(19) DANMARK

(10) DK/EP 2370463 T3

Patent- og Varemærkestyrelsen

(12) Oversættelse af europæisk patentskrift

(51) Int.Cl.: *C 07 K 14/495 (2006.01)* *C 07 K 14/71 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2016-11-28**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2016-08-31**

(86) Europæisk ansøgning nr.: **09761055.4**

(86) Europæisk indleveringsdag: **2009-11-24**

(87) Den europæiske ansøgnings publiceringsdag: **2011-10-05**

(86) International ansøgning nr.: **US2009006252**

(87) Internationalt publikationsnr.: **WO2010062383**

(30) Prioritet: **2008-11-26 US 200250 P** **2009-11-06 US 259060 P**

(84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR**

(73) Patenthaver: **Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799, USA**

(72) Opfinder: **SUN, Jeonghoon, 4340 Camino De La Rosa, Thousand Oaks, CA 91320, USA**
TAM, Lei-Ting Tony, 3427 Indian Ridge Circle, Thousand Oaks, CA 91362, USA
MICHAELS, Mark Leo, 5007 Texhoma Avenue, Encino, CA 91316, USA
BOONE, Thomas C., 2715 Kelly Knoll Lane, Newbury Park, CA 91320, USA
DESHPANDE, Rohini, 251 Bellafonte Court, Camarillo, CA 93012, USA
LI, Yue-Sheng, 3025 Shadow Hill Circle, Thousand Oaks, CA 91360, USA
HAN, HQ, 3353 Crossland Street, Thousand Oaks, CA 91362, USA

(74) Fuldmægtig i Danmark: **NORDIC PATENT SERVICE A/S, Bredgade 30, 1260 København K, Danmark**

(54) Benævnelse: **STABILISERET VARIANT AF ACTIVIN-IIB-RECEPTOR**

(56) Fremdragne publikationer:
WO-A-2008/097541
WO-A-2008/109167
WO-A1-2007/053775
WO-A1-2008/113185
US-B1- 6 812 339
THOMPSON T B ET AL: "Structures of an ActRIIB:activin A complex reveal a novel binding mode for TGF-beta ligand:receptor interactions" EMBO JOURNAL, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 22, no. 7, 1 April 2003 (2003-04-01), pages 1555-1566, XP002366299 ISSN: 0261-4189

DESCRIPTION

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application Serial No. 61/200,250, filed on November 26, 2008, and U.S. Provisional Application Serial No. 61/259,060, filed on November 6, 2009.

TECHNICAL FIELD OF THE INVENTION

[0002] The technical field of this invention relates to transforming growth factor-β (TGF-β) family members and soluble TGF-β receptors with improved properties, as well as methods of modulating the activities of TGF-β family members for the treatment of various disorders.

BACKGROUND OF THE INVENTION

[0003] The transforming growth factor β (TGF-β) family of proteins includes the transforming growth factors-β (TGF-β), activins, bone morphogenic proteins (BMP), nerve growth factors (NGFs), brain-derived neurotrophic factor (BDNF), and growth/ differentiation factors (GDFs). These family members are involved in the regulation of a wide range of biological processes including cell proliferation, differentiation, and other functions.

[0004] Growth/differentiation factor 8 (GDF-8), also referred to as myostatin, is a TGF-ß family member expressed for the most part in the cells of developing and adult skeletal muscle tissue. Myostatin appears to play an essential role in negatively controlling skeletal muscle growth (McPherron et al., Nature (London) 387, 83-90 (1997), Zimmers et al., Science 296:1486-1488 (2002)). Antagonizing myostatin has been shown to increase lean muscle mass in animals.

[0005] Another member of the TGF-β family of proteins is a related growth/differentiation factor, growth/differentiation factor 11 (GDF-11). GDF-11 has approximately 90 % sequence identity to the amino acid sequence of myostatin. GDF-11 has a role in the axial patterning in developing animals (Oh et al., Genes Dev 11:1812-26 (1997)), and also appears to play a role in skeletal muscle development and growth.

[0006] Activins A, B and AB are the homodimers and heterdimer respectively of two polypeptide chains, βA and βB (Vale et al., Nature 321, 776-779 (1986), Ling et al., Nature 321, 779-782 (1986)). Activins were originally discovered as gonadal peptides involved in the regulation of follicle stimulating hormone synthesis, and are now believed to be involved in the regulation of a number of biological activities. Activin A is a predominant form of activin.

[0007] Activin, myostatin, GDF-11 and other members of the TGF-β superfamily bind and signal through a combination of activin type II and activin type IIB receptors, both of which are transmembrane serine/threonine kinases (Harrison et al., J. Biol. Chem. 279, 28036-28044 (2004)). Cross-linking studies have determined that myostatin is capable of binding the activin type II receptors ActRIIA and ActRIIB *in vitro* (Lee et al., PNAS USA 98:9306-11 (2001)). There is also evidence that GDF-11 binds to both ActRIIA and ActRIIB (Oh et al., Genes Dev 16:2749-54 (2002)).

[0008] TGF-β protein expression is known to be associated with a variety of diseases and disorders. Therefore, therapeutic molecules capable of antagonizing several TGF-β proteins simultaneously may be particularly effective for treating these diseases and disorders.

[0009] WO 2008/109167 A discloses variant activin IIB soluble receptor polypeptides and proteins capable of binding and inhibiting the activities of activin A, myostatin, or GDF-11, comprising substitutions at position 28, 40 and/or 64 (with respect to SEQ ID NO: 2 of the present disclosure), and uses thereof.WO 2008/097541 A discloses variant activin IIB soluble receptor polypeptides and methods for treating diseases associated with abnormal activity of an activin IIB protein and/or ligand. The polypeptides comprise N-X-S/T motifs.

[0010] Production of therapeutic proteins on a commercial scale requires proteins that can be efficiently expressed and purified without disruption of the integrity of the protein. Manufacturability can be described as the ability to express and purify a protein in a sufficiently efficient manner to allow for cost-effective production of the protein. In a commercial setting, manufacturability must be determined for each potential therapeutic protein. Although protein expression and purification processes can be optimized for a protein, manufacturability appears to be a function of the intrinsic properties of the protein as well. The present invention provides biologically active therapeutic proteins having improved manufacturability properties, capable of effectively antagonizing TGF-β proteins.

SUMMARY OF THE INVENTION

[0011] The present invention provides isolated proteins comprising stabilized human activin receptor IIB (designated svActRIIB) polypeptides capable of binding and inhibiting the activities of activin, GDF-11 and myostatin, and characterized by improved manufacturability properties. The stabilized ActRIIB polypeptides are characterized by having amino acid substitutions at both positions 28 and 44 with respect to SEQ ID NO: 2.

[0012] In one embodiment, the isolated protein comprises a polypeptide having the sequence set forth in SEQ ID NO: 2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO: 2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO: 2, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has the sequence set forth in amino acids 19 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO: 2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO:2, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has the sequence set forth in amino acids 23 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO: 2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO: 2, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has the sequence set forth in amino acids 25 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO:2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO: 2, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has an amino acid sequence with at least 80 %, 85 %, 90 %, 95 %, 98 % or 99 % identity to any of the polypeptides above, wherein the polypeptide has a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO:2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO: 2, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In one embodiment, the substitution of the above polypeptides at position 28 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

[0013] In another embodiment, the isolated protein comprises a stabilized activin IIB receptor polypeptide, wherein the polypeptide has a sequence selected from the group consisting of SEQ ID NO: 4, 6, 12 and 14. In another embodiment the protein comprises a polypeptide having at least 90 % sequence identity to SEQ ID NO: 4, 6, 12 or 14, wherein the polypeptide has a W or Y at the position corresponding to position 28 of SEQ ID NO: 2 and a T at the position corresponding to position 44 of SEQ ID NO: 2, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In another embodiment, the protein comprises a polypeptide having at least 95 % sequence identity to SEQ ID NO: 4, 6, 12, or 14, wherein the polypeptide has a W or Y at the position corresponding to position 28 of SEQ ID NO: 2 and a T at the position corresponding to position 44 of SEQ ID NO: 2, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In one embodiment, the substitution at position 28 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

[0014] In a further embodiment the svActRIIB protein further comprises a heterologous protein. In one embodiment, the heterologous protein is an Fc domain. In a further embodiment, the Fc domain is a human IgG Fc domain. In a further embodiment the heterologous protein is attached by a linker or a hinge linker peptide. In one embodiment, the linker or hinge linker is selected from the group consisting of the amino acid sequences set forth in the group consisting of SEQ ID NO: 25, 27, 38, 40, 42, 44, 45, 46, 48, 49 and 50. In a further embodiment the hinge linkers set forth in SEQ ID NO: 27, 38, 40, 42, 44, 45, or 46 link the human IgG2 Fc (SEQ ID NO: 22) to an svActRIIB polypeptide. In another embodiment, the hinge linkers set forth in SEQ ID NO: 48, 49, or 50 link the human IgG1 Fc (SEQ ID NO: 23) or the modified IgG1 Fc (SEQ ID NO: 47) to an svActRIIB polypeptide.

[0015] In a further embodiment, the protein comprises a polypeptide having a sequence selected from the group consisting of SEQ ID NO: 8, 10, 16 and 18. In another embodiment the protein comprises a polypeptide having at least 90 % sequence identity to SEQ ID NO: 8, 10, 16 or 18, wherein the polypeptide has a W or Y at the position corresponding to position 28 of SEQ ID NO: 2 and a T at the position corresponding to position 44 of SEQ ID NO: 2, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In another embodiment, the protein comprises a polypeptide having at least 95 % sequence identity to SEQ ID NO: 8, 10, 16, or 18, wherein the polypeptide has a W or Y at the position corresponding to position 28 of SEQ ID NO: 2 and a T at the position corresponding to position 44 of SEQ ID NO: 2, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In a further embodiment, the substitution of the above polypeptides at position 28 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

[0016] In a further embodiment, the protein comprises a polypeptide recited above, wherein the amino acid residue at position 64 is alanine.

[0017] The invention also provides an isolated protein consisting of the sequence set forth in SEQ ID NO: 10.

[0018] In another aspect the present invention provides an isolated nucleic acid molecule comprising a polynucleotide encoding a stabilized ActRIIB polypeptide. In one embodiment, the polynucleotide encodes the polypeptide sequence set forth in SEQ ID NO: 2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO: 2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO: 2, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes the polypeptide having the sequence set forth in amino acids 19 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO: 2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO: 2, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes the polypeptide having the sequence set forth in amino acids 23 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO: 2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO: 2, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes the polypeptide having the sequence set forth in amino acids 25 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO: 2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO: 2, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes a polypeptide having an amino acid sequence at least 80 %, 85 %, 90 %, 95 %, 98 % or 99 % identity to any one of the polypeptides above, wherein the polypeptide has a single amino acid substitution at the position corresponding to position 28 of SEQ ID NO: 2, and a single amino acid substitution at the position corresponding to position 44 of SEQ ID NO: 2, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In one embodiment, the above polynucleotides encode a polypeptide wherein the substitution at position 28 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

[0019] In one embodiment, the nucleic acid molecule comprises a polynucleotide encoding a polypeptide having a sequence selected from the group consisting of SEQ ID NO: 4, 6, 12 and 14. In another embodiment, the nucleic acid molecule comprises a polynucleotide encoding a polypeptide having at least 90 % sequence identity to SEQ ID NO: 4, 6, 12 or 14, wherein the polypeptide has a W or Y at the position corresponding to position 28 of SEQ ID NO: 2 and a T at the position corresponding to position 44 of SEQ ID NO: 2, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In another embodiment, the nucleic acid molecule comprises a polynucleotide encoding a polypeptide having at least 95 % sequence identity to SEQ ID NO: 4, 6, 12 or 14, wherein the polypeptide has a W or Y at the position corresponding to position 28 of SEQ ID NO: 2 and a T at the position corresponding to position 44 of SEQ ID NO: 2, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In one embodiment, the above polynucleotides encode a polypeptide wherein the substitution at position 28 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

[0020] In another embodiment, the nucleic acid molecule comprises a polynucleotide having a sequence selected from the group consisting of SEQ ID NO: 3, 5, 11 and 13, or its complement.

[0021] In another embodiment, the isolated nucleic acid molecule comprises a polynucleotide set forth above, and further comprises a polynucleotide encoding at least one heterologous protein. In one embodiment, the nucleic acid molecule comprises a polynucleotide encoding a polypeptide having the sequence selected from the group consisting of SEQ ID NO: 8, 10, 16 and 18. In another embodiment, the nucleic acid molecule comprises a polynucleotide encoding a polypeptide having at least 90% sequence identity to SEQ ID NO: 8, 10, 16 or 18, wherein the polypeptide has a W or Y at the position corresponding to position 28 of SEQ ID NO: 2 and a T at the position corresponding to position 44 of SEQ ID NO: 2, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In another embodiment, the nucleic acid molecule comprises a polynucleotide encoding a polypeptide having at least 95 % sequence identity to SEQ ID NO: 8, 10, 16 or 18, wherein the polypeptide has a W or Y at the position corresponding to position 28 of SEQ ID NO: 2 and a T at the position corresponding to position 44 of SEQ ID NO: 2, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In one embodiment, the above polynucleotides encode a polypeptide wherein the substitution at position 28 is W and the substitution at position S44 is T, wherein the encoded polypeptide is capable of binding myostatin, activin A or GDF-11. In a further embodiment, the nucleic acid molecule comprises a polynucleotide having a sequence selected from the group consisting of SEQ ID NO: 7, 9, 15 and 17, or its complement.

[0022] In another embodiment, the nucleic acid molecule further comprises polynucleotides encoding the linkers and hinge linkers set forth in the group consisting of SEQ ID NO: 25, 27, 38, 40, 42, 44, 45, 46, 48, 49 and 50.

[0023] In a further embodiment, the nucleic acid molecule further comprises a transcriptional or translational regulatory sequence. In another aspect a recombinant vector comprising a polynucleotide encoding a stabilized ActRIIB protein or polypeptide of the invention is provided. In another aspect, host cells comprising the recombinant vectors of the invention are provided, and methods of producing the stabilized ActRIIB proteins and polypeptides of the invention are provided by culturing the host cells under conditions promoting expression of the proteins or polypeptides, and recovering the protein.

[0024] The present invention further provides a pharmaceutical composition containing at least one stabilized ActRIIB polypeptide or protein of the present invention in admixture with a pharmaceutically acceptable carrier, and use of the pharmaceutical composition as a medicament.

[0025] Also described herein is said composition for use in a method of inhibiting myostatin or activin activity in a subject in need of such treatment.

[0026] In another aspect, the invention provides use of said composition in a method of increasing lean muscle mass or increasing the ratio of lean muscle mass to fat mass in a subject in need of such treatment, wherein the method is not a method of treatment of the human or animal body by therapy.

[0027] In another aspect, the invention provides said composition for use in a method of treating or preventing muscle wasting or a metabolic disorder selected from diabetes, obesity, hyperglycemia and bone loss in a subject in need of such treatment. The muscle wasting may be due to a disease selected from, but not limited to, the following conditions: cancer cachexia, muscular dystrophy, amyotrophic lateral sclerosis, congestive obstructive pulmonary disease, chronic heart failure, chemical cachexia, cachexia from HIV/AIDS, renal failure, uremia, rheumatoid arthritis, age-related sarcopenia, age-related frailty, organ atrophy, carpal tunnel syndrome, androgen deprivation, and muscle-wasting due to inactivity from prolonged bed rest, spinal chord injury, stroke, bone fracture, burns, aging, insulin resistance, and other disorders. The muscle wasting may also result from weightlessness due to space flight.

[0028] In another aspect, the present invention provides said composition for use in a method of treating a cancer in which activin is overexpressed in a subject in need of such treatment. In another aspect, the present invention provides said composition for use in a method of treating a metabolic disorder in a subject in need of such treatment, wherein the metabolic disorder is selected from bone loss, diabetes, obesity, and hyperglycemia. In another aspect, the present invention provides a vector encoding an svActRIIB polypeptide or protein of the present invention for use in a method of gene therapy for treating muscle wasting, or metabolic or activin-related disorders as described above, wherein the vector is capable of expressing the svActRIIB protein or polypeptide in the subject.

BRIEF DESCRIPTION OF THE FIGURES

[0029]

- Figure 1 shows a comparison between ActRIIB-Fc (E28W) and svActRIIB-Fc (E28W, S44T) on an SEC column. svActRIIB-Fc (E28W, S44T) shows a single peak compared with ActRIIB-Fc (E28W), which shows three peaks.

- Figure 2 shows the increase in body mass over a 14 day period in 10 C57B1/6 mice administered a single dose of 10 mg/kg svActRIIB-Fc (E28W, S44T) compared with 10 mice administered 10 mg/kg of PBS.

- Figure 3 shows the dose-related change in lean body mass over time for C57B1/6 receiving a single dose of 0.3 mg/kg, 3 mg/kg, 10 mg/kg, and 30 mg/kg of svActRIIB-Fc (E28W, S44T).

DETAILED DESCRIPTION

[0030] The present invention provides an isolated protein comprising a stabilized human activin IIB receptor (svActRIIB) polypeptide. The protein and polypeptide of the invention are characterized by their ability to bind to at least one of three TGF-β proteins, myostatin (GDF-8), activin A, or GDF-11, to inhibit the activities of at least one of these proteins, and to have improved manufacturability properties compared with other ActRIIB soluble receptors. The stabilized human activin IIB receptor polypeptide is characterized by amino acid substitutions at both positions E28 and S44 with reference to the extracellular domain of ActRIIB, as set forth in SEQ ID NO: 2. In one embodiment, a stabilized human activin IIB receptor polypeptide can have a further substitution of alanine at position 64 with respect to SEQ ID NO: 2.

[0031] As used herein the term "TGF-β family members" or "TGF-β proteins" refers to the structurally related growth factors of the transforming growth factor family including activins, and growth and differentiation factor (GDF) proteins (Kingsley et al. Genes Dev. 8: 133-146 (1994), McPherron et al., Growth factors and cytokines in health and disease, Vol. 1B, D. LeRoith and C. Bondy. ed., JAI Press Inc., Greenwich, Conn, USA: pp 357-393).

[0032] GDF-8, also referred to as myostatin, is a negative regulator of skeletal muscle tissue (McPherron et al. PNAS USA 94:12457-12461 (1997)). Myostatin is synthesized as an inactive protein approximately 375 amino acids in length, having GenBank Accession No: AAB86694 (SEQ ID NO: 35) for human. The precursor protein is activated by proteolytic cleavage at a tetrabasic processing site to produce an N-terminal inactive prodomain and an approximately 109 amino acid C-terminal protein which dimerizes to form a homodimer of about 25 kDa. This homodimer is the mature, biologically active protein (Zimmers et al., Science 296, 1486 (2002)).

[0033] As used herein, the term "prodomain" or "propeptide" refers to the inactive N-terminal protein which is cleaved off to release the active C-terminal protein. As used herein the term "myostatin" or "mature myostatin" refers to the mature, biologically active C-terminal polypeptide, in monomer, dimer or other form, as well as biologically active fragments or related polypeptides including allelic variants, splice variants, and fusion peptides and polypeptides. The mature myostatin has been reported to have 100% sequence identity among many species including human, mouse, chicken, porcine, turkey, and rat (Lee et al., PNAS 98, 9306 (2001)).

