

SUMMARY

This disclosure provides, at least in part, polypeptides (*e.g.*, antibody molecules or fusion proteins) that comprise an Fc region of an immunoglobulin, and that comprise one or more of the structural or functional properties disclosed herein. In an embodiment, nucleic acid molecules encoding the polypeptides, expression vectors, host cells, compositions (*e.g.*, pharmaceutical compositions), kits, containers, and methods for making the polypeptides (*e.g.*, antibody molecules or fusion proteins), are also provided. The polypeptides (*e.g.*, antibody molecules or fusion proteins) disclosed herein can be used (alone or in combination with other agents or therapeutic modalities) to treat, prevent, and/or diagnose disorders, such as disorders and conditions disclosed herein.

The disclosure features a polypeptide, *e.g.*, an antibody molecule or fusion protein, comprising an Fc region, wherein the Fc region comprises a mutation, and wherein the polypeptide has one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all) of the following properties:

a) has an increased binding affinity (*e.g.*, a decreased dissociation constant (K_d)) for a neonatal Fc receptor (FcRn), *e.g.*, at a pH between 6.0 and 6.5 (*e.g.*, at pH 6.0), *e.g.*, at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, or 50-fold increase, compared to a reference polypeptide, *e.g.*, as determined by an octet-based assay or a cell-based assay;

b) has a higher binding affinity (*e.g.*, a lower dissociation constant (K_d)) for an FcRn at a pH between 6.0 and 6.5 (*e.g.*, at pH 6.0), *e.g.*, at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 50-fold higher, than the binding affinity at a pH between 7.0 and 7.4 (*e.g.*, at pH 7.4), *e.g.*, as determined by an octet-based assay or a cell-based assay;

c) binds to an FcRn with high affinity, *e.g.*, at a pH between 6.0 and 6.5 (*e.g.*, at pH 6.0), *e.g.*, with a dissociation constant (K_d) of 300 nM or less, *e.g.*, 250 nM or less, 200 nM or less, 150 nM or less, 100 nM or less, 50 nM or less, *e.g.*, 25 nM or less, 10 nM or less, 5 nM or less, 2 nM or less, 1 nM or less, 0.5 nM or less, 0.2 nM or less, 0.1 nM or less, 0.05 nM or less, 0.02 nM or less, or 0.01 nM or less, *e.g.*, between 25 nM and 0.1 nM, between 20 nM and 0.5 nM, between 15 nM and 1 nM, between 10 nM and 5 nM, or between 20 nM and 10 nM, *e.g.*, as determined by an octet-based assay or a cell-based assay;

d) binds to an FcRn with low affinity *e.g.*, at a pH between 7.0 and 7.4 (*e.g.*, at pH 7.4), *e.g.*, with a K_d of 50 nM or more, *e.g.*, 60 nM or more, 80 nM or more, 100 nM or more, 150 nM or more, 200 nM or more, 500 nM or more, *e.g.*, between 50 nM and 500 nM or between 100 nM and 250 nM, *e.g.*, as determined by an octet-based assay or a cell-based assay;

e) has the same binding affinity, does not substantially alter (*e.g.*, decreases or increases by no more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) the binding affinity, or increases the binding affinity (*e.g.*, by at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 50-fold), for an Fcγ receptor

n) increases mucosal uptake, *e.g.*, by at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, or 50-fold, compared to a reference polypeptide, *e.g.*, as determined by a transcytosis assay.

In one aspect, the present disclosure features a polypeptide comprising an Fc region, wherein the Fc region comprises a mutation, wherein the polypeptide has 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all of the following properties:

a) has an increased binding affinity for a neonatal Fc receptor (FcRn) at a pH between 6.0 and 6.5, compared to a reference polypeptide;

b) has a higher binding affinity for FcRn at a pH between 6.0 and 6.5 than the binding affinity at a pH between 7.0 and 7.4;

c) binds to an FcRn at a pH between 6.0 and 6.5 with a dissociation constant (K_d) of 300 nM or less;

d) binds to the FcRn at a pH between 7.0 and 7.4 with a K_d of 50 nM or more;

e) has the same, substantially the same, or increased binding affinity for an Fcγ receptor, compared to a reference polypeptide;

f) has the same, or substantially the same, thermal stability, compared to a reference polypeptide;

g) has the same, substantially the same, or increased binding affinity for C1q, compared to a reference polypeptide;

h) has the same, substantially the same, or increased binding affinity for TRIM21, compared to a reference polypeptide.

