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(54) **Title:** ANTI-MYOSTATIN ANTIBODIES

(57) **Abstract:** A neutralizing epitope is identified within amino acids 40-64 of the mature form of human myostatin. Antibodies that bind this epitope with high affinity preferentially bind GDF-8 over GDF-11 and may be chimeric, humanized or fully human antibodies, immunoconjugates of the antibodies or antigen-binding fragments thereof. The antibodies of the invention are useful for increasing muscle mass, increasing bone density, or for the treatment of various disorders in mammalian and avian species.

ANTI-MYOSTATIN ANTIBODIES

FIELD OF THE INVENTION

5   The invention is in the field of medicine, particularly in the field of monoclonal

antibodies against myostatin. More specifically the invention relates to high affinity

chimeric, humanized or human anti-myostatin antibodies that preferentially bind

myostatin over GDF-11 and use of the antibodies for therapy, prophylaxis or diagnosis of

various disorders or conditions in mammalian and avian species.

10

BACKGROUND OF THE INVENTION

          Members of the transforming growth factor beta (TGF-β) superfamily of proteins

are involved in embryonic development and adult tissue homeostasis. The TGF-β

15   superfamily members share a common structure including a peptide signal sequence

required for secretion of the protein and an amino-terminal fragment that is proteolytically

cleaved about 105–140 amino acids from the carboxy-terminus of the large precursor

protein to produce the mature protein. The mature protein is characterized by highly

conserved cysteine residues, while the active form of the mature protein is a disulfide-

20   linked homodimer of the proteolytically-cleaved proprotein (Gray, A., and Maston, A.,

Science, 247:1328, 1990).

          Myostatin, also referred to as growth differentiation factor-8 (GDF-8), is a

member of the TGF-β superfamily of proteins. Myostatin shares structural similarities

with other TGF-β family members. It contains a hydrophobic amino-terminus that acts as

25   a secretory signal and a conserved RSRR domain that is important for proteolytic

processing. Cleavage of the protein gives rise to an amino-terminal latency associated

peptide and a carboxy-terminal mature signaling peptide which forms the biologically

active homodimer. Myostatin is expressed largely in developing and adult skeletal

muscle and functions as a negative regulator of skeletal muscle. Systemic over-

30   expression of myostatin in adult mice leads to muscle wasting (Zimmers, et al., *Science*,

296:1486-1488, 2002) while conversely, a myostatin knock-out mouse is characterized by

hypertrophy and hyperplasia of the skeletal muscle resulting in two- to threefold greater

muscle mass than their wild type littermates and a decrease in fat accumulation
(McPherron, et al. *Nature*, 387:83-90, 1997). A human with a myostatin knock-out
mutation was reported to be associated with gross muscle hypertrophy (Scheulke, et al.,
New Eng. J. Med. 350:2682, 2004).

5   There are presently limited treatments available for muscle wasting or for
disorders or conditions which would benefit from an increase in muscle mass and/or
muscle strength including, for example, muscular dystrophy, frailty, disuse atrophy and,
cachexia, as well as disorders which are associated with muscle wasting, for example,
renal disease, cardiac failure or disease, and liver disease. Due to its role as a negative

10   regulator of skeletal muscle growth, myostatin is a desirable target for therapeutic or
prophylactic intervention for such disorders or conditions or for monitoring progression of
such disorders or conditions. Apart from its direct role in skeletal muscle regulation,
myostatin may also be involved in other physiological processes including preadipocyte
differentiation to adipocytes (Kim et al. *BBRC*, 281:902-906, 2001), and, indirectly, with

15   glucose homeostasis (McPherron, A and Lee S-J. *JCI* 109:595, 2002) and inhibition of
bone formation (Hamrick, M. *Mol. Cell Evol. Biol.* 272 388-91, 2003; Hamrick et al.
Calcif Tissue Int. 71:63, 2002). Therefore, myostatin-specific antagonists, e.g.,
myostatin-specific antibodies, may also prove useful for treating, preventing or
monitoring disorders or conditions such as those which benefit from increasing bone

20   density (*e.g.*, osteoporosis), Type II diabetes, metabolic syndrome, obesity and
osteoarthritis.

         Myostatin is highly conserved across species; the amino acid sequence of the
mature form of myostatin in human, mouse, rat, chicken, turkey and cow are 100%
identical (See Figs. 2 and 3). There are naturally occurring myostatin mutations in cattle,

25   which have been linked to a double-muscled phenotype (McPherron, et al. *PNAS*,
94:12457-61, 1997). Since myostatin is highly conserved in sequence and in function
across species, not only does an anti-myostatin antibody provide a promising means of
increasing muscle mass, or treatment or prevention of such disorders or conditions listed
above in humans, but also in other mammals including, *e.g.*, domestic animals (*e.g.*,

30   canine and feline), sports animals (*e.g.*, equine), food-source animals (*e.g.*, bovine,
porcine and ovine) and in avian species (*e.g.*, chicken, turkey, duck and other game birds
and poultry).

Growth differentiation factor-11, also referred to as GDF-11 or BMP-11, is the member of the TGF-β superfamily of proteins that is most homologous to myostatin. The amino acid sequence of the mature forms of human myostatin and GDF-11 are about 90% identical; however, GDF-11 is expressed in a wider range of tissues than is GDF-8

5   including dental pulp, brain, heart, kidney and lung as well as muscle and adipose tissue (Nakashima, et al. *Mech. of Development* 80:185, 1999). GDF-11 knock-out mice die within 24 hours of birth with multiple abnormalities. In particular, the mice exhibit extra pairs of ribs, lack kidneys and show defects in the stomach, spleen and pancreas (McPherron et al., *Nature Genetics* 22:260, 1999; Esquela and Lee, *Dev. Biol.* 257:356,

10   2003; Harmon et al., *Devpt.* 131:6163, 2004). Human GDF-11 has recently been found to govern the temporal windows during which multipotent progenitors retain competence to produce distinct neural progeny (Kim, J. et al. *Science* 308:1927-1930, 2005).

There is a therapeutic need to specifically inhibit a myostatin activity while not inhibiting, or minimally inhibiting, an activity of other TGF-β superfamily proteins,

15   particularly GDF-11, in order to minimize the possibility of undesirable side effects resulting from binding of the myostatin antagonist to another TGF-β superfamily protein. Furthermore, there is a diagnostic need for an anti-myostatin antibody that does not cross-react, or minimally cross-reacts, with another TGF-β superfamily protein, particularly GDF-11, in order to more accurately monitor or determine myostatin levels in a sample.

20   Furthermore, there is a need for myostatin-specific antibodies which specifically and preferentially bind myostatin with a high affinity and thereby allow the dosage level that patients receive to be minimized which may thereby result in less frequent dosing with such an antibody than with an antibody that binds myostatin with a lesser affinity (i.e., a higher K_D). A high affinity antibody is also desirable in that it may allow for more

25   flexibility in the route of administration of the antibody to a patient since it is less desirable for a drug to be administered intravenously than subcutaneously for example. There is also a need for myostatin-specific antibodies with a low or otherwise favorable IC_{50} value in a myostatin bioactivity assay in order to generate a therapeutic anti-myostatin antibody with a minimum effective therapeutic dose. It is also desirable to

30   provide antibodies specific to myostatin where any immune response to the antibody evoked by a patient receiving the antibody is reduced to a minimum. The present invention satisfies these needs and provides related advantages.

SUMMARY OF THE INVENTION

Antibodies of the invention are chimeric, humanized, or fully human anti-
5 myostatin monoclonal antibodies, and antigen-binding portions thereof, that antagonize or
neutralize at least one in vitro or in vivo biological activity or property associated with
myostatin or a portion thereof.

In one embodiment, antibodies of the invention that specifically and/or
preferentially bind myostatin are significantly less reactive with GDF-11 than with
10 myostatin, i.e., a monoclonal antibody of the invention binds myostatin at least about 2, 3,
5, 10, 20, 22, 24, or 25-times greater than it binds GDF-11 as measured by a technique in
the art e.g., by competition ELISA, or by BIACORE or KINEXA assay to demonstrate
higher affinity (i.e., lower K_D) of the antibody to GDF-8 than GDF-11. Most preferably
the antibodies of the invention, when expressed as Fabs, do not bind GDF-11 above
15 background levels in any binding assay available in the art. Preferably antibodies of the
invention specifically bind myostatin within the domain spanning amino acids 40-64
[ANYCSGECEFVFLQKYPHTHLVHQA for human], 43-57 [CSGECEFVFLQKYPH
OR CSGESEFVFLQKYPH for human] and/or 45-59 [GECEFVFLQKYPHTH for
human] of mature myostatin or they specifically bind a polypeptide consisting of amino
20 acids 40-64, 43-57 and/or amino acids 45-59 of mature myostatin.

In one embodiment, antibodies of the invention have an IC_{50} of less than, or equal
to, about 25 nM, 20 nM, 16 nM, 14 nM, 10 nM, 9 nM, 6 nM or 5.2 nM in an in vitro
myostatin/SBE reporter assay (see Example 6). Preferably, such antibodies of the
invention are further characterized in that they are significantly less reactive with GDF-11
25 than with GDF-8, i.e., binds myostatin at least about 2, 3, 5, 10, 20, 22, 24, or 25-times
greater than it binds GDF-11 as measured by a technique in the art e.g., by competition
ELISA, or by BIACORE or KINEXA assay to demonstrate higher affinity (i.e., lower K_D)
of the antibody to GDF-8 than GDF-11. and even more preferably they bind myostatin
within the domain spanning amino acids 40-64, 43-57 and/or 45-59 of mature myostatin
30 or they bind a polypeptide consisting of amino acids 40-64, 43-57 and/or 45-59 of mature
myostatin.

5

In one embodiment, antibodies of the invention are characterized by a strong binding affinity (K_D) for myostatin, i.e., less than about 3×10^{-8} M, 1×10^{-8} M or 1×10^{-9} M, preferably less than about 9×10^{-10} M, 8.7×10^{-10} M or most preferably, less than about 8×10^{-11} M. Alternatively, the antibodies of the invention are characterized by a K_D for

5   myostatin of no greater than about 3×10^{-8} M, 1×10^{-8} M, 1×10^{-9} M or 9×10^{-10} M, more preferably no greater than about 8.7×10^{-10} M and most preferably no greater than about 8×10^{-11} M. Preferably, antibodies of the invention characterized by a strong binding affinity as described above also have an IC_{50} of less than 25 nM, 20 nM, 16 nM, 14 nM, 10 nM, 9 nM, 6 nM, or 5.2 nM in an in vitro myostatin/SBE reporter assay and/or are

10   significantly less reactive with GDF-11 than with GDF-8. Even more preferably they bind myostatin within the domain spanning amino acids 40-64, 43-57 and/or 45-59 of mature myostatin and/or they bind a polypeptide consisting of amino acids 40-64, 43-57 and/or 45-59 of mature myostatin.

In another embodiment, an anti-myostatin monoclonal antibody of the invention

15   comprises a light chain variable region ("LCVR") polypeptide with an amino acid sequence selected from the group consisting of SEQ ID NOs: 5-12. In another embodiment, an anti-myostatin monoclonal antibody of the invention comprises a heavy chain variable region ("HCVR") polypeptide with an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-26, 55 and 56. The sequences associated with

20   each SEQ ID Number are shown in Figures 5-9 herein.

In another embodiment, a monoclonal antibody of the invention is one which can compete for binding to human myostatin with a competing antibody as demonstrated by an available assay in the art (e.g., a competition ELISA), wherein the competing antibody comprises two polypeptides with the sequences selected from the group consisting of: (i)

25   SEQ ID NOs: 5 and 15, (ii) SEQ ID NOs: 5 and 16, and (iii) SEQ ID NOs: 10 and 26. Preferably, an antibody of the invention which competes with the competing antibody defined above is further characterized by preferentially binding GDF-8 over GDF-11; even more preferably they bind myostatin within the domain spanning amino acids 40-64, 43-57 and/or 45-59 of mature myostatin and/or they bind a polypeptide consisting of

30   amino acids 40-64, 43-57 and/or 45-59 of mature myostatin. Preferably, an antibody of the invention which competes with the competing antibody defined above is further characterized by having a K_D for myostatin of no greater than about 3×10^{-8} M, 1×10^{-8}

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M, 1×10^{-9} M or 9×10^{-10} M, 8.7×10^{-10} M or 8×10^{-11} M. Preferably, an antibody of the invention which competes with the competing antibody defined above is further characterized by having an IC_{50} of less than 25 nM, 20 nM, 16 nM, 14 nM, 10 nM, 9 nM, 6 nM, or 5.2 nM in an in vitro myostatin/SBE reporter assay.

5   In one embodiment, an anti-myostatin antibody of the invention has a heavy and a light chain variable region, wherein the heavy chain variable region comprises CDR regions with the following amino acid sequences: CDRH1 (SEQ ID NO: 59), CDRH2 (SEQ ID NO: 60) and CDRH3 (SEQ ID NO: 61); and/or wherein the light chain variable region comprises CDR regions with the following amino acid sequences: CDRL1 (SEQ

10   ID NO: 57), CDRL2 (SEQ ID NO: 30) and CDRL3 (SEQ ID NO: 58).

An anti-myostatin monoclonal antibody of the invention may further comprise a heavy chain constant region selected from the group consisting of human (or substantially of human origin) IgG_1, IgG_2, IgG_3, IgG_4, IgA, IgE, IgM and IgD, preferably IgG_1 or IgG_4. An anti-myostatin monoclonal antibody of the invention may further comprise a human

15   kappa or lambda light chain constant region. When the antibody is to be used as a therapeutic in a human, the constant region is preferably substantially of human origin. When the antibody is to be used as a therapeutic in a non-human animal, or egg of a non-human animal, the constant region preferably substantially originates from the animal in which the antibody is to be used as a therapeutic. (see, e.g., Clarkson, C. et al., *Mol. Imm.*

20   30:1195-1204, 1993; U.S. application number 2002/01651350; and Genbank accession numbers X69797, U03778, X16701, X07174, AB016711).

Various forms of the antibody are contemplated herein. For example, an anti-myostatin monoclonal antibody of the invention may comprise or consist of an intact antibody (*i.e.*, full-length, having an intact Fc region), a substantially intact antibody, an

25   antigen-binding portion thereof (e.g., a Fab, Fab', $F(ab')_2$) or a single chain Fv fragment. It is understood that all such forms of the antibodies are encompassed herein and throughout within the term "antibody." Furthermore, an antibody of the invention may be labeled with a detectable label, immobilized on a solid phase and/or conjugated with a heterologous compound, e.g., an enzyme or polyethylene glycol molecule. Furthermore,

30   antibodies of the invention are contemplated to be "monoclonal" even though they may differ in glycosylation pattern.

Diagnostic, therapeutic and prophylactic uses for the monoclonal antibodies of the invention are contemplated herein. In one diagnostic application, the invention provides a method for determining the presence and/or quantity of myostatin protein comprising exposing a test sample suspected of containing the myostatin protein to an anti-myostatin antibody of the invention and determining specific binding of the antibody to the sample. An anti-myostatin antibody of the invention may be used to determine the levels of myostatin in test samples by comparing test sample values to a standard curve generated by binding said antibody to samples with known amounts of myostatin using any method available in the art, e.g., an ELISA assay. The invention further provides a kit comprising an antibody of the invention and, preferably, instructions for using the antibody to detect myostatin protein in e.g., a test sample.

In another embodiment, the invention provides a pharmaceutical composition comprising an anti-myostatin monoclonal antibody of the invention. The pharmaceutical composition of the invention may further comprise a pharmaceutically acceptable carrier. In said pharmaceutical composition, the anti-myostatin monoclonal antibody of the invention is the active ingredient. Preferably the pharmaceutical composition comprises a homogeneous or substantially homogeneous population of an anti-myostatin monoclonal antibody of the invention. The composition for therapeutic use is sterile and may be lyophilized, preferably supplied with an appropriate diluent.

The invention provides a method of inhibiting at least one myostatin biological activity in an animal, preferably a mammalian or avian species, preferably a human, in need thereof, comprising administering a therapeutically effective amount, or prophylactically effective amount, or myostatin-neutralizing or myostatin-inhibiting amount of an anti-myostatin monoclonal antibody of the invention to said mammalian or avian species. The invention further provides a method of enhancing muscle mass or treating or preventing a disease or disorder or condition ameliorated by neutralizing or antagonizing a myostatin bioactivity that comprises administering to a patient (e.g., a human) in need of such treatment or prevention a therapeutically or prophylactically effective amount of a monoclonal antibody of the invention.

The invention embodies an anti-myostatin monoclonal antibody of the invention for use in the manufacture of a medicament for administration to a mammal, preferably a human, for the treatment of e.g., frailty, cachexia, age-related sarcopenia, muscle wasting

or weakness, myopathy, muscular dystrophy, osteoporosis, obesity, COPD, renal failure
or disease, liver failure or disease, cardiac failure or disease, metabolic syndrome and
Type II diabetes in a mammal, preferably a human, in need thereof by administering to
said mammal a therapeutically effective or prophylactically effective amount of an anti-
5   myostatin monoclonal antibody of the invention.

The invention embodies an article of manufacture comprising a packaging
material and an antibody of the invention contained within said packaging material and
wherein the packaging material comprises a package insert which preferably indicates that
the antibody specifically neutralizes or antagonizes a myostatin activity or decreases the
10   level of myostatin. Optionally, the package insert further indicates that the antibody
preferentially-neutralizes or antagonizes a myostatin activity with respect to a GDF-11
activity or preferentially decreases the level of myostatin with respect to decreasing the
level of GDF-11 by preferentially binding myostatin with respect to binding GDF-11.

The invention further provides isolated nucleic acid encoding an antibody of the
15   invention; a vector (or vectors) comprising that nucleic acid, optionally operably linked to
control sequences recognized by a host cell transformed with the vector; a host cell
comprising that vector; a process for producing an antibody of the invention comprising
culturing the host cell so that the nucleic acid is expressed and, optionally, recovering the
antibody from the host cell medium.
20

BRIEF DESRIPTION OF THE DRAWINGS

FIG. 1 shows the amino acid sequence of human promyostatin with the signal
sequence underlined and the portion of the protein at the carboxy-terminus that makes up
a monomer of the mature form of myostatin in bold letters.
25   FIG. 2 shows the amino acid sequence of the monomeric human mature myostatin.
Active human myostatin is a homodimer of this polypeptide associated by disulfide
bonds. The antigenic epitope of the present invention is underlined. This sequence is
identical to that of the mature myostatin in mouse, rat, chicken, turkey, dog, horse, and
pig.
30   FIG. 3 shows an alignment of the amino acid sequence of mature myostatin of
various mammalian and avian species.

FIG. 4 shows the alignment of the amino acid sequence of the mature form of human myostatin and human GDF-11 with the antigenic epitope of the present invention underlined and the residues within the antigenic epitope that differ between myostatin and GDF-11 in bold print.

5   FIG. 5 shows the alignment of the amino acid sequence of HCVRs with a VH2-70 framework. The CDR domains are in bold print.

FIG. 6 shows the alignment of the amino acid sequence of HCVRs with a VH4-39 framework. The CDR domains are in bold print.

FIGS. 7 and 8 show the alignment of the amino acid sequence of LCVRs with an

10   O2 framework. The CDR domains are in bold print.

FIG. 9 shows the amino acid sequence of CDRs of the LCVR and HCVR of various antibodies of the invention.

FIG. 10 shows the amino acid sequence of the LCVR of various murine anti-myostatin antibodies, any of which may be an exemplary parent molecule for antibodies

15   of the invention. See U.S. patent applications 60/559,621 and 60/555,456 incorporated herein.

FIG. 11 shows the amino acid sequence of the HCVR of various murine anti-myostatin antibodies, any of which may be an exemplary parent molecule for antibodies of the invention. See U.S. patent applications 60/559,621 and 60/555,456.

20

DETAILED DESCRIPTION OF THE INVENTION

The invention presents anti-myostatin monoclonal antibodies able to specifically bind to mammalian or avian myostatin (proprotein or mature protein or a portion thereof; monomeric or homodimeric), wherein the antibodies are further characterized as being

25   chimeric, humanized or fully human antibodies or antigen-binding portions thereof able to neutralize or antagonize at least one myostatin activity in vitro and/or in vivo; preferably demonstrating an IC50 less than about 25 nM, 20 nM, 16 nM, 14 nM, 10 nM, 9 nM, 6 nM, or 5.2 nM in a myostatin/SBE reporter assay and/or preferably demonstrating a high

30   binding affinity with myostatin. The antibodies of the invention are further characterized in that they bind myostatin within the domain spanning the amino acids at residues 40-64

of the mature form of myostatin (*e.g.*, SEQ ID NO: 53) and are significantly less reactive with myostatin's nearest homologue, GDF-11.

Definitions

5   When used herein, the term "mature myostatin" (see SEQ ID NO: 2 for human, murine, rat, chicken, turkey, canine, equine and porcine species) refers to the monomeric or the homodimeric form of the protein resulting after proteolytic cleavage at Arg 266 of the 375 amino acid proprotein form of myostatin. When used herein, the term "myostatin" refers to mature myostatin. When used herein, the term "promyostatin" or

10   "proprotein form of myostatin" when used with reference to the human protein refers to a protein comprising the sequence shown in SEQ ID NO: 1 either as a monomer or homodimer.

         A full-length antibody as it exists naturally is an immunoglobulin molecule comprised of four peptide chains, two heavy (H) chains (about 50-70 kDa when full

15   length) and two light (L) chains (about 25 kDa when full length) interconnected by disulfide bonds. The amino terminal portion of each chain includes a variable region of about 100-110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

20   Light chains are classified as kappa or lambda and characterized by a particular constant region as known in the art. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD, and IgE, respectively and several of these may be further divided into subclasses (isotypes) e.g., IgG$_1$, IgG$_2$, IgG$_3$, IgG$_4$, IgA$_1$, and IgA$_2$. Each heavy chain type is characterized by a

25   particular constant region known in the art. The subunit structures and three-dimensional configurations of different classes of antibodies are well known in the art. Each heavy chain is comprised of an N-terminal heavy chain variable region (herein "HCVR") and a heavy chain constant region. The heavy chain constant region is comprised of three domains (CH1, CH2, and CH3) for IgG, IgD, and IgA; and 4 domains (CH1, CH2, CH3,

30   and CH4) for IgM and IgE. Each light chain is comprised of a light chain variable region (herein "LCVR") and a light chain constant region, CL. The HCVR and LCVR regions can be further subdivided into regions of hypervariability, termed complementarity

determining regions (CDRs), interspersed with regions that are more conserved, termed
framework regions (FR). Each HCVR and LCVR is composed of three CDRs and four
FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1,
CDR1, FR2, CDR2, FR3, CDR3, FR4. Herein the 3 CDRs of the heavy chain are

5   referred to as "CDRH1, CDRH2, and CDRH3" and the 3 CDRs of the light chain are
referred to as "CDRL1, CDRL2 and CDRL3." The CDRs contain most of the residues
which form specific interactions with the antigen. Assignment of amino acids to each
domain is in accordance with well-known conventions [e.g., Kabat, "Sequences of
Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991)

10   or Chothia numbering scheme as described in Al-Lazikani *et al., J. Mol. Biol.* 273:927-
948, 1997, see also the internet site http:www.rubic.rdg.ac.uk/~andrew/bioinf.org/abs.
The functional ability of an antibody to bind a particular antigen is largely influenced by
the six CDRs.

