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(54) Titre : ANTICORPS ANTI-MYOSTATINE, POLYPEPTIDES CONTENANT DES VARIANTS DE REGIONS FC, ET
PROCEDES D'UTILISATION
(54) Title: ANTI-MYOSTATIN ANTIBODIES, POLYPEPTIDES CONTAINING VARIANT FC REGIONS, AND METHODS
OF USE

(57) **Abrégé/Abstract:**

The disclosure provides anti-myostatin antibodies and methods of making and using the same. Nucleic acids encoding the anti-myostatin antibodies and host cells comprising the nucleic acids are also provided. The disclosure also provides polypeptides containing a variant Fc region and methods of making and using the same. Nucleic acids encoding the polypeptides and host cells comprising the nucleic acids are also provided.

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(54) Title: ANTI-MYOSTATIN ANTIBODIES, POLYPEPTIDES CONTAINING VARIANT FC REGIONS, AND METHODS OF USE

(57) Abstract: The disclosure provides anti-myostatin antibodies and methods of making and using the same. Nucleic acids encoding the anti-myostatin antibodies and host cells comprising the nucleic acids are also provided. The disclosure also provides polypeptides containing a variant Fc region and methods of making and using the same. Nucleic acids encoding the polypeptides and host cells comprising the nucleic acids are also provided.



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Description

Title of Invention: ANTI-MYOSTATIN ANTIBODIES, POLYPEPTIDES CONTAINING VARIANT FC REGIONS, AND METHODS OF USE

Technical Field

- [0001] The present invention relates to anti-myostatin antibodies and methods of using the same. The present invention also relates to polypeptides containing variant Fc regions and methods of using the same.

Background Art

- [0002] Myostatin, also referred to as growth differentiation factor-8 (GDF8), is a secreted protein and is a member of the transforming growth factor- beta (TGF-beta) superfamily of proteins. Members of this superfamily possess growth-regulatory and morphogenetic properties (see, e.g., NPL 1, NPL 2, and PTL 1). Myostatin is expressed primarily in the developing and adult skeletal muscle and functions as a negative regulator of muscle growth. Systemic overexpression of myostatin in adult mice leads to muscle wasting (see, e.g., NPL 3) while, conversely, a myostatin knockout mouse is characterized by hypertrophy and hyperplasia of the skeletal muscle resulting in two- to threefold greater muscle mass than their wild type littermates (see, e.g., NPL 4).
- [0003] Like other members of the TGF-beta family, myostatin is synthesized as a large precursor protein containing an N-terminal propeptide domain, and a C-terminal domain considered as the active molecule (see, e.g., NPL 5; PTL 2). Two molecules of myostatin precursor are covalently linked via a single disulfide bond present in the C-terminal growth factor domain. Active mature myostatin (disulfide-bonded homodimer consisting of the C-terminal growth factor domain) is liberated from myostatin precursor through multiple steps of proteolytic processing. In the first step of the myostatin activation pathway, a peptide bond between the N-terminal propeptide domain and the C-terminal growth factor domain, Arg266-Asp267, is cleaved by a furin-type proprotein convertase in both chains of the homodimeric precursor. But the resulting three peptides (two propeptides and one mature myostatin (i.e., a disulfide-bonded homodimer consisting of the growth factor domains)) remain associated, forming a noncovalent inactive complex that is referred to as "latent myostatin." Mature myostatin can then be liberated from latent myostatin through degradation of the propeptide. Members of the bone morphogenetic protein 1 (BMP1) family of metalloproteinases cleave a single peptide bond within the propeptide, Arg98-Asp99, with concomitant release of mature, active myostatin, a homodimer (see, e.g., NPL 6).

Moreover, the latent myostatin can be activated in vitro by dissociating the complex with either acid or heat treatment as well (see, e.g., NPL 7).

[0004] Myostatin exerts its effects through a transmembrane serine/threonine kinase heterotetramer receptor family, activation of which enhances receptor transphosphorylation, leading to the stimulation of serine/threonine kinase activity. It has been shown that the myostatin pathway involves an active myostatin dimer binding to the activin receptor type IIB (ActRIIB) with high affinity, which then recruits and activates the transphosphorylation of the low affinity receptor, the activin-like kinase 4 (ALK4) or activin-like kinase 5 (ALK5). It has also been shown that the proteins Smad 2 and Smad 3 are subsequently activated and form complexes with Smad 4, which are then translocated to the nucleus for the activation of target gene transcription. It has been demonstrated that ActRIIB is able to mediate the influence of myostatin in vivo, as expression of a dominant negative form of ActRIIB in mice mimics myostatin gene knockout (see, e.g., NPL 8).

[0005] A number of disorders or conditions are associated with muscle wasting (i.e., loss of or functional impairment of muscle tissue), such as muscular dystrophy (MD; including Duchenne muscular dystrophy), amyotrophic lateral sclerosis (ALS), muscle atrophy, organ atrophy, frailty, congestive obstructive pulmonary disease (COPD), sarcopenia, and cachexia resulting from cancer or other disorders, as well as renal disease, cardiac failure or disease, and liver disease. Patients will benefit from an increase in muscle mass and/or muscle strength; however, there are presently limited treatments available for these disorders. Thus, due to its role as a negative regulator of skeletal muscle growth, myostatin becomes a desirable target for therapeutic or prophylactic intervention for such disorders or conditions, or for monitoring the progression of such disorders or conditions. In particular, agents that inhibit the activity of myostatin may be therapeutically beneficial.

[0006] Inhibition of myostatin expression leads to both muscle hypertrophy and hyperplasia (NPL 4). Myostatin negatively regulates muscle regeneration after injury and lack of myostatin in myostatin null mice results in accelerated muscle regeneration (see, e.g., NPL 9). Anti-myostatin (GDF8) antibodies described in, e.g., PTL 3, PTL 4, PTL 5, PTL 6, and PTL 7, and PTL 8, PTL 9, and PTL 10 have been shown to bind to myostatin and inhibit myostatin activity in vitro and in vivo, including myostatin activity associated with the negative regulation of skeletal muscle mass. Myostatin-neutralizing antibodies increase body weight, skeletal muscle mass, and muscle size and strength in the skeletal muscle of wild type mice (see, e.g., NPL 10) and the mdx mice, a model for muscular dystrophy (see, e.g., NPL 11; NPL 12). However, these prior art antibodies are all specific for mature myostatin but not for latent myostatin, and the strategies described for inhibiting myostatin activity have utilized antibodies

that can bind to and neutralize mature myostatin.

- [0007] Antibodies are drawing attention as pharmaceuticals since they are highly stable in blood and have few side effects (see, e.g., NPL 13 and NPL 14). Almost all therapeutic antibodies currently on the market are antibodies of the human IgG1 subclass. One of the known functions of IgG class antibodies is antibody-dependent cell-mediated cytotoxicity (hereinafter denoted as ADCC activity) (see, e.g., NPL 15). For an antibody to exhibit ADCC activity, the antibody Fc region must bind to an Fc gamma receptor (hereinafter denoted as Fc gamma R) which is an antibody-binding receptor present on the surface of effector cells such as killer cells, natural killer cells, and activated macrophages.
- [0008] In humans, the Fc gamma RIa (CD64A), Fc gamma RIIa (CD32A), Fc gamma RIIb (CD32B), Fc gamma RIIIa (CD16A), and Fc gamma RIIIb (CD16B) isoforms have been reported as the Fc gamma R protein family, and the respective allotypes have also been reported (see, e.g., NPL 16). Fc gamma RIa, Fc gamma RIIa, and Fc gamma RIIIa are called activating Fc gamma R since they have immunologically active functions, and Fc gamma RIIb is called inhibitory Fc gamma R since it has immunosuppressive functions (see, e.g., NPL 17).
- [0009] In the binding between the Fc region and Fc gamma R, several amino acid residues in the antibody hinge region and CH2 domain, and a sugar chain attached to Asn at position 297 (EU numbering) bound to the CH2 domain have been shown to be important (see, e.g., NPL 18, NPL 19, and NPL 20). Various variants having Fc gamma R-binding properties, mainly antibodies with mutations introduced into these sites, have been studied so far; and Fc region variants having higher binding activities towards activating Fc gamma R have been obtained (see, e.g., PTL 11, PTL 12, PTL 13, and PTL 14).
- [0010] When an activating Fc gamma R is cross-linked with an immune complex, it phosphorylates immunoreceptor tyrosine-based activating motifs (ITAMs) contained in the intracellular domain or FcR common gamma-chain (an interaction partner), activates a signal transducer SYK, and triggers an inflammatory immune response by initiating an activation signal cascade (see, e.g., NPL 21).
- [0011] Fc gamma RIIb is the only Fc gamma R expressed on B cells (see, e.g., NPL 22). Interaction of the antibody Fc region with Fc gamma RIIb has been reported to suppress the primary immune response of B cells (see, e.g., NPL 23). Furthermore, it is reported that when Fc gamma RIIb on B cells and B cell receptor (BCR) are cross-linked via an immune complex in blood, B cell activation and antibody production by B cells is suppressed (see, e.g., NPL 24). In this immunosuppressive signal transduction mediated by BCR and Fc gamma RIIb, the immunoreceptor tyrosine-based inhibitory motif (ITIM) contained in the intracellular domain of Fc gamma RIIb is necessary (see,

e.g., NPL 25 and NPL 26). When ITIM is phosphorylated upon signaling, SH2-containing inositol polyphosphate 5-phosphatase (SHIP) is recruited, transduction of other activating Fc gamma R signal cascades is inhibited, and inflammatory immune response is suppressed (see, e.g., NPL 27). Furthermore, aggregation of Fc gamma RIIB alone has been reported to transiently suppress calcium influx due to BCR cross-linking and B cell proliferation in a BCR-independent manner without inducing apoptosis of IgM-producing B cells (see, e.g., NPL 28).

[0012] Fc gamma RIIB is also expressed on dendritic cells, macrophages, activated neutrophils, mast cells, and basophils. Fc gamma RIIB inhibits the functions of activating Fc gamma R such as phagocytosis and release of inflammatory cytokines in these cells, and suppresses inflammatory immune responses (see, e.g., NPL 17).

[0013] The importance of immunosuppressive functions of Fc gamma RIIB has been elucidated so far through studies using Fc gamma RIIB knockout mice. There are reports that in Fc gamma RIIB knockout mice, humoral immunity is not appropriately regulated (see, e.g., NPL 29), sensitivity towards collagen-induced arthritis (CIA) is increased (see, e.g., NPL 30), lupus-like symptoms are presented, and Goodpasture's syndrome-like symptoms are presented (see, e.g., NPL 31).

[0014] Additionally, regulatory inadequacy of Fc gamma RIIB has been reported to be related to human autoimmune diseases. For example, the relationship between genetic polymorphism in the transmembrane region and promoter region of Fc gamma RIIB, and the frequency of development of systemic lupus erythematosus (SLE) (see, e.g., NPL 32, NPL 33, NPL 34, NPL 35, and NPL 36), and decrease of Fc gamma RIIB expression on the surface of B cells in SLE patients (see, e.g., NPL 37 and NPL 38) have been reported.

[0015] From mouse models and clinical findings as such, Fc gamma RIIB is considered to play the role of controlling autoimmune diseases and inflammatory diseases through involvement in particular with B cells, and it is a promising target molecule for controlling autoimmune diseases and inflammatory diseases.

[0016] IgG1, mainly used as a commercially available therapeutic antibody, is known to bind not only to Fc gamma RIIB, but also strongly to activating Fc gamma R (see, e.g., NPL 39). It may be possible to develop therapeutic antibodies having greater immunosuppressive properties compared with those of IgG1, by utilizing an Fc region with enhanced Fc gamma RIIB binding, or improved Fc gamma RIIB-binding selectivity compared with activating Fc gamma R. For example, it has been suggested that the use of an antibody having a variable region that binds to BCR and an Fc with enhanced Fc gamma RIIB binding may inhibit B cell activation (see, e.g., NPL 40). It has been reported that crosslinking Fc gamma RIIB on B cells and IgE bound to a B-cell receptor suppresses differentiation of B cells into plasma cells, which as a result causes

suppression of IgE production; and in human PBMC-transplanted mice, human IgG and IgM concentrations are maintained whereas the human IgE concentration is decreased (see, e.g., NPL 41). Besides IgE, it has been reported that when Fc gamma RIIb and CD79b which is a constituent molecule of a B-cell receptor complex are cross-linked by an antibody, B cell proliferation is suppressed in vitro, and arthritis symptoms are alleviated in the collagen arthritis model (see, e.g., NPL 42).

- [0017] Besides B cells, it has been reported that crosslinking of Fc epsilon RI and Fc gamma RIIb on mast cells using molecules, in which the Fc portion of an IgG with enhanced Fc gamma RIIb binding is fused to the Fc portion of IgE that binds to an IgE receptor Fc epsilon RI, causes phosphorylation of Fc gamma RIIb, thereby suppressing Fc epsilon RI-dependent calcium influx. This suggests that inhibition of degranulation via Fc gamma RIIb stimulation is possible by enhancing Fc gamma RIIb binding (see, e.g., NPL 43).
- [0018] Accordingly, an antibody having an Fc with improved Fc gamma RIIb-binding activity is suggested to be promising as a therapeutic agent for inflammatory diseases such as an autoimmune disease.
- [0019] Furthermore, it has been reported that activation of macrophages and dendritic cells via Toll-like receptor 4 due to LPS stimulation is suppressed in the presence of an antibody-antigen immune complex, and this effect is also suggested to be actions of the immune complex via Fc gamma RIIb (see, e.g., NPL 44 and NPL 45). Therefore, use of antibodies with enhanced Fc gamma RIIb binding is expected to enable enhancement of TLR-mediated activation signal-suppressing actions; thus such antibodies have been suggested as being promising as therapeutic agents for inflammatory diseases such as autoimmune diseases.
- [0020] Additionally, mutants with enhanced Fc gamma RIIb binding have been suggested to be promising therapeutic agents for cancer, as well as therapeutic agents for inflammatory diseases such as autoimmune diseases. So far, Fc gamma RIIb has been found to play an important role in the agonistic activity of agonist antibodies against the anti-TNF receptor superfamily. Specifically, it has been suggested that interaction with Fc gamma RIIb is required for the agonistic activity of antibodies against CD40, DR4, DR5, CD30, and CD137, which are included in the TNF receptor family (see, e.g., NPL 46, NPL 47, NPL 48, NPL 49, NPL 50, NPL 51 and NPL 52). NPL 46 shows that the use of antibodies with enhanced Fc gamma RIIb binding enhances the anti-tumor effect of anti-CD40 antibodies. Accordingly, antibodies with enhanced Fc gamma RIIb are expected to have an effect of enhancing agonistic activity of agonist antibodies including antibodies against the anti-TNF receptor superfamily.
- [0021] In addition, it has been shown that cell proliferation is suppressed when using an antibody that recognizes Kit, a type of receptor tyrosine kinase (RTK), to crosslink Fc

gamma RIIb and Kit on Kit-expressing cells. Similar effects have been reported even in cases where this Kit is constitutively activated and has mutations that cause oncogenesis (see, e.g., NPL 53). Therefore, it is expected that use of antibodies with enhanced Fc gamma RIIb binding may enhance inhibitory effects on cells expressing RTK having constitutively activated mutations.

[0022] Antibodies having an Fc with improved Fc gamma RIIb-binding activity have been reported (see, e.g., NPL 40). In this Literature, Fc gamma RIIb-binding activity was improved by adding alterations such as S267E/L328F, G236D/S267E, and S239D/S267E to an antibody Fc region. Among them, the antibody introduced with the S267E/L328F mutation most strongly binds to Fc gamma RIIb, and maintains the same level of binding to Fc gamma RIIa and Fc gamma RIIa type H in which a residue at position 131 of Fc gamma RIIa is His as that of a naturally-occurring IgG1. However, another report shows that this alteration enhances the binding to Fc gamma RIIa type R in which a residue at position 131 of Fc gamma RIIa is Arg several hundred times to the same level of Fc gamma RIIb binding, which means the Fc gamma RIIb-binding selectivity is not improved in comparison with type-R Fc gamma RIIa (see, e.g., PTL 15).

[0023] Only the effect of enhancing Fc gamma RIIa binding and not the enhancement of Fc gamma RIIb binding is considered to have influence on cells such as platelets which express Fc gamma RIIa but do not express Fc gamma RIIb (see, e.g., NPL 17). For example, the group of patients who were administered bevacizumab, an antibody against VEGF, is known to have an increased risk for thromboembolism (see, e.g., NPL 54). Furthermore, thromboembolism has been observed in a similar manner in clinical development tests of antibodies against the CD40 ligand, and the clinical study was discontinued (see, e.g., NPL 55). In both cases of these antibodies, later studies using animal models and such have suggested that the administered antibodies aggregate platelets via Fc gamma RIIa binding on the platelets, and form blood clots (see, e.g., NPL 56 and NPL 57). In systemic lupus erythematosus which is an autoimmune disease, platelets are activated via an Fc gamma RIIa-dependent mechanism, and platelet activation has been reported to correlate with the severity of symptoms (see, e.g., NPL 58). Administering an antibody with enhanced Fc gamma RIIa binding to such patients who already have a high risk for developing thromboembolism will increase the risk for developing thromboembolism, thus is extremely dangerous.

[0024] Furthermore, antibodies with enhanced Fc gamma RIIa binding have been reported to enhance macrophage-mediated antibody dependent cellular phagocytosis (ADCP) (see, e.g., NPL 59). When antigens to be bound by the antibodies are phagocytized by macrophages, antibodies themselves are considered to be also phagocytized at the same time. When antibodies are administered as pharmaceuticals, it is supposed that

peptide fragments derived from the administered antibodies are likely to be also presented as an antigen, thereby increasing the risk of production of antibodies against therapeutic antibodies (anti-therapeutic antibodies). More specifically, enhancing Fc gamma RIIa binding will increase the risk of production of antibodies against the therapeutic antibodies, and this will remarkably decrease their value as pharmaceuticals. Furthermore, Fc gamma RIIb on dendritic cells have been suggested to contribute to peripheral tolerance by inhibiting dendritic cell activation caused by immune complexes formed between antigens and antibodies, or by suppressing antigen presentation to T cells via activating Fc gamma receptors (see, e.g., NPL 60). Since Fc gamma RIIa is also expressed on dendritic cells, when antibodies having an Fc with enhanced selective binding to Fc gamma RIIb are used as pharmaceuticals, antigens are not readily presented by dendritic cells and such due to enhanced selective binding to Fc gamma RIIb, and risk of anti-drug antibody production can be relatively decreased. Such antibodies may be useful in that regard as well.

[0025] More specifically, the value as pharmaceuticals will be considerably reduced when Fc gamma RIIa binding is enhanced, which leads to increased risk of thrombus formation via platelet aggregation and increased risk of anti-therapeutic antibody production due to an increased immunogenicity.

[0026] From such a viewpoint, the aforementioned Fc variant with enhanced Fc gamma RIIb binding shows significantly enhanced type-R Fc gamma RIIa binding compared with that of a naturally-occurring IgG1. Therefore, its value as a pharmaceutical for patients carrying type-R Fc gamma RIIa is considerably reduced. Types H and R of Fc gamma RIIa are observed in Caucasians and African-Americans with approximately the same frequency (see, e.g., NPL 61 and NPL 62). Therefore, when this Fc variant was used for treatment of autoimmune diseases, the number of patients who can safely use it while enjoying its effects as a pharmaceutical will be limited.

[0027] Furthermore, in dendritic cells deficient in Fc gamma RIIb or dendritic cells in which the interaction between Fc gamma RIIb and the antibody Fc portion is inhibited by an anti-Fc gamma RIIb antibody, dendritic cells have been reported to mature (see, e.g., NPL 63 and NPL 64). This report suggests that Fc gamma RIIb is actively suppressing maturation of dendritic cells in a steady state where inflammation and such are not taking place and activation does not take place. Fc gamma RIIa is expressed on the dendritic cell surface in addition to Fc gamma RIIb; therefore, even if binding to inhibitory Fc gamma RIIb is enhanced and if binding to activating Fc gamma R such as Fc gamma RIIa is also enhanced, maturation of dendritic cells may be promoted as a result. More specifically, improving not only the Fc gamma RIIb-binding activity but also the ratio of Fc gamma RIIb-binding activity relative to Fc gamma RIIa-binding activity is considered to be important in providing antibodies with an immunosup-

pressive action.

[0028] Therefore, when considering the generation of a pharmaceutical that utilizes the Fc gamma RIIb binding-mediated immunosuppressive action, there is a need for an Fc variant that not only has enhanced Fc gamma RIIb-binding activity, but also has binding to both Fc gamma RIIa types H and R allotypes, which is maintained at a similar level or is weakened to a lower level than that of a naturally-occurring IgG1.

[0029] Meanwhile, cases where amino acid alterations were introduced into the Fc region to increase the Fc gamma RIIb-binding selectivity have been reported so far (see, e.g., NPL 65). However, all variants said to have improved Fc gamma RIIb selectivity as reported in this literature showed decreased Fc gamma RIIb binding compared with that of a naturally-occurring IgG1. Therefore, it is considered to be difficult for these variants to actually induce an Fc gamma RIIb-mediated immunosuppressive reaction more strongly than IgG1.

[0030] Furthermore, since Fc gamma RIIb plays an important role in the agonist antibodies mentioned above, enhancing their binding activity is expected to enhance the agonistic activity. However, when Fc gamma RIIa binding is similarly enhanced, unintended activities such as ADCC activity and ADCP activity will be exhibited, and this may cause side effects. Also from such viewpoint, it is preferable to be able to selectively enhance Fc gamma RIIb-binding activity.

[0031] From these results, in producing therapeutic antibodies to be used for treating autoimmune diseases and cancer utilizing Fc gamma RIIb, it is important that compared with those of a naturally-occurring IgG, the activities of binding to both Fc gamma RIIa allotypes are maintained or decreased, and Fc gamma RIIb binding is enhanced. However, Fc gamma RIIb shares 93% sequence identity in the extracellular region with that of Fc gamma RIIa which is one of the activating Fc gamma Rs, and they are very similar structurally. There are allotypes of Fc gamma RIIa, H type and R type, in which the amino acid at position 131 is His (type H) or Arg (type R), and yet each of them reacts differently with the antibodies (see, e.g., NPL 66). Therefore, the difficult problem may be producing an Fc region variant with enhanced selective Fc gamma RIIb binding as compared to each allotype of Fc gamma RIIa, which involves distinguishing highly homologous sequences between Fc gamma RIIa and Fc gamma RIIb. In spite of those difficulties, several Fc region variants have been identified so far, which has selective binding activity to Fc gamma RIIb as compared to Fc gamma RIIa, by conducting comprehensive amino acid modification analysis in the Fc region (see, e.g., PTL 16, PTL 17, PTL 18, PTL 19 and PTL 20).

[0032] There has been a report on an Fc region variant with binding selectivity for Fc gamma RIIb in relation to human Fc gamma R so far, whereas there has been no report on an Fc region variant with binding selectivity for Fc gamma RIIb in relation to

monkey Fc gamma R. Owing to the absence of such an Fc variant, the effects of the Fc variant selectively binding to Fc gamma RIIB have not been thoroughly tested yet in monkey.

[0033] Apart from the above, it is reported that by modifying the charge of amino acid residues which may be exposed on the surface of an antibody so as to increase or decrease the isoelectric point (pI) of the antibody, it is possible to regulate the half-life of the antibody in blood (see, e.g., PTL 21 and PTL 22). They show that it is possible to prolong the plasma half-life of an antibody by reducing the antibody's pI and vice versa.

[0034] Further, it is reported that incorporation of an antigen into cells can be promoted by modifying the charge of specified amino acid residues particularly in its CH3 domain to increase the antibody's pI (see, e.g., PTL 23). Also, it has been reported that modifying the charge of amino acid residues in the constant region (mainly CH1 domain) of an antibody to reduce pI can prolong the half-life of the antibody in plasma (see, e.g., PTL 24).

Citation List

Patent Literature

- [0035] PTL 1: US Patent No. 5,827,733
 PTL 2: WO 1994/021681
 PTL 3: US Patent No. 6,096,506
 PTL 4: US Patent No. 7,261,893
 PTL 5: US Patent No. 7,320,789
 PTL 6: US Patent No. 7,807,159
 PTL 7: US Patent No. 7,888,486
 PTL 8: WO 2005/094446
 PTL 9: WO 2007/047112
 PTL 10: WO 2010/070094
 PTL 11: WO 2000/042072
 PTL 12: WO 2006/019447
 PTL 13: WO 2004/099249
 PTL 14: WO 2004/029207
 PTL 15: US Appl. Publ. No. US2009/0136485
 PTL 16: WO 2012/115241
 PTL 17: WO 2013/047752
 PTL 18: WO 2013/125667
 PTL 19: WO 2014/030728
 PTL 20: WO 2014/163101

PTL 21: WO 2007/114319

PTL 22: WO 2009/041643

PTL 23: WO 2014/145159

PTL 24: WO 2012/016227

Non-Patent Literature

- [0036] NPL 1: Kingsley et al., Genes Dev. 8(2):133-146 (1994)
- NPL 2: Hoodless et al., Curr. Top. Microbiol. Immunol. 228:235-272 (1998)
- NPL 3: Zimmers et al., Science 296(5572):1486-1488 (2002)
- NPL 4: McPherron et al., Nature 387(6628):83-90 (1997)
- NPL 5: McPherron and Lee, Proc. Natl. Acad. Sci. USA 94(23):12457-12461 (1997)
- NPL 6: Szlama et al., FEBS J 280(16):3822-3839 (2013)
- NPL 7: Lee, PloS One 3(2):e1628 (2008)
- NPL 8: Lee, Proc. Natl. Acad. Sci. USA 98(16):9306-9311 (2001)
- NPL 9: McCroskery et al., J Cell Sci. 118(15):3531-3541 (2005)
- NPL 10: Whittemore et al., Biochem. Biophys. Res. Commun. 300(4):965-971 (2003)
- NPL 11: Bogdanovich et al., Nature 420(6914):418-421 (2002)
- NPL 12: Wagner., Ann. Neurol. 52(6):832-836 (2002)
- NPL 13: Reichert et al., Nat. Biotechnol. 23:1073-1078 (2005)
- NPL 14: Pavlou et al., Eur. J. Pharm. Biopharm. 59:389-396 (2005)
- NPL 15: Clark et al., Chem. Immunol. 65:88-110 (1997)
- NPL 16: Jefferis et al., Immunol. Lett. 82:57-65 (2002)
- NPL 17: Smith et al., Nat. Rev. Immunol. 10:328-343 (2010)
- NPL 18: Radaev et al., J. Biol. Chem. 276:16478-16483 (2001)
- NPL 19: Greenwood et al., Eur. J. Immunol. 23:1098-1104 (1993)
- NPL 20: Morgan et al., Immunology 86:319-324 (1995)
- NPL 21: Nimmerjahn et al., Nat. Rev. Immunol. 8:34-47 (2008)
- NPL 22: Amigorena et al., Eur. J. Immunol. 19:1379-1385 (1989)[NPL 23] Sinclair, J. Exp. Med. 129:1183-1201 (1969)
- NPL 24: Heyman, Immunol. Lett. 88:157-161 (2003)
- NPL 25: Amigorena et al., Science 256:1808-1812 (1992)
- NPL 26: Muta et al., Nature 368:70-73 (1994)
- NPL 27: Ravetch, Science 290:84-89 (2000)
- NPL 28: Fournier et al., J. Immunol. 181:5350-5359 (2008)
- NPL 29: J. Immunol. 163:618-622 (1999)
- NPL 30: Yuasa et al., J. Exp. Med. 189:187-194 (1999)
- NPL 31: Nakamura et al., J. Exp. Med. 191:899-906 (2000)
- NPL 32: Blank, Hum. Genet. 117:220-227 (2005)
- NPL 33: Olfieriev et al., J. Biol. Chem. 282:1738-1746 (2007)

- NPL 34: Chen et al., Arthritis Rheum. 54:3908-3917 (2006)
- NPL 35: Floto et al., Nat. Med. 11:1056-1058 (2005)
- NPL 36: Li et al., J. Immunol. 176:5321-5328 (2006)
- NPL 37: Mackay et al., J. Exp. Med. 203:2157-2164 (2006)
- NPL 38: Yang et al., J. Immunol. 178:3272-3280 (2007)
- NPL 39: Bruhns et al., Blood 113:3716-3725 (2009)
- NPL 40: Chu et al., Mol. Immunol. 45:3926-3933 (2008)
- NPL 41: Chu et al., J. Allergy Clin. Immunol. 129:1102-1115 (2012)
- NPL 42: Veri et al., Arthritis Rheum. 62:1933-1943 (2010)
- NPL 43: Cemerski et al., Immunol. Lett. 143:34-43 (2012)
- NPL 44: Wenink et al., J. Immunol. 183:4509-4520 (2009)
- NPL 45: Zhang et al., J. Immunol. 182:554-562 (2009)
- NPL 46: Ravetch, Science 333:1030-1034 (2011)
- NPL 47: Wilson et al., Cancer Cell 19:101-113 (2011)
- NPL 48: Kohrt et al., J. Clin. Invest. 122:1066-1075 (2012)
- NPL 49: Xu et al., J. Immunol. 171:562-568 (2003)
- NPL 50: Zhang et al., Blood 108:705-710 (2006)
- NPL 51: Chuntharapai et al., J. Immunol. 166:4891-4898 (2001)
- NPL 52: Ravetch et al., Proc. Natl. Acad. Sci. USA 109:10966-10971 (2012)
- NPL 53: Malbec et al., Immunol. Lett. 143:28-33 (2012)
- NPL 54: Scappaticci et al., J. Natl. Cancer. Inst. 99:1232-1239 (2007)
- NPL 55: Arthritis Rheum. 48:719-727 (2003)
- NPL 56: Meyer et al., J. Thromb. Haemost. 7:171-181 (2008)
- NPL 57: Robles-Carrillo et al., J. Immunol. 185:1577-1583 (2010)
- NPL 58: Duffau et al., Sci. Transl. Med. 2:47ra63 (2010)
- NPL 59: Richards et al, Mol. Cancer Ther. 7:2517-2527 (2008)
- NPL 60: Desai et al., J. Immunol. 178:6217-6226 (2007)
- NPL 61: Salmon et al., J. Clin. Invest. 97:1348-1354 (1996)
- NPL 62: Manger et al., Arthritis Rheum. 41:1181-1189 (1998)
- NPL 63: Boruchov et al., J. Clin. Invest. 115:2914-2923 (2005)
- NPL 64: Dhodapkar et al., Proc. Natl. Acad. Sci. USA 102:2910-2915 (2005)
- NPL 65: Armour et al., Mol. Immunol. 40:585-593 (2003)
- NPL 66: Warmerdam et al., J. Exp. Med. 172:19-25 (1990)

Summary of Invention

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 invention provides anti-myostatin antibodies and methods of using the same. The invention also provides proteins containing a variant Fc region and methods of using the same.

[0039] In some embodiments, an isolated anti-myostatin antibody of the present invention binds to latent myostatin. In further embodiments, the antibody binds to an epitope within a fragment consisting of amino acids 21-100 of myostatin propeptide (SEQ ID NO: 78). In some embodiments, an isolated anti-myostatin antibody of the present invention inhibits activation of myostatin. In further embodiments, the antibody blocks the release of mature myostatin from latent myostatin. In further embodiments, the antibody blocks the proteolytic release of mature myostatin. In further embodiments, the antibody blocks the spontaneous release of mature myostatin. In further embodiments, the antibody does not bind to mature myostatin. In further embodiments, the antibody binds to the same epitope as an antibody described in Table 13. In further embodiments, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 13. In further embodiments, the antibody binds to the same epitope as an antibody described in Table 2a. In further embodiments, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 2a. In further embodiments, the antibody binds to the same epitope as an antibody described in Table 11a. . In further embodiments, the antibody binds to the same epitope as an antibody comprising a VH and a VL pair described in Table 11a. In further embodiments, the antibody binds to the same epitope as an antibody described in Table 2a, 11a, or 13. In further embodiments, 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 embodiments, an isolated anti-myostatin antibody of the present invention binds to latent myostatin with higher affinity at neutral pH than at acidic pH. In some embodiments, the anti-myostatin antibody binds to latent myostatin with higher affinity at pH7.4 than at pH5.8. In some embodiments, an isolated anti-myostatin antibody of the present invention 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, an isolated anti-myostatin antibody of the present invention competes for binding latent myostatin with an antibody provided herein. In some embodiments, an isolated anti-myostatin antibody of the present invention competes for binding latent myostatin with an antibody described in Table 13. In some embodiments, an isolated anti-myostatin antibody of the present invention competes for binding latent myostatin with an antibody comprising a VH and VL pair described in Table 13. In some embodiments, the antibody competes for binding latent myostatin with an antibody described in Table 2a. In some embodiments, an isolated anti-myostatin antibody of the present invention competes for binding latent myostatin with an antibody comprising a VH and VL pair described in Table 2a. In some embodiments, the antibody competes for binding latent myostatin with an antibody described in Table 11a. In some embodiments, an isolated anti-myostatin antibody of the present invention competes for binding latent myostatin with an antibody comprising a VH and VL pair described in Table 11a. In additional embodiments, an anti-myostatin antibody competes for binding latent myostatin with an antibody described in Table 2a, 11a, or 13. In additional embodiments, 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 embodiments, the anti-myostatin antibody binds to latent myostatin with a higher affinity at neutral pH than at acidic

pH. In further embodiments, the anti-myostatin antibody binds to latent myostatin with a higher affinity at pH7.4 than at pH5.8. In further embodiments, 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 embodiments, an isolated anti-myostatin antibody of the present invention is a monoclonal antibody. In some embodiments, an isolated anti-myostatin antibody of the present invention is a human, humanized, or chimeric antibody. In some embodiments, an isolated anti-myostatin antibody of the present invention is an antibody fragment that binds to myostatin. In some embodiments, an isolated anti-myostatin antibody of the present invention is an antibody fragment that binds to latent myostatin. In some embodiments, an isolated anti-myostatin antibody of the present invention 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 embodiments, an isolated anti-myostatin antibody of the present invention is a full length IgG antibody.

[0043] In some embodiments, a anti-myostatin antibody of the invention comprises:

(a) (i) a HVR-H3 comprising the amino acid sequence GVPAX₁SX₂GGDX₃, wherein X₁ is Y or H, X₂ is T or H, X₃ is L or K (SEQ ID NO: 128), (ii) a HVR-L3 comprising the amino acid sequence AGGYGGGX₁YA, wherein X₁ is L or R (SEQ ID NO: 131), and (iii) a HVR-H2 comprising the amino acid sequence IISX₁AGX₂X₃YX₄X₅X₆WAKX₇, wherein X₁ is Y or H, X₂ is S or K, X₃ is T, M or K, X₄ is Y or K, X₅ is A, M or E, X₆ is S or E, X₇ is G or K (SEQ ID NO: 127);

(b) (i) a HVR-H1 comprising the amino acid sequence X₁X₂DIS, wherein X₁ is S or H, X₂ is Y, T, D or E (SEQ ID NO: 126), (ii) a HVR-H2 comprising the amino acid sequence IISX₁AGX₂X₃YX₄X₅X₆WAKX₇, wherein X₁ is Y or H, X₂ is S or K, X₃ is T, M or K, X₄ is Y or K, X₅ is A, M or E, X₆ is S or E, X₇ is G or K (SEQ ID NO: 127), and (iii) a HVR-H3 comprising the amino acid sequence GVPAX₁SX₂GGDX₃, wherein X₁ is Y or H, X₂ is T or H, X₃ is L or K (SEQ ID NO: 128);

(c) (i) a HVR-H1 comprising the amino acid sequence X₁X₂DIS, wherein X₁ is S or H, X₂ is Y, T, D or E (SEQ ID NO: 126), (ii) a HVR-H2 comprising the amino acid sequence IISX₁AGX₂X₃YX₄X₅X₆WAKX₇, wherein X₁ is Y or H, X₂ is S or K, X₃ is T, M or K, X₄ is Y or K, X₅ is A, M or E, X₆ is S or E, X₇ is G or K (SEQ ID NO: 127), (iii) a HVR-H3 comprising the amino acid sequence GVPAX₁SX₂GGDX₃, wherein X₁ is Y or H, X₂ is T or H, X₃ is L or K (SEQ ID NO: 128), (iv) a HVR-L1 comprising the amino acid sequence X₁X₂SQX₃VX₄X₅X₆NWLS, wherein X₁ is Q or T, X₂ is S or T, X₃ is S or E, X₄ is Y or F, X₅ is D or H, X₆ 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 embodiments 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 embodiments, 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 embodiments, an isolated anti-myostatin antibody of the present invention 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 embodiments, an isolated anti-myostatin antibody of the present invention 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 embodiments, 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 embodiments, the antibody additionally comprises (a) a HVR-L1 comprising the amino acid sequence $X_1X_2SQX_3VX_4$

X₅X₆NWLS, wherein X₁ is Q or T, X₂ is S or T, X₃ is S or E, X₄ is Y or F, X₅ is D or H, X₆ is N, D, A or E (SEQ ID NO: 129); (b) a HVR-L2 comprising the amino acid sequence WAX₁TLAX₂, wherein X₁ is S or E, X₂ is S, Y, F or W (SEQ ID NO: 130); and (c) a HVR-L3 comprising the amino acid sequence AGGYGGGX₁YA, wherein X₁ is L or R (SEQ ID NO: 131).

[0046] In some embodiments, an isolated anti-myostatin antibody of the present invention comprises (a) a HVR-L1 comprising the amino acid sequence X₁X₂SQX₃VX₄X₅X₆NWLS, wherein X₁ is Q or T, X₂ is S or T, X₃ is S or E, X₄ is Y or F, X₅ is D or H, X₆ is N, D, A or E (SEQ ID NO: 129); (b) a HVR-L2 comprising the amino acid sequence WAX₁TLAX₂, wherein X₁ is S or E, X₂ is S, Y, F or W (SEQ ID NO: 130); and (c) a HVR-L3 comprising the amino acid sequence AGGYGGGX₁YA, wherein X₁ is L or R (SEQ ID NO: 131). In a further embodiment, 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 embodiments, an isolated anti-myostatin antibody of the present invention 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 embodiments, an isolated anti-myostatin antibody of the present invention 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 embodiments, an isolated anti-myostatin antibody of the present invention 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 embodiments, the antibody comprises a VH sequence of any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95. In further embodiments, the antibody comprises a VL sequence of any one of SEQ ID NOs: 15, 31, 35-38, and 96-99. In some embodiments, the antibody comprises a VH sequence of any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95. In further embodiments, 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] The invention also provides isolated nucleic acids encoding an anti-myostatin antibody of the present invention. The invention also provides host cells comprising a nucleic acid of the present invention. The invention also provides a method of producing an antibody comprising culturing a host cell of the present invention so that the antibody is produced.

[0050] In some aspects the invention provides a method of producing an anti-myostatin antibody comprising: (a) culturing a host cell of the present invention 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 invention further provides a method of producing an anti-myostatin antibody. In some embodiments, 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] The invention also provides a pharmaceutical formulation comprising an anti-myostatin antibody of the present invention and a pharmaceutically acceptable carrier.

[0053] Anti-myostatin antibodies of the present invention may be for use as a medicament. In some embodiments, 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 embodiments, anti-myostatin antibodies of the present invention may be for use in treating a muscle wasting disease. Anti-myostatin antibodies of the present invention may be for use in increasing mass of muscle tissue. Anti-myostatin antibodies of the present invention may be for use in increasing strength of muscle tissue. Anti-myostatin antibodies of the present invention may be for use in reducing body fat accumulation.

[0054] In some embodiments, 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 invention may be used in the manufacture of a medicament. In some embodiments, 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 embodiments, the medicament is for treatment of a muscle wasting disease. In some embodiments, the medicament is for increasing mass of muscle tissue. In some embodiments, the medicament is for increasing strength of muscle tissue. In some embodiments, the medicament is for reducing body fat accumulation.

- [0056] The invention also provides a method of treating an individual having a muscle wasting disease. In some embodiments, the method comprises administering to the individual an effective amount of an anti-myostatin antibody of the present invention. The invention also provides a method of increasing mass of muscle tissue in an individual. In some embodiments, the method comprises administering to the individual an effective amount of an anti-myostatin antibody of the present invention to increase mass of muscle tissue. The invention also provides a method of increasing strength of muscle tissue in an individual. In some embodiments, the method comprises administering to the individual an effective amount of an anti-myostatin antibody of the present invention to increase strength of muscle tissue. The invention also provides a method of reducing body fat accumulation in an individual. In some embodiments, the method comprises administering to the individual an effective amount of an anti-myostatin antibody of the present invention to reduce body fat accumulation.
- [0057] The invention provides polypeptides comprising variant Fc regions and methods of making and using the same.
- [0058] In one embodiment, the invention provides Fc gamma RIIB-binding polypeptides comprising variant Fc regions and methods of using the same. In some embodiments, a variant Fc region with enhanced Fc gamma RIIB-binding activity of the present invention comprises at least one amino acid alteration in a parent Fc region. In further embodiments, 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 embodiments, the ratio of [KD value of the parent Fc region for monkey Fc gamma RIIBa]/[KD value of the variant Fc region for monkey Fc gamma RIIBa] is 0.5 or smaller. In further embodiments, 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 embodiments, the ratio of [KD value of the parent Fc region for human Fc gamma RIIBa]/[KD value of the variant Fc region for human Fc gamma RIIBa] is 0.5 or smaller. In further embodiments, the ratio of [KD value of the parent Fc region for human Fc gamma RIIBa (type H)]/[KD value of the variant Fc region for human Fc gamma RIIBa (type H)] is 5.0 or smaller. In further embodiments, the ratio of [KD value of the parent Fc region for human Fc gamma RIIBa (type R)]/[KD value of the variant Fc region for human Fc gamma RIIBa (type R)] is 5.0 or smaller. In another embodiment, the KD value of the variant Fc region for monkey Fc gamma RIIB is 1.0×10^{-6} M or smaller. In another embodiment, the KD value of the variant Fc region for monkey Fc gamma RIIBa is 5.0×10^{-7} M or greater. In another embodiment, the KD value of the variant Fc region for human Fc gamma RIIB is 2.0×10^{-6} M or smaller. In another embodiment, the KD value of the variant Fc region for human Fc gamma RIIBa is 1.0×10^{-6} M or greater. In another embodiment, the KD value of the variant Fc region

for human Fc gamma RIIa (type H) is 1.0×10^{-7} M or greater. In another embodiment, the KD value of the variant Fc region for human Fc gamma RIIa (type R) is 2.0×10^{-7} M or greater.

- [0059] In some embodiments, a variant Fc region with enhanced Fc gamma RIIb-binding activity of the present invention 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, a variant Fc region with enhanced Fc gamma RIIb-binding activity of the present invention 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 embodiments, 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 embodiment, the invention provides a polypeptide comprising an isoelectric point (pI)-increased variant Fc region and a method of using the same. In some embodiments, a polypeptide comprising a variant Fc region with increased pI comprises at least two amino acid alterations in a parent Fc region. In further embodiments, 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 embodiments, the amino acid can be exposed on the surface of the variant Fc region. In further embodiments, a polypeptide comprises the variant Fc region and an antigen-binding domain. In further embodiments, antigen-binding activity of the antigen-binding domain changes according to ion concentration conditions. In further embodiments, the variant Fc region with increased pI of the present invention 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 embodiments, the variant Fc region with increased pI comprises Arg or Lys at each of the positions selected.

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

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

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

- [0070] The invention also provides isolated nucleic acid encoding the polypeptide comprising a variant Fc region of the present invention. The invention also provides a host cell comprising the nucleic acid of the present invention. The invention also provides a method of producing a polypeptide comprising a variant Fc region comprising culturing the host of the present invention so that the polypeptide is produced.
- [0071] The invention further provides a pharmaceutical formulation comprising the polypeptide comprising a variant Fc region of the present invention 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.

[fig.6C]Figure 6C 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 gastrocnemius and quadriceps muscle mass was measured.

[fig.7A]Figure 7A illustrates comparison of in vivo efficacy among several anti-myostatin antibodies, as described in Example 9. Anti-latent myostatin antibody (MST1032-G1m; described as MST1032 in the Figures) or anti-mature myostatin antibody (41C1E4, REGN, OGD, or MYO-029) was administered to SCID mice, and full body lean mass was measured.

[fig.7B]Figure 7B illustrates comparison of in vivo efficacy among several anti-myostatin antibodies, as described in Example 9. Anti-latent myostatin antibody (MST1032-G1m; described as MST1032 in the Figures) or anti-mature myostatin antibody (41C1E4, REGN, OGD, or MYO-029) was administered to SCID mice, and grip strength was measured.

[fig.7C]Figure 7C illustrates comparison of in vivo efficacy among several anti-myostatin antibodies, as described in Example 9. Anti-latent myostatin antibody (MST1032-G1m; described as MST1032 in the Figures) or anti-mature myostatin antibody (41C1E4, REGN, OGD, or MYO-029) was administered to SCID mice, and change of full body fat mass from Day 0 to Day 14 was measured.

[fig.8]Figure 8 illustrates inhibition of proteolytic and spontaneous activation of latent myostatin by humanized anti-latent myostatin antibody, as described in Example 10. The activity of active myostatin released from latent myostatin by BMP1 protease (proteolytic) or by 37 degrees C incubation without BMP1 (spontaneous) was measured in the presence of anti-latent myostatin antibody, using HEK Blue Assay.

[fig.9]Figure 9 illustrates BIAcore (registered trademark) sensorgrams of histidine substituted variants of anti-latent myostatin antibody, as described in Example 11. The antibody/antigen complexes were allowed to dissociate at pH7.4, followed by additional dissociation at pH5.8 (pointed by an arrow) to assess the pH-dependent interactions. Antibodies tested in this experiment are: Ab001 (black solid curve), Ab002 (black short-dashed curve), Ab003 (black dotted curve), Ab004 (gray short-dashed curve), Ab005 (gray solid curve), Ab006 (gray long-dashed curve), and Ab007 (black long-dashed curve).

[fig.10]Figure 10 illustrates inhibition of proteolytic and spontaneous activation of latent myostatin by pH-dependent anti-latent myostatin antibodies, as described in Example 13. The activity of active myostatin released from latent myostatin by BMP1 protease (proteolytic) or by 37 degrees C incubation without BMP1 (spontaneous) was measured in the presence of anti-latent myostatin antibodies, using HEK Blue Assay.

Antibodies MS1032LO01-SG1, MS1032LO02-SG1, MS1032LO03-SG1, and MS1032LO04-SG1 are described respectively as MSLO-01, MSLO-02, MSLO-03, and MSLO-04 in the Figure. Comparable inhibition of proteolytic and spontaneous activation of latent myostatin to MS1032LO00-SG1 was achieved by MS1032LO01-SG1, MS1032LO02-SG1, MS1032LO03-SG1, and MS1032LO04-SG1.

[fig.11]Figures 11A-11F illustrate BIACORE (registered trademark) sensorgrams of pH-dependent anti-latent myostatin antibody, as described in Example 14. Kinetic parameters of MST1032-SG1 (A), MS1032LO00-SG1 (B), MS1032LO01-SG1 (C), MS1032LO02-SG1 (D), MS1032LO03-SG1 (E), and MS1032LO04-SG1 (F), were measured at neutral pH and acidic pH.

[fig.12]Figure 12 illustrates the time course of plasma myostatin concentration after intravenous administration of an anti-myostatin antibody in mice, as described in Example 15. The effects of Fc gamma R-mediated cellular uptake of antibody/antigen complexes on myostatin clearance in vivo was assessed by comparing anti-myostatin antibodies with Fc gamma R binding (MS1032LO00-SG1) and anti-myostatin antibodies with abolished Fc gamma R binding (MS1032LO00-F760).

[fig.13]Figure 13 illustrates the time course of plasma myostatin concentration after intravenous administration of an anti-myostatin antibody in mice, as described in Example 16. The effects of pH-dependent binding of anti-myostatin antibody on myostatin clearance in vivo was assessed by comparing pH-dependent anti-myostatin antibody (MS1032LO01-SG1 or MS1032LO01-F760) and non pH-dependent anti-myostatin antibody (MS1032LO00-SG1 or MS1032LO00-F760).

[fig.14]Figures 14A-14E illustrate in vivo efficacy of pH-dependent and non pH-dependent anti-latent myostatin antibodies, as described in Example 17. pH-dependent anti-latent myostatin antibody (MS1032LO01-SG1; described as MSLO1 in the Figure) or non pH-dependent anti-latent myostatin antibody (MS1032LO00-SG1; described as MSLO0 in the Figure) was administered to SCID mice, and full body lean mass (A), full body fat mass (B), quadriceps muscle mass (C), gastrocnemius muscle mass (D), and grip strength (E) were measured.

[fig.15]Figure 15 illustrates binding activity of anti-latent myostatin antibody MST1032 to latent myostatin and GDF11, as described in Example 19.

[fig.16]Figure 16 illustrates the inhibitory activity of anti-latent myostatin antibody MST1032 against proteolytic and spontaneous activation of GDF11, as described in Example 20. The activity of active GDF11 released by BMP1 protease (proteolytic) or by 37 degrees C incubation without BMP1 (spontaneous) was measured in the presence of anti-latent myostatin antibody, using HEK Blue Assay.

[fig.17]Figure 17 illustrates inhibition of proteolytic activation of latent myostatin by

anti-latent myostatin antibody, as described in Example 22. 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.18]Figure 18 illustrates the time course of plasma myostatin concentration after intravenous administration of anti-latent myostatin antibodies in mice, as described in Example 23. The effects of pH dependency on myostatin clearance in vivo was assessed by comparing non-pH dependent anti-latent myostatin antibody (MS1032LO00-SG1) and different pH-dependent anti-latent myostatin antibodies (MS1032LO01-SG1, MS1032LO06-SG1, MS1032LO11-SG1, MS1032LO18-SG1, MS1032LO19-SG1, MS1032LO21-SG1 and MS1032LO25-SG1).

[fig.19]Figures 19A and 19B illustrate the time course of plasma myostatin concentration after intravenous administration of anti-latent myostatin antibodies in cynomolgus monkey, as described in Example 24. (A) The effect of pH-dependency and Fc engineering on myostatin clearance in vivo was assessed by comparing non pH-dependent anti-latent myostatin antibody (MS1032LO00-SG1) and pH-dependent anti-latent myostatin antibodies with Fc engineering (MS1032LO06-SG1012, MS1032LO06-SG1016, MS1032LO06-SG1029, MS1032LO06-SG1031, MS1032LO06-SG1033, MS1032LO06-SG1034). (B) The effect of Fc engineering on myostatin clearance in vivo was assessed by comparing anti-latent myostatin antibodies (MS1032LO19-SG1079, MS1032LO19-SG1071, MS1032LO19-SG1080, MS1032LO19-SG1074, MS1032LO19-SG1081, and MS1032LO19-SG1077).

[fig.20A]Figure 20A, together with Figures 20B-20I, illustrates in vivo efficacy of anti-latent myostatin antibodies (MS1032 variants) on lean body mass (LBM), grip strength, and body fat mass, as described in Example 25. MS1032LO06-SG1, MS1032LO11-SG1, and MS1032LO18-SG1 were administered to Scid mice, and lean body mass (A) was measured.

[fig.20B]Figure 20B, together with Figures 20A, 20C-20I, illustrates in vivo efficacy of anti-latent myostatin antibodies (MS1032 variants) on lean body mass (LBM), grip strength, and body fat mass, as described in Example 25. MS1032LO06-SG1, MS1032LO19-SG1, and MS1032LO25-SG1 were administered to Scid mice, and lean body mass (B) was measured.

[fig.20C]Figure 20C, together with Figures 20A-B, 20D-I, illustrates in vivo efficacy of anti-latent myostatin antibodies (MS1032 variants) on lean body mass (LBM), grip strength, and body fat mass, as described in Example 25. MS1032LO01-SG, MS1032LO06-SG1, and MS1032LO11-SG1 were administered to Scid mice, and lean body mass (C) was measured.

[fig.20D]Figure 20D, together with Figures 20A-C, 20E-I, illustrates in vivo efficacy of anti-latent myostatin antibodies (MS1032 variants) on lean body mass (LBM), grip

strength, and body fat mass, as described in Example 25. MS1032LO06-SG1, MS1032LO11-SG1, and MS1032LO18-SG1 were administered to Scid mice, and grip strength (D) was measured.

[fig.20E]Figure 20E, together with Figures 20A-D, 20F-20I, illustrates in vivo efficacy of anti-latent myostatin antibodies (MS1032 variants) on lean body mass (LBM), grip strength, and body fat mass, as described in Example 25. MS1032LO06-SG1, MS1032LO19-SG1, and MS1032LO25-SG1 were administered to Scid mice, and grip strength (E) was measured.

[fig.20F]Figure 20F, together with Figures 20A-E, 20G-I, illustrates in vivo efficacy of anti-latent myostatin antibodies (MS1032 variants) on lean body mass (LBM), grip strength, and body fat mass, as described in Example 25. MS1032LO01-SG, MS1032LO06-SG1, and MS1032LO11-SG1 were administered to Scid mice, and grip strength (F) was measured.

[fig.20G]Figure 20G, together with Figures 20A-F, 20H-I, illustrates in vivo efficacy of anti-latent myostatin antibodies (MS1032 variants) on lean body mass (LBM), grip strength, and body fat mass, as described in Example 25. MS1032LO06-SG1, MS1032LO11-SG1, and MS1032LO18-SG1 were administered to Scid mice, and body fat mass (G) was measured.

[fig.20H]Figure 20H, together with Figures 20A-G, 20I, illustrates in vivo efficacy of anti-latent myostatin antibodies (MS1032 variants) on lean body mass (LBM), grip strength, and body fat mass, as described in Example 25. MS1032LO06-SG1, MS1032LO19-SG1, and MS1032LO25-SG1 were administered to Scid mice, and body fat mass (H) was measured.

[fig.20I]Figure 20I, together with Figures 20A-H, illustrates in vivo efficacy of anti-latent myostatin antibodies (MS1032 variants) on lean body mass (LBM), grip strength, and body fat mass, as described in Example 25. MS1032LO01-SG, MS1032LO06-SG1, and MS1032LO11-SG1 were administered to Scid mice, and body fat mass (I) was measured.

[fig.21]Figure 21 illustrates inhibitory activity on latent myostatin activation by anti-latent myostatin antibodies, as described in Example 26. The amounts of mature myostatin released from latent myostatin by BMP1 protease were measured in the presence of anti-latent myostatin antibodies (MST1032, MST1504, MST1538, MST1551, MST1558, MST1572, and MST1573).

[fig.22A]Figure 22A illustrates a schematic diagram of latent myostatin fragments of 100 amino acid each, designed for epitope mapping of anti-latent myostatin antibodies, as described in Example 26.

[fig.22B]Figure 22B illustrates Western blotting analysis against GST tagged human latent myostatin fragments (GST-hMSTN) by an anti-GST antibody, as described in

Example 26. Each lane indicates: 1, GST-hMSTN 1-100aa; 2, GST-hMSTN 21-120aa; 3, GST-hMSTN 41-140aa; 4, GST-hMSTN 61-160aa; 5, GST-hMSTN 81-180aa; 6, GST-hMSTN 101-200aa; 7, GST-hMSTN 121-220aa; 8, GST-hMSTN 141-241aa; 9, GST control.

[fig.22C]Figure 22C illustrates Western blotting analysis against GST tagged human latent myostatin fragments (GST-hMSTN) by anti-latent myostatin antibodies (MST1032, MST1538, MST1572, and MST1573), as described in Example 26. Each lane indicates: 1, GST-hMSTN 1-100aa; 2, GST-hMSTN 21-120aa; 3, GST-hMSTN 41-140aa; 4, GST-hMSTN 61-160aa; 5, GST-hMSTN 81-180aa; 6, GST-hMSTN 101-200aa; 7, GST-hMSTN 121-220aa; 8, GST-hMSTN 141-241aa; 9, GST control; 10, human latent myostatin (100ng).

[fig.22D]Figure 22D illustrates summarized results of Western blotting analysis and deduced epitope positions for anti-latent myostatin antibodies (MST1032, MST1538, MST1572, and MST1573), as described in Example 26.

[fig.23]Figure 23 illustrates an alignment of amino acid sequences of cynomolgus (cyno) Fc gamma RIIa1, Fc gamma RIIa2, Fc gamma RIIa3, Fc gamma RIIb, human Fc gamma RIIaH, Fc gamma RIIaR, and Fc gamma RIIb. Squared regions indicate putative residues which interact with Fc domain.

[fig.24]Figure 24 illustrates the time course of total myostatin concentration in plasma after intravenous administration of an anti-myostatin antibody with an Fc gamma RIIb-enhanced Fc variant in all human Fc gamma R transgenic mice, as described in Example 28. The effects of Fc gamma RIIb-enhanced Fc variants on antigen elimination through human Fc gamma RIIb were evaluated.

[fig.25]Figure 25 illustrates the time course of antibody concentration in plasma after intravenous administration of an anti-myostatin antibody with an Fc gamma RIIb-enhanced Fc variant in all human Fc gamma R transgenic mice, as described in Example 28. The effects of Fc gamma RIIb-enhanced Fc variants on antibody pharmacokinetics were evaluated.

[fig.26]Figures 26A and 26B illustrate the time course of plasma myostatin concentration after intravenous administration of anti-latent myostatin antibodies in cynomolgus monkey, as described in Example 29. (A) The effect of pH-dependency and Fc engineering on myostatin clearance in vivo was assessed by comparing non pH-dependent anti-latent myostatin antibody (MS1032LO00-SG1) and pH-dependent anti-latent myostatin antibodies with Fc engineering (MS1032LO06-SG1012, MS1032LO06-SG1016, MS1032LO06-SG1029, MS1032LO06-SG1031, MS1032LO06-SG1033, MS1032LO06-SG1034). (B) The effect of Fc engineering on myostatin clearance in vivo was assessed by comparing anti-latent myostatin antibodies (MS1032LO19-SG1079, MS1032LO19-SG1071, MS1032LO19-SG1080,

MS1032LO19-SG1074, MS1032LO19-SG1081, and MS1032LO19-SG1077).

[fig.27A]Figure 27A illustrates the time course of total myostatin concentration in plasma after intravenous administration of an anti-myostatin antibody with a pI-increased Fc variant in human FcRn transgenic mice, as described in Example 30. The effects of pI-increased Fc variants on antigen elimination were evaluated.

[fig.27B]Figure 27B illustrates the time course of antibody concentration in plasma after intravenous administration of an anti-myostatin antibody with a pI-increased Fc variant in human FcRn transgenic mice, as described in Example 30. The effects of pI-increased Fc variants on antibody pharmacokinetics were evaluated.

[fig.28A]Figure 28A illustrates the time course of total myostatin concentration in plasma after intravenous administration of an anti-myostatin antibody with a pI-increased Fc variant in human FcRn transgenic mice, as described in Example 30. The effects of pI-increased Fc variants on antigen elimination were evaluated. In this assay, an excess of human normal immunoglobulin were co-administered with the anti-myostatin antibody in order to mimic the situation of human plasma.