i) has an effector function that is the same, substantially the same, or increased, compared to a reference polypeptide;

j) has an increased half-life *in vivo*, compared to a reference polypeptide;

k) has a biological function, *in vitro*, *ex vivo*, or *in vivo*, that is the same, substantially the same, or increased, compared to a reference polypeptide;

l) has a developability characteristic that is the same or substantially the same, compared to a reference polypeptide;

m) has the same, substantially the same, or increased binding affinity, specificity, or both, for an epitope, compared to a reference polypeptide; or

n) increases mucosal uptake, compared to a reference polypeptide, and

wherein the polypeptide has at least properties a), b), and one, two, three, four, or all of properties e), f), g), h), or i);

optionally, wherein the reference polypeptide comprises a wild-type Fc region, an Fc region comprising the amino acid sequence of

PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVF

SCSVMHEALHNHYTQKSLSLSPGK (amino acids 121-330 of SEQ ID NO: 1 or amino acids 238-447 according to Fc numbering), or an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 1, 2, 5, 10, or 15 amino acid residues, and/or optionally, wherein the polypeptide is an isolated polypeptide and/or a synthetic polypeptide.

5 In an embodiment, the polypeptide has an increased binding affinity (*e.g.*, a decreased dissociation constant (K_d)) for a neonatal Fc receptor (FcRn), *e.g.*, at a pH between 6.0 and 6.5 (*e.g.*, at pH 6.0), *e.g.*, at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, or 50-fold increase, compared to a reference polypeptide, *e.g.*, as determined by an octet-based assay or a cell-based assay.

10 In an embodiment, the polypeptide has a higher binding affinity (*e.g.*, a lower dissociation constant (K_d)) for an FcRn at a pH between 6.0 and 6.5 (*e.g.*, at pH 6.0), *e.g.*, at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 50-fold higher, than the binding affinity at a pH between 7.0 and 7.4 (*e.g.*, at pH 7.4), *e.g.*, as determined by an octet-based assay or a cell-based assay.

15 In an embodiment, the polypeptide binds to an FcRn with high affinity *e.g.*, at a pH between 6.0 and 6.5 (*e.g.*, at pH 6.0), *e.g.*, with a dissociation constant (K_d) of 50 nM or less, *e.g.*, 25 nM or less, 10 nM or less, 5 nM or less, 2 nM or less, 1 nM or less, 0.5 nM or less, 0.2 nM or less, 0.1 nM or less, 0.05 nM or less, 0.02 nM or less, or 0.01 nM or less, *e.g.*, between 25 nM and 0.1 nM, between 20 nM and 0.5 nM, between 15 nM and 1 nM, between 10 nM and 5 nM, or between 20 nM and 10 nM, *e.g.*, as determined by an octet-based assay or a cell-based assay.

20 In an embodiment, the polypeptide binds to an FcRn with low affinity *e.g.*, at a pH between 7.0 and 7.4 (*e.g.*, at pH 7.4), *e.g.*, with a K_d of 50 nM or more, *e.g.*, 60 nM or more, 80 nM or more, 100 nM or more, 150 nM or more, 200 nM or more, 500 nM or more, *e.g.*, between 50 nM and 500 nM or between 100 nM and 250 nM, *e.g.*, as determined by an octet-based assay or a cell-based assay.

25 In an embodiment, the polypeptide has the same binding affinity, does not substantially alter (*e.g.*, decreases or increases by no more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) the binding affinity, or increases the binding affinity (*e.g.*, by at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 50-fold), for an Fc γ receptor (*e.g.*, one, two, or all of Fc γ RI, Fc γ RIIa/b, or Fc γ RIII), compared to a reference polypeptide, *e.g.*, as determined by an octet-based assay or a cell-based assay.

30 In an embodiment, the polypeptide has the same thermal stability, or does not substantially alter (*e.g.*, increases or decreases the melting temperature by no more than 1°C, 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, 8°C, 9°C, or 10°C), the thermal stability, compared to a reference polypeptide, *e.g.*, as determined by a sypro orange assay.