The term "antibody," in reference to an anti-myostatin monoclonal antibody of the

15   invention (or simply, "monoclonal antibody of the invention"), as used herein, refers to a
monoclonal antibody. A "monoclonal antibody" as used herein refers to a chimeric
antibody, a humanized antibody or a fully human antibody, unless otherwise indicated
herein. Preferably a monoclonal antibody of the invention exists in a homogeneous or
substantially homogeneous population. Monoclonal antibodies of the invention can be

20   produced using *e.g.,* hybridoma techniques well known in the art, as well as recombinant
technologies, phage display technologies, synthetic technologies or combinations of such
technologies or other technologies readily known in the art. "Monoclonal antibody"
refers to an antibody that is derived from a single copy or clone, including *e.g.,* any
eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. A

25   "monoclonal antibody" can be an intact antibody (comprising a complete or full length Fc
region), a substantially intact antibody, or a portion or fragment of an antibody comprising
an antigen-binding portion, *e.g.,* a Fab fragment, Fab' fragment or F(ab')$_2$ fragment of a
chimeric, humanized or human antibody.

The variable regions of each light/heavy chain pair form the antigen-binding sites

30   of the antibody. Thus, an intact IgG antibody has two binding sites. Except in
bifunctional or bispecific antibodies, the two binding sites are the same. As used herein,
the "antigen-binding portion" or "antigen-binding region" or "antigen-binding fragment"

refers interchangeably herein to that portion of an antibody molecule, within the variable

region, which contains the amino acid residues that interact with an antigen and confer on

the antibody its specificity and affinity for the antigen. The antigen-binding portion of the

antibody includes framework amino acid residues necessary to maintain the proper

5   conformation of the antigen-binding residues. Preferably, the CDRs of the antigen-

binding region, or the entire antigen-binding region, of the antibodies of the invention will

be of murine origin or substantially of murine origin with certain amino acids residues

altered to improve a particular activity (see e.g., Fig. 9). Preferably, the framework

regions of antibodies of the invention are of human origin or substantially of human

10   origin (at least 85%, 90%, 95%, 97% or 99% of human origin). In other embodiments,

the antigen-binding region, or the CDRs of the antigen-binding region, can be derived

from other non-human species including, but not limited to, rabbit, rat or hamster. In

other embodiments, the antigen-binding region can be entirely of human origin or

substantially of human origin with certain amino acids residues altered to improve a

15   particular activity, e.g., affinity or specificity (see e.g., the amino acid positions of Fig. 9

which are in bold print and underlined).

　　　　Furthermore, a "monoclonal antibody" as used herein can be a single chain Fv

fragment that may be produced by joining the DNA encoding the LCVR and HCVR with

a linker sequence. (See, Pluckthun, *The Pharmacology of Monoclonal Antibodies,* vol.

20   113, Rosenburg and Moore eds., Springer-Verlag, New York, pp 269-315, 1994). It is

understood that regardless of whether fragments or portions are specified, the term

"antibody" as used herein includes such fragments or portions as well as single chain

forms. As long as the protein retains the ability to specifically or preferentially bind its

intended target (*i.e.,* epitope or antigen), it is included within the term "antibody."

25   - - A "population of monoclonal antibodies," refers to a homogeneous or

substantially homogeneous antibody population (*i.e.,* at least about 85%, 87%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, more preferably at least about 97% or 98% or most

preferably at least 99% of the antibodies in the population would compete in an ELISA

assay for the same antigen or epitope). Antibodies may or may not be glycosylated and

30   still fall within the bounds of the invention. Monoclonal antibodies may be homogeneous

if they have identical amino acid sequence although they may differ in a post-translational

modification, e.g., glycosylation pattern.

A "variant" anti-myostatin antibody, refers herein to a molecule which differs in amino acid sequence from a "parent" anti-myostatin antibody amino acid sequence by virtue of addition, deletion and/or substitution of one or more amino acid residue(s) in the parent antibody sequence. In the preferred embodiment, the variant comprises one or

5   more amino acid substitution(s) in one or more CDR region(s) of the parent antibody. For example, the variant may comprise at least one (e.g., from about one to about ten, and preferably from about two to about five) substitution in one or more CDR regions of the parent antibody. Identity or homology with respect to the variant sequence is defined herein as the percentage of amino acid residues in the variant sequence that are identical

10   with the parent antibody residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions or insertions in the antibody sequence shall be construed as affecting sequence identity or homology. The variant retains the ability to bind myostatin and preferably has properties which are superior to those of the parent

15   antibody. For example, the variant may have stronger binding affinity, lower IC_{50} in a SBE/reporter assay or enhanced ability to inhibit a myostatin bioactivity. The variant antibody of particular interest herein is one which displays at least about 2 fold, 5 fold, preferably at least about 10 fold, and more preferably at least about 20, 30, or 50 fold enhancement in a biological activity when compared to the parent antibody.

20   The "parent" antibody herein is one which is encoded by an amino acid sequence used for the preparation of the variant. The parent antibody may have a murine framework, but preferably has a human framework region. The parent antibody may be a murine (see e.g., Figs 10 and 11 herein), chimeric, humanized or human antibody.

The term "specifically binds" as used herein refers to the situation in which one

25   member of a specific binding pair does not significantly bind to molecules other than its specific binding partner(s) (i.e., cross-reactivity of less than about 35%, 33%, 30%, 20%, or 10%) as measured by a technique in the art e.g., by competition ELISA or by measurement of K_D with BIACORE or KINEXA assay. The term is also applicable where e.g., an antigen-binding domain of an antibody of the invention is specific for a

30   particular epitope that is carried by a number of antigens, in which case the specific antibody carrying the antigen-binding domain will be able to specifically bind to the various antigens carrying the epitope. Accordingly a monoclonal antibody of the present

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invention specifically binds an epitope localized within amino acids 40-64, 43-57 and/or 45-59 of the mature form of myostatin.

The term "preferentially binds" as used herein refers to the situation in which an antibody binds a specific antigen at least about 2, 3, 5, 10, 20, 22, 24, or 25-times greater

5   than it binds a different antigen as measured by a technique in the art *e.g.,* by competition ELISA or by measurement of K_D with BIACORE or KINEXA assay. Accordingly, a monoclonal antibody of the present invention preferentially binds GDF-8 over GDF-11. Similarly, an antibody may preferentially bind one epitope within an antigen over a different epitope within the same antigen.

10   The term "epitope" refers to that portion of a molecule capable of being recognized by and bound by an antibody at one or more of the antibody's antigen-binding regions. Epitopes often consist of a chemically active surface grouping of molecules such as amino acids or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. By "inhibiting epitope" and/or

15   "neutralizing epitope" is intended an epitope, which when in the context of the intact molecule (in this case, myostatin) and when bound by antibody specific to the epitope, results in loss or diminution of a biological activity of the molecule or organism containing the molecule, in vivo or in vitro.

The term "epitope," as used herein, further refers to a portion of a polypeptide

20   having antigenic and/or immunogenic activity in an animal, preferably a mammal, e.g., a mouse or a human. The term "antigenic epitope," as used herein, is defined as a portion of a polypeptide to which an antibody can specifically bind as determined by any method well known in the art, for example, by conventional immunoassays. Antigenic epitopes need not necessarily be immunogenic, but may be immunogenic. An "immunogenic

25   epitope," as used herein, is defined as a portion of a polypeptide that elicits an antibody response in an animal, as determined by any method known in the art. (See, *e.g.*, Geysen *et al., Proc. Natl. Acad. Sci. USA* 81:3998-4002 (1983)).

The phrases "biological property" or "bioactivity," "activity" or "biological activity," in reference to an antibody of the present invention, are used interchangeably

30   herein and include, but are not limited to, epitope/antigen affinity and specificity, ability to neutralize or antagonize an activity of myostatin in vivo or in vitro, IC_{50} in a myostatin/SBE reporter assay or other in vitro activity assay, the in vivo stability of the

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antibody and the immunogenic properties of the antibody. Other identifiable biological properties of an antibody include, for example, cross-reactivity, (*i.e.*, with non-human homologs of the targeted peptide, or with other proteins or tissues, generally), and ability to preserve high expression levels of protein in mammalian cells. The aforementioned

5   properties or characteristics can be observed or measured or assessed using art-recognized techniques including, but not limited to, ELISA, competitive ELISA, surface plasma resonance analysis, *in vitro* and *in vivo* neutralization assays without limit, receptor binding, cytokine or growth factor production and/or secretion, Xenopus animal cap development, signal transduction and immunohistochemistry with tissue sections from

10   different sources including human, primate, or any other source as the need may be.

The term "myostatin activity" as used herein refers to one or more of physiologically growth-regulatory or morphogenetic activities associated with active myostatin protein. For example, active myostatin is a negative regulator of skeletal muscle mass. Active myostatin can also modulate the production of muscle-specific

15   enzymes (e.g., creatine kinase), stimulate myoblast proliferation, and modulate preadipocyte differentiation to adipocytes.

The term "inhibit" or "neutralize" as used herein with respect to an activity of an antibody 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

20   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.

The term "isolated" when used in relation to a nucleic acid or protein (*e.g.*, an antibody) refers to a nucleic acid sequence or protein that is identified and separated from

25   -- at least one contaminant with which it is ordinarily associated in its natural source. Preferably, an "isolated antibody" is an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, pharmaceutical compositions of the invention comprise an isolated antibody that specifically binds myostatin and is substantially free of antibodies that specifically bind antigens other than myostatin).

30   The terms "Kabat numbering" and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (*i.e.*, hypervariable) than other amino acid

residues in the heavy and light chain variable regions of an antibody (Kabat, *et al., Ann. NY Acad. Sci.* 190:382-93 (1971); Kabat, *et al., Sequences of Proteins of Immunological Interest,* Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991)).

5   A polynucleotide is "operably linked" when it is placed into a functional relationship with another polynucleotide. For example, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence. A peptide is "operably linked" to another peptide when the polynucleotides encoding them are operably linked, preferably they are in the same open reading frame.

10   The terms "individual," "subject," and "patient," used interchangeably herein, refer to an animal, preferably a mammalian (including a nonprimate 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

15   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

20   disorder, disease or condition that would benefit from a decreased level of myostatin or a decreased bioactivity of myostatin.

          The term "vector" includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked including, but not limited to, plasmids and viral vectors. Certain vectors are capable of autonomous replication in a host cell into

25   which they are introduced while other vectors can be integrated into the genome of a host cell upon introduction into the host cell, and thereby, are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operably linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply "expression vectors") and exemplary vectors are well

30   known in the art.

          As used herein, the expressions "cell," "host cell," "cell line," and "cell culture" are used interchangeably and include an individual cell or cell culture that is a recipient of

any isolated polynucleotide of the invention or any recombinant vector(s) comprising a sequence encoding a HCVR, LCVR or monoclonal antibody of the invention. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in total DNA complement) to the original parent cell due to

5   natural, accidental, or deliberate mutation and/or change. A host cell includes cells transformed, transduced or infected in vivo or in vitro with one or more a recombinant vectors or a polynucleotide expressing a monoclonal antibody of the invention or a light chain or heavy chain thereof. A host cell which comprises a recombinant vector of the invention (either stably incorporated into the host chromosome or not) may also be

10   referred to as a "recombinant host cell". Preferred host cells for use in the invention are CHO cells (e.g., ATCC CRL-9096), NSO cells, SP2/0 cells and COS cells (ATCC e.g., CRL-1650, CRL-1651), HeLa (ATCC CCL-2). Additional host cells for use in the invention include plant cells, yeast cells, other mammalian cells and prokaryotic cells.

15   Antibody Characterization

        The present invention relates to isolated, monoclonal antibodies that specifically bind myostatin with high affinity. The antibodies of the invention are preferably chimeric, humanized or human antibodies or antigen-binding portions thereof and preferably bind to myostatin within the region of the mature form of myostatin spanning

20   amino acids 40-64 or more preferably within the region of the mature form of myostatin spanning amino acids 43-57 and/or 45-59. Furthermore, antibodies of the invention neutralize a myostatin biological activity in vivo or in vitro. Specific binding of anti-myostatin monoclonal antibodies of the invention to myostatin allows the antibodies of the invention to be used as therapeutics or prophylactics for myostatin-associated

25   conditions, diseases or disorders, i.e., conditions, diseases or disorders which benefit from lowering myostatin levels or antagonizing or inhibiting a myostatin biological activity. Further, antibodies of the invention may be used to diagnose or monitor conditions, diseases or disorders which benefit from an altered level or bioactivity of myostatin or to determine the level of myostatin in a sample.

30   In a preferred embodiment, the invention provides an anti-myostatin monoclonal antibody that binds myostatin or a portion thereof with a binding affinity (K_D) for myostatin of less than or equal to about 3×10^{-8} M, 1×10^{-8} M or 1×10^{-9} M, preferably

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less than about 9×10^{-10} M or 8.7×10^{-10} M or 8×10^{-11} M. Preferably the antibodies of the invention are characterized by a K_D for myostatin of no greater than about 3×10^{-8} M, 1×10^{-8} M, 1×10^{-9} M or 9×10^{-10} M and most preferably by a K_D for myostatin of no greater than about 8.7×10^{-10} M or 8×10^{-11} M. Antibody affinities may be determined as

5   described in the examples hereinbelow or by use of any suitable method available in the art.

In another preferred embodiment, the invention provides an anti-myostatin monoclonal antibody that has an IC_{50} of less than or equal to about 25 nM, 20 nM, 16 nM, 14 nM, 10 nM, 9 nM, 6 nM, 5.2 nM or less in an in vitro myostatin/SBE reporter assay as

10   described in the examples hereinbelow. Such antibodies may be further characterized by a K_D for myostatin of no greater than about 3×10^{-8} M, 1×10^{-8} M, 1×10^{-9} M or 9×10^{-10} M and most preferably by a K_D for myostatin of no greater than about 8.7×10^{-10} M or 8×10^{-11} M. Such antibodies may be further characterized by preferentially binding myostatin when compared to their ability to bind GDF-11 using any art-available method, e.g.,

15   ELISA assay or competitive ELISA assay or a K_D value measured e.g., with a BIACORE or KINEXA assay.

In one embodiment, a monoclonal antibody of the invention has less than about 35, 33, 30, 28, 25, 23, or 20% cross-reactivity (more preferably, less than about 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7 or 6, even more preferably, less than about 5 or 4 per

20   cent cross-reactivity) with a non-myostatin protein (such as, e.g., GDF-11) or with a non-myostatin peptide consisting of at least 15, 14, 13, 12, 11, 10 or 9 contiguous amino acids, as measured by a standard technique in the art such as an ELISA assay, a competitive ELISA assay or K_D values as measured, e.g., with a BIACORE or KINEXA assay. Preferably an antibody of the invention binds myostatin at least 2, 3, 5, 10, 20, 22, 24 or

25   25-fold greater than with which it binds GDF-11, as determined e.g., by competition ELISA or BIACORE or KINEXA assay. Most preferably, the antibodies of the invention do not bind GDF-11 at levels greater than background levels when using any binding assay available to one or ordinary skill in the art. A monoclonal antibody of the invention binds a monomeric or dimeric form of myostatin or a portion thereof comprising amino

30   acids spanning residues 40-64, 43-57 and/or 45-59 or mature myostatin.

Preferably the myostatin and GDF-11 polypeptides tested for preferential binding of an antibody of the invention are both homodimeric forms of the mature protein,

preferably of mammalian or avian origin, even more preferably of human origin.
However, the myostatin and GDF-11 polypeptides tested for preferential binding of an
antibody of the invention may be the monomeric form of the mature protein or proprotein
form or a polypeptide comprising a portion of the mature protein spanning amino acids

5   40-64, 43-57 and/or 45-59 of the mature form of the protein.

The anti-myostatin monoclonal antibodies of the invention bind an antigenic
epitope discovered to be localized within amino acids 40-64 (SEQ ID NO: 53 for human)
of mature myostatin preferably within amino acids 43-57 and/or 45-59 of mature
myostatin. Furthermore, a myostatin immunogenic epitope of the invention is localized

10   within amino acids 40-64 of mature myostatin (SEQ ID NO: 53 for human), preferably
within amino acids 43-57 and/or 45-59 of mature myostatin of any mammalian or avian
species. An immunogenic epitope of the invention is also contemplated to be an antigenic
epitope. The antigenic epitope may possess additional myostatin residues outside of
amino acids 40-64 of mature myostatin, but the monoclonal antibodies of the invention do

15   not require these additional residues to specifically bind myostatin. Additionally, residues
of myostatin outside of the amino acids 40-64 may affect the conformational structure of
the antigenic domain and thereby alter binding of an antibody of the invention to the
antigenic epitope. Preferably the monoclonal antibodies of the invention do not
significantly react (i.e., bind) with GDF-11 compared to the level with which they bind

20   GDF-8. More preferably, the monoclonal antibodies of the invention bind myostatin at
least 2, 3, 5, 10, 20, 22, 24 or 25-fold greater (e.g., greater affinity or greater specificity)
than with which it binds GDF-11 as determined e.g., by ELISA assay, competition ELISA
assay or K_D values in a BIACORE or KINEXA assay.

A peptide consisting of amino acids 40-64 (inclusive), 43-57 or 45-59 of mature

25   myostatin or any peptide consisting of an immunogenic epitope as described herein may
be used as an immunogenic peptide, preferably conjugated to a carrier protein e.g., KLH,
to generate monoclonal antibodies of the invention using methods available to one of
ordinary skill in the art. It is contemplated that cysteine residues within these peptides
may be changed to serine residues and still function as an immunogenic epitope. The

30   immunogenic peptide may be used to immunize a non-human animal, preferably a
mammal, more preferably a mouse. For a fully human antibody, the immunogenic
peptide may be used to immunize a transgenic strain of mouse in which mouse antibody

gene expression is suppressed and effectively replaced with human antibody gene expression. Then anti-myostatin antibodies are isolated from the immunized animal and screened by methods well known in the art to isolate those antibodies that specifically bind within the domain spanning amino acids 40-64 of mature myostatin, preferably

5   within the domain spanning amino acids 43-57 and/or 45-59 preferably screened for a binding affinity less than about 3×10^{-8} M, 1×10^{-8} M or 1×10^{-9} M, preferably less than about 9×10^{-10} M, 8.7×10^{-10} M or 8×10^{-11} M and/or screened for an IC_{50} of less than or equal to about 25 nM, 20 nM, 16 nM, 14 nM, 10 nM, 9 nM, 6 nM, 5.2 nM or less in an in vitro myostatin/SBE assay (see the assay described in Example 6 herein).

10   Monoclonal antibodies may be made using the hybridoma method widely known in the art (see e.g., Kohler et al., *Nature*, 256:495, 1975) or may be made by recombinant DNA methods (e.g., as in U.S. Patent No. 4,816,567) or other methods available in the art. Generally, a hybridoma can be produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as SP2/0) with antibody producing cells of the immunized

15   animal The antibody producing cell, preferably those of the spleen or lymph nodes, are obtained from animals immunized with the antigen of interest. The fused cells (hybridomas) can be isolated using selective culture conditions, and cloned by limiting dilution. Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity

20   of mabs produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or ELISA. Cells which produce antibodies with the desired binding properties can be selected by a suitable screening assay. Methods for such isolation and screening are well known in the art.

        Other suitable methods of producing or isolating antibodies which bind within the

25   domain spanning-amino acids 40-64, 43-57 and/or 45-59 of mature myostatin, including human or artificial antibodies, can be used, including, for example, methods which select a recombinant antibody (*e.g.,* single chain Fv or Fab) from a library, or which rely upon immunization of transgenic animals (*e.g.,* mice) capable of producing a repertoire of human antibodies (see e.g., Jakobovits *et al., Proc. Natl. Acad. Sci. USA*, 90:2551-2555,

30   1993; Jakobovits *et al., Nature,* 362:255-258, 1993; Lonberg et al., U.S. Patent Number 5,545,806; Surani *et al.,* U.S. Patent Number 5,545,807).

Single chain antibodies, and chimeric, humanized or primatized (CDR-grafted) antibodies, as well as chimeric or CDR-grafted single chain antibodies, and the like, comprising portions derived from different species, are also encompassed by the present invention and the term "antibody". The various portions of these antibodies can be joined

5   together chemically by conventional techniques, synthetically, or can be prepared as a contiguous protein using genetic engineering techniques. For example, nucleic acids encoding a chimeric or humanized chain can be expressed to produce a contiguous protein. See e.g., U.S. Patent No. 4,816,567; European Patent No. 0,125,023 B1; U.S. Patent No. 4,816,397; European Patent No. 0,120,694 B1; WO 86/01533; European

10   Patent No. 0,194,276 B1; U.S. Patent No. 5,225, 539; European Patent No. 0,239,400 B1 and U.S. Patent Nos. 5,585,089 and 5,698,762. See also, Newman, R. *et al.* *BioTechnology,* 10:1455-1460, 1993, regarding primatized antibody, and Ladner *et al.,* U.S. Patent No. 4,946,778 and Bird, R.E. *et al., Science,* 242:423-426, 1988, regarding single chain antibodies.

15   In addition, functional portions of antibodies, including antigen-binding portions of chimeric, humanized, human or single chain antibodies, can also be produced. Functional portions of the foregoing antibodies retain at least one antigen-binding function and/or biological function or bioactivity of the full-length antibody from which they are derived. Preferred functional portions retain an antigen-binding function of a

20   corresponding full-length antibody (*e.g.,* the ability to bind a mammalian mature form of myostatin). Particularly preferred functional portions or fragments retain the ability to inhibit one or more functions or bioactivities characteristic of a mammalian mature myostatin, such as a binding activity, a signaling activity, and/or stimulation of a cellular response. For example, in one embodiment, a functional portion or fragment can inhibit

25   the interaction of mature myostatin with one or more of its ligands-and/or can inhibit one or more receptor-mediated functions.

