Figure 5 presents Peptide N primary structure showing disulfide bonds linking four chymotryptic peptides together to form a cystine knot and a disulfide bond linking two 63-82 sequences together at Cys73. Peptide N does not bind the antibody.

Figure 6 represents results of the BIAcore® competition assay for non-reduced and TCEP-reduced peptide samples. Peptides A, C, E, G, O and P can all bind to antibody 12A5-5 thereby preventing 12A5-5 from binding to mature myostatin. None of other peptides tested, including the ones from TCEP reduction of peptide G and Peptide N, the cystine knot chymotryptic peptide, showed binding with the antibody.

Figure 7 illustrates results of the direct BIAcore® binding assay for Peptides A, C, E, and G, along with myostatin.

Figure 8 represents the structures of myostatin dimer and monomer derived from the co-crystal structure of myostatin/follistatin complex (Cash et al., infra). The Cys residues involved in disulphide bonds are indicated, as are additional amino acid residues in the regions that are believed to be important for binding of 12A5-5 (indicated in the shaded rhomboids).

Summary of the Invention

In one aspect, the disclosure provides an isolated myostatin-specific antibody comprising at least one light chain and at least one heavy chain, wherein the light chain comprises a constant region and a variable region that comprises three complementarity determining regions (CDRs) and the heavy chain comprises a constant region and a variable region that comprises three CDRs, wherein the light chain CDRs are those disclosed in SEQ ID NO:39, SEQ ID NO:43, and SEQ ID NO:48 , and the heavy chain CDRs are those disclosed in in SEQ ID NO:30, SEQ ID NO:33, and SEQ ID NO:36.

The ~~invention~~disclosure provides an isolated myostatin-specific antibody that binds myostatin with a K_d of less than 100 pM. In one embodiment, the ~~invention~~disclosure provides an isolated myostatin-specific antibody that binds myostatin with a K_d of less than 100 pM and binds GDF-11 with a K_d of greater than 10nM. In another embodiment, the ~~invention~~disclosure provides an isolated myostatin-specific antibody that binds myostatin with an affinity at least 5,000 times greater than its affinity for GDF-11. In a further embodiment, the ~~invention~~disclosure provides an isolated myostatin-specific antibody that exhibits selectivity for myostatin that is at least 5,000 times greater than for GDF-11.

In one ~~aspect~~embodiment of the ~~invention~~disclosure, there is provided an isolated myostatin-specific antibody that binds myostatin and blocks the interaction of myostatin with ALK4. In another ~~aspect~~embodiment, there is provided an isolated myostatin-specific antibody that binds myostatin and blocks the interaction of myostatin with ALK4 but co-binds with a myostatin/ActRIIA complex and/or a myostatin/ActRIIB complex.

In another ~~aspect~~embodiment of the ~~invention~~disclosure, there is provided an isolated myostatin-specific antibody that binds myostatin, wherein two regions in myostatin that are required for binding of myostatin to the myostatin-specific antagonist locate at sequence near position 21 to 31 and position 50 to 60 of mature myostatin (SEQ ID NO:25). Also provided is an isolated myostatin-specific antibody that interacts with two regions in myostatin, located at sequence near position 21 to 31 and position 50 to 60 of mature

myostatin, so as to prevent chymotrypsin cleavage of peptide bonds within these regions.

In one embodiment of the ~~invention~~disclosure, the myostatin-specific antagonist is an antibody comprising at least one light chain and at least one heavy chain, wherein the light chain comprises a constant region and a variable region that comprises three complementarity determining regions (CDRs) and the heavy chain comprises a constant region and a variable region that comprises three complementarity determining regions (CDRs). This embodiment may incorporate one or more of the previously described embodiments and/or aspects of the ~~invention~~disclosure. In certain embodiments, the sequences of the heavy and light chain CDRs are as disclosed herein. In one embodiment, the light chain CDRs are those disclosed in SEQ ID NO:10, and the heavy chain CDRs are those disclosed in SEQ ID NO:20. In another embodiment, the light chain CDRs are selected from the group consisting of the light chain CDRs disclosed in SEQ ID NO:1; the light chain CDRs disclosed in SEQ ID NO:2; the light chain CDRs disclosed in SEQ ID NO:3; the light chain CDRs disclosed in SEQ ID NO:4; the light chain CDRs disclosed in SEQ ID NO:5; the light chain CDRs disclosed in SEQ ID NO:6; the light chain CDRs disclosed in SEQ ID NO:7; the light chain CDRs disclosed in SEQ ID NO:8; and the light chain CDRs disclosed in SEQ ID NO:9;and the heavy chain CDRs are selected from the group consisting of: the heavy chain CDRs disclosed in SEQ ID NO:11; the heavy chain CDRs disclosed in SEQ ID NO:12; the heavy chain CDRs disclosed in SEQ ID NO:13; the heavy chain CDRs disclosed in SEQ ID NO:14; the heavy chain CDRs disclosed in SEQ ID NO:15; the heavy chain CDRs disclosed in SEQ ID NO:16; the heavy chain CDRs disclosed in SEQ ID NO:17; the heavy chain CDRs disclosed in SEQ ID NO:18; and the heavy chain CDRs disclosed in SEQ ID NO:19.

