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CA 2654944 C 2017/01/03

(11)(21) 2 654 944

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2007/06/06
(87) Date publication PCT/PCT Publication Date: 2007/12/21
(45) Date de délivrance/Issue Date: 2017/01/03
(85) Entrée phase nationale/National Entry: 2008/12/08
(86) N° demande PCT/PCT Application No.: US 2007/070468
(87) N° publication PCT/PCT Publication No.: 2007/146689
(30) Priorités/Priorities: 2006/06/09 (US60/812,349); 2006/10/20 (US60/862,244); 2007/01/24 (US60/897,187)

(51) Cl.Int./Int.Cl. *A61K 38/30* (2006.01), *C07K 14/65* (2006.01)

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(54) Titre : POLYPEPTIDES DU FACTEUR DE CROISSANCE DE TYPE INSULINE STABILISES
(54) Title: STABILIZED INSULIN-LIKE GROWTH FACTOR POLYPEPTIDES

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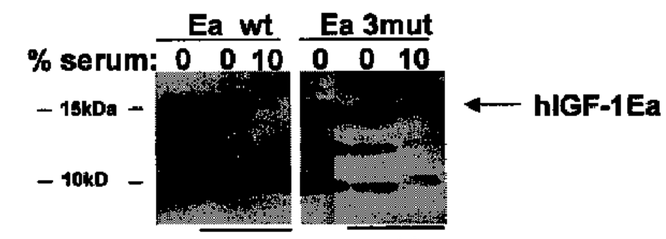

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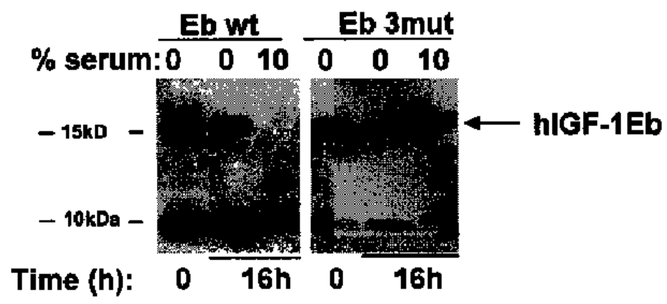

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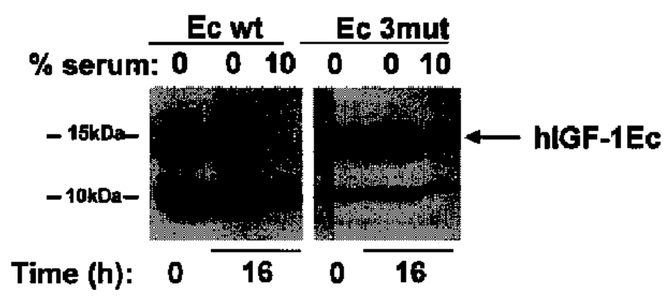

(57) Abrégé/Abstract:
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 December 2007 (21.12.2007)

PCT

(10) International Publication Number
WO 2007/146689 A3

(51) International Patent Classification:
A61K 38/30 (2006.01) *C07K 14/65* (2006.01)

(21) International Application Number:
PCT/US2007/070468

(22) International Filing Date: 6 June 2007 (06.06.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/812,349	9 June 2006 (09.06.2006)	US
60/862,244	20 October 2006 (20.10.2006)	US
60/897,187	24 January 2007 (24.01.2007)	US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,

[Continued on next page]

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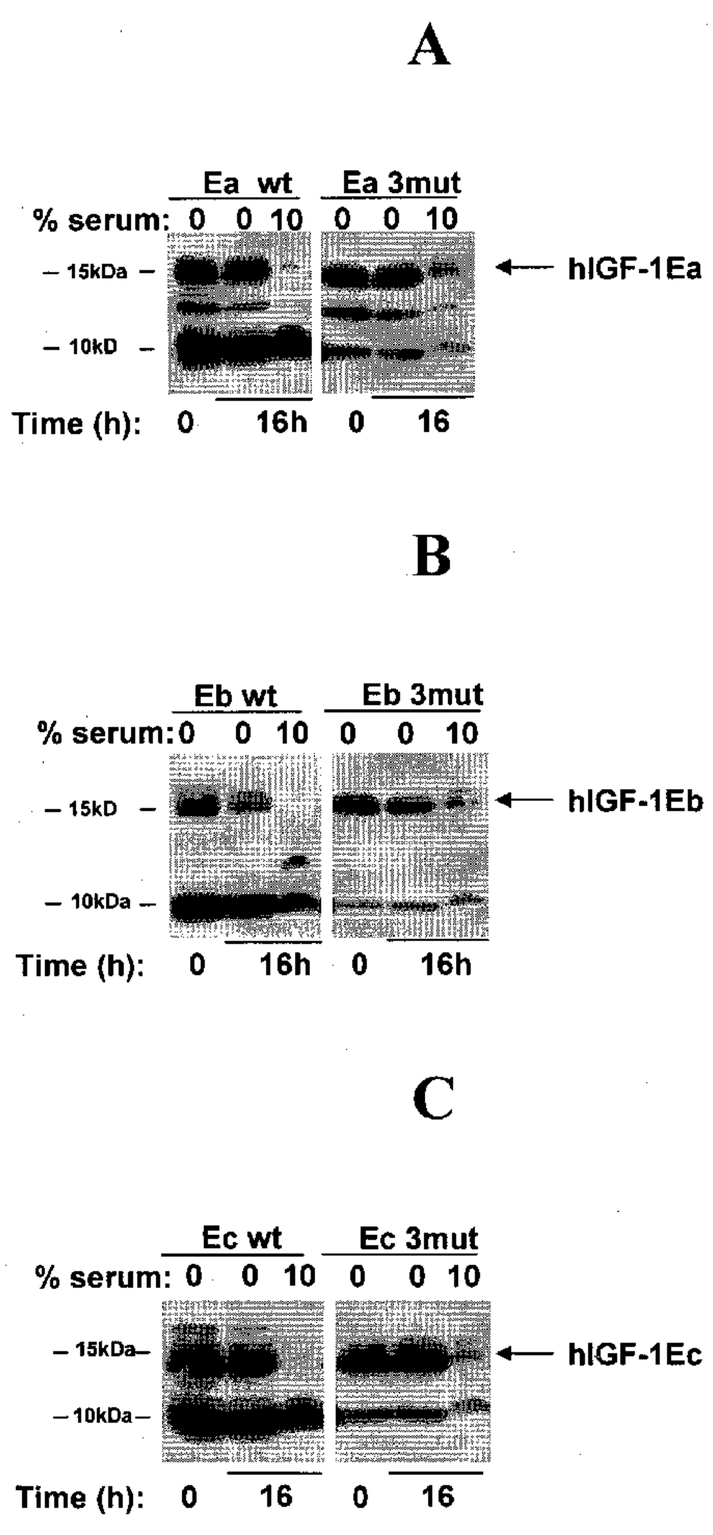

WO 2007/146689 A3

WO 2007/146689 A3

ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
10 April 2008

STABILIZED INSULIN-LIKE GROWTH FACTOR POLYPEPTIDES

Background of the Invention

Insulin-like growth factors (IGFs) are part of a complex system that cells use to communicate with their physiologic environment. This complex system (often referred to as the insulin-like growth factor axis) consists of two cell-surface receptors (IGF-1R and IGF-2R), two ligands (IGF-1 and IGF-2), a family of six high-affinity IGF-binding proteins (IGFBP 1-6), and associated IGFBP degrading enzymes (proteases). This system is important not only for the regulation of normal physiology but also for a number of pathological states (Glass, Nat Cell Biol 5:87-90, 2003).

The IGF axis has been shown to play roles in the promotion of cell proliferation and the inhibition of cell death (apoptosis). IGF-1 is mainly secreted by the liver as a result of stimulation by human growth hormone (hGH). Almost every cell in the human body is affected by IGF-1, especially cells in muscles, cartilage, bones, liver, kidney, nerves, skin and lungs. In addition to the insulin-like effects, IGF-1 can also regulate cell growth. IGF-1 and IGF-2 are regulated by a family of gene products known as the IGF-binding proteins. These proteins help to modulate IGF action in complex ways that involve both inhibiting IGF action by preventing binding to the IGF receptors as well as promoting IGF action through aiding delivery to the receptors and increasing IGF half life in the blood stream. There are at least six characterized binding proteins (IGFBP1-6).

In its mature form, human IGF-1 (gpetlcgaelvdalqfvcgdrgfyfnkptgygsssrrapqtgivdeccfrscdlrrlem ycaplkpaksa; SEQ ID NO:1), also called somatomedin, is a small protein of 70 amino acids that has been shown to stimulate growth of a wide range of cells in culture. The mature protein is initially encoded by three known splice variant mRNAs. The open reading frame of each mRNA encodes a precursor protein containing the 70 amino acid IGF-1 and a particular E-peptide at the C-terminus, depending on the particular IGF-1 mRNA. These E-peptides have been termed the Ea (rsvraqrhtdmpktqkevhlknasrgsagnknyrm; SEQ ID NO:2), Eb (rsvraqrhtdmpktqkyqppstnknt ksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk; SEQ ID NO:3), and Ec (rsvraqrhtdm pktqkyqppstnkntksqrrkgstfeerk; SEQ ID NO:4) peptides and range from 35 to 87 amino acids in

length and encompass a common sequence region at the N-terminus and a variable sequence region at the C-terminus. For example, the wild-type open reading frame for the IGF-1-Ea encodes a polypeptide of 105 amino acids (gpetlcgaelvdalqfvcgdrgfyfnkptgygsssrrapqtgivde ccfrscdlrrlemycaplkpaksa rsvraqrhtdmpktqkevhlknasrgsagnknyrm; SEQ ID NO:5). In physiological expression, the E-peptides are cleaved off of the precursor by endogenous proteases to yield the mature 70 amino acid IGF-1 known to be bioactive. In certain contexts, one to three of the N-terminal amino acids of IGF-1 are known to be cleaved under physiological conditions, yielding active IGF-1 having between 67-70 amino acids. IGF-2 gene expression and processing is characterized by similar attributes except that only one E-peptide (rdvstpptvlpdnfprypvgkffqydtwkqstqrlrrglpallrarrghvlakeleafreakrhrplialptqdpahggappemasnrk; SEQ ID NO:6) for human IGF-2 has been identified for the 156 amino acid precursor (ayrpsetlcggelvdtlqfvcgdrgfyfsrpasrvsrrsrgiveeccfrscdlalletycatpakserdvstpptvlpdnfprypvgkffqy dtwkqstqrlrrglpallrarrghvlakeleafreakrhrplialptqdpahggappemasnrk; SEQ ID NO:7). Both IGF-1 and IGF-2 appear to be poor drug candidates, since these proteins are quickly degraded by endogenous proteases in the serum of patients. One strategy that has been contemplated is to stabilize IGF-1 as a drug by forming a complex with one of its binding proteins.

Summary of the Invention

The invention is based on the discovery that a precursor IGF-1 or IGF-2 protein containing substantially its E-peptide is bioactive and stabilized in the presence of serum, resulting in an IGF-1 or IGF-2 polypeptide that is useful as a pharmaceutical. In the compositions of the invention, the normal cleavage of the E-peptide from IGF-1 is avoided, for example, by mutating or deleting either of the arginine at position 1 or the serine at position 2 of the E-peptides (corresponding to positions 71 and 72 in the wild-type precursor IGF-1). In IGF-2, the cleavage is avoided, for example, by mutating or deleting either the arginine at position 1 or the aspartic acid at position 2 of the E-peptide (corresponding to positions 68 and 69 in the wild-type precursor IGF-2). Other modifications of an IGF precursor protein can avoid or reduce this cleavage.

In addition, further modifications of the IGF-1 precursor amino acid sequence can confer additional pharmaceutical benefits. For example, the polypeptides of the invention can exhibit

increased affinity for the IGF-1 receptor or decreased binding ability to an inhibitory IGF-1 or IGF-2 binding protein.

For the sake of clarity and consistency, the numbering of amino acid residues in IGF-1 or IGF-2 precursor or mature proteins throughout this application and in the claims is based on the wild-type precursor protein sequence numbering without signal peptide.

Accordingly, the invention includes a polypeptide containing a human IGF-1 precursor protein, where the cleavage of the E-peptide from IGF-1 by a protease is reduced by modification of the precursor protein. The E-peptide can be the Ea, Eb, or Ec peptide. At the N-terminus of the precursor, amino acids G1, P2, or E3 of the precursor protein can be deleted or mutated, as can R36 (e.g., R36A) and R37 (e.g., R37A).

The precursor protein can further include the N-linked glycosylation consensus sequence NXS/T, for example by insertion of amino acids 93-102 of Ea between amino acids N95 and T96 of the Eb. In general, the precursor protein can include an oligosaccharide covalently linked to an amino acid side chain of the precursor protein, such as an arginine side chain of the precursor protein.

In addition, a residue of the precursor protein can be replaced by a non-natural amino acid (e.g., one that includes an acetylene or azido group). Such non-natural amino acids can facilitate linkage of a poly(ethylene glycol) moiety to a side-chain of the precursor protein, though typical protein pegylation strategies are well known in the art.

The precursor protein can further include one or more additional E-peptides linked to the C-terminus of the precursor protein. For example, a polypeptide can include, from N-terminus to C-terminus, (1) an IGF-1 precursor protein having a first Eb peptide, where G1, P1, and E1 are deleted, either R36 or R37 or both are mutated, R71 and S72 are deleted, and the last seven C-terminal amino acids of the first Eb peptide are deleted; (2) a second Eb peptide, where R71, S72, and the last seven C-terminal amino acids of the second Eb peptide are deleted; (3) a third

Eb peptide, where R71, S72, and the last seven C-terminal amino acids of the third Eb peptide are deleted; and (4) a fourth Eb peptide, where R71 and S72 are deleted.

An effective means of preventing cleavage of the E-peptide from the IGF-1 is the deletion or mutation of R71 or S72.

Similarly, the invention includes a human IGF-2 precursor protein where the cleavage of the E-peptide from IGF-2 by a protease is reduced by modification of the precursor protein. In particular, deletion or mutation of R68 or D69 can be an effective means of avoiding protease digestion of the IGF-2 precursor protein.

In addition, any E-peptide of IGF-1 can be combined with an IGF-2 and any E-peptide of IGF-2 can be combined with IGF-1 to provide the benefits described herein.

The invention further includes a method of treating a musculoskeletal disease, diabetes, neuronal cell death by administering a therapeutically effective amount of a polypeptide of the invention. Likewise, the invention includes the use of a polypeptide of the invention for the manufacture of a medicament for the treatment of a musculoskeletal disease, diabetes, neuronal cell death, or anemia.

In another embodiment, the invention includes a pegylated IGF-1 without an E-peptide but having introduced therein a non-natural amino acid as the site of pegylation. Any of the modified, pegylated IGF-1 containing a non-natural amino acid as disclosed herein, without an E-peptide, is also included in the invention.

The invention also includes veterinary methods and uses of administering an effective amount of the polypeptide of the invention to obtain a desired effect.

The veterinary uses include (i) enhancing the rate and/or extent of growth in an animal, (ii) enhancing the efficiency of their conversion of feed into body tissue, (iii) enhancing milk production in lactating animals, (iv) treating animal wasting symptoms associated with cachexia,

trauma, or other consumption diseases, and (v) treating lactating animals for improvement in neonatal health.

The invention as claimed relates to:

- a human IGF-1 precursor protein, wherein: (i) one or more of the following precursor protein residues is deleted or mutated: G1, P2, E3, R36 and R37; and (ii) the cleavage of the E-peptide from IGF-1 by a protease is reduced by modification of the precursor protein, wherein the modification is the deletion or mutation of R71 or S72 of the precursor protein, and wherein the numbering of the amino acids G1, P2, E3, R36, R37, R71 and S72 corresponds to SEQ ID NO: 5;

- use of the protein as described herein for the treatment of a musculoskeletal disease, diabetes, neuronal cell death, chronic obstructive pulmonary disease, burn injury, or anemia;

- use of the protein as described herein for the manufacture of a medicament for the treatment of a musculoskeletal disease, diabetes, neuronal cell death, chronic obstructive pulmonary disease, burn injury, or anemia;

- the protein as described herein for use in the treatment of a musculoskeletal disease, diabetes, neuronal cell death, chronic obstructive pulmonary disease, burn injury, or anemia;

- a nucleic acid encoding the protein as described herein;

- a vector comprising the nucleic acid as described herein; and

- a cell transfected with the vector as described herein.

Brief Description of the Drawings

Figs. 1A-1C are Western blots of polypeptides of the invention and wild-type IGF-1 precursor after zero or 16-hour incubation in the presence or absence of 10 % human serum at 37°C. Expression vectors encoding various IGF-1 constructs were transfected into Cos7 cells, and the conditioned culture medium obtained. The "3mut" refers to a hIGF-1-E-peptide precursor having the following three sets of modifications: deletion of G1, P2, and E3; mutation of Arg 37 to Ala (R37A); and deletion of R71 and S72. Fig. 1A shows the Western blot results (using antibody to IGF-1) for the wild-type and 3mut precursor containing Ea. Fig. 1B shows the Western blot results (using antibody to hIGF-1) for the wild-type and 3mut precursor containing Eb. Fig. 1C shows the Western blot results (using antibody to hIGF-1) for the wild-type and 3mut precursor containing Ec.

Figs. 2A-2D are line graphs showing the biological activity of various IGF-1 polypeptides ("ligands"). Biological activity was measured by stimulation of C2C12 myoblasts with Cos7-expressed polypeptides. The stimulated C2C12 cells were then assayed for the relative amounts of total AKT and phosphorylated AKT. Long-R3-IGF-1 is a commercially available reagent (Sigma Product No. I-1271) that consists of the mature human IGF-1 amino acid sequence, with an E3R mutation and an additional 13 amino acid N-terminal extension peptide. Fig. 2A shows the activity of IGF-1-Ea3mut. Fig. 2B shows the activity of IGF-1-Eb3mut. Fig. 2C shows the activity of IGF-1-Eab3mut, which is a 3mut construct in which Ea amino acids 93 to 102 were inserted between amino acids 95 and 96 of Eb. Fig. 2D shows the activity of IGF-1-Ec3mut.

Figs. 3A-3D and **4A-4D** are line graphs showing whether IGF-1 precursor polypeptides of the invention maintain selectivity to the appropriate receptor by assaying for receptor phosphorylation in response to ligand binding. Figs 3A and 3B test the receptor selectivity of IGF-1-Ea3mut against the IGF-1 receptor (Fig. 3A) and the insulin receptor (Fig. 3B). Figs. 3C

and 3D tests the receptor selectivity of IGF-1-Eb3mut against the IGF-1 receptor (Fig. 3C) and the insulin receptor (Fig. 3D). Figs. 4A and 4B test the receptor selectivity of IGF-1-Ec3mut against the IGF-1 receptor (Fig. 4A) and the insulin receptor (Fig. 4B). Figs. 4C and 4D tests the receptor selectivity of IGF-1-Eab3mut against the IGF-1 receptor (Fig. 4C) and the insulin receptor (Fig. 4D). "IGF1-R3" refers to the Long-R3-IGF-1 described above. The polypeptide listed as "IGF1Eab" refers to construct in which Ea amino acids 93 to 102 were inserted between amino acids 95 and 96 of Eb.

Fig. 5 is a Western blot showing relative AKT phosphorylation upon stimulation of C2C12 myotubes (as a result of 3 to 4 days of differentiation of C2C12 myocytes) by different ligands. The IGF-1Eb multimer refers to the construct schematically shown in Fig. 6A.

Figs. 6A and **6B** are schematic representations of two of the polypeptides of the invention. Fig. 6A shows an IGF-1-Eb precursor polypeptide with four sets of modifications: deletion of G1, P2, and E3; mutation of R37 to A; deletion of R71 and S72; and deletion of the last seven C-terminal amino acids. In addition, the polypeptide is lengthened by the addition of two more Eb peptides (but without R71 and S72 and without the last seven C-terminal amino acids) and the addition of a final Eb peptide (buth without R71 and S72) at the C-terminus of the polypeptide. This construct is often referred to as the IGF-1-Eb multimer. Fig. 6B shows an IGF-1-Eab precursor polypeptide with four sets of modifications: deletion of G1, P2, and E3; mutation of R37 to A; deletion of R71 and S72; and insertion of Ea amino acids 93 to 102 between amino acids 95 and 96 of Eb.

Fig. 7A is a sequence alignment of the human IGF-1 (SEQ ID NO:1) with corresponding animal IGF-1. All animal species analyzed and their corresponding GenBank accession numbers for the sequence are given. G1, P2, E3 is conserved in all analyzed species except Sterlet (where S2 replaces P2). R36 and R37 are conserved in all analyzed species.

Fig. 7B is a graph showing the phylogeny of the analyzed amino acid sequences compared to human IGF-1 (SEQ ID NO:1). Below the tree is a scale indicating the number of "Amino Acid Substitutions" per 100 residues for protein sequences. The Kimura distance formula is used to

calculate distance values, derived from the number of non-gap mismatches and corrected for silent substitutions. The values computed are the mean number of differences per site and fall between zero and 1. Zero represents complete identity and 1 represents no identity. The phylogenetic tree scale uses these values multiplied by 100.

Fig. 8A is a sequence alignment of the human Ea peptide (SEQ ID NO:2) with various animal Ea peptides. All animal species analyzed and their corresponding GenBank accession numbers for the sequence are given. R71 and S72 are conserved in all analyzed species.

Fig. 8B is a graph showing the phylogeny of the analyzed amino acid sequences compared to human IGF-1 Ea peptide (SEQ ID NO:2).

Fig. 9A is a sequence alignment of the human Eb peptide (SEQ ID NO:3) with various animal Eb peptides. All animal species analyzed and their corresponding GenBank accession numbers for the sequence are given. R71 and S72 are conserved in all analyzed species.

