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Summary

Technical Problem

[0037] An objective of the invention is to provide anti-myostatin antibodies, polypeptides containing variant Fc regions, and methods of using the same.

Solution to Problem

[0038] The disclosure provides anti-myostatin antibodies and methods of using the same. The disclosure also provides proteins containing a variant Fc region and methods of using the same. In one aspect, the present disclosure refers to an isolated antibody that binds to latent myostatin, wherein the antibody comprises:
(a) (i) a HVR-H3 comprising the amino acid sequence $GVPAX_1SX_2GGDX_3$, wherein X_1 is Y or H, X_2 is T or H, X_3 is L or K (SEQ ID NO: 128), (ii) a HVR-L3 comprising the amino acid sequence $AGGYGGGX_1YA$, wherein X_1 is L or R (SEQ ID NO: 131), and (iii) a HVR-H2 comprising the amino acid sequence $IISX_1AGX_2X_3YX_4X_5X_6WAKX_7$, wherein X_1 is Y or H, X_2 is S or K, X_3 is T, M or K, X_4 is Y or K, X_5 is A, M or E, X_6 is S or E, X_7 is G or K (SEQ ID NO: 127);
(b) (i) a HVR-H1 comprising the amino acid sequence X_1X_2DIS, wherein X_1 is S or H, X_2 is Y, T, D or E (SEQ ID NO: 126), (ii) a HVR-H2 comprising the amino acid sequence $IISX_1AGX_2X_3YX_4X_5X_6WAKX_7$, wherein X_1 is Y or H, X_2 is S or K, X_3 is T, M or K, X_4 is Y or K, X_5 is A, M or E, X_6 is S or E, X_7 is G or K (SEQ ID NO: 127), and (iii) a HVR-H3 comprising the amino acid sequence $GVPAX_1SX_2GGDX_3$, wherein X_1 is Y or H, X_2 is T or H, X_3 is L or K (SEQ ID NO: 128);
(c) (i) a HVR-H1 comprising the amino acid sequence X_1X_2DIS, wherein X_1 is S or H, X_2 is Y, T, D or E (SEQ ID NO: 126), (ii) a HVR-H2 comprising the amino acid sequence $IISX_1AGX_2X_3YX_4X_5X_6WAKX_7$, wherein X_1 is Y or H, X_2 is S or K, X_3 is T, M or K, X_4 is Y or K, X_5 is A, M or E, X_6 is S or E, X_7 is G or K (SEQ ID NO: 127), (iii) a HVR-H3 comprising the amino acid sequence $GVPAX_1SX_2GGDX_3$, wherein X_1 is Y or H, X_2 is T or H, X_3 is L or K (SEQ ID NO: 128), (iv) a HVR-L1 comprising the amino acid sequence $X_1X_2SQX_3VX_4X_5X_6NWLS$, wherein X_1 is Q or T, X_2 is S or T, X_3 is S or E, X_4 is Y or F, X_5 is D or H, X_6 is N, D, A or E (SEQ ID NO: 129); (v) a HVR-L2 comprising the amino acid sequence WAX_1TLAX_2, wherein X_1 is S or E, X_2 is S, Y, F or W (SEQ ID NO: 130); and (vi) a HVR-L3 comprising the amino acid sequence $AGGYGGGX_1YA$, wherein X_1 is L or R (SEQ ID NO: 131); or
(d) (i) a HVR-L1 comprising the amino acid sequence $X_1X_2SQX_3VX_4X_5X_6NWLS$, wherein X_1 is Q or T, X_2 is S or T, X_3 is S or E, X_4 is Y or F, X_5 is D or H, X_6 is N, D, A or E (SEQ ID NO: 129); (ii) a HVR-L2 comprising the amino acid sequence WAX_1TLAX_2, wherein X_1 is S or E, X_2 is S, Y, F or W (SEQ ID NO: 130); and (iii) a HVR-L3 comprising the amino acid sequence $AGGYGGGX_1YA$, wherein X_1 is L or R (SEQ ID NO: 131). In another aspect, the present disclosure refers to an isolated antibody that binds to latent myostatin, comprising (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95; (b) a VL sequence having at least 95% sequence identity to the amino acid

sequence of any one of SEQ ID NOs: 15, 31, 35-38, and 96-99; or (c) the VH sequence of (a) and the VL sequence of (b).

