

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 a polypeptide 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 nomenclature.

In an example, 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.

5 In an example, 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.

10 In an example, 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.

15 In an example, 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.

20 In an example, 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 Fcγ receptor (*e.g.*, one, two, or all of FcγRI, FcγRIIa/b, or

FcγRIII), compared to a reference polypeptide, *e.g.*, as determined by an octet-based assay or a cell-based assay.

In an example, 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.

In an example, 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 example, 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.

In an example, the polypeptide has the same effector function, or does not substantially alter (*e.g.*, decreases or increases by more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) an effector function, or increases an effector function (*e.g.*, by at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 50-fold), *e.g.*, one or more (*e.g.*, 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), compared to a reference polypeptide.

In an example, the polypeptide has an increased half-life *in vivo*, *e.g.*, at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold increase, compared to a reference polypeptide, *e.g.*, as determined in an animal model.

In an example, the polypeptide has the same biological function, does not substantially alter (*e.g.*, decreases or increases by no more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) a biological function, or increases a biological function (*e.g.*, by at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 50-fold), *in vitro*, *ex vivo*, or *in vivo*, compared to a reference polypeptide. In an example, the biological function comprises an inhibitory (*e.g.*, neutralizing) activity. In an example, the biological function comprises inhibiting (*e.g.*, neutralizing) a pathogen, *e.g.*, a virus, a bacterium, or a fungus. In an example, the biological function comprising an anti-tumor activity. In an example, the biological function comprises inhibiting an immune response. In an example, the biological function comprises an agonistic activity. In an example, the biological function comprises activating or restoring an immune response.

In an example, the polypeptide has the same developability characteristic, does not substantially alter (*e.g.*, decreases or increases by no more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) a developability characteristic, or increases a developability characteristic (*e.g.*, by at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 50-fold), *e.g.*, one or more (*e.g.*, two, three, or all) of stability, solubility, aggregation, or expression level, compared to a reference polypeptide;

In an example, the polypeptide has the same binding affinity, specificity, or both, or 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, specificity, or both, or increases the binding affinity, specificity, or both (*e.g.*, by at least 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 50-fold), for an epitope, compared to a reference polypeptide.

In an example, the polypeptide 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 example, the polypeptide has properties a) or b) above. In an example, the polypeptide has properties a) and b) above. In an example, the polypeptide has one or both of properties a) or b) above, and one or both of properties c) or d) above. In an example, the polypeptide has one or both of properties a) or b) above, and one, two, three, four, or all of properties

e), f), g), h), or i) above. In an example, the polypeptide has one or both of properties a) or b) above, and one or both of properties c) or d) above. In an example, the polypeptide has one or both of properties a) or b) above, and one, two, three, four, five, six, or all of properties c), d), j), k), l), m), or n) above. In an example, the polypeptide has one or both of properties a) or b) above, one, two, three, 5 four, or all of properties e), f), g), h), or i) above, and one, two, three, four, five, six, or all of properties c), d), j), k), l), m), or n) above. In an example, the polypeptide has one, two, three, or all of properties a), b), c), or d) above, one, two, three, four, or all of properties e), f), g), h), or i) above, and one, two, three, four, or all of properties j), k), l), m), or n) above. In an example, the polypeptide has one or both of properties a) or c) above, one or both of properties b) or d) above, one, two, three, 10 four, or all of properties e), f), g), h), or i) above, and one, two, three, four, or all of properties j), k), l), m), or n) above.

In an example, the reference polypeptide is an otherwise identical polypeptide without the mutation, *e.g.*, comprising a wild-type Fc region, *e.g.*, having the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or 15 which differs by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 15 amino acid residues.

In an example, the mutation is in a residue in a CH2 domain. In another example, the mutation is in a residue in a CH3 domain. In an example, the polypeptide comprises at least one mutation in a residue in a CH2 domain and at least one mutation in a residue in a CH3 domain. In an example, the polypeptide further comprises a mutation in a residue in a region other than a CH2 20 domain and/or a CH3 domain.