Fig. 9B is a graph showing the phylogeny of the analysed amino acid sequences compared to human IGF-1 Eb peptide (SEQ ID NO:3).

Fig. 10A is a sequence alignment of the human Ec peptide (SEQ ID NO:4) with various animal Ec peptides. All animal species analyzed and their corresponding GenBank accession numbers for the sequence are given. R71 and S72 are conserved in all analyzed species.

Fig. 10B is a graph showing the phylogeny of the analyzed amino acid sequences compared to human IGF-1 Ec peptide (SEQ ID NO:4).

Fig. 11A is a sequence alignment of the human IGF-2 (SEQ ID NO:7) with corresponding animal IGF-2. All animal species analyzed and their corresponding GenBank accession numbers for the sequence are given. R68 is conserved in all analyzed species; D69 is conserved except for chimpanzee, where a histidine resides in that position.

Fig. 11B is a graph showing the phylogeny of the analyzed amino acid sequences compared to human IGF-2 (SEQ ID NO:7).

Fig. 12A is a sequence alignment of the human IGF-2 E-peptide (SEQ ID NO:6) with various animal IGF-2 E-peptides. All animal species analyzed and their corresponding GenBank accession numbers for the sequence are given. R68 is conserved in all analyzed species; D69 is conserved except for Chimpanzee, where a histidine resides in that position.

Fig. 12B is a graph showing the phylogeny of the analyzed amino acid sequences compared to human IGF-2 E peptide (SEQ ID NO:6).

Detailed Description of the Invention

The invention relates to new IGF-1 and IGF-2 precursor polypeptides containing substantially an E-peptide that has been modified to prevent, reduce, or avoid the typical protease cleavage responsible for releasing the active IGF-1 or IGF-2 from its E-peptides. The utility of the polypeptides of the invention is based on the surprising discovery that such precursor polypeptides are biologically active, stable and beneficial as pharmaceuticals.

Screening for Active IGF Precursor Polypeptides

The usefulness of any polypeptide of the invention can be assessed using the following assays.

Stability A polypeptide of the invention should have sufficient stability in the presence of endogenous proteases, such as in human serum, to be an effective drug. To assess stability, an expression vector encoding the polypeptide can be transfected into Cos7 cells (ATCC) in a DMEM medium containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. The culture medium containing secreted polypeptides can be applied to further analysis, or in the alternative, the expression vector can encode readily available tags, such as a hexa-histidine tag, in the polypeptide to facilitate efficient purification of the expressed polypeptides in the Cos7 cultures. However prepared, the polypeptide sample is incubated in normal human serum (Sigma) or in PBS for various times (e.g., 0, 1, 5, 10, and 16 hours), subjected to polyacrylamide gel electrophoresis, blotted onto nitrocellulose, and the relevant

proteins visualized using a primary antibody against human IGF-1 or IGF-2 and a secondary antibody, e.g., conjugated to horseradish peroxidase. Any number of similar blotting and detection techniques, some using fluorescent dyes or even radionuclides, can be used. The intensity of the precursor band versus the intensity of the IGF-1 or IGF-2 band should indicate the degree to which the precursor polypeptide is cleaved under various conditions. A polypeptide of the invention that is exposed to human serum for 16 hours at 37°C can exhibit a ratio of uncleaved precursor to cleaved mature IGF of about 1:2 to 1:0.1, e.g., about 1:1 to 1:0.5, particularly a ratio of about 1:1 or a ratio of about 1:0.5. Typically, the precursor should exhibit a ratio of at least 1:1.

AKT Phosphorylation A polypeptide of the invention should maintain the ability to signal through the IGF-1 receptor. (Both IGF-1 and IGF-2 signal through the IGF-1 receptor.) To determine this signaling ability, one can assess whether a downstream intracellular target, AKT, is phosphorylated in response to ligand binding at the cell surface. For analysis of AKT phosphorylation, C2C12 myoblasts are starved in serum-free medium and then stimulated with different ligands. Cells are lysed and cleared by centrifugation. AKT phosphorylation and total AKT levels are analyzed by ELISA using PathScan phospho AKT (Ser473) sandwich ELISA kit and PathScan AKT sandwich ELISA kit (Cell Signaling), respectively.

IGF-1 Receptor Specificity A polypeptide of the invention preferably maintains the specificity for the IGF-1 receptor and should bind to the related insulin receptor with low affinity. To assess receptor specificity, polypeptide samples are added to serum-starved NIH3T3 cells overexpressing the IGF-1 receptor or the insulin receptor, and the level of IGF-1 receptor phosphorylation or insulin receptor phosphorylation is determined by lysing the cells and subjecting the lysates to ELISA using the DuoSet IC human phosphor-IGF-1 receptor and insulin receptor ELISA kit (R&D Systems).

In Vivo Testing in Mouse Models of Hypertrophy To determine whether a polypeptide of the invention can act to increase skeletal muscle mass under a context that already leads to muscle hypertrophy, one can subject treated and untreated animals to exercise and determine whether animals receiving the polypeptide have developed larger muscles than untreated animals.

Exercise Models

One model known in the art is based on the use of a voluntary running wheel with user-variable loads (see, e.g., Konhilas et al., Am J Physiol Heart Circ Physiol 289:H455-H465, 2005). The voluntary cage wheel eliminates physical and psychological insults that are common in forced exercised models, and are therefore more appropriate for evaluating candidate drugs that are used in relatively healthy individuals for whom increases in muscle mass is desirable.

Any suitable mouse strain can be used. For example, male C57Bl/6J mice can be randomly assigned to experimental (e.g., receiving IGF precursor polypeptide) and control groups. Animals are individually housed in a cage containing an exercise wheel; sedentary control animals are housed in identical cages without a wheel. The exercise wheels are described in Allen et al., J Appl Physiol 90:1900-1908, 2001. Briefly, the system consists of an 11.5 cm-diameter wheel with a 5.0 cm-wide running surface (model 6208, Petsmart, Phoenix, AZ) equipped with a digital magnetic counter (model BC 600, Sigma Sport, Olney, IL) that is activated by wheel rotation. In addition, each wheel is engineered with a resistance mechanism allowing adjustment of the load. This is accomplished by attaching stainless steel fishing line to the cage top and wrapping the wire around an immovable pulley that is secured to the cage wheel at the axis of rotation so as to not contribute to the wheel load. The wire is again secured to the cage top with a spring and screw. This design permits fine adjustments of the wheel load, which is evenly distributed throughout the rotation of the wheel. Daily exercise values for time and distance run are recorded for each exercised animal throughout the duration of the exercise period. All animals are given water and standard hard rodent chow ad libitum. Voluntary running (cage wheel exposure) can begin at an average age of about 12 weeks for all groups. Each group continues running under varying resistance, depending on experimental group, for 50 days until the animals are about 19 weeks of age. The load on the wheel is determined by hanging known weights on the wheel until the wheel was slightly displaced. All exercise groups begin with no load on the cage wheel for the first week. However, the "no-load" condition is actually 2 g, which is determined as the load necessary to maintain wheel inertia and frictional load. Considering a wheel acclimatization period of 1 week, wheel loads can be changed at one-week intervals, except for higher loads, which can be changed after 2 weeks. The range of loads

can be anywhere from 2 g to up to 12 g. Exercised and sedentary control animals are euthanized by cervical dislocation under inhaled anesthesia immediately after the end of the specific exercise period. Body mass is measured, and specific muscles are rapidly excised, washed, and frozen for histological or biochemical assays at a future date.

Alternative exercise hypertrophy models are also available to the skilled artisan. See, e.g., the treadmill exercise model described in Lerman et al., J Appl Physiol 92:2245-2255, 2002.

Clenbuterol Injection Model

Clenbuterol is a β_2-adrenergic agonist with growth-promoting properties that cause a documented increase in muscle mass. The precise mechanism of clenbuterol action remains unclear, although a reduction in muscle protein degradation has been proposed. In the clinic, clenbuterol is used as an anti-asthma drug, but it appears to be mostly misused as a body-building agent to increase muscle mass in both humans and show animals.

Five mice are given a daily injection of clenbuterol (3 mg/kg, subcutaneous (s.c.)) for 3, 7, or 14 days to induce muscle hypertrophy. Mice injected with PBS serves as negative control. The animals are monitored daily (visual inspection) for any adverse reactions (i.e. unkempt coat, lethargic) to the treatment. Clenbuterol treatment has the potential to make mice more fearful or aggressive, so mice should be especially monitored for fighting if housed in groups. Mice are mobile, and can eat and drink normally. Mice are monitored daily until they are euthanized on day 3, 7, or 14, and tissue collected for further analysis.

In Vivo Testing in Muscle Atrophy Models In various skeletal muscle atrophy models, an IGF precursor polypeptide of the invention can be tested for the ability to maintain muscle mass under conditions that generally reduce muscle mass. With the example models described below, the skilled artisan can readily design and implement controlled experiments involving the administration and use of IGF precursor polypeptides to determine whether such polypeptides can increase muscle mass.

For example, C57Bl6/2 male mice are purchased from The Jackson Laboratories. Mice are purchased so that they are about 9 weeks at the start of each experiment. Generally mice are housed in microisolator cages with normal rodent chow. At the start of each experiment mice are weighed. At the end of each experiment, generally mice are euthanized by CO_2 inhalation followed by cervical dislocation, and muscle tissues harvested for further processing. Mice are weighed to provide "end body weight." Skeletal muscles that can be harvested are tibialis anterior, extensor digitorum longus, soleus, and gastrocnemius muscles. Other tissues harvested occasionally are: heart, liver, spleen, kidneys, testes, and brain. All muscles and tissues are completely dissected and weighed on a balance capable of measuring to 0.0001g. Tissues are then snap-frozen in liquid nitrogen for later RNA and protein extraction, or snap-frozen embedded in OCT on a cork disc. Muscles frozen on a cork disc for later cryosectioning are immersed in isopentane cooled to a thick slush by liquid nitrogen. All samples are stored at -80°C.

Dexamethasone Treatment

A pharmacological method of inducing muscle wasting in mice is daily intraperitoneal injection with dexamethasone at 20mg/kg. Dexamethasone is a synthetic member of the glucocorticoid class of hormones. It acts as an anti-inflammatory and immunosuppressant, with a potency of about 40 times that of hydrocortisone. Dexamethasone is used to treat many inflammatory and autoimmune conditions, e.g. rheumatoid arthritis. It is also given to cancer patients undergoing chemotherapy, to counteract certain side-effects of their antitumor treatment. Dexamethasone causes muscle atrophy both in mice and in human patients.

Mice are injected intraperitoneally (ip) with dexamethasone for 3, 7, or 14 days. On the terminal day subjects are euthanized using CO_2, and the leg muscles harvested. The animals are monitored daily (visual inspection) for any adverse reactions (i.e. unkempt coat, lethargic) to the treatment. Mice are usually mobile, and can eat and drink normally. Mice injected with PBS are the negative control.

Cast Immobilization

Physical disuse of various muscle groups results in atrophy of those muscles. Ankle joint fixation ("pinned heel" or casting) has proven to be a highly useful and reproducible way to induce physical immobilization of rat and mouse hindlimb musculature.

Mice are anesthetized with isofluorane for immobilization. The ankle and knee joints are fixed at 90 degrees with a light-weight casting material (VET-LITE) around the joints. The material is soaked in warm water and then wrapped around the limb, leaving the toes and hip joint free. The joints are maintained in at 90° positions until the casting material has dried. The contralateral leg serves as control. The mice are then allowed to recover from anesthesia and housed in normal micro isolator cages. Casting has not been observed to cause excessive stress, and animals freely move about the cage to feed and drink. The mice are however monitored daily for any adverse events affecting body weight, activity, and irritations.

Once a cast is applied to a mouse, the animal is monitored daily to make sure that the cast remains in place, as chewing can occur. The animals can move, drink, and feed after recovery of anesthesia, and they do not require special bedding, caging or other assistance.

Denervation

Generally, mice are anesthetized with isofluorane gas for denervation. Using aseptic surgical procedures (three washes of betadine with a final ethanol wash), the right sciatic nerve is isolated in the mid-thigh and a 2 to 5 mm piece cut out. The contralateral leg serves as control.

More specifically, the skin incision is closed with a suture clip, and the animals injected with a single dose of buprenorphine before being allowed to recover from the anesthesia. Three, seven, or 14 days after surgery animals are euthanized by CO_2 inhalation followed by cervical dislocation, and muscles (gastrocnemius complex, tibialis anterior, extensor digitorum longus, soleus) are removed for histological and biochemical analyses.

Given that the sciatic nerve is transected, the effected limb is rendered immobile to induce skeletal muscle atrophy of the muscles involved. The animal can otherwise move, drink, and feed after recovery of anesthesia and they do not require special bedding, caging or other

assistance. Nonetheless, animals are monitored immediately post-surgery and through recovery (1-2 hrs). In addition, the incision sites and general animal health are monitored for 3 days post-surgery. The suture clip is removed 7 to 10 days after surgery.

Genetic Models

Genetically manipulated transgenic mice can also be used as models of muscle atrophy. For example, the so-called Mini Mice (The Jackson Laboratory, Stock No. 003258) contains a knock out mutation in the IGF-1 gene that results in abnormally decreased postnatal growth, as well as low body weight and size. For additional information, see Powell-Braxton et al., Genes Dev 7:2609-2617, 1993. In addition, the so-called Midi Mice (The Jackson Laboratory, Stock No. 003259) contains a different mutation in the IGF-1 gene that results in a hypomorph exhibiting low adult body weight and other cardiovascular phenotypes. For additional information, see Lembo et al., J Clin Invest 98:2648-2655, 1996.

Critical and Optional Mutations or Modificatons in the IGF Precursors

Critical Mutations The invention is based in part on the observation that an IGF precursor polypeptide that contains substantially its E-peptide remains bioactive and stable in the presence of serum. To ensure that the E-peptide is not cleaved by endogenous proteases targeting the dibasic protease site, in general either of the two N-terminal dibasic amino acids of the E-peptide in the precursor is deleted, mutated, or otherwise masked. In the case of hIGF-1, these two amino acids are R71 and S72, while in the case of hIGF-2, these first two amino acids are R68 and D69.

A variety of modifications enables this prevention of cleavage:

(1) Deletion of one or both dibasic residues

(2) Mutate one or both dibasic residues to a non-basic amino acid, such as alanine

(3) Insert one or more non-basic amino acids between the dibasic residues

(4) Place a glycosylation site near the dibasic residues sufficient to mask the protease site

(5) Site-directed pegylation using replacement of either dibasic residue, or insertion near or between the dibasic residues, with a non-natural amino acid, as described below.

In addition, residues K68 and K65 appear to play a role in IGF-1/E-peptide cleavage; accordingly, mutations or deletions of these residues can be incorporated into any tactic directed to the dibasic amino acids as described above.

Mutations at the N-terminus of Mature IGF In certain embodiments of the invention, the IGF precursor polypeptides have deletions or mutations of the first few N-terminal amino acids. In the case of IGF-1, any of the first three N-terminal amino acids can be deleted or mutated, whereas in the case of IGF-2, any of the first six N-terminal amino acids can be deleted or mutated. It has been observed that certain N-terminal amino acids are naturally cleaved in vivo, and the introduction of these mutations or deletions minimizes the in vivo associations of the polypeptides of the invention with IGF binding proteins (IGFBPs). The interaction of IGF-1 and IGF-2 with the IGF-1 receptor is regulated by IGFBPs. All six IGFBPs have been shown to inhibit IGF action (particularly IGFBP5), but in some instances a stimulatory effect has been observed. At least 99% of the IGF in the circulation is normally bound to IGFBPs. The most abundant IGFBP in the circulation after the neonatal period is IGFBP3 which can bind both IGF-1 and IGF-2 with similar affinities. The naturally occurring truncated IGF-1 (bearing deletion of G1, P2, and E3) binds to IGFBP3 with several times lower affinity than natural IGF-1. In addition, G3 is important for IGFBP binding, and G6 plays a similar role in the IGF-2 peptide.

Accordingly, in the case of the hIGF-1 precursor, any of G1, P2, or E3 can be deleted or mutated either alone or in combination. When a mutation is desired, a mutation to alanine can be introduced. In another example, in the case of hIGF-2 precursor, any of P4, S5, and E6 can be deleted or mutated either alone or in combination. When a mutation is desired, a mutation to alanine can be introduced.

Mutations at Residues 36 and 37 IGF-1 can e cleaved by serine proteases present in human serum. Mutation of either R36 or R37 to A can prevent cleavage of IGF-1 at this predicted cleavage site between R36 and R37. In the case of hIGF-2, R38 can be mutated or deleted to prevent this deleterious cleavage.

Use of Glycosylation The in vivo half-life of the polypeptides of the invention can be improved by the addition of N-linked glycosylation sites into either the IGF or the E-peptide portions of the precursor when expressed in mammalian or other eukaryotic cells capable of N-linked glycosylation. It has been shown in vitro that human IGF-1 Ea is glycosylated at N92 and N100, as these portions of Ea fits the consensus N-linked glycosylation sequence of N-X-S/T, where X can be any amino acid and the third amino acid of the triplet is either S or T. It is also know that the adjacent amino acid context of the consensus will affect how strongly the asparagine is glycosylated. Therefore, one strategy to introduce a glycosylation site into Eb or Ec is to insert Ea amino acids around the consensus sequence into roughly the same part of Eb or Ec. A particular implementation of this strategy is illustrated in the Examples below. In any event, any other consensus N-linked glycosylation site, including surrounding context amino acids, known to the skilled artisan can be inserted into a precursor polypeptide of the invention. In addition, O-linked glycosylation of a polypeptide of the invention can be accomplished by choosing the particular host used for production of the polypeptide. For example, use of certain yeast strains for IGF-1 expression results in the addition of oligosaccharides on a serines or threonines. See, e.g., US Patent No. 5,273,966.

Addition of Poly(ethylene glycol) Conjugation to poly(ethylene glycol) (PEG; pegylation) have proven to be beneficial in prolonging the half-life of therapeutic proteins drugs. It is expected that pegylation of the IGF precursor polypeptides of the invention may result in similar pharmaceutical advantages. Methods of pegylation of IGF-1 are well known in the art. See, for example, US Patent Application Publication 2006/0154865, which describes the beneficial properties of lysine-monopegylated IGF-1. Such lysine-monopegylation can be adapted for the precursor IGF polypeptides of the invention. In addition, pegylation can be achieved in any part of a polypeptide of the invention by the introduction of a nonnatural amino acid. Certain nonnatural amino acids can be introduced by the technology described in Deiters et al., J Am Chem Soc 125:11782-11783, 2003; Wang and Schultz, Science 301:964-967, 2003; Wang et al., Science 292:498-500, 2001; Zhang et al., Science 303:371-373, 2004 or in US Patent No. 7,083,970. Briefly, some of these expression systems involve site-directed mutagenesis to introduce a nonsense codon, such as an amber TAG, into the open reading frame encoding a polypeptide of the invention. Such expression vectors are then introduced into a host that can

utilize a tRNA specific for the introduced nonsense codon and charged with the nonnatural amino acid of choice. Particular nonnatural amino acids that are beneficial for purpose of conjugating moieties to the polypeptides of the invention include those with acetylene and azido side chains. The IGF precursor polypeptides containing these novel amino acids can then be pegylated at these chosen sites in the protein. In addition, such pegylated IGF molecules without the E-peptide are also useful as therapeutics.

Multimers of E-Peptides In certain pharmacological contexts, it is beneficial to increase the size of a peptide or protein drug to ensure that the drug remains on one side of the blood- brain barrier or the other. Since mature IGF molecules are relatively short peptides, even if the E-peptide remains attached, it can be beneficial to increase the size of the polypeptides of the invention. One means of doing so is to provide multimers of E-peptides at the C-terminus of the IGF precursor polypeptide, as illustrated in certain Examples described below.

C-Terminal Deletion of E-Peptides It is suspected that the free cysteine at position 81 of Eb may result in homodimerization or other effects that, when present in the polypeptides of the invention, might lead to lower activity drugs. Thus, deletion or mutation of C81 in Eb can optimize drug activity. In a particular example, deletion of the last seven amino acids of Eb (i.e., amino acids 81-87) is beneficial.

Other Mutations or Modifications Additional mutations or modifications of IGF that can be incorporated into the IGF precursor polypeptides of the invention are described in US Patent No. 5,077,276; and US Patent Application Publication Nos. 2005/0287151, 2006/0211606, and 2006/0166328.

The invention should be construed, in addition to human IGF-1 and IGF-2, to include all known and unknown non-human animal precursor IGF-1 or IGF-2 sequences containing substantially its E-peptide wherein the normal cleavage of the E-peptide is avoided or reduced according to modifications of the present invention.

The preferred type of IGF to be used depends upon the species of the subject being treated.

It is preferred that the IGF is species-matched, for example, when a cow is being treated, the preferred type of IGF is bovine IGF.

Although all forms of IGF are likely to have an effect in different subjects due to the high sequence homologies, species matching will avoid potential adverse immunological complications stemming from the induction of an immune response to an IGF from a different species.

In one embodiment of the invention, modified non-human animal precursor IGF-1 sequences are provided.

Preferred are precursor IGF-1 sequences containing substantially its E-peptide wherein the normal cleavage of the E-peptide is avoided or reduced according to modifications of the present invention from a vertebrate animal.

For example, such sequences include but are not limited to sequences from a mouse, rat, cow, pig, horse, sheep, goat, bird, dog, cat, fish and the like, from any source whether natural, synthetic, or recombinant.

In another embodiment of the invention, modified non-human animal precursor IGF-2 sequences are provided.