[0039] In some examples, an isolated anti-myostatin antibody of the present disclosure binds to latent myostatin. In further examples, the antibody binds to an epitope within a fragment consisting of amino acids 21-100 of myostatin propeptide (SEQ ID NO: 78). In some examples, an isolated anti-myostatin antibody of the present disclosure inhibits activation of myostatin. In further examples, the antibody blocks the release of mature myostatin from latent myostatin. In further examples, the antibody blocks the proteolytic release of mature myostatin. In further examples, the antibody blocks the spontaneous release of mature myostatin. In further examples, the antibody does not bind to mature myostatin. In further examples, the antibody binds to the same epitope as an antibody described in Table 13. In further examples, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 13. In further examples, the antibody binds to the same epitope as an antibody described in Table 2a. In further examples, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 2a. In further examples, the antibody binds to the same epitope as an antibody described in Table 11a. . In further examples, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 11a. In further examples, the antibody binds to the same epitope as an antibody described in Table 2a, 11a, or 13. In further examples, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 2a, 11a, or 13.

[0040] In some examples, an isolated anti-myostatin antibody of the present disclosure binds to latent myostatin with higher affinity at neutral pH than at acidic pH. In some examples, the anti-myostatin antibody binds to latent myostatin with higher affinity at pH7.4 than at pH5.8. In some examples, an isolated anti-myostatin antibody of the present disclosure binds to a polypeptide fragment consisting of amino acids 21-100 of myostatin propeptide (SEQ ID NO: 78) with higher affinity at pH7.4 than at pH5.8. In some examples, the antibody binds to the same myostatin epitope as an antibody described in Table 13 with a higher affinity at neutral pH than at acidic pH. In additional examples, an anti-myostatin antibody binds to the same epitope as an antibody described in Table 13 with a higher affinity at pH7.4 than at pH5.8. In further examples, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 13 with a higher affinity at pH7.4

than at pH5.8. In some examples, the antibody binds to the same myostatin epitope as an antibody described in Table 2a with a higher affinity at neutral pH than at acidic pH. In some examples, the antibody binds to the same myostatin epitope as an antibody described in Table 2a with a higher affinity at pH7.4 than at pH5.8. In further examples, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 2a with a higher affinity at pH7.4 than at pH5.8. In additional examples, an anti-myostatin antibody binds to the same epitope as an antibody described in Table 11a with a higher affinity at neutral pH than at acidic pH. In further examples, the antibody binds to the same myostatin epitope as an antibody described in Table 11a with a higher affinity at pH7.4 than at pH5.8. In further examples, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 11a with a higher affinity at pH7.4 than at pH5.8. In additional examples, an anti-myostatin antibody binds to the same epitope as an antibody described in Table 2a, 11a, or 13 with a higher affinity at neutral pH than at acidic pH. In further examples, the antibody binds to the same myostatin epitope as an antibody described in Table 2a, 11a, or 13 with a higher affinity at pH7.4 than at pH5.8. In further examples, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 2a, 11a, or 13 with a higher affinity at pH7.4 than at pH5.8.

[0041] In some examples, an isolated anti-myostatin antibody of the present disclosure competes for binding latent myostatin with an antibody provided herein.In some examples, an isolated anti-myostatin antibody of the present disclosure competes for binding latent myostatin with an antibody described in Table 13. In some examples, an isolated anti-myostatin antibody of the present disclosure competes for binding latent myostatin with an antibody comprising a VH and VL pair described in Table 13. In some examples, the antibody competes for binding latent myostatin with an antibody described in Table 2a. In some examples, an isolated anti-myostatin antibody of the present disclosure competes for binding latent myostatin with an antibody comprising a VH and VL pair described in Table 2a. In some examples, the antibody competes for binding latent myostatin with an antibody described in Table 11a. In some examples, an isolated anti-myostatin antibody of the present disclosure competes for binding latent myostatin with an antibody comprising a VH and VL pair described in Table 11a. In additional examples, an anti-myostatin antibody competes for binding latent myostatin with an antibody described in Table 2a, 11a, or 13. In additional examples, an anti-myostatin antibody competes for binding latent myostatin with an antibody comprising a VH and VL pair described in Table 2a, 11a, or 13. In further examples, the anti-myostatin antibody binds to latent myostatin with a higher affinity at neutral pH than at acidic

pH. In further examples, the anti-myostatin antibody binds to latent myostatin with a higher affinity at pH7.4 than at pH5.8. In further examples, the anti-myostatin antibody binds to a polypeptide fragment consisting of amino acids 21-100 of myostatin propeptide (SEQ ID NO: 78) with higher affinity at pH7.4 than at pH5.8. Methods for assessing the ability of an antibody to compete with a reference antibody for binding latent myostatin are described herein and known in the art.