[0034] As used herein GDF-11 refers to the BMP (bone morphogenic protein) having Swissprot accession number 095390 (SEQ ID NO: 36), as well as variants and species homologs of that protein. GDF-11 is involved in the regulation of anterior/posterior patterning of the axial skeleton (McPherron et al, Nature Genet. 22 (93): 260-264 (1999); Gamer et al, Dev. Biol. 208 (1), 222-232 (1999)) but postnatal functions are unknown.

[0035] Activin A is the homodimer of the polypeptide chains βA. As used herein the term "activin A" refers to the activin protein having GenBank Accession No: NM_002192 (SEQ ID NO: 34). Activins A, B, and AB are the homodimers and heterodimer respectively of two polypeptide chains, βA and βB. As used herein, "activin" refers to activin A, B, and AB, as well as variants and species homo logs of that protein.

Receptor Polypeptides

[0036] As used herein, the term activin type II B receptors (ActRIIB) refers to human activin receptors having accession number NP_001097 or variants thereof, such as those having the arginine at position 64 substituted with alanine. The term soluble ActRIIB (wild type) refers to the extracellular domain of ActRIIB, amino acids 1 to 134 (with signal sequence), or amino acids 19 through 134 of SEQ ID NO: 2 (without signal sequence).

Stabilized receptor polypeptides

[0037] The present invention provides an isolated protein comprising a stabilized ActIIB receptor polypeptide (referred herein as "svActRIIB polypeptide"). As used herein the term "svActRIIB protein" refers to a protein comprising a stabilized ActRIIB polypeptide. As used herein the term "isolated" refers to a protein or polypeptide molecule purified to some degree from endogenous material. These polypeptides and proteins are characterized as having the ability to bind and inhibit the activity of any one of activin A, myostatin, or GDF-11, in addition to having improved manufacturability characteristics.

[0038] The stabilized ActRIIB polypeptide is characterized by having an amino acid substitution at both position 28 and 44 with respect to SEQ ID NO: 2. For consistency, the amino acid positions on the stabilized ActRIIB polypeptides and proteins are always referred to with respect to the positions in SEQ ID NO: 2, regardless of whether the polypeptide is mature or truncated. As used herein, the term "mature" refers to a polypeptide or peptide without its signal sequence. As used herein, the term "truncated" refers to polypeptides having N terminal amino acids or C terminal amino acids removed.

[0039] In one embodiment, the isolated stabilized activin IIB receptor polypeptide (svActRIIB) has the polypeptide sequence set forth in SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has the sequence set forth in amino acids 19 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has the sequence set forth in amino acids 23 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has the sequence set forth in amino acids 25 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polypeptide has an amino acid sequence with at least 80 %, 85 %, 90 %, 95 %, 96 %, 97 %, 98 % or 99 % identity to any one of the polypeptides above, wherein the polypeptide has a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In one embodiment, the substitution of the above polypeptides at position 28 is W, and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11.

[0040] In one embodiment, the svActRIIB polypeptide includes a signal sequence, for example, SEQ ID NO: 4, 8, 12, and 16. However, various signal peptides can be used in the preparation of the polypeptides of the instant application. The signal peptides can have the sequence set forth in amino acids 1 to 19 of SEQ ID NO: 4, for example, or the signal sequences set forth in SEQ ID NO: 31 and 32. Any other signal peptides useful for expressing svActRIIB polypeptides may be used. In other embodiments, the signal sequence is removed, leaving the mature peptide. Examples of svActRIIB polypeptides lacking a signal sequence includes, for example, SEQ ID NO: 6, 10, 14 and 18.

[0041] In one embodiment, the protein comprises a stabilized activin IIB receptor polypeptide, wherein the polypeptide is selected from the group consisting of polypeptides having the sequence set forth in the group consisting of SEQ ID NO: 4, 6, 12 and 14. These polypeptides represent amino acids 25 to 134 of SEQ ID NO: 2, wherein the polypeptide has a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11, with and without a signal sequence different from that shown in SEQ ID NO: 2. In another embodiment the protein comprises a polypeptide having at least 90 %, 95 %, 96 %, 97 %, 98 %, or 99 % sequence identity to SEQ ID NO: 4, 6, 12 or 14, wherein the polypeptide has a W or Y at position 28 and a T at position 44, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In one embodiment, the substitution at position 28 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A or GDF-11.

[0042] In a further embodiment the svActRIIB protein further comprises a heterologous protein. In one embodiment, the heterologous protein is an Fc domain. In a further embodiment, the Fc domain is a human IgG Fc domain. In one embodiment, the protein comprises a polypeptide having the sequence selected from the group consisting of SEQ ID NO: 8, 10, 16 and 18. In another embodiment, the protein comprises a polypeptide having at least 90 %, 95 %, 96 %, 97 %, 98 %, or 99 % sequence identity to SEQ ID NO: 8, 10, 16 or 18, wherein the polypeptide has a W or Y at position 28 and a T at position 44, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In one embodiment, the substitution at position 28 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A or GDF-11.

[0043] In a further embodiment, the protein comprises any one of the polypeptides described above, wherein the amino acid residue at position 64 is alanine.

[0044] In another embodiment, the term svActRIIB polypeptide and protein encompasses proteins comprising fragments of SEQ ID NO: 2, 4, 6, 12 and 14, including N and C terminal truncations, wherein position 28 is W or Y, and position 44 is T, and wherein the polypeptide is capable of binding myostatin, activin A or GDF-11.

[0045] As used herein the term "derivative" of the svActRIIB polypeptide refers to the attachment of at least one additional chemical moiety, or at least one additional polypeptide to form covalent or aggregate conjugates such as glycosyl groups, lipids, acetyl groups, or C-terminal or N-terminal fusion polypeptides, conjugation to PEG molecules, and other modifications which are described more fully below. Stabilized ActRIIB receptor polypeptides can also include additional modifications and derivatives, including modifications to the C and N termini which arise from processing due to expression in various cell types such as mammalian cells, E. coli, yeasts and other recombinant host cells.

[0046] The svActRIIB proteins of the present invention may further comprise heterologous polypeptides attached to the svActRIIB polypeptide either directly or through a linker sequence to form a fusion protein. As used herein the term "fusion protein" refers to a protein having a heterologous polypeptide attached via recombinant DNA techniques. Heterologous polypeptides include but are not limited to Fc polypeptides, his tags, and leucine zipper domains to promote oligomerization and further stabilization of the stabilized ActRIIB polypeptides as described in, for example, WO 00/29581. In one embodiment, the heterologous polypeptide is an Fc polypeptide or domain. In one embodiment, the Fc domain is selected from a human IgG1 Fc (SEQ ID NO: 23), modified IgG1 Fc (SEQ ID NO: 47), IgG2 Fc (SEQ ID NO: 22), and IgG4 Fc (SEQ ID NO: 24) domain. The svActRIIB protein can further comprise all or a portion of the hinge sequence of the IgG1 (SEQ ID NO: 29), IgG2 (SEQ ID NO: 28), or IgG4 (SEQ ID NO: 30). Exemplary svActRIIB polypeptides are selected from polypeptides consisting of the sequences as set forth in SEQ ID NO: 8, 10, 16 and 18, as well as those polypeptides having substantial similarity to these sequences, wherein the substitutions at positions 28 and 44 are retained. As used herein, "substantial similarity" refers to sequences that are at least 90 % identical, 95 % identical, 96 % identical, 97 % identical, 98 % identical, 99 % identical to any of SEQ ID NO: 8, 10, 16, and 18, wherein the polypeptides retain W or Y at position 28 and T at position 44, and wherein the polypeptide is capable of binding myostatin, activin A or GDF-11. In one embodiment, the substitution at position 28 is W and the substitution at position 44 is T, wherein the polypeptide is capable of binding myostatin, activin A or GDF-11.

[0047] The svActRIIB polypeptide can optionally further comprise a "linker" sequence. Linkers serve primarily as a spacer between a polypeptide and a second heterologous polypeptide or other type of fusion or between two or more stabilized ActRIIB polypeptides. In one embodiment, the linker is made up of amino acids linked together by peptide bonds, preferably from 1 to 20 amino acids linked by peptide bonds, wherein the amino acids are selected from the 20 naturally occurring amino acids. One or more of these amino acids may be glycosylated, as is understood by those of skill in the art. In one embodiment, the 1 to 20 amino acids may be selected from glycine, alanine, proline, asparagine, glutamine, and lysine. In one embodiment, a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. Exemplary linkers are polyglycines (particularly $(Gly)_5$, $(Gly)_8$, poly(Gly-Ala), and polyalanines. One exemplary suitable linker as shown in the Examples below is $(Gly)_4$Ser (SEQ ID NO: 25). In a further embodiment, svActRIIB can comprise a "hinge linker", that is a linker sequence provided adjacent to a hinge region or a partial hinge region of an IgG, as exemplified in SEQ ID NO: 27. Hinge sequences include IgG2Fc (SEQ ID NO: 28), IgG1Fc (SEQ ID NO: 29), and IgG4Fc (SEQ ID NO: 30).

[0048] Hinge linker sequences may also be designed to improve manufacturability and stability of the svActRIIB-Fc proteins. In one embodiment, the hinge linkers of SEQ ID NO: 27, 38, 40, 42, 44, 45, and 46 are designed to improve manufacturability with the IgG2 Fc (SEQ ID NO: 22) when attached to svActRIIB polypeptides. In one embodiment, the hinge linker sequences is designed to improve manufacturability when attaching svActRIIB polypeptides to a human IgG1 Fc (SEQ ID NO: 23) or a modified human IgG1 Fc (SEQ ID NO: 47), for example, the hinge linkers having SEQ ID NO: 48, SEQ ID NO: 49 and SEQ ID NO: 50. The improved manufacturability of these polypeptides is described below in Example 4.

[0049] Linkers may also be non-peptide linkers. For example, alkyl linkers such as - NH-(CH_2)s-C(O)-, wherein s = 2-20 can be used. These alkyl linkers may further be substituted by any non-sterically hindering group such as lower alkyl (e.g., C_1-C_6) lower acyl, halogen (e.g., Cl, Br), CN, NH_2, phenyl, etc.

[0050] The svActRIIB polypeptides disclosed herein can also be attached to a non-polypeptide molecule for the purpose of conferring desired properties such as reducing degradation and/or increasing half-life, reducing toxicity, reducing immunogenicity, and/or increasing the biological activity of the svActRIIB polypeptides. Exemplary molecules include but are not limited to linear polymers such as polyethylene glycol (PEG), polylysine, a dextran; a lipid; a cholesterol group (such as a steroid); a carbohydrate, or an oligosaccharide molecule.

[0051] The svActRIIB proteins and polypeptides have improved manufacturability properties when compared to other ActRIIB soluble polypeptides. As used herein, the term "manufacturability" refers to the stability of a particular protein during recombinant expression and purification of that protein. Manufacturability is believed to be due to the intrinsic properties of the molecule under conditions of expression and purification. Examples of improved manufacturability characteristics are set forth in the Examples below and include uniform glycosylation of a protein (Example 2), increased cell titer, growth and protein expression during recombinant production of the protein (Example 1), improved purification properties (Example 2), and improved stability at low pH (Example 2). The svActRIIB proteins and polypeptides of the present invention demonstrate the improved manufacturability, along with retention of *in vitro* and *in vivo* activity (Examples 2 and 3), compared with other soluble ActRIIB polypeptides. Further, additional hinge linker sequences may confer additional manufacturability benefits, as shown in Example 4 below.

[0052] As used herein, the term a "svActRIIB polypeptide activity" or "a biological activity of a soluble ActRIIB polypeptide" refers to one or more *in vitro* or *in vivo* activities of the svActRIIB polypeptides including but not limited to those demonstrated in the Example below. Activities of the svActRIIB polypeptides include, but are not limited to, the ability to bind to myostatin or activin A or GDF-11, and the ability to inhibit or neutralize an activity of myostatin or activin A or GDF-11. As used herein, the term "capable of binding" to myostatin, activin A, or GDF-11 refers to binding measured by methods known in the art, such as the KinExA™ method shown in the Examples below. *In vitro* inhibition of myostatin, activin A, or GDF-11 can be measured using, for example, the pMARE C2C12 cell-based assay described in the Examples below. *In vivo* activity, demonstrated in Example 3 below, is demonstrated by increased lean muscle mass in mouse models. *In vivo* activities of the svActRIIB polypeptides and proteins include but are not limited to increasing body weight, increasing lean muscle mass, and increasing the ratio of lean muscle to fat mass. Therapeutic activities further include reducing or preventing cachexia caused by certain types of tumors, preventing the growth of certain types of tumors, and increasing survival of certain animal models. Further discussion of the svActRIIB protein and polypeptide activities is provided below.

[0053] In another aspect, the present invention provides an isolated nucleic acid molecule comprising a polynucleotide encoding an svActRIIB polypeptide of the present invention. As used herein the term "isolated" refers to nucleic acid molecules purified to some degree from endogenous material.

[0054] In one embodiment, the polynucleotide encodes a polypeptide having the sequence set forth in SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes a polypeptide having the sequence set forth in amino acids 19 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes a polypeptide having the sequence set forth in amino acids 23 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes a polypeptide having the sequence set forth in amino acids 25 through 134 of SEQ ID NO: 2, except for a single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T. In another embodiment, the polynucleotide encodes a polypeptide having an amino acid sequence at least 80 %, 85 %, 90 %, 95 %, 98 % or 99 % identity to any one of the polypeptides above, wherein the polypeptide has single amino acid substitution at position 28, and a single amino acid substitution at position 44, wherein the substitution at position 28 is selected from W or Y, and the substitution at position 44 is T, and wherein the polypeptide is capable of binding myostatin, activin A, or GDF-11. In one embodiment, the polynucleotide of the above embodiments encodes a polypeptide wherein the substitution at position 28 is W and the substitution at position 44 is T.

[0055] In one embodiment, the isolated nucleic acid molecule of the present invention comprises a polynucleotide encoding a polypeptide having the sequence selected from the group consisting of SEQ ID NO: 4, 6, 12, and 14. In another embodiment, the nucleic acid comprises a polynucleotide encoding a polypeptide having at least 90 %, 95 %, 96 %, 97 %, 98 %, 99 % sequence identity to SEQ ID NO: 4, 6, 12 or 14, wherein the polypeptide has a W or Y at position 28 and a T at position 44, and wherein the polypeptide is capable of binding activin A, GDF-11, or myostatin. In one embodiment, the polynucleotide of the above embodiments encodes a polypeptide wherein the substitution at position 28 is W and the substitution at position 44 is T, and wherein the polypeptide is capable of binding activin A, GDF-11 or myostatin.

[0056] In another embodiment, the isolated nucleic acid molecule further comprises a polynucleotide encoding at least one heterologous protein. In one embodiment, the heterologous protein is an Fc domain, in a further embodiment, the Fc domain is a human IgG Fc domain. In another embodiment, the nucleic acid molecule further comprises polynucleotides encoding the linkers and hinge linkers set forth in SEQ ID NO: 25, 27, 38, 40, 42, 44, 45, 46, 48, 49 or 50. In a further embodiment, such polynucleotides have sequences selected from the group consisting of SEQ ID NO: 26, 37, 39, 41, and 43.

[0057] In one embodiment, the nucleic acid molecule comprises a polynucleotide encoding a polypeptide consisting of the sequence selected from the group consisting of SEQ ID NO: 8, 10, 16 and 18. In another embodiment, the nucleic acid comprises a polynucleotide encoding a polypeptide having at least 90 %, 95 %, 96 %, 97 %, 98 %, 99 % sequence identity to the group consisting of SEQ ID NO: 8, 10, 16 and 18, wherein the polypeptide has a W or Y at position 28 and a T at position 44, and wherein the polypeptide is capable of binding activin A, GDF-11, or myostatin. In one embodiment, the polynucleotide of the above embodiments encodes a polypeptide wherein the substitution at position 28 is W and the substitution at position 44 is T, and wherein the polypeptide is capable of binding myostatin, activin A or GDF-11.

[0058] In one embodiment, the isolated nucleic acid molecule comprises a polynucleotide having the sequence selected from the group consisting of SEQ ID NO: 3, 5, 11 or 13, or its complement. In another embodiment, the isolated nucleic acid molecule comprises a polynucleotide having the sequence selected from the group consisting of the sequence SEQ ID NO: 7, 9, 15 and 17, or its complement.

[0059] Nucleic acid molecules of the invention include DNA in both single-stranded and double-stranded form, as well as the RNA complement thereof. DNA includes, for example, cDNA, genomic DNA, synthetic DNA, DNA amplified by PCR, and combinations thereof. Genomic DNA may be isolated by conventional techniques, such as by using the DNA of SEQ ID NO: 3, 5, 11 or 13, or a suitable fragment thereof, as a probe. Genomic DNA encoding ActRIIB polypeptides is obtained from genomic libraries which are available for a number of species. Synthetic DNA is available from chemical synthesis of overlapping oligonucleotide fragments followed by assembly of the fragments to reconstitute part or all of the coding regions and flanking sequences. RNA may be obtained from procaryotic expression vectors which direct high-level synthesis of mRNA, such as vectors using T7 promoters and RNA polymerase. cDNA is obtained from libraries prepared from mRNA isolated from various tissues that express ActRIIB. The DNA molecules of the invention include full length genes as well as polynucleotides and fragments thereof. The full length gene may also include sequences encoding the N-terminal signal sequence.

[0060] The invention further provides the nucleic acid molecule describe above, wherein the polynucleotide is operably linked to a transcriptional or translational regulatory sequence.