In certain embodiments, the sequences of the heavy and light chain variable regions are as disclosed herein. In one embodiment, the light chain the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO:10, and the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO:20. In another embodiment, the light chain variable region is selected from the group consisting of the light chain variable region disclosed in SEQ ID NO:1; the light chain variable region disclosed in SEQ ID NO:2; the light chain variable region disclosed in SEQ ID NO:3; the light chain variable region disclosed in SEQ ID NO:4; the light chain variable region disclosed in SEQ ID NO:5; the light chain variable region disclosed in SEQ ID NO:6; the light chain variable region disclosed in SEQ ID NO:7; the light chain variable region disclosed in SEQ ID NO:8; and the light chain variable region disclosed in SEQ ID NO:9; and the heavy chain variable region is selected from the group consisting of: the heavy chain variable region disclosed in SEQ ID NO:11; the heavy chain variable

region disclosed in SEQ ID NO:12; the heavy chain variable region disclosed in SEQ ID NO:13; the heavy chain variable region disclosed in SEQ ID NO:14; the heavy chain variable region disclosed in SEQ ID NO:15; the heavy chain variable region disclosed in SEQ ID NO:16; the heavy chain variable region disclosed in SEQ ID NO:17; the heavy chain variable region disclosed in SEQ ID NO:18; and the heavy chain variable region disclosed in SEQ ID NO:19.

Variants of the afore-mentioned antibodies are also provided. In one embodiment a variant antibody is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to one of the antibodies, for example, antibody 12A5-5. In another embodiment, a variant antibody differs from the aforementioned antibodies (for example, 12A5-5) at one, two, three, four fine, six, seven, eight, nine or ten amino acid residues (by substitution or deletion of the amino acid(s). In a further embodiment, one (or more) amino acid is modified post-translationally (for example, by cyclization or conversion to another amino acid; and/or by deamidation, isomerization, glycation and/or oxidation).

In a further ~~aspect~~embodiment of the ~~invention~~disclosure, the antibody light chain constant region is selected from the group consisting of a kappa and a lambda light chain, and the heavy chain constant region is selected from the group consisting of a mu, a delta, a gamma, an alpha, and an epsilon constant region. A further embodiment provides an antibody that antibody belongs to a subclass selected from the group consisting of IgG1, IgG2, IgG3 and IgG4. It is understood that these aspects of the ~~invention~~disclosure apply equally to the previously described aspects and embodiments.

In one aspect, the disclosure provides a composition comprising the myostatin-specific antibody as defined herein and a physiologically acceptable diluent, excipient or carrier.

In one aspect, the disclosure provides an isolated nucleic acid encoding the myostatin-specific antibody as defined herein.

In one aspect, the disclosure provides a vector comprising the nucleic acid as defined herein.

In one aspect, the disclosure provides an isolated host cell transfected or transformed with the vector as defined herein.

In one aspect, the disclosure provides a method for the production of a myostatin-specific antibody comprising culturing the host cell as defined herein under conditions promoting expression and recovering the myostatin-specific antibody from the culture medium.

In one aspect, the disclosure provides the use of a composition as defined herein in the manufacture of a medicament for inhibiting at least one activity of myostatin, wherein the medicament is to be administered to an individual such that at least one activity of myostatin is partially or fully inhibited.

The ~~invention~~disclosure also provides isolated nucleic acid encoding any of the afore-mentioned myostatin-specific antagonists, as well as a vector comprising such nucleic acid, an isolated host cell transfected or transformed with such vector, and a method for the production of a myostatin-specific antagonist comprising culturing such a host cell under conditions promoting expression and recovering the myostatin-specific antagonist from the culture medium. A composition comprising the myostatin-specific antagonist as previously described and a physiologically acceptable diluent, excipient or carrier is also provided, as is method of inhibiting at least one activity of myostatin, comprising administering such a composition to an individual such that at least one activity of myostatin is partially or fully inhibited.