[0042] In some examples, an isolated anti-myostatin antibody of the present disclosure is a monoclonal antibody. In some examples, an isolated anti-myostatin antibody of the present disclosure is a human, humanized, or chimeric antibody. In some examples, an isolated anti-myostatin antibody of the present disclosure is an antibody fragment that binds to myostatin. In some examples, an isolated anti-myostatin antibody of the present disclosure is an antibody fragment that binds to latent myostatin. In some examples, an isolated anti-myostatin antibody of the present disclosure is an antibody fragment that binds to a polypeptide fragment consisting of amino acids 21-100 of myostatin propeptide (SEQ ID NO: 78). In some examples, an isolated anti-myostatin antibody of the present disclosure is a full length IgG antibody.

[0043] In some examples, a anti-myostatin antibody of the disclosure comprises:
(a) (i) a HVR-H3 comprising the amino acid sequence $GVPAX_1SX_2GGDX_3$, wherein X_1 is Y or H, X_2 is T or H, X_3 is L or K (SEQ ID NO: 128), (ii) a HVR-L3 comprising the amino acid sequence $AGGYGGGX_1YA$, wherein X_1 is L or R (SEQ ID NO: 131), and (iii) a HVR-H2 comprising the amino acid sequence $IISX_1AGX_2X_3YX_4X_5X_6WAKX_7$, wherein X_1 is Y or H, X_2 is S or K, X_3 is T, M or K, X_4 is Y or K, X_5 is A, M or E, X_6 is S or E, X_7 is G or K (SEQ ID NO: 127);
(b) (i) a HVR-H1 comprising the amino acid sequence X_1X_2DIS, wherein X_1 is S or H, X_2 is Y, T, D or E (SEQ ID NO: 126), (ii) a HVR-H2 comprising the amino acid sequence $IISX_1AGX_2X_3YX_4X_5X_6WAKX_7$, wherein X_1 is Y or H, X_2 is S or K, X_3 is T, M or K, X_4 is Y or K, X_5 is A, M or E, X_6 is S or E, X_7 is G or K (SEQ ID NO: 127), and (iii) a HVR-H3 comprising the amino acid sequence $GVPAX_1SX_2GGDX_3$, wherein X_1 is Y or H, X_2 is T or H, X_3 is L or K (SEQ ID NO: 128);
(c) (i) a HVR-H1 comprising the amino acid sequence X_1X_2DIS, wherein X_1 is S or H, X_2 is Y, T, D or E (SEQ ID NO: 126), (ii) a HVR-H2 comprising the amino acid sequence $IISX_1AGX_2X_3YX_4X_5X_6WAKX_7$, wherein X_1 is Y or H, X_2 is S or K, X_3 is T, M or K, X_4 is Y or K, X_5 is A, M or E, X_6 is S or E, X_7 is G or K (SEQ ID NO: 127), (iii) a HVR-H3 comprising the amino acid sequence $GVPAX_1SX_2GGDX_3$, wherein X_1 is Y or H, X_2 is T or H, X_3 is L or K (SEQ ID NO: 128), (iv) a HVR-L1 comprising the amino acid sequence $X_1X_2SQX_3VX_4X_5X_6NWLS$, wherein X_1 is Q or T, X_2 is S or T, X_3 is S or E, X_4 is Y or F, X_5 is D or H, X_6 is N, D, A or E (SEQ ID NO: 129); (v) a

HVR-L2 comprising the amino acid sequence WAX_1TLAX_2, wherein X_1 is S or E, X_2 is S, Y, F or W (SEQ ID NO: 130); and (vi) a HVR-L3 comprising the amino acid sequence $AGGYGGGX_1YA$, wherein X_1 is L or R (SEQ ID NO: 131); (d) (i) a HVR-L1 comprising the amino acid sequence $X_1X_2SQX_3VX_4X_5X_6NWLS$, wherein X_1 is Q or T, X_2 is S or T, X_3 is S or E, X_4 is Y or F, X_5 is D or H, X_6 is N, D, A or E (SEQ ID NO: 129); (ii) a HVR-L2 comprising the amino acid sequence WAX_1TLAX_2, wherein X_1 is S or E, X_2 is S, Y, F or W (SEQ ID NO: 130); and (iii) a HVR-L3 comprising the amino acid sequence $AGGYGGGX_1YA$, wherein X_1 is L or R (SEQ ID NO: 131). In some examples the antibody of (b) further comprises a heavy chain variable domain framework FR1 comprising the amino acid sequence of any one of SEQ ID NOs: 132-134; FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 135-136; FR3 comprising the amino acid sequence of SEQ ID NO: 137; and FR4 comprising the amino acid sequence of SEQ ID NO: 138. In some examples, the antibody of (d), further comprises a light chain variable domain framework FR1 comprising the amino acid sequence of SEQ ID NO: 139; FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 140-141; FR3 comprising the amino acid sequence of any one of SEQ ID NOs: 142-143; and FR4 comprising the amino acid sequence of SEQ ID NO: 144.

