MYOSTATIN-BINDING PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to, and the benefit of, U.S. Provisional Application No. 63/557,574, filed on February 25, 2024. The entire contents of the aforementioned application are incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on February 24, 2025, is named BHJ-003PC.xml and is 181 kilobytes in size.

FIELD OF THE INVENTION

The present invention relates to polypeptides that bind myostatin with improved PK properties. The invention also relates to the use of the polypeptides in therapeutic applications to treat neurodegenerative diseases and metabolic disorders.

BACKGROUND

Myostatin, also known as growth and differentiation factor-8 (GDF-8), is a member of the transforming growth factor-β (TGF-β) superfamily of secreted growth factors. Myostatin expression is limited primarily to skeletal muscle and adipose tissue, where it has been shown to be a negative regulator of skeletal muscle development (Lee LS, *Immunol. Endocr. Metab. Agents Med. Chem*. 2010;10:183-194). Both genetic and pharmacological findings indicate that myostatin regulates energy metabolism and that its inhibition can significantly attenuate the progression of metabolic diseases, including overweight, obesity, and related health conditions. For example, myostatin null mice exhibit decreased body fat accumulation (McPherron & Lee, J. *JCI* 109:595, 2002) when compared with wild type mice of the same age. This reduction in body fat is a manifestation of reduced adipocyte number and size, implicating a significant role of myostatin in adipogenesis as well as in myogenesis. In addition, increases in skeletal muscle mass and strength are associated with metabolic adaptations which positively affect body composition, energy expenditure, glucose homeostasis and insulin requirements.

Over the past two decades, recombinant DNA technology has led to the discovery of a significant number of protein therapeutics. For example, anti-myostatin Adnectins which effectively inhibit myostatin activity *in vitro* and *in vivo* have been described (US Patents 8,933,199; 8,993,265; 8,853,154; and 9,493,546). These anti-myostatin Adnectins are useful for the treatment of disorders, diseases, and conditions for which inhibition of myostatin activity is beneficial, including, for example, neurological disorders, muscle wasting diseases, metabolic disorders, and conditions resulting in muscle atrophy.

There is a growing need, however, to develop an inhibitor of myostatin having a prolonged exposure and improved PK properties by reducing FcγR and C1q binding and improving FcRn-binding.

SUMMARY

The present invention relates to innovative polypeptides which bind to myostatin (e.g., human myostatin) and comprise an anti-myostatin Adnectin and an engineered Fc domain, wherein the engineered Fc domain differs in amino acid sequence from a wild-type Fc domain (e.g., an IgG1 Fc domain or IgG4 Fc domain) or S228P IgG4 Fc domain by at least one amino acid but no more than 15 amino acids. Such polypeptides exhibit improved PK properties and increased half-life.

Accordingly, in one aspect, provided herein is a polypeptide comprising (a) a fibronectin type III tenth (^{10}Fn3) domain which binds to myostatin (e.g., human myostatin) and comprises AB, BC, CD, DE, EF, and FG loops and (b) an Fc region, wherein:

(i) the BC, DE, and FG loops of the ^{10}Fn3 domain comprise the amino acid sequences of SEQ ID NOs: 5, 6, and 7, respectively, or at least one loop of the BC, DE, and FG loops of the ^{10}Fn3 domain has 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops of SEQ ID NOs: 5, 6, and 7, respectively; and

(ii) the Fc region differs in amino acid sequence from a reference human IgG1 Fc region comprising the amino acid sequence of SEQ ID NO: 78 or a reference human or S228P IgG4 Fc region comprising the amino acid sequence of SEQ ID NO: 79 (or a wild-type human IgG4 Fc region) by at least one amino acid but no more than 15 amino acids.

In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 8 or 10, or an amino acid sequence which is at least 90%, 95%, 98%, or 99% identical to

SEQ ID NO: 8 or 10, or an amino acid sequence which differs from SEQ ID NO: 8 or 10 by at least one amino acid but no more than 15 amino acids. In some embodiments, the Fc region differs from the amino acid sequence of SEQ ID NO: 78 or 79 by at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids. In some embodiments, the Fc region comprises a combination of amino acid substitutions relative to SEQ ID NO: 78 selected from the group consisting of: (a) L234A/L235A, (b) L234A/L235A/P329A, (c) L234A/L235A/P329A/M248L/N434S, (d) L234A/L235A/P329A/M252Y/S254T/T256E, (e) M248L/N434S, (f) M252Y/S254T/T256E, (g) M252Y/S254T/T256E/N297Q, and (h) N297Q/M248L/N434S, wherein the numbering of positions is according to the EU Index. In some embodiments, the Fc region comprises a combination of amino acid substitutions relative to SEQ ID NO: 79 selected from the group consisting of: (a) F234A/L235A/M252Y/S254T/T256E, and (b) M252Y/S254T/T256E/N297Q, wherein the numbering of positions is according to the EU Index. In some embodiments, the Fc region comprises the amino acid sequence of any one of SEQ ID NOs: 83-91 and 92-101. In some embodiments, the Fc region comprises a hinge comprising the amino acid sequence of any one of SEQ ID NOs: 64-72. In some embodiments, the polypeptide comprises a signal peptide (e.g., a signal peptide comprising the amino acid sequence of SEQ ID NO: 74 or 75). In some embodiments, the polypeptide comprises a linker between the ^{10}Fn3 domain to the Fc region, e.g., a linker comprises the amino acid sequence of any one of SEQ ID NOs: 35-63. In some embodiments, the Fc region is located N-terminal to the ^{10}Fn3 domain. In other embodiments, the Fc region is located C-terminal to the ^{10}Fn3 domain. In some embodiments, the polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 102-131, or a polypeptide comprising an amino acid sequence which is at least 90%, 95%, 98%, or 99% identical to any one of SEQ ID NOs: 102-131, or a polypeptide which differs from the amino acid sequence of any one of SEQ ID NOs: 102-131 by at least one amino acid but no more than 30, 20, or 10 amino acids.

In some embodiments, the polypeptide (a) binds to active myostatin with a K_D of 15 nM or less, such as 10 nM or less, 5 nM or less, 1 nM or less, 1-15 nM, 5-15 nM, or 10-15 nM, (b) binds to FcRn at pH 6.0 with a 40-60 fold higher affinity than a polypeptide comprising the amino acid sequence of SEQ ID NO: 81, (c) has a lower affinity for FcγRI relative to a polypeptide comprising the amino acid sequence of SEQ ID NO: 81, and/or (d) has a lower affinity for C1q relative to a polypeptide comprising the amino acid sequence of SEQ ID NO:

81, e.g., as assessed using biolayer interferometry. In some embodiments, the polypeptide has increased FcRn-mediated recycling relative to a polypeptide comprising the amino acid sequence of SEQ ID NO: 81, e.g., as assessed by the cell-based assay described in Example 6. In some embodiments, the polypeptide has a longer half-life relative to a polypeptide comprising the amino acid sequence of SEQ ID NO: 81. In some embodiments, the polypeptide inhibits SMAD2/3 signaling.

In another aspect, provided are nucleic acid molecules (e.g., isolated nucleic acid molecules) and expression vectors which encode the polypeptides described herein, as well as cells (e.g., host cells) which comprise the nucleic acid molecules or expression vectors. Also provided is a method of producing a myostatin-binding polypeptide comprising culturing the cells (e.g., host cells) under conditions suitable for expressing the polypeptide, and purifying the polypeptide.

In another aspect, provided are pharmaceutical compositions and kits comprising the polypeptides described herein.

In another aspect, provided are stable formulations comprising:

(i) at least 10 mg/mL of a polypeptide described herein;

(ii) a disaccharide at a concentration of at least 5%;

(iii) a histidine buffer at a concentration of between about 20 to about 60 mM; and

(iv) a pharmaceutically acceptable aqueous carrier,

wherein the formulation has a pH range of about 6.5 to about 7.8.

In some embodiments, the protein concentration of the polypeptide in the formulation is between about 10 mg/mL and 200 mg/mL. In some embodiments, the disaccharide is present at weight (w/w) ratio of at least 5:1 protein to sugar. In some embodiments, the formulation comprises about 5-30% disaccharide (e.g., trehalose, e.g., trehalose dihydrate). In some embodiments, the concentration of the disaccharide (e.g., trehalose dihydrate) is about 150-800 mM. In some embodiments, histidine is present at a concentration of at least 20 mM, the viscosity of the formulation is from about 5 to 20 cps, and/or the pH is about 6.6 to 7.6. In some embodiments, a surfactant is present in the formulation at a concentration of between about 0.01% and 0.5%. In some embodiments, the formulation comprises a chelator (e.g., DPTA, EDTA, EGTA) at a concentration of about 0.01-0.5 mM.

In some embodiments, the stable pharmaceutical formulation comprises:

(a) about 10-140 mg/mL of a polypeptide described herein;
about 5-25% trehalose dihydrate;
about 20-30 mM histidine; and
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 6.8 to 7.3;

(b) about 10-140 mg/mL of a polypeptide described herein;
about 5-25% trehalose dihydrate;
about 20-30 mM histidine;
about 0.02-0.06 mM DTPA;
about 0.01-0.05% polysorbate 80; and
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 6.8 to 7.3;

(c) about 10-140 mg/mL of a polypeptide described herein;
about 600 mM trehalose dihydrate;
25-30 mM histidine; and
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 7.0 to 7.3;

(d) about 10-140 mg/mL of a polypeptide described herein;
about 600 mM trehalose dihydrate;
25-30 mM histidine;
about 0.02-0.06 mM DTPA;
about 0.01-0.05% polysorbate 80; and
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 7.0 to 7.3;

(e) about 10-75 mg/mL of a polypeptide described herein;
about 5-25% trehalose dihydrate;
about 20-30 mM histidine; and
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 6.8 to 7.3;

(f) about 10-75 mg/mL of a polypeptide described herein;
about 5-25% trehalose dihydrate;

about 20-30 mM histidine;
about 0.02-0.06 mM DTPA;
about 0.01-0.05% polysorbate 80; and
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 6.8 to 7.3;

(g) about 10-75 mg/mL of a polypeptide described herein;
about 600 mM trehalose dihydrate;
about 30 mM histidine;
about 0.05 mM DTPA;
about 0.02% polysorbate 80;
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 7.1.

In another aspect, provided is a unit dosage form comprising about 1.0 mL or less of a formulation comprising (i) about 10-75 mg/mL of a polypeptide described herein; (ii) about 5-25% trehalose dihydrate; (iii) about 20-30 mM histidine; (iv) about 0.02-0.06 mM DTPA; (v) about 0.01-0.05% polysorbate 80; and (vi) a pharmaceutically acceptable aqueous carrier, wherein the pH of the formulation is about 6.8 to 7.3.

In another aspect, provided is a method of treating a myostatin-related disease or disorder in a subject comprising administering to the subject an effective amount of a polypeptide, composition, stable formulation, or unit dosage form described herein.

In another aspect, provided is a method of attenuating or inhibiting a disorder associated with degeneration or wasting of muscle in a subject comprising administering to the subject an effective amount of a polypeptide, composition, stable formulation, or unit dosage form described herein.

In another aspect, provided is a method of treating a metabolic disease, disorder, or condition in a subject, comprising administering to the subject an effective amount of a polypeptide, composition, stable formulation, or unit dosage form described herein.

In another aspect, provided is a method of enhancing lean muscle mass in a subject, comprising administering to the subject an effective amount of a polypeptide, composition, stable formulation, or unit dosage form described herein.

In another aspect, provided is a method of increasing the ratio of lean muscle mass to fat in a subject, comprising administering to the subject an effective amount of a polypeptide, composition, stable formulation, or unit dosage form described herein.

In another aspect, provided is a method of improving glycemic control in a subject, comprising administering to the subject an effective amount of a polypeptide, composition, stable formulation, or unit dosage form described herein.

In another aspect, provided is a method of treating, preventing, or reducing overweight or obesity and related comorbidities in a subject, comprising administering to the subject an effective amount of a polypeptide, composition, stable formulation, or unit dosage form described herein.

In another aspect, provided is a treating or preventing type II diabetes in a subject, comprising administering to the subject an effective amount of a polypeptide, composition, stable formulation, or unit dosage form described herein.

In another aspect, provided is a treating or preventing a neurodegenerative disease in a subject, comprising administering to the subject an effective amount of a polypeptide, composition, stable formulation, or unit dosage form described herein.

Also provided are polypeptides, compositions, stable formulations, or unit dosage forms as described herein for use in a method of (a) treating a myostatin-related disease or disorder, (b) attenuating or inhibiting a disorder associated with degeneration or wasting of muscle, (c) treating a metabolic disease, disorder, or condition, (d) enhancing lean muscle mass, (e) increasing the ratio of lean muscle mass to fat, (f) improving glycemic control, (g) treating, preventing, or reducing overweight or obesity and related comorbidities, (h) treating or preventing type II diabetes, and/or (i) treating or preventing a neurodegenerative disease.

Also provided herein are uses of a polypeptide, composition, stable formulation, or unit dosage form described herein in the manufacture of a medicament for (a) treating a myostatin-related disease or disorder, (b) attenuating or inhibiting a disorder associated with degeneration or wasting of muscle, (c) treating a metabolic disease, disorder, or condition, (d) enhancing lean muscle mass, (e) increasing the ratio of lean muscle mass to fat, (f) improving glycemic control, (g) treating, preventing, or reducing overweight or obesity and related comorbidities, (h) treating or preventing type II diabetes, and/or (i) treating or preventing a neurodegenerative disease.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing the percentage of high molecular weight (HMW) species after 0-5 freeze/thaw cycles for Fc engineered Adnectin Compounds 3189, 3190, 3191, 3192, and 3195.

FIGs. 2A-2B are graphs showing concentrations of Fc engineered Adnectins after 0-5 freeze/thaw cycles as assessed by A280 measurements (**FIG. 2A**) s and size exclusion chromatography (SEC) (**FIG. 2B**). Assessed Fc engineered Adnectins include Compounds 3189, 3190, 3191, 3192, and 3195.

FIG. 3 is a graph showing the concentration of Fc engineered Adnectins after storage at 4°C for 6 weeks by A280 measurements. Assessed Fc engineered Adnectins include Compounds 3189, 3190, 3191, 3192, and 3195.

FIG. 4 is a graph showing binding of Fc engineered Adnectins, BHV-2000, and assay controls to baculovirus particles to assess non-specific binding.

FIG. 5 is a schematic of a free-ligand assay based on a SMAD binding element luciferase reporter to assess SMAD2/3 signaling activity.

FIGs. 6A-6F are graphs showing the effects of Fc engineered Adnectins and BHV-2000 on SMAD2/3 signaling using the free-ligand assay in the presence of increasing amounts of BHV-2000 or Fc engineered Adnectins.

FIG. 7 is a schematic of a pre-complexed ligand assay based on a SMAD binding element luciferase reporter to assess SMAD2/3 signaling activity.

FIGs. 8A-8L are graphs showing the effects of Fc engineered Adnectins and BHV-2000 on SMAD2/3 signaling using the pre-complexed ligand assay in the presence of increasing amounts of Activin A. **FIGs. 8A-8D** correspond to Adnectin-only experiments (i.e., without pre-complexes). **FIGs. 8E-8H** correspond to assays with pre-complexes of myostatin and BHV-2000, Compound 3189, Compound 3190, or Compound 3195, respectively. **FIGs. 8I-8L** correspond to assays with pre-complexes of GDF11 and BHV-2000, Compound 3189, Compound 3190, or Compound 3195, respectively.

FIG. 9 is a schematic showing the cell-based FcRn recycling assay.

FIG. 10 is a graph showing the amount of Fc engineered Adnectins and BHV-2000 recycled via FcRn in a cell-based assay. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001, as assessed by one-way ANOVA.

DETAILED DESCRIPTION

I. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by the skilled artisan. Although any methods and compositions similar or equivalent to those described herein can be used in practice or testing of the present invention, the preferred methods and compositions are described herein.

The singular form "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

The term "about", particularly in reference to a given quantity or number, is meant to encompass deviations within plus or minus ten percent (± 10%), (*e.g.*, ± 5%).

"Full-length myostatin" as used herein refers to the full-length polypeptide sequence described in McPherron et al. (1997), *supra*, as well as related full-length polypeptides including allelic variants and interspecies homologs. The term "myostatin" or "mature myostatin" refers to fragments of the biologically active mature myostatin, as well as related polypeptides including allelic variants, splice variants, and fusion peptides and polypeptides. The mature C-terminal protein has been reported to have 100% sequence identity among many species including human, mouse, chicken, porcine, turkey, and rat (Lee *et al.*, *PNAS* 2001;98:9306). The sequence for human prepromyostatin is:

MQKLQLCVYIYLFMLIVAGPVDLNENSEQKENVEKEGLCNACTWRQNTK
SSRIEAIKIQILSKLRLETAPNISKDVIRQLLPKAPPLRELIDQYDVQRDDSS
DGSLEDDDYHATTETIITMPTESDFLMQVDGKPKCCFFKFSSKIQYNKVV
KAQLWIYLRPVETPTTVFVQILRLIKPMKDGTRYTGIRSLKLDMNPGTGIW
QSIDVKTVLQNWLKQPESNLGIEIKALDENGHDLAVTFPGPGEDGLNPFLE
VKVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRY
KANYCSGECEFVFLQKYPHTHLVHQANPRGSAGPCCTPTKMSPINMLYF
NGKEQIIYGKIPAMVVDRCGCS (SEQ ID NO: 1).

The sequence for human pro-myostatin is:

NENSEQKENVEKEGLCNACTWRQNTKSSRIEAIKIQILSKLRLETAPNISKD
VIRQLLPKAPPLRELIDQYDVQRDDSSDGSLEDDDYHATTETIITMPTESDF
LMQVDGKPKCCFFKFSSKIQYNKVVKAQLWIYLRPVETPTTVFVQILRLIK
PMKDGTRYTGIRSLKLDMNPGTGIWQSIDVKTVLQNWLKQPESNLGIEIK
ALDENGHDLAVTFPGPGEDGLNPFLEVKVTDTPKRSRRDFGLDCDEHSTE
SRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFLQKYPHTHLV
HQANPRGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPAMVVDRCGCS
(SEQ ID NO: 2).

The sequence for mature myostatin (conserved in human, murine, rat, chicken, turkey, dog, horse, and pig) is:

DFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFV
FLQKYPHTHLVHQANPRGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPA
MVVDRCGCS (SEQ ID NO: 3).

As used herein, a "fibronectin based scaffold" or "FBS" protein or moiety refers to proteins or moieties that are based on a fibronectin type III ("Fn3") repeat. Fibronectin has 18 Fn3 repeats, and while the sequence homology between the repeats is low, they all share a high similarity in tertiary structure. For reviews see Bork et al., *Proc. Natl. Acad. Sci. USA*, 89(19):8990-8994 (1992); Bork et al., *J. Mol. Biol.*, 242(4):309-320 (1994); Campbell et al., *Structure*, 2(5):333-337 (1994); Harpez et al., *J. Mol. Biol.*, 238(4):528-539 (1994)). An Fn3 domain is small, monomeric, soluble, and stable. It lacks disulfide bonds and, therefore, is stable under reducing conditions. Fn3 domains comprise, in order from N-terminus to C-terminus, a beta or beta-like strand, A; a loop, AB; a beta or beta-like strand, B; a loop, BC; a beta or beta-like strand, C; a loop, CD; a beta or beta-like strand, D; a loop, DE; a beta or beta-like strand, E; a loop, EF; a beta or beta-like strand, F; a loop, FG; and a beta or beta-like strand, G. The seven antiparallel β-strands are arranged as two beta sheets that form a stable core, while creating two "faces" composed of the loops that connect the beta or beta-like strands. Loops AB, CD, and EF are located at one face ("the south pole") and loops BC, DE, and FG are located on the opposing face ("the north pole").

Adnectins are a class of therapeutic FBS proteins with high-affinity and specific target-binding properties that are derived from the tenth human fibronectin type III domain (^{10}Fn3):

VSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQEFTV
PGSKSTATISGLKPGVDYTITVYAVTGRGDSPASSKPISINYRT
(SEQ ID NO: 4) (BC, DE, and FG loops are underlined).

Accordingly, as used herein, a "^{10}Fn3 domain" or "^{10}Fn3 moiety" or "^{10}Fn3 molecule" refers to wild-type ^{10}Fn3 and biologically active variants thereof, *e.g.*, biologically active variants that specifically bind to a target, such as a target protein.

A "region" of a ^{10}Fn3 domain (or moiety or molecule) as used herein refers to either a loop (AB, BC, CD, DE, EF and FG), a β-strand (A, B, C, D, E, F and G), the N-terminus (corresponding to amino acid residues 1-7 of SEQ ID NO: 4), or the C-terminus (corresponding to amino acid residues 93-94 of SEQ ID NO: 4).

A "scaffold region" refers to any non-loop region of a human ^{10}Fn3 domain. The scaffold region includes the A, B, C, D, E, F and G β-strands as well as the N-terminal region (amino acids corresponding to residues 1-7 of SEQ ID NO: 4) and the C-terminal region (amino acids corresponding to residues 93-94 of SEQ ID NO: 4).

The term "anti-myostatin Adnectin" refers to a protein molecule that binds to and antagonizes myostatin and that comprises at least a one ^{10}Fn3 domain derived from the human wild-type ^{10}Fn3 domain (SEQ ID NO: 4). The anti-myostatin Adnectin can further comprise additional protein domains (*e.g.,* an Fc domain), and can also refer to multimer forms of the polypeptide, such as dimers, tetramers and hexamers. The term “anti-myostatin Fc engineered Adnectin” refers to an anti-myostatin Adnectin which is linked to an engineered Fc region which comprises an amino acid sequence that differs from a wild-type Fc region by at least one amino acid (e.g., an amino acid modification, such as an amino acid substitution). In some embodiments, the engineered Fc region is a variant IgG1 Fc region or a variant IgG4 Fc region.

"Polypeptide" as used herein refers to any sequence of two or more amino acids, regardless of length, post-translation modification, or function. "Polypeptide," "peptide," and "protein" are used interchangeably herein. Polypeptides can include natural amino acids and non-natural amino acids such as those described in U.S. Pat. No. 6,559,126, incorporated herein by reference. Polypeptides can also be modified in any of a variety of standard chemical ways (*e.g.*, an amino acid can be modified with a protecting group; the carboxy-terminal amino acid can be made into a terminal amide group; the amino-terminal residue can be modified with groups to, *e.g.*, enhance lipophilicity; or the polypeptide can be chemically glycosylated or otherwise

modified to increase stability or *in vivo* half-life). Polypeptide modifications can include the attachment of another structure such as a cyclic compound or other molecule to the polypeptide and can also include polypeptides that contain one or more amino acids in an altered configuration (*i.e.*, R or S; or, L or D). The peptides of the invention are proteins derived from the tenth type III domain of fibronectin that have been modified to bind to myostatin and are referred to herein as, "anti-myostatin Adnectin" or "myostatin Adnectin."

A "polypeptide chain", as used herein, refers to a polypeptide wherein each of the domains thereof is joined to other domain(s) by peptide bond(s), as opposed to non-covalent interactions or disulfide bonds.

An "isolated" polypeptide is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to greater than 95% by weight of polypeptide as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing condition using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes the polypeptide in situ within recombinant cells since at least one component of the polypeptide's natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

"Percent (%) amino acid sequence identity" herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in a selected 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, ALIGN-2 or Megalign (DNASTAR™) software. Those skilled in the art can readily determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For example, 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.

As used herein, "conservative substitution" denotes the replacement of an amino acid residue by another, without altering the overall conformation and function of the peptide, including, but not limited to, replacement of an amino acid with one having similar properties (such as, for example, polarity, hydrogen bonding potential, acidic, basic, shape, hydrophobic, aromatic, and the like). Exemplary conservative substitutions include those fulfilling the criteria defined for an accepted point mutation in Dayhoff *et al.*, *Atlas of Protein Sequence and Structure*, 5:345-352 (1978 and Supp.) Examples of conservative substitutions include substitutions within the following groups: (a) valine, glycine; (b) glycine, alanine; (c) valine, isoleucine, leucine; (d) aspartic acid, glutamic acid; (e) asparagine, glutamine; (f) serine, threonine; (g) lysine, arginine, methionine; and (h) phenylalanine, tyrosine. By "substituted"' or "modified" the present invention includes those amino acids that have been altered or modified from naturally occurring amino acids. As such it should be understood that in the context of the present invention a conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties.