In additional embodiments of the ~~invention~~disclosure, the individual is afflicted with a condition selected from the group consisting of: hypogonadism (including hypogonadism resulting from androgen deprivation therapy, and hypogonadism resulting from age-related decrease in gonadal functioning), cachexia; cardiac cachexia, renal cachexia, cardiac atrophy; cardiac hypotrophy; heat failure; sarcopenia; traumatic bone fracture; osteoporotic fracture; bone loss (for example, osteoporosis or osteopenia); Addison's

Also provided by the present invention are methods of facilitating the healing of traumatic bone fracture, and repair of osteoporotic fracture, as well as treatment of bone loss in general (for example, osteoporosis and/or osteopenia) and as a result of concomitant prolonged inactivity or bed-rest, and/or immobilization of limbs.

Other conditions for which adminstration of a myostatin antagonist would prove beneficial include Addison's disease, amyotrophic lateral sclerosis or motor neuron disease (ALS; MND; Lou Gehrig's disease), Bell's palsy (and/or facial nerve problems), botulism, cerebral palsy, Charcot-Marie-Tooth disease and other peripheral neuropathies, Cushing's syndrome, diabetic neuropathy, Guillan-Barre syndrome, multiple sclerosis, muscular atrophy (including progressive and spinal muscular atrophy), muscular dystrophy (of which there are numerous forms; including myotonic dystrophy), myasthenia gravis, poliomyelitis, polymyositis, sprains and strains of muscles, tendons and/or ligaments, stroke, and other conditions that result in muscle wasting, such as prolonged inactivity or bed-rest, immobilization of limbs (for example, by casting and/or splinting), and space flight.

The myostatin binding proteins of the present invention may also find uses in diagnostic methods. For example, antigen binding proteins of the invention may be "labeled" by association with a detectable marker substance (e.g., radiolabeled with ^{125}I or conjugated to another detectable moiety) to provide reagents useful in detection and quantification of myostatin in solid tissue and fluid samples such as blood or urine. Contemplated herein are kits containing such labeled materials.

The following examples are provided for the purpose of illustrating specific embodiments or features of the instant invention and do not limit its scope.

Example 1

Anti-myostatin Antibodies

Several variant anti-myostatin antibodies were prepared, and tested for activity; their sequences are shown in Tables 1 and 2 below.

Table 1: Anti-Myostatin Antibody Heavy Chain Sequences

HC	FR1	CDR1	FR2
12A5-1	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	NYWMN	WVRQAPGKGLEWVA
12A5-3	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	NYWCN	WVRQAPGKGLEWVA
12A5-5	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	RYWMN	WVRQAPGKGLEWVA
12A5-6	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	RYWMN	WVRQAPGKGLEWVA
12A5-8	EVQLVESGGGLVQPGGSLRLSCAASGFTFR	NYWCN	WVRQAPGKGLEWVA
12A5-9	EVQLVESGGGLVQPGGSLRLSCAASGFTFR	NYWCN	WVRQAPGKGLEWVA

12A5-10	EVQLVESGGGLVQPGGSLRLSCAASGFTFR	NYWCN	WVRQAPGKGLEWVA
12A5-12	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	NYWLN	WVRQAPGKGLEWVA
12A5-18	EVQLVESGGGLVQPGGSLRLSCAASGFTFR	NYWCN	WVRQAPGKGLEWVA

HC	CDR2	FR3
12A5-1	QIRLKSDNYATHYAESVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR
12A5-3	QIRLKSDNYATHYAESVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE
12A5-5	QIRLKSDNYATHYAESVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE
12A5-6	QIRLKSDNYATHYAESVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE
12A5-8	QIRLKSDNYATHYAESVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE
12A5-9	QIRLKSDNYATHYAESVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE
12A5-10	QIRLKSDNYATHYAESVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE
12A5-12	QIRLKSDNYATHYAESVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE
12A5-18	QIRLKSDNYATHYAESVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE

HC	CDR3	FR4
12A5-1	GLDY	WGQGTTVTVSS
12A5-3	GLDY	WGQGTTVTVSS
12A5-5	GLDY	WGQGTTVTVSS
12A5-6	GLDY	WGQGTTVTVSS
12A5-8	GLDY	WGQGTTVTVSS
12A5-9	GLDY	WGQGTTVTVSS
12A5-10	GLDY	WGQGTTVTVSS
12A5-12	GLDY	WGQGTTVTVSS
12A5-18	GLDY	WGQGTTVTVSS

