434Y, H436G, H436K, H436M, H436R, H436Y, T437A, and T437R.

[00091] In a particular example, the invention comprises one or more mutations, described herein, in the wild-type amino acid sequence set forth in SEQ ID NO.: 1, 3, or 4. In some embodiments, the mutant IgG constant domain is a homologue, a variant, an isomer, or a functional fragment having one or more mutations, described herein. Each possibility represents a separate embodiment of the present invention.

[00092] The amino acid sequence of the mutant constant domain is encoded by its corresponding mutant nucleic acid sequence.

Methods for Making Antibody Molecules of the Invention

[00093] Methods for making antibody molecules are well known in the art and fully described in U.S. Patents 8,394,925; 8,088,376; 8,546,543; 10,336,818; and 9,803,023 and U.S. Patent Application Publication 20060067930, which are incorporated by reference herein in their entirety. Any suitable method, process, or technique, known to one of skilled in the art, can be used. An antibody molecule having a variant Fc region of the invention may be generated according to the methods well known in the art. In some embodiments, the variant Fc region can be fused to a heterologous polypeptide of choice, such as an antibody variable domain or binding domain of a receptor or ligand.

[00094] With the advent of methods of molecular biology and recombinant technology, a person of skilled in the art can produce antibody and antibody-like molecules by recombinant means and thereby generate gene sequences that code for specific amino acid sequences found in the polypeptide structure of the antibodies. Such antibodies can be produced by either cloning the gene sequences encoding the polypeptide chains of said antibodies or by direct synthesis of said polypeptide chains, with assembly of the synthesized chains to form active tetrameric (H2L2) structures with affinity for specific epitopes and antigenic determinants. This has

permitted the ready production of antibodies having sequences characteristic of neutralizing antibodies from different species and sources.

[00095] Regardless of the source of the antibodies, or how they are recombinantly constructed, or how they are synthesized, in vitro or in vivo, using transgenic animals, large cell cultures of laboratory or commercial size, using transgenic plants, or by direct chemical synthesis employing no living organisms at any stage of the process, all antibodies have a similar overall 3 dimensional structure. This structure is often given as H₂L₂ and refers to the fact that antibodies commonly comprise two light (L) amino acid chains and 2 heavy (H) amino acid chains. Both chains have regions capable of interacting with a structurally complementary antigenic target. The regions interacting with the target are referred to as "variable" or "V" regions and are characterized by differences in amino acid sequence from antibodies of different antigenic specificity. The variable regions of either H or L chains contain the amino acid sequences capable of specifically binding to antigenic targets.

[00096] As used herein, the term "antigen binding region" refers to that portion of an antibody molecule which contains the amino acid residues that interact with an antigen and confer on the antibody its specificity and affinity for the antigen. The antibody binding region includes the "framework" amino acid residues necessary to maintain the proper conformation of the antigen-binding residues. Within the variable regions of the H or L chains that provide for the antigen binding regions are smaller sequences dubbed "hypervariable" because of their extreme variability between antibodies of differing specificity. Such hypervariable regions are also referred to as "complementarity determining regions" or "CDR" regions. These CDR regions account for the basic specificity of the antibody for a particular antigenic determinant structure.

[00097] The CDRs represent non-contiguous stretches of amino acids within the variable regions but, regardless of species, the positional locations of these critical amino acid sequences within the variable heavy and light chain regions have been found to have similar locations within the amino acid sequences of the variable chains. The variable heavy and light chains of all antibodies each have three CDR regions, each non-contiguous with the others. In all mammalian species, antibody peptides contain constant (i.e., highly conserved) and variable regions, and, within the latter, there are the CDRs and the so-called "framework regions" made up of amino acid sequences within the variable region of the heavy or light chain but outside the CDRs.

[00098] The present invention further provides a vector including at least one of the nucleic acids described above. Because the genetic code is degenerate, more than one codon can be used to encode a particular amino acid. Using the genetic code, one or more different nucleotide sequences can be identified, each of which would be capable of encoding the amino acid. The probability that a particular oligonucleotide will, in fact, constitute the actual encoding sequence can be estimated by considering abnormal base pairing relationships and the frequency with which a particular codon is actually used (to encode a particular amino acid) in eukaryotic or prokaryotic cells expressing an antibody or portion. Such "codon usage rules" are disclosed by Lathe, et al., 183 J. Molec. Biol. 1-12 (1985). Using the "codon usage rules" of Lathe, a single nucleotide sequence, or a set of nucleotide sequences that contains a theoretical "most probable" nucleotide sequence capable of encoding feline IgG sequences can be identified. It is also intended that the antibody coding regions for use in the present invention could also be provided by altering existing antibody genes using standard molecular biological techniques that result in variants of the antibodies and peptides described herein. Such variants include, but are not limited to deletions, additions and substitutions in the amino acid sequence of the antibodies or peptides.

[00099] For example, one class of substitutions is conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a feline antibody peptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu, and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg, replacements among the aromatic residues Phe, Tyr, and the like. Guidance concerning which amino acid changes are likely to be phenotypically silent is found in Bowie *et al.*, 247 *Science* 1306-10 (1990).

[00100] Variant feline antibodies or peptides may be fully functional or may lack function in one or more activities. Fully functional variants typically contain only conservative variations or variations in non-critical residues or in non-critical regions. Functional variants can also contain substitution of similar amino acids that result in no change or an insignificant change in function. Alternatively, such substitutions may positively or negatively affect function to some degree. Non-functional variants typically contain one or more non-conservative amino acid substitutions, deletions, insertions, inversions, or truncation or a substitution, insertion, inversion, or deletion in a critical residue or critical region.

[000101] Amino acids that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis. Cunningham *et al.*, 244 *Science* 1081-85 (1989). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity
5 such as epitope binding or in vitro ADCC activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallography, nuclear magnetic resonance, or photoaffinity labeling. Smith *et al.*, 224 *J. Mol. Biol.* 899-904 (1992); de Vos *et al.*, 255 *Science* 306-12 (1992).

[000102] Moreover, polypeptides often contain amino acids other than the twenty "naturally
10 occurring" amino acids. Further, many amino acids, including the terminal amino acids, may be modified by natural processes, such as processing and other post-translational modifications, or by chemical modification techniques well known in the art. Known modifications include, but are not limited to, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent
15 attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic
20 processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. Such modifications are well known to those of skill in the art and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP
25 ribosylation, for instance, are described in most basic texts, such as *Proteins-Structure and Molecular Properties* (2nd ed., T. E. Creighton, W. H. Freeman & Co., N.Y., 1993). Many detailed reviews are available on this subject, such as by Wold, *Posttranslational Covalent Modification of proteins*, 1-12 (Johnson, ed., Academic Press, N.Y., 1983); Seifter *et al.* 182 *Meth. Enzymol.* 626-46 (1990); and Rattan *et al.* 663 *Ann. NY Acad. Sci.* 48-62 (1992).

[000103] In another aspect, the invention provides antibody derivatives. A "derivative" of an
30 antibody contains additional chemical moieties not normally a part of the protein. Covalent modifications of the protein are included within the scope of this invention. Such modifications may be introduced into the molecule by reacting targeted amino acid residues of the antibody

with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. For example, derivatization with bifunctional agents, well-known in the art, is useful for cross-linking the antibody or fragment to a water-insoluble support matrix or to other macromolecular carriers.

- 5 [000104] Derivatives also include radioactively labeled monoclonal antibodies that are labeled. For example, with radioactive iodine (251,1311), carbon (4C), sulfur (35S), indium, tritium (H³) or the like; conjugates of monoclonal antibodies with biotin or avidin, with enzymes, such as horseradish peroxidase, alkaline phosphatase, beta-D-galactosidase, glucose oxidase, glucoamylase, carboxylic acid anhydrase, acetylcholine esterase, lysozyme, malate
- 10 dehydrogenase or glucose 6-phosphate dehydrogenase; and also conjugates of monoclonal antibodies with bioluminescent agents (such as luciferase), chemoluminescent agents (such as acridine esters) or fluorescent agents (such as phycobiliproteins).

- [000105] Another derivative bifunctional antibody of the invention is a bispecific antibody, generated by combining parts of two separate antibodies that recognize two different antigenic
- 15 groups. This may be achieved by crosslinking or recombinant techniques. Additionally, moieties may be added to the antibody or a portion thereof to increase half-life in vivo (e.g., by lengthening the time to clearance from the blood stream. Such techniques include, for example, adding PEG moieties (also termed pegylation), and are well-known in the art. See U.S. Patent. Appl. Pub. No. 20030031671.

- 20 [000106] In some embodiments, the nucleic acids encoding a subject antibody are introduced directly into a host cell, and the cell is incubated under conditions sufficient to induce expression of the encoded antibody. After the subject nucleic acids have been introduced into a cell, the cell is typically incubated, normally at 37° C., sometimes under selection, for a period of about 1-24 hours in order to allow for the expression of the antibody. In one embodiment,
- 25 the antibody is secreted into the supernatant of the media in which the cell is growing. Traditionally, monoclonal antibodies have been produced as native molecules in murine hybridoma lines. In addition to that technology, the present invention provides for recombinant DNA expression of the antibodies. This allows the production of antibodies, as well as a spectrum of antibody derivatives and fusion proteins in a host species of choice.

- 30 [000107] A nucleic acid sequence encoding at least one antibody, portion or polypeptide of the invention may be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to

provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulations are disclosed, e.g., by Maniatis et al., MOLECULAR CLONING, LAB. MANUAL, (Cold Spring Harbor Lab. Press, NY, 1982 and 1989), and Ausubel et al. 1993
5 supra, may be used to construct nucleic acid sequences which encode an antibody molecule or antigen binding region thereof.

[000108] A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences
10 which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression as peptides or antibody portions in recoverable amounts. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, as is well known in the analogous art. See, e.g., Sambrook *et al.*, 2001 supra; Ausubel
15 *et al.*, 1993 supra.

[000109] The present invention accordingly encompasses the expression of an antibody or peptide, in either prokaryotic or eukaryotic cells. Suitable hosts include bacterial or eukaryotic hosts including bacteria, yeast, insects, fungi, bird and mammalian cells either in vivo, or in situ, or host cells of mammalian, insect, bird or yeast origin. The mammalian cell or tissue may
20 be of human, primate, hamster, rabbit, rodent, cow, pig, sheep, horse, goat, dog or cat origin. Any other suitable mammalian cell, known in the art, may also be used.

[000110] In one embodiment, the nucleotide sequence of the invention will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. See, e.g., Ausubel *et al.*, 1993 supra.
25 Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

30 [000111] Example prokaryotic vectors known in the art include plasmids such as those capable of replication in *E. coli* (such as, for example, pBR322, ColE1, pSC101, pACYC 184, .pi.vX). Such plasmids are, for example, disclosed by Maniatis et al., 1989 supra; Ausubel et al, 1993

supra. Bacillus plasmids include pC194, pC221, pT127, etc. Such plasmids are disclosed by Gryczan, in THE MOLEC. BIO. OF THE BACILLI 307-329 (Academic Press, NY, 1982). Suitable Streptomyces plasmids include p1J101 (Kendall et al., 169 J. Bacteriol. 4177-83 (1987), and Streptomyces bacteriophages such as phLC31 (Chater et al., in SIXTH INT'L SYMPOSIUM ON ACTINOMYCETALES BIO. 45-54 (Akademiai Kiado, Budapest, Hungary 1986). Pseudomonas plasmids are reviewed in John et al., 8 Rev. Infect. Dis. 693-704 (1986); Izaki, 33 Jpn. J. Bacteriol. 729-42 (1978); and Ausubel et al., 1993 supra.

[000112] Alternatively, gene expression elements useful for the expression of cDNA encoding antibodies or peptides include, but are not limited to, (a) viral transcription promoters and their enhancer elements, such as the SV40 early promoter (Okayama et al., 3 Mol. Cell. Biol. 280 (1983), Rous sarcoma virus LTR (Gorman et al., 79 Proc. Natl. Acad. Sci., USA 6777 (1982), and Moloney murine leukemia virus LTR (Grosschedl et al., 41 Cell 885 (1985); (b) splice regions and polyadenylation sites such as those derived from the SV40 late region (Okayama et al., 1983), and (c) polyadenylation sites such as in SV40 (Okayama et al., 1983).

[000113] Immunoglobulin cDNA genes can be expressed as described by Weidle *et al.*, 51 Gene 21 (1987), using as expression elements the SV40 early promoter and its enhancer, the mouse immunoglobulin H chain promoter enhancers, SV40 late region mRNA splicing, rabbit S-globin intervening sequence, immunoglobulin and rabbit S-globin polyadenylation sites, and SV40 polyadenylation elements. For immunoglobulin genes comprised of part cDNA, part genomic DNA (Whittle et al., 1 Protein Engin. 499 (1987)), the transcriptional promoter can be human cytomegalovirus, the promoter enhancers can be cytomegalovirus and mouse/human immunoglobulin, and mRNA splicing and polyadenylation regions can be the native chromosomal immunoglobulin sequences.

[000114] In one embodiment, for expression of cDNA genes in rodent cells, the transcriptional promoter is a viral LTR sequence, the transcriptional promoter enhancers are either or both the mouse immunoglobulin heavy chain enhancer and the viral LTR enhancer, the splice region contains an intron of greater than 31 bp, and the polyadenylation and transcription termination regions are derived from the native chromosomal sequence corresponding to the immunoglobulin chain being synthesized. In other embodiments, cDNA sequences encoding other proteins are combined with the above-recited expression elements to achieve expression of the proteins in mammalian cells.