The terms “specifically binds,” “specific binding,” “selective binding,” and “selectively binds,” as used interchangeably herein refers to an Adnectin that exhibits affinity for a myostatin, but does not significantly bind (e.g., less than about 10% binding) to a different polypeptide as measured by a technique available in the art such as, but not limited to, Scatchard analysis and/or competitive binding assays (e.g., competition ELISA, BIACORE assay). The term is also applicable where e.g., a binding domain of an Adnectin of the invention is specific for myostatin.

The term "preferentially binds" as used herein refers to the situation in which an Adnectin described herein binds myostatin at least about 20% greater than it binds a different polypeptide

as measured by a technique available in the art such as, but not limited to, Scatchard analysis and/or competitive binding assays (e.g., competition ELISA, BIACORE assay).

The term "K_D," as used herein, is intended to refer to the dissociation equilibrium constant of a particular Adnectin-protein (e.g., myostatin) interaction or the affinity of an Adnectin for a protein (e.g., myostatin), as measured using a surface plasmon resonance assay or a cell binding assay. A "desired K_D," as used herein, refers to a K_D of an Adnectin that is sufficient for the purposes contemplated. For example, a desired K_D may refer to the K_D of an Adnectin required to elicit a functional effect in an *in vitro* assay, e.g., a cell-based luciferase assay.

The term "k_{ass}", as used herein, is intended to refer to the association rate constant for the association of an Adnectin into the Adnectin/protein complex.

The term "k_{diss}", as used herein, is intended to refer to the dissociation rate constant for the dissociation of an Adnectin from the Adnectin/protein complex.

The term "IC_{50}", as used herein, refers to the concentration of an Adnectin that inhibits a response, either in an *in vitro* or an *in vivo* assay, to a level that is 50% of the maximal inhibitory response, i.e., halfway between the maximal inhibitory response and the untreated response.

The term "myostatin activity" as used herein refers to one or more of growth-regulatory or morphogenetic activities associated with the binding of active myostatin protein to ActRIIb and the subsequent recruitment of Alk4 or Alk5. For example, active myostatin is a negative regulator of skeletal muscle mass. Active myostatin can also modulate the production of muscle-specific enzymes (e.g., creatine kinase), stimulate myoblast proliferation, and modulate preadipocyte differentiation to adipocytes. Myostatin activity can be determined using art-recognized methods, such as those described herein.

The phrases "inhibit myostatin activity" or "antagonize myostatin activity" or "antagonize myostatin" are used interchangeably to refer to the ability of the anti-myostatin Adnectins of the present invention to neutralize or antagonize an activity of myostatin *in vivo* or *in vitro*. The terms "inhibit" or "neutralize" as used herein with respect to an activity of an Adnectin of the invention means the ability to substantially antagonize, prohibit, prevent, restrain, slow, disrupt, eliminate, stop, reduce or reverse e.g., progression or severity of that which is being inhibited including, but not limited to, a biological activity or property, a disease

or a condition. The inhibition or neutralization is preferably at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or higher.

For example, an anti-myostatin Fc engineered Adnectin may reduce circulating levels of biologically active myostatin normally found in a vertebrate subject, or a reduction of circulating levels of biologically active myostatin in subjects with disorders that result in elevated circulating levels of myostatin. A reduction of myostatin activity may be determined using *in vitro* assays, e.g., binding assays, as described herein.

The term "PK" is an acronym for "pharmacokinetic" and encompasses properties of a compound including, by way of example, absorption, distribution, metabolism, and elimination by a subject. A "PK modulation protein" or "PK moiety" as used herein refers to any protein, peptide, or moiety that affects the pharmacokinetic properties of a biologically active molecule when fused to or administered together with the biologically active molecule. Examples of a PK modulation protein or PK moiety include PEG, human serum albumin (HSA) binders (as disclosed in U.S. Publication Nos. 2005/0287153 and 2007/0003549, PCT Publication Nos. WO 2009/083804 and WO 2009/133208), human serum albumin, Fc or Fc fragments and variants thereof, and sugars (e.g., sialic acid).

The "half-life" of an amino acid sequence or compound can generally be defined as the time taken for the serum concentration of the polypeptide to be reduced by 50%, *in vivo*, for example due to degradation of the sequence or compound and/or clearance or sequestration of the sequence or compound by natural mechanisms. The half-life can be determined in any manner known per se, such as by pharmacokinetic analysis. Suitable techniques will be clear to the person skilled in the art, and may for example generally involve the steps of suitably administering to a subject a suitable dose of the amino acid sequence or compound of the invention; collecting blood samples or other samples from the subject at regular intervals; determining the level or concentration of the amino acid sequence or compound of the invention in said blood sample; and calculating, from (a plot of) the data thus obtained, the time until the level or concentration of the amino acid sequence or compound of the invention has been reduced by 50% compared to the initial level upon dosing. Reference is, for example, made to the standard handbooks, such as Kenneth, A. et al., Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and in Peters et al., Pharmacokinetic Analysis: A Practical Approach

(1996). Reference is also made to Gibaldi, M. et al., Pharmacokinetics, 2nd Rev. Edition, Marcel Dekker (1982).

Half-life can be expressed using parameters such as the $t_{1/2}$-alpha, $t_{1/2}$-beta, HL_Lambda_z, and the area under the curve (AUC). In the present specification, an "increase in half-life" refers to an increase in any one of these parameters, any two of these parameters, any three of these parameters or all four of these parameters. An "increase in half-life" in particular refers to an increase in the $t_{1/2}$-beta, and/or HL_Lambda_z, either with or without an increase in the $t_{1/2}$-alpha and/or the AUC or both.

The notations "mpk", "mg/kg", or "mg per kg" refer to milligrams per kilogram. All notations are used interchangeably throughout the present disclosure.

A "stable" formulation or drug product is one in which an anti-myostatin Fc engineered Adnectin described herein in the formulation essentially retains its physical and chemical stability and integrity upon storage. Stability of the anti-myostatin Fc engineered Adnectin molecule formulations can be measured at selected temperatures after selected time periods. For example, an increase in aggregate formation following lyophilization and storage is an indicator for instability of a lyophilized anti-myostatin Fc engineered Adnectin molecule formulation. In addition to aggregate formation, retention of original clarity, color and odor throughout shelf life are indicators utilized to monitor stability of anti-myostatin Fc engineered Adnectin molecule solutions. HMW species are multimers (i.e., tetramers, hexamers, etc.), which have a higher molecular weight than anti-myostatin Fc engineered Adnectin molecule monomers or dimers. Typically a "stable" drug product may be one wherein the increase in aggregation, as measured by an increase in the percentage of high molecular weight species (% HMW), is less than about 5% and preferably less than about 3%, when the formulation is stored at 2-8ºC for one year. Preferably, the manufactured drug product comprises less than about 25% HMW species, preferably less than about 15% HMW species, more preferably less than about 10% HMW species, most preferred less than about 5% HMW species.

"Shelf-life" of a pharmaceutical product, e.g., a protein comprising an anti-myostatin Fc engineered Adnectin, is the length of time the product is stored before decomposition occurs. For

example, shelf-life may be defined as the time for decomposition of 0.1%, 0.5%, 1%, 5%, or 10% of the product.

The terms “lyophilized” and “freeze-dried” are used interchangeably herein and refer to a material that is dehydrated by first freezing and then reducing the surrounding pressure to allow the frozen water in the material to sublimate.

A "reconstituted" formulation is one which has been prepared by dissolving a lyophilized formulation in an aqueous carrier such that the anti-myostatin Fc engineered Adnectin molecule is dissolved in the reconstituted formulation. The reconstituted formulation is suitable for intravenous administration (IV) or subcutaneous (SC) administration to a patient in need thereof.

An "isotonic" formulation is one which has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsmol/KgH2O. The term "hypertonic" is used to describe a formulation with an osmotic pressure above that of human blood. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

The term "buffering agent" refers to one or more components that when added to an aqueous solution is able to protect the solution against variations in pH when adding acid or alkali, or upon dilution with a solvent. Pharmaceutically acceptable buffers include, but are not limited to, histidine, TRIS® (tris (hydroxymethyl) aminomethane), citrate, succinate, glycolate and the like, as described herein.

The term "pKa" refers to the negative logarithm (p) of the ionization (acid dissociation) constant (Ka) of an acid which is equal to the pH value at which equal concentrations of the acid and conjugate base forms of a buffer are present (in which half of the acid molecules are ionized). When the p of a buffering agent equals the pH of the solution to be buffered, the buffering system is most effective.

An "acid" is a substance that yields hydrogen ions in aqueous solution. A "pharmaceutically acceptable acid" includes inorganic and organic acids which are nontoxic at the concentration and manner in which they are formulated.

A "base" is a substance that yields hydroxyl ions in aqueous solution. "Pharmaceutically acceptable bases" include inorganic and organic bases which are non-toxic at the concentration and manner in which they are formulated.

A "preservative" is an agent that reduces bacterial action and may be optionally added to the formulations herein. The addition of a preservative may, for example, facilitate the production of a multi-use (multiple-dose) formulation. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3pentanol, and m-cresol.

A "surfactant" is a surface active molecule containing both a hydrophobic portion (e.g., alkyl chain) and a hydrophilic portion (e.g., carboxyl and carboxylate groups). Surfactants suitable for use in the formulations of the present invention include, but are not limited to, polysorbates (e.g. polysorbates 20 or 80); poloxamers (e.g. poloxamer 188); sorbitan esters and derivatives; Triton; sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetadine; lauryl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauramidopropyl-cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropylbetaine (e.g., lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUATTM series (Mona Industries, Inc., Paterson, N.J.), polyethylene glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (e.g., Pluronics, PF68 etc.).

A "drug substance" refers to the starting material utilized in formulation of the final drug product. Typical anti-myostatin Fc engineered Adnectin drug substance compositions comprise a protein concentration from 10 mg/mL and 200 mg/mL, pH from 6.6 to 7.6 and % HMW species of < 5%.

A "formulated bulk solution" refers to the final formulation prior to filling of the container such as the formulated solution prior to filling the vials for lyophilization, or the formulated solution prior to filling the syringe for IV and/or SC injection.

A "drug product" refers to the final formulation packaged in a container which may be reconstituted before use, such as with a lyophilized drug product; diluted further before use, such as with a liquid drug product; or utilized as is, such as with a SC solution drug product.

The terms "individual," "subject," and "patient," used interchangeably herein, refer to an animal, preferably a mammalian (including a non-primate and a primate) or avian species,

including, but not limited to, murines, simians, humans, mammalian farm animals (e.g., bovine, porcine, ovine), mammalian sport animals (e.g., equine), and mammalian pets (e.g., canine and feline); preferably the term refers to humans. The term also refers to avian species, including, but not limited to, chickens and turkeys. In a certain embodiment, the subject, preferably a mammal, preferably a human, is further characterized with a disease or disorder or condition that would benefit from a decreased level or decreased bioactivity of myostatin. In another embodiment the subject, preferably a mammal, preferably a human, is further characterized as being at risk of developing a disorder, disease or condition that would benefit from a decreased level of myostatin or a decreased bioactivity of myostatin. In some embodiments, the subject is an obese patient, patient having obesity-related comorbidities, and/or a patient having type II diabetes.

The term "effective amount" refers to an amount of an agent sufficient to achieve the stated purpose or otherwise achieve the effects for which it is administered.

The term "therapeutically effective amount" refers to at least the minimal dose, but less than a toxic dose, of an agent which is necessary to impart a therapeutic benefit to a subject. For example, a therapeutically effective amount of an anti-myostatin Fc engineered Adnectin of the invention is an amount which in mammals, preferably humans, results in one or more of the following: an increase in muscle volume and/or muscle strength, a decrease in body fat, an increase in insulin sensitivity, or the treatment of conditions wherein the presence of myostatin causes or contributes to an undesirable pathological effect or a decrease in myostatin levels results in a beneficial therapeutic effect.

The term “frail” or “frailty” as used herein refers to a condition that can be characterized by two or more symptoms from weakness, weight loss, slowed mobility, fatigue, low activity levels, poor endurance, and impaired behavioral response to sensory cues. One hallmark of frailty is “sarcopenia,” or the age-related loss of muscle mass.

The term “cachexia” as used herein refers to the condition of accelerated muscle wasting and loss of lean body mass that can result from various diseases.

The term “obesity” is defined as a condition of abnormal or excessive fat accumulation in adipose tissue. The amount of excess fat in absolute terms, and its regional distribution between different fat depots both play an important role in determining the health impact of obesity. Obesity can be categorized into central/android obesity and peripheral/gynoid obesity, android obesity being more typical for men while gynoid obesity being more characteristic for women.

Obesity is based on Body Mass Index (BMI) for both youth and adults, but the definitions are not directly comparable. Among adults, there is a set cut point based on health risk, while among children the definition is statistical and is based on a comparison to a reference population. BMI is calculated as weight in kilograms divided by height in meters squared, rounded to one decimal place. Obesity in adults is defined as a BMI of greater than or equal to 30 kg/m^2. Obesity in youth is defined as a BMI of greater than or equal to the age- and sex-specific 95th percentile of the 2000 CDC growth charts.

The terms "overweight" or "overweight condition" are based on a BMI ≥ 25 - < 30 kg/m^2. Overweight condition can also be associated with at least one additional risk factor for fatal diseases (e.g., stroke, heart failure, sudden death), such as diabetes, hypertension, family history of premature coronary artery disease, etc.

Because different subjects can have same BMI, but different percentage of fat and muscle mass, BMI is not always a good index to classify overweight and obesity. A high percentage of muscle mass can lead to a high BMI even with a small percentage of fat. In this case the subject can be wrongly considered overweight or obese, based on the BMI classification. Other indexes in addition to BMI are used, namely waist circumference and a body shape index. Imaging, by DXA and MRI, is often used in clinical trials to quantify the percentage of muscle, fat and the fat distribution.

The term "body composition" is used herein to describe the percentages of fat and muscle in human bodies. Because muscular tissue takes up less space in our body than fat tissue, body composition, as well as our weight, determines leanness. Two people of same sex and body weight may look completely different from each other because they have a different body composition.

"Lean body mass" (LBM) is a component of body composition, calculated by subtracting body fat weight from total body weight. Total body weight is lean plus fat. Lean body mass equals body weight minus body fat. Lean body mass plus body fat equals body weight.

The percentage of total body mass that is lean is usually not quoted, but would typically be 60-90%. Instead, the body fat percentage, which is the complement, is computed, and is typically 10-40%. The lean body mass (LBM) has been described as an index superior to total body weight for prescribing proper levels of medications and for assessing metabolic disorders, as body fat is less relevant for metabolism.

The term "fat mass" refers to that portion of the human body that is composed strictly of fat. It can be measured with dual energy absorptiometry DXA, MRI or bioelectrical impedance techniques.

The term "central adiposity" refers to the following:

The term "type II diabetes" referred as type 2 diabetes, previously referred to as "non-insulin-dependent diabetes" or "adult-onset diabetes," accounts for 90-95% of all diabetes, encompasses individuals who have insulin resistance and usually relative (rather than absolute) insulin deficiency. At least initially, and often throughout their lifetime, these individuals may not need insulin treatment to survive. There are various causes of type 2 diabetes. Although the specific etiologies are not known, autoimmune destruction of B-cells does not occur, and patients do not have any of the other known causes of diabetes. Most, but not all, patients with type 2 diabetes are overweight or obese. Excess weight itself causes some degree of insulin resistance. Patients who are not obese or overweight by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Type 2 diabetes frequently goes undiagnosed for many years because hyperglycemia develops gradually and, at earlier stages, is often not severe enough for the patient to notice the classic diabetes symptoms. Nevertheless, even undiagnosed patients are at increased risk of developing macrovascular and microvascular complications.

The term "comorbidities of obesity or overweight" is associated with serious chronic disorders including, but not limited to: type 2 diabetes, glucose intolerance, prediabetes, insulin resistance, hypertension, dyslipidemia, increased waist circumference, cardiovascular disease, non-alcoholic fatty liver disease, obstructive sleep apnea, physical impairment, osteoarthritis, osteoporosis, renal disease, sexual hormone(s) impairment, endocrine reproductive disorders such as polycystic ovary syndrome or male hypogonadism, stroke, and gallstones.

"Glucose intolerance" is defined as the inability to properly metabolize glucose.

"Insulin sensitivity" describes how sensitive the body is to the effects of insulin. Someone said to be insulin sensitive will require smaller amounts of insulin to lower blood glucose levels than someone who has low sensitivity. Insulin sensitivity varies from person to person and doctors can perform tests to determine how sensitive an individual is to insulin.

"Insulin resistance" is defined as a condition of tolerance to insulin, making the hormone less effective, causing decreased glucose uptake in muscle tissue that result in impaired glucose

oxidation and glycogen synthesis, and a deficient suppression of hepatic glucose production in the liver. In obese, increased visceral fat mass with elevation of plasma free fatty acid (FFA) caused by the intensified lipolytic activity, worsen insulin resistance through the impairment of insulin action (Reaven, G.M., *et al.*, (1988), *Diabetes.* 37:1020-1024), a mechanism known as lipotoxicity (DeFronzo R.A. (2004), *Int. J. Clin. Pract. Suppl.*; (143):9-21). Herein the terms "improving insulin sensitivity" and "treating/lowering insulin resistance" shall be construed as equivalent.

High concentrations of plasma free fatty acid (FFA) in skeletal muscle cells lead to reduction in insulin-stimulated intracellular transport of glucose through the Glut4 transporter (see, *e.g.*, Dresner A., *et al.* (1999), *J. Clin. Invest.* 103(2): 253-259), in the hepatocytes lead to enhanced rate of gluconeogenesis and glucose release from the liver and augmented insulin secretion from β cells in response to a transient increase of FFA or an inhibition effect in response to chronic elevated levels (see, *e.g.,* Boden G. (1997), *Diabetes.* 46(1):3-10). As a result of insulin resistance and lipotoxicity, more insulin is needed to induce glucose uptake from fat and muscle cells, and glycogen synthesis in the liver (see, *e.g.*, Boden G. (1997), *Diabetes.* 46(1):3-10). The overproduction of insulin by pancreatic β cells is the physiologic reaction to insulin resistance and can lead to decline of β cell function and, eventually to prediabetes and type 2 diabetes (Donath M.Y., *et al.*, (2005), *Diabetes*, 54: S108-S1 13.).

The term "improving insulin sensitivity" refers to the systemic responsiveness to glucose, which can be measured by the insulin sensitivity index (which measures the ability of endogenous insulin to lower glucose in extracellular fluids by inhibiting glucose release from the liver and stimulating the peripheral consumption of glucose) and the glucose-clamp technique (which measures the effect of changes in insulin concentration on glucose clearance-glucose uptake rate divided by plasma glucose concentration per unit of body surface area).

"Prediabetes" is the precursor stage before diabetes mellitus in which blood sugar is abnormally high (*e.g.*, 100-125 mg/dL). Impaired fasting glycemia and impaired glucose tolerance are two aspects of prediabetes that are similar in clinical definition (glucose levels too high for their context) but are physiologically distinct. Insulin resistance, metabolic syndrome (or syndrome X), and prediabetes are closely related to one another and have overlapping aspects.

Various aspects of the present invention are described in further detail in the following subsections.

II. Anti-myostatin Fc engineered Adnectins

Provided herein are polypeptides which comprise a ^{10}Fn3 domain which binds to myostatin (e.g., human myostatin) ("anti-myostatin Adnectins") and include an Fc region (e.g., human IgG1 Fc region or human IgG4 Fc region), wherein the Fc region has at least one amino acid modification (e.g., amino acid substitutions) relative to a wild-type Fc region (e.g., a wild-type IgG1 Fc region or IgG4 Fc region) or S228P IgG4 Fc domain. Such polypeptides are referred to herein as "anti-myostatin Fc engineered Adnectins."

A. Adnectin portion

The Adnectin portion of anti-myostatin Fc engineered Adnectins comprises an Fn3 domain derived from the wild-type tenth module of the human fibronectin type III domain (^{10}Fn3) (SEQ ID NO: 4).

In some embodiments, the anti-myostatin Fc engineered Adnectin comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6, and 7, respectively.

In some embodiments, the anti-myostatin Fc engineered Adnectin comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6, and 7, respectively, wherein the BC loop comprises 1, 2 or 3 amino acid substitutions, such as conservative amino acid substitutions, which allow the anti-myostatin Fc engineered Adnectin to maintain binding to myostatin.

In some embodiments, the anti-myostatin Fc engineered Adnectin comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6, and 7, respectively, wherein at least one loop of the BC, DE, and FG loops of the ^{10}Fn3 domain has 1 amino acid substitution relative to the respective BC, DE, and FG loops of SEQ ID NOs: 5, 6, and 7.

In some embodiments, the anti-myostatin Fc engineered Adnectin comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6, and 7, respectively, wherein one loop from the BC, DE, or FG loop of the ^{10}Fn3 domain has 1 amino acid substitution relative to the respective BC, DE, or FG loop of SEQ ID NOs: 5, 6, and 7.

In some embodiments, the anti-myostatin Fc engineered Adnectin comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6, or 7, respectively, wherein (i) the serine at position 3 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of A, C, D, F, H, I, K, L, N, Q, R, T, V, W, or Y; (ii) the leucine at position 4 of

the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from M or V; (iii) the proline at position 5 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of A, C, D, E, I, K, L, M, N, Q, R, S, T, V, or Y; (vi) the histidine at position 6 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of A, C, D, E, F, G, I, K, L, M, N, Q, R, S, T, V, W, or Y; (vii) the glutamine at position 7 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, R, S, T, V, W, or Y; (viii) the glycine at position 8 of the BC loop (SEQ ID NO: 5) is substituted with the amino acid S; (ix) the lysine at position 9 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, L, M, N, Q, R, S, T, V, W, or Y; (x) the alanine at position 10 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of C, G, L, M, S, or T; or (xi) the asparagine at position 11 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of A, C, F, H, P, Q, R, S, or Y.

In some embodiments, the anti-myostatin Fc engineered Adnectin comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6, or 7, respectively, wherein (i) the serine at position 3 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of C, F, I, V, W, or Y; (ii) the histidine at position 6 of the BC loop (SEQ ID NO: 6) is substituted with an amino acid selected from the group consisting of C, D, E, F, G, I, K, L, M, N, Q, R, S, T, V, W, or Y; (iii) the lysine at position 9 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of A, C, G, H, I, L, M, N, Q, R, S, V, W, or Y; (iv) the alanine at position 10 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of G, L, M, or S; or (v) the asparagine at position 11 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of C, H, Q, S, or Y.

In some embodiments, the anti-myostatin Fc engineered Adnectin comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6, or 7, respectively, wherein (i) the serine at position 3 of the BC loop (SEQ ID NO: 5) is substituted with the amino acid F or W; (ii) the histidine at position 6 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of C, F, G, I, K, L, M, N, R, S, T, V, W, or Y; (iii) the glutamine at position 7 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the

group consisting of A, C, E, F, H, I, K, L, M, P, R, S, T, V, or Y; (iii) the lysine at position 9 of the BC loop (SEQ ID NO: 5) is substituted with an amino acid selected from the group consisting of A, C, H, L, M, N, R, V, W, or Y; (iv) the alanine at position 10 of the BC loop (SEQ ID NO: 5) is substituted with the amino acid G or L; or (v) the asparagine at position 11 of the BC loop (SEQ ID NO: 5) is substituted with the amino acid H or Q.

In some embodiments, the anti-myostatin Fc engineered Adnectin comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6, or 7, respectively, wherein the valine at position 5 of the DE loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, D, E, F, I, K, L, M, N, Q, S, or T. In some embodiments, the valine at position 5 of the DE loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of C, E, I, L, M, Q, or T. In some embodiments, the valine at position 5 of the DE loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of C, E, I, L, or M.