Table 2: Anti-Myostatin Antibody Light Chain Sequences

LC	FR1	CDR1	FR2	CDR2
12A5-1	DIQMTQSPSSLSASVGDRVTITC	KASQDINKYVA	WYQQKPGKAPKLLIY	YTSTLQP
12A5-3	DIQMTQSPSSLSASVGDRVTITC	KASQDINKYVA	WYQQKPGKAPKLLIY	YTSFLQP
12A5-5	DIQMTQSPSSLSASVGDRVTITC	KASQDINKYVA	WYQQKPGKAPKLLIY	YTSWLQP
12A5-6	DIQMTQSPSSLSASVGDRVTITC	KASQDINKYVA	WYQQKPGKAPKLLIY	YTSFLQP
12A5-8	DIQMTQSPSSLSASVGDRVTITC	KASQDINKYVA	WYQQKPGKAPKLLIY	YTKTLQP
12A5-9	DIQMTQSPSSLSASVGDRVTITC	KASQDINKYVA	WYQQKPGKAPKLLIY	YTRTLQP
12A5-10	DIQMTQSPSSLSASVGDRVTITC	KASQDINKYVA	WYQQKPGKAPKLLIY	YTSTLQP
12A5-12	DIQMTQSPSSLSASVGDRVTITC	KASQDINKYVA	WYQQKPGKAPKLLIY	YTSWLQP
12A5-18	DIQMTQSPSSLSASVGDRVTITC	KASQDINKYVA	WYQQKPGKAPKLLIY	YTSHLQP

LC	FR3	CDR3	FR4
12A5-1	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	LQYDNLLYT	FGQGTKLEIK
12A5-3	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	LQYDNLLYT	FGQGTKLEIK
12A5-5	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	LQYDNLLYT	FGQGTKLEIK

TABLE 1

Anti-Myostatin Antibody Heavy Chain Sequences

COMPLETE HEAVY CHAIN (HC) SEQ ID NO:	ANTI-MYOSTATIN ANTIBODY
11	12A5-1
12	12A5-3
13	12A5-5
14	12A5-6
15	12A5-8
16	12A5-9
17	12A5-10
18	12A5-12
19	12A5-18
20	CONSENSUS

ANTI-MYOSTATIN ANTIBODY	HC FR1	HC CDR1	HC FR2
12A5-1	EVQLVESGGGLVQPGGSLRLSCAASGFTFS (SEQ ID NO: 26)	NYWMN (SEQ ID NO: 28)	WVRQAPGKGLEWVA (SEQ ID NO: 32)
12A5-3	EVQLVESGGGLVQPGGSLRLSCAASGFTFS (SEQ ID NO: 26)	NYWCN (SEQ ID NO: 29)	WVRQAPGKGLEWVA (SEQ ID NO: 32)
12A5-5	EVQLVESGGGLVQPGGSLRLSCAASGFTFS (SEQ ID NO: 26)	RYWMN (SEQ ID NO: 30)	WVRQAPGKGLEWVA (SEQ ID NO: 32)

TABLE 1-continued

Anti-Myostatin Antibody Heavy Chain Sequences

12A5-6	EVQLVESGGGLVQPGGSLRLSCAASGFTFS (SEQ ID NO: 26)	RYWMN (SEQ ID NO: 30)	WVRQAPGKGLEWVA (SEQ ID NO: 32)
12A5-8	EVQLVESGGGLVQPGGSLRLSCAASGFTFR (SEQ ID NO: 27)	NYWCN (SEQ ID NO: 29)	WVRQAPGKGLEWVA (SEQ ID NO: 32)
12A5-9	EVQLVESGGGLVQPGGSLRLSCAASGFTFR (SEQ ID NO: 27)	NYWCN (SEQ ID NO: 29)	WVRQAPGKGLEWVA (SEQ ID NO: 32)
12A5-10	EVQLVESGGGLVQPGGSLRLSCAASGFTFR (SEQ ID NO: 27)	NYWCN (SEQ ID NO: 29)	WVRQAPGKGLEWVA (SEQ ID NO: 32)
12A5-12	EVQLVESGGGLVQPGGSLRLSCAASGFTFS (SEQ ID NO: 27)	NYWLN (SEQ ID NO: 31)	WVRQAPGKGLEWVA (SEQ ID NO: 32)
12A5-18	EVQLVESGGGLVQPGGSLRLSCAASGFTFR (SEQ ID NO: 27)	NYWCN (SEQ ID NO: 29)	WVRQAPGKGLEWVA (SEQ ID NO: 32)