[000115] Each fused gene can be assembled in, or inserted into, an expression vector. Recipient cells capable of expressing the immunoglobulin chain gene product are then transfected singly with a peptide or H or L chain-encoding gene, or are co-transfected with H and L chain gene. The transfected recipient cells are cultured under conditions that permit expression of the incorporated genes and the expressed immunoglobulin chains or intact antibodies or fragments are recovered from the culture.

[000116] In one embodiment, the fused genes encoding the peptide or H and L chains, or portions thereof are assembled in separate expression vectors that are then used to cotransfect a recipient cell. Alternatively the fused genes encoding the H and L chains can be assembled on the same expression vector. For transfection of the expression vectors and production of the antibody, the recipient cell line may be a myeloma cell. Myeloma cells can synthesize, assemble and secrete immunoglobulins encoded by transfected immunoglobulin genes and possess the mechanism for glycosylation of the immunoglobulin. Myeloma cells can be grown in culture or in the peritoneal cavity of a mouse, where secreted immunoglobulin can be obtained from ascites fluid. Other suitable recipient cells include lymphoid cells such as B lymphocytes of feline or non-feline origin, hybridoma cells of feline or non-feline origin, or interspecies heterohybridoma cells.

[000117] The expression vector carrying an antibody construct or polypeptide of the invention can be introduced into an appropriate host cell by any of a variety of suitable means, including such biochemical means as transformation, transfection, conjugation, protoplast fusion, calcium phosphate-precipitation, and application with polycations such as diethylaminoethyl (DEAE) dextran, and such mechanical means as electroporation, direct microinjection, and microprojectile bombardment. Johnston *et al.*, 240 *Science* 1538 (1988).

[000118] Yeast may provide substantial advantages over bacteria for the production of immunoglobulin H and L chains. Yeasts carry out post-translational peptide modifications including glycosylation. A number of recombinant DNA strategies now exist which utilize strong promoter sequences and high copy number plasmids which can be used for production of the desired proteins in yeast. Yeast recognizes leader sequences of cloned mammalian gene products and secretes peptides bearing leader sequences (i.e., pre-peptides). Hitzman *et al.*, 11th Int'l Conference on Yeast, Genetics & Molec. Biol. (Montpelier, France, 1982).

[000119] Yeast gene expression systems can be routinely evaluated for the levels of production, secretion and the stability of peptides, antibodies, fragments and regions thereof. Any of a

series of yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeasts are grown in media rich in glucose can be utilized. Known glycolytic genes can also provide very efficient transcription control signals. For example, the promoter and terminator
5 signals of the phosphoglycerate kinase (PGK) gene can be utilized. A number of approaches can be taken for evaluating optimal expression plasmids for the expression of cloned immunoglobulin cDNAs in yeast. See Vol. II DNA Cloning, 45-66, (Glover, ed.) IRL Press, Oxford, UK 1985).

[000120] Bacterial strains can also be utilized as hosts for the production of antibody molecules
10 or peptides described by this invention. Plasmid vectors containing replicon and control sequences which are derived from species compatible with a host cell are used in connection with these bacterial hosts. The vector carries a replication site, as well as specific genes which are capable of providing phenotypic selection in transformed cells. A number of approaches can be taken for evaluating the expression plasmids for the production of antibodies, fragments
15 and regions or antibody chains encoded by the cloned immunoglobulin cDNAs in bacteria (see Glover, 1985 supra; Ausubel, 1993 supra; Sambrook, 2001 supra; Colligan et al., eds. Current Protocols in Immunology, John Wiley & Sons, NY, N.Y. (1994-2001); Colligan et al., eds. Current Protocols in Protein Science, John Wiley & Sons, NY, N.Y. (1997-2001).

[000121] Host mammalian cells may be grown in vitro or in vivo. Mammalian cells provide
20 posttranslational modifications to immunoglobulin protein molecules including leader peptide removal, folding and assembly of Heavy L chains, glycosylation of the antibody molecules, and secretion of functional antibody protein. Mammalian cells which can be useful as hosts for the production of antibody proteins, in addition to the cells of lymphoid origin described above, include cells of fibroblast origin, such as Vero (ATCC CRL 81) or CHO-K1 (ATCC CRL 61)
25 cells. Many vector systems are available for the expression of cloned peptides Heavy L chain genes in mammalian cells (see Glover, 1985 supra). Different approaches can be followed to obtain complete H2L2 antibodies. It is possible to co-express Heavy L chains in the same cells to achieve intracellular association and linkage of Heavy L chains into complete tetrameric H2L2 antibodies and/or peptides. The co-expression can occur by using either the same or
30 different plasmids in the same host. Genes for both Heavy L chains and/or peptides can be placed into the same plasmid, which is then transfected into cells, thereby selecting directly for cells that express both chains. Alternatively, cells can be transfected first with a plasmid encoding one chain, for example the L chain, followed by transfection of the resulting cell line with an

H chain plasmid containing a second selectable marker. cell lines producing peptides and/or H2L2 molecules via either route could be transfected with plasmids encoding additional copies of peptides, H, L, or H plus L chains in conjunction with additional selectable markers to generate cell lines with enhanced properties, such as higher production of assembled H2L2 antibody molecules or enhanced stability of the transfected cell lines.

[000122] For long-term, high-yield production of recombinant antibodies, stable expression may be used. For example, cell lines, which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with immunoglobulin expression cassettes and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into a chromosome and grow to form foci which in turn can be cloned and expanded into cell lines. Such engineered cell lines may be particularly useful in screening and evaluation of compounds/components that interact directly or indirectly with the antibody molecule.

[000123] Once an antibody of the invention has been produced, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In many embodiments, antibodies are secreted from the cell into culture medium and harvested from the culture medium.

Pharmaceutical and Veterinary Applications

[000124] The invention also provides a pharmaceutical composition comprising molecules of the invention and one or more pharmaceutically acceptable carriers. More specifically, the invention provides for a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, an antibody or peptide according to the invention.

[000125] "Pharmaceutically acceptable carriers" include any excipient which is nontoxic to the cell or animal being exposed thereto at the dosages and concentrations employed. The pharmaceutical composition may include one or additional therapeutic agents.

[000126] "Pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for contact with the tissues of animals without excessive toxicity, irritation, allergic response, or other problem complications commensurate with a reasonable benefit/risk ratio.

5 [000127] Pharmaceutically acceptable carriers include solvents, dispersion media, buffers, coatings, antibacterial and antifungal agents, wetting agents, preservatives, buffers, chelating agents, antioxidants, isotonic agents and absorption delaying agents.

[000128] Pharmaceutically acceptable carriers include water; saline; phosphate buffered saline; dextrose; glycerol; alcohols such as ethanol and isopropanol; phosphate, citrate and other
10 organic acids; ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; EDTA; salt forming counterions such as sodium; and/or nonionic surfactants such as
15 TWEEN, polyethylene glycol (PEG), and PLURONICS; isotonic agents such as sugars, polyalcohols such as mannitol and sorbitol, and sodium chloride; as well as combinations thereof.

[000129] The pharmaceutical compositions of the invention may be formulated in a variety of ways, including for example, liquid, semi-solid, or solid dosage forms, such as liquid solutions
20 (e.g., injectable and infusible solutions), dispersions or suspensions, liposomes, suppositories, tablets, pills, or powders. In some embodiments, the compositions are in the form of injectable or infusible solutions. The composition can be in a form suitable for intravenous, intraarterial, intramuscular, subcutaneous, parenteral, transmucosal, oral, topical, or transdermal administration. The composition may be formulated as an immediate, controlled, extended or
25 delayed release composition.

[000130] The compositions of the invention can be administered either as individual therapeutic agents or in combination with other therapeutic agents. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice. Administration of the
30 antibodies disclosed herein may be carried out by any suitable means, including parenteral injection (such as intraperitoneal, subcutaneous, or intramuscular injection), orally, or by topical administration of the antibodies (typically carried in a pharmaceutical formulation) to

an airway surface. Topical administration to an airway surface can be carried out by intranasal administration (e.g., by use of dropper, swab, or inhaler). Topical administration of the antibodies to an airway surface can also be carried out by inhalation administration, such as by creating respirable particles of a pharmaceutical formulation (including both solid and liquid particles) containing the antibodies as an aerosol suspension, and then causing the subject to inhale the respirable particles. Methods and apparatus for administering respirable particles of pharmaceutical formulations are well known, and any conventional technique can be employed.

[000131] In some desired embodiments, the antibodies are administered by parenteral injection. For parenteral administration, antibodies or molecules can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable parenteral vehicle. For example, the vehicle may be a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, such as an aqueous carrier such vehicles are water, saline, Ringer's solution, dextrose solution, trehalose or sucrose solution, or 5% serum albumin, 0.4% saline, 0.3% glycine and the like. Liposomes and nonaqueous vehicles such as fixed oils can also be used. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjustment agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, for example from less than about 0.5%, usually at or at least about 1% to as much as 15% or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, REMINGTON'S PHARMA. SCI. (15th ed., Mack Pub. Co., Easton, Pa., 1980).

[000132] The antibodies or molecules of the invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immune globulins. Any suitable lyophilization and reconstitution techniques

can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss and that use levels may have to be adjusted to compensate. The compositions containing the present antibodies or a cocktail thereof can be administered for prevention of recurrence and/or therapeutic treatments for existing disease. Suitable pharmaceutical carriers are described in the most recent edition of REMINGTON'S PHARMACEUTICAL SCIENCES, a standard reference text in this field of art. In therapeutic application, compositions are administered to a subject already suffering from a disease, in an amount sufficient to cure or at least partially arrest or alleviate the disease and its complications.

10 [000133] Effective doses of the compositions of the present invention, for treatment of conditions or diseases as described herein vary depending upon many different factors, including, for example, but not limited to, the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; target site; physiological state of the animal; other medications administered; whether treatment is prophylactic or therapeutic; age, 15 health, and weight of the recipient; nature and extent of symptoms kind of concurrent treatment, frequency of treatment, and the effect desired.

[000134] Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating veterinarian. In any event, the pharmaceutical formulations should provide a quantity of the antibody(ies) of this invention sufficient to 20 effectively treat the subject.

[000135] Treatment dosages may be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

[000136] The pharmaceutical compositions of the invention may include a “therapeutically effective amount.” A “therapeutically effective amount” refers to an amount effective, at 25 dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of a molecule may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the molecule to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the molecule are outweighed by the therapeutically beneficial effects. 30

[000137] In another aspect, the compositions of the invention can be used, for example, in the treatment of various diseases and disorders in cats. As used herein, the terms “treat” and

“treatment” refer to therapeutic treatment, including prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change associated with a disease or condition. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of the extent of a disease or condition, stabilization of a disease or condition (i.e., where the disease or condition does not worsen), delay or slowing of the progression of a disease or condition, amelioration or palliation of the disease or condition, and remission (whether partial or total) of the disease or condition, whether detectable or undetectable. Those in need of treatment include those already with the disease or condition as well as those prone to having the disease or condition or those in which the disease or condition is to be prevented.

[000138] All patents and literature references cited in the present specification are hereby incorporated by reference in their entirety.

[000139] The following examples are provided to supplement the prior disclosure and to provide a better understanding of the subject matter described herein. These examples should not be considered to limit the described subject matter. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be apparent to persons skilled in the art and are to be included within, and can be made without departing from, the true scope of the invention.

EXAMPLES

EXAMPLE 1

Construction of feline IgG Fc mutants

[000140] Construction of all feline IgGs (**Fig. 1**) was carried out as described by Strietzel *et al.* (Strietzel *et al.*, 2014, *Vet Immunol Immunopathol.*, vol. 158(3-4), pages 214-223), in which plasmids containing sequence encoding for feline constant regions for the IgG sub-class 1 Allele a (IgG1a) were utilized and VH/VL sequences for each mAb investigated herein were inserted upstream and in frame with the nucleotides encoding for the constant domains. Mutations were incorporated into each respective position of the CH1, CH2 or CH3 domain (**Fig. 2**) of each plasmid by direct DNA synthesis of the constant region as gene fragment and were subsequently sub-cloned into respective variable region of interest.

Expression and Purification

[000141] The monoclonal antibody (mAbs) mutants were expressed in mammalian suspension cell systems, EXPICHO-S (Chinese Hamster Ovary) cells, obtained from Thermo Fisher. Suspension EXPICHO-S cells were maintained in EXPICHO expression medium (Gibco) between 0.14 and 8.0x10⁶ cells/ml. Cells were diluted following the ExpiCHO Protocol user manual on Day -1 and transfection day. Diluted cells were transfected as described in the protocol using reagents sourced from ExpiFectamine CHO Transfection Kit (Gibco) following Max Titer conditions. Following 12-14 days of incubation, the cultures were harvested and clarified. Antibodies were purified from the clarified supernatant via Protein A chromatography over MabSelect Sure LX (GE Healthcare) which had been pre-equilibrated with PBS. Following sample load, the resin was washed with PBS and then with 20 mM sodium acetate, pH 5.5. Samples were eluted from the column with 20 mM acetic acid, pH 3.5. Following elution, pools were made and neutralized with the addition of 1 M sodium acetate to 4%. Depending on available volume and intended use, samples were sometimes exchanged into a final buffer (e.g. PBS, other). Concentration was measured by absorbance at 280 nm.