In an example, the mutation does not alter, or does not substantially alter, the conformation of the linker region between the CH2 and CH3 domains. In an example, the mutation does not introduce a cluster (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, or more consecutive) of hydrophobic or aromatic residues, *e.g.*, on a surface region (*e.g.*, a region defined as the area covered by amino acid residues that have more than 25 20% solvent accessible area).

In an example, the polypeptide is an antibody molecule, *e.g.*, an antibody molecule described herein.

In an example, the polypeptide is an IgG, *e.g.*, IgG1, IgG2, IgG3, or IgG4. In an example, the polypeptide is an IgG1. In an example, the polypeptide is an IgG4.

30 In an example, the polypeptide comprises a heavy chain immunoglobulin variable region, a light chain immunoglobulin variable region, or both. In an example, the polypeptide comprises a tetramer of two heavy chain immunoglobulin variable regions and two light chain immunoglobulin variable regions. In an example, the polypeptide comprises a full length antibody molecule. In an example, the polypeptide comprises a fragment (*e.g.*, an antigen-binding fragment) of an antibody 35 molecule.

In an example, the polypeptide comprises a chimeric antibody molecule. In an example, the polypeptide comprises a humanized antibody molecule. In an example, the

polypeptide comprises a human antibody molecule. In an example, the polypeptide comprises a murine antibody molecule. In an example, the polypeptide comprises a bispecific or multispecific antibody molecule.

In another example, the polypeptide is a fusion protein, *e.g.*, a fusion protein described herein. In an example, the polypeptide comprises a fragment (*e.g.*, functional fragment) of the fusion polypeptide.

In an example, the polypeptide comprises one or more (*e.g.*, 2, 3, 4, or all) of the following:

(i) a mutation in a residue in a surface region (*e.g.*, a region defined as the area covered by amino acid residues that have more than 20% solvent accessible area) that interacts with the FcRn, *e.g.*, an FcRn contact residue;

(ii) a mutation in a residue that is a peripheral residue along the Fc-FcRn interface (*e.g.*, any amino acid residues on the surface of the Fc region that is less than 7 Angstroms from the FcRn in the Fc-FcRn complex);

(iii) a mutation is in a residue that is non-contact residue in Fc-FcRn binding;

(iv) a mutation in a residue which is a helix contact residue that enhances the conformational dynamics of 250-helix, *e.g.*, a helix comprising one or more (*e.g.*, 2, 3, 4, 5, or all) of P247, K248, D249, T250, L251, or M252; or

(v) a mutation, which modulates pK of a histidine and/or is an introduction of a histidine along the Fc-FcRn interface (*e.g.*, any amino acid residues on the surface of the Fc region that is less than 7 Angstroms from the FcRn in the Fc-FcRn complex).

In an example, the polypeptide comprises (i) and (ii) above. In an example, the polypeptide comprises (i) and (iii) above. In an example, the polypeptide comprises (i) and (iv) above. In an example, the polypeptide comprises (i) and (v) above. In an example, the polypeptide comprises (ii) and (iii) above. In an example, the polypeptide comprises (ii) and (iv) above. In an example, the polypeptide comprises (ii) and (v) above. In an example, the polypeptide comprises (iii) and (iv) above. In an example, the polypeptide comprises (iii) and (v) above. In an example, the polypeptide comprises (iv) and (v) above.

In an example, the polypeptide comprises (i), (ii) and (iii) above. In an example, the polypeptide comprises (i), (ii) and (iv) above. In an example, the polypeptide comprises (i), (ii) and (v) above. In an example, the polypeptide comprises (i), (iii) and (iv) above. In an example, the polypeptide comprises (i), (iv) and (v) above. In an example, the polypeptide comprises (ii), (iii) and (iv) above. In an example, the polypeptide comprises (ii), (iii) and (v) above. In an example, the polypeptide comprises (ii), (iv) and (v) above. In an example, the polypeptide comprises (iii), (iv) and (v) above.