Antibody portions or fragments capable of binding to mature myostatin or a portion thereof (preferably within amino acids 40-64, 43-57 and/or 45-59 of mature myostatin), include, but are not limited to, Fv, Fab, Fab' and F(ab')$_2$ fragments and are

30   encompassed by the invention. Such fragments can be produced by enzymatic cleavage or by recombinant techniques. For instance, papain or pepsin cleavage can generate Fab or F(ab')$_2$ fragments, respectively. The smallest antigen-binding fragment is the Fv,

which consist of the HCVR and the LCVR domains. The Fab fragment consists of the
HCVR-CH1 and LCVR-CL domains covalently linked by a disulfide bond between the
constant regions. To overcome the tendency of non-covalently linked HCVR and LCVR
domains in the Fv to dissociate when co-expressed in a host cell, a so-called single chain

5   (sc) Fv fragment (scFv) can be constructed, in which a flexible and adequately long
polypeptide links either the C-terminus of the HCVR to the N-terminus of the LCVR or
the C-terminus of the LCVR to the N-terminus of the HCVR. The most commonly used
linker has been a 15-residue (Gly$_4$Ser)$_3$ peptide, but other linkers are also known in the art.
Antibodies can also be produced in a variety of truncated forms using antibody genes in

10   which one or more stop codons has been introduced upstream of the natural stop site. For
example, a chimeric gene encoding a F(ab')$_2$ heavy chain portion can be designed to
include DNA sequences encoding the CH$_1$ domain and hinge region of the heavy chain.

Selection of antibody fragments from libraries using enrichment technologies such
as phage-display (Matthews DJ and Wells JA. *Science. 260:*1113-7, 1993), ribosome

15   display (Hanes, *et al., Proc. Natl. Acad. Sci. (USA)* 95:14130-5, 1998), bacterial display
(Samuelson P., *et al., Journal of Biotechnology.* 96:129-54, 2002) or yeast display (Kieke
MC, *et al., Protein Engineering,* 10:1303-10, 1997) has proven to be successful
alternatives to classical hybridoma technology (recent reviews: Little M. *et al.,*
Immunology Today, 21:364-70, 2000;).

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Variant Antibodies

A murine monoclonal antibody or a human antibody (produced e.g., in a
transgenic mouse) raised against an immunogenic epitope (amino acids 40-64, 43-57 or
45-59 of mature myostatin) of the invention or against a protein comprising an

25   immunogenic epitope of the invention is a parent antibody. A murine parent antibody
may be further altered to create a chimeric or humanized form of the antibody using
methods well known in the art. Such chimeric or humanized antibodies, may serve as
parent antibodies for further variation or mutagenesis. Parent antibodies of the invention
may be further mutagenized e.g., within the CDR domain(s) (see, e.g., Fig. 9) to create a

30   variant antibody with an optimized property of interest, e.g., binding affinity, IC$_{50}$,
specificity, etc. An amino acid substitution variant antibody is preferred and has at least
one amino acid residue of the parent antibody molecule removed and a different residue

inserted in its place. The sites of greatest interest for substitutional mutagenesis include the CDR regions, but FR alterations are also contemplated. Conservative amino acid substitutions are preferred. If such substitutions result in a change in a biological activity of the antibody, then more substantial changes, i.e., non-conservative amino acid changes,

5   may be introduced and the products screened.

A convenient way for generating substitutional variants is affinity maturation using phage display. Briefly, several CDR region sites are mutated to generate all possible amino acid substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene

10   III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g., binding affinity, specificity, IC_{50}) as herein disclosed. In order to identify candidate CDR region sites for modification, alanine scanning mutagens can be performed to identify CDR region residues contributing significantly to antigen binding. Alternatively, or in addition, it may be beneficial to

15   analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and myostatin. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein or known in the art. Alternatively, or in addition, random mutagenesis may be performed on one or more CDR sequences at one or more residue positions, either while the CDR is operably linked

20   to the variable region or while the CDR is independent of other variable region sequence and then the altered CDR returned to a variable region using recombinant DNA technology. Once such variant antibodies are generated and expressed, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

25   Any cysteine residue not involved in maintaining the proper conformation of an anti-myostatin antibody of the invention may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

30   Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties

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found in the antibody, and/or adding one or more glycosylation sites that are not present in the parent antibody.

Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagines

5   residue. The tripeptide sequence asparagines-X-serine and asparagines-X-threonine, where X is any amino acid except praline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagines side chain. Thus the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-

10   aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made

15   by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

<u>Sequence</u>

A preferred monoclonal antibody of the invention has a LCVR comprising a

20   peptide with a sequence selected from the group consisting of SEQ ID NOs: 5-12 and/or a HCVR comprising a peptide with a sequence selected from the group consisting of SEQ ID NOs: 13-26, 55 and 56 (See Figs. 5-9 herein). Furthermore, a monoclonal antibody of the invention is one that is competitively inhibited from binding mature human myostatin (or a portion thereof) by a monoclonal antibody comprising two polypeptides with the

25   sequences shown in the group consisting of (i) SEQ ID NOs: 5 and 15; (ii) SEQ ID NOs: 5 and 16; and (iii) SEQ ID NOs: 10 and 26. Such competitive inhibition between antibodies may be measured by assays readily known to one of skill in the art, e.g., a competition ELISA assay.

In another embodiment, an anti-myostatin monoclonal antibody of the invention

30   comprises (i) a LCVR polypeptide with an amino acid sequence selected from the group consisting of SEQ ID NOs: 5-12 and (ii) a HCVR polypeptide with an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-26, 55 and 56.

Preferably, an antibody comprising an LCVR polypeptide with an amino acid sequence of SEQ ID NO: 5 further comprises a HCVR polypeptide with an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-20. Preferably, an antibody comprising an LCVR polypeptide with an amino acid sequence of SEQ ID NO: 6 further

5   comprises a HCVR polypeptide with an amino acid sequence of SEQ ID NO: 14. Preferably, an antibody comprising an LCVR polypeptide with an amino acid sequence of SEQ ID NO: 7 further comprises a HCVR polypeptide with an amino acid sequence selected from the group consisting of SEQ ID NOs: 21-25 and 56. Preferably, an antibody comprising an LCVR polypeptide with an amino acid sequence of SEQ ID NO:

10   10 further comprises a HCVR polypeptide with an amino acid sequence of SEQ ID NO: 26 or 55. Preferably, an antibody comprising an HCVR polypeptide with an amino acid sequence of SEQ ID NO: 55 further comprises a LCVR polypeptide with an amino acid sequence selected from the group consisting of SEQ ID NOs: 8-12.

       The skilled artisan will appreciate that the antibodies of the invention are not

15   limited to the specific sequences of HCVR and LCVR as stated in Figs 5-9 herein, but also include variants of these sequences that retain antigen binding ability. Such variants may be derived from the provided sequences using techniques known in the art as described above.

       In another embodiment, an anti-myostatin antibody of the invention has a heavy

20   and a light chain variable region, wherein the heavy chain variable region comprises CDR regions with the following amino acid sequences: CDRH1 (SEQ ID NO: 59), CDRH2 (SEQ ID NO: 60) and CDRH3 (SEQ ID NO: 61); and/or wherein the light chain variable region comprises CDR regions with the following amino acid sequences: CDRL1 (SEQ ID NO: 57), CDRL2 (SEQ ID NO: 30 or 52) and CDRL3 (SEQ ID NO: 58). Preferably,

25   the heavy chain CDRs of an antibody of the invention are as shown in Figs. 5, 6 or 9 and the light chain CDRs of an antibody of the invention are as shown in Figs. 7, 8 or 9 and the CDRs exist in the antibody at the CDR location defined in the Figures. For example, one antibody of the invention has CDRL1 with SEQ ID NO: 27, CDRL2 with SEQ ID NO: 30, CDRL3 with SEQ ID NO: 31, CDRH1 with SEQ ID NO: 36, CDRH2 with SEQ

30   ID NO: 43 and CDRH3 with SEQ ID NO: 47 (as does antibody IC7.1 in Figure 9).

       It is further contemplated that an anti-myostatin antibody of the invention comprises a heavy chain variable region comprising a CDRH1 region with a sequence

selected from the group consisting of that as shown in SEQ ID NOs: 36-42 and/or a
CDRH2 region with a sequence with a sequence selected from the group consisting of
that as shown in SEQ ID NOs: 43-46 and/or a CDRH3 region with a sequence selected
from the group consisting of that as shown in SEQ ID NOs: 47-51. In another

5   embodiment, an anti-myostatin antibody of the invention comprises a light chain variable
region comprising a CDRL1 region with a sequence selected from the group consisting of
that as shown in SEQ ID NOs: 27-29 and/or a CDRL2 region with a sequence selected
from the group consisting of that as shown in SEQ ID NOs 30 or 52 and/or a CDRL3
region with a sequence selected from the group consisting of that as shown in SEQ ID

10   NOs: 31-35. In a preferred embodiment, an anti-myostatin antibody of the invention
comprises a heavy chain variable region comprising a CDRH1 region with a sequence
selected from the group consisting of that as shown in SEQ ID NOs: 36-42 and/or a
CDRH2 region with a sequence selected from the group consisting of that as shown in
SEQ ID NOs: 43-46 and/or a CDRH3 region with a sequence selected from the group

15   consisting of that as shown in SEQ ID NOs: 47-51 and further comprises a light chain
variable region comprising a CDRL1 region with a sequence selected from the group
consisting of that as shown in SEQ ID NOs: 27-29 and/or a CDRL2 region with a
sequence selected from the group consisting of that as shown in SEQ ID NOs 30 or 52
and/or a CDRL3 region with a sequence selected from the group consisting of that as

20   shown in SEQ ID NOs: 31-35.

       The structure for carrying a CDR of the invention will generally be an antibody
heavy or light chain sequence or a substantial portion thereof, in which the CDR is
located at a location corresponding to the CDR of naturally occurring HCVR and LCVR
(Kabat et al, Sequences of Proteins of Immunological Interest, US Dept of HHS, 1991).

25   Preferably, the three hypervariable regions (CDRs) for each chain, light and heavy, are
provided in a human framework region, e.g., as a contiguous sequence represented by the
following formula: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. The heavy chain or light
chain FR1, FR2, FR3 and FR4 combine to form the complete framework when arranged
as a contiguous sequence with the CDRs in the order stated.

30   In addition to CDR sequences, the LCVR and HCVR further comprise framework
sequence. In a humanized antibody for therapeutic use in humans, the framework
sequence is preferably entirely or substantially of human origin (i.e., at least 85%, 87%,

90%, 92%, 95%, 96%, 97%, 98% or 99% of human origin). Preferably the light chain
framework region of a humanized, human or chimeric antibody of the invention is O2 as
shown in Fig. 7, comprised of FR1 with SEQ ID NO: 65, FR2 with SEQ ID NO: 66, FR3
with SEQ ID NO: 67 and FR4 with SEQ ID NO: 68. Preferably the heavy chain
framework region of a humanized, human or chimeric antibody of the invention is VH2-
70 or VH4-39 as shown in Figs. 5 and 6 respectively. VH2-70 framework is comprised of
FR1 with SEQ ID NO: 69, FR2 with SEQ ID NO: 70, FR3 with SEQ ID NO: 71, and FR4
with SEQ ID NO: 72. VH4-39 is comprised of FR1 with SEQ ID NO: 73, FR2 with SEQ
ID NO: 74, FR3 with SEQ ID NO: 75 and FR4 with SEQ ID NO: 76. In an antibody for
use in a non-human animal, the framework region sequence may substantially originate
from the human genome (preferably used in a non-human animal when it is an embryo or
newly born) or from the genome of the animal in which it is to be used therapeutically.

In one embodiment, an anti-myostatin antibody of the invention wherein all or a
portion of the variable region is limited by a particular sequence as shown by a SEQ ID
NO herein is further characterized by being a chimeric, humanized, or fully human
antibody or antigen-binding portion thereof that antagonizes or neutralizes at least one
myostatin activity in vivo or in vitro. An anti-myostatin antibody of the invention
wherein all or a portion of the variable region is limited by a particular sequence as shown
by a SEQ ID NO herein is preferably further characterized by preferentially binding GDF-
8 over GDF-11. More preferably such antibodies bind myostatin at least about 2, 3, 5, 10,
20, 22, 24, or 25-times greater than it binds GDF-11. Most preferably such antibodies,
when expressed as Fabs, do not bind GDF-11 above background levels.

An anti-myostatin antibody of the invention wherein all or a portion of the
variable region is limited by a particular sequence as shown by a SEQ ID NO herein are
preferably further characterized by (i) binding a peptide consisting of an amino acid
sequence as shown in SEQ ID NO: 53, preferably a peptide consisting of an amino acid
sequence as shown in SEQ ID NO: 62; and/or (ii) having an IC_{50} less than or equal to
about 25 nM, 20 nM, 16 nM, 10 nM, 9 nM, 6 nM, 5.7 nM in an in vitro myostatin/SBE
reporter assay and/or (iii) having a binding affinity (K_D) for myostatin less than about 3 x
10^{-8} M, 1 x 10^{-8} M or 1 x 10^{-9} M, preferably less than about 9 x 10^{-10} M or 8.7 x 10^{-10} M or
8 x 10^{-11} M; alternatively characterized as a K_D for myostatin of no greater than about 3 x

10^{-8} M, 1 x 10^{-8} M, 1 x 10^{-9} M or 9 x 10^{-10} M; more preferably a K_D no greater than about 8.7 x 10^{-10} M and most preferably a K_D no greater than about 8 x 10^{-11} M.

Antibody Expression

The present invention is also directed to cell lines that express an anti-myostatin monoclonal antibody of the invention or portion thereof. Creation and isolation of cell lines producing a monoclonal antibody of the invention can be accomplished using standard techniques known in the art. Preferred cell lines include COS, CHO, SP2/0, NS0 and yeast (available from public repositories such as ATCC, American Type Culture Collection, Manassas, VA).

‒ ‒A wide variety of host expression systems can be used to express an antibody of the present invention including prokaryotic (bacterial) and eukaryotic expression systems (such as yeast, baculovirus, plant, mammalian and other animal cells, transgenic animals, and hybridoma cells), as well as phage display expression systems. An example of a suitable bacterial expression vector is pUC119 and a suitable eukaryotic expression vector is a modified pcDNA3.1 vector with a weakened DHFR selection system. Other antibody expression systems are also known in the art and are contemplated herein.

An antibody of the invention can be prepared by recombinant expression of immunoglobulin light and heavy chain genes in a host cell. To express an antibody recombinantly, a host cell is transformed, transduced, infected or the like with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and/or heavy chains of the antibody such that the light and/or heavy chains are expressed in the host cell. The heavy chain and the light chain may be expressed independently from different promoters to which they are operably linked in one vector or, alternatively, the heavy chain and the light chain may be expressed independently from different promoters to which they are operably linked in two vectors – one expressing the heavy chain and one expressing the light chain. Optionally the heavy chain and light chain may be expressed in different host cells. Preferably, the recombinant antibodies are secreted into the medium in which the host cells are cultured, from which the antibodies can be recovered or purified. Standard recombinant DNA methodologies are used to obtain antibody heavy and light chain genes, incorporate these genes into recombinant expression vectors, and introduce the vectors into host cells. Such

standard recombinant DNA technologies are described, for example, in Sambrook,

Fritsch, and Maniatis (Eds.), *Molecular Cloning; A Laboratory Manual,* Second Edition,

Cold Spring Harbor, N.Y., 1989; Ausubel, *et al* (Eds.) *Current Protocols in Molecular*

Biology, Greene Publishing Associates, 1989.

5   An isolated DNA encoding a HCVR region can be converted to a full-length

heavy chain gene by operably linking the HCVR-encoding DNA to another DNA

molecule encoding heavy chain constant regions (CH1, CH2, and CH3). The sequences

of human heavy chain constant region genes are known in the art. See, *e.g.,* Kabat, *et al.,*

Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of

10   Health and Human Services, NIH Publication No. 91-3242 (1991). DNA fragments

encompassing these regions can be obtained *e.g.,* by standard PCR amplification. The

heavy chain constant region can be of any type, (*e.g.,* IgG, IgA, IgE, IgM or IgD), class

(*e.g.,* IgG_1, IgG_2, IgG_3 and IgG_4) or subclass constant region and any allotypic variant

thereof as described in Kabat (*supra*). Alternatively, the antigen binding portion can be a

15   Fab fragment, Fab' fragment, $F(ab')_2$ fragment, Fd, or a single chain Fv fragment (scFv).

For a Fab fragment heavy chain gene, the HCVR-encoding DNA may be operably linked

to another DNA molecule encoding only a heavy chain CH1 constant region.

An isolated DNA encoding a LCVR region may be converted to a full-length light

chain gene (as well as to a Fab light chain gene) by operably linking the LCVR-encoding

20   DNA to another DNA molecule encoding a light chain constant region, CL. The

sequences of human light chain constant region genes are known in the art. See, *e.g.,*

Kabat, *supra.* DNA fragments encompassing these regions can be obtained by standard

PCR amplification. The light chain constant region can be a kappa or lambda constant

region.

25   To create an scFv gene, the HCVR- and LCVR-encoding DNA fragments are

operably linked to another fragment encoding a flexible linker, *e.g.,* encoding the amino

acid sequence $(Gly_4-Ser)_3$, such that the HCVR and LCVR sequences can be expressed as

a contiguous single-chain protein, with the LCVR and HCVR regions joined by the

flexible linker. See, *e.g.,* Bird, *et al., Science* 242:423-6, 1988; Huston, *et al., Proc. Natl.*

30   *Acad. Sci. USA* 85:5879-83, 1988; McCafferty, *et al., Nature* 348:552-4, 1990.

To express an antibody of the invention, a DNA encoding a partial or full-length

light and/or heavy chain, obtained as described above, are inserted into an expression

30

vector such that the gene is operably linked to transcriptional and translational control

sequences. The expression vector and expression control sequences are chosen to be

compatible with the expression host cell used. The antibody light chain gene and the

antibody heavy chain gene can be inserted into separate vectors or, more typically, both

5   genes are inserted into the same expression vector. The antibody genes are inserted into

the expression vector by standard methods. Additionally, the recombinant expression

vector can encode a signal peptide that facilitates secretion of the anti-myostatin

monoclonal antibody light and/or heavy chain from a host cell. The anti-myostatin

monoclonal antibody light and/or heavy chain gene can be cloned into the vector such that

10   the signal peptide is operably linked in-frame to the amino terminus of the antibody chain

gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous

signal peptide.

In addition to the antibody heavy and/or light chain gene(s), a recombinant

expression vector of the invention carries regulatory sequences that control the expression

15   of the antibody chain gene(s) in a host cell. The term "regulatory sequence" is intended to

include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation

signals), as needed, that control the transcription or translation of the antibody chain

gene(s). The design of the expression vector, including the selection of regulatory

sequences may depend on such factors as the choice of the host cell to be transformed, the

20   level of expression of protein desired. Preferred regulatory sequences for mammalian

host cell expression include viral elements that direct high levels of protein expression in

mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus

(CMV), Simian Virus 40 (SV40), adenovirus, (*e.g.*, the adenovirus major late promoter

(AdMLP)) and polyoma virus.

25   In addition to the antibody heavy and/or light chain genes and regulatory

sequences, the recombinant expression vectors of the invention may carry additional

sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*,

origins of replication) and one or more selectable marker genes. The selectable marker

gene facilitates selection of host cells into which the vector has been introduced. For

30   example, typically the selectable marker gene confers resistance to drugs, such as G418,

hygromycin, or methotrexate, on a host cell into which the vector has been introduced.

Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for

use in DHFR-minus host cells with methotrexate selection/amplification), the *neo* gene
(for G418 selection), and glutamine synthetase (GS) in a GS-negative cell line (such as
NS0) for selection/amplification.

For expression of the light and/or heavy chains, the expression vector(s) encoding

5   the heavy and/or light chains is introduced into a host cell by standard techniques *e.g.,*
electroporation, calcium phosphate precipitation, DEAE-dextran transfection,
transduction, infection and the like. Although it is theoretically possible to express the
antibodies of the invention in either prokaryotic or eukaryotic host cells, eukaryotic cells
are preferred, and most preferably mammalian host cells, because such cells, are more

10   likely to assemble and secrete a properly folded and immunologically active antibody.
Preferred mammalian host cells for expressing the recombinant antibodies of the
invention include Chinese Hamster Ovary (CHO cells) (including DHFR-CHO cells,
described in Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA* 77:4216-20, 1980, used with
a DHFR selectable marker, *e.g.,* as described in Kaufman and Sharp, *J. Mol. Biol.*

15   159:601-21, 1982, NS0 myeloma cells, COS cells, and SP2/0 cells. When recombinant
expression vectors encoding antibody genes are introduced into mammalian host cells, the
antibodies are produced by culturing the host cells for a period of time sufficient to allow
for expression of the antibody in the host cells or, more preferably, secretion of the
antibody into the culture medium in which the host cells are grown. Antibodies can be

20   recovered from the host cell and/or the culture medium using standard purification
methods.

Host cells can also be used to produce portions, or fragments, of intact antibodies,
e.g., Fab fragments or scFv molecules by techniques that are conventional. It will be
understood by a skilled artisan that variations on the above procedure are within the scope

25   of the present invention. For example, it may be desirable to transfect a host cell with
DNA encoding either the light chain or the heavy chain of an antibody of this invention.
Recombinant DNA technology may also be used to remove some or all the DNA
encoding either or both of the light and heavy chains that is not necessary for binding to
myostatin. The molecules expressed from such truncated DNA molecules are also

30   encompassed by the antibodies of the invention.

In a preferred system for recombinant expression of an antibody of the invention, a
recombinant expression vector encoding both the antibody heavy chain and the antibody

light chain is introduced into DHFR-CHO cells by e.g., calcium phosphate-mediated

transfection. Within the recombinant expression vector, the antibody heavy and light

chain genes are each operably linked to enhancer/promoter regulatory elements (e.g.,

derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP

5   promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element)

to drive high levels of transcription of the genes. The recombinant expression vector also

carries a DHFR gene, which allows for selection of CHO cells that have been transfected

with the vector using methotrexate selection/amplification. The selected transformant

host cells are cultured to allow for expression of the antibody heavy and light chains and

10   intact antibody is recovered from the culture medium. Standard molecular biology

techniques are used to prepare the recombinant expression vector, transfect the host cells,

select for transformants, culture the host cells and recover the antibody from the culture

medium. Antibodies, or antigen-binding portions thereof, of the invention can be

expressed in an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes

15   (see, e.g., Taylor, et al., Nucleic Acids Res. 20:6287-95, 1992).