ANTI-MYOSTATIN ANTIBODY	HC CDR2	HC FR3
12A5-1	QIRLKSDNYATHYAESVKG (SEQ ID NO: 33)	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR (SEQ ID NO: 34)
12A5-3	QIRLKSDNYATHYAESVKG (SEQ ID NO: 33)	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE (SEQ ID NO: 35)
12A5-5	QIRLKSDNYATHYAESVKG (SEQ ID NO: 33)	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE (SEQ ID NO: 35)
12A5-6	QIRLKSDNYATHYAESVKG (SEQ ID NO: 33)	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE (SEQ ID NO: 35)
12A5-8	QIRLKSDNYATHYAESVKG (SEQ ID NO: 33)	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE (SEQ ID NO: 35)
12A5-9	QIRLKSDNYATHYAESVKG (SEQ ID NO: 33)	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE (SEQ ID NO: 35)
12A5-10	QIRLKSDNYATHYAESVKG (SEQ ID NO: 33)	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE (SEQ ID NO: 35)
12A5-12	QIRLKSDNYATHYAESVKG (SEQ ID NO: 33)	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE (SEQ ID NO: 35)
12A5-18	QIRLKSDNYATHYAESVKG (SEQ ID NO: 33)	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCTE (SEQ ID NO: 35)

ANTI-MYOSTATIN ANTIBODY	HC CDR3	HC FR4
12A5-1	GLDY (SEQ ID NO: 36)	WGQGTTVTVSS (SEQ ID NO: 37)
12A5-3	GLDY (SEQ ID NO: 36)	WGQGTTVTVSS (SEQ ID NO: 37)
12A5-5	GLDY (SEQ ID NO: 36)	WGQGTTVTVSS (SEQ ID NO: 37)
12A5-6	GLDY (SEQ ID NO: 36)	WGQGTTVTVSS (SEQ ID NO: 37)
12A5-8	GLDY (SEQ ID NO: 36)	WGQGTTVTVSS (SEQ ID NO: 37)
12A5-9	GLDY (SEQ ID NO: 36)	WGQGTTVTVSS (SEQ ID NO: 37)
12A5-10	GLDY (SEQ ID NO: 36)	WGQGTTVTVSS (SEQ ID NO: 37)
12A5-12	GLDY (SEQ ID NO: 36)	WGQGTTVTVSS (SEQ ID NO: 37)
12A5-18	GLDY (SEQ ID NO: 36)	WGQGTTVTVSS (SEQ ID NO: 37)

TABLE 2

Anti-Myostatin Antibody Light Chain Sequences

COMPLETE LIGHT CHAIN (LC) SEQ ID NO:	ANTI-MYOSTATIN ANTIBODY
1	12A5-1
2	12A5-3

TABLE 2-continued

Anti-Myostatin Antibody Light Chain Sequences

3	12A5-5
4	12A5-6
5	12A5-8
6	12A5-9
7	12A5-10
8	12A5-12
9	12A5-18
10	CONSENSUS

ANTI-MYOSTATIN ANTIBODY	LC FR1	LC CDR1	LC FR2	LC CDR2
12A5-1	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 38)	KASQDINKYVA (SEQ ID NO: 39)	WYQQKPGKAPKLLIY (SEQ ID NO: 40)	YTSTLQP (SEQ ID NO: 41)
12A5-3	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 38)	KASQDINKYVA (SEQ ID NO: 39)	WYQQKPGKAPKLLIY (SEQ ID NO: 40)	YTSFLQP (SEQ ID NO: 42)
12A5-5	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 38)	KASQDINKYVA (SEQ ID NO: 39)	WYQQKPGKAPKLLIY (SEQ ID NO: 40)	YTSWLQP (SEQ ID NO: XX43)
12A5-6	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 38)	KASQDINKYVA (SEQ ID NO: 39)	WYQQKPGKAPKLLIY (SEQ ID NO: 40)	YTSFLQP (SEQ ID NO: 42)
12A5-8	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 38)	KASQDINKYVA (SEQ ID NO: 39)	WYQQKPGKAPKLLIY (SEQ ID NO: 40)	YTKTLQP (SEQ ID NO: 44)
12A5-9	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 38)	KASQDINKYVA (SEQ ID NO: 39)	WYQQKPGKAPKLLIY (SEQ ID NO: 40)	YTRTLQP (SEQ ID NO: 45)
12A5-10	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 38)	KASQDINKYVA (SEQ ID NO: 39)	WYQQKPGKAPKLLIY (SEQ ID NO: 40)	YTSTLQP (SEQ ID NO: 41)
12A5-12	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 38)	KASQDINKYVA (SEQ ID NO: 39)	WYQQKPGKAPKLLIY (SEQ ID NO: 40)	YTSWLQP (SEQ ID NO: 43)
12A5-18	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 38)	KASQDINKYVA (SEQ ID NO: 39)	WYQQKPGKAPKLLIY (SEQ ID NO: 40)	YTSHLQP (SEQ ID NO: 46)