35 In an embodiment, the polypeptide has the same binding affinity, does not substantially alter (*e.g.*, decreases or increases by no more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) the binding affinity, or increases the binding affinity (*e.g.*, by at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 50-fold), for C1q, compared to a reference polypeptide, *e.g.*, as determined by ELISA.

In an embodiment, the polypeptide has the same binding affinity, does not substantially alter (*e.g.*, decreases or increases by no more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%)

the binding affinity, or increases the binding affinity (*e.g.*, by at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 50-fold), for TRIM21, compared to a reference polypeptide, *e.g.*, as determined by ELISA.

following mutation or mutations: (i) M252Y, S254T, and T256E; (ii) L309N; (iii) T250Q and M428L; (iv) M428L and N434A; (v) N434A; (vi) T307A, E380A, and N434A; (vii) M252W; (viii) V308F; (ix) V308F and N434Y; or (x) H435A.

5 In an embodiment, the polypeptide comprises a first mutation chosen from M252Y, S254T, T256E, L309N, T250Q, M428L, N434S, N434A, T307A, E380A, N434A, M252Y, S254T, or T256E, and a second mutation chosen from a mutation in **Table 1** other than M252Y, S254T, T256E, L309N, T250Q, M428L, N434S, N434A, T307A, E380A, N434A, M252Y, S254T, and T256E.

10 In an embodiment, the polypeptide comprises a combination of mutations chosen from M252Y, S254T, T256E, L309N, T250Q, M428L, N434S, N434A, T307A, E380A, N434A, M252Y, S254T, or T256E, wherein the combination is other than (i) M252Y, S254T, and T256E; (ii) L309N; (iii) T250Q and M428L; (iv) M428L and N434A; (v) N434A; (vi) T307A, E380A, and N434A; (vii) M252W; (viii) V308F; (ix) V308F and N434Y; or (x) H435A.

15 In an embodiment, the polypeptide further comprises a mutation in the Fc region that increases an effector function. In an embodiment, the mutation is in a residue chosen from S239 (*e.g.*, S239D), A330 (*e.g.*, A330L), I332 (*e.g.*, I332E), F243 (*e.g.*, F243L), G236 (*e.g.*, G236A), or a combination thereof, *e.g.*, to increase an effector function.

20 In an embodiment, the polypeptide further comprises a mutation in the Fc region that decreases an effector function. In an embodiment, the mutation is in a residue chosen from K322 (*e.g.*, K322A), L234 (*e.g.*, L234A or L234F), L235 (*e.g.*, L235A or L235E), P331 (*e.g.*, P331S), N297, or a combination thereof, *e.g.*, to decrease an effector function.

In an embodiment, the polypeptide further comprises a mutation in a region other than the Fc region, *e.g.*, in a Fab region.

In an embodiment, the polypeptide further comprises a plurality of mutations, wherein at least one mutation is a compensating mutation, *e.g.*, a compensating mutation described herein.

25 In an embodiment, the polypeptide is an isolated polypeptide. In an embodiment, the polypeptide is a synthetic polypeptide.

In an aspect, the disclosure features a composition, *e.g.*, pharmaceutical composition, comprising a polypeptide described herein. In an embodiment, the composition further comprises a pharmaceutical acceptable carrier.

30 In an aspect, the disclosure features a nucleic acid molecule encoding a polypeptide described herein. In an aspect, the disclosure features a vector comprising a nucleic acid molecule described herein. In an aspect, the disclosure features a cell, *e.g.*, an isolated cell, comprising a nucleic acid molecule described herein or a vector described herein. In an aspect, the disclosure features a kit comprising a polypeptide described herein and instructions to use of the polypeptide. In an aspect, the disclosure features a container comprising a polypeptide described herein.

35 The disclosure features a method of producing a polypeptide described herein, the method comprising culturing a cell described herein under conditions that allow production of an

antibody molecule, thereby producing the polypeptide. In an embodiment, the method further comprises isolating or purifying the polypeptide. In another aspect, the present disclosure features a method of producing a polypeptide, the method comprising culturing a cell described herein under conditions that allow production of an antibody molecule, thereby producing the polypeptide, optionally, wherein the method further comprises isolating or purifying the polypeptide.