Once expressed, the intact antibodies, their dimers, individual light and heavy

chains, or other immunoglobulin forms of the present invention can be purified according

to standard procedures of the art, including ammonium sulfate precipitation, ion

-exchange, affinity, reverse phase, hydrophobic interaction column chromatography, gel

20   electrophoresis and the like. Substantially pure immunoglobulins of at least about 90%,

92%, 94% or 96% homogeneity are preferred, and 98 to 99% or more homogeneity most

preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired,

the peptides may then be used therapeutically or prophylactically, as directed herein.

25   Chimeric antibody

As used herein, the term "chimeric antibody" includes monovalent, divalent or

polyvalent immunoglobulins. A monovalent chimeric antibody is a dimer formed by a

chimeric heavy chain associated through disulfide bridges with a chimeric light chain. A

divalent chimeric antibody is a tetramer formed by two heavy chain-light chain dimers

30   associated through at least one disulfide bridge.

A chimeric heavy chain of an antibody comprises an antigen-binding region

derived from the heavy chain of a non-human antibody specific for myostatin, which is

operably linked to at least a portion of a human or substantially human (or species different from that from which the antigen-binding region was derived) heavy chain constant region. A chimeric light chain of an antibody comprises an antigen binding region derived entirely or substantially from the light chain of a non-human antibody

5   operably linked to at least a portion of a human or substantially human (or species different from that from which the antigen-binding region was derived) light chain constant region (CL). Antibodies, fragments or derivatives having chimeric heavy chains and light chains of the same or different variable region binding specificity, can also be prepared by appropriate association of the individual polypeptide chains, according to

10   known method steps.

With this approach, hosts expressing chimeric heavy chains are separately cultured from hosts expressing chimeric light chains, and the immunoglobulin chains are separately recovered and then associated. Alternatively, the hosts can be co-cultured and the chains allowed to associate spontaneously in the culture medium, followed by

15   recovery of the assembled immunoglobulin or fragment. Methods for producing chimeric antibodies are known in the art (see, e.g., U.S. Patent Nos.: 6,284,471; 5,807,715; 4,816,567; and 4,816,397).

Humanized antibodies

20   Preferably an antibody of the invention to be used for therapeutic purposes, would have the sequence of the framework and constant region (to the extent it exists in the antibody) derived from the mammal in which it would be used as a therapeutic so as to decrease the possibility that the mammal would illicit an immune response against the therapeutic antibody. Humanized antibodies are of particular interest since they are

25   considered to be valuable for therapeutic application and avoid the human anti-mouse antibody response frequently observed with rodent antibodies. Additionally, in humanized antibodies the effector portion is human so it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement-dependent cytotoxicity or antibody-dependent cellular cytotoxicity). Also,

30   injected humanized antibodies may have a half-life more like that of naturally occurring human antibodies than do e.g., murine antibodies, thereby allowing smaller and less frequent doses to be given. The term "humanized antibody" as used herein refers to an

antibody comprising portions of antibodies of different origin, wherein at least one portion is of human origin. For example, the humanized antibody can comprise portions derived from an antibody of nonhuman origin with the requisite specificity, such as a mouse, and from an antibody of human origin, joined together chemically by conventional

5 techniques (e.g., synthetic) or prepared as a contiguous polypeptide using genetic engineering techniques.

Preferably, a "humanized antibody" has CDRs that originate from a non-human antibody (preferably a mouse monoclonal antibody) while framework and constant region, to the extent it is present, (or a significant or substantial portion thereof, i.e., at least about

10 85%, 87%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99%) are encoded by nucleic acid sequence information that occurs in the human germline immunoglobulin region (see, e.g., the International ImMunoGeneTics Database) or in recombined or mutated forms thereof whether or not said antibodies are produced in a human cell. The CDRs of a humanized antibody may be optimized from the CDRs of non-human parent antibody

15 from which they originated to generate desired properties, e.g., specificity, affinity and capacity. Optimized CDRs may have amino acid substitutions, additions and/or deletions when compared to the parent CDRs. For example, the amino acid positions of SEQ ID NOs: 57, 30, 58, 59, 60, and 61 that are underlined and in bold print in Fig. 9 are positions which have been optimized from the parent CDRs as shown in Figs 10 and 11. However,

20 it is contemplated that any non-human anti-myostatin antibody (i) raised against a peptide consisting of a sequence shown in SEQ ID NO: 53 or 62 or (ii) raised against a peptide comprising the sequence shown in SEQ ID NO: 53 or 62 and reactive to a peptide consisting of the sequence shown in SEQ ID NO: 53 or 62, may serve as a parent antibody which may be converted to a humanized or chimeric antibody using standard

25 methods available in the art.

Humanized forms of non-human (e.g., murine) antibodies include an intact antibody, a substantially intact antibody, a portion of an antibody comprising an antigen-binding site, or a portion of an antibody comprising a Fab fragment, Fab' fragment, F(ab')2, or a single chain Fv fragment. Humanized antibodies preferably contain minimal

30 sequence derived from non-human immunoglobulin. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise

substantially all of at least one, and typically two, variable domains, in which all or

substantially all of the amino acids in the CDR regions correspond to those of a non-

human immunoglobulin and all or substantially all of the amino acids in the FR regions

are those of a human immunoglobulin consensus sequence. The humanized antibody

5   optimally also comprises at least a portion of an immunoglobulin constant region (Fc),

typically that of a human immunoglobulin. [Jones et al., Nature, 321:522-525 (1986);

Riechmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-

596 (1992).]

           Humanized antibodies may be subjected to *in vitro* mutagenesis using methods of

10   routine use in the art (or, when an animal transgenic for human Ig sequences is used, *in*

vivo somatic mutagenesis) and, thus, the framework region amino acid sequences of the

HCVR and LCVR regions of the humanized recombinant antibodies are sequences that,

while derived from those related to human germline HCVR and LCVR sequences, may

not naturally exist within the human antibody germline repertoire *in vivo*. It is

15   contemplated that such amino acid sequences of the HCVR and LCVR framework

regions of the humanized recombinant antibodies are at least 85%, 87%, 90%, 92%, 94%,

95%, 96%, 98% or most preferably at least 99% identical to a human germline sequence.

Preferably, those framework residues of the parent antibody (*e.g.,* murine antibody or

generally the antibody from which the humanized antibody is derived) which maintain or

20   affect combining-site structures will be retained. These residues may be identified e.g., by

X-ray crystallography of the parent antibody or Fab fragment, thereby identifying the

three-dimensional structure of the antigen-binding site.

           The humanized antibody of the present invention may comprise or be derived

from a human germline light chain framework. In particular embodiments, the light chain

25   germline sequence is selected from human VK sequences including, but not limited to,

A1, A10, A11, A14, A17, A18, A19, A2, A20, A23, A26, A27, A3, A30, A5, A7, B2, B3,

L1, L10, L11, L12, L14, L15, L16, L18, L19, L2, L20, L22, L23, L24, L25, L4/18a, L5,

L6, L8, L9, O1, O11, O12, O14, O18, O2, O4, and O8. In certain embodiments, this light

chain human germline framework is selected from V1-11, V1-13, V1-16, V1-17, V1-18,

30   V1-19, V1-2, V1-20, V1-22, V1-3, V1-4, V1-5, V1-7, V1-9, V2-1, V2-11, V2-13, V2-14,

V2-15, V2-17, V2-19, V2-6, V2-7, V2-8, V3-2, V3-3, V3-4, V4-1, V4-2, V4-3, V4-4,

V4-6, V5-1, V5-2, V5-4, and V5-6. See PCT WO 2005/005604 for a description of the different germline sequences.

In other embodiments, the humanized antibody of the present invention may comprise or be derived from a human germline heavy chain framework. In particular embodiments, this heavy chain human germline framework is selected from VH1-18, VH1-2, VH1-24, VH1-3, VH1-45, VH1-46, VH1-58, VH1-69, VH1-8, VH2-26, VH2-5, VH2-70, VH3-11, VH3-13, VH3-15, VH3-16, VH3-20, VH3-21, VH3-23, VH3-30, VH3-33, VH3-35, VH3-38, VH3-43, VH3-48, VH3-49, VH3-53, VH3-64, VH3-66, VH3-7, VH3-72, VH3-73, VH3-74, VH3-9, VH4-28, VH4-31, VH4-34, VH4-39, VH4-4, VH4-59, VH4-61, VH5-51, VH6-1, and VH7-81. See PCT WO 2005/005604 for a description of the different germline sequences.

In particular embodiments, the light chain variable region and/or heavy chain variable region comprises a framework region or at least a portion of a framework region (e.g., containing 2 or 3 subregions, such as FR2 and FR3). In certain embodiments, at least FRL1, FRL2, FRL3, or FRL4 is fully human. In other embodiments, at least FRH1, FRH2, FRH3, or FRH4 is fully human. In some embodiments, at least FRL1, FRL2, FRL3, or FRL4 is a germline sequence (e.g., human germline) or comprises human consensus sequences for the particular framework. In other embodiments, at least FRH1, FRH2, FRH3, or FRH4 is a germline sequence (e.g., human germline) or comprises human consensus sequences for the particular framework. In preferred embodiments, the framework region is a human framework region.

In general, humanized antibodies may be produced by obtaining nucleic acid sequences encoding the HCVR and LCVR of an antibody, e.g., a murine antibody or antibody made by a hybridoma, which binds a myostatin epitope of the invention, identifying the CDRs in said HCVR and LCVR (nonhuman), and grafting such CDR-encoding nucleic acid sequences onto selected human framework-encoding nucleic acid sequences. Optionally, a CDR region may be optimized by mutagenizing randomnly or at particular locations in order to substitute, delete, or add one or more amino acids in the CDR prior to grafting the CDR region into the framework region. Alternatively, a CDR region may be optimized subsequent to insertion into the human framework region using methods available to one of skill in the art. Preferably, the human framework amino acid sequences are selected such that the resulting antibody is likely to be suitable for in vivo

administration in humans. This can be determined, *e.g.,* based on previous usage of antibodies containing such human framework sequence. Preferably, the human framework sequence will not itself be significantly immunogenic.

5   Alternatively, the amino acid sequences of the frameworks for the antibody to be humanized may be compared to those of known human framework sequences the human framework sequences to be used for CDR-grafting and selected based on their comprising sequences highly similar to those of the parent antibody, e.g., a murine antibody which binds myostatin. Numerous human framework sequences have been isolated and their sequences reported in the art. This enhances the likelihood that the resultant CDR-grafted

10   humanized antibody, which contains CDRs of the parent *(e.g.,* murine) or optimized CDRs of the parent antibody grafted onto selected human frameworks (and possibly also the human constant region) will substantially retain the antigen binding structure and thus retain the binding affinity of the parent antibody. To retain a significant degree of antigen binding affinity, the selected human framework regions will preferably be those that are

15   expected to be suitable for *in vivo* administration, *i.e.,* not immunogenic.

In either method, the DNA sequence encoding the HCVR and LCVR regions of the preferably murine anti-myostatin antibody are obtained. Methods for cloning nucleic acid sequences encoding immunoglobulins are well known in the art. Such methods may, for example, involve the amplification of the immunoglobulin-encoding sequences to be

20   cloned using appropriate primers by polymerase chain reaction (PCR). Primers suitable for amplifying immunoglobulin nucleic acid sequences, and specifically murine HCVR and LCVR sequences have been reported in the literature. After such immunoglobulin-encoding sequences have been cloned, they will be sequences by methods well known in the art.

25   After the CDR-encoding sequences are grafted onto the selected human framework encoding sequences, the resultant DNA sequences encoding the "humanized" variable heavy and variable light sequences are then expressed to produce a humanized Fv or humanized antibody that binds myostatin. The humanized HCVR and LCVR may be expressed as part of a whole anti-myostatin antibody molecule, *i.e.,* as a fusion protein

30   with human constant domain sequences whose encoding DNA sequences have been obtained from a commercially available library or which have been obtained using, e.g., one of the above described methods for obtaining DNA sequences, or are in the art.

However, the HCVR and LCVR sequences can also be expressed in the absence of constant sequences to produce a humanized anti-myostatin Fv. Nevertheless, fusion of human constant sequences onto the variable region is potentially desirable because the resultant humanized anti-myostatin antibody may possess human effector functions.

5   Methods for synthesizing DNA encoding a protein of known sequence are well known in the art. Using such methods, DNA sequences which encode the subject humanized HCVR and LCVR sequences (with or without constant regions) are synthesized, and then expressed in a vector system suitable for expression of recombinant antibodies. This may be effected in any vector system which provides for the subject

10   humanized HCVR and LCVR sequences to be expressed as a fusion protein with human constant domain sequences and to associate to produce functional (antigen binding) antibodies or antibody fragments.

        Human constant domain sequences are well known in the art, and have been reported in the literature. Preferred human constant light chain sequences include the

15   kappa and lambda constant light chain sequences. Preferred human constant heavy chain sequences include human IgG$_1$, human IgG$_2$, human IgG$_3$, human IgG$_4$, and mutated versions thereof which provide for altered effector function, e.g., enhanced in vivo half-life, reduced Fc receptor binding or altered deamidation profile.

        If present, human framework regions are preferably derived from a human

20   antibody variable region having sequence similarity to the analogous or equivalent region of the antigen binding region donor (i.e., the parent antibody). Other sources of framework regions for portions of human origin of a humanized antibody include human variable consensus sequences (see e.g., Kettleborough, C.A. et al. Protein Engineering 4:773-783 (1991); Carter et al., WO 94/04679. For example, the sequence of the

25   antibody or variable region used to obtain the nonhuman portion can be compared to human sequences as described in Kabat et al. Sequences of Proteins of Immunological Interest, Fifth Edition, NIH, U.S. Government Printing Office (1991). In a particularly preferred embodiment, the framework regions of a humanized antibody chain are derived from a human variable region having at least about 60% overall sequence identity,

30   preferably at least about 70% overall sequence identity and more preferably at least about 85% overall sequence identity, with the variable region of the nonhuman donor. A human portion can also be derived from a human antibody having at least about 65% sequence

identity, and preferably at least about 70% sequence identity, within the particular portion (*e.g.*, FR) being used, when compared to the equivalent portion (*e.g.*, FR) of the nonhuman donor.

References further describing methods involved in humanizing a mouse antibody that may be used are *e.g.*, Queen *et al.*, *Proc. Natl. Acad. Sci. USA* 88:2869, 1991; U.S. Pat. No. 5,693,761; U.S. Pat. No. 4,816,397; U.S. Pat. No. 5,225,539; computer programs ABMOD and ENCAD as described in Levitt, M., *J. Mol. Biol.* 168:595-620, 1983; humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)],

Human Antibodies

As an alternative to humanization, human antibodies can be generated. Human antibodies can be produced using various techniques known in the art, including phage display libraries. Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991). The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies. Cole et al., Monoclonal Antibodies and *Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or complete inactivated. Upon immunization, e.g., with an antigen comprising an immunogenic epitope of the invention, a full repertoire of human antibody production is obtained, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,589,369; 5,591,669; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., *BioTechnology* 10:779-783, 1992; Lonberg et al., *Nature* 368: 856-859, 1994; Morrison, *Nature* 368: 812-13, 1994; Fishwild et al., *Nature Biotechnology* 14:845-51, 1996; Neuberger, *Nature Biotechnology* 14: 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995) and Jobkobovits et al., *Proc. Natl. Acad. Sci. USA*, 90:2551, 1993.

Human immunoglobulin genes introduced into the mouse thus creating transgenic

mice capable of responding to antigens with antibodies having human sequences are also

described in Bruggemann et al. *Proc. Nat'l. Acad. Sci.* USA 86:6709-6713 (1989)]. There

are several strategies that exist for the generation of mammals that produce human

5   antibodies. In particular, there is the "minilocus" approach in which an exogenous Ig locus

is mimicked through the inclusion of pieces (individual genes) from the Ig locus (see, e.g.,

U.S. Pat. Nos. 5,545,807, 5,545,806, 5,625,825, 5,625,126, 5,633,425, 5,661,016,

5,770,429, 5,789,650, and 5,814,318, 5,612,205, 5,721,367, 5,789,215), YAC

introduction of large and substantially germline fragments of the Ig loci [See Mendez et

10   al. Nature Genetics 15:146-156 (1997), Green and Jakobovits J. Exp. Med. 188:483-495

(1998)], and introduction of entire or substantially entire loci through the use of microcell

fusion (see European Patent Application No. EP 0 843 961 A1).

Any transgenic mouse capable of responding to immunization with antibodies

having human sequences may be used to produce an anti-myostatin antibody of the

15   invention when using methods available to one skilled in the art, e.g., when such mouse is

immunized with a polypeptide comprising an immunogenic epitope of the invention.

Uses

Antibodies of the present invention are useful in therapeutic, prophylactic,

20   diagnostic and research applications as described herein. An antibody of the invention

may be used to diagnose a disorder or disease associated with the expression of human

myostatin. In a similar manner, the antibody of the invention can be used in an assay to

monitor myostatin levels in a subject being treated for a myostatin-associated condition.

Diagnostic assays include methods that utilize the antibody of the invention and a label to

25   detect myostatin in a sample, *e.g.,* in a human body fluid or in a cell or tissue extract.

Antibodies of the invention may be used with or without modification, and are labeled by

covalent or non-covalent attachment of a detectable moiety. The detectable moiety can be

any one that is capable of producing, either directly or indirectly, a detectable signal. For

example, the detectable moiety may be a radioisotope such as, e.g., ^{3}H, ^{14}C, ^{32}P, ^{35}S, or

30   ^{125}I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate,

rhodamine, or luciferin; or an exzyme, such as alkaline phosphatase, beta-galactosidase,

or horseradish peroxidase. Any method known in the art for separately conjugating the

antibody to the detectable moiety may be employed, including those methods described by Hunter, *et al., Nature* 144:945, 1962; David, *et al., Biochemistry* 13: 1014, 1974; Pain, *et al., J. Immunol. Meth.* 40: 219, 1981; and Nygren, J. *Histochem. And Cytochem.* 30: 407, 1982.

5   A variety of conventional protocols for measuring myostatin, including *e.g.,* ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of myostatin expression. Normal or standard expression values are established using any art known technique, *e.g.,* by combining a sample comprising a myostatin polypeptide with, *e.g.,* antibodies under conditions suitable to form a

10   antigen:antibody complex. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of

15   suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of a radioactive material include ^{125}I, ^{131}I, ^{35}S, or ^{3}H. (See, *e.g.,* Zola, *Monoclonal Antibodies:*

20   *A Manual of Techniques*, CRC Press, Inc. (1987)).

The amount of a standard complex formed is quantitated by various methods, such as, *e.g.,* photometric means. Amounts of myostatin polypeptide expressed in subject, control, and samples (*e.g.,* from biopsied tissue) are then compared with the standard values. Deviation between standard and subject values establishes parameters for

25   correlating a particular disorder, state, condition, syndrome, or disease with a certain level of expression (or lack thereof) for a myostatin polypeptide.

Once the presence of a disorder, state, condition, syndrome, or disease is established and a treatment protocol is initiated, assays are repeated on a regular basis to monitor the level of myostatin expression. The results obtained from successive assays

30   are used to show the efficacy of treatment over a period ranging from several days to months. With respect to a particular disorders (*e.g.,* frailty or cachexia) the presence of an altered amount of myostatin in biopsied tissue or fluid (*e.g.,* serum or urine) from a

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subject may indicate a predisposition for the development of a disorder, state, condition, syndrome, or disease or it may provide a means for detecting such a disorder, state, condition, syndrome, or disease prior to the appearance of actual clinical symptoms or it may define a population more likely to respond therapeutically to an antibody of the

5   invention. A more definitive initial detection may allow earlier treatment thereby preventing and/or ameliorating further progression of a disease or disorder associated with myostatin expression, e.g., muscle or bone deterioration.

As a matter of convenience, the antibody of the present invention can be provided in a kit, a packaged combination of reagents in predetermined amounts with instructions

10   for performing the diagnostic assay. Where the antibody is labeled with an enzyme, the kit will include substrates and cofactors required by the enzyme (e.g., a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives may be included such as stabilizers, buffers (e.g., a blocking buffer or lysis buffer) and the like. The relative amounts of the various reagents may be varied widely to

15   provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

20   Therapeutic Uses for the Antibody

Myostatin plays a role in muscle development and a number of related disorders or diseases. In adults, myostatin mRNA is primarily detected in skeletal muscle although lower concentrations are also found in adipose tissue and cardiac tissue (Sharma, M., *et al, J. Cell Physiol.* 180:1, 1999). Myostatin knockout mice have two- to three-fold greater

25   muscle mass than their wild type littermates. The increased muscle mass is the result of fiber hypertrophy and hyperplasia (McPherron, A., *et al. Nature* 387:83-90, 1997 and Zhu, X. et al., *FEBS Letters* 474:71). In addition, the myostatin knockout mice accumulate less fat than their wild type littermates but otherwise appear normal and healthy. Myostatin has also been recently shown to be an important regulator of

30   adipogenesis (Rebbapragada, A., *et al., Mol. and Cell. Bio.* 23:7230-7242, 2003). Additionally, bone structure and content has been recently studied in myostatin deficient

mice (Hamrick M.W., *et al., J. Orthopaedic Research* 21:1025, 2003; Hamrick, M.W., *et al., Calcif Tissue Int* 71:63, 2002.

Therefore, a pharmaceutical composition comprising an anti-myostatin monoclonal antibody of the invention may be used to increase muscle mass, increase bone density, decrease muscle wasting, or may be useful for the treatment or prevention of conditions wherein the presence of myostatin causes or contributes to undesirable pathological effects or decrease of myostatin levels has a therapeutic benefit in mammals, preferably humans, including, but not limited to, muscle wasting, muscle injury, surgery, repair of damaged muscle, frailty, age-related sarcopenia, disuse atrophy, osteoporosis, osteoarthritis, ligament growth and repair, obesity, suppression of body fat accumulation, obesity, muscular dystrophy of any type, critical care myopaythy, alcoholic myopathy, cachexia (*e.g.*, cancer-related or HIV-induced, or resulting from COPD, chronic lung disease, recovery from sepsis, renal failure, liver failure, cardiac failure or disease), metabolic syndrome, post-burn muscle wasting, and Type II diabetes. Disuse atrophy may result from numerous causes or incidents including any disorder or disease or state which leads to prolonged immobility or disuse or bed rest including, but not limited to, solid organ transplant, joint replacement, stroke, spinal cord injury, recovery from severe burn, sedentary chronic hemodialysis, post-sepsis recovery and exposure to microgravity. Since myostatin is highly conserved in sequence and function across species, the antibodies of the invention may be used to increase muscle mass, increase bone density or treat or prevent conditions in non-human mammals or avian species [e.g., domestic animals (e.g., canine and feline), sports animals (e.g., equine), food-source animals (e.g., bovine, porcine and ovine), avian species (e.g., chicken, turkey, other game birds or poultry)] wherein the presence of myostatin causes or contributes to undesirable pathological effects or decrease of myostatin levels has a therapeutic benefit.