ANTI-MYOSTATIN ANTIBODY	LC FR3	LC CDR3	LC FR4
12A5-1	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC (SEQ ID NO: 47)	LQYDNLLYT (SEQ ID NO: 48)	FGQGTKLEIK (SEQ ID NO: 51)
12A5-3	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC (SEQ ID NO: 47)	LQYDNLLYT (SEQ ID NO: 48)	FGQGTKLEIK (SEQ ID NO: 51)
12A5-5	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC (SEQ ID NO: 47)	LQYDNLLYT (SEQ ID NO: 48)	FGQGTKLEIK (SEQ ID NO: 51)
12A5-6	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC (SEQ ID NO: 47)	LQYDALLYT (SEQ ID NO: 49)	FGQGTKLEIK (SEQ ID NO: 51)
12A5-8	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC (SEQ ID NO: 47)	LQYDNLLYT (SEQ ID NO: 48)	FGQGTKLEIK (SEQ ID NO: 51)
12A5-9	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC (SEQ ID NO: 47)	LQYDNLLYT (SEQ ID NO: 48)	FGQGTKLEIK (SEQ ID NO: 51)
12A5-10	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC (SEQ ID NO: 47)	LQYDNKLYT (SEQ ID NO: 50)	FGQGTKLEIK (SEQ ID NO: 51)
12A5-12	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC (SEQ ID NO: 47)	LQYDNLLYT (SEQ ID NO: 48)	FGQGTKLEIK (SEQ ID NO: 51)
12A5-18	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC (SEQ ID NO: 47)	LQYDNLLYT (SEQ ID NO: 48)	FGQGTKLEIK (SEQ ID NO: 51)

12A5-6	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	LQYDALLYT	FGQGTKLEIK
12A5-8	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	LQYDNLLYT	FGQGTKLEIK
12A5-9	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	LQYDNLLYT	FGQGTKLEIK
12A5-10	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	LQYDNKLYT	FGQGTKLEIK
12A5-12	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	LQYDNLLYT	FGQGTKLEIK
12A5-18	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	LQYDNLLYT	FGQGTKLEIK

Example 2

***In vitro* Assays**

The activity(ies) of anti-myostatin antibodies are evaluated in several assays.

C2C12 Cell Based Myostatin Activity Assay

This assay demonstrates the myostatin neutralizing capability of the inhibitor being tested by measuring the extent that binding of myostatin to its receptor is inhibited.

A myostatin-responsive reporter cell line was generated by transfection of C2C12 myoblast cells (ATCC No: CRL-1772) with a pMARE-luc construct. The pMARE-luc construct was made by cloning twelve repeats of the CAGA sequence, representing the myostatin/activin response elements (Dennler et al. EMBO 17: 3091-3100 (1998)) into a pLuc-MCS reporter vector (Stratagene cat # 219087) upstream of the TATA box. The myoblast C2C12 cells naturally express myostatin/activin receptors on the cell surface. When myostatin binds the cell receptors, the Smad pathway is activated, and phosphorylated Smad binds to the response element (Macias-Silva et al. Cell 87:1215 (1996)), resulting in the expression of the luciferase gene. Luciferase activity is then measured using a commercial luciferase reporter assay kit (cat # E4550, Promega, Madison, WI) according to manufacturer's protocol. A stable line of C2C12 cells that had been transfected with pMARE-luc (C2C12/pMARE clone #44) was used to measure myostatin activity according to the following procedure.

Equal numbers of the reporter cells (C2C12/pMARE clone #44) are plated into 96 well cultures. Recombinant mature myostatin is pre-incubated for one hour at room temperature with antibodies to be tested. The reporter cell culture is treated with the myostatin with or without antibodies for six hours. Myostatin activity is measured by determining the luciferase activity in the treated cultures. This assay can be used to initially identify antibodies that inhibit myostatin signaling activity; titration curves can be generated using varied concentrations of antibody with fixed concentration of myostatin. Such titration curves are used to determine IC_{50} values for a number of the antibodies, as shown in Table 3 below.

Claims

We claim:

1. An isolated myostatin-specific antibody comprising at least one light chain and at least one heavy chain, wherein the light chain comprises a constant region and a variable region that comprises three complementarity determining regions (CDRs) and the heavy chain comprises a constant region and a variable region that comprises three CDRs, wherein the light chain CDRs are those disclosed in SEQ ID NO:10, and the heavy chain CDRs are those disclosed in SEQ ID NO:20.