The disclosure also features a method of treating a disorder (*e.g.*, a disorder described herein), the method comprising administering to a subject in need thereof an effective amount of a polypeptide described herein or a composition described herein, thereby treating the disorder.

The disclosure also features a polypeptide described herein for use in a method of treating a disorder (*e.g.*, a disorder described herein). The disclosure further features use of a polypeptide described herein in the manufacture of a medicament in the treatment of a disorder (*e.g.*, a disorder described herein). In another aspect, the present disclosure features use of a polypeptide described herein or a composition described herein, in the manufacture of a medicament for the treatment of a disorder in a subject.

In an aspect, the disclosure features a method of detecting a molecule, the method comprising contacting a cell or a sample from a subject with polypeptide described herein, thereby detecting the molecule.

The disclosure contemplates all combinations of any one or more of the foregoing aspects and/or embodiments, as well as combinations with any one or more of the embodiments set forth in the detailed description and examples.

Other features, objects, and advantages of the compositions and methods herein will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the binding between FcRn and IgG1.

FIG. 2 depicts various binding sites in the Fc region.

FIG. 3 depicts the structural basis for pH specific engagement of FcRn.

FIG. 4 depicts mapping of Fc-FcRn interaction interface. CH2 and CH3 domains are indicated and shown by different gray shades.

FIG. 5 depicts a network view of Fc-FcRn engagement.

FIG. 6 depicts FcRn binding to anti-CH1 immobilized antibody and pH specific enhancement.

FIG. 7 depicts the results of a cell-based FcRn binding competition assay.

FIG. 8 depicts the binding of exemplary antibody molecules to FcγRI and FcγRIIIa.

FIG. 9 depicts the thermal stability of exemplary antibody molecules.

FIG. 10 depicts the half-lives of exemplary antibody molecules in Tg32 mice.

FIG. 11A depicts K_d , k_{on} and k_{off} of exemplary Fc engineered antibodies. Values presented as fold change as compared to IgG containing WT Fc.

FIG. 11B depicts improved binding to FcRn at pH 6.0 and poor binding to FcRn at pH 7.4 of a representative Fc engineered antibody.

5 **FIG. 11C** depicts the interaction of the CH2 domain of the antibody Fc with the FcRn molecule.

FIG. 12 depicts biophysical properties of Fc engineered variants.

What is claimed is:

1. A polypeptide comprising an Fc region, wherein the Fc region comprises a mutation, wherein the polypeptide has 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all of the following properties:

5 a) has an increased binding affinity for a neonatal Fc receptor (FcRn) at a pH between 6.0 and 6.5, compared to a reference polypeptide;

b) has a higher binding affinity for FcRn at a pH between 6.0 and 6.5 than the binding affinity at a pH between 7.0 and 7.4;

10 c) binds to an FcRn at a pH between 6.0 and 6.5 with a dissociation constant (K_d) of 300 nM or less;

d) binds to the FcRn at a pH between 7.0 and 7.4 with a K_d of 50 nM or more;

e) has the same, substantially the same, or increased binding affinity for an Fc γ receptor, compared to a reference polypeptide;

15 f) has the same, or substantially the same, thermal stability, compared to a reference polypeptide;

g) has the same, substantially the same, or increased binding affinity for C1q, compared to a reference polypeptide;

h) has the same, substantially the same, or increased binding affinity for TRIM21, compared to a reference polypeptide.

20 i) has an effector function that is the same, substantially the same, or increased, compared to a reference polypeptide;

j) has an increased half-life *in vivo*, compared to a reference polypeptide;

k) has a biological function, *in vitro*, *ex vivo*, or *in vivo*, that is the same, substantially the same, or increased, compared to a reference polypeptide;

25 l) has a developability characteristic that is the same or substantially the same, compared to a reference polypeptide;

m) has the same, substantially the same, or increased binding affinity, specificity, or both, for an epitope, compared to a reference polypeptide; or

n) increases mucosal uptake, compared to a reference polypeptide, and

30 wherein the polypeptide has at least properties a), b), and one, two, three, four, or all of properties e), f), g), h), or i);

optionally, wherein the reference polypeptide comprises a wild-type Fc region, an Fc region comprising the amino acid sequence of

35 PSVFLFPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVF

SCSVMHEALHNHYTQKSLSLSPGK (amino acids 121-330 of SEQ ID NO: 1 or amino acids 238-447 according to Fc numbering), or an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 1, 2, 5, 10, or 15 amino acid residues, and/or optionally, wherein the polypeptide is an isolated polypeptide and/or a synthetic polypeptide.