The use of an anti-myostatin monoclonal antibody of the present invention for treating or preventing of at least one of the aforementioned disorders in which myostatin activity is detrimental or which benefits for decreased levels of bioactive myostatin is contemplated herein. Additionally, the use of an anti-myostatin monoclonal antibody of the present invention for use in the manufacture of a medicament for the treatment of at least one of the aforementioned disorders is contemplated.

As used herein, the terms "treatment", "treating", and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse affect

5   attributable to the disease. "Treatment", as used herein, includes administration of a compound of the present invention for treatment of a disease or condition in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *i.e.*, arresting its development; and (c) relieving the disease,

10   i.e., causing regression of the disease or disorder or alleviating symptoms or complications thereof. Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic

15   situation.

Pharmaceutical Composition

An antibody of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. The compounds of the invention

20   may be administered alone or in combination with a pharmaceutically acceptable carrier, diluent, and/or excipients, in single or multiple doses. The pharmaceutical compositions for administration are designed to be appropriate for the selected mode of administration, and pharmaceutically acceptable diluents, carrier, and/or excipients such as dispersing agents, buffers, surfactants, preservatives, solubilizing agents, isotonicity agents,

25   stabilizing agents and the like are used as appropriate. Said compositions are designed in accordance with conventional techniques as in *e.g.*, Remington, *The Science and Practice of Pharmacy*, 19th Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA 1995 which provides a compendium of formulation techniques as are generally known to practitioners.

A pharmaceutical composition comprising an anti-myostatin monoclonal antibody

30   of the present invention can be administered to a subject at risk for or exhibiting pathologies as described herein using standard administration techniques including oral,

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intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration.

A pharmaceutical composition of the invention preferably is a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody of the

5   invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective

10   amount is also one in which any toxic or detrimental effect of the antibody, are outweighed-by-the therapeutically-beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount

15   will be less than the therapeutically effective amount.

A therapeutically-effective or prophylactically-effective amount is at least the minimal dose, but less than a toxic dose, of an active agent which is necessary to impart therapeutic benefit to a subject. Stated another way, a therapeutically-effective amount of an antibody of the invention is an amount which in mammals, preferably humans,

20   increases muscle mass, increases bone density, or treats conditions wherein the presence of myostatin causes or contributes to undesirable pathological effects or decrease in myostatin levels results in a beneficial therapeutic effect in a mammal, preferably a human, including, but not limited to, muscle wasting, muscle injury, surgery frailty, age-related sarcopenia, disuse atrophy, osteoporosis, osteoarthritis, ligament growth and

25   repair, obesity, suppression of body fat accumulation, muscular dystrophy of any type, critical care myopaythy, cachexia (e.g., cancer-related or HIV-induced, or resulting from COPD, renal failure, liver failure, cardiac failure or disease), metabolic syndrome and Type II diabetes. Disuse atrophy may result from numerous causes or incidents including any disorder or disease or state which leads to prolonged immobility or disuse or bed rest

30   including, but not limited to, solid organ transplant, joint replacement, stroke, spinal cord injury, recovery from severe burn, sedentary chronic hemodialysis, post-sepsis recovery and exposure to microgravity.

The route of administration of an antibody of the present invention may be oral, parenteral, by inhalation, or topical. Preferably, the antibodies of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. The term parenteral as used herein includes intravenous, intramuscular, subcutaneous,

5   rectal, vaginal, or intraperitoneal administration. Peripheral systemic delivery by intravenous or intraperitoneal or subcutaneous injection is preferred. Suitable vehicles for such injections are straightforward in the art.

The pharmaceutical composition typically must be sterile and stable under the conditions of manufacture and storage in the container provided, including *e.g.,* a sealed

10 vial or syringe. Therefore, pharmaceutical compositions may be sterile filtered after making the formulation, or otherwise made microbiologically acceptable. A typical composition for intravenous infusion could have a volume as much as 250-1000 ml of fluid, such as sterile Ringer's solution, physiological saline, dextrose solution and Hank's solution and a therapeutically effective dose, (*e.g.,* 1 to 100 mg/mL, or more) of antibody

15 concentration. Dose may vary depending on the type and severity of the disease. As is well known in the medical arts, dosages for any one subject depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. A typical dose can be, for example, in the range of 0.001 to

20 1000 μg; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. The daily parenteral dosage regimen can be about 0.1 μg/kg to about 100 mg/kg of total body weight, preferably from about 0.3 μg/kg to about 10 mg/kg and more preferably from about 1 μg/kg to 1 mg/kg, even more preferably from about 0.5 to 10 mg/kg body weight per day. Progress may be monitored

25 by periodic assessment. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and are not excluded herefrom. The desired dosage can be delivered by a single bolus administration, by multiple bolus administrations, or by continuous infusion administration of antibody,

30 depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve.

These suggested amounts of antibody are subject to a great deal of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result

obtained. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the antibody, the particular type of antibody, the method of administration, the scheduling of administration, and other

5   factors known to medical practitioners.

Therapeutic agents of the invention may be frozen or lyophilized for storage and reconstituted in a suitable sterile carrier prior to use. Lyophilization and reconstitution can lead to varying degrees of antibody activity loss. Dosages may have to be adjusted to compensate. Generally, pH between 6 and 8 is preferred.

10

Articles of Manufacture.

In another embodiment of the invention, 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

15   containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition of the invention which is effective for preventing or treating the disorder or condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection

20   needle). The active agent in the composition is an anti-myostatin antibody of the invention. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other

25   materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

30   **EXAMPLES**

Example 1: ELISA Assays

A. <u>Anti-myostatin Fabs preferentially bind myostatin over GDF-11.</u>

Anti-myostatin Fabs (or full-length mAbs) of the present invention are tested in an

ELISA assay, in which binding of the Fab to mature myostatin (dimeric form) coated at

various concentrations on a 96-well plate is measured. Binding of the Fabs to GDF-11 is

5   also tested.

Each well of two 96-well plates is coated with 200 ng/well recombinant mouse

myostatin (R&D systems, Cat. #788-G8/CF, carrier-free, in carbonate buffer, pH 9.6) or

200 ng/well recombinant human GDF-11 (Peprotech, Inc., Cat. # 120-11, carrier-free, in

carbonate buffer, pH 9.6). The plates are incubated at 4°C overnight. The wells are

10   aspirated and washed twice with washing buffer (20 mM Tris (hydroxymethyl)

aminomethane, pH 7.4, 0.15 M NaCl, 0.1% Tween-20). The plates are blocked with 200

µl blocking buffer per well (5% Carnation Instant milk in the above washing buffer) for 5

hours.

Fabs or mabs to be tested are diluted into blocking buffer at various

15   concentrations, e.g., 10 µg/ml, 2 µg/ml, 0.4 µg/ml, 0.08 µg/ml, and 0.016 µg/ml. Fifty

microliters of each Fab solution is added to the GDF-8 and GDF-11 coated wells in

duplicate. The plates are incubated for 1 hour at room temperature. The wells are then

washed 3 times with washing buffer. AP-conjugated secondary antibody (50 µl goat anti-

kappa AP (Southern Biotech), diluted 1:1000 in blocking buffer) is added to each well

20   and incubated for 1 hour at room temperature. The wells are then washed 3 times with

washing buffer. Fifty microliters of chromogenic substrate (*i.e.,* AP substrate) is added to

each well and allowed to develop at room temperature. The absorbance of the wells is

read at OD of 560 nm. The average absorbance from duplicate wells is determined.

All antibodies of the invention are contemplated to bind to plate-bound human

25   mature myostatin and preferentially bind to myostatin when compared to GDF-11

binding. In a GDF-8 down ELISA, Fab C12 has an IC_{50} of 0.25 µg/ml and Fab 510C2

has an IC_{50} of 0.27 µg/ml.

B. <u>Humanized-optimized anti-myostatin antibodies bind myostatin peptide</u>

30   In an ELISA assay, antibodies of the invention bind a purified, biotinylated

peptide with the sequence that spans amino acids 40-64 of mature human myostatin (SEQ

ID NO: 53) with the exception that an aminobutyric acid is at position 47 instead of the

cysteine residue. Antibodies of the invention also bind a peptide with the sequence shown in SEQ ID NO: 62 (with cysteine or serine at position 47 of mature myostatin) as well as the mature form of myostatin in monomeric and dimeric forms.

In an exemplary ELISA assay, 50 μl of a 1 μg/ml solution of goat anti-human
5   kappa antibody (UNLB, Southern Biotech 2060-01) in 50mM sodium bicarbonate buffer, pH 8.0 is used to coat each well of a high-bind microtiter plate (Greiner EK 20061). The wells are aspirated and washed with PBST (PBS, 0.1% Tween) and then blocked for 1 hour with PBST-BSA (PBS, 1% BSA, 0.1% Tween). The wells are then washed again with PBST. All steps of the assay are performed at room temperature. Titrations of
10   humanized-optimized anti-myostatin antibody (full-length antibody comprising an IgG4 Fc-region, transiently expressed in 293 cells), diluted in PBST-BSA beginning at 50 ng/well are added to the wells and the plate is further incubated for 1 hr, washed as above and probed with 50 μl of the biotinylated peptide described above at a concentration of 50 nM (diluted in PBST-BSA). Then 50 μl of a 2 μg/ml NeutrAvidin-AP (1:1000 in PBST-
15   BSA, Pierce 31002) is added and detection is achieved by addition of AP substrate according to manufacturer's instructions (Pierce 31002). Buffer alone is used as a control. Absorbance is read at 560 nm. The 510C2 and C12 Fabs bind the peptide with IC_{50} values of 6.73 and 5.42 ng/well respectively.

Antibodies of the invention are able to bind a peptide consisting of amino acids
20   40-64 (SEQ ID NO: 53) of mature myostatin and/or a peptide consisting of amino acids 43-57 (SEQ ID NO: 62) of mature myostatin (with a cysteine or serine amino acid at position 47). However, antibodies of the invention are not able to bind (at levels significantly greater than background), peptides consisting of amino acids 60-73, 74-86, 87-97, 96-108, 16-29, 1-15 or 30-42 of mature myostatin. The murine antibody named
25   Mab-3 (see U.S. patent applications 60/559,621 and 60/555,456 incorporated herein), comprising murine variable regions (see Figs 10 and 11) operably attached to murine IgG1 Fc region - does not bind peptides consisting of amino acids 60-73, 74-86, 87-97, 96-108, 16-29, 1-15 or 30-42 of mature myostatin and is the parent antibody for antibodies of the invention.

30   Humanized antibodies 510C2 and C12 bind peptides consisting of amino acids 43-57 and 45-59 of mature myostatin (as shown in Table 1 below) to a similar degree. This demonstrates that the epitope of the invention is more specifically localized within

the peptide spanning amino acids 45-57 of mature myostatin. Binding is slightly reduced

when the antibodies are tested for binding to a peptide consisting of amino acids 47-61 of

mature myostatin, demonstrating importance of amino acids 45-46 of mature myostatin

for binding or for preserving conformation of the peptide that allows for binding. Binding

5   is further reduced when antibodies are tested for binding to a peptide consisting of amino

acids 49-63 of mature myostatin, demonstrating importance of amino acids 47-48 of

mature myostatin for binding or for preserving conformation of the peptide that allows for

binding. Binding is reduced to background levels when the antibodies are tested for

binding a peptide consisting of amino acids 39-53 of mature myostatin, suggesting the

10   importance of the region from residues 54-57.

Table 1

Peptide	Residues	Relative Binding	Seq Id No:
ANYCSGECEFVFLQKYPHTHLVHQA	40-64	+++	53
CSGES*EFVFLQKYPH	43-57	+++	62
GECEFVFLQKYPHTH	45-59	+++	92
CEFVFLQKYPHTHLV	47-61	++	93
FVFLQKYPHTHLVHQ	49-63	+	94
KANYCSGECEFVFLQ	39-53	–	95

(* residue is C in myostatin)

C. Myostatin Humanized-Optimized Mab ELISA Assay

25   Humanized anti-human myostatin full-length monoclonal antibodies 510C2 and

C12 are tested in an ELISA assay, in which binding of the antibody to the GDF-8 antigen

coated on a plate is measured. Cross-reactivity of the Mabs to GDF-11 is also tested. A

no Mab condition, an isotype control antibody, and an irrelevant protein (HGF) are used

as negative controls.

30   Each well of 96-well plates is coated with 70 μl of human GDF-8 (carrier-free,

1ug/ml in carbonate buffer, pH 9.6), recombinant human GDF-11 (from Peprotech, Inc.,

catalog # 120-11, carrier-free, 1ug/ml in carbonate buffer, pH 9.6), or recombinant human

HGF (from R&D Systems, catalog # 294-HG/CF, carrier-free, 1ug/ml in carbonate buffer,

pH 9.6). The plates are incubated at 4°C overnight. The wells are aspirated and washed

35   twice with washing buffer (20 mM Tris (hydroxymethyl) aminomethane, pH 7.4, 0.15 M

NaCl, 0.1% Tween-20). The plates are blocked with 200ul blocking buffer per well (5%

51

Carnation Instant milk in the above washing buffer) for 4 hours at room temperature.
Plates are washed twice with washing buffer.

Mabs are diluted into blocking buffer at 1 µg/ml, 0.2 µg/ml, 0.04 ug/ml, 0.008
ug/ml, 0.0016 µg/ml, and 0.00032 µg/ml. A no Mab control is used, which consists of
5   blocking buffer alone. An isotype control antibody is also used as a negative control, at
1ug/ml. Then 50 µl of each Mab solution is added to the GDF-8 and GDF-11 coated
wells in duplicate. The plates are incubated for 1 hour at room temperature. The wells
are then washed 3 times with washing buffer.

Then 50 µl peroxidase-conjugated secondary antibody (goat anti-human IgG HRP,
10   Fc gamma fragment specific, Jackson ImmunoResearch, catalog # 109-035-008), diluted
1:2000 in blocking buffer, is added to each well and incubated for 1 hour at room
temperature. The wells are then washed 3 times with washing buffer. Then 50 µl of
chromogenic substrate (i.e., OPD substrate) is added to each well and allowed to develop
at room temperature for 2.5 minutes. The reaction is stopped by adding 100 µl 1N HCl to
15   each well.   The absorbance of the wells is read at 490 nm. The average absorbance from
duplicate wells is determined, and these values are listed in Table 2, below.

These data demonstrate that Mabs 510C2 and C12 bind to plate-bound myostatin.
With regards to specificity, these Mabs preferentially bind GDF-8 over GDF-11. Mab
510C2 shows approximately 25-fold greater binding to myostatin (GDF-8) than to GDF-
20   11. Mab C12 shows approximately 5-fold greater binding to myostatin than to GDF-11.

Table 2

	510C2	510C2	510C2	C12	C12	C12
	GDF-8	GDF-11	HGF	GDF-8	GDF-11	HGF
1ug/ml	1.62275	0.67645	0.0723	1.5785	1.3021	0.0665
0.2ug/ml	1.20585	0.24605	0.071	1.37095	0.87605	0.0543
0.04ug/ml	0.55695	0.11485	0.07525	0.8177	0.34495	0.0564
0.008ug/ml	0.2482	0.09125	0.07105	0.32805	0.11915	0.0507
0.0016ug/ml	0.11425	0.09385	0.07255	0.1257	0.07	0.058
0.00032ug/ml	0.0876	0.08245	0.0722	0.07415	0.05475	0.0554
no Mab	0.08645	0.0936	0.0721	0.0544	0.05185	0.0522
isotype control	0.09075	0.0919	0.0804	0.05185	0.0487	0.049

Furthermore, full-length mab C12 was demonstrated in an ELISA assay to not
bind other TGF-β superfamily members BMP-2, BMP-3, BMP-3b/GDF-10, BMP-4,
25   BMP-7, BMP-8B, Activin A, Activin B, TGF-α, TGF- β1, TGF- β2 and GDF-3; it
demonstrated minimal binding to BMP-5. It is contemplated that other anti-myostatin

52

antibodies of the invention similarly do not bind or minimally bind these other TGF-β superfamily members.

Example 3 Myostatin Neutralization Assay

Ectodermal explants are removed from stage 8-9 blastula Xenopus embryos by standard procedures and cultured in 0.5X MBS (1X MBS: 88 mM NaCl, 1 mM KCl, 0.7 mM CaCl₂, 1 mM MgSO₄, 5 mM HEPES, 2.5 mM NaHCO₃, 1:1000 v/v gentamycin, 0.1% bovine serum albumin) with the addition of growth factor (GDF8 or GDF11) plus or indicated, for 18 hours at 18°C, by which time control embryos reach the early neurula stage (stage 15-16). Explants are photographed and the length of each explant is measured using an image analysis algorithm designed for animal cap quantitation. Explants not treated with either growth factor or Fab (controls), round into balls of epidermis. Myostatin and GDF-11 induce mesoderm in these ectodermal explants which causes the explants to elongate and form dumbbell-like structures. Antibodies or Fabs, when tested for neutralizing activity, are added to the culture medium containing myostatin for the entire length of the culture period and their ability to inhibit the growth factor-induced elongation movements is assessed. Myostatin is added to the explants at 25 ng/ml. Antibodies or Fabs to be tested are added at 20 μg/ml. A Fab generated to an irrelevant antigen is used as a control. A commercially available monoclonal anti-mouse GDF8 antibody may be tested as a control, this antibody is produced in goats immunized with purified mouse GDF8 and demonstrated by the manufacturer to neutralize elongation of Xenopus animal caps elicited by 25 ng/ml of murine GDF8 when present at about 10-20 μg/ml (R&D Systems Cat. #MAB788).

ImagePro (v4.5.1.22, from Media Cybernetics) is used for the image processing. A macro is written to automate the image processing. The macro processes the image and records length in units of bits. Alternative measuring methods may be used as known in the art. Antibodies of the invention are contemplated to neutralize GDF8 activity in the animal cap assay and have no GDF-11 neutralization activity.

Example 4: Affinity Measurement of Fabs

The affinity (K_D) and k_{on} and k_{off} rates of anti-myostatin Fabs of the present invention are measured using a BIAcore® 2000 instrument containing a CM4 sensor chip.

The BIAcore® utilizes the optical properties of surface plasmon resonance to detect
alterations in protein concentration of interacting molecules within a dextran biosensor
matrix. Except where noted, all reagents and materials are purchased from BIAcore® AB
(Upsala, Sweden). All measurements are performed at 25°C. Samples containing Fabs

5   are dissolved in HBS-EP buffer (150 mM sodium chloride, 3 mM EDTA, 0.05% (w/v)
surfactant P-20, and 10 mM HEPES, pH 7.4). Myostatin or GDF-11 (R&D Systems) is
immobilized onto flow cells of a CM4 chip using amine-coupling chemistry. Flow cells
(1-4) are activated with a 1:1 mixture of 0.1 M N-hydroxysuccinimide and 0.1 M 3-(N,N-
dimethylamino)propyl-N-ethylcarbodiimide at a flow rate of 20 µl/min. Myostatin or

10   GDF-11 (2.5 µg/mL in 10mM sodium acetate, pH 4.5) is manually injected over
individual flow cells at a flow rate of 10 µL/min. The surface density is monitored and
until each flow cell reaches a surface density of ~150 response units (RU). Surfaces are
blocked with a 50 µl injection of 1 M ethanolamine-HCl, pH 8.5 (10 µL/min). To ensure
complete removal of any noncovalently bound myostatin or GDF-11, 15 µl of 10 mM

15   glycine, pH 1.5 is injected twice. Running buffer used for kinetic experiments contained
10mM HEPES, pH 7.4, 150mM NaCl, 0.005% P20.

       Collection of kinetic binding data is performed at maximum flow rate (100
µl/min). Each analysis cycle consists of (i) 250 µl injection of a Fab (concentration range
of 50 nM to 0.4 nM in 2-fold dilution increments) over all 4 flow cells with flow cell 1 as

20   the reference flow cell, (ii) 20 min dissociation (buffer flow), (iii) regeneration of the
GDF-8 or GDF-11 surface with a two 15 µl injection of 10 mM glycine, pH 1.5, (iv) a 15
µl blank injection of running buffer, and (v) a 2 min stabilization time before start of next
cycle. Signal is monitored as flow cell 2 minus flow cell 1, flow cell 3 minus flow cell 1
and flow cell 4 minus flow cell 1. Samples and a buffer blank are injected in duplicate in

25   a random order. Data are processed using SCRUBBER (Center for Biomolecular
Interaction Analysis, Univ. of Utah) software. Association and dissociation rates for each
cycle are determined by fitting of the biosensor data using to a simple association model
using ClampXP (Center for Biomolecular Interaction Analysis, Univ. of Utah) to extract
the k_{on} and k_{off} rate constants; the equilibrium binding constant K_d is calculated using the

30   relationship $K_d = k_{off}/k_{on}$. The affinity data measured for myostatin binding to the 510C2

Fab are: k_{on} of 5.4×10^5 $M^{-1}s^{-1}$, k_{off} of 4.67×10^{-4} sec^{-1}, and K_d (calc) of 0.87 nM. Binding to GDF-11 by 510C2 Fab was not observed.

Example 5: Affinity Measurement of Mabs

Binding affinity measurements for full-length monoclonal antibodies of the invention are determined using a Sapidyne KINEXA assay. NHS-activated fast-flow sepharose beads (GE Healthcare) are pre-coated with an antibody of the invention (50 µg anti-myostatin antibody per ml of beads) and blocked with 10 mg/ml BSA in 1 M Tris-HCl, pH 8.0. Then 20 pM and 50 pM of an antibody of the invention is incubated with various concentration (e.g., 2.4 pM to 20 nM, serial dilutions) of myostatin in running buffer (PBS, 0.005% (v/v) Tween-20, 1 mg/ml ovalbumin) for 10 hours at room temperature. To determine the free antibody present at equilibrium, each sample is passed through the myostatin-coated beads. The amount of bead-bound antibody is then quantified by passing a solution of fluorescent (Cy5) labeled goat ant-human Fc antibody (Jackson Immuno Research) diluted 1:4000 in running buffer over the beads. The measured fluorescence signal is proportional to the concentration of free antibody at equilibrium. Each concentration of myostatin is measured in duplicate. The equilibrium dissociation constant (K_D) is obtained from non-linear regression of the competition curves using a multiple-curve, one-site homogeneous binding model (KINEXA software) and data is presented with 95% confidence intervals (CI).