In some embodiments, the anti-myostatin Fc engineered Adnectin comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6, or 7, respectively, wherein (i) the valine at position 2 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, F, I, L, M, Q, T, W, or Y; (ii) the threonine at position 3 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, F, G, H, I, K, L, M, N, Q, R, S, V, W, or Y; (iii) the aspartic acid at position 4 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; (iv) the threonine at position 5 of the FG loop (SEQ ID NO: 7) is substituted to with an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, or Y; (v) the glycine at position 6 of the FG loop (SEQ ID NO: 7) is substituted to with an amino acid selected from the group consisting of A, C, D, E, F, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; (vi) the tyrosine at position 7 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, F, H, I, L, M, N, P, S, T, V, or W; (vii) the leucine at position 8 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, E, F, H, I, K, M, N, Q, R, S, T, V, W, or Y; (viii) the lysine at position 9 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; (ix) the tyrosine at position 10 of the FG loop (SEQ ID NO: 7) is substituted

with the amino acid F or W; or (x) the lysine at position 11 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y.

In some embodiments, the anti-myostatin Fc engineered Adnectin comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6, or 7, respectively, wherein (i) the valine at position 2 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, I, L, or M; (ii) the threonine at position 3 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of C, F, H, I, L, M, Q, R, S, V, W, or Y; (iii) the aspartic acid at position 4 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, E, F, G, H, I, L, M, N, P, Q, S, T, V, W, or Y; (iv) the threonine at position 5 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, V, W, or Y; (v) the glycine at position 6 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, D, E, F, H, I, L, M, N, Q, S, T, V, W, or Y; (vi) the tyrosine at position 7 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of C, F, I, L, M, P, T, V, or W; (vii) the leucine at position 8 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of C, F, H, I, K, M, N, Q, R, T, V, W, or Y; (viii) the lysine at position 9 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, E, F, G, I, L, M, N, P, Q, R, S, T, V, W, or Y; (ix) the tyrosine at position 10 of the FG loop (SEQ ID NO: 7) is substituted with the amino acid W; or (x) the lysine at position 11 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, D, E, G, H, L, M, N, P, Q, R, S, T, or V.

In some embodiments, the anti-myostatin Fc engineered Adnectin comprises the BC, DE, and FG loops as set forth in SEQ ID NOs: 5, 6, or 7, respectively, wherein (i) the valine at position 2 of the FG loop (SEQ ID NO: 7) is substituted with the amino acid I; (ii) the threonine at position 3 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of C, F, I, L, M, V, W, or Y; (iii) the aspartic acid at position 4 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, E, F, G, H, I, L, M, N, Q, S, T, or V; (iv) the threonine at position 5 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, D, F, G, I, L, M, N,

Q, S, V, W, or Y; (v) the glycine at position 6 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, S, T, or W; (vi) the tyrosine at position 7 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of F, I, V, or W; (vii) the leucine at position 8 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of F, H, I, M, V, W, or Y; (viii) the lysine at position 9 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, C, F, G, I, L, M, T, V, or W; or (x) the lysine at position 11 of the FG loop (SEQ ID NO: 7) is substituted with an amino acid selected from the group consisting of A, G, L, M, P, Q, or R.

In certain embodiments, the anti-myostatin Fc engineered Adnectin comprises an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 8, or an amino acid sequence which differs from SEQ ID NO: 8 by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., by amino acid modifications, such as amino acid substitutions):

EVVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT (SEQ ID NO: 8)

In some embodiments, the anti-myostatin Fc engineered Adnectin contains a ^{10}Fn3 domain which binds myostatin comprising an amino acid sequence which is at least 90%, 95%, 98%, 99% or 100% identical to the non-BC, DE, and FG loop regions of SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In some embodiments, the anti-myostatin Fc engineered Adnectin contains a ^{10}Fn3 domain which binds myostatin comprising an amino acid sequence which differs from the non-BC, DE, and FG loop regions of SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10 by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., by amino acid modifications, such as amino acid substitutions). For example, in some embodiments, the non-ligand binding sequences of ^{10}Fn3, i.e., the "^{10}Fn3 scaffold", may also be altered provided that the ^{10}Fn3 retains ligand binding function and/or structural stability. A variety of mutant ^{10}Fn3 scaffolds have been reported. In some embodiments, one or more of Asp 7, Glu 9, and Asp 23 is replaced by another amino acid, such as, for example, a non-negatively charged amino acid residue (e.g., Asn, Lys, etc.). These mutations have been reported to have the effect of promoting greater stability of the mutant ^{10}Fn3 at neutral pH as compared to the wild-type form (see, e.g., PCT Publication No. WO 02/04523). A variety of additional alterations in the ^{10}Fn3 scaffold that are either beneficial or neutral have been disclosed. See, for example, Batori et al.,

Protein Eng., 15(12):1015-1020 (December 2002); Koide et al., Biochemistry, 40(34):10326-10333 (Aug. 28, 2001).

In some embodiments, the ^{10}Fn3 domain of the anti-myostatin Fc engineered Adnectin comprises SEQ ID NO: 8. In some embodiments, the ^{10}Fn3 domain of the anti-myostatin Fc engineered Adnectin comprises SEQ ID NO: 9. In some embodiments, the ^{10}Fn3 domain of the anti-myostatin Fc engineered Adnectin comprises SEQ ID NO: 10.

In some embodiments, the anti-myostatin Fc engineered Adnectins are modified to comprise an N-terminal extension sequence and/or a C-terminal extension sequence. For example, an MG sequence may be placed at the N-terminus of the ^{10}Fn3 defined by SEQ ID NO: 4. The M will usually be cleaved off, leaving a G at the N-terminus. In some embodiments, the anti-myostatin Fc engineered Adnectin may comprise the amino acid sequence of SEQ ID NO: 8, and an N-terminal extension sequence (exemplary leader) as shown in **Table 1** (i.e., any one of SEQ ID NOs: 11-17, or RDL or DL). In addition, an M, G or MG may also be placed N-terminal to any of the N-terminal extensions shown in **Table 1** (i.e., any one of SEQ ID NOs: 11-17, or RDL or DL). In some embodiments, the anti-myostatin Fc engineered Adnectin may be truncated at the threonine corresponding to T94 of SEQ ID NO: 4. Alternatively, C-terminal extensions may be added after the C-terminal residue of SEQ ID NO: 8. Exemplary C-terminal extension sequences are shown in **Table 1** (i.e., any one of SEQ ID NOs: 18-34, or EI).

Table 1: Summary of N-terminal and C-terminal Extension Sequences

SEQ ID NO	Description	Name	Sequence
11	Exemplary leader	AdNT1	MGVSDVPRDL
12	Exemplary leader	AdNT2	GVSDVPRDL
13	Exemplary leader	AdNT3	VSDVPRDL
14	Exemplary leader	AdNT4	SDVPRDL
15	Exemplary leader	AdNT5	DVPRDL
16	Exemplary leader	AdNT6	VPRDL
17	Exemplary leader	AdNT7	PRDL
-----	Exemplary leader	AdNT8	RDL
-----	Exemplary leader	AdNT9	DL
18	Exemplary tail	AdCT1	EIDKPSQ
-----	Exemplary tail	AdCT2	EI
19	Exemplary tail	AdCT3	EIEPKSS
20	Exemplary tail	AdCT4	EIDKPC
21	Exemplary tail	AdCT5	EIDKP

22	Exemplary tail	AdCT6	EIDK
23	Exemplary tail	AdCT7	EIDKPS
24	Exemplary tail	AdCT8	EIEKPSQ
25	Exemplary tail	AdCT9	EIDKPSQLE
26	Exemplary tail	AdCT10	EIEDEDEDEDED
27	Exemplary tail	AdCT11	EGSGS
28	Exemplary tail	AdCT12	EIDKPCQ
29	Exemplary tail	AdCT13	GSGC
30	Exemplary tail	AdCT14	EGSGC
31	Exemplary tail	AdCT15	EIDKPCQLE
32	Exemplary tail	AdCT16	EIDKPSQHHHHHH
33	Exemplary tail	AdCT17	GSGCHHHHHH
34	Exemplary tail	AdCT18	EGSGCHHHHHH

In some embodiments, the C-terminal extension sequences (also called "tails"), comprise E and D residues, and may be between 8 and 50, 10 and 30, 10 and 20, 5 and 10, and 2 and 4 amino acids in length. In some embodiments, tail sequences include ED-based linkers in which the sequence comprises tandem repeats of ED. In exemplary embodiments, the tail sequence comprises 2-10, 2-7, 2-5, 3-10, 3-7, 3-5, 3, 4 or 5 ED repeats. In certain embodiments, the ED-based tail sequences may also include additional amino acid residues, such as, EI, EID, ES, EC, EGS, and EGC. Such sequences are based, in part, on known Adnectin tail sequences, such as EIDKPSQ (SEQ ID NO: 18), in which residues D and K have been removed. In exemplary embodiments, the ED-based tail comprises an E, I or EI residues before the ED repeats.

B. Fc variants

The anti-myostatin Fc engineered Adnectins described herein comprise a ^{10}Fn3 domain and a Fc region, wherein the Fc region comprises at least one amino acid modification (e.g., amino acid substitution), but no more than 15 amino acid modifications (e.g., amino acid substitutions), relative to a wild-type Fc region (e.g., a wild-type IgG1 or IgG4 Fc region) or S228P IgG4 Fc domain. Accordingly, in some embodiments, the Fc region differs from a reference human Fc region (e.g., a wild-type IgG1 Fc region or IgG4 Fc region or S228P IgG4 Fc domain) by at least one amino acid, but no more than 15 amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 differences relative to the reference IgG1 Fc region or IgG4 Fc region or S228P IgG4 Fc domain). In some embodiments, such engineered Fc regions differ in sequence from wild-type Fc regions and thus do not comprise the amino acid sequence of a wild-

type Fc region (e.g., do not comprise the amino acid sequence of a wild-type human IgG1 Fc region, human IgG2 Fc region, human IgG3 Fc region, human IgG4 Fc region). In some embodiments, such engineered Fc regions differ in sequence from a S228P IgG4 Fc domain (e.g., a human S228P IgG4 Fc domain).

The engineered Fc portion of anti-myostatin Fc engineered Adnectins comprises a variant functional immunoglobulin Fc region, or a fragment thereof. As used herein, a "functional Fc region" is an Fc region or fragment thereof which retains the ability to bind FcRn. In some embodiments, a functional Fc region binds to FcRn, but does not possess effector function. The ability of the Fc region or fragment thereof to bind to FcRn can be determined by standard binding assays known in the art. In other embodiments, the Fc region or fragment thereof binds to FcRn and possesses at least one “effector function” of a native Fc region. Exemplary "effector functions" include C1q binding; 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; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g., an anti-myostatin Adnectin) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

A "native sequence Fc region" or "wild-type Fc region" are used interchangeably to refer to an Fc region having an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. A "variant Fc region" or "engineered 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. 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 15 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% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% sequence identity therewith, more preferably at least about 95% sequence identity therewith.

In some embodiments, the engineered Fc region of an anti-myostatin Fc engineered Adnectin comprises the amino acid sequence of any one of SEQ ID NOs: 83-91, or an amino

acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to any one of SEQ ID NOs: 83-91, or an amino acid sequence which differs from any one of SEQ ID NOs: 83-91 by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

In an exemplary embodiment, the Fc region is derived from an IgG1 subclass, however, other subclasses (e.g., IgG2, IgG3, and IgG4) may also be used. Shown below is the sequence of a human IgG1 immunoglobulin Fc region:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 76)

The core hinge sequence is underlined, and the CH2 and CH3 regions are in regular text. It should be understood that the C-terminal lysine (K) is optional. In some embodiments, the C-terminal "GK" is optional.

In some embodiments, the engineered Fc region does not comprise the amino acid sequence of a wild-type immunoglobulin Fc region. In some embodiments, the engineered Fc region does not comprise the amino acid sequence of a wild-type IgG Fc region (or a wild-type IgG Fc region without the C-terminal lysine, or a wild-type IgG Fc region without the C-terminal "GK"). In some embodiments, the engineered Fc region does not comprise the amino acid sequence of a wild-type IgG1 Fc region (or a wild-type IgG1 Fc region without the C-terminal lysine, or a wild-type IgG1 Fc region without the C-terminal "GK"). In some embodiments, the engineered Fc region does not comprise the amino acid sequence of a wild-type IgG2 Fc region (or a wild-type IgG2 Fc region without the C-terminal lysine, or a wild-type IgG2 Fc region without the C-terminal "GK"). In some embodiments, the engineered Fc region does not comprise the amino acid sequence of a wild-type IgG3 Fc region (or a wild-type IgG3 Fc region without the C-terminal lysine, or a wild-type IgG3 Fc region without the C-terminal "GK"). In some embodiments, the engineered Fc region does not comprise the amino acid sequence of a wild-type IgG4 Fc region (or a wild-type IgG4 Fc region without the C-terminal lysine, or a wild-type IgG4 Fc region without the C-terminal "GK"). In some embodiments, the engineered Fc region does not comprise the amino acid sequence of a S228P IgG4 Fc region (or a S228P

IgG4 Fc domain without the C-terminal lysine, or a S228P IgG4 Fc region without the C-terminal "GK").

The fusion of the anti-myostatin Adnectin with the engineered Fc region may be formed by attaching an anti-myostatin Adnectin to either end of the engineered Fc molecule, i.e., Fc-anti-myostatin Adnectin or anti-myostatin Adnectin-Fc arrangements. In certain embodiments, the Fc and anti-myostatin Adnectin are fused via a linker. Exemplary linker sequences include GAGGGGSG (SEQ ID NO: 35), EPKSSD (SEQ ID NO: 36), D, ESPKAQASSVPTAQPQAEGLA (SEQ ID NO: 37), ELQLEESAAEAQDGELD (SEQ ID NO: 38), GQPDEPGGS (SEQ ID NO: 39), GGSGSGSGSGSGS (SEQ ID NO: 40), ELQLEESAAEAQEGELE (SEQ ID NO: 41), GSGSG (SEQ ID NO: 42), GSGC (SEQ ID NO: 43), AGGGGSG (SEQ ID NO: 44), GSGS (SEQ ID NO: 45), QPDEPGGS (SEQ ID NO: 46), GSGSGS (SEQ ID NO: 47), TVAAPS (SEQ ID NO: 48), KAGGGGSG (SEQ ID NO: 49), KGSGSGSGSGSGS (SEQ ID NO: 50), KQPDEPGGS (SEQ ID NO: 51), KELQLEESAAEAQDGELD (SEQ ID NO: 52), KTVAAPS (SEQ ID NO: 53), KAGGGGSGG (SEQ ID NO: 54), KGSGSGSGSGSGSG (SEQ ID NO: 55), KQPDEPGGSG (SEQ ID NO: 56), KELQLEESAAEAQDGELDG (SEQ ID NO: 57), KTVAAPSG (SEQ ID NO: 58) AGGGGSGG (SEQ ID NO: 59), AGGGGSG (SEQ ID NO: 60), GSGSGSGSGSGSG (SEQ ID NO: 61), QPDEPGGSG (SEQ ID NO: 62), and TVAAPSG (SEQ ID NO: 63). In some embodiments, the linker comprises the amino acid sequence of any one of SEQ ID NOs: 35-63, or an amino acid sequence which differs from SEQ ID NOs: 64-72 by 1, 2, or 3 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

In some embodiments, the engineered Fc region used in the anti-myostatin Fc engineered Adnectin comprises the hinge region of an Fc molecule. In some embodiments, the hinge region comprises the core hinge residues spanning positions 1-16 of SEQ ID NO: 76 (DKTHTCPPCPAPELLG; SEQ ID NO: 64) of the IgG1 Fc region. In some embodiments, the hinge region comprises the amino acid of any one of SEQ ID NOs: 64-72, or an amino acid sequence which differs from SEQ ID NOs: 64-72 by 1, 2, or 3 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

In certain embodiments, the anti-myostatin Adnectin – engineered Fc region fusion adopts a multimeric structure (e.g., dimer) owing, in part, to the cysteine residues at positions 6 and 9 of SEQ ID NO: 76 within the hinge region. In other embodiments, the hinge region as

used herein, may further include residues derived from the CH1 and CH2 regions that flank the core hinge sequence, as shown in SEQ ID NO: 76. In yet other embodiments, the hinge sequence is GSTHTCPPCPAPELLG (SEQ ID NO: 65).

In some embodiments, the hinge sequence, may include substitutions that confer desirable pharmacokinetic, biophysical, and/or biological properties. Some exemplary hinge sequences include EPKSSDKTHTCPPCPAPELLGGPS (SEQ ID NO: 66; core hinge region underlined), EPKSSDKTHTCPPCPAPELLGGSS (SEQ ID NO 67; core hinge region underlined), EPKSSGSTHTCPPCPAPELLGGSS (SEQ ID NO: 68; core hinge region underlined), DKTHTCPPCPAPELLGGPS (SEQ ID NO: 69; core hinge region underlined), and DKTHTCPPCPAPELLGGSS (SEQ ID NO: 70; core hinge region underlined). In one embodiment, the residue P at position 18 of SEQ ID NO: 76 has been replaced with S to ablate Fc effector function; this replacement is exemplified in hinges having any one of SEQ ID NOs: 67, 68, and 70. In another embodiment, the residues DK at positions 1-2 of SEQ ID NO: 76 have been replaced with GS to remove a potential clip site; this replacement is exemplified in SEQ ID NO: 68. In another embodiment, the C at position 103 of SEQ ID NO: 79, which corresponds to the heavy chain constant region of human IgG1 (i.e., domains CH1-CH3), has been replaced with S to prevent improper cysteine bond formation in the absence of a light chain; this replacement is exemplified in SEQ ID NOs: 66-68.

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 80)

In some embodiments, the engineered Fc region of an anti-myostatin Fc engineered Adnectin comprises the amino acid sequence of any one of SEQ ID NOs: 92-101, or an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to any one of SEQ ID NOs: 92-101, or an amino acid sequence which differs from any one of SEQ ID NOs: 92-101 by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

In certain embodiments, an anti-myostatin Adnectin-Fc fusion may have the following configurations: 1) anti-myostatin Adnectin-hinge-Fc or 2) hinge-Fc-anti-myostatin Adnectin.

Therefore, any anti-myostatin Adnectin described herein can be fused to an Fc region comprising a hinge sequence according to these configurations. In some embodiments, a linker may be used to join the anti-myostatin Adnectin to the hinge-Fc moiety, for example, an exemplary fusion protein may have the configuration anti-myostatin Adnectin-linker-hinge-Fc or hinge-Fc-linker-anti-myostatin Adnectin. Additionally, depending on the system in which the fusion polypeptide is produced, a leader sequence may be placed at the N-terminus of the fusion polypeptide. For example, if the fusion is produced in a mammalian system, a leader sequence such as MRAWIFFLLCLAGRALA (SEQ ID NO: 74) or METDTLLLWVLLLWVPGSTG (SEQ ID NO: 75) may be added to the N-terminus of the fusion molecule. If the fusion is produced in *E. coli*, the fusion sequence will be preceded by a methionine.

In some embodiments, the polypeptide is an Fc engineered variant of the Fc-anti-myostatin Adnectin construct known as BHV-2000, wherein the Fc engineered variant comprises the sequence of SEQ ID NO: 81, with the exception of at least one amino acid modification (e.g., at least one amino acid substitution) but no more than 15 amino acid modifications (e.g., no more than 15 amino acid substitutions) in the Fc region. BHV-2000, which comprises BC, DE, and FG loop sequences of SEQ ID NOs: 5, 6, and 7, respectively, has the following sequence (hinge region is underlined, the linker is in italics, the leader sequence is in bold, and the anti-myostatin Adnectin sequence is underlined and in italics):

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPR**
DL*EVVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTATI*
*SGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT*EI (SEQ ID NO: 81).

In some embodiments, the anti-myostatin Fc engineered Adnectins described herein bind to active myostatin with a K_D of about 5-15 nM, for example, as assessed using biolayer interferometry.

In some embodiments, the anti-myostatin Fc engineered Adnectins described herein binds with a stronger affinity to FcRn at pH. 6.0 compared to a polypeptide comprising the amino acid sequence of SEQ ID NO: 81, e.g., as assessed by biolayer interferometry.

In some embodiments, the anti-myostatin Fc engineered Adnectins described herein binds to FcRn at pH 6.0 with a 40-60 fold (e.g., 40-55 fold, 40-50 fold, 40-45 fold, 45-60 fold,

45-55 fold, 45-50 fold, 50-60 fold, 55-60 fold) higher affinity than a polypeptide comprising the amino acid sequence of SEQ ID NO: 81, e.g., as assessed by biolayer interferometry.

In some embodiments, the anti-myostatin Fc engineered Adnectins described herein have a lower (i.e., weaker) affinity for FcγRI relative to a polypeptide comprising the amino acid sequence of SEQ ID NO: 81, e.g., as assessed by biolayer interferometry.

In some embodiments, the anti-myostatin Fc engineered Adnectins described herein have a lower (i.e., weaker) affinity for C1q relative to a polypeptide comprising the amino acid sequence of SEQ ID NO: 81, e.g., as assessed by biolayer interferometry.

In some embodiments, the anti-myostatin Fc engineered Adnectins described herein inhibit SMAD2/3 signaling.

In some embodiments, the anti-myostatin Fc engineered Adnectins described herein have increased FcRn-mediated recycling relative to a polypeptide comprising the amino acid sequence of SEQ ID NO: 81, e.g., as assessed by the cell-based assay described in Example 6. In some embodiments, the increase in FcRn-mediated recycling over a polypeptide comprising the amino acid sequence of SEQ ID NO: 81 is at least 2-fold, at least 3-rold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 11-fold, at least 12 fold, at least 13-fold, at least 14-fold, at least 15-fold, about 2-fold to about 15-fold, about 5-fold to about 15-fold, about 8-fold to about 15-fold, about 10-fold to about 15-fold, about 13-fold to about 15-fold, about 2-fold to about 13-fold, about 5-fold to about 13-fold, about 8-fold to about 13 fold, about 10-fold to about 13-fold, about 2-fold to about 10-fold, about 5-fold to about 10-fold, about 8-fold to about 10-fold, about 2-fold to about 8-fold, about 5-fold to about 8-fold, or about 2-fold to about 5-fold.

In some embodiments, the anti-myostatin Fc engineered Adnectins described herein have a longer half-life (e.g., in circulation) relative to a polypeptide comprising the amino acid sequence of SEQ ID NO: 81. The half-life of the anti-myostatin Fc engineered Adnectins can be determined using art-recognized methods.

C. Exemplary anti-myostatin Fc engineered Adnectins

In some embodiments, the anti-myostatin Fc engineered Adnectin comprises (a) a ^{10}Fn3 domain which binds to myostatin described herein (e.g., the ^{10}Fn3 domain of BHV-2000 or a variant thereof), and (b) an Fc region which differs in amino acid sequence from a reference

human IgG1 Fc region comprising the amino acid sequence of SEQ ID NO: 78 or a reference human S228P IgG4 Fc region comprising the amino acid sequence of SEQ ID NO: 79 or wild-type human IgG4 Fc region (SEQ ID NO: 79 without the S228P substitution) by at least one amino acid but no more than 15 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

In some embodiments, the ^{10}Fn3 domain of the anti-myostatin Fc engineered Adnectin comprises BC, DE, and FG loops comprising the amino acid sequences of SEQ ID NOs: 5, 6, and 7, respectively, or a ^{10}Fn3 domain wherein at least one loop of the BC, DE, and FG loops has 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops of SEQ ID NOs: 5, 6, and 7, respectively. Exemplary substitutions to the BC, DE, and/or FG loops of SEQ ID NOs: 5, 6, and 7, respectively, are described *supra*.

In some embodiments, the ^{10}Fn3 domain of the anti-myostatin Fc engineered Adnectin comprises the amino acid sequence of SEQ ID NO: 8, or an amino acid sequence which is at least 90%, 95%, 98%, or 99% identical to SEQ ID NO: 8, or an amino acid sequence which differs from the amino acid sequence of SEQ ID NO: 8 by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids (e.g., by amino acid modifications, such as amino acid substitutions). In some embodiments, the ^{10}Fn3 domain comprises an N-terminal leader sequence and/or a C-terminal tail sequence. In some embodiments, the N-terminal leader sequence comprises an amino acid sequence selected from any one of SEQ ID NOs: 11-17, or the amino acid sequence RDL or DL. In some embodiments, the C-terminal leader sequence comprises an amino acid sequence selected from any one of SEQ ID NOs: 18-34, or the amino acid sequence EI. In some embodiments, the ^{10}Fn3 domain comprises both an N-terminal leader sequence and a C-terminal tail sequence. Accordingly, in some embodiments, the ^{10}Fn3 domain comprises an N-terminal leader sequence comprises an amino acid sequence selected from any one of SEQ ID NOs: 11-17, RDL, or DL, and the C-terminal leader sequence comprises an amino acid sequence selected from any one of SEQ ID NOs: 18-34 or EI.