Preferred are precursor IGF-2 sequences containing substantially its E-peptide wherein the normal cleavage of the E-peptide is avoided or reduced according to modifications of the present invention from a vertebrate animal.

For example, such sequences include but are not limited to sequences from a mouse, rat, cow, pig, horse, sheep, goat, bird, dog, cat, fish and the like from any source, whether natural, synthetic, or recombinant.

Therapeutic Use of IGF Precursor Polypeptides

Indications The invention also includes the use of an IGF precursor polypeptide of the invention in the manufacture of a medicament for the treatment or prevention of a musculoskeletal disease. In addition, the invention includes use of IGF precursor polypepides to increase muscle or bone mass in an individual, whether or not such an individual is at risk for or has a musculoskeletal disease.

In particular, the musculoskeletal disease can be muscle atrophy. There are many causes of muscle atrophy, including as a result of treatment with a glucocorticoid such as cortisol, dexamethasone, betamethasone, prednisone, methylprednisolone, or prednisolone. The muscle atrophy can also be a result of denervation due to nerve trauma or a result of degenerative, metabolic, or inflammatory neuropathy (e.g., Guillian-Barré syndrome, peripheral neuropathy, or exposure to environmental toxins or drugs). In addition, the muscle atrophy can be a result of an adult motor neuron disease, infantile spinal muscular atrophy, juvenile spinal muscular atrophy, autoimmune motor neuropathy with multifocal conductor block, paralysis due to stroke or spinal cord injury, skeletal immobilization due to trauma, prolonged bed rest, voluntary inactivity, involuntary inactivity, metabolic stress or nutritional insufficiency, cancer, AIDS, fasting, rhabdomyolysis, a thyroid gland disorder, diabetes, benign congenital hypotonia, central core disease, nemalene myopathy, myotubular (centronuclear) myopathy, burn injury, chronic obstructive pulmonary disease, liver disease, sepsis, renal failure, congestive heart failure, or ageing.

The musculoskeletal disease can also be a muscular dystrophy syndrome, such as Duchenne, Becker, myotonic, fascioscapulohumeral, Emery-Deifuss, oculopharyngeal, scapulohumeral, limb girdle, a congenital muscular dystrophy, or hereditary distal myopathy. The musculoskeletal disease can also be osteoporosis, a bone fracture, short stature, or dwarfism.

IGF-1 is suggested as a treatment for insulin-insensitive diabetes, since IGF-1 can also bind heterodimers of IGF-1 receptor and insulin receptor. Accordingly, the polypeptides of the invention can be used to treat diabetes.

IGF-1 is neurotrophic and increases survival of neurons. It has been suggested that IGF-1 can be used to treat instances of motor-neuron death such as seen in amyotrophic lateral sclerosis (ALS), brain atrophy, ageing, and dementia. Accordingly, the polypeptides of the invention can be used to treat conditions associated with neuronal death, such as ALS, brain atrophy, or dementia.

IGF-1 increases both white and red blood cell populations and has an additive effect to administration of erythropoietin. Accordingly, the polypeptides of the invention can be used to treat anemia.

Since IGF-1 and IGF-2 are ubiquitous and essential regulators of cell division and vertebrate growth, they may be advantageously used in a variety of veterinary methods to exogenously enhance or maintain growth in an animal. Some examples include, but are not limited to:

(i) enhancing rate and/or extent of growth in an animal, for example, enhancing muscle growth in swine, cattle, poultry and fish;

(ii) enhancing the efficiency of their conversion of feed into body tissue (lean to fat ratio), for example, in swine, cattle, sheep, poultry and fish; and

(iii) enhancing milk production in lactating animals, for example, dairy cattle, sheep, goats.

Other veterinary therapeutic applications include, but are not limited to:

(iv) treating animal wasting symptoms associated with cachexia, trauma or other consumption diseases, for example, in companion animals such as dogs, cats, and horses; and

(v) treating lactating animals for improvement in neonatal health, for example, lactating sows for improvement in neonatal performance.

Methods of Administration The polypeptides of the invention can be delivered in a variety of ways, including the use of gene delivery vehicles. Methods known in the art for the therapeutic delivery of agents such as proteins or nucleic acids can be used for the therapeutic delivery of a polypeptide of the invention, e.g., cellular transfection, gene therapy, direct administration with a delivery vehicle, or pharmaceutically acceptable carrier, indirect delivery by providing recombinant cells containing a nucleic acid encoding the polypeptide.

Various delivery systems are known and can be used to administer the polypeptide of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the protein, receptor-mediated endocytosis (see, e.g., Wu and Wu, J Biol Chem 262:4429-4432, 1987), construction of a nucleic acid as part of a retroviral, adeno-associated viral, adenoviral, poxviral (e.g., avipoxviral, particularly fowlpoxviral) or other vector, etc. Methods of introduction can be enteral or parenteral and include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, pulmonary, intranasal, intraocular, epidural, and oral routes. The polypeptides can be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, e.g., by injection, by means of a catheter, or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, fibers, or commercial skin substitutes.

In another embodiment, the active agent can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533, 1990). In yet another embodiment, the active agent can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used (see Howard et al., J Neurosurg 71:105, 1989). In another embodiment where the active agent of the invention is a nucleic acid encoding a polypeptide of the invention, the nucleic acid can be administered in vivo to promote expression

of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see, for example, US Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868, 1991), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

Cellular Transfection and Gene Therapy The present invention encompasses the use of nucleic acids encoding polypeptides of the invention for transfection of cells in vitro and in vivo. These nucleic acids can be inserted into any of a number of well-known vectors for transfection of target cells and organisms. The nucleic acids are transfected into cells ex vivo and in vivo, through the interaction of the vector and the target cell. The compositions are administered (e.g., by injection into a muscle) to a subject in an amount sufficient to elicit a therapeutic response.

In another aspect, the invention provides a method of treating a target site, i.e., a target cell or tissue, in a human or other animal including transfecting a cell with a nucleic acid encoding a polypeptide of the invention, wherein the nucleic acid includes an inducible promoter operably linked to the nucleic acid encoding the targeting fusion polypeptide. For gene therapy procedures in the treatment or prevention of human disease, see for example, Van Brunt Biotechnology 6:1149-1154, 1998.

Combination Therapies In numerous embodiments, the polypeptides of the present invention can be administered in combination with one or more additional compounds or therapies. For example, multiple polypeptides can be co-administered in conjunction with one or more therapeutic compounds. The combination therapy may encompass simultaneous or alternating administration. In addition, the combination may encompass acute or chronic administration. The polypeptides of the invention can be administered in combination with anabolic agents such as testosterone or specific androgen receptor modulators (SARMs). Additional anabolic agents include growth hormone (GH) or molecules that induce GH release. Ghrelin is particularly

useful in a combination therapy for cachexia, since Ghrelin can cause an increase in appetite. In a similar vein, the polypeptides of the invention can be combined with protein supplements to increase anabolism, or combined with physical therapy or exercise to increase body weight. Any molecule that inhibits myostatin is also a candidate for combination therapy.

Pharmaceutical Compositions. The present invention also provides pharmaceutical compositions comprising a IGF precursor protein of the invention and a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals or humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin, et al. (Mack Publishing Company; 13th Edition, January 1, 1965).

In some embodiments, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water

for injection or saline can be provided so that the ingredients can be mixed prior to administration.

The polypeptides of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of a polypeptide of the invention which will be effective in the treatment of a condition or disease can be determined by standard clinical techniques based on the present description. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each subject's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-5000 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems. In particular, a possible dosage regimen can be about 60 to 120 μg/kg body weight, subcutaneous injection, twice daily.

Veterinary Uses

In addition to the aforementioned methods of administration in humans, there may be additional considerations for veterinary administration.

The dosage may differ when administered to a healthy animal versus those animals suffering from a disease. An assessment of the appropriate dosage can easily be made by those skilled in the art using assays known in the art, for example, the myoblast proliferation assay (Example 79) or the mammary epithelial tissue assay (Example 80) as described below. General assays to measure IGF are also known in the art, such as those in Example 81.

Those skilled in the art will recognize that some species of animal exhibit seasonal fertility influenced by the length of the photoperiod. Any embodiment of a veterinary method or use may optionally include starting the treatment method at a specific time within the animal's reproductive cycle in order to achieve the desired effect. Those skilled in the art will know that reproductive status and cycle can easily be determined, and, if desired, synchronized by the use of an appropriate regimen.

When used for veterinary indications, in addition to methods previously mentioned for human use, the IGF-1 or IGF-2 peptide of the present invention can also be used as an oral drench, or a supplement to oral or solid feeds for animals.

The invention is further described but not limited by the following Examples.

Examples

Example 1

A DNA expression vector encoding the hIGF-1-Ea precursor polypeptide containing the following modifications was constructed: deletion of G1, deletion of P2, and deletion of E3; mutation of R37 to A; and deletion of R71 and deletion of S72. These mutations are sometimes referred to as "3mut" throughout the present disclosure. This results in the following secreted protein sequence:

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkevhlknasr gsagnknyrm (SEQ ID NO:8)

Cos7 cells (available from ATCC) were maintained in DMEM containing 10% fetal bovine serum, 100U/ml penicillin, and 100 µg/ml streptomycin and plated at a density of 1 x 10^6 cells per 10-cm plate. These cell cultures were transfected with 8 µg of expression plasmid using Fugene (Roche) according to manufacturer's instructions. Twenty-four hours post-transfection, cells were washed once and cultured in serum-free medium for 48 hours. Supernatants were collected and stored at -80°C.

In order to assess polypeptide stability in human serum, supernatants collected from the Cos7 cells transfected with wild-type (wt) hIGF-1Ea, and hIGF-1Ea3mut were incubated for 16 hours at 37°C either in the absence or presence of 10% human serum (Sigma). Samples were separated by 18% SDS-PAGE, and imunoblotting was performed using goat polyclonal antibody to human IGF-1. The results in Fig. 1A indicate that, while the wt hIGF-1Ea was substantially degraded after incubation with serum for 16 hours, the hIGF-1Ea3mut was stabilized. Densitometry indicated that the ratio of uncleaved to cleaved IGF-1 was about 1:6.2, while the ratio for hIGF-1Ea3mut was about 1:0.68, showing that these mutations result in a stabilized polypeptide.

To confirm that the hIGF-1Ea3mut was able to signal through the IGF-1R, AKT phosphorylation of cells in contact with the polypeptide was measured. C2C12 were purchased from ATCC and maintained in Dulbecco's modified Eagle's medium (DMEM) with high glucose (Invitrogen) containing 10% fetal bovine serum (AMIMED), 100U/ml penicillin (Invitrogen), 100 µg/ml streptomycin (Invitrogen) and 2 mM glutamine (Invitrogen). For analysis of AKT phosphorylation, the C2C12 cells were plated at a density of 0.15×10^6 cells per well of a 6-well plate and were cultured in growth medium for 72 hours. Cells were starved for four hours in serum-free medium and then stimulated with different ligands at 37°C for 30 minutes. Cells were lysed with PhosphoSafe buffer (Cell Signaling) containing various protease inhibitors and cleared by centrifugation at 14,000 x g for 15 minutes at 4°C. AKT phosphorylation and total AKT levels were analyzed by ELISA using PathScan phospho AKT (Ser473) sandwich ELISA kit and Pathscan™ AKT sandwich ELISA kit (Cell Signaling), respectively. The AKT phosphorylation results are summarized in Fig. 2A, which indicate that the hIGF-1Ea3mut was able to activate the IGF-1R cellular pathway to a similar extent as the long-R3-IGF-1 positive control reagent and the recombinant IGF-1. In addition, the data in Fig. 5 directly shows that hIGF-1Ea3mut led to AKT phosphorylation.

Next, to ensure that the receptor specificity of the hIGF-1Ea3mut remained with the IGF-1R, various ligands were added to cultures of NIH3T3 overexpressing either IGF-1R or insulin receptor (InsR). These cells were cultured under the same conditions as described above for Cos7 cells. For analysis of IGF-1R and InsR phosphorylation, NIH3T3-IGF1R and NIH3T3-

InsR cells were plated at a density of 0.2 x 10^6 cells per well of a 6-well plate and were cultured in growth medium for 24 hours. Cells were starved for 18 hours in serum-free medium and then stimulated with different ligands at 37°C for 10 minutes. Cells were lysed as described above for the AKT experiment, and IGF-1R and InsR phosphorylation levels were analyzed by ELISA using DuoSet IC human phosphor-IGF1R and –InsR ELISA kit (R&D Systems). The results summarized in Figs. 3A and 3B indicate that this IGF-1 precursor polypeptide retains specificity for the IGF-1 receptor and should bind to the related insulin receptor with low affinity.

Example 2

A DNA expression vector encoding the hIGF-1-Eb precursor polypeptide containing the following mutations was constructed: deletion of G1, deletion of P2, and deletion of E3; mutation of R37 to A; and deletion of R71 and deletion of S72 (i.e., the "3mut"). This results in the following secreted protein sequence:

tlcgaelvdalqfvcgdrgfyfnkptgygsssarapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnk ntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:9)

The polypeptide was assayed in accordance with the procedures described in Example 1 above. Fig. 1B and use of densitometry indicated that the ratio of uncleaved to cleaved IGF-1 was about 1:9, while the ratio for hIGF-1Eb3mut was about 1:1, showing that these modifications result in a stabilized polypeptide. Fig. 2B indicates that the hIGF-1Eb3mut was able to activate the IGF-1R cellular pathway to a similar extent as the long-R3-IGF-1 positive control reagent and the recombinant IGF-1. In addition, the data in Fig. 5 directly shows that hIGF-1Eb3mut led to AKT phosphorylation. The results summarized in Figs. 3C and 3D indicate that this IGF-1 precursor polypeptide retains specificity for the IGF-1 receptor and should bind to the related insulin receptor with low affinity.

Example 3

A DNA expression vector encoding the hIGF-1-Ec precursor polypeptide containing the following mutations was constructed: deletion of G1, deletion of P2, and deletion of E3;

mutation of R37 to A; and deletion of R71 and deletion of S72 (i.e., the "3mut"). This results in the following secreted protein sequence:

tlcgaelvdalqfvcgdrgfyfnkptgygsssrapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnkn tksqrrkgstfeerk (SEQ ID NO:10)

The polypeptide was assayed in accordance with the procedures described in Example 1 above. Fig. 1C and use of densitometry indicated that the ratio of uncleaved to cleaved IGF-1 was about 1:5, while the ratio for hIGF-1Ec3mut was about 1:0.96, showing that these modifications result in a stabilized polypeptide. Fig. 2D indicates that the hIGF-1Ec3mut was able to activate the IGF-1R cellular pathway to a similar extent as the long-R3-IGF-1 positive control reagent and the recombinant IGF-1. In addition, the data in Fig. 5 directly shows that hIGF-1Ec3mut led to AKT phosphorylation. The results summarized in Figs. 4A and 4B indicate that this IGF-1 precursor polypeptide retains specificity for the IGF-1 receptor and should bind to the related insulin receptor with low affinity.

Example 4

A DNA expression vector encoding the hIGF-1-Eab chimeric precursor polypeptide containing the following modifications to the hIGF-1-Eb peptide was constructed: deletion of G1, deletion of P2, and deletion of E3; mutation of R37 to A; deletion of R71 and deletion of S72 (i.e., the "3mut"); and insertion of Ea amino acids 93 to 102 between amino acids 95 and 96 of Eb. The insertion creates a putative N-linked glycosylation signal at N92. This results in the following secreted protein sequence:

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnk nasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:11)

The polypeptide was assayed in accordance with some of the procedures described in Example 1 above. Fig. 2C indicates that the hIGF-1Eab3mut was able to activate the IGF-1R cellular pathway to a similar extent as the long-R3-IGF-1 positive control reagent and the recombinant

IGF-1. The results summarized in Figs. 4C and 4D indicate that this IGF-1 precursor polypeptide retains specificity for the IGF-1 receptor and does not activate the insulin receptor.

Example 5

A DNA expression vector encoding the hIGF-1-Eb multimer precursor polypeptide containing the following mutations was constructed: deletion of G1, deletion of P2, deletion of E3, deletion of R36, deletion of R37, deletion of R71, deletion of S72, deletion of the last seven C-terminal amino acids of Eb; and insertion to the C-terminus of this precursor of two additional Eb peptides both without the R71 and S72 and last seven C-terminal amino acids and a fourth and final Eb peptide without the R71 and S72. Fig. 6A shows a schematic drawing of this construct. This results in the following secreted protein sequence:

tlcgaelvdalqfvcgdrgfyfnkptgygsssapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnknt ksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaersvraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeq kegteaslqirgkkkeqrreigsrnaersvraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrr eigsrnaersvraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:12)

This polypeptide was subjected to an AKT phosphorylation assay as described in Example 1. Fig. 5 indicates that this hIGF-1-Eb multimer was able to signal through the IGF-1R pathway.

Example 6

A hIGF-1-Eb precursor polypeptide of the invention as shown schematically in Fig. 6B can be expressed. This construct contains the following modifications: deletion of G1, deletion of P2, deletion of E3, deletion of R36, deletion of R37, deletion of R71, deletion of S72; and the insertion of Ea amino acids 93-102 between amino acids 95 and 96 of Eb, thereby creating an N-linked glycosylation site at position N92 and N100. This results in the following secreted protein sequence:

tlcgaelvdalqfvcgdrgfyfnkptgygsssapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnkna srgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:13)

Example 7

A hIGF-2-E precursor polypeptide of the invention having the following modifications can be expressed: deletion of P4, deletion of S5, and deletion of E6; mutation of R38 to A; and deletion of R68 and deletion of D69. This results in the following secreted protein sequence:

ayrtlcggelvdtlqfvcgdrgfyfsrpasrvsrasrgiveeccfrscdlalletycatpaksevstpptvlpdnfprypvgkffqydtwkq stqrlrrglpallrarrghvlakeleafreakrhrplialptqdpahggappemasnrk (SEQ ID NO:14)

Example 8

A hIGF-1-Ea precursor polypeptide of the invention having the following mutations can be expressed: deletion of G1 and deletion of P2; mutation of E3 to X where X is a nonnatural amino acid that is pegylated; mutation of R37 to A; and deletion of R71 and deletion of S72. This results in the following secreted protein sequence:

Xtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkevhlkna srgsagnknyrm (SEQ ID NO:15)

Examples 9-78 (Δ = deletion)

9) hIGF-1-Ea: ΔG1, ΔP2, ΔE3; R36A; ΔR71

tlcgaelvdalqfvcgdrgfyfnkptgygsssarapqtgivdeccfrscdlrrlemycaplkpaksasvraqrhtdmpktqkevhlknas rgsagnknyrm (SEQ ID NO:16)

10) hIGF-1-Ea: ΔG1, ΔP2, ΔE3; R36A; ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssarapqtgivdeccfrscdlrrlemycaplkpaksarvraqrhtdmpktqkevhlknas rgsagnknyrm (SEQ ID NO:17)

10) hIGF-1-Ea: ΔG1, ΔP2, ΔE3; R36A; ΔR71, ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssarapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkevhlknasr gsagnknyrm (SEQ ID NO:18)

11) hIGF-1-Ea: ΔG1, ΔP2, ΔE3; R37A; ΔR71

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksasvraqrhtdmpktqkevhlknas rgsagnknyrm (SEQ ID NO:19)

12) hIGF-1-Ea: ΔG1, ΔP2, ΔE3; R37A; ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksarvraqrhtdmpktqkevhlknas rgsagnknyrm (SEQ ID NO:20)

13) hIGF-1-Ea: ΔG1, ΔP2, ΔE3, ΔR37; ΔR71

tlcgaelvdalqfvcgdrgfyfnkptgygsssrapqtgivdeccfrscdlrrlemycaplkpaksasvraqrhtdmpktqkevhlknasr gsagnknyrm (SEQ ID NO:21)

14) hIGF-1-Ea: ΔG1, ΔP2, ΔE3, ΔR37; ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssrapqtgivdeccfrscdlrrlemycaplkpaksarvraqrhtdmpktqkevhlknasr gsagnknyrm (SEQ ID NO:22)

15) hIGF-1-Ea: ΔG1, ΔP2, ΔE3; ΔR37; ΔR71, ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssrapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkevhlknasrg sagnknyrm (SEQ ID NO:23)

16) hIGF-1-Eb: ΔG1, ΔP2, ΔE3; R36A; ΔR71

tlcgaelvdalqfvcgdrgfyfnkptgygsssarapqtgivdeccfrscdlrrlemycaplkpaksasvraqrhtdmpktqkyqppstn kntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:24)

17) hIGF-1-Eb: ΔG1, ΔP2, ΔE3; R36A; ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssarapqtgivdeccfrscdlrrlemycaplkpaksarvraqrhtdmpktqkyqppstn kntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:25)

18) hIGF-1-Eb: ΔG1, ΔP2, ΔE3; R37A; ΔR71

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksasvraqrhtdmpktqkyqppstn kntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:26)

19) hIGF-1-Eb: ΔG1, ΔP2, ΔE3; R37A; ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksarvraqrhtdmpktqkyqppstn kntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:27)

20) hIGF-1-Eb: ΔG1, ΔP2, ΔE3; R37A; ΔR71, ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnk ntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:28)

21) hIGF-1-Eb: ΔG1, ΔP2, ΔE3, ΔR37; ΔR71

tlcgaelvdalqfvcgdrgfyfnkptgygsssrapqtgivdeccfrscdlrrlemycaplkpaksasvraqrhtdmpktqkyqppstnk ntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:29)

22) hIGF-1-Eb: ΔG1, ΔP2, ΔE3, ΔR37; ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssrapqtgivdeccfrscdlrrlemycaplkpaksarvraqrhtdmpktqkyqppstnk ntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:30)

23) hIGF-1-Eb: ΔG1, ΔP2, ΔE3, ΔR37; ΔR71, ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssrapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnkn tksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:31)