In an example, the polypeptide comprises (i), (ii), (iii) and (iv) above. In an example, the polypeptide comprises (i), (ii), (iii) and (v) above. In an example, the polypeptide comprises (i), (ii), (iv) and (v) above. In an example, the polypeptide comprises (i), (iii), (iv) and (v) above. In an example, the polypeptide comprises (ii), (iii), (iv) and (v) above.

5 In an example, the polypeptide comprises (i), (ii), (iii), (iv), and (v) above.

In an example, the polypeptide comprises a mutation in a residue in a surface region (*e.g.*, a region defined as the area covered by amino acid residues that have more than 20% solvent accessible area) that interacts with the FcRn, *e.g.*, an FcRn contact residue.

In an example, the mutation is in a residue chosen from: L251, I253, R255, P257, H285,
10 N286, K288, T307, V308, L309, Q311, L314, H310, H433, N434, H435, or Y436. In an example, the polypeptide comprises a plurality of mutations in two or more (*e.g.*, 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.

In an example, the polypeptide comprises a mutation in a residue that is a peripheral residue
15 along the Fc-FcRn interface (*e.g.*, any amino acid residues on the surface of the Fc region that is less than 7 Angstroms from the FcRn in the Fc-FcRn complex).

In an example, the mutation is in a residue chosen from one or more (*e.g.*, 2, 3, 4, 5, 6, or all) of T256, H285, N286, T307, Q311, N315, or A378. In an example, the polypeptide comprises a plurality of mutations in two or more (*e.g.*, 2, 3, 4, 5, 6, or all) of the residues chosen from T256, H285, N286,
20 T307, Q311, N315, or A378. In an example, the polypeptide comprises one or more (*e.g.*, 2, 3, 4, 5, 6, or all) of the mutations chosen from T256D, H285N, N286D, T307Q, Q311V, N315D, or A378V.

In an example, the mutation is in a residue chosen from T256, Q311, or A378. In an example, the polypeptide comprises a plurality of mutations in two or all of the residues chosen from T256, Q311, or A378. In an example, the polypeptide comprises one, two, or all of the mutations chosen
25 from T256D, Q311V, or A378V.

In an example, the mutation is in a residue chosen from H285, T307, or N315. In an example, the polypeptide comprises a plurality of mutations in two or all of the residues chosen from H285, T307, or N315. In an example, the polypeptide comprises one, two, or all of the mutations chosen from H285N, T307Q, or N315D.

30 In an example, the mutation is in a residue chosen from H285, T307, or A378. In an example, the polypeptide comprises a plurality of mutations in two or all of the residues chosen from H285, T307, or A378. In an example, the polypeptide comprises one, two, or all of the mutations chosen from H285D, T307Q, or A378V.

In an example, the mutation is in a residue chosen from T307, Q311, or A378. In an example, the polypeptide comprises a plurality of mutations in two or all of the residues chosen

from T307, Q311, or A378. In an example, the polypeptide comprises one, two, or all of the mutations chosen from T307Q, Q311V, or A378V.

In an example, the mutation is in a residue chosen from T256, N286, T307, Q311, or A378. In an example, the polypeptide comprises a plurality of mutations in two, three, four, or all of the residues chosen T256, N286, T307, Q311, or A378. In an example, the polypeptide comprises one, two, three, four, or all of the mutations chosen from T256D, N286D, T307R, Q311V, or A378V.

In an example, the mutation is in a residue chosen from T256, H285, T307, Q311, or A378. In an example, the polypeptide comprises a plurality of mutations in two, three, four, or all of the residues chosen T256, H285, T307, Q311, or A378. In an example, the polypeptide comprises one, two, three, four, or all of the mutations chosen from T256D, H285D, T307R, Q311V, or A378V.