<u>Exemplary polynucleotide and polypeptide sequences.</u>

[0061]

svActRIIB (E28W, S44T) with signal sequence

atggagtttgggctgagctgggttttcctcgttgctcttttaagaggtgtccagtgtgagacacggtggtgcatctactacaac
gccaactgggagctggagcgcaccaaccagaccggcctggagcgctgcgaaggcgagcaggacaagcggctgcact
gctacgcctcctggcgcaacagctctggcaccatcgagctcgtgaagaagggctgctggctagatgacttcaactgctacg
ataggcaggagtgtgtggccactgaggagaacccccaggtgtacttctgctgctgtgagggcaacttctgcaacgagcgct
tcactcatttgccagaggctgggggcccggaagtcacgtacgagccacccccgacagcccccacc (SEQ ID NO: 3)

svActRIIB (E28W, S44T) with signal sequence

mefglswvflvallrgvqcetrwciyynanwelertnqtglercegeqdkrlhcyaswrnssgtielvkkgcwlddfn
cydrqccvatccnpqvyfccccgnfcncrfthlpcaggpcvtycppptapt (SEQ ID NO: 4)

svActRIIB (E28W, S44T) without signal sequence

gagacacggtggtgcatctactacaacgccaactgggagctggagcgcaccaaccagaccggcctggagcgctgcgaa
ggcgagcaggacaagcggctgcactgctacgcctcctggcgcaacagctctggcaccatcgagctcgtgaagaagggc
tgctggctagatgacttcaactgctacgataggcaggagtgtgtggccactgaggagaacccccaggtgtacttctgctgct
gtgagggcaacttctgcaacgagcgcttcactcatttgccagaggctgggggcccggaagtcacgtacgagccaccccc
gacagcccccacc (SEQ ID NO: 5)

svActRIIB (E28W, S44T) without signal sequence

etrwciyynanwelertnqtglercegeqdkrlhcyaswrnssgtielvkkgcwlddfncydrqecvateenpqvyfc
ccegnfcnerfthlpeaggpevtyeppptapt (SEQ ID NO: 6)

svActRIIB-Fc (E28W, S44T) polynucleotide sequence with signal sequence

atggagtttgggctgagctgggttttcctcgttgctcttttaagaggtgtccagtgtgagacacggtggtgcatctactacaac
gccaactgggagctggagcgcaccaaccagaccggcctggagcgctgcgaaggcgagcaggacaagcggctgcact
gctacgcctcctggcgcaacagctctggcaccatcgagctcgtgaagaagggctgctggctagatgacttcaactgctacg
ataggcaggagtgtgtggccactgaggagaacccccaggtgtacttctgctgctgtgagggcaacttctgcaacgagcgct
tcactcatttgccagaggctgggggcccggaagtcacgtacgagccacccccgacagcccccaccggagggggaggat
ctgtcgagtgcccaccgtgcccagcaccacctgtggcaggaccgtcagtcttcctcttccccccaaaacccaaggacacc
ctcatgatctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccacgaagaccccgaggtccagttcaactg
gtacgtggacggcgtggaggtgcataatgccaagacaaagccacgggaggagcagttcaacagcacgttccgtgtggtc
agcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagtgcaaggtctccaacaaaggcctcccag
cccccatcgagaaaaccatctccaaaaccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccggg
aggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtggga
gagcaatgggcagccggagaacaactacaagaccacacctcccatgctggactccgacggctccttcttcctctacagca
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccac
tacacgcagaagagcctctccctgtctccgggtaaa (SEQ ID NO: 7)

svActRIIB-Fc (E28W, S44T) polypeptide sequence with signal sequence
mefglswvflvallrgvqcetrwciyynanwelertnqtglercegeqdkrlhcyaswrnssgtielvkkgcwlddfn
cydrqecvateenpqvyfccegnfcnerfthlpeaggpevtyeppptaptggggsvecppcpappvagpsvflfpp
kpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykc
kvsnkglpapiektisktkgqprepqvytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppml
dsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk (SEQ ID NO: 8)

svActRIIB-Fc (E28W, S44T) polynucleotide sequence without signal sequence
gagacacggtggtgcatctactacaacgccaactgggagctggagcgcaccaaccagaccggcctggagcgctgcgaa
ggcgagcaggacaagcggctgcactgctacgcctcctggcgcaacagctctggcaccatcgagctcgtgaagaagggc
tgctggctagatgacttcaactgctacgataggcaggagtgtgtggccactgaggagaacccccaggtgtacttctgctgct
gtgagggcaacttctgcaacgagcgcttcactcatttgccagaggctgggggcccggaagtcacgtacgagccaccccc
gacagcccccaccggagggggaggatctgtcgagtgcccaccgtgcccagcaccacctgtggcaggaccgtcagtcttc
ctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacgtgcgtggtggtggacgtgagcca
cgaagaccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccacgggagga
gcagttcaacagcacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagt
gcaaggtctccaacaaaggcctcccagcccccatcgagaaaaccatctccaaaaccaaagggcagccccgagaaccac
aggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctat
cccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacacctcccatgctggac
tccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctcc
gtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaa (SEQ ID NO: 9)

svActRIIB-Fc (E28W, S44T), polypeptide sequence without signal sequence
etrwciyynanwelertnqtglercegeqdkrlhcyaswrnssgtielvkkgcwlddfncydrqecvateenpqvyfc
ccegnfcnerfthlpeaggpevtyeppptaptggggsvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvd
vshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkg
qprepqvytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwq
qgnvfscsvmhealhnhytqkslslspgk (SEQ ID NO: 10)

svActRIIB (E28Y, S44T) with signal sequence
atggagtttgggctgagctgggttttcctcgttgctcttttaagaggtgtccagtgtgagacacggtactgcatctactacaac
gccaactgggagctggagcgcaccaaccagaccggcctggagcgctgcgaaggcgagcaggacaagcggctgcact
gctacgcctcctggcgcaacagctctggcaccatcgagctcgtgaagaagggctgctggctagatgacttcaactgctacg
ataggcaggagtgtgtggccactgaggagaacccccaggtgtacttctgctgctgtgagggcaacttctgcaacgagcgct
tcactcatttgccagaggctgggggcccggaagtcacgtacgagccacccccgacagcccccacc (SEQ ID NO: 11)

svActRIIB (E28Y,S44T) with signal sequence
mefglswvflvallrgvqcetryciyynanwelertnqtglercegeqdkrlhcyaswrnssgtielvkkgcwlddfnc
ydrqecvateenpqvyfccegnfcnerfthlpeaggpevtyeppptapt (SEQ ID NO: 12)

svActRIIB (E28Y,S44T) without signal sequence
gagacacggtactgcatctactacaacgccaactgggagctggagcgcaccaaccagaccggcctggagcgctgcgaa
ggcgagcaggacaagcggctgcactgctacgcctcctggcgcaacagctctggcaccatcgagctcgtgaagaagggc
tgctggctagatgacttcaactgctacgataggcaggagtgtgtggccactgaggagaacccccaggtgtacttctgctgct
gtgagggcaacttctgcaacgagcgcttcactcatttgccagaggctgggggcccggaagtcacgtacgagccaccccc
gacagcccccacc (SEQ ID NO: 13)

svActRIIB (E28Y,S44T) without signal sequence
etryciyynanwelertnqtglercegeqdkrlhcyaswrnssgtielvkkgcwlddfncydrqecvateenpqvyfc
ccegnfcnerfthlpeaggpevtyeppptapt (SEQ ID NO: 14)

svActRIIB-Fc (E28Y, S44T) polynucleotide sequence with signal sequence
atggagtttgggctgagctgggttttcctcgttgctcttttaagaggtgtccagtgtgagacacggtactgcatctactacaac
gccaactgggagctggagcgcaccaaccagaccggcctggagcgctgcgaaggcgagcaggacaagcggctgcact
gctacgcctcctggcgcaacagctctggcaccatcgagctcgtgaagaagggctgctggctagatgacttcaactgctacg
ataggcaggagtgtgtggccactgaggagaacccccaggtgtacttctgctgctgtgagggcaacttctgcaacgagcgct
tcactcatttgccagaggctgggggcccggaagtcacgtacgagccacccccgacagcccccaccggagggggaggat
ctgtcgagtgcccaccgtgcccagcaccacctgtggcaggaccgtcagtcttcctcttccccccaaaacccaaggacacc

ctcatgatctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccacgaagaccccgaggtccagttcaactg
gtacgtggacggcgtggaggtgcataatgccaagacaaagccacgggaggagcagttcaacagcacgttccgtgtggtc
agcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagtgcaaggtctccaacaaaggcctcccag
cccccatcgagaaaaccatctccaaaaccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccggg
aggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtggga
gagcaatgggcagccggagaacaactacaagaccacacctcccatgctggactccgacggctccttcttcctctacagca
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccac
tacacgcagaagagcctctccctgtctccgggtaaa (SEQ ID NO: 15)

svActRIIB-Fc (E28Y, S44T) polypeptide sequence with signal sequence
mcfglswvflvallrgvqcctryciyynanwelertnqtglercegeqdkrlhcyaswrnssgtielvkkgcwlddfnc
ydrqecvateenpqvyfcccegnfcnerfthlpeaggpevtyeppptaptggggsvecppcpappvagpsvflfppk
pkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykck
vsnkglpapiektisktkgqprepqvytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppmlds
dgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk (SEQ ID NO: 16)

svActRIIB-Fc (E28Y, S44T) polynucleotide sequence without signal sequence
gagacacggtactgcatctactacaacgccaactgggagctggagcgcaccaaccagaccggcctggagcgctgcgaa
ggcgagcaggacaagcggctgcactgctacgcctcctggcgcaacagctctggcaccatcgagctcgtgaagaagggc
tgctggctagatgacttcaactgctacgataggcaggagtgtgtggccactgaggagaacccccaggtgtacttctgctgct
gtgagggcaacttctgcaacgagcgcttcactcatttgccagaggctgggggcccggaagtcacgtacgagccacccccc
gacagcccccaccggagggggaggatctgtcgagtgcccaccgtgcccagcaccacctgtggcaggaccgtcagtcttc
ctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacgtgcgtggtggtggacgtgagcca
cgaagaccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccacgggagga
gcagttcaacagcacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagt
gcaaggtctccaacaaaggcctcccagcccccatcgagaaaaccatctccaaaaccaaagggcagccccgagaaccac
aggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctat
cccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacacctcccatgctggac
tccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctcc
gtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaa (SEQ ID NO: 17)

svActRIIB-Fc (E28Y, S44T) polypeptide sequence without signal sequence
etryciyynanwelertnqtglercegeqdkrlhcyaswrnssgtielvkkgcwlddfncydrqecvateenpqvyfc
ccegnfcnerfthlpeaggpevtyeppptaptggggsvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvd
vshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkg
qprepqvytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwq
qgnvfscsvmhealhnhytqkslslspgk (SEQ ID NO: 18)

[0062] In another aspect of the present invention, expression vectors containing the nucleic acid molecules and polynucleotides of the present invention are also provided, and host cells transformed with such vectors, and methods of producing the svActRIIB polypeptides are also provided. The term "expression vector" refers to a plasmid, phage, virus or vector for expressing a polypeptide from a polynucleotide sequence. Vectors for the expression of the svActRIIB polypeptides contain at a minimum sequences required for vector propagation and for expression of the cloned insert. An expression vector comprises a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a sequence that encodes svActRIIB polypeptides and proteins to be transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences. These sequences may further include a selection marker. Vectors suitable for expression in host cells are readily available and the nucleic acid molecules are inserted into the vectors using standard recombinant DNA techniques. Such vectors can include promoters which function in specific tissues, and viral vectors for the expression of svActRIIB polypeptides in targeted human or animal cells. An exemplary expression vector suitable for expression of svActRIIB is the pDSRa, (described in WO 90/14363) and its derivatives, containing svActRIIB polynucleotides, as well as any additional suitable vectors known in the art or described below.

[0063] The invention further provides methods of making svActRIIB polypeptides. A variety of other expression/host systems may be utilized. These systems include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transfected with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (e.g., Ti or pBR322 plasmid); or animal cell systems. Mammalian cells useful in recombinant protein production include but are not limited to VERO cells, HeLa cells, Chinese hamster ovary (CHO) cell lines, or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (see Rasmussen et al., 1998, Cytotechnology 28:31) or CHO strain DX-B11, which is deficient in DHFR (see Urlaub et al., 1980, Proc. Natl. Acad. Sci. USA 77:4216-20) COS cells such as the COS-7 line of monkey kidney cells (ATCC CRL 1651) (see Gluzman et al., 1981, Cell 23:175), W138, BHK, HepG2, 3T3 (ATCC CCL 163), RIN, MDCK, A549, PC12, K562, L cells, C127 cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line

CV1 (ATCC CCL 70) (see McMahan et al., 1991, EMBO J. 10:2821), human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Mammalian expression allows for the production of secreted or soluble polypeptides which may be recovered from the growth medium.

[0064] Using an appropriate host-vector system, svActRIIB polypeptides are produced recombinantly by culturing a host cell transformed with an expression vector containing the nucleic acid molecules of the present invention under conditions allowing for production. Transformed cells can be used for long-term, high-yield polypeptide production. Once such cells are transformed with vectors that contain selectable markers as well as the desired expression cassette, the cells can be allowed to grow in an enriched media before they are switched to selective media, for example. The selectable marker is designed to allow growth and recovery of cells that successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell line employed. An overview of expression of recombinant proteins is found in Methods of Enzymology, v. 185, Goeddell, D.V., ed., Academic Press (1990).

[0065] In some cases, such as in expression using procaryotic systems, the expressed polypeptides of this invention may need to be "refolded" and oxidized into a proper tertiary structure and disulfide linkages generated in order to be biologically active. Refolding can be accomplished using a number of procedures well known in the art. Such methods include, for example, exposing the solubilized polypeptide to a pH usually above 7 in the presence of a chaotropic agent. The selection of chaotrope is similar to the choices used for inclusion body solubilization, however a chaotrope is typically used at a lower concentration. Exemplary chaotropic agents are guanidine and urea. In most cases, the refolding/oxidation solution will also contain a reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential which allows for disulfide shuffling to occur for the formation of cysteine bridges. Some commonly used redox couples include cysteine/cystamine, glutathione/dithiobisGSH, cupric chloride, dithiothreitol DTT/dithiane DTT, and 2-mercaptoethanol (bME)/dithio-bME. In many instances, a co-solvent may be used to increase the efficiency of the refolding. Commonly used cosolvents include glycerol, polyethylene glycol of various molecular weights, and arginine.

[0066] In addition, the polypeptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, Solid Phase Peptide Synthesis, 2d.Ed., Pierce Chemical Co. (1984); Tam et al., J Am Chem Soc, 105:6442, (1983); Merrifield, Science 232:341-347 (1986); Barany and Merrifield, The Peptides, Gross and Meienhofer, eds, Academic Press, New York, 1-284; Barany et al., Int J Pep Protein Res, 30:705-739 (1987).

[0067] The polypeptides and proteins of the present invention can be purified according to protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the proteinaceous and non-proteinaceous fractions. Having separated the peptide polypeptides from other proteins, the peptide or polypeptide of interest can be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). The term "isolated polypeptide" or "purified polypeptide" as used herein, is intended to refer to a composition, isolatable from other components, wherein the polypeptide is purified to any degree relative to its naturally-obtainable state. A purified polypeptide therefore also refers to a polypeptide that is free from the environment in which it may naturally occur. Generally, "purified" will refer to a polypeptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation will refer to a peptide or polypeptide composition in which the polypeptide or peptide forms the major component of the composition, such as constituting about 50 %, about 60 %, about 70 %, about 80 %, about 85 %, or about 90 % or more of the proteins in the composition.

[0068] Various techniques suitable for use in purification will be well known to those of skill in the art. These include, for example, precipitation with ammonium sulphate, PEG, antibodies (immunoprecipitation) and the like or by heat denaturation, followed by centrifugation; chromatography such as affinity chromatography (Protein-A columns), ion exchange, gel filtration, reverse phase, hydroxylapatite, hydrophobic interaction chromatography, isoelectric focusing, gel electrophoresis, and combinations of these techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified polypeptide. Exemplary purification steps are provided in the Examples below.

[0069] Various methods for quantifying the degree of purification of polypeptide will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific binding activity of an active fraction, or assessing the amount of peptide or polypeptide within a fraction by SDS/PAGE analysis. A preferred method for assessing the purity of a polypeptide fraction is to calculate the binding activity of the fraction, to compare it to the binding activity of the initial extract, and to thus calculate the degree of purification, herein assessed by a "-fold purification number." The actual units used to represent the amount of binding activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the polypeptide or peptide exhibits a detectable binding activity.

[0070] Stabilized activin type IIB polypeptides bind to ligands that activate muscle-degradation cascades. svActRIIB polypeptides capable of binding and inhibiting the activity of the ligands activin A, myostatin, and/or GDF-11, and have the ability to treat diseases that involve muscle atrophy, as well as the treatment of certain cancers, and other diseases.

[0071] The Examples below show improved properties for svActRIIB polypeptides and proteins having the amino acid substitutions described herein, while retaining the ability to bind and neutralize myostatin, activin A, or GDF-11 in *in vitro* assays, as well as retaining *in vivo* activity. These properties result in proteins and polypeptides having improved manufacturability in comparison to other soluble receptors.

Pharmaceutical Compositions

[0072] Pharmaceutical compositions containing the svActRIIB proteins and polypeptides of the present invention are also provided. Such compositions comprise a therapeutically or prophylactically effective amount of the polypeptide or protein in admixture with pharmaceutically acceptable materials, and physiologically acceptable formulation materials. The pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, other organic acids); bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides and other carbohydrates (such as glucose, mannose, or dextrins); proteins (such as serum albumin, gelatin or immunoglobulins); coloring; flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides (preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. (Remington's Pharmaceutical Sciences, 18th Edition, A.R. Gennaro, ed., Mack Publishing Company, 1990).

[0073] The optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format, and desired dosage. See for example, Remington's Pharmaceutical Sciences, *supra*. Such compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the polypeptide. For example, suitable compositions may be water for injection, physiological saline solution for parenteral administration.

[0074] The primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffers, or acetate buffers, which may further include sorbitol or a suitable substitute thereof. In one embodiment of the present invention, compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (Remington's Pharmaceutical Sciences, supra) in the form of a lyophilized cake or an aqueous solution. Further, the therapeutic composition may be formulated as a lyophilizate using appropriate excipients such as sucrose.

[0075] The formulations can be delivered in a variety of methods, for example, by inhalation therapy, orally, or by injection. When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the desired polypeptide in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which a polypeptide is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (polylactic acid, polyglycolic acid), beads, or liposomes, that provides for the controlled or sustained release of the product which may then be delivered via a depot injection.

Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

[0076] In another aspect, pharmaceutical formulations suitable for injectable administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions. In another embodiment, a pharmaceutical composition may be formulated for inhalation. Inhalation solutions may also be formulated with a propellant for aerosol delivery. In yet another embodiment, solutions may be nebulized. Pulmonary administration is further described in PCT Application No. PCT/US94/001875, which describes pulmonary delivery of chemically modified proteins.

[0077] It is also contemplated that certain formulations may be administered orally. In one embodiment of the present invention, molecules that are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the therapeutic molecule. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed. Pharmaceutical compositions for oral administration can also be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

[0078] Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate

[0079] Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

[0080] Pharmaceutical preparations that can be used orally also include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

[0081] Additional pharmaceutical compositions will be evident to those skilled in the art, including formulations involving polypeptides in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See for example, PCT/US93/00829 that describes controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions. Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., Biopolymers, 22:547-556 (1983), poly (2-hydroxyethyl-methacrylate) (Langer et al., J. Biomed. Mater. Res., 15:167-277, (1981); Langer et al., Chem. Tech.,12:98-105(1982)), ethylene vinyl acetate (Langer et al., supra) or poly-D(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomes, which can be prepared by any of several methods known in the art. See e.g., Eppstein et al., PNAS (USA), 82:3688 (1985); EP 36,676; EP 88,046; EP 143,949.

[0082] The pharmaceutical composition to be used for *in vivo* administration typically must be sterile. This may be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0083] Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

[0084] In a specific embodiment, the present invention is directed to kits for producing a single-dose administration unit. The kits may each contain both a first container having a dried protein and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing single and multi-chambered pre-filled syringes (*e.g.*, liquid syringes and lyosyringes).

[0085] An effective amount of a pharmaceutical composition to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which the polypeptide is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician may titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1mg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. Polypeptide compositions may be preferably injected or administered intravenously. Long-acting pharmaceutical compositions may be administered every three to four days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation. The frequency of dosing will depend upon the pharmacokinetic parameters of the polypeptide in the formulation used. Typically, a composition is administered until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as multiple doses (at the same or different concentrations/dosages) over time, or as a continuous infusion. Further refinement of the appropriate dosage is routinely made. Appropriate dosages may be ascertained through use of appropriate dose-response data.

[0086] The route of administration of the pharmaceutical composition is in accord with known methods, e.g. orally, through injection by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intra-ocular, intraarterial, intraportal, intralesional routes, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, or intraperitoneal; as well as intranasal, enteral, topical, sublingual, urethral, vaginal, or rectal means, by sustained release systems or by implantation devices. Where desired, the compositions may be administered by bolus injection or continuously by infusion, or by implantation device. Alternatively or additionally, the composition may be administered locally via implantation of a membrane, sponge, or another appropriate material on to which the desired molecule has been absorbed or encapsulated. Where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of the desired molecule may be via diffusion, timed-release bolus, or continuous administration.