SDS-PAGE

[000142] Non-reduced (nr) and reduced sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) was performed using 4-12% Bis-Tris NuPAGE gels in MES-SDS running buffer, and SeeBlue Plus 2 standards, all from Invitrogen. For non-reduced samples, 1mM of alkylating agent N-ethylmaleimide (NEM) was added, for reduced samples reducing agent dithiothreitol (DTT) was added. Gels were stained with Coomassie Blue to detect the protein bands.

Analytical SEC

[000143] Analytical SEC was conducted using a TSK gel SuperSW3000, 4.6mm, 10x30 cm, 4µm column from TOSOH BioScience, in 200mM NaPhosphate pH 7.2 running buffer at 0.25ml/minute.

NR-CGE

[000144] Non-reduced capillary gel electrophoresis (nrCGE) was performed using a Beckman Coulter PA800 plus analyzer using an A55625 capillary cartridge per the manufacture's instructions.

SMAC

[000145] Standup monolayer absorption chromatography (SMAC) was conducted using a Sepax Zenix SEC-300, 4.6X300mm column in 200mM Na Phosphate pH 7.2 running buffer at 0.35ml/min.

5 HIC

[000146] Hydrophobic interaction chromatography (HIC) was conducted using a Sepax Proteomix HIC Butyl-NP5, 4.6x100mm column. A linear gradient from 100% 1.8M Ammonium Sulfate in 0.1M Na Phosphate pH 6.5 to 100% 0.1M Na Phosphate pH 6.5 was applied at 0.75ml/min for 20min.

10 Octet – BLI

[000147] This assay was conducted using Forte Bio's Octet QKe with Amine Reactive Second-Generation Biosensors. Samples are exchanged into 1x Gibco PBS without calcium and magnesium and diluted to a concentration of 0.5mg/mL. After establishing the biosensor's baseline, a biosensor is submerged into 100uL of the sample for 600sec.

15 [000148] Antibodies were screened for binding to protein A sensors via Octet QKe quantitation (Pall ForteBio Corp, Menlo Park, CA, USA). Constructs which bound to protein A were purified and quantified as described in Strietzel *et al.* for protein quality.

EXAMPLE 2

FcRn Binding assay

20 [000149] Feline FcRn was isolated, prepared and mutant Fc IgGs were assayed against feline FcRn according to Strietzelg *et.al*. Standard RACE PCR was used to amplify feline FcRn- α subunit and β -microglobulin. FcRn- α subunit and β -microglobulin were co-transfected into HEK 293 cells and the FcRn complex was purified by IMAC affinity purification via the c-terminal His tag. FcRn complex was biotin labeled through BirA enzymatic biotinylation reaction. KD's were measured by Biacore 3000 or Biacore T200 (GE Healthcare, Pittsburgh, PA, USA) using a SA sensor chip.

[000150] FcRn was captured on the surface of the sensor using a modified SA capture method. 10mM MES; 150mM NaCl; 0.005% Tween20; 0.5 mg/mL BSA; pH6 was used as capture, method running running buffer and titrations. 1x HBS-P, 0.5 mg/mL BSA; pH7.4 was also
30 used for method running buffer and titrations. Fc mutant IgGs were flowed over receptor

surfaces and affinity was determined using Scrubber2 software analysis (BioLogic Software Pty, Ltd., Campbell, Australia) or T200 evaluation software (**Tables 1 and 4**). Blank runs containing buffer only were subtracted out from all runs. Flow cells were regenerated using 50 mM Tris pH8. Runs were performed at 15 °C.

- 5 [000151] Mutations made at respective positions have a marked effect on the affinity of the IgG to FcRn at pH6. Increase in FcRn affinity for IgG is not dependent on the VHVL domains, and is universal for any feline IgG1a.

[000152] Binding of wild-type (WTs) and mutant IgGs to Feline FcRn were measured by surface plasmon resonance (Biacore). Biophysical Characterizations were also performed.

- 10 Table 1. Effect of mutants on FcRn binding affinity.

ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	KD at pH6	KD at pH7.4
0	WT						2.02E-08	NBO
	Buffer						NBO	NBO
1	S252R						1.48E-09	5.06E-09
2	S434A						4.49E-09	NBO
3	S434C						1.18E-08	NBO
4	S434D						5.66E-09	NBO
5	S434E						3.35E-09	NBO
6	S434F						7.45E-10	2.10E-08
7	S434G						9.58E-09	2.16E-08
8	S434H						1.02E-09	NBO
9	S434I						1.44E-08	NBO
10	S434K						6.76E-09	NBO
11	S434L						8.67E-09	NBO
12	S434M						2.80E-09	NBO
13	S434N						6.94E-09	NBO
14	S434P						5.64E-09	NBO
15	S434Q						4.80E-09	NBO
16	S434R						1.19E-09	1.63E-08
17	S434T						1.06E-08	NBO
18	S434V						3.71E-09	NBO
19	S434W						7.71E-10	2.16E-08
20	S434Y						4.33E-10	1.44E-08
21	D312A						6.95E-09	8.18E-06
22	D312H						7.33E-09	NBO
23	D312K						5.86E-09	5.61E-08
24	D312R						6.91E-09	2.50E-08
25	D399M						5.33E-09	4.68E-08

ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	KD at pH6	KD at pH7.4
26	D399T						6.75E-09	NBO
27	D399V						8.29E-09	2.88E-06
28	D401R						4.77E-09	NBO
29	G402R						6.10E-09	1.20E-06
30	L309G						9.12E-09	5.45E-08
31	L309I						6.81E-09	4.53E-06
32	Q311F						8.85E-09	1.37E-06
33	Q311H						5.84E-09	9.37E-08
34	Q311I						7.00E-09	7.71E-08
35	Q311K						4.85E-09	1.75E-06
36	Q311L						6.38E-09	NBO
37	Q311M						4.97E-09	5.10E-06
38	Q311R						4.56E-09	6.72E-08
39	T403R						6.22E-09	8.40E-07
40	Y404Q						9.96E-09	9.94E-06
41	A431Q						6.64E-09	NBO
42	A431R						6.73E-09	NBO
43	A431V						7.08E-09	NBO
44	D249A						5.41E-09	NBO
45	D249E						5.84E-09	NBO
46	D249S						5.24E-09	NBO
47	E430I						5.89E-09	NBO
48	E430Q						6.58E-09	NBO
49	H436G						1.09E-08	NBO
50	H436K						6.91E-09	NBO
51	H436M						8.25E-09	NBO
52	H436R						5.69E-09	NBO
53	L432S						6.58E-09	NBO
54	P247I						4.51E-09	NBO
55	P247L						4.72E-09	NBO
56	P247V						5.21E-09	NBO
57	T250E						6.07E-09	NBO
58	T250I						4.66E-09	NBO
59	T250Q						4.35E-09	NBO
60	T250S						4.94E-09	NBO
61	T250V						6.87E-09	NBO
62	T437A						8.79E-09	NBO
63	T437R						6.85E-09	NBO
64	Y285A						8.01E-09	NBO
65	A378C						7.73E-08	ND
66	A378D						1.00E-07	ND
67	A378E						6.36E-08	ND
68	A378F						7.48E-08	ND

ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	KD at pH6	KD at pH7.4
69	A378G						8.83E-08	ND
70	A378H						4.93E-08	ND
71	A378I						5.83E-08	ND
72	A378K						1.04E-07	ND
73	A378L						3.47E-08	ND
74	A378M						2.66E-08	ND
75	A378N						2.86E-08	ND
76	A378P						6.84E-08	ND
77	A378Q						4.90E-08	ND
78	A378R						3.62E-08	ND
79	A378S						4.83E-08	ND
80	A378T						5.92E-08	ND
81	A378V						4.29E-08	ND
82	A378W						9.10E-08	ND
83	A378Y						6.96E-08	ND
84	S252A						2.71E-08	ND
85	S252C						9.22E-07	ND
86	S252D						9.35E-08	ND
87	S252E						1.63E-07	ND
88	S252F						1.23E-07	5.96E-06
89	S252G						4.98E-08	ND
90	S252H						4.99E-08	8.41E-06
91	S252I						1.56E-07	ND
92	S252K						5.37E-08	ND
93	S252L						7.07E-08	ND
94	S252N						4.00E-07	ND
95	S252P						7.03E-06	ND
96	S252Q						1.92E-07	ND
97	S252R	S428L	S434F				6.43E-09	7.74E-07
98	S252T						1.19E-06	ND
99	S252V						4.47E-06	ND
100	S252Y						8.93E-07	1.27E-05
101	S254A						5.30E-08	ND
102	S254D						4.74E-07	ND
103	S254E						7.11E-10	ND
104	S254F						5.68E-07	ND
105	S254G						6.42E-08	ND
106	S254H						1.85E-06	ND
107	S254K						4.65E-08	ND
108	S254L						4.27E-08	ND
109	S254M						1.01E-07	ND
110	S428A						1.19E-07	ND
111	S428C						6.14E-08	ND

ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	KD at pH6	KD at pH7.4
112	S428D						3.62E-07	ND
113	S428E						1.84E-07	ND
114	S428F						8.39E-08	ND
115	S428G						4.63E-08	ND
116	S428H						4.16E-08	ND
117	S428I						4.08E-08	ND
118	S428K						7.02E-08	ND
119	S434Y	S434F					3.61E-09	9.48E-08
120	S428L	S434Y					2.75E-09	4.69E-08
121	S428N						4.29E-08	ND
122	S428P						1.80E-08	ND
123	S428Q						3.83E-08	ND
124	S428R						6.86E-08	ND
125	S428T						2.73E-08	ND
126	S428V						1.92E-08	ND
127	S428W						3.64E-08	ND
128	S428Y						1.64E-08	1.87E-05
129	A431K						1.01E-08	9.39E-09
130	D312P						3.96E-09	2.96E-06
131	H436Y						1.02E-08	4.34E-08
132	L314K						8.43E-09	4.15E-08
133	Q311W						4.18E-09	2.07E-07
134	Q311Y						5.53E-09	4.14E-08
135	S252F	S434H					1.69E-09	1.81E-08
136	S252M						5.50E-08	NBO
137	S252R	S434H					2.21E-09	4.13E-08
138	S252W						8.03E-08	NBO
139	S252Y	S434H					7.74E-10	1.17E-08
140	S254C						4.40E-08	2.16E-06
141	S254I						1.24E-08	1.61E-05
142	S428L						8.15E-08	NBO
143	S428M						2.18E-08	NBO
144	S254N						2.57E-08	NBO
145	S254P						2.73E-08	5.69E-06
146	S254Q						2.47E-08	NBO
147	S254R						2.15E-08	NBO
148	S254T						1.61E-08	NBO
149	S254V						9.91E-09	4.98E-05
150	S254W						6.09E-08	NBO
151	S254Y						7.85E-08	NBO
152	T256A						4.06E-08	NBO
153	T256C						1.54E-07	NBO
154	T256D						2.44E-07	NBO

ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	KD at pH6	KD at pH7.4
155	T256E						5.09E-08	NBO
156	T256F						5.03E-08	4.27E-07
157	T256G						7.69E-08	NBO
158	T256H						8.74E-08	NBO
159	T256I						1.15E-07	NBO
160	T256K						1.07E-07	NBO
161	T256L						8.65E-08	NBO
162	T256M						1.93E-08	NBO
163	T256N						7.74E-08	NBO
164	T256P						5.76E-08	NBO
165	T256Q						5.86E-08	NBO
166	T256R						2.77E-08	NBO
167	T256V						1.95E-08	NBO
168	T256W						2.52E-08	NBO
169	T256Y						2.20E-08	NBO
170	D312P	S434Y					2.26E-08	1.80E-06
171	L314K	S434Y					2.44E-07	7.43E-06
172	Q311W	D312P					2.76E-09	5.03E-08
173	Q311W	D312P	L314K				1.23E-06	NBO
174	Q311W	D312P	S434Y				1.53E-09	3.77E-08
175	Q311W	L314K					3.47E-09	5.58E-08
176	Q311W	S434Y					2.70E-08	NBO
177	S252F	S434F					1.73E-07	2.35E-06
178	S252F	S434W					6.15E-09	1.37E-06
179	S252F	S434Y					9.28E-09	1.47E-06
180	S252Y	D312P	L314K				2.67E-08	1.82E-06
181	S252Y	L314K					1.09E-08	NBO
182	S252Y	Q311W					2.69E-08	9.53E-07
183	S252Y	Q311W	D312P				3.52E-08	1.13E-06
184	S252Y	Q311W	L314K				1.08E-08	1.79E-07
185	S252Y	Q311W	S434Y				9.72E-08	NBO
186	S252Y	S428Y					1.72E-08	NBO
187	S252Y	S428Y	D312P				1.51E-08	4.08E-06
188	S252Y	S428Y	L314K				7.93E-09	2.33E-05
189	S252Y	S428Y	Q311W				6.77E-08	NBO
190	S252Y	S428Y	S434Y				7.81E-08	NBO
191	S252Y	S434F					1.97E-06	NBO
192	S252Y	S434F	Q311W				1.64E-07	NBO
193	S252Y	S434H	Q311W				8.39E-09	9.89E-07
194	S252Y	S434R	Q311W				1.43E-10	4.44E-09
195	S252Y	S434W					1.02E-08	8.38E-06
196	S252Y	S434W	Q311W				6.59E-09	2.19E-06
197	S252Y	S434Y					1.16E-07	NBO

ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	KD at pH6	KD at pH7.4
198	S428Y	D312P	L314K				1.52E-08	6.86E-06
199	S428Y	L314K					7.63E-09	1.85E-06
200	S428Y	L314K	S434Y				7.93E-10	1.72E-08
201	S428Y	Q311W					1.27E-08	7.63E-06
202	S428Y	Q311W	D312P				2.60E-08	4.19E-06
203	S428Y	Q311W	L314K				1.01E-08	3.48E-07
204	S428Y	S434Y					4.65E-09	2.35E-07
205	T256S						2.14E-06	NBO
206	D312P	L314K					7.24E-09	3.11E-07
207	S252F	S434R					3.59E-09	3.98E-07
208	S252Y	D312P					1.05E-08	NBO
209	S252Y	D312P	S434Y				2.21E-11	2.26E-09
210	S252Y	L314K	S434Y				4.96E-11	2.85E-09
211	S252Y	S434R					4.12E-09	1.09E-05
212	S428Y	D312P					3.41E-08	2.76E-06
213	S428Y	D312P	S434Y				1.48E-09	8.64E-07
214	S428Y	Q311W	S434Y				3.80E-09	1.51E-06
215	S252H	S428M	D312P				3.24E-09	4.12E-08
216	S252H	D312P					3.90E-09	2.67E-08
217	S252H	L314K					6.37E-09	2.00E-08
218	S252H	Q311W					6.04E-09	2.50E-08
219	S252H	Q311Y					1.21E-08	3.65E-08
220	S252H	S428M					2.65E-09	1.01E-07
221	S252H	S428M	D312P				8.22E-09	3.30E-07
222	S252H	S428M	Q311W				3.14E-09	5.46E-08
223	S252H	S428M	Q311Y				5.73E-09	1.41E-07
224	S252H	S428M,	S434F				1.21E-10	2.69E-08
225	S252H	S428M	S434L				8.59E-09	3.55E-09
226	S252H	S428M	S434P				7.45E-09	5.56E-08
227	S252H	S428M	S434W				1.35E-10	2.08E-08
228	S252H	S428M	S434Y				1.38E-10	2.07E-08
229	S252H	S434F					3.91E-10	2.41E-08
230	S252H	S434L					6.08E-09	3.79E-09
231	S252H	S434P					9.32E-09	1.28E-08
232	S252H	S434W					4.69E-10	3.11E-08
233	S252H	S434Y					1.4E-10	1.25E-08
234	S252M	S428M					7.27E-09	1.95E-08
235	S252M	S428M	S434N				8.51E-09	1.53E-08
236	S252M	S428M	S434N	H436Y			2.05E-08	2.87E-08
237	S252M	S434N					1.48E-08	3.38E-08
238	S252Y	S428M					2.58E-09	3.92E-08

ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	KD at pH6	KD at pH7.4
239	S252Y	S428M	D312P				7.96E-10	2.53E-08
240	S252Y	S428M	L314K				2.13E-09	2.50E-08
241	S252Y	S428M	Q311W				5.06E-10	1.88E-08
242	S252Y	S428M	Q311Y				2.40E-09	3.99E-08
243	S252Y	S428M	S434F				2.07E-11	9.13E-09
244	S252Y	S428M	S434L				6.91E-09	2.89E-08
245	S252Y	S428M	S434P				4.86E-09	4.44E-08
246	S252Y	S428M	S434W				4.06E-11	7.91E-09
247	S252Y	S428M	S434Y				9.02E-11	7.65E-09
248	S428M	D312P					5.70E-09	2.37E-08
249	S428M	L314K					9.78E-10	1.93E-05
250	S428M	Q311W					5.56E-09	3.32E-08
251	S428M	Q311Y					5.83E-09	2.03E-08
252	S428M	S434F					1.90E-10	2.74E-08
253	S428M	S434L					8.00E-09	NBO
254	S428M	S434N					1.06E-08	8.83E-09
255	S428M	S434P					7.53E-09	NBO
256	S428M	S434W					1.93E-10	3.16E-08
257	S428M	S434Y					2.64E-10	2.19E-08
258	WT 2						3.92E-08	NBO
259	S428L						2.45E-08	NBO
260	S252Y	S254T	T256E				1.32E-08	6.65E-08
261	WT 3						8.18E-08	NBO
262	S428L						2.54E-08	NBO
263	S252Y	S254T	T256E				1.40E-08	NBO
264	D312P	L314K	S434Y				7.17E-08	NBO
265	Q311W	D312P	L314K	S434Y			7.95E-09	NBO
266	Q311W	L314K	S434Y				2.80E-09	3.88E-07
267	S252F	S434F	Q311W				4.15E-09	4.03E-06
268	S252F	S434H	Q311W				1.43E-08	1.07E-05
269	S252F	S434R	Q311W				1.13E-08	1.52E-06
270	S252F	S434W	Q311W				3.68E-09	2.64E-06
271	S252F	S434Y	Q311W				9.69E-11	6.21E-09
272	S252R	S434F					7.96E-09	1.19E-05
273	S252R	S434F	Q311W				4.77E-09	4.76E-06
274	S252R	S434H	Q311W				5.62E-09	3.85E-06
275	S252R	S434R					7.86E-08	NBO
276	S252R	S434R	Q311W				2.87E-08	1.48E-05
277	S252R	S434W					7.10E-09	6.60E-06
278	S252R	S434H	Q311W				5.45E-09	5.91E-06
279	S252R	S434Y					1.08E-08	1.21E-05
280	S252R	S434Y	Q311W				2.87E-09	4.40E-06
281	S252Y	D312P	L314K	S434Y			2.35E-09	1.59E-06

ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	KD at pH6	KD at pH7.4
282	S252Y	Q311W	D312P	L314K			3.00E-08	1.19E-07
283	S252Y	Q311W	D312P	L314K	S434Y		1.14E-09	3.00E-07
284	S252Y	Q311W	D312P	S434Y			7.27E-09	NBO
285	S252Y	Q311W	L314K	S434Y			1.21E-09	1.73E-06
286	S252Y	S428Y	D312P	L314K			2.70E-08	NBO
287	S252Y	S428Y	D312P	L314K	S434Y		6.33E-10	1.27E-07
288	S252Y	S428Y	D312P	S434Y			2.68E-11	3.22E-09
289	S252Y	S428Y	L314K	S434Y			8.08E-10	1.32E-07
290	S252Y	S428Y	Q311W	D312P			3.08E-09	2.54E-07
291	S252Y	S428Y	Q311W	D312P	L314K		3.97E-08	1.67E-05
292	S252Y	S428Y	Q311W	D312P	L314K	S434Y	4.01E-09	2.72E-06
293	S252Y	S428Y	Q311W	D312P	S434Y		1.07E-09	2.93E-06
294	S252Y	S428Y	Q311W	L314K			6.92E-10	1.28E-08
295	S252Y	S428Y	Q311W	L314K	S434Y		6.75E-10	2.01E-07
296	S252Y	S428Y	Q311W	S434Y			5.44E-10	1.48E-06
297	S428Y	D312P	L314K	S434Y			8.98E-10	8.29E-08
298	S428Y	Q311W	D312P	L314K			2.53E-08	NBO
299	S428Y	Q311W	D312P	L314K	S434Y		1.17E-09	7.96E-08
300	S428Y	Q311W	D312P	S434Y			2.35E-10	1.82E-07
301	S428Y	Q311W	L314K	S434Y			1.11E-09	4.94E-07
302	A431K	S434P					2.17E-09	NBO
303	A431K	S434Y					1.33E-09	2.63E-08
304	D399M	D401R					5.43E-09	NBO
305	D399M	D401R	T403R				3.11E-03	NBO
306	D399M	T403R					1.28E-08	NBO
307	D401R	T403R					1.59E-08	NBO
308	P247V	S252H					5.61E-09	1.79E-08
309	P247V	T250Q					9.60E-09	NBO
310	P247V	T250Q	S252H				8.21E-09	2.44E-08
311	Q311H	D312P	L314K				1.44E-09	NBO
312	Q311K	D312P	L314K				6.07E-09	NBO
313	Q311M	D312P	L314K				1.89E-08	NBO
314	Q311R	D312P	L314K				2.13E-06	NBO
315	Q311Y	D312P	L314K				1.14E-08	NBO
316	S428M	A431K					4.87E-09	2.42E-06
317	S428M	A431K	S434P				2.22E-08	1.56E-08
318	S428M	A431K	S434Y				1.35E-10	2.53E-08
319	S428Y	D312P	A431K	S434P			6.96E-09	1.08E-06
320	S428Y	D312P	S434P				2.27E-08	NBO
321	S428Y	D312P	S434Y	A431K			5.39E-11	1.02E-08
322	S428Y	D312P	S434Y	D399M	D401R		4.02E-12	NBO
323	S428Y	D312P	S434Y	D399M	D401R	T403R	1.29E-13	1.30E-06
324	S428Y	D312P	S434Y	D399M	T403R		1.13E-10	2.16E-08

ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	KD at pH6	KD at pH7.4
325	S428Y	D312P	S434Y	D401R	T403R		7.20E-12	1.11E-08
326	S428Y	D312P	S434Y	P247V	S252H		6.27E-11	1.37E-08
327	S428Y	D312P	S434Y	P247V	T250Q		2.21E-10	2.74E-08
328	S428Y	D312P	S434Y	P247V	T250Q	S252H	4.18E-11	8.68E-09
329	S428Y	D312P	S434Y	Q311H	L314K		3.38E-10	1.89E-08
330	S428Y	D312P	S434Y	Q311K	L314K		9.06E-12	NBO
331	S428Y	D312P	S434Y	Q311M	L314K		2.59E-10	1.46E-08
332	S428Y	D312P	S434Y	Q311R	L314K		3.32E-10	2.86E-08
333	S428Y	D312P	S434Y	Q311Y	L314K		9.08E-10	2.59E-08
334	S428Y	D312P	S434Y	T250Q	S252H		1.82E-10	1.99E-08
335	T250Q	S252H					8.02E-09	3.69E-08
336	S252Y	S428M	S434F	A431K			3.59E-11	7.28E-09
337	S252Y	S428M	S434F	D312H			2.72E-11	8.96E-09
338	S252Y	S428M	S434F	D399T			2.72E-11	1.38E-08
339	S252Y	S428M	S434F	P247L			1.68E-11	6.73E-09
340	S252Y	S428M	S434F	Q311R			2.50E-11	3.30E-09
341	S252Y	S428M	S434F	D312A			3.53E-11	6.50E-09
342	S252Y	S428M	S434F	D312P			6.30E-12	3.29E-09
343	S252Y	S428M	S434F	D399M			4.10E-11	1.09E-08
344	S252Y	S428M	S434F	D401R			1.28E-11	1.10E-08
345	S252Y	S428M	S434F	E430I			2.27E-11	1.15E-08
346	S252Y	S428M	S434F	G402R			4.04E-11	9.95E-09
347	S252Y	S428M	S434F	L314K			5.67E-11	1.05E-08
348	S252Y	S428M	S434F	Q311H			9.49E-12	1.08E-08
349	S252Y	S428M	S434F	Q311K			1.92E-11	4.78E-09
350	S252Y	S428M	S434F	Q311M			8.79E-15	4.89E-09
351	S252Y	S428M	S434F	Q311W			1.10E-11	3.35E-09
352	S252Y	S428M	S434F	Q311Y			2.71E-11	6.48E-09
353	S252Y	S428M	S434F	S254V			1.97E-13	6.68E-09
354	S252Y	S428M	S434F	T250Q			1.92E-11	4.46E-09
355	S252Y	S428M	S434F	T256M			9.64E-11	1.57E-08
356	S252Y	S428M	S434F	T403R			2.49E-11	9.98E-09
357	S252Y	S434R	A431K				7.69E-10	2.59E-08
358	S252Y	S434R	A431K				7.48E-10	2.83E-08
359	S252Y	S434R	D312A				6.26E-10	2.53E-08
360	S252Y	S434R	D312H				1.09E-09	4.31E-08
361	S252Y	S434R	D312P				1.88E-10	2.07E-08
362	S252Y	S434R	D399M				1.14E-09	4.36E-08
363	S252Y	S434R	D399T				9.23E-10	3.89E-08
364	S252Y	S434R	D401R				7.32E-10	2.44E-08
365	S252Y	S434R	E430I				2.96E-10	2.89E-08
366	S252Y	S434R	E430I				3.22E-10	2.61E-08
367	S252Y	S434R	G402R				8.85E-10	4.48E-08

ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	KD at pH6	KD at pH7.4
368	S252Y	S434R	L314K				1.30E-09	2.56E-08
369	S252Y	S434R	P247L				4.42E-10	2.65E-08
370	S252Y	S434R	Q311H				9.74E-10	3.13E-08
371	S252Y	S434R	Q311K				4.88E-10	1.07E-08
372	S252Y	S434R	Q311M				4.43E-10	2.19E-08
373	S252Y	S434R	Q311R				3.65E-10	1.29E-08
374	S252Y	S434R	Q311Y				7.15E-10	3.03E-08
375	S252Y	S434R	S254V				5.51E-10	3.41E-08
376	S252Y	S434R	T250Q				3.94E-10	2.46E-08
377	S252Y	S434R	T256M				1.55E-09	2.56E-08
378	S252Y	S434R	T403R				8.59E-10	2.58E-08

Mutants are numbered according to the EU Index as in Kabat. NBO=no binding observed. ND=not determined/no data.

Table 2. Biophysical Characterization.

