

[0008] Also disclosed is a fusion molecule comprising a canine IgG Fc region variant disclosed herein and a polypeptide. In some examples, the canine IgG Fc region variant is covalently attached to the polypeptide (e.g., through a hinge region or a linker). In some instances, the polypeptide is a ligand binding domain of a canine receptor protein, an extracellular domain of a canine receptor protein, or an antigen-binding domain. In some instances, the polypeptide is selected from the ligand binding domain or extracellular domain of canine IL-13R α 1, or IL-13R α 2, canine EPO, canine CTLA4, canine LFA3, canine VEGFR1/VEGFR3, canine IL-1R, canine GLP-1 receptor agonist, and canine Thrombopoietin binding peptide. In some instances, the polypeptide is a scFv, a nanobody, or single domain antibody. In some instances, the IgG Fc region variant is a variant of a canine IgG.B antibody Fc region. In some instances, the IgG Fc region variant is a variant of a canine IgG.A antibody Fc region. In some instances, the IgG Fc region variant is a variant of a canine IgG.C antibody Fc region. In some instances, the IgG Fc region variant is a variant of a canine IgG.D antibody Fc region.

[0008a] In one aspect, the present disclosure refers to a polypeptide comprising a canine IgG Fc region variant, or a canine FcRn-binding region thereof, wherein the polypeptide comprises at least one amino acid substitution at a position selected from the group consisting of:

- an amino acid position that corresponds to amino acid position 250 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 251 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 252 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 254 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 256 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 285 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 286 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 307 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 308 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 309 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 311 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 315 of a wild type canine IgG,

an amino acid position that corresponds to amino acid position 378 of a wild type canine IgG,
an amino acid position that corresponds to amino acid position 380 of a wild type canine IgG,
an amino acid position that corresponds to amino acid position 428 of a wild type canine IgG,
an amino acid position that corresponds to amino acid position 430 of a wild type canine IgG,
an amino acid position that corresponds to amino acid position 433 of a wild type canine IgG,
an amino acid position that corresponds to amino acid position 434 of a wild type canine IgG,
an amino acid position that corresponds to amino acid position 435 of a wild type canine IgG, and
an amino acid position that corresponds to amino acid position 436 of a wild type canine IgG,

wherein the amino acid positions are based on EU numbering, and wherein the polypeptide has increased binding to canine FcRn compared to an Fc domain of the wild type canine IgG.

[0009] In one example, the disclosure provides a polypeptide or polypeptides comprising a canine IgG Fc CH2 region variant, the CH2 region variant comprising an amino acid sequence that is at least 75% identical to the sequence set forth in SEQ ID NO:1, and comprises at least one of the following:

an amino acid other than Ile at amino acid position 250,
an amino acid other than Leu at amino acid position 251,
an amino acid other than Arg at amino acid position 252,
an amino acid other than Thr at amino acid position 254,
an amino acid other than Thr at amino acid position 256,
an amino acid other than His at amino acid position 285,
an amino acid other than Thr at amino acid position 286,
an amino acid other than Pro at amino acid position 307,
an amino acid other than Ile at amino acid position 308,
an amino acid other than Glu at amino acid position 309,
an amino acid other than Gln at amino acid position 311, or
an amino acid other than Thr at amino acid position 315.

[0010] In some examples, the polypeptide or polypeptides has/have: (1) increased half-life in a dog than a control polypeptide or control polypeptides, wherein the control polypeptide or control polypeptides are identical to the polypeptide or polypeptides except for having the corresponding wild type canine IgG Fc CH2 region in place of the IgG Fc CH2

region variant; and/or (2) increased binding to canine FcRn than the control polypeptide or polypeptides; and wherein the amino acid positions are based on EU numbering.

[0011] In some examples, the amino acid sequence is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequence set forth in SEQ ID NO:1.

[0012] In one example, the disclosure provides a polypeptide or polypeptides comprising a canine IgG Fc CH2 region variant, the CH2 region variant comprising an amino acid sequence that is at least 75% identical to the sequence set forth in SEQ ID NO:2, and comprises at least one of the following:

- an amino acid other than Thr at amino acid position 250,
- an amino acid other than Leu at amino acid position 251,
- an amino acid other than Leu at amino acid position 252,
- an amino acid other than Ala at amino acid position 254,
- an amino acid other than Thr at amino acid position 256,
- an amino acid other than Gln at amino acid position 285,
- an amino acid other than Thr at amino acid position 286,
- an amino acid other than Pro at amino acid position 307,
- an amino acid other than Ile at amino acid position 308,
- an amino acid other than Gly at amino acid position 309,
- an amino acid other than Gln at amino acid position 311, or
- an amino acid other than Lys at amino acid position 315.

[0013] In some examples, the polypeptide or polypeptides has/have: (1) increased half-life in a dog than a control polypeptide or control polypeptides, wherein the control polypeptide or control polypeptides are identical to the polypeptide or polypeptides except for having the corresponding wild type canine IgG Fc CH2 region in place of the IgG Fc CH2 region variant; and/or (2) increased binding to canine FcRn than the control polypeptide or polypeptides; and wherein the amino acid positions are based on EU numbering.