[0087] In some cases, the svActRIIB polypeptides of the present invention can be delivered by implanting certain cells that have been genetically engineered, using methods such as those described herein, to express and secrete the polypeptide. Such cells may be animal or human cells, and may be autologous, heterologous, or xenogeneic. Optionally, the cells may be immortalized. In order to decrease the chance of an immunological response, the cells may be encapsulated to avoid infiltration of surrounding tissues. The encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the polypeptide product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

[0088] svActRIIB gene therapy *in vivo* is also envisioned wherein a nucleic acid molecule encoding svActRIIB, or a derivative of svActRIIB is introduced directly into the subject. For example, a nucleic acid sequence encoding a svActRIIB is introduced into target cells via local injection of a nucleic acid construct with or without an appropriate delivery vector, such as an adeno-associated virus vector. Alternative viral vectors include, but are not limited to, retroviruses, adenovirus, herpes simplex, virus and papilloma virus vectors. Physical transfer of the virus vector may be achieved *in vivo* by local injection of the desired nucleic acid construct or other appropriate delivery vector containing the desired nucleic acid sequence, liposome-mediated transfer, direct injection (naked DNA), or microparticle bombardment (gene-gun).

[0089] The compositions of the present disclosure may be used alone or in combination with other therapeutic agents to enhance their therapeutic effects or decrease potential side effects.

<u>Uses of svActRIIB Compositions</u>

[0090] The present invention provides pharmaceutical compositions for use in methods of reducing or neutralizing the amount or activity of myostatin, activin A, or GDF-11 *in vivo* and *in vitro.* svActRIIB polypeptides have a high binding affinity for myostatin, activin A, and GDF-11, and are capable of reducing and inhibiting the biological activities of at least one of myostatin, activin A and GDF-11.

[0091] In one aspect, the present invention provides compositions for use in methods and reagents for treating myostatin-related and/or activin A related disorders in a subject in need of such a treatment by administering an effective dosage of an svActRIIB composition to the subject. As used herein the term "subject" refers to any animal, such as mammals including humans.

[0092] The compositions of the present invention are useful for increasing lean muscle mass in a subject. The compositions may also be useful to increase lean muscle mass in proportion to fat mass, and thus decrease fat mass as percentage of body weight in a subject. Example 3 demonstrates that the svActRIIB polypeptides and proteins of the invention can increase lean muscle mass in animals.

[0093] The disorders that can be treated by an svActRIIB composition include but are not limited to various forms of muscle wasting, as well as metabolic disorders such as diabetes and related disorders, and bone degenerative diseases such as osteoporosis.

[0094] Muscle wasting disorders also include dystrophies such as Duchenne's muscular dystrophy, progressive muscular dystrophy, Becker's type muscular dystrophy, Dejerine-Landouzy muscular dystrophy, Erb's muscular dystrophy, and infantile neuroaxonal muscular dystrophy. Additional muscle wasting disorders arise from chronic diseases or disorders such as amyotrophic lateral sclerosis, congestive obstructive pulmonary disease, cancer, AIDS, renal failure, organ atrophy, androgen deprivation, and rheumatoid arthritis.

[0095] Over-expression of myostatin and/or activin may contribute to cachexia, a severe muscle wasting syndrome. Cachexia results from cancers, and also arises due to rheumatoid arthritis, diabetic nephropathy, renal failure, chemotherapy, injury due to burns, as well as other causes. In another example, serum and intramuscular concentrations of myostatin-immunoreactive protein was found to be increased in men exhibiting AIDS-related muscle wasting and was inversely related to fat-free mass (Gonzalez-Cadavid et al., PNAS USA 95: 14938-14943 (1998)). Myostatin levels have also been shown to increase in response to burns injuries, resulting in a catabolic muscle effect (Lang et al, FASEB J 15, 1807-1809 (2001)). Additional conditions resulting in muscle wasting may arise from inactivity due to disability such as confinement in a wheelchair, prolonged bed rest due to stroke, illness, spinal chord injury, bone fracture or trauma, and muscular atrophy in a microgravity environment (space flight). For example, plasma myostatin immunoreactive protein was found to increase after prolonged bed rest (Zachwieja et al. J Gravit Physiol. 6(2):11(1999). It was also found that the muscles of rats exposed to a microgravity environment during a space shuttle flight expressed an increased amount of myostatin compared with the muscles of rats which were not exposed (Lalani et al., J.Endocrin 167 (3):417-28 (2000)).

[0096] In addition, age-related increases in fat to muscle ratios, and age-related muscular atrophy appear to be related to myostatin. For example, the average serum myostatin-immunoreactive protein increased with age in groups of young (19-35 yr. old), middle-aged (36-75 yr. old), and elderly (76-92 yr old) men and women, while the average muscle mass and fat-free mass declined with age in these groups (Yarasheski et al. J Nutr Aging 6(5):343-8 (2002)). In addition, myostatin has now been found to be expressed at low levels in heart muscle and expression is upregulated in cardiomyocytes after infarct (Sharma et al., J Cell Physiol. 180 (1):1-9 (1999)). Therefore, reducing myostatin levels in the heart muscle may improve recovery of heart muscle after infarct.

[0097] Myostatin also appears to influence metabolic disorders including type 2 diabetes, noninsulin-dependent diabetes mellitus, hyperglycemia, and obesity. For example, lack of myostatin has been shown to improve the obese and diabetic phenotypes of two mouse models (Yen et al. FASEB J. 8:479 (1994). The svActRIIB polypeptides of the present disclosure are suitable for treating such metabolic disorders. Therefore, administering the compositions of the present invention will improve diabetes, obesity, and hyperglycemic conditions in suitable subjects. In addition, compositions containing the svActRIIB polypeptides may decrease food intake in obese individuals.

[0098] Administering the stabilized ActRIIB polypeptides of the present invention may improve bone strength and reduce osteoporosis and other degenerative bone diseases. It has been found, for example, that myostatin-deficient mice showed increased mineral content and density of the mouse humerus and increased mineral content of both trabecular and cortical bone at the regions where the muscles attach, as well as increased muscle mass (Hamrick et al. Calcif Tissue Int 71(1):63-8 (2002)). In addition, the svActRIIB compositions of the present invention can be used to treat the effects of androgen deprivation in cases such as androgen deprivation therapy used for the treatment of prostate cancer, for example.

[0099] The present invention also provides compositions for use in methods of increasing muscle mass in food animals by administering an effective dosage of the svActRIIB proteins to the animal. Since the mature C-terminal myostatin polypeptide is similar or identical in all species tested, svActRIIB polypeptides would be expected to be effective for increasing lean muscle mass and reducing fat in any agriculturally important species including cattle, chicken, turkeys, and pigs.

[0100] The svActRIIB polypeptides and compositions of the present invention also antagonize the activity of activin A, as shown in the *in vitro* assays below. Activin A is known to be expressed in certain types of cancers, particularly gonadal tumors such as ovarian carcinomas, and to cause severe cachexia. (Ciprano et al. Endocrinol 141 (7):2319-27 (2000), Shou et al., Endocrinol 138 (11):5000-5 (1997); Coerver et al, Mol Endocrinol 10(5):534-43 (1996); Ito et al. British J Cancer 82(8):1415-20 (2000), Lambert-Messerlian, et al, Gynecologic Oncology 74:93-7 (1999). Therefore, the compositions of the present disclosure may be used to treat conditions related to activin A overexpression, as well as myostatin expression, such as cachexia from certain cancers and the treatment of certain gonadal type tumors.

[0101] In addition, the svActRIIB polypeptides of the present invention are useful for detecting and quantitating myostatin, activin A, or GDF-11 in any number of assays. In general, the stabilized ActRIIB polypeptides of the present invention are useful as capture agents to bind and immobilize myostatin, activin A, or GDF-11 in a variety of assays, similar to those described, for example, in Asai, ed., Methods in Cell Biology, 37, Antibodies in Cell Biology, Academic Press, Inc., New York (1993). The polypeptides may be labeled in some manner or may react with a third molecule such as an antibody which is labeled to enable myostatin to be detected and quantitated. For example, a polypeptide or a third molecule can be modified with a detectable moiety, such as biotin, which can then be bound by a fourth molecule, such as enzyme-labeled streptavidin, or other proteins. (Akerstrom, J Immunol 135:2589 (1985); Chaubert, Mod Pathol 10:585 (1997)).

[0102] The invention having been described, the following examples are offered by way of illustration, and not limitation.

Example 1

<u>Expression and Purification of svActRIIB Polypeptides</u>

[0103] The following methods were used for expressing and purifying the stabilized ActRIIB polypeptides.

[0104] The cDNA of the human activin type IIB receptor was isolated from a cDNA library of human testis origin (Clontech, Inc.) and cloned as described in U.S. application serial no: 11/590,962, U.S. application publication No: 2007/0117130.

[0105] The following method was used to produce the svActRIIB-Fc (E28W, S44T) polypeptide (SEQ ID NO: 10), and the ActRIIB-Fc (E28W) (SEQ ID NO: 21). Polynucleotides encoding the svActRIIB, (E28W, S44T) (SEQ ID NO: 5), or polynucleotides encoding ActRIIB (E28W) (SEQ ID NO: 19) were fused to polynucleotides encoding the human IgG2 Fc (SEQ ID NO: 22), via polynucleotides encoding hinge linker sequence (SEQ ID NO: 26) using PCR overlap extension using primers containing the mutation resulting in the amino acid substitutions at position 28 of E to W, and at position 44 of S to T. The full polynucleotide sequence is SEQ ID NO: 9 for svActRIIB-IgG Fc (E28W, S44T), and SEQ ID NO: 20 for ActRIIB-ActRIIB-IgG Fc (E28W). Double stranded DNA fragments were subcloned into vectors pTT5 (Biotechnology Research Institute, National Research Council Canada (NRCC), 6100 Avenue Royalmount, Montréal (Quebec) Canada H4P 2R2), pDSRα described in WO/9014363) and/or derivatives of pDSRα.

[0106] Transient expression of stabilized ActRIIB-Fc polypeptides was carried out as follows.

[0107] The svActRIIB-IgG Fc (E28W, S44T) (SEQ ID NO: 10), and ActRIIB-IgG Fc (E28W) (SEQ ID NO: 21) polypeptides were expressed transiently in serum-freesuspension adapted 293-6E cells (National Research Council of Canada, Ottawa, Canada) maintained in FreeStyle™ medium (Invitrogen Corporation, Carlsbad, CA) supplemented with 250 µg/ml geneticin (Invitrogen) and 0.1% Pluronic F68 (Invitrogen). Transfections were performed as 1L cultures. Briefly, the cell inoculum was grown to 1.1×10^6 cells/ml in a 4L fernbach shake flask (Corning, Inc.). The shake flask culture was maintained on an Innova 2150 shaker platform (News Brunswick Scientific, Edison, NJ) at 65 RPM which was placed in a humidified incubator maintained at 37°C and 5% CO_2. At the time of transfection, the 293-6E cells were diluted to 1.0 × 10^6 cells/ml.

[0108] The transfection complexes were formed in 100 ml FreeStyle™ 293 Media (Invitrogen). 1 mg plasmid DNA was first added to the medium followed by 3 ml of FuGene HD transfection reagent (Roche Applied Science, Indianapolis, IN). The transfection complex was incubated at room temperature for approximately 15 minutes and then added to the cells in the shake flask. Twenty hours post transfection, 20% (w/v) of peptone TN1 (OrganoTechnie S.A., TeknieScience, QC, Canada) was added to reach a final concentration of 0.5% (w/v). The transfection/expression was performed for 4-7 days, after which the conditioned medium was harvested by centrifugation at 4,000 RPM for 60 minutes at 4°C.

[0109] Stable transfection and expression was carried out as follows. The svActRIIB-IgG-Fc cell lines were created by transfecting stable CHO host cells with the expression plasmids containing polynucleotides encoding svActRIIB-IgG Fc (E28W, S44T) (SEQ ID NO: 9) or ActRIIB-IgG Fc (E28W) (SEQ ID NO: 20) using a standard electroporation procedure. After transfection of the host cell line with the expression plasmids the cells were grown in serum-free selection medium without GHT for 2-3 weeks to allow for selection of the plasmid and recovery of the cells. Cells are selected until they achieved greater than 85% viability. This pool of transfected cells was then cultured in medium containing 150 nM methotrexate.

[0110] In a six-day expression assay, pools of svActRIIB-Fc (E28W, S44T) expressing cells showed higher cell titer, growth performance, and improved specific productivity (picogram/cell/day) of protein produced compared with pools of ActRIIB-Fc (E28W) expressing cells. Select pools, for example, produced about 1.2 g/liter for svActRIIB-Fc (E28W, S44T) compared with 0.9 g/liter for ActRIIB-Fc (E28W).

[0111] Each of an svActRIIB-Fc (E28W, S44T) and an ActRIIB-Fc (E28W) expressing cell line was scaled up using a typical fed-batch process. Cells were inoculated into a Wave bioreactor (Wave Biotech LLC). Cultures were fed three times with bolus feeds. 10 L were harvested on day 10, the remainder was harvested on day 11; both harvests underwent depth filtration followed by sterile filtration. The conditioned media was filtered through a 10 inch 0.45/0.2 micron pre filter, followed by a filtration through a 6 inch 0.2 micron filter.

Protein Purification

[0112] Approximately 5 L of conditioned media was directly loaded onto a 220mL MabSelect™ column Protein A column (GE Healthcare). The column was pre-equilibrated in PBS (phosphate-buffered saline: 2.67 mM potassium chloride, 138 mM sodium chloride, 1.47 mM potassium phosphate monobasic, 8.1 mM sodium phosphate dibasic, pH 7.4). The column was washed with the equilibration buffer until the reading at OD280 was approximately zero, and then the protein was eluted with 0.1M acetic acid.

[0113] The Mabselect™ Pool was applied to a 300mL SP-HP column (GE Healthcare) (5 x 15 cm). The column was pre-equilibrated with 10mM NaOAC, pH 5. The column was then washed with the equilibration buffer until the reading at OD280 was approximately 0. The column was eluted with 20 column volumes of a gradient buffer from 0-150 mM NaCl in 10 mM NaOAC, pH 5. The SP-HP pool was concentrated, and filtered with a 0.2uM cellulose acetate (Corning) filter.

[0114] The sequences of the proteins used are set forth in the Table below.

ActRIIB-Fc	ActRIIB sequence	Linker-Hinge	IgG2 Fc
svActRIIB-IgG_2Fc (E28W, S44T)	ETRWCIYYNANWELERT NQTGLERCEGEQDKRLH CYASWRNSSGTIELVKKG CWLDDFNCYDRQECVAT EENPQVYFCCCEGNFCNE RFTHLPEAGGPEVTYEPP	GGGGSV ECPPCP (SEQ ID NO: 27)	APPVAGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTFR VVSVLTVVHQDWLNGKEYKCKV SNKGLPAPIEKTISKTKGQPREPQ VYTLPPSREEMTKNQVSLTCLVK
(SEQ ID NO: 10)	PTAPT (SEQ ID NO: 6)		GFYPSDIAVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:22)

ActRIIB-Fc	ActRIIB sequence	Linker-Hinge	IgG2 Fc
ActRIIB-IgG$_2$Fc (E28W) (SEQ ID NO: 21)	ETRWCIYYNANWELERT NQSGLERCEGEQDKRLH CYASWRNSSGTIELVKKG CWLDDFNCYDRQECVAT EENPQVYFCCCEGNFCNE RFTHLPEAGGPEVTYEPP PTAPT (SEQ ID NO: 19)	GGGGSV ECPPCP (SEQ ID NO: 27)	APPVAGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTFR VVSVLTVVHQDWLNGKEYKCKV SNKGLPAPIEKTISKTKGQPREPQ VYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO: 22)

Example 2

Characterization of polypeptides

[0115] Samples of the svActRIIB-Fc (E28W, S44T) (SEQ ID NO: 10) purified through the MabSelect™ step, and ActRIIB-Fc (E28W) (SEQ ID NO: 21) polypeptides purified through the SP-HP column step, as described above, were diluted with PBS, pH 7.4 to 0.2 mg/ml. The glycosylation profile of the polypeptides were then determined using SEC as described below.

[0116] Size exclusion chromatography (SEC). Experiments were performed on an Agilent 1100 HPLC system with two columns (TOSOHAAS G3000swxl, 7.8 x 300 mm) in tandem. 2x PBS was used as the mobile phase at 0.5 ml/minute.

[0117] Figure 1 shows a comparison between ActRIIB-Fc (E28W) and svActRIIB-Fc (E28W, S44T) on an SEC column using the protocols described above. svActRIIB-Fc (E28W, S44T) shows a single peak compared with ActRIIB-Fc (E28W), which shows three peaks. These correspond to the degree of N-linked glycosylation at the N42 position of the Fc dimers of both proteins. The single peak of the svActRIIB-Fc (E28W, S44T) polypeptide corresponds to fully glycosylated N-linked asparagines at position N42 of the dimer. The three peaks of ActRIIB-Fc (E28W) corresponds to (from left to right), fully glycosylated asparagines at N42, partially glycosylated asparagines at N42, and non-glycosylated asparagines at N42. Therefore, this demonstrates that the svActRIIB-Fc (E28W, S44T) molecule is fully glycosylated compared to ActRIIB-Fc (E28W), which is heterogeneous with respect to this glycosylation site, and thus more difficult to purify. In addition, preliminary studies indicate that the svActRIIB-Fc (E28W, S44T) molecule has addition improved manufacturability properties as set forth below. Additional studies also demonstrated that the least glycosylated peak of the ActRIIB-Fc (E28W) has lower physical and thermal stability than partially and fully glycosylated molecules.

[0118] Determination of K_D and IC_{50} values of the receptor polypeptides for activin A, myostatin, and GDF-11 were obtained as described below.

KinEx A™ Equilibrium Assays

[0119] Solution-based equilibrium-binding assays using KinExA™ technology (Sapidyne Instruments, Inc.) were used to determine the dissociation equilibrium (K_D) of ligand binding to ActRIIB-Fc polypeptides. UltraLink Biosupport beads (Pierce) was pre-coated with about 100 µg/ml each of myostatin, GDF-11, and activin A overnight, and then blocked with BSA. 1 pM and 3 pM of ActRIIB-Fc (E28W) (SEQ ID NO: 21) and svActRIIB-Fc (E28W, S44T) (SEQ ID NO: 10) samples were incubated with various concentrations (0.7 fM to 160 pM) of myostatin, activin A, and GDF-11 respectively in sample buffer at room temperature for 8 hours before being run through the ligand-coated beads. The amount of the bead-bound soluble receptor was quantified by fluorescent (Cy5) labeled goat anti-human-Fc antibody at 1 mg/ml in superblock. The binding signal is proportional to the concentration of free soluble receptor at equilibrium with a given myostatin, activin A, or GDF-11 concentration. K_D was obtained from the nonlinear regression of the competition curves using a dual-curve one-site homogeneous binding model provided in the KinEx A™ software (Sapidyne Instruments, Inc.). The K_D values obtained for each are given in the table below.

	Myostatin	GDF-11	Activin A
ActRIIB-Fc (E28W)	0.1 pM	0.1 pM	0.2 pM

	Myostatin	GDF-11	Activin A
sv ActRIIB-Fc (E28W, S44T)	0.1pM	0.1 pM	0.1 pM

C2C12 Cell Based Activity Assay

[0120] The ability of ActRIIB-Fc (E28W) (SEQ ID NO: 21) and svActRIIB-Fc (E28W, S44T) (SEQ ID NO: 10) to inhibit the binding of activin A, GDF-11, or myostatin to the wild type activin IIB receptor-Fc was tested using a cell based activity assay as described below.