[0044] In some examples, an isolated anti-myostatin antibody of the present disclosure comprises (a) a HVR-H3 comprising the amino acid sequence $GVPAX_1SX_2GGDX_3$, wherein X_1 is Y or H, X_2 is T or H, X_3 is L or K (SEQ ID NO: 128), (b) a HVR-L3 comprising the amino acid sequence $AGGYGGGX_1YA$, wherein X_1 is L or R (SEQ ID NO: 131), and (c) a HVR-H2 comprising the amino acid sequence $IISX_1AGX_2X_3YX_4X_5X_6WAKX_7$, wherein X_1 is Y or H, X_2 is S or K, X_3 is T, M or K, X_4 is Y or K, X_5 is A, M or E, X_6 is S or E, X_7 is G or K (SEQ ID NO: 127).

[0045] In some examples, an isolated anti-myostatin antibody of the present disclosure comprises (a) a HVR-H1 comprising the amino acid sequence X_1X_2DIS, wherein X_1 is S or H, X_2 is Y, T, D or E (SEQ ID NO: 126), (b) a HVR-H2 comprising the amino acid sequence $IISX_1AGX_2X_3YX_4X_5X_6WAKX_7$, wherein X_1 is Y or H, X_2 is S or K, X_3 is T, M or K, X_4 is Y or K, X_5 is A, M or E, X_6 is S or E, X_7 is G or K (SEQ ID NO: 127), and (c) a HVR-H3 comprising the amino acid sequence $GVPAX_1SX_2GGDX_3$, wherein X_1 is Y or H, X_2 is T or H, X_3 is L or K (SEQ ID NO: 128). In further examples, the antibody comprises a heavy chain variable domain framework FR1 comprising the amino acid sequence of any one of SEQ ID NOs: 132-134; FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 135-136; FR3 comprising the amino acid sequence of SEQ ID NO: 137; and FR4 comprising the amino acid sequence of SEQ ID NO:138. In further examples, the antibody additionally comprises (a) a HVR-L1 comprising the amino acid sequence $X_1X_2SQX_3VX_4$

X_5X_6NWLS, wherein X_1 is Q or T, X_2 is S or T, X_3 is S or E, X_4 is Y or F, X_5 is D or H, X_6 is N, D, A or E (SEQ ID NO: 129); (b) a HVR-L2 comprising the amino acid sequence WAX_1TLAX_2, wherein X_1 is S or E, X_2 is S, Y, F or W (SEQ ID NO: 130); and (c) a HVR-L3 comprising the amino acid sequence AGGYGGGX_1YA, wherein X_1 is L or R (SEQ ID NO: 131).

[0046] In some examples, an isolated anti-myostatin antibody of the present disclosure comprises (a) a HVR-L1 comprising the amino acid sequence X_1X_2SQX_3V$X_4X_5X_6$NWLS, wherein X_1 is Q or T, X_2 is S or T, X_3 is S or E, X_4 is Y or F, X_5 is D or H, X_6 is N, D, A or E (SEQ ID NO: 129); (b) a HVR-L2 comprising the amino acid sequence WAX_1TLAX_2, wherein X_1 is S or E, X_2 is S, Y, F or W (SEQ ID NO: 130); and (c) a HVR-L3 comprising the amino acid sequence AGGYGGGX_1YA, wherein X_1 is L or R (SEQ ID NO: 131). In a further example, the antibody further comprises a light chain variable domain framework FR1 comprising the amino acid sequence of SEQ ID NO: 139; FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 140-141; FR3 comprising the amino acid sequence of any one of SEQ ID NOs: 142-143; and FR4 comprising the amino acid sequence of SEQ ID NO: 144.

[0047] In some examples, an isolated anti-myostatin antibody of the present disclosure comprises a heavy chain variable domain framework FR1 comprising the amino acid sequence of any one of SEQ ID NOs: 132-134; FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 135-136; FR3 comprising the amino acid sequence of SEQ ID NO: 137; and FR4 comprising the amino acid sequence of SEQ ID NO: 138. In some examples, an isolated anti-myostatin antibody of the present disclosure comprises a light chain variable domain framework FR1 comprising the amino acid sequence of SEQ ID NO: 139; FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 140-141; FR3 comprising the amino acid sequence of any one of SEQ ID NOs: 142-143; and FR4 comprising the amino acid sequence of SEQ ID NO: 144.