In some embodiments, the Fc region of the anti-myostatin Fc engineered Adnectin differs from the amino acid sequence of SEQ ID NO: 78 by at least 2 amino acids, at least 3 amino acids, at least 4 amino acids, at least 5 amino acids, at least 6 amino acids, at least 7 amino acids, at least 8 amino acids, at least 9 amino acids, or at least 10 amino acids, but by no more than 15 amino acids (e.g., by amino acid modifications, such as amino acid substitutions). In some

embodiments, the Fc region of the anti-myostatin Fc engineered Adnectin differs from the amino acid sequence of SEQ ID NO: 78 by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids (e.g., by amino acid modifications, such as amino acid substitutions). The difference in amino acid sequences relative to reference sequence SEQ ID NO: 78 may be introduced by site-directed mutagenesis using art-recognized methods, or the Fc region may be chemically synthesized to have the variant sequence.

In some embodiments, the Fc region of the anti-myostatin Fc engineered Adnectin differs from the amino acid sequence of SEQ ID NO: 79 by at least 2 amino acids, at least 3 amino acids, at least 4 amino acids, at least 5 amino acids, at least 6 amino acids, at least 7 amino acids, at least 8 amino acids, at least 9 amino acids, or at least 10 amino acids, but by no more than 15 amino acids (e.g., by amino acid modifications, such as amino acid substitutions). In some embodiments, the Fc region of the anti-myostatin Fc engineered Adnectin differs from the amino acid sequence of SEQ ID NO: 79 by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids (e.g., by amino acid modifications, such as amino acid substitutions). The difference in amino acid sequences relative to reference sequence SEQ ID NO: 79 may be introduced by site-directed mutagenesis using art-recognized methods, or the Fc region may be chemically synthesized to have the variant sequence.

In some embodiments, the Fc region of the anti-myostatin Fc engineered Adnectin does not comprise a wild-type Fc region (e.g., does not comprise a wild-type IgG1, IgG2, IgG3, or IgG4 Fc region, or does not comprise a wild-type IgG1, IgG2, IgG3, or IgG4 Fc region without the C-terminal lysine, or does not comprise a wild-type IgG1, IgG2, IgG3, or IgG4 Fc region without the C-terminal “GK” sequence). In some embodiments, the Fc region of the anti-myostatin Fc engineered Adnectin does not comprise a S228P IgG4 Fc region (e.g., does not comprise a S228P IgG4 Fc region without the C-terminal lysine, or does not comprise a S228P IgG4 Fc region without the C-terminal “GK” sequence).

In some embodiments, the Fc region of the anti-myostatin Fc engineered Adnectin comprises an engineered IgG1 Fc region. In some embodiments, the engineered IgG1 Fc region comprises one or more of the following: an alanine at position 234 (e.g., a L234A substitution), an alanine at position 235 (e.g., a L235A substitution), a leucine at position 248 (e.g., an M248L substitution), a tyrosine at position 252 (e.g., an M252Y substitution), a threonine at position 254 (e.g., a S254T substitution), a glutamic acid at position 256 (e.g., a T256E substitution), a

glutamine at position 297 (e.g., a N297Q substitution), an alanine at position 329 (e.g., a P329A substitution), and a serine at position 434 (e.g., a N434S substitution). In some embodiments, the engineered IgG1 Fc region comprises an alanine at position 234 and an alanine at position 235 (e.g., a L234A/L235A substitution). In some embodiments, the engineered IgG1 Fc region comprises an alanine at position 234, an alanine at position 235, and an alanine at position 329 (e.g., a L234A/L235A/P329A substitution). In some embodiments, the engineered IgG1 Fc region comprises an alanine at position 234, an alanine at position 235, an alanine at position 329, a leucine at position 248, and a serine at position 434 (e.g., a L234A/L235A/P329A/M248L/N434S substitution). In some embodiments, the engineered IgG1 Fc region comprises an alanine at position 234, an alanine at position 235, an alanine at position 329, a tyrosine at position 252, a threonine at position 254, and a glutamic acid at position 256 (e.g., a L234A/L235A/P329A/M252Y/S254T/T256E substitution). In some embodiments, the engineered IgG1 Fc region comprises a leucine at position 248 and a serine at position 434 (e.g., a M248L/N434S substitution). In some embodiments, the engineered IgG1 Fc region comprises a tyrosine at position 252, a threonine at position 254, and a glutamic acid at position 256 (e.g., a M252Y/S254T/T256E substitution). In some embodiments, the engineered IgG1 Fc region comprises a tyrosine at position 252, a threonine at position 254, a glutamic acid at position 256, and a glutamine at position 297 (e.g., a M252Y/S254T/T256E/N297Q substitution). In some embodiments, the engineered IgG1 Fc region comprises a leucine at position 248, a glutamine at position 297, and a serine at position 424 (e.g., a N297Q/M248L/N434S substitution). In some embodiments, the engineered IgG1 Fc region comprises the amino acid sequence of any one of SEQ ID NOs: 83-89, or an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to any one of SEQ ID NOs: 83-89, or an amino acid sequence which differs from any one of SEQ ID NOs: 83-89 by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

In some embodiments, the Fc region of the anti-myostatin Fc engineered Adnectin comprises an engineered IgG4 Fc region. In some embodiments, the engineered IgG4 Fc region comprises one or more of the following: an alanine at position 234 (e.g., a F234A substitution), an alanine at position 235 (e.g., a L235A substitution), a tyrosine at position 252 (e.g., an M252Y substitution), a threonine at position 254 (e.g., a S254T substitution), a glutamic acid at position 256 (e.g., a T256E substitution), and a glutamine at position 297 (e.g., a N297Q

substitution). In some embodiments, the engineered IgG4 Fc region comprises an alanine at position 234, an alanine at position 235, a tyrosine at position 252, a threonine at position 254, and a glutamic acid at position 256 (e.g., a F234A/L235A/M252Y/S254T/T256E substitution). In some embodiments, the engineered IgG4 Fc region comprises a tyrosine at position 252, a threonine at position 254, and a glutamic acid at position 256, and a glutamine at position 297 (e.g., a M252Y/S254T/T256E/N297Q substitution). In some embodiments, the engineered IgG4 Fc region comprises the amino acid sequence of SEQ ID NO: 90 or 91, or an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 90 or 91, or an amino acid sequence which differs from SEQ ID NO: 90 or 91 by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

In some embodiments, the numbering of positions in the Fc region for embodiments described herein is according to the EU Index.

In some embodiments, the engineered Fc region of the anti-myostatin Fc engineered Adnectin comprises a hinge sequence, for example, a hinge sequence comprising the amino acid sequence selected from any one of SEQ ID NOs: 64-72, or an amino acid sequence which is at least 90% identical (e.g., at least 95% identical) to an amino acid sequence selected from any one of SEQ ID NOs: 64-72, or an amino acid sequence which differs from any one of SEQ ID NOs: 64-72 by 1, 2, or 3 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

In some embodiments, the engineered Fc region of the anti-myostatin Fc engineered Adnectin comprises the amino acid sequence of any one of SEQ ID NOs: 92-99. In some embodiments, the engineered Fc region of the anti-myostatin Fc engineered Adnectin comprises an amino acid sequence which is at least 95%, 96%, 97%, 98%, or 99% identity to any one of SEQ ID NOs: 92-99. In some embodiments, the engineered Fc region of the anti-myostatin Fc engineered Adnectin comprises an amino acid sequence which differs from any one of SEQ ID NOs: 92-99 by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

In some embodiments, the anti-myostatin Fc engineered Adnectin comprises a signal peptide at the N-terminus. In some embodiments, the signal peptide comprises the amino acid sequence of SEQ ID NO: 74 or 75, or an amino acid sequence which differs from SEQ ID NO:

74 or 75 by 1, 2, or 3 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

In some embodiments, the engineered Fc region is linked to the ^{10}Fn3 domain of the anti-myostatin Fc engineered Adnectin via a linker (e.g., a peptide linker). In some embodiments, the linker comprises the amino acid sequence of any one of SEQ ID NOs: 35-63, or an amino acid sequence which differs from any one of SEQ ID NOs: 35-63 by 1, 2, or 3 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

In some embodiments, the Fc region is located N-terminal to the ^{10}Fn3 domain and thus has the configuration Fc domain – ^{10}Fn3 domain, Fc domain – linker – ^{10}Fn3 domain, or signal peptide – Fc domain – linker – ^{10}Fn3 domain.

In some embodiments, the Fc region is located C-terminal to the ^{10}Fn3 domain and thus has the configuration ^{10}Fn3 domain – Fc region, ^{10}Fn3 domain– linker – Fc region, or signal peptide – ^{10}Fn3 domain – linker – Fc region.

In some embodiments, provided herein is a polypeptide (e.g., an anti-myostatin Fc engineered Adnectin) which comprises the amino acid sequence of any one of SEQ ID NO: 102-111, or a polypeptide comprising an amino acid sequence which is at least 90%, 95%, 98%, or 99% identical to any one of SEQ ID NOs: 102-111, or a polypeptide which differs from the amino acid sequence of any one of SEQ ID NOs: 102-111 by at least one amino acid but no more than 30, 20, or 10 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

In some embodiments, provided herein is a polypeptide (e.g., an anti-myostatin Fc engineered Adnectin) which comprises the amino acid sequence of any one of SEQ ID NO: 112-121, or a polypeptide comprising an amino acid sequence which is at least 90%, 95%, 98%, or 99% identical to any one of SEQ ID NOs: 102-111, or a polypeptide which differs from the amino acid sequence of any one of SEQ ID NOs: 102-111 by at least one amino acid but no more than 30, 20, or 10 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

In some embodiments, provided herein is a polypeptide (e.g., an anti-myostatin Fc engineered Adnectin) which comprises the amino acid sequence of any one of SEQ ID NO: 122-131, or a polypeptide comprising an amino acid sequence which is at least 90%, 95%, 98%, or 99% identical to any one of SEQ ID NOs: 102-111, or a polypeptide which differs from the

amino acid sequence of any one of SEQ ID NOs: 102-111 by at least one amino acid but no more than 30, 20, or 10 amino acids (e.g., by amino acid modifications, such as amino acid substitutions).

III. Nucleic Acid Molecules and Vectors

Provided herein are nucleic acid molecules which encode the anti-myostatin Fc engineered Adnectins described herein, as well as vectors (e.g., expression vectors) which comprise the nucleic acid molecules encoding the anti-myostatin Fc engineered Adnectins described herein. Nucleic acids encoding the anti-myostatin Fc engineered Adnectins may be synthesized chemically, enzymatically or recombinantly. Codon usage may be selected so as to improve expression in a cell. Such codon usage will depend on the cell type selected. Specialized codon usage patterns have been developed for *E. coli* and other bacteria, as well as mammalian cells, plant cells, yeast cells and insect cells. See for example: Mayfield et al., *Proc. Natl. Acad. Sci. USA*, 100(2):438-442 (Jan. 21, 2003); Sinclair et al., *Protein Expr. Purif.*, 26(1):96-105 (Oct. 2002); Connell, N.D., *Curr. Opin. Biotechnol.*, 12(5):446-449 (Oct. 2001); Makrides et al., *Microbiol. Rev.*, 60(3):512-538 (Sep. 1996); and Sharp et al., *Yeast*, 7(7):657-678 (Oct. 1991).

General techniques for nucleic acid manipulation are described for example in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Vols. 1-3, Cold Spring Harbor Laboratory Press (1989), or Ausubel, F. et al., *Current Protocols in Molecular Biology*, Green Publishing and Wiley-Interscience, New York (1987) and periodic updates, herein incorporated by reference. The DNA encoding the polypeptide is operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, viral, or insect genes. Such regulatory elements include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences that control the termination of transcription and translation. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants are additionally incorporated.

Accordingly, the anti-myostatin Fc engineered Adnectins described herein may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific

cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected preferably is one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. An exemplary N-terminal leader sequence for production of polypeptides in a mammalian system is: MRAWIFFLLCLAGRALA (SEQ ID NO: 74) or METDTLLLWVLLLWVPGSTG (SEQ ID NO: 75), which is removed by the host cell following expression. For prokaryotic host cells that do not recognize and process a native signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, a factor leader (including Saccharomyces and Kluyveromyces alpha-factor leaders), or acid phosphatase leader, the *C. albicans* glucoamylase leader, or the signal described in PCT Publication No. WO 90/13646. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available. The DNA for such precursor regions may be ligated in reading frame to DNA encoding the protein.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 micron plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for Bacilli.

A suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7 (Stinchcomb et al., *Nature*, 282:39 (1979)). The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC® No. 44076 or PEP4-1. Jones, *Genetics*, 85:12 (1977). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, Leu2-deficient yeast strains (ATCC® 20,622 or 38,626) are complemented by known plasmids bearing the Leu2 gene.

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the nucleic acid encoding the protein of the invention, e.g., a fibronectin-based scaffold protein. Promoters suitable for use with prokaryotic hosts include the phoA promoter, beta-lactamase and lactose promoter systems, alkaline phosphatase, a tryptophan (trp) promoter system, and hybrid promoters such as the tan promoter. However, other known bacterial promoters are suitable. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the protein of the invention.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP Patent

Publication No. 73,657 and PCT Publication Nos. WO 2011/124718 and WO 2012/059486. Yeast enhancers also are advantageously used with yeast promoters.

Transcription from vectors in mammalian host cells can be controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

Transcription of a DNA encoding protein of the invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the peptide-encoding sequence, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (e.g., yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of mRNA encoding the protein of the invention. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO 94/11026 and the expression vector disclosed therein.

The recombinant DNA can also include any type of protein tag sequence that may be useful for purifying the protein. Examples of protein tags include, but are not limited to, a histidine tag, a FLAG tag, a myc tag, an HA tag, or a GST tag. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts can be

found in Cloning Vectors: A Laboratory Manual, (Elsevier, New York (1985)), the relevant disclosure of which is hereby incorporated by reference.

The expression construct is introduced into the host cell using a method appropriate to the host cell, as will be apparent to one of skill in the art. A variety of methods for introducing nucleic acids into host cells are known in the art, including, but not limited to, electroporation; transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (where the vector is an infectious agent).

Suitable host cells include prokaryotes, yeast, mammalian cells, or bacterial cells. Suitable bacteria include gram negative or gram positive organisms, for example, *E. coli* or Bacillus spp. Yeast, preferably from the Saccharomyces species, such as *S. cerevisiae*, may also be used for production of polypeptides. Various mammalian or insect cell culture systems can also be employed to express recombinant proteins. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow et al. (Bio/Technology, 6:47 (1988)). Examples of suitable mammalian host cell lines include endothelial cells, COS-7 monkey kidney cells, CV-1, L cells, C127, 3T3, Chinese hamster ovary (CHO), human embryonic kidney cells, HeLa, 293, 293T, and BHK cell lines. Purified polypeptides are prepared by culturing suitable host/vector systems to express the recombinant proteins. For many applications, the small size of many of the polypeptides disclosed herein would make expression in *E. coli* as the preferred method for expression. The protein is then purified from culture media or cell extracts.

IV. Protein Production

Host cells are transformed with the herein-described expression or cloning vectors for protein production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The host cells used to produce the anti-myostatin Fc engineered Adnectins may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma)) are suitable for culturing the host cells. In addition, many of the media described in Ham et al., Meth. Enzymol., 58:44 (1979), Barites et al., Anal. Biochem.,

102:255 (1980), U.S. Pat. Nos. 4,767,704, 4,657,866, 4,927,762, 4,560,655, 5,122,469, 6,048,728, 5,672,502, or U.S. Pat. No. RE 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as Gentamycin drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Proteins disclosed herein can also be produced using cell-translation systems. For such purposes the nucleic acids encoding the polypeptide must be modified to allow *in vitro* transcription to produce mRNA and to allow cell-free translation of the mRNA in the particular cell-free system being utilized (eukaryotic such as a mammalian or yeast cell-free translation system or prokaryotic such as a bacterial cell-free translation system.

Proteins of the invention can also be produced by chemical synthesis (e.g., by the methods described in Solid Phase Peptide Synthesis, 2nd Edition, The Pierce Chemical Co., Rockford, Ill. (1984)). Modifications to the protein can also be produced by chemical synthesis.

The proteins can be purified by isolation/purification methods for proteins generally known in the field of protein chemistry. Non-limiting examples include extraction, recrystallization, salting out (e.g., with ammonium sulfate or sodium sulfate), centrifugation, dialysis, ultrafiltration, adsorption chromatography, ion exchange chromatography, hydrophobic chromatography, normal phase chromatography, reversed-phase chromatography, get filtration, gel permeation chromatography, affinity chromatography, electrophoresis, countercurrent distribution or any combinations of these. After purification, polypeptides may be exchanged into different buffers and/or concentrated by any of a variety of methods known to the art, including, but not limited to, filtration and dialysis.

The purified polypeptide is preferably at least 85% pure, or preferably at least 95% pure, and most preferably at least 98% pure. Regardless of the exact numerical value of the purity, the polypeptide is sufficiently pure for use as a pharmaceutical product.

Monomer, dimer and HMW species of anti-myostatin Fc engineered Adnectin molecule may be separated by size exclusion chromatography (SEC). SEC separates molecules based on the molecular size. Separation is achieved by the differential molecular exclusion or inclusion as the molecules migrate along the length of the column. Thus, resolution increases as a function of column length. Anti-myostatin Fc engineered Adnectins samples may be separated using a 2695 Alliance HPLC (Waters, Milford, Mass.) equipped with TSK Gel G3000SWxL (300 mm.x7.8 mm, 5 microns) and TSK Gel SWxL (40 mm x 6.0 mm, 7 microns) columns (Tosoh Bioscience, Montgomery, Pa.) in tandem. Neat samples with injection weight of 200 µg are separated using a mobile phase consisting of 40 mM NaH_2PO_4, 60 mM Na_2HPO_4, 0.1 M Na_2SO_4, pH 6.8, at a flow rate of 0.5 ml/min. Samples are monitored at an absorbance of 280 nm using Water's 2487 Dual Wavelength detector. Using this system, the HMW species has a retention time of 16.0 min ± 1.0 min. Each peak is integrated for area under the peak. The % HMW species calculated by dividing the HMW peak area by the total peak area.

V. Compositions and Stable Formulations

Provided herein are compositions (e.g., pharmaceutical compositions) comprising the anti-myostatin Fc engineered Adnectins described herein and a carrier (e.g., a pharmaceutically acceptable carrier). Suitable carriers for the composition are known in the art, for example, in U.S. Patent No. 8,853,154, the content of which are herein incorporated by reference in its entirety.

Also provided herein are stable formulations comprising the anti-myostatin Fc engineered Adnectins described herein, which can be administered to a subject (e.g., a human patient).

A. Exemplary Formulations

In some embodiments, the concentration of the anti-myostatin Fc engineered Adnectin in the formulation is between about 10 mg/mL and 200 mg/mL. In other embodiments, the concentration of the anti-myostatin Fc engineered Adnectin in the formulation is between about 10 mg/mL and 140 mg/mL. In other embodiments, the concentration of the anti-myostatin Fc engineered Adnectin in the formulation is least about 10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL, 30 mg/mL, 35 mg/mL, 40 mg/mL, 45 mg/mL, 50, mg/mL, 60 mg/mL, 65 mg/mL, 70 mg/mL, 75 mg/mL, 80 mg/mL, 85 mg/mL, 90 mg/mL, 95 mg/mL or higher. In certain

embodiments, the concentration of the anti-myostatin Fc engineered Adnectin in the formulation is at least about 110 mg/mL, 115 mg/mL, 120 mg/mL, 125 mg/mL, 130 mg/mL, 135 mg/mL, 140, mg/mL or 145, mg/m L. In certain embodiments, the concentration of the anti-myostatin Fc engineered Adnectin in the formulation is 10.7 mg/mL, 21.4 mg/mL, 50.0 mg/mL or 71.4 mg/mL.

The stabilizing sugar in the formulation is a disaccharide in a weight (w/w) ratio of at least 5:1 protein to sugar. In some embodiments, the protein:sugar weight ratio is between about 5:1 to 10:1. In some embodiments, the protein:sugar ratio is about 6:1, 7:1, 8:1, 9:1 or 10:1. In some embodiments, the protein:sugar ratio is about 6.75:1.

In some embodiments, the formulation comprises about 5% to about 30% of the disaccharide. In some embodiments, the formulation comprises about 10% to about 28% of the disaccharide. In some embodiments, the formulation comprises about 15% to about 25% of the disaccharide. In some embodiments, the formulation comprises about 20% to about 25% of the disaccharide. In some embodiments, the formulation comprises about 18%, 19%, 20%, 21%, 22%, 23%, 24% or about 25% of the disaccharide.

In some embodiments, the concentration of the sugar in the formulation is about 150 mM to about 800 mM. In some embodiments, the concentration of the sugar in the formulation is about 300 to about 700 mM. In other embodiments, the concentration of the sugar in the formulation is about 150 mM, about 200 mM, about 250 mM, about 300 mM, about 350 mM, about 400 mM, about 450 mM, about 500 mM, about 550 mM, about 575 mM, about 600, about 625 mM, about 650 mM, about 675 mM or about 700 mM.

In some embodiments, the disaccharide is trehalose. In some embodiments, the formulation comprises about 5 to about 30% trehalose. In some embodiments, the formulation comprises about 10% to about 28% trehalose. In some embodiments, the formulation comprises about 15% to about 25% trehalose. In some embodiments, the formulation comprises about 20% to about 25% trehalose. In some embodiments, the formulation comprises about 18%, 19%, 20%, 21%, 22%, 23%, 24% or about 25% trehalose. In one embodiment, the formulation comprises 22% trehalose. In another embodiment, the formulation comprises 23% trehalose.

In some embodiments, the disaccharide is trehalose dihydrate. In some embodiments, concentration of trehalose dihydrate in the formulation is about 150 mM to about 800 mM. In some embodiments, the concentration of the trehalose dihydrate in the formulation is about 300

to about 700 mM. In other embodiments, the concentration of the trehalose dihydrate in the formulation is about 150 mM, about 200 mM, about 250 mM, about 300 mM, about 350 mM, about 400 mM, about 450 mM, about 500 mM, about 550 mM, about 575 mM, about 600, about 625 mM, about 650 mM, about 675 mM or about 700 mM. In one embodiment, the concentration of trehalose dihydrate in the formulation is 600 nM.

The stabilizing sugar in the formulation is employed in an amount no greater than that which may result in a viscosity undesirable or unsuitable for administration via SC syringe. In some embodiments, the viscosity of the formulation is from about 5 to 20 cps. In some embodiments, the viscosity of the formulation is from about 7 to 12 cps. In some embodiments, the viscosity is about 7-10 cps. In some embodiments, the viscosity of the formulation is less than 8 cps.

The buffering agent in the formulation is present in an amount of at least 20 mM, and is preferably between about 20 mM and about 40 mM. In some embodiments, the buffering agent is histidine at a concentration of about 20 mM, about 25 mM, about 30 mM or about 35 mM. In one embodiment, the formulation comprises about 30 mM histidine.

The pH of the formulation is maintained at a range from about 6.5 to about 7.8. In certain embodiments, the pH is maintained at a range from about pH from 6.6 to 7.6. In certain embodiments, the pH of the formulation is about 6.8 to 7.4. In certain embodiments, the pH of the formulation is about 7.0 to 7.3. In some embodiments, the pH of the formulation is 6.9, 7.0, 7.1, 7.2 or 7.3. In some embodiments, the pH of the formulation is about 7.1.