In an example, the mutation is in a residue chosen from M252, T256, T307, L309, Q311, H433, N434, Y436, N286, or K288. In an example, the polypeptide comprises a plurality of mutations in two or more (*e.g.*, 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.

In an example, the polypeptide comprises a mutation is in a residue that is non-contact residue in Fc-FcRn binding.

In an example, the mutation is in a residue chosen from A287, V308, N315, L314, L432, H429, E430, or A431. In an example, the polypeptide comprises a plurality of mutations in two or more (*e.g.*, 3, 4, 5, 6, 7, or all) of the residues chosen from A287, V308, N315, L314, L432, H429, E430, or A431.

In an example, the polypeptide comprises a mutation in a residue which is a helix contact residue that enhances the conformational dynamics of 250-helix (*e.g.*, a helix comprising one or more (*e.g.*, 2, 3, 4, 5, or all) of P247, K248, D249, T250, L251, or M252), *e.g.*, a lateral displacement or conformational flexibility exhibited by the 250-helix (*e.g.*, as shown by a comparison of the crystal structures of the Fc domain crystallized at pH5.0 (PDB ID: 4J12) and at pH 6.5 (PDB ID: 4Q7D).

In an example, the mutation is in a residue chosen from P244, P245, T250, L251, P247, E380, M428, A378, D376, P257, V308, A287, L306, or H427. In an example, the polypeptide comprises a plurality of mutations in two or more (*e.g.*, 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.

In an example, the polypeptide comprises a mutation which is the introduction of a histidine along the Fc-FcRn interface (*e.g.*, any amino acid residues on the surface of the Fc region that is less than 7 Angstroms from the FcRn in the Fc-FcRn complex).

In an example, the mutation introduces a polar amino acid residue. In another example, the mutation introduces a non-polar amino acid residue. In an example, the

mutation introduces a charged amino acid residue. In another example, the mutation introduces a non-charge amino acid residue. In an example, the mutation introduces a positively charged (or basic) amino acid residue. In another example, the mutation introduces a negatively charged (or acidic) amino acid residue. In an example, the mutation introduces a hydrophobic amino acid residue. In another example, the mutation introduces a hydrophilic amino acid residue.

In an example, the polypeptide comprises a mutation in at least one (*e.g.*, 2, 3, 4, 5, or more) FcRn contact residue. In an example, the polypeptide comprises a mutation in one or more (*e.g.*, 2 or all) of residues T256, T307, or N286. In an example, the polypeptide comprises mutations in residues T256 and T307, optionally, further comprises a mutation in residue N286. In an example, the mutation in residue T256 is a polar residue, *e.g.*, chosen from T256D, T256E, or T256R. In an example, the mutation in residue T256 is T256D. In an example, the mutation in residue T307 is a polar residue, *e.g.*, chosen from T307D, T307E, or T307R. In an example, the mutation in residue T307 is T307R. In an example, the mutation in residue N286 is N286I. In an example, the polypeptide comprises mutations T256D and T307R. In an example, the polypeptide comprises mutations T256D, T307R, and N286I.

In an example, the polypeptide comprises a mutation in at least one (*e.g.*, 2, 3, 4, 5, or more) FcRn contact residue. In an example, the polypeptide comprises a mutation in one or more (*e.g.*, 2 or all) of residues L309, D312, or N315. In an example, the polypeptide comprises a mutation in residue L309, optionally, further comprises mutations in residues D312 and N315. In an example, the mutation in residue L309 is L309N. In an example, the mutation in residue D312 is D312A. In an example, the mutation in residue N315 is N315D. In an example, the polypeptide comprises mutation L309N. In an example, the polypeptide comprises mutations L309N, D312A and N315D.