[0014] In some examples, the amino acid sequence is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequence set forth in SEQ ID NO:2.

[0015] In one example, the disclosure further provides a polypeptide or polypeptides comprising a canine IgG Fc CH2 region variant, the CH2 region variant comprising an amino acid sequence that is at least 75% identical to the sequence set forth in SEQ ID NO:3, and comprises at least one of the following:

an amino acid other than Ile at amino acid position 250,
an amino acid other than Leu at amino acid position 251,
an amino acid other than Val at amino acid position 252,
an amino acid other than Ala at amino acid position 254,
an amino acid other than Thr at amino acid position 256,
an amino acid other than Gln at amino acid position 285,
an amino acid other than Thr at amino acid position 286,
an amino acid other than Pro at amino acid position 307,
an amino acid other than Ile at amino acid position 308,
an amino acid other than Gly at amino acid position 309,
an amino acid other than Gln at amino acid position 311, or
an amino acid other than Ser at amino acid position 315,

[0016] In some examples, the polypeptide or polypeptides has/have: increased half-life in a dog than a control polypeptide or control polypeptides, wherein the control polypeptide or control polypeptides are identical to the polypeptide or polypeptides except for having the corresponding wild type canine IgG Fc CH2 region in place of the IgG Fc CH2 region variant.

[0017] In some examples, the amino acid sequence is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequence set forth in SEQ ID NO:3.

[0018] In one example, the disclosure is related to a polypeptide or polypeptides comprising a canine IgG Fc CH2 region variant, the CH2 region variant comprising an amino acid sequence that is at least 75% identical to the sequence set forth in SEQ ID NO:4, and comprises at least one of the following:

an amino acid other than Ile at amino acid position 250,
an amino acid other than Leu at amino acid position 251,
an amino acid other than Arg at amino acid position 252,
an amino acid other than Thr at amino acid position 254,
an amino acid other than Thr at amino acid position 256,
an amino acid other than His at amino acid position 285,
an amino acid other than Thr at amino acid position 286,
an amino acid other than Pro at amino acid position 307,
an amino acid other than Ile at amino acid position 308,
an amino acid other than Glu at amino acid position 309,

an amino acid other than Gln at amino acid position 311, or

an amino acid other than Thr at amino acid position 315.

[0019] In some examples, the polypeptide or polypeptides has/have: (1) increased half-life in a dog than a control polypeptide or control polypeptides, wherein the control polypeptide or control polypeptides are identical to the polypeptide or polypeptides except for having the corresponding wild type canine IgG Fc CH2 region in place of the IgG Fc CH2 region variant; and/or (2) increased binding to canine FcRn than the control polypeptide or polypeptides; and wherein the amino acid positions are based on EU numbering.

[0020] In some examples, the amino acid sequence is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequence set forth in SEQ ID NO:4.

[0021] In some examples, the polypeptide or polypeptides comprise(s) at least one of the following:

Glu or Gln at amino acid position 250,
Asp or Glu at amino acid position 251,
Tyr at amino acid position 252,
Thr at amino acid position 254,
Asp, Glu, or Phe at amino acid position 256,
Asn or Asp at amino acid position 285,
Asp at amino acid position 286,
Arg, Gln, or Ala at amino acid position 307,
Pro at amino acid position 308,
Pro at amino acid position 309,
Val at amino acid position 311, or
Asp at amino acid position 315.

[0022] In some examples, the polypeptide or polypeptides comprise(s) at least one of the following:

Tyr or Met at amino acid position 252,
Thr at amino acid position 254, or
Glu at amino acid position 256.

[0023] In some examples, the canine IgG Fc CH2 region variant comprises at least one of the following:

(i) Tyr at amino acid position 252, Thr at amino acid position 254, and Glu at amino acid position 256;

- (ii) Leu at amino acid position 428 and Ser at amino acid position 434;
- (iii) Asp at amino acid position 256, Arg at amino acid position 307, and Val at amino acid position 311;
- (iv) Asp at amino acid position 256, Asp at amino acid position 315, and Val at amino acid position 378;
- (v) Asp at amino acid position 256, Asp at amino acid position 286, Arg at amino acid position 307, and Val at amino acid position 311;
- (vi) Asn at amino acid position 285, Gln at amino acid position 307, and Asp at amino acid position 315; or
- (vii) Asp at amino acid position 251, and Pro at amino acid position 309.

[0024] In one example, the disclosure provides a polypeptide or polypeptides comprising a canine IgG Fc CH3 region variant comprising an amino acid sequence that is at least 75% identical to the sequence set forth in SEQ ID NO:5, and comprises at least one of the following:

- an amino acid other than Asp at amino acid position 378,
- an amino acid other than Glu at amino acid position 380,
- an amino acid other than Met at amino acid position 428,
- an amino acid other than Glu at amino acid position 430,
- an amino acid other than Gln at amino acid position 433,
- an amino acid other than Asn at amino acid position 434,
- an amino acid other than His at amino acid position 435, or
- an amino acid other than Tyr at amino acid position 436.