The association rate constant (k_{on}) for GDF-8 binding is also determined using a Sapidyne KINEXA assay. Twenty pM antibody is mixed with 1 nM GDF-8 using the same conditions described above. At various time points, samples are probed for free antibody using the conditions described above for equilibrium binding, and then the resulting time dependence is fit using the KINEXA software to determine the association rate (k_{on}). The dissociation rate constant (k_{off}) is calculated using the expression $k_{off} = K_D \times k_{on}$. When full-length 510C2 and C12 (operably linked to a IgG$_4$ Fc region) is measured using the described assay, the results are as stated in Table 3 below.

Table 3

Mab	Kd, pM (95% CI)	k_{on}, M^{-1}, s^{-1} (95% CI)	koff, s^{-1}, calculated
510C2	80 (41-134)	1.38×10^5 (1.08-1.75×10^5)	1.11×10^{-5}
C12	108 (23-294)	7.42×10^4	8.02×10^{-6}

		$(6.83\text{-}8.01 \times 10^4)$	

Example 6 Myostatin/SBE Reporter Assay

In this reporter assay, a plasmid encoding a reporter gene, i.e., luciferase gene, downstream of a SMAD binding element ("SBE"), more specifically $(CAGA)_{12}$ expresses

5   luciferase protein when a molecule such as myostatin, GDF-11, or other TGF-β superfamily member binds its own receptor, thereby triggering SMAD signaling which results in a phosphorylated SMAD complex which is capable of binding the SBE. The CAGA sequence was previously reported to be a TGF-β responsive sequence within the promoter of the TGF-β induced gene PAI-1 (Denner et al., *EMBO J.*, 17:3091-3100,

10   1998). The amount of active myostatin exposed to the cells is directly proportional to the quantity of luciferase enzyme produced which is directly proportional to the quantity of light produced and measurable. The presence of an inhibitor (e.g., an antibody that binds myostatin) reduces the quantity of myostatin able to activate the SBE which ultimately results in a reduced production of light. This assay is also described in International

15   Publication Number WO 2004/037861 incorporated herein.

It is contemplated that a Myostatin/SBE Reporter Assay not be limited to the exact conditions described herein, other types of cells may be used, e.g., 293HEK (ATCC) or A204 Rhabsomyosarcoma cells (see, e.g., Whittemore, et al. *BBRC*, 200:965-71, 2003); other types of reporters may be used, e.g., CAT, β-gal, GFP, and other growth conditions

20   for the cells and assay conditions including varying amounts of myostatin in the reaction may be used. One of skill in the art would readily be able to discern if an assay falls within the scope of a myostatin/SBE reporter assay for it would have a vector comprising an SBE element upstream of a reporter gene introduced into a host cell, wherein the SBE element used is responsive to the SMAD produced in response to myostatin binding the

25   myostatin receptor. R.S. Thies, et al., *Growth Factors*, 18:251-259, 2001, describes a similar assay while, Wittemore, L. et al., *BBRC*, 300:965-971, 2003 describes the SBE element response to the SMAD produced in response to myostatin binding its receptor.

In this assay, 293E cells (Edge Biosystems) in DMEM/F12 media (3:1) (Gibco 93-0152DK), 10% FBS, 20mM Hepes, 4mM L-glutamine are seeded at about 25000 cells per

30   well in poly-lysine coated inner wells of a 96-well plate (BD Biocoat 35-4461) and incubated overnight at 37°C. The following day, the cells are washed in PBS and 50 μl OptiMEM I (Gibco 31985-070) is added per well. The cells are transfected with 50 μl of

the following SBE-luciferase DNA mixture: (i) 80 μl Lipofectamine (Gibco 11668-019

combined with 1.5 ml OptiMEM and allowed to sit for 5 minutes then added to (ii) a tube

in which 20 μg SBE luciferase DNA is combined with 1.5 ml OptiMEM and 200 μl Plus

reagent, mixed and allowed to sit for 5 minutes. After the two mixtures are added

5   together, the solution is mixed vigorously and allowed to stand for 30 minutes before 50

μl is added to each well. The cells are then incubated overnight at 37°C in 5% CO_2. For

each plate of cells, 5 ml of myostatin (R&D Systems 788-G8) is diluted to 20 ng/ml in

complete medium. Each antibody of the invention to be tested is titrated in complete

medium, e.g., from about 40 μg/ml to about 50 ng/ml. The transfection media is removed

10   from the wells and 50 μl of an antibody dilution is added per well and 50 μl of GDF-8

(myostatin) or GDF-11 (R&D Systems) is added per well. The plate of cells is then

incubated overnight at 37°C in 5% CO_2. The following day, the media is aspirated, the

cells are washed in PBS and 75 μl lysis buffer (Promega E266A) is added. The luciferase

activity in the cell lysate is measured using Luciferase Reagent according to

15   manufacturer's instructions (Promega E2620). Luminescence is plotted against Log_{10}

Mab concentration (μg/ml) and the IC_{50} for each Mab for myostatin and GDF-11 is

calculated.

Monoclonal antibodies 510C2, C12 when tested in these conditions with GDF-8

yield IC_{50} values (from an average of two tests) of 5.15 nM (5.74 and 4.57 nM) and 16.07

20   nM (23.04 and 9.12 nM) respectively. Neither 510C2 nor C12 mAbs demonstrate

neutralization activity in this assay when tested with GDF-11 instead of GDF-8.

Example 7 Pharmacokinetics

The pharmacokinetics (PK) of the antibodies of the invention may be evaluated in

25   C57B6/SCID mice at a dose of 1 mg/kg after a single intravenous (IV) or intraperitoneal

(IP) administration. The animals receive a mixture of unlabeled and ^{125}I-labeled antibody

at a dose described above and serum concentration is determined based on [125]I

radioactivity in the serum and the specific activity of the injected dose. Serum

concentration of the antibody administered either IV or IP versus time is plotted.

30

Example 8 In vivo effect on Muscle Mass and Strength

To determine whether an antibody of the invention blocks myostatin activity in vivo, an antibody of the invention may be tested in adult SCID mice. SCID mice suffer from severe combined immune deficiency, and therefore do not generate an immunological reaction following injection of an antibody of the invention. Muscle mass

5   is used as an indicator for myostatin activity in mice treated with an antibody of the invention.

Male C57B6 SCID eight weeks old mice are weighed and evenly distributed with respect to body weight into groups of eight. An antibody of the invention in PBS buffer is injected into the mice IP at various doses (e.g., 60, 10, 5 and 1 mg/kg) weekly. PBS-

10   treated or untreated mice are used as controls. The treatments continue for 4-12 weeks. Muscle mass is assessed by dissecting and weighing the gastrocnemious and quadriceps muscles following treatment.

To determine muscle strength, the strength of the front limb is measured with a grip strength test meter (e.g., model 1027 csx, Columbus Instruments).

CLAIMS

What we claim is:

1. An anti-myostatin monoclonal antibody that specifically binds myostatin with an
   affinity no greater than about 3×10^{-8} M, wherein said antibody neutralizes or
   antagonizes at least one myostatin activity.

2. The monoclonal antibody of Claim 1, wherein the monoclonal antibody binds
   myostatin with an affinity no greater than about 9×10^{-10} M.

3. An anti-myostatin monoclonal antibody that has an IC_{50} of less than or equal to
   about 25 nM in an in vitro myostatin/SBE assay.

4. The monoclonal antibody according to any one of Claims 1 to 3, wherein the
   monoclonal antibody binds myostatin within the domain spanning amino acids 40
   to 64 of mature myostatin.

5. The monoclonal antibody according to any one of Claims 1 to 3 wherein the
   monoclonal antibody binds a polypeptide consisting of an amino acid sequence as
   shown in the group consisting of: ANYCSGECEFVFLQKYPHTHLVHQA (SEQ
   ID NO: 53) and CSGECEFVFLQKYPH (SEQ ID NO: 62).

6. A monoclonal antibody according to any one of Claims 1 to 5, which
   preferentially binds a myostatin polypeptide over a GDF-11 polypeptide.

7. The monoclonal antibody according to any one of Claims 1 to 6, wherein said
   antibody is cross-reactive at 35% or less with a GDF-11 polypeptide.

8. The monoclonal antibody according to any one of claims 1 to 7, wherein said
   antibody is a chimeric, humanized or fully human antibody.

9. The monoclonal antibody according to any one of claims 1 to 8, wherein said antibody competes for binding myostatin in a competition ELISA assay with an antibody comprising two polypeptides with the sequences shown in the group consisting of: (i) SEQ ID NOs: 5 and 15, (ii) SEQ ID NOs: 5 and 16, and (iii) SEQ ID NOs: 10 and 26.

10. The monoclonal antibody according to any one of claims 1 to 9 comprising two polypeptides with a sequence selected from the group consisting of:

   (i)   SEQ ID NO: 6 and SEQ ID NO: 14;
   (ii)   SEQ ID NO: 5 and SEQ ID NO: 13, 14, 15, 16, 17, 18, 19 or 20;
   (iii) SEQ ID NO: 7 and SEQ ID NO: 21, 22, 23, 24, 25 or 56;
   (iv)   SEQ ID NO: 8, 9, 10, 11 or 12 and SEQ ID NO: 55.

11. The monoclonal antibody according to any one of Claims 1 to 9, wherein said antibody comprises a HCVR and a LCVR, wherein the HCVR comprises a peptide at CDRH1 with a sequence as shown in SEQ ID NO: 59, a peptide at CDRH2 with a sequence as shown in SEQ ID NO: 60, and a peptide at CDRH3 with a sequence as shown in SEQ ID NO: 61, and wherein the LCVR comprises a peptide at CDRL1 with a sequence as shown in SEQ ID NO: 57, a peptide at CDRL2 with a sequence as shown in SEQ ID NO: 30 or SEQ ID NO: 52, and a peptide at CDRL3 with a sequence as shown in SEQ ID NO: 58.

12. The monoclonal antibody of Claim 11, further comprising a human framework region.

13. The monoclonal antibody of either of Claims 11 or 12, wherein said CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3 comprise a peptide as shown in Figure 9 herein in the corresponding CDR position.

14. The monoclonal antibody according to any one of claims 1 to 13, wherein the monoclonal antibody is a full-length antibody, a substantially intact antibody, a Fab fragment, a F(ab')₂ fragment or a single chain Fv fragment.

15. The monoclonal antibody according to any one of claims 1 to 13, wherein the monoclonal antibody further comprises a heavy chain constant region selected from the group consisting of IgG_1, IgG_2, IgG_3, IgG_4, IgA, IgE, IgM and IgD.

16. The monoclonal antibody according to any one of claims 1 to 15 wherein the constant region present in the antibody originates from the genome of an animal selected from the group consisting of domestic animals, sports animals and food-source animals.

17. A pharmaceutical composition comprising the antibody according to any one of claims 1 to 16.

18. The pharmaceutical composition of claim 17 further comprising a pharmaceutically acceptable carrier.

19. An antibody according to any one of claims 1 to 16 for use as a medicament.

20. Use of an effective amount of antibody according to any one of claims 1 to 16 in the preparation of a medicament for increasing muscle mass or increasing bone density in a subject in need thereof.

21. Use of an effective amount of antibody according to any one of claims 1 to 14 in the preparation of a medicament for the treatment or prevention of one or more conditions selected from frailty, cachexia, muscle wasting, muscle weakness, myopathy, muscular dystrophy, osteoporosis, COPD, renal failure or disease, liver failure or disease, cardiac failure, type II diabetes or metabolic syndrome.

FIG. 1   Promyostatin

1	MQKLQLCVYI	YLFMLIVAGP	VDLNENSEQK	ENVEKEGLCN	40
41	ACTWRQNTKS	SRIEAIKIQI	LSKLRLETAP	NISKDVIRQL	80
81	LPKAPPLREL	IDQYDVQRDD	SSDGSLEDDD	YHATTETIIT	120
121	MPTESDFLMQ	VDGKPKCCFF	KFSSKIQYNK	VVKAQLWIYL	160
161	RPVETPTTVF	VQILRLIKPM	KDGTRYTGIR	SLKLDMNPGT	200
201	GIWQSIDVKT	VLQNWLKQPE	SNLGIEIKAL	DENGHDLAVT	240
241	FPGPGEDGLN	PFLEVKVTDT	PKRSRRDFGL	DCDEHSTESR	280
281	CCRYPLTVDF	EAFGWDWIIA	PKRYKANYCS	GECEFVFLQK	320
321	YPHTHLVHQA	NPRGSAGPCC	TPTKMSPINM	LYFNGKEQII	360
361	YGKIPAMVVD	RCGCS 376	(SEQ ID NO:1)		

FIG. 2 Mature Myostatin (Human, murine, rat, chicken, turkey, dog, horse, pig)

```
1      DFGLDCDEHS TESRCCRYPL TVDFEAFGWD WIIAPKRYKA     40
41     NYCSGECEFV FLQKYPHTHL VHQANPRGSA GPCCTPTKMS     80
81     PINMLYFNGK EQIIYGKIPA MVVDRCGCS 109 (SEQ ID NO:2)
```

FIG. 3　　　**Mature myostatin**

```
                                                                      58
chicken   DFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFLQKYPHTH
dog       DFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFLQKYPHTH
horse     DFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFLQKYPHTH
sheep     DFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFLFLQKYPHTH
cow       DFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFLQKYPHTH
pig       DFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFLQKYPHTH
```

```
                                                        109    SEQ ID
chicken    LVHQANPRGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPAMVVDRCGCS       2
dog        LVHQANPRGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPAMVVDRCGCS       2
horse      LVHQANPRGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPAMVVDRCGCS       2
sheep      LVHQANPKGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPGMVVDRCGCS       3
cow        LVHQANPRGSAGPCCTPTKMSPINMLYFNGEGQIIYGKIPAMVVDRCGCS       2
pig        LVHQANPRGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPAMVVDRCGCS       2
```

FIG. 4   Myostatin:GDF-11 Homology

```
Myostatin    DFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYK
GDF-11       NLGLDCDEHSSESRCCRYPLTVDFEAFGWDWIIAPKRYK


Myostatin    ANYCSGECEFVFLQKYPHTHLVHQANPRGSAGPCCTPTK
GDF-11       ANYCSGQCEYMFMQKYPHTHLVQQANPRGSAGPCCTPTK


Myostatin    MSPINMLYFNGKEQIIYGKIPAMVVDRCGCS    (SEQ ID NO:2)
GDF-11       MSPINMLYFNDKQQIIYGKIPGMVVDRCGCS    (SEQ ID NO:4)
```

FIG. 5 Heavy Chain Variable Regions - <u>Framework: VH2-70</u>:

IC7.1 (SEQ ID NO: 13)
QVTLRESGPALVKPTQTLTLTCTFS<u>GFSLRKVGSSVS</u>WIRQPPGKALEWLA<u>HIYWDDDKRYNPS</u>
<u>LRN</u>RLTISKDTSKNQVVLTMTNMDPVDTATYYCARR<u>AITTVIGGGTMDY</u>WGQGTTVTVSS

329D2 (SEQ ID NO: 14)
QVTLRESGPALVKPTQTLTLTCTFS<u>GFSLRKVGSSVS</u>WIRQPPGKALEWLA<u>HIYWDDDKRYNPS</u>
<u>LRN</u>RLTISKDTSKNQVVLTMTNMDPVDTATYYCARR<u>AITTVIGGGTFDY</u>WGQGTTVTVSS

510C2 (SEQ ID NO: 15)
QVTLRESGPALVKPTQTLTLTCTFS<u>GFSLRKVGRSVS</u>WIRQPPGKALEWLA<u>HIYWDDDKRYNPS</u>
<u>LRN</u>RLTISKDTSKNQVVLTMTNMDPVDTATYYCARR<u>AITTVIGGGTFDY</u>WGQGTTVTVSS

518H3 (SEQ ID NO: 16)
QVTLRESGPALVKPTQTLTLTCTFS<u>GFSWRKVGSSVS</u>WIRQPPGKALEWLA<u>HIYWDDDKRYNPS</u>
<u>LRN</u>RLTISKDTSKNQVVLTMTNMDPVDTATYYCARR<u>AITTVIGGGTFDY</u>WGQGTTVTVSS

525D8 (SEQ ID NO: 17)
QVTLRESGPALVKPTQTLTLTCTFS<u>GFSLRKVGSSVS</u>WIRQPPGKALEWLA<u>HIYWDDDLRYNPS</u>
<u>LRN</u>RLTISKDTSKNQVVLTMTNMDPVDTATYYCARR<u>AITTVIGGGTFDY</u>WGQGTTVTVSS

LC14 (SEQ ID NO: 14)
QVTLRESGPALVKPTQTLTLTCTFS<u>GFSLRKVGSSVS</u>WIRQPPGKALEWLA<u>HIYWDDDKRYNPS</u>
<u>LRN</u>RLTISKDTSKNQVVLTMTNMDPVDTATYYCARR<u>AITTVIGGGTFDY</u>WGQGTTVTVSS

HC5 (SEQ ID NO: 18)
QVTLRESGPALVKPTQTLTLTCTFS<u>GFSMRKVGSSVS</u>WIRQPPGKALEWLA<u>HIYWDDDKRYNPS</u>
<u>LRN</u>RLTISKDTSKNQVVLTMTNMDPVDTATYYCARR<u>AITTVIGGGTFDY</u>WGQGTTVTVSS

HC8 (SEQ ID NO: 19)
QVTLRESGPALVKPTQTLTLTCTFS<u>GFSLRKVGSSIS</u>WIRQPPGKALEWLA<u>HIYWDDDKRYNPS</u>
<u>LRN</u>RLTISKDTSKNQVVLTMTNMDPVDTATYYCARR<u>AITTVIGGGTFDY</u>WGQGTTVTVSS

HC16 (SEQ ID NO: 20)
QVTLRESGPALVKPTQTLTLTCTFS<u>GFSLRKVGSSVS</u>WIRQPPGKALEWLA<u>HIYWDDDKRYNPS</u>
<u>LRN</u>RLTISKDTSKNQVVLTMTNMDPVDTATYYCARR<u>KITTVIGGGTFDY</u>WGQGTTVTVSS

VH2-70 FR1 (SEQ ID NO: 69)   QVTLRESGPALVKTQTLTLTCTFS
VH2-70 FR2 (SEQ ID NO: 70)   WIRQPPGKALEWLA
VH2-70 FR3 (SEQ ID NO: 71)   RLTISKDTSKNQVVLTMTNMDPVDTATYYCAR
VH2-70 FR4 (SEQ ID NO: 72)   WGQGTTVTVSS

FIG. 6 Heavy Chain Variable Regions – Framework VH4-39

DM1 (SEQ ID NO: 21)
QLQLQESGPGLVKPSETLSLTCTVS**GFSLRRVGSSVS**WIRQPPGKGLEWIG**HIYWDDDKRYNPSLRN**RVTISVDTS
KNQFSLKLSSVTAADTAVYYCAR**RAITTVIGGGTFDY**WGQGTLVTVSS

DM6 (SEQ ID NO: 22)
QLQLQESGPGLVKPSETLSLTCTVS**GFSLRMVGSSVS**WIRQPPGKGLEWIG**HIYWDDDKRLNPSLRN**RVTISVDTS
KNQFSLKLSSVTAADTAVYYCAR**RAITTVIGGGTFDY**WGQGTLVTVSS

DM37 (SEQ ID NO: 56)
QLQLQESGPGLVKPSETLSLTCTVS**GFSLRKVGSSVS**WIRQPPGKGLEWIG**HIYWDDDKRLNPSLRN**RVTISVDTS
KNQFSLKLSSVTAVYYCAR**RAITTVIGGGTFDY**WGQGTLVTVSS

DM41 (SEQ ID NO: 23)
QLQLQESGPGLVKPSETLSLTCTVS**GFSLRKVGSSVS**WIRQPPGKGLEWIG**HIYWDDDLRYNPSLRN**RVTISVDTS
KNQFSLKLSSVTAADTAVYYCAR**RAITTVIGGGTFDY**WGQGTLVTVSS

DM14 (SEQ ID NO: 24)
QLQLQESGPGLVKPSETLSLTCTVS**GFSLRKVGSSVS**WIRQPPGKGLEWIG**HIYWDDDKRYNPSLRN**RVTISVDTS
KNQFSLKLSSVTAADTAVYYCAR**RAITTVIGGGTFDM**WGQGTLVTVSS

DM15 (SEQ ID NO: 25)
QLQLQESGPGLVKPSETLSLTCTVS**GFSLRKVGSSVS**WIRQPPGKGLEWIG**HIYWDDDKRYNPSLRN**RVTISVDTS
KNQFSLKLSSVTAADTAVYYCAR**RAITTVIGGGTFDL**WGQGTLVTVSS

DM26 (SEQ ID NO: 55)
QLQLQESGPGLVKPSETLSLTCTVS**GFSLRKVGSSVS**WIRQPPGKGLEWIG**HIYWDDDKRYNPSLRN**RVTISVDTS
KNQFSLKLSSVTAADTAVYYCAR**RAITTVIGGGTFDY**WGQGTLVTVSS

DM27 (SEQ ID NO: 55)
QLQLQESGPGLVKPSETLSLTCTVS**GFSLRKVGSSVS**WIRQPPGKGLEWIG**HIYWDDDKRYNPSLRN**RVTISVDTS
KNQFSLKLSSVTAADTAVYYCAR**RAITTVIGGGTFDY**WGQGTLVTVSS

DM16 (SEQ ID NO: 55)
QLQLQESGPGLVKPSETLSLTCTVS**GFSLRKVGSSVS**WIRQPPGKGLEWIG**HIYWDDDKRYNPSLRN**RVTISVDTS
KNQFSLKLSSVTAADTAVYYCAR**RAITTVIGGGTFDY**WGQGTLVTVSS

DM18 (SEQ ID NO: 55)
QLQLQESGPGLVKPSETLSLTCTVS**GFSLRKVGSSVS**WIRQPPGKGLEWIG**HIYWDDDKRYNPSLRN**RVTISVDTS
KNQFSLKLSSVTAADTAVYYCAR**RAITTVIGGGTFDY**WGQGTLVTVSS

DM21 (SEQ ID NO: 55)
QLQLQESGPGLVKPSETLSLTCTVS**GFSLRKVGSSVS**WIRQPPGKGLEWIG**HIYWDDDKRYNPSLRN**RVTISVDTS
KNQFSLKLSSVTAADTAVYYCAR**RAITTVIGGGTFDY**WGQGTLVTVSS

C12 (SEQ ID NO: 26)
QLQLQESGPGLVKPSETLSLTCTVS**GFSLRKVGSSVS**WIRQPPGKGLEWIG**HIYWDDDKRLNPSLRN**RVTISVDTS
KNQFSLKLSSVTAADTAVYYCAR**RAITTVIGGGTFDL**WGQGTLVTVSS