[fig.28B]Figure 28B illustrates the time course of antibody concentration in plasma after intravenous administration of an anti-myostatin antibody with a pI-increased Fc variant in human FcRn transgenic mice, as described in Example 30. The effects of pI-increased Fc variants on antibody pharmacokinetics were evaluated. In this assay, an excess of human normal immunoglobulin were co-administered with the anti-myostatin antibody in order to mimic the situation of human plasma.

[fig.29]Figure 29 illustrates the time course of total myostatin concentration in plasma after intravenous administration of an anti-myostatin antibody with an Fc gamma RIIb-enhanced Fc variant in human Fc gamma RIIb transgenic mice, as described in Example 31. The effects of Fc gamma RIIb-enhanced Fc variants on antigen elimination through human Fc gamma RIIb were evaluated.

[fig.30]Figure 30 illustrates the time course of antibody concentration in plasma after intravenous administration of an anti-myostatin antibody with an Fc gamma RIIb-enhanced Fc variant in human Fc gamma RIIb transgenic mice, as described in Example 31. The effects of Fc gamma RIIb-enhanced Fc variants on antibody pharmacokinetics were evaluated.

[fig.31]Figure 31 illustrates results of cell imaging analysis of anti-myostatin antibodies with an Fc gamma RIIb-enhanced Fc variant, as described in Example 33. Each antibody was complexed with fluorescence-labelled myostatin and intracellular uptake of the antigen-antibody complex into cells expressing human Fc gamma RIIb was measured.

Description of Embodiments

[0073] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 3d edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (F.M. Ausubel, et al., eds., (2003)); the series *Methods in Enzymology* (Academic Press, Inc.): *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *Antibodies, A Laboratory Manual*, and *Animal Cell Culture* (R.I. Freshney, ed. (1987)); *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J.E. Cellis, ed., 1998) Academic Press; *Animal Cell Culture* (R.I. Freshney), ed., 1987); *Introduction to Cell and Tissue Culture* (J. P. Mather and P.E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; *Handbook of Experimental Immunology* (D.M. Weir and C.C. Blackwell, eds.); *Gene Transfer Vectors for Mammalian Cells* (J.M. Miller and M.P. Calos, eds., 1987); *PCR: The Polymerase Chain Reaction*, (Mullis et al., eds., 1994); *Current Protocols in Immunology* (J.E. Coligan et al., eds., 1991); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999); *Immunobiology* (C.A. Janeway and P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: A Practical Approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal Antibodies: A Practical Approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using Antibodies: A Laboratory Manual* (E. Harlow and D. Lane, Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and *Cancer: Principles and Practice of Oncology* (V.T. DeVita et al., eds., J.B. Lippincott Company, 1993).

I. DEFINITIONS

[0074] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton et al., *Dictionary of Microbiology and Molecular Biology* 2nd ed., J. Wiley & Sons (New York, N.Y. 1994), and March, *Advanced Organic Chemistry Reactions, Mechanisms and Structure* 4th ed., John Wiley & Sons (New York, N.Y. 1992), provide one skilled in the art with a general guide to many of the terms used in the present application.

[0075] For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. It is to be understood that the terminology used herein is for the purpose of

describing particular embodiments only, and is not intended to be limiting. In the event that any definition set forth below conflicts with any document cited, the definition set forth below shall control.

- [0076] An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.
- [0077] "Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.
- [0078] An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.
- [0079] The terms "anti-myostatin antibody" and "an antibody that binds to myostatin" refer to an antibody that is capable of binding myostatin with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting myostatin. In one embodiment, the extent of binding of an anti-myostatin antibody to an unrelated, non-myostatin protein is less than about 10% of the binding of the antibody to myostatin as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to myostatin has a dissociation constant (K_d) of 1 micro M or less, 100 nM or less, 10 nM or less, 1 nM or less, 0.1 nM or less, 0.01 nM or less, or 0.001 nM or less (e.g., 10^{-8} M or less, e.g., from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti myostatin antibody binds to an epitope of myostatin that is conserved among myostatin from different species.

- [0080] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.
- [0081] An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g., scFv); and multispecific antibodies formed from antibody fragments.
- [0082] An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay, and/or conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay. An exemplary competition assay is provided herein.
- [0083] The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.
- [0084] The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively.
- [0085] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.
- [0086] "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor); and B cell activation.

- [0087] An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.
- [0088] The term "epitope" includes any determinant capable of being bound by an antibody. An epitope is a region of an antigen that is bound by an antibody that targets that antigen, and includes specific amino acids that directly contact the antibody. Epitope determinants can include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl or sulfonyl groups, and can have specific three dimensional structural characteristics, and/or specific charge characteristics. Generally, antibodies specific for a particular target antigen will preferentially recognize an epitope on the target antigen in a complex mixture of proteins and/or macromolecules.
- [0089] "Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. In some embodiments, an FcR is a native human FcR. In some embodiments, an FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the Fc gamma RI, Fc gamma RII, and Fc gamma RIII subclasses, including allelic variants and alternatively spliced forms of those receptors. Fc gamma RII receptors include Fc gamma RIIA (an "activating receptor") and Fc gamma RIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor Fc gamma RIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor Fc gamma RIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (See, e.g., Daeron, *Annu. Rev. Immunol.* 15:203-234 (1997).) FcRs are reviewed, for example, in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-92 (1991); Capel et al., *Immunomethods* 4:25-34 (1994); and de Haas et al., *J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.
- [0090] The term "Fc receptor" or "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)) and regulation of homeostasis of immunoglobulins. Methods of measuring binding to FcRn are known (see, e.g., Ghetie and Ward., *Immunol. Today* 18(12):592-598 (1997); Ghetie et al., *Nature Biotechnology* 15(7):637-640 (1997); Hinton et al., *J. Biol. Chem.* 279(8):6213-6216 (2004); WO 2004/92219 (Hinton et al.). Binding to human FcRn in vivo and serum half life of human FcRn high affinity binding polypeptides can be assayed, e.g., in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides with a variant Fc region are administered. WO 2000/42072 (Presta) describes antibody variants with improved or di-

minished binding to FcRs. See also, e.g., Shields et al., J. Biol. Chem. 9(2):6591-6604 (2001).

- [0091] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) or glycine-lysine (residues 446-447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.
- [0092] The term "Fc region-comprising antibody" refers to an antibody that comprises an Fc region. The C-terminal lysine (residue 447 according to the EU numbering system) or C-terminal glycine-lysine (residues 446-447) of the Fc region may be removed, for example, during purification of the antibody or by recombinant engineering of the nucleic acid encoding the antibody. Accordingly, a composition comprising an antibody having an Fc region according to this invention can comprise an antibody with G446-K447, with G446 and without K447, with all G446-K447 removed, or a mixture of three types of antibodies described above .
- [0093] "Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.
- [0094] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.
- [0095] A "functional Fc region" possesses an "effector function" of a native sequence Fc region. Exemplary "effector functions" include C1q binding; CDC; Fc receptor binding; ADCC; phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g., an antibody variable domain) and can be assessed using various assays as disclosed, for example, in definitions herein.
- [0096] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and

"transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0097] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0098] A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., supra.

[0099] A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

[0100] The term "hypervariable region" or "HVR" as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence ("complementarity determining regions" or "CDRs") and/or form structurally defined loops ("hypervariable loops") and/or contain the antigen-contacting residues ("antigen contacts"). Generally, antibodies comprise six HVRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include: (a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987)); (b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., Sequences of Proteins of Im-

munological Interest, 5th Ed. Public Health Service, NIH, Bethesda, MD (1991)); (c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al., J. Mol. Biol. 262: 732-745 (1996)); and (d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

- [0101] Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.
- [0102] An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.
- [0103] An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.
- [0104] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., J. Chromatogr. B 848:79-87 (2007).
- [0105] An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.
- [0106] "Isolated nucleic acid encoding an anti-myostatin antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.
- [0107] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the

character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

- [0108] A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.
- [0109] "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (kappa) and lambda (lambda), based on the amino acid sequence of its constant domain.
- [0110] A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof.
- [0111] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.
- [0112] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within

the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, Megalign (DNASTAR) software, or GENETYX (registered trademark) (Genetyx Co., Ltd.). Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the US Copyright Office, Washington D.C., 20559, where it is registered under US Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

- [0113] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows: 100 times the fraction X/Y; where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.
- [0114] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.
- [0115] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.
- [0116] The term "myostatin", as used herein, may refer to any native myostatin from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats). Unless otherwise indicated, the term "myostatin" refers to a human myostatin protein having the amino acid sequence shown in SEQ ID NO: 1 and

containing the terminal propeptide domain of human myostatin as shown in SEQ ID NO: 75 or 78. The term encompasses "full-length", unprocessed myostatin as well as any form of myostatin that results from processing in the cell. The term also encompasses naturally occurring variants of myostatin, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human myostatin (promyostatin) is shown in SEQ ID NO: 1. The amino acid sequence of an exemplary C-terminal growth factor domain of human myostatin is shown in SEQ ID NO: 2. The amino acid sequence of an exemplary N-terminal propeptide domain of human myostatin is shown in SEQ ID NO: 75 or 78. Active mature myostatin is a disulfide-bonded homodimer consisting of two C-terminal growth factor domains. Inactive latent myostatin is a non-covalently-associated complex of two propeptides and the mature myostatin. As disclosed herein, the antibodies of the invention bind inactive latent myostatin, but do not bind the mature active myostatin homodimer. In some embodiments, the antibodies of the invention bind an epitope within a fragment consisting of amino acids 21-100 of myostatin propeptide (SEQ ID NO:78), but do not bind the mature active myostatin homodimer. The amino acid sequence of an exemplary cynomolgus monkey and murine myostatin (promyostatin) are shown in SEQ ID NO: 3 and 5, respectively. The amino acid sequence of an exemplary C-terminal growth factor domain of cynomolgus monkey and murine myostatin are shown in SEQ ID NO: 4 and 6, respectively. The amino acid sequence of an exemplary N-terminal propeptide domain of cynomolgus monkey and murine myostatin are shown in SEQ ID NO: 76 or 79, and 77 or 80, respectively. GDF-11 (BMP-11) is a closely related molecule to myostatin, both of which are members of TGF-beta superfamily. Similarly to myostatin, GDF11 is synthesized as a precursor polypeptide first, and then cleaved into an N-terminal prodomain and C-terminal mature GDF11. The amino acid sequence of human GDF11 (precursor) is shown in SEQ ID NO: 81. The amino acid sequence of C-terminal mature human GDF11 is shown in SEQ ID NO: 82. The amino acid sequence of an N-terminal prodomain of human GDF11 is shown in SEQ ID NO: 83 or 84. Amino acid sequences of SEQ ID NOs: 1, 3, 5, 78, 79, 80, 81, and 84 include a signal sequence. Amino acids 1-24 of them correspond to it and they are removed during processing in the cell.

[0117] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or

palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

[0118] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al., *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

[0119] A "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification (alteration), preferably one or more amino acid substitution(s). Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g., from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% homology therewith, more preferably at least about 95% homology therewith.

[0120] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors".

II. COMPOSITIONS AND METHODS

[0121] In one aspect, the invention is based, in part, on anti-myostatin antibodies and uses thereof. In certain embodiments, antibodies that bind to myostatin are provided. Antibodies of the invention are useful, e.g., for the diagnosis or treatment of a muscle wasting disease.

[0122] In another aspect, the invention is based, in part, on polypeptides comprising variant Fc regions and uses thereof. In one embodiment, polypeptides comprising variant Fc

regions with enhanced Fc gamma RIIB-binding activity are provided. In another embodiment, polypeptides comprising variant Fc regions with increased pI are provided. In particular embodiments, the polypeptides of the invention are antibodies. Polypeptides comprising a variant Fc region of the invention are useful, e.g., for the diagnosis or treatment of diseases.

A. Exemplary Anti-myostatin Antibodies and Polypeptides comprising variant Fc regions

[0123] In one aspect, the invention provides isolated antibodies that bind to myostatin. In certain embodiments, an anti-myostatin antibody of the present invention binds to latent myostatin. In further embodiments, an anti-myostatin antibody of the present invention binds to the myostatin propeptide (human: SEQ ID NO: 75 or 78; cynomolgus monkey: SEQ ID NO: 76 or 79; mouse: SEQ ID NO: 77 or 80). In further embodiments, the antibody binds to an epitope within a fragment consisting of amino acids 21-100 of myostatin propeptide (SEQ ID NO: 78). Propeptide is contained in latent myostatin as one of the components, as described above. In certain embodiments, an anti-myostatin antibody of the present invention inhibits activation of myostatin. In certain embodiments, the anti-myostatin antibody blocks the release of mature myostatin from latent myostatin. It has been reported that mature myostatin is released via proteolytic and non-proteolytic processes from latent myostatin. The anti-myostatin antibody of the present invention may block the proteolytic and/or non-proteolytic release of mature myostatin from latent myostatin. In certain embodiments, the anti-myostatin antibody blocks the proteolytic cleavage of latent myostatin. In certain embodiments, the anti-myostatin antibody blocks access of a protease to latent myostatin (especially, to the proteolytic cleavage site (Arg98-Asp99) of latent myostatin). In further embodiments, the protease may be a BMP1/TLD family metalloprotease such as BMP1, TED, tolloid-like protein-1 (TLL-1), or tolloid-like protein-2 (TLL-2). In another embodiment, the anti-myostatin antibody blocks the non-proteolytic release of mature myostatin from latent myostatin. The non-proteolytic release as used herein means a spontaneous release of mature myostatin from latent myostatin, which is not accompanied by the proteolytic cleavage of latent myostatin. The non-proteolytic release includes, for example, a release of mature myostatin by incubating latent myostatin e.g., at 37 degrees C in the absence of a protease that cleaves the latent myostatin. In certain embodiments, the anti-myostatin antibody of the present invention does not bind to mature myostatin. In some embodiments, the anti-myostatin antibody binds to the same epitope as an antibody described in Table 2a. In some embodiments, the anti-myostatin antibody competes for binding latent myostatin with an antibody described in Table 2a. In additional embodiments, an anti-myostatin antibody competes for binding latent myostatin with an antibody comprising a VH and

VL pair described in Table 2a. In some embodiments, the anti-myostatin antibody competes for binding a fragment consisting of amino acids 21-100 of myostatin propeptide (SEQ ID NO: 78) with an antibody described in Table 2a. In further embodiments, the anti-myostatin antibody binds to the same epitope as an antibody described in Table 11a or 13. In some embodiments, the anti-myostatin antibody competes for binding latent myostatin with an antibody described in Table 11a or 13. In some embodiments, the anti-myostatin antibody competes for binding a fragment consisting of amino acids 21-100 of myostatin propeptide (SEQ ID NO: 78) with an antibody described in Table 11a or 13.

In some embodiments, an anti-myostatin antibody of the present invention binds to latent myostatin and inhibits the activation of myostatin. In further embodiments, the antibody: (a) blocks the release of mature myostatin from latent myostatin; (b) blocks the proteolytic release of mature myostatin; (c) blocks the spontaneous release of mature myostatin; or (d) does not bind to mature myostatin; or binds to an epitope within a fragment consisting of amino acids 21-100 of myostatin propeptide (SEQ ID NO: 78). In further embodiments, the antibody competes for binding latent myostatin with, or binds the same epitope as, an antibody comprising a VH and VL pair described in Tables 2a, 11a, or 13. In further embodiments, the antibody binds to latent myostatin with higher affinity at neutral pH (e.g., pH7.4) than at acidic pH (e.g., pH5.8). In further embodiments, the antibody is (a) a monoclonal antibody, (b) a human, humanized, or chimeric antibody; (c) a full length IgG antibody or (d) an antibody fragment that binds to latent myostatin or myostatin propeptide.

[0124] In another embodiment, an anti-myostatin antibody of the present invention does not bind to GDF11. In certain embodiments, an anti-myostatin antibody of the present invention does not inhibit activation of GDF11. In certain embodiments, the anti-myostatin antibody does not block the release of mature GDF11 from latent GDF11. The anti-myostatin antibody of the present invention does not block either proteolytic or non-proteolytic release of mature GDF11 from latent GDF11. In certain embodiments, the anti-myostatin antibody does not block the proteolytic cleavage of latent GDF11. In certain embodiments, the anti-myostatin antibody does not block access of a protease to latent GDF11 (especially, to the proteolytic cleavage site of latent GDF11). In further embodiments, the protease may be a BMP1/TLD family metalloprotease such as BMP1, TED, tolloid-like protein-1 (TLL-1), or tolloid-like protein-2 (TLL-2). The non-proteolytic release as used herein means a spontaneous release of mature GDF11 from latent GDF11, which is not accompanied by the proteolytic cleavage of latent GDF11. The non-proteolytic release includes, for example, a release of mature GDF11 by incubating latent GDF11 e.g., at 37 degrees C in the absence of a protease that cleaves the latent GDF11. Most of the anti-myostatin an-

antibodies known to date were not specific for myostatin. These antibodies have high affinity for other members of the TGF-beta superfamily, such as GDF11 and neutralize the biological activities of them. GDF11 plays an important role during embryogenesis, and is responsible for homeotic transformation of the axial skeleton. Homozygous GDF11 knockout mice are perinatal lethal, mice with one wild type copy of the GDF11 gene are viable but have skeletal defects. Since GDF11 plays an important role during embryogenesis, an antagonist that inhibits GDF11 poses theoretical safety risks that could present either as toxicity in treated patients or as reproductive toxicity in, e.g., women of childbearing potential. Thus, there is a need for specific inhibition of myostatin activity in treatments of myostatin-associated disorders for which it is desirable to increase muscle mass, size, strength, etc., particularly in women with childbearing potential.

[0125] In another aspect, the invention provides anti-myostatin antibodies that exhibit pH-dependent binding characteristics. As used herein, the expression "pH-dependent binding" means that the antibody exhibits "reduced binding to myostatin at acidic pH as compared to its binding at neutral pH" (for purposes of the present disclosure, both expressions may be used interchangeably). For example, antibodies "with pH-dependent binding characteristics" include antibodies that bind to myostatin with higher affinity at neutral pH than at acidic pH. In certain embodiments, the antibodies of the present invention bind to myostatin with at least 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 400, 1000, 10000, or more times higher affinity at neutral pH than at acidic pH. In some embodiments, the antibodies bind to myostatin (e.g., latent myostatin or propeptide myostatin) with higher affinity at pH7.4 than at pH5.8. In further embodiments, the antibodies bind to myostatin with at least 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 400, 1000, 10000, or more times higher affinity at pH7.4 than at pH5.8.

[0126] When an antigen is a soluble protein, the binding of an antibody to the antigen can result in an extended half-life of the antigen in plasma (i.e., reduced clearance of the antigen from plasma), since the antibody can have a longer half-life in plasma than the antigen itself and may serve as a carrier for the antigen. This is due to the recycling of the antigen-antibody complex by FcRn through the endosomal pathway in cell (Roopenian, Nat. Rev. Immunol. 7(9): 715-725 (2007)). However, an antibody with pH-dependent binding characteristics, which binds to its antigen in neutral extracellular environment while releasing the antigen into acidic endosomal compartments following its entry into cells, is expected to have superior properties in terms of antigen neutralization and clearance relative to its counterpart that binds in a pH-independent manner (Igawa et al., Nature Biotechnol. 28(11):1203-1207 (2010); Devanaboyina et

al., mAbs 5(6):851-859 (2013); WO 2009/125825).

[0127] The "affinity" of an antibody for myostatin, for purposes of the present disclosure, is expressed in terms of the KD of the antibody. The KD of an antibody refers to the equilibrium dissociation constant of an antibody-antigen interaction. The greater the KD value is for an antibody binding to its antigen, the weaker its binding affinity is for that particular antigen. Accordingly, as used herein, the expression "higher affinity at neutral pH than at acidic pH" (or the equivalent expression "pH-dependent binding") means that the KD of the antibody binding to myostatin at acidic pH is greater than the KD of the antibody binding to myostatin at neutral pH. For example, in the context of the present invention, an antibody is considered to bind to myostatin with higher affinity at neutral pH than at acidic pH if the KD of the antibody binding to myostatin at acidic pH is at least 2 times greater than the KD of the antibody binding to myostatin at neutral pH. Thus, the present invention includes antibodies that bind to myostatin at acidic pH with a KD that is at least 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 400, 1000, 10000, or more times greater than the KD of the antibody binding to myostatin at neutral pH. In another embodiment, the KD value of the antibody at neutral pH can be 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or less. In another embodiment, the KD value of the antibody at acidic pH can be 10^{-9} M, 10^{-8} M, 10^{-7} M, 10^{-6} M, or greater.

[0128] In further embodiments an antibody is considered to bind to myostatin (e.g., latent myostatin or propeptide myostatin) with a higher affinity at neutral pH than at acidic pH if the KD of the antibody binding to myostatin at pH5.8 is at least 2 times greater than the KD of the antibody binding to myostatin at pH7.4. In some embodiments the provided antibodies bind to myostatin at pH5.8 with a KD that is at least 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 400, 1000, 10000, or more times greater than the KD of the antibody binding to myostatin at pH7.4. In another embodiment, the KD value of the antibody at pH7.4 can be 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or less. In another embodiment, the KD value of the antibody at pH5.8 can be 10^{-9} M, 10^{-8} M, 10^{-7} M, 10^{-6} M, or greater.

[0129] The binding properties of an antibody for a particular antigen may also be expressed in terms of the kd of the antibody. The kd of an antibody refers to the dissociation rate constant of the antibody with respect to a particular antigen and is expressed in terms of reciprocal seconds (i.e., sec^{-1}). An increase in kd value signifies weaker binding of an antibody to its antigen. The present invention therefore includes antibodies that bind to myostatin with a higher kd value at acidic pH than at neutral pH. The present invention includes antibodies that bind to myostatin at acidic pH with a kd that is at least 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 400, 1000, 10000, or more times greater than the kd of the antibody binding to

myostatin at neutral pH. In another embodiment, the k_d value of the antibody at neutral pH can be 10^{-2} 1/s, 10^{-3} 1/s, 10^{-4} 1/s, 10^{-5} 1/s, 10^{-6} 1/s, or less. In another embodiment, the k_d value of the antibody at acidic pH can be 10^{-3} 1/s, 10^{-2} 1/s, 10^{-1} 1/s, or greater. The invention also includes antibodies that bind to myostatin (e.g., latent myostatin or propeptide myostatin) with a higher k_d value at pH5.8 than at pH7.4. The invention includes antibodies that bind to myostatin at pH5.8 with a k_d that is at least 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 400, 1000, 10000, or more times greater than the k_d of the antibody binding to myostatin at pH7.4. In another embodiment, the k_d value of the antibody at pH7.4 can be 10^{-2} 1/s, 10^{-3} 1/s, 10^{-4} 1/s, 10^{-5} 1/s, 10^{-6} 1/s, or less. In another embodiment, the k_d value of the antibody at pH5.8 can be 10^{-3} 1/s, 10^{-2} 1/s, 10^{-1} 1/s, or greater.

[0130] In certain instances, a "reduced binding to myostatin at acidic pH as compared to its binding at neutral pH" is expressed in terms of the ratio of the K_D value of the antibody binding to myostatin at acidic pH to the K_D value of the antibody binding to myostatin at neutral pH (or vice versa). For example, an antibody may be regarded as exhibiting "reduced binding to myostatin at acidic pH as compared to its binding at neutral pH", for purposes of the present invention, if the antibody exhibits an acidic/neutral K_D ratio of 2 or greater. In certain embodiments, the pH5.8/pH7.4 K_D ratio for an anti-myostatin antibody of the present invention is 2 or greater. In certain exemplary embodiments, the acidic/neutral K_D ratio for an antibody of the present invention can be 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 400, 1000, 10000, or greater. In another embodiment, the K_D value of the antibody at neutral pH can be 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or less. In another embodiment, the K_D value of the antibody at acidic pH can be 10^{-9} M, 10^{-8} M, 10^{-7} M, 10^{-6} M, or greater. In further instances an antibody may be regarded as exhibiting "reduced binding to myostatin (e.g., latent myostatin) at acidic pH as compared to its binding at neutral pH", if the antibody exhibits an pH5.8/pH7.4 K_D ratio of 2 or greater. In certain exemplary embodiments, the pH5.8/pH7.4 K_D ratio for the antibody can be 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 400, 1000, 10000, or greater. In another embodiment, the K_D value of the antibody at pH7.4 can be 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or less. In another embodiment, the K_D value of the antibody at pH5.8 can be 10^{-9} M, 10^{-8} M, 10^{-7} M, 10^{-6} M, or greater.

[0131] In certain instances, a "reduced binding to myostatin at acidic pH as compared to its binding at neutral pH" is expressed in terms of the ratio of the k_d value of the antibody binding to myostatin at acidic pH to the k_d value of the antibody binding to myostatin at neutral pH (or vice versa). For example, an antibody may be regarded as exhibiting "reduced binding to myostatin at acidic pH as compared to its binding at neutral pH",

for purposes of the present invention, if the antibody exhibits an acidic/neutral k_d ratio of 2 or greater. In certain exemplary embodiments, the pH5.8/pH7.4 k_d ratio for an antibody of the present invention is 2 or greater. In certain exemplary embodiments, the acidic/neutral k_d ratio for an antibody of the present invention can be 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 400, 1000, 10000, or greater. In another embodiment, the k_d value of the antibody at neutral pH can be 10^{-2} 1/s, 10^{-3} 1/s, 10^{-4} 1/s, 10^{-5} 1/s, 10^{-6} 1/s, or less. In another embodiment, the k_d value of the antibody at acidic pH can be 10^{-3} 1/s, 10^{-2} 1/s, 10^{-1} 1/s, or greater. In certain exemplary embodiments, the pH5.8/pH7.4 k_d ratio for an antibody of the present invention can be 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 400, 1000, 10000, or greater. In another embodiment, the k_d value of the antibody at pH7.4 can be 10^{-2} 1/s, 10^{-3} 1/s, 10^{-4} 1/s, 10^{-5} 1/s, 10^{-6} 1/s, or less. In another embodiment, the k_d value of the antibody at pH5.8 can be 10^{-3} 1/s, 10^{-2} 1/s, 10^{-1} 1/s, or greater.

- [0132] As used herein, the expression "acidic pH" means a pH of 4.0 to 6.5. The expression "acidic pH" includes pH values of any one of 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, and 6.5. In particular aspects, the "acidic pH" is 5.8.
- [0133] As used herein, the expression "neutral pH" means a pH of 6.7 to about 10.0. The expression "neutral pH" includes pH values of any one of 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, and 10.0. In particular aspects, the "neutral pH" is 7.4.
- [0134] K_D values, and k_d values, as expressed herein, may be determined using a surface plasmon resonance-based biosensor to characterize antibody-antigen interactions. (See, e.g., Example 7, herein.) K_D values, and k_d values can be determined at 25 degrees C or 37 degrees C.
- [0135] In certain embodiments, an anti-myostatin antibody of the present invention binds to myostatin from more than one species. In further embodiments, the anti-myostatin antibody binds to myostatin from a human and non-human animal. In further embodiments, the anti-myostatin antibody binds to myostatin from human, mouse, and monkey (e.g., cynomolgus, rhesus macaque, marmoset, chimpanzee, or baboon).
- [0136] In certain embodiments, an anti-myostatin antibody of the present invention binds to latent myostatin from more than one species. In further embodiments, the anti-myostatin antibody binds to latent myostatin from a human and non-human animal. In further embodiments, the anti-myostatin antibody binds to latent myostatin from human, mouse, and monkey.
- [0137] In certain embodiments, an anti-myostatin antibody of the present invention binds to propeptide myostatin from more than one species. In further embodiments, the anti-

myostatin antibody binds to propeptide myostatin from a human and non-human animal. In further embodiments, the anti-myostatin antibody binds to propeptide myostatin from human, mouse, and monkey.

- [0138] In a further aspect, the invention provides an anti-myostatin antibody that forms an immune complex (i.e. antigen-antibody complex) with myostatin. In certain embodiments, two or more anti-myostatin antibodies bind to two or more myostatin molecules to form an immune complex. This is possible because myostatin exists as a homodimer containing two myostatin molecules while an antibody has two antigen-binding sites. The anti-myostatin antibody may bind to the same epitope on a myostatin molecule or may bind to different epitopes on a myostatin molecule, much like a bispecific antibody. Generally speaking, when two or more antibodies form an immune complex with two or more antigens, the resulting immune complex can strongly bind to Fc receptors existing on cell surfaces due to avidity effects through the Fc regions of the antibodies in the complex and can then be taken up into the cell with high efficiency. Thus, the above-mentioned anti-myostatin antibody capable of forming an immune complex containing two or more anti-myostatin antibodies and two or more myostatin molecules can lead to a rapid clearance of myostatin from plasma in a living body, via the strong binding to Fc receptors due to avidity effects.
- [0139] Furthermore, an antibody with pH-dependent binding characteristics is thought to have superior properties in terms of antigen neutralization and clearance relative to its counterpart that binds in a pH-independent manner (Igawa et al., Nature Biotech. 28(11):1203-1207 (2010); Devanaboyina et al. mAbs 5(6):851-859 (2013); WO 2009/125825). Therefore, an antibody having both properties above, that is, an antibody which has pH-dependent binding characteristics and which forms an immune complex containing two or more antibodies with two or more antigens, is expected to have even more superior properties for highly accelerated elimination of antigens from plasma (WO 2013/081143).
- [0140] In another aspect, the invention provides an anti-myostatin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 55-57, 114-115, 126; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58-60, 116-120, 127; (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64, 121, 128; (d) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 65-69, 122-124, 129; (e) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 70-72, 125, 130; and (f) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74, 131.
- [0141] In another aspect, the invention provides an anti-myostatin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) a HVR-H1 comprising

the amino acid sequence of any one of SEQ ID NOs: 55-57; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58-60; (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64; (d) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 65-69; (e) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 70-72; and (f) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74.

[0142] In one aspect, the invention provides an anti-myostatin antibody comprising at least one, two, three, four, five, or six hypervariable regions (HVRs) selected from (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 114-115; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 116-120; (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 121; (d) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 122-124; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 125; and (f) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74.

[0143] In another aspect, the invention provides an anti-myostatin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114; (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58; (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63; (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 122; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71; and (f) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74. In another aspect, the invention provides an anti-myostatin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114; (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58; (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63; (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 123; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71; and (f) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74.

[0144] In another aspect, the invention provides an anti-myostatin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 126; (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 127; (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 128; (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 129; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 130; and (f) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 131.

[0145] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 55-57, 114-115, 126; (b) a HVR-H2

comprising the amino acid sequence of any one of SEQ ID NOs: 58-60, 116-120, 127; (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64, 121, 128. In one embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64, 121, 128. In another embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64, 121, 128 and HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74, 131. In a further embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64, 121, 128, HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74, 131, and HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58-60, 116-120, 127. In a further embodiment, the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 55-57, 114-115, 126; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58-60, 116-120, 127; and (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64, 121, 128.

[0146] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 55-57; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58-60; and (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64. In one embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64. In another embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64 and HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74. In a further embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64, HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74, and HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58-60. In a further embodiment, the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 55-57; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58-60; and (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64.

[0147] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 114-115; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 116-120; and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 121. In one embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 121. In another embodiment, the antibody comprises a HVR-H3 comprising the amino

acid sequence of SEQ ID NO: 121 and HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74. In a further embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 121, HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74, and HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 116-120. In a further embodiment, the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 114-115; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 116-120; and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 121.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114; (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58; and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63. In one embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63. In another embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63 and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74. In a further embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58. In a further embodiment, the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114; (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58; and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63.

[0148] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 126; (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 127; and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 128. In one embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 128. In another embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 128 and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 131. In a further embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 128, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 131, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 127. In a further embodiment, the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 126; (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 127; and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 128.

[0149] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 65-69, 122-124, 129; (b) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 70-72, 125, 130; and (c) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74, 131. In one embodiment, the antibody comprises (a) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 65-69, 122-124, 129; (b) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 70-72, 125, 130; and (c) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74, 131.

[0150] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 65-69; (b) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 70-72; and (c) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74. In one embodiment, the antibody comprises (a) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 65-69; (b) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 70-72; and (c) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74.

[0151] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 122-124; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 125; and (c) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74. In one embodiment, the antibody comprises (a) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 122-124; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 125; and (c) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 122; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71; and (c) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74. In one embodiment, the antibody comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 122; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71; and (c) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74. In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 123; (b) a

HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71; and (c) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74. In one embodiment, the antibody comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 123; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71; and (c) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74.

[0152] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 129; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 130; and (c) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 131. In one embodiment, the antibody comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 129; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 130; and (c) a HVR-L3 comprising the amino acid sequence of SEQ ID NO 131.

[0153] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 55-57, 114, 115, 126 (ii) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58-60, 116-120, 127, and (iii) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64, 121, 128; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 65-69, 122-124, 129, (ii) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 70-72, 125, 130 and (iii) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74, 131.

[0154] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 55-57, (ii) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58-60, and (iii) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 65-69, (ii) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 70-72, and (iii) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74.

[0155] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 114-115, (ii) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs:

116-120, and (iii) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 121; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 122-124, (ii) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 125, and (iii) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114, (ii) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58, and (iii) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 122, (ii) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71, and (iii) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74. In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114, (ii) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58, and (iii) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 123, (ii) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71, and (iii) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74.

[0156] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 126, (ii) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 127, and (iii) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 128; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 129, (ii) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 130, and (iii) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 131.

[0157] In another aspect, the invention provides an antibody comprising (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 55-57, 114-115, 126; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58-60, 116-120, 127; (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64, 121, 128; (d) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 65-69, 122-124, 129; (e) a HVR-L2 comprising the amino acid

sequence of any one of SEQ ID NOs: 70-72, 125, 130; and (f) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74, 131.

[0158] In another aspect, the invention provides an antibody comprising (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 55-57, 114-115; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58-60, 116-120; (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64, 121; (d) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 65-69, 122-124; (e) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 70-72, 125; and (f) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74.

[0159] In another aspect, the invention provides an antibody comprising (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 114-115; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 116-120; (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 121; (d) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 122-124; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 125; and (f) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74.

In another aspect, the invention provides an antibody comprising (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114; (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58; (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63; (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 122; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71; and (f) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74. In another aspect, the invention provides an antibody comprising (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114; (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58; (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63; (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 123; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71; and (f) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74.

[0160] In another aspect, the invention provides an antibody comprising (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 126; (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 127; (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 128; (d) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 129; (e) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 130; and (f) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 131.

[0161] In certain embodiments, any one or more amino acids of an anti-myostatin antibody as provided above are substituted at the following HVR positions: (a) in HVR-H1 (SEQ ID NO: 55), at positions 1, and 2; (b) in HVR-H2 (SEQ ID NO: 58), at positions

4, 7, 8, 10, 11, 12, and 16; (c) in HVR-H3 (SEQ ID NO: 61), at positions 5, 7, and 11; (d) in HVR-L1 (SEQ ID NO: 65), at positions 1, 2, 5, 7, 8, and 9; (e) in HVR-L2 (SEQ ID NO: 70), at positions 3, and 7; and (f) in HVR-L3 (SEQ ID NO: 73), at position 8.

[0162] In certain embodiments, the one or more amino acid substitutions of an anti-myostatin antibody are conservative substitutions, as provided herein. In certain embodiments, any one or more of the following substitutions may be made in any combination: (a) in HVR-H1 (SEQ ID NO: 55), S1H; Y2T, D, or E; (b) in HVR-H2 (SEQ ID NO: 58), Y4H; S7K; T8M or K; Y10K; A11M or E; S12E; G16K; (c) in HVR-H3 (SEQ ID NO: 61), Y5H; T7H; L11K; (d) in HVR-L1 (SEQ ID NO: 65), Q1T; S2T; S5E; Y7F; D8H; N9D or A or E; (e) in HVR-L2 (SEQ ID NO: 70), S3E; S7Y, F or W; and (f) in HVR-L3 (SEQ ID NO: 73), L8R.

[0163] All possible combinations of the above substitutions are encompassed by the consensus sequences of SEQ ID NOs: 126, 127, 128, 129, 130, and 131 for HVR-H1, HVR-H2, HVR-H3, HVR-L1, HVR-L2, and HVR-L3, respectively.

[0164] In any of the above embodiments, an anti-myostatin antibody can be humanized. In one embodiment, an anti-myostatin antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g., a human immunoglobulin framework or a human consensus framework. In another embodiment, an anti-myostatin antibody comprises HVRs as in any of the above embodiments, and further comprises a VH or VL comprising an FR sequence. In a further embodiment, the anti-myostatin antibody comprises the following heavy chain and/or light chain variable domain FR sequences: For the heavy chain variable domain, the FR1 comprises the amino acid sequence of any one of SEQ ID NOs: 132-134, FR2 comprises the amino acid sequence of any one of SEQ ID NOs: 135-136, FR3 comprises the amino acid sequence of SEQ ID NO: 137, FR4 comprises the amino acid sequence of SEQ ID NO: 138. For the light chain variable domain, FR1 comprises the amino acid sequence of SEQ ID NO: 139, FR2 comprises the amino acid sequence of any one of SEQ ID NOs: 140-141, FR3 comprises the amino acid sequence of any one of SEQ ID NOs: 142-143, FR4 comprises the amino acid sequence of SEQ ID NO: 144.

[0165] In one aspect, the invention provides an anti-myostatin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 157-162; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 163-168; (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 169-174; (d) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 175-180; (e) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 181-186; and (f) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs:

187-192.

- [0166] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 157-162; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 163-168; and (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 169-174. In one embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 169-174. In another embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 169-174 and HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 187-192. In a further embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 169-174, HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 187-192, and a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 163-168. In a further embodiment, the antibody comprises (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 157-162; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 163-168; and (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 169-174.
- [0167] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 175-180; (b) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 181-186; and (c) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 187-192. In one embodiment, the antibody comprises (a) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 175-180; (b) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 181-186; and (c) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 187-192.
- [0168] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 157-162, (ii) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 163-168, and (iii) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 169-174; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 175-180, (ii) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 181-186, and (iii) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 187-192.
- [0169] In another aspect, the invention provides an antibody comprising (a) a HVR-H1

comprising the amino acid sequence of any one of SEQ ID NOs: 157-162; (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 163-168; (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 169-174; (d) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 175-180; (e) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 181-186; and (f) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 187-192.

[0170] In another aspect, an anti-myostatin antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-myostatin antibody comprising that sequence retains the ability to bind to myostatin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-myostatin antibody comprises the VH sequence in any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 55-57, 114-115, 126, (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58-60, 116-120, 127, and (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64, 121, 128. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0171] In another aspect, an anti-myostatin antibody comprises a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 13, 16-30, 32, 33, and 34. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-myostatin antibody comprising that sequence retains the ability to bind to myostatin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in any one of SEQ ID NOs: 13, 16-30, 32, 33 and 34. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-myostatin antibody comprises the VH sequence

in any one of SEQ ID NOs: 13, 16-30, 32, 33, and 34, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 55-57, (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58-60, and (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61-64. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0172] In another aspect, an anti-myostatin antibody comprises a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 86-95. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-myostatin antibody comprising that sequence retains the ability to bind to myostatin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in any one of SEQ ID NOs: 86-95. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-myostatin antibody comprises the VH sequence in any one of SEQ ID NOs: 86-95, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 57, 114-115, 126, (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58, 116-120, 127, and (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 63, 121, 128. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

In another aspect, an anti-myostatin antibody comprises a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 86. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-myostatin antibody comprising that sequence retains the ability to bind to myostatin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 86. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-myostatin antibody comprises the VH sequence in SEQ ID NO: 86, including post-translational modifications of that

sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation. In another aspect, an anti-myostatin antibody comprises a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 92. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-myostatin antibody comprising that sequence retains the ability to bind to myostatin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 92. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-myostatin antibody comprises the VH sequence in SEQ ID NO: 92, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114, (b) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0173] In another aspect, an anti-myostatin antibody is provided, wherein the antibody comprises a VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 15, 31, 35-38, and 96-99. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-myostatin antibody comprising that sequence retains the ability to bind to myostatin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in any one of SEQ ID NOs: 15, 31, 35-38, and 96-99. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-myostatin antibody comprises the VL sequence in any one of SEQ ID NOs: 15, 31, 35-38, and 96-99, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 65-69, 122-124, 129;

(b) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 70-72, 125, 130; and (c) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74, 131. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0174] In another aspect, an anti-myostatin antibody is provided, wherein the antibody comprises a VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 15, 31, 35, 36, 37, and 38. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-myostatin antibody comprising that sequence retains the ability to bind to myostatin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in any one of SEQ ID NOs: 15, 31, 35, 36, 37, and 38. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-myostatin antibody comprises the VL sequence in any one of SEQ ID NOs: 15, 31, 35, 36, 37, and 38, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 65-69; (b) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 70-72; and (c) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73-74. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0175] In another aspect, an anti-myostatin antibody is provided, wherein the antibody comprises a VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 96-99. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-myostatin antibody comprising that sequence retains the ability to bind to myostatin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in any one of SEQ ID NOs: 96-99. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs. Optionally, the anti-myostatin antibody comprises the VL sequence in any one of SEQ ID NOs: 96-99, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) a

HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 122-124, 129; (b) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 71, 125, 130; and (c) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74, 131. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

In another aspect, an anti-myostatin antibody is provided, wherein the antibody comprises a VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 96. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-myostatin antibody comprising that sequence retains the ability to bind to myostatin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 96. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs. Optionally, the anti-myostatin antibody comprises the VL sequence in SEQ ID NO: 96, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 122; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71; and (c) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation. In another aspect, an anti-myostatin antibody is provided, wherein the antibody comprises a VL having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 97. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-myostatin antibody comprising that sequence retains the ability to bind to myostatin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 97. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs. Optionally, the anti-myostatin antibody comprises the VL sequence in SEQ ID NO: 97, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 123; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71; and (c) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74. Post-

translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0176] In another aspect, an anti-myostatin antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in any one of SEQ ID NOs: 13, 16-30, 32-34, and 86-95 and any one of SEQ ID NOs: 15, 31, 35-38, and 96-99, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in any one of SEQ ID NOs: 13, 16-30, and 32-34 and any one of SEQ ID NOs: 15, 31, and 35-38, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in any one of SEQ ID NOs: 86-95 and any one of SEQ ID NOs: 96-99, respectively, including post-translational modifications of those sequences. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0177] In another aspect, an anti-myostatin antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 86 and SEQ ID NO: 96, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 92 and SEQ ID NO: 97, respectively, including post-translational modifications of those sequences. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0178] In another aspect, an anti-myostatin antibody comprises a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 12, 145-150. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-myostatin antibody comprising that sequence retains the ability to bind to myostatin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in any one of SEQ ID NOs: 12, 145-150. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-myostatin antibody comprises the VH sequence in any one of SEQ ID NOs:

12, 145-150, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) a HVR-H1 comprising the amino acid sequence of any one of SEQ ID NOs: 55, 157-162, (b) a HVR-H2 comprising the amino acid sequence of any one of SEQ ID NOs: 58, 163-168, and (c) a HVR-H3 comprising the amino acid sequence of any one of SEQ ID NOs: 61, 169-174. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0179] In another aspect, an anti-myostatin antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 14, 151-156. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-myostatin antibody comprising that sequence retains the ability to bind to myostatin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in any one of SEQ ID NOs: 14, 151-156. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-myostatin antibody comprises the VL sequence in any one of SEQ ID NOs: 14, 151-156, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) a HVR-L1 comprising the amino acid sequence of any one of SEQ ID NOs: 65, 175-180; (b) a HVR-L2 comprising the amino acid sequence of any one of SEQ ID NOs: 70, 181-186; and (c) a HVR-L3 comprising the amino acid sequence of any one of SEQ ID NOs: 73, 187-192. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0180] In another aspect, an anti-myostatin antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in any one of SEQ ID NOs: 12, 145-150 and any one of SEQ ID NOs: 14, 151-156, respectively, including post-translational modifications of those sequences. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0181] In certain embodiments, an anti-myostatin antibody of the present invention comprises a VH as in any of the embodiments provided above and a heavy chain

constant region comprising the amino acid sequence of any one of SEQ ID NOs: 7, 9, 11, 193, 195-198, 227, 228, 229-381. In certain embodiments, an anti-myostatin antibody of the present invention comprises a VL as in any of the embodiments provided above, and a light chain constant region comprising the amino acid sequence of any one of SEQ ID NOs: 8 and 10.

- [0182] In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-myostatin antibody provided herein. In a further aspect, the invention provides an antibody that binds to the same epitope as an antibody described in Table 2a. In a further aspect, the invention provides an antibody that binds to the same epitope as an antibody described in Tables 11a or 13. In certain embodiments, an antibody is provided that binds to an epitope within a fragment of myostatin propeptide consisting of amino acids 21-100 of SEQ ID NO: 78. Alternatively, the antibody binds a myostatin propeptide fragment consisting of amino acids 21-80, 41-100, 21-60, 41-80, 61-100, 21-40, 41-60, 61-80, or 81-100, of SEQ ID NO: 78.
- [0183] In a further aspect of the invention, an anti-myostatin antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-myostatin antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')₂ fragment. In another embodiment, the antibody is a full length IgG antibody, e.g., an intact IgG1 or IgG4 antibody or other antibody class or isotype as defined herein.
- [0184] In a further aspect, an anti-myostatin antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below.

1. Antibody Affinity

- [0185] In certain embodiments, an antibody provided herein has a dissociation constant (K_d) of 1 micro M or less, 100 nM or less, 10 nM or less, 1 nM or less, 0.1 nM or less, 0.01 nM or less, or 0.001 nM or less (e.g., 10⁻⁸ M or less, e.g., from 10⁻⁸ M to 10⁻¹³ M, e.g., from 10⁻⁹ M to 10⁻¹³ M).
- [0186] In one embodiment, K_d is measured by a radiolabeled antigen binding assay (RIA). In one embodiment, an RIA is performed with the Fab version of an antibody of interest and its antigen. For example, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (¹²⁵I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., J. Mol. Biol. 293:865-881 (1999)). To establish conditions for the assay, MICROTITER (registered trademark) multi-well plates (Thermo Scientific) are coated overnight with 5 micro g/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five

hours at room temperature (approximately 23 degrees C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [¹²⁵I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., Cancer Res. 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20 (registered trademark)) in PBS. When the plates have dried, 150 micro l/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0187] According to another embodiment, K_d is measured using a BIACORE (registered trademark) surface plasmon resonance assay. For example, an assay using a BIACORE (registered trademark)-2000 or a BIACORE (registered trademark)-3000 (BIACORE (registered trademark), Inc., Piscataway, NJ) is performed at 25 degrees C with immobilized antigen CM5 chips at ~10 response units (RU). In one embodiment, carboxymethylated dextran biosensor chips (CM5, BIACORE (registered trademark), Inc.) are activated with N-ethyl-N'- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH4.8, to 5 micro g/ml (~0.2 micro M) before injection at a flow rate of 5 micro l/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetic measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25 degrees C at a flow rate of approximately 25 micro l/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE (registered trademark) Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_d) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al., J. Mol. Biol. 293:865-881 (1999). If the on-rate exceeds 10⁶ M⁻¹ s⁻¹ by the surface plasmon resonance assay above, then the on-rate can be determined using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25 degrees C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow

equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

- [0188] In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')₂, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. Nat. Med. 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also, WO 1993/16185; and US Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')₂ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see US Patent No. 5,869,046.
- [0189] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat. Med. 9:129-134 (2003); and Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat. Med. 9:129-134 (2003).
- [0190] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., US Patent No. 6,248,516 B1).
- [0191] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g., E. coli or phage), as described herein.

3. Chimeric and Humanized Antibodies

- [0192] In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in US Patent No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.
- [0193] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or

portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

- [0194] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al., *Methods* 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).
- [0195] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al., *Proc. Natl. Acad. Sci. USA* 89:4285 (1992); and Presta et al. *J. Immunol.* 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

- [0196] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5:368-374 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).
- [0197] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.*

23:1117-1125 (2005). See also, e.g., US Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; US Patent No. 5,770,429 describing HUMAB (registered trademark) technology; US Patent No. 7,041,870 describing K-M MOUSE (registered trademark) technology, and US Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE (registered trademark) technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

[0198] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., J. Immunol. 147:86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., Proc. Natl. Acad. Sci. USA 103:3557-3562 (2006). Additional methods include those described, for example, in US Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, Xiandai Mianyixue 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, Histology and Histopathology 20(3):927-937 (2005) and Vollmers and Brandlein, Methods and Findings in Experimental and Clinical Pharmacology 27(3):185-191 (2005).

[0199] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

[0200] Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in Methods in Molecular Biology 178:1-37 (2000); O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., Nature 348:552-554; Clackson et al., Nature 352:624-628 (1991); Marks et al., J. Mol. Biol. 222:581-597 (1992); Marks and Bradbury, in Methods in Molecular Biology 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., J. Mol. Biol. 338(2):299-310 (2004); Lee et al., J. Mol. Biol. 340(5):1073-1093 (2004); Fellouse, Proc. Natl. Acad. Sci. USA 101(34):12467-12472 (2004); and Lee et al., J. Immunol. Methods 284(1-2): 119-132 (2004).

[0201] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.* 12:433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J.* 12:725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter, J. *Mol. Biol.* 227:381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Publ. Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0202] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

[0203] In certain embodiments, an antibody provided herein is a multispecific antibody, e.g., a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for myostatin and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of myostatin. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express myostatin. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[0204] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see, Milstein and Cuello, *Nature* 305:537 (1983)), WO 1993/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., US Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., *Science*, 229:81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.* 148(5):1547-1553 (1992)); using "diabody" technology for making

bispecific antibody fragments (see, e.g., Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see, e.g., Gruber et al., J. Immunol. 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al., J. Immunol. 147:60 (1991).

[0205] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g., US 2006/0025576A1).

[0206] The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to myostatin as well as another, different antigen (see, US 2008/0069820, for example).

7. Antibody Variants

[0207] In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

a. Substitution, Insertion, and Deletion Variants

[0208] In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

[Table 1]

Original Residue	Exemplary Substitutions	Preferred Substitution
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0209] Amino acids may be grouped according to common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe. Non-conservative substitutions will entail exchanging a member of one of these groups for a member of another group.

[0210] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g., a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g., binding affinity).

[0211] Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process

(see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or residues that contact antigen, with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0212] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may, for example, be outside of antigen contacting residues in the HVRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0213] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex may be analyzed to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0214] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N-

or C-terminus of the antibody to an enzyme (e.g., for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b. Glycosylation variants

[0215] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0216] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al., TIBTECH 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0217] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about +/- 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Publ.Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO 2005/053742; WO 2002/031140; Okazaki et al., J. Mol. Biol. 336:1239-1249 (2004); Yamane-Ohnuki et al., Biotech. Bioeng. 87:614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka

et al., Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki et al., Biotech. Bioeng. 87:614 (2004); Kanda et al., Biotechnol. Bioeng. 94(4):680-688 (2006); and WO 2003/085107).

[0218] Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana et al.). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c. Fc region variants

[0219] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g., a substitution) at one or more amino acid positions.

[0220] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks Fc gamma R binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc gamma RIII only, whereas monocytes express Fc gamma RI, Fc gamma RII and Fc gamma RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9:457-492 (1991). Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest is described in US Patent No. 5,500,362 (see, e.g., Hellstrom, et al. Proc. Nat'l Acad. Sci. USA 83:7059-7063 (1986)) and Hellstrom, I et al., Proc. Nat'l Acad. Sci. USA 82:1499-1502 (1985); 5,821,337 (see Bruggemann, et al., J. Exp. Med. 166:1351-1361 (1987)). Alternatively, non-radioactive assays

methods may be employed (see, for example, ACT1™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA); and CytoTox 96 (registered trademark) non-radioactive cytotoxicity assay (Promega, Madison, WI)). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al. Proc. Nat'l Acad. Sci. USA 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996); Cragg et al., Blood 101:1045-1052 (2003); and Cragg, Blood 103:2738-2743 (2004)). FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova et al., Int'l. Immunol. 18(12):1759-1769 (2006)).

- [0221] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (US Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).
- [0222] Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., US Patent No. 6,737,056; WO 2004/056312, and Shields et al., J. Biol. Chem. 9(2): 6591-6604 (2001).)
- [0223] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).
- [0224] In some embodiments, alterations are made in the Fc region that result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 1999/51642, and Idusogie et al., J. Immunol. 164:4178-4184 (2000).
- [0225] Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US

Patent No. 7,371,826). See also, Duncan, Nature 322:738-40 (1988); US Patent Nos. 5,648,260 and 5,624,821; and WO 1994/29351 concerning other examples of Fc region variants.

d. Cysteine engineered antibody variants

[0226] In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in US Patent No. 7,521,541.

e. Antibody Derivatives

[0227] In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

[0228] In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., Proc. Natl. Acad. Sci.

USA 102:11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

8. Variant Fc regions

- [0229] In one aspect, the invention provides an isolated polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. In certain embodiments, the variant Fc region comprises at least one amino acid residue alteration (e.g., substitution) compared to the corresponding sequence in the Fc region of a native or reference variant sequence (sometimes collectively referred to herein as a "parent" Fc region). In certain embodiments, the variant Fc region of the invention has enhanced binding activity for monkey Fc gamma RIIb compared to the parent Fc region. In particular embodiments, the monkey Fc gamma RIIb is cynomolgus monkey Fc gamma RIIb (SEQ ID NO: 223).
- [0230] In certain embodiments, the ratio of [KD value of a parent Fc region for monkey Fc gamma RIIb]/[KD value of a variant Fc region for monkey Fc gamma RIIb] can be 2.0 or greater, 3.0 or greater, 4.0 or greater, 5.0 or greater, 6.0 or greater, 7.0 or greater, 8.0 or greater, 9.0 or greater, 10 or greater, 15 or greater, 20 or greater, 25 or greater, 30 or greater, 40 or greater, or 50 or greater. In further embodiments, the variant Fc region has decreased binding activity for monkey Fc gamma RIIIa. In certain embodiments, the ratio of [KD value of a parent Fc region for monkey Fc gamma RIIIa]/[KD value of a variant Fc region for monkey Fc gamma RIIIa] can be 0.50 or smaller, 0.40 or smaller, 0.30 or smaller, 0.20 or smaller, 0.10 or smaller, 0.09 or smaller, 0.08 or smaller, 0.07 or smaller, 0.06 or smaller, 0.05 or smaller, 0.04 or smaller, 0.03 or smaller, 0.02 or smaller, or 0.01 or smaller. In certain embodiments, the monkey Fc gamma RIIb has the sequence of SEQ ID NO:223 (cynomolgus monkey). In certain embodiments, the monkey Fc gamma RIIIa has the sequence of SEQ ID NO:224 (cynomolgus monkey).
- [0231] In further embodiments, the variant Fc region has enhanced binding activity for human Fc gamma RIIb. In certain embodiments, the ratio of [KD value of a parent Fc region for human Fc gamma RIIb]/[KD value of a variant Fc region for human Fc gamma RIIb] can be 2.0 or greater, 3.0 or greater, 4.0 or greater, 5.0 or greater, 6.0 or greater, 7.0 or greater, 8.0 or greater, 9.0 or greater, 10 or greater, 15 or greater, 20 or greater, 25 or greater, 30 or greater, 40 or greater, or 50 or greater. In further embodiments, the variant Fc region has decreased binding activity for human Fc gamma RIIIa. In certain embodiments, the ratio of [KD value of a parent Fc region for human Fc gamma RIIIa]/[KD value of a variant Fc region for human Fc gamma RIIIa] can be

0.50 or smaller, 0.40 or smaller, 0.30 or smaller, 0.20 or smaller, 0.10 or smaller, 0.09 or smaller, 0.08 or smaller, 0.07 or smaller, 0.06 or smaller, 0.05 or smaller, 0.04 or smaller, 0.03 or smaller, 0.02 or smaller, or 0.01 or smaller. In certain embodiments, the human Fc gamma RIIb has the sequence of SEQ ID NOS:212, 213, or 214. In certain embodiments, the human Fc gamma RIIIa has the sequence of SEQ ID NO:215, 216, 217, or 218.

[0232] In further embodiments, the variant Fc region has decreased binding activity for human Fc gamma RIIa (type H) than for human Fc gamma RIIb. In certain embodiments, the ratio of [KD value of a parent Fc region for human Fc gamma RIIa (type H)]/[KD value of a variant Fc region for human Fc gamma RIIa (type H)] can be 5.0 or smaller, 4.0 or smaller, 3.0 or smaller, 2.0 or smaller, 1.0 or smaller, 0.9 or smaller, 0.8 or smaller, 0.7 or smaller, 0.6 or smaller, 0.5 or smaller, 0.4 or smaller, 0.3 or smaller, 0.2 or smaller, or 0.1 or smaller. In further embodiments, the variant Fc region has decreased binding activity for human Fc gamma RIIa (type R) than for human Fc gamma RIIb. In certain embodiments, the ratio of [KD value of a parent Fc region for human Fc gamma RIIa (type R)]/[KD value of a variant Fc region for human Fc gamma RIIa (type R)] can be 5.0 or smaller, 4.0 or smaller, 3.0 or smaller, 2.0 or smaller, 1.0 or smaller, 0.9 or smaller, 0.8 or smaller, 0.7 or smaller, 0.6 or smaller, 0.5 or smaller, 0.4 or smaller, 0.3 or smaller, 0.2 or smaller, or 0.1 or smaller. In certain embodiments, the human Fc gamma RIIa (type H) has the sequence of SEQ ID NO:211. In certain embodiments, the human Fc gamma RIIa (type R) has the sequence of SEQ ID NO:210.

[0233] In certain embodiments, the ratio of [KD value of a parent Fc region for monkey Fc gamma RIIa]/[KD value of a variant Fc region for monkey Fc gamma RIIa] can be 2.0 or greater, 3.0 or greater, 4.0 or greater, 5.0 or greater, 6.0 or greater, 7.0 or greater, 8.0 or greater, 9.0 or greater, 10 or greater, 15 or greater, 20 or greater, 25 or greater, 30 or greater, 40 or greater, or 50 or greater. In certain embodiments, monkey Fc gamma RIIa is selected from monkey Fc gamma RIIa1 (e.g., cynomolgus Fc gamma RIIa1 (SEQ ID NO:220)), monkey Fc gamma RIIa2 (e.g., cynomolgus Fc gamma RIIa2 (SEQ ID NO:221)), and monkey Fc gamma RIIa3 (e.g., cynomolgus Fc gamma RIIa3 (SEQ ID NO:222)).