[0025] In some examples, the polypeptide or polypeptides has/have: (1) increased half-life in a dog than a control polypeptide or control polypeptides, wherein the control polypeptide or control polypeptides are identical to the polypeptide or polypeptides except for having the corresponding wild type canine IgG Fc CH3 region in place of the IgG Fc CH3 region variant; and/or (2) increased binding to canine FcRn than the control polypeptide or polypeptides; and wherein the amino acid positions are based on EU numbering.

[0026] In some examples, the amino acid sequence is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequence set forth in SEQ ID NO:5.

[0027] In one example, the disclosure provides a polypeptide or polypeptides comprising a canine IgG Fc CH3 region variant comprising an amino acid sequence that is at

least 75% identical to the sequence set forth in SEQ ID NO:6, and comprises at least one of the following:

- an amino acid other than Asp at amino acid position 378,
- an amino acid other than Glu at amino acid position 380,
- an amino acid other than Met at amino acid position 428,
- an amino acid other than Glu at amino acid position 430,
- an amino acid other than His at amino acid position 433,
- an amino acid other than Asn at amino acid position 434,
- an amino acid other than His at amino acid position 435, or
- an amino acid other than Tyr at amino acid position 436.

[0028] In some examples, the polypeptide or polypeptides has/have: (1) increased half-life in a dog than a control polypeptide or control polypeptides, wherein the control polypeptide or control polypeptides are identical to the polypeptide or polypeptides except for having the corresponding wild type canine IgG Fc CH3 region in place of the IgG Fc CH3 region variant; and/or (2) increased binding to canine FcRn than the control polypeptide or polypeptides; and wherein the amino acid positions are based on EU numbering.

[0029] In some examples, the amino acid sequence is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequence set forth in SEQ ID NO:6.

[0030] In one example, the disclosure provides a polypeptide or polypeptides comprising a canine IgG Fc CH3 region variant comprising an amino acid sequence that is at least 75% identical to the sequence set forth in SEQ ID NO:7, and comprises at least one of the following:

- an amino acid other than Asp at amino acid position 378,
- an amino acid other than Glu at amino acid position 380,
- an amino acid other than Met at amino acid position 428,
- an amino acid other than Glu at amino acid position 430,
- an amino acid other than His at amino acid position 433,
- an amino acid other than Asn at amino acid position 434,
- an amino acid other than His at amino acid position 435, or
- an amino acid other than Tyr at amino acid position 436.

[0031] In some examples, the polypeptide or polypeptides has/have: increased half-life in a dog than a control polypeptide or control polypeptides, wherein the control polypeptide or control polypeptides are identical to the polypeptide or polypeptides except for

having the corresponding wild type canine IgG Fc CH3 region in place of the IgG Fc CH3 region variant.

[0032] In some examples, the amino acid sequence is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequence set forth in SEQ ID NO:7.

[0033] In one example, the disclosure provides a polypeptide or polypeptides comprising a canine IgG Fc CH3 region variant comprising an amino acid sequence that is at least 75% identical to the sequence set forth in SEQ ID NO:8, and comprises at least one of the following:

- an amino acid other than Asp at amino acid position 378,
- an amino acid other than Glu at amino acid position 380,
- an amino acid other than Met at amino acid position 428,
- an amino acid other than Glu at amino acid position 430,
- an amino acid other than Gln at amino acid position 433,
- an amino acid other than Asn at amino acid position 434,
- an amino acid other than His at amino acid position 435, or
- an amino acid other than Tyr at amino acid position 436.

[0034] In some examples, the polypeptide or polypeptides has/have: (1) increased half-life in a dog than a control polypeptide or control polypeptides, wherein the control polypeptide or control polypeptides are identical to the polypeptide or polypeptides except for having the corresponding wild type canine IgG Fc CH3 region in place of the IgG Fc CH3 region variant; and/or (2) increased binding to canine FcRn than the control polypeptide or polypeptides; and wherein the amino acid positions are based on EU numbering.

[0035] In some examples, the amino acid sequence is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequence set forth in SEQ ID NO:8.

[0036] In some examples, the polypeptide or polypeptides comprise(s) at least one of the following:

- Val at amino acid position 378,
- Ala at amino acid position 380,
- Leu at amino acid position 428,
- Ala or Lys at amino acid position 430,
- Lys at amino acid position 433,
- Ser, Ala, or Phe at amino acid position 434,

Try at amino acid position 435,

His at amino acid position 436.

In some examples, the canine IgG Fc CH3 region variant comprises:

(i) Leu at amino acid position 428, and Ser at amino acid position 434;

(ii) Leu at amino acid position 428, and Ala at amino acid position 434;

(iii) Lys at amino acid position 430, and Lys at amino acid position 433; or

(iv) Tyr at amino acid position 435, and His at amino acid position 436.

[0037] In some examples, the polypeptide or polypeptides comprise(s) Tyr, Trp, Arg, or His at amino acid position 434.