24) hIGF-1-Ec: ΔG1, ΔP2, ΔE3; R36A; ΔR71

tlcgaelvdalqfvcgdrgfyfnkptgygsssarapqtgivdeccfrscdlrrlemycaplkpaksasvraqrhtdmpktqkyqppstn kntksqrrkgstfeerk (SEQ ID NO:32)

25) hIGF-1-Ec: ΔG1, ΔP2, ΔE3; R36A; ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssarapqtgivdeccfrscdlrrlemycaplkpaksarvraqrhtdmpktqkyqppstn kntksqrrkgstfeerk (SEQ ID NO:33)

26) hIGF-1-Ec: ΔG1, ΔP2, ΔE3; R36A; ΔR71, ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssarapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnk ntksqrrkgstfeerk (SEQ ID NO:34)

27) hIGF-1-Ec: ΔG1, ΔP2, ΔE2; R37A; ΔR71

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksasvraqrhtdmpktqkyqppstn kntksqrrkgstfeerk (SEQ ID NO:35)

28) hIGF-1-Ec: ΔG1, ΔP2, ΔE3; R37A; ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksarvraqrhtdmpktqkyqppstn
kntksqrrkgstfeerk (SEQ ID NO:36)

29) hIGF-1-Ec: ΔG1, ΔP2, ΔE3; R37A; ΔR71, ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnk
ntksqrrkgstfeerk (SEQ ID NO:37)

30) hIGF-1-Ec: ΔG1, ΔP2, ΔE3, ΔR37, ΔR71

tlcgaelvdalqfvcgdrgfyfnkptgygsssrapqtgivdeccfrscdlrrlemycaplkpaksasvraqrhtdmpktqkyqppstnk
ntksqrrkgstfeerk (SEQ ID NO:38)

31) hIGF-1-Ec: ΔG1, ΔP2, ΔE3, ΔR37, ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssrapqtgivdeccfrscdlrrlemycaplkpaksarvraqrhtdmpktqkyqppstnk
ntksqrrkgstfeerk (SEQ ID NO:39)

32) hIGF-1-Eab: ΔG1, ΔP2, ΔE3; R36A; ΔR71; insertion of Ea aa 93-102 between aa 95 and 96 of Eb (i.e., "Eab")

tlcgaelvdalqfvcgdrgfyfnkptgygsssarapqtgivdeccfrscdlrrlemycaplkpaksasvraqrhtdmpktqkyqppstn
knasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:40)

33) hIGF-1-Eab: ΔG1, ΔP2, ΔE3; R37A; ΔR71

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksasvraqrhtdmpktqkyqppstn
knasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:41)

34) hIGF-1-Eab: ΔG1, ΔP2, ΔE3, ΔR37, ΔR71

tlcgaelvdalqfvcgdrgfyfnkptgygsssrapqtgivdeccfrscdlrrlemycaplkpaksasvraqrhtdmpktqkyqppstnk nasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:42)

35) hIGF-1-Eab: ΔG1, ΔP2, ΔE3; R36A; ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssarapqtgivdeccfrscdlrrlemycaplkpaksarvraqrhtdmpktqkyqppstn knasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:43)

36) hIGF-1-Eab: ΔG1, ΔP2, ΔE3; R37A; ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksarvraqrhtdmpktqkyqppstn knasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:44)

37) hIGF-1-Eab: ΔG1, ΔP2, ΔE3, ΔR37, ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssrapqtgivdeccfrscdlrrlemycaplkpaksarvraqrhtdmpktqkyqppstnk nasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:45)

38) hIGF-1-Eab: ΔG1, ΔP2, ΔE3; R36A; ΔR71, ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssarapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnk nasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:46)

39) hIGF-1-Eab: ΔG1, ΔP2, ΔE3, ΔR37, ΔR71, ΔS72

tlcgaelvdalqfvcgdrgfyfnkptgygsssrapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnkn asrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:47)

40) hIGF-1-Ea: ΔP2, ΔE3; R37A; ΔR71, ΔS72

gtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkevhlkna srgsagnknyrm (SEQ ID NO:48)

41) hIGF-1-Eb: ΔP2, ΔE3; R37A; ΔR71, ΔS72

gtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstn kntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:49)

42) hIGF-1-Eb multimer: (ΔG1, ΔP2, ΔE3; R37A)-3xEb(ΔR71, ΔS72, ΔC-term 7 aa)-Eb(ΔR71, ΔS72)

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnk ntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeq kegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrrei gsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:50)

43) hIGF-1-Eb multimer: (ΔP2, ΔE3; R37A)-3xEb(ΔR71, ΔS72, ΔC-term 7 aa)-Eb(ΔR71, ΔS72)

gtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstn kntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpgge qkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrr eigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:51)

44) hIGF-1-Ec: ΔP2, ΔE3; R37A; ΔR71, ΔS72

gtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstn kntksqrrkgstfeerk (SEQ ID NO:52)

45) hIGF-1-Ea: ΔE3; R37A; ΔR71, ΔS72

gptlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkevhlkn asrgsagnknyrm (SEQ ID NO:53)

46) hIGF-1-Eb: ΔE3; R37A; ΔR71, ΔS72

gptlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppst nkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:54)

47) hIGF-1-Eb multimer: (ΔG1, ΔP2, ΔE3; R37A)-3xEb(ΔR71, ΔS72, ΔC-term 7 aa)-Eb(ΔR71, ΔS72)

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnk ntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeq kegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrrei gsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:55)

48) hIGF-1-Eb multimer: (ΔE3; R37A)-3xEb(ΔR71, ΔS72, ΔC-term 7 aa)-Eb(ΔR71, ΔS72)

gptlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppst nkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpg geqkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeq rreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:56)

49) hIGF-1-Ec: ΔE3; R37A; ΔR71, ΔS72

gptlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppst nkntksqrrkgstfeerk (SEQ ID NO:57)

50) hIGF-1-Ea: ΔE3; R37A; ΔR71, ΔS72

gptlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkevhlkn asrgsagnknyrm (SEQ ID NO:58)

51) hIGF-1-Eb: ΔE3; R37A; ΔR71, ΔS72

gptlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppst nkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:59)

52) hIGF-1-Ec: ΔE3; R37A; ΔR71, ΔS72

gptlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppst nkntksqrrkgstfeerk (SEQ ID NO:60)

53) hIGF-1-Eab: ΔE3; R37A; Δ R71, ΔS72

gptlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppst nknasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:61)

54) hIGF-1-Eb multimer: (ΔE3; R37A)-3xEb(ΔR71, ΔS72, ΔC-term 7 aa)-Eb(ΔR71, ΔS72)

gptlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppst nkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpg geqkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeq rreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:62)

55) hIGF-1-Ea: E3A; R37A; ΔR71, ΔS72

gpatlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkevhlk nasrgsagnknyrm (SEQ ID NO:63)

56) hIGF-1-Eb: E3A; R37A; ΔR71, ΔS72

gpatlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqpps tnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:64)

57) hIGF-1-Ec: E3A; R37A; ΔR71, ΔS72

gpatlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqpps tnkntksqrrkgstfeerk (SEQ ID NO:65)

58) hIGF-1-Eab: E3A; R37A; ΔR71, ΔS72

gpatlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqpps tnknasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:66)

59) hIGF-1-Eb multimer: (E3A; R37A)-3xEb(ΔR71, ΔS72, ΔC-term 7 aa)-Eb(ΔR71, ΔS72)

gpatlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqpps tnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpg geqkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeq rreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:67)

60) hIGF-1-Ea: ΔP2, ΔE3; R37A; ΔR71, ΔS72

gtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkevhlkna srgsagnknyrm (SEQ ID NO:68)

61) hIGF-1-Eb: ΔP2, ΔE3; R37A; ΔR71, ΔS72

gtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstn knktksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:69)

62) hIGF-1-Ec: ΔP2, ΔE3; R37A; ΔR71, ΔS72

gtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstn knktksqrrkgstfeerk (SEQ ID NO:181)

63) hIGF-1-Eab: ΔP2, ΔE3; R37A; ΔR71, ΔS72

gtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstn knasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:70)

64) hIGF-1-Eb multimer: (ΔP2, ΔE3; R37A)-3xEb(ΔR71, ΔS72, ΔC-term 7 aa)-Eb(ΔR71, ΔS72)

gtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstn knktksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpgge qkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrr eigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:71)

65) hIGF-1-Eb: ΔG1, ΔP2; E3X; R37A; ΔR71, ΔS72

Xtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstn knktksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:72)

66) hIGF-1-Ec: ΔG1, ΔP2; E3X; R37A; ΔR71, ΔS72

Xtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstn knktksqrrkgstfeerk (SEQ ID NO:73)

67) hIGF-1-Eab: ΔG1, ΔP2; E3X; R37A; ΔR71, ΔS72

Xtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstn knasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:74)

68) hIGF-1-Eb multimer: (ΔG1, ΔP2; E3X; R37A)-3xEb(ΔR71, ΔS72, ΔC-term 7 aa)-Eb(ΔR71, ΔS72)

Xtlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstn knktksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpgge qkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrr eigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:75)

69) hIGF-1-Ea: ΔG1, ΔP2, ΔE3; R37A; ΔR71; S72X

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksaXvraqrhtdmpktqkevhlkna srgsagnknyrm (SEQ ID NO:76)

70) hIGF-1-Eb: ΔG1, ΔP2, ΔE3; R37A; ΔR71; S72X

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksaXvraqrhtdmpktqkyqppstn knktksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:77)

71) hIGF-1-Ec: ΔG1, ΔP2, ΔE3; R37A; ΔR71; S72X

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksaXvraqrhtdmpktqkyqppstn knktksqrrkgstfeerk (SEQ ID NO:78)

72) hIGF-1-Eab: ΔG1, ΔP2, ΔE3; R37A; ΔR71; S72X

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksaXvraqrhtdmpktqkyqppstn knasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:79)

73) hIGF-1-Eb multimer: (ΔG1, ΔP2, ΔE3; R37A)-Eb(ΔR71; S72X; ΔC-term 7 aa)-2xEb(ΔR71, ΔS72, ΔC-term 7 aa)-Eb(ΔR71, ΔS72)

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpakXsavraqrhtdmpktqkyqppstn knktksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpgge qkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrr eigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaecrgkkgk (SEQ ID NO:80)

74) hIGF-1-Ea: ΔG1, ΔP2, ΔE3; R37A; ΔR71, ΔS72; N92X

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkevhlkXas rgsagnknyrm (SEQ ID NO:81)

75) hIGF-1-Eb: ΔG1, ΔP2, ΔE3; R37A; ΔR71, ΔS72; C142X

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnk ntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaeXrgkkgk (SEQ ID NO:82)

76) hIGF-1-Eab: ΔG1, ΔP2, ΔE3; R37A; ΔR71, ΔS72; C151X

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnk nasrgsagnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaeXrgkkgk (SEQ ID NO:83)

78) hIGF-1-Eb multimer (ΔG1, ΔP2, ΔE3; R37A)-3xEb(ΔR71, ΔS72, ΔC-term 7 aa)-Eb(ΔR71, ΔS72; C71X)

tlcgaelvdalqfvcgdrgfyfnkptgygsssraapqtgivdeccfrscdlrrlemycaplkpaksavraqrhtdmpktqkyqppstnk ntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeq kegteaslqirgkkkeqrreigsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrrei gsrnaevraqrhtdmpktqkyqppstnkntksqrrkgwpkthpggeqkegteaslqirgkkkeqrreigsrnaeXrgkkgk (SEQ ID NO:84)

Example 79: Myoblast Proliferation Assay

The myoblast proliferation assay provides a reliable in vitro indicator of IGF activity and is used as a model for factors affecting embryonic myoblasts and adult satellite cells. Factors active in this system behave similarly in primary cultures of myoblasts. The enhancement of myoblast proliferation in vitro by a peptide of this invention indicates its activity in causing increased myoblast proliferation and, therefore, an increase in ultimate myofiber number in utero. In addition, similar enhancement of myoblast proliferation indicates that peptides of this invention can be used to enhance adult muscle hypertrophy, e.g. via stimulation of satellite muscle cell proliferation.

Example 80: Mammary Epithelial Tissue Assay

In lactating animals, the amount of mammary epithelial tissue is a limiting factor in milk production, as these are the cells which produce and secrete milk. Employing in vitro systems, epithelial cells obtained from mammary glands of animals can be stimulated by the modified IGF-1 or IGF-2 of the present invention to proliferate and produce increased quantities of milk constituents. It can further be demonstrated that mammary epithelial cells stimulated to proliferate in one such in vitro cell system can be reimplanted in cleared mammary fat pads and be stimulated to proliferate and/or produce milk in lactating female animals.

Example 81: Measurement of IGF-1 or IGF-2 in Blood or Other Body Fluids

The effective amount of the peptide administered parenterally per dose can be measured by a dose-response curve. For example, modified IGF peptides of the invention can be measured in

the blood or body fluids of the subject to be treated to determine the dosing. Alternatively, one can administer increasing amounts of the peptide to the subject and check the serum levels of the subject for modified IGF-1 and IGF-2. The amount of peptide to be employed can be calculated on a molar basis based on these serum levels of modified IGF-1 or IGF-2.

One method for determining appropriate dosing of the peptide entails measuring an IGF peptide of the invention in a biological fluid such as a body or blood fluid. Measuring such levels can be done by any means, including RIA and ELISA. After measuring IGF levels, the fluid is contacted with the peptide using single or multiple doses. After this contacting step, the IGF levels are re-measured in the fluid. If the fluid IGF levels have fallen by an amount sufficient to produce the desired efficacy for which the molecule is to be administered, then the dose of the molecule can be adjusted to produce maximal efficacy. This method may be carried out in vitro or in vivo. Preferably, this method is carried out in vivo, i.e., after the fluid is extracted from a subject and the IGF levels measured, the peptide herein is administered to the mammal using single or multiple doses (that is, the contacting step is achieved by administration to an animal), and then the IGF levels are re-measured from fluid extracted from the animal.

Another method for determining dosing is to use antibodies to the peptide or another detection method for the peptide in the LIFA format.

Example 82: In Vivo Pharmacokinetics of hIGF-1-Ec 3mut

Adult male mice (n = 3/group) received an intravenous (i.v.) bolus injection of rhIGF-1 at 1 mg/kg, and hIGF-1-Ec 3mut (described in Example 3) at 1.55 mg/kg. Serial blood specimens were collected at 5, 15, 30 and 60 minutes after administration of test material. Serum concentrations of rhIGF-1 and hIGF-1-Ec 3mut were determined by ELISA. This assay is specific for hIGF-1.

Equimolar doses of rhIGF-1 and hIGF-1-Ec 3mut were administered i.v. in mice. The results show significantly higher levels of the hIGF-1-Ec 3mut protein as compared to rhIGF-1 at all examined time points, indicating that the hIGF-1-Ec 3mut is metabolically more stable than the 70 amino acid-long IGF-1.

time (min)	IGF-1-Ec 3mut (nM)	IGF-1 (nM)
5	201.4	54.7
15	65.3	14.3
30	12	2.4
60	0.76	0.2

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 21489-11012 Seq 08-JAN-09 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

<110> Novartis AG

<120> STABILIZED INSULIN-LIKE GROWTH FACTOR
POLYPEPTIDES

<130> 50210

<140> PCT/US07/070468
<141> 2007-06-06

<150> 60/812,349
<151> 2006-06-09

<150> 60/862,244
<151> 2006-10-20

<150> 60/897,187
<151> 2007-01-24

<160> 181

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 70
<212> PRT
<213> Homo sapiens

<220>
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<222> (1)...(70)
<223> Human IGF-1 also called Somatomedin

<400> 1
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
 1   5   10   15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20   25   30
Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35   40   45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50   55   60
Lys Pro Ala Lys Ser Ala
65   70

<210> 2
<211> 35
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> IGF-1 mRNA E-peptide (Ea)

<400> 2
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
 1   5   10   15
Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn Lys Asn
            20   25   30
Tyr Arg Met
        35

<210> 3
<211> 77
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(77)
<223> IGF-1 mRNA E-peptide (Eb)

<400> 3
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
 1   5   10   15
Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys
            20   25   30
Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu
        35   40   45
Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile
    50   55   60
Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
65   70   75

<210> 4
<211> 40
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(40)
<223> IGF-1 mRNA E-peptide (Ec)

<400> 4
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
1 5 10 15
Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys
20 25 30
Gly Ser Thr Phe Glu Glu Arg Lys
35 40

<210> 5
<211> 105
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(105)
<223> Wild type IGF-1 e-peptide (Ea)

<400> 5
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1 5 10 15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
20 25 30
Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
35 40 45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
50 55 60
Lys Pro Ala Lys Ser Ala Arg Ser Val Arg Ala Gln Arg His Thr Asp
65 70 75 80
Met Pro Lys Thr Gln Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly
85 90 95
Ser Ala Gly Asn Lys Asn Tyr Arg Met
100 105

<210> 6
<211> 89
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(89)
<223> E-peptide Human IGF-2

<400> 6
Arg Asp Val Ser Thr Pro Pro Thr Val Leu Pro Asp Asn Phe Pro Arg
1 5 10 15
Tyr Pro Val Gly Lys Phe Phe Gln Tyr Asp Thr Trp Lys Gln Ser Thr
20 25 30
Gln Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala Arg Arg Gly
35 40 45
His Val Leu Ala Lys Glu Leu Glu Ala Phe Arg Glu Ala Lys Arg His
50 55 60
Arg Pro Leu Ile Ala Leu Pro Thr Gln Asp Pro Ala His Gly Gly Ala
65 70 75 80
Pro Pro Glu Met Ala Ser Asn Arg Lys
85

<210> 7
<211> 156
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(156)
<223> IGF-2 precursor to E-peptide in Sequence 6

<400> 7
Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
1 5 10 15
Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Arg Pro Ala
20 25 30
Ser Arg Val Ser Arg Arg Ser Arg Gly Ile Val Glu Glu Cys Cys Phe
35 40 45
Arg Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Thr Pro Ala
50 55 60
Lys Ser Glu Arg Asp Val Ser Thr Pro Pro Thr Val Leu Pro Asp Asn
65 70 75 80
Phe Pro Arg Tyr Pro Val Gly Lys Phe Phe Gln Tyr Asp Thr Trp Lys
85 90 95
Gln Ser Thr Gln Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala
100 105 110
Arg Arg Gly His Val Leu Ala Lys Glu Leu Glu Ala Phe Arg Glu Ala
115 120 125
Lys Arg His Arg Pro Leu Ile Ala Leu Pro Thr Gln Asp Pro Ala His
130 135 140
Gly Gly Ala Pro Pro Glu Met Ala Ser Asn Arg Lys
145 150 155

<210> 8
<211> 100
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(100)
<223> 3MUT HIGF-1 Ea

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Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
35 40 45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
50 55 60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65 70 75 80
Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn Lys
85 90 95
Asn Tyr Arg Met
100

<210> 9
<211> 142
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(142)
<223> 3MUT HIGF-1-EB Precursor

<400> 9
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1   5   10   15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20   25   30
Ala Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35   40   45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50   55   60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65   70   75   80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg
                85   90   95
Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr
            100   105   110
Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu
        115   120   125
Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
    130   135   140

<210> 10
<211> 104
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(104)
<223> 3 MUT HIGF-1 (Ec) Precursor

<400> 10
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1   5   10   15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20   25   30
Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys
        35   40   45
Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys
    50   55   60
Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
65   70   75   80
Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys
                85   90   95
Gly Ser Thr Phe Glu Glu Arg Lys
            100

<210> 11
<211> 152
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(152)
<223> HIGF-1-EAB CHIMERIC PRECURSOR creates CREATES A
PUTATIVE N-LINKED GLYCOSYLATION SIGNAL AT N92 by
deletion

<400> 11
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
35 40 45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
50 55 60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65 70 75 80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser Ala
85 90 95
Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr
100 105 110
His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile
115 120 125
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130 135 140
Glu Cys Arg Gly Lys Lys Gly Lys
145 150

<210> 12
<211> 350
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(350)
<223> HIGF-1-EB MULTIMER

<400> 12
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys Asp
35 40 45
Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys Ser
50 55 60
Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr
65 70 75 80
Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly
85 90 95
Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala
100 105 110
Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly
115 120 125
Ser Arg Asn Ala Glu Arg Ser Val Arg Ala Gln Arg His Thr Asp Met
130 135 140
Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys
145 150 155 160
Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln
165 170 175
Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu
180 185 190
Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Arg Ser Val Arg Ala
195 200 205
Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser
210 215 220
Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr
225 230 235 240

His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile
245 250 255
Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala
260 265 270
Glu Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
275 280 285
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg
290 295 300
Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr
305 310 315 320
Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu
325 330 335
Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
340 345 350

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<213> Homo sapiens

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<222> (1)...(150)
<223> HIGF-1-EB: N-LINKED GLYCOSYLATION SITE AT POSITION
N92 AND N100

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20 25 30
Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys Asp
35 40 45
Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys Ser
50 55 60
Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr
65 70 75 80
Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn
85 90 95
Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro
100 105 110
Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly
115 120 125
Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Cys
130 135 140
Arg Gly Lys Lys Gly Lys
145 150

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<211> 151
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(151)
<223> HIGF-2-E PRECURSOR DELETION OF P4, DELETION OF S5,
AND DELETION OF E6; MUTATION OF R38 TO A; AND
DELETION OF R68 AND DELETION OF D69

<400> 14
Ala Tyr Arg Thr Leu Cys Gly Gly Glu Leu Val Asp Thr Leu Gln Phe
1 5 10 15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Arg Pro Ala Ser Arg Val
20 25 30
Ser Arg Ala Ser Arg Gly Ile Val Glu Glu Cys Cys Phe Arg Ser Cys
35 40 45
Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Thr Pro Ala Lys Ser Glu
50 55 60
Val Ser Thr Pro Pro Thr Val Leu Pro Asp Asn Phe Pro Arg Tyr Pro
65 70 75 80
Val Gly Lys Phe Phe Gln Tyr Asp Thr Trp Lys Gln Ser Thr Gln Arg
85 90 95
Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala Arg Arg Gly His Val
100 105 110
Leu Ala Lys Glu Leu Glu Ala Phe Arg Glu Ala Lys Arg His Arg Pro
115 120 125
Leu Ile Ala Leu Pro Thr Gln Asp Pro Ala His Gly Gly Ala Pro Pro
130 135 140
Glu Met Ala Ser Asn Arg Lys
145 150

<210> 15
<211> 101
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(101)
<223> Xaa is non-natural amino acid that is pegylated.