[0234] In another embodiment, the KD value of the variant Fc region for monkey Fc gamma RIIb can be 1.0×10^{-6} M or smaller, 9.0×10^{-7} M or smaller, 8.0×10^{-7} M or smaller, 7.0×10^{-7} M or smaller, 6.0×10^{-7} M or smaller, 5.0×10^{-7} M or smaller, 4.0×10^{-7} M or smaller, 3.0×10^{-7} M or smaller, 2.0×10^{-7} M or smaller, or 1.0×10^{-7} M or smaller. In another embodiment, the KD value of the variant Fc region for monkey Fc gamma RIIIa can be 5.0×10^{-7} M or greater, 6.0×10^{-7} M or greater, 7.0×10^{-7} M or greater, 8.0×10^{-7} M or greater, 9.0×10^{-7} M or greater, 1.0×10^{-6} M or greater, 2.0×10^{-6} M or greater,

3.0x10⁻⁶ M or greater, 4.0x10⁻⁶ M or greater, 5.0x10⁻⁶ M or greater, 6.0x10⁻⁶ M or greater, 7.0x10⁻⁶ M or greater, 8.0x10⁻⁶ M or greater, 9.0x10⁻⁶ M or greater, or 1.0x10⁻⁵ M or greater. In another embodiment, the KD value of the variant Fc region for human Fc gamma RIIb can be 2.0x10⁻⁶ M or smaller, 1.0x10⁻⁶ M or smaller, 9.0x10⁻⁷ M or smaller, 8.0x10⁻⁷ M or smaller, 7.0x10⁻⁷ M or smaller, 6.0x10⁻⁷ M or smaller, 5.0x10⁻⁷ M or smaller, 4.0x10⁻⁷ M or smaller, 3.0x10⁻⁷ M or smaller, 2.0x10⁻⁷ M or smaller, or 1.0x10⁻⁷ M or smaller. In another embodiment, the KD value of the variant Fc region for human Fc gamma RIIIa can be 1.0x10⁻⁶ M or greater, 2.0x10⁻⁶ M or greater, 3.0x10⁻⁶ M or greater, 4.0x10⁻⁶ M or greater, 5.0x10⁻⁶ M or greater, 6.0x10⁻⁶ M or greater, 7.0x10⁻⁶ M or greater, 8.0x10⁻⁶ M or greater, 9.0x10⁻⁶ M or greater, 1.0x10⁻⁵ M or greater, 2.0x10⁻⁵ M or greater, 3.0x10⁻⁵ M or greater, 4.0x10⁻⁵ M or greater, or 5.0x10⁻⁵ M or greater. In another embodiment, the KD value of the variant Fc region for human Fc gamma RIIa (type H) can be 1.0x10⁻⁷ M or greater, 2.0x10⁻⁷ M or greater, 3.0x10⁻⁷ M or greater, 4.0x10⁻⁷ M or greater, 5.0x10⁻⁷ M or greater, 6.0x10⁻⁷ M or greater, 7.0x10⁻⁷ M or greater, 8.0x10⁻⁷ M or greater, 9.0x10⁻⁷ M or greater, 1.0x10⁻⁶ M or greater, 2.0x10⁻⁶ M or greater, 3.0x10⁻⁶ M or greater, 4.0x10⁻⁶ M or greater, or 5.0x10⁻⁶ M or greater. In another embodiment, the KD value of the variant Fc region for human Fc gamma RIIa (type R) can be 2.0x10⁻⁷ M or greater, 3.0x10⁻⁷ M or greater, 4.0x10⁻⁷ M or greater, 5.0x10⁻⁷ M or greater, 6.0x10⁻⁷ M or greater, 7.0x10⁻⁷ M or greater, 8.0x10⁻⁷ M or greater, 9.0x10⁻⁷ M or greater, 1.0x10⁻⁶ M or greater, 2.0x10⁻⁶ M or greater, 3.0x10⁻⁶ M or greater, 4.0x10⁻⁶ M or greater, or 5.0x10⁻⁶ M or greater.

[0235] In another embodiment, the KD value of the variant Fc region for monkey Fc gamma RIIa can be 1.0x10⁻⁶ M or smaller, 9.0x10⁻⁷ M or smaller, 8.0x10⁻⁷ M or smaller, 7.0x10⁻⁷ M or smaller, 6.0x10⁻⁷ M or smaller, 5.0x10⁻⁷ M or smaller, 4.0x10⁻⁷ M or smaller, 3.0x10⁻⁷ M or smaller, 2.0x10⁻⁷ M or smaller, or 1.0x10⁻⁷ M or smaller. In certain embodiments, monkey Fc gamma RIIa can be selected from any one of monkey Fc gamma RIIa1, monkey Fc gamma RIIa2, and monkey Fc gamma RIIa3.

[0236] When we develop a pharmaceutical product for the treatment of human diseases, evaluation of its efficacy and safety in monkey are important because of its biological proximity to human. From such viewpoints, a pharmaceutical product to be developed is preferable to have cross-reactivity to both human and monkey in the target binding activity.

[0237] "Fc gamma receptors" (herein, referred to as Fc gamma receptors, Fc gamma R or FcγR) refers to receptors that may bind to the Fc region of IgG1, IgG2, IgG3, and IgG4 monoclonal antibodies, and practically means any member of the family of proteins encoded by the Fc gamma receptor genes. In humans, this family includes Fc gamma RI (CD64) including isoforms Fc gamma RIa, Fc gamma RIb, and Fc gamma RIc; Fc gamma RII (CD32) including isoforms Fc gamma RIIa (including allotypes

H131 (type H) and R131 (type R)), Fc gamma RIIb (including Fc gamma RIIb-1 and Fc gamma RIIb-2), and Fc gamma RIIC; and Fc gamma RIII (CD16) including isoforms Fc gamma RIIIA (including allotypes V158 and F158), and Fc gamma RIIIB (including allotypes Fc gamma RIIIB-NA1 and Fc gamma RIIIB-NA2), and any human Fc gamma Rs, Fc gamma R isoforms or allotypes yet to be discovered, but is not limited thereto. Fc gamma RIIb1 and Fc gamma RIIb2 have been reported as splicing variants of human Fc gamma RIIb. In addition, a splicing variant named Fc gamma RIIb3 has been reported (J Exp Med, 1989, 170: 1369-1385). In addition to these splicing variants, human Fc gamma RIIb includes all splicing variants registered in NCBI, which are NP_001002273.1, NP_001002274.1, NP_001002275.1, NP_001177757.1, and NP_003992.3. Furthermore, human Fc gamma RIIb includes every previously-reported genetic polymorphism, as well as Fc gamma RIIb (Arthritis Rheum. 48:3242-3252 (2003); Kono et al., Hum. Mol. Genet. 14:2881-2892 (2005); and Kyogoku et al., Arthritis Rheum. 46:1242-1254 (2002)), and every genetic polymorphism that will be reported in the future.

- [0238] In Fc gamma RIIa, there are two allotypes, one where the amino acid at position 131 of Fc gamma RIIa is histidine (type H) and the other where the amino acid at position 131 is substituted with arginine (type R) (Warrmerdam, J. Exp. Med. 172:19-25 (1990)).
- [0239] The Fc gamma R includes human, mouse, rat, rabbit, and monkey-derived Fc gamma Rs but is not limited thereto, and may be derived from any organism. Mouse Fc gamma Rs include Fc gamma RI (CD64), Fc gamma RII (CD32), Fc gamma RIII (CD16), and Fc gamma RIII-2 (CD16-2), and any mouse Fc gamma Rs, or Fc gamma R isoforms, but are not limited thereto. Unless otherwise specified, the term "monkey Fc gamma R" or variation thereof, refers to cynomolgus Fc gamma RIIa1 (SEQ ID NO:220), Fc gamma RIIa2 (SEQ ID NO:221), Fc gamma RIIa3 (SEQ ID NO:222), Fc gamma RIIb (SEQ ID NO:223), or Fc gamma RIIaS (SEQ ID NO:224).
- [0240] The polynucleotide sequence of human Fc gamma RI is set forth in SEQ ID NO: 199 (NM_000566.3); the polynucleotide sequence of human Fc gamma RIIa is set forth in SEQ ID NO: 200 (BC020823.1) or SEQ ID NO:201 (NM_001136219.1); the polynucleotide sequence of human Fc gamma RIIb is set forth in SEQ ID NO: 202 (BC146678.1) or SEQ ID NO:203 (NM_004001.3); the polynucleotide sequence of human Fc gamma RIIIA is set forth in SEQ ID NO: 204 (BC033678.1) or SEQ ID NO:205 (NM_001127593.1); and the polynucleotide sequence of human Fc gamma RIIIB is set forth in SEQ ID NO: 206 (BC128562.1).
- [0241] The amino acid sequence of human Fc gamma RI is set forth in SEQ ID NO: 207 (NP_000557.1); the amino acid sequence of human Fc gamma RIIa is set forth in SEQ ID NO: 208 (AAH20823.1), SEQ ID NO:209, SEQ ID NO:210 or SEQ ID NO:211;

the amino acid sequence of human Fc gamma RIIb is set forth in SEQ ID NO: 212 (AAI46679.1), SEQ ID NO: 213 or SEQ ID NO:214; the amino acid sequence of human Fc gamma RIIIa is set forth in SEQ ID NO: 215 (AAH33678.1), SEQ ID NO:216, SEQ ID NO:217 or SEQ ID NO:218; and the amino acid sequence of human Fc gamma RIIIb is set forth in SEQ ID NO: 219 (AAI28563.1).

[0242] The amino acid sequence of cynomolgus monkey Fc gamma RIIa is set forth in SEQ ID NO: 220 (Fc gamma RIIa1), SEQ ID NO:221 (Fc gamma RIIa2) or SEQ ID NO:222 (Fc gamma RIIa3); the amino acid sequence of cynomolgus Fc gamma RIIb is set forth in SEQ ID NO: 223; and the amino acid sequence of cynomolgus monkey Fc gamma RIIIa is set forth in SEQ ID NO: 224.

[0243] In one aspect, the invention provides polypeptides containing variant Fc regions with enhanced Fc gamma RIIb-binding activity compared to a corresponding reference Fc gamma RIIb-binding polypeptide. In further aspects, the polypeptide of the invention 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. In certain embodiments, the Fc gamma RIIb has the sequence of cynomolgus monkey Fc gamma RIIb (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIb has the sequence of human Fc gamma RIIb (e.g., SEQ ID NOS:212, 213, or 214).

[0244] In one aspect, the invention provides polypeptides comprising variant Fc regions with enhanced Fc gamma RIIb-binding activity comprising 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. In certain embodiments, the Fc gamma RIIb has the sequence of cynomolgus monkey Fc gamma RIIb (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIb has the sequence of human Fc gamma RIIb (e.g., SEQ ID NOS:212, 213, or 214).

[0245] In one aspect, the invention provides polypeptides comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity comprising an amino acid alteration at position 236 according to EU numbering.

[0246] In one aspect, the invention provides polypeptides comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity comprising 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. In a further embodiment, the variant Fc region comprises an 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. In a further embodiment, the variant Fc region comprises an amino acid alteration of at least one position selected from the group consisting of: 268, 295, 326, and 330, according to EU numbering. In certain embodiments, the Fc gamma RIIb has the sequence of cynomolgus monkey Fc gamma RIIb (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIb has the sequence of human Fc gamma RIIb (e.g., SEQ ID NOS:212, 213, or 214).

[0247] In another aspect, the invention provides polypeptides comprising variant Fc regions with enhanced Fc gamma RIIb-binding activity comprising amino acid alterations of any one of the following (1)-(37): (1) positions 231, 236, 239, 268 and 330; (2) positions 231, 236, 239, 268, 295 and 330; (3) positions 231, 236, 268 and 330; (4) positions 231, 236, 268, 295 and 330; (5) positions 232, 236, 239, 268, 295 and 330; (6) positions 232, 236, 268, 295 and 330; (7) positions 232, 236, 268 and 330; (8) positions 235, 236, 268, 295, 326 and 330; (9) positions 235, 236, 268, 295 and 330; (10) positions 235, 236, 268 and 330; (11) positions 235, 236, 268, 330 and 396; (12) positions 235, 236, 268 and 396; (13) positions 236, 239, 268, 295, 298 and 330; (14) positions 236, 239, 268, 295, 326 and 330; (15) positions 236, 239, 268, 295 and 330; (16) positions 236, 239, 268, 298 and 330; (17) positions 236, 239, 268, 326 and 330; (18) positions 236, 239, 268 and 330; (19) positions 236, 239, 268, 330 and 396; (20) positions 236, 239, 268 and 396; (21) positions 236 and 268; (22) positions 236, 268 and 295; (23) positions 236, 268, 295, 298 and 330; (24) positions 236, 268, 295, 326 and 330; (25) positions 236, 268, 295, 326, 330 and 396; (26) positions 236, 268, 295 and 330; (27) positions 236, 268, 295, 330 and 396; (28) positions 236, 268, 298 and 330; (29) positions 236, 268, 298 and 396; (30) positions 236, 268, 326 and 330; (31) positions 236, 268, 326, 330 and 396; (32) positions 236, 268 and 330; (33) positions 236, 268, 330 and 396; (34) positions 236, 268 and 396; (35) positions 236 and 295; (36) positions 236, 330 and 396; and (37) positions 236 and 396, according to EU numbering. In certain embodiments, the Fc gamma RIIb has the sequence of cynomolgus monkey Fc gamma RIIb (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIb has the sequence of human Fc gamma RIIb (e.g., SEQ ID NOS:212, 213, or 214).

[0248] In a further embodiment, the variant Fc region with enhanced Fc gamma RIIb-binding activity 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. In certain embodiments, the Fc gamma RIIB has the sequence of cynomolgus monkey Fc gamma RIIB (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIB has the sequence of human Fc gamma RIIB (e.g., SEQ ID NOS:212, 213, or 214).

[0249] In a further embodiment, the variant Fc region with enhanced Fc gamma RIIB-binding activity comprises at least one amino acid alteration (e.g., substitution) 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. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIB-binding activity comprises amino acid alterations (e.g., substitutions) of: Asn at position 236, Glu at position 268, Lys at position 330, and Met at position 396; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIB-binding activity comprises amino acid alterations (e.g., substitutions) of: Asn at position 236, Asp at position 268, and Lys at position 330; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIB-binding activity comprises amino acid alterations (e.g., substitutions) of: Asn at position 236, Asp at position 268, Leu at position 295, and Lys at position 330; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIB-binding activity comprises amino acid alterations (e.g., substitutions) of: Thr at position 236, Asp at position 268, and Lys at position 330; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIB-binding activity comprises amino acid alterations (e.g., substitutions) of: Asn at position 236, Asp at position 268, Leu at position 295, Thr at position 326, and Lys at position 330; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIB-binding activity comprises amino acid alterations (e.g., substitutions) of: Trp at position 235, Asn at position 236, Asp at position 268, Leu at position 295, Thr at position 326, and Lys at position 330; according to EU numbering.

[0250] In one aspect, the invention provides polypeptides comprising variant Fc regions

with enhanced Fc gamma RIIb-binding activity comprising an amino acid alteration at position 238 according to EU numbering.

[0251] In one aspect, the invention provides polypeptides comprising variant Fc regions with enhanced Fc gamma RIIb-binding activity comprising at least one amino acid alteration of at least one position selected from the group consisting of: 234, 238, 250, 264, 267, 307, and 330 according to EU numbering. In further embodiments, the polypeptide comprises at least one amino acid alteration of at least one position selected from the group consisting of: 234, 250, 264, 267, 307, and 330 according to EU numbering. In certain embodiments, the Fc gamma RIIb has the sequence of cynomolgus monkey Fc gamma RIIb (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIb has the sequence of human Fc gamma RIIb (e.g., SEQ ID NOS:212, 213, or 214).

[0252] In another aspect, the invention provides polypeptides comprising variant Fc regions with enhanced Fc gamma RIIb-binding activity comprising amino acid alterations of any one of the following (1)-(9): (1) positions 234, 238, 250, 307 and 330; (2) positions 234, 238, 250, 264, 307 and 330; (3) positions 234, 238, 250, 264, 267, 307 and 330; (4) positions 234, 238, 250, 267, 307 and 330; (5) positions 238, 250, 264, 307 and 330; (6) positions 238, 250, 264, 267, 307 and 330; (7) positions 238, 250, 267, 307 and 330; (8) positions 238, 250 and 307; and (9) positions 238, 250, 307 and 330, according to EU numbering. In certain embodiments, the Fc gamma RIIb has the sequence of cynomolgus monkey Fc gamma RIIb (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIb has the sequence of human Fc gamma RIIb (e.g., SEQ ID NOS:212, 213, or 214).

[0253] In a further embodiment, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises at least one amino acid alteration (e.g., substitution) selected from the group consisting of: (a) Tyr at position 234; (b) Asp at position 238; (c) Val at position 250, (d) Ile at position 264; (e) Ala at position 267; (f) Pro at position 307; and (g) Lys at position 330; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises amino acid alterations (e.g., substitutions) of: Asp at position 238; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises amino acid alterations (e.g., substitutions) of: Asp at position 238, Val at position 250, and Pro at position 307; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises amino acid alterations (e.g., substitutions) of: Asp at position 238, Val at position 250, Pro at position 307, and Lys at position 330; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIb-binding activity comprises amino acid alterations (e.g., sub-

stitutions) of: Asp at position 238, Val at position 250, Ile at position 264, Pro at position 307, and Lys at position 330; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIB-binding activity comprises amino acid alterations (e.g., substitutions) of: Asp at position 238, Val at position 250, Ala at position 267, Pro at position 307, and Lys at position 330; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIB-binding activity comprises amino acid alterations (e.g., substitutions) of: Tyr at position 234, Asp at position 238, Val at position 250, Pro at position 307, and Lys at position 330; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIB-binding activity comprises amino acid alterations (e.g., substitutions) of: Tyr at position 234, Asp at position 238, Val at position 250, Ala at position 267, Pro at position 307, and Lys at position 330; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIB-binding activity comprises amino acid alterations (e.g., substitutions) of: Asp at position 238, Val at position 250, Ile at position 264, Ala at position 267, Pro at position 307, and Lys at position 330; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIB-binding activity comprises amino acid alterations (e.g., substitutions) of: Tyr at position 234, Asp at position 238, Val at position 250, Ile at position 264, Pro at position 307, and Lys at position 330; according to EU numbering. In a further embodiment, the variant Fc region with enhanced Fc gamma RIIB-binding activity comprises amino acid alterations (e.g., substitutions) of: Tyr at position 234, Asp at position 238, Val at position 250, Ile at position 264, Ala at position 267, Pro at position 307, and Lys at position 330; according to EU numbering. In certain embodiments, the Fc gamma RIIB has the sequence of cynomolgus monkey Fc gamma RIIB (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIB has the sequence of human Fc gamma RIIB (e.g., SEQ ID NOS:212, 213, or 214).

[0254] In another aspect, the invention provides isolated polypeptides comprising variant Fc regions with increased isoelectric point (pI). In certain embodiments, a variant Fc region described herein comprises at least two amino acid alterations in a parent Fc region. In certain embodiments, each of the amino acid alterations increases the isoelectric point (pI) of the variant Fc region compared with that of the parent Fc region. They are based on the findings that antigen elimination from plasma can be promoted with an antibody whose pI has been increased by modification of at least two amino acid residues, for example when the antibody is administered in vivo.

[0255] In the present invention, pI may be either a theoretical or an experimentally determined pI. The value of pI can be determined, for example, by isoelectric focusing known to those skilled in the art. The value of a theoretical pI can be calculated, for

example, using gene and amino acid sequence analysis software (Genetyx, etc.).

[0256] In one embodiment, the pI value may be increased, for example, at least by 0.01, 0.03, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, or more, at least by 0.6, 0.7, 0.8, 0.9, or more, at least by 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, or more, or at least by 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 3.0 or more, as compared to before modification.

[0257] In certain embodiments, the amino acid for increased pI can be exposed on the surface of the variant Fc region. In the present invention, an amino acid that can be exposed on the surface generally refers to an amino acid residue located on the surface of a polypeptide constituting a variant Fc region. An amino acid residue located on the surface of a polypeptide refers to an amino acid residue whose side chain can be in contact with solvent molecules (which in general are mostly water molecules). However, the side chain does not necessarily have to be wholly in contact with solvent molecules, and when even a portion of the side chain is in contact with the solvent molecules, the amino acid is defined as an "amino acid residue located on the surface". The amino acid residues located on the surface of a polypeptide also include amino acid residues located close to the surface and thereby can have an electric charge influence from another amino acid residue whose side chain, even partly, is in contact with the solvent molecules. Those skilled in the art can prepare a homology model of a polypeptide for example, using commercially available softwares. Alternatively, it is possible to use methods known to those skilled in the art, such as X-ray crystallography. The amino acid residues that can be exposed on the surface are determined, for example, using coordinates from a three-dimensional model using a computer program such as InsightII program (Accelrys). Surface-exposable sites may be determined using algorithms known in the technical field (for example, Lee and Richards (J. Mol. Biol. 55:379-400 (1971)); Connolly (J. Appl. Cryst. 16:548-558 (1983)). Surface-exposable sites can be determined using software suitable for protein modeling and three-dimensional structure information. Software available for such purposes includes, for example, the SYBYL Biopolymer Module software (Tripos Associates). When an algorithm requires a user input size parameter, the "size" of a probe which is used in the calculation may be set to about 1.4 Angstrom (Å) or less in radius. Furthermore, methods for determining surface-exposable regions using software for personal computers have been described by Pacios (Comput. Chem. 18(4):377-386 (1994); J. Mol. Model. 1:46-53 (1995)). Based on such information as described above, appropriate amino acid residues located on the surface of a polypeptide that constitutes a variant Fc region can be selected.

[0258] In certain embodiments, a polypeptide comprises both the variant Fc region and an antigen-binding domain. In further embodiments, the antigen is a soluble antigen. In one embodiment, the antigen is present in biological fluids (for example, plasma, in-

terstitial fluid, lymphatic fluid, ascitic fluid, and pleural fluid) of subjects. The antigen may also be a membrane antigen.

[0259] In further embodiments, antigen-binding activity of the antigen-binding domain changes according to ion concentration conditions. In one embodiment, ion concentration is not particularly limited and refers to hydrogen ion concentration (pH) or metal ion concentration. Herein, metal ions refer to ions of group I elements except hydrogen, such as alkaline metals and the copper group elements, group II elements such as alkaline earth metals and zinc group elements, group III elements except boron, group IV elements except carbon and silicon, group VIII elements such as iron group and platinum group elements, elements belonging to subgroup A of groups V, VI, and VII, and metal elements such as antimony, bismuth, and polonium. In the present invention, metal ions include, for example, calcium ion, as described in WO 2012/073992 and WO 2013/125667. In one embodiment, "ion concentration condition" may be a condition that focuses on differences in the biological behavior of an antigen-binding domain between a low ion concentration and a high ion concentration. Furthermore, "antigen-binding activity of an antigen-binding domain changes according to ion concentration conditions" means that the antigen-binding activity of an antigen-binding domain changes between a low ion concentration and a high ion concentration (such an antigen-binding domain is referred to herein as "ion concentration-dependent antigen-binding domain"). The antigen-binding activity of an antigen-binding domain under a high ion concentration condition may be higher (stronger) or lower (weaker) than that under a low ion concentration condition. In one embodiment, ion concentration-dependent antigen-binding domains (such as pH-dependent antigen-binding domains or calcium ion concentration-dependent antigen-binding domains) can be obtained by known methods, for example, described in WO 2009/125825, WO 2012/073992, and WO 2013/046722.

[0260] In the present invention, the antigen-binding activity of an antigen-binding domain under a high calcium ion concentration condition may be higher than under a low calcium ion concentration condition. The high calcium ion concentration is not particularly limited to but may be a concentration selected between 100 micro M and 10 mM, between 200 micro M and 5 mM, between 400 micro M and 3 mM, between 200 micro M and 2 mM, between 400 micro M and 1 mM, or between 500 micro M and 2.5 mM, which is preferable to be close to the plasma (blood) concentration of calcium ion in vivo. Meanwhile, the low calcium ion concentration is not particularly limited to but may be a concentration selected between 0.1 micro M and 30 micro M, between 0.2 micro M and 20 micro M, between 0.5 micro M and 10 micro M, between 1 micro M and 5 micro M, or between 2 micro M and 4 micro M, which is preferable to be close to the concentration of calcium ion in early endosomes in vivo.

- [0261] In one embodiment, the ratio between the antigen-binding activities under a low calcium ion concentration condition and a high calcium ion concentration condition is not limited but the ratio of the dissociation constant (KD) under a low calcium ion concentration condition to the KD under a high calcium ion concentration condition, i.e., $KD(\text{low calcium ion concentration condition})/KD(\text{high calcium ion concentration condition})$, is 2 or more, 10 or more, or 40 or more. The upper limit of the ratio may be 400, 1000, or 10000, as long as such an antigen-binding domain can be produced by techniques known to those skilled in the art. Alternatively, for example, the dissociation rate constant (kd) can be used instead of the KD. In this case, the ratio of the kd under a low calcium ion concentration condition to the kd under a high calcium ion concentration condition, i.e., $kd(\text{low calcium ion concentration condition})/kd(\text{high calcium ion concentration condition})$, is 2 or more, 5 or more, 10 or more, or 30 or more. The upper limit of the ratio may be 50, 100, or 200, as long as the antigen-binding domain can be produced based on the common technical knowledge of those skilled in the art.
- [0262] In the present invention, the antigen-binding activity of an antigen-binding domain under a low hydrogen ion concentration (neutral pH) may be higher than under a high hydrogen ion concentration (acidic pH). The acidic pH may be, for example, a pH selected from pH4.0 to pH6.5, selected from pH4.5 to pH6.5, selected from pH5.0 to pH6.5, or selected from pH5.5 to pH6.5, which is preferable to be close to the in vivo pH in early endosomes. The acidic pH may also be, for example, pH5.8 or pH6.0. In particular embodiments, the acidic pH is pH5.8. Meanwhile, the neutral pH may be, for example, a pH selected from pH6.7 to pH10.0, selected from pH6.7 to pH9.5, selected from pH7.0 to pH9.0, or selected from pH7.0 to pH8.0, which is preferable to be close to the in vivo pH in plasma (blood). The neutral pH may also be, for example, pH7.4 or pH7.0. In particular embodiments, the neutral pH is pH7.4.
- [0263] In one embodiment, the ratio between the antigen-binding activities under an acidic pH condition and a neutral pH condition is not limited but the ratio of the dissociation constant (KD) under an acidic pH condition to the KD under a neutral pH condition, i.e., $KD(\text{acidic pH condition})/KD(\text{neutral pH condition})$, is 2 or more, 10 or more, or 40 or more. The upper limit of the ratio may be 400, 1000, or 10000, as long as such an antigen-binding domain can be produced by techniques known to those skilled in the art. Alternatively, for example, the dissociation rate constant (kd) can be used instead of the KD. In this case, the ratio of the kd under an acidic pH condition to the kd under a neutral pH condition, i.e., $kd(\text{acidic pH condition})/kd(\text{neutral pH condition})$ is 2 or more, 5 or more, 10 or more, or 30 or more. The upper limit of the ratio may be 50, 100, or 200, as long as the antigen-binding domain can be produced based on the common technical knowledge of those skilled in the art.

- [0264] In one embodiment, for example, at least one amino acid residue is substituted with an amino acid residue with a side-chain pKa of 4.0-8.0, and/or at least one amino acid with a side-chain pKa of 4.0-8.0 is inserted in the antigen-binding domain, as described in WO 2009/125825. The amino acid may be substituted and/or inserted at any site as long as the antigen-binding activity of the antigen-binding domain becomes weaker under an acidic pH condition than under a neutral pH condition as compared to before the substitution or insertion. When the antigen-binding domain has a variable region or CDR, the site may be within the variable region or CDR. The number of amino acids that are substituted or inserted can be appropriately determined by those skilled in the art; and the number may be one or more. Amino acids with a side-chain pKa of 4.0-8.0 can be used to change the antigen-binding activity of the antigen-binding domain according to the hydrogen ion concentration condition. Such amino acids include, for example, natural amino acids such as His (H) and Glu (E), and unnatural amino acids such as histidine analogs (US2009/0035836), m-NO₂-Tyr (pKa 7.45), 3,5-Br₂-Tyr (pKa 7.21), and 3,5-I₂-Tyr (pKa 7.38) (Heyl et al., Bioorg. Med. Chem. 11(17):3761-3768 (2003)). Amino acids with a side-chain pKa of 6.0-7.0 can also be used, which include, e.g., His (H).
- [0265] In another embodiment, preferable antigen-binding domains for the variant Fc region with increased pI are described and can be obtained by methods described in Japanese patent applications JP2015-021371 and JP2015-185254.
- [0266] In certain embodiments, the variant Fc region with increased pI 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.
- [0267] In further embodiments, the variant Fc region with increased pI comprises at least two amino acid alterations of at least two positions selected from the group consisting of: 311, 341, 343, 384, 399, 400, 401, 402, and 413, according to EU numbering.
- [0268] In another aspect, the invention provides polypeptides comprising variant Fc regions with increased pI comprising amino acid alterations of any one of the following (1)-(10): (1) positions 311 and 341; (2) positions 311 and 343; (3) positions 311, 343 and 413; (4) positions 311, 384 and 413; (5) positions 311 and 399; (6) positions 311 and 401; (7) positions 311 and 413; (8) positions 400 and 413; (9) positions 401 and 413; and (10) positions 402 and 413; according to EU numbering.
- [0269] A method for increasing the pI of a protein is, for example, to reduce the number of amino acids with a negatively charged side chain (for example, aspartic acid and glutamic acid) and/or to increase the number of amino acids with a positively charged side chain (for example, arginine, lysine and histidine) at a neutral pH condition. Amino acids with a negatively charged side chain have a negative charge represented

as -1 at a pH condition that is sufficiently higher than their side chain pKa, which is a theory well known to those skilled in the art. For example, the theoretical pKa for the side chain of aspartic acid is 3.9, and the side chain has a negative charge represented as -1 at a neutral pH condition (for example, in a solution of pH7.0). Conversely, amino acids with a positively charged side chain have a positive charge represented as +1 at a pH condition that is sufficiently lower than their side chain pKa. For example, the theoretical pKa for the side chain of arginine is 12.5, and the side chain has a positive charge represented as +1 at a neutral pH condition (for example, in a solution of pH7.0). Meanwhile, amino acids whose side chain has no charge at a neutral pH condition (for example, in a solution of pH7.0) are known to include 15 types of natural amino acids, i.e., alanine, cysteine, phenylalanine, glycine, isoleucine, leucine, methionine, asparagine, proline, glutamine, serine, threonine, valine, tryptophan, and tyrosine. As a matter of course, it is understood that amino acids for increasing the pI may be unnatural amino acids.

[0270] From the above, a method for increasing the pI of a protein at a neutral pH condition (for example, in a solution of pH7.0) can confer a charge alteration of +1 to a protein of interest, for example, by substituting amino acids with non-charged side chains for aspartic acid or glutamic acid (whose side chain has a negative charge of -1) in the amino acid sequence of the protein. Furthermore, a charge alteration of +1 can be conferred to the protein, for example, by substituting arginine or lysine (whose side chain has a positive charge of +1) for amino acids whose side chain has no charge. Moreover, a charge alteration of +2 can be conferred at a time to the protein by substituting arginine or lysine (whose side chain has a positive charge of +1) for aspartic acid or glutamic acid (whose side chain has a negative charge of -1). Alternatively, to increase the pI of a protein, amino acids with a side chain having no charge and/or preferably amino acids having a positively charged side chain can be added or inserted into the amino acid sequence of the protein, or amino acids with a side chain having no charge and/or preferably amino acids with a negatively charged side chain present in the amino acid sequence of the protein can be deleted. It is understood that, for example, the N-terminal and C-terminal amino acid residues of a protein have a main chain-derived charge (NH_3^+ of the amino group at the N-terminus and COO^- of the carbonyl group at the C-terminus) in addition to their side chain-derived charges. Thus, the pI of a protein can also be increased by performing to the main chain-derived functional groups some addition, deletion, substitution, or insertion.

[0271] The substitution of an amino acid to increase the pI includes, for example, substitution of an amino acid whose side chain has no charge for an amino acid having a negatively charged side chain, substitution of an amino acid having a positively charged side chain for an amino acid whose side chain has no charge, and substitution

of an amino acid having a positively charged side chain for an amino acid having a negatively charged side chain in the amino acid sequence of a parent Fc region, which are performed alone or in appropriate combinations.

[0272] The insertion or addition of an amino acid to increase the pI includes, for example, insertion or addition of an amino acid whose side chain has no charge, and/or insertion or addition of an amino acid having a positively charged side chain in the amino acid sequence of a parent Fc region, which are performed alone or in appropriate combinations.

[0273] The deletion of an amino acid to increase the pI includes, for example, deletion of an amino acid whose side chain has no charge, and/or deletion of an amino acid having a negatively charged side chain in the amino acid sequence of a parent Fc region, which are performed alone or in appropriate combinations.

[0274] In one embodiment, natural amino acids used for increasing pI can be classified as follows: (a) an amino acid with a negatively charged side chain can be Glu (E) or Asp (D); (b) an amino acid whose side chain has no charge can be Ala (A), Asn (N), Cys (C), Gln (Q), Gly (G), His (H), Ile (I), Leu (L), Met (M), Phe (F), Pro (P), Ser (S), Thr (T), Trp (W), Tyr (Y), or Val (V); and (c) an amino acid with a positively charged side chain can be His (H), Lys (K), or Arg (R). In one embodiment, the amino acid insertion or substitution after modification is Lys (K) or Arg (R).

[0275] In another aspect, the invention provides isolated polypeptides comprising variant Fc regions with enhanced Fc gamma RIIb-binding activity and increased pI. In certain embodiments, a variant Fc region described herein comprises at least two amino acid alterations in a parent Fc region.

[0276] In one aspect, the invention provides polypeptides comprising variant Fc regions with enhanced Fc gamma RIIb-binding activity and increased pI comprising at least three amino acid alterations comprising: (a) 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, and (b) 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.

[0277] In one aspect, the invention provides polypeptides comprising variant Fc regions with enhanced Fc gamma RIIb-binding activity and increased pI, and that comprise at least three amino acid alterations comprising: (a) at least one amino acid alteration of at least one position selected from the group consisting of: 231, 232, 235, 236, 239, 268, 295, 298, 326, 330, and 396, according to EU numbering, and (b) at least two amino acid alterations of at least two positions selected from the group consisting of:

311, 341, 343, 384, 399, 400, 401, 402, and 413, according to EU numbering.

[0278] In another aspect, the invention provides polypeptides comprising variant Fc regions with enhanced Fc gamma RIIB-binding activity and increased pI comprising amino acid alterations of any one of the following (1)-(9): (1) positions 235, 236, 268, 295, 311, 326, 330 and 343; (2) positions 236, 268, 295, 311, 326, 330 and 343; (3) positions 236, 268, 295, 311, 330 and 413; (4) positions 236, 268, 311, 330, 396 and 399; (5) positions 236, 268, 311, 330 and 343; (6) positions 236, 268, 311, 330, 343 and 413; (7) positions 236, 268, 311, 330, 384 and 413; (8) positions 236, 268, 311, 330 and 413; and (9) positions 236, 268, 330, 396, 400 and 413; according to EU numbering. In certain embodiments, the Fc gamma RIIB has the sequence of cynomolgus monkey Fc gamma RIIB (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIB has the sequence of human Fc gamma RIIB (e.g., SEQ ID NOS:212, 213, or 214).

[0279] In one aspect, the invention provides polypeptides comprising variant Fc regions with enhanced Fc gamma RIIB-binding activity and increased pI comprising at least three amino acid alterations comprising: (a) at least one amino acid alteration of at least one position selected from the group consisting of: 234, 238, 250, 264, 267, 307, and 330, and (b) 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 embodiments, the polypeptides comprise at least two amino acid alterations of at least two positions selected from the group consisting of: 311, 341, 343, 384, 399, 400, 401, 402, and 413, according to EU numbering. In certain embodiments, the Fc gamma RIIB has the sequence of cynomolgus monkey Fc gamma RIIB (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIB has the sequence of human Fc gamma RIIB (e.g., SEQ ID NOS:212, 213, or 214).

[0280] In another aspect, the invention provides polypeptides comprising variant Fc regions with enhanced Fc gamma RIIB-binding activity and increased pI comprising amino acid alterations of any one of the following (1)-(16): (1) positions 234, 238, 250, 264, 307, 311, 330 and 343; (2) positions 234, 238, 250, 264, 307, 311, 330 and 413; (3) positions 234, 238, 250, 264, 267, 307, 311, 330 and 343; (4) positions 234, 238, 250, 264, 267, 307, 311, 330 and 413; (5) positions 234, 238, 250, 267, 307, 311, 330 and 343; (6) positions 234, 238, 250, 267, 307, 311, 330 and 413; (7) positions 234, 238, 250, 307, 311, 330 and 343; (8) positions 234, 238, 250, 307, 311, 330 and 413; (9) positions 238, 250, 264, 267, 307, 311, 330 and 343; (10) positions 238, 250, 264, 267, 307, 311, 330 and 413; (11) positions 238, 250, 264, 307, 311, 330 and 343; (12) positions 238, 250, 264, 307, 311, 330 and 413; (13) positions 238, 250, 267, 307, 311, 330 and 343; (14) positions 238, 250, 267, 307, 311, 330 and 413; (15) positions 238,

250, 307, 311, 330 and 343; and (16) positions 238, 250, 307, 311, 330 and 413; according to EU numbering.

- [0281] In a further embodiment, the variant Fc region comprises amino acid alterations selected from any single alteration, combination of single alterations, or combination alterations described in Tables 14-30.
- [0282] In some embodiments, a polypeptide comprises a variant Fc region of the present invention. In a further embodiment, the polypeptide is an antibody heavy chain constant region. In a further embodiment, the polypeptide is an antibody heavy chain. In a further embodiment, the polypeptide is an antibody. In a further embodiment, the polypeptide is an Fc fusion protein.
- [0283] In a further embodiment, the invention provides a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 229-381.
- [0284] A "parent Fc region" as used herein refers to an Fc region prior to introduction of amino acid alteration(s) described herein. Preferred examples of the parent Fc region include Fc regions derived from native antibodies. Antibodies include, for example, IgA (IgA1, IgA2), IgD, IgE, IgG (IgG1, IgG2, IgG3, IgG4), and IgM, or such. Antibodies may be derived from human or monkey (e.g., cynomolgus, rhesus macaque, marmoset, chimpanzee, or baboon). Native antibodies may also include naturally-occurring mutations. A plurality of allotype sequences of IgGs due to genetic polymorphism are described in "Sequences of proteins of immunological interest", NIH Publication No. 91-3242, and any of them may be used in the present invention. In particular, for human IgG1, the amino acid sequence at positions 356 to 358 (EU numbering) may be either DEL or EEM. Preferred examples of the parent Fc region include Fc regions derived from a heavy chain constant region of human IgG1 (SEQ ID NO: 195), human IgG2 (SEQ ID NO: 196), human IgG3 (SEQ ID NO: 197), and human IgG4 (SEQ ID NO: 198). Another preferred example of the parent Fc region is an Fc region derived from a heavy chain constant region SG1 (SEQ ID NO: 9). Furthermore, the parent Fc region may be an Fc region produced by adding an amino acid alteration(s) other than the amino acid alteration(s) described herein to an Fc region derived from a native antibody.
- [0285] In addition, amino acid alterations performed for other purpose(s) can be combined in a variant Fc region described herein. For example, amino acid substitutions that improve FcRn-binding activity (Hinton et al., J. Immunol. 176(1):346-356 (2006); Dall'Acqua et al., J. Biol. Chem. 281(33):23514-23524 (2006); Petkova et al., Intl. Immunol. 18(12):1759-1769 (2006); Zalevsky et al., Nat. Biotechnol. 28(2):157-159 (2010); WO 2006/019447; WO 2006/053301; and WO 2009/086320), and amino acid substitutions for improving antibody heterogeneity or stability (WO 2009/041613) may be added. Alternatively, polypeptides with the property of promoting antigen

clearance, which are described in WO 2011/122011, WO 2012/132067, WO 2013/046704 or WO 2013/180201, polypeptides with the property of specific binding to a target tissue, which are described in WO 2013/180200, polypeptides with the property for repeated binding to a plurality of antigen molecules, which are described in WO 2009/125825, WO 2012/073992 or WO 2013/047752, can be combined with a variant Fc region described herein. Alternatively, with the objective of conferring binding ability to other antigens, the amino acid alterations disclosed in EP1752471 and EP1772465 may be combined in CH3 of a variant Fc region described herein. Alternatively, with the objective of increasing plasma retention, amino acid alterations that decrease the pI of the constant region (WO 2012/016227) may be combined in a variant Fc region described herein. Alternatively, with the objective of promoting uptake into cells, amino acid alterations that increase the pI of the constant region (WO 2014/145159) may be combined in a variant Fc region described herein. Alternatively, with the objective of promoting elimination of a target molecule from plasma, amino acid alterations that increase the pI of the constant region (Japanese patent application numbers JP2015-021371 and JP2015-185254) may be combined in a variant Fc region described herein. In one embodiment, such alteration may include, for example, substitution at at least one position selected from the group consisting of 311, 343, 384, 399, 400, and 413 according to EU numbering. In a further embodiment, such substitution may be a replacement of an amino acid with Lys or Arg at each position.

[0286] Amino acid alterations of enhancing human FcRn-binding activity under acidic pH can also be combined in a variant Fc region described herein. Specifically, such alterations may include, for example, substitution of Leu for Met at position 428 and substitution of Ser for Asn at position 434, according to EU numbering (Zalevsky et al., *Nat. Biotechnol.* 28:157-159 (2010)); substitution of Ala for Asn at position 434 (Deng et al., *Metab. Dispos.* 38(4):600-605 (2010)); substitution of Tyr for Met at position 252, substitution of Thr for Ser at position 254 and substitution of Glu for Thr at position 256 (Dall'Acqua et al., *J. Biol. Chem.* 281:23514-23524 (2006)); substitution of Gln for Thr at position 250 and substitution of Leu for Met at position 428 (Hinton et al., *J. Immunol.* 176(1):346-356 (2006)); substitution of His for Asn at position 434 (Zheng et al., *Clin. Pharmacol. Ther.* 89(2):283-290 (2011), and alterations described in WO 2010/106180, WO 2010/045193, WO 2009/058492, WO 2008/022152, WO 2006/050166, WO 2006/053301, WO 2006/031370, WO 2005/123780, WO 2005/047327, WO 2005/037867, WO 2004/035752, or WO 2002/060919. Such alterations may include, for example, at least one alteration selected from the group consisting of substitution of Leu for Met at position 428, substitution of Ala for Asn at position 434 and substitution of Thr for Tyr at position 436. Those alterations may further include substitution of Arg for Gln at position 438 and/or

substitution of Glu for Ser at position 440 (Japanese patent application numbers JP2015-021371 and JP2015-185254).

[0287] Two or more polypeptides comprising a variant Fc region described herein can be included in one molecule, wherein two polypeptides comprising variant Fc regions are associated, much like in an antibody. The type of antibody is not limited, and IgA (IgA1, IgA2), IgD, IgE, IgG (IgG1, IgG2, IgG3, IgG4), and IgM, or such can be used.

[0288] The two associated polypeptides comprising variant Fc regions may be polypeptides comprising variant Fc regions into which the same amino acid alteration(s) have been introduced (hereinafter, referred to as homologous variant Fc regions), or polypeptides comprising variant Fc regions into which different amino acid alteration(s) have been introduced, or alternatively polypeptides comprising variant Fc regions where amino acid alteration(s) have been introduced into only one of the Fc regions (hereinafter, referred as a heterologous polypeptides comprising variant Fc regions). One of the preferable amino acid alterations is an alteration in the loop structure from positions 233 to 239 (EU numbering) in the CH2 domain of the Fc region, which is involved in binding with Fc gamma RIIb and Fc gamma RIIa. Preferably, an alteration is introduced in the loop structure of the CH2 domain of one of the Fc regions that enhances Fc gamma RIIb-binding activity and/or selectivity, and another alteration is introduced in the loop structure of the CH2 domain of the other Fc region that destabilizes it. Examples of amino acid alterations that can destabilize the loop structure of the CH2 domain may be substitution of at least one amino acid selected from amino acids at positions 235, 236, 237, 238, and 239 to another amino acid. Specifically, it can be destabilized, for example, by altering the amino acid at position 235 to Asp, Gln, Glu, or Thr, altering the amino acid at position 236 to Asn, altering the amino acid at position 237 to Phe or Trp, altering the amino acid at position 238 to Glu, Gly, or Asn, and altering the amino acid at position 239 to Asp or Glu, according to EU numbering.

[0289] For association of heterologous polypeptides comprising variant Fc regions, a technique of suppressing unintended association of homologous polypeptides comprising variant Fc regions by introducing electrostatic repulsion into the interface of the CH2 or CH3 domain of the Fc region can be applied, as described in WO 2006/106905.

[0290] Examples of amino acid residues in contact at the interface of the CH2 or CH3 domain of the Fc region include the residue at position 356 (EU numbering), the residue at position 439 (EU numbering), the residue at position 357 (EU numbering), the residue at position 370 (EU numbering), the residue at position 399 (EU numbering), and the residue at position 409 (EU numbering) in the CH3 domain.

[0291] More specifically, for example, the Fc region in which one to three pairs of amino

acid residues selected from (1) to (3) shown below have the same charge can be produced: (1) amino acid residues at positions 356 and 439 (EU numbering) in the CH3 domain; (2) amino acid residues at positions 357 and 370 (EU numbering) in the CH3 domain; and (3) amino acid residues at positions 399 and 409 (EU numbering) in the CH3 domain.

- [0292] Furthermore, heterologous polypeptides comprising variant Fc regions can be produced, wherein one to three pairs of amino acid residues selected from (1) to (3) indicated above have the same charge in the CH3 domain of the first Fc region, and the pairs of amino acid residues selected in the aforementioned first Fc region also have the same charge in the CH3 domain of the second Fc region, provided that the charges in the first and second Fc regions are opposite.
- [0293] In the above-mentioned Fc regions, for example, negatively-charged amino acid residues are preferably selected from glutamic acid (E) and aspartic acid (D), and positively-charged amino acid residues are preferably selected from lysine (K), arginine (R), and histidine (H).
- [0294] Other known techniques can be used additionally for association of heterologous polypeptides comprising variant Fc regions. Specifically, such a technique is conducted by substituting an amino acid side chain present in one of the Fc regions with a larger side chain (knob; which means "bulge"), and substituting an amino acid side chain present in the Fc region with a smaller side chain (hole; which means "void"), to place the knob within the hole. This can promote efficient association between Fc-region-containing polypeptides having different amino acid sequences from each other (WO 1996/027011; Ridgway et al., *Prot. Eng.* 9:617-621 (1996); Merchant et al., *Nat. Biotech.* 16, 677-681 (1998)).
- [0295] In addition, other known techniques can also be used for heterologous association of polypeptides comprising variant Fc regions. Association of polypeptides comprising an Fc region can be induced efficiently using strand-exchange engineered domain CH3 heterodimers (Davis et al., *Prot. Eng. Des. & Sel.*, 23:195-202 (2010)). This technique can also be used to efficiently induce association between Fc region-containing polypeptides having different amino acid sequences.
- [0296] In addition, heterodimerized antibody production techniques that use association of antibody CH1 and CL, and association of VH and VL, which are described in WO 2011/028952, can also be used.
- [0297] As with the method described in WO 2008/119353 and WO 2011/131746, it is also possible to use the technique of producing heterodimerized antibodies by producing two types of homodimerized antibodies in advance, incubating the antibodies under reducing conditions to dissociate them, and allowing them to associate again.
- [0298] As with the method described in Strop (*J. Mol. Biol.* 420:204-219 (2012)), it is also

possible to use the technique of producing heterodimerized antibodies by introducing charged residues such as Lys, Arg, Glu, and Asp so that electrostatic repulsion is introduced into CH3 domains.

[0299] Furthermore, as with the method described in WO 2012/058768, it is also possible to use the technique of producing heterodimerized antibodies by adding alterations to the CH2 and CH3 domains.

[0300] When simultaneously expressing two polypeptides comprising a variant Fc region which have different amino acid sequences, in order to produce polypeptides comprising heterologous variant Fc regions, polypeptides comprising homologous variant Fc regions are also usually produced as impurities. In such cases, polypeptides comprising heterologous variant Fc regions can be efficiently obtained by separating and purifying them from polypeptides comprising homologous variant Fc regions using known technologies. A method has been reported to efficiently separate and purify heterodimerized antibodies from a homodimerized antibodies using ion exchange chromatography, by introducing amino acid alterations into the variable regions of the two types of antibody heavy chains to create a difference in isoelectric points between the homodimerized antibodies and the heterodimerized antibodies (WO 2007/114325). Another method has been reported to purify heterodimerized antibodies using Protein A chromatography, by constructing a heterodimerized antibody comprising two types of heavy chains derived from mouse IgG2a that binds to Protein A and rat IgG2b that does not bind to Protein A (WO 1998/050431 and WO 1995/033844).

[0301] Furthermore, a heterodimerized antibody can be efficiently purified using Protein A chromatography, by substituting amino acid residues at positions 435 and 436 (EU numbering), which are located in the Protein A binding site of an antibody heavy chain, with amino acids such as Tyr or His, to yield different Protein A binding affinities.

[0302] In the present invention, amino acid alteration means any of substitution, deletion, addition, insertion, and modification, or a combination thereof. In the present invention, amino acid alteration may be rephrased as amino acid mutation.

[0303] When substituting amino acid residues, substitution to a different amino acid residue can be carried out with the objective of altering aspects such as (a)-(c) described below: (a) polypeptide backbone structure in the sheet-structure or helical-structure region; (b) electric charge or hydrophobicity at the target site; or (c) size of the side chain.

[0304] Amino acid residues are classified into the following groups based on their general side chain properties: (a) hydrophobic: Norleucine, Met, Ala, Val, Leu, and Ile; (b) neutral hydrophilic: Cys, Ser, Thr, Asn, and Gln; (c) acidic: Asp and Glu; (d) basic:

His, Lys, and Arg; (e) residues that affect the chain orientation: Gly and Pro; and (f) aromatic: Trp, Tyr, and Phe.

- [0305] Amino acid alterations are produced by various methods known to those skilled in the art. Such methods include the site-directed mutagenesis method (Hashimoto-Gotoh et al., *Gene* 152:271-275 (1995); Zoller, *Meth. Enzymol.* 100:468-500 (1983); Kramer et al., *Nucleic Acids Res.* 12: 9441-9456 (1984)); Kramer and Fritz, *Methods Enzymol.* 154: 350-367 (1987); and Kunkel, *Proc. Natl. Acad. Sci. USA* 82:488-492 (1985)), the PCR mutation method, and the cassette mutation method, but are not limited thereto.
- [0306] The number of amino acid alterations introduced into an Fc region is not limited. In certain embodiments, it can be 1, 2 or less, 3 or less, 4 or less, 5 or less, 6 or less, 8 or less, 10 or less, 12 or less, 14 or less, 16 or less, 18 or less, or 20 or less.
- [0307] Amino acid modification includes post-translational modification. A specific post-translational modification may be addition or deletion of a sugar chain. For example, the amino acid residue at position 297 (EU numbering) in the IgG1 constant region may be sugar chain-modified. The sugar chain structure for the modification is not limited. For example, sialic acid may be added to the sugar chain of an Fc region (*MAbs* 2010 Sep-Oct, 2(5): 519-527). Generally, antibodies expressed in eukaryotic cells comprise glycosylation in the constant region. For example, it is known that some type of sugar chain are normally added to antibodies expressed in cells such as naturally-occurring antibody-producing cells of mammals or eukaryotic cells transformed with an expression vector comprising a DNA encoding an antibody.
- [0308] Eukaryotic cells shown here include yeast and animal cells. For example, CHO cells and HEK293 cells are representative animal cells used in transformation with an expression vector comprising an antibody-encoding DNA. On the other hand, constant regions without glycosylation are also included in the present invention. Antibodies whose constant region is not glycosylated can be obtained by expressing an antibody-encoding gene in prokaryotic cells such as *Escherichia coli*.
- [0309] Furthermore, a polypeptide comprising a variant Fc region of the present invention may be chemically modified with various molecules such as polyethylene glycol (PEG) and cytotoxic substances. Methods for such chemical modification of a polypeptide are established in the art.
- [0310] In one aspect, the invention provides an isolated polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. In certain embodiments, an antibody is a chimeric antibody, or a humanized antibody. The origin of an antibody is not particularly limited, but examples include a human antibody, a mouse antibody, a rat antibody, and a rabbit antibody. In some aspects, the

polypeptide is an Fc fusion protein.

- [0311] The variable regions of antibodies that comprise a variant Fc region provided herein and the protein binding motifs of Fc fusion proteins comprising a variant Fc region can recognize any antigen. Examples of antigens that can be bound by such antibodies and fusion proteins include, but are not limited to ligands (cytokines, chemokines, and such), receptors, cancer antigens, MHC antigens, differentiation antigens, immunoglobulins, and immune complexes partly containing immunoglobulins.
- [0312] Examples of cytokines that can be bound by an antibody or fusion protein containing a variant Fc region of the invention, and/or recombinantly fused with a polypeptide comprising a disclosed variant Fc region include but are not limited to, interleukins 1 to 18, colony stimulating factors (G-CSF, M-CSF, GM-CSF, etc.), interferons (IFN-alpha, IFN-beta, IFN-gamma, etc.), growth factors (EGF, FGF, IGF, NGF, PDGF, TGF, HGF, etc.), tumor necrosis factors (TNF-alpha and TNF-beta), lymphotoxin, erythropoietin, leptin, SCF, TPO, MCAF, and BMP.
- [0313] Examples of chemokines that can be bound by an antibody or fusion protein containing a variant Fc region of the invention, and/or recombinantly fused with a polypeptide comprising a disclosed variant Fc region include but are not limited to, CC chemokines such as CCL1 to CCL28, CXC chemokines such as CXCL1 to CXCL17, C chemokines such as XCL1 to XCL2, and CX3C chemokines such as CX3CL1.
- [0314] Examples of receptors that can be bound by an antibody or fusion protein containing a variant Fc region of the invention, and/or recombinantly fused with a polypeptide comprising a disclosed variant Fc region include but are not limited to, receptors belonging to receptor families such as the hematopoietic growth factor receptor family, cytokine receptor family, tyrosine kinase-type receptor family, serine/threonine kinase-type receptor family, TNF receptor family, G protein-coupled receptor family, GPI anchor-type receptor family, tyrosine phosphatase-type receptor family, adhesion factor family, and hormone receptor family. The receptors belonging to these receptor families and their characteristics have been described in many documents such as Cooke, ed. New Comprehensive Biochemistry Vol.18B "Hormones and their Actions Part II" pp.1-46 (1988) Elsevier Science Publishers BV; Patthy (Cell 61(1):13-14 (1990)); Ullrich (Cell 61(2):203-212 (1990)); Massague (Cell 69(6):1067-1070 (1992)); Miyajima et al. (Annu. Rev. Immunol. 10:295-331 (1992)); Taga et al. (FASEB J. 6:3387-3396 (1992)); Fantl et al. (Annu. Rev. Biochem. 62:453-481 (1993)); Smith et al. (Cell 76(6):959-962 (1994)); and Flower (Biochim. Biophys. Acta 1422(3): 207-234 (1999)).
- [0315] Examples of specific receptors belonging to the above-mentioned receptor families include human or mouse erythropoietin (EPO) receptors (Jones et al., Blood 76(1):31-35 (1990); D'Andrea et al., Cell 57(2):277-285 (1989)), human or mouse

granulocyte-colony stimulating factor (G-CSF) receptors (Fukunaga et al., Proc. Natl. Acad. Sci. USA 87(22):8702-8706 (1990), mG-CSFR; Fukunaga et al., Cell 61(2): 341-350 (1990)), human or mouse thrombopoietin (TPO) receptors (Vigon et al., Proc. Natl. Acad. Sci. USA. 89(12):5640-5644 (1992); Skoda et al., EMBO J. 12(7):2645-2653 (1993)), human or mouse insulin receptors (Ullrich et al., Nature 313(6005):756-761 (1985)), human or mouse Flt-3 ligand receptors (Small et al., Proc. Natl. Acad. Sci. USA. 91(2):459-463 (1994)), human or mouse platelet-derived growth factor (PDGF) receptors (Gronwald et al., Proc. Natl. Acad. Sci. USA. 85(10):3435-3439 (1988)), human or mouse interferon (IFN)-alpha and beta receptors (Uze et al., Cell 60(2): 225-234 (1990); Novick et al., Cell 77(3):391-400 (1994)), human or mouse leptin receptors, human or mouse growth hormone (GH) receptors, human or mouse interleukin (IL)-10 receptors, human or mouse insulin-like growth factor (IGF)-I receptors, human or mouse leukemia inhibitory factor (LIF) receptors, and human or mouse ciliary neurotrophic factor (CNTF) receptors.

- [0316] Cancer antigens are antigens that are expressed as cells become malignant, and they are also called tumor-specific antigens. Abnormal sugar chains that appear on cell surfaces or protein molecules when cells become cancerous are also cancer antigens, and they are also called sugar-chain cancer antigens. Examples of cancer antigens that can be bound by an antibody or fusion protein containing a variant Fc region of the invention include but are not limited to, GPC3 which is a receptor belonging to the GPI anchor-type receptor family mentioned above, and is also expressed in several cancers including liver cancer (Midorikawa et al., Int. J. Cancer 103(4):455-465 (2003)), as well as EpCAM which is expressed in several cancers including lung cancer (Linnenbach et al., Proc. Natl. Acad. Sci. USA 86(1):27-31 (1989)), CA19-9, CA15-3, and sialyl SSEA-1 (SLX).
- [0317] MHC antigens are roughly classified into MHC class I antigens and MHC class II antigens. MHC class I antigens include HLA-A, -B, -C, -E, -F, -G, and -H, and MHC class II antigens include HLA-DR, -DQ, and -DP.
- [0318] Examples of differentiation antigens that can be bound by an antibody or fusion protein containing a variant Fc region of the invention, and/or recombinantly fused with a polypeptide comprising a disclosed variant Fc region include but are not limited to, CD1, CD2, CD4, CD5, CD6, CD7, CD8, CD10, CD11a, CD11b, CD11c, CD13, CD14, CD15s, CD16, CD18, CD19, CD20, CD21, CD23, CD25, CD28, CD29, CD30, CD32, CD33, CD34, CD35, CD38, CD40, CD41a, CD41b, CD42a, CD42b, CD43, CD44, CD45, CD45RO, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD51, CD54, CD55, CD56, CD57, CD58, CD61, CD62E, CD62L, CD62P, CD64, CD69, CD71, CD73, CD95, CD102, CD106, CD122, CD126, and CDw130.
- [0319] Immunoglobulins include IgA, IgM, IgD, IgG, and IgE. Immune complexes include

a component of at least any of the immunoglobulins.