VH4-39 FR1 (SEQ ID NO: 73)   QLQLQESGPGLVKPSETLSLTCTVS
VH4-39 FR2 (SEQ ID NO: 74)   WIRQPPGKGLEWIG
VH4-39 FR3 (SEQ ID NO: 75)   RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
VH4-39 FR4 (SEQ ID NO: 76)   WGQGTLVTVSS

FIG. 7 Light Chain Variable Region - <u>Framework: O2</u>

IC7.1 (SEQ ID NO: 5)
DIQMTQSPSSLSASVGDRVTITC**SASSSISYMH**WYQQKPGKAPKLLIY**DTSKLAR**GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC**QQWYRNPLT**FGGGTKVEIK

329D2 (SEQ ID NO: 5)
DIQMTQSPSSLSASVGDRVTITC**SASSSISYMH**WYQQKPGKAPKLLIY**DTSKLAR**GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC**QQWYRNPLT**FGGGTKVEIK

510C2 (SEQ ID NO: 5)
DIQMTQSPSSLSASVGDRVTITC**SASSSISYMH**WYQQKPGKAPKLLIY**DTSKLAR**GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC**QQWYRNPLT**FGGGTKVEIK

518H3 (SEQ ID NO: 5)
DIQMTQSPSSLSASVGDRVTITC**SASSSISYMH**WYQQKPGKAPKLLIY**DTSKLAR**GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC**QQWYRNPLT**FGGGTKVEIK

525D8 (SEQ ID NO: 5)
DIQMTQSPSSLSASVGDRVTITC**SASSSISYMH**WYQQKPGKAPKLLIY**DTSKLAR**GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC**QQWYRNPLT**FGGGTKVEIK

LC14 (SEQ ID NO: 6)
DIQMTQSPSSLSASVGDRVTITC**SASSSISYMH**WYQQKPGKAPKLLIY**DTSKLAV**GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC**QQWYRNPLT**FGGGTKVEIK

HC5 (SEQ ID NO: 5)
DIQMTQSPSSLSASVGDRVTITC**SASSSISYMH**WYQQKPGKAPKLLIY**DTSKLAR**GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC**QQWYRNPLT**FGGGTKVEIK

HC8 (SEQ ID NO: 5)
DIQMTQSPSSLSASVGDRVTITC**SASSSISYMH**WYQQKPGKAPKLLIY**DTSKLAR**GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC**QQWYRNPLT**FGGGTKVEIK

HC16 (SEQ ID NO: 5)
DIQMTQSPSSLSASVGDRVTITC**SASSSISYMH**WYQQKPGKAPKLLIY**DTSKLAR**GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC**QQWYRNPLT**FGGGTKVEIK

O2 FR1 (SEQ ID NO: 65)   DIQMTQSPSSLSASVGDRVTITC
O2 FR2 (SEQ ID NO: 66)   WYQQKPGKAPKLLIY
O2 FR3 (SEQ ID NO: 67)   GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
O2 FR4 (SEQ ID NO: 68)   FGGGTKVEIK

FIG. 8 Light Chain Variable Regions - <u>Framework O2</u>

DM1 (SEQ ID NO: 7)
DIQMTQSPSSLSASVGDRVTITC<u>SASSSISYMH</u>WYQQKPGKAPKLLIY<u>DTSKLAR</u>GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC<u>QQWYSNPLT</u>FGGGTKVEIK

DM6 (SEQ ID NO: 7)
DIQMTQSPSSLSASVGDRVTITC<u>SASSSISYMH</u>WYQQKPGKAPKLLIY<u>DTSKLAR</u>GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC<u>QQWYSNPLT</u>FGGGTKVEIK

DM37 (SEQ ID NO: 7)
DIQMTQSPSSLSASVGDRVTITC<u>SASSSISYMH</u>WYQQKPGKAPKLLIY<u>DTSKLAR</u>GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC<u>QQWYSNPLT</u>FGGGTKVEIK

DM41 (SEQ ID NO: 7)
DIQMTQSPSSLSASVGDRVTITC<u>SASSSISYMH</u>WYQQKPGKAPKLLIY<u>DTSKLAR</u>GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC<u>QQWYSNPLT</u>FGGGTKVEIK

DM14 (SEQ ID NO: 7)
DIQMTQSPSSLSASVGDRVTITC<u>SASSSISYMH</u>WYQQKPGKAPKLLIY<u>DTSKLAR</u>GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC<u>QQWYSNPLT</u>FGGGTKVEIK

DM15 (SEQ ID NO: 7)
DIQMTQSPSSLSASVGDRVTITC<u>SASSSISYMH</u>WYQQKPGKAPKLLIY<u>DTSKLAR</u>GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC<u>QQWYSNPLT</u>FGGGTKVEIK

DM26 (SEQ ID NO: 8)
DIQMTQSPSSLSASVGDRVTITC<u>SASSSISYSH</u>WYQQKPGKAPKLLIY<u>DTSKLAR</u>GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC<u>QQWYSNPLT</u>FGGGTKVEIK

DM27 (SEQ ID NO: 9)
DIQMTQSPSSLSASVGDRVTITC<u>SASSSISYAH</u>WYQQKPGKAPKLLIY<u>DTSKLAR</u>GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC<u>QQWYSNPLT</u>FGGGTKVEIK

DM16 (SEQ ID NO: 10)
DIQMTQSPSSLSASVGDRVTITC<u>SASSSISYMH</u>WYQQKPGKAPKLLIY<u>DTSKLAR</u>GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC<u>QQWYLNPLT</u>FGGGTKVEIK

DM18 (SEQ ID NO: 11)
DIQMTQSPSSLSASVGDRVTITC<u>SASSSISYMH</u>WYQQKPGKAPKLLIY<u>DTSKLAR</u>GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC<u>QQWYENPLT</u>FGGGTKVEIK

DM21 (SEQ ID NO: 12)
DIQMTQSPSSLSASVGDRVTITC<u>SASSSISYMH</u>WYQQKPGKAPKLLIY<u>DTSKLAR</u>GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC<u>QQWYFNPLT</u>FGGGTKVEIK

C12 (SEQ ID NO: 10)
DIQMTQSPSSLSASVGDRVTITC<u>SASSSISYMH</u>WYQQKPGKAPKLLIY<u>DTSKLAR</u>GVPSRFSGSGSGTDFTLTISS
LQPEDFATYYC<u>QQWYLNPLT</u>FGGGTKVEIK

FIG. 9

Name	CDRL1	CDRL2	CDRL3	CDRH1	CDRH2	CDRH3
IC7.1	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYRNPLT (SEQ ID NO: 31)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTMDY (SEQ ID NO: 47)
329D2	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYRNPLT (SEQ ID NO: 31)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDY (SEQ ID NO: 48)
510C2	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYRNPLT (SEQ ID NO: 31)	GFSLRKVGRSVS (SEQ ID NO: 37)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDY (SEQ ID NO: 48)
518H3	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYRNPLT (SEQ ID NO: 31)	GFSWRKVGSSVS (SEQ ID NO: 38)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDY (SEQ ID NO: 48)
525D8	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYRNPLT (SEQ ID NO: 31)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDLRYNPSLRN (SEQ ID NO: 44)	RAITTVIGGGTFDY (SEQ ID NO: 48)
LC14	SASSSISYMH (SEQ ID NO:27)	DTSKLAV (SEQ ID NO:52)	QQWYRNPLT (SEQ ID NO: 31)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDY (SEQ ID NO: 48)
HC5	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYRNPLT (SEQ ID NO: 31)	GFSMRKVGSSVS (SEQ ID NO: 39)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDY (SEQ ID NO: 48)
HC8	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYRNPLT (SEQ ID NO: 31)	GFSLRKVGSSIS (SEQ ID NO: 40)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDY (SEQ ID NO: 48)
HC16	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYRNPLT (SEQ ID NO: 31)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RKITTVIGGGTFDY (SEQ ID NO: 49)
DM1	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYSNPLT (SEQ ID NO: 32)	GFSLRRVGSSVS (SEQ ID NO: 41)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDY (SEQ ID NO: 48)
DM6	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYSNPLT (SEQ ID NO: 32)	GFSLRMVGSSVS (SEQ ID NO: 42)	HIYWDDDKRLNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDY (SEQ ID NO: 48)
DM37	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYSNPLT (SEQ ID NO: 32)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDKRLNPSLRN (SEQ ID NO: 45)	RAITTVIGGGTFDY (SEQ ID NO: 48)
DM41	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYSNPLT (SEQ ID NO: 32)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDLRYNPSLRN (SEQ ID NO: 46)	RAITTVIGGGTFDY (SEQ ID NO: 48)
DM14	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYSNPLT (SEQ ID NO: 32)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDM (SEQ ID NO: 50)
DM15	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYSNPLT (SEQ ID NO: 32)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDL (SEQ ID NO: 51)
DM26	SASSSISYSH (SEQ ID NO:28)	DTSKLAR (SEQ ID NO:30)	QQWYSNPLT (SEQ ID NO: 32)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDY (SEQ ID NO: 48)

FIG. 9 continued

DM27	SASSSISYAH (SEQ ID NO:29)	DTSKLAR (SEQ ID NO:30)	QQWYSNPLT (SEQ ID NO: 32)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDY (SEQ ID NO: 48)
DM16	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYLNPLT (SEQ ID NO: 33)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDY (SEQ ID NO: 48)
DM18	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYENPLT (SEQ ID NO: 34)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDY (SEQ ID NO: 48)
DM21	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYFNPLT (SEQ ID NO: 35)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDKRYNPSLRN (SEQ ID NO: 43)	RAITTVIGGGTFDY (SEQ ID NO: 48)
C12	SASSSISYMH (SEQ ID NO:27)	DTSKLAR (SEQ ID NO:30)	QQWYLNPLT (SEQ ID NO: 33)	GFSLRKVGSSVS (SEQ ID NO: 36)	HIYWDDDKRLNPSLRN (SEQ ID NO: 45)	RAITTVIGGGTFDL (SEQ ID NO: 51)
Consensus	SASSSISYXH X is S or A (SEQ ID NO: 57)	DTSKLAR (SEQ ID NO:30)	QQWYXNPLT X is S, R, L, E or F (SEQ ID NO: 58)	GFSX$_1$RX$_2$VGX$_3$SVS X1 is L, W or M X2 is K, R or M X3 is S or R (SEQ ID NO:59)	HIYWDDDX$_1$RX$_2$NPSLRN X1 is K or L X2 is Y or L (SEQ ID NO: 60)	RX$_3$IITTVIGGGTX$_1$DX$_2$ X1 is A or K X2 is M or F X3 is M or L (SEQ ID NO: 61)

FIG. 10 Figure 10 Murine Parent Antibodies LCVR

```
FAb  1                               CDR1          40
3    QIVLTQSPAI MSASPGEKVT MTCSASSSIS YMHWYQQKPG
5    QVVLTQSPAI MSASLGEKVT MTCSASSSVH YMHWYQQKSG
7    QIVLTQSPAI MSASPGEKVT MTCSASSSIS YMHWYQQKPG
8    QIVLTQSPAI MSASPGEKVT MTCSASSSVS YMHWYQQKSG
9    QIVLTQSPAI MSASPGEKVT MTCSASSSVS YMHWYQQKSG
10   QVVLTQSPAI MSASPGEKVT MTCSASSSIS YMHWYQQKPG
11   QIVLTQSPAI MSASPGEKVT MTCSASSSIS YMHWYQQKPG
12   QVVLTQSPAI MSASPGEKVT MTCSASSSVY YMHWYQQRSG
14   QIVLTQSPAI MSASPGEKVT MTCSASSSVS YMHWYQQKPG
15   QIVLTQSPAI MSASPGEEVT MTCSASSSIN YMHWYQQKSG


FAb  41        CDR2                               80
3    TSPKRWIYDT SKLASGVPAR FSGSGSGTSY SLTISSMEAE
5    TSPKRWIYDT SKLASGVPAR FSGSGSGTSY SLTISSMEAE
7    TSPKRWIYDT SKLASGVPAR FSGSGSGTSY SLTISSMEAE
8    TSPKRWIYDT SKLASGVPAR FSGSGSGTSY SLTISSMEAE
9    TSPKRWIYDT SKLASGVPVR FSGSGSGTSY SLTISSMEAE
10   TSPKRWIYDT SKLASGVPAR FSGSGSGTSY SLTISSMEAE
11   TSPKRWIYDT SKLASGVPAR FSGSGSGTSY SLTISSMEAE
12   ASPKRWIYDT SKLASGVPAR FSGSGSGTSY SLTISSMEAE
14   TSPKRWIYDT SKLASGVPAR FSGSGSGTSY SLTISSMEAE
15   TSPKRWIYDT SKLASGVPAR FSGSGSGTSY SLTISSMEAE


FAb  81        CDR3              108
3    DAATYYCQQW YSNPLTFGAG TKLELKRAD (SEQ ID NO:77)
5    DAATYYCQQW SSNPLTFGAG TKLELKRAD (SEQ ID NO:78)
7    DAATYYCQQW YSNPLTFGAG TKLELKRAD (SEQ ID NO:77)
8    DAATYYCQQW SSNPLTFGAG TKLELKRAD (SEQ ID NO:79)
9    DAATYYCQQW SRNPLTFGAG TKLELKRAD (SEQ ID NO:80)
10   DAATYYCQQW YSNPLTFGAG TKLELKRAD (SEQ ID NO:81)
11   DAATYYCQQW NSNPLTFGAG TKLELKRAD (SEQ ID NO:82)
12   DAATYYCQQW TYNPLTFGAG TKLELKRAD (SEQ ID NO:83)
14   DAATYYCQQW YSNPLTFGAG TKLELKRAD (SEQ ID NO:84)
15   DAATYYCQQW NSNPLTFGGG TKLELKRAD (SEQ ID NO:85)
```

FIG. 11 Murine Parent Antibodies HCVR

```
FAb    1                                          CDR1      40
3      QVTLKESGPGILQSSQTLSLTCSLSGFSLRTSGMSVSWIR
5      QVTLKESGPGILQSSQTLSLTCSFSGFSLSTSGMSVSWIR
7      QVTLK-SGPGILQSSQTLTLTCSLSGFSLTTSGMIVSWIR
8      QVTLKESGPGILQSSQTLSLTCSLSGFSLRTSGMSVSWIR
9      QVTLKESGPGILQSSQTLSLTCSVSGFSLSTSGMSVSWIR
10     QVTLKESGPGILQPSQTLSLTCSLSGFSLRTSGMSVSWIR
11     QVTLKESGPGILQSSQTLSLTCSLSGFSLRTSGMSVSWIR
12     QVTLKESGPGMLQSSQTLSLTCSLSGFSLRTSGMSVSWIR
14     QVTLKESGPGILQSSQTLSLTCSLSGFSLRTSGMSVSWIR
15     QVTLKESGPGILQSSQTLSLTCSLSGFSLRTSGMSVSWIR


FAb    41              CDR2                          80
3      QSSGKGLEWLAHIYWDDDKRYNPSLRNRLTISKDTLRNQV
5      QSSGKGLEWLAHIYWDDDKRYNPSLRSRLTISKDTSRNQV
7      QSSGRGLEWLAHIYWDDDKRYNPSLRNRLTISKDTLRNQV
8      QSSGKGLEWLAHIYWDDDKRYNPSLRNRLTISKDTLRNQV
9      QPSGKGLEWLAHIYWDDDKRYNPSLKSRLTISKDTSRNQV
10     QSSGKGLEWLAHIYWDDDERYNPSLRNRLTISKDTLRNQV
11     QSSGKGLEWLAHIYWDDDKRYNPSLRNRLTISKDTLRNQV
12     QSSGKGLEWLAHIYWDDDKRYNPSLRNRLTISKDTLRNQV
14     QSSGKGLEWLAHIYWDDDKRYNPSLRNRLTISKDTLRNQV
15     QSSGKGLEWLAHIYWDDDKRYNPSLRNRLTISKDTLRNQV


FAb    81                    CDR3
3      FLKITSVGTADTATYYCARRAITTVIGGGTMDYWGQGTSVTVSS
5      FLKITSVDTADTATYYCARRGITTVLGGGTMDYWGQGTSVTVSS
7      FLWISSVGTADTATYYCARRAITTVIGGGTMDYWGQGTSVTVSS
8      FLKITSVGTADTATYYCARRAITTVIGGGTMDYWGQGTSVTVSS
9      FLKITSVDTADTATYYCARRAITTVLGGGTMDYWGQGTSVTVSS
10     FLKITSVGTADTATYYCARRAITTVIGGGTMDYWGQGTSVTVSS
11     FLKITSVGTADTATYYCARRAITTVIGGGTMDYWGQGTSVTVSS
12     FLKITSVGTADTATYYCARRAITTVIGGGTMDYWGQGTSVTVSS
14     FLKITSVGTADTATYYCARRAITTVIGGGTMDYWGQGTSVTVSS
15     FLKITSVGTADTATYYCARRAITTVIGGGTMDYWGQGTSVTVSS


3      (SEQ ID NO:86)
5      (SEQ ID NO:29)
7      (SEQ ID NO:87)
8      (SEQ ID NO:86)
9      (SEQ ID NO:54)
10     (SEQ ID NO:63)
11     (SEQ ID NO:86)
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14     (SEQ ID NO:86)
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<141> 2005-10-12

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            20   25   30

Val Glu Lys Glu Gly Leu Cys Asn Ala Cys Thr Trp Arg Gln Asn Thr
        35   40   45

Lys Ser Ser Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu
    50   55   60

Arg Leu Glu Thr Ala Pro Asn Ile Ser Lys Asp Val Ile Arg Gln Leu
65   70   75   80

Leu Pro Lys Ala Pro Pro Leu Arg Glu Leu Ile Asp Gln Tyr Asp Val
                85   90   95

Gln Arg Asp Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr His
            100   105   110

Ala Thr Thr Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe Leu
        115   120   125

Met Gln Val Asp Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser
    130   135   140

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Lys Ile Gln Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr Leu
145               150               155               160

Arg Pro Val Glu Thr Pro Thr Thr Val Phe Val Gln Ile Leu Arg Leu
              165               170               175

Ile Lys Pro Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu
              180               185               190

Lys Leu Asp Met Asn Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val
              195               200               205

Lys Thr Val Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly
    210               215               220

Ile Glu Ile Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr
225               230               235               240

Phe Pro Gly Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys
              245               250               255

Val Thr Asp Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys
              260               265               270

Asp Glu His Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val
              275               280               285

Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr
    290               295               300

Lys Ala Asn Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys
305               310               315               320

Tyr Pro His Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala
              325               330               335

Gly Pro Cys Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr
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Phe Asn Gly Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val
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Val Asp Arg Cys Gly Cys Ser
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Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr Cys Ser Gly Glu Cys Glu
            35   40   45

Phe Val Phe Leu Gln Lys Tyr Pro His Thr His Leu Val His Gln Ala
        50   55   60

Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys Thr Pro Thr Lys Met Ser
65   70   75   80

Pro Ile Asn Met Leu Tyr Phe Asn Gly Lys Glu Gln Ile Ile Tyr Gly
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Lys Ile Pro Ala Met Val Val Asp Arg Cys Gly Cys Ser
            100   105

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1   5   10   15

Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile
                20   25   30

Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr Cys Ser Gly Glu Cys Glu
            35   40   45

Phe Leu Phe Leu Gln Lys Tyr Pro His Thr His Leu Val His Gln Ala
        50   55   60

Asn Pro Lys Gly Ser Ala Gly Pro Cys Cys Thr Pro Thr Lys Met Ser
65   70   75   80

Pro Ile Asn Met Leu Tyr Phe Asn Gly Lys Glu Gln Ile Ile Tyr Gly
                85   90   95

Lys Ile Pro Gly Met Val Val Asp Arg Cys Gly Cys Ser
            100   105

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<212>  PRT
<213>  Homo sapiens

<400>  4
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Asn Leu Gly Leu Asp Cys Asp Glu His Ser Ser Glu Ser Arg Cys Cys
1   5   10   15

Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile
                20   25   30

Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr Cys Ser Gly Gln Cys Glu
            35   40   45

Tyr Met Phe Met Gln Lys Tyr Pro His Thr His Leu Val Gln Gln Ala
        50   55   60

Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys Thr Pro Thr Lys Met Ser
65   70   75   80

Pro Ile Asn Met Leu Tyr Phe Asn Asp Lys Gln Gln Ile Ile Tyr Gly
                85   90   95

Lys Ile Pro Gly Met Val Val Asp Arg Cys Gly Cys Ser
            100   105


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<220>
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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr Met
                20   25   30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
            35   40   45

Asp Thr Ser Lys Leu Ala Arg Gly Val Pro Ser Arg Phe Ser Gly Ser
        50   55   60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu

X17158.ST25.txt

```
        65                      70                      75                      80
```

Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Tyr Arg Asn Pro Leu Thr
                85   90   95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100   105

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr Met
            20   25   30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35   40   45

Asp Thr Ser Lys Leu Ala Val Gly Val Pro Ser Arg Phe Ser Gly Ser
    50   55   60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65   70   75   80

Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Tyr Arg Asn Pro Leu Thr
                85   90   95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100   105

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<220>
<223> Synthetic construct

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr Met

X17158.ST25.txt

| | | 20 | | | | | 25 | | | | | 30 | |

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35   40   45

Asp Thr Ser Lys Leu Ala Arg Gly Val Pro Ser Arg Phe Ser Gly Ser
50   55   60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65   70   75   80

Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Tyr Ser Asn Pro Leu Thr
85   90   95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100   105

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<220>
<223>   Synthetic construct

<400>   8

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr Ser
20   25   30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35   40   45

Asp Thr Ser Lys Leu Ala Arg Gly Val Pro Ser Arg Phe Ser Gly Ser
50   55   60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65   70   75   80

Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Tyr Ser Asn Pro Leu Thr
85   90   95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100   105

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X17158.ST25.txt

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr Ala
            20   25   30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35   40   45

Asp Thr Ser Lys Leu Ala Arg Gly Val Pro Ser Arg Phe Ser Gly Ser
    50   55   60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65   70   75   80

Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Tyr Ser Asn Pro Leu Thr
                85   90   95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100   105

<210> 10
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<213> Artificial

<220>
<223> Synthetic construct

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr Met
            20   25   30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35   40   45

Asp Thr Ser Lys Leu Ala Arg Gly Val Pro Ser Arg Phe Ser Gly Ser
    50   55   60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65   70   75   80

X17158.ST25.txt

```
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Tyr Leu Asn Pro Leu Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
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Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr Met
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
            35                  40                  45

Asp Thr Ser Lys Leu Ala Arg Gly Val Pro Ser Arg Phe Ser Gly Ser
            50                  55                  60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Tyr Glu Asn Pro Leu Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
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<400> 12

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr Met
            20                  25                  30
```

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X17158.ST25.txt

```
His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35              40              45