[0038] In one example, the disclosure provides a polypeptide or polypeptides comprising a canine IgG Fc region variant comprising an amino acid sequence that is at least 75% identical to the sequence set forth in SEQ ID NO:9, and that comprises at least one of the following:

an amino acid other than Ile at amino acid position 250,

an amino acid other than Leu at amino acid position 251,

an amino acid other than Arg at amino acid position 252,

an amino acid other than Thr at amino acid position 254,

an amino acid other than Thr at amino acid position 256,

an amino acid other than His at amino acid position 285,

an amino acid other than Thr at amino acid position 286,

an amino acid other than Pro at amino acid position 307,

an amino acid other than Ile at amino acid position 308,

an amino acid other than Glu at amino acid position 309,

an amino acid other than Gln at amino acid position 311,

an amino acid other than Thr at amino acid position 315,

an amino acid other than Asp at amino acid position 378,

an amino acid other than Glu at amino acid position 380,

an amino acid other than Met at amino acid position 428,

an amino acid other than Glu at amino acid position 430,

an amino acid other than Gln at amino acid position 433,

an amino acid other than Asn at amino acid position 434,

an amino acid other than His at amino acid position 435, or

an amino acid other than Tyr at amino acid position 436.

[0039] In some examples, the polypeptide or polypeptides has/have: (1) increased half-life in a dog than a control polypeptide or control polypeptides, wherein the control polypeptide or control polypeptides are identical to the polypeptide or polypeptides except for having the corresponding wild type canine IgG Fc region in place of the IgG Fc region variant; and/or (2) increased binding to canine FcRn than the control polypeptides; and wherein the amino acid positions are based on EU numbering.

[0040] In some examples, the amino acid sequence is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequence set forth in SEQ ID NO:9.

[0041] In one example, the disclosure also provides a polypeptide or polypeptides comprising a canine IgG Fc region variant comprising an amino acid sequence that is at least 75% identical to the sequence set forth in SEQ ID NO:10, and that comprises at least one of the following:

- an amino acid other than Thr at amino acid position 250,
- an amino acid other than Leu at amino acid position 251,
- an amino acid other than Leu at amino acid position 252,
- an amino acid other than Ala at amino acid position 254,
- an amino acid other than Thr at amino acid position 256,
- an amino acid other than Gln at amino acid position 285,
- an amino acid other than Thr at amino acid position 286,
- an amino acid other than Pro at amino acid position 307,
- an amino acid other than Ile at amino acid position 308,
- an amino acid other than Gly at amino acid position 309,
- an amino acid other than Gln at amino acid position 311,
- an amino acid other than Lys at amino acid position 315,
- an amino acid other than Asp at amino acid position 378,
- an amino acid other than Glu at amino acid position 380,
- an amino acid other than Met at amino acid position 428,
- an amino acid other than Glu at amino acid position 430,
- an amino acid other than His at amino acid position 433,
- an amino acid other than Asn at amino acid position 434,
- an amino acid other than His at amino acid position 435, or
- an amino acid other than Tyr at amino acid position 436.

[0042] In some examples, the polypeptide or polypeptides has/have: (1) increased half-life in a dog than a control polypeptide or control polypeptides, wherein the control polypeptide or control polypeptides are identical to the polypeptide or polypeptides except for having the corresponding wild type canine IgG Fc region in place of the IgG Fc region variant; and/or (2) increased binding to canine FcRn than the control polypeptides; and wherein the amino acid positions are based on EU numbering.

[0043] In some examples, the amino acid sequence is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequence set forth in SEQ ID NO:10.

[0044] In one example, the disclosure provides a polypeptide or polypeptides comprising a canine IgG Fc region variant comprising an amino acid sequence that is at least 75% identical to the sequence set forth in SEQ ID NO:11, and that comprises at least one of the following:

- an amino acid other than Ile at amino acid position 250,
- an amino acid other than Leu at amino acid position 251,
- an amino acid other than Val at amino acid position 252,
- an amino acid other than Ala at amino acid position 254,
- an amino acid other than Thr at amino acid position 256,
- an amino acid other than Gln at amino acid position 285,
- an amino acid other than Thr at amino acid position 286,
- an amino acid other than Pro at amino acid position 307,
- an amino acid other than Ile at amino acid position 308,
- an amino acid other than Gly at amino acid position 309,
- an amino acid other than Gln at amino acid position 311,
- an amino acid other than Ser at amino acid position 315,
- an amino acid other than Asp at amino acid position 378,
- an amino acid other than Glu at amino acid position 380,
- an amino acid other than Met at amino acid position 428,
- an amino acid other than Glu at amino acid position 430,
- an amino acid other than His at amino acid position 433,
- an amino acid other than Asn at amino acid position 434,
- an amino acid other than His at amino acid position 435, or
- an amino acid other than Tyr at amino acid position 436.

[0045] In some examples, the polypeptide or polypeptides has/have: increased half-life in a dog than a control polypeptide or control polypeptides, wherein the control polypeptide or control polypeptides are identical to the polypeptide or polypeptides except for having the corresponding wild type canine IgG Fc region in place of the IgG Fc region variant.

[0046] In some examples, the amino acid sequence is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequence set forth in SEQ ID NO:11.