[0320] Other examples of antigens that can be bound by an antibody or fusion protein containing a variant Fc region of the invention, and/or recombinantly fused with a polypeptide comprising a disclosed variant Fc region include but are not limited to, 17-IA, 4-1BB, 4Dc, 6-keto-PGF1a, 8-iso-PGF2a, 8-oxo-dG, A1 adenosine receptor, A33, ACE, ACE-2, activin, activin A, activin AB, activin B, activin C, activin RIA, activin RIA ALK-2, activin RIB ALK-4, activin RIIA, activin RIIB, ADAM, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADAM8, ADAM9, ADAMTS, ADAMTS4, ADAMTS5, addressin, aFGF, ALCAM, ALK, ALK-1, ALK-7, alpha-1-antitrypsin, alpha-V/beta-1 antagonist, ANG, Ang, APAF-1, APE, APJ, APP, APRIL, AR, ARC, ART, artemin, anti-Id, ASPARTIC, atrial natriuretic peptide, av/b3 integrin, Axl, b2M, B7-1, B7-2, B7-H, B-lymphocyte stimulating factor (BlyS), BACE, BACE-1, Bad, BAFF, BAFF-R, Bag-1, BAK, Bax, BCA-1, BCAM, Bcl, BCMA, BDNF, b-ECGF, bFGF, BID, Bik, BIM, BLC, BL-CAM, BLK, BMP, BMP-2 BMP-2a, BMP-3 Osteogenin, BMP-4, BMP-2b, BMP-5, BMP-6 Vgr-1, BMP-7 (OP-1), BMP-8 (BMP-8a, OP-2), BMPR, BMPR-IA (ALK-3), BMPR-IB (ALK-6), BRK-2, RPK-1, BMPR-II (BRK-3), BMP, b-NGF, BOK, bombesin, bone-derived neurotrophic factor, BPDE, BPDE-DNA, BTC, complement factor 3 (C3), C3a, C4, C5, C5a, C10, CA125, CAD-8, calcitonin, cAMP, carcinoembryonic antigen (CEA), cancer associated antigen, cathepsin A, cathepsin B, cathepsin C/DPPI, cathepsin D, cathepsin E, cathepsin H, cathepsin L, cathepsin O, cathepsin S, cathepsin V, cathepsin X/Z/P, CBL, CCI, CCK2, CCL, CCL1, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/10, CCR, CCR1, CCR10, CCR11, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CD1, CD2, CD3, CD3E, CD4, CD5, CD6, CD7, CD8, CD10, CD11a, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD25, CD27L, CD28, CD29, CD30, CD30L, CD32, CD33 (p67 protein), CD34, CD38, CD40, CD40L, CD44, CD45, CD46, CD49a, CD52, CD54, CD55, CD56, CD61, CD64, CD66e, CD74, CD80 (B7-1), CD89, CD95, CD123, CD137, CD138, CD140a, CD146, CD147, CD148, CD152, CD164, CEACAM5, CFTR, cGMP, CINC, Botulinum toxin, Clostridium perfringens toxin, CKb8-1, CLC, CMV, CMV UL, CNTF, CNTN-1, COX, C-Ret, CRG-2, CT-1, CTACK, CTGF, CTLA-4, CX3CL1, CX3CR1, CXCL, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCR, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, cytokeratin tumor associated antigen, DAN, DCC, DcR3, DC-SIGN, complement regulatory factor (Decay accelerating factor), des(1-3)-IGF-I (brain IGF-

1), Dhh, digoxin, DNAM-1, Dnase, Dpp, DPPIV/CD26, Dtk, ECAD, EDA, EDA-A1, EDA-A2, EDAR, EGF, EGFR (ErbB-1), EMA, EMMPRIN, ENA, endothelin receptor, enkephalinase, eNOS, Eot, eotaxin 1, EpCAM, ephrin B2/EphB4, EPO, ERCC, E-selectin, ET-1, factor IIa, factor VII, factor VIIIc, factor IX, fibroblast activation protein (FAP), Fas, FcR1, FEN-1, ferritin, FGF, FGF-19, FGF-2, FGF3, FGF-8, FGFR, FGFR-3, fibrin, FL, FLIP, Flt-3, Flt-4, follicle stimulating hormone, fractalkine, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, G250, Gas6, GCP-2, GCSF, GD2, GD3, GDF, GDF-1, GDF-3 (Vgr-2), GDF-5 (BMP-14, CDMP-1), GDF-6 (BMP-13, CDMP-2), GDF-7 (BMP-12, CDMP-3), GDF8 (myostatin), GDF-9, GDF-15 (MIC-1), GDNF, GFAP, GFRA-1, GFR-alpha1, GFR-alpha2, GFR-alpha3, GITR, glucagon, Glut4, glycoprotein IIb/IIIa (GPIIb/IIIa), GM-CSF, gp130, gp72, GRO, growth hormone releasing hormone, hapten (NP-cap or NIP-cap), HB-EGF, HCC, HCMV gB envelope glycoprotein, HCMV gH envelope glycoprotein, HCMV UL, hematopoietic growth factor (HGF), Hep B gp120, heparanase, Her2, Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), herpes simplex virus (HSV) gB glycoprotein, HSV gD glycoprotein, HGFA, high molecular weight melanoma-associated antigen (HMW-MAA), HIV gp120, HIV IIIB gp 120 V3 loop, HLA, HLA-DR, HM1.24, HMFG PEM, HRG, Hrk, human cardiac myosin, human cytomegalovirus (HCMV), human growth hormone (HGH), HVEM, I-309, IAP, ICAM, ICAM-1, ICAM-3, ICE, ICOS, IFNg, Ig, IgA receptor, IgE, IGF, IGF binding protein, IGF-1R, IGFBP, IGF-I, IGF-II, IL, IL-1, IL-1R, IL-2, IL-2R, IL-4, IL-4R, IL-5, IL-5R, IL-6, IL-6R, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-18, IL-18R, IL-23, interferon (IFN)-alpha, IFN-beta, IFN-gamma, inhibin, iNOS, insulin A chain, insulin B chain, insulin-like growth factor1, integrin alpha2, integrin alpha3, integrin alpha4, integrin alpha4/beta1, integrin alpha4/beta7, integrin alpha5 (alpha V), integrin alpha5/beta1, integrin alpha5/beta3, integrin alpha6, integrin beta1, integrin beta2, interferon gamma, IP-10, I-TAC, JE, kallikrein 2, kallikrein 5, kallikrein 6, kallikrein 11, kallikrein 12, kallikrein 14, kallikrein 15, kallikrein L1, kallikrein L2, kallikrein L3, kallikrein L4, KC, KDR, keratinocyte growth factor (KGF), laminin 5, LAMP, LAP, LAP (TGF-1), latent TGF-1, latent TGF-1 bp1, LBP, LDGF, LECT2, lefty, Lewis-Y antigen, Lewis-Y associated antigen, LFA-1, LFA-3, Lfo, LIF, LIGHT, lipoprotein, LIX, LKN, Lptn, L-selectin, LT-a, LT-b, LTB4, LTBP-1, lung surface, luteinizing hormone, lymphotoxin beta receptor, Mac-1, MAdCAM, MAG, MAP2, MARC, MCAM, MCK-2, MCP, M-CSF, MDC, Mer, METALLOPROTEASES, MGDF receptor, MGMT, MHC (HLA-DR), MIF, MIG, MIP, MIP-1-alpha, MK, MMAC1, MMP, MMP-1, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-2, MMP-24, MMP-3, MMP-7, MMP-8, MMP-9, MPIF, Mpo, MSK, MSP, mucin (Muc1), MUC18, Mullerian-inhibiting substance, Mug, MuSK, NAIP, NAP, NCAD, N-Cadherin, NCA

90, NCAM, neprilysin, neurotrophin-3, -4, or -6, neurturin, nerve growth factor (NGF), NGFR, NGF-beta, nNOS, NO, NOS, Npn, NRG-3, NT, NTN, OB, OGG1, OPG, OPN, OSM, OX40L, OX40R, p150, p95, PADPr, parathyroid hormone, PARC, PARP, PBR, PBSF, PCAD, P-cadherin, PCNA, PDGF, PDK-1, PECAM, PEM, PF4, PGE, PGF, PGI2, PGJ2, PIN, PLA2, placental alkaline phosphatase (PLAP), PIGF, PLP, PP14, proinsulin, prorelaxin, protein C, PS, PSA, PSCA, prostate-specific membrane antigen (PSMA), PTEN, PTHrp, Ptk, PTN, R51, RANK, RANKL, RANTES, relaxin A chain, relaxin B chain, renin, respiratory syncytial virus (RSV) F, RSV Fgp, Ret, Rheumatoid factor, RLIP76, RPA2, RSK, S100, SCF/KL, SDF-1, SERINE, serum albumin, sFRP-3, Shh, SIGIRR, SK-1, SLAM, SLPI, SMAC, SMDF, SMOH, SOD, SPARC, Stat, STEAP, STEAP-II, TACE, TACI, TAG-72 (tumor-associated glycoprotein-72), TARC, TCA-3, T-cell receptor (for example, T-cell receptor alpha/beta), TdT, TECK, TEM1, TEM5, TEM7, TEM8, TERT, testis PLAP-like alkaline phosphatase, TfR, TGF, TGF-alpha, TGF-beta, TGF-beta Pan Specific, TGF-betaRI (ALK-5), TGF-betaRII, TGF-betaRIIb, TGF-betaRIII, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5, thrombin, thymus Ck-1, thyroid-stimulating hormone, Tie, TIMP, TIQ, tissue factor, TMEFF2, Tmpo, TNFRSF2, TNF, TNF-alpha, TNF-alphabeta, TNF-beta2, TNFc, TNF-RI, TNF-RII, TNFRSF10A (TRAIL R1 Apo-2, DR4), TNFRSF10B (TRAIL R2 DR5, KILLER, TRICK-2A, TRICK-B), TNFRSF10C (TRAIL R3 DcR1, LIT, TRID), TNFRSF10D (TRAIL R4 DcR2, TRUND), TNFRSF11A (RANK ODF R, TRANCE R), TNFRSF11B (OPG OCIF, TR1), TNFRSF12 (TWEAK R FN14), TNFRSF13B (TACI), TNFRSF13C (BAFF R), TNFRSF14 (HVEM ATAR, HveA, LIGHT R, TR2), TNFRSF16 (NGFR p75NTR), TNFRSF17 (BCMA), TNFRSF18 (GITR AITR), TNFRSF19 (TROY TAJ, TRADE), TNFRSF19L (RELT), TNFRSF1A (TNF RI CD120a, p55-60), TNFRSF1B (TNF RII CD120b, p75-80), TNFRSF26 (TNFRH3), TNFRSF3 (LTbR TNF RIII, TNFC R), TNFRSF4 (OX40 ACT35, TXGP1 R), TNFRSF5 (CD40 p50), TNFRSF6 (Fas Apo-1, APT1, CD95), TNFRSF6B (DcR3 M68, TR6), TNFRSF7 (CD27), TNFRSF8 (CD30), TNFRSF9 (4-1BB CD137, ILA), TNFRSF21 (DR6), TNFRSF22 (DcTRAIL R2 TNFRH2), TNFRST23 (DcTRAIL R1 TNFRH1), TNFRSF25 (DR3 Apo-3, LARD, TR-3, TRAMP, WSL-1), TNFSF10 (TRAIL Apo-2 ligand, TL2), TNFSF11 (TRANCE/RANK ligand ODF, OPG ligand), TNFSF12 (TWEAK Apo-3 ligand, DR3 ligand), TNFSF13 (APRIL TALL2), TNFSF13B (BAFF BLYS, TALL1, THANK, TNFSF20), TNFSF14 (LIGHT HVEM ligand, LTg), TNFSF15 (TL1A/VEGI), TNFSF18 (GITR ligand AITR ligand, TL6), TNFSF1A (TNF-a Conectin, DIF, TNFSF2), TNFSF1B (TNF-b LTa, TNFSF1), TNFSF3 (LTb TNFC, p33), TNFSF4 (OX40 ligand gp34, TXGP1), TNFSF5 (CD40 ligand CD154, gp39, HIGM1, IMD3, TRAP), TNFSF6 (Fas ligand Apo-1 ligand, APT1 ligand), TNFSF7 (CD27 ligand

CD70), TNFSF8 (CD30 ligand CD153), TNFSF9 (4-1BB ligand CD137 ligand), TP-1, t-PA, Tpo, TRAIL, TRAIL R, TRAIL-R1, TRAIL-R2, TRANCE, transferrin receptor, TRF, Trk, TROP-2, TSG, TSLP, tumor associated antigen CA125, tumor associated antigen expressing Lewis-Y associated carbohydrates, TWEAK, TXB2, Ung, uPAR, uPAR-1, urokinase, VCAM, VCAM-1, VECAD, VE-Cadherin, VE-cadherin-2, VEGFR-1 (flt-1), VEGF, VEGFR, VEGFR-3 (flt-4), VEGI, VIM, virus antigen, VLA, VLA-1, VLA-4, VNR integrin, von Willebrand factor, WIF-1, WNT1, WNT2, WNT2B/13, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, WNT16, XCL1, XCL2, XCR1, XCR1, XEDAR, XIAP, XPD, HMGB1, IgA, A beta, CD81, CD97, CD98, DDR1, DKK1, EREG, Hsp90, IL-17/IL-17R, IL-20/IL-20R, oxidized LDL, PCSK9, prekallikrein, RON, TMEM16F, SOD1, Chromogranin A, Chromogranin B, tau, VAP1, high molecular weight kininogen, IL-31, IL-31R, Nav1.1, Nav1.2, Nav1.3, Nav1.4, Nav1.5, Nav1.6, Nav1.7, Nav1.8, Nav1.9, EPCR, C1, C1q, C1r, C1s, C2, C2a, C2b, C3, C3a, C3b, C4, C4a, C4b, C5, C5a, C5b, C6, C7, C8, C9, factor B, factor D, factor H, properdin, sclerostin, fibrinogen, fibrin, prothrombin, thrombin, tissue factor, factor V, factor Va, factor VII, factor VIIa, factor VIII, factor VIIa, factor IX, factor IXa, factor X, factor Xa, factor XI, factor XIa, factor XII, factor XIIa, factor XIII, factor XIIIa, TFPI, antithrombin III, EPCR, thrombomodulin, TAPI, tPA, plasminogen, plasmin, PAI-1, PAI-2, GPC3, Syndecan-1, Syndecan-2, Syndecan-3, Syndecan-4, LPA, and S1P; and receptors for hormone and growth factors.

[0321] As discussed herein, one or more amino acid residue alterations are allowed in the amino acid sequences constituting the variable regions as long as their antigen-binding activities are maintained. When altering a variable region amino acid sequence, there is no particularly limitation on the site of alteration and number of amino acids altered. For example, amino acids present in CDR and/or FR can be altered appropriately. When altering amino acids in a variable region, the binding activity is preferably maintained without particular limitation; and for example, as compared to before alteration, the binding activity may be 50% or more, 80% or more, and 100% or more. Furthermore, the binding activity may be increased by amino acid alterations. For example, the binding activity may be 2-, 5-, 10-times higher or such than that before alteration. Alteration of amino acid sequence may be at least one of amino acid residue substitution, addition, deletion, and modification.

[0322] For example, the modification of the N-terminal glutamine of a variable region into pyroglutamic acid by pyroglutamylation is a modification well known to those skilled in the art. Thus, when the heavy-chain N terminus is glutamine, antibodies described herein may comprise the variable regions in which the glutamine is modified to pyroglutamic acid.

- [0323] Antibody variable regions described herein may have any sequences, and they may be antibody variable regions of any origin, such as mouse antibodies, rat antibodies, rabbit antibodies, goat antibodies, camel antibodies, humanized antibodies produced by humanizing these non-human antibodies, and human antibodies. Furthermore, these antibodies may have various amino acid substitutions introduced into their variable regions to improve their antigen binding, pharmacokinetics, stability, and immunogenicity. Variable regions may be able to bind antigens repeatedly due to their pH dependency in antigen binding (WO 2009/125825).
- [0324] kappa chain and lambda chain are present in antibody light-chain constant regions, and either one is acceptable. Furthermore, they may have some amino acid alterations such as substitutions, deletions, additions, and/or insertions.
- [0325] Furthermore, polypeptides comprising variant Fc regions described herein may be made into Fc fusion proteins by linking to other proteins such as physiologically active peptides. Such fusion proteins may be a multimer of at least two polypeptides comprising the variant Fc regions. Examples of the other proteins include receptors, adhesion molecules, ligands, and enzymes, but are not limited thereto.
- [0326] Examples of Fc fusion proteins include proteins fused with an Fc region to a receptor that binds to a target molecule, including TNFR-Fc fusion protein, IL1R-Fc fusion protein, VEGFR-Fc fusion protein, and CTLA4-Fc fusion protein (Economides et al., Nat. Med. 9(1):47-52 (2003); Dumont et al., BioDrugs. 20(3):151-60 (2006)). Furthermore, proteins to be fused may be other molecules that have a target-binding activity, for example, scFvs (WO 2005/037989), single-domain antibodies (WO 2004/058821; WO 2003/002609), antibody-like molecules (Davinder, Curr. Op. Biotech. 17:653-658 (2006); Current Opinion in Biotechnology 18:1-10 (2007); Nygren et al., Curr. Op. Struct. Biol. 7:463-469 (1997); and Hoss. Protein Science 15:14-27 (2006)) such as DARPins (WO 2002/020565), Affibody (WO 1995/001937), Avimer (WO 2004/044011; WO 2005/040229), and Adnectin (WO 2002/032925). Furthermore, antibodies and Fc fusion proteins may be multispecific and may bind to multiple types of target molecules or epitopes.

B. Recombinant Methods and Compositions

- [0327] Antibodies may be produced using recombinant methods and compositions, e.g., as described in US Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-myostatin antibody described herein is provided. In another embodiment, isolated nucleic acid encoding a polypeptide comprising a variant Fc region or a parent Fc region described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic

acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, e.g., a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., a YO, NS0, and Sp20 cell). In one embodiment, a method of making an anti-myostatin antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium). In another embodiment, a method of making a polypeptide comprising a variant Fc region or a parent Fc region is provided, wherein the method comprises culturing a host cell comprising the nucleic acid(s) encoding a polypeptide such as, an antibody, Fc region, or variant Fc region, as provided above, under conditions suitable for expression of the polypeptide, and optionally recovering the polypeptide from the host cell (or host cell culture medium).

[0328] For recombinant production of an anti-myostatin antibody, nucleic acids encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. For recombinant production of an Fc region, nucleic acid encoding an Fc region is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0329] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., US Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also, Charlton, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0330] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi

and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

- [0331] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.
- [0332] Plant cell cultures can also be utilized as hosts. See, e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).
- [0333] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR- CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).
- [0334] Antibodies with pH-dependent characteristics may be obtained using screening methods and/or mutagenesis methods e.g., as described in WO 2009/125825. The screening methods may comprise any process by which an antibody having pH-dependent binding characteristics is identified within a population of antibodies specific for a particular antigen. In certain embodiments, the screening methods may comprise measuring one or more binding parameters (e.g., KD or kd) of individual antibodies within an initial population of antibodies both at acidic and neutral pH. The binding parameters of the antibodies may be measured using, e.g., surface plasmon resonance, or any other analytic method that allows for the quantitative or qualitative assessment of the binding characteristics of an antibody to a particular antigen. In

certain embodiments, the screening methods may comprise identifying an antibody that binds to an antigen with an acidic/neutral KD ratio of 2 or greater. In further embodiments, the screening methods may comprise identifying an antibody that binds to an antigen with a pH5.8/pH7.4 KD ratio of 2 or greater. Alternatively, the screening methods may comprise identifying an antibody that binds to an antigen with an acidic/neutral kd ratio of 2 or greater. In further embodiments, the screening methods may comprise identifying an antibody that binds to an antigen with a pH5.8/pH7.4 kd ratio of 2 or greater.

[0335] In another embodiment, the mutagenesis methods may comprise incorporating a deletion, substitution, or addition of an amino acid within the heavy and/or light chain of the antibody to enhance the pH-dependent binding of the antibody to an antigen. In certain embodiments, the mutagenesis may be carried out within one or more variable domains of the antibody, e.g., within one or more HVRs (e.g., CDRs). For example, the mutagenesis may comprise substituting an amino acid within one or more HVRs (e.g., CDRs) of the antibody with another amino acid. In certain embodiments, the mutagenesis may comprise substituting one or more amino acids in at least one HVR (e.g., CDR) of the antibody with a histidine. In certain embodiments, "enhanced pH-dependent binding" means that the mutated version of the antibody exhibits a greater acidic/neutral KD ratio, or a greater acidic/neutral kd ratio, than the original "parent" (i.e., the less pH-dependent) version of the antibody prior to mutagenesis. In certain embodiments, the mutated version of the antibody has an acidic/neutral KD ratio of 2 or greater. In certain embodiments, the mutated version of the antibody has a pH5.8/pH7.4 KD ratio of 2 or greater. Alternatively, the mutated version of the antibody has an acidic/neutral kd ratio of 2 or greater. In further embodiments, the mutated version of the antibody has a pH5.8/pH7.4 kd ratio of 2 or greater.

[0336] Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl_2 , or $\text{R}^1\text{N}=\text{C}=\text{NR}$, where R and R^1 are different alkyl groups.

[0337] Animals (usually non-human mammals) are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 micro g or 5 micro g of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or

conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

- [0338] Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translational modifications (e.g., isomerizations, amidations) that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.
- [0339] For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., *Nature* 256(5517):495-497 (1975). In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro.
- [0340] The immunizing agent will typically include the antigenic protein or a fusion variant thereof. Generally either peripheral blood lymphocytes (PBLs) are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press (1986), pp. 59-103).
- [0341] Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which are substances that prevent the growth of HGPRT-deficient cells.
- [0342] Preferred immortalized myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred are murine

myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 cells (and derivatives thereof, e.g., X63-Ag8-653) available from the American Type Culture Collection, Manassas, Virginia USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor et al. J. Immunol. 133(6):3001-3005 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, pp. 51-63 (1987)).

- [0343] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). Such techniques and assays are known in the art. For example, binding affinity may be determined by the Scatchard analysis of Munson, Anal. Biochem. 107(1):220-239 (1980).
- [0344] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, supra). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as tumors in a mammal.
- [0345] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxyapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.
- [0346] Antibodies may be produced by immunizing an appropriate host animal against an antigen. In one embodiment, the antigen is a polypeptide comprising a full-length myostatin. In one embodiment, the antigen is a polypeptide comprising latent myostatin. In one embodiment, the antigen is a polypeptide comprising a myostatin propeptide. In one embodiment, the antigen is a polypeptide comprising the region corresponding to the amino acids at positions 21 to 100 of myostatin propeptide (SEQ ID NO: 78). In one embodiment, the antigen is a polypeptide comprising amino acids at positions 21-80, 41-100, 21-60, 41-80, 61-100, 21-40, 41-60, 61-80, or 81-100 of myostatin propeptide (SEQ ID NO: 78). Also included in the present invention are antibodies produced by immunizing an animal against the antigen. The antibodies may incorporate any of the features, singly or in combination, as described in "Exemplary Anti-myostatin Antibodies" above.
- [0347] An Fc region may be obtained by re-eluting the fraction adsorbed onto protein A column after partially digesting IgG1, IgG2, IgG3, IgG4 monoclonal antibodies or

such using a protease such as pepsin. The protease is not particularly limited as long as it can digest a full-length antibody so that Fab and F(ab')₂ will be produced in a restrictive manner by appropriately setting the enzyme reaction conditions such as pH, and examples include pepsin and papain.

[0348] Furthermore, the present invention provides a method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity in comparison with a polypeptide comprising a parent Fc region, which comprises introducing at least one amino acid alteration to the parent Fc region. In some aspects, the produced polypeptide is an antibody. In certain embodiments, an antibody is a chimeric antibody, or a humanized antibody. In some aspects, the produced polypeptide is an Fc fusion protein.

[0349] In certain embodiments, the production method comprises the following steps: (a) preparing a polypeptide comprising a parent Fc region; (b) introducing at least one amino acid alteration to the parent Fc region within the polypeptide; (c) measuring the monkey Fc gamma RIIb-binding activities of the polypeptide comprising the parent Fc region and the polypeptide comprising the Fc region in step (b); and (d) selecting a polypeptide comprising a variant Fc region with enhanced monkey Fc gamma RIIb-binding activity in comparison with the polypeptide comprising the parent Fc region.

[0350] In certain embodiments, the production method comprises the following steps: (a) preparing a polypeptide comprising a parent Fc region; (b) introducing at least one amino acid alteration to the parent Fc region within the polypeptide; (c) measuring the monkey Fc gamma RIIIa-binding activities of the polypeptide comprising the parent Fc region and the polypeptide comprising the Fc region in step (b); and (d) selecting a polypeptide comprising a variant Fc region with decreased monkey Fc gamma RIIIa-binding activity in comparison with the polypeptide comprising the parent Fc region.

[0351] In certain embodiments, the production method comprises the following steps: (a) preparing a polypeptide comprising a parent Fc region; (b) introducing at least one amino acid alteration to the parent Fc region within the polypeptide; (c) measuring the human Fc gamma RIIb-binding activities of the polypeptide comprising the parent Fc region and the polypeptide comprising the Fc region in step (b); and (d) selecting a polypeptide comprising a variant Fc region with enhanced human Fc gamma RIIb-binding activity in comparison with the polypeptide comprising the parent Fc region.

[0352] In certain embodiments, the production method comprises the following steps: (a) preparing a polypeptide comprising a parent Fc region; (b) introducing at least one amino acid alteration to the parent Fc region within the polypeptide; (c) measuring the human Fc gamma RIIIa-binding activities of the polypeptide comprising the parent Fc region and the polypeptide comprising the Fc region in step (b); and (d) selecting a polypeptide comprising a variant Fc region with decreased human Fc gamma RIIIa-

- binding activity in comparison with the polypeptide comprising the parent Fc region.
- [0353] In certain embodiments, the production method comprises the following steps: (a) preparing a polypeptide comprising a parent Fc region; (b) introducing at least one amino acid alteration to the parent Fc region within the polypeptide; (c) measuring the human Fc gamma RIIa (type H)-binding activities of the polypeptide comprising the parent Fc region and the polypeptide comprising the Fc region in step (b); and (d) selecting a polypeptide comprising a variant Fc region with decreased human Fc gamma RIIa (type H)-binding activity in comparison with the polypeptide comprising the parent Fc region.
- [0354] In certain embodiments, the production method comprises the following steps: (a) preparing a polypeptide comprising a parent Fc region; (b) introducing at least one amino acid alteration to the parent Fc region within the polypeptide; (c) measuring the human Fc gamma RIIa (type R)-binding activities of the polypeptide comprising the parent Fc region and the polypeptide comprising the Fc region in step (b); and (d) selecting a polypeptide comprising a variant Fc region with decreased human Fc gamma RIIa (type R)-binding activity in comparison with the polypeptide comprising the parent Fc region.
- [0355] In one aspect, at least one amino acid is altered in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, 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. In certain embodiments, the Fc gamma RIIb has the sequence of cynomolgus monkey Fc gamma RIIb (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIb has the sequence of human Fc gamma RIIb (e.g., SEQ ID NOS:212, 213, or 214).
- [0356] In another aspect, one amino acid is altered in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, at position 236.
- [0357] In another aspect, at least two amino acids are altered in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, the 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. In certain embodiments, the Fc gamma RIIb has the sequence of cynomolgus monkey Fc gamma RIIb (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIb has the sequence of human Fc gamma RIIb (e.g., SEQ ID NOS:212, 213, or 214).

- [0358] In another aspect, at least two amino acids are altered in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, the 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. In certain embodiments, the Fc gamma RIIB has the sequence of cynomolgus monkey Fc gamma RIIB (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIB has the sequence of human Fc gamma RIIB (e.g., SEQ ID NOS:212, 213, or 214).
- [0359] In another aspect, at least two amino acids are altered in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, the 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. In certain embodiments, the Fc gamma RIIB has the sequence of cynomolgus monkey Fc gamma RIIB (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIB has the sequence of human Fc gamma RIIB (e.g., SEQ ID NOS:212, 213, or 214).
- [0360] In another aspect, amino acids are altered in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, at positions of any one of the following (1)-(37): (1) at positions 231, 236, 239, 268 and 330; (2) at positions 231, 236, 239, 268, 295 and 330; (3) at positions 231, 236, 268 and 330; (4) at positions 231, 236, 268, 295 and 330; (5) at positions 232, 236, 239, 268, 295 and 330; (6) at positions 232, 236, 268, 295 and 330; (7) at positions 232, 236, 268 and 330; (8) at positions 235, 236, 268, 295, 326 and 330; (9) at positions 235, 236, 268, 295 and 330; (10) at positions 235, 236, 268 and 330; (11) at positions 235, 236, 268, 330 and 396; (12) at positions 235, 236, 268 and 396; (13) at positions 236, 239, 268, 295, 298 and 330; (14) at positions 236, 239, 268, 295, 326 and 330; (15) at positions 236, 239, 268, 295 and 330; (16) at positions 236, 239, 268, 298 and 330; (17) at positions 236, 239, 268, 326 and 330; (18) at positions 236, 239, 268 and 330; (19) at positions 236, 239, 268, 330 and 396; (20) at positions 236, 239, 268 and 396; (21) at positions 236 and 268; (22) at positions 236, 268 and 295; (23) at positions 236, 268, 295, 298 and 330; (24) at positions 236, 268, 295, 326 and 330; (25) at positions 236, 268, 295, 326, 330 and 396; (26) at positions 236, 268, 295 and 330; (27) at positions 236, 268, 295, 330 and 396; (28) at positions 236, 268, 298 and 330; (29) at positions 236, 268, 298 and 396; (30) at positions 236, 268, 326 and 330; (31) at positions 236, 268, 326, 330 and 396; (32) at positions 236, 268 and 330; (33) at positions 236, 268, 330 and 396; (34) at positions 236, 268 and 396; (35)

at positions 236 and 295; (36) at positions 236, 330 and 396; and (37) at positions 236 and 396; according to EU numbering. In certain embodiments, the Fc gamma RIIb has the sequence of cynomolgus monkey Fc gamma RIIb (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIb has the sequence of human Fc gamma RIIb (e.g., SEQ ID NOS:212, 213, or 214).

[0361] In a further aspect, an amino acid alteration in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, is selected at each position 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. In certain embodiments, the Fc gamma RIIb has the sequence of cynomolgus monkey Fc gamma RIIb (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIb has the sequence of human Fc gamma RIIb (e.g., SEQ ID NOS:212, 213, or 214).

[0362] In a further aspect, an amino acid alteration in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, is selected at each position 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. In certain embodiments, the Fc gamma RIIb has the sequence of cynomolgus monkey Fc gamma RIIb (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIb has the sequence of human Fc gamma RIIb (e.g., SEQ ID NOS:212, 213, or 214).

In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, are: Asn at position 236, Glu at position 268, Lys at position 330, and Met at position 396; according to EU numbering. In a further aspect, amino

acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, are: Asn at position 236, Asp at position 268, and Lys at position 330; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, are: Asn at position 236, Asp at position 268, Leu at position 295, and Lys at position 330; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, are: Thr at position 236, Asp at position 268, and Lys at position 330; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, are: Asn at position 236, Asp at position 268, Leu at position 295, Thr at position 326, and Lys at position 330; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, are: Trp at position 235, Asn at position 236, Asp at position 268, Leu at position 295, Thr at position 326, and Lys at position 330; according to EU numbering.

[0363] In one aspect, at least one amino acid is altered in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, of at least one position selected from the group consisting of: 234, 238, 250, 264, 267, 307, and 330 according to EU numbering. In certain embodiments, the Fc gamma RIIb has the sequence of cynomolgus monkey Fc gamma RIIb (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIb has the sequence of human Fc gamma RIIb (e.g., SEQ ID NOS:212, 213, or 214).

[0364] In another aspect, one amino acid is altered in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, at position 238.

[0365] In another aspect, at least two amino acids are altered in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIb-binding activity, the alterations comprising: (i) one amino acid alteration at position 238, and (ii) at least one amino acid alteration of at least one position selected from the group consisting of: 234, 250, 264, 267, 307, and 330 according to EU numbering. In certain embodiments, the Fc gamma RIIb has the sequence of cynomolgus monkey Fc gamma RIIb (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIb has the sequence of human Fc gamma RIIb (e.g., SEQ ID NOS:212, 213, or 214).

[0366] In another aspect, amino acids are altered in the above-mentioned method for

producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, at positions of any one of the following (1)-(9): (1) at positions 234, 238, 250, 307 and 330; (2) at positions 234, 238, 250, 264, 307 and 330; (3) at positions 234, 238, 250, 264, 267, 307 and 330; (4) at positions 234, 238, 250, 267, 307 and 330; (5) at positions 238, 250, 264, 307 and 330; (6) at positions 238, 250, 264, 267, 307 and 330; (7) at positions 238, 250, 267, 307 and 330; (8) at positions 238, 250 and 307; and (9) at positions 238, 250, 307 and 330; according to EU numbering.

[0367] In a further aspect, an amino acid alteration in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, is selected at each position from the group consisting of: (a) Tyr at position 234; (b) Asp at position 238; (c) Val at position 250; (d) Ile at position 264; (e) Ala at position 267; (f) Pro at position 307; and (g) Lys at position 330; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, are: Asp at position 238; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, are: Asp at position 238, Val at position 250, and Pro at position 307; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, are: Asp at position 238, Val at position 250, Pro at position 307, and Lys at position 330; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, are: Asp at position 238, Val at position 250, Ile at position 264, Pro at position 307, and Lys at position 330; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, are: Asp at position 238, Val at position 250, Ala at position 267, Pro at position 307, and Lys at position 330; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, are: Tyr at position 234, Asp at position 238, Val at position 250, Pro at position 307, and Lys at position 330; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, are: Tyr at position 234, Asp at position 238, Val at position 250, Ala at position 267, Pro at position 307, and Lys at

position 330; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, are: Asp at position 238, Val at position 250, Ile at position 264, Ala at position 267, Pro at position 307, and Lys at position 330; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, are: Tyr at position 234, Asp at position 238, Val at position 250, Ile at position 264, Pro at position 307, and Lys at position 330; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with enhanced Fc gamma RIIB-binding activity, are: Tyr at position 234, Asp at position 238, Val at position 250, Ile at position 264, Ala at position 267, Pro at position 307, and Lys at position 330; according to EU numbering. In certain embodiments, the Fc gamma RIIB has the sequence of cynomolgus monkey Fc gamma RIIB (SEQ ID NO:223). In certain embodiments, the Fc gamma RIIB has the sequence of human Fc gamma RIIB (e.g., SEQ ID NOS:212, 213, or 214).

- [0368] Furthermore, the present invention provides a method for producing a polypeptide comprising a variant Fc region with increased pI in comparison with a polypeptide comprising a parent Fc region, which comprises introducing at least two amino acid alterations to the parent Fc region.
- [0369] In certain embodiments, the production method for producing a polypeptide comprising a variant Fc region provided herein comprises the following steps: (a) preparing a polypeptide comprising a parent Fc region; (b) introducing at least two amino acid alterations to the parent Fc region within the polypeptide; (c) measuring the pI's of the polypeptide comprising the parent Fc region and the polypeptide comprising the Fc region in step (b); and (d) selecting a polypeptide comprising a variant Fc region with increased pI in comparison with the corresponding polypeptide comprising the parent Fc region.
- [0370] In one aspect, at least two amino acids are altered in the above-mentioned method for producing a polypeptide comprising a variant Fc region with increased pI, 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 a further aspect, the produced polypeptide comprises a variant Fc region with increased pI, which comprises at least two amino acid alterations of at least two positions selected from the group consisting of: 311, 341, 343, 384, 399, 400, 401, 402, and 413, according to EU numbering.
- [0371] In another aspect, amino acids are altered in the above-mentioned method for producing a polypeptide comprising a variant Fc region with increased pI, at positions

of any one of the following (1)-(10): (1) at positions 311 and 341; (2) at positions 311 and 343; (3) at positions 311, 343 and 413; (4) at positions 311, 384 and 413; (5) at positions 311 and 399; (6) at positions 311 and 401; (7) at positions 311 and 413; (8) at positions 400 and 413; (9) at positions 401 and 413; and (10) at positions 402 and 413; according to EU numbering.

In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with increased pI, are: Arg at position 400 and Lys at position 413; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with increased pI, are: Arg at position 311 and Lys at position 413; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with increased pI, are: Arg at position 311 and Arg at position 399; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with increased pI, are: Arg at position 311 and Arg at position 343; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with increased pI, are: Arg at position 311 and Arg at position 413; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with increased pI, are: Arg at position 311, Arg at position 343, and Arg at position 413; according to EU numbering. In a further aspect, amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with increased pI, are: Arg at position 311, Arg at position 384, and Arg at position 413; according to EU numbering.

[0372] In a further aspect, the amino acid alterations in the above-mentioned method for producing a polypeptide comprising a variant Fc region with increased pI, are selected from Lys or Arg at each position.

[0373] In a further aspect, the amino acid alterations in the above-mentioned production methods are selected from any single alteration, combination of single alterations, or combination alterations described in Tables 14-30.

[0374] Optionally, the following additional steps can be included in the above-mentioned methods for producing a polypeptide comprising a variant Fc region, when the polypeptide further comprises an antigen-binding domain: (e) assessing pharmacokinetic of the antigen in plasma, after administration of the polypeptide comprising the parent Fc region and the polypeptide comprising the variant Fc region in animals such as mice, rats, rabbits, dogs, monkeys, and humans; and (f) selecting a polypeptide comprising a variant Fc region with enhanced ability of antigen elimination from

plasma in comparison with the polypeptide comprising the parent Fc region.

- [0375] Polypeptides comprising a variant Fc region produced by any of the above-mentioned methods or other methods known in the art are included in the present invention.

C. Assays

- [0376] Anti-myostatin antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

- [0377] Variant Fc regions provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays described herein or otherwise known in the art.

1. Binding assays and other assays

- [0378] In one aspect, an antibody of the invention is tested for its antigen binding activity, by known methods such as ELISA, Western blot, BIAcore (registered trademark), etc. In one aspect, a polypeptide comprising a variant Fc region of the invention is tested for its Fc receptor binding activity, by known methods such as BIAcore (registered trademark), etc.
- [0379] In another aspect, competition assays may be used to identify an antibody that competes for binding to myostatin with any anti-myostatin antibody described herein. In certain embodiments, when such a competing antibody is present in excess, it blocks (e.g., reduces) the binding of a reference antibody to myostatin by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or more. In some instances, binding is inhibited by at least 80%, 85%, 90%, 95%, or more. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by an anti-myostatin antibody described herein (e.g., an anti-myostatin antibody described in Tables 2a, 11a, or 13). In further aspects, the reference antibody has a VH and a VL pair described in Table 2a, 11a, or 13. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, NJ).
- [0380] In an exemplary competition assay, immobilized latent myostatin or myostatin propeptide is incubated in a solution comprising a first labeled antibody that binds to the myostatin and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to the myostatin. The second antibody may be present in a hybridoma supernatant. As a control, immobilized myostatin is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to the myostatin, excess unbound antibody is removed, and the amount

of label associated with immobilized myostatin is measured. If the amount of label associated with immobilized myostatin is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to the myostatin. See, Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

- [0381] Assays for determining the binding activity of a polypeptide containing a variant Fc region towards one or more Fc gamma R family members are described herein or otherwise known in the art. Such binding assays include but are not limited to BIACORE (registered trademark) analysis, which utilizes the surface plasmon resonance (SPR) phenomena, Amplified Luminescent Proximity Homogeneous Assay (ALPHA) screening, ELISA, and fluorescence activated cell sorting (FACS) (Lazar et al., *Proc. Natl. Acad. Sci. USA* (2006) 103(11): 4005-4010).
- [0382] In one embodiment, BIACORE (registered trademark) analysis can be used to evaluate whether the binding activity of a polypeptide comprising a variant Fc region is enhanced, or maintained or decreased with respect to a particular Fc gamma R family member, for example, by observing whether there is a decrease or an increase in the dissociation constant (KD) value obtained from sensorgram analysis, where various Fc gamma Rs are subjected to interaction as an analyte with polypeptides comprising a variant Fc region immobilized or captured onto the sensor chip using known methods and reagents (such as Protein A, Protein L, Protein A/G, Protein G, anti-lambda chain antibodies, anti-kappa chain antibodies, antigenic peptides, antigenic proteins). Alterations in binding activity can also be determined by comparing changes in the resonance unit (RU) value on the sensorgram before and after the one or more types of Fc gamma Rs are subjected to interaction as analytes with the captured polypeptides comprising the variant Fc region. Alternatively, Fc gamma R can be immobilized or captured onto the sensor chips, and the polypeptides comprising the variant Fc region are used as an analyte.
- [0383] In BIACORE (registered trademark) analysis, one of the substances (the ligand) in observation of an interaction is immobilized onto a gold thin film on a sensor chip, and by shining light from the reverse side of the sensor chip so that total reflection takes place at the interface between the gold thin film and glass, a portion of reduced reflection intensity is formed in part of the reflected light (SPR signal). When the other one of the substances (the analyte) in observation of an interaction is made to flow on the sensor chip surface and the ligand binds to the analyte, the mass of the immobilized ligand molecule increases and the refractive index of the solvent on the sensor chip surface changes. The position of the SPR signal shifts as a result of this change in refractive index (on the other hand, the signal position returns when this binding dis-

sociates). The BIACORE (registered trademark) system indicates the amount of shift mentioned above, or more specifically the time variable of mass by plotting the change in mass on the sensor chip surface on the ordinate as the measurement data (sensorgram). The amount of analyte bound to the ligand trapped on the sensor chip surface is determined from the sensorgram. Kinetic parameters such as association rate constants (k_a) and dissociation rate constants (k_d) are determined from the curves of the sensorgram, and the dissociation constants (K_D) are determined from the ratio of these constants. In the BIACORE (registered trademark) method, a method for measuring inhibition is preferably used. An example of the method for measuring inhibition is described in Lazar et al., Proc. Natl. Acad. Sci. USA 103(11):4005-4010 (2006).

[0384] ALPHA screening is performed by ALPHA technology which uses two beads, a donor and an acceptor, based on the following principles. Luminescent signals are detected only when molecules bound to donor beads physically interact with molecules bound to the acceptor beads, and the two beads are in close proximity to each other. Laser-excited photosensitizer in the donor beads converts ambient oxygen to excited-state singlet oxygen. Singlet oxygen is dispersed around the donor beads, and when it reaches the adjacent acceptor beads, chemiluminescent reaction is induced in the beads, and light is ultimately emitted. When the molecules bound to the donor beads do not interact with the molecules bound to the acceptor beads, the chemiluminescent reaction does not take place because singlet oxygen produced by the donor beads does not reach the acceptor beads.

[0385] For example, a biotinylated polypeptide complex is bound to the donor beads, and Fc gamma receptor tagged with glutathione S transferase (GST) is linked to the acceptor beads. In the absence of a competing polypeptide complex comprising a variant Fc region, the polypeptide complex comprising a parent Fc region interacts with the Fc gamma receptor and produces 520-620 nm signals. The polypeptide complex comprising an untagged variant Fc region competes with the polypeptide complex comprising a parent Fc region for interaction with the Fc gamma receptor. Relative binding activities can be determined by quantifying the decrease in fluorescence observed as a result of the competition. Biotinylation of polypeptide complexes such as antibodies using Sulfo-NHS-biotin and such is well known. The method of expressing the Fc gamma receptor and GST in a cell carrying a fusion gene produced by fusing a polynucleotide encoding the Fc gamma receptor in frame with a polynucleotide encoding GST in an expressible vector, and performing purification using a glutathione column is appropriately adopted as a method for tagging an Fc gamma receptor with GST. The obtained signals are preferably analyzed, for example, by fitting them to a one-site competition model which uses a non-linear regression analysis using software

such as GRAPHPAD PRISM (GraphPad, San Diego).

- [0386] A variant Fc region with decreased Fc gamma R-binding activity refers to an Fc region which binds to Fc gamma R with essentially weaker binding activity than a parent Fc region when assays are performed using substantially the same amount of a corresponding parent Fc region and a variant Fc region. Furthermore, a variant Fc region with enhanced Fc gamma R-binding activity refers to an Fc region which binds to Fc gamma R with essentially stronger binding activity than a corresponding parent Fc region when assays are performed using substantially the same amount of a parent Fc region and a variant Fc region. A variant Fc region with maintained Fc gamma R-binding activity refers to an Fc region that binds to Fc gamma R with binding activity equivalent to or essentially not different from that of a parent Fc region when assays are performed using substantially the same amount of the corresponding parent Fc region and the polypeptide containing the variant Fc region.
- [0387] Whether or not the binding activities of an Fc region towards various Fc gamma Rs were enhanced or decreased can be determined from the increase or decrease in the amount of binding of the various Fc gamma Rs to the Fc region, which were determined according to the above-mentioned measurement method. Here, the amount of binding of the various Fc gamma Rs to the Fc region can be evaluated as a value obtained by dividing the difference in the RU values of sensorgrams that changed before and after interaction of various Fc gamma Rs as the analyte with the Fc region, by the difference in the RU values of sensorgrams that changed before and after capturing the Fc regions to the sensor chips.
- [0388] In the present invention, enhanced Fc gamma RIIB-binding activity preferably means, for example, that the ratio of [KD value of a parent Fc region for Fc gamma RIIB]/[KD value of a variant Fc region for Fc gamma RIIB] in the KD values measured by the above-mentioned measurement method preferably becomes 2.0 or greater, 3.0 or greater, 4.0 or greater, 5.0 or greater, 6.0 or greater, 7.0 or greater, 8.0 or greater, 9.0 or greater, 10 or greater, 15 or greater, 20 or greater, 25 or greater, 30 or greater, 35 or greater, 40 or greater, 45 or greater, or even 50 or greater, 55 or greater, 60 or greater, 65 or greater, 70 or greater, 75 or greater, 80 or greater, 85 or greater, 90 or greater, 95 or greater, or 100 or greater.
- [0389] The KD value (mol/L) of a variant Fc region described herein for Fc gamma RIIB is preferably less than that of a parent Fc region for Fc gamma RIIB, and may be, for example, 2.0×10^{-6} M or smaller, 1.0×10^{-6} M or smaller, 9.0×10^{-7} M or smaller, 8.0×10^{-7} M or smaller, 7.0×10^{-7} M or smaller, 6.0×10^{-7} M or smaller, 5.0×10^{-7} M or smaller, 4.0×10^{-7} M or smaller, 3.0×10^{-7} M or smaller, 2.0×10^{-7} M or smaller, 1.0×10^{-7} M or smaller, 9.0×10^{-8} M or smaller, 8.0×10^{-8} M or smaller, 7.0×10^{-8} M or smaller, 6.0×10^{-8} M or smaller, 5.0×10^{-8} M or smaller.

[0390] Furthermore, a variant Fc region with enhanced binding selectivity to Fc gamma RIIb compared to Fc gamma RIIa refers to an Fc region where: (a) Fc gamma RIIb-binding activity is enhanced, and Fc gamma RIIa-binding activity is maintained or decreased; (b) Fc gamma RIIb-binding activity is enhanced and Fc gamma RIIa-binding activity is also enhanced, but the degree of enhancement of Fc gamma RIIa-binding activity is lower than the degree of enhancement of Fc gamma RIIb-binding activity; or (c) Fc gamma RIIb-binding activity is decreased and Fc gamma RIIa-binding activity is also decreased, but the degree of decrease of Fc gamma RIIb-binding activity is less than the degree of decrease of Fc gamma RIIa-binding activity. Whether or not a variant Fc region has improved binding selectivity for Fc gamma RIIb rather than for Fc gamma RIIa can be determined, for example, by comparing the ratio of the KD value for Fc gamma RIIa to the KD value for Fc gamma RIIb of the variant Fc region (the ratio of [KD value of the variant Fc region for Fc gamma RIIa]/[KD value of the variant Fc region for Fc gamma RIIb]), with the ratio of the KD value for Fc gamma RIIa to the KD value for Fc gamma RIIb of the parent Fc region (the ratio of [KD value of the parent Fc region for Fc gamma RIIa]/[KD value of the parent Fc region for Fc gamma RIIb]), which were determined according to the above-mentioned examples. Specifically, when the KD ratio for the variant Fc region is greater than that of the parent Fc region, the variant Fc region can be determined to have an improved binding selectivity for Fc gamma RIIb rather than for Fc gamma RIIa in comparison with the parent Fc region. Especially in human, the Fc gamma RIIb-binding activity is likely to correlate with binding activity to Fc gamma RIIa (type R) than to Fc gamma RIIa (type H) since the amino acid sequence of Fc gamma RIIb shares higher identity with Fc gamma RIIa (type R) than Fc gamma RIIa (type H). Therefore, finding amino acid alteration(s) that can enhance binding selectivity to human Fc gamma RIIb compared to human Fc gamma RIIa (type R) is important for enhancing binding selectivity to Fc gamma RIIb compared to Fc gamma RIIa in humans.

[0391] When a variant Fc region of the present invention has higher binding activity against Fc gamma RIIb and lower binding activity against Fc gamma RIII (such as Fc gamma RIIIa or Fc gamma RIIIb) compared to those of a parent Fc region, the variant Fc region can be said to be Fc gamma RIIb-specific.

2. Activity assays

[0392] In one aspect, assays are provided for identifying anti-myostatin antibodies having biological activity. Biological activity may include, e.g., inhibiting the activation of myostatin, blocking the release of mature myostatin from latent myostatin, inhibiting the proteolytic cleavage of latent myostatin, blocking the access of a protease to latent myostatin, etc. Antibodies having such biological activity in vivo and/or in vitro are

also provided. In certain embodiments, an antibody of the invention is tested for such biological activity.

[0393] In certain embodiments, whether a test antibody inhibits the cleavage of latent myostatin is determined by detecting the cleavage product of latent myostatin (myostatin) using a method known in the art such as electrophoresis, chromatography, immunoblot analysis, an enzyme-linked immunosorbent assay (ELISA), or mass spectrometry, after a protease that can cleave latent myostatin is contacted with the latent myostatin in the presence or absence of the test antibody (see, for example, Thies et al., Growth Factors 18(4):251-259 (2001)). Where a decreased amount of the cleavage product of latent myostatin (e.g., human myostatin propeptide) is detected in the presence of (or following contact with) the test antibody, the test antibody is identified as an antibody that can inhibit the cleavage of latent myostatin. In certain embodiments, whether a test antibody blocks access of a protease to latent myostatin is determined by methods for the detection of protein interactions between the protease and latent myostatin, e.g., ELISAs or BIACORE (registered trademark). Where a decreased interaction between the protease and latent myostatin is detected in the presence of (or following contact with) the test antibody, the test antibody is identified as an antibody that can block access of the protease to latent myostatin.

[0394] In certain embodiments, whether a test antibody blocks the release of mature myostatin from latent myostatin is determined by detecting mature myostatin activity, for example, the activity of binding to a myostatin receptor, or the activity of mediating signal transduction in a cell expressing a myostatin receptor (e.g., ActRIIb). Cells useful for such an assay can be those that express an endogenous myostatin receptor, for example, L6 myocytes, or can be those that are genetically modified, transiently or stably, to express a transgene encoding a myostatin receptor, for example, an activin receptor such as an activin type II receptor (Thies et al, Supra). Binding of myostatin to a myostatin receptor can be detected using a receptor binding assay. Myostatin mediated signal transduction can be detected at any level in the signal transduction pathway, for example, by examining phosphorylation of a Smad polypeptide, examining expression of a myostatin regulated gene including a reporter gene, or measuring proliferation of a myostatin-dependent cell. Where a decreased mature myostatin activity is detected in the presence of (or following contact with) the test antibody, the test antibody is identified as an antibody that can block the release of mature myostatin from latent myostatin.

[0395] Inhibition of myostatin activation can also be detected and/or measured using the methods set forth and exemplified in the working examples. Using assays of these or other suitable types, test antibodies can be screened for those capable of inhibiting the activation of myostatin. In certain embodiments, inhibition of myostatin activation

includes at least a 5%, 10%, 15%, 20%, 25%, 30%, 35%, or 40% or greater decrease in myostatin activation in the assay as compared to a negative control under similar conditions. In some embodiments, it refers to the inhibition of myostatin activation of at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or greater.

[0396] In another aspect, assays for identifying anti-myostatin antibodies that form an immune complex (i.e., an antigen-antibody complex) with myostatin are provided. Antibodies having such biological activity in vivo and/or in vitro are also provided.

[0397] In certain embodiments, an antibody of the invention is tested for such biological activity.

[0398] In certain embodiments, the formation of an immune complex is evaluated by a method such as size exclusion (gel filtration) chromatography, ultracentrifugation, light scattering, electron microscope, or mass spectrometry (Mol. Immunol. 39:77-84 (2002), Mol. Immunol. 47:357-364 (2009)). These methods make use of the property that an immune complex is a larger molecule than an antibody alone, or an antigen alone. Where a large complex containing two or more antibodies and two or more antigens (e.g., myostatin molecules) is detected in the presence of a test antibody and an antigen, the test antibody is identified as an antibody that can form an immune complex containing two or more antibodies and two or more molecules of myostatin. In another embodiment, formation of an immune complex is evaluated by a method such as, ELISA, FACS, or SPR (surface plasmon resonance assay; for example, using BIACORE (registered trademark)) (Shields et al., J. Biol. Chem. 276(9):6591-6604 (2001); Singh et al., J. Immunol. Methods 50:109-114 (1982); Suzuki et al., J. Immunol. 184(4):1968-1976 (2010); Luo et al., mAbs 1(5):491-504 (2009)). These methods make use of the property that an immune complex containing two or more antibodies and two or more antigens can bind more strongly to an Fc receptor or a complement component than an antibody or antigen alone can. Where an increased binding to an Fc receptor or a complement component is detected in the presence of both a test antibody and an antigen compared to in the presence of an antibody alone, the test antibody is identified as an antibody that can form an immune complex containing two or more antibodies and two or more molecules of myostatin. In another embodiment, formation of an immune complex is evaluated by administering a test antibody to an animal (e.g., a mouse) and measuring the clearance of antigen from plasma. As described above, an antibody which forms an immune complex containing two or more antibodies with two or more antigens is expected to accelerate the elimination of antigens from plasma. Therefore, where an accelerated elimination of myostatin from plasma is observed in a test antibody-administered mouse compared to in a reference antibody-administered mouse, the test antibody is identified as an antibody that can form an immune complex containing two or more antibodies and two

or more molecules of myostatin more efficiently than the reference antibody.

D. Immunoconjugates

- [0399] In some embodiments, the invention provides immunoconjugates comprising an anti-myostatin antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.
- [0400] In some embodiments, the invention provides immunoconjugates comprising a polypeptide comprising a variant Fc region herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.
- [0401] In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see US Patent Nos. 5,208,020, 5,416,064 and EP Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see US Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see US Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., Cancer Res. 53:3336-3342 (1993); and Lode et al., Cancer Res. 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., Current Med. Chem. 13:477-523 (2006); Jeffrey et al., Bioorganic & Med. Chem. Letters 16:358-362 (2006); Torgov et al., Bioconj. Chem. 16:717-721 (2005); Nagy et al., Proc. Natl. Acad. Sci. USA 97:829-834 (2000); Dubowchik et al., Bioorg. & Med. Chem. Letters 12:1529-1532 (2002); King et al., J. Med. Chem. 45:4336-4343 (2002); and US Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.
- [0402] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and a tricothecene.
- [0403] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples

include At^{211} , I^{131} , I^{125} , Y^{90} , Re^{186} , Re^{188} , Sm^{153} , Bi^{212} , P^{32} , Pb^{212} and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I^{123} , or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese and iron.

[0404] Conjugates of an antibody and cytotoxic agent may be made using a variety of bi-functional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO 1994/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., Cancer Res. 52:127-131 (1992); US Patent No. 5,208,020) may be used.

[0405] The immunoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

E. Methods and Compositions for Diagnostics and Detection

[0406] In certain embodiments, any of the anti-myostatin antibodies provided herein is useful for detecting the presence of myostatin in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as serum, whole blood, plasma, biopsy sample, tissue sample, cell suspension, saliva, sputum, oral fluid, cerebrospinal fluid, amniotic fluid, ascites fluid, milk, colostrum, mammary gland secretion, lymph, urine, sweat, lacrimal fluid, gastric fluid, synovial fluid, peritoneal fluid, ocular lens fluid or mucus.

- [0407] In one embodiment, an anti-myostatin antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of latent myostatin or myostatin propeptide in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-myostatin antibody as described herein under conditions permissive for binding of the anti-myostatin antibody to myostatin, and detecting whether a complex is formed between the anti-myostatin antibody and myostatin. Such method may be an in vitro or in vivo method. In one embodiment, an anti-myostatin antibody is used to select subjects eligible for therapy with an anti-myostatin antibody, e.g., where myostatin is a biomarker for selection of patients.
- [0408] Exemplary disorders that may be diagnosed using an antibody of the invention include but are not limited to, muscular dystrophy (MD; including Duchenne muscular dystrophy), amyotrophic lateral sclerosis (ALS), muscle atrophy, organ atrophy, carpal tunnel syndrome, frailty, congestive obstructive pulmonary disease (COPD), sarcopenia, cachexia, muscle wasting syndromes, HIV-induced muscle wasting, type 2 diabetes, impaired glucose tolerance, metabolic syndrome (including syndrome X), insulin resistance (including resistance induced by trauma, e.g., burns or nitrogen imbalance), adipose tissue disorders (e.g., obesity, dyslipidemia, nonalcoholic fatty liver disease, etc.), osteoporosis, osteopenia, osteoarthritis, and metabolic bone disorders (including low bone mass, premature gonadal failure, androgen suppression, vitamin D deficiency, secondary hyperparathyroidism, nutritional deficiencies, and anorexia nervosa).
- [0409] In certain embodiments, labeled anti-myostatin antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P , ^{14}C , ^{125}I , ^3H , and ^{131}I , fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (US Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, beta-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

F. Pharmaceutical Formulations

- [0410] Pharmaceutical formulations of an anti-myostatin antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions.
- [0411] Pharmaceutical formulations of a polypeptide comprising a variant Fc region as described herein are prepared by mixing such polypeptide having the desired degree of purity with one or more optional pharmaceutically acceptable carriers in the form of lyophilized formulations or aqueous solutions.
- [0412] Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counterions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX (registered trademark), Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Publ. Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.
- [0413] Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO 2006/044908, the latter formulations including a histidine-acetate buffer.
- [0414] The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose

intended.

[0415] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethyl-cellulose or gelatin-microcapsules and poly-(methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0416] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules.

[0417] The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

G. Therapeutic Methods and Compositions

[0418] Any of the anti-myostatin antibodies provided herein may be used in therapeutic methods. Likewise, any of the polypeptides comprising an variant Fc region provided herein may be used in therapeutic methods.

[0419] In one aspect, an anti-myostatin antibody for use as a medicament is provided. In further aspects, an anti-myostatin antibody for use in treating a muscle wasting disease is provided. In certain embodiments, an anti-myostatin antibody for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-myostatin antibody for use in a method of treating an individual having a muscle wasting disease comprising administering to the individual an effective amount of the anti-myostatin antibody. In one embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent. In further embodiments, the invention provides an anti-myostatin antibody for use in increasing mass of muscle tissue. In certain embodiments, the invention provides an anti-myostatin antibody for use in a method of increasing mass of muscle tissue in an individual comprising administering to the individual an effective amount of the anti-myostatin antibody to increase mass of muscle tissue. In further embodiments, the invention provides an anti-myostatin antibody for use in increasing strength of muscle tissue. In certain embodiments, the invention provides an anti-myostatin antibody for use in a method of increasing strength of muscle tissue in an individual comprising administering to the individual an effective amount of the anti-myostatin antibody to increase strength of muscle tissue. In further embodiments, the invention provides an anti-myostatin antibody for use in reducing body fat accumulation. In certain embodiments, the invention provides an anti-myostatin antibody

for use in a method of reducing body fat accumulation in an individual comprising administering to the individual an effective amount of the anti-myostatin antibody to reduce body fat accumulation. An "individual" according to any of the above embodiments is preferably a human.