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2. The polypeptide of claim 1, which has one of both of the following properties (a) or (b):

(a) at least properties a), b), c), d), and one, two, three, four, or all of properties e), f), g), h), or i); or

10 (b) at least properties a), b), one, two, three, four, or all of properties e), f), g), h), or i), and one, two, three, four, five, six, or all of properties c), d), j), k), l), m), or n).

3. The polypeptide of any of claims 1-2, which has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all of the following properties (a)-(n):

15 (a) has at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, or 50-fold increase in the binding affinity for the FcRn at pH 6.0, compared to a reference polypeptide, as determined by an octet-based assay or a cell-based assay.

(b) has at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 50-fold higher in the binding affinity for the FcRn at pH 6.0 than that at pH 7.4, as determined by an octet-based assay or a cell-based assay.

20 (c) binds to the FcRn at pH 6.0 with a dissociation constant (K_d) of 250 nM or less, 200 nM or less, 150 nM or less, 100 nM or less, 50 nM or less, 25 nM or less, 10 nM or less, 5 nM or less, 2 nM or less, 1 nM or less, 0.5 nM or less, 0.2 nM or less, 0.1 nM or less, 0.05 nM or less, 0.02 nM or less, 0.01 nM or less, between 25 nM and 0.1 nM, between 20 nM and 0.5 nM, between 15 nM and 1 nM, between 10 nM and 5 nM, or between 20 nM and 10 nM, as determined by an octet-based assay or a cell-based assay.

25 (d) binds to the FcRn at pH 7.4 with a K_d of 60 nM or more, 80 nM or more, 100 nM or more, 150 nM or more, 200 nM or more, 500 nM or more, between 50 nM and 500 nM, or between 100 nM and 250 nM, as determined by an octet-based assay or a cell-based assay.

30 (e) decreases the binding affinity for one, two, or, all of FcγRI, FcγRIIa/b, or FcγRIII by no more than 10%, 20%, 30%, 40%, or 50%, or increases the binding affinity for one, two, or all of FcγRI, FcγRIIa/b, or FcγRIII by at least 1.5, 2, 3, 4, or 5-fold, compared to a reference polypeptide, as determined by an octet-based assay or a cell-based assay;

(f) increases or decreases the melting temperature by no more than 1°C, 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, 8°C, 9°C, or 10°C, compared to a reference polypeptide, as determined by a sypro orange assay.

35 (g) decreases the binding affinity for C1q by no more than 10%, 20%, 30%, 40%, or 50%, or increases the binding affinity for C1q by at least 1.5, 2, 3, 4, or 5-fold, compared to a reference polypeptide, as determined by ELISA;

(h) decreases the binding affinity for TRIM21 by no more than 10%, 20%, 30%, 40%, or 50%, or increases the binding affinity for TRIM21 by at least 1.5, 2, 3, 4, or 5-fold, compared to a reference polypeptide, as determined by ELISA;

5 (i) decreases one, two, three, or all of a complement dependent cytotoxicity (CDC), an antibody dependent cell mediated cytotoxicity (ADCC), an antibody dependent cell mediated phagocytosis (ADCP), or an antibody dependent intracellular neutralization (ADIN) by no more than 10%, 20%, 30%, 40%, or 50%, or increases one, two, three, or all of a complement dependent cytotoxicity (CDC), an antibody dependent cell mediated cytotoxicity (ADCC), an antibody
10 dependent cell mediated phagocytosis (ADCP), or an antibody dependent intracellular neutralization (ADIN) by at least 1.5, 2, 3, 4, or 5-fold, compared to a reference polypeptide;

(j) has at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold increase in the half-life *in vivo*, compared to a reference polypeptide, as determined in an animal model;

(k) decreases the biological function, *in vitro*, *ex vivo*, or *in vivo* by no more than 10%, 20%,
15 30%, 40%, or 50%, or increases the biological function, *in vitro*, *ex vivo*, or *in vivo* by at least 1.5, 2, 3, 4, or 5-fold, compared to a reference polypeptide;

(l) alters one, two, three, or all of stability, solubility, aggregation, or expression level by no more than 10%, 20%, 30%, 40%, or 50%, compared to a reference polypeptide.