ID No.	Yield (mg/L)	SEC % Monomer	SEC RT (min)	CGE % Monomer	Polyreactivity	SMAC RT (min)
0	175.15	93.8	11.6	93.47	0.712	
6	49.984	94.55	12.1	92.4	0.264	8.538
16	83.264	95.25	11.6	95.6	0.610	8.285
19	27.968	91.14	12.1	92.55	0.296	9.214
20	34.56	97.11	11.7	91.99	0.291	8.5
88	27.264	95.97	11.6	91.64	0.433	8.328
90	30.464	96.91	11.6	88.95	0.404	8.322
100	63.36	91.93	11.6	93.74	0.944	8.312
128	64.64	96.77	11.6	93.57	0.408	8.331
130	41.792	97.93	11.6	93.02	0.529	8.306
132	0.832	N/A	N/A	N/A	N/A	N/A
133	42.816	96.11	11.8	94.08	0.219	8.547
143	154.98	94.75	11.6	93.31	0.707	
170	79.536	98.5	11.64		0.637	
170	44.8	98.08	11.68	94.27	N/A	
172	49.2	96.54	11.79		0.601	
172	29.696	98.5	11.84	94.28	N/A	
174	4.608	N/A	N/A	N/A	N/A	N/A
174	9.536	N/A	N/A	N/A	N/A	N/A
175	0.704	N/A	N/A	N/A	N/A	N/A
175	2.048	N/A	N/A	N/A	N/A	N/A
177	38.4	92.99	12.18	94.73	0.450	
178	14.336	N/A	N/A	N/A	N/A	N/A
178	73.984	98.24	13.12	94.5	0.455	
179	54.66	97.99	11.59		0.677	
179	54.784	99.05	11.6	94.28	N/A	

ID No.	Yield (mg/L)	SEC % Monomer	SEC RT (min)	CGE % Monomer	Polyreactivity	SMAC RT (min)
180	3.264	N/A	N/A	N/A	N/A	N/A
180	3.456	N/A	N/A	N/A	N/A	N/A
181	67.26	97.09	11.61		0.536	
181	25.536	98.3	11.63	95.54	N/A	
182	45.568	97.5	11.86		0.269	
182	44.608	98.94	11.86	95.2	N/A	
183	2.88	N/A	N/A	N/A	N/A	N/A
183	6.592	N/A	N/A	N/A	N/A	N/A
184	79.3	95.47	11.87		0.736	
184	37.76	97.63	11.9	95.02	N/A	
185	7.2	N/A	N/A	N/A	N/A	N/A
185	60.096	98.4	12.05	95.62	0.426	
186	115.38	95.85	11.63	94.26	0.735	
186	5.504	N/A	N/A	N/A	N/A	N/A
187	9.24	N/A	N/A	N/A	N/A	N/A
187	41.6	85.19	11.65	95.21	0.549	
188	40.384	94.23	11.7	93.22	0.187	8.313
188	18.112	N/A	N/A	N/A	N/A	N/A
188	36.544	98.5	11.64	95.52	0.372	
189	9.28	N/A	N/A	N/A	N/A	N/A
189	43.456	98.9	11.98	94.93	0.421	
190	9.9	96.93	11.8		N/A	
190	63.104	98.24	11.77	95.62	N/A	
193	85.1	96.03	12.03		0.773	
193	68.672	98.57	12.06	94.92	N/A	
194	98.56	97.28	11.91		0.723	
194	31.68	99.11	11.95	94.88	N/A	
195	51.2	89.02	13.1	95.28	0.729	
195	1.28	N/A	N/A	N/A	N/A	N/A
196	11.584	N/A	N/A	N/A	N/A	N/A
196	6.336	N/A	N/A	N/A	N/A	N/A
199	11.2	N/A	N/A		N/A	
199	7.232	N/A	N/A	N/A	N/A	N/A
200	23.744	N/A	N/A	93.56	N/A	
200	11.136	N/A	N/A	N/A	N/A	N/A
201	156.736	96.47	11.86		0.552	
201	7.808	N/A	N/A	N/A	N/A	N/A
202	16.704	N/A	N/A	93.15	N/A	
202	32.704	97.26	11.85	95.18	0.492	
203	20.48	N/A	N/A	91.26	N/A	
203	11.776	N/A	N/A	N/A	N/A	N/A
204	9.408	N/A	N/A	N/A	N/A	N/A
204	16.448	N/A	N/A	93.68	N/A	

ID No.	Yield (mg/L)	SEC % Monomer	SEC RT (min)	CGE % Monomer	Polyreactivity	SMAC RT (min)
206	0.448	N/A	N/A	N/A	N/A	N/A
207	65.984	97.9	11.6	95.68	0.905	8.271
208	41.344	98.71	11.6	95.68	1.000	8.311
209	27.968	97.74	11.7	92.98	0.557	8.434
210	65.728	96.76	11.7	92.64	0.728	8.44
211	42.368	97.7	11.6	95.18	0.918	8.275
213	28.48	95.2	11.8	90.61	0.721	8.512
214	39.232	96.94	12.3	92.13	0.353	10.394
215	46.2	96.8	11.6	93.3	0.596	
216	140.952	95.85	11.6	95.28	0.642	
217	144.15	97.44	11.6	95.29	0.529	
218	132.308	95.19	11.8	94.44	0.365	
220	191.464	96.59	11.6	93.61	0.488	
222	45.42	97.3	11.8	91.82	0.191	
224	29.952	91.18	12.2	91.02	0.480	8.584
226	73.92	95.79	11.6	84.17	0.148	
227	42.816	75.37	12.8	93.28	0.713	8.917
228	31.168	91.76	11.7	91.69	0.589	8.483
229	66.112	96.04	12.1	91.2	0.162	
231	86.4	97.34	11.6	91.95	0.203	
232	35.648	89.81	13	91.21	0.254	
233	82.56	96.8	11.6	91.63	0.115	
234	40.14	95.52	11.6	92.46	0.317	
238	110.72	95.17	11.6	92.25	0.465	
239	194.112	96.79	11.6	94.92	0.888	
241	235.328	94.51	11.8	94.72	0.549	
243	2.688	N/A	N/A	N/A	N/A	N/A
245	48.64	96.14	11.6	93	0.197	
246	2.88	N/A	N/A	N/A	N/A	N/A
247	21.312	N/A	N/A	95.4	N/A	N/A
248	37.62	97.44	11.6	91.91	0.313	
249	167.832	96.69	11.6	93.31	0.410	
250	140.096	95.88	11.8	92.63	0.385	
251	138.81	95.62	11.7	91.26	0.333	
252	72.768	93.2	12.2	92.86	0.683	8.561
253	80.384	95.81	11.7	91.9	0.259	
255	123.264	85.91	11.6	92.57	0.415	
256	20.672	N/A	N/A	92.46	N/A	N/A
257	47.936	95.97	11.7	93.72	0.243	8.471
266	111.42	98.34	12	95.15	0.435	
267	155.712	96.78	13.5	94.98	0.716	
268	20.9	N/A	N/A	94.52	N/A	
270	108.16	77.05	16.5	94.75	0.330	

ID No.	Yield (mg/L)	SEC % Monomer	SEC RT (min)	CGE % Monomer	Polyreactivity	SMAC RT (min)
271	105.12	97.25	11.9	94.77	0.396	
272	154.02	98.33	12.1	83.24	0.590	
273	114.05	89.38	13.7	88.03	0.530	
274	19.712	N/A	N/A	76.99	0.405	
276	113.45	98.35	11.8	85.09	0.562	
277	77.16	79.69	13.1	81.63	0.253	
278	126.85	53.21	16.6	86.53	0.448	
279	134.1	97.67	11.7	82.4	0.594	
280	119.424	98.8	12	88.95	0.355	
281	45.568	98.14	11.7	94.28	0.786	
282	31.04	99.29	11.8	93.67	0.829	
284	74.58	98.73	12	94.41	0.643	
285	61.8	95.96	12	92.86	0.424	
286	2.176	N/A	N/A	N/A	N/A	N/A
288	135.488	97.87	11.7	94.6	0.733	
289	63.36	97.42	11.7	93.84	0.217	
290	56.704	98.83	11.9	94.1	0.394	
294	146.64	98.83	11.9	94.71	0.731	
296	26.368	99.37	13.3	92.88	0.180	

For specific mutation numbers, refer to the corresponding ID numbers in Table 1. SEC = Size Exclusion Chromatography; CGE = Capillary Gel Electrophoresis; RT = Retention Time; SMAC = Standup Monolayer Absorption Chromatography.

- [000153] The data on biophysical characterization shows that a plurality of mutations exhibited improved biophysical properties, particularly on polyreactivity.

Table 3. Codons of Mutations in Tables 1 and 2.

ID No.	Mutations as per EU numbering system						Codon Usage					
	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
0	WT											
	Buffer											
1	S252R						CGG					
2	S434A						GCT					
3	S434C						TGT					
4	S434D						GAT					
5	S434E						GAA					
6	S434F						TTC					
7	S434G						GGT					
8	S434H						CAT					
9	S434I						ATT					
10	S434K						AAA					
11	S434L						CTG					
12	S434M						ATG					
13	S434N						AAC					
14	S434P						CCC					
15	S434Q						CAA					
16	S434R						AGG					
17	S434T						ACT					
18	S434V						GTT					
19	S434W						TGG					
20	S434Y						TAC					
21	D312A						GCC					
22	D312H						CAC					

Mutations as per EU numbering system							Codon Usage					
ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
23	D312K						AAG					
24	D312R						AGG					
25	D399M						ATG					
26	D399T						ACC					
27	D399V						GTG					
28	D401R						AGG					
29	G402R						AGG					
30	L309G						GGC					
31	L309I						ATC					
32	Q311F						TTC					
33	Q311H						CAC					
34	Q311I						ATC					
35	Q311K						AAG					
36	Q311L						CTG					
37	Q311M						ATG					
38	Q311R						AGG					
39	T403R						AGG					
40	Y404Q						CAG					
41	A431Q						CAA					
42	A431R						CGT					
43	A431V						GTT					
44	D249A						GCT					
45	D249E						GAA					
46	D249S						AGT					
47	E430I						ATT					
48	E430Q						CAA					

Mutations as per EU numbering system							Codon Usage					
ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
49	H436G						GGT					
50	H436K						AAA					
51	H436M						ATG					
52	H436R						CGT					
53	L432S						AGT					
54	P247I						ATT					
55	P247L						CTG					
56	P247V						GTT					
57	T250E						GAA					
58	T250I						ATT					
59	T250Q						CAG					
60	T250S						AGT					
61	T250V						GTT					
62	T437A						GCT					
63	T437R						CGT					
64	Y285A						GCC					
65	A378C						TGC					
66	A378D						GAC					
67	A378E						GAG					
68	A378F						TTC					
69	A378G						GGC					
70	A378H						CAC					
71	A378I						ATC					
72	A378K						AAG					
73	A378L						CTG					
74	A378M						ATG					

ID No.	Mutations as per EU numbering system						Codon Usage					
	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
75	A378N						AAC					
76	A378P						CCC					
77	A378Q						CAG					
78	A378R						CGG					
79	A378S						AGC					
80	A378T						ACC					
81	A378V						GTG					
82	A378W						TGG					
83	A378Y						TAC					
84	S252A						GCC					
85	S252C						TGC					
86	S252D						GAC					
87	S252E						GAG					
88	S252F						TTC					
89	S252G						GGC					
90	S252H						CAC					
91	S252I						ATC					
92	S252K						AAG					
93	S252L						CTG					
94	S252N						AAC					
95	S252P						CCC					
96	S252Q						CAG					
97	S252R	S428L	S434F				CGG	CTG	TTC			
98	S252T						ACC					
99	S252V						GTG					
100	S252Y						TAC					

Mutations as per EU numbering system							Codon Usage					
ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
101	S254A						GCC					
102	S254D						GAC					
103	S254E						GAG					
104	S254F						TTC					
105	S254G						GGC					
106	S254H						CAC					
107	S254K						AAG					
108	S254L						CTG					
109	S254M						ATG					
110	S428A						GCC					
111	S428C						TGC					
112	S428D						GAC					
113	S428E						GAG					
114	S428F						TTC					
115	S428G						GGC					
116	S428H						CAC					
117	S428I						ATC					
118	S428K						AAG					
119	S434Y	S434F					CTG	TTC				
120	S428L	S434Y					CTG	TAC				
121	S428N						AAC					
122	S428P						CCC					
123	S428Q						CAG					
124	S428R						CGG					
125	S428T						ACC					
126	S428V						GTG					

Mutations as per EU numbering system							Codon Usage					
ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
127	S428W						TGG					
128	S428Y						TAC					
129	A431K						AAA					
130	D312P						CCC					
131	H436Y						TAT					
132	L314K						AAG					
133	Q311W						TGG					
134	Q311Y						TAC					
135	S252F	S434H					TTC	CAT				
136	S252M						ATG					
137	S252R	S434H					CGG	CAT				
138	S252W						TGG					
139	S252Y	S434H					TAC	CAT				
140	S254C						TGC					
141	S254I						ATC					
142	S428L						CTG					
143	S428M						ATG					
144	S254N						AAC					
145	S254P						CCC					
146	S254Q						CAG					
147	S254R						CGG					
148	S254T						ACC					
149	S254V						GTG					
150	S254W						TGG					
151	S254Y						TAC					
152	T256A						GCC					