<400> 15
Xaa Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys
1 5 10 15
Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser
20 25 30
Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
35 40 45
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro
50 55 60
Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn
85 90 95
Lys Asn Tyr Arg Met
100

<210> 16
<211> 101
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(101)
<223> HIGF-1-EA:Deletions: at G1, at P2, at E3; R36A;at
R71

<400> 16
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15

Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Ala Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
35 40 45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
50 55 60
Lys Ser Ala Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn
85 90 95
Lys Asn Tyr Arg Met
100

<210> 17
<211> 101
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(101)
<223> HIGF-1-EA: deletions at G1, P2, E3; R36A; S72

<400> 17
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Ala Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
35 40 45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
50 55 60
Lys Ser Ala Arg Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn
85 90 95
Lys Asn Tyr Arg Met
100

<210> 18
<211> 100
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(100)
<223> HIGF-1-EA: deletions at G1, P2, E3; R36A; R71, S72

<400> 18
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Ala Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
35 40 45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
50 55 60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65 70 75 80

Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn Lys
85 90 95
Asn Tyr Arg Met
100

<210> 19
<211> 101
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(101)
<223> HIGF-1-EA: deletions at G1, P2, E3; R37A; R71

<400> 19
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
35 40 45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
50 55 60
Lys Ser Ala Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn
85 90 95
Lys Asn Tyr Arg Met
100

<210> 20
<211> 101
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(101)
<223> HIGF-1-EA: deletions at G1, P2, E3; R37A; S72

<400> 20
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
35 40 45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
50 55 60
Lys Ser Ala Arg Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn
85 90 95
Lys Asn Tyr Arg Met
100

<210> 21
<211> 100
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(100)
<223> HIGF-1-EA: deletions G1, P2, E3, R37; R71

<400> 21
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys
35 40 45
Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys
50 55 60
Ser Ala Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65 70 75 80
Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn Lys
85 90 95
Asn Tyr Arg Met
100

<210> 22
<211> 100
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(100)
<223> HIGF-1-EA: deletions at G1, P2, E3, R37; S72

<400> 22
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys
35 40 45
Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys
50 55 60
Ser Ala Arg Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65 70 75 80
Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn Lys
85 90 95
Asn Tyr Arg Met
100

<210> 23
<211> 99
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(99)
<223> HIGF-1-EA: deletions at G1, P2, E3; R37; R71, S72

<400> 23
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30

```
Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys
        35                  40                  45
Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys
    50                  55                  60
Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
65                  70                  75                  80
Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn Lys Asn
                85                  90                  95
Tyr Arg Met
```

<210> 24
<211> 143
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(143)
<223> HIGF-1-EB: deletions G1, P2, E3; R36A; R71

<400> 24

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Ala Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65                  70                  75                  80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
                85                  90                  95
Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly
            100                 105                 110
Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg
        115                 120                 125
Glu Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
    130                 135                 140
```

<210> 25
<211> 143
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(143)
<223> HIGF-1-EB: deletions at G1, P2, E3; R36A; S72

<400> 25

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Ala Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Arg Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65                  70                  75                  80
```

Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
85 90 95
Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly
100 105 110
Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg
115 120 125
Glu Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
130 135 140

<210> 26
<211> 143
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(143)
<223> HIGF-1-EB: deletions at G1, P2, E3; R37A; R71

<400> 26
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
35 40 45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
50 55 60
Lys Ser Ala Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
85 90 95
Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly
100 105 110
Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg
115 120 125
Glu Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
130 135 140

<210> 27
<211> 143
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(143)
<223> HIGF-1-EB: deletions at G1, P2, E3; R37A; S72

<400> 27
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
35 40 45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
50 55 60
Lys Ser Ala Arg Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
85 90 95

```
Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly
            100                 105                 110
Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg
        115                 120                 125
Glu Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
    130                 135                 140
```

<210> 28
<211> 142
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(143)
<223> HIGF-1-EB: deletions at G1, P2, E3; R37A; R71, S72

<400> 28

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65                  70                  75                  80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg
                85                  90                  95
Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr
            100                 105                 110
Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu
        115                 120                 125
Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
    130                 135                 140
```

<210> 29
<211> 142
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(143)
<223> HIGF-1-EB: deletions at G1, P2, E3, R37; R71

<400> 29

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys
        35                  40                  45
Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys
    50                  55                  60
Ser Ala Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65                  70                  75                  80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg
                85                  90                  95
```

Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr
100 105 110
Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu
115 120 125
Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
130 135 140

<210> 30
<211> 142
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(142)
<223> HIGF-1-EB: deletions at G1, P2, E3, R37; S72

<400> 30
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys
35 40 45
Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys
50 55 60
Ser Ala Arg Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65 70 75 80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg
85 90 95
Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr
100 105 110
Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu
115 120 125
Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
130 135 140

<210> 31
<211> 141
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(141)
<223> HIGF-1-EB: deletions at G1, P2, E3, R37; R71, S72

<400> 31
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys
35 40 45
Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys
50 55 60
Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
65 70 75 80
Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys
85 90 95
Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu
100 105 110

```
Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile
        115                 120                 125
Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
    130                 135                 140
```

<210> 32
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(106)
<223> HIGF-1-EC: deletions at G1, P2, E3; R36A; R71

<400> 32

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Ala Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65                  70                  75                  80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
                85                  90                  95
Arg Lys Gly Ser Thr Phe Glu Glu Arg Lys
            100                 105
```

<210> 33
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(106)
<223> HIGF-1-EC: deletions at G1, P2, E3; R36A; S72

<400> 33

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Ala Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Arg Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65                  70                  75                  80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
                85                  90                  95
Arg Lys Gly Ser Thr Phe Glu Glu Arg Lys
            100                 105
```

<210> 34
<211> 105
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(105)
<223> HIGF-1-EC: deletions at G1, P2, E3; R36A; R71, S72

<400> 34
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1   5   10   15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20   25   30
Ala Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35   40   45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50   55   60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65   70   75   80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg
                85   90   95
Lys Gly Ser Thr Phe Glu Glu Arg Lys
            100   105

<210> 35
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(106)
<223> HIGF-1-EC: deletions at G1, P2, E2; R37A; R71

<400> 35
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1   5   10   15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20   25   30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35   40   45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50   55   60
Lys Ser Ala Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65   70   75   80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
                85   90   95
Arg Lys Gly Ser Thr Phe Glu Glu Arg Lys
            100   105

<210> 36
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(106)
<223> HIGF-1-EC: deletions at G1, P2, E3; R37A; S72

<400> 36
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1   5   10   15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20   25   30

```
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Arg Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65                  70                  75                  80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
                85                  90                  95
Arg Lys Gly Ser Thr Phe Glu Glu Arg Lys
            100                 105
```

<210> 37
<211> 105
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(105)
<223> HIGF-1-EC: deletions at G1, P2, E3; R37A; R71, S72

<400> 37

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65                  70                  75                  80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg
                85                  90                  95
Lys Gly Ser Thr Phe Glu Glu Arg Lys
            100                 105
```

<210> 38
<211> 105
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(105)
<223> HIGF-1-EC: deletions at G1, P2, E3, R37, R71

<400> 38

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys
        35                  40                  45
Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys
    50                  55                  60
Ser Ala Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65                  70                  75                  80
```

```
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg
                85                  90                  95
Lys Gly Ser Thr Phe Glu Glu Arg Lys
            100                 105
```

<210> 39
<211> 105
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(105)
<223> HIGF-1-EC: deletions at G1, P2, E3, R37, S72

<400> 39

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys
        35                  40                  45
Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys
    50                  55                  60
Ser Ala Arg Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65                  70                  75                  80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg
                85                  90                  95
Lys Gly Ser Thr Phe Glu Glu Arg Lys
            100                 105
```

<210> 40
<211> 153
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(153)
<223> HIGF-1-EAB: deletions at G1, P2, E3; R36A; R71;
INSERTION OF EA AA 93-102 BETWEEN AA 95 AND 96 OF
EB (I.E., "EAB")

<400> 40

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Ala Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65                  70                  75                  80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser
                85                  90                  95
Ala Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys
            100                 105                 110
Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln
        115                 120                 125
```

Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn
130 135 140
Ala Glu Cys Arg Gly Lys Lys Gly Lys
145 150

<210> 41
<211> 153
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(153)
<223> HIGF-1-EAB: deletions at G1, P2, E3; R37A; R71

<400> 41
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
35 40 45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
50 55 60
Lys Ser Ala Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser
85 90 95
Ala Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys
100 105 110
Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln
115 120 125
Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn
130 135 140
Ala Glu Cys Arg Gly Lys Lys Gly Lys
145 150

<210> 42
<211> 152
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(152)
<223> HIGF-1-EAB: deletions at G1, P2, E3, R37, R71

<400> 42
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys
35 40 45
Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys
50 55 60
Ser Ala Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65 70 75 80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser Ala
85 90 95
Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr
100 105 110

```
His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile
        115                 120                 125
Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala
    130                 135                 140
Glu Cys Arg Gly Lys Lys Gly Lys
145                 150
```

<210> 43
<211> 153
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(153)
<223> HIGF-1-EAB: deletions at G1, P2, E3; R36A; S72

<400> 43

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Ala Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Arg Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65                  70                  75                  80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser
                85                  90                  95
Ala Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys
            100                 105                 110
Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln
        115                 120                 125
Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn
    130                 135                 140
Ala Glu Cys Arg Gly Lys Lys Gly Lys
145                 150
```

<210> 44
<211> 153
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(153)
<223> HIGF-1-EAB: G1, P2, E3; R37A; S72

<400> 44

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Arg Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65                  70                  75                  80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser
                85                  90                  95
```

```
Ala Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys
            100                 105                 110
Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln
        115                 120                 125
Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn
    130                 135                 140
Ala Glu Cys Arg Gly Lys Lys Gly Lys
145                 150
```

<210> 45
<211> 152
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(152)
<223> HIGF-1-EAB: G1, P2, E3, R37, S72

<400> 45

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys
        35                  40                  45
Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys
    50                  55                  60
Ser Ala Arg Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65                  70                  75                  80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser Ala
                85                  90                  95
Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr
            100                 105                 110
His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile
        115                 120                 125
Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala
    130                 135                 140
Glu Cys Arg Gly Lys Lys Gly Lys
145                 150
```

<210> 46
<211> 152
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(152)
<223> HIGF-1-EAB: G1, P2, E3; R36A; R71, S72

<400> 46

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Ala Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65                  70                  75                  80
```

```
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser Ala
                    85                  90                  95
Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr
               100                 105                 110
His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile
           115                 120                 125
Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala
       130                 135                 140
Glu Cys Arg Gly Lys Lys Gly Lys
145                 150
```

<210> 47
<211> 151
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(151)
<223> HIGF-1-EAB: G1, P2, E3, R37, R71, S72

<400> 47

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys
        35                  40                  45
Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala Lys
    50                  55                  60
Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
65                  70                  75                  80
Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser Ala Gly
                85                  90                  95
Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His
            100                 105                 110
Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg
        115                 120                 125
Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu
    130                 135                 140
Cys Arg Gly Lys Lys Gly Lys
145                 150
```

<210> 48
<211> 101
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(101)
<223> HIGF-1-EA: P2, E3; R37A; R71, S72

<400> 48

```
Gly Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys
 1               5                  10                  15
Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser
            20                  25                  30
Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
        35                  40                  45
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro
    50                  55                  60
```

Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn
85 90 95
Lys Asn Tyr Arg Met
100

<210> 49
<211> 143
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(143)
<223> HIGF-1-EB: P2, E3; R37A; R71, S72

<400> 49
Gly Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys
1 5 10 15
Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser
20 25 30
Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
35 40 45
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro
50 55 60
Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
85 90 95
Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly
100 105 110
Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg
115 120 125
Glu Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
130 135 140

<210> 50
<211> 346
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(346)
<223> HIGF-1-EB MULTIMER: (G1, P2, E3; R37A)-3XEB(R71, S72, C-TERM 7 AA)-EB(R71, S72)

<400> 50
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
35 40 45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
50 55 60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65 70 75 80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg
85 90 95

Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr
100 105 110
Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu
115 120 125
Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln Arg His Thr Asp Met
130 135 140
Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys
145 150 155 160
Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln
165 170 175
Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu
180 185 190
Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln Arg
195 200 205
His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn
210 215 220
Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro
225 230 235 240
Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly
245 250 255
Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Val
260 265 270
Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro
275 280 285
Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro
290 295 300
Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu
305 310 315 320
Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg
325 330 335
Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
340 345

<210> 51
<211> 347
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(347)
<223> HIGF-1-EB MULTIMER: (P2, E3; R37A)-3XEB(R71, S72, C-TERM 7 AA)-EB R71, S72)

<400> 51
Gly Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys
1 5 10 15
Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser
20 25 30
Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
35 40 45
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro
50 55 60
Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
85 90 95
Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly
100 105 110
Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg
115 120 125
Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln Arg His Thr Asp
130 135 140

Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr
145 150 155 160
Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu
165 170 175
Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys
180 185 190
Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln
195 200 205
Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr
210 215 220
Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His
225 230 235 240
Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg
245 250 255
Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu
260 265 270
Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln
275 280 285
Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp
290 295 300
Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser
305 310 315 320
Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser
325 330 335
Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
340 345

<210> 52
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(106)
<223> HIGF-1-EC: P2, E3; R37A; R71, S72

<400> 52
Gly Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys
1 5 10 15
Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser
20 25 30
Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
35 40 45
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro
50 55 60
Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
85 90 95
Arg Lys Gly Ser Thr Phe Glu Glu Arg Lys
100 105

<210> 53
<211> 102
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(102)
<223> HIGF-1-EA: E3; R37A; R71, S72

<400> 53
Gly Pro Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val
1 5 10 15
Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser
20 25 30
Ser Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe
35 40 45
Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys
50 55 60
Pro Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys
65 70 75 80
Thr Gln Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly
85 90 95
Asn Lys Asn Tyr Arg Met
100

<210> 54
<211> 144
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(144)
<223> HIGF-1-EB: E3; R37A; R71, S72

<400> 54
Gly Pro Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val
1 5 10 15
Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser
20 25 30
Ser Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe
35 40 45
Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys
50 55 60
Pro Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys
65 70 75 80
Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln
85 90 95
Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu
100 105 110
Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg
115 120 125
Arg Glu Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
130 135 140

<210> 55
<211> 346
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(346)
<223> HIGF-1-EB MULTIMER: (G1, P2, E3; R37A)-3XEB(R71,
S72, C-TERM 7 AA)-EB(R71,S72)

<400> 55
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30

```
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65                  70                  75                  80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg
                85                  90                  95
Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr
            100                 105                 110
Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu
        115                 120                 125
Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln Arg His Thr Asp Met
    130                 135                 140
Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys
145                 150                 155                 160
Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln
                165                 170                 175
Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu
            180                 185                 190
Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln Arg
        195                 200                 205
His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn
    210                 215                 220
Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro
225                 230                 235                 240
Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly
                245                 250                 255
Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Val
            260                 265                 270
Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro
        275                 280                 285
Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro
    290                 295                 300
Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu
305                 310                 315                 320
Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg
                325                 330                 335
Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
            340                 345
```

<210> 56
<211> 348
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(346)
<223> HIGF-1-EB MULTIMER: (E3; R37A)-3XEB(R71, S72, C-TERM 7 AA)-EB(R71, S72)

<400> 56

```
Gly Pro Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val
 1               5                  10                  15
Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser
            20                  25                  30
Ser Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe
        35                  40                  45
Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys
    50                  55                  60
Pro Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys
65                  70                  75                  80
```

45aa

Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln
85 90 95
Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu
100 105 110
Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg
115 120 125
Arg Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln Arg His Thr
130 135 140
Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn
145 150 155 160
Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly
165 170 175
Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys
180 185 190
Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala
195 200 205
Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser
210 215 220
Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr
225 230 235 240
His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile
245 250 255
Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala
260 265 270
Glu Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr
275 280 285
Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly
290 295 300
Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala
305 310 315 320
Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly
325 330 335
Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
340 345

<210> 57
<211> 107
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(107)
<223> HIGF-1-EC: E3; R37A; R71, S72

<400> 57
Gly Pro Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val
1 5 10 15
Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser
20 25 30
Ser Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe
35 40 45
Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys
50 55 60
Pro Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys
65 70 75 80
Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln
85 90 95
Arg Arg Lys Gly Ser Thr Phe Glu Glu Arg Lys
100 105

<210> 58
<211> 102
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(102)
<223> HIGF-1-EA: E3; R37A; R71, S72

<400> 58
Gly Pro Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val
1 5 10 15
Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser
20 25 30
Ser Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe
35 40 45
Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys
50 55 60
Pro Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys
65 70 75 80
Thr Gln Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly
85 90 95
Asn Lys Asn Tyr Arg Met
100

<210> 59
<211> 144
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(144)
<223> HIGF-1-EB: E3; R37A; R71, S72

<400> 59
Gly Pro Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val
1 5 10 15
Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser
20 25 30
Ser Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe
35 40 45
Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys
50 55 60
Pro Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys
65 70 75 80
Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln
85 90 95
Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu
100 105 110
Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg
115 120 125
Arg Glu Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
130 135 140

<210> 60
<211> 107
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(107)
<223> HIGF-1-EC: E3; R37A; R71, S72

<400> 60
Gly Pro Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val
 1   5   10   15
Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser
            20   25   30
Ser Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe
        35   40   45
Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys
    50   55   60
Pro Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys
65   70   75   80
Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln
                85   90   95
Arg Arg Lys Gly Ser Thr Phe Glu Glu Arg Lys
            100   105

<210> 61
<211> 154
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(154)
<223> HIGF-1-EAB: E3; R37A; R71, S72

<400> 61
Gly Pro Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val
 1   5   10   15
Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser
            20   25   30
Ser Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe
        35   40   45
Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys
    50   55   60
Pro Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys
65   70   75   80
Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly
                85   90   95
Ser Ala Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro
            100   105   110
Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu
        115   120   125
Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg
    130   135   140
Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
145   150

<210> 62
<211> 348
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE

<222> (1)...(348)
<223> HIGF-1-EB MULTIMER: (E3; R37A)-3XEB(R71, S72, C-TERM 7 AA)-EB(R71, S72)

<400> 62

Gly	Pro	Thr	Leu	Cys	Gly	Ala	Glu	Leu	Val	Asp	Ala	Leu	Gln	Phe	Val
1				5				10						15	
Cys	Gly	Asp	Arg	Gly	Phe	Tyr	Phe	Asn	Lys	Pro	Thr	Gly	Tyr	Gly	Ser
			20					25					30		
Ser	Ser	Arg	Ala	Ala	Pro	Gln	Thr	Gly	Ile	Val	Asp	Glu	Cys	Cys	Phe
		35					40					45			
Arg	Ser	Cys	Asp	Leu	Arg	Arg	Leu	Glu	Met	Tyr	Cys	Ala	Pro	Leu	Lys
	50					55					60				
Pro	Ala	Lys	Ser	Ala	Val	Arg	Ala	Gln	Arg	His	Thr	Asp	Met	Pro	Lys
65					70					75					80
Thr	Gln	Lys	Tyr	Gln	Pro	Pro	Ser	Thr	Asn	Lys	Asn	Thr	Lys	Ser	Gln
				85					90					95	
Arg	Arg	Lys	Gly	Trp	Pro	Lys	Thr	His	Pro	Gly	Gly	Glu	Gln	Lys	Glu
			100					105					110		
Gly	Thr	Glu	Ala	Ser	Leu	Gln	Ile	Arg	Gly	Lys	Lys	Lys	Glu	Gln	Arg
		115					120					125			
Arg	Glu	Ile	Gly	Ser	Arg	Asn	Ala	Glu	Val	Arg	Ala	Gln	Arg	His	Thr
	130					135					140				
Asp	Met	Pro	Lys	Thr	Gln	Lys	Tyr	Gln	Pro	Pro	Ser	Thr	Asn	Lys	Asn
145					150					155					160
Thr	Lys	Ser	Gln	Arg	Arg	Lys	Gly	Trp	Pro	Lys	Thr	His	Pro	Gly	Gly
				165					170					175	
Glu	Gln	Lys	Glu	Gly	Thr	Glu	Ala	Ser	Leu	Gln	Ile	Arg	Gly	Lys	Lys
			180					185					190		
Lys	Glu	Gln	Arg	Arg	Glu	Ile	Gly	Ser	Arg	Asn	Ala	Glu	Val	Arg	Ala
		195					200					205			
Gln	Arg	His	Thr	Asp	Met	Pro	Lys	Thr	Gln	Lys	Tyr	Gln	Pro	Pro	Ser
	210					215					220				
Thr	Asn	Lys	Asn	Thr	Lys	Ser	Gln	Arg	Arg	Lys	Gly	Trp	Pro	Lys	Thr
225					230					235					240
His	Pro	Gly	Gly	Glu	Gln	Lys	Glu	Gly	Thr	Glu	Ala	Ser	Leu	Gln	Ile
				245					250					255	
Arg	Gly	Lys	Lys	Lys	Glu	Gln	Arg	Arg	Glu	Ile	Gly	Ser	Arg	Asn	Ala
			260					265					270		
Glu	Val	Arg	Ala	Gln	Arg	His	Thr	Asp	Met	Pro	Lys	Thr	Gln	Lys	Tyr
		275					280					285			
Gln	Pro	Pro	Ser	Thr	Asn	Lys	Asn	Thr	Lys	Ser	Gln	Arg	Arg	Lys	Gly
	290					295					300				
Trp	Pro	Lys	Thr	His	Pro	Gly	Gly	Glu	Gln	Lys	Glu	Gly	Thr	Glu	Ala
305					310					315					320
Ser	Leu	Gln	Ile	Arg	Gly	Lys	Lys	Lys	Glu	Gln	Arg	Arg	Glu	Ile	Gly
				325					330					335	
Ser	Arg	Asn	Ala	Glu	Cys	Arg	Gly	Lys	Lys	Gly	Lys				
			340					345							

<210> 63
<211> 103
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(103)
<223> HIGF-1-EA: E3A; R37A; R71, S72

<400> 63

Gly	Pro	Ala	Thr	Leu	Cys	Gly	Ala	Glu	Leu	Val	Asp	Ala	Leu	Gln	Phe
1				5				10						15	

Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
20 25 30
Ser Ser Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
35 40 45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
50 55 60
Lys Pro Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro
65 70 75 80
Lys Thr Gln Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala
85 90 95
Gly Asn Lys Asn Tyr Arg Met
100

<210> 64
<211> 145
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(145)
<223> HIGF-1-EB: E3A; R37A; R71, S72

<400> 64
Gly Pro Ala Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1 5 10 15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
20 25 30
Ser Ser Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
35 40 45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
50 55 60
Lys Pro Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro
65 70 75 80
Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser
85 90 95
Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys
100 105 110
Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln
115 120 125
Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly
130 135 140
Lys
145

<210> 65
<211> 108
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(108)
<223> HIGF-1-EC: E3A; R37A; R71, S72

<400> 65
Gly Pro Ala Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1 5 10 15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
20 25 30
Ser Ser Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
35 40 45