In an example, the polypeptide comprises a mutation in at least one (*e.g.*, 1, 2, 3, 4, 5, or more) FcRn non-contact residue. In an example, the polypeptide comprises a mutation in one or more (*e.g.*, 2 or all) of residues L209R, D312, or Q311. In an example, the polypeptide comprises mutations in residues L309 and D312, optionally, further comprising a mutation in residue Q311. In an example, the polypeptide comprises a mutation in residue Q311. In an example, the mutation in residue L309 is L309R. In an example, the mutation in residue D312 is D312E. In an example, the mutation in residue Q311 is Q311P. In an example, the polypeptide comprises mutations L309R and D312E. In an example, the polypeptide comprises mutations L309R, D312E, and Q311P. In an example, the polypeptide comprises mutation Q311P.

In an example, the polypeptide comprises a mutation in at least one (*e.g.*, 2, 3, 4, 5, or more) FcRn contact residue. In an example, the polypeptide comprises a mutation in one or more (*e.g.*, 2, 3, or 4) of residues chosen from I253, S254, M252, or R255. In an example, the polypeptide comprises a mutation in residue I253. In an example, the mutation in residue I253 is I253M. In an example, the polypeptide comprises a mutation in residue S254. In an example,

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the mutation in residue S254 is S254H or S254M. In an example, the polypeptide comprises mutations in residues M252 and S254. In an example, the mutation in residue M252 is M252E. In an example, the mutation in residue S254 is S254R. In an example, the polypeptide comprises mutations in residues M252, S254, and R255. In an example, the mutation in residue M252 is M252E. In an example, the mutation in residue S254 is S254R. In an example, the mutation in residue R255 is R255Y.

In an example, the polypeptide comprises a mutation in at least one (*e.g.*, 2, 3, 4, 5, or more) FcRn non-contact residue. In an example, the polypeptide comprises mutations that are equivalent of T250Q and M34L. In an example, the polypeptide comprises a mutation in one or more (*e.g.*, 2, 3, 4, or all) of residues chosen from D376, K248, E380, M428, or A328. In an example, the polypeptide comprises a mutation in residue D376 and a mutation in a residue chosen from K248, E380, M428, or A328. In an example, the polypeptide comprises a mutation in residue D376 and a mutation in residue K248. In an example, the polypeptide comprises a mutation in residue D376 and a mutation in residue E380. In an example, the polypeptide comprises a mutation in residue D376 and a mutation in residue M428. In an example, the polypeptide comprises a mutation in residue D376 and a mutation in residue A328. In an example, the mutation in residue D376 is D376Q or D376N. In an example, the mutation in residue K248 is K248S. In an example, the mutation in residue E380 is E380A. In an example, the mutation in residue D376 is D376Q. In an example, the mutation in residue M428 is M428L. In an example, the mutation in residue A328 is A328I. In an example, the polypeptide comprises mutation D376Q or D376N, and mutation K248S. In an example, the polypeptide comprises mutation D376Q or D376N, and mutation E380A. In an example, the polypeptide comprises mutations D376Q and M428L. In an example, the polypeptide comprises mutations D376Q and A328I.

In an example, the polypeptide comprises a mutation in at least one (*e.g.*, 2, 3, 4, 5, or more) FcRn non-contact residue. In an example, the polypeptide comprises a mutation in one or more (*e.g.*, 2 or all) of residues chosen from K246, P247, or D376. In an example, the polypeptide comprises a mutation in residue K246 and a mutation in residue P247, optionally, further comprising a mutation in residue D376. In an example, the mutation in residue K246 is K246N. In an example, the mutation in residue P247 is P247A. In an example, the mutation in residue D376 is D376N. In an example, the polypeptide comprises mutations K246N and P247A, optionally, further comprising mutation D376N.