[0048] In some examples, an isolated anti-myostatin antibody of the present disclosure comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 15, 31, 35-38, and 96-99; or (c) a VH sequence as in (a) and a VL sequence as in (b). In further examples, the antibody comprises a VH sequence of any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95. In further examples, the antibody comprises a VL sequence of any one of SEQ ID NOs: 15, 31, 35-38, and 96-99. In some embodimments, the antibody comprises a VH sequence of any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95. In further examples, the antibody comprises a VH sequence of any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95; and a VL

sequence of any one of SEQ ID NOs: 15, 31, 35-38, and 96-99.

[0049] In another aspect, the present disclosure also provides isolated nucleic acids encoding an anti-myostatin antibody of the present disclosure. In another aspect, the present disclosure also provides host cells comprising a nucleic acid of the present disclosure. In yet another aspect, the present disclosure also provides a method of producing an antibody comprising culturing a host cell of the present disclosure so that the antibody is produced.

[0050] In some examples the disclosure provides a method of producing an anti-myostatin antibody comprising: (a) culturing a host cell of the present disclosure so that the antibody is produced; or (b) immunizing an animal against a polypeptide, wherein the polypeptide comprises the region corresponding to amino acids at positions 21 to 100 of myostatin propeptide (SEQ ID NO: 78).

[0051] The disclosure further provides a method of producing an anti-myostatin antibody. In some examples, the method comprises immunizing an animal against a polypeptide, wherein the polypeptide comprises the region corresponding to amino acids at positions 21-100 of myostatin propeptide (SEQ ID NO: 78).

[0052] In yet another aspect, the present disclosure also provides a pharmaceutical formulation comprising an anti-myostatin antibody of the present disclosure and a pharmaceutically acceptable carrier.

[0053] Anti-myostatin antibodies of the present disclosure may be for use as a medicament. In some examples, the antibody is used in the manufacture of a medicament for: (a) treatment of a muscle wasting disease; (b) increasing mass of muscle tissue; (c) increasing strength of muscle tissue; or (d) reducing body fat accumulation. In some examples, anti-myostatin antibodies of the present disclosure may be for use in treating a muscle wasting disease. Anti-myostatin antibodies of the present disclosure may be for use in increasing mass of muscle tissue. Anti-myostatin antibodies of the present disclosure may be for use in increasing strength of muscle tissue. Anti-myostatin antibodies of the present disclosure may be for use in reducing body fat accumulation.

[0054] In some examples, an anti-myostatin antibody provided herein has use in: (a) treating a muscle wasting disease; (b) increasing mass of muscle tissue; (c) increasing strength of muscle tissue; or (d) reducing body fat accumulation.

[0055] Anti-myostatin antibodies of the present disclosure may be used in the manufacture of a medicament. In some examples, the antibody is used in the manufacture of a medicament for: (a) treatment of a muscle wasting disease; (b) increasing mass of muscle tissue; (c) increasing strength of muscle tissue; or (d) reducing body fat accumulation. In some examples, the medicament is for treatment of a muscle wasting disease. In some examples, the medicament is for increasing mass of muscle tissue. In some examples, the medicament is for increasing strength of muscle tissue. In some examples, the medicament is for reducing body fat accumulation.

[0056] The disclosure also provides a method of treating an individual having a muscle wasting disease. In some examples, the method comprises administering to the individual an effective amount of an anti-myostatin antibody of the present disclosure. The disclosure also provides a method of increasing mass of muscle tissue in an individual. In some examples, the method comprises administering to the individual an effective amount of an anti-myostatin antibody of the present disclosure to increase mass of muscle tissue. The disclosure also provides a method of increasing strength of muscle tissue in an individual. In some examples, the method comprises administering to the individual an effective amount of an anti-myostatin antibody of the present disclosure to increase strength of muscle tissue. The disclosure also provides a method of reducing body fat accumulation in an individual. In some examples, the method comprises administering to the individual an effective amount of an anti-myostatin antibody of the present disclosure to reduce body fat accumulation.

[0057] The disclosure provides polypeptides comprising variant Fc regions and methods of making and using the same.