The aqueous carrier used in the formulations herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a liquid formulation. Illustrative carriers include sterile water for injection (SWFI), bacteriostatic water for injection (BWFI), a pH buffered solution (e.g., phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

The formulations may further comprise a surfactant to further reduce the formation of visible particulates. Preferred surfactants include poloxamer and polysorbate at a concentration of between about 0.01% and 0.5%. In some embodiments, the concentration of the surfactant is between about 0.02% and about 0.1%. In one embodiment, the surfactant is poloxamer 188. In some embodiments, the surfactant is polysorbate 20 or polysorbate 80. In one embodiment, the surfactant is polysorbate 80.

The formulations may further comprise a chelator at a concentration between about 0.01 mM and about 0.5 mM, preferably, between about 0.05 mM and 0.2 mM. Preferred chelators include, but are not limited to DPTA, EDTA and EGTA. In one embodiment, the chelator in the formulation is DPTA at a concentration of about 0.05 mM.

A preservative may be optionally added to the formulations herein to reduce bacterial action. The addition of a preservative may, for example, facilitate the production of a multi-use (multiple-dose) formulation.

In some embodiments, the formulation provided herein comprises:

(i) about 10-140 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 5-25% trehalose dihydrate; and

(iii) about 20-30 mM histidine,

wherein the pH of the formulation is about 6.8 to 7.3.

In some embodiments, the formulation provided herein consists essentially of:

(i) about 10-140 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 5-25% trehalose dihydrate; and

(iii) about 20-30 mM histidine,

wherein the pH of the formulation is about 6.8 to 7.3.

In some embodiments, the formulation provided herein comprises:

(i) about 10-140 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 5-25% trehalose dihydrate;

(iii) about 20-30 mM histidine;

(iv) about 0.02-0.06 mM DTPA; and

(v) about 0.01-0.05% polysorbate 80

wherein the pH of the formulation is about 6.8 to 7.3.

In some embodiments, the formulation provided herein consists essentially of:

(i) about 10-140 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 5-25% trehalose dihydrate;

(iii) about 20-30 mM histidine;

(iv) about 0.02-0.06 mM DTPA; and

(v) about 0.01-0.05% polysorbate 80

wherein the pH of the formulation is about 6.8 to 7.3.

In some embodiments, the formulation comprises:

(i) about 10-75 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 600 mM trehalose dihydrate; and

(iii) 25-30 mM histidine,

wherein the pH of the formulation is about 7.0 to 7.3.

In some embodiments, the formulation consists essentially of:

(i) about 10-75 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 600 mM trehalose dihydrate; and

(iii) 25-30 mM histidine,

wherein the pH of the formulation is about 7.0 to 7.3.

In some embodiments, the formulation comprises:

(i) about 10-75 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 600 mM trehalose dihydrate;

(iii) 25-30 mM histidine;

(iv) about 0.02-0.06 mM DTPA; and

(v) about 0.01-0.05% polysorbate 80,

wherein the pH of the formulation is about 7.0 to 7.3.

In some embodiments, the formulation consists essentially of:

(i) about 10-75 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 600 mM trehalose dihydrate;

(iii) 25-30 mM histidine;

(iv) about 0.02-0.06 mM DTPA; and

(v) about 0.01-0.05% polysorbate 80,

wherein the pH of the formulation is about 7.0 to 7.3.

In some embodiments, the formulation comprises

(i) about 10-75 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 600 mM trehalose dihydrate;

(iii) about 30 mM histidine;

(iv) about 0.05 mM DTPA; and

(v) about 0.02% polysorbate 80,

wherein the pH of the formulation is about 7.1.

In some embodiment, the formulation consists essentially of:

(i) about 10-75 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 600 mM trehalose dihydrate;

(iii) about 30 mM histidine;

(iv) about 0.05 mM DTPA; and

(v) about 0.02% polysorbate 80,

wherein the pH of the formulation is about 7.1.

In one embodiment, the formulation comprises or consists essentially of:

(i) about 10.7 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 600 mM trehalose dihydrate;

(iii) about 30 mM histidine;

(iv) about 0.05 mM DTPA; and

(v) about 0.02% polysorbate 80,

wherein the pH of the formulation is about 7.1.

In one embodiment, the formulation comprises or consists essentially of:

(i) about 21.4 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 600 mM trehalose dihydrate;

(iii) about 30 mM histidine;

(iv) about 0.05 mM DTPA; and

(v) about 0.02% polysorbate 80,

wherein the pH of the formulation is about 7.1.

In one embodiment, the formulation comprises or consists essentially of:

(i) about 50 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 600 mM trehalose dihydrate;

(iii) about 30 mM histidine;

(iv) about 0.05 mM DTPA; and

(v) about 0.02% polysorbate 80,

wherein the pH of the formulation is about 7.1.

In one embodiment, the formulation comprises or consists essentially of:

(i) about 71.4 mg/mL of the anti-myostatin Fc engineered Adnectin;

(ii) about 600 mM trehalose dihydrate;

(iii) about 30 mM histidine;

(iv) about 0.05 mM DTPA; and

(v) about 0.02% polysorbate 80,

wherein the pH of the formulation is about 7.1.

The recommended storage condition for the liquid formulation is from 2-8°C, with a recommended shelf life of at least 12 months. In order to ensure efficacy and safety during the time course of the shelf life of pharmaceutical formulations, the composition is stability tested. Typically, the stability tests include but are not limited to tests regarding identity, purity and potency of the composition. The stability is tested both at the intended storage temperature and at elevated temperature or temperatures. Purity tests may include but are not limited to SDS-PAGE, CE-SDS, isoelectrofocusing, immunoelectrophoresis, Western blot, reversed-phase chromatography, size-exclusion chromatography (SEC), ion exchange and affinity chromatography. Other tests may include, but are not limited to: visual appearance such as color and transparency, particulates, pH, protein concentration measurement, moisture and reconstitution time.

The degradation profile regarding, in particular, purity and potency, during the stability time course is intimately coupled to the composition and/or the formulation of the pharmaceutical product. In particular, proper choice of formulation may significantly change the degradation profile. Typical degradation profiles for protein molecules products derived from FBS includes the formation of covalent and non-covalent high molecular weight aggregates, fragments, deamidation and oxidation products. Particularly, de-amidation and oxidation products as well as other acidic species usually develop during the time course of the stability testing. In some cases, the acidic species limits the acceptable shelf life of the pharmaceutical composition. The formation of acidic species due to, for example, deamidation, can be tested by, *e.g.*, imaged capillary isoelectrofocusing (icIEF). In other cases, the formation of high molecular weight aggregates limits the acceptable shelf life of the pharmaceutical composition. The formation of aggregates may be tested by for example SEC (size exclusion chromatography), DLS, MFI, SDS-PAGE or CE-SDS.

For example, an anti-myostatin Fc engineered Adnectin formulation with pharmaceutically acceptable stability can be one wherein, when stored at a temperature of about 5 ± 3°C or 25 ± 2°C for a period of least about 6 weeks, for example, about 3 months, preferably

about 6 months, and more preferably about 12 months or longer, such as 18 months or longer, such as for at least 24 or even 36 months, the percentage of aggregates is less than about 10%, preferably less than about 5%, more preferably less than about 2%, when determined using SEC analysis. Additionally or alternatively, a stable anti-myostatin Fc engineered Adnectin formulation of the invention can be one wherein, when stored at a temperature of about 5 ± 3°C or 25 ± 2°C for a period of at least about 6 weeks, for example, about 3 months, preferably about 6 months, and more preferably about 12 months or longer, the changes of main isoform are less than 15%, preferably less than 10%, more preferably less than 8%, most preferably less than 5%, when determined using icIEF analysis.

B. Preparation of the Formulation

The manufacturing process developed for SC formulations typically involves compounding with sugar, chelating agent and surfactant, followed by aseptic sterile filtration and filling into vials or syringes, optionally preceded by diafiltration (buffer exchange) and concentration of drug substance using an ultrafiltration unit. The protein purification is the first stage after production in the fermentation bioreactor. The protein is purified using multiple column and filtration steps and concentrated using tangential flow filtration into the formulation buffer. The concentrated drug substance is diluted with the formulation buffer at the target concentrations and this solution is sterile filtered and filled into sterile vials/syringes for patient use. One skilled in the art would be aware of the need to overfill the container so as to compensate for vial, needle, syringe hold-up during preparation and injection. For example, a 5-10% overage of drug product is incorporated into each vial of liquid formulation to account for withdrawal losses and guarantee that required dose (label claim) of drug product can be withdrawn from the vial.

Preparation of unit dosage forms for the formulation comprising the anti-myostatin Fc engineered Adnectin involves protein production in a recombinant cell line, purification vial multiple column steps, concentration and buffer exchange into formulation buffer using tangential flow filtration. The concentrated protein for the tangential flow filtration is further processed by dilution with formulation buffer to the target protein concentrations and the diluted product, after filtration, is filled into 1 mL syringes (*e.g.,* insulin syringe, tuberculin syringe,

BioPak syringe, NeoPak syringe). In one embodiment, the syringes are then equipped with an UltraSafe Passive needle guard.

The unit dosage form of the formulation typically contains about 0.3 to 1.5 mL of the formulation. In certain embodiments, the unit dosage form contains a volume of 0.3, 0.5, 0.7, 0.8, 1.0, 1.2, 1.4 or 1.4 mL. In certain embodiments, the unit dosage form is provided at a volume of 0.7 mL. In some embodiments, the unit dosage form contains 5-100 mg of the anti-myostatin Fc engineered Adnectin. In some embodiments, the unit dosage form comprises 7.5 mg, 15 mg, 35 mg or 50 mg of the anti-myostatin Fc engineered Adnectin.

In some embodiments, the formulations are manufactured as disclosed herein and are stored in bulk at -60°C, for example, in 12L FFTp bags at polypeptide concentration of 85-150 mg/mL. In some embodiments, the formulations are stored at -60°C at an anti-myostatin Fc engineered Adnectin concentration of 85 mg/mL. The bulk formulations are then thawed and diluted to the appropriate anti-myostatin Fc engineered Adnectin concentration for preparation of the unit dosage forms. In some embodiments, the anti-myostatin Fc engineered Adnectin concentration of the formulation in the unit dosage form is about 10 mg/mL to about 140 mg/mL. In some embodiments, the anti-myostatin Fc engineered Adnectin concentration of the formulation in the unit dosage form is about 10 mg/mL to about 75 mg/mL. In certain embodiments, the anti-myostatin Fc engineered Adnectin concentration of the formulation in the unit dosage form is 10.7 mg/mL, 20.4 mg/ml, 50 mg/mL or 71.4 mg/mL.

C. Administration

A pharmaceutical formulation comprising an anti-myostatin Fc engineered Adnectins described herein can be administered to a subject at risk for or exhibiting pathologies as described herein. The formulations provided herein are particularly useful for peripheral systemic delivery by intravenous, intraperitoneal or subcutaneous injection. In some embodiments, the formulations are delivered by subcutaneous injection.

A therapeutically effective dose refers to a dose that produces the therapeutic effects for which it is administered. An effective amount of a pharmaceutical composition to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which the binding agent

molecule is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient.

The exact dosage is determined based on factors related to the subject requiring treatment and can be ascertained using standard techniques. Dosage and administration are adjusted to provide sufficient levels of the active compound or to maintain the desired effect. Factors that can be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and sex of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy.

The polypeptides (anti-myostatin Fc engineered Adnectins) are administered in the target population at any suitable dose. In one embodiment, the polypeptides are administered as repeated subcutaneous injections. In one embodiment, the polypeptides are administered at a dosing interval of once a week. In one embodiment, the polypeptides are administered at a dosing interval of once every two weeks. In one embodiment, the polypeptides are administered at a dosing interval of once a month.

In one embodiment, the therapeutic dose level(s) and dosing interval(s) are determined by the pharmacokinetic:pharmacodynamic (PK/PD) correlation of the anti-myostatin Fc engineered Adnectin (e.g., anti-myostatin Fc engineered Adnectin) in the target population, as determined in a controlled clinical trial. Desired pharmacodynamic changes include, but are not limited to, reduction in free (unbound) myostatin, improvements in body composition and total body weight.

The frequency of dosing depends upon the pharmacokinetic:pharmacodynamic parameters of the binding agent molecule in the formulation used. Typically, a composition is administered until a dosage is reached that achieves the desired effect. The composition can therefore, be administered as a single dose or as multiple doses (at the same or different concentrations/dosages) over time. Further refinement of the appropriate dosage is routinely made. Appropriate dosages can be ascertained through use of appropriate dose-response data. For example, the anti-myostatin Fc engineered Adnectin can be administered less frequently (*e.g.*, bi-weekly, or monthly). In addition, as is known in the art, adjustments for age as well as the body weight, general health, sex, time of administration, drug interaction, and the severity of the disease may be necessary, and re ascertainable with routine experimentation by those skilled

in the art. The anti-myostatin Fc engineered Adnectin can be suitably administered to the patient at one time or over a series of treatments.

VI. Kits and Articles of Manufacture

The anti-myostatin Fc engineered Adnectin described herein can be provided in a kit, a packaged combination of reagents in predetermined amounts with instructions for use in the therapeutic or diagnostic methods of the invention.

In some embodiments, an article of manufacture containing materials useful for the treatment or prevention of the disorders or conditions described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The container may be formed from a variety of materials such as glass, plastic or metals.

The container holds liquid formulations provided herein. The label on, or associated with, the container may indicate directions for storage and/or use. For instance, for a subcutaneous formulation, the label may further indicate that the SC formulation is useful or intended for subcutaneous administration. The container holding the formulation may be a multi-use vial, which allows for repeat administrations (e.g. from 2-6 administrations) of, for example, the subcutaneous formulation. Alternatively, the container may be a pre-filled syringe containing, for example, the subcutaneous formulation in unit dosage form.

The article of manufacture may further comprise other materials desirable from a commercial and user standpoint, including diluents, filters, needles, syringes, and package inserts with instructions for use.

VII. Methods of Use

Provided herein are methods of treating myostatin-related disease or disorders, e.g., muscle wasting disorders, muscle atrophy, metabolic disorders, and bone degenerative disorders, using the anti-myostatin Fc engineered Adnectins described herein. Accordingly, in certain embodiments the invention provides methods for attenuating or inhibiting a myostatin-related disease or disorder in a subject comprising administering an effective amount of myostatin-binding polypeptide, i.e., an anti-myostatin Fc engineered Adnectin, to a subject. In some embodiments, the subject is a human. In some embodiments, the anti-myostatin Fc engineered

Adnectins are pharmaceutically acceptable to a mammal, in particular a human. A "pharmaceutically acceptable" polypeptide refers to a polypeptide that is administered to an animal without significant adverse medical consequences, such as essentially endotoxin free, or very low endotoxin levels.

The anti-myostatin Fc engineered Adnectins described herein can be used to treat muscular, neurological and metabolic disorders associated with muscle wasting and/or muscle atrophy. For example, myostatin overexpression *in vivo* induces signs and symptoms characteristic of cachexia, and myostatin binding agents can partially resolve the muscle wasting effect of myostatin (Zimmers et al., *Science* 2002;296:1486-8). Patients with AIDS also exhibit increased serum levels of myostatin immunoreactive material compared to patients without AIDS or to AIDS patients who do not exhibit weight loss (Gonzalez-Cadavid et al., *PNAS* 1998;95:14938-43). It has also been observed that heart-specific elimination of myostatin reduces skeletal muscle atrophy in mice with heart failure, and conversely, specifically overexpressing myostatin in the heart is sufficient to induce muscle wasting (Breitbart et al., *AJP-Heart*; 2011;300:H1973-82). In contrast, myostatin knockout mice show increased muscle mass, and an age-dependent decrease in fat accumulation compared to their wild type counterparts (McPherron et al., *J. Clin. Invest.* 2002;109:595-601).

Exemplary disorders that can be treated according to the methods of the invention include myopathies and neuropathies, including, for example, motor neuron disease, neuromuscular and neurological disorders.

For example, anti-myostatin Fc engineered Adnectins can be used to treat inherited myopathies and neuromuscular disorders (e.g., muscular dystrophy (Gonzalez-Kadavid et al., *PNAS*, 1998;95:14938-43), motor neuron disorders, congenital myopathies, inflammatory myopathies and metabolic myopathies), as well as acquired myopathies (e.g., drug induced myopathy, toxin induced myopathy, infection induced myopathy, paraneoplastic myopathy and other myopathies associated with critical illnesses).

Such disorders include, but are not limited to, Duchenne's muscular dystrophy (DMD), progressive muscular dystrophy, Becker's type muscular dystrophy (BMD), Dejerine-Landouzy muscular dystrophy, Erb's muscular dystrophy, Emery Dreifuss muscular dystrophy, limb girdle muscular dystrophy, oculopharyngeal muscular dystrophy (OPMD), facioscapulohumeral muscular dystrophy, congenital muscular dystrophy, infantile neuroaxonal muscular dystrophy,

myotonic dystrophy (Steinert's disease), distal muscular dystrophy, nemaline myopathy, familial periodic paralysis, nondystrophic myotonia, periodic paralyses, spinal muscular atrophy, spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), progressive muscular atrophy (PMA), distal myopathy, myotubular/centronuclear myopathy, nemaline myopathy, mini core disease, central core disease, desminopathy, inclusion body myositis, dermatomyositis, polymyositis, mitochondrial myopathy, congenital myasthenic syndrome, myasthenia gravis, post-polio muscle dysfunction, steroid myopathy, alcoholic myopathy, perioperative muscular atrophy and ICU neuromyopathy.

Inherited and acquired neuropathies and radiculopathies which can be treated with anti-myostatin Fc engineered Adnectins include, but are not limited to, rigid spine syndrome, muscle-eye-brain disease, heredity motor and sensory neuropathy, Carcot-Marie-Tooth disease, chronic inflammatory neuropathy, progressive hypertrophic neuropathy, tomaculous neuropathy, lupus, Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, multiple sclerosis, sarcoidosis, diabetic neuropathy, alcoholic neuropathy, disease related neuropathies (e.g., HIV/AIDS, Lyme disease), toxin related neuropathies (e.g., heavy metal, chemotherapy), compression neuropathies (e.g., tumors, entrapment neuropathy), and neuropathies associated with injury or trauma (e.g., cauda equine syndrome, paraplegia, quadriplegia).

In some embodiments, the anti-myostatin Fc engineered Adnectins described herein can be used to treat muscular dystrophies (e.g., Duchenne's muscular dystrophy, Becker's type muscular dystrophy), ALS, and sarcopenia.

Additional disorders associated with muscle wasting that can be treated with the anti-myostatin Fc engineered Adnectins described herein include achexia, wasting syndrome, sarcopenia, congestive obstructive pulmonary disease, fibrosis, cystic fibrosis (pulmonary cachexia), cardiac disease or failure (cardiac cachexia), chronic heart disease, cancer, wasting due to AIDS, AIDS, wasting due to renal failure, renal disease, renal failure, chronic kidney disease, claudication, cachexia associated with dialysis, uremia, rheumatoid arthritis, muscle injury, surgery, repair of damaged muscle, frailty, disuse atrophy, osteoporosis, osteoarthritis, ligament growth and repair.

The anti-myostatin Fc engineered Adnectins described herein can also be used to increase muscle volume in subjects who suffer from muscle atrophy due to disuse. Disuse atrophy may result from numerous causes including any disorder or state which leads to prolonged immobility

or disuse, including, but not limited to aging, bone fracture, prolonged bedrest, being wheelchair bound, limb immobilization, unloading of the diaphragm via mechanical ventilation, solid organ transplant, joint replacement, stroke, CNS damage related weakness, spinal cord injury, recovery from severe burn, sedentary chronic hemodialysis, post-trauma recovery, post-sepsis recovery and exposure to microgravity (Powers et al., *Am J Physiol Regul Integr Comp Physiol* 2005;288:R337-44). In some embodiments, administration of the anti-myostatin Fc engineered Adnectins to the subject results in at least one (1, 2, 3, or all 4) of the following biological effects: (a) an increase in muscle mass; (b) an increase in the number of muscle cells; (c) an increase in the size of muscle cells; and (d) an increase in muscle strength.

In addition, age-related increases in fat to muscle ratios, and age-related muscular atrophy appear to be related to myostatin. For example, the average serum myostatin-immunoreactive protein increased with age in groups of young (19-35 yr old), middle-aged (36-75 yr old), and elderly (76-92 yr old) men and women, while the average muscle mass and fat-free mass declined with age in these groups (Yarasheski et al. J Nutr Aging 6(5):343-8 (2002)). Accordingly, Subjects with muscle atrophy due to aging, and/or subjects who are frail due to, for example, sarcopenia, would also benefit from treatment with the anti-myostatin Fc engineered Adnectins of the invention.

Also contemplated are methods for increasing muscle mass in food animals by administering an effective dosage of the anti-myostatin Fc engineered Adnectins described herein to these animals. Since the mature C-terminal myostatin polypeptide is identical in all species, anti-myostatin Fc engineered Adnectins would be expected to effectively increase muscle mass and reducing fat in any agriculturally important species, for example, but not limited to, cattle, chicken, turkeys, and pigs.

The efficacy of the anti-myostatin Fc engineered Adnectin in the treatment of muscle wasting disorders or muscle atrophy can be determined, for example, by one or more methods for measuring an increase in muscle mass or volume, an increase in the number of muscle cells (hyperplasia), an increase in muscle cell size (hypertrophy) and/or an increase in muscle strength. For example, the muscle volume increasing effects of the anti-myostatin Fc engineered Adnectins of the present invention are demonstrated in the Examples described *infra*. Methods for determining "increased muscle mass" are well known in the art. For example, muscle content can be measured before and after administration of an anti-myostatin Fc engineered Adnectin of

the invention using standard techniques, such as underwater weighing (see, e.g., Bhasin et al. New Eng. J. Med. (1996) 335:1-7) and dual-energy x-ray absorptiometry (see, e.g., Bhasin et al. Mol. Endocrinol. (1998) 83:3155-3162). An increase in muscle size may be evidenced by weight gain of at least about 5-10%, preferably at least about 10-20% or more.

In another aspect, provided herein is a method for enhancing lean muscle mass in a subject, the method comprising administering an effective amount of the anti-myostatin Fc engineered Adnectins described herein to enhance lean muscle mass.

In another aspect, provided herein is a method for increasing the ratio of lean muscle mass to fat in a subject, the method comprising administering an effective amount of the anti-myostatin Fc engineered Adnectins described herein to increase the ratio of lean muscle mass to fat.

The anti-myostatin Fc engineered Adnectins described herein are also useful for treating metabolic disorders, such as obesity, obesity-related complications, type II diabetes mellitus, diabetes associated disorders, metabolic syndrome, and hyperglycemia. Accordingly, in one aspect, provided herein is a method of treating or preventing a metabolic disorder in a subject, comprising administering to the subject an effective amount of an anti-myostatin Fc engineered Adnectin described herein to treat or prevent the metabolic disorder. In some embodiments, the subject has a disease or disorder selected from the group consisting of diabetes (e.g., type II diabetes), hyperglycemia, hyperinsulinemia, hyperlipidemia, insulin resistance, impaired glucose metabolism, obesity, metabolic syndrome, glucose intolerance, prediabetes, hypertension, dyslipidemia, increased waist circumference, cardiovascular disease, non-alcoholic fatty liver disease, obstructive sleep apnea, physical impairment, osteoarthritis, osteoporosis, renal disease, sexual hormone(s) impairment, endocrine reproductive disorders such as polycystic ovary syndrome or male hypogonadism, stroke, and gallstones.

In another aspect, provided herein are methods for improving glycemic control in a human patient, methods for treating, preventing, or reducing obesity and related comorbidities, and methods of treating or preventing type II diabetes, by administering to the patient an anti-myostatin Fc engineered Adnectin described herein. In some embodiments, the anti-myostatin Fc engineered Adnectin is administered a subcutaneous injection.

In another aspect, provided herein are methods of treating, preventing, or reducing overweight or obesity and related comorbidities in a subject, the method comprising

administering to the subject an effective amount of an anti-myostatin Fc engineered Adnectin described herein to treat, prevent, or reduce overweight or obesity and related comorbidities.

Obesity is a condition in which excess body fat has accumulated to such an extent that health may be negatively affected. It is commonly defined as a body mass index (BMI) of 30 kg/m^2 or higher which distinguishes it from being overweight as defined by a BMI of 25 kg/m^2 or higher (see, e.g., World Health Organization (2000) (PDF). Technical report series 894: Obesity: Preventing and managing the global epidemic. Geneva: World Health Organization). Excessive body weight is associated with various diseases, particularly cardiovascular diseases, diabetes mellitus type II, obstructive sleep apnea, certain types of cancer, and osteoarthritis.