[0420] An anti-myostatin antibody of the present invention may exhibit pH-dependent binding characteristics. In further embodiments, the invention provides an anti-myostatin antibody for use in enhancing the clearance of myostatin from plasma. In certain embodiments, the invention provides an anti-myostatin antibody for use in a method of enhancing the clearance of myostatin from plasma in an individual comprising administering to the individual an effective amount of the anti-myostatin antibody to enhance the clearance of myostatin from plasma. In one embodiment, an anti-myostatin antibody with pH-dependent binding characteristics enhances the clearance of myostatin from plasma, compared to a conventional anti-myostatin antibody which does not have pH-dependent binding characteristics. In a further embodiment, an anti-myostatin antibody with a pH-dependent binding characteristic between binding at pH5.8 and pH7.4 enhances the clearance of myostatin from plasma, compared to a conventional anti-myostatin antibody which does not have pH-dependent binding characteristics. An "individual" according to any of the above embodiments is preferably a human.

[0421] In a further aspect, the invention provides the use of an anti-myostatin antibody in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of a muscle wasting disease. In a further embodiment, the medicament is for use in a method of treating a muscle wasting disease comprising administering to an individual having a muscle wasting disease an effective amount of the medicament. In one embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent. In a further embodiment, the medicament is for increasing mass of muscle tissue. In a further embodiment, the medicament is for use in a method of increasing mass of muscle tissue in an individual comprising administering to the individual an effective amount of the medicament to increase mass of muscle tissue. In a further embodiment, the medicament is for increasing strength of muscle tissue. In a further embodiment, the medicament is for use in a method of increasing strength of muscle tissue in an individual comprising administering to the individual an effective amount of the medicament to increase strength of muscle tissue. In a further embodiment, the medicament is for reducing body fat accumulation. In a further embodiment, the medicament is for use in a method of reducing body fat accumulation in an individual comprising administering to the individual an effective amount of the medicament to reduce body fat accumulation. An "individual" according to any of the above embodiments may be a human.

- [0422] An anti-myostatin antibody of the present invention may exhibit pH-dependent binding characteristics. In a further embodiment, the medicament is for enhancing the clearance of myostatin from plasma. In a further embodiment, the medicament is for use in a method of enhancing the clearance of myostatin from plasma in an individual comprising administering to the individual an effective amount of the medicament to enhance the clearance of myostatin from plasma. In one embodiment, an anti-myostatin antibody with pH-dependent binding characteristics enhances the clearance of myostatin from plasma, compared to a conventional anti-myostatin antibody which does not have pH-dependent binding characteristics. In a further embodiment, the anti-myostatin antibody exhibits different pH-dependent binding characteristics between pH5.8 and pH7.4. An "individual" according to any of the above embodiments is preferably a human.
- [0423] In another aspect, the invention provides a method for treating a muscle wasting disease. In one embodiment, the method comprises administering to an individual having such a muscle wasting disease an effective amount of an anti-myostatin antibody provided herein. In one embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent. The "individual" according to any of the above embodiments may be a human.
- [0424] In another aspect, the invention provides a method for increasing mass of muscle tissue in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-myostatin antibody provided herein to increase mass of muscle tissue. In one embodiment, the "individual" is a human.
- [0425] In another aspect, the invention provides a method for increasing strength of muscle tissue in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-myostatin antibody provided herein to increase strength of muscle tissue. In one embodiment, the "individual" is a human.
- [0426] In another aspect, the invention provides a method for reducing body fat accumulation in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-myostatin antibody provided herein to reduce body fat accumulation. In one embodiment, the "individual" is a human.
- [0427] An anti-myostatin antibody of the present invention may exhibit pH-dependent binding characteristics. In a further embodiment, the invention provides a method for enhancing the clearance of myostatin from plasma in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-myostatin antibody provided herein to enhance the clearance of myostatin from plasma. In one embodiment, an anti-myostatin antibody with pH-dependent binding characteristics enhances the clearance of myostatin from plasma, compared to a conventional anti-myostatin antibody which does not have pH-dependent binding charac-

teristics. In a further embodiment, the anti-myostatin antibody exhibits different pH-dependent binding characteristics between pH5.8 and pH7.4. In one embodiment, an "individual" is a human.

[0428] In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-myostatin antibodies provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-myostatin antibodies provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-myostatin antibodies provided herein and at least one additional therapeutic agent.

[0429] In a further aspect, the pharmaceutical formulation is for treatment of a muscle wasting disease. In a further embodiment, the pharmaceutical formulation is for increasing mass of muscle tissue. In a further embodiment, the pharmaceutical formulation is for increasing strength of muscle tissue. In a further embodiment, the pharmaceutical formulation is for reducing body fat accumulation. An anti-myostatin antibody of the present invention may exhibit pH-dependent binding characteristics. In a further embodiment, the pharmaceutical formulation is for enhancing the clearance of myostatin from plasma. In one embodiment, the pharmaceutical formulation is administered to an individual having a muscle wasting disease. An "individual" according to any of the above embodiments is preferably a human.

[0430] In a further aspect, the invention provides methods for preparing a medicament or a pharmaceutical formulation, comprising mixing any of the anti-myostatin antibodies provided herein with a pharmaceutically acceptable carrier, e.g., for use in any of the above therapeutic methods. In one embodiment, the methods for preparing a medicament or a pharmaceutical formulation further comprise adding at least one additional therapeutic agent to the medicament or pharmaceutical formulation.

[0431] In certain embodiments, a muscle wasting disease is selected from the group consisting of muscular dystrophy (MD; including Duchenne muscular dystrophy), amyotrophic lateral sclerosis (ALS), muscle atrophy, organ atrophy, carpal tunnel syndrome, frailty, congestive obstructive pulmonary disease (COPD), sarcopenia, cachexia, muscle wasting syndromes, HIV-induced muscle wasting, type 2 diabetes, impaired glucose tolerance, metabolic syndrome (including syndrome X), insulin resistance (including resistance induced by trauma, e.g., burns or nitrogen imbalance), adipose tissue disorders (e.g., obesity, dyslipidemia, nonalcoholic fatty liver disease, etc.), osteoporosis, osteopenia, osteoarthritis, and metabolic bone disorders (including low bone mass, premature gonadal failure, androgen suppression, vitamin D deficiency, secondary hyperparathyroidism, nutritional deficiencies, and anorexia nervosa).

[0432] Any of the polypeptide comprising a variant Fc region provided herein may be used

in therapeutic methods. In a further aspect, the invention provides pharmaceutical formulations comprising a polypeptide comprising any of the polypeptides comprising variant Fc regions provided herein, e.g., for use in therapeutic methods. In one embodiment, a pharmaceutical formulation comprises a polypeptide comprising any of the polypeptides comprising variant Fc regions provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises a polypeptide comprising any of the variant Fc regions provided herein and at least one additional therapeutic agent.

[0433] In one aspect, a polypeptide comprising a variant Fc region for use as a medicament is provided. In further aspects, a polypeptide comprising a variant Fc region for use in treating a disorder is provided. In certain embodiments, a polypeptide comprising a variant Fc region for use in a method of treatment is provided. In certain embodiments, the invention provides a polypeptide comprising a variant Fc region for use in a method of treating an individual having a disorder comprising administering to the individual an effective amount of the polypeptide comprising a variant Fc region provided herein. In one embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent. In one embodiment, the "individual" is a human.

[0434] In a further aspect, the invention provides the use of a polypeptide comprising a variant Fc region in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of a disorder. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. In a further embodiment, the medicament is for use in a method of treating a disorder comprising administering to an individual having the disorder to be treated an effective amount of the medicament. In one embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent. In one embodiment, the "individual" is a human.

[0435] In a further aspect, the invention provides a method for treating a disorder. In one embodiment, the method comprises administering to an individual having such a disorder an effective amount of a polypeptide comprising a variant Fc region. In one embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent. In one embodiment, the "individual" is a human.

[0436] In a further aspect, the invention provides pharmaceutical formulations comprising a polypeptide comprising a variant Fc region provided herein, for use in a therapeutic method such as any of the therapeutic methods described herein. In one embodiment, a pharmaceutical formulation comprises a polypeptide comprising a variant Fc region provided herein and a pharmaceutically acceptable carrier. In another embodiment, a

pharmaceutical formulation comprises a polypeptide comprising a variant Fc region provided herein and at least one additional therapeutic agent.

[0437] In a further aspect, the pharmaceutical formulation is for treatment of a disorder. In one embodiment, the pharmaceutical formulation is administered to an individual having a disorder. In one embodiment, the "individual" is a human.

[0438] In further embodiments, the invention provides a polypeptide comprising a variant Fc region for use in suppressing the activation of B cells, mast cells, dendritic cells, and/or basophils. In certain embodiments, the invention provides a polypeptide comprising a variant Fc region for use in a method of suppressing the activation of B cells, mast cells, dendritic cells, and/or basophils in an individual comprising administering to the individual an effective amount of the polypeptide comprising a variant Fc region to suppress the activation of B cells, mast cells, dendritic cells, and/or basophils. In one embodiment, the "individual" is a human.

[0439] In a further aspect, the invention provides the use of a polypeptide comprising a variant Fc region in the manufacture or preparation of a medicament. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. In a further embodiment, the medicament is for suppressing the activation of B cells, mast cells, dendritic cells, and/or basophils. In a further embodiment, the medicament is for use in a method of suppressing the activation of B cells, mast cells, dendritic cells, and/or basophils in an individual comprising administering to the individual an effective amount of the medicament to suppress the activation of B cells, mast cells, dendritic cells, and/or basophils. In one embodiment, the "individual" is a human.

[0440] In a further aspect, the invention provides a method for suppressing the activation of B cells, mast cells, dendritic cells, and/or basophils in an individual. In one embodiment, the method comprises administering to the individual an effective amount of a polypeptide comprising a variant Fc region to suppress the activation of B cells, mast cells, dendritic cells, and/or basophils. In one embodiment, the "individual" is a human.

[0441] In a further aspect, the invention provides pharmaceutical formulations comprising a polypeptide comprising a variant Fc region. In a further embodiment, the pharmaceutical formulation is for suppressing the activation of B cells, mast cells, dendritic cells, and/or basophils.

[0442] Polypeptides comprising a variant Fc region of the present invention can suppress the activation of B cells, mast cells, dendritic cells, and/or basophils. Without wishing to be bound by theory, it is believed that this suppressed activation is the result of the selective binding of the provided variant Fc regions to Fc gamma RIIB without activating activating Fc gamma R. As used herein, "B cell activation" includes pro-

liferation, IgE production, IgM production, and IgA production. Without wishing to be bound by theory, it is believed that the suppressed activation of B cells is the result of the ability of the provided variant Fc regions on polypeptides comprising a variant Fc region of the present invention to crosslink Fc gamma RIIb with IgE to suppress IgE production of B cells, with IgM to suppress IgM production of B cells, and with IgA to suppress IgA production. Other than the above, suppressive effects similar to those mentioned above are exhibited by directly or indirectly cross-linking Fc gamma RIIb with molecules that are expressed on B cells and comprise the ITAM domain inside the cell or interact with the ITAM domain such as BCR, CD19, and CD79b. As used herein, "activation of mast cells" includes proliferation, activation by IgE, and degranulation. Without wishing to be bound by theory, it is believed that the suppressed activation of mast cells is the result of the ability of the provided variant Fc regions on polypeptides comprising a variant Fc region of the present invention to directly or indirectly cross-link Fc gamma RIIb with IgE receptor molecules that are expressed on mast cells and comprise the ITAM domain or interact with the ITAM domain such as Fc epsilon RI, DAP12, and CD200R3. As used herein, "activation of basophils" includes proliferation and degranulation of basophils. Without wishing to be bound by theory, it is believed that the suppressed activation of basophils is the result of the ability of the provided variant Fc regions on polypeptides comprising a variant Fc region of the present invention to directly or indirectly cross-link Fc gamma RIIb with molecules on the cell membrane, which comprise the ITAM domain inside the cell or interact with the ITAM domain. As used herein, "activation of dendritic cells" includes proliferation and degranulation of dendritic cells. Without wishing to be bound by theory, it is believed that the suppressed activation of dendritic cells is the result of the ability of the provided variant Fc regions on polypeptides comprising a variant Fc region of the present invention to directly or indirectly cross-link Fc gamma RIIb with molecules on the cell membrane, which comprise the ITAM domain inside the cell or interact with the ITAM domain.

[0443] In further embodiments, the invention provides a polypeptide comprising a variant Fc region provided herein for use in treating an immunological inflammatory disease. In certain embodiments, the invention provides a polypeptide comprising a variant Fc region for use in a method of treating an immunological inflammatory disease in an individual comprising administering to the individual an effective amount of the polypeptide comprising a variant Fc region to treat an immunological inflammatory disease. In one embodiment, the "individual" is a human.

[0444] In a further aspect, the invention provides the use of a polypeptide comprising a variant Fc region provided herein in the manufacture or preparation of a medicament. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an

Fc fusion protein. In a further embodiment, the medicament is for treating an immunological inflammatory disease. In a further embodiment, the medicament is for use in a method of treating an immunological inflammatory disease in an individual comprising administering to the individual an effective amount of the medicament to treat immunological inflammatory diseases. An "individual" according to any of the above embodiments may be a human.

- [0445] In a further aspect, the invention provides a method for treating an immunological inflammatory disease in an individual. In one embodiment, the method comprises administering to the individual an effective amount of a polypeptide comprising a variant Fc region to treat immunological inflammatory diseases. In one embodiment, an "individual" is a human.
- [0446] In a further aspect, the invention provides pharmaceutical formulations comprising any of the polypeptides comprising a variant Fc region provided herein. In a further embodiment, the pharmaceutical formulation is for treating an immunological inflammatory disease.
- [0447] As described above, since polypeptides comprising a variant Fc region of the present invention can suppress activation of B cells, mast cells, dendritic cells and/or basophils, administration of the polypeptides comprising a variant Fc region of the present invention as a result can treat or prevent immunological inflammatory diseases.
- [0448] In certain embodiments, the treated immunological inflammatory disease is selected from the group consisting of rheumatoid arthritis, autoimmune hepatitis, autoimmune thyroiditis, autoimmune blistering diseases, autoimmune adrenocortical disease, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, megalocytic anemia, autoimmune atrophic gastritis, autoimmune neutropenia, autoimmune orchitis, autoimmune encephalomyelitis, autoimmune receptor disease, autoimmune infertility, chronic active hepatitis, glomerulonephritis, interstitial pulmonary fibrosis, multiple sclerosis, Paget's disease, osteoporosis, multiple myeloma, uveitis, acute and chronic spondylitis, gouty arthritis, inflammatory bowel disease, adult respiratory distress syndrome (ARDS), psoriasis, Crohn's disease, Basedow's disease, juvenile diabetes, Addison's disease, myasthenia gravis, lens-induced uveitis, systemic lupus erythematosus, allergic rhinitis, allergic dermatitis, ulcerative colitis, hypersensitivity, muscle degeneration, cachexia, systemic scleroderma, localized scleroderma, Sjogren's syndrome, Behchet's disease, Reiter's syndrome, type I and type II diabetes, bone resorption disorder, graft-versus-host reaction, ischemia-reperfusion injury, atherosclerosis, brain trauma, cerebral malaria, sepsis, septic shock, toxic shock syndrome, fever, malgias due to staining, aplastic anemia, hemolytic anemia, idiopathic thrombocytopenia, Goodpasture's syndrome, Guillain-Barre syndrome, Hashimoto's thyroiditis, pemphigus, IgA nephropathy, pollinosis, antiphospholipid

antibody syndrome, polymyositis, Wegener's granulomatosis, arteritis nodosa, mixed connective tissue disease, fibromyalgia, asthma, atopic dermatitis, chronic atrophic gastritis, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune pancreatitis, aortitis syndrome, rapidly progressive glomerulonephritis, megaloblastic anemia, idiopathic thrombocytopenic purpura, primary hypothyroidism, idiopathic Addison's disease, insulin-dependent diabetes mellitus, chronic discoid lupus erythematosus, pemphigoid, herpes gestationis, linear IgA bullous dermatosis, epidermolysis bullosa acquisita, alopecia areata, vitiligo vulgaris, leukoderma acquisitum centrifugum of Sutton, Harada's disease, autoimmune optic neuropathy, idiopathic azoospermia, habitual abortion, hypoglycemia, chronic urticaria, ankylosing spondylitis, psoriatic arthritis, enteropathic arthritis, reactive arthritis, spondyloarthropathy, enthesopathy, irritable bowel syndrome, chronic fatigue syndrome, dermatomyositis, inclusion body myositis, Schmidt's syndrome, Graves' disease, pernicious anemia, lupoid hepatitis, presenile dementia, Alzheimer's disease, demyelinating disorder, amyotrophic lateral sclerosis, hypoparathyroidism, Dressler's syndrome, Eaton-Lambert syndrome, dermatitis herpetiformis, alopecia, progressive systemic sclerosis, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), sarcoidosis, rheumatic fever, erythema multiforme, Cushing's syndrome, transfusion reaction, Hansen's disease, Takayasu arteritis, polymyalgia rheumatica, temporal arteritis, giant cell arthritis, eczema, lymphomatoid granulomatosis, Kawasaki disease, endocarditis, endomyocardial fibrosis, endophthalmitis, fetal erythroblastosis, eosinophilic fasciitis, Felty syndrome, Henoch-Schonlein purpura, transplant rejection, mumps, cardiomyopathy, purulent arthritis, familial Mediterranean fever, Muckle-Wells syndrome, and hyper-IgD syndrome.

[0449] In further embodiments, the invention provides a polypeptide comprising a variant Fc region for use in treating or preventing an autoimmune diseases which may be caused by or associated with the production of antibodies against autoantigens (autoantibodies). In certain embodiments, the invention provides a polypeptide comprising a variant Fc region for use in a method of treating or preventing an autoimmune disease which may be caused by, or associated with, the production of antibodies against an autoantigen (autoantibody) in an individual comprising administering to the individual an effective amount of the polypeptide comprising a variant Fc region to treat or prevent the autoimmune disease. An "individual" according to any of the above embodiments is preferably a human.

[0450] In a further aspect, the invention provides the use of a polypeptide comprising a variant Fc region in the manufacture or preparation of a medicament. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. In a further embodiment, the medicament is for treating or preventing an au-

toimmune disease which may be caused by or associated with production of antibodies against autoantigens (autoantibodies). In a further embodiment, the medicament is for use in a method of treating or preventing an autoimmune disease which may be caused by or associated with production of antibodies against autoantigens (autoantibodies) in an individual comprising administering to the individual an effective amount of the medicament to treat or prevent the autoimmune disease. An "individual" according to any of the above embodiments may be a human.

[0451] In a further aspect, the invention provides a method for treating or preventing an autoimmune disease which may be caused by or associated with production of antibodies against autoantigens (autoantibodies) in an individual. In one embodiment, the method comprises administering to the individual an effective amount of a polypeptide comprising a variant Fc region to treat or prevent the autoimmune disease. In one embodiment, an "individual" is a human.

[0452] In a further aspect, the invention provides pharmaceutical formulations comprising any of the polypeptides comprising a variant Fc region provided herein. In a further embodiment, the pharmaceutical formulation is for treating or preventing an autoimmune disease which may be caused by or associated with production of antibodies against an autoantigen (autoantibodies).

[0453] The polypeptides comprising a variant Fc region of the present invention can treat or prevent an autoimmune disease which may be caused by or associated with production of antibodies against an autoantigen (autoantibodies) by suppressing production of those autoantibodies. Use of a fusion molecule of an antibody Fc portion with AchR (an autoantigen of myasthenia gravis) has been reported to suppress proliferation of B cells which express AchR-recognizing BCR, and induce apoptosis (J. Neuroimmunol 227: 35-43 (2010)). Use of a fusion protein formed between a variant Fc region of the present invention and an antigen recognized by an autoantibody enables crosslinking of Fc gamma RIIb with BCR for that autoantigen on a B cell, which causes suppression of proliferation of the B cells and/or causes induction of apoptosis of the B cells.

[0454] In certain embodiments, an autoimmune disease that can be treated or or prevented is selected from the group consisting of Guillain-Barre syndrome, myasthenia gravis, chronic atrophic gastritis, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune pancreatitis, aortitis syndrome, Goodpasture's syndrome, rapidly progressive glomerulonephritis, megaloblastic anemia, autoimmune hemolytic anemia, autoimmune neutropenia, idiopathic thrombocytopenic purpura, Basedow's disease, Hashimoto's thyroiditis, primary hypothyroidism, idiopathic Addison's disease, insulin-dependent diabetes mellitus, chronic discoid lupus erythematosus, localized scleroderma, pemphigus, pemphigoid, herpes gestationis, linear

IgA bullous dermatosis, epidermolysis bullosa acquisita, alopecia areata, vitiligo vulgaris, leukoderma acquisitum centrifugum of Sutton, Harada's disease, autoimmune optic neuropathy, idiopathic azoospermia, habitual abortion, type II diabetes, hypoglycemia, and chronic urticarial.

[0455] In further embodiments, the invention provides a polypeptide comprising a variant Fc region for use in treating a disease associated with a deficiency of a biologically essential protein. In certain embodiments, the invention provides a polypeptide comprising a variant Fc region for use in a method of treating a disease associated with a deficiency of a biologically essential protein in an individual comprising administering to the individual an effective amount of the polypeptide comprising a variant Fc region to treat the disease. An "individual" according to any of the above embodiments is preferably a human.

[0456] In a further aspect, the invention provides the use of a polypeptide comprising a variant Fc region in the manufacture or preparation of a medicament. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. In a further embodiment, the medicament is for treating a disease associated with a deficiency of a biologically essential protein. In a further embodiment, the medicament is for use in a method of treating a disease associated with a deficiency of a biologically essential protein in an individual comprising administering to the individual an effective amount of the medicament to treat the disease. An "individual" according to any of the above embodiments may be a human.

[0457] In a further aspect, the invention provides a method of treating a disease associated with a deficiency of a biologically essential protein in an individual. In one embodiment, the method comprises administering to the individual an effective amount of a polypeptide comprising a variant Fc region to treat the disease. In one embodiment, an "individual" is a human.

[0458] In a further aspect, the invention provides pharmaceutical formulations comprising any of the polypeptides comprising a variant Fc region provided herein. In a further embodiment, the pharmaceutical formulation is for treating a disease associated with a deficiency of a biologically essential protein.

[0459] For diseases with deficiency of a biologically essential protein, therapeutic methods that administer and supplement the protein as a pharmaceutical agent are used. However, since the patient lacks the protein from the beginning, the externally supplemented protein is recognized as a foreign substance and antibodies against that protein are produced. As a result, the protein becomes easily removed, and the effect as a pharmaceutical is reduced. Use of a fusion protein comprising such a protein and a variant Fc region of the present invention enables crosslinking between Fc gamma RIIB and BCR that recognizes the protein on B cells, which causes suppression of

antibody production against the protein.

- [0460] In certain embodiments, the protein to be supplemented is selected from the group consisting of Factor VIII, Factor IX, TPO, EPO, alpha-iduronidase, iduronate sulfatase, A-type heparan N-sulfatase, B type alpha-N-acetylglucosaminidase, C type acetyl CoA: alpha-glucosaminidase acetyltransferase, D type N-acetylglucosamine 6-sulfatase, galactose 6-sulfatase, N-acetylgalactosamine 4-sulfatase, beta-glucuronidase, alpha-galactosidase, acidic alpha-galactosidase, and glucocerebrosidase. These proteins may be supplemented for diseases such as hemophilia, idiopathic thrombocytopenic purpura, renal anemia, and lysosomal disease (mucopolysaccharidosis, Fabry's disease, Pompe disease, and Gaucher's disease), without being limited thereto.
- [0461] In further embodiments, the invention provides a polypeptide comprising a variant Fc region for use in treating a viral infection. In certain embodiments, the invention provides a polypeptide comprising a variant Fc region for use in a method of treating a viral infection in an individual comprising administering to the individual an effective amount of the polypeptide comprising a variant Fc region to treat a viral infection. An "individual" according to any of the above embodiments is preferably a human.
- [0462] In a further aspect, the invention provides the use of a polypeptide comprising a variant Fc region provided herein in the manufacture or preparation of a medicament. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. In a further embodiment, the medicament is for treating a viral infection. In a further embodiment, the medicament is for use in a method of treating a viral infection in an individual comprising administering to the individual an effective amount of the medicament to treat a viral infection. An "individual" according to any of the above embodiments may be a human.
- [0463] In a further aspect, the invention provides a method for treating a viral infection in an individual. In one embodiment, the method comprises administering to the individual an effective amount of a polypeptide comprising a variant Fc region to treat a viral infection. In one embodiment, an "individual" is a human.
- [0464] In a further aspect, the invention provides pharmaceutical formulations comprising any of the polypeptides comprising a variant Fc region provided herein. In a further embodiment, the pharmaceutical formulation is for treating a viral infection.
- [0465] Anti-virus antibodies that comprise a variant Fc region of the present invention can suppress antibody-dependent enhancement observed with conventional anti-virus antibodies. Antibody-dependent enhancement is a phenomenon where a virus bound to an antibody is phagocytosed via activating Fc gamma Rs so that infection of the virus to a cell is enhanced. Binding of anti-dengue-virus antibodies to Fc gamma RIIB has been reported to play an important role in suppressing antibody-dependent en-

hancement (Proc. Natl. Acad. Sci. USA 108:12479-12484, (2011)). Crosslinking Fc gamma RIIB molecules by an immune complex of the anti-dengue-virus antibodies and dengue virus, inhibits Fc gamma R-mediated phagocytosis, resulting in the suppression of antibody-dependent enhancement. Examples of such viruses include dengue virus (DENV1, DENV2, DENV3, and DENV4) and HIV, but are not limited thereto.

[0466] In further embodiments, the invention provides a polypeptide comprising a variant Fc region for use in preventing or treating arteriosclerosis. In certain embodiments, the invention provides a polypeptide comprising a variant Fc region for use in a method of preventing or treating arteriosclerosis in an individual comprising administering to the individual an effective amount of the polypeptide comprising a variant Fc region to prevent or treat arteriosclerosis. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. An "individual" according to any of the above embodiments is preferably a human.

[0467] In a further aspect, the invention provides the use of a polypeptide comprising a variant Fc region in the manufacture or preparation of a medicament. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. In a further embodiment, the medicament is for preventing or treating arteriosclerosis. In a further embodiment, the medicament is for use in a method of preventing or treating arteriosclerosis in an individual comprising administering to the individual an effective amount of the medicament to prevent or treat arteriosclerosis. An "individual" according to any of the above embodiments may be a human.

[0468] In a further aspect, the invention provides a method for preventing or treating arteriosclerosis in an individual. In one embodiment, the method comprises administering to the individual an effective amount of a polypeptide comprising a variant Fc region to prevent or treat arteriosclerosis. In one embodiment, an "individual" is a human.

[0469] In a further aspect, the invention provides pharmaceutical formulations comprising any of the polypeptides comprising a variant Fc region provided herein. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. In a further embodiment, the pharmaceutical formulation is for preventing or treating arteriosclerosis.

[0470] Antibodies against oxidized LDL, i.e., a cause for arteriosclerosis, comprising a variant Fc region of the present invention can prevent Fc gamma RIIa-dependent adhesion of inflammatory cells. It has been reported that while anti-oxidized LDL antibodies inhibit the interaction between oxidized LDL and CD36, anti-oxidized LDL antibodies bind to endothelial cells, and monocytes recognize their Fc portion in an Fc gamma RIIa-dependent or Fc gamma RI-dependent manner (Immunol. Lett. 108:52-61, (2007)). Using antibodies comprising a variant Fc region of the present invention may inhibit Fc gamma RIIa-dependent binding and suppress monocyte

adhesion by Fc gamma RIIB-mediated inhibitory signals.

[0471] In further embodiments, the invention provides a polypeptide comprising a variant Fc region for use in preventing or treating cancer. In certain embodiments, the invention provides a polypeptide comprising a variant Fc region for use in a method of preventing or treating cancer in an individual comprising administering to the individual an effective amount of the polypeptide comprising a variant Fc region to prevent or treat cancer. An "individual" according to any of the above embodiments is preferably a human.

[0472] In a further aspect, the invention provides the use of a polypeptide comprising a variant Fc region provided herein in the manufacture or preparation of a medicament. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. In a further embodiment, the medicament is for preventing or treating cancer. In a further embodiment, the medicament is for use in a method of preventing or treating cancer in an individual comprising administering to the individual an effective amount of the medicament to prevent or treat cancer. An "individual" according to any of the above embodiments may be a human.

[0473] In a further aspect, the invention provides a method for preventing or treating cancer in an individual. In one embodiment, the method comprises administering to the individual an effective amount of a polypeptide comprising a variant Fc region to prevent or treat cancer. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. In one embodiment, an "individual" is a human.

[0474] In a further aspect, the invention provides pharmaceutical formulations comprising any of the polypeptides comprising a variant Fc region provided herein. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. In a further embodiment, the pharmaceutical formulation is for preventing or treating cancer.

[0475] As described above, it is known that enhancing the Fc gamma RIIB binding increases the agonistic activity of an agonist antibody, and enhances the antitumor effect of the antibody. Therefore, agonist antibodies comprising a variant Fc region of the present invention are useful for treatment or prevention of cancer. Specifically, a variant Fc region of the present invention enhances the agonistic activity of antibodies against, for example, receptors of the TNF receptor family such as, CD120a, CD120b, Lymphotoxin beta receptor, CD134, CD40, FAS, TNFRSF6B, CD27, CD30, CD137, TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, RANK, Osteoprotegerin, TNFRSF12A, TNFRSF13B, TNFRSF13C, TNFRSF14, Nerve growth factor receptor, TNFRSF17, TNFRSF18, TNFRSF19, TNFRSF21, TNFRSF25, and Ectodysplasin A2 receptor, and can be used for treating or preventing cancer. Furthermore, agonistic

activity is also enhanced for antibodies against other molecules, which exhibit agonistic activity through interaction with Fc gamma RIIb. In addition, by incorporating a variant Fc region of the present invention into an antibody against a receptor tyrosine kinase (RTK) such as Kit, which suppresses cell proliferation upon crosslinking with Fc gamma RIIb, the inhibitory effect of the antibody against cell proliferation may be enhanced.

- [0476] In some embodiments, the invention provides a method for preventing or treating a cancer including but not limited to a member selected from: small cell lung cancer, non-small cell lung cancer, pulmonary adenocarcinoma, and squamous cell carcinoma of the lung), large intestine cancer, rectal cancer, colon cancer, breast cancer, liver cancer, gastric cancer, pancreatic cancer, renal cancer, prostate cancer, ovarian cancer, thyroid cancer, cholangiocarcinoma, peritoneal cancer, mesothelioma, squamous cell carcinoma, cervical cancer, endometrial cancer, bladder cancer, esophageal cancer, head and neck cancer, nasopharyngeal cancer, salivary gland tumor, thymoma, skin cancer, basal cell tumor, malignant melanoma, anal cancer, penile cancer, testicular cancer, Wilms' tumor, acute myeloid leukemia (including acute myeloleukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, and acute monocytic leukemia), chronic myelogenous leukemia, acute lymphoblastic leukemia, chronic lymphatic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma (Burkitt's lymphoma, chronic lymphocytic leukemia, mycosis fungoides, mantle cell lymphoma, follicular lymphoma, diffuse large-cell lymphoma, marginal zone lymphoma, pilocytic leukemia plasmacytoma, peripheral T-cell lymphoma, and adult T cell leukemia/lymphoma), Langerhans cell histiocytosis, multiple myeloma, myelodysplastic syndrome, brain tumor (including glioma, astrogloma, glioblastoma, meningioma, and ependymoma), neuroblastoma, retinoblastoma, osteosarcoma, Kaposi's sarcoma, Ewing's sarcoma, angiosarcoma, and hemangiopericytoma.
- [0477] Antibodies which have been modified to have enhanced binding activity to Fc gamma Rs including Fc gamma RIIb by modification of at least one amino acid residue can promote antigen elimination from the plasma, as described or suggested in, for example, WO 2013/047752, WO 2013/125667, WO 2014/030728, or WO 2014/163101.
- [0478] Without being bound by a particular theory, an antibody having an increased Fc gamma R-binding activity under a neutral pH condition is rapidly taken up into cells together with its antigen complexed with the antibody, and as a result, antigen elimination from plasma can be promoted when the antibody is administered in vivo.
- [0479] Antibodies which have been modified to have an increased pI by modification of at least one amino acid residue that can be exposed on the antibody surface can be in-

corporated more rapidly into cells or can promote antigen elimination from the plasma, as described or suggested in, for example, WO 2007/114319, WO 2009/041643, WO 2014/145159, or WO 2012/016227.

[0480] Without being bound by a particular theory, it is believed that the pH of biological fluids (for example, plasma) is in a neutral pH range. In biological fluids, the net positive charge of a pI-increased antibody is increased due to the increased pI, and as a result the antibody is more strongly attracted by physicochemical Coulomb interaction to the endothelial cell surface that has a net negative charge compared to an antibody not having an increased pI. That means, via such non-specific binding, uptake into cells of the antibody complexed with its antigen is enhanced, which results in enhancement of antigen elimination from plasma. These phenomena are expected to occur commonly in vivo, regardless of cell type, tissue type, organ type, etc.

[0481] Herein, enhancement of antigen elimination from plasma means, for example, that the rate of antigen elimination from plasma is increased as compared to that when a polypeptide comprising a corresponding Fc region that has not been so modified has been administered. The increase of antigen elimination from plasma can be assessed, for example, by measuring the concentration of the antigen in plasma. Various methods for measuring antigen concentration in plasma are known in the art.

[0482] In further embodiments, the invention provides a polypeptide comprising a variant Fc region for use in promoting elimination of an antigen from plasma. In certain embodiments, the invention provides a polypeptide comprising a variant Fc region for use in a method of promoting elimination of an antigen from plasma in an individual comprising administering to the individual an effective amount of the polypeptide comprising a variant Fc region to promote elimination of an antigen from plasma. In those embodiments, it is preferable that the polypeptide further comprises an antigen-binding domain. An "individual" according to any of the above embodiments is preferably a human.

[0483] In a further aspect, the invention provides the use of a polypeptide comprising a variant Fc region in the manufacture or preparation of a medicament. In some aspects, the polypeptide is an antibody. In some aspects, the polypeptide is an Fc fusion protein. In a further embodiment, the medicament is for promoting elimination of an antigen from plasma. In a further embodiment, the medicament is for use in a method of promoting elimination of an antigen from plasma in an individual comprising administering to the individual an effective amount of the medicament to promote elimination of an antigen from plasma. In those embodiments, it is preferable that the polypeptide further comprises an antigen-binding domain. An "individual" according to any of the above embodiments may be a human.

[0484] In a further embodiment, the invention provides a method for promoting elimination

of an antigen from plasma in an individual. In one embodiment, the method comprises administering to the individual an effective amount of a polypeptide comprising a variant Fc region to promote elimination of an antigen from plasma. In those embodiments, it is preferable that the polypeptide further comprises an antigen-binding domain. In one embodiment, an "individual" is a human.

[0485] In a further aspect, the invention provides pharmaceutical formulations comprising any of the polypeptides comprising a variant Fc region provided herein. In a further embodiment, the pharmaceutical formulation is for promoting elimination of an antigen from plasma. In that embodiment, it is preferable that the polypeptide further comprises an antigen-binding domain.

[0486] In one embodiment, when compared to before amino acid modification, an antibody having a variant Fc region of the present invention enhances antigen elimination from plasma, for example, by at least 1.1-fold, 1.25-fold, 1.5-fold, 1.75-fold, 2.0-fold, 2.25-fold, 2.5-fold, 2.75-fold, 3.0-fold, 3.25-fold, 3.5-fold, 3.75-fold, 4.0-fold, 4.25-fold, 4.5-fold, 4.75-fold, 5.0-fold, 5.5-fold, 6.0-fold, 6.5-fold, 7.0-fold, 7.5-fold, 8.0-fold, 8.5-fold, 9.0-fold, 9.5-fold, or 10.0-fold or more, when the antibody is administered in vivo.

[0487] In a further aspect, the invention provides methods for preparing a medicament or a pharmaceutical formulation, comprising mixing any of the polypeptides comprising a variant Fc region provided herein with a pharmaceutically acceptable carrier, e.g., for use in any of the above therapeutic methods. In one embodiment, the methods for preparing a medicament or a pharmaceutical formulation further comprise adding at least one additional therapeutic agent to the medicament or pharmaceutical formulation.

[0488] Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent.

[0489] Likewise, polypeptides comprising a variant Fc region of the invention can be used either alone or in combination with other agents in a therapy. For instance, a polypeptide comprising a variant Fc region of the invention may be co-administered with at least one additional therapeutic agent.

[0490] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody or polypeptide comprising a variant Fc region of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In one embodiment, the administration of the anti-myostatin antibody and the administration of an additional therapeutic agent occur within about one month, or within about one,

two or three weeks, or within about one, two, three, four, five, or six days, of each other.

- [0491] In another embodiment, the administration of the polypeptide comprising the variant Fc region and the administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other.
- [0492] An antibody or a polypeptide comprising a variant Fc region of the invention (and optionally any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g., by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.
- [0493] Antibodies or polypeptides comprising a variant Fc region of the invention can be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.
- [0494] For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the type of polypeptide comprising a variant Fc region, the severity and course of the disease, whether the antibody or polypeptide comprising the variant Fc region, is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody or polypeptide comprising the variant Fc region, and the discretion of the attending physician. The antibody or polypeptide comprising a variant Fc region of the invention is suitably administered to the patient at one time or over a series of treatments. Depending on the type and

severity of the disease, about 1 micro g/kg to 15 mg/kg (e.g., 0.1mg/kg-10mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 micro g/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody or polypeptide comprising the variant Fc region would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g., every week or every three weeks (e.g., such that the patient receives from about two to about twenty, or e.g., about six doses of the antibody or polypeptide comprising the variant Fc region). An initial higher loading dose, followed by one or more lower doses may be administered. The progress of this therapy is easily monitored by conventional techniques and assays.

[0495] It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-myostatin antibody.

[0496] It is likewise understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to a polypeptide comprising a variant Fc region provided herein.

H. Articles of Manufacture

[0497] In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic

or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0498] It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-myostatin antibody.

Examples

[0499] The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1

[0500] Expression and purification of human, cynomolgus monkey, and mouse myostatin latent and mature form

Human latent myostatin (also described herein as human myostatin latent form) (SEQ ID NO: 1) was expressed transiently using FreeStyle293-F cells (FS293-F cells) (Thermo Fisher, Carlsbad, CA, USA). Conditioned media containing expressed human myostatin latent form was acidified to pH6.8 and diluted with 1/2 vol of milliQ water, followed by application to a Q-sepharose FF anion exchange column (GE healthcare, Uppsala, Sweden). The flow-through fraction was adjusted to pH5.0 and applied to a SP-sepharose HP cation exchange column (GE healthcare, Uppsala, Sweden), and then eluted with a NaCl gradient. Fractions containing the human myostatin latent form were collected and subsequently subjected to a Superdex 200 gel filtration column (GE healthcare, Uppsala, Sweden) equilibrated with 1x PBS. Fractions containing the human myostatin latent form were then pooled and stored at -80 degrees C.

[0501] Human mature myostatin (also described herein as human myostatin mature form) (SEQ ID NO: 2) was purified from the purified human myostatin latent form. The human myostatin latent form was acidified by addition of 0.1% trifluoroacetic acid (TFA) and applied to a Vydac 214TP C4 reverse phase column (Grace, Deerfield, IL, USA) and eluted with a TFA/CH₃CN gradient. Fractions containing human mature myostatin were pooled, dried and stored at -80 degrees C. To reconstitute, human mature myostatin was dissolved in 4mM HCl.

[0502] Expression and purification of myostatin latent and mature form from cynomolgus

monkey (cynomolgus or cyno) (SEQ ID NOs: 3 and 4, respectively) and mouse (SEQ ID NOs: 5 and 6, respectively) were all performed exactly the same way as the human counterpart. The sequence homology of mature form among human, cyno, and mouse are 100% identical, therefore, any of mature myostatin regardless of species were used as mature myostatin in all the necessary experiments.

Example 2

[0503] Identification of anti-latent myostatin antibody

Anti-latent myostatin antibodies were prepared, selected, and assayed as follows.

[0504] Twelve to sixteen week old NZW rabbits were immunized intradermally with mouse latent myostatin and/or human latent myostatin (50-100 micro g/dose/rabbit). This dose was repeated 3-4 times over a one month period. One week after the final immunization, the spleen and blood from the immunized rabbit were collected. Antigen-specific B-cells were stained with labelled antigen, sorted with FCM cell sorter (FACS aria III, BD), and plated in 96-well plates at a one cell/well density together with 25,000 cells/well of EL4 cells (European Collection of Cell Cultures) and with rabbit T-cell conditioned medium diluted 20 times, and were cultured for 7-12 days. EL4 cells were treated with mitomycin C (Sigma) for 2 hours and washed 3 times in advance. The rabbit T-cell conditioned medium was prepared by culturing rabbit thymocytes in RPMI-1640 containing Phytohemagglutinin-M (Roche), phorbol 12-myristate 13-acetate (Sigma) and 2% FBS. After cultivation, B-cell culture supernatants were collected for further analysis and pellets were cryopreserved.

[0505] An ELISA assay was used to test the specificity of antibodies in a B-cell culture supernatant. Streptavidin (GeneScript) was coated onto a 384-well MAXISorp (Nunc) at 50nM in PBS for 1 hour at room temperature. Plates were then blocked with Blocking One (Nacalai Tesque) diluted 5 times. Human or mouse latent myostatin was labelled with NHS-PEG4-Biotin (PIERCE) and was added to the blocked ELISA plates, incubated for 1 hour, and washed with Tris-buffered saline with 0.05% Tween-20 (TBS-T). B-cell culture supernatants were added to the ELISA plates, incubated for 1 hour, and washed with TBS-T. Binding was detected by goat anti-rabbit IgG-horseradish peroxidase (BETHYL) followed by the addition of ABTS (KPL).

[0506] A total of 17,818 B-cell lines were screened for binding specificity to mouse and/or human latent myostatin and 299 lines were selected and designated MST0255-287, 630-632, 677-759, 910, 932-1048, 1050-1055, 1057-1066, 1068, 1070-1073, 1075-1110, 1113-1119. RNA was purified from corresponding cell pellets using ZR-96 Quick-RNA kits (ZYMO RESEARCH).

[0507] DNA encoding the variable regions of the heavy and light chain of each selected cell line was amplified by reverse transcription PCR and cloned into expression vectors

with the heavy chain constant region G1m sequence (SEQ ID NO: 50 (the amino acid sequence is shown in SEQ ID NO: 7)) and with the light chain constant region k0MTC or k0MC sequence (SEQ ID NO: 53 or 194 (the amino acid sequence (both are the same) is shown in SEQ ID NO: 8)), respectively. Recombinant antibodies were expressed transiently using the FreeStyle FS293-F cells and 293fectin (Life technologies), according to the manufacturer's instructions. Culture supernatant or recombinant antibodies were used for screening. Recombinant antibodies were purified with protein A (GE Healthcare) and eluted in D-PBS or His buffer (20mM Histidine, 150mM NaCl, pH6.0). Size exclusion chromatography was further conducted to remove high molecular weight and/or low molecular weight component, if necessary.

Example 3

[0508] Characterization of anti-latent myostatin antibody (HEK Blue Assay (BMP1 activation))

A reporter gene assay was used to assess the biological activity of active myostatin in vitro. HEK-Blue™ TGF-beta cells (Invivogen) which express a Smad3/4-binding elements (SBE)-inducible SEAP reporter genes, allow the detection of bioactive myostatin by monitoring the activation of the activin type 1 and type 2 receptors. Active myostatin stimulates the production of SEAP which is secreted into the cell supernatant. The quantity of SEAP secreted is then assessed using QUANTIBLue™ (Invivogen).

[0509] HEK-Blue™ TGF-beta cells were maintained in DMEM medium (Gibco) supplemented with 10% fetal bovine serum, 50 micro g/mL streptomycin, 50 U/mL penicillin, 100 micro g/mL Normocin™, 30 micro g/mL of Blasticidin, 200 micro g/mL of HygroGold™ and 100 micro g/mL of Zeocin™. During the functional assay, cells were changed to assay medium (DMEM with 0.1% bovine serum albumin, streptomycin, penicillin and Normocin™) and seeded to a 96-well plate. Human, cynomolgus, or mouse latent myostatin was incubated with recombinant human BMP1 (R&D Systems) and anti-latent antibody at 37degrees C overnight. The sample mixtures were transferred to cells. After 24-hour incubation, the cell supernatants were mixed with QUANTIBLue™ and the optical density at 620 nm was measured in a colorimetric plate reader. As shown as Figure 1, mAb MST1032-G1m (see Table 2a for amino acid and nucleotide sequences) prevented protease-mediated activation of human, cynomolgus, and mouse latent myostatin, as reflected by lowered concentrations of secreted SEAP. MST1032-G1m showed almost comparable inhibition activity against human, cynomolgus, and mouse latent myostatin.

[Table 2a]

Anti-latent myostatin antibodies and their DNA and amino acid sequences (shown as SEQ ID NOs)

Antibody name	Abbreviation	Variable region				Constant region			
		Heavy		Light		Heavy		Light	
		DNA	PROTEIN	DNA	PROTEIN	DNA	PROTEIN	DNA	PROTEIN
MS_MST1032Ha-G1m/MST1032La-k0MC	MST1032-G1m	039	012	041	014	050	007	194	008
MS_MST1032Ha-F760/MST1032La-k0MC	MST1032-F760	039	012	041	014	051	011	194	008
MS_M103205H000-SG1/M103202L000-SK1	MS1032L000-SG1 or Ab001	040	013	042	015	052	009	054	010
MS_M103205H000-F760/M103202L000-SK1	MS1032L000-F760	040	013	042	015	051	011	054	010
MS_M103205H714-SG1/M103202L861-SK1	MS1032L001-SG1	043	032	046	035	052	009	054	010
MS_M103205H781-SG1/M103202L719-SK1	MS1032L002-SG1	044	033	047	036	052	009	054	010
MS_M103205H781-SG1/M103202L813-SK1	MS1032L003-SG1	044	033	048	037	052	009	054	010
MS_M103205H707-SG1/M103202L802-SK1	MS1032L004-SG1	045	034	049	038	052	009	054	010
MS_M103205H714-F760/M103202L861-SK1	MS1032L001-F760	043	032	046	035	051	011	054	010
MS_M103205H781-F760/M103202L719-SK1	MS1032L002-F760	044	033	047	036	051	011	054	010
MS_M103205H781-F760/M103202L813-SK1	MS1032L003-F760	044	033	048	037	051	011	054	010
MS_M103205H707-F760/M103202L802-SK1	MS1032L004-F760	045	034	049	038	051	011	054	010

[Table 2b]

HVR amino acid sequences of anti-latent myostatin antibodies (shown as SEQ ID NOs)

Antibody name	Abbreviation	Hyper Variable region (HVR)					
		H1	H2	H3	L1	L2	L3
MS_MST1032Ha-G1m/MST1032La-k0MC	MST1032-G1m	055	058	061	065	070	073
MS_MST1032Ha-F760/MST1032La-k0MC	MST1032-F760	055	058	061	065	070	073
MS_M103205H000-SG1/M103202L000-SK1	MS1032L000-SG1 or Ab001	055	058	061	065	070	073
MS_M103205H000-F760/M103202L000-SK1	MS1032L000-F760	055	058	061	065	070	073
MS_M103205H714-SG1/M103202L861-SK1	MS1032L001-SG1	057	058	063	067	071	074
MS_M103205H781-SG1/M103202L719-SK1	MS1032L002-SG1	057	060	063	066	072	073
MS_M103205H781-SG1/M103202L813-SK1	MS1032L003-SG1	057	060	063	068	070	074
MS_M103205H707-SG1/M103202L802-SK1	MS1032L004-SG1	056	058	063	069	071	073
MS_M103205H714-F760/M103202L861-SK1	MS1032L001-F760	057	058	063	067	071	074
MS_M103205H781-F760/M103202L719-SK1	MS1032L002-F760	057	060	063	066	072	073
MS_M103205H781-F760/M103202L813-SK1	MS1032L003-F760	057	060	063	068	070	074
MS_M103205H707-F760/M103202L802-SK1	MS1032L004-F760	056	058	063	069	071	073

Example 4

[0510] Characterization of anti-latent myostatin antibody (HEK Blue Assay (Spontaneous activation))

Human, cynomolgus, or mouse latent myostatin was incubated with the anti-latent myostatin antibody at 37 degrees C overnight and the sample mixtures were transferred to HEK-Blue™ TGF-beta cells. After 24-hour incubation, cell supernatant was mixed with QUANTIBLue™ and the optical density at 620 nm was measured in a colorimetric plate reader. As shown as Figure 2, the liberation of active myostatin from its latent form was detected after incubation at 37 degrees C. In the presence of mAb MST1032-G1m, myostatin activation was inhibited and thus a lower SEAP level was detected in the cell supernatant. MST1032-G1m inhibited spontaneous activation of latent myostatin, and showed almost comparable inhibition activity against human, cynomolgus, and mouse latent myostatin.

Example 5

[0511] Characterization of anti-latent myostatin antibody (ELISA)

Uncoated ELISA plates (NUNC-IMMUNO plate MAXISORP surface, Nalge Nunc International) were coated with 20 micro L of 50nM streptavidin (GenScript) for 1 hour at room temperature. Plates were then washed with PBST three times and blocked with 50 micro L 20% Blocking One (Nacalai Tesque) overnight. On the next day, each well of each plate was incubated with biotinylated mature myostatin, human or mouse biotinylated latent myostatin, or biotinylated recombinant mouse myostatin propeptide-Fc chimera protein (R&D Systems) at 4nM/well/20 micro L for 2 hours. After washing, 20 micro L of antibody sample was added to the wells and the plates were left for 1 hour. The plates were washed and 20 micro L of anti-human IgG-horseradish peroxidase (HRP) (Abcam) diluted in HEPES-buffered saline was added, and the plates were left for another hour. The plates were then washed again, and then 50 micro L ABTS (KPL) was added to each well, and the plates were incubated for 1 hour. The signal was detected at 405 nm in a colorimetric plate reader. The results of the binding experiment are shown in Figure 3. MST1032-G1m bound to latent myostatin (i.e., the non-covalent complex of mature myostatin and propeptides) and propeptide, but did not bind to mature myostatin. These results show that MST1032-G1m specifically binds to the myostatin propeptide (e.g., the propeptide portion), but not to active myostatin (e.g., mature region).

Example 6

[0512] Characterization of anti-latent myostatin antibody (Western Blot)

Mouse latent myostatin was incubated with recombinant human BMP1 (R&D Systems), with or without MST1032-G1m at 37 degrees C overnight. The samples

were then mixed with 4x reducing SDS-PAGE sample buffer (Wako) and heated at 95 degrees C for 5 minutes and then loaded for SDS gel electrophoresis. Proteins were transferred to membrane by Trans-Blot (registered trademark) Turbo™ Transfer System (Bio-rad). Myostatin propeptide was detected using a sheep anti-mouse GDF8 propeptide antibody (R&D Systems), which was then detected by anti-sheep IgG-HRP (Santa Cruz). The membrane was incubated with ECL substrate, and imaged using an ImageQuant LAS 4000 (GE Healthcare). As shown in Figure 4, propeptide cleavage by BMP1 was inhibited by MST1032-G1m.

Example 7

[0513] Characterization of anti-latent myostatin antibody (BIAcore (registered trademark))

The kinetic parameters of anti-latent myostatin antibodies against human, cynomolgus monkey (cyno), and mouse latent myostatin were assessed at 37 degrees C at pH7.4 using a BIAcore (registered trademark) T200 instrument (GE Healthcare). ProA/G (Pierce) was immobilized onto all flow cells of a CM4 chip using amine coupling kit (GE Healthcare). Anti-latent myostatin antibody and analytes were prepared in ACES pH7.4 (20 mM ACES, 150 mM NaCl, 1.2 mM CaCl₂, 0.05% Tween 20, 0.005% NaN₃). Antibody was captured onto the sensor surface by ProA/G. Antibody capture levels were typically 150 to 220 resonance units (RU). Then human, cyno, or mouse latent myostatin was injected at 3.125 to 50 nM prepared by two-fold serial dilution, followed by dissociation. The sensor surface was regenerated with 25 mM NaOH. Kinetic parameters were determined by processing and fitting the data to a 1:1 binding model using BIAcore (registered trademark) T200 Evaluation software, version 2.0 (GE Healthcare). The sensorgrams are shown in Figure 5. Association rate (ka), dissociation rate (kd), and binding affinity (KD) are listed in Table 3. The kinetic parameters of MST1032-G1m toward human, cyno, and mouse latent myostatin were comparable.

[Table 3]

ka, kd, and KD of anti-latent myostatin antibody MST1032-G1m

	ka (M ⁻¹ s ⁻¹)	kd (s ⁻¹)	KD (M)
Human latent myostatin	1.13E+06	8.65E-05	7.66E-11
Mouse latent myostatin	1.41E+06	8.16E-05	5.79E-11
Cyno latent myostatin	1.09E+06	7.79E-05	7.17E-11

Example 8

[0514] In vivo efficacy of anti-latent myostatin antibody on muscle mass and fat mass

The in vivo efficacy of mAb MST1032-G1m was evaluated in mice. Anti-mature

myostatin antibody 41C1E4 (as described in US Patent No. 7,632,499) was used as positive control in this study. To avoid potential immunomodulation due to a mouse anti-human antibody response, in vivo studies were performed in immune-deficient Severe Combined Immunodeficient (SCID) mice. Five-week-old SCID (C.B-17 SCID) mice (Charles River Laboratories Japan, Inc.(Kanagawa, JAPAN)) were treated with monoclonal antibodies at various doses or vehicle (PBS) given intravenously once per week for two weeks. On day 0, 7 and 14, full body lean mass and fat mass of the mice were assessed by nuclear magnetic resonance (NMR) (the minispec LF-50, Bruker Bio Spin, (Kanagawa, JAPAN)). The animals were euthanized on day 14, and the gastrocnemius and quadriceps muscles were dissected and weighed.

- [0515] Statistical significance was determined by ANOVA, a Student's t-test and a Dunnett's test with JMP 9 software (SAS, Inc.). A p value of less than 0.05 was considered significant.
- [0516] The results of this experiment are shown in Figures 6A-C. Both antibodies (MST1032-G1m and 41C1E4) increased lean body mass dose-dependently, and MST1032-G1m, when administered at 40 mg/kg, significantly decreased fat mass compared with the vehicle (PBS) group on day 14.
- [0517] After two-week treatment, the antibodies administrated at 10 mg/kg and 40 mg/kg resulted in significant increment of quadriceps and gastrocnemius wet weight relative to the vehicle group.

Example 9

- [0518] Comparison of in vivo efficacy of various myostatin related antibodies

All experimental settings for the comparison were the same as in Example 8. Various myostatin related antibodies, 41C1E4, REGN, OGD, and MYO-029 were tested in five-week-old SCID mice for two weeks. REGN, OGD, and MYO-029 are anti-mature myostatin antibodies described in WO 2011/150008 as H4H1657N2, WO 2013/186719 as OGD1.0.0., and WO 2004/037861 as MYO-029, respectively. These antibodies were administrated intravenously once per week. Lean body mass and fat mass were examined by full-body NMR scanning on day 0 and 14. Grip strength was measured with a grip strength test meter (e.g., GPM-100B, MELQUEST Ltd., (Toyama, JAPAN)) on day 14. As shown in the results presented in Figures 7A-C, the lean body mass was increased by these antibodies except for MYO-029 ($P>0.05$). Grip strength of the MST1032-G1m-administered group was significantly increased relative to the vehicle (PBS) group, at 10 mg/kg ($P<0.05$). 41C1E4 and REGN also significantly increased grip strength compared with the vehicle (PBS). Full body fat mass tended to be reduced by 10 mg/kg of MST1032-G1m. Fat mass reduction efficacy of MST1032-G1m was stronger than that of other anti-mature myostatin antibodies.

Example 10**[0519] Humanization of anti-latent myostatin antibody**

Amino acid residues of an antibody variable region are numbered according to Kabat (Kabat et al., Sequence of proteins of immunological interest, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)).

[0520] Variable regions of the heavy and light chains for humanized MST1032 antibodies were designed. Some of the humanized MST1032 antibodies contain back mutation in framework region. The polynucleotides of the designed heavy and light chain variable regions were synthesised by GenScript Inc., and were cloned into expression vectors containing the heavy chain constant region SG1 sequence (SEQ ID NO: 52 (the amino acid sequence is shown in SEQ ID NO: 9)) and the light chain constant region SK1 sequence (SEQ ID NO: 54 (the amino acid sequence is shown in SEQ ID NO: 10)), respectively. Humanized antibodies were transiently expressed in FS293-F cells, and HEK Blue Assay and BIACORE (registered trademark) analysis were carried out as described above. As shown in Figure 8 and Table 4, and compared to Figures 1 and 2, humanized antibody (MS1032LO00-SG1) showed comparable inhibition activity and affinity to chimeric antibody (MST1032-G1m).

[Table 4]

Kinetic parameters of humanized anti-latent myostatin antibody

	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	KD (M)
MST1032-G1m	1.13E+06	8.65E-05	7.66E-11
MS1032LO00-SG1	1.27E+06	9.52E-05	7.50E-11

Example 11**[0521] Generation of pH-dependent anti-latent myostatin antibody**

To generate pH-dependent anti-latent myostatin antibodies, histidine scanning mutagenesis was conducted for all CDRs of mAb MS1032LO00-SG1. Each amino acid in the CDRs was individually mutated to histidine using In-Fusion HD Cloning Kit (Clontech Inc. or Takara Bio company) according to the manufacturer's instructions. After confirming through sequencing that each variant was mutated correctly, variants were transiently expressed and purified by the method described above. All histidine-substituted variants were evaluated by a modified BIACORE (registered trademark) assay as compared to that described above. Briefly, an additional dissociation phase at pH5.8 was integrated into the BIACORE (registered trademark) assay immediately after the dissociation phase at pH7.4. This is to assess the pH-dependent dissociation between antibody (Ab) and antigen (Ag) from the complexes formed at pH7.4 as opposed to the corresponding dissociation at pH5.8. The dissociation rate at pH5.8

buffer was determined by processing and fitting data using the Scrubber 2.0 (BioLogic Software) curve fitting software.

[0522] As shown in Figure 9, the parental antibody (Ab001) showed no reduction in binding response at pH5.8 compared to the pH7.4 dissociation phase. Several of the single-histidine substitutions resulted in a moderate to strong reduction in binding response at pH5.8 compared to the pH7.4 dissociation phase. The dissociation rate at pH5.8 for each of the single-histidine substitution variants are shown in Table 5. As shown in Table 5, Ab002 showed the fastest Ab/Ag complex dissociation rate at pH5.8, more than 200 fold faster than parental antibody (Ab001). Antibodies with a combination of these mutations in the CDRs were then generated. The CDR sequences of the antibodies containing these various mutations are shown in Table 6.