```
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50                  55                  60
Lys Pro Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro
65                  70                  75                  80
Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser
                85                  90                  95
Gln Arg Arg Lys Gly Ser Thr Phe Glu Glu Arg Lys
            100                 105
```

<210> 66
<211> 155
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(155)
<223> HIGF-1-EAB: E3A; R37A; R71, S72

<400> 66

```
Gly Pro Ala Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
 1               5                  10                  15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20                  25                  30
Ser Ser Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35                  40                  45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50                  55                  60
Lys Pro Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro
65                  70                  75                  80
Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg
                85                  90                  95
Gly Ser Ala Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp
            100                 105                 110
Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser
        115                 120                 125
Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser
    130                 135                 140
Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
145                 150                 155
```

<210> 67
<211> 348
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(348)
<223> HIGF-1-EB MULTIMER: (E3A; R37A)-3XEB(R71, S72,
C-TERM 7 AA)-EB(R71, S72

<400> 67

```
Gly Pro Ala Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
 1               5                  10                  15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20                  25                  30
Ser Ser Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35                  40                  45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50                  55                  60
```

Lys Pro Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro
65 70 75 80
Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser
85 90 95
Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys
100 105 110
Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln
115 120 125
Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln Arg His
130 135 140
Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys
145 150 155 160
Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly
165 170 175
Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys
180 185 190
Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Val Arg
195 200 205
Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro
210 215 220
Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys
225 230 235 240
Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln
245 250 255
Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn
260 265 270
Ala Glu Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
275 280 285
Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys
290 295 300
Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu
305 310 315 320
Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile
325 330 335
Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly
340 345

<210> 68
<211> 101
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(101)
<223> HIGF-1-EA: P2, E3; R37A; R71, S72

<400> 68
Gly Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys
1 5 10 15
Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser
20 25 30
Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
35 40 45
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro
50 55 60
Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn
85 90 95
Lys Asn Tyr Arg Met
100

<210> 69
<211> 143
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(143)
<223> HIGF-1-EB: P2, E3; R37A; R71, S72

<400> 69
Gly Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys
1 5 10 15
Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser
20 25 30
Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
35 40 45
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro
50 55 60
Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
85 90 95
Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly
100 105 110
Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg
115 120 125
Glu Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
130 135 140

<210> 70
<211> 153
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(153)
<223> HIGF-1-EAB: P2, E3; R37A; R71, S72

<400> 70
Gly Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys
1 5 10 15
Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser
20 25 30
Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
35 40 45
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro
50 55 60
Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser
85 90 95
Ala Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys
100 105 110
Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln
115 120 125
Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn
130 135 140
Ala Glu Cys Arg Gly Lys Lys Gly Lys
145 150

<210> 71
<211> 347
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(347)
<223> HIGF-1-EB MULTIMER: (P2, E3; R37A)-3XEB(R71, S72, C-TERM 7 AA)-EB(R71, S72)

<400> 71
Gly Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys
1 5 10 15
Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser
20 25 30
Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
35 40 45
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro
50 55 60
Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65 70 75 80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
85 90 95
Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly
100 105 110
Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg
115 120 125
Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln Arg His Thr Asp
130 135 140
Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr
145 150 155 160
Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu
165 170 175
Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys
180 185 190
Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln
195 200 205
Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr
210 215 220
Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His
225 230 235 240
Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg
245 250 255
Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu
260 265 270
Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln
275 280 285
Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp
290 295 300
Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser
305 310 315 320
Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser
325 330 335
Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
340 345

<210> 72
<211> 143
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(143)
<223> Xaa is non-natural amino acid that is pegylated.

<400> 72
Xaa Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys
 1   5   10   15
Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser
            20   25   30
Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
        35   40   45
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro
    50   55   60
Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65   70   75   80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
                85   90   95
Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly
            100   105   110
Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg
        115   120   125
Glu Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
    130   135   140

<210> 73
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(106)
<223> Xaa is non-naturaly amino acid that is pegylated.

<400> 73
Xaa Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys
 1   5   10   15
Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser
            20   25   30
Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
        35   40   45
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro
    50   55   60
Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65   70   75   80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
                85   90   95
Arg Lys Gly Ser Thr Phe Glu Glu Arg Lys
            100   105

<210> 74
<211> 153
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(153)
<223> Xaa is non-natural amino acid that is pegylated.

<400> 74
Xaa Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys
 1   5   10   15
Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser
            20   25   30
Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
        35   40   45
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro
    50   55   60
Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65   70   75   80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser
                85   90   95
Ala Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys
            100   105   110
Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln
        115   120   125
Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn
    130   135   140
Ala Glu Cys Arg Gly Lys Lys Gly Lys
145   150

<210> 75
<211> 347
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(347)
<223> Xaa is non-natural amino acid that is pegylated.

<400> 75
Xaa Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys
 1   5   10   15
Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser
            20   25   30
Ser Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
        35   40   45
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro
    50   55   60
Ala Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65   70   75   80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
                85   90   95
Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly
            100   105   110
Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg
        115   120   125
Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln Arg His Thr Asp
    130   135   140
Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr
145   150   155   160
Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu
                165   170   175
Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys
            180   185   190
Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln
        195   200   205
Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr
    210   215   220
Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His
225   230   235   240

```
Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg
                245                 250                 255
Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu
            260                 265                 270
Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln
        275                 280                 285
Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp
    290                 295                 300
Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser
305                 310                 315                 320
Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser
                325                 330                 335
Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
            340                 345
```

<210> 76
<211> 101
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(101)
<223> Xaa is non-natural amino acid that is pegylated.

<400> 76

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Xaa Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65                  70                  75                  80
Gln Lys Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn
                85                  90                  95
Lys Asn Tyr Arg Met
            100
```

<210> 77
<211> 143
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(143)
<223> Xaa is non-natural amino acid that is pegylated

<400> 77

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Xaa Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65                  70                  75                  80
```

```
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
                85                  90                  95
Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly
            100                 105                 110
Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg
        115                 120                 125
Glu Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
    130                 135                 140
```

<210> 78
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(106)
<223> Xaa is non-natural amino acid that is pegylated.

<400> 78

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Xaa Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65                  70                  75                  80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
                85                  90                  95
Arg Lys Gly Ser Thr Phe Glu Glu Arg Lys
            100                 105
```

<210> 79
<211> 153
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(153)
<223> Xaa is non-natural amino acid that is pegylated

<400> 79

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Ser Ala Xaa Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65                  70                  75                  80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser
                85                  90                  95
Ala Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys
            100                 105                 110
Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln
        115                 120                 125
```

```
Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn
    130                 135                 140
Ala Glu Cys Arg Gly Lys Lys Gly Lys
145                 150
```

<210> 80
<211> 347
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(347)
<223> HIGF-1-EB MULTIMER: (G1, P2, E3; R37A)-EB(R71; S72X; C-TERM 7 AA)-2XEB(R71, Xaa is non-natural amino acid that is pegylated.
<223> Xaa is a non-naturaly amino acid pegylated

<400> 80

```
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1               5                  10                  15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20                  25                  30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35                  40                  45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50                  55                  60
Lys Xaa Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr
65                  70                  75                  80
Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg
                85                  90                  95
Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly
            100                 105                 110
Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg
        115                 120                 125
Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln Arg His Thr Asp
    130                 135                 140
Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr
145                 150                 155                 160
Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu
                165                 170                 175
Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys
            180                 185                 190
Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln
        195                 200                 205
Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr
    210                 215                 220
Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His
225                 230                 235                 240
Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg
                245                 250                 255
Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu
            260                 265                 270
Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln
        275                 280                 285
Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp
    290                 295                 300
Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser
305                 310                 315                 320
Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser
                325                 330                 335
Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys
            340                 345
```

<210> 81
<211> 100
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(100)
<223> Xaa is non-natural amino acid that is pegylated.

<400> 81
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
35 40 45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
50 55 60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65 70 75 80
Lys Glu Val His Leu Lys Xaa Ala Ser Arg Gly Ser Ala Gly Asn Lys
85 90 95
Asn Tyr Arg Met
100

<210> 82
<211> 142
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(142)
<223> Xaa is non-natural amino acid that is pegylated.

<400> 82
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1 5 10 15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
20 25 30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
35 40 45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
50 55 60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65 70 75 80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg
85 90 95
Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr
100 105 110
Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu
115 120 125
Ile Gly Ser Arg Asn Ala Glu Xaa Arg Gly Lys Lys Gly Lys
130 135 140

<210> 83
<211> 152
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(152)
<223> Xaa is non natural amino acid that is pegylated.

<400> 83
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1   5   10   15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20   25   30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35   40   45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50   55   60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65   70   75   80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Ala Ser Arg Gly Ser Ala
                85   90   95
Gly Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr
            100   105   110
His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile
        115   120   125
Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala
    130   135   140
Glu Xaa Arg Gly Lys Lys Gly Lys
145   150

<210> 84
<211> 346
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(346)
<223> HIGF-1-EB MULTIMER (G1, P2, E3; R37A)-3XEB(R71, S72, Xaa is non-natural amino acid that is pegylated.
<223> Xaa is a non-naturaly amino acid pegylated

<400> 84
Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
 1   5   10   15
Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20   25   30
Arg Ala Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35   40   45
Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50   55   60
Lys Ser Ala Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln
65   70   75   80
Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg
                85   90   95
Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr
            100   105   110
Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu
        115   120   125
Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln Arg His Thr Asp Met
    130   135   140
Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys
145   150   155   160
Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln
                165   170   175

```
Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu
            180                 185                 190
Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Val Arg Ala Gln Arg
        195                 200                 205
His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn
    210                 215                 220
Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro Lys Thr His Pro
225                 230                 235                 240
Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu Gln Ile Arg Gly
                245                 250                 255
Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg Asn Ala Glu Val
            260                 265                 270
Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys Tyr Gln Pro
        275                 280                 285
Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys Gly Trp Pro
    290                 295                 300
Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr Glu Ala Ser Leu
305                 310                 315                 320
Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu Ile Gly Ser Arg
                325                 330                 335
Asn Ala Glu Xaa Arg Gly Lys Lys Gly Lys
            340                 345
```

<210> 85
<211> 70
<212> PRT
<213> Ovis aries

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> SHEEP REF NP_001009774.1

```
<400> 85
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
 1               5                  10                  15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20                  25                  30
Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35                  40                  45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50                  55                  60
Lys Ala Ala Lys Ser Ala
65                  70
```

<210> 86
<211> 61
<212> PRT
<213> Ovis aries

<220>
<221> PEPTIDE
<222> (1)...(61)
<223> ABB02295.1

```
<400> 86
Leu Val Asp Ala Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe
 1               5                  10                  15
Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser Arg Arg Ala Pro Gln Thr
            20                  25                  30
Gly Ile Val Asp Glu Cys Cys Phe Arg Ser Cys Asp Leu Arg Arg Leu
        35                  40                  45
```

Glu Met Tyr Cys Ala Pro Leu Lys Ala Ala Lys Ser Ala
50 55 60

<210> 87
<211> 70
<212> PRT
<213> Capra hircus

<220>
<221> PEPTIDE
<222> (1)...(60)
<223> P51457

<400> 87
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1 5 10 15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
20 25 30
Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
35 40 45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
50 55 60
Lys Pro Thr Lys Ser Ala
65 70

<210> 88
<211> 70
<212> PRT
<213> Ailuropoda melanoleuca

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> Q6JLX1

<400> 88
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1 5 10 15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
20 25 30
Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
35 40 45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
50 55 60
Lys Pro Ala Lys Ser Ala
65 70

<210> 89
<211> 70
<212> PRT
<213> Cervus elaphus

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> ABL98032.1

<400> 89
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1 5 10 15

```
Val Cys Gly Asp Arg Gly Ser Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20                  25                  30
Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35                  40                  45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50                  55                  60
Lys Pro Thr Lys Ala Ala
65                  70
```

<210> 90
<211> 70
<212> PRT
<213> Oryctolagus cuniculus

<220>
<221> PEPTIDE
<222> (1)...(90)
<223> AAB48032.1

<400> 90

```
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
 1               5                  10                  15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20                  25                  30
Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35                  40                  45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50                  55                  60
Lys Pro Ala Lys Ala Ala
65                  70
```

<210> 91
<211> 70
<212> PRT
<213> Rattus norvegicus

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> REF NP_849197.1

<400> 91

```
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
 1               5                  10                  15
Val Cys Gly Pro Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20                  25                  30
Ser Ser Ile Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35                  40                  45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Val Arg Cys
    50                  55                  60
Lys Pro Thr Lys Ser Ala
65                  70
```

<210> 92
<211> 70
<212> PRT
<213> Rattus norvegicus

<220>
<221> PEPTIDE

<222> (1)...(70)
<223> RAT CAA29436.1

<400> 92
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
 1   5   10   15
Val Cys Gly Pro Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20   25   30
Ser Ser Ile Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35   40   45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50   55   60
Lys Pro Thr Lys Ser Ala
65   70

<210> 93
<211> 70
<212> PRT
<213> Mus musculus

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> MOUSE ISO1 REF NP_034642.1

<400> 93
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
 1   5   10   15
Val Cys Gly Pro Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20   25   30
Ser Ser Ile Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35   40   45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50   55   60
Lys Pro Thr Lys Ala Ala
65   70

<210> 94
<211> 70
<212> PRT
<213> Anser anser

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> Goose ABF 57993.1

<400> 94
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
 1   5   10   15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Lys Pro Thr Gly Tyr Gly
            20   25   30
Ser Ser Ser Arg Arg Leu His His Lys Gly Ile Val Asp Glu Cys Cys
        35   40   45
Phe Gln Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Ile
    50   55   60
Lys Pro Pro Lys Ser Ala
65   70

<210> 95
<211> 70

<212> PRT
<213> Oncorhynchus tshawytscha

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> CHINOOK SALMON AAA67268.1

<400> 95
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Thr Leu Gln Phe
 1   5   10   15
Val Cys Gly Glu Arg Gly Phe Tyr Phe Ser Lys Pro Thr Gly Tyr Gly
            20   25   30
Pro Ser Ser Arg Arg Ser His Asn Arg Gly Ile Val Asp Glu Cys Cys
        35   40   45
Phe Gln Ser Cys Glu Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Val
    50   55   60
Lys Ser Gly Lys Ala Ala
65   70

<210> 96
<211> 70
<212> PRT
<213> Acipenser ruthenus

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> STERLET ABC54785.1

<400> 96
Gly Ser Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Thr Leu Gln Phe
 1   5   10   15
Val Cys Gly Glu Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20   25   30
Ala Ser Ser Arg Arg Pro His His Arg Gly Ile Val Asn Glu Cys Cys
        35   40   45
Phe Gln Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Val
    50   55   60
Lys Pro Ala Lys Ala Ser
65   70

<210> 97
<211> 68
<212> PRT
<213> Paralichthys olivaceus

<220>
<221> PEPTIDE
<222> (1)...(68)
<223> HALIBUT CAA09267.1

<400> 97
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Thr Leu Gln Phe
 1   5   10   15
Val Cys Gly Glu Arg Gly Phe Tyr Phe Ser Lys Pro Thr Gly Tyr Gly
            20   25   30
Pro Asn Ala Arg Arg Ser Arg Gly Ile Val Asp Glu Cys Cys Phe Gln
        35   40   45

Ser Cys Glu Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Ala Lys Thr
50 55 60
Ser Lys Ala Ala
65

<210> 98
<211> 70
<212> PRT
<213> Oncorhynchus mykiss

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> RAINBOW TROUT Q02815

<400> 98
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Thr Leu Gln Phe
1 5 10 15
Val Cys Gly Glu Arg Gly Phe Tyr Phe Ser Lys Pro Thr Gly Tyr Gly
20 25 30
Pro Ser Ser Arg Arg Ser His Asn Arg Gly Ile Val Asp Glu Cys Cys
35 40 45
Phe Gln Ser Cys Glu Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Val
50 55 60
Lys Ser Gly Lys Ala Ala
65 70

<210> 99
<211> 70
<212> PRT
<213> Ictalurus punctatus

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> CHANNEL CATFISH AAQ56592.1

<400> 99
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Thr Leu Gln Phe
1 5 10 15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Lys Pro Thr Gly Tyr Gly
20 25 30
Pro Asn Ser Arg Arg Leu His Asn Arg Gly Ile Val Asp Glu Cys Cys
35 40 45
Phe Gln Ser Cys Glu Leu Lys Arg Leu Glu Met Tyr Cys Ala Pro Val
50 55 60
Lys Ser Gly Lys Ala Pro
65 70

<210> 100
<211> 70
<212> PRT
<213> Cyprinus carpio

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> CARP AAY21902.1

<400> 100
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Thr Leu Gln Phe
1 5 10 15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Lys Pro Thr Gly Tyr Gly
20 25 30
Pro Ser Ser Arg Arg Ser His Asn Arg Gly Ile Val Asp Glu Cys Cys
35 40 45
Phe Gln Ser Cys Glu Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Val
50 55 60
Lys Pro Gly Lys Thr Pro
65 70

<210> 101
<211> 70
<212> PRT
<213> Cyprinus carpio

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> CARP AAP78926.1

<400> 101
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Thr Leu Gln Phe
1 5 10 15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Lys Pro Thr Gly Tyr Gly
20 25 30
Pro Ser Ser Arg Arg Ser His Asn Arg Gly Ile Val Asp Glu Cys Cys
35 40 45
Phe Gln Ser Cys Glu Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Val
50 55 60
Lys Pro Gly Lys Thr Pro
65 70

<210> 102
<211> 70
<212> PRT
<213> Danio rerio

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> ZEBRAFISH NP_571900.1

<400> 102
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Thr Leu Gln Phe
1 5 10 15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Lys Pro Thr Gly Tyr Gly
20 25 30
Pro Ser Ser Arg Arg Ser His Asn Arg Gly Ile Val Asp Glu Cys Cys
35 40 45
Phe Gln Ser Cys Glu Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Val
50 55 60
Lys Thr Gly Lys Ser Pro
65 70

<210> 103
<211> 70
<212> PRT
<213> Myxocyprinus asiaticus

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> CHINESE SUCKER ABH12114.1

<400> 103
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Thr Leu Gln Phe
 1   5   10   15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Lys Pro Thr Gly Tyr Gly
            20   25   30
Pro Ser Ser Arg Arg Ser His Asn Arg Gly Ile Val Asp Glu Cys Cys
        35   40   45
Phe Gln Ser Cys Glu Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Val
    50   55   60
Lys Pro Gly Lys Ala Pro
65   70

<210> 104
<211> 70
<212> PRT
<213> Pimephales promelas

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> FATHEADMINNOW AAT02176.1

<400> 104
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Thr Leu Gln Phe
 1   5   10   15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Ala Gly Tyr Gly
            20   25   30
Ser Asn Ser Arg Arg Ser Asn Asn Tyr Gly Ile Val Asp Glu Cys Cys
        35   40   45
Phe Gln Ser Cys Glu Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Val
    50   55   60
Lys Thr Gly Lys Thr Pro
65   70

<210> 105
<211> 70
<212> PRT
<213> Carassius auratus

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> GOLDFISH AAC83443.1

<400> 105
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Thr Leu Gln Phe
 1   5   10   15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Lys Pro Thr Gly Tyr Gly
            20   25   30
Pro Asn Ser Arg Arg Ser His Asn Arg Gly Ile Val Asp Glu Cys Cys
        35   40   45
Phe Gln Ser Cys Glu Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Val
    50   55   60
Lys Pro Gly Lys Thr Pro
65   70

<210> 106
<211> 70
<212> PRT
<213> Xenopus laevis

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> African clawed frog AAA70330.1

<400> 106
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Thr Leu Gln Phe
 1   5   10   15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Lys Pro Thr Gly Tyr Gly
            20   25   30
Ser Asn Asn Arg Arg Ser His His Arg Gly Ile Val Asp Glu Cys Cys
        35   40   45
Phe Gln Ser Cys Asp Phe Arg Arg Leu Glu Met Tyr Cys Ala Pro Ala
    50   55   60
Lys Pro Ala Lys Ser Ala
65   70

<210> 107
<211> 70
<212> PRT
<213> Xenopus laevis

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> African clawed frog 1A P16501

<400> 107
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Thr Leu Gln Phe
 1   5   10   15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Lys Pro Thr Gly Tyr Gly
            20   25   30
Ser Asn Asn Arg Arg Ser His His Arg Gly Ile Val Asp Glu Cys Cys
        35   40   45
Phe Gln Ser Cys Asp Phe Arg Arg Leu Glu Met Tyr Cys Ala Pro Ala
    50   55   60
Lys Gln Ala Lys Ser Ala
65   70

<210> 108
<211> 70
<212> PRT
<213> Monodelphis domestica

<220>
<221> PEPTIDE
<222> (1)...(70)
<223> Gray short-tailed opossum XP_001373491.1

<400> 108
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
 1   5   10   15
Val Cys Gly Glu Arg Gly Phe Tyr Phe Ser Lys Pro Thr Gly Tyr Gly
            20   25   30
Ser Ser Ser Arg Arg Leu His His Thr Gly Ile Val Asp Glu Cys Cys
        35   40   45

