

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 an aspect, there is provided an antibody molecule comprising the CH2 and CH3 domains of an Fc region, wherein the CH2 and CH3 domains of the Fc region comprises mutations chosen

5 from:

(i) T256D, Q311V, and A378V;

(ii) H285N, T307Q, and N315D;

(iii) H285D, T307Q, and A378V;

(iv) T307Q, Q311V, and A378V;

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

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

according to EU numbering.

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.

In an example, 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.

In an example, 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.

In an example, the polypeptide further comprises a mutation in the Fc region that increases an effector function. In an example, 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.

In an example, the polypeptide further comprises a mutation in the Fc region that decreases an effector function. In an example, 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 example, the polypeptide further comprises a mutation in a region other than the Fc region, *e.g.*, in a Fab region.

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

In an example, the polypeptide is an isolated polypeptide. In an example, the polypeptide is a synthetic polypeptide.

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

In another aspect, the disclosure features a composition comprising an antibody molecule as described above, optionally further comprising a pharmaceutical acceptable carrier.

In an example, the disclosure features a nucleic acid molecule encoding a polypeptide described herein. In another aspect, the disclosure features a nucleic acid molecule encoding an antibody molecule as described above. 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 example, the disclosure features a kit comprising a polypeptide described herein and instructions to use of the polypeptide. In another aspect, the disclosure features a kit comprising an antibody molecule as described above and

instructions to use of the antibody molecule. In an example, the disclosure features a container comprising a polypeptide described herein. In another aspect, the disclosure features a container comprising an antibody molecule as described above. 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 example, the method further comprises isolating or purifying the polypeptide. In another example, 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. In another aspect, the disclosure features a method of producing an antibody molecule, the method comprising culturing a cell as described above under conditions that allow production of an antibody molecule, thereby producing the antibody molecule, optionally, wherein the method, further comprises isolating or purifying the antibody molecule.

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; wherein the disorder is an immune disorder, a cardiovascular disorder, a metabolic disorder, a neurological disorder, an infectious disease, an eye disorder, an inflammatory disorder, an organ transplant, or a cancer.

In an example, 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. In another aspect, the disclosure features a method of detecting a molecule, the method comprising contacting a cell or a sample from a subject with an antibody molecule as described above, thereby detecting the molecule.

The disclosure contemplates all combinations of any one or more of the foregoing aspects and/or examples, as well as combinations with any one or more of the examples 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.

What is claimed is:

1. An antibody molecule comprising the CH2 and CH3 domains of an Fc region, wherein the CH2 and CH3 domains of the Fc region comprises mutations chosen from:

- 5 (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
10 (vi) T256D, H285D, T307R, Q311V, and A378V;
according to EU numbering.

2. The antibody molecule of claim 1, further comprising a mutation in a region other than the Fc region and/or further comprising a hinge region between the CH2 and CH3
15 domains of the Fc region.

3. The antibody molecule of claim 1 or 2, which is an isolated antibody molecule or a synthetic antibody molecule.

20 4. The antibody molecule of any of claims 1-3, which:

- (i) is a chimeric antibody molecule, a murine antibody molecule, human antibody molecule, or a humanized antibody molecule;
(ii) further comprises a heavy chain immunoglobulin variable region, a light chain immunoglobulin variable region, or both;
25 (iii) is an immunoglobulin chain or a fragment thereof; or
(iv) is a fusion protein.

5. The antibody molecule of any of claims 1-4, wherein the antibody molecule has 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all of the following properties:

- 30 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 antibody molecule;
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;

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

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

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

10 h) has the same, substantially the same, or increased binding affinity for TRIM21, compared to a reference antibody molecule;

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

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

15 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 antibody molecule;

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

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

n) increases mucosal uptake, compared to a reference antibody molecule, and

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

25 6. The antibody molecule of any of claims 1-5,

(i) which 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 antibody molecule, as determined by an octet-based assay or a cell-based assay;

30 (ii) which 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;

(iii) which 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;

(iv) which 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;

(v) which 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 antibody molecule, as determined by an octet-based assay or a cell-based assay;

(vi) which 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 antibody molecule, as determined by a sypro orange assay;

(vii) which 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 antibody molecule, as determined by ELISA;

(viii) which 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 antibody molecule, as determined by ELISA;

(ix) which 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 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 antibody molecule;

(x) which 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 antibody molecule, as determined in an animal model;

(xi) which decreases the biological function, *in vitro*, *ex vivo*, or *in vivo* by no more than 10%, 20%, 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 antibody molecule;

(xii) which 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 antibody molecule;

(xiii) which decreases the binding affinity, specificity, or both by no more than 10%, 20%, 30%, 40%, 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 antibody molecule; and/or

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

10 7. The antibody molecule of claim 5 or 6, wherein the reference antibody molecule comprises a wild-type Fc region, an Fc region comprising the amino acid sequence of PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
15 KLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (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.

20 8. A composition comprising an antibody molecule of any of claims 1-7, optionally further comprising a pharmaceutical acceptable carrier.

9. A nucleic acid molecule encoding an antibody molecule of any of claims 1-7.

25 10. A vector comprising a nucleic acid molecule of claim 9.

11. A cell comprising a nucleic acid molecule of claim 9 or a vector of claim 10.

12. A kit comprising an antibody molecule of any of claims 1-7 and instructions to
30 use of the antibody molecule.

13. A container comprising an antibody molecule of any of claims 1-7.

14. A method of producing an antibody molecule, the method comprising culturing a cell of claim 11 under conditions that allow production of an antibody molecule, thereby producing the antibody molecule,

optionally, wherein the method, further comprises isolating or purifying the antibody
5 molecule.

15. A method of detecting a molecule, the method comprising contacting a cell or a sample from a subject with an antibody molecule of any of claims 1-7, thereby detecting the molecule.