[Table 5]

pH5.8 dissociation rate for single histidine substituted anti-latent myostatin antibodies

Name of variable region		Abbreviation	kd (1/s)
Heavy chain	Light chain		
M103205H000	M103202L000	Ab001	2.92E-05
M103205H001	M103202L000	Ab002	6.09E-03
M103205H009	M103202L000	Ab003	8.80E-04
M103205H026	M103202L000	Ab004	1.86E-04
M103205H028	M103202L000	Ab005	7.63E-05
M103205H000	M103202L008	Ab006	8.58E-05
M103205H028	M103202L008	Ab007	4.15E-04

[Table 6]

CDR sequences and their SEQ ID NOs of histidine substituted anti-latent myostatin antibodies

Heavy chain																																				
Variable region										Kabat No.																										
Antibody name	H1	H2	H3	31	32	33	34	35	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	95	96	97	98	99	100	100a	100b	100c	101	102	
M103205H000	013	055	058	061	S	Y	D	I	S	I	I	S	Y	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	Y	S	T	G	G	D	L
M103205H001	016	056	058	061	H	Y	D	I	S	I	I	S	Y	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	Y	S	T	G	G	D	L
M103205H009	017	055	059	061	S	Y	D	I	S	I	I	S	H	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	Y	S	T	G	G	D	L
M103205H026	018	055	058	062	S	Y	D	I	S	I	I	S	Y	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	H	S	T	G	G	D	L
M103205H028	019	055	058	063	S	Y	D	I	S	I	I	S	Y	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	Y	S	H	G	G	D	L
M103205H033	020	056	059	061	H	Y	D	I	S	I	I	S	H	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	Y	S	T	G	G	D	L
M103205H034	021	055	059	062	S	Y	D	I	S	I	I	S	H	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	H	S	T	G	G	D	L
M103205H035	022	056	058	062	H	Y	D	I	S	I	I	S	Y	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	H	S	T	G	G	D	L
M103205H036	023	056	059	062	H	Y	D	I	S	I	I	S	H	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	H	S	T	G	G	D	L
M103205H707	024	056	058	063	H	Y	D	I	S	I	I	S	Y	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	Y	S	H	G	G	D	L
M103205H708	025	055	059	063	S	Y	D	I	S	I	I	S	H	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	Y	S	H	G	G	D	L
M103205H709	026	055	058	064	S	Y	D	I	S	I	I	S	Y	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	H	S	H	G	G	D	L
M103205H710	027	056	059	063	H	Y	D	I	S	I	I	S	H	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	Y	S	H	G	G	D	L
M103205H711	028	055	059	064	S	Y	D	I	S	I	I	S	H	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	H	S	H	G	G	D	L
M103205H712	029	056	058	064	H	Y	D	I	S	I	I	S	Y	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	H	S	H	G	G	D	L
M103205H713	030	056	059	064	H	Y	D	I	S	I	I	S	H	A	G	S	T	Y	Y	A	S	W	A	K	G	G	V	P	A	H	S	H	G	G	D	L

Light chain																																	
Variable region										Kabat No.																							
Antibody name	L1	L2	L3	24	25	26	27	27a	27b	28	29	30	31	32	33	34	50	51	52	53	54	55	56	89	90	91	92	93	94	95	95a	96	97
M103202L000	015	065	070	073	Q	S	S	Q	S	V	Y	D	N	N	W	L	S	W	A	S	T	L	A	S	A	G	G	Y	G	G	L	Y	A
M103202L008	031	066	070	073	Q	S	S	Q	S	V	Y	H	N	N	W	L	S	W	A	S	T	L	A	S	A	G	G	Y	G	G	L	Y	A

Example 12

[0523] Affinity improvement of pH-dependent anti-latent myostatin antibody

To identify mutations which improve affinity at pH7.4 and/or in vitro inhibition activity, more than 500 variants were generated for heavy and light chain respectively, using at least one variant generated in Example 11 as a template. These variants had each amino acid in the CDRs substituted with 18 other amino acids, excluding the original amino acid and Cysteine. The binding ability of variants to human latent myostatin was assessed at 37 degrees C under pH7.4 using BIACORE (registered trademark) 4000 instrument (GE Healthcare). Culture supernatant containing variants expressed in FS293-F cells was prepared with ACES pH7.4 buffer containing 10mg/ml BSA and 0.1mg/ml carboxymethyl dextran (CMD). Each antibody was captured onto the flow cells until the capture level reached around 200 RU. Then 25 nM human latent myostatin was injected over the flow cells. An additional dissociation phase at pH5.8 was integrated into the BIACORE (registered trademark) assay immediately after the dissociation phase at pH7.4. This additional dissociation phase assesses the pH-dependent dissociation between antibody (Ab) and antigen (Ag) from the complexes formed at pH7.4. The flow cell surface was regenerated with 25 mM NaOH. Kinetic parameters were determined using BIACORE (registered trademark) 4000 Evaluation software, version 2.0 (GE Healthcare). Analysis of additional dissociation at pH5.8 was performed using Scrubber2 (BioLogic Software).

[0524] Variants with improved affinity at pH7.4 and/or in vitro inhibition activity were selected, and combined with mutations improving pH dependency identified in Example 11. After combination of these mutations, four variants (MS1032LO01, 02, 03, and 04) were selected, and transiently expressed with SG1 or F760 (SEQ ID NO: 11 and 51) for BIACORE (registered trademark) binding kinetic analysis, in vitro and/or in vivo assays. Both SG1 and F760 are human heavy chain constant regions. The binding affinities of SG1 against human Fc gamma Rs are comparable to that for the natural IgG1 constant region, but that of F760 is abolished by Fc modification (also described herein as silent Fc). Amino acid and nucleotide sequences of the four anti-myostatin variants are shown in Table 2a.

Example 13

[0525] Characterization of pH-dependent MS1032 variants (HEK Blue Assay (BMP1 and Spontaneous activation))

All experimental settings were the same in Examples 3 and 4. As shown in Figure 10, all variants showed comparable inhibition activity against human latent myostatin to MS1032LO00-SG1.

Example 14

[0526] Characterization of pH-dependent MS1032 variants (BIAcore (registered trademark))

All experimental settings were the same as in Example 7, except that measurements were also performed in ACES pH5.8 buffer in addition to ACES pH7.4 condition. Some antibodies were only measured against human latent myostatin. Antibody capture level was aimed at 185 RU and 18.5 RU, for avidity and affinity assay, respectively. Kinetic parameters were determined using 1:1 Binding fitting using BIAcore (registered trademark) T200 Evaluation software, version 2.0 (GE Healthcare). The sensorgrams of all antibodies against human latent myostatin with avidity condition are shown in Figure 11, and k_a , k_d , and K_D calculated from different conditions are listed in Tables 7-10. Under the avidity condition, all antibodies except MS1032LO00-SG1 showed a faster dissociation rate under acidic pH than neutral pH. Under the affinity condition, all antibodies except MS1032LO00-SG1 showed a weak interaction with latent myostatin under acidic pH and therefore, kinetic parameters were not determined.

[Table 7]

k_a , k_d , and K_D of avidity condition assay under neutral pH

	Human latent myostatin			Mouse latent myostatin			Cyno latent myostatin		
	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
MS1032LO00-SG1	1.27E+06	9.52E-05	7.50E-11	1.52E+06	7.18E-05	4.71E-11	1.21E+06	8.08E-05	6.66E-11
MS1032LO01-SG1	1.27E+06	2.16E-04	1.70E-10	1.45E+06	2.17E-04	1.49E-10	1.19E+06	1.97E-04	1.65E-10
MS1032LO02-SG1	8.36E+05	2.49E-04	2.98E-10	N/T	N/T	N/T	N/T	N/T	N/T
MS1032LO03-SG1	9.81E+05	1.92E-04	1.96E-10	N/T	N/T	N/T	N/T	N/T	N/T
MS1032LO04-SG1	1.33E+06	2.17E-04	1.63E-10	N/T	N/T	N/T	N/T	N/T	N/T

N/T: not tested

[Table 8]

k_a , k_d , and K_D of avidity condition assay under acidic pH

	Human latent myostatin			Mouse latent myostatin			Cyno latent myostatin		
	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
MS1032LO00-SG1	1.60E+06	1.28E-04	7.97E-11	2.32E+06	2.02E-05	8.70E-12	1.79E+06	1.58E-05	8.83E-12
MS1032LO01-SG1	1.22E+06	2.42E-02	1.99E-08	1.02E+06	6.25E-03	6.15E-09	7.64E+05	6.80E-03	8.90E-09
MS1032LO02-SG1	1.70E+06	4.14E-02	2.43E-08	N/T	N/T	N/T	N/T	N/T	N/T
MS1032LO03-SG1	5.43E+05	5.24E-03	9.66E-09	N/T	N/T	N/T	N/T	N/T	N/T
MS1032LO04-SG1	7.81E+05	7.13E-03	9.13E-09	N/T	N/T	N/T	N/T	N/T	N/T

N/T: not tested

[Table 9]

k_a , k_d , and K_D of affinity condition assay under neutral pH

	Human latent myostatin			Mouse latent myostatin			Cyno latent myostatin		
	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
MS1032LO00-SG1	1.07E+06	2.45E-04	2.30E-10	1.31E+06	1.60E-04	1.22E-10	1.02E+06	2.13E-04	2.09E-10
MS1032LO01-SG1	1.64E+06	1.29E-03	7.88E-10	1.50E+06	1.07E-03	7.13E-10	1.15E+06	1.10E-03	9.54E-10
MS1032LO02-SG1	1.09E+06	1.79E-03	1.64E-09	N/T	N/T	N/T	N/T	N/T	N/T
MS1032LO03-SG1	1.08E+06	1.17E-03	1.08E-09	N/T	N/T	N/T	N/T	N/T	N/T
MS1032LO04-SG1	1.54E+06	1.25E-03	8.12E-10	N/T	N/T	N/T	N/T	N/T	N/T

N/T: not tested

[Table 10]

ka, kd, and KD of affinity condition assay under acidic pH

	Human latent myostatin			Mouse latent myostatin			Cyno latent myostatin		
	<i>ka</i> (M ⁻¹ s ⁻¹)	<i>kd</i> (s ⁻¹)	<i>KD</i> (M)	<i>ka</i> (M ⁻¹ s ⁻¹)	<i>kd</i> (s ⁻¹)	<i>KD</i> (M)	<i>ka</i> (M ⁻¹ s ⁻¹)	<i>kd</i> (s ⁻¹)	<i>KD</i> (M)
MS1032LO00-SG1	1.09E+06	2.35E-04	2.15E-10	1.62E+06	7.08E-05	4.38E-11	1.41E+06	1.96E-04	1.39E-10
MS1032LO01-SG1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MS1032LO02-SG1	n.d.	n.d.	n.d.	N/T	N/T	N/T	N/T	N/T	N/T
MS1032LO03-SG1	n.d.	n.d.	n.d.	N/T	N/T	N/T	N/T	N/T	N/T
MS1032LO04-SG1	n.d.	n.d.	n.d.	N/T	N/T	N/T	N/T	N/T	N/T

n.d.: not determined, N/T: not tested

Example 15

[0527] Comparison of plasma total myostatin concentration between antibodies with Fc gamma R binding and an abolished Fc gamma R binding in mice

In vivo test using C.B-17 SCID mice

The accumulation of endogenous myostatin was assessed in vivo upon administration of anti-latent myostatin antibody in C.B-17 SCID mice (In Vivos, Singapore). An anti-latent myostatin antibody (3mg/ml) was administered at a single dose of 10 ml/kg into the caudal vein. Blood was collected 5 minutes, 7 hours, 1 day, 2 days, 3 days, 7 days, 14 days, 21 days, and 28 days after administration. The collected blood was centrifuged immediately at 14,000 rpm in 4 degrees C for 10 minutes to separate the plasma. The separated plasma was stored at or below -80 degrees C until measurement. The anti-latent myostatin antibodies used were MS1032LO00-SG1 and MS1032LO00-F760.

Measurement of total myostatin concentration in plasma by electrochemiluminescence (ECL)

[0528] The concentration of total myostatin in mouse plasma was measured by ECL. Anti-mature myostatin-immobilized plates were prepared by dispensing anti-mature myostatin antibody RK35 (as described in WO 2009/058346) onto a MULTI-ARRAY 96-well plate (Meso Scale Discovery) and incubated overnight at 4 degrees C. Mature myostatin calibration curve samples and mouse plasma samples diluted 40-fold or more were prepared. The samples were mixed in an acidic solution (0.2 M Glycine-HCl, pH2.5) to dissociate mature myostatin from its binding protein (such as propeptide). Subsequently, the samples were added onto an anti-mature myostatin-immobilized plate, and allowed to bind for 1 hour at room temperature before washing. Next, SULFO TAG labelled anti-mature myostatin antibody RK22 (as described in WO 2009/058346) was added and the plate was incubated for 1 hour at room temperature before washing. Read Buffer T (x4) (Meso Scale Discovery) was immediately added to the plate and signal was detected by SECTOR Imager 2400 (Meso Scale Discovery). The mature myostatin concentration was calculated based on the response of the calibration curve using the analytical software SOFTmax PRO (Molecular

Devices). The time course of total myostatin concentration in plasma after intravenous administration of anti-latent myostatin antibody measured by this method is shown in Figure 12.

Effect of Fc gamma R binding on myostatin accumulation in vivo

- [0529] After administration of antibody MS1032LO00-F760, the plasma total myostatin concentration at day 28 accumulated 248 fold compared to plasma total myostatin concentration at 5 minutes. In contrast, after administration of MS1032LO00-SG1, plasma total myostatin concentration at day 28 accumulated 37 fold compared to plasma total myostatin concentration at 5 minutes. An approximately 7 fold difference of plasma total myostatin concentration at day28 was observed between MS1032LO00-F760 (silent Fc) and MS1032LO00-SG1 due to Fc gamma R binding. In human soluble IL-6R (hsIL-6R), no significant difference in plasma hsIL-6R concentration was observed between anti-hsIL-6R antibody-F760 (silent Fc) and -SG1 as described in WO 2013/125667. Since hsIL-6R is a monomeric antigen, antibody-hsIL-6R complex contains only 1 Fc. Therefore, the result in WO 2013/125667 suggested that an antibody-antigen complex with 1 Fc has no significant binding to Fc gamma R in vivo to accelerate the uptake of immune complex by cells. On the other hand, with a multimeric antigen (such as myostatin), antibody can make a large immune complex and the antibody-antigen complex contains more than 2 Fc. Therefore, a significant difference in plasma antigen concentration can be observed between F760 and SG1 due to strong avidity binding against Fc gamma R. The result suggests that MST1032 can form a large immune complex which contains more than 2 antibodies with myostatin.

Example 16

- [0530] Comparison of plasma total myostatin concentration between non-pH dependent anti-latent myostatin antibody and pH-dependent anti-latent myostatin antibody in mice

In vivo test using C.B-17 SCID mice

The in vivo accumulation of endogenous mouse myostatin was assessed after administering anti-latent myostatin antibody in C.B-17 SCID mice (In Vivos, Singapore) as described in Example 15. The anti-latent myostatin antibodies used were MS1032LO01-SG1 and MS1032LO01-F760.

Measurement of total myostatin concentration in plasma by ECL

- [0531] The concentration of total myostatin in mouse plasma was measured by ECL as described in Example 15. The time course of plasma total myostatin concentration after intravenous administration of anti-latent myostatin antibody as measured by this method is shown in Figure 13.

Effect of pH-dependent myostatin binding on myostatin accumulation in vivo

[0532] The pH-dependent anti-latent myostatin antibodies (MS1032LO01-SG1 and MS1032LO01-F760) were tested in vivo and comparison of plasma total myostatin concentration was made. The total myostatin concentration measurement results after administration of MS1032LO00-SG1 and MS1032LO00-F760 described in Example 15 are also shown in Figure 13. MS1032LO00 is non pH-dependent antibody and MS1032LO01 is pH-dependent antibody. As shown in Figure 13, total myostatin concentration after administration of MS1032LO01-F760 was reduced compared to MS1032LO00-F760 due to pH-dependent binding. Moreover, total myostatin concentration after administration of MS1032LO01-SG1 was dramatically reduced compared to that of MS1032LO00-SG1 due to pH-dependent binding and increased cellular uptake by Fc gamma R binding. It is expected that MS1032LO01-SG1 has a superior property of enhancing myostatin clearance from plasma as a "sweeping antibody".

Example 17

[0533] In vivo efficacy of pH-dependent anti-latent myostatin antibody

All experimental settings were the same as in Example 8. As shown in Figure 14, when administrated intravenously to SCID mice at doses of 0.2, 1, and 5 mg/kg, MS1032LO00-SG1 (non-sweeping antibody) and MS1032LO01-SG1 (sweeping antibody) increased skeletal muscle mass dose-dependently after 2 weeks. MS1032LO01-SG1 significantly increased quadriceps wet weight and grip strength at 1 and 5 mg/kg, and significantly increased lean body mass and gastrocnemius wet weight at 5 mg/kg relative to the vehicle group (PBS). MS1032LO01-SG1 also significantly decreased fat mass at 1 and 5 mg/kg relative to the vehicle group (PBS). MS1032LO00-SG1 showed no increase in lean body mass at 0.2 mg/kg. On the other hand, MS1032LO01-SG1 drastically increased lean body mass, quadriceps wet weight, gastrocnemius wet weight and grip strength at 0.2 mg/kg. Hence, a pH-dependent binding antibody shows greater muscle mass growth and muscle power improvement relative to a non pH-dependent binding antibody.

Example 18

[0534] Expression and purification of human and mouse latent GDF11

Human GDF11 with Flag-tag on N-terminus (also described herein as Flag-hGDF11, human latent GDF11, hGDF11, or human GDF11, SEQ ID NO: 85) were expressed transiently using FreeStyle293-F cells (Thermo Fisher). Conditioned media expressing Flag-hGDF11 was applied to a column packed with anti-Flag M2 affinity resin (Sigma) and eluted with Flag peptide (Sigma). Fractions containing Flag-hGDF11 were collected and subsequently subjected to a Superdex 200 gel filtration column (GE

healthcare) equilibrated with 1x PBS. Fractions containing the Flag-hGDF11 were then pooled and stored at -80 degrees C.

Example 19

[0535] Characterization of anti-latent myostatin antibody (ELISA)

Uncoated ELISA plates (NUNC-IMMUNO plate MAXISORP surface, Nalge Nunc International) were coated with 20 micro L of 50nM streptavidin (GenScript) for 2 hour at room temperature. Plates were then washed with PBST three times and blocked with 50 micro L 20% Blocking One (Nacalai Tesque) for overnight. On the next day, each well of the plates was incubated with biotinylated human latent myostatin at 2nM/well/20 micro L or biotinylated human latent GDF11 at 20nM/well/20 micro L for 2 hours. After washing, 20 micro L of antibody sample was added to the wells and the plates were left for 1 hour. The plates were washed and 20 micro L of anti-human IgG-horseradish peroxidase (HRP) (Abcam) diluted in HEPES-buffered saline was added, and the plates were left for another hour. The wells were washed again, and then 50 micro L ABTS (KPL) was added to each well, and the plates were incubated for 1 hour. The signal was detected at 405 nm in a colorimetric plate reader. The results of the binding experiment are shown in Figure 15. MST1032-G1m bound to latent myostatin (i.e., non-covalent complex of mature myostatin and propeptides), but didn't bind to hGDF11. These results show that MST1032-G1m specifically binds to myostatin, not to hGDF11.

Example 20

[0536] Characterization of anti-latent myostatin antibody (HEK Blue Assay (BMP1 and spontaneous activation))

A reporter gene assay was used to assess the biological activity of active GDF11 in vitro. HEK-Blue™ TGF-beta cells (Invivogen) that express a Smad3/4-binding element (SBE)-inducible SEAP reporter gene, allow the detection of bioactive GDF11 by monitoring the activation of the activin type 1 and type 2 receptors. Active GDF11 stimulates the production and secretion of SEAP into the cell supernatant. The quantity of SEAP secreted is then assessed using QUANTIBLue™ (Invivogen).

[0537] HEK-Blue™ TGF-beta cells were maintained in DMEM medium (Gibco) supplemented with 10% fetal bovine serum, 50 micro g/mL streptomycin, 50U/mL penicillin, 100 micro g/mL Normocin™, 30 micro g/mL of Blasticidin, 200 micro g/mL of HygroGold™ and 100 micro g/mL of Zeocin™. During the functional assay, cells were changed to assay medium (DMEM with 0.1% bovine serum albumin, streptomycin, penicillin and Normocin™) and seeded to a 96-well plate. Human GDF11 was incubated with or without recombinant human BMP1 (Calbiochem) and anti-latent antibody (MST1032-G1m) at 37 degrees C overnight. The sample mixtures

were transferred to cells. After 24-hour incubation, the cell supernatant was mixed with QUANTIBLue™ and the optical density at 620 nm was measured in a colorimetric plate reader. As shown as Figure 16, MST1032-G1m did not prevent protease-mediated or spontaneous activation of human GDF11 and thus failed to inhibit the secretion of SEAP.

Example 21

[0538] Further optimization of MS1032 variants to enhance the sweeping effect

As the pH dependent antibody, MS1032LO01-SG1, showed superior efficacy in mouse, further optimization was conducted to increase pH dependency, to enhance uptake into cells, to increase stability, and so on by introducing mutations into the antibody CDRs or by changing framework regions. More than a thousand variants were assessed in a BIAcore (registered trademark) and/or HEK Blue Assay as described above, and MS1032LO06-SG1, MS1032LO07-SG1, MS1032LO10-SG1, MS1032LO11-SG1, MS1032LO12-SG1, MS1032LO18-SG1, MS1032LO19-SG1, MS1032LO21-SG1, MS1032LO23-SG1, MS1032LO24-SG1, MS1032LO25-SG1, and MS1032LO26-SG1 were generated. Their amino acid and nucleotide sequences of these generated antibodies are shown in Table 11.

[Table 11a]

MS1032 variants and their DNA and amino acid sequences (shown as SEQ ID NOs)

Antibody name	Abbreviation	Variable region				Constant region			
		Heavy		Light		Heavy		Light	
		DNA	PROTEIN	DNA	PROTEIN	DNA	PROTEIN	DNA	PROTEIN
MS_M103205H795-SG1/M103202L889-SK1	MS1032L006-SG1	100	86	110	96	052	009	054	010
MS_M103205H1004-SG1/M103202L889-SK1	MS1032L007-SG1	101	87	110	96	052	009	054	010
MS_M103205H1046-SG1/M103202L889-SK1	MS1032L010-SG1	102	88	110	96	052	009	054	010
MS_M103205H1047-SG1/M103202L889-SK1	MS1032L011-SG1	103	89	110	96	052	009	054	010
MS_M103205H1057-SG1/M103202L889-SK1	MS1032L012-SG1	104	90	110	96	052	009	054	010
MS_M103205H1186-SG1/M103202L889-SK1	MS1032L018-SG1	105	91	110	96	052	009	054	010
MS_M103240H795-SG1/M103202L1045-SK1	MS1032L019-SG1	106	92	111	97	052	009	054	010
MS_M103245H795-SG1/M103202L1045-SK1	MS1032L021-SG1	107	93	111	97	052	009	054	010
MS_M103245H1233-SG1/M103202L1045-SK1	MS1032L023-SG1	108	94	111	97	052	009	054	010
MS_M103205H795-SG1/M103202L1060-SK1	MS1032L024-SG1	100	86	112	98	052	009	054	010
MS_M103240H1246-SG1/M103202L1045-SK1	MS1032L025-SG1	109	95	111	97	052	009	054	010
MS_M103240H795-SG1/M103209L1045-SK1	MS1032L026-SG1	106	92	113	99	052	009	054	010

[Table 11b]

HVR amino acid sequences of MS1032 variants (shown as SEQ ID NOs)

Antibody name	Abbreviation	Hyper Variable region (HVR)					
		H1	H2	H3	L1	L2	L3
MS_M103205H795-SG1/M103202L889-SK1	MS1032LO06-SG1	114	58	63	122	71	74
MS_M103205H1004-SG1/M103202L889-SK1	MS1032LO07-SG1	114	116	121	122	71	74
MS_M103205H1046-SG1/M103202L889-SK1	MS1032LO10-SG1	57	117	63	122	71	74
MS_M103205H1047-SG1/M103202L889-SK1	MS1032LO11-SG1	57	118	63	122	71	74
MS_M103205H1057-SG1/M103202L889-SK1	MS1032LO12-SG1	57	119	63	122	71	74
MS_M103205H1186-SG1/M103202L889-SK1	MS1032LO18-SG1	114	118	63	122	71	74
MS_M103240H795-SG1/M103202L1045-SK1	MS1032LO19-SG1	114	58	63	123	71	74
MS_M103245H795-SG1/M103202L1045-SK1	MS1032LO21-SG1	114	58	63	123	71	74
MS_M103245H1233-SG1/M103202L1045-SK1	MS1032LO23-SG1	115	58	63	123	71	74
MS_M103205H795-SG1/M103202L1060-SK1	MS1032LO24-SG1	114	58	63	124	125	74
MS_M103240H1246-SG1/M103202L1045-SK1	MS1032LO25-SG1	115	120	63	123	71	74
MS_M103240H795-SG1/M103209L1045-SK1	MS1032LO26-SG1	114	58	63	123	71	74

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 166

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 166

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

[CLAIMS]

[Claim 1]

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

- (1) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 122, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74; or
- (ii) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 116, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 121, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 122, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74; or
- (iii) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 57, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 117, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 122, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74; or
- (iv) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 57, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 118, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 122, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74; or
- (v) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 57, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 119, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 122, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74; or
- (vi) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 118,

HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63,
 HVR-L1 comprising the amino acid sequence of SEQ ID NO: 122,
 HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71, and
 HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74; or
 (vii) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114,
 HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58,
 HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63,
 HVR-L1 comprising the amino acid sequence of SEQ ID NO: 123,
 HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71, and
 HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74; or
 (viii) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 115,
 HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58,
 HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63,
 HVR-L1 comprising the amino acid sequence of SEQ ID NO: 123,
 HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71, and
 HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74; or
 (ix) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 114,
 HVR-H2 comprising the amino acid sequence of SEQ ID NO: 58,
 HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63,
 HVR-L1 comprising the amino acid sequence of SEQ ID NO: 124,
 HVR-L2 comprising the amino acid sequence of SEQ ID NO: 125, and
 HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74; or
 (x) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 115,
 HVR-H2 comprising the amino acid sequence of SEQ ID NO: 120,
 HVR-H3 comprising the amino acid sequence of SEQ ID NO: 63,
 HVR-L1 comprising the amino acid sequence of SEQ ID NO: 123,
 HVR-L2 comprising the amino acid sequence of SEQ ID NO: 71, and
 HVR-L3 comprising the amino acid sequence of SEQ ID NO: 74.

[Claim 2]

The antibody of claim 1(i) to (vi) and (ix) comprising
 a heavy chain variable domain framework FR1 (H-FR1) comprising the amino acid
 sequence of SEQ ID NO: 132;
 a heavy chain variable domain framework FR2 (H-FR2) comprising the amino acid
 sequence of SEQ ID NO: 135;
 a heavy chain variable domain framework FR3 (H-FR3) comprising the amino acid
 sequence of SEQ ID NO: 137;

a heavy chain variable domain framework FR4 (H-FR4) comprising the amino acid sequence of SEQ ID NO: 138,
 a light chain variable domain framework FR1 (L-FR1) comprising the amino acid sequence of SEQ ID NO: 139;
 a light chain variable domain framework FR2 (L-FR2) comprising the amino acid sequence of SEQ ID NO: 140;
 a light chain variable domain framework FR3 (L-FR3) comprising the amino acid sequence of SEQ ID NO: 142; and
 a light chain variable domain framework FR4 (L-FR4) comprising the amino acid sequence of SEQ ID NO: 144; or
 the antibody of claim 1 (vii) or (x), comprising
 a H-FR1 comprising the amino acid sequence of SEQ ID NO: 133;
 a H-FR2 comprising the amino acid sequence of SEQ ID NO: 136;
 a H-FR3 comprising the amino acid sequence of SEQ ID NO: 137;
 a H-FR4 comprising the amino acid sequence of SEQ ID NO: 138;
 a L-FR1 comprising the amino acid sequence of SEQ ID NO: 139;
 a L-FR2 comprising the amino acid sequence of SEQ ID NO: 140;
 a L-FR3 comprising the amino acid sequence of SEQ ID NO: 142; and
 a L-FR4 comprising the amino acid sequence of SEQ ID NO: 144; or
 the antibody of claim 1 (vii) or (viii), comprising
 a H-FR1 comprising the amino acid sequence of SEQ ID NO: 134;
 a H-FR2 comprising the amino acid sequence of SEQ ID NO: 136;
 a H-FR3 comprising the amino acid sequence of SEQ ID NO: 137;
 a H-FR4 comprising the amino acid sequence of SEQ ID NO: 138;
 a L-FR1 comprising the amino acid sequence of SEQ ID NO: 139;
 a L-FR2 comprising the amino acid sequence of SEQ ID NO: 140;
 a L-FR3 comprising the amino acid sequence of SEQ ID NO: 142; and
 a L-FR4 comprising the amino acid sequence of SEQ ID NO: 144; or
 the antibody of claim 1 (vii), comprising
 a H-FR1 comprising the amino acid sequence of SEQ ID NO: 133;
 a H-FR2 comprising the amino acid sequence of SEQ ID NO: 136;
 a H-FR3 comprising the amino acid sequence of SEQ ID NO: 137;
 a H-FR4 comprising the amino acid sequence of SEQ ID NO: 138;
 a L-FR1 comprising the amino acid sequence of SEQ ID NO: 139;
 a L-FR2 comprising the amino acid sequence of SEQ ID NO: 141;
 a L-FR3 comprising the amino acid sequence of SEQ ID NO: 143; and

a L-FR4 comprising the amino acid sequence of SEQ ID NO: 144.

[Claim 3]

The antibody of claim 1 or 2, comprising:

- (i) a VH sequence of SEQ ID NO: 86 and a VL sequence of SEQ ID NO: 96; or
- (ii) a VH sequence of SEQ ID NO: 86 and a VL sequence of SEQ ID NO: 98; or
- (iii) a VH sequence of SEQ ID NO: 87 and a VL sequence of SEQ ID NO: 96; or
- (iv) a VH sequence of SEQ ID NO: 88 and a VL sequence of SEQ ID NO: 96; or
- (v) a VH sequence of SEQ ID NO: 89 and a VL sequence of SEQ ID NO: 96; or
- (vi) a VH sequence of SEQ ID NO: 90 and a VL sequence of SEQ ID NO: 96; or
- (vii) a VH sequence of SEQ ID NO: 91 and a VL sequence of SEQ ID NO: 96; or
- (viii) a VH sequence of SEQ ID NO: 92 and a VL sequence of SEQ ID NO: 97; or
- (ix) a VH sequence of SEQ ID NO: 92 and a VL sequence of SEQ ID NO: 99; or
- (x) a VH sequence of SEQ ID NO: 93 and a VL sequence of SEQ ID NO: 97; or
- (xi) a VH sequence of SEQ ID NO: 94 and a VL sequence of SEQ ID NO: 97; or
- (xii) a VH sequence of SEQ ID NO: 95 and a VL sequence of SEQ ID NO: 97.

[Claim 4]

The antibody of any one of claims 1 to 3, which comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 352, and a light chain constant region comprising the amino acid sequence of SEQ ID NO: 10.

[Claim 5]

The antibody of claim 1, comprising a variant Fc region comprising at least one amino acid alteration in a parent Fc region, wherein 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, and 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.

[Claim 6]

The antibody of claim 5, wherein the KD value of the variant Fc region for monkey Fc gamma RIIB is 1.0×10^{-6} M or smaller, and the KD value of the variant Fc region for monkey Fc gamma RIIIA is 5.0×10^{-7} M or greater.

[Claim 7]

The antibody of claim 5 or 6, wherein the variant Fc region 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.

[Claim 8]

The antibody of claim 7, wherein the variant Fc region 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.

[Claim 9]

The antibody according to claim 7 or 8, wherein the variant Fc region 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.

[Claim 10]

The antibody according to claim 9, wherein the variant Fc region 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.

[Claim 11]

The antibody of claim 1, comprising a variant Fc region comprising at least two amino acid alterations in a parent Fc region, wherein each of the amino acid alterations increases the isoelectric point (pI) of the variant Fc region compared with that of the parent Fc region.

[Claim 12]

The antibody of claim 11, wherein the variant Fc region 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.

[Claim 13]

The antibody of claim 12, wherein the variant Fc region comprises Arg or Lys at each of the positions selected.

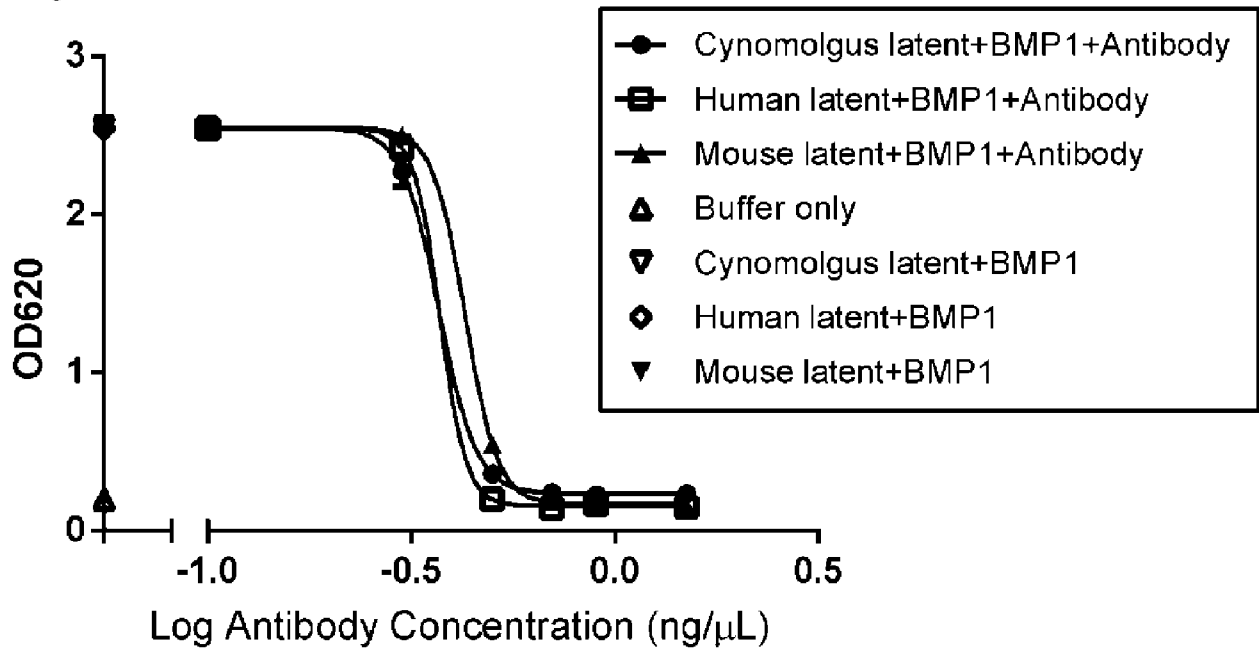
[Claim 14]

The antibody of claim 1, comprising a variant Fc region comprising amino acid alterations described in Tables 14-30.

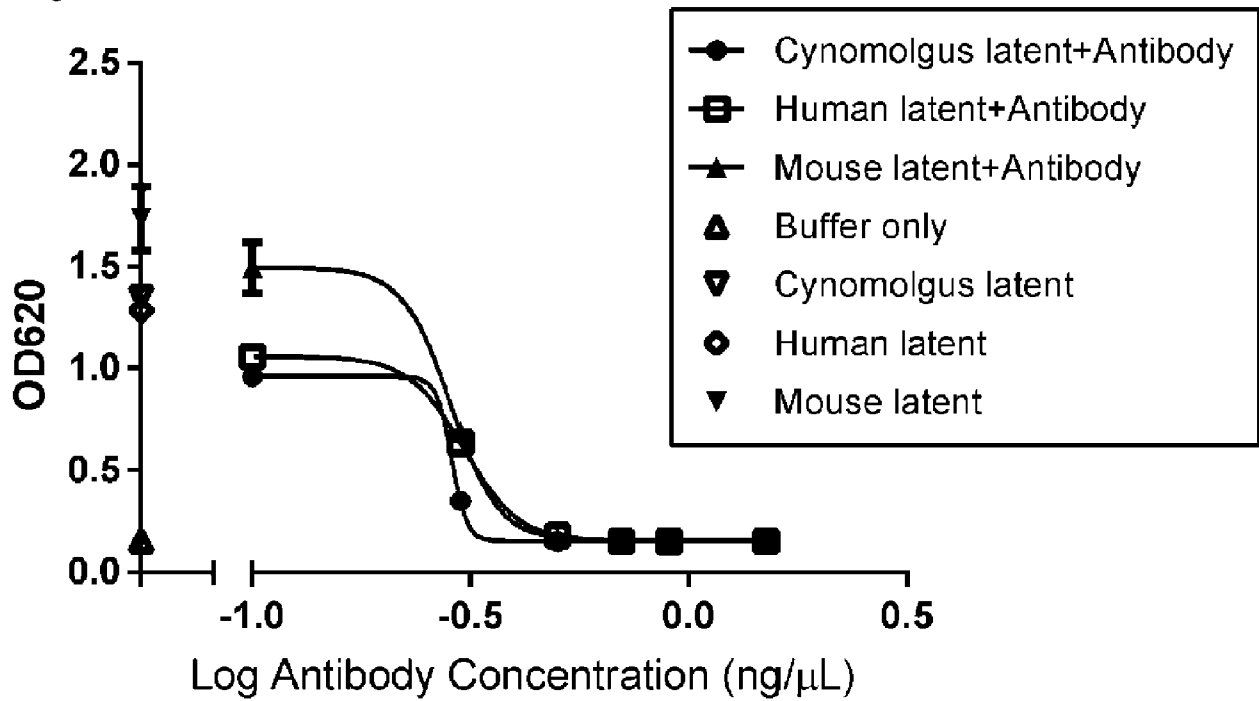
[Claim 15]

The antibody of claim 1, comprising the amino acid sequence of any one of SEQ ID NOs: 229-381.

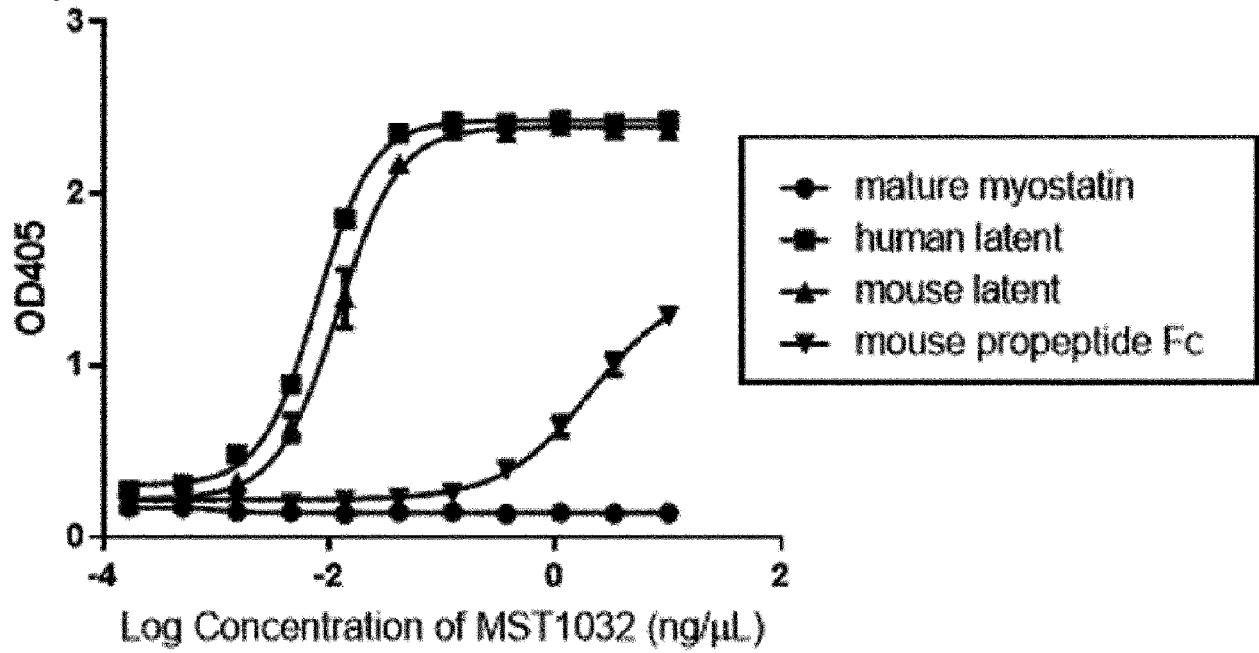
[Fig. 1]



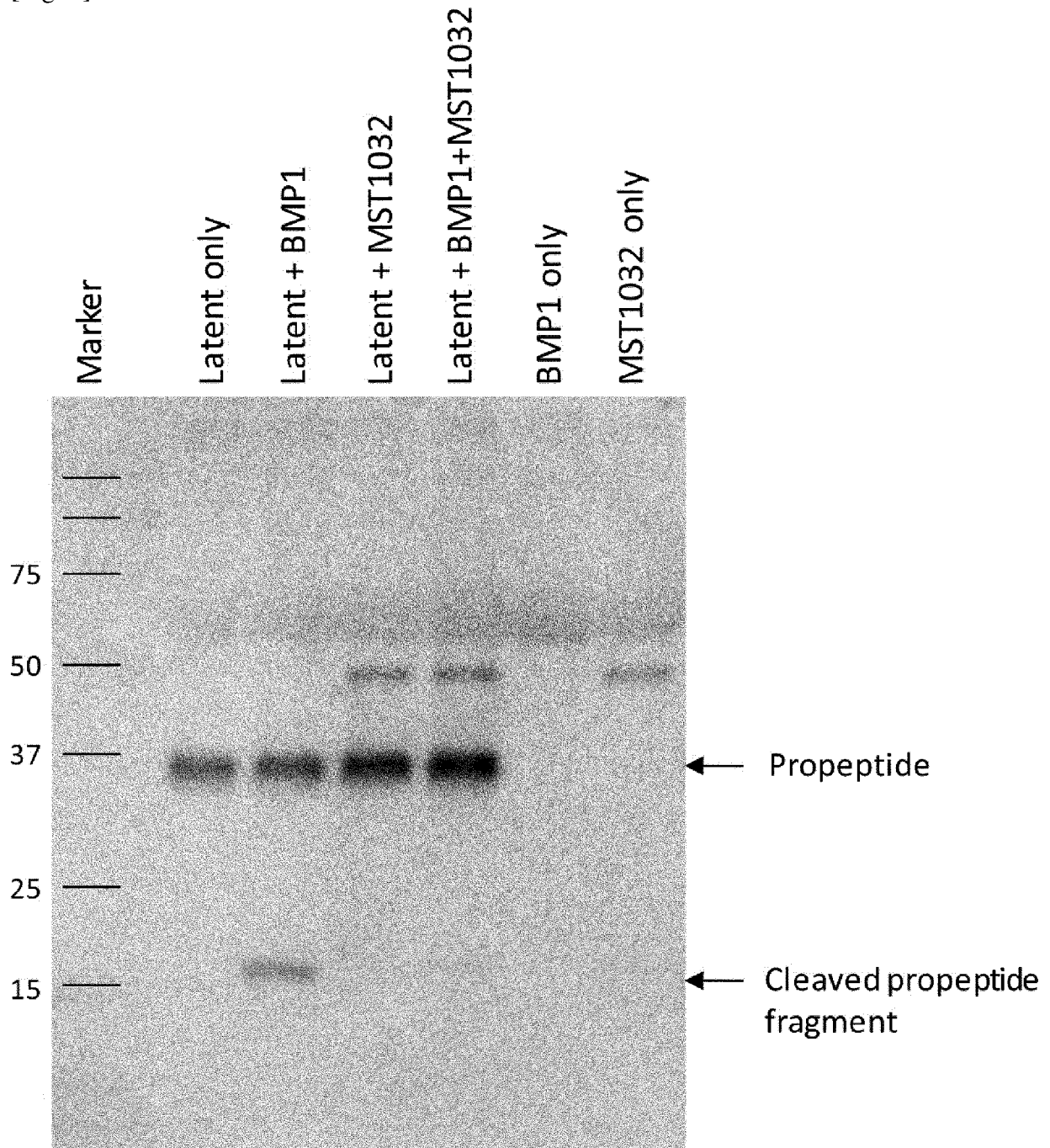
[Fig. 2]



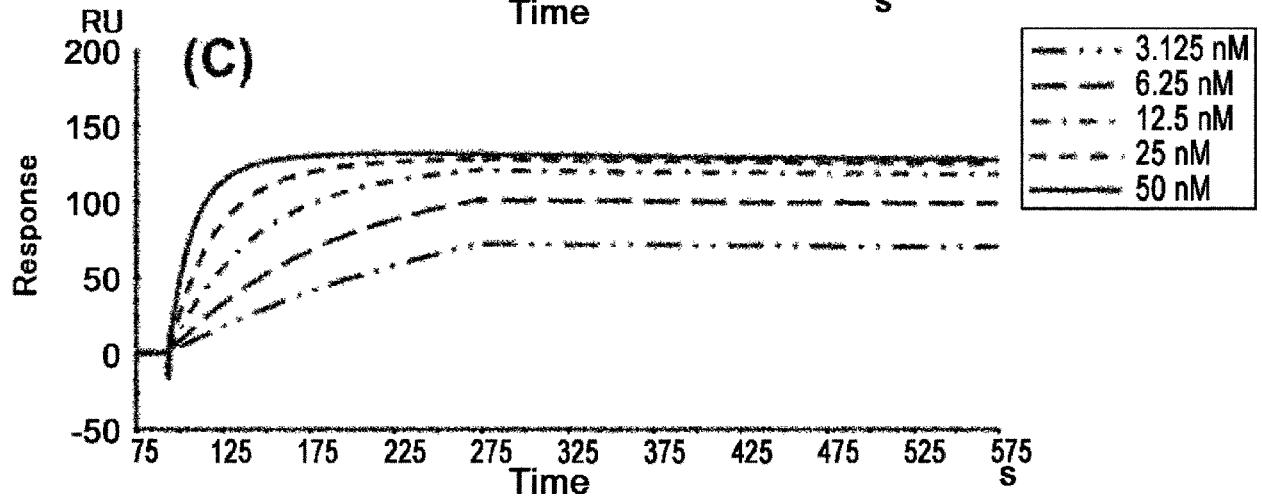
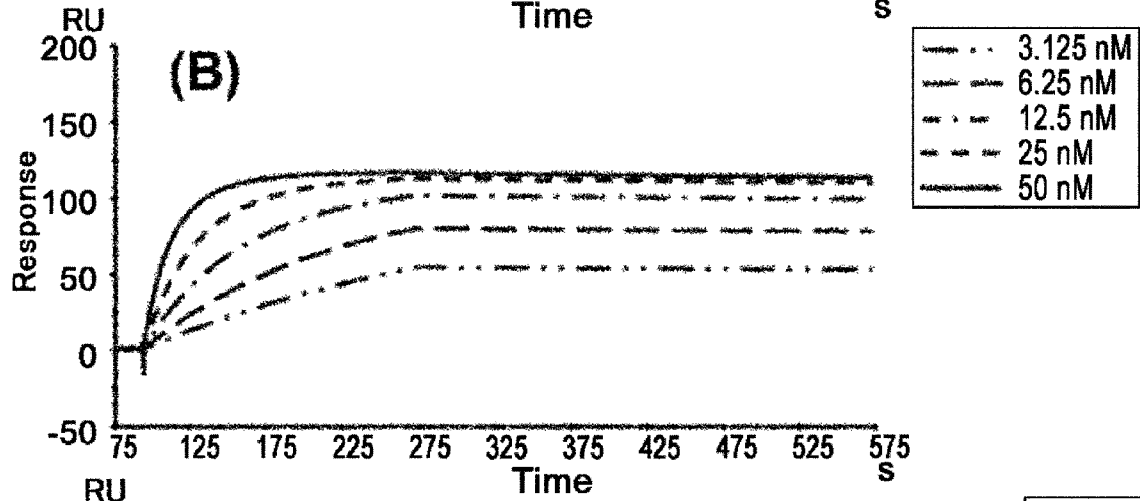
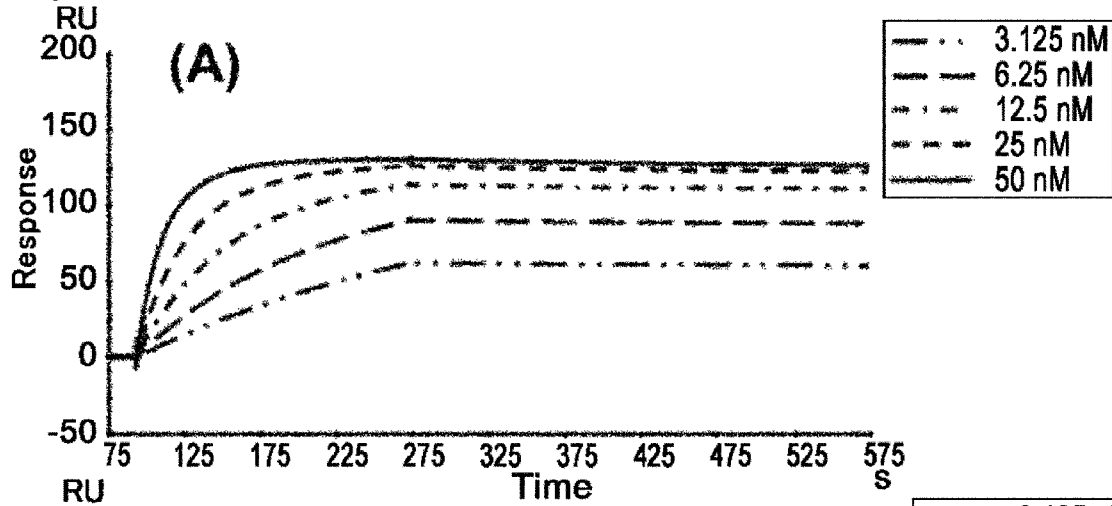
[Fig. 3]



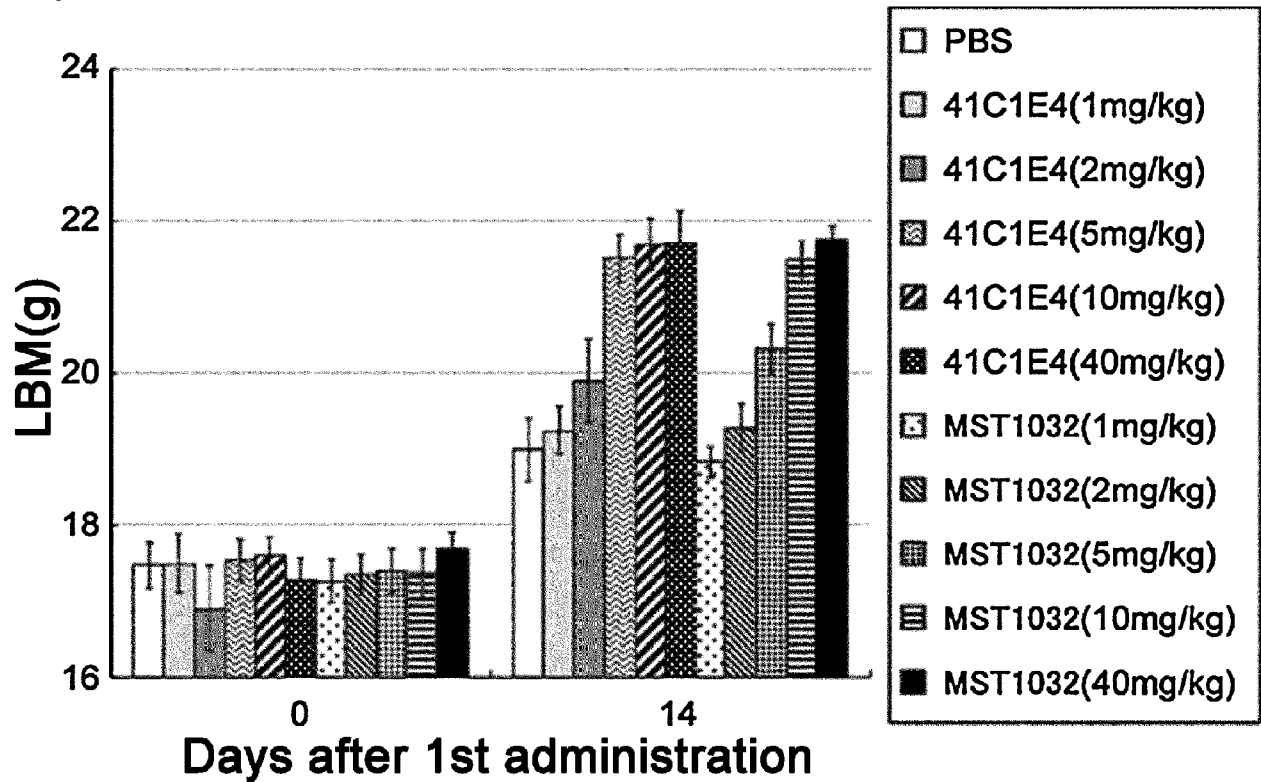
[Fig. 4]



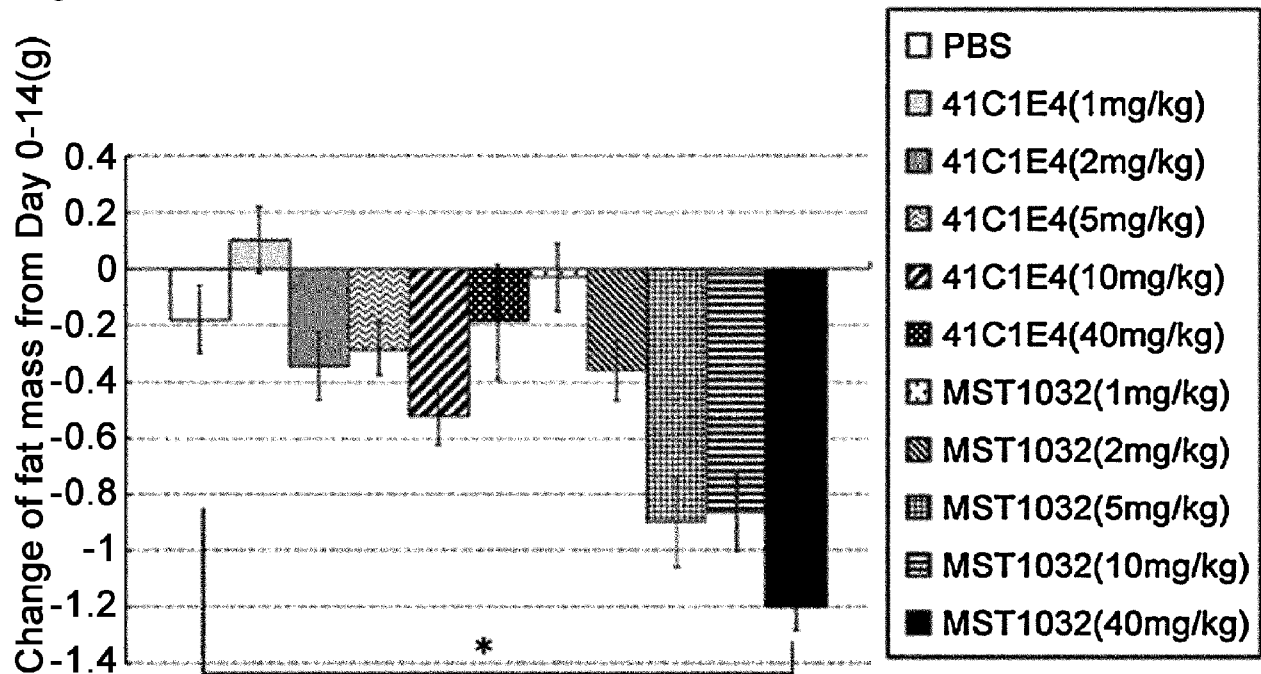
[Fig. 5]



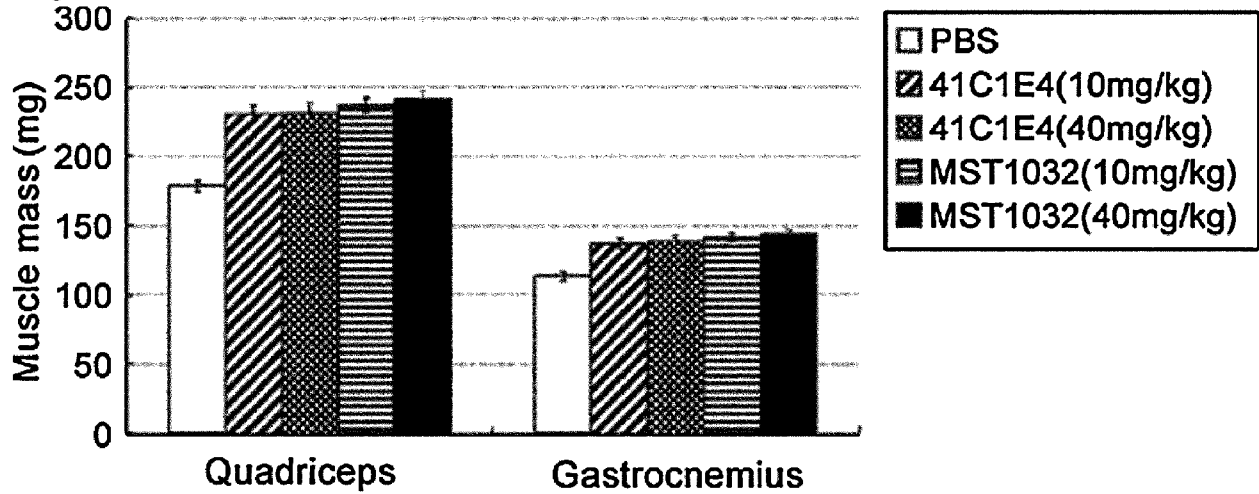
[Fig. 6A]