```
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Ile
    50                  55                  60
Lys Pro Ala Lys Ser Ala
65                  70
```

<210> 109
<211> 35
<212> PRT
<213> Sus scrofa

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> PIG REF NP_999421.1

<400> 109

```
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
 1               5                  10                  15
Glu Val His Leu Lys Asn Thr Ser Arg Gly Ser Ser Gly Asn Lys Asn
            20                  25                  30
Tyr Arg Met
        35
```

<210> 110
<211> 35
<212> PRT
<213> Sus scrofa

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> PIG CLASS1 ABG88023.1

<400> 110

```
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Arg Lys
 1               5                  10                  15
Glu Val His Leu Lys Asn Thr Ser Arg Gly Ser Ser Gly Asn Lys Asn
            20                  25                  30
Tyr Arg Met
        35
```

<210> 111
<211> 35
<212> PRT
<213> Bos taurus

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> COW CAA33746.1

<400> 111

```
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
 1               5                  10                  15
Glu Val His Leu Lys Asn Thr Ser Arg Gly Ser Ala Gly Asn Lys Asn
            20                  25                  30
Tyr Arg Met
        35
```

<210> 112
<211> 33
<212> PRT
<213> Canis familiaris

<220>
<221> PEPTIDE
<222> (1)...(33)
<223> DOG P33712

<400> 112
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
 1   5   10   15
Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn Lys Thr
            20   25   30
Tyr

<210> 113
<211> 35
<212> PRT
<213> Canis familiaris

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> DOG ISO2 XP_853117.1

<400> 113
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
 1   5   10   15
Glu Val His Leu Lys Asn Ala Ser Arg Gly Ser Ala Gly Asn Lys Asn
            20   25   30
Tyr Arg Met
        35

<210> 114
<211> 35
<212> PRT
<213> Oryctolagus cuniculus

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> RABBIT AAB48032.1

<400> 114
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
 1   5   10   15
Glu Val His Leu Lys Asn Thr Ser Arg Gly Ser Ala Gly Asn Lys Asn
            20   25   30
Tyr Arg Met
        35

<210> 115
<211> 35
<212> PRT
<213> Rattus norvegicus

<220>
<221> PEPTIDE

<222> (1)...(35)
<223> RAT REF NP_849197.1

<400> 115
Arg Ser Ile Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
 1   5   10   15
Glu Val His Leu Lys Asn Thr Ser Arg Gly Ser Ala Gly Asn Lys Thr
            20   25   30
Tyr Arg Met
        35

<210> 116
<211> 35
<212> PRT
<213> Gallus gallus

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> CHICKEN NP_001004384.1

<400> 116
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
 1   5   10   15
Glu Val His Leu Lys Asn Thr Ser Arg Gly Asn Thr Gly Asn Arg Asn
            20   25   30
Tyr Arg Met
        35

<210> 117
<211> 35
<212> PRT
<213> Meleagris gallopavo

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> TURKEY AAC26006.1

<400> 117
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
 1   5   10   15
Glu Leu His Leu Lys Asn Thr Ser Arg Gly Asn Thr Gly Asn Arg Asn
            20   25   30
Tyr Arg Met
        35

<210> 118
<211> 30
<212> PRT
<213> Anser anser

<220>
<221> PEPTIDE
<222> (1)...(30)
<223> GOOSE ABF57993.1

<400> 118
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
 1   5   10   15

Glu Val His Leu Lys Asn Thr Ser Arg Gly Asn Thr Glu Asn
20 25 30

<210> 119
<211> 35
<212> PRT
<213> Oncorhynchus tshawytscha

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> CHINOOK SALMON AAA67268.1

<400> 119
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Arg Thr Pro Lys
1 5 10 15
Glu Val His Gln Lys Asn Ser Ser Arg Gly Asn Thr Gly Gly Arg Asn
20 25 30
Tyr Arg Met
35

<210> 120
<211> 35
<212> PRT
<213> Acipenser ruthenus

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> STERLET ABC54785.1

<400> 120
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
1 5 10 15
Glu Val His Ser Lys Asn Ser Ser Arg Gly Asn Thr Gly Asn Arg Asn
20 25 30
Tyr Arg Ile
35

<210> 121
<211> 35
<212> PRT
<213> Perca fluviatilis

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> PERCH CAE52916.2

<400> 121
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Arg Ala Pro Lys
1 5 10 15
Glu Val His Gln Lys Asn Ser Ser Arg Gly Asn Thr Gly Gly Arg Asn
20 25 30
Tyr Arg Met
35

<210> 122
<211> 35

<212> PRT
<213> Paralichthys olivaceus

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> HALIBUT CAA09267.1

<400> 122
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Arg Ala Pro Lys
 1   5   10   15
Glu Val His Gln Lys Asn Ser Ser Arg Gly Thr Thr Gly Gly Arg Asn
            20   25   30
Tyr Arg Met
        35

<210> 123
<211> 35
<212> PRT
<213> Ictalurus punctatus

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> CHANNEL CATFISH AAQ56592.1

<400> 123
Arg Ser Val Arg Glu Gln Arg His Thr Asp Thr Pro Lys Thr Pro Lys
 1   5   10   15
Glu Val His Gln Lys Asn Ser Ser Arg Gly Asn Thr Gly Gly Arg Asn
            20   25   30
Tyr Arg Met
        35

<210> 124
<211> 35
<212> PRT
<213> Cirrhinus molitorella

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> MUD CARP AAY21902.1

<400> 124
Arg Ser Ile Arg Ala Gln Arg His Thr Asp Ser Pro Lys Thr Ala Lys
 1   5   10   15
Glu Val His Gln Lys Asn Ser Ser Arg Gly Asn Thr Gly Gly Arg Asn
            20   25   30
Tyr Arg Met
        35

<210> 125
<211> 35
<212> PRT
<213> Cirrhinus molitorella

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> MUD CARP AAP78926.1

<400> 125
Arg Ser Val Arg Ala Gln Arg His Thr Asp Ser Pro Arg Thr Ala Lys
 1   5   10   15
Glu Val His Gln Lys Asn Ser Ser Arg Gly Asn Thr Gly Gly Arg Asn
            20   25   30
Tyr Arg Ile
        35

<210> 126
<211> 35
<212> PRT
<213> Danio aequipinnatus

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> GIANT DANIO ABB05519.1

<400> 126
Arg Ser Leu Arg Ala Gln Arg His Thr Asp Ile Pro Arg Thr Ala Lys
 1   5   10   15
Glu Val His Gln Lys Asn Ser Ser Arg Gly Asn Thr Gly Gly Arg Asn
            20   25   30
Tyr Arg Met
        35

<210> 127
<211> 35
<212> PRT
<213> Danio rerio

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> ZEBRAFISH NP_571900.1

<400> 127
Arg Ser Leu Arg Ala Gln Arg His Thr Asp Ile Pro Arg Thr Pro Lys
 1   5   10   15
Glu Val His Gln Lys Asn Ser Ser Arg Gly Asn Thr Gly Gly Arg Asn
            20   25   30
Tyr Arg Met
        35

<210> 128
<211> 35
<212> PRT
<213> Myxocyprinus asiaticus

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> CHINESE SUCKER ABH12114.1

<400> 128
Arg Ser Leu Arg Ala Gln Arg His Thr Asp Ile Pro Arg Thr Pro Lys
 1   5   10   15
Asp Val His Gln Lys Asn Ser Ser Arg Gly Asn Thr Gly Gly Arg Asn
            20   25   30
Tyr Arg Met
        35

<210> 129
<211> 35
<212> PRT
<213> Barbus barbus

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> SPINYBARBUS ABE03747.1

<400> 129
Arg Ser Leu Arg Ala Gln Arg His Thr Asp Ser Pro Arg Thr Ala Lys
 1   5   10   15
Glu Val His Gln Lys Asn Ser Ser Arg Gly Asn Thr Gly Gly Arg Asn
            20   25   30
Tyr Arg Ile
        35

<210> 130
<211> 35
<212> PRT
<213> Pimephales promelas

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> FATHEADMINNOW AAT02176.1

<400> 130
Arg Ser Leu Arg Ala Gln Arg His Thr Asp Ile Thr Arg Thr Ala Lys
 1   5   10   15
Glu Val His Gln Lys Asn Ser Ser Arg Gly Ile Thr Gly Gly Arg Asn
            20   25   30
Tyr Arg Met
        35

<210> 131
<211> 35
<212> PRT
<213> Carassius auratus

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> GOLDFISH AAC83443.1

<400> 131
Arg Ser Leu Arg Ala Gln Arg His Thr Asp Gly Thr Arg Thr Ala Lys
 1   5   10   15
Glu Val His Gln Lys Asn Ser Ser Arg Gly Asn Thr Gly Gly Arg Asn
            20   25   30
Tyr Arg Met
        35

<210> 132
<211> 35
<212> PRT
<213> Xenopus laevis

<220>
<221> PEPTIDE

<222> (1)...(35)
<223> African clawed frog AAA70330.1

<400> 132
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
 1   5   10   15
Glu Val His Pro Lys Asn Thr Ser Arg Gly Asn Thr Gly Ser Arg Gly
            20   25   30
Phe Arg Met
        35

<210> 133
<211> 35
<212> PRT
<213> Kuhlia rupestris

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> BREAM AAK16727.1

<400> 133
Arg Ser Leu Arg Ala Gln Arg His Thr Asp Ile Thr Arg Thr Ala Lys
 1   5   10   15
Glu Val His Gln Lys Asn Ser Ser Arg Gly Asn Thr Gly Gly Arg Asn
            20   25   30
Tyr Arg Ile
        35

<210> 134
<211> 35
<212> PRT
<213> Xenopus laevis

<220>
<221> PEPTIDE
<222> (1)...(35)
<223> African clawed frog 1A P16501

<400> 134
Arg Ser Val Arg Thr Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
 1   5   10   15
Glu Val His Pro Lys Asn Thr Ser Arg Gly Asn Thr Gly Ser Arg Gly
            20   25   30
Phe Arg Met
        35

<210> 135
<211> 46
<212> PRT
<213> Artificial Sequence

<220>
<223> majority consensus sequence for Seq. ID. Nos.
      136-147

<400> 135
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
 1   5   10   15
Tyr Gln Pro Pro Ser Thr Asn Lys Lys Thr Lys Ser Gln Arg Arg Arg
            20   25   30

Lys Gly Gly Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu
35 40 45

<210> 136
<211> 41
<212> PRT
<213> Sus scrofa

<220>
<221> PEPTIDE
<222> (1)...(41)
<223> PIG AAT47735.1

<400> 136
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
1 5 10 15
Tyr Gln Pro Pro Ser Thr Asn Lys Lys Thr Lys Ser Gln Arg Arg Arg
20 25 30
Lys Gly Ser Thr Phe Glu Glu His Lys
35 40

<210> 137
<211> 30
<212> PRT
<213> Bos indicus

<220>
<221> PEPTIDE
<222> (1)...(31)
<223> COW INDIA AAU93628.1

<400> 137
Tyr Gln Pro Pro Ser Thr Asn Lys Lys Met Lys Ser Gln Arg Arg Arg
1 5 10 15
Lys Gly Gly Pro Lys Lys Arg Pro Gly Gly Glu Gln Lys Glu
20 25 30

<210> 138
<211> 50
<212> PRT
<213> Bos taurus

<220>
<221> PEPTIDE
<222> (1)...(50)
<223> CATTLE AAA03497.1

<400> 138
His Ala Gln Gly Ser Glu Gly Lys Pro Ala Arg Gly Gly Gly Glu Gly
1 5 10 15
Arg Pro Ser Ser Tyr Gln Pro Pro Ser Thr Asn Lys Lys Met Lys Ser
20 25 30
Gln Arg Arg Arg Lys Gly Gly Pro Lys Lys Arg Pro Gly Gly Glu Gln
35 40 45
Lys Glu
50

<210> 139
<211> 30

<212> PRT
<213> Bubalus bubalis

<220>
<221> PEPTIDE
<222> (1)...(30)
<223> WATER BUFFALO AAU93630.1

<400> 139
Tyr Gln Pro Pro Ser Thr Asn Lys Lys Met Lys Ser Gln Arg Arg Arg
 1   5   10   15
Lys Gly Gly Pro Lys Lys His Pro Gly Gly Glu Gln Lys Glu
            20   25   30

<210> 140
<211> 30
<212> PRT
<213> Ovis aries

<220>
<221> PEPTIDE
<222> (1)...(30)
<223> SHEEP AAU93626.1

<400> 140
Tyr Gln Leu Pro Ser Thr Asn Lys Lys Met Lys Ser Gln Arg Arg Arg
 1   5   10   15
Lys Gly Gly Pro Lys Lys His Pro Gly Gly Glu Gln Lys Glu
            20   25   30

<210> 141
<211> 41
<212> PRT
<213> Canis familiaris

<220>
<221> PEPTIDE
<222> (1)...(41)
<223> DOG XP_866946.

<400> 141
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
 1   5   10   15
Tyr His Pro Pro Ser Thr Thr Lys Arg Met Lys Ser Gln Arg Arg Arg
            20   25   30
Lys Gly Ser Thr Phe Glu Glu Cys Lys
        35   40

<210> 142
<211> 41
<212> PRT
<213> Oryctolagus cuniculus

<220>
<221> PEPTIDE
<222> (1)...(41)
<223> RABBIT Q95222

<400> 142
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
 1   5   10   15

Tyr Gln Pro Pro Ser Thr Asn Lys Lys Met Lys Ser Gln Arg Arg Arg
20 25 30
Lys Gly Ser Thr Phe Glu Glu His Lys
35 40

<210> 143
<211> 76
<212> PRT
<213> Pan troglodytes

<220>
<221> PEPTIDE
<222> (1)...(76)
<223> CHIMPANZEE XP_001156459.1

<400> 143
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
1 5 10 15
Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Arg
20 25 30
Lys Gly Gly Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr
35 40 45
Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu
50 55 60
Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys
65 70 75

<210> 144
<211> 90
<212> PRT
<213> Macaca mulatta

<220>
<221> PEPTIDE
<222> (1)...(90)
<223> RHESUS MONKEY XP_001094129.1

<400> 144
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
1 5 10 15
Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Arg
20 25 30
Lys Gly Gly Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr
35 40 45
Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys Lys Glu Gln Arg Arg Glu
50 55 60
Ile Gly Ser Arg Asn Ala Glu Cys Arg Gly Lys Lys Gly Lys Trp Arg
65 70 75 80
Thr Gly Gly Leu Ser Arg Gln Arg Gln Gly
85 90

<210> 145
<211> 63
<212> PRT
<213> Mus musculus

<220>
<221> PEPTIDE
<222> (1)...(60)
<223> MOUSE NP_908941.1

<400> 145
Arg Ser Ile Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
1 5 10 15
Ser Pro Ser Leu Ser Thr Asn Lys Lys Thr Lys Leu Gln Arg Arg Arg
20 25 30
Lys Gly Glu Pro Lys Thr His Pro Glu Gly Glu Gln Glu Glu Val Thr
35 40 45
Glu Ala Thr Arg Lys Ile Arg Gly Pro Arg Glu Lys Arg Leu Gly
50 55 60

<210> 146
<211> 63
<212> PRT
<213> Rattus norvegicus

<220>
<221> PEPTIDE
<222> (1)...(63)
<223> RAT AAA41214

<400> 146
Arg Ser Ile Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
1 5 10 15
Ser Gln Pro Leu Ser Thr His Lys Lys Arg Lys Leu Gln Arg Arg Arg
20 25 30
Lys Gly Glu Ser Lys Ala His Pro Gly Gly Glu Gln Glu Glu Gly Ala
35 40 45
Glu Ala Thr Gln Lys Ile Arg Gly Asp Arg Glu Arg Arg Pro Ser
50 55 60

<210> 147
<211> 41
<212> PRT
<213> Mus musculus

<220>
<221> PEPTIDE
<222> (1)...(41)
<223> MOUSE AAX61180.

<400> 147
Arg Ser Ile Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
1 5 10 15
Ser Pro Ser Leu Ser Thr Asn Lys Lys Thr Lys Leu Gln Arg Arg Arg
20 25 30
Lys Gly Ser Thr Phe Glu Glu His Lys
35 40

<210> 148
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Majority consensus sequence for Seq. ID Nos.
149-155.

<400> 148
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
1 5 10 15

Tyr Gln Pro Pro Ser Thr Asn Lys Lys Thr Lys Ser Gln Arg Arg Arg
20 25 30
Lys Gly Ser Thr Phe Glu Glu His
35 40

<210> 149
<211> 39
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(39)
<223> HUMAN EAW97695.1

<400> 149
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
1 5 10 15
Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Lys
20 25 30
Gly Ser Thr Phe Glu Glu Arg
35

<210> 150
<211> 40
<212> PRT
<213> Sus scrofa

<220>
<221> PEPTIDE
<222> (1)...(40)
<223> PIG AAT47735.1

<400> 150
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
1 5 10 15
Tyr Gln Pro Pro Ser Thr Asn Lys Lys Thr Lys Ser Gln Arg Arg Arg
20 25 30
Lys Gly Ser Thr Phe Glu Glu His
35 40

<210> 151
<211> 40
<212> PRT
<213> Canis familiaris

<220>
<221> PEPTIDE
<222> (1)...(40)
<223> DOG IB XP_866946.1

<400> 151
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Ala Gln Lys
1 5 10 15
Tyr His Pro Pro Ser Thr Thr Lys Arg Met Lys Ser Gln Arg Arg Arg
20 25 30
Lys Gly Ser Thr Phe Glu Glu Cys
35 40

<210> 152
<211> 40
<212> PRT
<213> Oryctolagus cuniculus

<220>
<221> PEPTIDE
<222> (1)...(40)
<223> RABBIT Q95222

<400> 152
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
 1   5   10   15
Tyr Gln Pro Pro Ser Thr Asn Lys Lys Met Lys Ser Gln Arg Arg Arg
            20   25   30
Lys Gly Ser Thr Phe Glu Glu His
        35   40

<210> 153
<211> 40
<212> PRT
<213> Macaca mulatta

<220>
<221> PEPTIDE
<222> (1)...(40)
<223> RHESUS MONKEY XP_001094016.1

<400> 153
Arg Ser Val Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
 1   5   10   15
Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr Lys Ser Gln Arg Arg Arg
            20   25   30
Lys Gly Ser Thr Phe Glu Glu Arg
        35   40

<210> 154
<211> 40
<212> PRT
<213> Mus musculus

<220>
<221> PEPTIDE
<222> (1)...(40)
<223> MOUSE IB AAX61180.1

<400> 154
Arg Ser Ile Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
 1   5   10   15
Ser Pro Ser Leu Ser Thr Asn Lys Lys Thr Lys Leu Gln Arg Arg Arg
            20   25   30
Lys Gly Ser Thr Phe Glu Glu His
        35   40

<210> 155
<211> 40
<212> PRT
<213> Rattus norvegicus

<220>
<221> PEPTIDE

<222> (1)...(40)
<223> RAT IB A40912

<400> 155
Arg Ser Ile Arg Ala Gln Arg His Thr Asp Met Pro Lys Thr Gln Lys
1 5 10 15
Ser Gln Pro Leu Ser Thr His Lys Lys Arg Lys Leu Gln Arg Arg Arg
20 25 30
Lys Gly Ser Thr Leu Glu Glu His
35 40

<210> 156
<211> 156
<212> PRT
<213> Artificial Sequence

<220>
<223> Majority consensus sequence for Seq. ID Nos. 157-169

<400> 156
Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
1 5 10 15
Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Arg Pro Ala
20 25 30
Ser Arg Val Asn Arg Arg Ser Arg Gly Ile Val Glu Glu Cys Cys Phe
35 40 45
Arg Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Thr Pro Ala
50 55 60
Lys Ser Glu Arg Asp Val Ser Thr Pro Pro Thr Val Leu Pro Asp Asn
65 70 75 80
Phe Pro Arg Tyr Pro Val Gly Lys Phe Phe Gln Tyr Asp Thr Trp Lys
85 90 95
Gln Ser Ala Gln Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala
100 105 110
Arg Arg Gly Arg Met Leu Ala Lys Glu Leu Glu Ala Phe Arg Glu Ala
115 120 125
Lys Arg His Arg Pro Leu Ile Ala Leu Pro Thr Gln Asp Pro Ala His
130 135 140
Gly Gly Ala Ser Pro Glu Ala Ser Ser Asn Arg Lys
145 150 155

<210> 157
<211> 156
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)...(156)
<223> HUMAN NP_000603.1

<400> 157
Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
1 5 10 15
Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Arg Pro Ala
20 25 30
Ser Arg Val Ser Arg Arg Ser Arg Gly Ile Val Glu Glu Cys Cys Phe
35 40 45
Arg Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Thr Pro Ala
50 55 60

Lys Ser Glu Arg Asp Val Ser Thr Pro Pro Thr Val Leu Pro Asp Asn
65 70 75 80
Phe Pro Arg Tyr Pro Val Gly Lys Phe Phe Gln Tyr Asp Thr Trp Lys
85 90 95
Gln Ser Thr Gln Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala
100 105 110
Arg Arg Gly His Val Leu Ala Lys Glu Leu Glu Ala Phe Arg Glu Ala
115 120 125
Lys Arg His Arg Pro Leu Ile Ala Leu Pro Thr Gln Asp Pro Ala His
130 135 140
Gly Gly Ala Pro Pro Glu Met Ala Ser Asn Arg Lys
145 150 155

<210> 158
<211> 157
<212> PRT
<213> Sus scrofa

<220>
<221> PEPTIDE
<222> (1)...(157)
<223> PIG NP_999048.1

<400> 158
Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
1 5 10 15
Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Arg Pro Ala
20 25 30
Ser Arg Val Asn Arg Arg Ser Arg Gly Ile Val Glu Glu Cys Cys Phe
35 40 45
Arg Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Thr Pro Ala
50 55 60
Lys Ser Glu Arg Asp Val Ser Thr Pro Pro Thr Val Leu Pro Asp Asn
65 70 75 80
Phe Pro Arg Tyr Pro Val Gly Lys Phe Phe Arg Tyr Asp Thr Trp Lys
85 90 95
Gln Ser Ala Gln Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala
100 105 110
Arg Arg Gly Arg Thr Leu Ala Lys Glu Leu Glu Ala Val Arg Glu Ala
115 120 125
Lys Arg His Arg Pro Leu Thr Ala Arg Pro Thr Arg Asp Pro Ala Ala
130 135 140
His Gly Gly Ala Ser Pro Glu Ala Ser Gly His Arg Lys
145 150 155

<210> 159
<211> 155
<212> PRT
<213> Bos taurus

<220>
<221> PEPTIDE
<222> (1)...(155)
<223> CATTLE NP_776512.2

<400> 159
Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
1 5 10 15
Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Arg Pro Ser
20 25 30
Ser Arg Ile Asn Arg Arg Ser Arg Gly Ile Val Glu Glu Cys Cys Phe
35 40 45

Arg Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Thr Pro Ala
50 55 60
Lys Ser Glu Arg Asp Val Ser Ala Ser Thr Thr Val Leu Pro Asp Asp
65 70 75 80
Val Thr Ala Tyr Pro Val Gly Lys Phe Phe Gln Tyr Asp Ile Trp Lys
85 90 95
Gln Ser Thr Gln Arg Leu Arg Arg Gly Leu Pro Ala Phe Leu Arg Ala
100 105 110
Arg Arg Gly Arg Thr Leu Ala Lys Glu Leu Glu Ala Leu Arg Glu Ala
115 120 125
Lys Ser His Arg Pro Leu Ile Ala Leu Pro Thr Gln Asp Pro Ala Thr
130 135 140
His Gly Gly Ala Ser Ser Lys Ala Ser Ser Asp
145 150 155

<210> 160
<211> 155
<212> PRT
<213> Ovis aries

<220>
<221> PEPTIDE
<222> (1)...(155)
<223> SHEEP NP_001009311.1

<400> 160
Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
1 5 10 15
Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Arg Pro Ser
20 25 30
Ser Arg Ile Asn Arg Arg Ser Arg Gly Ile Val Glu Glu Cys Cys Phe
35 40 45
Arg Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Ala Pro Ala
50 55 60
Lys Ser Glu Arg Asp Val Ser Ala Ser Thr Thr Val Leu Pro Asp Asp
65 70 75 80
Phe Thr Ala Tyr Pro Val Gly Lys Phe Phe Gln Ser Asp Thr Trp Lys
85 90 95
Gln Ser Thr Gln Arg Leu Arg Arg Gly Leu Pro Ala Phe Leu Arg Ala
100 105 110
Arg Arg Gly Arg Thr Leu Ala Lys Glu Leu Glu Ala Leu Arg Glu Ala
115 120 125
Lys Ser His Arg Pro Leu Ile Ala Leu Pro Thr Gln Asp Pro Ala Thr
130 135 140
His Gly Gly Ala Ser Ser Glu Ala Ser Ser Asp
145 150 155

<210> 161
<211> 158
<212> PRT
<213> Canis familiaris

<220>
<221> PEPTIDE
<222> (1)...(158)
<223> DOG ISO1 XP_540785.2

<400> 161
Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
1 5 10 15
Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Arg Pro Ala
20 25 30

Ser Arg Val Thr Arg Arg Ser Ser Arg Gly Ile Val Glu Glu Cys Cys
35 40 45
Phe Arg Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Thr Pro
50 55 60
Ala Lys Ser Glu Arg Asp Val Ser Thr Pro Pro Thr Val Leu Pro Asp
65 70 75 80
Asn Phe Pro Arg Tyr Pro Val Gly Lys Phe Phe Gln Tyr Asp Thr Trp
85 90 95
Lys Gln Ser Ala Gln Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg
100 105 110
Ala Arg Arg Gly Arg Met Leu Ala Lys Glu Leu Glu Ala Phe Arg Glu
115 120 125
Ala Lys Arg His Arg Pro Leu Ile Ala Leu Pro Thr His Asp Pro Ala
130 135 140
Thr His Gly Gly Ala Ser Pro Glu Ala Ser Gly Asn Gln Lys
145 150 155

<210> 162
<211> 161
<212> PRT
<213> Canis familiaris

<220>
<221> PEPTIDE
<222> (1)...(161)
<223> DOG ISO2 XP_851137.1

<400> 162
Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
1 5 10 15
Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Asp Leu Ser
20 25 30
Arg Pro Ala Ser Arg Val Thr Arg Arg Ser Ser Arg Gly Ile Val Glu
35 40 45
Glu Cys Cys Phe Arg Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys
50 55 60
Ala Thr Pro Ala Lys Ser Glu Arg Asp Val Ser Thr Pro Pro Thr Val
65 70 75 80
Leu Pro Asp Asn Phe Pro Arg Tyr Pro Val Gly Lys Phe Phe Gln Tyr
85 90 95
Asp Thr Trp Lys Gln Ser Ala Gln Arg Leu Arg Arg Gly Leu Pro Ala
100 105 110
Leu Leu Arg Ala Arg Arg Gly Arg Met Leu Ala Lys Glu Leu Glu Ala
115 120 125
Phe Arg Glu Ala Lys Arg His Arg Pro Leu Ile Ala Leu Pro Thr His
130 135 140
Asp Pro Ala Thr His Gly Gly Ala Ser Pro Glu Ala Ser Gly Asn Gln
145 150 155 160
Lys

<210> 163
<211> 170
<212> PRT
<213> Canis familiaris

<220>
<221> PEPTIDE
<222> (1)...(170)
<223> DOG ISO3 XP_863200.1

<400> 163
Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
1 5 10 15
Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Asp Ala Ala
20 25 30
Leu Leu Pro Pro Val Gly Leu Pro Gly Arg Pro Ala Ser Arg Val Thr
35 40 45
Arg Arg Ser Ser Arg Gly Ile Val Glu Glu Cys Cys Phe Arg Ser Cys
50 55 60
Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Thr Pro Ala Lys Ser Glu
65 70 75 80
Arg Asp Val Ser Thr Pro Pro Thr Val Leu Pro Asp Asn Phe Pro Arg
85 90 95
Tyr Pro Val Gly Lys Phe Phe Gln Tyr Asp Thr Trp Lys Gln Ser Ala
100 105 110
Gln Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala Arg Arg Gly
115 120 125
Arg Met Leu Ala Lys Glu Leu Glu Ala Phe Arg Glu Ala Lys Arg His
130 135 140
Arg Pro Leu Ile Ala Leu Pro Thr His Asp Pro Ala Thr His Gly Gly
145 150 155 160
Ala Ser Pro Glu Ala Ser Gly Asn Gln Lys
165 170

<210> 164
<211> 164
<212> PRT
<213> Gallus gallus

<220>
<221> PEPTIDE
<222> (1)...(164)
<223> REDJUNGLEFOWL XP_421026.2

<400> 164
Ala Tyr Gly Thr Ala Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
1 5 10 15
Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Arg Pro Val
20 25 30
Gly Arg Asn Asn Arg Arg Ile Asn Arg Gly Ile Val Glu Glu Cys Cys
35 40 45
Phe Arg Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Lys Ser
50 55 60
Val Lys Ser Glu Arg Asp Leu Ser Ala Thr Ser Leu Ala Gly Leu Pro
65 70 75 80
Ala Leu Asn Lys Glu Ser Phe Gln Lys Pro Ser His Ala Lys Tyr Ser
85 90 95
Lys Tyr Asn Val Trp Gln Lys Lys Ser Ser Gln Arg Leu Gln Arg Glu
100 105 110
Val Pro Gly Ile Leu Arg Ala Arg Arg Tyr Arg Trp Gln Ala Glu Gly
115 120 125
Leu Gln Ala Ala Glu Glu Ala Arg Ala Met His Arg Pro Leu Ile Ser
130 135 140
Leu Pro Ser Gln Arg Pro Pro Ala Pro Arg Ala Ser Pro Glu Ala Thr
145 150 155 160
Gly Pro Gln Glu

<210> 165
<211> 156
<212> PRT
<213> Rattus norvegicus

<220>
<221> PEPTIDE
<222> (1)...(156)
<223> RAT NP_113699.1

<400> 165

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Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
 1               5                  10                  15
Leu Gln Phe Val Cys Ser Asp Arg Gly Phe Tyr Phe Ser Arg Pro Ser
            20                  25                  30
Ser Arg Ala Asn Arg Arg Ser Arg Gly Ile Val Glu Glu Cys Cys Phe
        35                  40                  45
Arg Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Thr Pro Ala
    50                  55                  60
Lys Ser Glu Arg Asp Val Ser Thr Ser Gln Ala Val Leu Pro Asp Asp
65                  70                  75                  80
Phe Pro Arg Tyr Pro Val Gly Lys Phe Phe Lys Phe Asp Thr Trp Arg
                85                  90                  95
Gln Ser Ala Gly Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala
            100                 105                 110
Arg Arg Gly Arg Met Leu Ala Lys Glu Leu Glu Ala Phe Arg Glu Ala
        115                 120                 125
Lys Arg His Arg Pro Leu Ile Val Leu Pro Pro Lys Asp Pro Ala His
    130                 135                 140
Gly Gly Ala Ser Ser Glu Met Ser Ser Asn His Gln
145                 150                 155
```