2. The antibody of claim 1, wherein the light chain CDRs are selected from the group consisting of:
   a) the light chain CDRs disclosed in SEQ ID NO:1;
   b) the light chain CDRs disclosed in SEQ ID NO:2;
   c) the light chain CDRs disclosed in SEQ ID NO:3;
   d) the light chain CDRs disclosed in SEQ ID NO:4;
   e) the light chain CDRs disclosed in SEQ ID NO:5;
   f) the light chain CDRs disclosed in SEQ ID NO:6;
   g) the light chain CDRs disclosed in SEQ ID NO:7;
   h) the light chain CDRs disclosed in SEQ ID NO:8; and
   i) the light chain CDRs disclosed in SEQ ID NO:9;
   and wherein the heavy chain CDRs are selected from the group consisting of:
   a') the heavy chain CDRs disclosed in SEQ ID NO:11;
   b') the heavy chain CDRs disclosed in SEQ ID NO:12;
   c') the heavy chain CDRs disclosed in SEQ ID NO:13;
   d') the heavy chain CDRs disclosed in SEQ ID NO:14;
   e') the heavy chain CDRs disclosed in SEQ ID NO:15;
   f') the heavy chain CDRs disclosed in SEQ ID NO:16;
   g') the heavy chain CDRs disclosed in SEQ ID NO:17;
   h') the heavy chain CDRs disclosed in SEQ ID NO:18; and
   i') the heavy chain CDRs disclosed in SEQ ID NO:19.

3. The antibody of claim 1, wherein the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO:10, and the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO:20.

4. The antibody of claim 3, wherein the light chain variable region is selected from the group consisting of:
   a) the light chain variable region disclosed in SEQ ID NO:1;
   b) the light chain variable region disclosed in SEQ ID NO:2;
   c) the light chain variable region disclosed in SEQ ID NO:3;
   d) the light chain variable region disclosed in SEQ ID NO:4;
   e) the light chain variable region disclosed in SEQ ID NO:5;
   f) the light chain variable region disclosed in SEQ ID NO:6;
   g) the light chain variable region disclosed in SEQ ID NO:7;
   h) the light chain variable region disclosed in SEQ ID NO:8; and
   i) the light chain variable region disclosed in SEQ ID NO:9;
   and wherein the heavy chain variable region is selected from the group consisting of:
   a') the heavy chain variable region disclosed in SEQ ID NO:11;
   b') the heavy chain variable region disclosed in SEQ ID NO:12;
   c') the heavy chain variable region disclosed in SEQ ID NO:13;
   d') the heavy chain variable region disclosed in SEQ ID NO:14;
   e') the heavy chain variable region disclosed in SEQ ID NO:15;
   f') the heavy chain variable region disclosed in SEQ ID NO:16;
   g') the heavy chain variable region disclosed in SEQ ID NO:17;
   h') the heavy chain variable region disclosed in SEQ ID NO:18; and
   i') the heavy chain variable region disclosed in SEQ ID NO:19.

5. An isolated myostatin-specific antibody that binds myostatin with a K_d of less than 100 pM.

6. The isolated myostatin-specific antibody of claim 5, that binds GDF-11 with a K_d of greater than 10nM.

7. An isolated myostatin-specific antibody that binds myostatin with an affinity at least 5,000 times greater than its affinity for GDF-11.

8. An isolated myostatin-specific antibody that exhibits selectivity for myostatin that is at least 5,000 times greater than for GDF-11.

9. An isolated myostatin-specific antibody that binds myostatin and blocks the interaction of myostatin with ALK4.

10. The isolated myostatin-specific antibody of claim 9 that binds myostatin and blocks the interaction of myostatin with ALK4 but co-binds with a myostatin/ActRIIA complex and/or a myostatin/ActRIIB complex.

11. An isolated myostatin-specific antibody that binds myostatin, wherein two regions in myostatin that are required for binding of myostatin to the myostatin-specific antibody locate at sequence near position 21 to 31 and position 50 to 60 of mature myostatin (SEQ ID NO:25)

12. An isolated myostatin-specific antibody that interacts with two regions in myostatin located at sequence near position 21 to 31 and position 50 to 60 of mature myostatin, so as to prevent chymotrypsin cleavage of peptide bonds within these regions.

13. The antibody of any one of claims 1 – 12, wherein the light chain constant region is selected from the group consisting of a kappa and a lambda light chain, and the heavy chain constant region is selected from the group consisting of a mu, a delta, a gamma, an alpha, and an epsilon constant region.