A subject with obesity may be identified, for example, by determining BMI (BMI is calculated by dividing the subject's mass by the square of his or her height), waist circumference and waist–hip ratio (the absolute waist circumference (>102 cm in men and >88 cm in women) and the waist–hip ratio (the circumference of the waist divided by that of the hips of >0.9 for men and >0.85 for women) (see, e.g., Yusuf S, et al., (2004). Lancet 364: 937–52), and/or body fat percentage (total body fat expressed as a percentage of total body weight: men with more than 25% body fat and women with more than 33% body fat are obese; body fat percentage can be estimated from a person's BMI by the following formula: Bodyfat% = (1.2 * BMI) + (0.23 * age) − 5.4 − (10.8 * gender), where gender is 0 if female and 1 if male). Body fat percentage measurement techniques include, for example, computed tomography (CT scan), magnetic resonance imaging (MRI), and dual energy X-ray absorptiometry (DEXA).

In another aspect, provided herein are methods of treating or preventing type II diabetes in a subject, the method comprising administering to the subject an effective amount of an anti-myostatin Fc engineered Adnectin described herein to treat or prevent type II diabetes.

Myostatin is involved in the pathogenesis of type II diabetes mellitus. Myostatin is expressed in adipose tissue and myostatin deficient mice exhibit reduced fat accumulation as they age. Moreover, glucose load, fat accumulation, and total body weight are reduced in myostatin lacking agouti lethal yellow and obese (Lep$^{ob/ob}$) mice (Yen et al., *FASEB J.* 8:479, 1994; McPherron et al., 2002). As disclosed in US2011/0008375, myostatin antagonists can decrease the fat to muscle ratio in an aged mouse model, preserve skeletal muscle mass and lean body mass, and attenuate kidney hypertrophy in STZ-induced diabetic mice.

The term “type II diabetes” refers to a chronic, life-long disease that results when the body’s insulin does not work effectively. A main component of type II diabetes is "insulin resistance," wherein the insulin produced by the pancreas cannot connect with fat and muscle cells to allow glucose inside to produce energy, causing hyperglycemia (high blood glucose). To compensate, the pancreas produces more insulin, and cells, sensing this flood of insulin, become even more resistant, resulting in a vicious cycle of high glucose levels and often high insulin levels.

The phrase “disorders associated with diabetes” or “diabetes associated disorders” or “diabetes related disorders,” as used herein, refers to conditions and other diseases which are commonly associated with or related to diabetes. Example of disorders associated with diabetes include, for example, hyperglycemia, hyperinsulinaemia, hyperlipidaemia, insulin resistance, impaired glucose metabolism, obesity, diabetic retinopathy, macular degeneration, cataracts, diabetic nephropathy, glomerulosclerosis, diabetic neuropathy, erectile dysfunction, premenstrual syndrome, vascular restenosis, ulcerative colitis, coronary heart disease, hypertension, angina pectoris, myocardial infarction, stroke, skin and connective tissue disorders, foot ulcerations, metabolic acidosis, arthritis, and osteoporosis. Accordingly, in another aspect, provided herein is a method of treating a diabetes associated disorder in a subject, the method comprising administering to the subject an effective amount of an anti-myostatin Fc engineered Adnectin described herein to treat or prevent the diabetes associated disorder.

The efficacy of the anti-myostatin Fc engineered Adnectins in the treatment of metabolic disorders can be determined, for example, by one or more methods of measuring an increase in insulin sensitivity, an increase in glucose uptake by cells from the subject, a decrease in blood glucose levels, and a decrease in body fat.

For example, in subjects having type II diabetes or who are at risk of developing diabetes, HbA1c levels can be monitored. The term “hemoglobin 1AC” or “HbA1c” as used herein refers to the product of a non-enzymatic glycation of the hemoglobin B chain. The desired target range of HbA1c levels for people with diabetes can be determined from American Diabetes Association (ADA) guidelines, i.e., the Standards of Medical Care in Diabetes (*Diabetes Care* 2012;35(Suppl 1):S511-563). Current HbA1c target levels are generally <7.0% for people with diabetes, and people who do not have diabetes typically have HbA1c values of less than 6%.

Accordingly, the efficacy of the anti-myostatin Fc engineered Adnectins of the present invention can be determined by an observed decrease in the HBA1c level in a subject.

Also provided herein are methods which include administration of an anti-myostatin Fc engineered Adnectin alone, or in combination with other agents that are known in the art for glycemic control (e.g., insulin, GLP1) or for treating art-recognized diabetes-related complications.

In one embodiment, the patient is overweight. In another embodiment, the patient is obese. In another embodiment, the patient has an obesity related comorbidity. Exemplary obesity related comorbidities include, but are not limited to, type 2 diabetes, glucose intolerance, prediabetes, insulin resistance, high triglycerides, physical impairment, osteoporosis, renal disease, obstructive sleep apnea, sexual hormones impairment, endocrine reproductive disorders such as polycystic ovary syndrome or male hypogonadism, osteoarthritis, gastrointestinal cancers, dyslipidaemia, hypertension, heart failure, coronary heart disease, stroke, gallstones, and altered gonadal hormone profile.

In some embodiments, administration of the anti-myostatin Fc engineered Adnectin to the subject results in at least one (1, 2, 3, or all 4) of the following biological effects: (a) an increase in insulin sensitivity; (b) an increase in glucose uptake by cells in the subject; (c) a decrease in blood glucose levels; and (d) a decrease in body fat.

The peptide or formulation comprising the peptide can be administered to a subject by any suitable means. In one embodiment, the peptide is formulated for intravenous administration. In one embodiment, the peptide is formulated for subcutaneous administration.

Exemplary Embodiments

1. A composition of matter comprising:

(a) a first peptide unit having the following sequence:

MRAWIFFLLCLAGRALA (SEQ ID NO: 74);

(b) a second peptide unit having the following sequence:

DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSVMHE ALHNHYTQKS LSLSP (SEQ ID NO: 78);

(c) a third peptide unit having the following sequence:

ELQLE ESAAEAQEGELE (SEQ ID NO: 41); and

(d) a fourth peptide unit having the following sequence:

GVSDVPRD LEVVAATPTS LLISWSLPHQ GKANYYRITY
GETGGNSPVQ EFTVPGRGVT ATISGLKPGV DYTITVYAVT
VTDTGYLKYK PISINYRTEI (SEQ ID NO: 10);

wherein the second peptide unit has at least one amino acid substitution.

2. The composition of matter of embodiment 1, wherein the second peptide unit has at least two amino acid substitutions.

3. The composition of matter of embodiment 2, wherein the at least two amino acid substitutions are as follows:

DKTHTCPPCP APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSVMHE ALHNHYTQKS LSLSP (SEQ ID NO: 92)

4. The composition of matter of embodiment 2, wherein the at least two amino acid substitutions are as follows:

DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSV**L**HE ALH**S**HYTQKS LSLSP (SEQ ID NO: 96)

5. The composition of matter of embodiment 1, wherein the second peptide unit has at least three amino acid substitutions.

6. The composition of matter of embodiment 5, wherein the at least three substitutions are as follows:

DKTHTCPPCP APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKAL**A**A PIEKTISKAK
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSVMHE ALHNHYTQKS LSLSP (SEQ ID NO: 93)

7. The composition of matter of embodiment 4, wherein the at least three substitutions are as follows:

DKTHTCPPCP APELLGGPSV FLFPPKPKDT L**Y**I**T**R**E**PEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSVMHE ALHNHYTQKS LSLSP (SEQ ID NO: 97)

8. The composition of matter of embodiment 1, wherein the second peptide unit has at least four amino acid substitutions.

9. The composition of matter of embodiment 1, wherein the second peptide unit has at least five amino acid substitutions.

10. The composition of matter of embodiment 4, wherein the at least five substitutions are as follows:

DKTHTCPPCP APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKAL**A**A PIEKTISKAK
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSV**L**HE ALH**S**HYTQKS LSLSP (SEQ ID NO: 94)

11. The composition of matter of embodiment 1, wherein the second peptide unit has at least six amino acid substitutions.

12. The composition of matter of embodiment 4, wherein the at least six substitutions are as follows:

DKTHTCPPCP APE**AA**GGPSV FLFPPKPKDT L**Y**I**T**R**E**PEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKAL**A**A PIEKTISKAK
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSVMHE ALHNHYTQKS LSLSP (SEQ ID NO: 95)

13. The composition of matter of embodiment 1 having the following sequence:

MRAWIFFLLCLAGRALA
DKTHTCPPCP APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKAL**A**A PIEKTISKAK
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSVMHE ALHNHYTQKS LSLSPELQLE ESAAEAQEGE
LEGVSDVPRD LEVVAATPTS LLISWSLPHQ GKANYYRITY
GETGGNSPVQ EFTVPGRGVT ATISGLKPGV DYTITVYAVT
VTDTGYLKYK PISINYRTEI (SEQ ID NO: 123)

14. The composition of matter of embodiment 1 having the following sequence:

MRAWIFFLLCLAGRALA
DKTHTCPPCP APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKAL**A**A PIEKTISKAK
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSV**L**HE ALH**S**HYTQKS LSLSPELQLE ESAAEAQEGE
LEGVSDVPRD LEVVAATPTS LLISWSLPHQ GKANYYRITY
GETGGNSPVQ EFTVPGRGVT ATISGLKPGV DYTITVYAVT
VTDTGYLKYK PISINYRTEI (SEQ ID NO: 124)

15. The composition of matter of embodiment 1 having the following sequence:

MRAWIFFLLCLAGRALA
DKTHTCPPCP APE**AA**GGPSV FLFPPKPKDT L**Y**I**T**R**E**PEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKAL**A**A PIEKTISKAK
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG

NVFSCSVMHE ALHNHYTQKS LSLSPELQLE ESAAEAQEGE
LEGVSDVPRD LEVVAATPTS LLISWSLPHQ GKANYYRITY
GETGGNSPVQ EFTVPGRGVT ATISGLKPGV DYTITVYAVT
VTDTGYLKYK PISINYRTEI (SEQ ID NO: 125)

16. The composition of matter of embodiment 1 having the following sequence:

MRAWIFFLLCLAGRALA
DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSV**L**HE ALH**S**HYTQKS LSLSPELQLE ESAAEAQEGE
LEGVSDVPRD LEVVAATPTS LLISWSLPHQ GKANYYRITY
GETGGNSPVQ EFTVPGRGVT ATISGLKPGV DYTITVYAVT
VTDTGYLKYK PISINYRTEI (SEQ ID NO: 126)

17. The composition of matter of embodiment 1 having the following sequence:

MRAWIFFLLCLAGRALA
DKTHTCPPCP APELLGGPSV FLFPPKPKDT L**Y**I**T**R**E**PEVT
CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSVMHE ALHNHYTQKS LSLSPELQLE ESAAEAQEGE
LEGVSDVPRD LEVVAATPTS LLISWSLPHQ GKANYYRITY
GETGGNSPVQ EFTVPGRGVT ATISGLKPGV DYTITVYAVT
VTDTGYLKYK PISINYRTEI (SEQ ID NO: 127)

18. A method of improving glycemic control in a human patient, comprising administering to the patient the composition of matter of any one of embodiments 1 to 17.

19. A method of treating, preventing, or reducing overweight or obesity and related comorbidities in a human patient, comprising administering to the patient the composition of matter of any one of embodiments 1 to 17.

20. A method of treating or preventing type II diabetes in a human patient, comprising administering to the patient the composition of matter of any one of embodiments 1 to 17.

21. A method of treating or preventing a neurodegenerative disease in a human patient, comprising administering to the patient the composition of matter of any one of embodiments 1 to 17.

22. The method of embodiment 21, wherein the neurodegenerative disease is spinal muscular atrophy.

The following examples are merely illustrative and should not be construed as limiting the scope of this disclosure in any way as many variations and equivalents will become apparent to those skilled in the art upon reading the present disclosure. The contents of all references, Genbank entries, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

EXAMPLES

Example 1. Generation of Adnectins with modified Fc regions

This Example describes the generation of variants of BHV-2000 with modified Fc regions ("Fc engineered Adnectins"). Briefly, the Adnectin 3116 A06 was fused to an Fc region via a linker, with the Fc region (either IgG1 or IgG4) modified to have the combination of mutations shown below (numbering of positions based on EU Index). In the sequences below, the osteonectin signal peptide is underlined, the mutations in the Fc region is bolded/italicized/underlined, the linker is italicized, and the Adnectin 3116 A06 sequence is bolded/underlined.

(a) L234A/L235A/P329A (LALA/PA) – IgG1

MRAWIFFLLCLAGRALADKTHTCPPCPAPE***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***A***AP
IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQEG*
*ELE***GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVT
ATISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI (SEQ ID NO: 123)

(b) **Compound 3189**: L234A/L235A/P329A/M248L/N434S (LALA/PA/LS) – IgG1

MRAWIFFLLCLAGRALADKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL**A**AP
IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV**L**HEALH**S**HYTQKSLSLSP*ELQLEESAAEAQEG*
*ELE***GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTATI**
SGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI (SEQ ID NO: 124)

(c) **Compound 3190**: L234A/L235A/P329A/M252Y/S254T/T256E (LALA/PA/YTE) – IgG1

MRAWIFFLLCLAGRALADKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL**A**AP
IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQEG*
*ELE***GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTATI**
SGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI (SEQ ID NO: 125)

(d) **Compound 3191**: M248L/N434S (LS) – IgG1

MRAWIFFLLCLAGRALADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV**L**HEALH**S**HYTQKSLSLSP*ELQLEESAAEAQEG*
*ELE***GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTATI**
SGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI (SEQ ID NO: 126)

(e) **Compound 3192**: M252Y/S254T/T256E (YTE) – IgG1

MRAWIFFLLCLAGRALADKTHTCPPCPAPELLGGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQEG*

*ELE***GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTATI**
SGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI (SEQ ID NO: 127)

(f) **Compound 3193**: M252Y/S254T/T256E/N297Q (N297Q/YTE) – IgG1

MRAWIFFLLCLAGRALADKTHTCPPCPAPELLGGPSVFLFPPKPKDTL***Y***I***T***R***E***PEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQY***Q***STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQEG*
*ELE***GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTATI**
SGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI (SEQ ID NO: 128)

(g) **Compound 3194**: N297Q/M248L/N434S (N297Q/LS) – IgG1

MRAWIFFLLCLAGRALADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQY***Q***STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV***L***HEALH***S***HYTQKSLSLSP*ELQLEESAAEAQEG*
*ELE***GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTATI**
SGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI (SEQ ID NO: 129)

(h) **Compound 3195**: F234A/L235A/M252Y/S254T/T256E (LALA/YTE) – IgG4

MRAWIFFLLCLAGRALAESKYGPPCPPCPAPE***AA***GGPSVFLFPPKPKDTL***Y***I***T***R***E***PEVTCVVVD
VSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP
SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQ*
*EGELE***GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA**
TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI (SEQ ID NO: 130)

(i) **Compound 3196**: M252Y/S254T/T256E/N297Q (N297Q/YTE) – IgG4

MRAWIFFLLCLAGRALAESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL***Y***I***T***R***E***PEVTCVVVD
VSQEDPEVQFNWYVDGVEVHNAKTKPREEQF***Q***STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP
SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT

TPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQ*
*EGELE*GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA
TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI (SEQ ID NO: 131)

Example 2. Biophysical properties of Fc engineered Adnectins

Biophysical properties of the Fc engineered Adnectins were assessed, as described below.

I. Biophysical properties

Briefly, the Fc engineered Adnectins described in Example 1 were produced, purified, and subjected to biophysical characterization. Adnectins were expressed by transiently transfecting Expi293 cells, incubating at 37°C for 4 days, centrifuging cells, and filtering supernatant. Clarified cell culture supernatant was purified using HiTrap MabSelect PrismA columns, washed, and eluted. The eluted fraction was dialyzed overnight into 25 mM sodium phosphate pH 7. Trehalose was added to 150 mM before filtration, and finally, poloxamer 188 was added to 0.1% w/v.

Protein concentrations were determined by measuring A280 using NanoDrop One. Main peak % was determined by SEC-HPLC, main band % by non-reduced and reduced SDS-PAGE, endotoxin levels using the EndoSafe Nexgen PTS endotoxin testing system, and apparent solution molecular weight by SEC-MALS.

The results of these analyses are summarized in **Table 2**. The pI of constructs was between 5 and 5.5.

Table 2. Biophysical properties of Adnectins

Sample (Compound)	Theoretical MW	SEC-MALS MW (kDA)	C_A280 (ng/ml)	Purity_SEC (%)	Endotoxin (EU/mg)	Volume (ml)	Total mass (mg)
3189	75.28	74.57	13.32	96.2%	<0.1 EU/ml or <0.008 EU/mg	26.3	350

3190	75.51	74.74	24	96.0%	<0.1 EU/ml or <0.004 EU/mg	14.5	348
3191	75.50	76.40	14.89	96.2%	<0.1 EU/ml or <0.007 EU/mg	27.5	409
3192	75.74	74.72	15.03	95.3%	<0.1 EU/ml or <0.007 EU/mg	18.5	278
3193	75.76	n.d.	5.08	99%	<0.152 EU/ml or <0.03 EU/mg	1.9	9.6
3194	75.5	n.d.	3.44	86%	<0.1 EU/ml or <0.029 EU/mg	10	34
3195	75.92	76.39	10.39	96%	<0.1 EU/ml or <0.01 EU/mg	19	200
3196	76.18	n.d.	0.48	62%	<0.1 EU/ml or <0.1 EU/mg	16	8
BHV-2000	75.59	74.58	n.d.	98.6%	n.d.	-	-

n.d.: not determined

II. Freeze/thaw stability

Freeze/thaw stability of Compounds 3189, 3190, 3191, 3192, and 3195 was tested. Briefly, a vial of each construct was thawed on ice. For each sample, 6 aliquots were prepared, and each aliquot was frozen at -70°C for 30 minutes and thawed at room temperature for up to 5 additional times. Samples were then spun down and evaluated by SEC-HPLC for % of high and low molecular weight species. A BSA standard curve was used to evaluate protein concentration for % mass recovery.

There was no increase in high or low molecular weight species after 5 freeze/thaw cycles for any of the Adnectins tested (**FIG. 1**). Protein concentration, as measured at A280 or with SEC-HPLC, did not change for Compounds 3189 and 3192, but decreased by 10-20% for Compounds 3190, 3191, and 3195 (**Table 3** and **FIGs. 2A-2B**).

Table 3. Freeze/thaw stability

Concentration measured by A280				**Concentration measured by SEC**		
Sample (Compound)	**Slope**	**% change (F/T-5 to F/T-0)***		**Sample (Compound)**	**Slope**	**% change (F/T-5 to F/T-0)***
3189	0.23	9.93		3189	0.18	9.14
3192	-0.27	-9.43		3192	0.02	8.59
3190	-0.46	-11.34		3191	-0.55	-14.27
3195	-0.57	-21.69		3195	-0.57	-15.19
3191	-0.62	-15.28		3190	-0.7	-15.46

*% change comparing concentration at fifth freeze/thaw (F/T-5) versus no freeze thaw (F/T-0)

An evaluation of protein concentration during storage found no change in protein concentration for Compounds 3189, 3190, 3191, 3192, and 3195 when these Adnectins were stored at 4°C for 6 weeks (**FIG. 3**).

III. Self-interaction

Self-interaction of the Fc engineered Adnectins was assessed by BLI. Briefly, Adnectin molecules were immobilized on anti-Fc probe at 5 ug/mL concentration, and Association at 1

μM concentration for 300 s was monitored by binding response. As shown in **Table 4**, the Fc-engineered Adnectins and BHV-2000 did not self-interact (shift <0.1 nm).

Table 4. Self-interaction analysis

Sample (Compound)	Self-interaction association height (nm)
1420*	0.0522
1421*	0.0098
3189	-0.006
3190	-0.026
3195	-0.032
BHV-2000	-0.01

*1420 and 1421 are assay controls

IV. High molecular weight species assessed by dynamic light scattering

Dynamic light scattering (DLS) was used to test for high molecular weight species in Fc engineered Adnectin samples.

Briefly, all Adnectin samples were diluted to the same concentration (10.39 mg/ml) prior to the experiment in formulation buffer (25 mM sodium phosphate, 150 mM Trehalose, 0.1% Poloxamer 188, pH7). PRO1422 and PRO1425 (technical assay controls) were diluted to 1 mg/ml. Samples were run in triplicate.

As shown in **Table 5**, Fc engineered Adnectins (Compounds 1389, 3190, and 3195) showed higher Polydispersity indices (PDIs) than BHV-2000, yet overall below the 0.4 cut-off of high polydispersity. Altogether, Fc engineered Adnectins and BHV-2000 present PDIs within the range of 0.1 to 0.4. Peak 1 contains more than 99% of the total mass, indicating the presence of dimers or similarly sized impurities and a low amount of HMW species or aggregates. Peak 1 diameter is similar for all samples, indicating a low number of dimers (or similarly sized impurities).

Table 5. DLS summary

Sample (Compound)	PDI	Z-Ave. Dia. (nm)	Pk 1 Polydispersity (%)	Pk 1 mass (%)	Pk 1 diameter (mm)
3189	0.32	29.23	30.50	99.63	9.88
3190	0.32	26.40	38.72	99.60	10.74
3195	0.27	11.59	31.96	99.68	9.18
BHV-2000	0.13	9.96	25.02	100.00	10.69
1422*	0.09	10.06	27.20	100.00	10.43
1425*	0.07	10.21	16.31	99.89	9.62

*1422 and 1425 are assay controls

V. Melting temperature

Melting temperatures of Fc engineered Adnectins 3189, 3190, 3195, and BHV-2000 were determined based on the ratio of 350/330 nm intrinsic fluorescence. IgG1 constructs (Compound 3189, Compound 3190, and BHV-2000) showed 3 melting temperatures, and the IgG4 construct (Compound 3195) showed a single melting temperature. Tm1 was similar for all IgG1 constructs. Tm2 for Compound 3190 had the lowest Tm2, and Compound 3189 had the lowest Tm3. The results are summarized in **Table 6**.

Table 6. Melting temperature

Sample (Compound)	Description	Average Tm1	Average Tm2	Average Tm3
3189	IgG1 Fc (LALA/PA/LS)	54.3°C	69.9°C	79.5°C
3190	IgG1 Fc (LALA/PA/YTE)	53.6°C	63.6°C	84°C
3195	IgG4 Fc (LALA/YTE)	60.8°C	N/A	N/A
BHV-2000	IgG1 Fc	52.7°C	71.7°C	85.3°C

VI. Aggregation

Static light scattering (SLS) was used to assess aggregation of the Fc engineered Adnectins. Curves for aggregation were read at 266 nm and 473 nm and are summarized in **Table 7**. BHV-2000 and Compound 3189 have similar aggregation levels, and Compound 3190 has slightly lower aggregation. The IgG4 construct (Compound 3195) had significantly lower aggregation compared to IgG1 constructs (Compound 3189, Compound 3190, and BHV-2000).

Table 7. Aggregation as assessed by SLS

Sample (Compound)	Tagg (266 nm)	Tagg (473 nm)
3189	71.3	71.6
3190	65.1	65.5
3195	57.5	57.5
BHV-2000	72.6	73.1

Example 3. Binding properties of Fc engineered Adnectins

This Example describes the binding of Fc engineered Adnectins to myostatin, FcRn, FcγRI, and C1q by biolayer interferometry (BLI) and surface plasmon resonance (SPR).

I. Myostatin binding

For myostatin binding, Fc engineered Adnectins and BHV-2000 were loaded onto anti-human Fc probes at 5 ug/mL concentration. Active myostatin was prepared at 400 nM, 200 nM, and 100 nM concentrations, and probes were incubated in solutions for 360 s Association, followed by 300 s Dissociation. Association and dissociation rate constants, k_a and k_d, respectively, were extracted to derive K_D values. As shown in **Table 8**, Fc engineered Adnectins bound to active myostatin with comparable affinity (5-12 nM) and to a similar extent as BHV-2000.