[0047] In one example, the disclosure provides a polypeptide or polypeptides comprising a canine IgG Fc region variant comprising an amino acid sequence that is at least 75% identical to the sequence set forth in SEQ ID NO:12, and that comprises at least one of the following:

- an amino acid other than Ile at amino acid position 250,
- an amino acid other than Leu at amino acid position 251,
- an amino acid other than Arg at amino acid position 252,
- an amino acid other than Thr at amino acid position 254,
- an amino acid other than Thr at amino acid position 256,
- an amino acid other than His at amino acid position 285,
- an amino acid other than Thr at amino acid position 286,
- an amino acid other than Pro at amino acid position 307,
- an amino acid other than Ile at amino acid position 308,
- an amino acid other than Glu at amino acid position 309,
- an amino acid other than Gln at amino acid position 311,
- an amino acid other than Thr at amino acid position 315,
- an amino acid other than Asp at amino acid position 378,
- an amino acid other than Glu at amino acid position 380,
- an amino acid other than Met at amino acid position 428,
- an amino acid other than Glu at amino acid position 430,
- an amino acid other than Gln at amino acid position 433,
- an amino acid other than Asn at amino acid position 434,
- an amino acid other than His at amino acid position 435, or
- an amino acid other than Tyr at amino acid position 436.

[0048] In some examples, the polypeptide or polypeptides has/have: (1) increased half-life in a dog than a control polypeptide or control polypeptides, wherein the control

polypeptide or control polypeptides are identical to the polypeptide or polypeptides except for having the corresponding wild type canine IgG Fc region in place of the IgG Fc region variant; and/or (2) increased binding to canine FcRn than the control polypeptides; and wherein the amino acid positions are based on EU numbering.

[0049] In some examples, the amino acid sequence is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequence set forth in SEQ ID NO:12.

[0050] In some examples, the polypeptide or polypeptides comprise at least one of the following:

- Glu or Gln at amino acid position 250,
- Asp or Glu at amino acid position 251,
- Tyr at amino acid position 252,
- Thr at amino acid position 254,
- Asp, Glu, or Phe at amino acid position 256,
- Asn or Asp at amino acid position 285,
- Asp at amino acid position 286,
- Arg, Gln, or Ala at amino acid position 307,
- Pro at amino acid position 308,
- Pro at amino acid position 309,
- Val at amino acid position 311,
- Asp at amino acid position 315,
- Val at amino acid position 378,
- Ala at amino acid position 380,
- Leu at amino acid position 428,
- Ala or Lys at amino acid position 430,
- Lys at amino acid position 433,
- Ser, Ala, or Phe at amino acid position 434,
- Try at amino acid position 435, or
- His at amino acid position 436.

[0051] In some examples, the polypeptide or polypeptides comprise(s) at least one of the following:

- Tyr or Met at amino acid position 252,
- Thr at amino acid position 254,
- Glu at amino acid position 256, or

Tyr, Trp, Arg, or His at amino acid position 434.

[0052] In some examples, the canine IgG Fc region variant comprises at least one of the following:

- (i) Tyr at amino acid position 252, Thr at amino acid position 254, and Glu at amino acid position 256;
- (ii) Leu at amino acid position 428 and Ser at amino acid position 434;
- (iii) Asp at amino acid position 256, Arg at amino acid position 307, and Val at amino acid position 311;
- (iv) Asp at amino acid position 256, Asp at amino acid position 315, and Val at amino acid position 378;
- (v) Asp at amino acid position 256, Asp at amino acid position 286, Arg at amino acid position 307, and Val at amino acid position 311;
- (vi) Asn at amino acid position 285, Gln at amino acid position 307, and Asp at amino acid position 315;
- (vii) Asp at amino acid position 256, Arg at amino acid position 307, Val at amino acid position 311, and Val at amino acid position 378;
- (viii) Asp at amino acid position 285, Val at amino acid position 311, and Val at amino acid position 378;
- (ix) Asp at amino acid position 256, Asp at amino acid position 285, and Val at amino acid position 378;
- (x) Asp at amino acid position 256, Val at amino acid position 311, and Val at amino acid position 378;
- (xi) Asp at amino acid position 256, Asp at amino acid position 285, Asp at amino acid position 286, Arg at amino acid position 307, and Val at amino acid position 378;
- (xii) Asp at amino acid position 256, Asp at amino acid position 286, Arg at amino acid position 307, Val at amino acid position 311, and Val at position 378;
- (xiii) Gln at amino acid position 307, Val at amino acid position 311, and Val at amino acid position 378;
- (xiv) Asp at amino acid position 285, Gln at amino acid position 307, and Val at amino acid position 378;
- (xv) Asp at amino acid position 256, Asp at amino acid position 285, Arg at amino acid position 307, Val at amino acid position 311, and Val at amino acid position 378;
- (xvi) Gln at amino acid position 307, Ala at amino acid position 380, Ser or Ala at amino acid position 434;

(xvii) Leu at amino acid position 428, and Ser or Ala at amino acid position 434; or

(xviii) Gln at amino acid position 250 and Leu at amino acid position 428.