[0058] In one example, the disclosure provides Fc gamma RIIB-binding polypeptides comprising variant Fc regions and methods of using the same. In some examples, a variant Fc region with enhanced Fc gamma RIIb-binding activity of the present disclosure comprises at least one amino acid alteration in a parent Fc region. In further examples, the ratio of [KD value of the parent Fc region for monkey Fc gamma RIIb]/[KD value of the variant Fc region for monkey Fc gamma RIIb] is 2.0 or greater. In further examples, the ratio of [KD value of the parent Fc region for monkey Fc gamma RIIIa]/[KD value of the variant Fc region for monkey Fc gamma RIIIa] is 0.5 or smaller. In further examples, the ratio of [KD value of the parent Fc region for human Fc gamma RIIb]/[KD value of the variant Fc region for human Fc gamma RIIb] is 2.0 or greater. In further examples, the ratio of [KD value of the parent Fc region for human Fc gamma RIIIa]/[KD value of the variant Fc region for human Fc gamma RIIIa] is 0.5 or smaller. In further examples, the ratio of [KD value of the parent Fc region for human Fc gamma RIIa (type H)]/[KD value of the variant Fc region for human Fc gamma RIIa (type H)] is 5.0 or smaller. In further examples, the ratio of [KD value of the parent Fc region for human Fc gamma RIIa (type R)]/[KD value of the variant Fc region for human Fc gamma RIIa (type R)] is 5.0 or smaller. In another example, the KD value of the variant Fc region for monkey Fc gamma RIIb is $1.0x10^{-6}$ M or smaller. In another example, the KD value of the variant Fc region for monkey Fc gamma RIIIa is $5.0x10^{-7}$ M or greater. In another example, the KD value of the variant Fc region for human Fc gamma RIIb is $2.0x10^{-6}$ M or smaller. In another example, the KD value of the variant Fc region for human Fc gamma RIIIa is $1.0x10^{-6}$ M or greater. In another example, the KD value of the variant Fc region

for human Fc gamma RIIa (type H) is $1.0x10^{-7}$ M or greater. In another example, the KD value of the variant Fc region for human Fc gamma RIIa (type R) is $2.0x10^{-7}$ M or greater.

[0059] In some examples, a variant Fc region with enhanced Fc gamma RIIb-binding activity of the present disclosure comprises at least one amino acid alteration of at least one position selected from the group consisting of: 231, 232, 233, 234, 235, 236, 237, 238, 239, 264, 266, 267, 268, 271, 295, 298, 325, 326, 327, 328, 330, 331, 332, 334, and 396, according to EU numbering.

[0060] In further examples, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises at least two amino acid alterations comprising: (a) one amino acid alteration at position 236, and (b) at least one amino acid alteration of at least one position selected from the group consisting of: (i) position 231, 232, 233, 234, 235, 237, 238, 239, 264, 266, 267, 268, 271, 295, 298, 325, 326, 327, 328, 330, 331, 332, 334, and 396; (ii) position: 231, 232, 235, 239, 268, 295, 298, 326, 330, and 396; or (iii) position 268, 295, 326, and 330; according to EU numbering.

[0061] In further examples, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises at least two amino acid alterations comprising: (a) one amino acid alteration at position 236, and (b) at least one amino acid alteration of at least one position selected from the group consisting of: 231, 232, 233, 234, 235, 237, 238, 239, 264, 266, 267, 268, 271, 295, 298, 325, 326, 327, 328, 330, 331, 332, 334, and 396, according to EU numbering.

[0062] In further examples, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises at least two amino acid alterations comprising: (a) one amino acid alteration at position 236, and (b) at least one amino acid alteration of at least one position selected from the group consisting of: 231, 232, 235, 239, 268, 295, 298, 326, 330, and 396, according to EU numbering.

[0063] In further examples, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises at least two amino acid alterations comprising: (a) one amino acid alteration at position 236, and (b) at least one amino acid alteration of at least one position selected from the group consisting of: 268, 295, 326, and 330 according to EU numbering.

[0064] In some examples, a variant Fc region with enhanced Fc gamma RIIb-binding activity of the present disclosure comprises at least one amino acid selected from the group consisting of: (a) Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr at position 231; (b) Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp, Tyr at position 232; (c) Asp at position 233; (d) Trp, Tyr at position 234; (e) Trp at position 235; (f) Ala, Asp, Glu, His, Ile, Leu, Met, Asn, Gln, Ser, Thr, Val at position 236; (g) Asp, Tyr at position 237; (h)

Glu, Ile, Met, Gln, Tyr at position 238; (i) Ile, Leu, Asn, Pro, Val at position 239; (j) Ile at position 264; (k) Phe at position 266; (l) Ala, His, Leu at position 267; (m) Asp, Glu at position 268; (n) Asp, Glu, Gly at position 271; (o) Leu at position 295; (p) Leu at position 298; (q) Glu, Phe, Ile, Leu at position 325; (r) Thr at position 326; (s) Ile, Asn at position 327; (t) Thr at position 328; (u) Lys, Arg at position 330; (v) Glu at position 331; (w) Asp at position 332; (x) Asp, Ile, Met, Val, Tyr at position 334; and (y) Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp, Tyr at position 396; according to EU numbering.