Table 8. Affinity of Fc engineered Adnectins for active myostatin

Sample (Compound)	K_D (nM)
BHV-2000	9.4
3189	4.9
3190	9,3
3191	5.7
3192	11.3
3195	5.9

II. FcRn binding

For FcRn binding, his-tagged FcRn was loaded onto anti-his probes and dipped into 1000 nM – 1.37 nM Adnectins (BHV-2000 and Compounds 3189, 3190, 3191, 3192, and 3195) at two pHs (pH 6.0 and pH 7.4) and subjected to biolayer interferometry. While BHV-2000 bound to FcRn at pH 6.0, it did not bind at pH 7.4. Fc engineered Adnectins bound to FcRn at pH 6.0, and showed lower response consistent with weak binding at pH 7.4. Binding affinity of Fc engineered Adnectins to FcRn at pH 6.0 are shown in **Table 9**. As shown in the table, Fc engineered Adnectins improved FcRn binding compared to BHV-2000 by about 50-fold.

Table 9. Affinity of Fc engineered Adnectins for FcRn at pH 6.0

Sample (Compound)	kd (s^{-1})	ka ($M^{-1}s^{-1}$)	K_D (nM)	Binding response (normalized for loading)
BHV-2000	1.13E-004	1.66E+005	1.21E-007	0.3
3189	7.54E-004	3.40E+005	2.22E-009	0.6
3190	7.39E-004	3.40E+005	2.18E-009	0.6
3191	4.43E-004	3.77E+005	1.17E-009	0.6
3192	7.75E-004	3.46E+005	2.24E-009	0.6
3195	7.47E-004	3.10E+005	2.41E-009	0.6

III. FcγRI binding

For FcγRI binding, his-tagged FcγRI was loaded onto anti-his probes and dipped into 1000 nM – 1.37 nM Adnectins (BHV-2000 and Compounds 3189, 3190, 3191, 3192, and 3195)

and subjected to biolayer interferometry. A summary of binding results is provided in **Table 10**. As shown in the table, Fc engineered Adnectins with LALA and PA mutations (Compounds 3189 and 3190) showed decreased FcγRI binding relative to BHV-2000.

Table 10. Affinity of Fc engineered Adnectins for FcγRI

Sample (Compound)	kd (s^{-1})	ka ($M^{-1}s^{-1}$)	KD (nM)	Binding response (normalized for loading)
BHV-2000	3.32E-004	7.16E+005	4.64E-010	0.8
3189	7.16E-004	1.33E+004	5.40E-008	0.3
3190	8.32E-004	1.25E+004	6.64E-008	0.3
3191	3.82E-004	8.77E+005	4.36E-010	0.8
3192	6.15E-004	8.45E+005	7.28E-010	0.8
3195	6.32E-004	8.78E+004	7.20E-009	0.5

IV. C1q binding

For C1q binding, biotinylated BHV-2000 or biotinylated Fc engineered Adnectins (Compounds 3189, 3190, 3191, 3192, and 3195) were loaded onto streptavidin XT probes, dipped into 1000 nM – 1.37 nM C1q, and subjected to biolayer interferometry. Binding affinity of Fc engineered Adnectins to C1q are shown in **Table 11**. Fc engineered Adnectins with LALA and PA Fc mutations (Compounds 3189, 3190, and 3195) showed decreased binding to C1q relative to BHV-2000.

Table 11. Affinity of Fc engineered Adnectins for C1q

Sample (Compound)	KD (nM)
BHV-2000	53.1
3189	No binding
3190	No binding
3191	45.6
3192	195
3195	No binding

V. Non-specific binding

To test for non-specific binding of the Fc engineered Adnectins, a baculovirus particle (BVP) binding assay was performed. The BVP assay is an ELISA-based approach to identify constructs with a high propensity for cross-interaction. Detection of non-specific binding to baculovirus particles can indicate an increased risk of fast clearance.

Briefly, plate wells were coated with BVPs overnight at 4°C. Wells were then blocked with BSA for one hour at room temperature. Fc engineered Adnectins, BHV-2000, and controls were then added to the wells and incubated for 1 hour at room temperature, followed by detection with anti-human IgG-HRP conjugated antibody (Fcγ fragment specific) and development with 1-Step TMB Ultra. Absorbance was measured at 450 nm. BVP score was calculated as sample absorbance divided by blank absorbance (n=8 for blank; n=2 for all samples and standards).

As shown in **FIG. 4**, Compounds 3189 and 3190 had moderate signals (BVP score) and BHV-2000 had a low signal, suggesting that Compounds 3189 and 3190 potentially have slightly more non-specific binding than BHV-2000.

Example 4. Inhibition of SMAD2/3 signaling by Fc engineered Adnectins in a free-ligand assay

A free-ligand assay using a SMAD binding element-based luciferase reporter was used to assess the inhibition of SMAD2/3 signaling by Fc engineered Adnectins.

FIG. 5 shows a schematic of the free-ligand assay. HEK293 cells stably expressing firefly luciferase under the control of a SMAD binding element allows for the detection and quantification of SMAD2/3 signaling. A reduction in luciferase signal indicates the prevention of TGFβ ligands from binding the ActRIIB receptor.

Briefly, 40,000 cells/well were plated in white-walled, clear bottom 96-well plates on Day 0. On Day 1, 2 nM of myostatin, 1.6 nM GDF11, 0.3 nM Activin A, or a combination of all 3 ligands plus a dose response of Adnectins (BHV-2000 and Compounds 3189, 3190, 3191,

3192, or 3195) were added to the wells. On Day 2, OneGlo Assay Reagent was added and luciferase signal was read using a Synergy Neo2 plate reader.

As shown in **FIGs. 6A-6F** and summarized in **Table 12**, the Fc engineered Adnectins (Compounds 3189, 3190, 3191, 3192, and 3195) suppressed SMAD2/3 signaling to a similar extent as BHV-2000 *in vitro* in the free-ligand assay, demonstrating that the Fc mutations have minimal or no impact on Adnectin behavior.

Table 12. Free-ligand assay (IC50 values)

Sample (Compound)	Myostatin	GDF-11	Activin A	Combination
BHV-2000	0.6599	0.08771	231.4	0.3200
3189	0.5481	0.07462	2.302	0.6267
3190	0.2719	0.0987	1236	0.2749
3191	0.4585	0.08763	0.01551	0.4203
3192	0.1726	0.07284	N/A	0.2663
BHV-2000[1]	0.6397	0.3878	N/A	1.049
3189	0.5329	0.2321	10.26	0.8148
3190	0.6567	0.2057	0.01155	2.248
3195	0.6995	0.1641	0.02527	0.7817

[1]Corresponds to a separate experiment.

Example 5. Impact on SMAD2/3 signaling of Fc engineered Adnectins in a pre-complexed ligand assay

A pre-complexed ligand assay using a SMAD binding element-based luciferase reporter was used to assess the effects of Fc engineered Adnectins on SMAD2/3 signaling.

FIG. 7 shows a schematic of the pre-complexed ligand assay. HEK293 cells stably expressing firefly luciferase under the control of a SMAD binding element allows for the detection and quantification of SMAD2/3 signaling.

Briefly, on Day 0, 40,000 cells/well were plated in white-walled, clear bottom 96-well plates in 50 µL, and pre-complexes of myostatin or GDF11 with Adnectins (i.e., BHV-2000 and

Compound 3189, 3190, or 3195) were formed by incubating the components at a 1:5 ratio overnight at 4°C. On Day 1, pre-complexed myostatin – Adnectin or GDF11 – Adnectin were added to the wells, followed by the addition of increasing doses (dose response) of Activin A. On Day 2, OneGlo Assay Reagent was added and luciferase signal was read using a Synergy Neo2 plate reader.

As shown in **FIGs. 8A-8D**, when no pre-complexes were added (i.e., only Adnectins were added), Compounds 3189, 3190, and 3195 had a minimal impact on SMAD2/3 signaling activity compared to when BHV-2000 was added. Addition of pre-complexes of myostatin with BHV-2000, Compound 3189, Compound 3190, or Compound 3195 (**FIGs. 8E-8H**) or pre-complexes of GDF11 with BHV-2000, Compound 3189, Compound 3190, or Compound 3195 (**FIGs. 8I-8L**) to cells had a minimal impact on SMAD2/3 signaling activity compared to when pre-complexes of myostatin or GDF11 with BHV-2000 were added. These results are summarized in **Table 13**, and demonstrate that the Fc mutations have minimal or no impact on Adnectin behavior *in vitro* in the pre-complexed ligand assay.

Table 13. Pre-complexed ligand assay (EC50 values)

Adnectin only	**125 nM**	**62.5 nM**	**31.25 nM**	**15.625 nM**	**Activin A only**
BHV-2000	4.548	3.671	1.414	1.353	2.556
3189	11.21	2.911	2.028	2.395	1.7
3190	3.803	4.8	2.733	1.554	0.8828
3195	5.018	8.63	1.436	4.417	2.269
Precomplexes with myostatin	**25/125 nM**	**12.5/62.5 nM**	**6.25/31.25 nM**	**3.125/15.625 nM**	**Activin A only**
BHV-2000	81.18	8.394	5.503	7.991	2.081
3189	10.49	3.843	4.005	17.24	2.995
3190	8.955	2.869	6.335	19.23	2.555
3195	2.837	1.981	4.074	4.074	1.38

Precomplexes with GDF11	25/125 nM	12.5/62.5 nM	6.25/31.25 nM	3.125/15.625 nM	Activin A only
BHV-2000	34.89	8.481	4.807	6.702	2.386
3189	8.347	5.83	4.996	4.722	2.302
3190	10.4	3.31	3.305	2.882	2.394
3195	6.105	1.928	3.528	3.282	0.9281

Example 6. Impact of FcRn-mediated recycling on Fc engineered Adnectins

A cell-based assay was used to determine FcRn-mediated recycling of Fc engineered Adnectins, as shown in **FIG. 9**.

Briefly, HMEC1 cells transfected with FcRn were seeded and grown for 24 h in low IgG media. The cells were then incubated with the Adnectins (Compounds 3189, 3190, 3191, 3192, 3195) and BHV-2000 in serum-free media for 4 hours, washed with PBS, and incubated in fresh media. The following day, the media was harvested for Adnectin quantification. To quantify the Adnectins, a streptavidin Meso Scale Discovery (MSD) plate was coated with biotinylated GDF-8 at room temperature (RT) for 1 hr. The plate was blocked with MSD blocker A for 1 hr, washed three times with PBS + 0.1% tween (PBST), and incubated with diluted media samples along with Adnectin standard curves for 2 hrs at RT. The plate was washed 3 times with PBST and incubated with 1 μg/mL SULFO-tagged anti-CH2 antibody (MA5-16929) for 1.5 hrs at RT. Finally, the plate was washed three times with PBST, added MSD read buffer B, and analyzed by MESO QuickPlex SQ 120MM.

As shown in **FIG. 10**, Compounds 3189, 3190, 3191, and 3195 all showed significantly more FcRn-mediated recycling than BHV-2000 with about 10-fold more being recycled back out to the media.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims. For example, pharmaceutically acceptable salts other than those specifically disclosed in the description and Examples herein can be employed. Furthermore, it is intended that specific items within lists of items, or subset groups of items within larger groups of items,

can be combined with other specific items, subset groups of items or larger groups of items whether or not there is a specific disclosure herein identifying such a combination.

Table 14. Summary of Sequences

SEQ ID	Description	Sequence
1	Human prepromyostatin	MQKLQLCVYIYLFMLIVAGPVDLNENSEQKENVEKEGLCNACT WRQNTKSSRIEAIKIQILSKLRLETAPNISKDVIRQLLPKAPP LRELIDQYDVQRDDSSDGSLEDDDYHATTETIITMPTESDFLM QVDGKPKCCFFKFSSKIQYNKVVKAQLWIYLRPVETPTTVFVQ ILRLIKPMKDGTRYTGIRSLKLDMNPGTGIWQSIDVKTVLQNW LKQPESNLGIEIKALDENGHDLAVTFPGPGEDGLNPFLEVKVT DTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAP KRYKANYCSGECEFVFLQKYPHTHLVHQANPRGSAGPCCTPTK MSPINMLYFNGKEQIIYGKIPAMVVDRCGCS
2	Human promyostatin	NENSEQKENVEKEGLCNACTWRQNTKSSRIEAIKIQILSKLRL ETAPNISKDVIRQLLPKAPPLRELIDQYDVQRDDSSDGSLEDD DYHATTETIITMPTESDFLMQVDGKPKCCFFKFSSKIQYNKVV KAQLWIYLRPVETPTTVFVQILRLIKPMKDGTRYTGIRSLKLD MNPGTGIWQSIDVKTVLQNWLKQPESNLGIEIKALDENGHDLA VTFPGPGEDGLNPFLEVKVTDTPKRSRRDFGLDCDEHSTESRC CRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFLQKYPHT HLVHQANPRGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPAM VVDRCGCS
3	Human mature myostatin	DFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYC SGECEFVFLQKYPHTHLVHQANPRGSAGPCCTPTKMSPINMLY FNGKEQIIYGKIPAMVVDRCGCS
4	Wild-type human ^{10}Fn3 domain (BC, DE, and FG loops are underlined)	VSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGETGGNS PVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPASSK PISINYRT
5	ADX 3116 A06 BC loop	SWSLPHQGKAN
6	ADX 3116 A06 DE loop	PGRGVT
7	ADX 3116 A06 FG loop	TVTDTGYLKYKP
8	ADX 3116 A06 core sequence	EVVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTV PGRGVTATISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYR T
9	ADX 3116 A06 with N-terminal leader sequence and his tag	MGVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETG GNSPVQEFTVPGRGVTATISGLKPGVDYTITVYAVTVTDTGYL KYKPISINYRT*EIDKPSQ*HHHHHH

10	ADX 3116 A06 N-terminal extension and C-terminal tail (EI)	GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETGG NSPVQEFTVPGRGVTATISGLKPGVDYTITVYAVTVTDTGYLK YKPISINYRT*EI*
11	Exemplary leader #1	MGVSDVPRDL
12	Exemplary leader #2	GVSDVPRDL
13	Exemplary leader #3	VSDVPRDL
14	Exemplary leader #4	SDVPRDL
15	Exemplary leader #5	DVPRDL
16	Exemplary leader #6	VPRDL
17	Exemplary leader #7	PRDL
----	Exemplary leader #8	RDL
----	Exemplary leader #9	DL
18	Exemplary tail #1	EIDKPSQ
----	Exemplary tail #2	EI
19	Exemplary tail #3	EIEPKSS
20	Exemplary tail #4	EIDKPC
21	Exemplary tail #5	EIDKP
22	Exemplary tail #6	EIDK
23	Exemplary tail #7	EIDKPS
24	Exemplary tail #8	EIEKPSQ
25	Exemplary tail #9	EIDKPSQLE
26	Exemplary tail #10	EIEDEDEDEDED
27	Exemplary tail #11	EGSGS
28	Exemplary tail #12	EIDKPCQ
29	Exemplary tail #13	GSGC
30	Exemplary tail #14	EGSGC
31	Exemplary tail #15	EIDKPCQLE
32	Exemplary tail #16	EIDKPSQHHHHHH
33	Exemplary tail #17	GSGCHHHHHH
34	Exemplary tail #18	EGSGCHHHHHH
35	Exemplary linker #1	GAGGGGSG
36	Exemplary linker #2	EPKSSD
37	Exemplary linker #3	ESPKAQASSVPTAQPQAEGLA
38	Exemplary linker #4	ELQLEESAAEAQDGELD
39	Exemplary linker #5	GQPDEPGGS
40	Exemplary linker #6	GGSGSGSGSGSGS
41	Exemplary linker #7	ELQLEESAAEAQEGELE
42	Exemplary linker #8	GSGSG
43	Exemplary linker #9	GSGC
44	Exemplary linker #10	AGGGGSG

45	Exemplary linker #11	GSGS
46	Exemplary linker #12	QPDEPGGS
47	Exemplary linker #13	GSGSGS
48	Exemplary linker #14	TVAAPS
49	Exemplary linker #15	KAGGGGSG
50	Exemplary linker #16	KGSGSGSGSGSGS
51	Exemplary linker #17	KQPDEPGGS
52	Exemplary linker #18	KELQLEESAAEAQDGELD
53	Exemplary linker #19	KTVAAPS
54	Exemplary linker #20	KAGGGGSGG
55	Exemplary linker #21	KGSGSGSGSGSGSG
56	Exemplary linker #22	KQPDEPGGSG
57	Exemplary linker #23	KELQLEESAAEAQDGELDG
58	Exemplary linker #24	KTVAAPSG
59	Exemplary linker #25	AGGGGSGG
60	Exemplary linker #26	AGGGGSG
61	Exemplary linker #27	GSGSGSGSGSGSG
62	Exemplary linker #28	QPDEPGGSG
63	Exemplary linker #29	TVAAPSG
64	Exemplary hinge #1 (core hinge underlined)	DKTHTCPPCPAPELLG
65	Exemplary hinge #2 (core hinge underlined)	GSTHTCPPCPAPELLG
66	Exemplary hinge #3 (core hinge underlined)	EPKSSDKTHTCPPCPAPELLGGPS
67	Exemplary hinge #4 (core hinge underlined)	EPKSSDKTHTCPPCPAPELLGGSS
68	Exemplary hinge #5 (core hinge underlined)	EPKSSGSTHTCPPCPAPELLGGSS
69	Exemplary hinge #6 (core hinge underlined)	DKTHTCPPCPAPELLGGPS
70	Exemplary hinge #7 (core hinge underlined)	DKTHTCPPCPAPELLGGSS
71	Exemplary hinge #8 (core hinge underlined)	DKTHTCPPCPAPE**AA**G
72	Exemplary hinge #9 (core hinge underlined)	ESKYGPPCP**P**CPAPE**AA**G
73	Linker	ELQLEESAAEAQEGELE
74	Signal peptide	MRAWIFFLLCLAGRALA
75	Signal peptide	METDTLLLWVLLLWVPGSTG
76	Human IgG1 Fc (core hinge is underlined)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL

		TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
77	Human IgG1 Fc without C-terminal “K”	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPG
78	Human IgG1 Fc without C-terminal “GK”	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP
79	Human S228P IgG4 Fc without C-terminal “GK”	ESKYGPPCP**P**CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSP
80	Heavy chain constant region of human IgG1 (domains CH1-CH3)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK
81	BHV-2000 (hinge region is underlined, linker is in italics, leader is in bold, and anti-myostatin Adnectin sequence is underlined and in italics)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDL***EVVAATP* *TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA* *TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT*EI
82	Human IgG1 Fc (without hinge) – L234A/L235A (LALA)	GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL

		TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
83	Human IgG1 Fc (without hinge) – L234A/L235A/P329A (LALA/PA)	GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKAL**A**APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
84	Human IgG1 Fc (without hinge) – L234A/L235A/P329A/ M248L/N434S (LALA/PA/LS)	GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKAL**A**APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSV**L**HEALH**S**HYTQKSLSLSP
85	Human IgG1 Fc (without hinge) – L234A/L235A/P329A/ M252Y/S254T/T256E (LALA/PA/YTE)	GPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKAL**A**APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
86	Human IgG1 Fc (without hinge) – M248L/N434S (LS)	GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSV**L**HEALH**S**HYTQKSLSLSP
87	Human IgG1 Fc (without hinge) – M252Y/S254T/T256E (YTE)	GPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
88	Human IgG1 Fc (without hinge) – M252Y/S254T/T256E/ N297Q (N297Q/YTE)	GPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQY**Q**STYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
89	Human IgG1 Fc (without hinge) – N297Q/M248L/N434S (N297Q/LS)	GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQY**Q**STYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSV**L**HEALH**S**HYTQKSLSLSP
90	Human IgG4 Fc (without hinge) – F234A/L235A/M252Y/ S254T/T256E (LALA*/YTE)	GPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL

	*F234A/L235A in IgG4 is referred to as LALA herein	TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSP
91	Human IgG4 Fc (without hinge) – M252Y/S254T/T256E/ N297Q (N297Q/YTE)	GPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQF**Q**STYRVVSVLTVLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSP
92	Human IgG1 Fc – L234A/L235A (LALA)	DKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP
93	Human IgG1 Fc – L234A/L235A/P329A (LALA/PA)	DKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKAL**A**APIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP
94	Human IgG1 Fc – L234A/L235A/P329A/ M248L/N434S (LALA/PA/LS)	DKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKAL**A**APIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV**L**HEALH**S**H YTQKSLSLSP
95	Human IgG1 Fc – L234A/L235A/P329A/ M252Y/S254T/T256E (LALA/PA/YTE)	DKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKAL**A**APIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP
96	Human IgG1 Fc – M248L/N434S (LS)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV**L**HEALH**S**H YTQKSLSLSP
97	Human IgG1 Fc – M252Y/S254T/T256E (YTE)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY

		TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP
98	Human IgG1 Fc – M252Y/S254T/T256E/ N297Q (N297Q/YTE)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY**Q**STYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP
99	Human IgG1 Fc – N297Q/M248L/N434S (N297Q/LS)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY**Q**STYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV**L**HEALH**S**H YTQKSLSLSP
100	Human IgG4 Fc – F234A/L235A/M252Y/ S254T/T256E (LALA/YTE)	ESKYGPPCPPCPAPE**AA**GGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSP
101	Human IgG4 Fc – M252Y/S254T/T256E/ N297Q (N297Q/YTE)	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF**Q**STYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSP
102	Compound 0001 – IgG1 Fc L234A/L235A/P329A (LALA) – without signal peptide and C-terminal EI	DKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT**
103	Compound 0002 – IgG1 Fc L234A/L235A/P329A (LALA/PA) – without signal peptide and C-terminal EI	DKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKAL**A**APIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP

		TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT
104	Compound 3189 – IgG1 Fc L234A/L235A/P329A/ M248L/N434S (LALA/PA/LS) – without signal peptide and C-terminal EI	DKTHTCPPCPAPE***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKAL***A***APIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV***L***HEALH***S***H YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP **TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT**
105	Compound 3190 – IgG1 Fc L234A/L235A/P329A/ M252Y/S254T/T256E (LALA/PA/YTE) – without signal peptide and C-terminal EI	DKTHTCPPCPAPE***AA***GGPSVFLFPPKPKDTL***Y***I***T***R***E***PEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKAL***A***APIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP **TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT**
106	Compound 3191 – IgG1 Fc M248L/N434S (LS) – without signal peptide and C-terminal EI	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV***L***HEALH***S***H YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP **TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT**
107	Compound 3192 – IgG1 Fc M252Y/S254T/T256E (YTE) – without signal peptide and C-terminal EI	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL***Y***I***T***R***E***PEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP **TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT**
108	Compound 3193 – IgG1 Fc M252Y/S254T/T256E/ N297Q (N297Q/YTE) – without signal peptide and C-terminal EI	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL***Y***I***T***R***E***PEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY***Q***STYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP

		TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT
109	Compound 3194 – IgG1 Fc N297Q/M248L/N434S (N297Q/LS) – without signal peptide and C-terminal EI	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY***Q***STYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV***L***HEALH***S***H YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP **TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT**
110	Compound 3195 – IgG4 Fc F234A/L235A/M252Y/ S254T/T256E (LALA/YTE) – without signal peptide and C-terminal EI	ESKYGPPCPPCPAPE***AA***GGPSVFLFPPKPKDTL***Y***I***T***R***E***PEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAA **TPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGV TATISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT**
111	Compound 3196 – IgG4 Fc M252Y/S254T/T256E/ N297Q (N297Q/YTE) – without signal peptide and C-terminal EI	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL***Y***I***T***R***E***PEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF***Q***STYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAA **TPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGV TATISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRT**
112	Compound 0001 – IgG1 Fc L234A/L235A (LALA) – without signal peptide	DKTHTCPPCPAPE***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP **TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI**
113	Compound 0002 – IgG1 Fc L234A/L235A/P329A (LALA/PA) – without signal peptide	DKTHTCPPCPAPE***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKAL***A***APIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP **TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI**
114	Compound 3189 – IgG1 Fc – L234A/L235A/P329A/ M248L/N434S	DKTHTCPPCPAPE***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKAL***A***APIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK

	(LALA/PA/LS) – without signal peptide	TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV**L**HEALH**S**H YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP** **TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA** **TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI**
115	Compound 3190 – IgG1 Fc L234A/L235A/P329A/ M252Y/S254T/T256E (LALA/PA/YTE) – without signal peptide	DKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKAL**A**APIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLE** **VVAATPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTV** **PGRGVTATISGLKPGVDYTITVYAVTVTDTGYLKYKPISINY** **RTEI**
116	Compound 3191 – IgG1 Fc M248L/N434S (LS) – without signal peptide	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV**L**HEALH**S**H YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP** **TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA** **TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI**
117	Compound 3192 – IgG1 Fc M252Y/S254T/T256E (YTE) – without signal peptide	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP** **TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA** **TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI**
118	Compound 3193 – IgG1 Fc M252Y/S254T/T256E/ N297Q (N297Q/YTE) – without signal peptide	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY**Q**STYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP** **TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA** **TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI**
119	Compound 3194 – IgG1 Fc N297Q/M248L/N434S (N297Q/LS) – without signal peptide	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY**Q**STYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV**L**HEALH**S**H YTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAATP** **TSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGVTA** **TISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI**
120	Compound 3195 – IgG4 Fc	ESKYGPPCPPCPAPE**AA**GGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS

	F234A/L235A/M252Y/ S254T/T256E (LALA/YTE) – without signal peptide	VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAA TPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGV TATISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI**
121	Compound 3196 – IgG4 Fc M252Y/S254T/T256E/ N297Q (N297Q/YTE) – without signal peptide	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL***Y***I***T***R***E***PEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF***Q***STYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSP*ELQLEESAAEAQEGELE***GVSDVPRDLEVVAA TPTSLLISWSLPHQGKANYYRITYGETGGNSPVQEFTVPGRGV TATISGLKPGVDYTITVYAVTVTDTGYLKYKPISINYRTEI**
122	Compound 0001 – IgG1 Fc L234A/L235A (LALA)	MRAWIFFLLCLAGRALADKTHTCPPCPAPE***AA***GGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQEGEL E***GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETG GNSPVQEFTVPGRGVTATISGLKPGVDYTITVYAVTVTDTGYL KYKPISINYRTEI**
123	Compound 0002 – IgG1 Fc L234A/L235A/P329A (LALA/PA)	MRAWIFFLLCLAGRALADKTHTCPPCPAPE***AA***GGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***A***API EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQEGEL E***GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETG GNSPVQEFTVPGRGVTATISGLKPGVDYTITVYAVTVTDTGYL KYKPISINYRTEI**
124	Compound 3189 – IgG1 Fc – L234A/L235A/P329A/ M248L/N434S (LALA/PA/LS)	MRAWIFFLLCLAGRALADKTHTCPPCPAPE***AA***GGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***A***API EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSV***L***HEALH***S***HYTQKSLSLSP*ELQLEESAAEAQEGEL E***GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETG GNSPVQEFTVPGRGVTATISGLKPGVDYTITVYAVTVTDTGYL KYKPISINYRTEI**
125	Compound 3190 – IgG1 Fc L234A/L235A/P329A/ M252Y/S254T/T256E (LALA/PA/YTE)	MRAWIFFLLCLAGRALADKTHTCPPCPAPE***AA***GGPSVFLFPPK PKDTL***Y***I***T***R***E***PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***A***API EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQEGEL*

		*E*GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETG GNSPVQEFTVPGRGVTATISGLKPGVDYTITVYAVTVTDTGYL KYKPISINYRTEI
126	Compound 3191 – IgG1 Fc M248L/N434S (LS)	MRAWIFFLLCLAGRALADKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSV*L*HEALH*S*HYTQKSLSLSP*ELQLEESAAEAQEGEL* *E*GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETG GNSPVQEFTVPGRGVTATISGLKPGVDYTITVYAVTVTDTGYL KYKPISINYRTEI
127	Compound 3192 – IgG1 Fc M252Y/S254T/T256E (YTE)	MRAWIFFLLCLAGRALADKTHTCPPCPAPELLGGPSVFLFPPK PKDTL*Y*I*T*R*E*PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQEGEL* *E*GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETG GNSPVQEFTVPGRGVTATISGLKPGVDYTITVYAVTVTDTGYL KYKPISINYRTEI
128	Compound 3193 – IgG1 Fc M252Y/S254T/T256E/ N297Q (N297Q/YTE)	MRAWIFFLLCLAGRALADKTHTCPPCPAPELLGGPSVFLFPPK PKDTL*Y*I*T*R*E*PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQY*Q*STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQEGEL* *E*GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETG GNSPVQEFTVPGRGVTATISGLKPGVDYTITVYAVTVTDTGYL KYKPISINYRTEI
129	Compound 3194 – IgG1 Fc N297Q/M248L/N434S (N297Q/LS)	MRAWIFFLLCLAGRALADKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQY*Q*STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSV*L*HEALH*S*HYTQKSLSLSP*ELQLEESAAEAQEGEL* *E*GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGETG GNSPVQEFTVPGRGVTATISGLKPGVDYTITVYAVTVTDTGYL KYKPISINYRTEI
130	Compound 3195 – IgG4 Fc F234A/L235A/M252Y/ S254T/T256E (LALA/YTE)	MRAWIFFLLCLAGRALAESKYGPPCPPCPAPE*AA*GGPSVFLFP PKPKDTL*Y*I*T*R*E*PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQEG* *ELE*GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGE

		TGGNSPVQEFTVPGRGVTATISGLKPGVDYTITVYAVTVTDTG YLKYKPISINYRTEI
131	Compound 3196 – IgG4 Fc M252Y/S254T/T256E/ N297Q (N297Q/YTE)	MRAWIFFLLCLAGRALAESKYGPPCPPCPAPEFLGGPSVFLFP PKPKDTL***Y***I***T***R***E***PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA KTKPREEQF***Q***STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSP*ELQLEESAAEAQEG ELE***GVSDVPRDLEVVAATPTSLLISWSLPHQGKANYYRITYGE TGGNSPVQEFTVPGRGVTATISGLKPGVDYTITVYAVTVTDTG YLKYKPISINYRTEI**

We claim:

1. A polypeptide comprising (a) a fibronectin type III tenth (10Fn3) domain which binds to myostatin and comprises AB, BC, CD, DE, EF, and FG loops and (b) an Fc region, wherein:

(i) the BC, DE, and FG loops of the ^{10}Fn3 domain comprise the amino acid sequences of SEQ ID NOs: 5, 6, and 7, respectively, or at least one loop of the BC, DE, and FG loops of the ^{10}Fn3 domain has 1, 2, or 3 amino acid substitutions relative to the respective BC, DE, and FG loops of SEQ ID NOs: 5, 6, and 7, respectively; and

(ii) the Fc region differs in amino acid sequence from a reference human IgG1 Fc region comprising the amino acid sequence of SEQ ID NO: 78 or a reference human S228P IgG4 Fc region comprising the amino acid sequence of SEQ ID NO: 79 by at least one amino acid but no more than 15 amino acids.

2. The polypeptide of claim 1, wherein the ^{10}Fn3 domain comprises the amino acid sequence of SEQ ID NO: 8, or an amino acid sequence which is at least 90%, 95%, 98%, or 99% identical to SEQ ID NO: 8, or an amino acid sequence which differs from SEQ ID NO: 8 by at least one amino acid but no more than 15 amino acids.

3. The polypeptide of claim 1 or 2, wherein the ^{10}Fn3 domain comprises the amino acid sequence of SEQ ID NO: 10, or an amino acid sequence which is at least 90%, 95%, 98%, or 99% identical to SEQ ID NO: 10, or an amino acid sequence which differs from SEQ ID NO: 10 by at least one amino acid but no more than 15 amino acids.

4. The polypeptide of any one of the preceding claims, wherein the Fc region differs from the amino acid sequence of SEQ ID NO: 78 by at least 2 amino acids, at least 3 amino acids, at least 4 amino acids, at least 5 amino acids, at least 6 amino acids, at least 7 amino acids, at least 8 amino acids, at least 9 amino acids, or at least 10 amino acids.

5. The polypeptide of any one of the preceding claims, wherein the Fc region comprises a combination of amino acid substitutions relative to SEQ ID NO: 78 selected from the group consisting of:

(a) L234A/L235A/P329A/M248L/N434S,

(b) L234A/L235A,
(c) L234A/L235A/P329A,
(d) L234A/L235A/P329A/M252Y/S254T/T256E,
(e) M248L/N434S,
(f) M252Y/S254T/T256E,
(g) M252Y/S254T/T256E/N297Q, and
(h) N297Q/M248L/N434S,
wherein the numbering of positions is according to the EU Index.

6. The polypeptide of any one of the preceding claims, wherein the Fc region comprises the amino acid sequence of any one of SEQ ID NOs: 84, 83, and 85-89.

7. The polypeptide of claim 6, wherein the Fc region further comprises the amino acid sequence of any one of SEQ ID NOs: 71, 64-70, and 72.

8. The polypeptide of any one of the preceding claims, wherein the Fc region comprises the amino acid sequence of any one of SEQ ID NOs: 94, 92, 93, and 95-99.

9. The polypeptide of any one of claims 1-3, wherein the Fc region differs from the amino acid sequence of SEQ ID NO: 79 by at least 2 amino acids, at least 3 amino acids, at least 4 amino acids, at least 5 amino acids, at least 6 amino acids, at least 7 amino acids, at least 8 amino acids, at least 9 amino acids, or at least 10 amino acids.

10. The polypeptide of any one of claims 1-3 and 9, wherein the Fc region comprises a combination of amino acid substitutions relative to SEQ ID NO: 79 selected from the group consisting of:
(a) F234A/L235A/M252Y/S254T/T256E, and
(b) M252Y/S254T/T256E/N297Q,
wherein the numbering of positions is according to the EU Index.

11. The polypeptide of any one of claims 1-3, 9, and 10, wherein the Fc region comprises the amino acid sequence of SEQ ID NO: 90 or 91.

12. The polypeptide of claim 11, wherein the Fc region further comprises the amino acid sequence of any one of SEQ ID NOs: 72 and 64-71.

13. The polypeptide of any one of claims 1-3 and 9-12, wherein the Fc region comprises the amino acid sequence of SEQ ID NO: 100 or 101.

14. The polypeptide of any one of the preceding claims, which comprises a signal peptide.

15. The polypeptide of claim 14, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO: 74 or 75, or an amino acid sequence which differs from SEQ ID NO: 74 or 75 by no more than 3 amino acids.

16. The polypeptide of any one of the preceding claims, which comprises a linker between the ^{10}Fn3 domain to the Fc region.

17. The polypeptide of claim 16, wherein the linker comprises the amino acid sequence of any one of SEQ ID NOs: 41, 35-40, and 42-63, or an amino acid sequence which differs from any one of SEQ ID NOs: 41, 35-40, and 42-63 by no more than 1, 2, or 3 amino acids.

18. The polypeptide of any one of the preceding claims, wherein the Fc region is located N-terminal to the ^{10}Fn3 domain.

19. The polypeptide of any one of the preceding claims, wherein the configuration of the polypeptide, from N-terminus to C-terminus, is as follows: Fc domain – linker – ^{10}Fn3 domain.

20. The polypeptide of claim 19, wherein the configuration of the polypeptide, from N-terminus to C-terminus, is as follows: signal peptide – Fc domain – linker – ^{10}Fn3 domain.

21. The polypeptide of any one of claims 1-17, wherein the Fc region is located C-terminal to the ^{10}Fn3 domain.

22. The polypeptide of any one of claims 1-17 and 21, wherein the configuration of the polypeptide, from N-terminus to C-terminus, is as follows: ^{10}Fn3 domain – linker – Fc region.

23. The polypeptide of claim 22, wherein the configuration of the polypeptide, from N-terminus to C-terminus, is as follows: signal peptide – ^{10}Fn3 domain – linker – Fc region.

24. The polypeptide of any one of claims 1-20, wherein the polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 124, 102-123, and 125-131, or a polypeptide comprising an amino acid sequence which is at least 90%, 95%, 98%, or 99% identical to any one of SEQ ID NOs: 124, 102-123, and 125-131, or a polypeptide which differs from the amino acid sequence of any one of SEQ ID NOs: 124, 102-123, and 125-131 by at least one amino acid but no more than 30, 20, or 10 amino acids.

25. The polypeptide of any one of the preceding claims, wherein the polypeptide binds to active myostatin with a K_D of 15 nM or less, e.g., as assessed using biolayer interferometry.

26. The polypeptide of any one of the preceding claims, wherein the polypeptide binds with a stronger affinity to FcRn at pH. 6.0 compared to a polypeptide comprising the amino acid sequence of SEQ ID NO: 81, e.g., as assessed by biolayer interferometry.

27. The polypeptide of any one of the preceding claims, wherein the polypeptide binds to FcRn at pH 6.0 with a 40-60 fold (e.g., 40-55 fold, 40-50 fold, 40-45 fold, 45-60 fold, 45-55 fold, 45-50 fold, 50-60 fold, 55-60 fold) higher affinity than a polypeptide comprising the amino acid sequence of SEQ ID NO: 81, e.g., as assessed by biolayer interferometry.

28. The polypeptide of any one of the preceding claims, wherein the polypeptide has a lower affinity for FcγRI relative to a polypeptide comprising the amino acid sequence of SEQ ID NO: 81, e.g., as assessed by biolayer interferometry.

29. The polypeptide of any one of the preceding claims, wherein the polypeptide has a lower affinity for C1q relative to a polypeptide comprising the amino acid sequence of SEQ ID NO: 81, e.g., as assessed by biolayer interferometry.

30. The polypeptide of any one of the preceding claims, wherein the polypeptide has increased FcRn-mediated recycling relative to a polypeptide comprising the amino acid sequence of SEQ ID NO: 81, e.g., as assessed by the cell-based assay described in Example 6.

31. The polypeptide of any one of the preceding claims, wherein the polypeptide has a longer half-life relative to a polypeptide comprising the amino acid sequence of SEQ ID NO: 81.

32. The polypeptide of any one of the preceding claims, wherein the polypeptide inhibits SMAD2/3 signaling.

33. An isolated nucleic acid molecule which encodes the polypeptide of any one of claims 1-32.

34. An expression vector comprising the nucleic acid molecule of claim 33.

35. A cell comprising the nucleic acid molecule of claim 33 or expression vector of claim 34.

36. A kit comprising the polypeptide of any one of claims 1-32, and instructions for use.

37. A pharmaceutical composition comprising the polypeptide of any one of claims 1-32, and a pharmaceutically acceptable carrier.

38. A stable pharmaceutical formulation comprising:

(i) at least 10 mg/mL of the polypeptide of any one of claims 1-32;

(ii) a disaccharide at a concentration of at least 5%;

(iii) a histidine buffer at a concentration of between about 20 to about 60 mM; and

(iv) a pharmaceutically acceptable aqueous carrier,

wherein the formulation has a pH range of about 6.5 to about 7.8.

39. The formulation of claim 38, wherein the protein concentration of the polypeptide in the formulation is between about 10 mg/mL and 200 mg/mL, between about 10 mg/mL and 150 mg/mL, or between about 10 mg/mL and 85 mg/mL.

40. The formulation of claim 38 or 39, wherein the disaccharide is present at weight (w/w) ratio of at least 5:1 protein to sugar.

41. The formulation of any one of claims 38-40, wherein the formulation comprises about 5% to about 30% of the disaccharide.

42. The formulation of any one of claims 38-41, wherein the concentration of the disaccharide is about 150 mM to about 800 mM, or about 300 to about 700 mM.

43. The formulation of any one of claims 38-42, wherein the disaccharide is trehalose, and the formulation comprises about 5 to about 30% trehalose, about 15% to about 25% trehalose, or about 20% to about 25% trehalose.

44. The formulation of any one of claims 38-43, wherein the disaccharide is trehalose dehydrate, and the concentration of trehalose dihydrate in the formulation is about 150 mM to about 800 mM, about 300 to about 700 mM, about 150 mM, about 200 mM, about 250 mM, about 300 mM, about 350 mM, about 400 mM, about 450 mM, about 500 mM, about 550 mM, about 575 mM, about 600, about 625 mM, about 650 mM, about 675 mM, or about 700 mM.

45. The formulation of any one of claims 38-44, wherein the histidine is present at a concentration of at least 20 mM.

46. The formulation of any one of claims 38-45, wherein the viscosity of the formulation is from about 5 to 20 cps, from about 5 to 15 cps, or from about 7 to 12 cps.

47. The formulation of any one of claims 38-46, wherein the pH is about 6.6 to 7.6, about 6.8 to 7.4, or about 7.0 to 7.3.

48. The formulation of any one of claims 38-47 comprising a surfactant at a concentration of between about 0.01% and 0.5%.

49. The formulation of any one of claims 38-48 comprising a chelator, wherein the concentration of the chelator is between about 0.01 mM and about 0.5 mM or between about 0.05 mM and 0.2 mM, and wherein the chelator is selected from the group consisting of DPTA, EDTA and EGTA.

50. A stable pharmaceutical formulation comprising,

(a) about 10-140 mg/mL of the polypeptide of any one of claims 1-32;
about 5-25% trehalose dihydrate;
about 20-30 mM histidine; and
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 6.8 to 7.3;

(b) about 10-140 mg/mL of the polypeptide of any one of claims 1-32;
about 5-25% trehalose dihydrate;
about 20-30 mM histidine;
about 0.02-0.06 mM DTPA;
about 0.01-0.05% polysorbate 80; and
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 6.8 to 7.3;

(c) about 10-140 mg/mL of the polypeptide of any one of claims 1-32;
about 600 mM trehalose dihydrate;
25-30 mM histidine; and
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 7.0 to 7.3;

(d) about 10-140 mg/mL of the polypeptide of any one of claims 1-32;
about 600 mM trehalose dihydrate;

25-30 mM histidine;
about 0.02-0.06 mM DTPA;
about 0.01-0.05% polysorbate 80; and
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 7.0 to 7.3;

(e) about 10-75 mg/mL of the polypeptide of any one of claims 1-32;
about 5-25% trehalose dihydrate;
about 20-30 mM histidine; and
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 6.8 to 7.3;

(f) about 10-75 mg/mL of the polypeptide of any one of claims 1-32;
about 5-25% trehalose dihydrate;
about 20-30 mM histidine;
about 0.02-0.06 mM DTPA;
about 0.01-0.05% polysorbate 80; and
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 6.8 to 7.3;

(g) about 10-75 mg/mL of the polypeptide of any one of claims 1-32;
about 600 mM trehalose dihydrate;
about 30 mM histidine;
about 0.05 mM DTPA;
about 0.02% polysorbate 80;
a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 7.1.

51. A unit dosage form comprising about 1.0 mL or less of a formulation comprising,
(i) about 10-75 mg/mL of the polypeptide of any one of claims 1-32;
(ii) about 5-25% trehalose dihydrate;
(iii) about 20-30 mM histidine;
(iv) about 0.02-0.06 mM DTPA;
(v) about 0.01-0.05% polysorbate 80; and

(vi) a pharmaceutically acceptable aqueous carrier,
wherein the pH of the formulation is about 6.8 to 7.3.

52. A method of producing a myostatin-binding polypeptide comprising culturing the cell of claim 35 under conditions suitable for expressing the polypeptide, and purifying the polypeptide.

53. A method of treating a myostatin-related disease or disorder in a subject comprising administering to the subject an effective amount of the polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51.

54. The method of claim 53, wherein the disease or disorder is selected from the group consisting of: muscular dystrophy, amyotrophic lateral sclerosis (ALS), Becker's muscular dystrophy (BMD), spinal muscular atrophy (SMA), Duchenne muscular dystrophy (DMD), congestive obstructive pulmonary disease, chronic heart failure, cancer, AIDs, renal failure, chronic kidney disease, uremia, rheumatoid arthritis, sarcopenia, muscle wasting due to prolonged bedrest, spinal cord injury, stroke, bone fracture, aging, diabetes, obesity, hyperglycemia, cachexia, osteoarthritis, osteoporosis, myocardial infarction, and fibrosis.

55. A method of attenuating or inhibiting a disorder associated with degeneration or wasting of muscle in a subject, comprising administering to the subject an effective amount of the polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51.

56. The method of claim 55, wherein the disorder is selected from the group consisting of muscular dystrophy, amyotrophic lateral sclerosis, congestive obstructive pulmonary disease, chronic heart failure, cancer, AIDs, cachexia, renal failure, chronic kidney disease, uremia, rheumatoid arthritis, sarcopenia, muscle wasting due to prolonged bedrest, spinal cord injury, traumatic injury, stroke, bone fracture, and aging.

57. The method of claim 55 or 56, wherein administration of the polypeptide to the subject results in at least one of the following biological effects:

(a) an increase in muscle mass;

(b) an increase in the number of muscle cells;

(c) an increase in the size of muscle cells; and

(d) an increase in muscle strength.

58. A method of treating a metabolic disease, disorder, or condition in a subject, comprising administering to the subject an effective amount of the polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51.

59. The method of claim 58, wherein the subject has a disease or disorder selected from the group consisting of diabetes, hyperglycemia, hyperinsulinemia, hyperlipidemia, insulin resistance, impaired glucose metabolism, obesity, metabolic syndrome, glucose intolerance, prediabetes, hypertension, dyslipidemia, increased waist circumference, cardiovascular disease, non-alcoholic fatty liver disease, obstructive sleep apnea, physical impairment, osteoarthritis, osteoporosis, renal disease, sexual hormone(s) impairment, endocrine reproductive disorders such as polycystic ovary syndrome or male hypogonadism, stroke, and gallstones.

60. The method of claim 58 or 59, wherein the disease or disorder is Type II diabetes.

61. The method of claim 59 or 60, further comprising administration of a second therapeutic composition for treating diabetes.

62. The method of any one of claims 58-61, wherein administration of the polypeptide to the subject results in at least one of the following biological effects:

(a) an increase in insulin sensitivity;

(b) an increase in glucose uptake by cells in the subject;

(c) a decrease in blood glucose levels; and

(d) a decrease in body fat.

63. A method for enhancing lean muscle mass in a subject, comprising administering to the subject an effective amount of the polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51.

64. A method of increasing the ratio of lean muscle mass to fat in a subject, comprising administering to the subject an effective amount of the polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51.

65. A method of improving glycemic control in a subject, comprising administering to the subject an effective amount of the polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51.

66. A method of treating, preventing, or reducing overweight or obesity and related comorbidities in a subject, comprising administering to the subject an effective amount of the polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51.

67. A method of treating or preventing type II diabetes in a subject, comprising administering to the subject an effective amount of the polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51.

68. A method of treating or preventing a neurodegenerative disease in a subject, comprising administering to the subject an effective amount of the polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51.

69. The method of claim 68, wherein the neurodegenerative disease is spinal muscular atrophy.

70. The polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51, for use in a method of treating a myostatin-related disease or disorder.

71. The polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51, for use in a method of attenuating or inhibiting a disorder associated with degeneration or wasting of muscle.

72. The polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51, for use in a method of treating a metabolic disease, disorder, or condition.

73. The polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51, for use in a method of enhancing lean muscle mass.

74. The polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51, for use in a method of increasing the ratio of lean muscle mass to fat.

75. The polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51, for use in a method of improving glycemic control.

76. The polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51, for use in a method of treating, preventing, or reducing overweight or obesity and related comorbidities.

77. The polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51, for use in a method of treating or preventing type II diabetes.

78. The polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51, for use in a method of treating or preventing a neurodegenerative disease.

79. Use of polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51, in the manufacture of a medicament.

80. Use of polypeptide of any one of claims 1-32, the pharmaceutical composition of claim 37, the formulation of any one of claims 38-50, or the unit dosage form of claim 51, in the manufacture of a medicament for treating a myostatin-related disease or disorder, attenuating or inhibiting a disorder associated with degeneration or wasting of muscle, treating a metabolic disease, disorder, or condition, enhancing lean muscle mass, increasing the ratio of lean muscle mass to fat, improving glycemic control, treating, preventing, or reducing overweight or obesity and related comorbidities, treating or preventing type II diabetes, or treating or preventing a neurodegenerative disease.