[0053] In some examples, the polypeptide or polypeptides is/are an antigen binding domain(s).

[0054] In some examples, the antigen binding domain(s) bind to an antigen selected from the group consisting of NGF, TrKA, ADAMTS, IL-1, IL-2, IL-4, IL-4R, Angiotensin type 1 (AT1) receptor, Angiotensin type 2 (AT2) receptor, IL-5, IL-12, IL-13, IL-31, IL-33, TNF-alpha, IgE, PD-1, PD-1 ligand, CD3, CD20, CD47, CD52, OX40, OX40 ligand, CTLA4, VEGF, EGFR, NAV 1.7, and complement system complex (e.g., C1 complex, C2 complex, C3 complex, C4 complex, C5 complex, C6 complex, C7 complex, C8 complex, C9 complex).

[0055] In some examples, the antigen binding domain(s) is/are a scFv, scFab, or nanobody.

[0056] In some examples, the polypeptide or polypeptides described herein further comprise a protein. In some examples, the protein is selected from the group consisting of EPO, CTLA4, LFA3, VEGFR1/VEGFR3, IL-1R, IL-4R, GLP-1 receptor agonist, and Thrombopoietin binding peptide.

[0057] In one example, the disclosure provides a pharmaceutical composition comprising (i) the polypeptide or polypeptides described herein, and (ii) a pharmaceutically acceptable excipient. In one aspect, the present disclosure refers to a pharmaceutical composition comprising (i) any one of the polypeptides described herein, and (ii) a pharmaceutically acceptable excipient.

[0058] In one example, the disclosure provides a nucleic acid or nucleic acids encoding the polypeptide or polypeptides described herein. In one aspect, the present disclosure refers to a nucleic acid or nucleic acids encoding any one of the polypeptides described herein.

[0059] In one example, the disclosure provides an expression vector or expression vectors comprising the nucleic acid or nucleic acids described herein. In one aspect, the present disclosure refers to an expression vector or expression vectors comprising the nucleic acid or nucleic acids described herein.

[0060] In one aspect, the disclosure provides a host cell comprising the nucleic acid or nucleic acids described herein or the expression vector or expression vectors described herein.

[0061] In one example, the disclosure provides a method of making a polypeptide or polypeptides, the method comprising

- (a) providing a nucleic acid or nucleic acids described herein;
- (b) expressing the nucleic acid or nucleic acids in a host cell culture, thereby producing the polypeptide or polypeptides; and
- (c) collecting the polypeptide or polypeptides produced in (b) from the host cell culture.

[0061a] In one aspect, the present disclosure refers to a method of making a polypeptide, the method comprising:

- (a) providing a nucleic acid or nucleic acids described herein;
- (b) expressing the nucleic acid or nucleic acids in a host cell culture, thereby producing the polypeptide; and
- (c) collecting the polypeptide produced in (b) from the host cell culture.

[0062] In some examples, the method further comprises formulating the polypeptide or polypeptides as a pharmaceutical formulation.

[0063] In one example, the disclosure provides a method of treating a canine disease or disorder in a dog in need thereof, the method comprising administering an effective amount of a composition comprising the pharmaceutical composition described herein to the dog. In one aspect, the present disclosure refers to use of a composition comprising the pharmaceutical composition described herein in the manufacture of a medicament for treating a canine disease or disorder in a dog in need thereof.

[0064] In one example, the disclosure provides a method of preventing a canine disease or disorder in a dog in need thereof, the method comprising administering an effective amount of a composition comprising the pharmaceutical composition described herein to the dog. In one aspect, the present disclosure refers to use of a composition comprising the pharmaceutical composition described herein in the manufacture of a medicament for preventing a canine disease or disorder in a dog in need thereof.

[0065] In some examples, the disease or disorder is an allergic disease, a chronic pain, an acute pain, an inflammatory disease, an autoimmune disease, an endocrine disease, a gastrointestinal disease, a cardiovascular disease, a renal disease, a fertility related disorder, an infectious disease or a cancer.

[0066] In some examples, the disease or disorder is atopic dermatitis, allergic dermatitis, osteoarthritic pain, arthritis, anemia, or obesity.

[0067] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, the exemplary methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present application, including definitions, will control. The materials, methods, and examples are illustrative only and not intended to be limiting.

[0068] Other features and advantages of the disclosure will be apparent from the following detailed description and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

[0069] FIG. 1 is an amino acid sequence alignment of canine IgG γ chains. These chains contain V_H, CH1, CH2, and CH3 domains and the hinge region between CH1 and CH2. An N-glycosylation site is shown in bold and marked in a block. These sequences are assigned SEQ ID NOs.: 13, 14, 15, and 16, respectively.