Asp Thr Ser Lys Leu Ala Arg Gly Val Pro Ser Arg Phe Ser Gly Ser
    50              55              60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65              70              75              80

Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Tyr Phe Asn Pro Leu Thr
            85              90              95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100             105
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<210> 13
<211> 124
<212> PRT
<213> Artificial

<220>
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<400> 13

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Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1               5               10              15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Arg Lys Val
            20              25              30

Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35              40              45

Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
    50              55              60

Leu Arg Asn Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65              70              75              80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
            85              90              95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Met Asp
            100             105             110

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
            115             120
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<210> 14

X17158.ST25.txt

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<220>
<223>  Synthetic construct

<400>  14

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1               5                   10                  15


Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Arg Lys Val
            20                  25                  30


Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35                  40                  45


Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
    50                  55                  60


Leu Arg Asn Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80


Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
                85                  90                  95


Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
            100                 105                 110


Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115                 120


<210>  15
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<220>
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<400>  15

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1               5                   10                  15


Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Arg Lys Val
            20                  25                  30


Gly Arg Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35                  40                  45


Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
```

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```
        50                      55                      60
```

```
Leu Arg Asn Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80
```

```
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
                85                  90                  95
```

```
Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
            100                 105                 110
```

```
Tyr Trp Gly Gln Gly Thr Thr Val Val Ser Ser
            115                 120
```

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<213>  Artificial

<220>
<223>  Synthetic construct

<400>  16
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```
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1               5                   10                  15
```

```
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Trp Arg Lys Val
                20                  25                  30
```

```
Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
            35                  40                  45
```

```
Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
    50                  55                  60
```

```
Leu Arg Asn Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80
```

```
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
                85                  90                  95
```

```
Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
            100                 105                 110
```

```
Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
            115                 120
```

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<210>  17
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<213>   Artificial

<220>
<223>   Synthetic construct

<400> 17

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1   5   10   15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Arg Lys Val
            20   25   30

Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35   40   45

Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Leu Arg Tyr Asn Pro Ser
    50   55   60

Leu Arg Asn Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65   70   75   80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
                85   90   95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
            100   105   110

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115   120

<210>   18
<211>   124
<212>   PRT
<213>   Artificial

<220>
<223>   Synthetic construct

<400> 18

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1   5   10   15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Met Arg Lys Val
            20   25   30

Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35   40   45

Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
    50   55   60

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Leu Arg Asn Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65   70   75   80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
            85   90   95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
        100   105   110

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115   120

<210> 19
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<212> PRT
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<220>
<223> Synthetic construct

<400> 19

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1   5   10   15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Arg Lys Val
        20   25   30

Gly Ser Ser Ile Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35   40   45

Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
    50   55   60

Leu Arg Asn Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65   70   75   80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
            85   90   95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
        100   105   110

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115   120

<210> 20
<211> 124
<212> PRT
<213> Artificial

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X17158.ST25.txt
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Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1                   5                   10                  15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Arg Lys Val
            20                  25                  30

Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
            35                  40                  45

Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
    50                  55                  60

Leu Arg Asn Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
                85                  90                  95

Cys Ala Arg Arg Lys Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
            100                 105                 110

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
            115                 120
```

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<210>  21
<211>  124
<212>  PRT
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<220>
<223>  Synthetic construct

<400>  21

Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1                   5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Arg Arg Val
            20                  25                  30

Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
            35                  40                  45

Trp Ile Gly His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
    50                  55                  60

Leu Arg Asn Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
```
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| 65 | | | | 70 | | | | 75 | | | | 80 |

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85   90   95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
            100   105   110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 22
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<220>
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<400> 22

Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1   5   10   15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Arg Met Val
            20   25   30

Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35   40   45

Trp Ile Gly His Ile Tyr Trp Asp Asp Asp Lys Arg Leu Asn Pro Ser
    50   55   60

Leu Arg Asn Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65   70   75   80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85   90   95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
            100   105   110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120

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<220>
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X17158.ST25.txt

<400> 23

Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1   5   10   15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Arg Lys Val
            20   25   30

Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35   40   45

Trp Ile Gly His Ile Tyr Trp Asp Asp Asp Leu Arg Tyr Asn Pro Ser
    50   55   60

Leu Arg Asn Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65   70   75   80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85   90   95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
            100   105   110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
            115   120

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<212>   PRT
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<220>
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<400>   24

Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1   5   10   15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Arg Lys Val
            20   25   30

Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35   40   45

Trp Ile Gly His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
    50   55   60

Leu Arg Asn Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65   70   75   80

X17158.ST25.txt

```
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85              90              95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
            100             105             110

Met Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115             120
```

```
<210>  25
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<213>  Artificial

<220>
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<400>  25
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```
Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1               5               10              15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Arg Lys Val
            20              25              30

Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35              40              45

Trp Ile Gly His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
    50              55              60

Leu Arg Asn Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65              70              75              80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85              90              95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
            100             105             110

Leu Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115             120
```

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<210>  26
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<220>
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<400>  26
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Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1   5   10   15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Arg Lys Val
            20   25   30

Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35   40   45

Trp Ile Gly His Ile Tyr Trp Asp Asp Asp Lys Arg Leu Asn Pro Ser
    50   55   60

Leu Arg Asn Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65   70   75   80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
            85   90   95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
        100   105   110

Leu Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210>   27
<211>   10
<212>   PRT
<213>   Mus sp.

<400>   27

Ser Ala Ser Ser Ser Ile Ser Tyr Met His
1   5   10

<210>   28
<211>   10
<212>   PRT
<213>   Artificial

<220>
<223>   Synthetic construct

<400>   28

Ser Ala Ser Ser Ser Ile Ser Tyr Ser His
1   5   10

<210>   29
<211>   123
<212>   PRT
<213>   Mus sp.

<400>   29

X17158.ST25.txt

```
Gln Val Thr Leu Lys Ser Gly Pro Gly Ile Leu Gln Ser Ser Gln Thr
1               5                   10                  15


Leu Thr Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Thr Thr Ser Gly
            20                  25                  30


Met Ile Val Ser Trp Ile Arg Gln Ser Ser Gly Lys Gly Leu Glu Trp
        35                  40                  45


Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser Leu
    50                  55                  60


Arg Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Arg Asn Gln Val Phe
65                  70                  75                  80


Leu Lys Ile Thr Ser Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr Cys
                85                  90                  95


Ala Arg Arg Gly Ile Thr Thr Val Leu Gly Gly Gly Thr Met Asp Tyr
            100                 105                 110


Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
        115                 120
```

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<210>   30
<211>   7
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<213>   Mus sp.

<400>   30

Asp Thr Ser Lys Leu Ala Arg
1               5


<210>   31
<211>   9
<212>   PRT
<213>   Mus sp.

<400>   31

Gln Gln Trp Tyr Arg Asn Pro Leu Thr
1               5


<210>   32
<211>   9
<212>   PRT
<213>   Artificial

<220>
<223>   Synthetic construct

<400>   32
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Gln Gln Trp Tyr Ser Asn Pro Leu Thr
1   5

<210>   33
<211>   9
<212>   PRT
<213>   Artificial

<220>
<223>   Synthetic construct

<400>   33

Gln Gln Trp Tyr Leu Asn Pro Leu Thr
1   5

<210>   34
<211>   9
<212>   PRT
<213>   Artificial

<220>
<223>   Synthetic construct

<400>   34

Gln Gln Trp Tyr Glu Asn Pro Leu Thr
1   5

<210>   35
<211>   9
<212>   PRT
<213>   Artificial

<220>
<223>   Synthetic construct

<400>   35

Gln Gln Trp Tyr Phe Asn Pro Leu Thr
1   5

<210>   36
<211>   12
<212>   PRT
<213>   Mus sp.

<400>   36

Gly Phe Ser Leu Arg Lys Val Gly Ser Ser Val Ser
1   5   10

<210>   37
<211>   12
<212>   PRT
<213>   Artificial

<220>

X17158.ST25.txt

<223>   Synthetic construct

<400>   37

Gly Phe Ser Leu Arg Lys Val Gly Arg Ser Val Ser
1   5   10

<210>   38
<211>   12
<212>   PRT
<213>   Artificial

<220>
<223>   Synthetic construct

<400>   38

Gly Phe Ser Trp Arg Lys Val Gly Ser Ser Val Ser
1   5   10

<210>   39
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<212>   PRT
<213>   Artificial

<220>
<223>   Synthetic construct

<400>   39

Gly Phe Ser Met Arg Lys Val Gly Ser Ser Val Ser
1   5   10

<210>   40
<211>   12
<212>   PRT
<213>   Artificial

<220>
<223>   Synthetic construct

<400>   40

Gly Phe Ser Leu Arg Lys Val Gly Ser Ser Ile Ser
1   5   10

<210>   41
<211>   12
<212>   PRT
<213>   Artificial

<220>
<223>   Synthetic construct

<400>   41

Gly Phe Ser Leu Arg Arg Val Gly Ser Ser Val Ser
1   5   10

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<210>  42
<211>  12
<212>  PRT
<213>  Artificial

<220>
<223>  Synthetic construct

<400>  42

Gly Phe Ser Leu Arg Met Val Gly Ser Ser Val Ser
1               5               10


<210>  43
<211>  16
<212>  PRT
<213>  Mus sp.

<400>  43

His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser Leu Arg Asn
1               5               10              15


<210>  44
<211>  16
<212>  PRT
<213>  Artificial

<220>
<223>  Synthetic construct

<400>  44

His Ile Tyr Trp Asp Asp Asp Leu Arg Tyr Asn Pro Ser Leu Arg Asn
1               5               10              15


<210>  45
<211>  16
<212>  PRT
<213>  Artificial

<220>
<223>  Synthetic construct

<400>  45

His Ile Tyr Trp Asp Asp Asp Lys Arg Leu Asn Pro Ser Leu Arg Asn
1               5               10              15


<210>  46
<211>  16
<212>  PRT
<213>  Artificial

<220>
<223>  Synthetic construct

<400>  46

His Ile Tyr Trp Asp Asp Asp Leu Arg Tyr Asn Pro Ser Leu Arg Asn
```

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1   5   10   15

```
<210>  47
<211>  14
<212>  PRT
<213>  Artificial

<220>
<223>  Synthetic construct

<400>  47

Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Met Asp Tyr
1                    5                    10


<210>  48
<211>  14
<212>  PRT
<213>  Mus sp.

<400>  48

Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp Tyr
1                    5                    10


<210>  49
<211>  14
<212>  PRT
<213>  Artificial

<220>
<223>  Synthetic construct

<400>  49

Arg Lys Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp Tyr
1                    5                    10


<210>  50
<211>  14
<212>  PRT
<213>  Artificial

<220>
<223>  Synthetic construct

<400>  50

Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp Met
1                    5                    10


<210>  51
<211>  14
<212>  PRT
<213>  Artificial

<220>
<223>  Synthetic construct
```

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```
<400>  51

Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp Leu
1               5                   10


<210>  52
<211>  7
<212>  PRT
<213>  Homo sapiens

<400>  52

Asp Thr Ser Lys Leu Ala Val
1               5


<210>  53
<211>  25
<212>  PRT
<213>  Homo sapiens

<400>  53

Ala Asn Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr
1               5                   10                  15


Pro His Thr His Leu Val His Gln Ala
            20                  25


<210>  54
<211>  124
<212>  PRT
<213>  Mus sp.

<400>  54

Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln Ser Ser Gln
1               5                   10                  15


Thr Leu Ser Leu Thr Cys Ser Val Ser Gly Phe Ser Leu Ser Thr Ser
            20                  25                  30


Gly Met Ser Val Ser Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu
        35                  40                  45


Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
    50                  55                  60


Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Arg Asn Gln Val
65                  70                  75                  80


Phe Leu Lys Ile Thr Ser Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr
                85                  90                  95


Cys Ala Arg Arg Ala Ile Thr Thr Val Leu Gly Gly Gly Thr Met Asp
```

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```
                  100                '105                    110
```

Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
            115   120

<210>   55
<211>   124
<212>   PRT
<213>   Artificial

<220>
<223>   Synthetic construct

<400>   55

Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1   5   10   15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Arg Lys Val
            20   25   30

Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
            35   40   45

Trp Ile Gly His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
        50   55   60

Leu Arg Asn Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65   70   75   80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85   90   95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
            100   105   110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
            115   120

<210>   56
<211>   124
<212>   PRT
<213>   Artificial

<220>
<223>   Synthetic construct

<400>   56

Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1   5   10   15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Arg Lys Val

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```
              20                      25                      30
```

Gly Ser Ser Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35   40   45

Trp Ile Gly His Ile Tyr Trp Asp Asp Asp Lys Arg Leu Asn Pro Ser
      50   55   60

Leu Arg Asn Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65   70   75   80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
              85   90   95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Phe Asp
            -100   105   110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
          115   120

```
<210>  57
<211>  10
<212>  PRT
<213>  Artificial

<220>
<223>  Synthetic construct


<220>
<221>  X
<222>  (9)..(9)
<223>  X is S or A

<400>  57
```

Ser Ala Ser Ser Ser Ile Ser Tyr Xaa His
1   5   10

```
<210>  58
<211>  9
<212>  PRT
<213>  Artificial

<220>
<223>  Synthetic construct


<220>
<221>  X
<222>  (5)..(5)
<223>  X is S, R, L, E, or F

<400>  58
```

Gln Gln Trp Tyr Xaa Asn Pro Leu Thr

```
<210>  59
<211>  12
<212>  PRT
<213>  Artificial

<220>
<223>  Synthetic construct


<220>
<221>  X
<222>  (4)..(4)
<223>  X is L, W, or M

<220>
<221>  X
<222>  (6)..(6)
<223>  X is K, R, or M

<220>
<221>  X
<222>  (9)..(9)
<223>  X is S or R

<400>  59

Gly Phe Ser Xaa Arg Xaa Val Gly Xaa Ser Val Ser
1               5                   10


<210>  60
<211>  16
<212>  PRT
<213>  Artificial

<220>
<223>  Synthetic construct


<220>
<221>  X
<222>  (8)..(8)
<223>  X is K or L

<220>
<221>  X
<222>  (10)..(10)
<223>  X is Y or L

<400>  60

His Ile Tyr Trp Asp Asp Asp Xaa Arg Xaa Asn Pro Ser Leu Arg Asn
1               5                   10                  15


<210>  61
<211>  14
<212>  PRT
<213>  Artificial

<220>
```

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<223>   Synthetic construct

<220>
<221>   X
<222>   (2)..(2)
<223>   X is A or K

<220>
<221>   X
<222>   (12)..(12)
<223>   X is M or F

<220>
<221>   X
<222>   (14)..(14)
<223>   X is M or L

<400>   61

Arg Xaa Ile Thr Thr Val Ile Gly Gly Gly Thr Xaa Asp Xaa
1   5   10

<210>   62
<211>   15
<212>   PRT
<213>   Homo sapiens

<400>   62

Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His
1   5   10   15

<210>   63
<211>   124
<212>   PRT
<213>   Mus sp.

<400>   63

Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln Pro Ser Gln
1   5   10   15

Thr Leu Ser Leu Thr Cys Ser Leu Ser Gly Phe Ser Leu Arg Thr Ser
            20   25   30

Gly Met Ser Val Ser Trp Ile Arg Gln Ser Ser Gly Lys Gly Leu Glu
            35   40   45

Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Glu Arg Tyr Asn Pro Ser
    50   55   60

Leu Arg Asn Arg Leu Thr Ile Ser Lys Asp Thr Leu Arg Asn Gln Val
65   70   75   80

Phe Leu Lys Ile Thr Ser Val Gly Thr Ala Asp Thr Ala Thr Tyr Tyr
                85   90   95

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```
Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Met Asp
            100                 105                 110

Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
            115                 120
```

```
<210>  64
<211>  124
<212>  PRT
<213>  Mus sp.
```

```
<400>  64
```

```
Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Met Leu Gln Ser Ser Gln
1               5               10                  15

Thr Leu Ser Leu Thr Cys Ser Leu Ser Gly Phe Ser Leu Arg Thr Ser
            20                  25                  30

Gly Met Ser Val Ser Trp Ile Arg Gln Ser Ser Gly Lys Gly Leu Glu
            35                  40                  45

Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
    50                  55                  60

Leu Arg Asn Arg Leu Thr Ile Ser Lys Asp Thr Leu Arg Asn Gln Val
65                  70                  75                  80

Phe Leu Lys Ile Thr Ser Val Gly Thr Ala Asp Thr Ala Thr Tyr Tyr
                85                  90                  95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Met Asp
            100                 105                 110

Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
            115                 120
```

```
<210>  65
<211>  23
<212>  PRT
<213>  Homo sapiens
```

```
<400>  65
```

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5               10                  15

Asp Arg Val Thr Ile Thr Cys
            20
```

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```
<210>  66
<211>  15
<212>  PRT
<213>  Homo sapiens

<400>  66

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1               5                   10                  15


<210>  67
<211>  32
<212>  PRT
<213>  Homo sapiens

<400>  67

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1               5                   10                  15

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
            20                  25                  30


<210>  68
<211>  10
<212>  PRT
<213>  Homo sapiens

<400>  68

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
1               5                   10


<210>  69
<211>  24
<212>  PRT
<213>  Homo sapiens

<400>  69

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Thr Gln Thr
1               5                   10                  15

Leu Thr Leu Thr Cys Thr Phe Ser
                20


<210>  70
<211>  14
<212>  PRT
<213>  Homo sapiens

<400>  70

Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala
1               5                   10


<210>  71
```

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```
<211>   32
<212>   PRT
<213>   Homo sapiens

<400>   71

Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr
1               5                   10                  15

Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Arg
                20                  25                  30


<210>   72
<211>   11
<212>   PRT
<213>   Homo sapiens

<400>   72

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
1               5                   10


<210>   73
<211>   25
<212>   PRT
<213>   Homo sapiens

<400>   73

Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1               5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser
                20                  25


<210>   74
<211>   14
<212>   PRT
<213>   Homo sapiens

<400>   74

Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile Gly
1               5                   10


<210>   75
<211>   32
<212>   PRT
<213>   Homo sapiens

<400>   75

Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
1               5                   10                  15

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
                20                  25                  30
```

X17158.ST25.txt

```
<210>  76
<211>  11
<212>  PRT
<213>  Homo sapiens

<400>  76

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1               5                   10


<210>  77
<211>  109
<212>  PRT
<213>  Mus sp.

<400>  77

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1               5                   10                  15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr Met
                20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Thr Ser Pro Lys Arg Trp Ile Tyr
            35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65                  70                  75                  80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Tyr Ser Asn Pro Leu Thr
                85                  90                  95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp
                100                 105


<210>  78
<211>  109
<212>  PRT
<213>  Mus sp.

<400>  78

Gln Val Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Leu Gly
1               5                   10                  15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val His Tyr Met
                20                  25                  30

His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
```

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X17158-ST25.txt
         35   40   45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
        50   55   60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65   70   75   80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr
                85   90   95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp
                100   105

<210>   79
<211>   109
<212>   PRT
<213>   Mus sp.

<400>   79

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1   5   10   15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
                20   25   30

His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
        35   40   45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
        50   55   60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65   70   75   80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr
                85   90   95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp
                100   105

<210>   80
<211>   109
<212>   PRT
<213>   Mus sp.

<400>   80

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1   5   10   15

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Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20   25   30

His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
        35   40   45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser
    50   55   60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65   70   75   80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Leu Thr
            85   90   95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp
            100   105

<210> 81
<211> 109
<212> PRT
<213> Mus sp.

<400> 81

Gln Val Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1   5   10   15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr Met
            20   25   30

His Trp Tyr Gln Gln Lys Pro Gly Thr Ser Pro Lys Arg Trp Ile Tyr
        35   40   45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50   55   60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65   70   75   80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Tyr Ser Asn Pro Leu Thr
            85   90   95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp
            100   105

<210> 82
<211> 109
<212> PRT
<213> Mus sp.

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<400> 82

```
Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1               5                   10                  15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr Met
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Thr Ser Pro Lys Arg Trp Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65                  70                  75                  80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Asn Ser Asn Pro Leu Thr
                85                  90                  95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp
            100                 105
```

<210>   83
<211>   109
<212>   PRT
<213>   Mus sp.

<400>   83

```
Gln Val Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1               5                   10                  15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Tyr Tyr Met
            20                  25                  30

His Trp Tyr Gln Gln Arg Ser Gly Ala Ser Pro Lys Arg Trp Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65                  70                  75                  80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Tyr Asn Pro Leu Thr
                85                  90                  95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp
            100                 105
```

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```
<210>   84
<211>   109
<212>   PRT
<213>   Mus sp.

<400>   84

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1               5                   10                  15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Thr Ser Pro Lys Arg Trp Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65                  70                  75                  80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Tyr Ser Asn Pro Leu Thr
                85                  90                  95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp
            100                 105


<210>   85
<211>   109
<212>   PRT
<213>   Mus sp.

<400>   85

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1               5                   10                  15

Glu Glu Val Thr Met Thr Cys Ser Ala Ser Ser Ser Ile Asn Tyr Met
            20                  25                  30

His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65                  70                  75                  80
```

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Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Asn Ser Asn Pro Leu Thr
                    85   90   95

Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp
            100   105

<210> 86
<211> 124
<212> PRT
<213> Mus sp.

<400> 86

Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln Ser Ser Gln
1   5   10   15

Thr Leu Ser Leu Thr Cys Ser Leu Ser Gly Phe Ser Leu Arg Thr Ser
            20   25   30

Gly Met Ser Val Ser Trp Ile Arg Gln Ser Ser Gly Lys Gly Leu Glu
        35   40   45

Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser
    50   55   60

Leu Arg Asn Arg Leu Thr Ile Ser Lys Asp Thr Leu Arg Asn Gln Val
65   70   75   80

Phe Leu Lys Ile Thr Ser Val Gly Thr Ala Asp Thr Ala Thr Tyr Tyr
                85   90   95

Cys Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Met Asp
            100   105   110

Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
        115   120

<210> 87
<211> 123
<212> PRT
<213> Mus sp.

<400> 87

Gln Val Thr Leu Lys Ser Gly Pro Gly Ile Leu Gln Ser Ser Gln Thr
1   5   10   15

Leu Thr Leu Thr Cys Ser Leu Ser Gly Phe Ser Leu Thr Thr Ser Gly
            20   25   30

Met Ile Val Ser Trp Ile Arg Gln Ser Ser Gly Arg Gly Leu Glu Trp
        35   40   45

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Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ser Leu
    50   55   60

Arg Asn Arg Leu Thr Ile Ser Lys Asp Thr Leu Arg Asn Gln Val Phe
65   70   75   80

Leu Trp Ile Ser Ser Val Gly Thr Ala Asp Thr Ala Thr Tyr Tyr Cys
                85   90   95

Ala Arg Arg Ala Ile Thr Thr Val Ile Gly Gly Gly Thr Met Asp Tyr
            100   105   110

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
            115   120