Mutations as per EU numbering system							Codon Usage					
ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
153	T256C						TGC					
154	T256D						GAC					
155	T256E						GAG					
156	T256F						TTC					
157	T256G						GGC					
158	T256H						CAC					
159	T256I						ATC					
160	T256K						AAG					
161	T256L						CTG					
162	T256M						ATG					
163	T256N						AAC					
164	T256P						CCC					
165	T256Q						CAG					
166	T256R						CGG					
167	T256V						GTG					
168	T256W						TGG					
169	T256Y						TAC					
170	D312P	S434Y					CCC	TAC				
171	L314K	S434Y					AAG	TAC				
172	Q311W	D312P					TGG	CCC				
173	Q311W	D312P	L314K				TGG	CCC	AAG			
174	Q311W	D312P	S434Y				TGG	CCC	TAC			
175	Q311W	L314K					TGG	AAG				
176	Q311W	S434Y					TGG	TAC				
177	S252F	S434F					TTC	TTC				
178	S252F	S434W					TTC	TGG				

Mutations as per EU numbering system							Codon Usage					
ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
179	S252F	S434Y					TTC	TAC				
180	S252Y	D312P	L314K				TAC	CCC	AAG			
181	S252Y	L314K					TAC	AAG				
182	S252Y	Q311W					TAC	TGG				
183	S252Y	Q311W	D312P				TAC	TGG	CCC			
184	S252Y	Q311W	L314K				TAC	TGG	AAG			
185	S252Y	Q311W	S434Y				TAC	TGG	TAC			
186	S252Y	S428Y					TAC	TAC				
187	S252Y	S428Y	D312P				TAC	TAC	CCC			
188	S252Y	S428Y	L314K				TAC	TAC	AAG			
189	S252Y	S428Y	Q311W				TAC	TAC	TGG			
190	S252Y	S428Y	S434Y				TAC	TAC	TAC			
191	S252Y	S434F					TAC	TTC				
192	S252Y	S434F	Q311W				TAC	TTC	TGG			
193	S252Y	S434H	Q311W				TAC	CAT	TGG			
194	S252Y	S434R	Q311W				TAC	AGG	TGG			
195	S252Y	S434W					TAC	TGG				
196	S252Y	S434W	Q311W				TAC	TGG	TGG			
197	S252Y	S434Y					TAC	TAC				
198	S428Y	D312P	L314K				TAC	CCC	AAG			
199	S428Y	L314K					TAC	AAG				
200	S428Y	L314K	S434Y				TAC	AAG	TAC			
201	S428Y	Q311W					TAC	TGG				
202	S428Y	Q311W	D312P				TAC	TGG	CCC			
203	S428Y	Q311W	L314K				TAC	TGG	AAG			
204	S428Y	S434Y					TAC	TAC				

ID No.	Mutations as per EU numbering system						Codon Usage					
	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
205	T256S						AGC					
206	D312P	L314K					CCC	AAG				
207	S252F	S434R					TTC	AGG				
208	S252Y	D312P					TAC	CCC				
209	S252Y	D312P	S434Y				TAC	CCC	TAC			
210	S252Y	L314K	S434Y				TAC	AAG	TAC			
211	S252Y	S434R					TAC	AGG				
212	S428Y	D312P					TAC	CCC				
213	S428Y	D312P	S434Y				TAC	CCC	TAC			
214	S428Y	Q311W	S434Y				TAC	TGG	TAC			
215	S252H	S428M	D312P				CAC	ATG	CCC			
216	S252H	D312P					CAC	CCC				
217	S252H	L314K					CAC	AAG				
218	S252H	Q311W					CAC	TGG				
219	S252H	Q311Y					CAC	TAC				
220	S252H	S428M					CAC	ATG				
221	S252H	S428M	D312P				CAC	ATG	CCC			
222	S252H	S428M	Q311W				CAC	ATG	TGG			
223	S252H	S428M	Q311Y				CAC	ATG	TAC			
224	S252H	S428M	S434F				CAC	ATG	TTC			
225	S252H	S428M	S434L				CAC	ATG	CTG			
226	S252H	S428M	S434P				CAC	ATG	CCC			
227	S252H	S428M	S434W				CAC	ATG	TGG			
228	S252H	S428M	S434Y				CAC	ATG	TAC			
229	S252H	S434F					CAC	TTC				
230	S252H	S434L					CAC	CTG				

ID No.	Mutations as per EU numbering system						Codon Usage					
	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
231	S252H	S434P					CAC	CCC				
232	S252H	S434W					CAC	TGG				
233	S252H	S434Y					CAC	TAC				
234	S252M	S428M					ATG	ATG				
235	S252M	S428M	S434N				ATG	ATG	AAC			
236	S252M	S428M	S434N	H436Y			ATG	ATG	AAC	TAT		
237	S252M	S434N					ATG	AAC				
238	S252Y	S428M					TAC	ATG				
239	S252Y	S428M	D312P				TAC	ATG	CCC			
240	S252Y	S428M	L314K				TAC	ATG	AAG			
241	S252Y	S428M	Q311W				TAC	ATG	TGG			
242	S252Y	S428M	Q311Y				TAC	ATG	TAC			
243	S252Y	S428M	S434F				TAC	ATG	TTC			
244	S252Y	S428M	S434L				TAC	ATG	CTG			
245	S252Y	S428M	S434P				TAC	ATG	CCC			
246	S252Y	S428M	S434W				TAC	ATG	TGG			
247	S252Y	S428M	S434Y				TAC	ATG	TAC			
248	S428M	D312P					ATG	CCC				
249	S428M	L314K					ATG	AAG				
250	S428M	Q311W					ATG	TGG				
251	S428M	Q311Y					ATG	TAC				
252	S428M	S434F					ATG	TTC				
253	S428M	S434L					ATG	CTG				
254	S428M	S434N					ATG	AAC				
255	S428M	S434P					ATG	CCC				
256	S428M	S434W					ATG	TGG				

ID No.	Mutations as per EU numbering system						Codon Usage					
	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
257	S428M	S434Y					ATG	TAC				
258	WT 2											
259	S428L						CTG					
260	S252Y	S254T	T256E				TAC					
261	WT 3											
262	S428L						CTG					
263	S252Y	S254T	T256E				TAC					
264	D312P	L314K	S434Y				CCC	AAG	TAC			
265	Q311W	D312P	L314K	S434Y			TGG	CCC	AAG	TAC		
266	Q311W	L314K	S434Y				TGG	AAG	TAC			
267	S252F	S434F	Q311W				TTC	TTC	TGG			
268	S252F	S434H	Q311W				TTC	CAT	TGG			
269	S252F	S434R	Q311W				TTC	AGG	TGG			
270	S252F	S434W	Q311W				TTC	TGG	TGG			
271	S252F	S434Y	Q311W				TTC	TAC	TGG			
272	S252R	S434F					CGG	TTC				
273	S252R	S434F	Q311W				CGG	TTC	TGG			
274	S252R	S434H	Q311W				CGG	CAT	TGG			
275	S252R	S434R					CGG	AGG				
276	S252R	S434R	Q311W				CGG	AGG	TGG			
277	S252R	S434W					CGG	TGG				
278	S252R	S434H	Q311W				CGG	CAT	TGG			
279	S252R	S434Y					CGG	TAC				
280	S252R	S434Y	Q311W				CGG	TAC	TGG			
281	S252Y	D312P	L314K	S434Y			TAC	CCC	AAG	TAC		
282	S252Y	Q311W	D312P	L314K			TAC	TGG	CCC	AAG		

Mutations as per EU numbering system							Codon Usage					
ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
283	S252Y	Q311W	D312P	L314K	S434Y		TAC	TGG	CCC	AAG	TAC	
284	S252Y	Q311W	D312P	S434Y			TAC	TGG	CCC	TAC		
285	S252Y	Q311W	L314K	S434Y			TAC	TGG	AAG	TAC		
286	S252Y	S428Y	D312P	L314K			TAC	TAC	CCC	AAG		
287	S252Y	S428Y	D312P	L314K	S434Y		TAC	TAC	CCC	AAG	TAC	
288	S252Y	S428Y	D312P	S434Y			TAC	TAC	CCC	TAC		
289	S252Y	S428Y	L314K	S434Y			TAC	TAC	AAG	TAC		
290	S252Y	S428Y	Q311W	D312P			TAC	TAC	TGG	CCC		
291	S252Y	S428Y	Q311W	D312P	L314K		TAC	TAC	TGG	CCC	AAG	
292	S252Y	S428Y	Q311W	D312P	L314K	S434Y	TAC	TAC	TGG	CCC	AAG	TAC
293	S252Y	S428Y	Q311W	D312P	S434Y		TAC	TAC	TGG	CCC	TAC	
294	S252Y	S428Y	Q311W	L314K			TAC	TAC	TGG	AAG		
295	S252Y	S428Y	Q311W	L314K	S434Y		TAC	TAC	TGG	AAG	TAC	
296	S252Y	S428Y	Q311W	S434Y			TAC	TAC	TGG	TAC		
297	S428Y	D312P	L314K	S434Y			TAC	CCC	AAG	TAC		
298	S428Y	Q311W	D312P	L314K			TAC	TGG	CCC	AAG		
299	S428Y	Q311W	D312P	L314K	S434Y		TAC	TGG	CCC	AAG	TAC	
300	S428Y	Q311W	D312P	S434Y			TAC	TGG	CCC	TAC		
301	S428Y	Q311W	L314K	S434Y			TAC	TGG	AAG	TAC		
302	A431K	S434P					AAA	CCC				
303	A431K	S434Y					AAA	TAC				
304	D399M	D401R					ATG	AGG				
305	D399M	D401R	T403R				ATG	AGG	AGG			
306	D399M	T403R					ATG	AGG				
307	D401R	T403R					AGG	AGG				
308	P247V	S252H					GTT	CAC				

ID No.	Mutations as per EU numbering system						Codon Usage					
	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
309	P247V	T250Q					GTT	CAG				
310	P247V	T250Q	S252H				GTT	CAG	CAC			
311	Q311H	D312P	L314K				CAC	CCC	AAG			
312	Q311K	D312P	L314K				AAG	CCC	AAG			
313	Q311M	D312P	L314K				ATG	CCC	AAG			
314	Q311R	D312P	L314K				AGG	CCC	AAG			
315	Q311Y	D312P	L314K				TAC	CCC	AAG			
316	S428M	A431K					ATG	AAA				
317	S428M	A431K	S434P				ATG	AAA	CCC			
318	S428M	A431K	S434Y				ATG	AAA	TAC			
319	S428Y	D312P	A431K	S434P			TAC	CCC	AAA	CCC		
320	S428Y	D312P	S434P				TAC	CCC	CCC			
321	S428Y	D312P	S434Y	A431K			TAC	CCC	TAC	AAA		
322	S428Y	D312P	S434Y	D399M	D401R		TAC	CCC	TAC	ATG	AGG	
323	S428Y	D312P	S434Y	D399M	D401R	T403R	TAC	CCC	TAC	ATG	AGG	AGG
324	S428Y	D312P	S434Y	D399M	T403R		TAC	CCC	TAC	ATG	AGG	
325	S428Y	D312P	S434Y	D401R	T403R		TAC	CCC	TAC	AGG	AGG	
326	S428Y	D312P	S434Y	P247V	S252H		TAC	CCC	TAC	GTT	CAC	
327	S428Y	D312P	S434Y	P247V	T250Q		TAC	CCC	TAC	GTT	CAG	
328	S428Y	D312P	S434Y	P247V	T250Q	S252H	TAC	CCC	TAC	GTT	CAG	CAC
329	S428Y	D312P	S434Y	Q311H	L314K		TAC	CCC	TAC	CAC	AAG	
330	S428Y	D312P	S434Y	Q311K	L314K		TAC	CCC	TAC	AAG	AAG	
331	S428Y	D312P	S434Y	Q311M	L314K		TAC	CCC	TAC	ATG	AAG	
332	S428Y	D312P	S434Y	Q311R	L314K		TAC	CCC	TAC	AGG	AAG	
333	S428Y	D312P	S434Y	Q311Y	L314K		TAC	CCC	TAC	TAC	AAG	
334	S428Y	D312P	S434Y	T250Q	S252H		TAC	CCC	TAC	CAG	CAC	

ID No.	Mutations as per EU numbering system						Codon Usage					
	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
335	T250Q	S252H					CAG	CAC				
336	S252Y	S428M	S434F				TAC	ATG	TTC	AAA		
				A431K								
337	S252Y	S428M	S434F				TAC	ATG	TTC	CAC		
				D312H								
338	S252Y	S428M	S434F	D399T			TAC	ATG	TTC	ACC		
339	S252Y	S428M	S434F	P247L			TAC	ATG	TTC	CTG		
340	S252Y	S428M	S434F	Q311R			TAC	ATG	TTC	AGG		
341	S252Y	S428M	S434F	D312A			TAC	ATG	TTC	GCC		
342	S252Y	S428M	S434F	D312P			TAC	ATG	TTC	CCC		
343	S252Y	S428M	S434F	D399M			TAC	ATG	TTC	ATG		
344	S252Y	S428M	S434F	D401R			TAC	ATG	TTC	AGG		
345	S252Y	S428M	S434F	E430I			TAC	ATG	TTC	ATT		
346	S252Y	S428M	S434F	G402R			TAC	ATG	TTC	AGG		
347	S252Y	S428M	S434F	L314K			TAC	ATG	TTC	AAG		
348	S252Y	S428M	S434F	Q311H			TAC	ATG	TTC	CAC		
349	S252Y	S428M	S434F	Q311K			TAC	ATG	TTC	AAG		
350	S252Y	S428M	S434F	Q311M			TAC	ATG	TTC	ATG		
351	S252Y	S428M	S434F	Q311W			TAC	ATG	TTC	IGG		
352	S252Y	S428M	S434F	Q311Y			TAC	ATG	TTC	IAC		
353	S252Y	S428M	S434F	S254V			TAC	ATG	TTC	GTG		
354	S252Y	S428M	S434F	T250Q			TAC	ATG	TTC	CAG		
355	S252Y	S428M	S434F	T256M			TAC	ATG	TTC	ATG		
356	S252Y	S428M	S434F	T403R			TAC	ATG	TTC	AGG		
357	S252Y	S434R	A431K				TAC	AGG	AAA			
358	S252Y	S434R	A431K				TAC	AGG	AAA			