[0065] In further examples, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises at least one amino acid selected from the group consisting of: (a) Gly, Thr at position 231; (b) Asp at position 232; (c) Trp at position 235; (d) Asn, Thr at position 236; (e) Val at position 239; (f) Asp, Glu at position 268; (g) Leu at position 295; (h) Leu at position 298; (i) Thr at position 326; (j) Lys, Arg at position 330, and (k) Lys, Met at position 396; according to EU numbering.

[0066] In another example, the disclosure provides a polypeptide comprising an isoelectric point (pI)-increased variant Fc region and a method of using the same. In some examples, a polypeptide comprising a variant Fc region with increased pI comprises at least two amino acid alterations in a parent Fc region. In further examples, each of the amino acid alterations increases the isoelectric point (pI) of the variant Fc region compared with that of the parent Fc region. In further examples, the amino acid can be exposed on the surface of the variant Fc region. In further examples, a polypeptide comprises the variant Fc region and an antigen-binding domain. In further examples, antigen-binding activity of the antigen-binding domain changes according to ion concentration conditions. In further examples, the variant Fc region with increased pI of the present disclosure comprises at least two amino acid alterations of at least two positions selected from the group consisting of: 285, 311, 312, 315, 318, 333, 335, 337, 341, 342, 343, 384, 385, 388, 390, 399, 400, 401, 402, 413, 420, 422, and 431 according to EU numbering. In further examples, the variant Fc region with increased pI comprises Arg or Lys at each of the positions selected.

[0067] In some examples, a variant Fc region of the present disclosure comprises amino acid alterations described in Tables 14-30.

[0068] In some examples, a polypeptide comprises a variant Fc region of the present disclosure. In further examples, the parent Fc region is derived from human IgG1. In further examples, the polypeptide is an antibody. In further examples, the polypeptpide is an Fc fusion protein.

[0069] The disclosure provides a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 229-381.

[0070] The disclosure also provides isolated nucleic acid encoding the polypeptide comprising a variant Fc region of the present disclosure. The disclosure also provides a host cell comprising the nucleic acid of the present disclosure. The disclosure also provides a method of producing a polypeptide comprising a variant Fc region comprising culturing the host of the present disclosure so that the polypeptide is produced.

[0071] The disclosure further provides a pharmaceutical formulation comprising the polypeptide comprising a variant Fc region of the present disclosure and a pharmaceutically acceptable carrier.

Brief Description of Drawings

[0072] [Fig. 1] Figure 1 illustrates inhibition of proteolytic activation of latent myostatin by anti-latent myostatin antibody, as described in Example 3. The activity of active myostatin released from latent myostatin by BMP1 protease was measured in the presence of anti-latent myostatin antibody, using HEK Blue Assay.

[Fig. 2] Figure 2 illustrates inhibition of spontaneous activation of latent myostatin by anti-latent myostatin antibody, as described in Example 4. The activity of active myostatin released from latent myostatin by 37 degrees C incubation was measured in the presence of anti-latent myostatin antibody, using HEK Blue Assay.

[Fig. 3] Figure 3 illustrates binding of anti-latent myostatin antibody to propeptide domain, as described in Example 5.

[Fig. 4] Figure 4 illustrates Western Blot analysis against myostatin propeptide, as described in Example 6. Proteolytic cleavage of myostatin propeptide by BMP1 was assessed in the presence and absence of anti-latent myostatin antibody.

[Fig. 5] Figures 5A-5C illustrate BIACORE (registered trademark) sensorgrams of anti-latent myostatin antibody MST1032-G1m towards human latent myostatin (A), cynomolgus monkey latent myostatin (B), and mouse latent myostatin (C), as described in Example 7.

[Fig. 6A] Figure 6A illustrates in vivo efficacy of anti-latent myostatin antibody and anti-mature myostatin antibody on muscle mass and fat mass, as described in Example 8. Anti-latent myostatin antibody (MST1032-G1m; described as MST1032 in the Figures) or anti-mature myostatin antibody (41C1E4) was administered to SCID mice, and full body lean mass (or lean body mass: LBM) was measured.

[Fig. 6B] Figure 6B illustrates in vivo efficacy of anti-latent myostatin antibody and anti-mature myostatin antibody on muscle mass and fat mass, as described in Example 8. Anti-latent myostatin antibody (MST1032-G1m; described as MST1032 in the Figures) or anti-mature myostatin antibody (41C1E4) was administered to SCID mice, and change of full body fat mass from Day 0 to Day 14 was measured.