[0121] A myostatin/activin/GDF-11-responsive reporter cell line was generated by transfection of C2C12 myoblast cells (ATCC No: CRL-1772) with a pMARE-luc construct. The pMARE-luc construct is made by cloning twelve repeats of the CAGA sequence, representing the myostatin/activin response elements (Dennler et al. EMBO 17: 3091-3100 (1998)) into a pLuc-MCS reporter vector (Stratagene cat # 219087) upstream of the TATA box. The C2C12 cells naturally express activin receptor IIB on their cell surface. When myostatin/activinA/GDF-11 binds the cell receptors, the Smad pathway is activated, and phosphorylated Smad binds to the response element (Macias-Silva et al. Cell 87:1215 (1996)), resulting in the expression of the luciferase gene. Luciferase activity was then measured using a commercial luciferase reporter assay kit (cat # E4550, Promega, Madison, WI) according to manufacturer's protocol. A stable line of C2C12 cells that has been transfected with pMARE-luc (C2C12/pMARE) was used to measure activity according to the following procedure. Reporter cells were plated into 96 well cultures. Screening using dilutions of the ActRIIB-IgG2 Fc fusions constructed as described above was performed with the concentration fixed at 4 nM activin A, myostatin, and GDF-11. Each of these ligands was pre-incubated with the receptors at several concentrations. Activity was measured by determining the luciferase activity in the treated cultures. The IC_{50} values were determined for each polypeptide. These are shown in the Table below. These values are given in Table below.

	Myostatin	GDF-11	Activin A
ActRIIB-Fc (E28W)	0.95 nM	2.4 nM	3.2 nM
svActRIIB-Fc (E28W, S44T)	1.07 nM	2.4 nM	3.6 nM

[0122] Thus the cell based activities are approximately the same for ActRIIB-Fc (E28W) and svActRIIB-Fc (E28W, S44T).

Stability at low pH

[0123] Stability of a protein at low pH is a useful parameter in considering the manufacturability of the protein, since the viral inactivation step of a commercial production process typically is carried out at low pH, such as between about pH 3.0 to 4.0.

[0124] To assess the short term protein stability effects at low pH experienced during the viral inactivation step of commercial protein purification the following test was performed. Each protein was diluted to 10 mg/ml of 100 mM sodium acetate, pH 3.5. This was stored at 25 °C and analyzed at time 0, at 2 hours and at 24 hours using SEC analysis. SEC analysis was performed as described above, and percentage of high molecular weight aggregates was determined.

% HMW aggregate

	T = 0	T = 2 hours	T = 4 hours
ActRIIB-Fc (E28W)	1.53	1.36	13.74
svActRIIB-Fc (E28W, S44T)	1.66	2.17	8.93

[0125] Thus the percentage of high molecular weight aggregates produced at pH 3.5 is substantially less for svActRIIB-Fc (E28W, S44T) than ActRIIB-Fc (E28W) at 4 hours.

[0126] Additional studies showed that svActRIIB-Fc (E28W, S44T) showed better reversibility than ActRIIB-Fc (E28W) from exposure to pH 3.0, 3.5 and 5.0, and that svActRIIB-Fc (E28, S44T) was more homogeneous that ActRIIB-Fc (E28W) at all pHs.

[0127] Thus, the svActRIIB-Fc (E28W, S44T) polypeptides are demonstrated to have improved manufacturability characteristics, in particular, improved stability at low pH, and greater homogeneity at all pHs compared with ActRIIB-Fc (E28W) while retaining the ability to inhibit activin A, myostatin, and GDF-11 activity.

Example 3

Determination of *in vivo* efficacy

[0128] 11-week-old female C57B1/6 mice were purchased from Charles River Laboratories. The mice (ten mice per group) were administered a single dose (10 mg/kg) of svActRIIB-Fc (E28W, S44T) (SEQ ID NO: 10) or vehicle (PBS). Lean body mass was determined by NMR (PIXImus, GE LUNAR Corporation) at 3, 7, 10 and 14 days after dose administration for the ten animals in each group. The results for each set of mice are shown in Figure 2. It can be seen that a single dose of svActRIIB-Fc (E28W, S44T) significantly increased lean body mass in the animals. ($P<0.001$, based on repeated measurement ANOVA. $n=10$ animals per group).

[0129] A study to determine dose-response efficacy was carried out as follows. Escalating single doses of 0, 0.3, 3, 10, and 30 mg/kg of svActRIIB-Fc (E28W, S44T) (SEQ ID NO: 10) in PBS was administered subcutaneously to female 10-12 week old C57B1/6 mice (Charles River Laboratories). Six animals were initially in each dosage group including the PBS control group. Lean body mass was determined by NMR (PIXImus, GE LUNAR Corporation) every two to four days for the forty-two days of the study. At the end of each week, one animal from each group was sacrificed to obtain additional data (six in total each week from all six groups), and the lean body mass determined for the remaining animals in subsequent weeks. The results are set out in Figure 3. It can be seen that the svActRIIB-Fc (E28W, S44T) polypeptide at all doses significantly increased muscle mass in the animals, in a dose-dependent manner.

[0130] In further studies, head to head comparisons between ActRIIB-Fc (E28W) (SEQ ID NO: 21) and svActRIIB-Fc (E28W, S44T) (SEQ ID NO: 10) were performed on female C57B1/6 mice (Charles River Laboratories, 10 animals per group) to measure the increase in lean muscle mass and body weight changes after a single dose of 10 mg/kg of each soluble receptor compared with a control group (administered PBS). Lean body mass was determined by NMR (PIXImus, GE LUNAR Corporation), and body weight change was determined by weighing the animals periodically for 37 days. The results at the end of this comparative study was that ActRIIB-Fc (E28W) (SEQ ID NO: 21) showed an increase of 24% in lean muscle mass and 25% in increase of body weight compared with an increase of 25% in lean muscle mass and 20% increase in body weight for svActRIIB-Fc (E28W, S44T) (SEQ ID NO: 10), compared with an increase of 5% lean muscle mass and 9% increase body weight for the control group.

[0131] Therefore, it can be seen that svActRIIB-Fc (E28W, S44T) retains comparable *in vivo* efficacy compared with ActRIIB-Fc (E28W) while having improved manufacturability characteristics.

Example 4

Improved Manufacturability with Modified Hinge Linkers

[0132] Additional linkers and modified hinge regions were constructed to test for further improvement of protein expression and manufacturability of the stabilized ActRIIB (E28W, S44T) polypeptides. Modified linker/hinge sequences based on modifications of hinge linker #1 were generated using overlap extension PRC mutagenesis methods, according to Mikaelian et al., Methods in Molecular Biology, 57, 193-202 (1996), and well known methodology.

[0133] The modified hinge linkers designed to perform well with IgG2 Fc fusions are hinge linker #2-7 set forth below (in comparison to hinge linker #1 sequences).

hinge linker #1 polynucleotide
ggagggggaggatctgtcgagtgcccaccgtgccca (SEQ ID NO: 26).

hinge linker #1 polypeptide
GGGGSVECPPCP (SEQ ID NO: 27)

hinge linker #2 polynucleotide
ggagggggaggatctgagcgcaaatgttgtgtcgagtgcccaccgtgc (SEQ ID NO: 37)

hinge linker #2 peptide
GGGGSERKCCVECPPC (SEQ ID NO: 38)

hinge linker #3 polynucleotide
ggagggggaggatctggtggaggtggttcaggtccaccgtgc (SEQ ID NO: 39)

hinge linker #3 peptide
GGGGSGGGGSGPPC (SEQ ID NO: 40)

hinge linker #4 polynucleotide
ggagggggaggatctggtggaggtggttcaggtccaccggga (SEQ ID NO: 41)

hinge linker #4 peptide
GGGGSGGGGSGPPG (SEQ ID NO: 42)

hinge linker #5 polynucleotide
ggagggggaggatctgagcgcaaatgtccaccttgtgtcgagtgcccaccgtgc (SEQ ID NO: 43)

hinge linker #5 peptide
GGGGSERKCPPCVECPPC (SEQ ID NO: 44)

hinge linker #6 peptide
GPASGGPASGPPCP (SEQ ID NO: 45)

hinge linker #7 peptide
GPASGGPASGCPPCVECPPCP (SEQ ID NO: 46)

[0134] The following hinge linkers #8 to #10 below were designed to perform well with an IgG1Fc (SEQ ID NO: 23) or the modified IgG1Fc given below (SEQ ID NO: 47 below).

modified IgG1 Fc
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK (SEQ ID NO: 47)

hinge linker #8 peptide
GGGGSVDKTHTCPPCP (SEQ ID NO: 48)

hinge linker #9 peptide
GGGGSVDKTHTGPPCP (SEQ ID NO: 49)

hinge linker #10 peptide
GGGGSGGGGSVDKTHTGPPCP (SEQ ID NO: 50)

[0135] Testing of modified hinge linker sequences with svActRIIB-Fc (28W, S44T) was performed as follows. Polynucleotides encoding svActRIIB (E28W, S44T) (SEQ ID NO: 5), polynucleotides encoding the modified hinge linkers shown above, and polynucleotides encoding IgG2 Fc (SEQ ID NO: 22) or polynucleotides encoding IgG1 Fc (SEQ ID NO: 23) or modified IgG1 Fc (SEQ ID NO: 47) were subcloned into vectors as described in Example 1 and expressed using the transient 293-6E expression system as described in Example 1, except for the following changes: F17 media (Invitrogen) supplemented with 1.1 mg/ml Pluronic, 6 mM L-glutamine and 25 µg/ml geneticin (Invitrogen) was used in place of Freestyle 293 medium as described in Durocher et al., Nucleic Acids Research 30, No. 3, e9 (2002)). The cultures were grown for seven days at 37 °C after transfection. Aliquots were centrifuged to remove cells, and the supernatant was mixed with loading buffer before being heated and loaded onto a 4-20% tris-glycine gel for analysis by Western Blot. After the protein was transferred to a nitrocellulose membrane, samples were probed with a hydrogen peroxidase conjugated anti-human Fc antibody (Pierce #31423) at a dilution of 1:1000.

[0136] Protein purification was performed using the following procedure. Approximately 0.25 L of the conditioned media containing the svActRIIB-Fc variants were concentrated using a 5 ft^2 10K membrane tangential flow filter. The concentrated material was applied to a 5 mL Protein A High Performance Column™ (GE Heathcare) which had been equilibrated with PBS (Dulbecco's with no magnesium chloride or calcium chloride). After washing the column with the equilibration buffer until the absorbance at 280 nm (OD_{280}) was less than 0.1, the bound protein was eluted with 0.1 M glycine-HCl, pH 2.7, and immediately neutralized with 1 M Tris-HCl, pH 8.5.

[0137] The portion of aggregate in percent and the portion of half molecule in percent were determined by the following method. Denaturing size exclusion chromatography experiments were performed by injecting a 50 µl aliquot of each sample onto an HPLC system with two size exclusion columns (TOSOHAAS G3000swxl) in tandem. The mobile phase contains 5 M GuHCl in phosphate buffered saline (PBS). All samples were diluted to 1 mg/mL in PBS with 7 M GuHCl. The portion of aggregate in percent is determined from the total peak areas of the peaks eluted before the main peak, whereas the portion of half-molecule in percent is determined from the total peak areas of the peaks eluted after the main peak. The half-molecule are believed to represent inactive half-molecules.

[0138] Aggregate and half-molecule distribution of svActRIIB-Fc (E28W, S44T) with the various hinge linkers are set forth in the following table.

Hinge linker sequence	% aggregate	% half molecule
GGGGSVECPPC (SEQ ID NO: 27)	0.63	15.12
GGGGSERKCCVECPPC (SEQ ID NO: 38)	15.01	7.19
GGGGSGGGGSGPPC (SEQ ID NO: 40)	0.56	3.83
GGGGSGGGGSGPPG (SEQ ID NO: 42)	0.00	99.03
GGGGSERKCPPCVECPPC (SEQ ID NO: 44)	1.09	3.81

[0139] Thus certain linkers may improve manufacturability of the stabilized ActRIIB-Fc (E28W, S44T) according to these preliminary tests by reducing the percentage of inactive half-molecules produced.

[0140] The table below identifies the sequences as listed in the sequence listing.

SEQ ID NO	Description
1	ActRIIB extracellular domain, polynucleotide
2	ActRIIB extracellular domain, polypeptide
3	svActRIIB (E28W, S44T) polynucleotide with signal sequence
4	svActRIIB (E28W, S44T) polypeptide with signal sequence
5	svActRIIB (E28W, S44T) polynucleotide without signal sequence
6	svActRIIB (E28W, S44T) polypeptide without signal sequence
7	svActRIIB-Fc (E28W, S44T) polynucleotide with signal sequence
8	svActRIIB-Fc (E28W, S44T) polypeptide with signal sequence
9	svActRIIB-Fc (E28W, S44T) polynucleotide without signal sequence
10	svActRIIB-Fc (E28W, S44T) polypeptide without signal sequence
11	svActRIIB (E28Y, S44T) polynucleotide with signal sequence
12	svActRIIB (E28Y, S44T) polypeptide with signal sequence
13	svActRIIB (E28Y, S44T) polynucleotide without signal sequence
14	svActRIIB (E28Y, S44T) polypeptide without signal sequence
15	svActRIIB-Fc (E28Y, S44T) polynucleotide with signal sequence
16	svActRIIB-Fc (E28Y, S44T) polypeptide with signal sequence
17	svActRIIB-Fc (E28Y, S44T) polynucleotide without signal sequence
18	svActRIIB-Fc (E28Y, S44T) polypeptide without signal sequence

SEQ ID NO	Description
19	ActRIIB (E28W) polypeptide, without signal sequence
20	ActRIIB-Fc (E28W) polynucleotide, without signal sequence
21	ActRIIB-Fc (E28W) polypeptide, without signal sequence
22	IgG2Fc polypeptide sequence
23	IgG1Fc polypeptide sequence
24	IgG4 Fc polypeptide sequence
25	Linker amino acid sequence
26	Hinge linker #1 polynucleotide sequence
27	Hinge linker #1 peptide sequence
28	Hinge region IgG2
29	Hinge region IgG1
30	Hinge region IgG4
31	Alternative signal sequence, polypeptide
32	Signal sequence, polypeptide
33	Wild type ActRIIB accession NP 001097
34	Activin polypeptide sequence
35	Myostatin polypeptide sequence
36	GDF-11 polypeptide sequence
37	Hinge linker sequence #2 polynucleotide
38	Hinge linker sequence #2 peptide
39	Hinge linker sequence #3 polynucleotide
40	Hinge linker sequence #3 peptide
41	Hinge linker sequence #4 polynucleotide
42	Hinge linker sequence #4 peptide
43	Hinge linker sequence #5 polynucleotide
44	Hinge linker sequence #5 peptide
45	Hinge linker sequence #6 peptide
46	Hinge linker sequence #7 peptide
47	Modified IgG1 Fc polypeptide sequence
48	Hinge linker sequence #8 peptide
49	Hinge linker sequence #9 peptide
50	Hinge linker sequence #10 peptide

[0141] The present invention is not to be limited in scope by the specific embodiments described herein, which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

SEQUENCE LISTING

[0142]

<110> AMGEN INC.
SUN, Jeonghoon

TAM, Lei-Ting Tony
MICHAELS, Mark Leo
BOONE, Thomas C.
DESHPANDE, Rohini
LI, Yue-Sheng
HAN, HQ

<120> STABILIZED RECEPTOR POLYPEPTIDES AND USES THEREOF

<130> A-1450-WO-PCT

<140> to be assigned
<141> 2009-11-24

<150> 61/200,250
<151> 2008-11-26

<150> 61/259,060
<151> 2009-11-06

<160> 50

<170> PatentIn version 3.4

<210> 1
<211> 402
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(402)

<400> 1

```
atg acg gcg ccc tgg gtg gcc ctc gcc ctc ctc tgg gga tcg ctg tgc        48
Met Thr Ala Pro Trp Val Ala Leu Ala Leu Leu Trp Gly Ser Leu Cys
1               5                   10                  15

gcc ggc tct ggg cgt ggg gag gct gag aca cgg gag tgc atc tac tac        96
Ala Gly Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr
            20                  25                  30

aac gcc aac tgg gag ctg gag cgc acc aac cag agc ggc ctg gag cgc       144
Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg
        35                  40                  45

tgc gaa ggc gag cag gac aag cgg ctg cac tgc tac gcc tcc tgg cgc       192
Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg
    50                  55                  60

aac agc tct ggc acc atc gag ctc gtg aag aag ggc tgc tgg cta gat       240
Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp
65                  70                  75                  80

gac ttc aac tgc tac gat agg cag gag tgt gtg gcc act gag gag aac       288

Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn
                85                  90                  95

ccc cag gtg tac ttc tgc tgc tgt gaa ggc aac ttc tgc aac gag cgc       336
Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg
            100                 105                 110

ttc act cat ttg cca gag gct ggg ggc ccg gaa gtc acg tac gag cca       384
Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro
        115                 120                 125

ccc ccg aca gcc ccc acc                                               402
Pro Pro Thr Ala Pro Thr
    130
```

<210> 2
<211> 134
<212> PRT
<213> Homo sapiens

<400> 2

```
Met Thr Ala Pro Trp Val Ala Leu Ala Leu Leu Trp Gly Ser Leu Cys
1               5                   10                  15

Ala Gly Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr
            20                  25                  30

Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg
        35                  40                  45

Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg
    50                  55                  60

Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp
65                  70                  75                  80

Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn
                85                  90                  95

Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg
            100                 105                 110

Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro
        115                 120                 125

Pro Pro Thr Ala Pro Thr
    130
```

<210> 3
<211> 387
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(387)

<400> 3

```
atg gag ttt ggg ctg agc tgg gtt ttc ctc gtt gct ctt tta aga ggt      48

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
1               5                   10                  15

gtc cag tgt gag aca cgg tgg tgc atc tac tac aac gcc aac tgg gag      96
Val Gln Cys Glu Thr Arg Trp Cys Ile Tyr Tyr Asn Ala Asn Trp Glu
            20                  25                  30

ctg gag cgc acc aac cag acc ggc ctg gag cgc tgc gaa ggc gag cag     144
Leu Glu Arg Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln
        35                  40                  45

gac aag cgg ctg cac tgc tac gcc tcc tgg cgc aac agc tct ggc acc     192
Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr
    50                  55                  60

atc gag ctc gtg aag aag ggc tgc tgg cta gat gac ttc aac tgc tac     240
Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr
65                  70                  75                  80

gat agg cag gag tgt gtg gcc act gag gag aac ccc cag gtg tac ttc     288
Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe
                85                  90                  95

tgc tgc tgt gag ggc aac ttc tgc aac gag cgc ttc act cat ttg cca     336
Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro
            100                 105                 110

gag gct ggg ggc ccg gaa gtc acg tac gag cca ccc ccg aca gcc ccc     384
Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro
        115                 120                 125

acc                                                                 387
Thr
```

<210> 4
<211> 129
<212> PRT
<213> Homo sapiens

<400> 4

```
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
1               5                   10                  15

Val Gln Cys Glu Thr Arg Trp Cys Ile Tyr Tyr Asn Ala Asn Trp Glu
            20                  25                  30

Leu Glu Arg Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln
        35                  40                  45

Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr
    50                  55                  60

Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr
65                  70                  75                  80

Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe
                85                  90                  95

Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro
            100                 105                 110

Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro
        115                 120                 125

Thr
```