(m) decreases the binding affinity, specificity, or both by no more than 10%, 20%, 30%, 40%,
20 or 50%, or increases the binding affinity, specificity, or both by at least 1.5, 2, 3, 4, or 5-fold, compared to a reference polypeptide; or

(n) increases mucosal uptake by at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold, compared to a reference polypeptide, as determined by a transcytosis assay.

25 4. The polypeptide of any of claims 1-3, which has one or more of the following:

(a) the mutation is in a residue in a CH2 domain;

(b) the mutation is in a residue in a CH3 domain;

(c) comprising at least one mutation in a residue in a CH2 domain and at least one mutation in a residue in a CH3 domain;

30 (d) further comprising a mutation in a residue in a region other than a CH2 domain and/or a CH3 domain;

(e) the mutation does not alter, or does not substantially alter, the conformation of the linker region between a CH2 domain and a CH3 domain; or

(f) the mutation does not introduce 3, 4, 5, 6, 7, 8, 9, 10, or more consecutive hydrophobic or
35 aromatic residues on a surface region.

5. The polypeptide of any of claims 1-4, which comprises one or more of:

- (a) an antibody molecule;
 - (b) an IgG;
 - (c) an IgG1, IgG2, IgG3, or IgG4;
 - (d) a heavy chain immunoglobulin variable region, a light chain immunoglobulin variable
 - 5 region, or both;
 - (e) a tetramer of two heavy chain immunoglobulin variable regions and two light chain immunoglobulin variable regions;
 - (f) a full length antibody molecule;
 - (g) a fragment of an antibody molecule;
 - 10 (h) a chimeric antibody molecule or a murine antibody molecule;
 - (i) a human antibody molecule or a humanized antibody molecule; or
 - (j) a fusion protein.
6. The polypeptide of any of claims 1-5, comprising 1, 2, 3, 4, or all of the following:
- 15 (i) a mutation in a residue in a surface region that interacts with FcRn, optionally wherein the mutation is in a residue chosen from: L251, I253, R255, P257, H285, N286, K288, T307, V308, L309, Q311, L314, H310, H433, N434, H435, or Y436;
 - (ii) a mutation in a residue that is a peripheral residue along the Fc-FcRn interface, optionally wherein the mutation is in a residue chosen from M252, T256, T307, L309, Q311, H433, N434,
 - 20 Y436, N286, or K288;
 - (iii) a mutation in a residue that is non-contact residue in Fc-FcRn binding, optionally wherein the mutation is in a residue chosen from A287, V308, N315, L314, L432, H429, E430, or A431;
 - (iv) a mutation in a residue which is a helix contact residue that enhances the conformational
 - 25 dynamics of a helix comprising 1, 2, 3, 4, 5, or all of P247, K248, D249, T250, L251, or M252, optionally wherein the mutation is in a residue chosen from P244, P245, T250, L251, P247, E380, M428, A378, D376, P257, V308, A287, L306, or H427; or
 - (v) a mutation, which modulates pK of a histidine or is an introduction of a histidine along the Fc-FcRn interface, optionally wherein the mutation is the introduction of a histidine along the Fc-
 - 30 FcRn interface;
- optionally wherein the polypeptide comprises one or more of the following:
- (i) a plurality of mutations in 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or all of the residues chosen from L251, I253, R255, P257, H285, N286, K288, T307, V308, L309, Q311, L314, H310, H433, N434, H435, or Y436;
 - 35 (ii) a plurality of mutations in 2, 3, 4, 5, 6, 7, 8, 9, or all of the residues chosen from M252, T256, T307, L309, Q311, H433, N434, Y436, N286, or K288;

(iii) a plurality of mutations in 2, 3, 4, 5, 6, 7, or all of the residues chosen from A287, V308, N315, L314, L432, H429, E430, or A431;

(iv) a plurality of mutations in 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all of the residues chosen from P244, P245, T250, L251, P247, E380, M428, A378, D376, P257, V308, A287, L306, or H427;

5 or

(v) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more mutations, or one or more combination of mutations, as described in Table 1.