In an example, the polypeptide comprises a mutation in one or more (*e.g.*, 2, 3, 4, 5, 6, or all) of residues chosen from T256, T307, N286, A287, P257, Q311, or P247. In an

example, the polypeptide comprises mutations in residues T256, T307, N286, and A287. In an example, the polypeptide comprises mutations in residues T256, T307, and P257. In an example, the polypeptide comprises mutations in residues T256, T307, and Q311. In an example, the

polypeptide comprises mutations in residues T256, T307, and P247. In an example, mutation in residue T256 is T256D. In an example, the mutation in residue T307 is T307R. In an example, the mutation in residue N286 is N286I. In an example, the mutation in residue A287 is A287S. In an example, the mutation in residue P257 is P257L. In an example, the mutation in residue Q311 is Q311V or Q311L. In an example, the mutation in residue P247 is P247D. In an example, the polypeptide comprises mutations T256D, T307R, N286D, and A287S. In an example, the polypeptide comprises mutations T256D, T307R, and P257L. In an example, the polypeptide comprises mutations T256D, T307R, and Q311V or Q311L. In an example, the polypeptide comprises mutations T256D, T307R, and P247D.

10 In an example, the polypeptide comprises a mutation in one or more (*e.g.*, 2, 3, 4, 5, 6, 7, or all) of residues chosen from N286, A287, P247, Q311, V308, P257, N315, or V279. In an example, the polypeptide comprises mutations in residues N286, A287, P247, and Q311. In an example, the mutation in residue N286 is N286D. In an example, the mutation in residue A287 is A287S. In an example, the mutation in residue P247 is P247D. In an example, the mutation in residue Q311 is Q311V. In an example, the polypeptide comprises mutations in residues V308 and P257. In an example, the mutation in residue V308 is V308N. In an example, the mutation in residue P257 is P257M. In an example, the polypeptide comprises mutations in residues Q311, N315, and V279. In an example, the mutation in residue Q311 is Q311L. In an example, the mutation in residue N315 is N315T. In an example, the mutation in residue V279 is V279I.

15 20 In an example, the polypeptide comprises a mutation in one or more (*e.g.*, 2, 3, or all) of residues chosen from G433 or H433, P434 or G434, G434a, or H435. In an example, the polypeptide comprises mutations in residues G433, P434, and H435. In an example, the polypeptide comprises mutations in residues G433, P434, G434a, and H435. In an example, the polypeptide comprises mutations in residues H433, G434, P434a, and H435.

25 In an example, the polypeptide comprises one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) mutations, or one or more combination of mutations, as described in **Table 1**. For example, a combination of mutations can include one or more mutations listed under the name FcMutX and one or more mutations listed under the name FcMutY, where X and Y are three-digit numbers shown in **Table 1**, and X is not equal to Y.

30 In an example, the polypeptide comprises mutation I253M. In an example, the polypeptide comprises mutations L309H, D312A and N315D. In an example, the polypeptide comprises mutation L309N. In an example, the polypeptide comprises mutations M252E and S254R. In an example, the polypeptide

comprises mutations M252E, S254R and R255Y. In an example, the polypeptide comprises mutation S254H. In an example, the polypeptide comprises mutation S254M. In an example, the polypeptide comprises mutations T256D and T307R. In an example, the polypeptide comprises mutations T256L, N286I and T307I. In an