<210> 5
<211> 330
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(330)

<400> 5

```
gag aca cgg tgg tgc atc tac tac aac gcc aac tgg gag ctg gag cgc      48
Glu Thr Arg Trp Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1               5                   10                  15

acc aac cag acc ggc ctg gag cgc tgc gaa ggc gag cag gac aag cgg      96
Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
            20                  25                  30

ctg cac tgc tac gcc tcc tgg cgc aac agc tct ggc acc atc gag ctc     144
Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
        35                  40                  45

gtg aag aag ggc tgc tgg cta gat gac ttc aac tgc tac gat agg cag     192
Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln
    50                  55                  60

gag tgt gtg gcc act gag gag aac ccc cag gtg tac ttc tgc tgc tgt     240
Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65                  70                  75                  80

gag ggc aac ttc tgc aac gag cgc ttc act cat ttg cca gag gct ggg     288
Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
                85                  90                  95

ggc ccg gaa gtc acg tac gag cca ccc ccg aca gcc ccc acc             330
Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr
            100                 105                 110
```

<210> 6
<211> 110
<212> PRT
<213> Homo sapiens

<400> 6

```
Glu Thr Arg Trp Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1               5                   10                  15
```

```
Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
            20                  25                  30

Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
        35                  40                  45

Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln
    50                  55                  60

Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65                  70                  75                  80

Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
                85                  90                  95

Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr
            100                 105                 110
```

<210> 7
<211> 1071
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1071)

<400> 7

```
atg gag ttt ggg ctg agc tgg gtt ttc ctc gtt gct ctt tta aga ggt       48
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
1               5                   10                  15

gtc cag tgt gag aca cgg tgg tgc atc tac tac aac gcc aac tgg gag       96
Val Gln Cys Glu Thr Arg Trp Cys Ile Tyr Tyr Asn Ala Asn Trp Glu
            20                  25                  30

ctg gag cgc acc aac cag acc ggc ctg gag cgc tgc gaa ggc gag cag      144
Leu Glu Arg Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln
        35                  40                  45

gac aag cgg ctg cac tgc tac gcc tcc tgg cgc aac agc tct ggc acc      192
Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr
    50                  55                  60

atc gag ctc gtg aag aag ggc tgc tgg cta gat gac ttc aac tgc tac      240
Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr
65                  70                  75                  80

gat agg cag gag tgt gtg gcc act gag gag aac ccc cag gtg tac ttc      288
Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe
                85                  90                  95

tgc tgc tgt gag ggc aac ttc tgc aac gag cgc ttc act cat ttg cca      336
Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro
            100                 105                 110

gag gct ggg ggc ccg gaa gtc acg tac gag cca ccc ccg aca gcc ccc      384
Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro
        115                 120                 125
```

```
acc gga ggg gga gga tct gtc gag tgc cca ccg tgc cca gca cca cct        432

Thr Gly Gly Gly Gly Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro
    130                 135                 140

gtg gca gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc        480
Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
145                 150                 155                 160

ctc atg atc tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac gtg        528
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
                165                 170                 175

agc cac gaa gac ccc gag gtc cag ttc aac tgg tac gtg gac ggc gtg        576
Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
            180                 185                 190

gag gtg cat aat gcc aag aca aag cca cgg gag gag cag ttc aac agc        624
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
        195                 200                 205

acg ttc cgt gtg gtc agc gtc ctc acc gtt gtg cac cag gac tgg ctg        672
Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
    210                 215                 220

aac ggc aag gag tac aag tgc aag gtc tcc aac aaa ggc ctc cca gcc        720
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
225                 230                 235                 240

ccc atc gag aaa acc atc tcc aaa acc aaa ggg cag ccc cga gaa cca        768
Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
                245                 250                 255

cag gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag        816
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
            260                 265                 270

gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc        864
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
        275                 280                 285

gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc aca        912
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
    290                 295                 300

cct ccc atg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc        960
Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
305                 310                 315                 320

acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc       1008
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
                325                 330                 335

gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc       1056
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
            340                 345                 350

ctg tct ccg ggt aaa                                                   1071
Leu Ser Pro Gly Lys
        355
```

<210> 8

<211> 357

<212> PRT

<213> Homo sapiens

<400> 8

```
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
1               5                   10                  15

Val Gln Cys Glu Thr Arg Trp Cys Ile Tyr Tyr Asn Ala Asn Trp Glu
            20                  25                  30

Leu Glu Arg Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln
        35                  40                  45

Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr
    50                  55                  60

Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr
65                  70                  75                  80

Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe
                85                  90                  95

Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro
            100                 105                 110

Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro
        115                 120                 125

Thr Gly Gly Gly Gly Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro
    130                 135                 140

Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
145                 150                 155                 160

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
                165                 170                 175

Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
            180                 185                 190

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
        195                 200                 205

Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
    210                 215                 220

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
225                 230                 235                 240

Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
                245                 250                 255

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
            260                 265                 270

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
        275                 280                 285

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
    290                 295                 300

Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
305                 310                 315                 320

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
                325                 330                 335

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
            340                 345                 350

Leu Ser Pro Gly Lys
            355
```

<210> 9
<211> 1014
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1014)

<400> 9

gag aca cgg tgg tgc atc tac tac aac gcc aac tgg gag ctg gag cgc 48
Glu Thr Arg Trp Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1 5 10 15

acc aac cag acc ggc ctg gag cgc tgc gaa ggc gag cag gac aag cgg 96
Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
20 25 30

ctg cac tgc tac gcc tcc tgg cgc aac agc tct ggc acc atc gag ctc 144
Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
35 40 45

gtg aag aag ggc tgc tgg cta gat gac ttc aac tgc tac gat agg cag 192
Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln
50 55 60

gag tgt gtg gcc act gag gag aac ccc cag gtg tac ttc tgc tgc tgt 240
Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65 70 75 80

gag ggc aac ttc tgc aac gag cgc ttc act cat ttg cca gag gct ggg 288
Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
85 90 95

ggc ccg gaa gtc acg tac gag cca ccc ccg aca gcc ccc acc gga ggg 336
Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr Gly Gly
100 105 110

gga gga tct gtc gag tgc cca ccg tgc cca gca cca cct gtg gca gga 384
Gly Gly Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
115 120 125

ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc 432

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
130 135 140

tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac gtg agc cac gaa 480
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
145 150 155 160

gac ccc gag gtc cag ttc aac tgg tac gtg gac ggc gtg gag gtg cat 528
Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
165 170 175

aat gcc aag aca aag cca cgg gag gag cag ttc aac agc acg ttc cgt 576
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
180 185 190

gtg gtc agc gtc ctc acc gtt gtg cac cag gac tgg ctg aac ggc aag 624
Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
195 200 205

gag tac aag tgc aag gtc tcc aac aaa ggc ctc cca gcc ccc atc gag 672
Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
210 215 220

aaa acc atc tcc aaa acc aaa ggg cag ccc cga gaa cca cag gtg tac 720
Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
225 230 235 240

acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg 768
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
245 250 255

acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg 816
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
260 265 270

gag agc aat ggg cag ccg gag aac aac tac aag acc aca cct ccc atg 864
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
275 280 285

ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac 912
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
290 295 300

aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat 960
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
305 310 315 320

gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg 1008
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
325 330 335

ggt aaa 1014
Gly Lys

<210> 10
<211> 338
<212> PRT

<213> Homo sapiens

<400> 10

```
Glu Thr Arg Trp Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1               5                   10                  15

Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
            20                  25                  30

Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
        35                  40                  45

Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln
    50                  55                  60

Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65                  70                  75                  80

Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
                85                  90                  95

Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr Gly Gly
            100                 105                 110

Gly Gly Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
        115                 120                 125

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
    130                 135                 140

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
145                 150                 155                 160

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
                165                 170                 175

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
            180                 185                 190

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
        195                 200                 205

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
    210                 215                 220

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
225                 230                 235                 240

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
                245                 250                 255

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
            260                 265                 270

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
        275                 280                 285

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
    290                 295                 300

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
305                 310                 315                 320

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
                325                 330                 335

Gly Lys
```

<210> 11
<211> 387
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(387)

<400> 11

```
atg gag ttt ggg ctg agc tgg gtt ttc ctc gtt gct ctt tta aga ggt      48
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
1               5                   10                  15

gtc cag tgt gag aca cgg tac tgc atc tac tac aac gcc aac tgg gag      96
Val Gln Cys Glu Thr Arg Tyr Cys Ile Tyr Tyr Asn Ala Asn Trp Glu
            20                  25                  30

ctg gag cgc acc aac cag acc ggc ctg gag cgc tgc gaa ggc gag cag      144
Leu Glu Arg Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln
        35                  40                  45

gac aag cgg ctg cac tgc tac gcc tcc tgg cgc aac agc tct ggc acc      192
Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr
    50                  55                  60

atc gag ctc gtg aag aag ggc tgc tgg cta gat gac ttc aac tgc tac      240
Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr
65                  70                  75                  80

gat agg cag gag tgt gtg gcc act gag gag aac ccc cag gtg tac ttc      288
Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe
                85                  90                  95

tgc tgc tgt gag ggc aac ttc tgc aac gag cgc ttc act cat ttg cca      336
Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro
            100                 105                 110

gag gct ggg ggc ccg gaa gtc acg tac gag cca ccc ccg aca gcc ccc      384
Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro
        115                 120                 125

acc                                                                  387
Thr
```

<210> 12
<211> 129
<212> PRT
<213> Homo sapiens

<400> 12

```
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
1               5                   10                  15

Val Gln Cys Glu Thr Arg Tyr Cys Ile Tyr Tyr Asn Ala Asn Trp Glu
            20                  25                  30

Leu Glu Arg Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln
        35                  40                  45

Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr
    50                  55                  60

Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr
65                  70                  75                  80

Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe
                85                  90                  95

Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro
            100                 105                 110

Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro
        115                 120                 125

Thr
```

<210> 13
<211> 330
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(330)

<400> 13

gag aca cgg tac tgc atc tac tac aac gcc aac tgg gag ctg gag cgc 48
Glu Thr Arg Tyr Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1 5 10 15

acc aac cag acc ggc ctg gag cgc tgc gaa ggc gag cag gac aag cgg 96
Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
20 25 30

ctg cac tgc tac gcc tcc tgg cgc aac agc tct ggc acc atc gag ctc 144
Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
35 40 45

gtg aag aag ggc tgc tgg cta gat gac ttc aac tgc tac gat agg cag 192
Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln
50 55 60

gag tgt gtg gcc act gag gag aac ccc cag gtg tac ttc tgc tgc tgt 240
Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65 70 75 80

gag ggc aac ttc tgc aac gag cgc ttc act cat ttg cca gag gct ggg 288

Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
85 90 95

ggc ccg gaa gtc acg tac gag cca ccc ccg aca gcc ccc acc 330
Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr
100 105 110

<210> 14
<211> 110
<212> PRT
<213> Homo sapiens

<400> 14
Glu Thr Arg Tyr Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1 5 10 15

Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
20 25 30

Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
35 40 45

Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln
50 55 60

Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65 70 75 80

Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
85 90 95

Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr
100 105 110

<210> 15
<211> 1071
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1071)

<400> 15
atg gag ttt ggg ctg agc tgg gtt ttc ctc gtt gct ctt tta aga ggt 48
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
1 5 10 15

gtc cag tgt gag aca cgg tac tgc atc tac tac aac gcc aac tgg gag 96
Val Gln Cys Glu Thr Arg Tyr Cys Ile Tyr Tyr Asn Ala Asn Trp Glu
20 25 30

ctg gag cgc acc aac cag acc ggc ctg gag cgc tgc gaa ggc gag cag 144
Leu Glu Arg Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln
35 40 45

gac aag cgg ctg cac tgc tac gcc tcc tgg cgc aac agc tct ggc acc 192

```
Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr
    50                  55                  60

atc gag ctc gtg aag aag ggc tgc tgg cta gat gac ttc aac tgc tac      240
Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr
65                  70                  75                  80

gat agg cag gag tgt gtg gcc act gag gag aac ccc cag gtg tac ttc      288
Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe
                85                  90                  95

tgc tgc tgt gag ggc aac ttc tgc aac gag cgc ttc act cat ttg cca      336
Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro
            100                 105                 110

gag gct ggg ggc ccg gaa gtc acg tac gag cca ccc ccg aca gcc ccc      384
Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro
        115                 120                 125

acc gga ggg gga gga tct gtc gag tgc cca ccg tgc cca gca cca cct      432
Thr Gly Gly Gly Gly Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro
    130                 135                 140

gtg gca gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc      480
Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
145                 150                 155                 160

ctc atg atc tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac gtg      528
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
                165                 170                 175

agc cac gaa gac ccc gag gtc cag ttc aac tgg tac gtg gac ggc gtg      576
Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
            180                 185                 190

gag gtg cat aat gcc aag aca aag cca cgg gag gag cag ttc aac agc      624
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
        195                 200                 205

acg ttc cgt gtg gtc agc gtc ctc acc gtt gtg cac cag gac tgg ctg      672
Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
    210                 215                 220

aac ggc aag gag tac aag tgc aag gtc tcc aac aaa ggc ctc cca gcc      720
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
225                 230                 235                 240

ccc atc gag aaa acc atc tcc aaa acc aaa ggg cag ccc cga gaa cca      768
Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
                245                 250                 255

cag gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag      816
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
            260                 265                 270

gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc      864
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
        275                 280                 285

gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc aca      912

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
    290                 295                 300

cct ccc atg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc      960
Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
305                 310                 315                 320

acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc      1008
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
                325                 330                 335

gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc      1056
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
            340                 345                 350

ctg tct ccg ggt aaa                                                  1071
Leu Ser Pro Gly Lys
        355
```

<210> 16

<211> 357

<212> PRT

<213> Homo sapiens

<400> 16

```
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
1               5                   10                  15

Val Gln Cys Glu Thr Arg Tyr Cys Ile Tyr Tyr Asn Ala Asn Trp Glu
            20                  25                  30

Leu Glu Arg Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln
        35                  40                  45

Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr
    50                  55                  60

Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr
65                  70                  75                  80

Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe
                85                  90                  95

Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro
            100                 105                 110

Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro
        115                 120                 125

Thr Gly Gly Gly Gly Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro
    130                 135                 140

Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
145                 150                 155                 160

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
                165                 170                 175

Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
            180                 185                 190

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
        195                 200                 205

Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
    210                 215                 220

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
225                 230                 235                 240

Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
                245                 250                 255

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
            260                 265                 270

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
        275                 280                 285

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
    290                 295                 300

Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
305                 310                 315                 320

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
                325                 330                 335

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
            340                 345                 350

Leu Ser Pro Gly Lys
        355
```

<210> 17
<211> 1014
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1014)

<400> 17

```
gag aca cgg tac tgc atc tac tac aac gcc aac tgg gag ctg gag cgc       48
Glu Thr Arg Tyr Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1               5                   10                  15

acc aac cag acc ggc ctg gag cgc tgc gaa ggc gag cag gac aag cgg       96
Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
            20                  25                  30

ctg cac tgc tac gcc tcc tgg cgc aac agc tct ggc acc atc gag ctc      144
Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
        35                  40                  45

gtg aag aag ggc tgc tgg cta gat gac ttc aac tgc tac gat agg cag      192

Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln
    50                  55                  60

gag tgt gtg gcc act gag gag aac ccc cag gtg tac ttc tgc tgc tgt      240
Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65                  70                  75                  80

gag ggc aac ttc tgc aac gag cgc ttc act cat ttg cca gag gct ggg      288
Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
                85                  90                  95

ggc ccg gaa gtc acg tac gag cca ccc ccg aca gcc ccc acc gga ggg      336
Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr Gly Gly
            100                 105                 110

gga gga tct gtc gag tgc cca ccg tgc cca gca cca cct gtg gca gga      384
Gly Gly Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
        115                 120                 125

ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc      432
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
    130                 135                 140

tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac gtg agc cac gaa      480
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
145                 150                 155                 160

gac ccc gag gtc cag ttc aac tgg tac gtg gac ggc gtg gag gtg cat      528
Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
                165                 170                 175

aat gcc aag aca aag cca cgg gag gag cag ttc aac agc acg ttc cgt      576
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
            180                 185                 190

gtg gtc agc gtc ctc acc gtt gtg cac cag gac tgg ctg aac ggc aag      624
Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
        195                 200                 205

gag tac aag tgc aag gtc tcc aac aaa ggc ctc cca gcc ccc atc gag      672
Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
    210                 215                 220

aaa acc atc tcc aaa acc aaa ggg cag ccc cga gaa cca cag gtg tac      720
Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
225                 230                 235                 240

acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg      768
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
                245                 250                 255

acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg      816
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
            260                 265                 270

gag agc aat ggg cag ccg gag aac aac tac aag acc aca cct ccc atg      864
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
        275                 280                 285

ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac      912

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
    290                 295                 300

aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat      960
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
305                 310                 315                 320

gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg     1008
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
                325                 330                 335

ggt aaa                                                             1014
Gly Lys
```

<210> 18

<211> 338

<212> PRT

<213> Homo sapiens

<400> 18

```
Glu Thr Arg Tyr Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1               5                   10                  15

Thr Asn Gln Thr Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
            20                  25                  30

Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
        35                  40                  45

Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln
    50                  55                  60

Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65                  70                  75                  80

Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
                85                  90                  95

Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr Gly Gly
            100                 105                 110

Gly Gly Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
        115                 120                 125

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
    130                 135                 140

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
145                 150                 155                 160

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
                165                 170                 175

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
            180                 185                 190

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
        195                 200                 205

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
    210                 215                 220

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
225                 230                 235                 240

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
                245                 250                 255

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
            260                 265                 270

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
        275                 280                 285

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
    290                 295                 300

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
305                 310                 315                 320

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
                325                 330                 335

Gly Lys
```

<210> 19
<211> 110
<212> PRT
<213> Homo sapiens

<400> 19

Glu Thr Arg Trp Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1   5   10   15

Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
            20   25   30

Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
        35   40   45

Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln
    50   55   60

Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65   70   75   80

Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
                85   90   95

Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr
            100   105   110

<210> 20
<211> 1014
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1014)

<400> 20
gag aca cgg tgg tgc atc tac tac aac gcc aac tgg gag ctg gag cgc   48
Glu Thr Arg Trp Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1   5   10   15

acc aac cag agc ggc ctg gag cgc tgc gaa ggc gag cag gac aag cgg   96
Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
            20   25   30

ctg cac tgc tac gcc tcc tgg cgc aac agc tct ggc acc atc gag ctc   144
Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
        35   40   45

gtg aag aag ggc tgc tgg cta gat gac ttc aac tgc tac gat agg cag   192
Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln
    50   55   60

gag tgt gtg gcc act gag gag aac ccc cag gtg tac ttc tgc tgc tgt   240
Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65   70   75   80

gag ggc aac ttc tgc aac gag cgc ttc act cat ttg cca gag gct ggg   288
Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
                85   90   95

ggc ccg gaa gtc acg tac gag cca ccc ccg aca gcc ccc acc gga gga   336
Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr Gly Gly
            100   105   110

gga gga tct gtc gag tgc cca ccg tgc cca gca cca cct gtg gca gga   384
Gly Gly Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
        115   120   125

ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc   432
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
    130   135   140

tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac gtg agc cac gaa   480
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
145   150   155   160

gac ccc gag gtc cag ttc aac tgg tac gtg gac ggc gtg gag gtg cat   528
Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
                165   170   175

aat gcc aag aca aag cca cgg gag gag cag ttc aac agc acg ttc cgt   576
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
            180   185   190

gtg gtc agc gtc ctc acc gtt gtg cac cag gac tgg ctg aac ggc aag   624
Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
        195   200   205

gag tac aag tgc aag gtc tcc aac aaa ggc ctc cca gcc ccc atc gag   672

```
Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
    210                 215                 220

aaa acc atc tcc aaa acc aaa ggg cag ccc cga gaa cca cag gtg tac      720
Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
225                 230                 235                 240

acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg      768
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
                245                 250                 255

acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg      816
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
            260                 265                 270

gag agc aat ggg cag ccg gag aac aac tac aag acc aca cct ccc atg      864
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
        275                 280                 285

ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac      912
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
    290                 295                 300

aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat      960
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
305                 310                 315                 320

gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg     1008
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
                325                 330                 335

ggt aaa                                                             1014
Gly Lys
```

<210> 21

<211> 338

<212> PRT

<213> Homo sapiens

<400> 21

```
Glu Thr Arg Trp Cys Ile Tyr Tyr Asn Ala Asn Trp Glu Leu Glu Arg
1               5                   10                  15

Thr Asn Gln Ser Gly Leu Glu Arg Cys Glu Gly Glu Gln Asp Lys Arg
            20                  25                  30

Leu His Cys Tyr Ala Ser Trp Arg Asn Ser Ser Gly Thr Ile Glu Leu
        35                  40                  45

Val Lys Lys Gly Cys Trp Leu Asp Asp Phe Asn Cys Tyr Asp Arg Gln
    50                  55                  60

Glu Cys Val Ala Thr Glu Glu Asn Pro Gln Val Tyr Phe Cys Cys Cys
65                  70                  75                  80

Glu Gly Asn Phe Cys Asn Glu Arg Phe Thr His Leu Pro Glu Ala Gly
                85                  90                  95
```

```
Gly Pro Glu Val Thr Tyr Glu Pro Pro Pro Thr Ala Pro Thr Gly Gly
            100                 105                 110

Gly Gly Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
        115                 120                 125

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
    130                 135                 140

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
145                 150                 155                 160

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
                165                 170                 175

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
            180                 185                 190

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
        195                 200                 205

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
    210                 215                 220

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
225                 230                 235                 240

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
                245                 250                 255

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
            260                 265                 270

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
        275                 280                 285

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
    290                 295                 300

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
305                 310                 315                 320

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
                325                 330                 335

Gly Lys
```

<210> 22
<211> 216
<212> PRT
<213> Homo sapiens

<400> 22

```
Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
1               5                   10                  15

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
            20                  25                  30
```

Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
35 40 45

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
50 55 60

Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln
65 70 75 80

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
85 90 95

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro
100 105 110

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
115 120 125

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
130 135 140

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
145 150 155 160

Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
165 170 175

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
180 185 190

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
195 200 205

Ser Leu Ser Leu Ser Pro Gly Lys
210 215

<210> 23
<211> 217
<212> PRT
<213 Homo sapiens

<400> 23
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
1 5 10 15

Pro Lys Asp Ile Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
20 25 30

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
35 40 45

Val Gly Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
50 55 60

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65 70 75 80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
85 90 95

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
100 105 110

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
115 120 125

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
130 135 140

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
145 150 155 160

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
165 170 175

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
180 185 190

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
195 200 205

Lys Ser Leu Ser Leu Ser Pro Gly Lys
210 215

<210> 24
<211> 217
<212> PRT

<213> Homo sapiens

<400> 24

```
Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
1               5                   10                  15

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            20                  25                  30

Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
        35                  40                  45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    50                  55                  60

Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65                  70                  75                  80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                85                  90                  95

Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
            100                 105                 110

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met
        115                 120                 125

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    130                 135                 140

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
145                 150                 155                 160

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                165                 170                 175

Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val
            180                 185                 190

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
        195                 200                 205

Lys Ser Leu Ser Leu Ser Leu Gly Lys
    210                 215
```

<210> 25
<211> 5
<212> PRT
<213 Artificial

<220>
<223> Linker

<400> 25

```
Gly Gly Gly Gly Ser
1               5
```

<210> 26
<211> 36
<212> DNA
<213> Artificial

<220>
<223> Hinge linker

<220>
<221> CDS
<222> (1)..(36)

<400> 26

```
gga ggg gga gga tct gtc gag tgc cca ccg tgc cca                       36
Gly Gly Gly Gly Ser Val Glu Cys Pro Pro Cys Pro
1               5                   10
```

<210> 27
<211> 12
<212> PRT

<213> Artificial

<220>
<223> Hinge linker

<400> 27
Gly Gly Gly Gly Ser Val Glu Cys Pro Pro Cys Pro
1 5 10

<210> 28
<211> 12
<212> PRT
<213> Homo sapiens

<400> 28
Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro
1 5 10

<210> 29
<211> 15
<212> PRT
<213> Homo sapiens

<400> 29
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10 15

<210> 30
<211> 12
<212> PRT
<213> Homo sapiens

<400> 30
Glu Ser Lys Thr Gly Pro Pro Cys Pro Ser Cys Pro
1 5 10

<210> 31
<211> 18
<212> PRT
<213> Homo sapiens

<400> 31
Met Thr Ala Pro Trp Val Ala Leu Ala Leu Leu Trp Gly Ser Leu Trp
1 5 10 15

Pro Gly

<210> 32
<211> 18
<212> PRT
<213> Homo sapiens

<400> 32
Met Thr Ala Pro Trp Val Ala Leu Ala Leu Leu Trp Gly Ser Leu Cys
1 5 10 15

Ala Gly

<210> 33
<211> 512
<212> PRT
<213> Homo sapiens

<400> 33

```
Met Thr Ala Pro Trp Val Ala Leu Ala Leu Leu Trp Gly Ser Leu Cys
1               5                   10                  15

Ala Gly Ser Gly Arg Gly Glu Ala Glu Thr Arg Glu Cys Ile Tyr Tyr
            20                  25                  30
Asn Ala Asn Trp Glu Leu Glu Arg Thr Asn Gln Ser Gly Leu Glu Arg
        35                  40                  45

Cys Glu Gly Glu Gln Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg
    50                  55                  60

Asn Ser Ser Gly Thr Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp
65                  70                  75                  80

Asp Phe Asn Cys Tyr Asp Arg Gln Glu Cys Val Ala Thr Glu Glu Asn
                85                  90                  95

Pro Gln Val Tyr Phe Cys Cys Cys Glu Gly Asn Phe Cys Asn Glu Arg
            100                 105                 110

Phe Thr His Leu Pro Glu Ala Gly Gly Pro Glu Val Thr Tyr Glu Pro
        115                 120                 125

Pro Pro Thr Ala Pro Thr Leu Leu Thr Val Leu Ala Tyr Ser Leu Leu
    130                 135                 140

Pro Ile Gly Gly Leu Ser Leu Ile Val Leu Leu Ala Phe Trp Met Tyr
145                 150                 155                 160

Arg His Arg Lys Pro Pro Tyr Gly His Val Asp Ile His Glu Asp Pro
                165                 170                 175

Gly Pro Pro Pro Pro Ser Pro Leu Val Gly Leu Lys Pro Leu Gln Leu
            180                 185                 190

Leu Glu Ile Lys Ala Arg Gly Arg Phe Gly Cys Val Trp Lys Ala Gln
        195                 200                 205

Leu Met Asn Asp Phe Val Ala Val Lys Ile Phe Pro Leu Gln Asp Lys
    210                 215                 220

Gln Ser Trp Gln Ser Glu Arg Glu Ile Phe Ser Thr Pro Gly Met Lys
225                 230                 235                 240

His Glu Asn Leu Leu Gln Phe Ile Ala Ala Glu Lys Arg Gly Ser Asn
                245                 250                 255
```

```
Leu Glu Val Glu Leu Trp Leu Ile Thr Ala Phe His Asp Lys Gly Ser
            260                 265                 270

Leu Thr Asp Tyr Leu Lys Gly Asn Ile Ile Thr Trp Asn Glu Leu Cys
        275                 280                 285

His Val Ala Glu Thr Met Ser Arg Gly Leu Ser Tyr Leu His Glu Asp
    290                 295                 300

Val Pro Trp Cys Arg Gly Glu Gly His Lys Pro Ser Ile Ala His Arg
305                 310                 315                 320

Asp Phe Lys Ser Lys Asn Val Leu Leu Lys Ser Asp Leu Thr Ala Val
                325                 330                 335

Leu Ala Asp Phe Gly Leu Ala Val Arg Phe Glu Pro Gly Lys Pro Pro
            340                 345                 350

Gly Asp Thr His Gly Gln Val Gly Thr Arg Arg Tyr Met Ala Pro Glu
        355                 360                 365

Val Leu Glu Gly Ala Ile Asn Phe Gln Arg Asp Ala Phe Leu Arg Ile
    370                 375                 380

Asp Met Tyr Ala Met Gly Leu Val Leu Trp Glu Leu Val Ser Arg Cys
385                 390                 395                 400

Lys Ala Ala Asp Gly Pro Val Asp Glu Tyr Met Leu Pro Phe Glu Glu
                405                 410                 415

Glu Ile Gly Gln His Pro Ser Leu Glu Glu Leu Gln Glu Val Val Val
            420                 425                 430

His Lys Lys Met Arg Pro Thr Ile Lys Asp His Trp Leu Lys His Pro
        435                 440                 445

Gly Leu Ala Gln Leu Cys Val Thr Ile Glu Glu Cys Trp Asp His Asp
    450                 455                 460

Ala Glu Ala Arg Leu Ser Ala Gly Cys Val Glu Glu Arg Val Ser Leu
465                 470                 475                 480

Ile Arg Arg Ser Val Asn Gly Thr Thr Ser Asp Cys Leu Val Ser Leu
                485                 490                 495

Val Thr Ser Val Thr Asn Val Asp Leu Pro Pro Lys Glu Ser Ser Ile
            500                 505                 510
```

<210> 34
<211> 426
<212> PRT
<213> Homo sapiens

<400> 34

```
Met Pro Leu Leu Trp Leu Arg Gly Phe Leu Leu Ala Ser Cys Trp Ile
1               5                   10                  15

Ile Val Arg Ser Ser Pro Thr Pro Gly Ser Glu Gly His Ser Ala Ala
            20                  25                  30
```

```
Pro Asp Cys Pro Ser Cys Ala Leu Ala Ala Leu Pro Lys Asp Val Pro
        35                  40                  45

Asn Ser Gln Pro Glu Met Val Glu Ala Val Lys Lys His Ile Leu Asn
    50                  55                  60

Met Leu His Leu Lys Lys Arg Pro Asp Val Thr Gln Pro Val Pro Lys
65                  70                  75                  80

Ala Ala Leu Leu Asn Ala Ile Arg Lys Leu His Val Gly Lys Val Gly
                85                  90                  95

Glu Asn Gly Tyr Val Glu Ile Glu Asp Asp Ile Gly Arg Arg Ala Glu
            100                 105                 110

Met Asn Glu Leu Met Glu Gln Thr Ser Glu Ile Ile Thr Phe Ala Glu
        115                 120                 125

Ser Gly Thr Ala Arg Lys Thr Leu His Phe Glu Ile Ser Lys Glu Gly
    130                 135                 140

Ser Asp Leu Ser Val Val Glu Arg Ala Glu Val Trp Leu Phe Leu Lys
145                 150                 155                 160

Val Pro Lys Ala Asn Arg Thr Arg Thr Lys Val Thr Ile Arg Leu Phe
                165                 170                 175

Gln Gln Gln Lys His Pro Gln Gly Ser Leu Asp Thr Gly Glu Glu Ala
            180                 185                 190

Glu Glu Val Gly Leu Lys Gly Glu Arg Ser Glu Leu Leu Leu Ser Glu
        195                 200                 205

Lys Val Val Asp Ala Arg Lys Ser Thr Trp His Val Phe Pro Val Ser
    210                 215                 220

Ser Ser Ile Gln Arg Leu Leu Asp Gln Gly Lys Ser Ser Leu Asp Val
225                 230                 235                 240

Arg Ile Ala Cys Glu Gln Cys Gln Glu Ser Gly Ala Ser Leu Val Leu
                245                 250                 255

Leu Gly Lys Lys Lys Lys Lys Glu Glu Glu Gly Glu Gly Lys Lys Lys
            260                 265                 270

Gly Gly Gly Glu Gly Gly Ala Gly Ala Asp Glu Glu Lys Glu Gln Ser
        275                 280                 285

His Arg Pro Phe Leu Met Leu Gln Ala Arg Gln Ser Glu Asp His Pro
    290                 295                 300

His Arg Arg Arg Arg Arg Gly Leu Glu Cys Asp Gly Lys Val Asn Ile
305                 310                 315                 320

Cys Cys Lys Lys Gln Phe Phe Val Ser Phe Lys Asp Ile Gly Trp Asn
                325                 330                 335

Asp Trp Ile Ile Ala Pro Ser Gly Tyr His Ala Asn Tyr Cys Glu Gly
            340                 345                 350

Glu Cys Pro Ser His Ile Ala Gly Thr Ser Gly Ser Ser Leu Ser Phe
        355                 360                 365

His Ser Thr Val Ile Asn His Tyr Arg Met Arg Gly His Ser Pro Phe
    370                 375                 380

Ala Asn Leu Lys Ser Cys Cys Val Pro Thr Lys Leu Arg Pro Met Ser
385                 390                 395                 400

Met Leu Tyr Tyr Asp Asp Gly Gln Asn Ile Ile Lys Lys Asp Ile Gln
                405                 410                 415

Asn Met Ile Val Glu Glu Cys Gly Cys Ser
            420                 425
```

<210> 35
<211> 375
<212> PRT
<213> Homo sapiens

<400> 35

```
Met Gln Lys Leu Gln Leu Cys Val Tyr Ile Tyr Leu Phe Met Leu Ile
1               5                   10                  15

Val Ala Gly Pro Val Asp Leu Asn Glu Asn Ser Glu Gln Lys Glu Asn
            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

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
            340                 345                 350

Phe Asn Gly Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val
        355                 360                 365

Val Asp Arg Cys Gly Cys Ser
    370                 375
```

<210> 36
<211> 217
<212> PRT
<213> Homo sapiens

<400> 36

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
1 5 10 15

Pro Lys Asp Ile Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
20 25 30

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
35 40 45

Val Gly Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
50 55 60

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65 70 75 80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
85 90 95

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
100 105 110

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
115 120 125

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
130 135 140

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
145 150 155 160

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
165 170 175

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
180 185 190

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
195 200 205

Lys Ser Leu Ser Leu Ser Pro Gly Lys
210 215

<210> 37
<211> 48
<212> DNA
<213> Artificial

<220>
<223> Hinge linker

<220>
<221> CDS
<222> (1)..(48)

<400> 37
gga ggg gga gga tct gag cgc aaa tgt tgt gtc gag tgc cca ccg tgc 48
Gly Gly Gly Gly Ser Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys
1 5 10 15

<210> 38
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Hinge linker

<400> 38
Gly Gly Gly Gly Ser Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys
1 5 10 15

<210> 39
<211> 42
<212> DNA
<213> Artificial

<220>

<223> Hinge linker

<220>
<221> CDS
<222> (1)..(42)

<400> 39
```
gga ggg gga gga tct ggt gga ggt ggt tca ggt cca ccg tgc                42
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Pro Pro Cys
1               5                   10
```

<210> 40
<211> 14
<212> PRT
<213 > Artificial

<220>
<223> Hinge linker

<400> 40
```
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Pro Pro Cys
1               5                   10
```

<210> 41
<211> 42
<212> DNA
<213> Artificial

<220>
<223> Hinge linker

<220>
<221> CDS
<222> (1)..(42)

<400> 41
```
gga ggg gga gga tct ggt gga ggt ggt tca ggt cca ccg gga                42
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Pro Pro Gly
1               5                   10
```

<210> 42
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Hinge linker

<400> 42
```
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Pro Pro Gly
1               5                   10
```

<210> 43
<211> 54
<212> DNA
<213> Artificial

<220>
<223> Hinge linker

<220>
<221> CDS
<222> (1)..(54)

<400> 43

```
gga ggg gga gga tct gag cgc aaa tgt cca cct tgt gtc gag tgc cca       48
Gly Gly Gly Gly Ser Glu Arg Lys Cys Pro Pro Cys Val Glu Cys Pro
1               5                   10                  15

ccg tgc                                                               54
Pro Cys
```

<210> 44
<211> 18
<212> PRT
<213> Artificial

<220>
<223> Hinge linker

<400> 44

```
Gly Gly Gly Gly Ser Glu Arg Lys Cys Pro Pro Cys Val Glu Cys Pro
1               5                   10                  15

Pro Cys
```

<210> 45
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Hinge linker

<400> 45

```
Gly Pro Ala Ser Gly Gly Pro Ala Ser Gly Pro Pro Cys Pro
1               5                   10
```

<210> 46
<211> 21
<212> PRT
<213> Artificial

<220>
<223> Hinge linker

<400> 46

```
Gly Pro Ala Ser Gly Gly Pro Ala Ser Gly Cys Pro Pro Cys Val Glu
1               5                   10                  15

Cys Pro Pro Cys Pro
            20
```

<210> 47
<211> 217
<212> PRT
<213> Homo sapiens

<400> 47

```
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
1               5                   10                  15

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            20                  25                  30

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
        35                  40                  45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    50                  55                  60

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65                  70                  75                  80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                85                  90                  95

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
            100                 105                 110

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
        115                 120                 125

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    130                 135                 140

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
145                 150                 155                 160

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                165                 170                 175

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
            180                 185                 190

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
        195                 200                 205

Lys Ser Leu Ser Leu Ser Pro Gly Lys
    210                 215
```

<210> 48
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Hinge linker

<400> 48

```
Gly Gly Gly Gly Ser Val Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1               5                   10                  15
```

<210> 49
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Hinge linker

<400> 49

```
Gly Gly Gly Gly Ser Val Asp Lys Thr His Thr Gly Pro Pro Cys Pro
1               5                   10                  15
```

<210> 50
<211> 21
<212> PRT
<213> Artificial

<220>
<223> Hinge linker

<400> 50

```
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Val Asp Lys Thr His Thr
1               5                   10                  15

Gly Pro Pro Cys Pro
            20
```

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US61200250A [0001]
- US61259060A [0001]
- WO2008109167A [0009]
- WO2008097541A [0009]
- WO0029581A [0046]
- WO90143363A [0062] [0105]
- US94001875W [0076]
- US9300829W [0081]
- US3773919A [0081]
- EP58481A [0081]
- EP133988A [0081]
- EP36676A [0081]
- EP88046A [0081]
- EP143949A [0081]
- US590962A [0104]
- US20070117130A [0104]
- WO61200250A [0142]
- WO61259060A [0142]

Non-patent literature cited in the description

- **MCPHERRON et al.**Nature, 1997, vol. 387, 83-90 [0004]
- **ZIMMERS et al.**Science, 2002, vol. 296, 1486-1488 [0004]
- **OH et al.**Genes Dev, 1997, vol. 11, 1812-26 [0005]
- **VALE et al.**Nature, 1986, vol. 321, 776-779 [0006]
- **LING et al.**Nature, 1986, vol. 321, 779-782 [0006]
- **HARRISON et al.**J. Biol. Chem., 2004, vol. 279, 28036-28044 [0007]
- **LEE et al.**PNAS USA, 2001, vol. 98, 9306-11 [0007]
- **OH et al.**Genes Dev, 2002, vol. 16, 2749-54 [0007]
- **KINGSLEY et al.**Genes Dev., 1994, vol. 8, 133-146 [0031]
- **MCPHERRON et al.**Growth factors and cytokines in health and diseaseJAI Press Inc.vol. 1B, 357-393 [0031]
- **MCPHERRON et al.**PNAS USA, 1997, vol. 94, 12457-12461 [0032]
- **ZIMMERS et al.**Science, 2002, vol. 296, 1486- [0032]
- **LEE et al.**PNAS, 2001, vol. 98, 9306- [0033]
- **MCPHERRON et al.**Nature Genet., 1999, vol. 22, 93260-264 [0034]
- **GAMER et al.**Dev. Biol., 1999, vol. 208, 1222-232 [0034]