WHAT IS CLAIMED IS:

1. A polypeptide comprising a canine IgG Fc region variant, or a canine FcRn-binding region thereof, wherein the polypeptide comprises at least one amino acid substitution at a position selected from the group consisting of:

- an amino acid position that corresponds to amino acid position 250 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 251 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 252 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 254 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 256 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 285 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 286 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 307 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 308 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 309 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 311 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 315 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 378 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 380 of a wild type canine IgG,
- an amino acid position that corresponds to amino acid position 428 of a wild type canine IgG,

an amino acid position that corresponds to amino acid position 430 of a wild type canine IgG,

an amino acid position that corresponds to amino acid position 433 of a wild type canine IgG,

an amino acid position that corresponds to amino acid position 434 of a wild type canine IgG,

an amino acid position that corresponds to amino acid position 435 of a wild type canine IgG, and

an amino acid position that corresponds to amino acid position 436 of a wild type canine IgG,

wherein the amino acid positions are based on EU numbering, and wherein the polypeptide has increased binding to canine FcRn compared to an Fc domain of the wild type canine IgG.

2. The polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 12.

3. The polypeptide of claim 1, wherein the wild type canine IgG is a canine IgG.A, and the canine IgG Fc region variant or the canine FcRn-binding region thereof comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 9.

4. The polypeptide of claim 1, wherein the wild type canine IgG is a canine IgG.B, and the canine IgG Fc region variant or the canine FcRn-binding region thereof comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 10.

5. The polypeptide of claim 1, wherein the wild type canine IgG is a canine IgG.C, and the canine IgG Fc region variant or the canine FcRn-binding region thereof comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 11.

6. The polypeptide of claim 1, wherein the wild type canine IgG is a canine IgG.D, and the canine IgG Fc region variant or the canine FcRn-binding region thereof comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 12.

7. The polypeptide of any one of claims 1 to 6, wherein the polypeptide comprises at least one of the following:

Glu or Gln at the amino acid position that corresponds to amino acid position 250 of the wild type canine IgG,

Asp or Glu at the amino acid position that corresponds to amino acid position 251 of the wild type canine IgG,

Tyr or Met at the amino acid position that corresponds to amino acid position 252 of the wild type canine IgG,

Thr or Ser at the amino acid position that corresponds to amino acid position 254 of the wild type canine IgG,

Asp, Glu or Phe at the amino acid position that corresponds to amino acid position 256 of the wild type canine IgG,

Asn or Asp at the amino acid position that corresponds to amino acid position 285 of the wild type canine IgG,

Asp at the amino acid position that corresponds to amino acid position 286 of the wild type canine IgG,

Arg, Gln or Ala at the amino acid position that corresponds to amino acid position 307 of the wild type canine IgG,

Pro at the amino acid position that corresponds to amino acid position 308 of the wild type canine IgG,

Pro at the amino acid position that corresponds to amino acid position 309 of the wild type canine IgG,

Val at the amino acid position that corresponds to amino acid position 311 of the wild type canine IgG,

Asp at the amino acid position that corresponds to amino acid position 315 of the wild type canine IgG,

Val at the amino acid position that corresponds to amino acid position 378 of the wild type canine IgG,

Ala at the amino acid position that corresponds to amino acid position 380 of the wild type canine IgG,

Leu at the amino acid position that corresponds to amino acid position 428 of the wild type canine IgG,

Ala or Lys at the amino acid position that corresponds to amino acid position 430 of the wild type canine IgG,

Lys at the amino acid position that corresponds to amino acid position 433 of the wild type canine IgG,

Trp, Tyr, Arg, His, Ser, Ala or Phe at the amino acid position that corresponds to amino acid position 434 of the wild type canine IgG,

Tyr at the amino acid position that corresponds to amino acid position 435 of the wild type canine IgG, or

His at the amino acid position that corresponds to amino acid position 436 of the wild type canine IgG.

8. The polypeptide of any one of claims 1 to 6, wherein the at least one amino acid substitution is at a position selected from the group consisting of:

an amino acid position that corresponds to amino acid position 250 of the wild type canine IgG,

an amino acid position that corresponds to amino acid position 252 of the wild type canine IgG,

an amino acid position that corresponds to amino acid position 254 of the wild type canine IgG,

an amino acid position that corresponds to amino acid position 256 of the wild type canine IgG,

an amino acid position that corresponds to amino acid position 285 of the wild type canine IgG,

an amino acid position that corresponds to amino acid position 307 of the wild type canine IgG,

an amino acid position that corresponds to amino acid position 309 of the wild type canine IgG,

an amino acid position that corresponds to amino acid position 311 of the wild type canine IgG,

an amino acid position that corresponds to amino acid position 315 of the wild type canine IgG,

an amino acid position that corresponds to amino acid position 433 of the wild type canine IgG,

an amino acid position that corresponds to amino acid position 434 of the wild type canine IgG, and

an amino acid position that corresponds to amino acid position 436 of the wild type canine IgG.