Mutations as per EU numbering system							Codon Usage					
ID No.	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6	Mut#1	Mut#2	Mut#3	Mut#4	Mut#5	Mut#6
359	S252Y	S434R	D312A				TAC	AGG	GCC			
360	S252Y	S434R	D312H				TAC	AGG	CAC			
361	S252Y	S434R	D312P				TAC	AGG	CCC			
362	S252Y	S434R	D399M				TAC	AGG	ATG			
363	S252Y	S434R	D399T				TAC	AGG	ACC			
364	S252Y	S434R	D401R				TAC	AGG	AGG			
365	S252Y	S434R	E430I				TAC	AGG	ATT			
366	S252Y	S434R	E430I				TAC	AGG	ATT			
367	S252Y	S434R	G402R				TAC	AGG	AGG			
368	S252Y	S434R	L314K				TAC	AGG	AAG			
369	S252Y	S434R	P247L				TAC	AGG	CTG			
370	S252Y	S434R	Q311H				TAC	AGG	CAC			
371	S252Y	S434R	Q311K				TAC	AGG	AAG			
372	S252Y	S434R	Q311M				TAC	AGG	ATG			
373	S252Y	S434R	Q311R				TAC	AGG	AGG			
374	S252Y	S434R	Q311Y				TAC	AGG	TAC			
375	S252Y	S434R	S254V				TAC	AGG	GTG			
376	S252Y	S434R	T250Q				TAC	AGG	CAG			
377	S252Y	S434R	T256M				TAC	AGG	ATG			
378	S252Y	S434R	T403R				TAC	AGG	AGG			

Table 4. Effect of mutants on FcRn binding affinity.

Mutations as per Eu numbering system				Feline FcRn Binding Affinity	Codon Usage				
Mut#1	Mut#2	Mut#3	Mut#4	KD at pH6	KD at pH7.4	Mut#1	Mut#2	Mut#3	Mut#4
	S428L	S252H		5.94E-09	LS		CTG	CAC	
	S428L	S252Y		4.88E-09	LS		CTG	TAC	
	S428L	S252F		6.52E-09	LS		CTG	TTC	
	S428L	S252W		6.14E-09	LS		CTG	TGG	
Q311Y	S428L			5.72E-09	LS	TAC	CTG		
Q311W	S428L			8.08E-09	LS	TGG	CTG		
Q311H	S428L			7.19E-09	LS	CAC	CTG		
Q311K	S428L			5.62E-09	LS	AAG	CTG		
Q311L	S428L			6.03E-09	LS	CTG	CTG		
Q311M	S428L			6.44E-09	LS	ATG	CTG		
Q311R	S428L			5.47E-09	LS	CGG	CTG		
	S428L		S434Y	9.33E-10	2.22E-08		CTG		TAC
	S428L		S434R	4.66E-09	LS		CTG		CGG
	S428L		S434H	2.58E-09	4.39E-08		CTG		CAC
	S428L		S434F	1.39E-09	4.64E-08		CTG		TTC
	S428L		S434W	1.02E-09	4.18E-08		CTG		TGG
	S428M	S252H		6.83E-09	NBO		ATG	CAC	
	S428M	S252Y		5.12E-09	1.24E-07		ATG	TAC	
	S428M	S252F		5.90E-09	LS		ATG	TTC	
	S428M	S252W		4.65E-09	LS		ATG	TGG	
Q311Y	S428M			5.73E-09	LS	TAC	ATG		
Q311W	S428M			6.51E-09	LS	TGG	ATG		
Q311H	S428M			6.58E-09	LS	CAC	ATG		
Q311K	S428M			6.16E-09	LS	AAG	ATG		
Q311L	S428M			5.70E-09	LS	CTG	ATG		
Q311M	S428M			5.66E-09	LS	ATG	ATG		
Q311R	S428M			5.28E-09	LS	CGG	ATG		
	S428M		S434Y	8.88E-10	2.52E-08		ATG		TAC
	S428M		S434R	5.46E-09	LS		ATG		CGG
	S428M		S434H	3.09E-09	LS		ATG		CAC
	S428M		S434F	1.21E-09	3.82E-08		ATG		TTC
	S428M		S434W	9.37E-10	2.53E-08		ATG		TGG
	S428Y	S252H		6.41E-09	LS		TAC	CAC	
	S428Y	S252Y		8.58E-09	LS		TAC	TAC	
	S428Y	S252F		6.62E-09	LS		TAC	TTC	
	S428Y	S252W		2.95E-09	LS		TAC	TGG	
Q311Y	S428Y			5.57E-09	LS	TAC	TAC		
Q311W	S428Y			6.35E-09	LS	TGG	TAC		
Q311H	S428Y			6.92E-09	LS	CAC	TAC		
Q311K	S428Y			3.31E-09	3.59E-08	AAG	TAC		
Q311L	S428Y			4.74E-09	LS	CTG	TAC		

Mutations as per Eu numbering system				Feline FcRn Binding Affinity	Codon Usage				
Mut#1	Mut#2	Mut#3	Mut#4	KD at pH6	KD at pH7.4	Mut#1	Mut#2	Mut#3	Mut#4
Q311M	S428Y			5.72E-09	LS	ATG	TAC		
Q311R	S428Y			4.81E-09	LS	CGG	TAC		
	S428Y		S434Y	1.12E-09	2.41E-08		TAC		TAC
	S428Y		S434R	3.85E-09	LS		TAC		CGG
	S428Y		S434H	2.27E-09	LS		TAC		CAC
	S428Y		S434F	1.57E-09	3.97E-08		TAC		TTC
	S428Y		S434W	1.39E-09	2.41E-08		TAC		TGG

LS = Low signal. NBO = No binding observed.

[000154] The results clearly show that mutations made at various positions have a marked effect on the affinity of the IgG to FcRn.

EXAMPLE 3

5

Fc mutant IgG PK Studies in cats

[000155] Pharmacokinetic (PK) studies were conducted to show the effect of the half-life extension of various feline IgG point mutations (1) S428L; (2) S252H, S428M; (3) S252Y, S428M; (4) S428M, Q311W; and (5) S428Y, Q311W.

10 [000156] Five Fc modified feline monoclonal antibodies, listed in Table 4, as well as a wildtype feline monoclonal antibody were used in the experiments. Each molecule was given to 3 cats at a single 1 mg/kg subcutaneous administration. Serum samples were collected at pre-dose, day 7, 14, 28, 42 and 56 from the animals. Exposure of each monoclonal antibody was assessed using ligand binding methods.

15 [000157] Pharmacokinetics properties of five Fc modified feline monoclonal antibodies were evaluated in cats using the noncompartmental approach (linear trapezoidal rule for AUC calculations) with the aid of Watson™. Additional calculations were performed with Excel™, including correction of the AUC for the overlap of the concentration-time profiles after the 2nd and 3rd injections of drug. Summaries of concentration-time data and pharmacokinetic data with simple statistics (mean, standard deviation, coefficient of variation) were calculated using
20 Excel™ or Watson™. No other statistical analyses were conducted.

[000158] The results are summarized in Table 4 below.

Table 4. Effect of mutations on increasing the half-life.

Mutations	mAb (1mg/kg)	AUC $\mu\text{g}^*\text{Days}/\text{mL}$	AUC Extrap $\mu\text{g}^*\text{Days}/\text{mL}$	Cmax $\mu\text{g}/\text{mL}$	Tmax Days	T1/2 Days
S428L	ZTS515	545 $\pm$ 75	782 $\pm$ 161	14.6 $\pm$ 0.2	7	28.7 $\pm$ 4.0
S252H, S428M	ZTS520	493 $\pm$ 72	698 $\pm$ 158	16.0 $\pm$ 1.3	7	29.4 $\pm$ 6.7
S252Y, S428M	ZTS524	445 $\pm$ 28	780 $\pm$ 52	11.2 $\pm$ 1.3	7	41.6 $\pm$ 2.5
S428M, Q311W	ZTS530	473 $\pm$ 115	695 $\pm$ 221	13.1 $\pm$ 3.6	9.3 $\pm$ 4.0	30.4 $\pm$ 6.3
S428Y, Q311W	ZTS534	447 $\pm$ 149	643 $\pm$ 300	14.1 $\pm$ 2.0	7	27.2 $\pm$ 12.2

ZTS515, ZTS520, ZTS524, ZTS530, and ZTS534 refer to Fc modified felinized anti-IL31 antibody. ZTS5864, discussed herein, is a wildtype felinized anti-IL31 antibody. Anti-IL31 antibody is well known in the art. See e.g., U.S. Patents 10,526,405; 10,421,807; 9,206,253; 8,790,651.

- 5 [000159] The half-life ($T_{1/2}$) of wildtype mAb ZTS5864 was 12.4 $\pm$ 2.2 days. However, the half-life ($T_{1/2}$) of Fc modified feline monoclonal antibodies, ZTS515, ZTS520, ZTS524, ZTS530, and ZTS534, were 28.7 $\pm$ 4.0, 29.4 $\pm$ 6.7, 41.6 $\pm$ 2.5, 30.4 $\pm$ 6.3, and 27.2 $\pm$ 12.2, respectively.

[000160] The results clearly show that feline IgG point mutations (1) S428L; (2) S252H, S428M; (3) S252Y, S428M; (4) S428M, Q311W; and (5) S428Y, Q311W are highly effective

- 10 to increase the half-life in domestic cats.

[000161] Having described preferred embodiments of the invention, it is to be understood that the invention is not limited to the precise embodiments, and that various changes and modifications may be effected therein by those skilled in the art without departing from the scope or spirit of the invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A modified IgG comprising: a feline IgG constant domain comprising at least one amino acid substitution relative to a wild-type feline IgG constant domain, wherein said substitution is at amino acid residue 247, 249, 250, 252, 254, 256, 285, 309, 311, 312,
5 314, 378, 399, 401, 402, 403, 404, 428, 430, 431, 432, 434, 436, or 437, numbered according to the Eu index as in Kabat.
2. The modified IgG of claim 1, wherein said constant domain comprising one or more of substitutions P247I, P247L, P247V, D249A, D249E, D249S, T250E, T250I, T250Q, T250S, T250V, S252A, S252C, S252D, S252E, S252F, S252G, S252H, S252I, S252K,
10 S252L, S252N, S252P, S252Q, S252R, S252T, S252V, S252Y, S252M, S252W, S254A, S254D, S254E, S254F, S254G, S254H, S254K, S254L, S254M, S254C, S254I, S254N, S254P, S254Q, S254R, S254T, S254V, S254W, S254Y, T256A, T256C, T256D, T256E, T256F, T256G, T256H, T256I, T256K, T256L, T256M, T256N, T256P, T256Q, T256R, T256V, T256W, T256Y, T256S, Y285A, L309G, L309I,
15 Q311F, Q311H, Q311I, Q311K, Q311L, Q311M, Q311R, Q311W, Q311Y, D312A, D312H, D312K, D312R, D312P, L314K, 316Q, A378C, A378D, A378E, A378F, A378G, A378H, A378I, A378K, A378L, A378M, A378N, A378P, A378Q, A378R, A378S, A378T, A378V, A378W, A378Y, D399M, D399T, D399V, D401R, G402R, T403R, Y404Q, S428A, S428C, S428D, S428E, S428F, S428G, S428H, S428I, S428K, S428L, S428N, S428P, S428R, S428T, S428V, S428W, S428Y, S428M, E430I, E430Q, A431Q, A431R, A431V, A431K, L432S, S434A, S434C, S434D, S434E, S434F, S434G, S434H, S434I, S434K, S434L, S434M, S434N, S434P, S434Q, S434R, S434T, S434V, S434W, S434Y, H436G, H436K, H436M, H436R, H436Y, T437A, and T437R.
20
3. The modified IgG of claim 1, wherein the modified IgG has a higher affinity for FcRn than the IgG having the wild-type feline IgG constant domain.
25
4. The modified IgG of claim 1, wherein the modified IgG is a feline or felinized IgG.
5. The modified IgG of claim 1, wherein the IgG is IgG1_a, IgG1_b, or IgG2.
6. The modified IgG of claim 1, wherein the IgG constant domain is a constant domain of IgG1_a, IgG1_b, or IgG2.