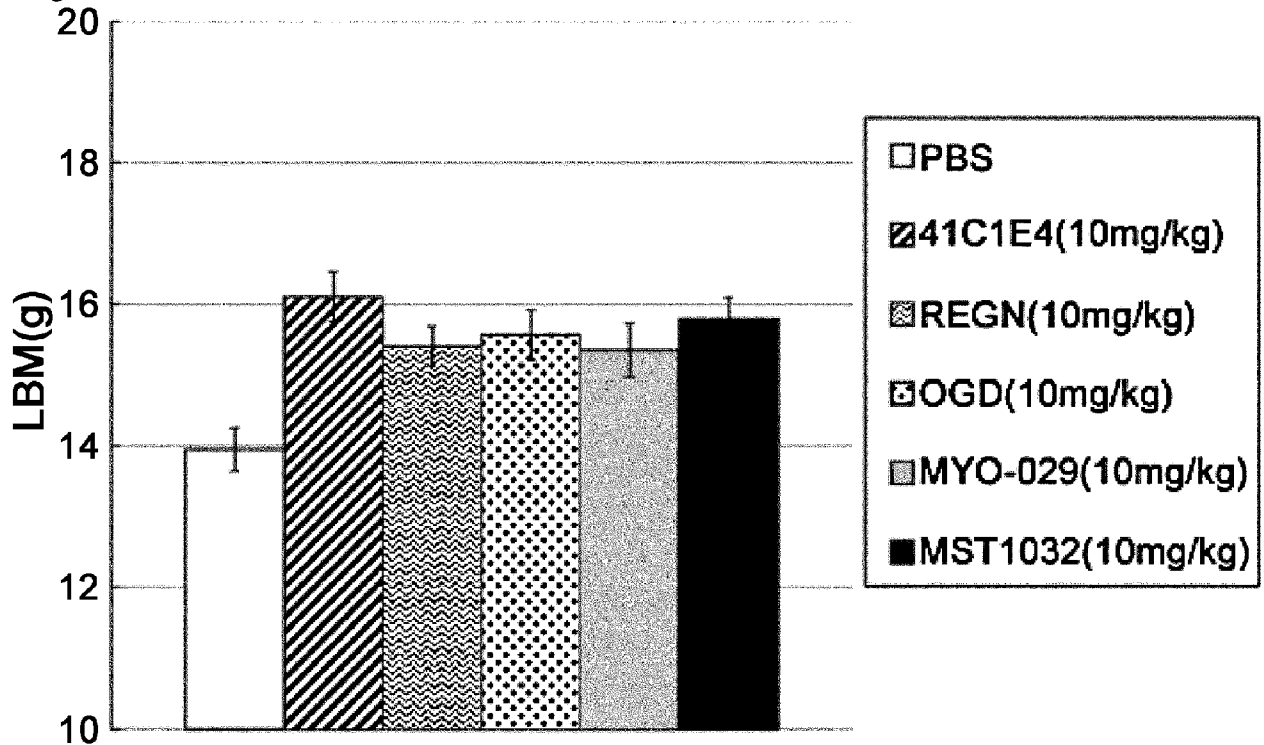
[Fig. 6B]



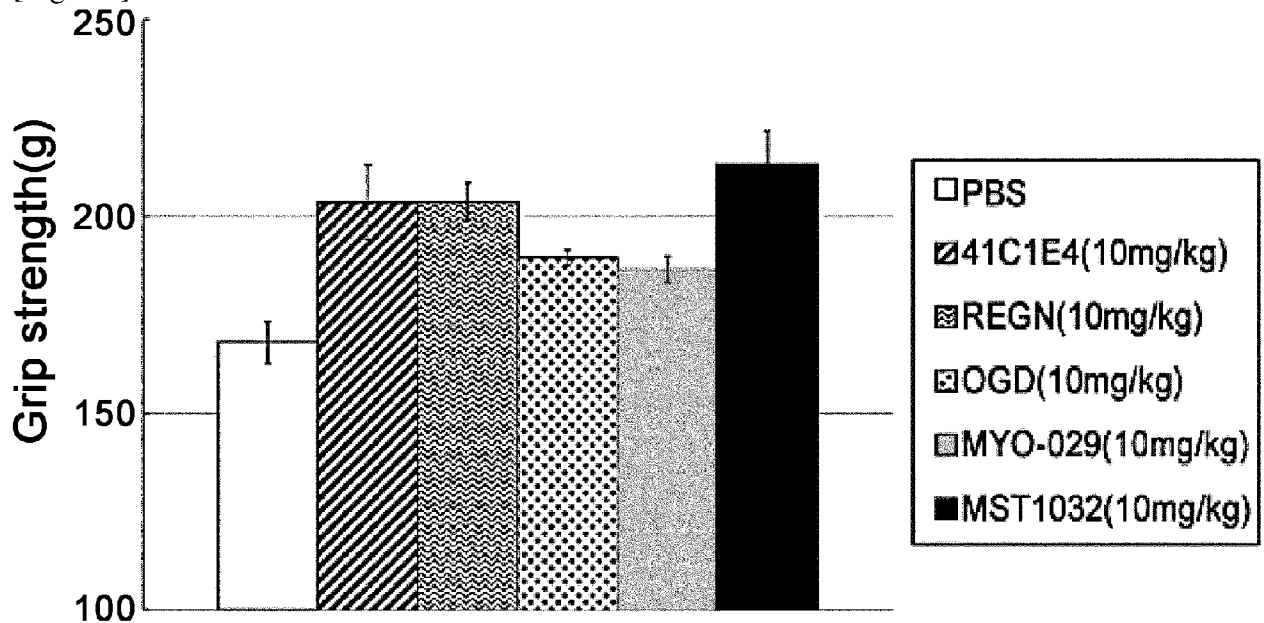
[Fig. 6C]



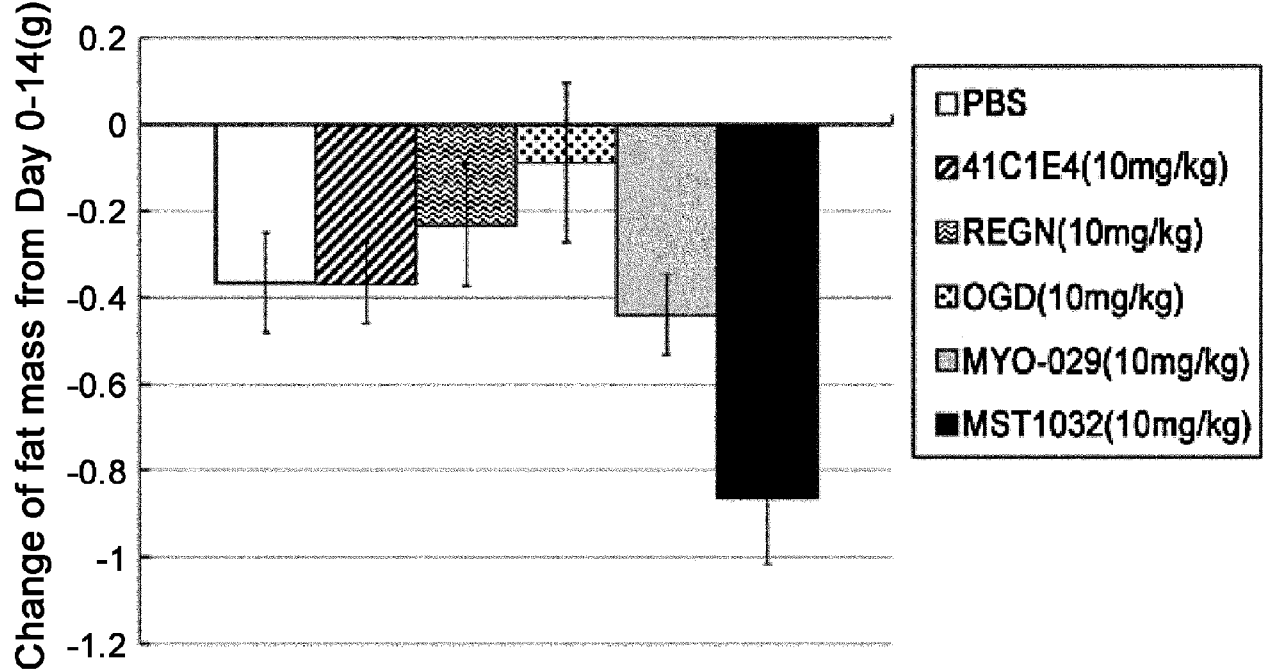
[Fig. 7A]



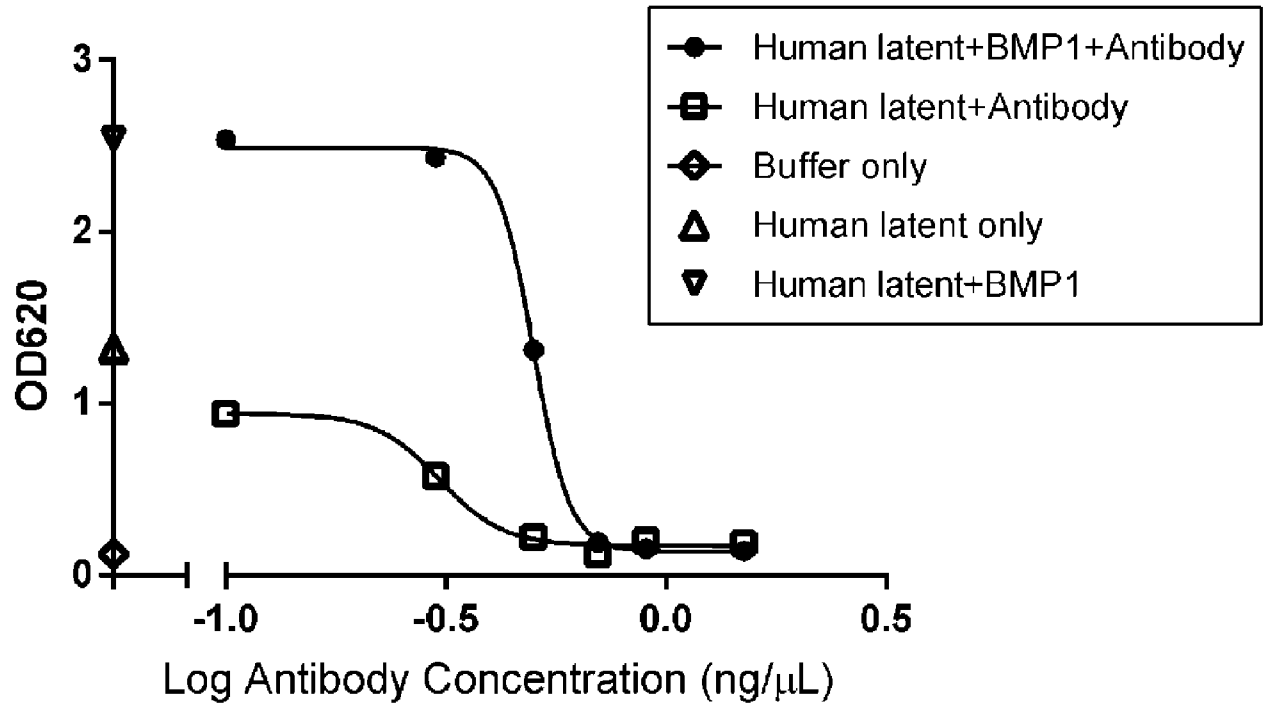
[Fig. 7B]



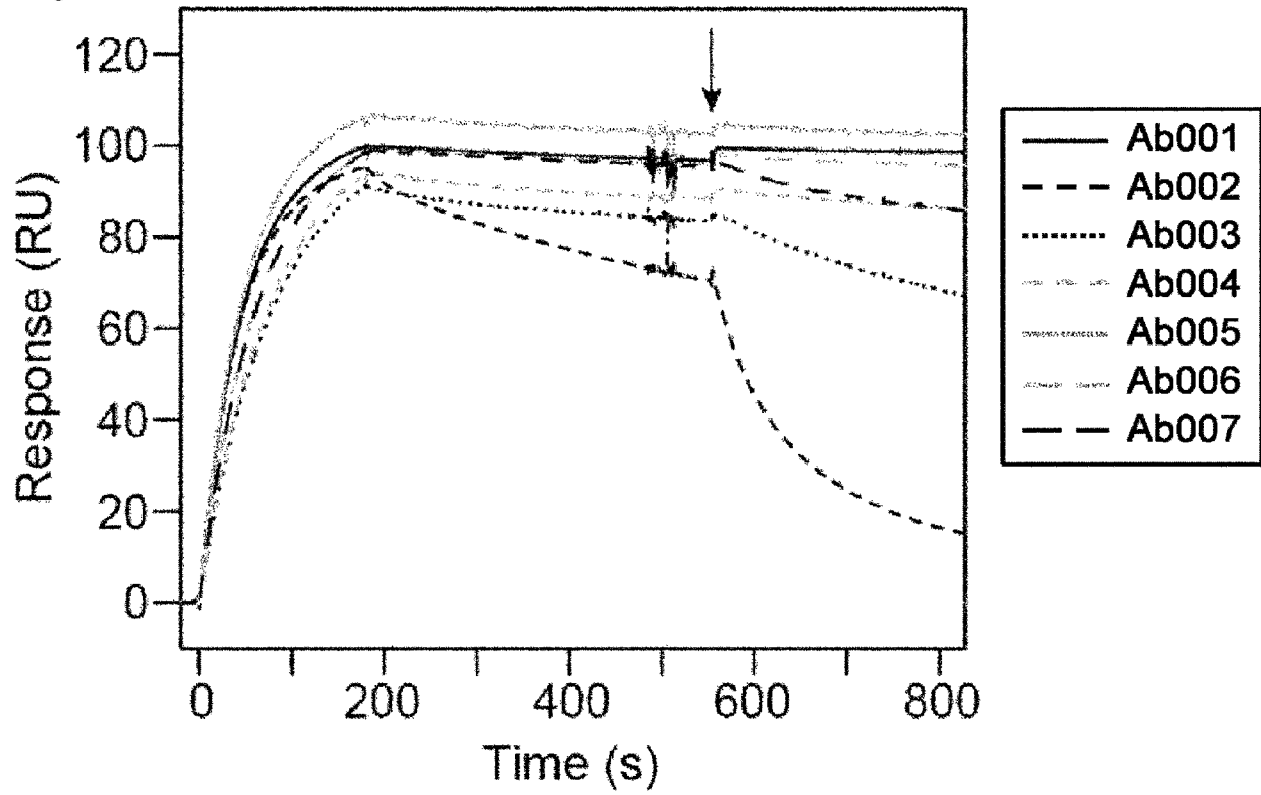
[Fig. 7C]



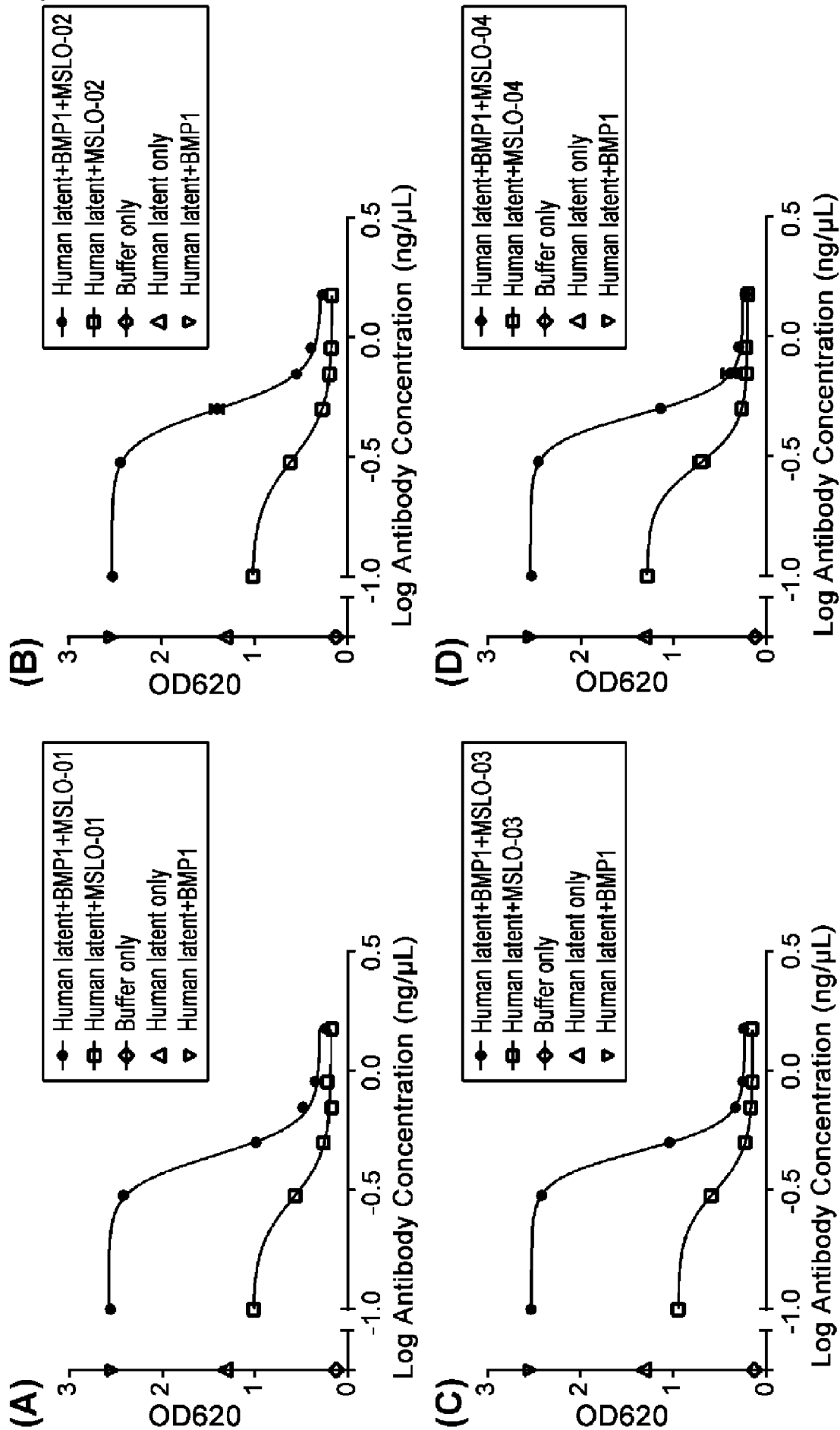
[Fig. 8]



[Fig. 9]



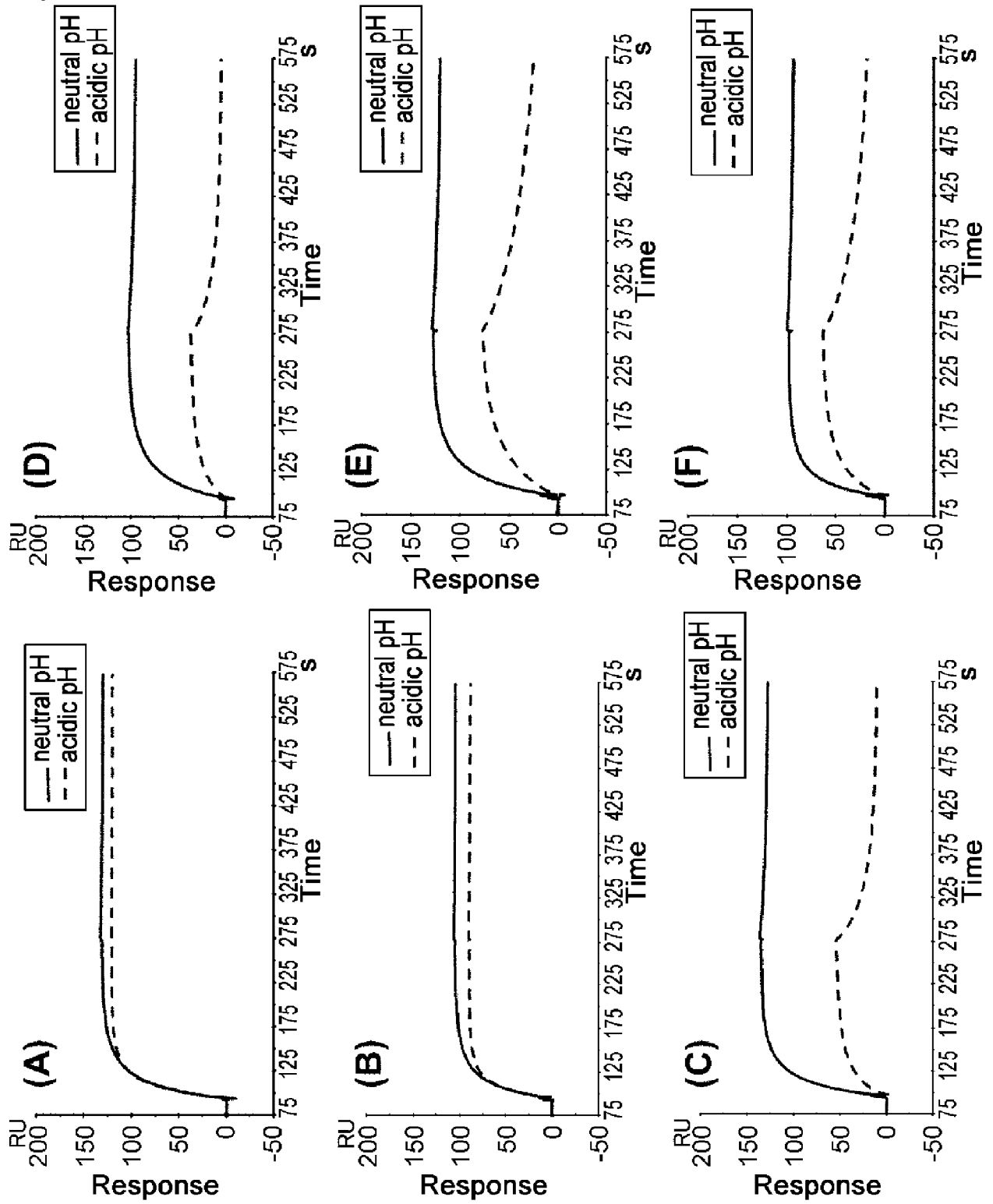
[Fig. 10]



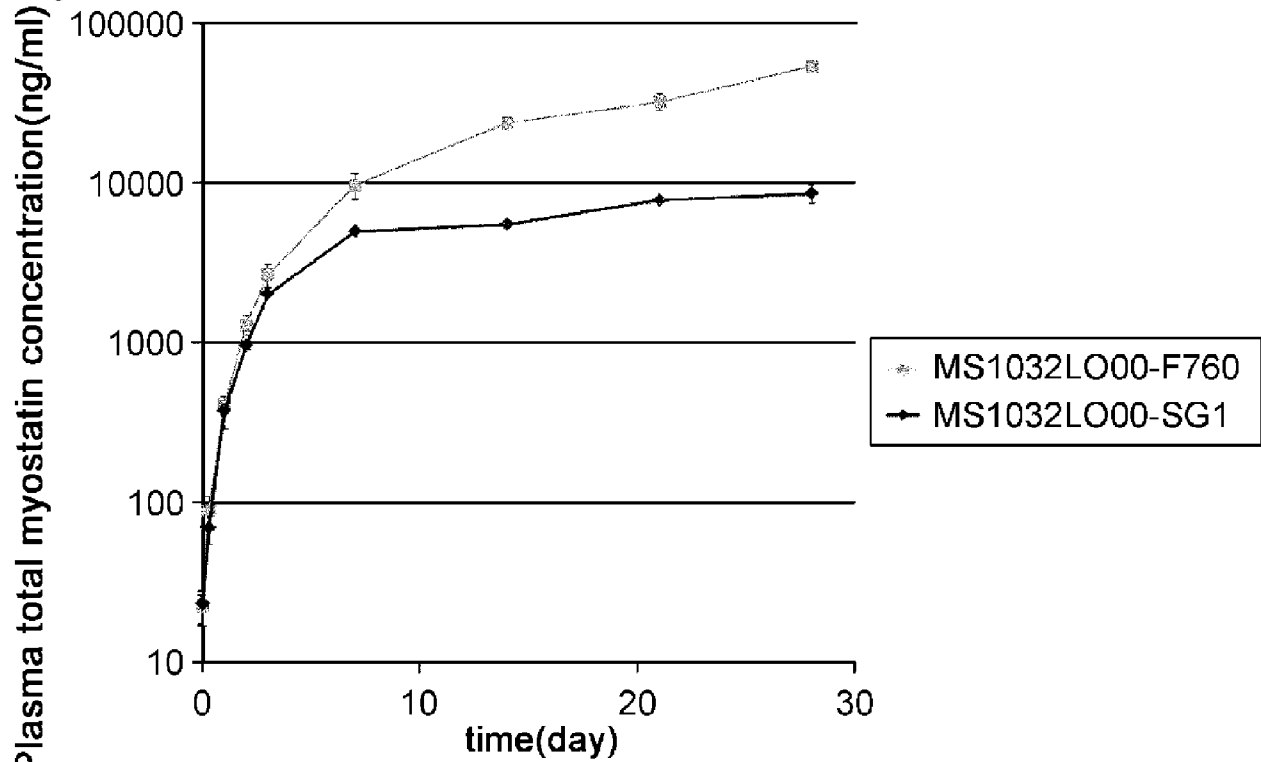
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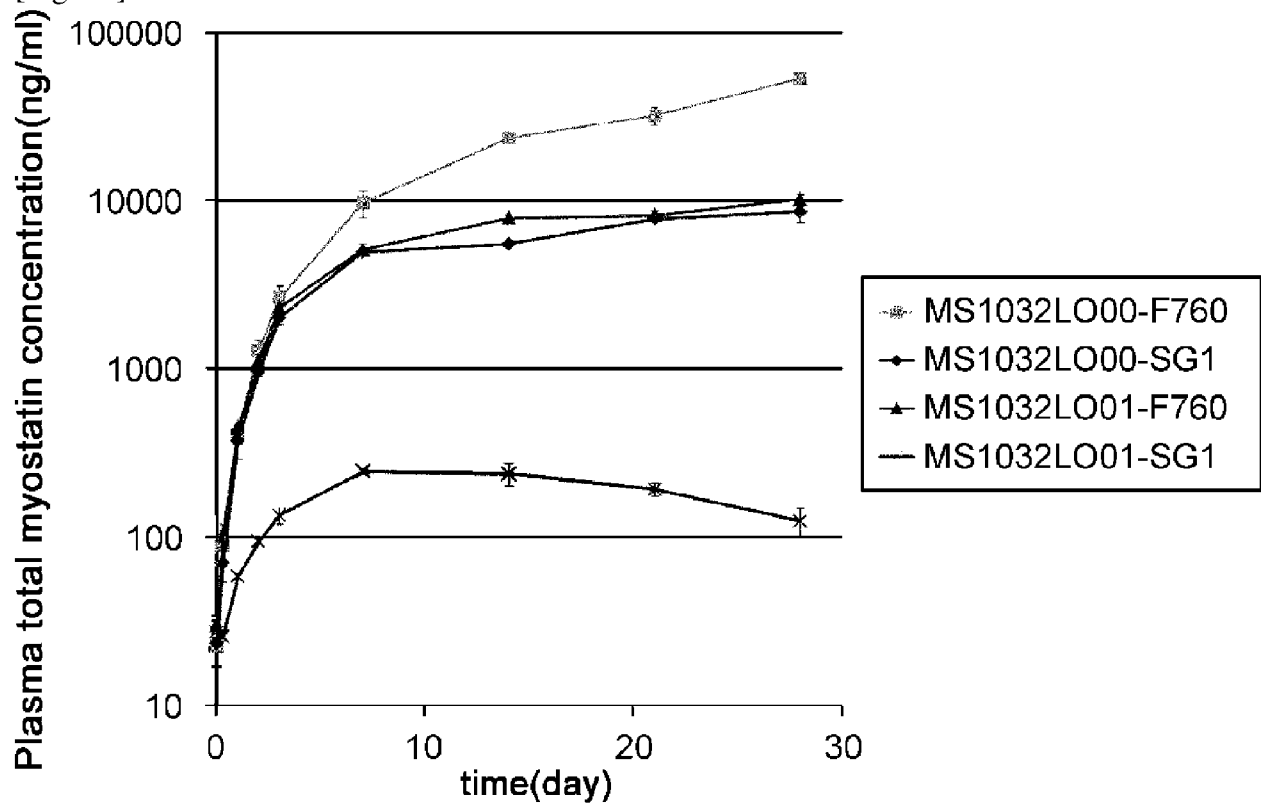
[Fig. 11]



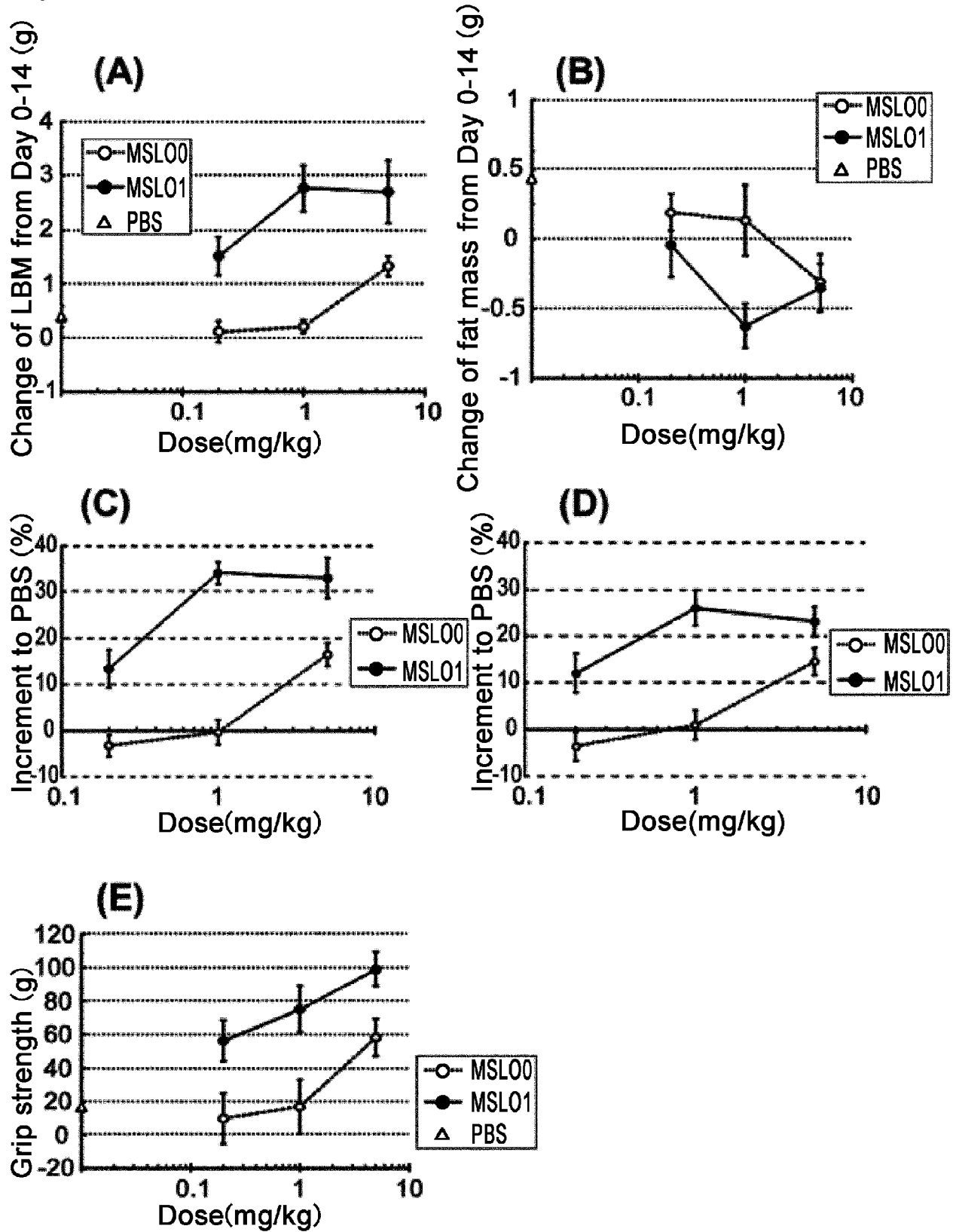
[Fig. 12]



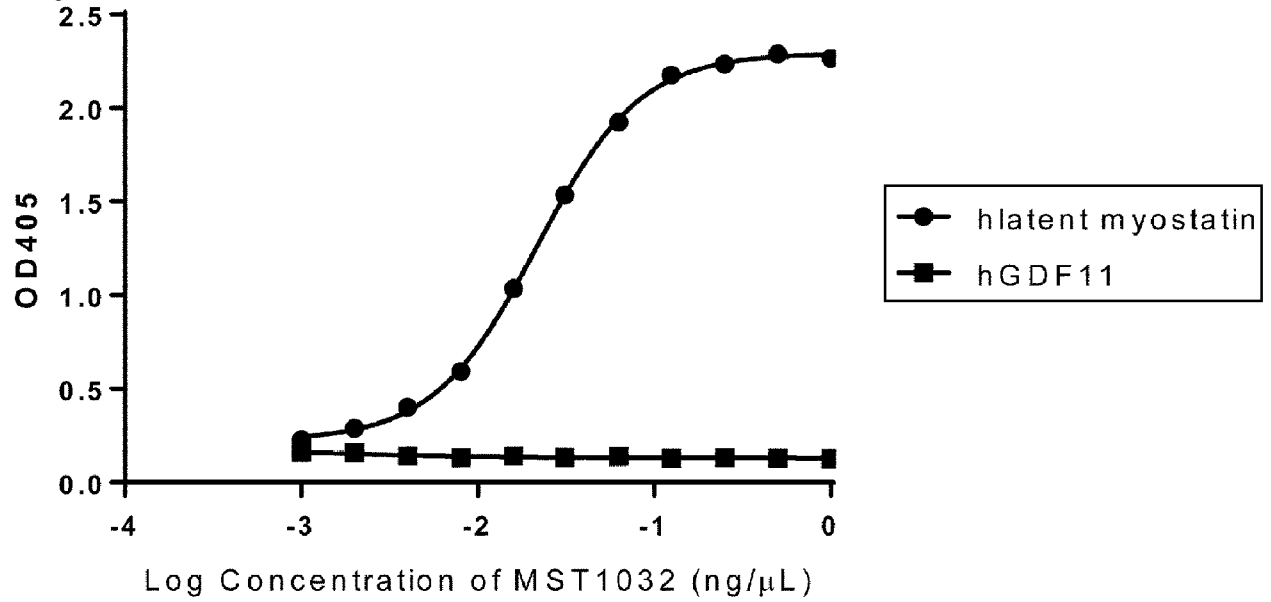
[Fig. 13]



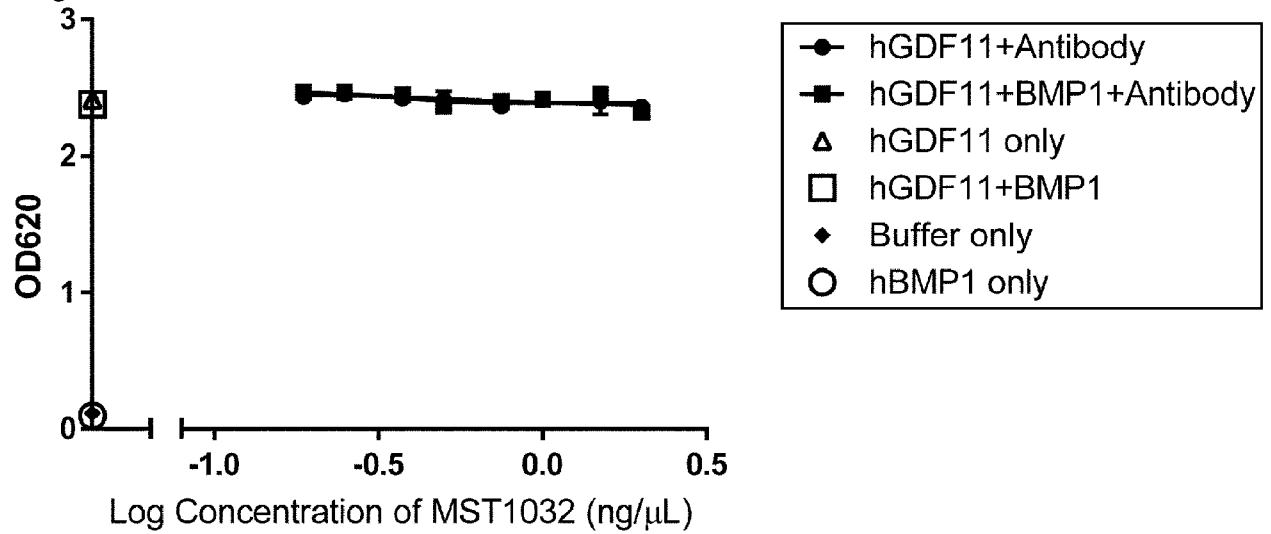
[Fig. 14]



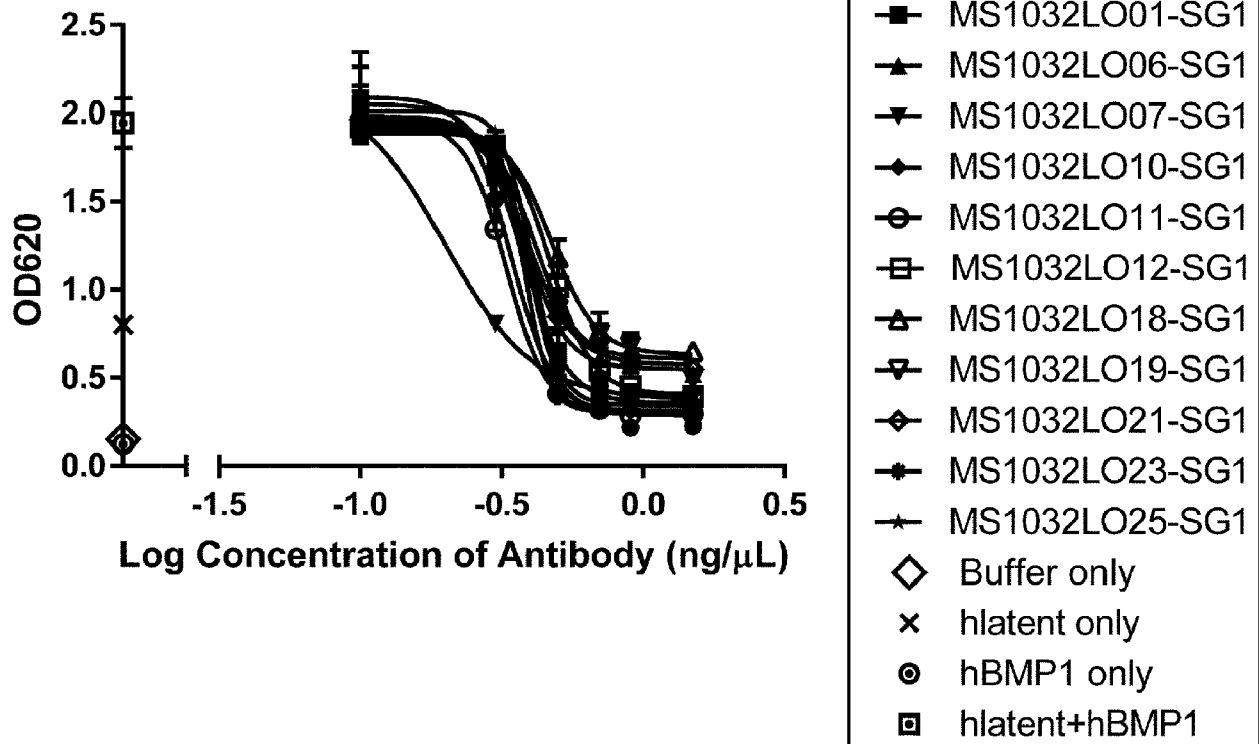
[Fig. 15]



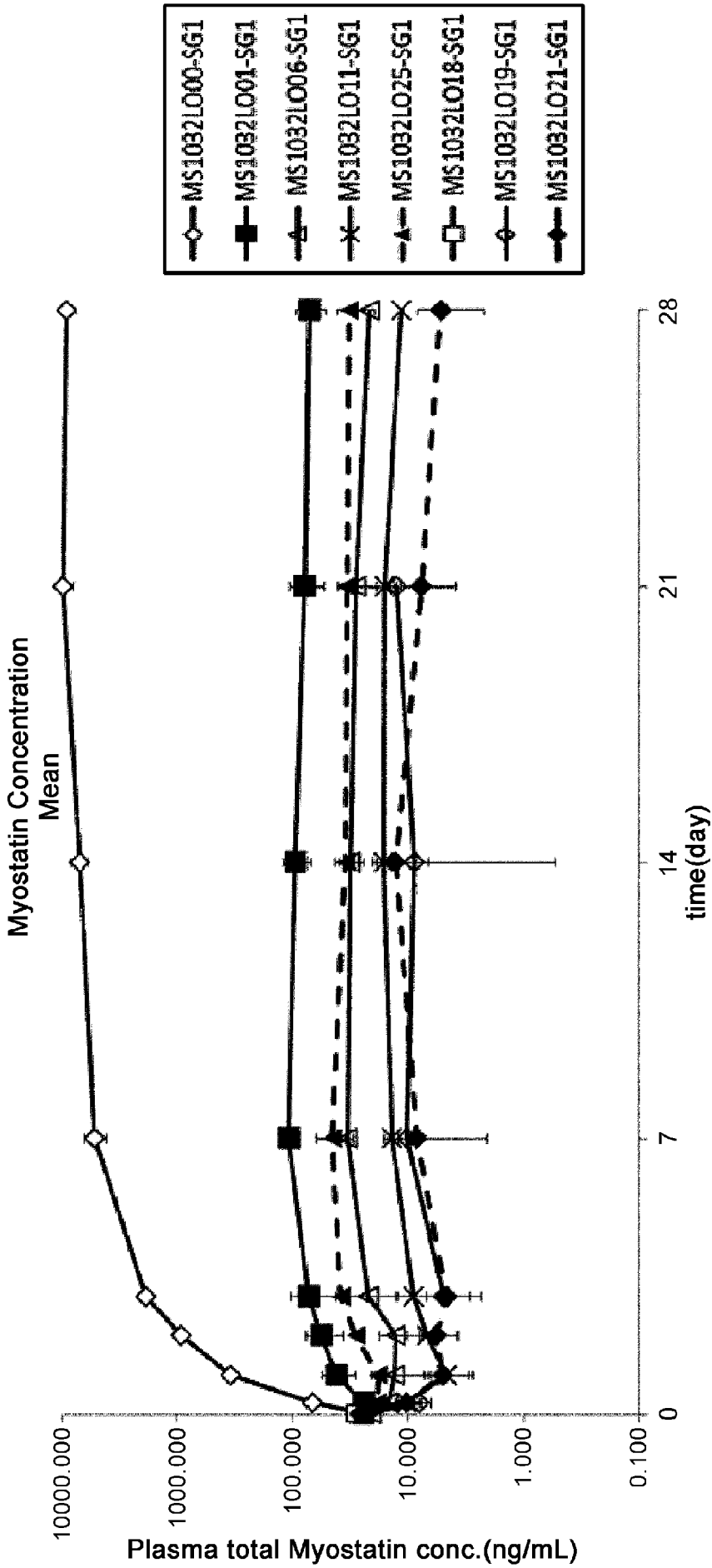
[Fig. 16]



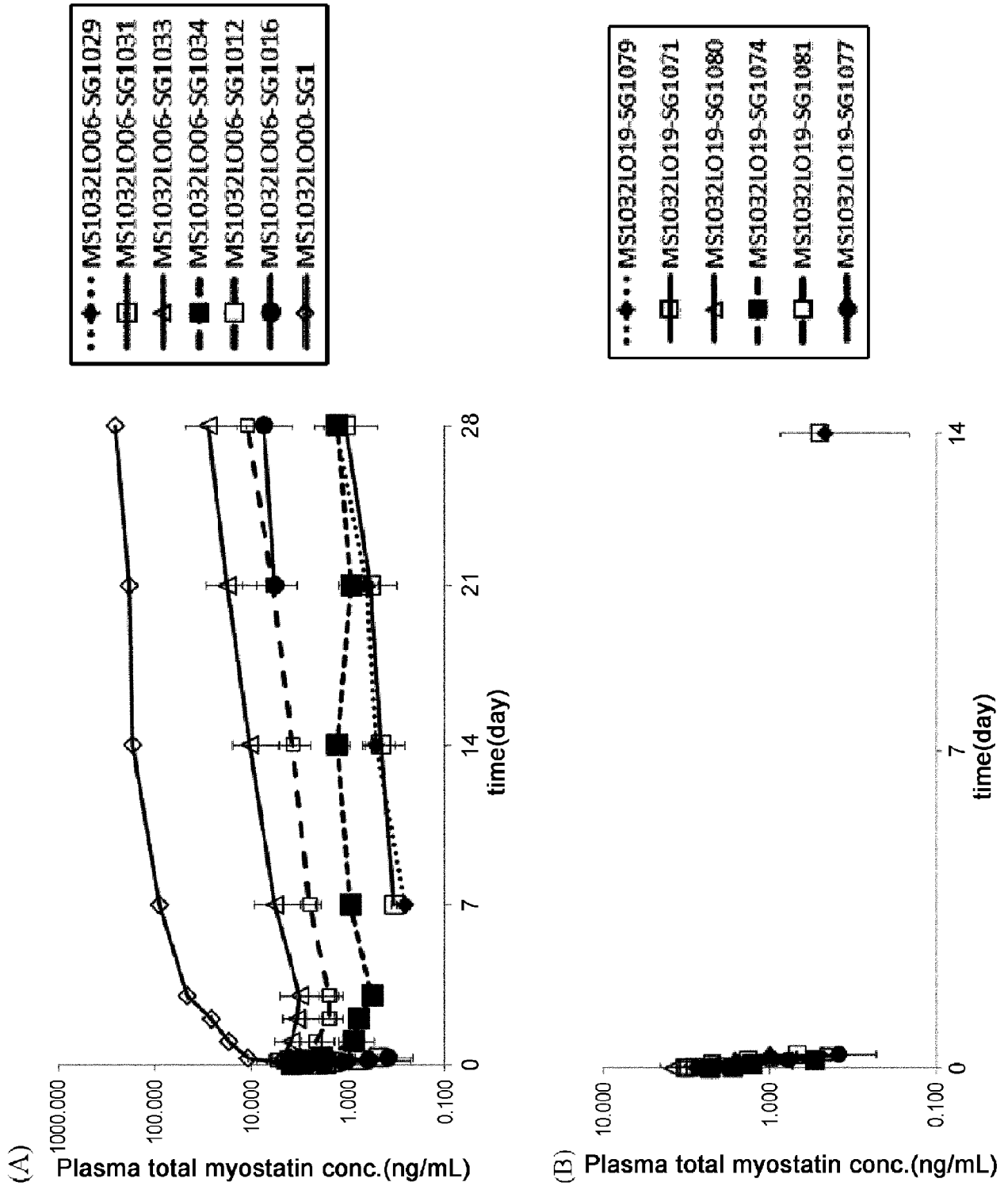
[Fig. 17]



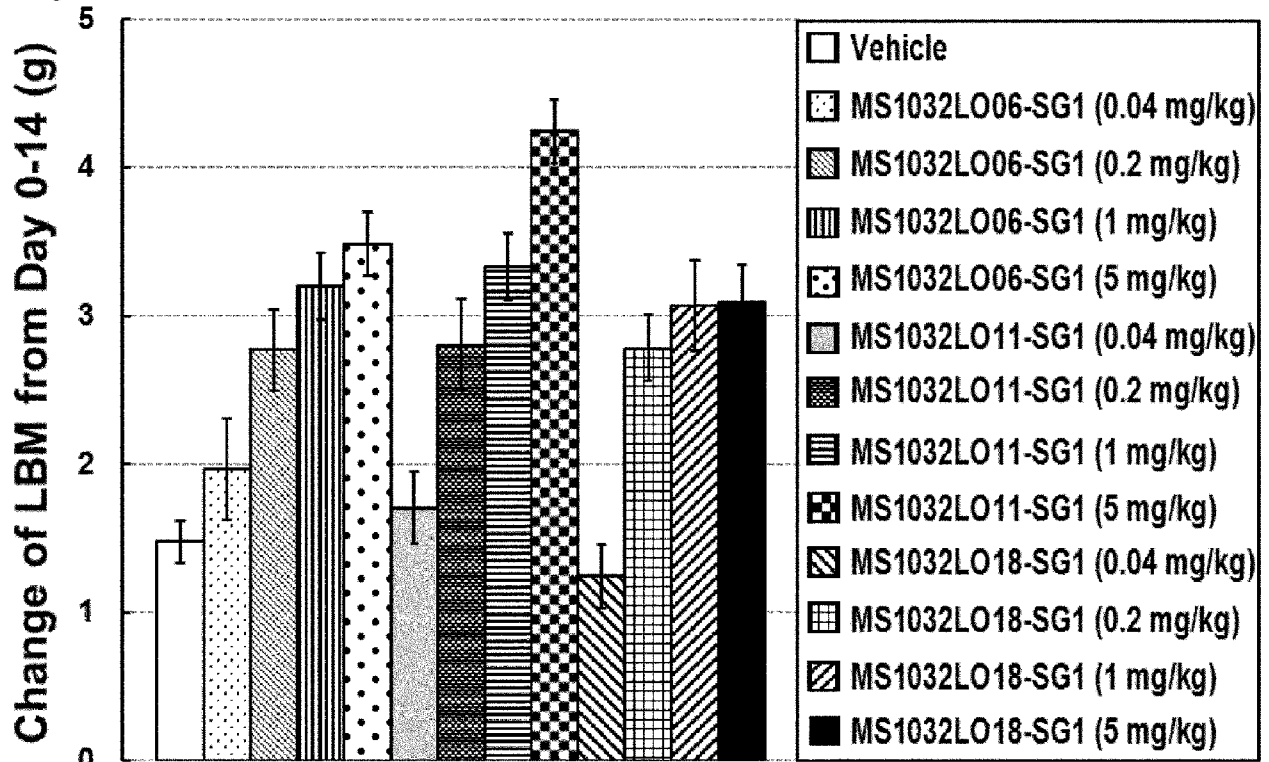
Myostatin Concentration



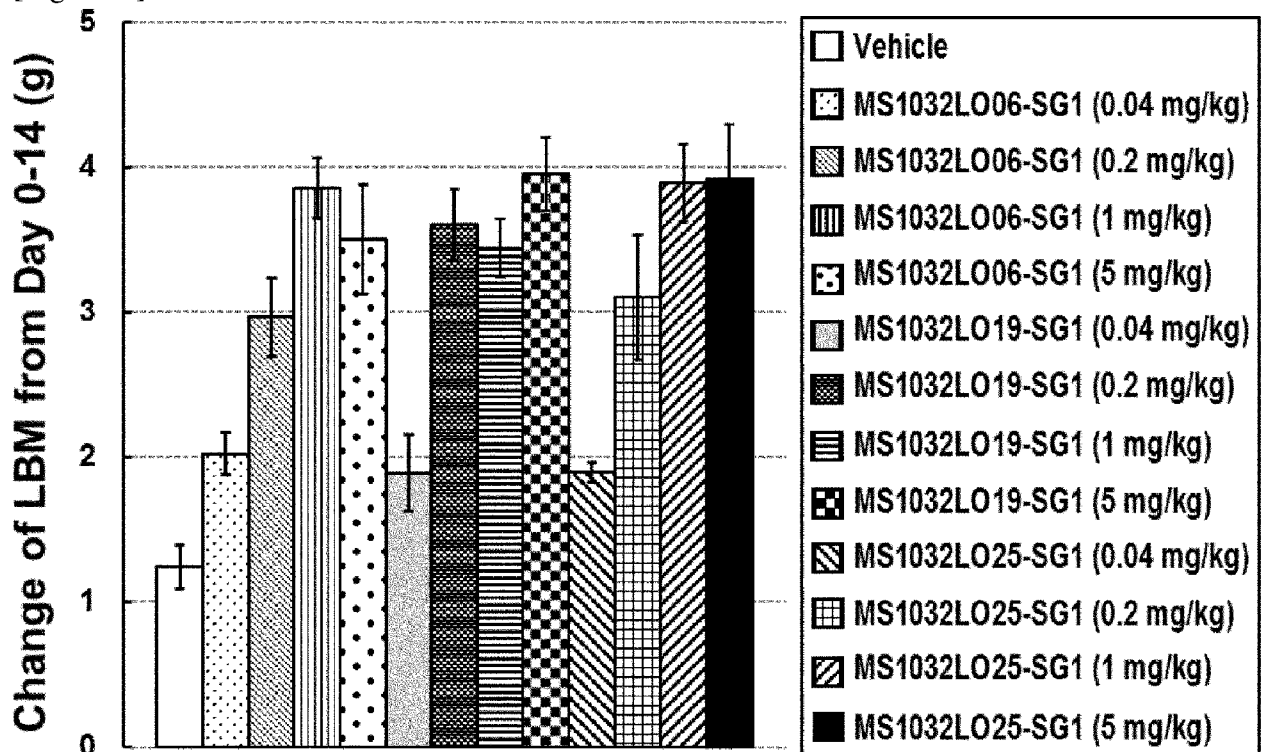
[Fig. 19]



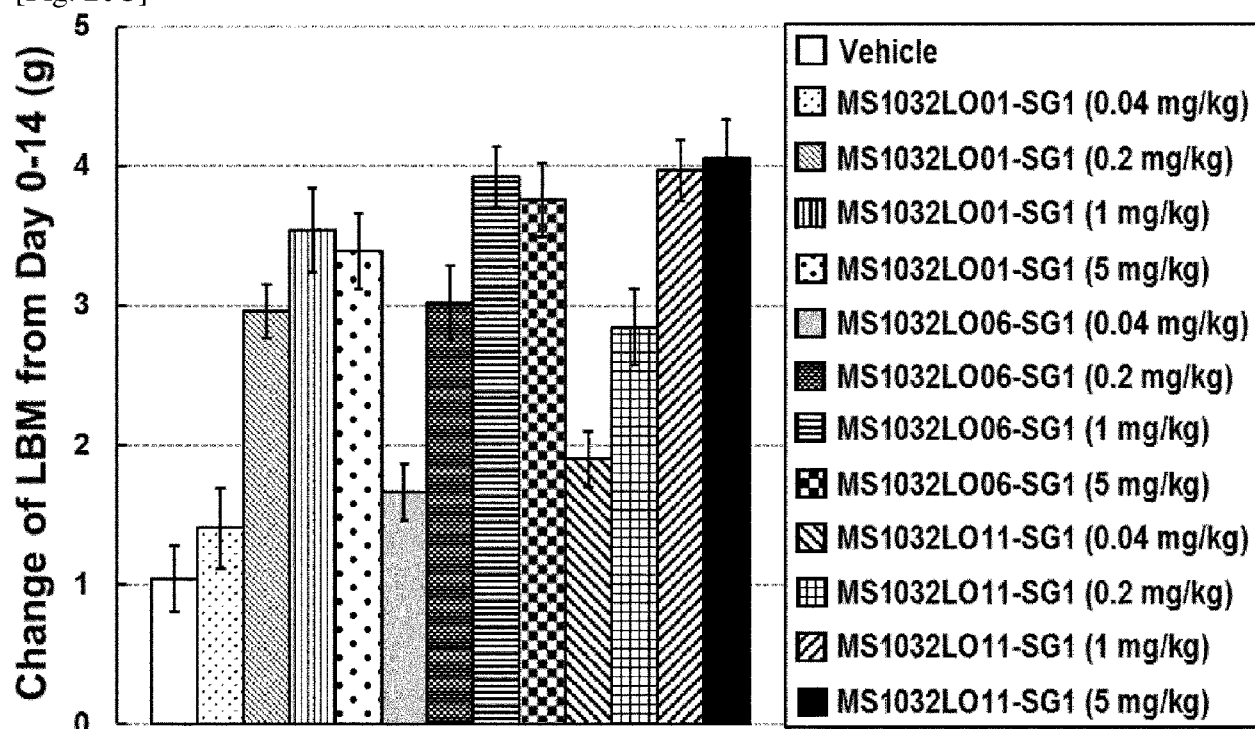
[Fig. 20A]



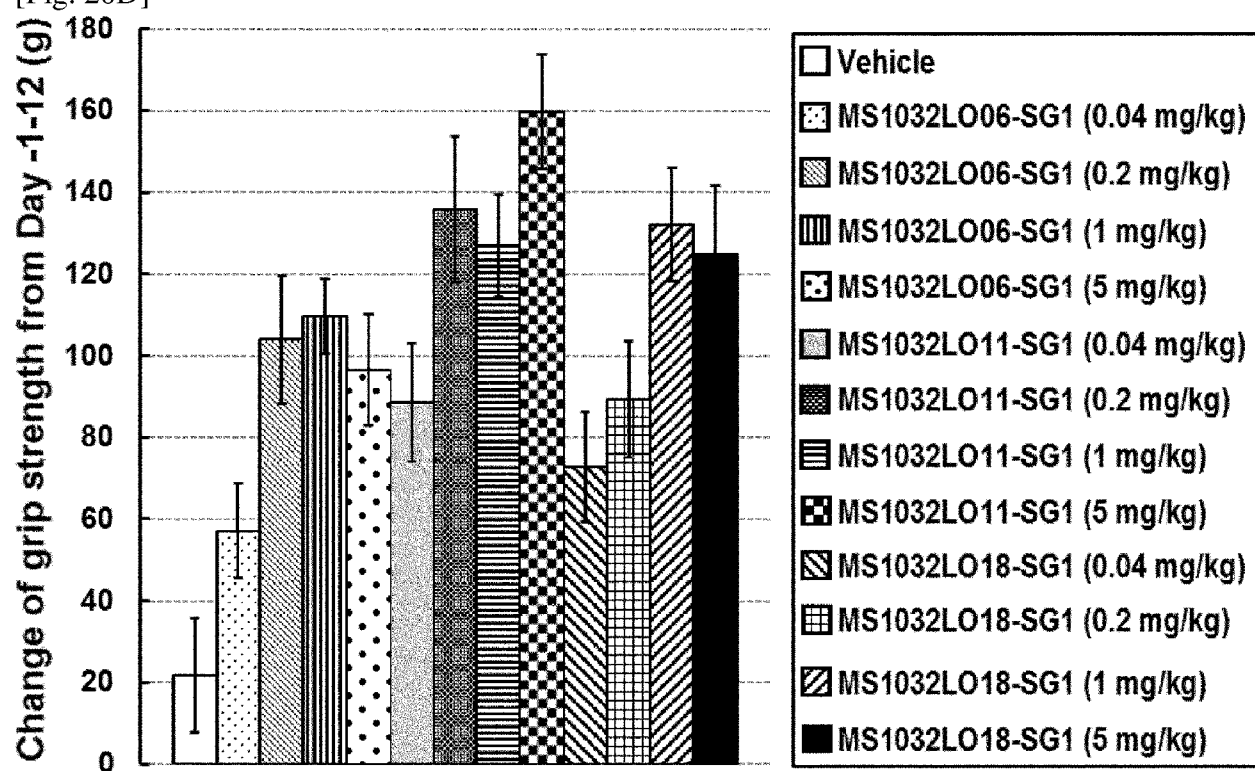
[Fig. 20B]