9. The polypeptide of claim 8, wherein the polypeptide comprises at least one of the following:

Glu or Gln at the amino acid position that corresponds to amino acid position 250 of the wild type canine IgG,

Tyr or Met at the amino acid position that corresponds to amino acid position 252 of the wild type canine IgG,

Thr or Ser at the amino acid position that corresponds to amino acid position 254 of the wild type canine IgG,

Asp, Glu or Phe at the amino acid position that corresponds to amino acid position 256 of the wild type canine IgG,

Asn or Asp at the amino acid position that corresponds to amino acid position 285 of the wild type canine IgG,

Arg, Gln or Ala at the amino acid position that corresponds to amino acid position 307 of the wild type canine IgG,

Pro at the amino acid position that corresponds to amino acid position 309 of the wild type canine IgG,

Val at the amino acid position that corresponds to amino acid position 311 of the wild type canine IgG,

Asp at the amino acid position that corresponds to amino acid position 315 of the wild type canine IgG,

Lys at the amino acid position that corresponds to amino acid position 433 of the wild type canine IgG,

Trp, Tyr, Arg, His, Ser, Ala or Phe at the amino acid position that corresponds to amino acid position 434 of the wild type canine IgG, or

His at the amino acid position that corresponds to amino acid position 436 of the wild type canine IgG.

10. The polypeptide of any one of claims 1 to 6, wherein the at least one amino acid substitution is at a position selected from the group consisting of:

an amino acid position that corresponds to amino acid position 252 of the wild type canine IgG,

an amino acid position that corresponds to amino acid position 254 of the wild type canine IgG,

an amino acid position that corresponds to amino acid position 256 of the wild type canine IgG, and

an amino acid position that corresponds to amino acid position 434 of the wild type canine IgG.

11. The polypeptide of claim 10, wherein the polypeptide comprises at least one of the following:

Tyr or Met at the amino acid position that corresponds to amino acid position 252 of the wild type canine IgG,

Thr or Ser at the amino acid position that corresponds to amino acid position 254 of the wild type canine IgG,

Asp, Glu or Phe at the amino acid position that corresponds to amino acid position 256 of the wild type canine IgG, or

Trp, Tyr, Arg, His, Ser, Ala or Phe at the amino acid position that corresponds to amino acid position 434 of the wild type canine IgG.

12. The polypeptide of claim 11, wherein the polypeptide comprises at least one of the following:

Tyr at the amino acid position that corresponds to amino acid position 252 of the wild type canine IgG,

Thr at the amino acid position that corresponds to amino acid position 254 of the wild type canine IgG,

Glu at the amino acid position that corresponds to amino acid position 256 of the wild type canine IgG, or

Trp, Tyr, Arg or His at the amino acid position that corresponds to amino acid position 434 of the wild type canine IgG.

13. The polypeptide of any one of claims 1 to 12, wherein the polypeptide further comprises a binding domain.

14. The polypeptide of claim 13, wherein the binding domain comprises (i) six complementarity determining regions (CDRs) of an immunoglobulin molecule; (ii) a ligand

binding domain of a canine receptor protein; (iii) a nanobody; or (iv) an extracellular domain of a canine receptor protein.

15. The polypeptide of claim 13 or claim 14, wherein the binding domain specifically binds to an antigen selected from the group consisting of NGF, TrKA, ADAMTS, IL-1, IL-2, IL-4, IL-4R, Angiotensin type 1 (AT1) receptor, Angiotensin type 2 (AT2) receptor, IL-5, IL-12, IL-13, IL-31, IL-33, CD3, CD20, CD47, CD52, and complement system complex.

16. The polypeptide of any one of claims 1 to 12, further comprising a protein selected from the group consisting of EPO, CTLA4, LFA3, VEGFR1/VEGFR3, IL-1R, IL-4R, GLP-1 receptor agonist, and Thrombopoietin binding peptide.

17. The polypeptide of any one of claims 1 to 16, wherein the polypeptide binds to a canine FcRn at a higher level at an acidic pH than at a neutral pH.

18. A pharmaceutical composition comprising (i) the polypeptide of any one of claims 1 to 17, and (ii) a pharmaceutically acceptable excipient.

19. A nucleic acid or nucleic acids encoding the polypeptide of any one of claims 1 to 17.

20. An expression vector or expression vectors comprising the nucleic acid or nucleic acids of claim 19.

21. A host cell comprising the nucleic acid or nucleic acids of claim 19 or the expression vector or expression vectors of claim 20.

22. A method of making a polypeptide, the method comprising:

- (a) providing a nucleic acid or nucleic acids of claim 19;
- (b) expressing the nucleic acid or nucleic acids in a host cell culture, thereby producing the polypeptide; and
- (c) collecting the polypeptide produced in (b) from the host cell culture.

23. The method of claim 22, further comprising formulating the polypeptide as a pharmaceutical formulation.

24. Use of a composition comprising the pharmaceutical composition of claim 18 in the manufacture of a medicament for treating a canine disease or disorder in a dog in need thereof.

25. Use of a composition comprising the pharmaceutical composition of claim 18 in the manufacture of a medicament for preventing a canine disease or disorder in a dog in need thereof.

26. The use of claim 24 or claim 25, wherein the canine disease or disorder is an allergic disease, chronic pain, acute pain, an inflammatory disease, an autoimmune disease, an endocrine disease, a gastrointestinal disease, a cardiovascular disease, a renal disease, a fertility related disorder, an infectious disease, or a cancer.

27. The use of claim 24 or claim 25, wherein the canine disease or disorder is atopic dermatitis, allergic dermatitis, osteoarthritic pain, arthritis, anemia, or obesity.