7. The modified IgG of claim 1, wherein the IgG constant domain comprises an Fc constant region having CH3 domain.
8. The modified IgG of claim 1, wherein the IgG constant domain comprises an Fc constant region having CH2 and CH3 domain.
- 5 9. The modified IgG of claim 1, wherein the wild-type feline IgG constant domain comprises the amino acid sequence set forth in SEQ ID NO.: 1, 3, or 4.
10. A pharmaceutical composition comprising the modified IgG of claim 1 and a pharmaceutically acceptable carrier.
11. A kit comprising the modified IgG of claim 1, in a container, and instructions for use.
- 10 12. A polypeptide comprising: a feline IgG constant domain comprising at least one amino acid substitution relative to a wild-type feline IgG constant domain, wherein said substitution is at amino acid residue 247, 249, 250, 252, 254, 256, 285, 309, 311, 312, 314, 378, 399, 401, 402, 403, 404, 428, 430, 431, 432, 434, 436, or 437, numbered according to the EU index as in Kabat.
- 15 13. The polypeptide of claim 12, wherein said constant domain comprising one or more of substitutions P247I, P247L, P247V, D249A, D249E, D249S, T250E, T250I, T250Q, T250S, T250V, S252A, S252C, S252D, S252E, S252F, S252G, S252H, S252I, S252K, S252L, S252N, S252P, S252Q, S252R, S252T, S252V, S252Y, S252M, S252W, S254A, S254D, S254E, S254F, S254G, S254H, S254K, S254L, S254M, S254C, S254I, S254N, S254P, S254Q, S254R, S254T, S254V, S254W, S254Y, T256A, T256C, 20 T256D, T256E, T256F, T256G, T256H, T256I, T256K, T256L, T256M, T256N, T256P, T256Q, T256R, T256V, T256W, T256Y, T256S, Y285A, L309G, L309I, Q311F, Q311H, Q311I, Q311K, Q311L, Q311M, Q311R, Q311W, Q311Y, D312A, D312H, D312K, D312R, D312P, L314K, 316Q, A378C, A378D, A378E, A378F, A378G, A378H, A378I, A378K, A378L, A378M, A378N, A378P, A378Q, A378R, 25 A378S, A378T, A378V, A378W, A378Y, D399M, D399T, D399V, D401R, G402R, T403R, Y404Q, S428A, S428C, S428D, S428E, S428F, S428G, S428H, S428I, S428K, S428L, S428N, S428P, S428R, S428T, S428V, S428W, S428Y, S428M, E430I, E430Q, A431Q, A431R, A431V, A431K, L432S, S434A, S434C, S434D, S434E, S434F,

S434G, S434H, S434I, S434K, S434L, S434M, S434N, S434P, S434Q, S434R, S434T, S434V, S434W, S434Y, H436G, H436K, H436M, H436R, H436Y, T437A, and T437R.

14. The polypeptide of claim 12, wherein the polypeptide has a higher affinity for FcRn than the polypeptide of the IgG having the wild-type feline IgG constant domain.
- 5 15. The polypeptide of claim 12, wherein the polypeptide is a polypeptide of a feline or felinized IgG.
16. The polypeptide of claim 12, wherein the IgG is IgG1a, IgG1b, or IgG2.
17. The polypeptide of claim 12, wherein the IgG constant domain is a constant domain of IgG1a, IgG1b, or IgG2.
- 10 18. The polypeptide of claim 12, wherein the IgG constant domain comprises an Fc constant region having CH3 domain.
19. The polypeptide of claim 12, wherein the IgG constant domain comprises an Fc constant region having CH2 and CH3 domain.
20. The polypeptide of claim 12, wherein the wild-type feline IgG constant domain comprises the amino acid sequence set forth in SEQ ID NO.: 1, 3, or 4.
- 15 21. An antibody comprising: a feline IgG constant domain comprising at least one amino acid substitution relative to a wild-type feline IgG constant domain, wherein said substitution is at amino acid residue 247, 249, 250, 252, 254, 256, 285, 309, 311, 312, 314, 378, 399, 401, 402, 403, 404, 428, 430, 431, 432, 434, 436, or 437, numbered according to the EU index as in Kabat.
- 20 22. The antibody of claim 21, wherein said constant domain comprising one or more of substitutions P247I, P247L, P247V, D249A, D249E, D249S, T250E, T250I, T250Q, T250S, T250V, S252A, S252C, S252D, S252E, S252F, S252G, S252H, S252I, S252K, S252L, S252N, S252P, S252Q, S252R, S252T, S252V, S252Y, S252M, S252W, S254A, S254D, S254E, S254F, S254G, S254H, S254K, S254L, S254M, S254C, S254I, S254N, S254P, S254Q, S254R, S254T, S254V, S254W, S254Y, T256A, T256C, T256D, T256E, T256F, T256G, T256H, T256I, T256K, T256L, T256M, T256N, T256P, T256Q, T256R, T256V, T256W, T256Y, T256S, Y285A, L309G, L309I,
- 25

- Q311F, Q311H, Q311I, Q311K, Q311L, Q311M, Q311R, Q311W, Q311Y, D312A, D312H, D312K, D312R, D312P, L314K, 316Q, A378C, A378D, A378E, A378F, A378G, A378H, A378I, A378K, A378L, A378M, A378N, A378P, A378Q, A378R, A378S, A378T, A378V, A378W, A378Y, D399M, D399T, D399V, D401R, G402R, T403R, Y404Q, S428A, S428C, S428D, S428E, S428F, S428G, S428H, S428I, S428K, S428L, S428N, S428P, S428R, S428T, S428V, S428W, S428Y, S428M, E430I, E430Q, A431Q, A431R, A431V, A431K, L432S, S434A, S434C, S434D, S434E, S434F, S434G, S434H, S434I, S434K, S434L, S434M, S434N, S434P, S434Q, S434R, S434T, S434V, S434W, S434Y, H436G, H436K, H436M, H436R, H436Y, T437A, and T437R.
23. The antibody of claim 21, wherein the polypeptide has a higher affinity for FcRn than the polypeptide of the IgG having the wild-type feline IgG constant domain.
24. The antibody of claim 21, wherein the polypeptide is a polypeptide of a feline or felinized IgG.
25. The antibody of claim 21, wherein the IgG is IgG1a, IgG1b, or IgG2.
26. The antibody of claim 21, wherein the IgG constant domain is a constant domain of IgG1a, IgG1b, or IgG2.
27. The antibody of claim 21, wherein the IgG constant domain comprises an Fc constant region having CH3 domain.
28. The antibody of claim 21, wherein the IgG constant domain comprises an Fc constant region having CH2 and CH3 domain.
29. The antibody of claim 21, wherein the wild-type feline IgG constant domain comprises the amino acid sequence set forth in SEQ ID NO.: 1, 3, or 4.
30. A pharmaceutical composition comprising the antibody of claim 29 and a pharmaceutically acceptable carrier.
31. A kit comprising the antibody of claim 29, in a container, and instructions for use.
32. A vector comprising the nucleic acid sequence encoding the amino acid sequence of antibody of claim 21, wherein the wild-type feline IgG constant domain comprises the amino acid sequence set forth in SEQ ID NO.: 1, 3, or 4.

33. An isolated cell comprising the vector of claim 32.
34. A method of manufacturing an antibody or a molecule, the method comprising: providing the cell of claim 33; and culturing said cell.
35. A method of manufacturing an antibody, the method comprising: providing an antibody
5 of any one of claims 21-19.
36. A fusion molecule comprising: a feline IgG constant domain comprising at least one amino acid substitution relative to a wild-type feline IgG constant domain, wherein said substitution is at amino acid residue 247, 249, 250, 252, 254, 256, 285, 309, 311, 312, 314, 378, 399, 401, 402, 403, 404, 428, 430, 431, 432, 434, 436, or 437, numbered
10 according to the EU index as in Kabat.
37. The molecule of claim 36, wherein said constant domain comprising one or more of substitutions P247I, P247L, P247V, D249A, D249E, D249S, T250E, T250I, T250Q, T250S, T250V, S252A, S252C, S252D, S252E, S252F, S252G, S252H, S252I, S252K, S252L, S252N, S252P, S252Q, S252R, S252T, S252V, S252Y, S252M, S252W,
15 S254A, S254D, S254E, S254F, S254G, S254H, S254K, S254L, S254M, S254C, S254I, S254N, S254P, S254Q, S254R, S254T, S254V, S254W, S254Y, T256A, T256C, T256D, T256E, T256F, T256G, T256H, T256I, T256K, T256L, T256M, T256N, T256P, T256Q, T256R, T256V, T256W, T256Y, T256S, Y285A, L309G, L309I, Q311F, Q311H, Q311I, Q311K, Q311L, Q311M, Q311R, Q311W, Q311Y, D312A,
20 D312H, D312K, D312R, D312P, L314K, 316Q, A378C, A378D, A378E, A378F, A378G, A378H, A378I, A378K, A378L, A378M, A378N, A378P, A378Q, A378R, A378S, A378T, A378V, A378W, A378Y, D399M, D399T, D399V, D401R, G402R, T403R, Y404Q, S428A, S428C, S428D, S428E, S428F, S428G, S428H, S428I, S428K, S428L, S428N, S428P, S428R, S428T, S428V, S428W, S428Y, S428M, E430I, E430Q,
25 A431Q, A431R, A431V, A431K, L432S, S434A, S434C, S434D, S434E, S434F, S434G, S434H, S434I, S434K, S434L, S434M, S434N, S434P, S434Q, S434R, S434T, S434V, S434W, S434Y, H436G, H436K, H436M, H436R, H436Y, T437A, and T437R.
38. The molecule of claim 36, wherein the polypeptide has a higher affinity for FcRn than the polypeptide of the IgG having the wild-type feline IgG constant domain.

39. The molecule of claim 36, wherein the polypeptide is a polypeptide of a feline or felinized IgG.
40. The molecule of claim 36, wherein the IgG is IgG1_a, IgG1_b, or IgG2.
41. The molecule of claim 36, wherein the IgG constant domain is a constant domain of IgG1_a, IgG1_b, or IgG2.
42. The molecule of claim 36, wherein the IgG constant domain comprises an Fc constant region having CH3 domain.
43. The molecule of claim 36, wherein the IgG constant domain comprises an Fc constant region having CH2 and CH3 domain.
44. The molecule of claim 36, wherein the wild-type feline IgG constant domain comprises the amino acid sequence set forth in SEQ ID NO.: 1, 3, or 4.
45. A pharmaceutical composition comprising the molecule of claim 36 and a pharmaceutically acceptable carrier.
46. A kit comprising the molecule of claim 36, in a container, and instructions for use.
47. The modified IgG of claim 1, wherein at least one of said mutations improves a biophysical property.
48. The modified IgG of claim 47, wherein said biophysical property is polyreactivity.
49. The polypeptide of claim 12, wherein at least one of said mutations improves a biophysical property.
50. The polypeptide of claim 49, wherein said biophysical property is polyreactivity.
51. The antibody of claim 21, wherein at least one of said mutations improves a biophysical property.
52. The antibody of claim 51, wherein said biophysical property is polyreactivity.
53. The molecule of claim 36, wherein at least one of said mutations improves a biophysical property.

54. The molecule of claim 53, wherein said biophysical property is polyreactivity.
55. A method for increasing an antibody serum half-life in a cat, the method comprising:
administering said cat a therapeutically effective amount of an antibody comprising a
feline IgG constant domain, said feline IgG constant domain comprising at least one
amino acid substitution relative to a wild-type feline IgG constant domain, wherein said
substitution is at amino acid residue 252, 311, or 428, numbered
according to the EU index as in Kabat.
56. The method of claim 55, wherein said feline IgG constant domain comprises one or more
of mutations S252H, S252Y, Q311W, S428L, S428M, and S428Y.
57. The method of claim 55, wherein said feline IgG constant domain comprises the
mutations selected from a group: (1) S428L; (2) S252H and S428M; (3) S252Y and
S428M; (4) S428M and Q311W; or (5) S428Y and Q311W.
58. The method of claim 55, wherein said feline IgG constant domain comprises the
mutation S428L.
59. The method of claim 55, wherein said feline IgG constant domain comprises the
combination of mutations S252H and S428M.
60. The method of claim 55, wherein said feline IgG constant domain comprises the
combination of mutations S252Y and S428M.
61. The method of claim 55, wherein said feline IgG constant domain comprises the
combination of mutations S428M and Q311W.
62. The method of claim 55, wherein said feline IgG constant domain comprises the
combination of mutations S428Y and Q311W.
63. The method of claim 55, wherein said feline IgG constant domain has a higher serum
half-life than an IgG having the wild-type feline IgG constant domain.
64. The method of claim 55, wherein the IgG is IgG1_a, IgG1_b, or IgG2.
65. The method of claim 55, wherein the IgG constant domain is a constant domain of
IgG1_a, IgG1_b, or IgG2.

66. The method of claim 55, wherein the IgG constant domain comprises an Fc constant region having CH3 domain.
67. The method of claim 55, wherein the IgG constant domain comprises an Fc constant region having CH2 and CH3 domain.
- 5 68. The method of claim 55, wherein the wild-type feline IgG constant domain comprises the amino acid sequence set forth in SEQ ID NO.: 1, 3, or 4.
69. The method of claim 55, wherein said antibody is an anti-IL31 antibody.
70. A method of treating an IL-31-mediated pruritic or allergic condition in a canine subject, the method comprising: administering to said subject a therapeutically effective amount
10 of the anti-IL31 antibody of claim 69, thereby treating said IL-31-mediated pruritic or allergic condition in said canine subject.
71. The method of claim 70, wherein the IL-31-mediated pruritic or allergic condition is a pruritic condition selected from the group consisting of atopic dermatitis, eczema, psoriasis, scleroderma, and pruritis.
- 15 72.