7. The polypeptide of any of claims 1-6, which has one or more of the following properties:

10 (a) the mutation is other than M252Y, S254T, T256E, L309N, T250Q, M428L, N434S, N434A, T307A, E380A, N434A, M252Y, S254T, T256E, or a combination thereof;

(b) the mutation is in a residue other than residue M252Y, S254T, T256E, L309N, T250, M428, N434, N434, T307, E380, N434, M252, S254, T256, or a combination thereof;

(c) does not have 1, 2, 3, 4, 5, 6, 7, 8, 9, or all of the following mutation or mutations:

15 (i) M252Y, S254T, and T256E;

(ii) L309N;

(iii) T250Q and M428L;

(iv) M428L and N434A;

(v) N434A;

20 (vi) T307A, E380A, and N434A;

(vii) M252W;

(viii) V308F;

(ix) V308F and N434Y; or

(x) H435A; or

25 (d) comprising a first mutation chosen from M252Y, S254T, T256E, L309N, T250Q, M428L, N434S, N434A, T307A, E380A, N434A, M252Y, S254T, or T256E, and a second mutation chosen from a mutation in Table 1 other than M252Y, S254T, T256E, L309N, T250Q, M428L, N434S, N434A, T307A, E380A, N434A, M252Y, S254T, and T256E.

30 8. The polypeptide of any of claims 1-7, further comprising one or more of the following properties:

(i) a mutation in the Fc region that increases an effector function, optionally wherein the mutation is in a residue chosen from S239, A330, I332, F243, G236, or a combination thereof;

35 (ii) a mutation in the Fc region that decreases an effector function, optionally wherein the mutation is in a residue chosen from K322, L234, L235, P331, N297, or a combination thereof;

(iii) a mutation in a region other than the Fc region; or

(iv) a plurality of mutations, wherein at least one mutation is a compensating or beneficial mutation.

9. The polypeptide of any of claims 1-8, wherein the Fc region comprises:

(a) 1, 2, 3, 4, 5, or all of the combinations of mutations chosen from: T256D/Q311V/A378V, H285N/T307Q/N315D, H285D/T307Q/A378V, T307Q/Q311V/A378V, T256D/N286D/T307R/Q311V/A378V, or T256D/T307R/Q311V;

(b) a mutation or a combination of mutations capable of disrupting an Fc effector function, or

(c) both (a) and (b).

10. The polypeptide of any of claims 1-9, which comprises mutations in residues chosen from:

(i) T256, Q311, and A378;

(ii) H285, T307, and N315;

(iii) H285, T307, and A378;

(iv) T307, Q311, and A378;

(v) T256, N286, T307, Q311, and A378; or

(vi) T256, H285, T307, Q311, and A378;

optionally, wherein the polypeptide comprises mutations chosen from:

(i) T256D, Q311V, and A378V;

(ii) H285N, T307Q, and N315D;

(iii) H285D, T307Q, and A378V;

(iv) T307Q, Q311V, and A378V;

(v) T256D, N286D, T307R, Q311V, and A378V; or

(vi) T256D, H285D, T307R, Q311V, and A378V.

11. A composition comprising a polypeptide of any of claims 1-10, optionally further comprising a pharmaceutical acceptable carrier.

12. A nucleic acid molecule encoding a polypeptide of any of claims 1-10.

13. A vector comprising a nucleic acid molecule of claim 12.

14. A cell comprising a nucleic acid molecule of claim 12 or a vector of claim 13.

15. A kit comprising a polypeptide of any of claims 1-10 and instructions to use of the polypeptide.

16. A container comprising a polypeptide of any of claims 1-10.

17. A method of producing a polypeptide, the method comprising culturing a cell of claim 14
under conditions that allow production of an antibody molecule, thereby producing the polypeptide,
5 optionally, wherein the method, further comprises isolating or purifying the polypeptide.

18. Use of a polypeptide of any of claims 1-10, or a composition of claim 11, in the
manufacture of a medicament for the treatment of a disorder in a subject.

10 19. A method of detecting a molecule, the method comprising contacting a cell or a sample
from a subject with a polypeptide of any of claims 1-10, thereby detecting the molecule.