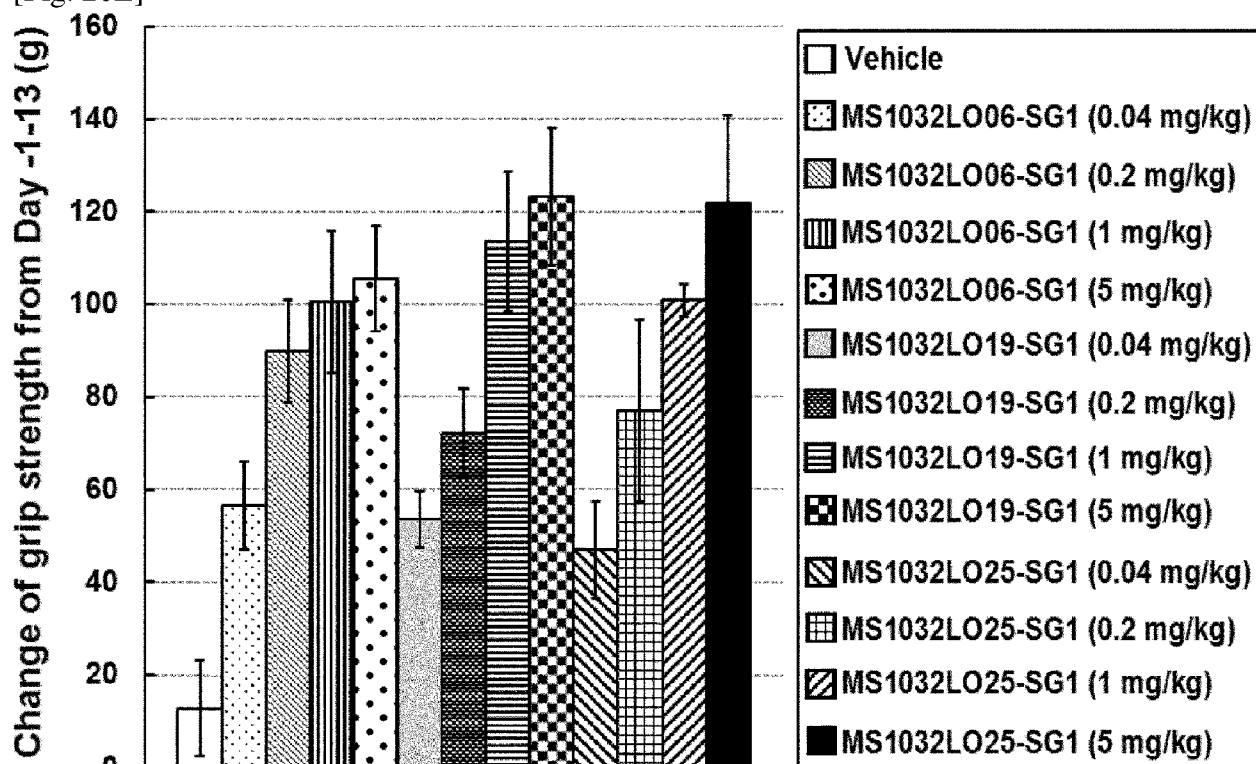
[Fig. 20C]



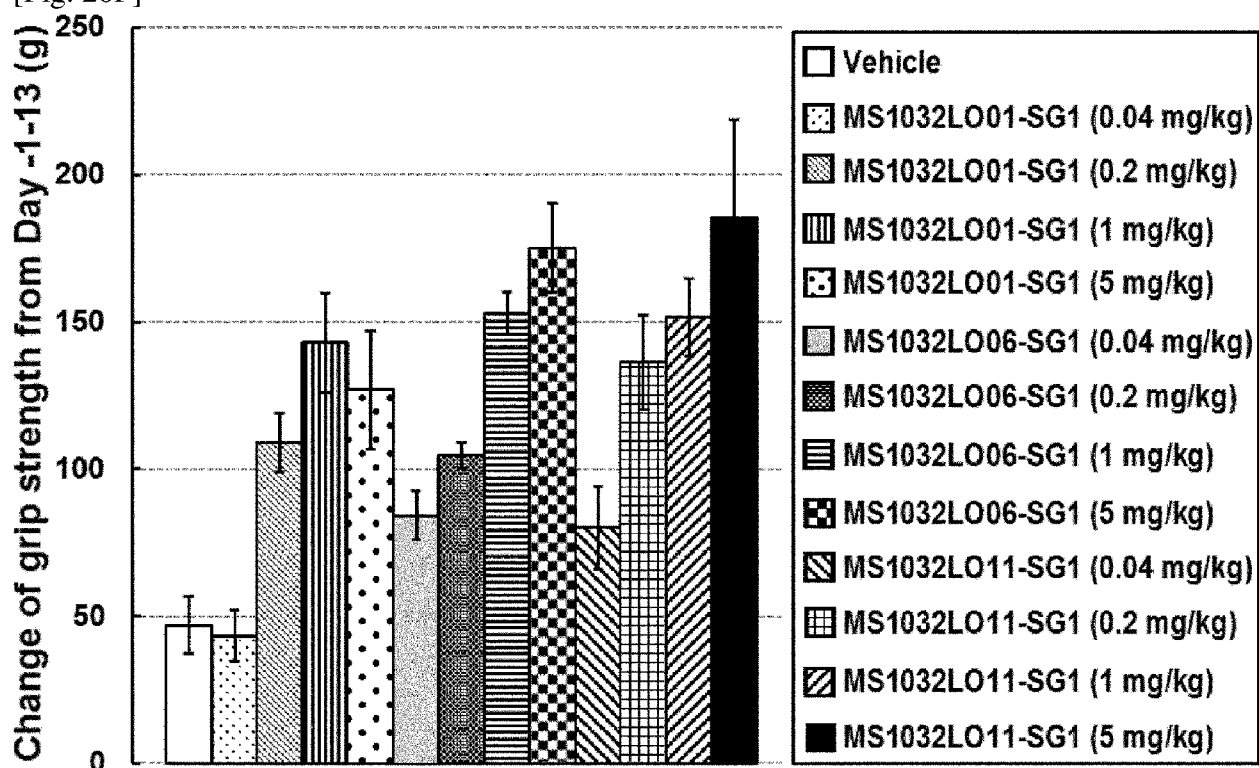
[Fig. 20D]



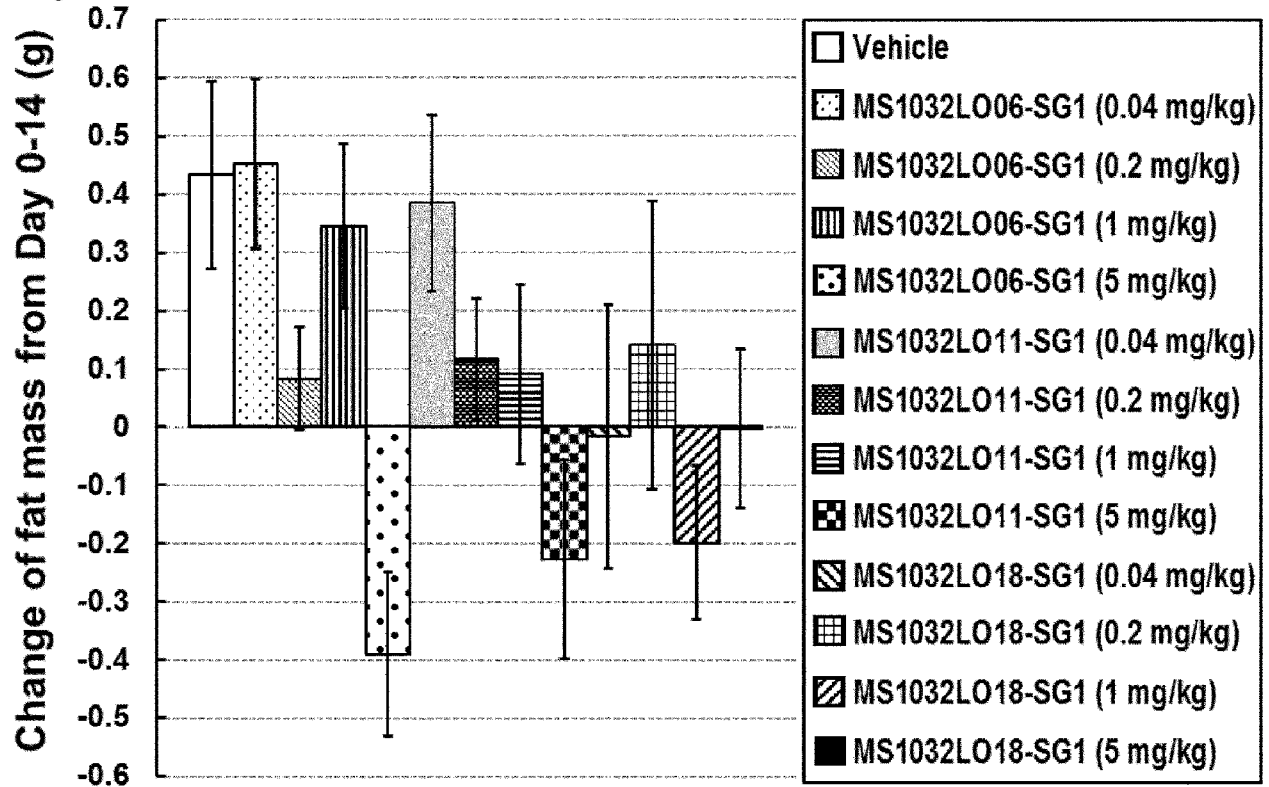
[Fig. 20E]



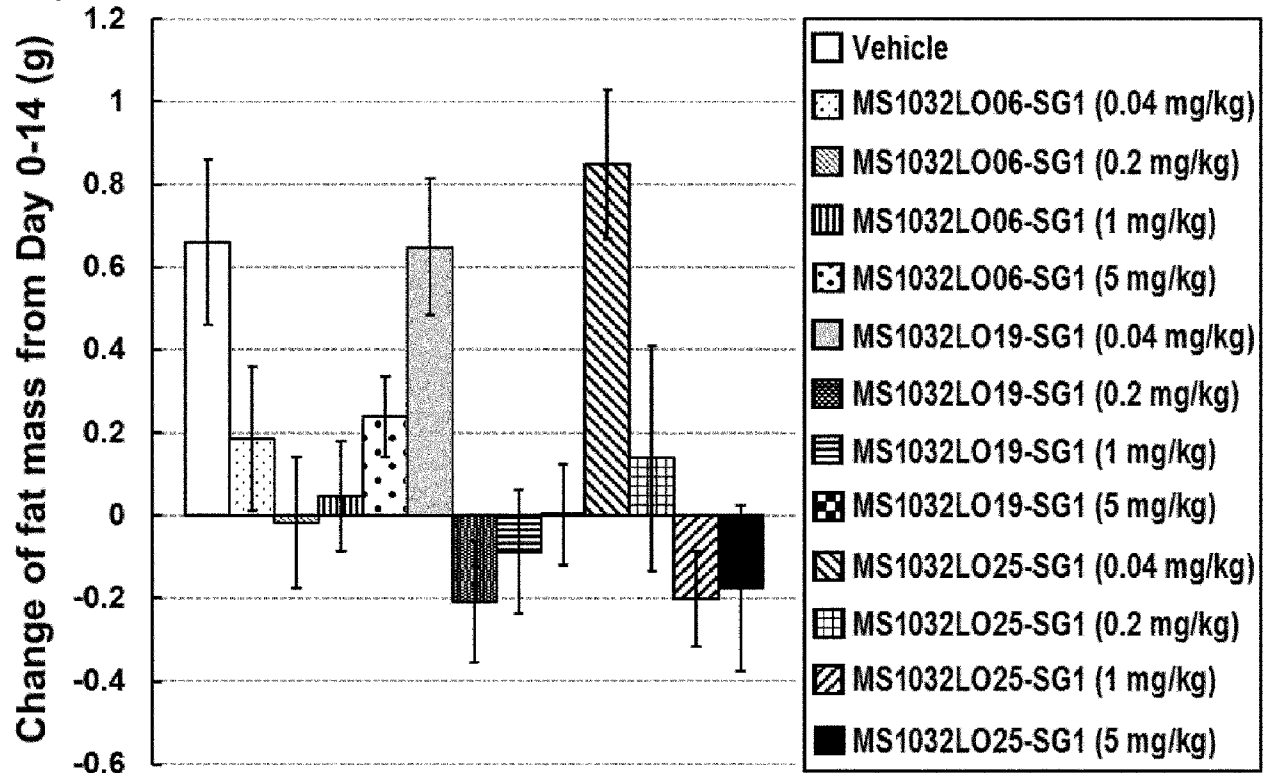
[Fig. 20F]



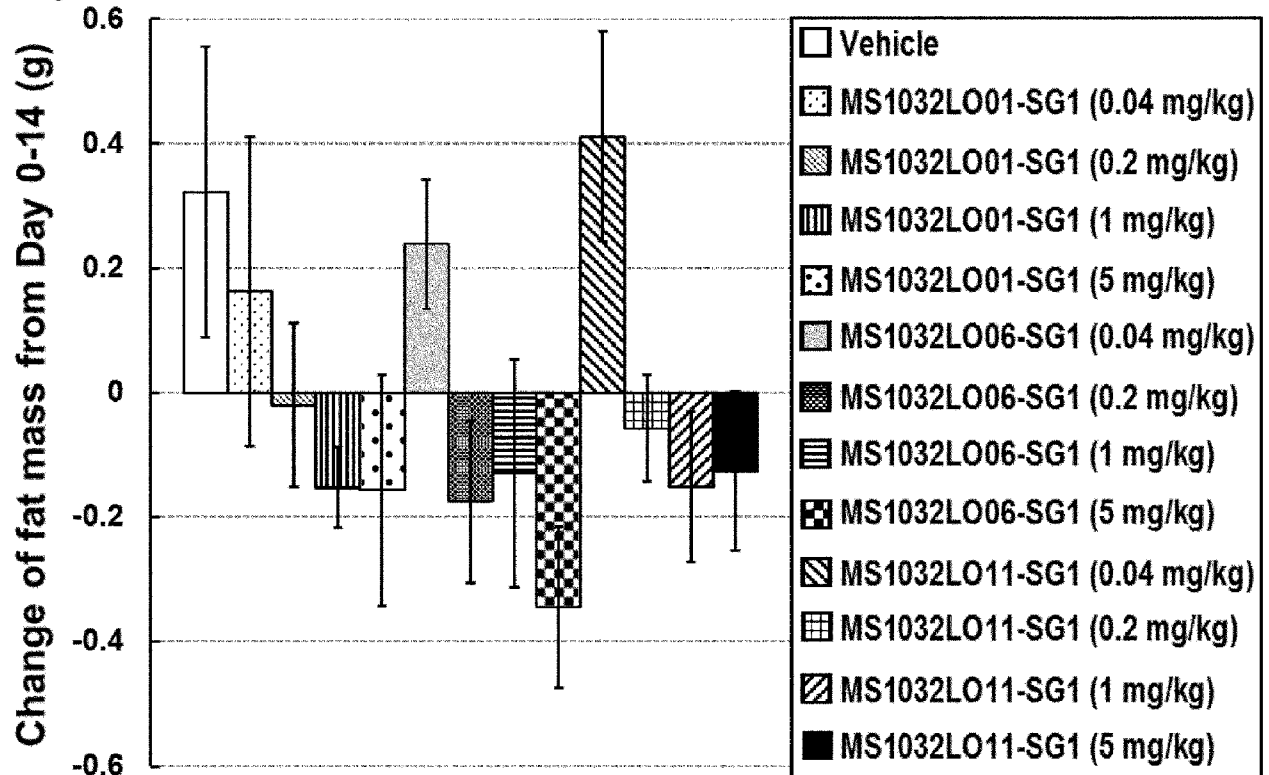
[Fig. 20G]



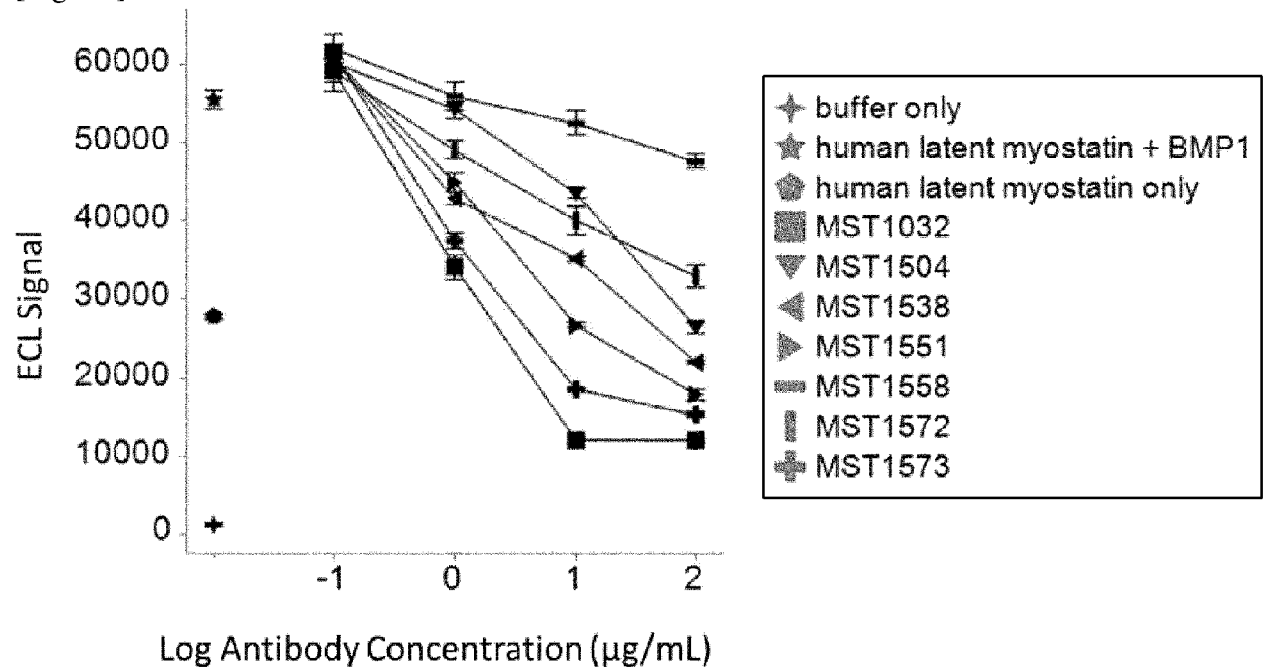
[Fig. 20H]



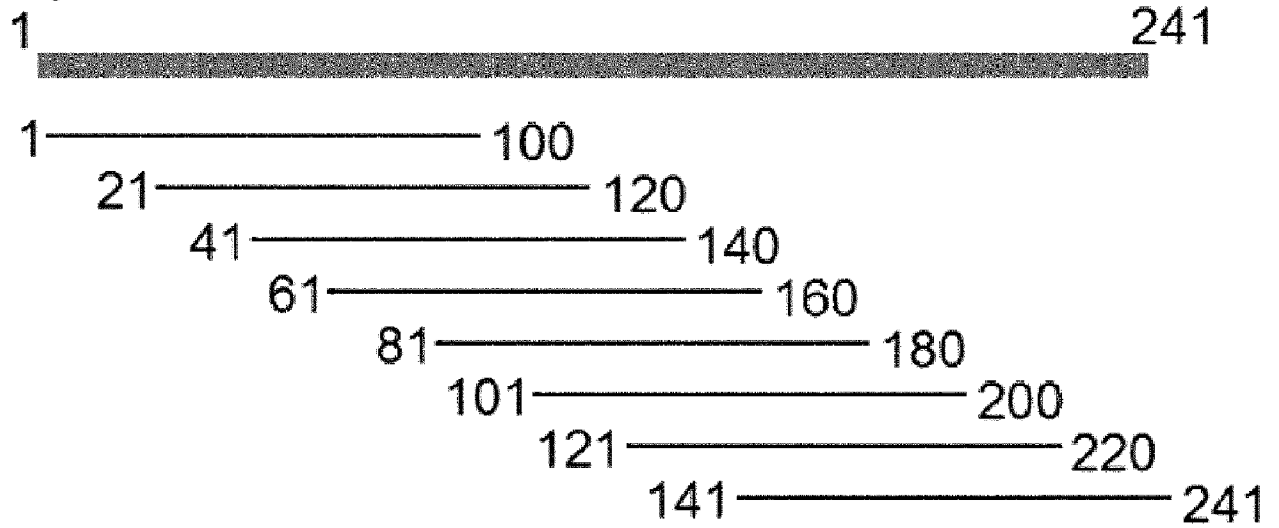
[Fig. 20I]



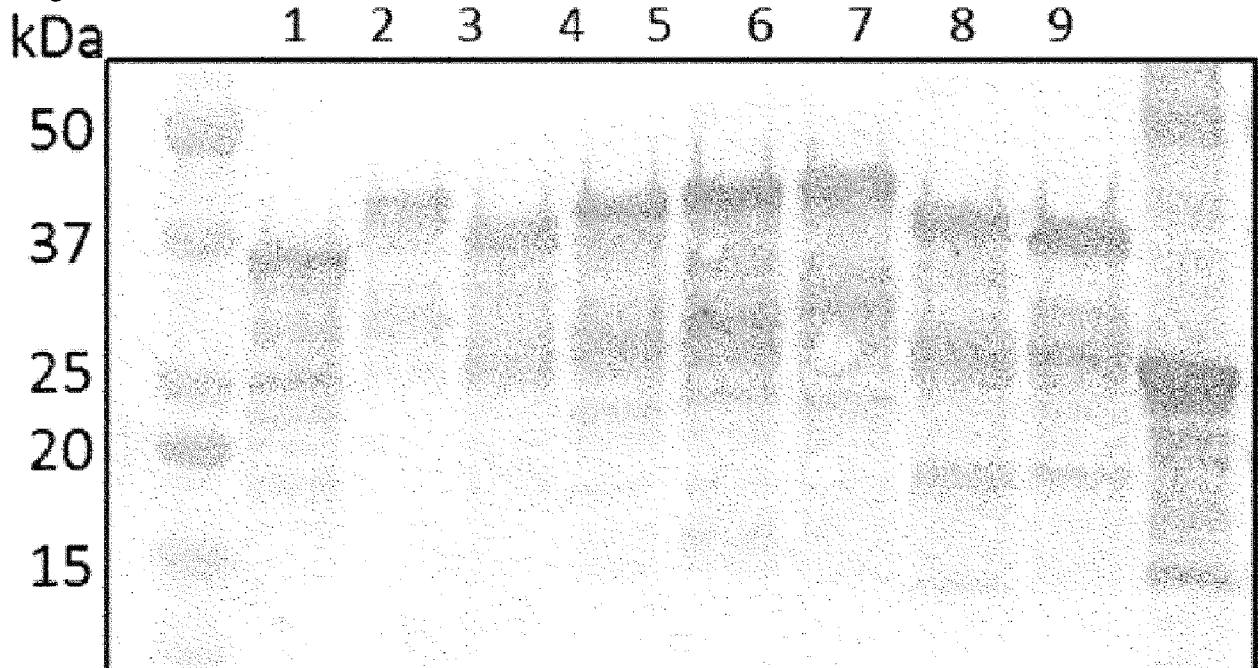
[Fig. 21]



[Fig. 22A]



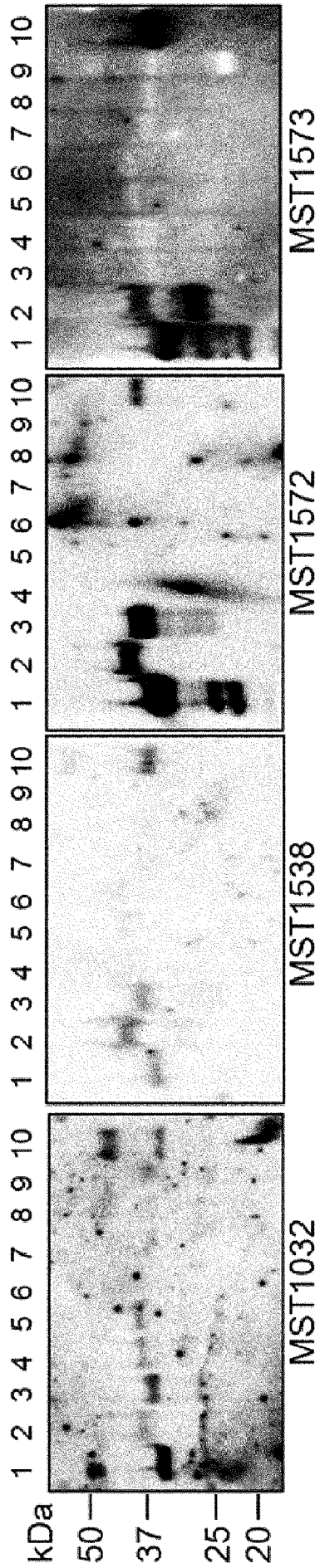
[Fig. 22B]



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PCT/JP2016/087487

[Fig. 22C]



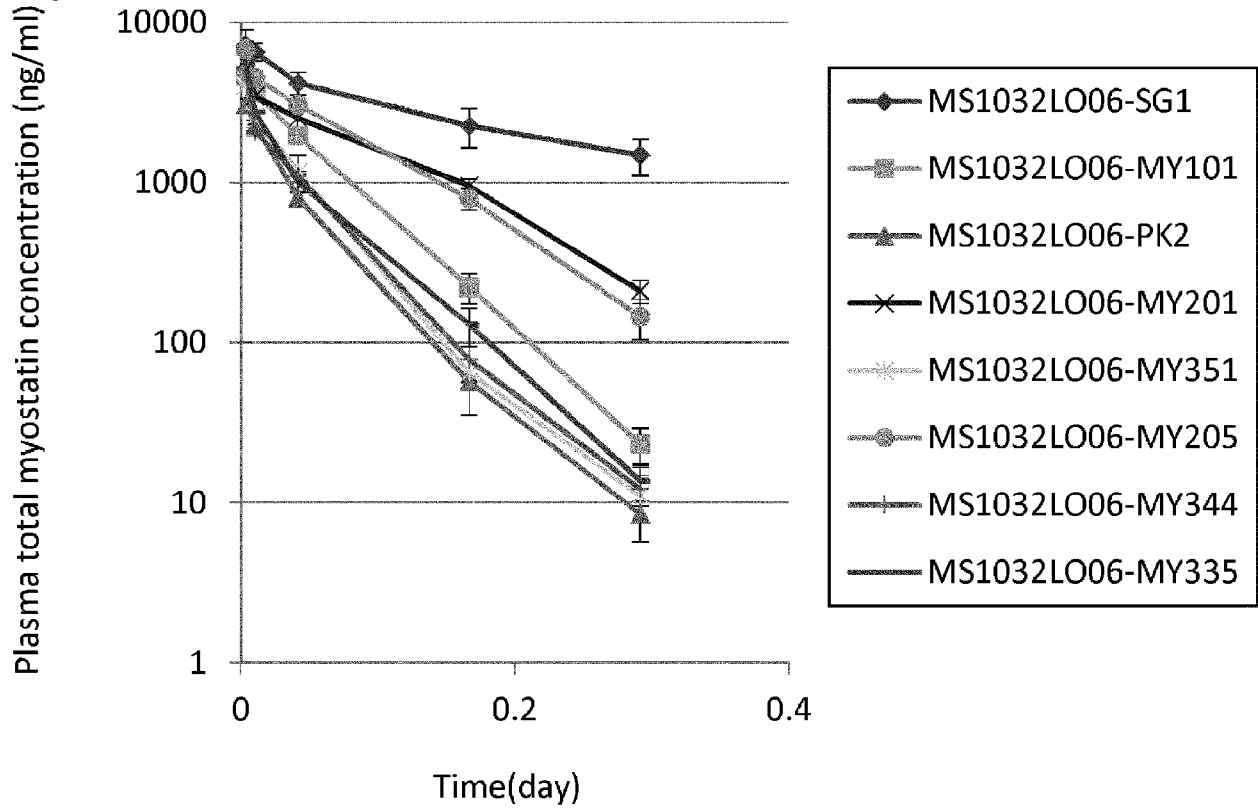
[Fig. 22D]

Ab	Main epitope	fragments							
		1	2	3	4	5	6	7	8
MST1032	81-100								
MST1538	41-60								
MST1572	41-60								
MST1573	21-40								

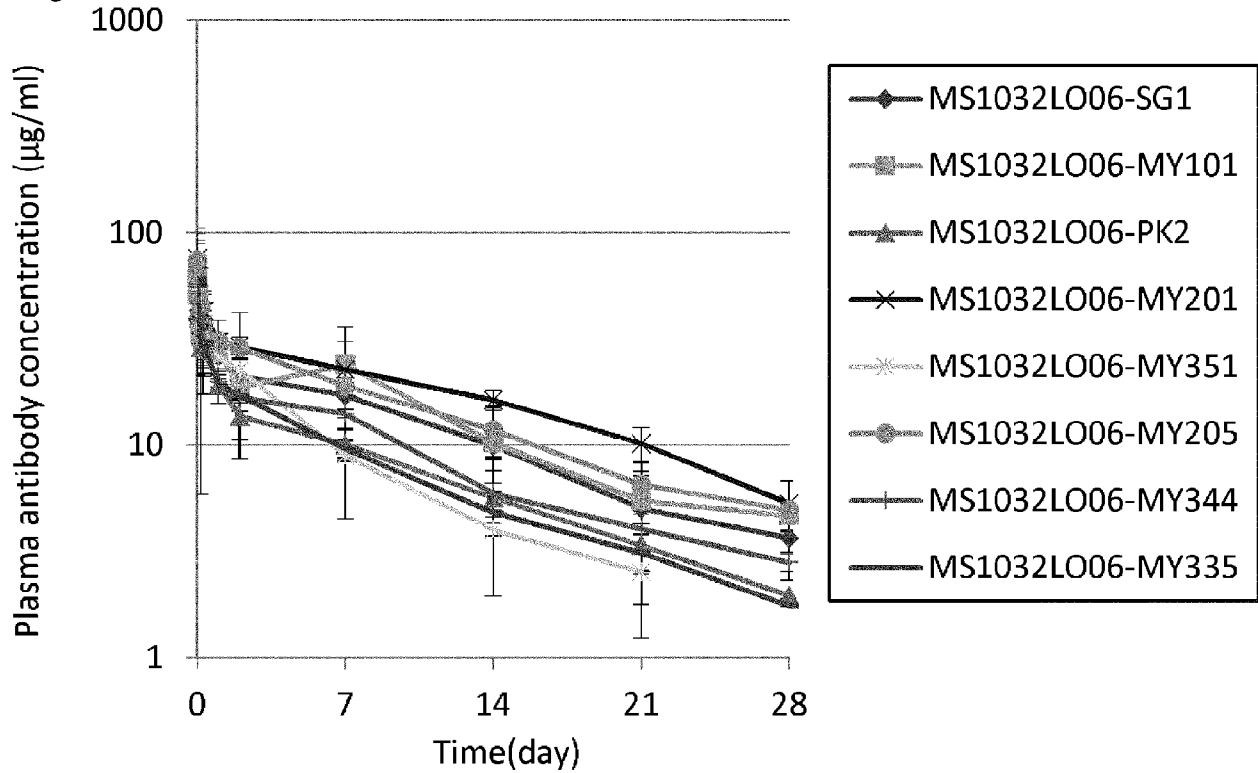
[Fig. 23]

cynoFcgrIIa1	-----MSQNVCPGNLWLLQPL-TVLLILLASADSQIA-PPKAVLKLEPPWINVLREDSVILTCGGAHSPDSDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYRCQTGRISLSDPVHLTVLSEWLALQIPHLEFREGETIMLRCHSNKDKPLIKVITFFQNGIAKKFESHMDPN	(SEQ ID NO: 220)
cynoFcgrIIa2	-----MSQNVCPGNLWLLQPL-TVLLILLASADSQIA-PPKAVLKLEPPWINVLREDSVILTCGGAHSPDSDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYRCQTGRISLSDPVHLTVLSEWLALQIITHLEFREGETIMLRCHSNKDKPLIKVATFFQNGKSKNFESHMDPN	(SEQ ID NO: 221)
cynoFcgrIIa3	-----MSQNVCPGNLWLLQPL-TVLLILLASADSQIA-PPKAVLKLEPPWINVLREDSVILTCGGAHSPDSDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYRCQTGRISLSDPVHLTVLSEWLALQIITHLEFREGETIMLRCHSNKDKPLIKVATFFQNGISKKFESHMDPN	(SEQ ID NO: 222)
cynoFcgrIIb	MGILSFLPVLATESDWADCKSSQPWGHMLLWIAVFLAPVAGIPAAPPKAVLKLEPPWINVLREDSVILTCGGAHSPDSDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYRCQTGRISLSDPVHLTVLSEWLALQIPHLEFREGETIMLRCHSNKDKPLIKVITFFQNGKSKFESHMDPN	(SEQ ID NO: 223)
humanFcgrIIaH	-----MTMETQMSQNVCPGNLWLLQPL-TVLLILLASADSQIAAPPKAVLKLEPPWINVLQEDSVILTCQGAARSPEDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYTCQTGTSLSDPVHLTVLSEMLVLQIPHLEFREGETIMLRCHSNKDKPLVLKVITFFQNGKSKFESHLDP	(SEQ ID NO: 211)
humanFcgrIIaR	-----MTMETQMSQNVCPGNLWLLQPL-TVLLILLASADSQIAAPPKAVLKLEPPWINVLQEDSVILTCQGAARSPEDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYTCQTGTSLSDPVHLTVLSEMLVLQIPHLEFREGETIMLRCHSNKDKPLVLKVITFFQNGKSKFESRLDPT	(SEQ ID NO: 210)
humanFcgrIIb	MGILSFLPVLATESDWADCKSPQPWGHMLLWIAVFLAPVAGIPAAPPKAVLKLEPPWINVLQEDSVILTCRGTHSPEDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYTCQTGTSLSDPVHLTVLSEMLVLQIPHLEFREGETIVLRCHSNKDKPLVLKVITFFQNGKSKFESRSDPN	(SEQ ID NO: 214)
cynoFcgrIIa1	-----MSQNVCPGNLWLLQPL-TVLLILLASADSQIA-PPKAVLKLEPPWINVLREDSVILTCGGAHSPDSDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYRCQTGRISLSDPVHLTVLSEWLALQIPHLEFREGETIMLRCHSNKDKPLIKVITFFQNGIAKKFESHMDPN	(SEQ ID NO: 220)
cynoFcgrIIa2	-----MSQNVCPGNLWLLQPL-TVLLILLASADSQIA-PPKAVLKLEPPWINVLREDSVILTCGGAHSPDSDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYRCQTGRISLSDPVHLTVLSEWLALQIITHLEFREGETIMLRCHSNKDKPLIKVATFFQNGKSKNFESHMDPN	(SEQ ID NO: 221)
cynoFcgrIIa3	-----MSQNVCPGNLWLLQPL-TVLLILLASADSQIA-PPKAVLKLEPPWINVLREDSVILTCGGAHSPDSDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYRCQTGRISLSDPVHLTVLSEWLALQIITHLEFREGETIMLRCHSNKDKPLIKVATFFQNGISKKFESHMDPN	(SEQ ID NO: 222)
cynoFcgrIIb	MGILSFLPVLATESDWADCKSSQPWGHMLLWIAVFLAPVAGIPAAPPKAVLKLEPPWINVLREDSVILTCGGAHSPDSDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYRCQTGRISLSDPVHLTVLSEWLALQIPHLEFREGETIMLRCHSNKDKPLIKVITFFQNGKSKFESHMDPN	(SEQ ID NO: 223)
humanFcgrIIaH	-----MTMETQMSQNVCPGNLWLLQPL-TVLLILLASADSQIAAPPKAVLKLEPPWINVLQEDSVILTCQGAARSPEDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYTCQTGTSLSDPVHLTVLSEMLVLQIPHLEFREGETIMLRCHSNKDKPLVLKVITFFQNGKSKFESHLDP	(SEQ ID NO: 211)
humanFcgrIIaR	-----MTMETQMSQNVCPGNLWLLQPL-TVLLILLASADSQIAAPPKAVLKLEPPWINVLQEDSVILTCQGAARSPEDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYTCQTGTSLSDPVHLTVLSEMLVLQIPHLEFREGETIMLRCHSNKDKPLVLKVITFFQNGKSKFESRLDPT	(SEQ ID NO: 210)
humanFcgrIIb	MGILSFLPVLATESDWADCKSPQPWGHMLLWIAVFLAPVAGIPAAPPKAVLKLEPPWINVLQEDSVILTCRGTHSPEDSIQWFHNGNL	IPHTIQPSYRFKANNNDSGEYTCQTGTSLSDPVHLTVLSEMLVLQIPHLEFREGETIVLRCHSNKDKPLVLKVITFFQNGKSKFESRSDPN	(SEQ ID NO: 214)

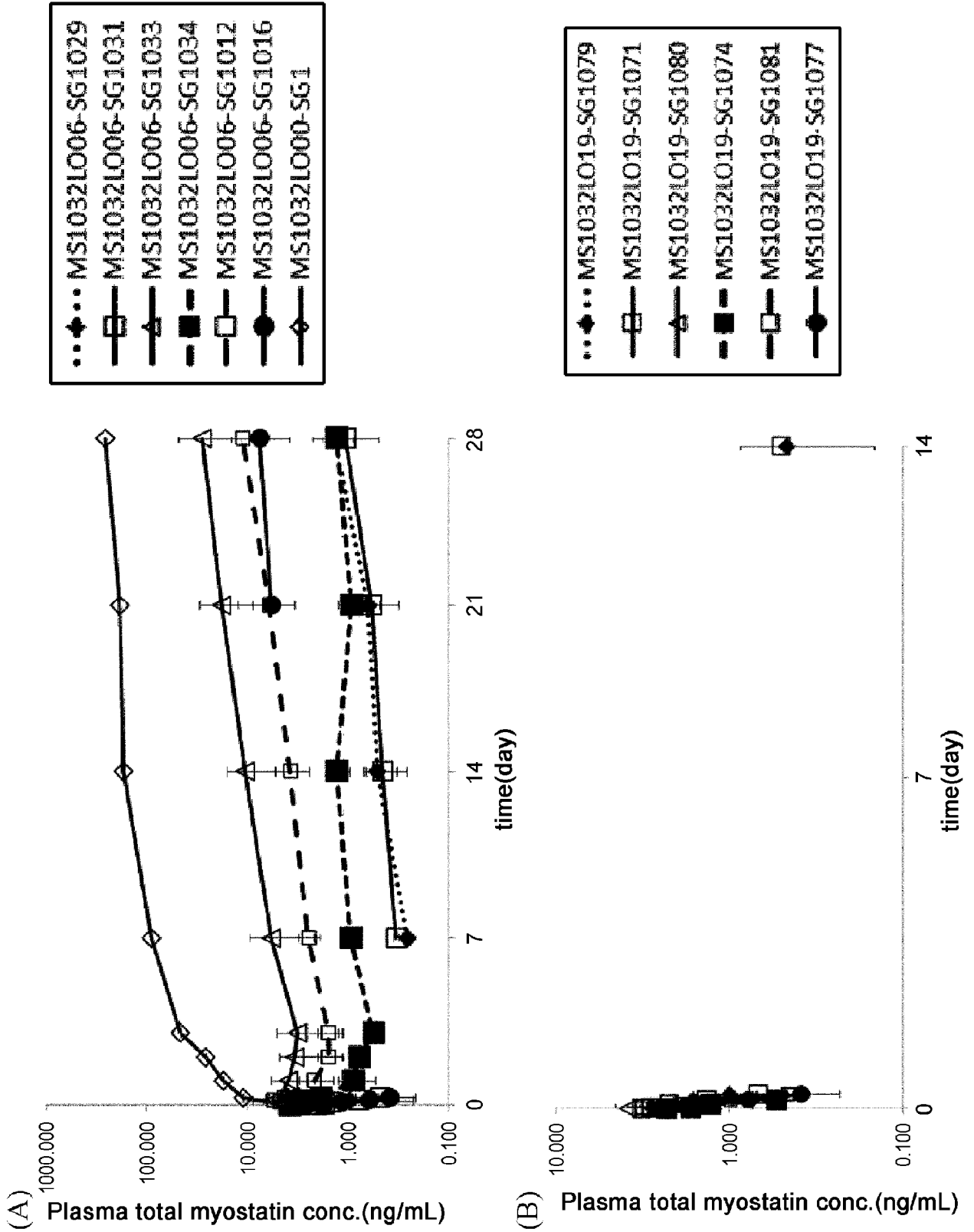
[Fig. 24]



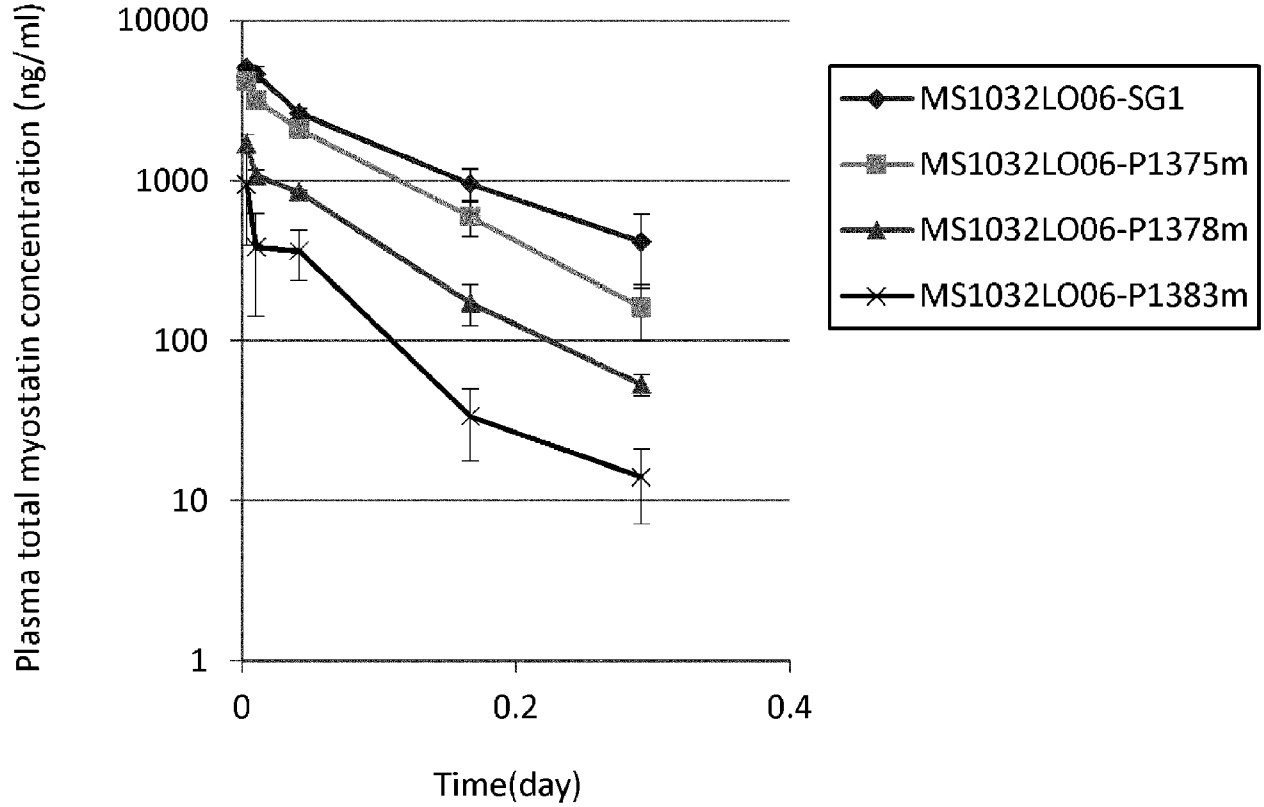
[Fig. 25]



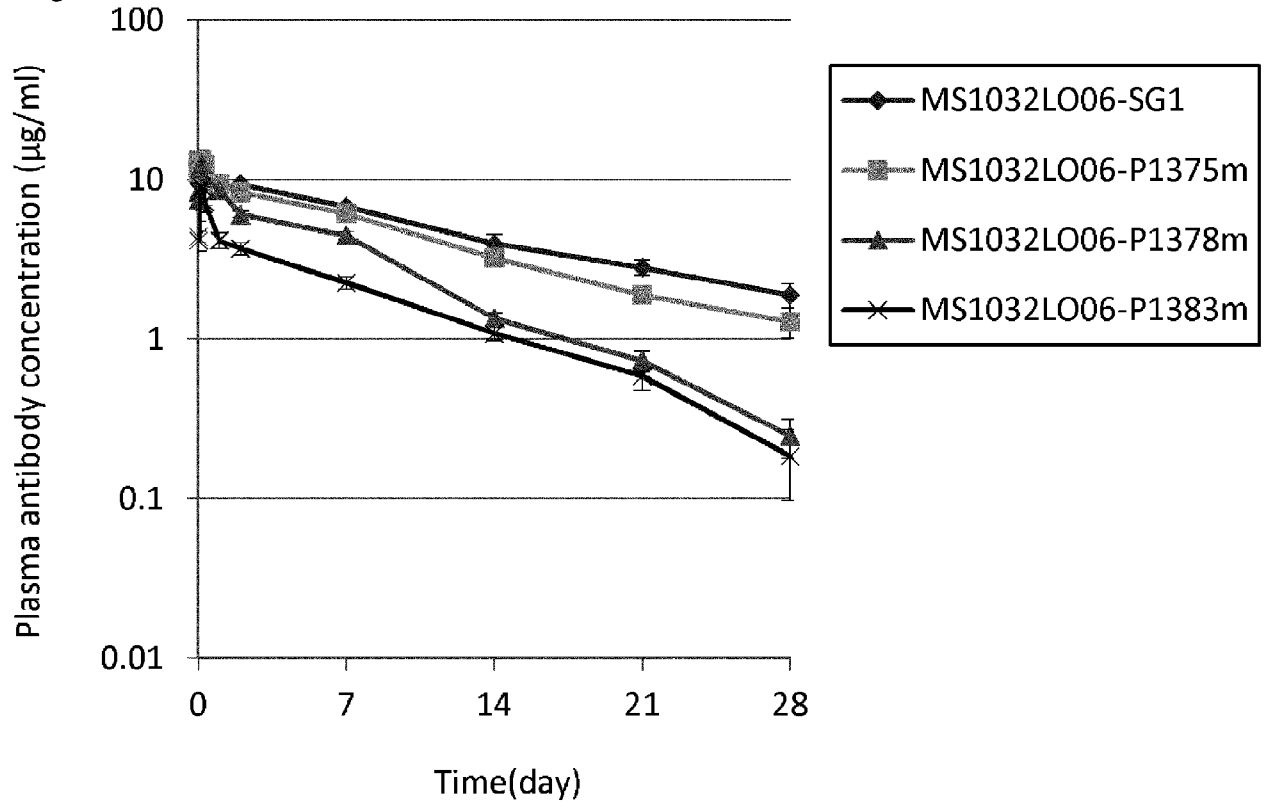
[Fig. 26]



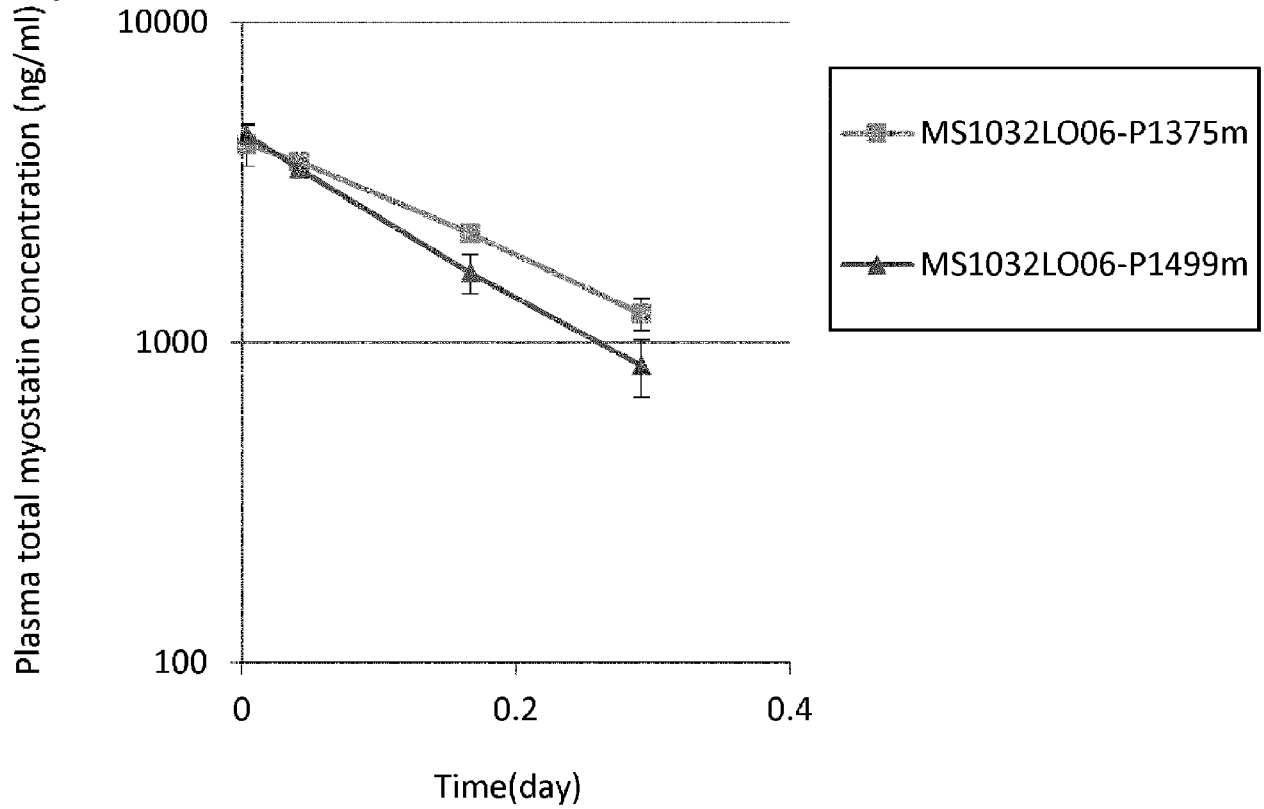
[Fig. 27A]



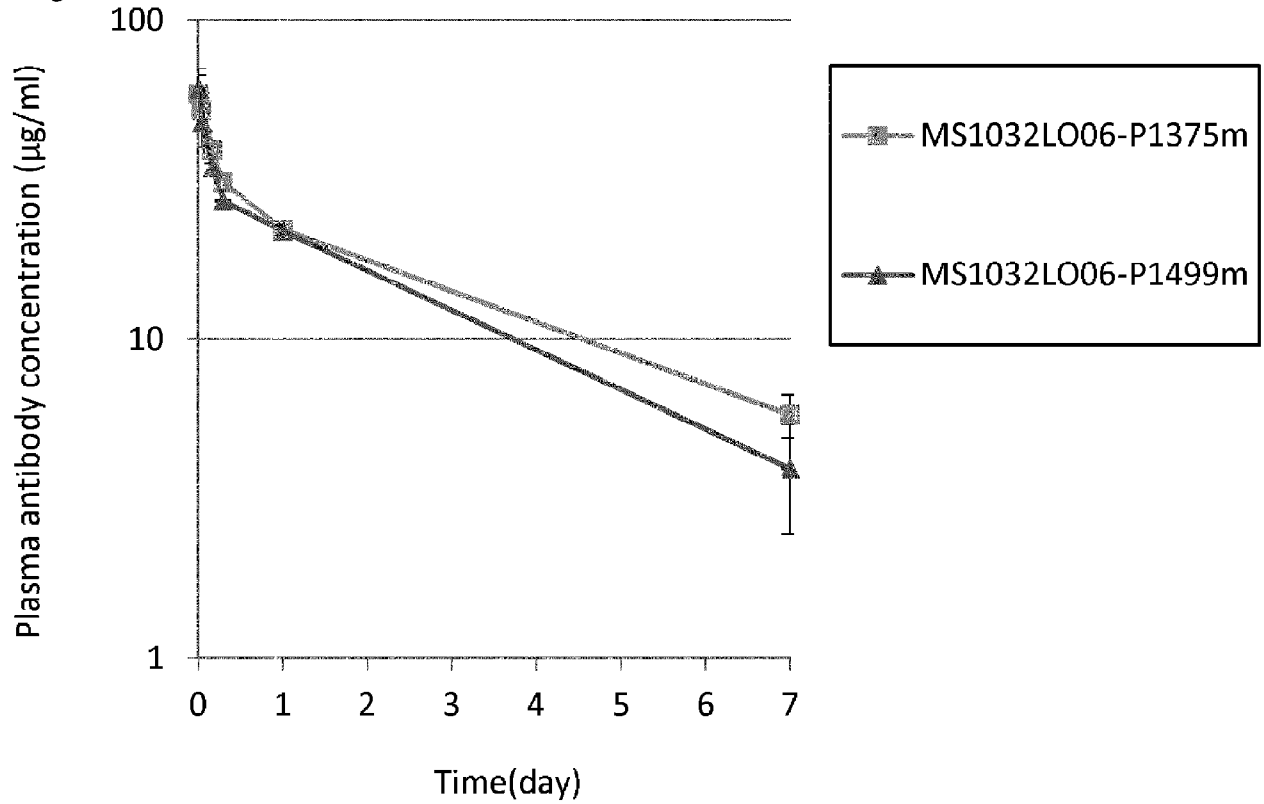
[Fig. 27B]



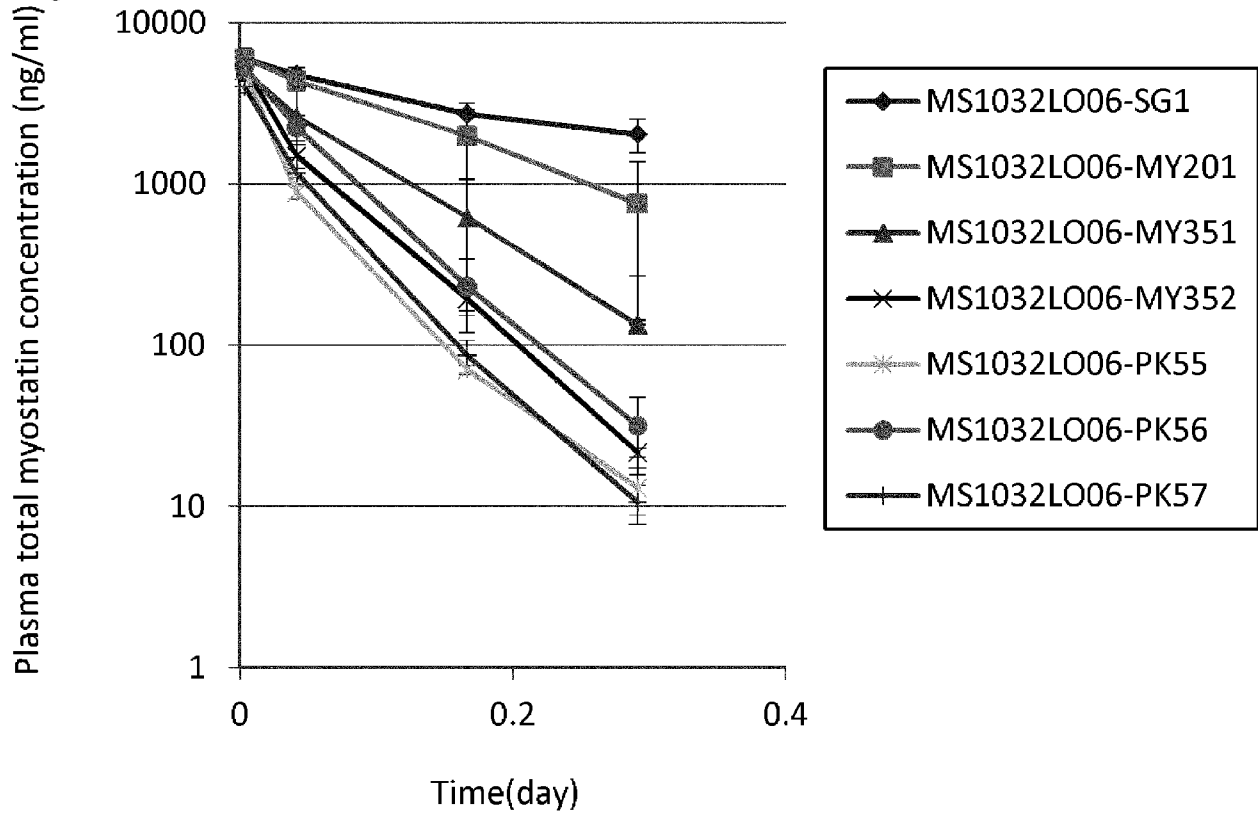
[Fig. 28A]



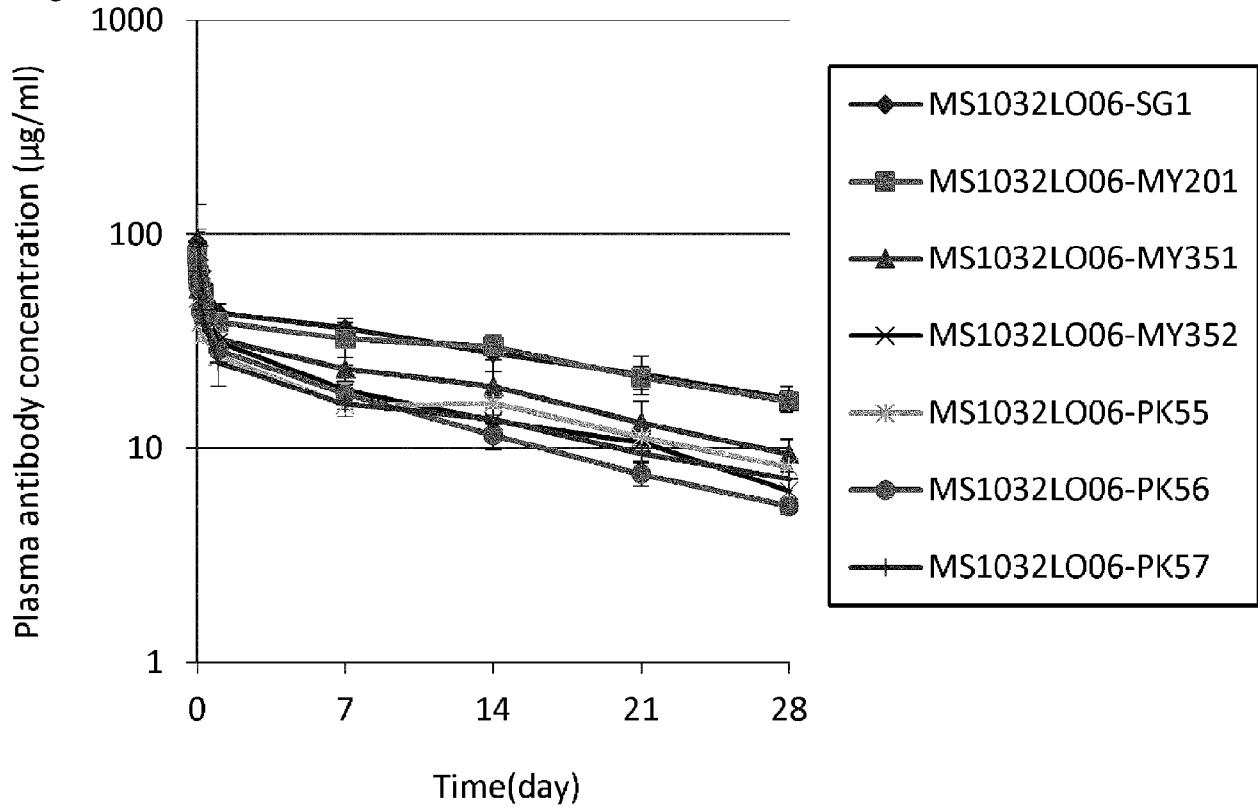
[Fig. 28B]



[Fig. 29]



[Fig. 30]



[Fig. 31]