14. The antibody of claim 13, wherein the antibody belongs to a subclass selected from the group consisting of IgG1, IgG2, IgG3 and IgG4.

15. An isolated nucleic acid encoding the myostatin-specific antagonist according to any one of claims 1 – 14.

16. A vector comprising the nucleic acid of claim 15.

17. An isolated host cell transfected or transformed with the vector of claim 16.

18. A method for the production of a myostatin-specific antagonist comprising culturing the host cell of claim 17 under conditions promoting expression and recovering the myostatin-specific antagonist from the culture medium.

19. A composition comprising the myostatin-specific antagonist of any one of claims 1 through 16 and a physiologically acceptable diluent, excipient or carrier.

20. A method of inhibiting at least one activity of myostatin, comprising administering a composition according to claim 19 to an individual such that at least one activity of myostatin is partially or fully inhibited.

21. The method of claim 20, wherein the individual is afflicted with a condition selected from the group consisting of: hypogonadism (including hypogonadism resulting from androgen deprivation therapy, and hypogonadism resulting from age-related decrease in gonadal functioning); cachexia; cardiac cachexia; renal cachexia; cardiac atrophy; cardiac hypotrophy; heart failure; sarcopenia; traumatic bone fracture; osteoporotic fracture; bone loss (for example, osteoporosis or osteopenia); Addison's disease; amyotrophic lateral sclerosis or motor neuron disease (ALS; MND; Lou Gehrig's disease); Bell's palsy (and/or facial nerve problems); botulism; cerebral palsy; Charcot-Marie-Tooth disease and other peripheral neuropathies; Cushing's syndrome; diabetic neuropathy; Guillan-Barre syndrome; multiple sclerosis; muscular atrophy (including progressive and spinal muscular atrophy); muscular dystrophy (including including Becker's muscular dystrophy, congenital muscular dystrophy, Duchenne muscular dystrophy, distal muscular dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophy, myotonic muscular dystrophy, oculopharyngeal muscular dystrophy, spinal muscular atrophy, Brown-Vialetto-Van Laere syndrome (BVVL), Fazio-Londe (FL) syndrome, and other syndromes characterized by progressive skeletal muscle weakness, defects in muscle proteins, and the death of muscle cells and tissue); myasthenia gravis; poliomyelitis; polymyositis; sprains and strains of muscles, tendons and/or ligaments; stroke (and other conditions that result in muscle wasting, such as prolonged inactivity or bed-rest, immobilization of limbs [for example, by casting and/or splinting] and space flight); and conditions treatable by growth hormone, insulin growth factor-1 (IGF-1), growth hormone secretagogues, and other agents related to the growth hormone- IGF-1 axis.

We claim:

1. An isolated myostatin-specific antibody comprising at least one light chain and at least one heavy chain, wherein the light chain comprises a constant region and a variable region that comprises three complementarity determining regions (CDRs) and the heavy chain comprises a constant region and a variable region that comprises three CDRs, wherein the light chain CDRs are those disclosed in SEQ ID NO:39, SEQ ID NO:43, and SEQ ID NO:48 , and the heavy chain CDRs are those disclosed in in SEQ ID NO:30, SEQ ID NO:33, and SEQ ID NO:36.

2. The antibody of claim 1, wherein the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO:3, and the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO:13 wherein residue 50 of SEQ ID NO:13 is the amino acid Glutamine.

3. The antibody of claim 1, wherein the light chain constant region is selected from the group consisting of a kappa and a lambda light chain, and the heavy chain constant region is selected from the group consisting of a mu, a delta, a gamma, an alpha, and an epsilon constant region.

4. The antibody of claim 3, wherein the antibody belongs to a subclass selected from the group consisting of IgG1, IgG2, IgG3 and IgG4.

5. A composition comprising the myostatin-specific antibody of claim 1and a physiologically acceptable diluent, excipient or carrier.

6. An isolated nucleic acid encoding the myostatin-specific antibody according to claim 1.

7. A vector comprising the nucleic acid of claim 6.

8. An isolated host cell transfected or transformed with the vector of claim 7.

9. A method for the production of a myostatin-specific antibody comprising culturing the host cell of claim 8 under conditions promoting expression and recovering the myostatin-specific antibody from the culture medium.

10. Use of a composition according to claim 5 in the manufacture of a medicament for inhibiting at least one activity of myostatin, wherein the medicament is to be administered to an individual such that at least one activity of myostatin is partially or fully inhibited.