- **RASMUSSEN et al.**Cytotechnology, 1998, vol. 28, 31- [0063]
- **URLAUB et al.**Proc. Natl. Acad. Sci. USA, 1980, vol. 77, 4216-20 [0063]
- **GLUZMAN et al.**Cell, 1981, vol. 23, 175- [0063]
- **MCMAHAN et al.**EMBO J., 1991, vol. 10, 2821- [0063]
- Methods of EnzymologyAcademic Press19900000vol. 185, [0064]
- **STEWARTYOUNG**Solid Phase Peptide SynthesisPierce Chemical Co.19840000 [0066]
- **TAM et al.**J Am Chem Soc, 1983, vol. 105, 6442- [0066]
- **MERRIFIELD**Science, 1986, vol. 232, 341-347 [0066]
- **BARANYMERRIFIELD**The PeptidesAcademic Press1-284 [0066]
- **BARANY et al.**Int J Pep Protein Res, 1987, vol. 30, 705-739 [0066]
- Remington's Pharmaceutical SciencesMack Publishing Company19900000 [0072]
- **SIDMAN et al.**Biopolymers, 1983, vol. 22, 547-556 [0081]
- **LANGER et al.**J. Biomed. Mater. Res., 1981, vol. 15, 167-277 [0081]
- **LANGER et al.**Chem. Tech., 1982, vol. 12, 98-105 [0081]
- **EPPSTEIN et al.**PNAS, 1985, vol. 82, 3688- [0081]
- **GONZALEZ-CADAVID et al.**PNAS USA, 1998, vol. 95, 14938-14943 [0095]
- **LANG et al.**FASEB J, 2001, vol. 15, 1807-1809 [0095]
- **ZACHWIEJA et al.**J Gravit Physiol., 1999, vol. 6, 211- [0095]
- **LALANI et al.**J.Endocrin, 2000, vol. 167, 3417-28 [0095]
- **YARASHESKI et al.**J Nutr Aging, 2002, vol. 6, 5343-8 [0096]
- **SHARMA et al.**J Cell Physiol., 1999, vol. 180, 1-9 [0096]
- **YEN et al.**FASEB J., 1994, vol. 8, 479- [0097]
- **HAMRICK et al.**Calcif Tissue Int, 2002, vol. 71, 163-8 [0098]
- **CIPRANO et al.**Endocrinol, 2000, vol. 141, 72319-27 [0100]
- **SHOU et al.**Endocrinol, 1997, vol. 138, 115000-5 [0100]
- **COERVER et al.**Mol Endocrinol, 1996, vol. 10, 5534-43 [0100]
- **ITO et al.**British J Cancer, 2000, vol. 82, 81415-20 [0100]
- **LAMBERT-MESSERLIAN et al.**Gynecologic Oncology, 1999, vol. 74, 93-7 [0100]
- Methods in Cell BiologyAntibodies in Cell BiologyAcademic Press, Inc.19930000vol. 37, [0101]
- **AKERSTROM**J Immunol, 1985, vol. 135, 2589- [0101]
- **CHAUBERT**Mod Pathol, 1997, vol. 10, 585- [0101]
- **DENNLER et al.**EMBO, 1998, vol. 17, 3091-3100 [0121]
- **MACIAS-SILVA et al.**Cell, 1996, vol. 87, 1215- [0121]
- **MIKAELIAN et al.**Methods in Molecular Biology, 1996, vol. 57, 193-202 [0132]
- **DUROCHER et al.**Nucleic Acids Research, 2002, vol. 30, 39- [0135]

Patentkrav

1. Isoleret protein, der omfatter et stabiliseret activin-IIB-receptorpolypeptid,
hvor polypeptidet er udvalgt fra gruppen, der består af:
(a) et polypeptid, der består af sekvensen ifølge gruppen, der består af SEQ ID NO: 4, 6, 12 og 14;
(b) et polypeptid, der har mindst 90 % sekvensidentitet med (a), hvor polypeptidet har en W eller en Y i positionen, der svarer til position 28 i SEQ ID NO: 2, og en T i positionen, der svarer til position 44 i SEQ ID NO: 2, hvor polypeptidet er i stand til at binde myostatin, activin A eller GDF-11, og
(c) polypeptider, der har mindst 95 % sekvensidentitet med (a), hvor polypeptidet har en W eller en Y i positionen, der svarer til position 28 i SEQ ID NO: 2, og en T i positionen, der svarer til position 44 i SEQ ID NO: 2, hvor polypeptidet er i stand til at binde myostatin, activin A eller GDF-11.

2. Protein ifølge krav 1, hvor polypeptidet er forbundet til mindst ét heterologt polypeptid.

3. Protein ifølge krav 2, hvor det heterologe polypeptid er et IgG-Fc-domæne.

4. Protein ifølge krav 2, hvor det heterologe polypeptid er forbundet til polypeptidet ved hjælp af en linkersekvens.

5. Protein ifølge krav 4, hvor linkersekvensen er udvalgt fra gruppen, der består af sekvenserne: SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 49 og SEQ ID NO: 50.

6. Protein ifølge krav 3, hvor proteinet omfatter et polypeptid, der er udvalgt fra gruppen, der består af:
(a) et polypeptid, der består af sekvensen ifølge gruppen, der består af SEQ ID NO: 8, 10, 16 og 18;
(b) et polypeptid, der har mindst 90 % sekvensidentitet med (a), hvor polypeptidet har en W eller en Y i positionen, der svarer til position 28 i SEQ ID NO: 2, og en T i positionen, der svarer til position 44 i SEQ ID NO: 2, hvor polypeptidet er i stand til at binde myostatin, activin A eller GDF-11, og
(c) polypeptider, der har mindst 95 % sekvensidentitet med (a), hvor polypeptidet har en W eller en Y i positionen, der svarer til position 28 i SEQ ID NO: 2, og en T i positionen, der svarer til position 44 i SEQ ID NO: 2, hvor polypeptidet er i stand til at binde myostatin, activin A eller GDF-11.

7. Isoleret protein, der består af sekvensen ifølge SEQ ID NO: 10.

8. Isoleret protein, der omfatter et stabiliseret activin-IIB-receptorpolypeptid (svActRIIB), hvor polypeptidet er udvalgt fra gruppen, der består af:

(a) et polypeptid, der består af sekvensen ifølge SEQ ID NO: 2 bortset fra en enkelt aminosyresubstitution i positionen, der svarer til position 28 i SEQ ID NO: 2, og en enkelt aminosyresubstitution i positionen, der svarer til position 44 i SEQ ID NO: 2, hvor substitutionen i position 28 er udvalgt fra gruppen, der består af W og Y, og substitutionen i position 44 er T;

(b) et polypeptid, der består af sekvensen ifølge aminosyrerne 19 til 134 i SEQ ID NO: 2 bortset fra en enkelt aminosyresubstitution i positionen, der svarer til position 28 i SEQ ID NO: 2, og en enkelt aminosyresubstitution i positionen, der svarer til position 44 i SEQ ID NO: 2, hvor substitutionen i position 28 er udvalgt fra gruppen, der består af W og Y, og substitutionen i position 44 er T;

(c) et polypeptid, der består af sekvensen ifølge aminosyrerne 23 til 134 i SEQ ID NO: 2 bortset fra en enkelt aminosyresubstitution i positionen, der svarer til position 28 i SEQ ID NO: 2, og en enkelt aminosyresubstitution i positionen, der svarer til position 44 i SEQ ID NO: 2, hvor substitutionen i position 28 er udvalgt fra gruppen, der består af W og Y, og substitutionen i position 44 er T;

(d) et polypeptid, der består af sekvensen ifølge aminosyrerne 25 til 134 i SEQ ID NO: 2 bortset fra en enkelt aminosyresubstitution i positionen, der svarer til position 28 i SEQ 10 NO: 2, og en enkelt aminosyresubstitution i positionen, der svarer til position 44 i SEQ ID NO: 2, hvor substitutionen i position 28 er udvalgt fra gruppen, der består af W og Y, og substitutionen i position 44 er T;

(e) et polypeptid, der har mindst 80 % sekvensidentitet med en hvilken som helst af (a) til (d) bortset fra en enkelt aminosyresubstitution i positionen, der svarer til position 28 i SEQ ID NO: 2, og en enkelt aminosyresubstitution i positionen, der svarer til position 44 i SEQ ID NO: 2, hvor substitutionen i position 28 er udvalgt fra gruppen, der består af W og Y, og substitutionen i position 44 er T, hvor polypeptidet er i stand til at binde myostatin, activin A eller GDF-11.

9. Isoleret nukleinsyremolekyle, der omfatter et polynukleotid, der koder for polypeptidet ifølge krav 8.

10. Isoleret nukleinsyremolekyle, der omfatter et polynukleotid, der er udvalgt fra gruppen, der består af:

(a) et polynukleotid, der koder for et polypeptid, der består af sekvensen ifølge gruppen, der består af SEQ ID NO: 4, 6, 12 og 14;

(b) et polynukleotid, der koder for et polypeptid, der har mindst 90 % sekvensidentitet med (a), hvor polypeptidet har en W eller en Y i positionen, der svarer til position 28 i SEQ ID NO: 2, og en T i positionen, der svarer til position 44 i SEQ ID NO: 2; og hvor polypeptidet er i stand til at binde myostatin, activin A eller GDF-11; og

(c) et polynukleotid, der koder for et polypeptid, der har mindst 95 % sekvensidentitet med (a), hvor polypeptidet har en W eller en Y i positionen, der svarer til position 28 i SEQ ID NO: 2, og en T i positionen, der svarer til position 44 i SEQ ID NO: 2, og hvor polypeptidet er i stand til at binde myostatin, activin A eller GDF-11.

11. Nukleinsyremolekyle ifølge krav 10, hvor polynukleotidet har sekvensen, der er udvalgt fra gruppen, der består af sekvensen ifølge SEQ ID NO: 3, 5, 11 og 13, eller dets komplement.

12. Nukleinsyremolekyle ifølge krav 10, hvor polynukleotidet yderligere omfatter et polynukleotid, der koder for mindst ét heterologt protein.

13. Nukleinsyremolekyle ifølge krav 12, der omfatter et polynukleotid, der er udvalgt fra gruppen, der består af:
(a) et polynukleotid, der koder for et polypeptid, der består af sekvensen ifølge gruppen, der består af SEQ ID NO: 8, 10, 16 og 18;
(b) et polynukleotid, der koder for et polypeptid, der har mindst 90 % sekvensidentitet med (a), hvor polypeptidet har en W eller en Y i positionen, der svarer til position 28 i SEQ ID NO: 2, og en T i positionen, der svarer til position 44 i SEQ ID NO: 2, og hvor polypeptidet er i stand til at binde myostatin, activin A eller GDF-11; og
(c) et polynukleotid, der koder for et polypeptid, der har mindst 95 % sekvensidentitet med (a), hvor polypeptidet har en W eller en Y i positionen, der svarer til position 28 i SEQ ID NO: 2, og en T i positionen, der svarer til position 44 i SEQ ID NO: 2, og hvor polypeptidet er i stand til at binde myostatin, activin A eller GDF-11.

14. Nukleinsyremolekyle ifølge krav 13, hvor polynukleotidet har sekvensen, der er udvalgt fra gruppen, der består af sekvensen ifølge SEQ ID NO: 7, 9, 15 og 17, eller dets komplement.

15. Nukleinsyremolekyle ifølge krav 10, hvor polynukleotidet er operabelt koblet til en transkriptions- eller translationsregulatorisk sekvens.

16. Rekombinant vektor, der omfatter et nukleinsyremolekyle ifølge krav 10.

17. Værtscelle, der omfatter den rekombinante vektor ifølge krav 16.

18. Værtscelle ifølge krav 17, hvor værtscellen er en mammaliacelle.

19. Fremgangsmåde til fremstilling af et svActRIIB-protein, der omfatter dyrkning af værtscellen ifølge krav 17 under betingelser, der fremmer ekspressionen af proteinet, og indvinding af proteinet.

20. Farmaceutisk sammensætning, der omfatter en effektiv mængde af proteinet ifølge et hvilket som helst af kravene 1-8 blandet med et farmaceutisk acceptabelt bæremateriale.

21. Sammensætning ifølge krav 20 til anvendelse som et medikament.

22. Sammensætning ifølge krav 20 til anvendelse i en fremgangsmåde til behandling af muskelsvind eller en metabolisk forstyrrelse, der er udvalgt blandt diabetes, obesitas, hyperglykæmi og knogletab, hos en person, der har brug for en sådan behandling.

23. Sammensætning til anvendelse ifølge krav 22, hvor muskelsvindet skyldes en muskelsvindssygdom, der er udvalgt blandt muskeldystrofi, amyotrofisk lateral sklerose, kongestiv obstruktiv lungesygdom, kronisk hjertesvigt, cancerkakeksi, AIDS, nyresvigt, uræmi, rheumatoid arthritis, aldersrelateret sarkopeni, organatrofi, karpaltunnelsyndrom, androgen deprivation, forbrændingsskade, diabetes, og muskelsvind som følge af sengeleje, rygmarvsskade, apopleksi, knoglefraktur, aldring eller eksponering for mikrotyngdekraft.

24. Sammensætning ifølge krav 20 til anvendelse i en fremgangsmåde til behandling af en sygdom, hvor activin overudtrykkes, hos en person, der har brug for en sådan behandling, og hvor sygdommen er cancer.

25. Vektor ifølge krav 16 til anvendelse i en fremgangsmåde til behandling af muskelsvind eller en metabolisk forstyrrelse, der er udvalgt blandt diabetes, obesitas, hyperglykæmi og knogletab, eller en cancer, hvor activin overtrykkes, hos en person, der har brug for en sådan behandling, hvor vektoren er i stand til at styre ekspressionen af et svActRIIB-polypeptid hos personen.

26. Sammensætning til anvendelse ifølge krav 24 eller vektor til anvendelse ifølge krav 25, hvor canceren er ovariecancer.

27. Anvendelse af proteinet ifølge et hvilket som helst af kravene 1 til 8 i en fremgangsmåde til forøgelse af mager muskelmasse eller forøgelse af forholdet mellem mager muskelmasse og fedtmasse, hvor fremgangsmåden ikke er en fremgangsmåde til behandling af menneske- eller dyrekroppen ved hjælp af terapi.

DRAWINGS

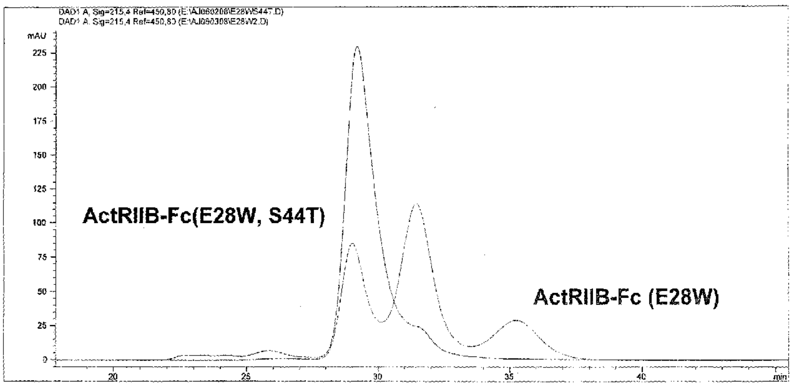


FIGURE 1

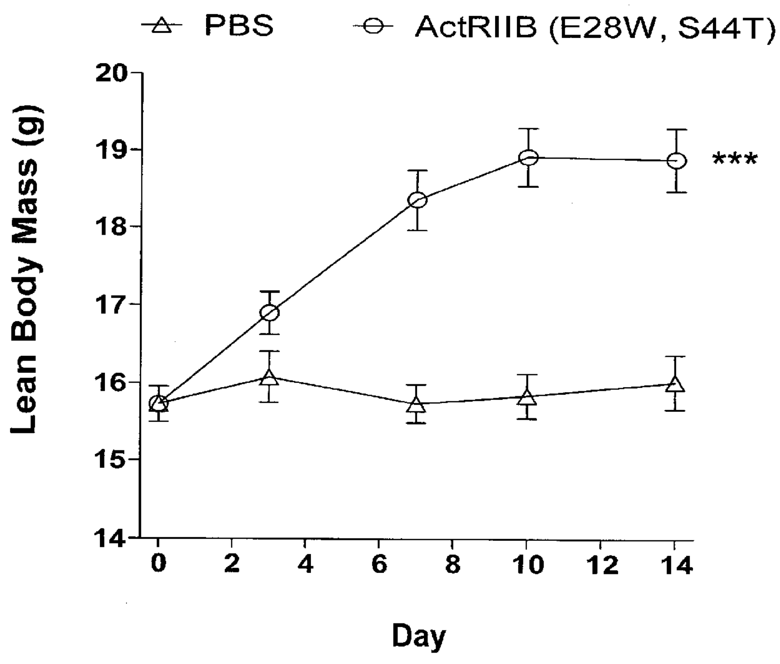
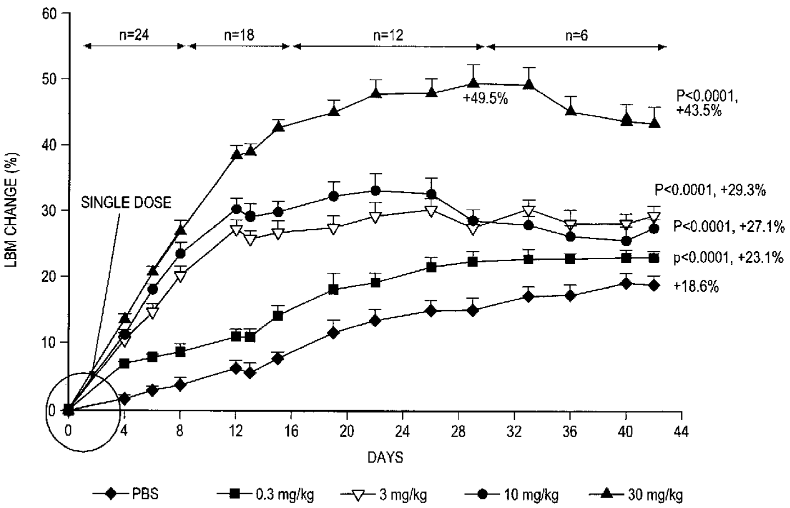

PBS
ActRIIB (E28W, S44T)
Lean Body Mass (g)
20
19
18
17
16
15
14
0
2
4
6
8
10
12
14
Day
***

FIGURE 2



60
50
40
30
20
10
0
LBM CHANGE (%)
n=24
n=18
n=12
n=6
SINGLE DOSE
+49.5%
P<0.0001,
+43.5%
P<0.0001, +29.3%
P<0.0001, +27.1%
p<0.0001, +23.1%
+18.6%
0
4
8
12
16
20
24
28
32
36
40
44
DAYS
PBS
0.3 mg/kg
3 mg/kg
10 mg/kg
30 mg/kg

FIGURE 3