CLAIMS

[Claim 1]

An isolated antibody that binds to latent myostatin, wherein the antibody comprises:

(a) (i) a HVR-H3 comprising the amino acid sequence $GVPAX_1SX_2GGDX_3$, wherein X_1 is Y or H, X_2 is T or H, X_3 is L or K (SEQ ID NO: 128), (ii) a HVR-L3 comprising the amino acid sequence $AGGYGGGX_1YA$, wherein X_1 is L or R (SEQ ID NO: 131), and (iii) a HVR-H2 comprising the amino acid sequence $IISX_1AGX_2X_3YX_4X_5X_6WAKX_7$, wherein X_1 is Y or H, X_2 is S or K, X_3 is T, M or K, X_4 is Y or K, X_5 is A, M or E, X_6 is S or E, X_7 is G or K (SEQ ID NO: 127);

(b) (i) a HVR-H1 comprising the amino acid sequence X_1X_2DIS, wherein X_1 is S or H, X_2 is Y, T, D or E (SEQ ID NO: 126), (ii) a HVR-H2 comprising the amino acid sequence $IISX_1AGX_2X_3YX_4X_5X_6WAKX_7$, wherein X_1 is Y or H, X_2 is S or K, X_3 is T, M or K, X_4 is Y or K, X_5 is A, M or E, X_6 is S or E, X_7 is G or K (SEQ ID NO: 127), and (iii) a HVR-H3 comprising the amino acid sequence $GVPAX_1SX_2GGDX_3$, wherein X_1 is Y or H, X_2 is T or H, X_3 is L or K (SEQ ID NO: 128);

(c) (i) a HVR-H1 comprising the amino acid sequence X_1X_2DIS, wherein X_1 is S or H, X_2 is Y, T, D or E (SEQ ID NO: 126), (ii) a HVR-H2 comprising the amino acid sequence $IISX_1AGX_2X_3YX_4X_5X_6WAKX_7$, wherein X_1 is Y or H, X_2 is S or K, X_3 is T, M or K, X_4 is Y or K, X_5 is A, M or E, X_6 is S or E, X_7 is G or K (SEQ ID NO: 127), (iii) a HVR-H3 comprising the amino acid sequence $GVPAX_1SX_2GGDX_3$, wherein X_1 is Y or H, X_2 is T or H, X_3 is L or K (SEQ ID NO: 128), (iv) a HVR-L1 comprising the amino acid sequence $X_1X_2SQX_3VX_4X_5X_6NWLS$, wherein X_1 is Q or T, X_2 is S or T, X_3 is S or E, X_4 is Y or F, X_5 is D or H, X_6 is N, D, A or E (SEQ ID NO: 129); (v) a HVR-L2 comprising the amino acid sequence WAX_1TLAX_2, wherein X_1 is S or E, X_2 is S, Y, F or W (SEQ ID NO: 130); and (vi) a HVR-L3 comprising the amino acid sequence $AGGYGGGX_1YA$, wherein X_1 is L or R (SEQ ID NO: 131); or

(d) (i) a HVR-L1 comprising the amino acid sequence $X_1X_2SQX_3VX_4X_5X_6NWLS$, wherein X_1 is Q or T, X_2 is S or T, X_3 is S or E, X_4 is Y or F, X_5 is D or H, X_6 is N, D, A or E (SEQ ID NO: 129); (ii) a HVR-L2 comprising the amino acid sequence WAX_1TLAX_2, wherein X_1 is S or E, X_2 is S, Y, F or W (SEQ ID NO: 130); and (iii) a HVR-L3 comprising the amino acid sequence $AGGYGGGX_1YA$, wherein X_1 is L or R (SEQ ID NO: 131).

[Claim 2]

The antibody of claim 1 (b), further comprising a heavy chain variable domain framework FR1 comprising the amino acid sequence of any one of SEQ ID NOs: 132-134; FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 135-136;

FR3 comprising the amino acid sequence of SEQ ID NO: 137; and FR4 comprising the amino acid sequence of SEQ ID NO: 138.

[Claim 3]
The antibody of claim 1 (d), further comprising a light chain variable domain framework FR1 comprising the amino acid sequence of SEQ ID NO: 139; FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 140-141; FR3 comprising the amino acid sequence of any one of SEQ ID NOs: 142-143; and FR4 comprising the amino acid sequence of SEQ ID NO: 144.

[Claim 4]
An isolated antibody that binds to latent myostatin, comprising (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 15, 31, 35-38, and 96-99; or (c) the VH sequence of (a) and the VL sequence of (b).

[Claim 5]
An isolated nucleic acid encoding the antibody of any one of claims 1 to 4.

[Claim 6]
A host cell comprising the nucleic acid of claim 5.

[Claim 7]
A method of producing an anti-myostatin antibody comprising culturing the host cell of claim 6 so that the antibody is produced.

[Claim 8]
A pharmaceutical formulation comprising the antibody of any one of claims 1 to 4 and a pharmaceutically acceptable carrier.