example, the polypeptide comprises mutations T256I, N286I and T307I. In an example, the polypeptide comprises mutations K248S and D376Q. In an example, the polypeptide comprises mutations K248S and D376N. In an example, the polypeptide comprises mutations D376Q and E380A. In an example, the polypeptide comprises mutations D376N and E380A. In an example, the polypeptide comprises mutations D376Q and M428L. In an example, the polypeptide comprises mutations K248S and A378I. In an example, the polypeptide comprises mutation L314K. In an example, the polypeptide comprises mutation M252W. In an example, the polypeptide comprises mutation V308F. In an example, the polypeptide comprises mutations V308F and N434Y. In an example, the polypeptide comprises mutations T256D, T307R and D376N. In an example, the polypeptide comprises mutations L309R and D312E. In an example, the polypeptide comprises mutations L309R, Q311P and D312E. In an example, the polypeptide comprises mutations K246N and P247A. In an example, the polypeptide comprises mutations K246N, P247A and D376N. In an example, the polypeptide comprises mutations T256E and T307R. In an example, the polypeptide comprises mutations T256R and T307D. In an example, the polypeptide comprises mutations T256R and T307E. In an example, the polypeptide comprises mutation Q311P. In an example, the polypeptide comprises mutation D376Q. In an example, the polypeptide comprises mutations T256D, N286D, A287S and T307R. In an example, the polypeptide comprises mutations T256D, P257L and T307R. In an example, the polypeptide comprises mutations T256D, T307R and Q311V. In an example, the polypeptide comprises mutations P247D, T256D and T307R. In an example, the polypeptide comprises mutations P247D, N286D, A287S and Q311V. In an example, the polypeptide comprises mutations P257M and V308N. In an example, the polypeptide comprises mutations V279I, Q311L and N315T. In an example, the polypeptide comprises mutations H433G and N434P. In an example, the polypeptide comprises mutations T256D, N286D and T307R. In an example, the polypeptide comprises mutations T256D, N286E and T307R. In an example, the polypeptide comprises mutations T256D, N286Q and T307R. In an example, the polypeptide comprises mutations T256D, P257T and T307R. In an example, the polypeptide comprises mutations T256D, P257V and T307R. In an example, the polypeptide comprises mutations T256D, T307R and Q311I. In an example, the polypeptide comprises mutations T256D, T307R and Q311L. In an example, the polypeptide comprises mutations T256D, T307R and Q311M. In an example, the polypeptide comprises mutations T256D, P257L, N286D, T307R and Q311V. In an example, the polypeptide comprises mutations T256D, T307R and M428L

In an example, the mutation is other than M252Y, S254T, T256E, L309N, T250Q, M428L, N434S, N434A, T307A, E380A, N434A, M252Y, S254T, T256E, or a combination thereof. In an example, the mutation is in a residue other than residue M252, S254, T256, L309, T250, M428, N434, N434, T307, E380, N434, M252, S254, T256, or a combination thereof. In an example, the

5 polypeptide does not have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or all) of the

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 aspect, 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 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.

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 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; 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 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 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. A polypeptide 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 nomenclature.
2. The polypeptide 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 domains of the Fc region.
- 15 3. The polypeptide of claim 1 or 2, which is an isolated polypeptide or a synthetic polypeptide.
4. The polypeptide of any of claims 1-3, which:
 - (i) is an antibody molecule, optionally wherein the antibody molecule is a chimeric
 - 20 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;
 - (iii) is an immunoglobulin chain or a fragment thereof; or
 - 25 (iv) is a fusion protein.
5. The polypeptide of any of claims 1-4, 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
 - 30 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;
- 5 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.
- 10 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;
- 15 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
- 20 wherein the polypeptide has at least properties a), b), and one, two, three, four, or all of properties e), f), g), h), or i).

6. The polypeptide 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
- 25 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;
- (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;
- 30 (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 polypeptide, 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 polypeptide, 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 polypeptide, 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 polypeptide, 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 polypeptide;

(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 polypeptide, 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 polypeptide;

(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 polypeptide;

(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 polypeptide; 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 polypeptide, as determined by a transcytosis assay.

10 7. The polypeptide of claim 5 or 6 wherein the reference polypeptide comprises a wild-type Fc region, an Fc region comprising the amino acid sequence of PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVTLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
15 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.

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

 9. A nucleic acid molecule encoding a polypeptide 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 a polypeptide of any of claims 1-7 and instructions to use of the
30 polypeptide.

 13. A container comprising a polypeptide of any of claims 1-7.

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

optionally, wherein the method, further comprises isolating or purifying the polypeptide.

15. Use of a polypeptide of any of claims 1-7, or a composition of claim 8, in the manufacture of a medicament for the treatment of a disorder in a subject; wherein the disorder is an immune
5 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.

16. 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 1-7, thereby detecting the molecule.