<210> 166
<211> 156
<212> PRT
<213> Mus musculus

<220>
<221> PEPTIDE
<222> (1)...(156)
<223> MOUSE NP_034644.1

<400> 166

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Ala Tyr Gly Pro Gly Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
 1               5                  10                  15
Leu Gln Phe Val Cys Ser Asp Arg Gly Phe Tyr Phe Ser Arg Pro Ser
            20                  25                  30
Ser Arg Ala Asn Arg Arg Ser Arg Gly Ile Val Glu Glu Cys Cys Phe
        35                  40                  45
Arg Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Thr Pro Ala
    50                  55                  60
Lys Ser Glu Arg Asp Val Ser Thr Ser Gln Ala Val Leu Pro Asp Asp
65                  70                  75                  80
Phe Pro Arg Tyr Pro Val Gly Lys Phe Phe Gln Tyr Asp Thr Trp Arg
                85                  90                  95
Gln Ser Ala Gly Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala
            100                 105                 110
Arg Arg Gly Arg Met Leu Ala Lys Glu Leu Lys Glu Phe Arg Glu Ala
        115                 120                 125
Lys Arg His Arg Pro Leu Ile Val Leu Pro Pro Lys Asp Pro Ala His
    130                 135                 140
Gly Gly Ala Ser Ser Glu Met Ser Ser Asn His Gln
145                 150                 155
```

<210> 167
<211> 160

<212> PRT
<213> Pan troglodytes

<220>
<221> PEPTIDE
<222> (1)...(160)
<223> CHIMPANZEE XP_001153640.1

<400> 167
Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
 1   5   10   15
Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Lys Ala Ser
            20   25   30
Thr Pro Ala Ala Phe Pro Ile Thr Arg Pro Leu Arg Arg Val Gly Gln
        35   40   45
Arg Cys Cys Arg Gly Gly Cys Pro Pro Ala Asp Leu Arg Asp Ala Ser
    50   55   60
Ala Phe Pro Arg Arg Glu Ser Arg His Leu Leu Thr Ser Pro Phe Pro
65   70   75   80
Ser Gln Asp Asn Phe Pro Arg Tyr Pro Val Gly Lys Phe Phe Gln Tyr
                85   90   95
Asp Thr Trp Lys Gln Ser Thr Gln Arg Leu Arg Arg Gly Leu Pro Ala
            100   105   110
Leu Leu Arg Ala Arg Arg Gly His Met Leu Ala Lys Glu Leu Glu Ala
        115   120   125
Phe Arg Glu Ala Lys Arg His Arg Pro Leu Ile Ala Leu Pro Thr Gln
    130   135   140
Asp Pro Ala His Gly Gly Ala Pro Pro Glu Met Ala Ser Asn Arg Lys
145   150   155   160

<210> 168
<211> 167
<212> PRT
<213> Danio rerio

<220>
<221> PEPTIDE
<222> (1)...(166)
<223> ZEBRAFISH ISO1 XP_001338042.1

<400> 168
Glu Val Ala Ser Ala Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Ala
 1   5   10   15
Leu Gln Phe Val Cys Glu Asp Arg Gly Phe Tyr Phe Ser Arg Pro Thr
            20   25   30
Ser Arg Ser Asn Ser Arg Arg Ser Gln Asn Arg Gly Ile Val Glu Glu
        35   40   45
Cys Cys Phe Ser Ser Cys Asn Leu Ala Leu Leu Glu Gln Tyr Cys Ala
    50   55   60
Lys Pro Ala Lys Ser Glu Arg Asp Val Ser Ala Thr Ser Leu Gln Val
65   70   75   80
Ile Pro Val Met Pro Ala Leu Lys Gln Glu Val Pro Arg Lys His Val
                85   90   95
Thr Val Lys Tyr Ser Lys Tyr Asp Val Trp Gln Arg Lys Ala Ala Gln
            100   105   110
Arg Leu Arg Arg Gly Ile Pro Ala Ile Leu Arg Ala Lys Lys Phe Arg
        115   120   125
Arg Gln Ala Glu Arg Ile Lys Ala Gln Glu Gln Leu Leu His His Arg
    130   135   140
Pro Leu Ile Thr Leu Pro Ser Lys Leu Pro Pro Ile Leu Leu Pro Thr
145   150   155   160
Glu Asn Tyr Val Ser His Lys
                165

<210> 169
<211> 161
<212> PRT
<213> Danio rerio

<220>
<221> PEPTIDE
<222> (1)...(167)
<223> ZEBRAFISH ISO2B NP_571508.1

<400> 169
Asn Val Thr Ala Gly Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
1 5 10 15
Leu Gln Phe Val Cys Gly Glu Asp Gly Phe Tyr Ile Ser Arg Pro Asn
20 25 30
Arg Ser Asn Ser Arg Arg Pro Gln Arg Gly Ile Val Glu Glu Cys Cys
35 40 45
Phe Arg Ser Cys Glu Leu His Leu Leu Gln Gln Tyr Cys Ala Lys Pro
50 55 60
Val Lys Ser Glu Arg Asp Val Ser Ser Thr Ser Leu Gln Val Phe Pro
65 70 75 80
Val Ser Gln Ala Leu His Lys Asp Thr Ile Asn Val Lys Tyr Ser Lys
85 90 95
Tyr Glu Val Trp Gln Gln Lys Ala Ala Gln Arg Leu Arg Arg Gly Val
100 105 110
Pro Ser Ile Leu Leu Ala Arg Lys Phe Arg Arg Gln Met Glu Lys Ile
115 120 125
Gln Asp Glu Glu Gln Thr Ser Phe His Arg Pro Leu Met Thr Leu Pro
130 135 140
Asn Arg Gln Pro Ala Ile Val Pro His Val Gln Ile Ser Thr Ser Arg
145 150 155 160
Lys

<210> 170
<211> 89
<212> PRT
<213> Artificial Sequence

<220>
<223> Majority consensus sequence for Seq. ID Nos.
171-180.

<400> 170
Arg Asp Val Ser Thr Pro Pro Thr Val Leu Pro Asp Asn Phe Pro Arg
1 5 10 15
Tyr Pro Val Gly Lys Phe Phe Gln Tyr Asp Thr Trp Lys Gln Ser Ala
20 25 30
Gln Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala Arg Arg Gly
35 40 45
Arg Met Leu Ala Lys Glu Leu Glu Ala Phe Arg Glu Ala Lys Arg His
50 55 60
Arg Pro Leu Ile Ala Leu Pro Thr Gln Asp Pro Ala His Gly Gly Ala
65 70 75 80
Ser Pro Glu Ala Ser Ser Asn Arg Lys
85

<210> 171
<211> 90
<212> PRT
<213> Sus scrofa

<220>
<221> PEPTIDE
<222> (1)...(90)
<223> PIG NP_999048.1

<400> 171
Arg Asp Val Ser Thr Pro Pro Thr Val Leu Pro Asp Asn Phe Pro Arg
 1   5   10   15
Tyr Pro Val Gly Lys Phe Phe Arg Tyr Asp Thr Trp Lys Gln Ser Ala
            20   25   30
Gln Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala Arg Arg Gly
        35   40   45
Arg Thr Leu Ala Lys Glu Leu Glu Ala Val Arg Glu Ala Lys Arg His
    50   55   60
Arg Pro Leu Thr Ala Arg Pro Thr Arg Asp Pro Ala Ala His Gly Gly
65   70   75   80
Ala Ser Pro Glu Ala Ser Gly His Arg Lys
                85   90

<210> 172
<211> 88
<212> PRT
<213> Bos taurus

<220>
<221> PEPTIDE
<222> (1)...(88)
<223> CATTLE NP_776512.2

<400> 172
Arg Asp Val Ser Ala Ser Thr Thr Val Leu Pro Asp Asp Val Thr Ala
 1   5   10   15
Tyr Pro Val Gly Lys Phe Phe Gln Tyr Asp Ile Trp Lys Gln Ser Thr
            20   25   30
Gln Arg Leu Arg Arg Gly Leu Pro Ala Phe Leu Arg Ala Arg Arg Gly
        35   40   45
Arg Thr Leu Ala Lys Glu Leu Glu Ala Leu Arg Glu Ala Lys Ser His
    50   55   60
Arg Pro Leu Ile Ala Leu Pro Thr Gln Asp Pro Ala Thr His Gly Gly
65   70   75   80
Ala Ser Ser Lys Ala Ser Ser Asp
                85

<210> 173
<211> 88
<212> PRT
<213> Ovis aries

<220>
<221> PEPTIDE
<222> (1)...(88)
<223> SHEEP NP_001009311.1

<400> 173
Arg Asp Val Ser Ala Ser Thr Thr Val Leu Pro Asp Asp Phe Thr Ala
 1   5   10   15
Tyr Pro Val Gly Lys Phe Phe Gln Ser Asp Thr Trp Lys Gln Ser Thr
            20   25   30
Gln Arg Leu Arg Arg Gly Leu Pro Ala Phe Leu Arg Ala Arg Arg Gly
        35   40   45
Arg Thr Leu Ala Lys Glu Leu Glu Ala Leu Arg Glu Ala Lys Ser His
    50   55   60

Arg Pro Leu Ile Ala Leu Pro Thr Gln Asp Pro Ala Thr His Gly Gly
65 70 75 80
Ala Ser Ser Glu Ala Ser Ser Asp
85

<210> 174
<211> 90
<212> PRT
<213> Canis familiaris

<220>
<221> PEPTIDE
<222> (1)...(90)
<223> DOG ISO1 XP_540785.2

<400> 174
Arg Asp Val Ser Thr Pro Pro Thr Val Leu Pro Asp Asn Phe Pro Arg
1 5 10 15
Tyr Pro Val Gly Lys Phe Phe Gln Tyr Asp Thr Trp Lys Gln Ser Ala
20 25 30
Gln Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala Arg Arg Gly
35 40 45
Arg Met Leu Ala Lys Glu Leu Glu Ala Phe Arg Glu Ala Lys Arg His
50 55 60
Arg Pro Leu Ile Ala Leu Pro Thr His Asp Pro Ala Thr His Gly Gly
65 70 75 80
Ala Ser Pro Glu Ala Ser Gly Asn Gln Lys
85 90

<210> 175
<211> 96
<212> PRT
<213> Gallus gallus

<220>
<221> PEPTIDE
<222> (1)...(96)
<223> REDJUNGLEFOWL XP_421026.2

<400> 175
Arg Asp Leu Ser Ala Thr Ser Leu Ala Gly Leu Pro Ala Leu Asn Lys
1 5 10 15
Glu Ser Phe Gln Lys Pro Ser His Ala Lys Tyr Ser Lys Tyr Asn Val
20 25 30
Trp Gln Lys Lys Ser Ser Gln Arg Leu Gln Arg Glu Val Pro Gly Ile
35 40 45
Leu Arg Ala Arg Arg Tyr Arg Trp Gln Ala Glu Gly Leu Gln Ala Ala
50 55 60
Glu Glu Ala Arg Ala Met His Arg Pro Leu Ile Ser Leu Pro Ser Gln
65 70 75 80
Arg Pro Pro Ala Pro Arg Ala Ser Pro Glu Ala Thr Gly Pro Gln Glu
85 90 95

<210> 176
<211> 89
<212> PRT
<213> Rattus norvegicus

<220>
<221> PEPTIDE

<222> (1)...(89)
<223> RAT NP_113699.1

<400> 176
Arg Asp Val Ser Thr Ser Gln Ala Val Leu Pro Asp Asp Phe Pro Arg
 1   5   10   15
Tyr Pro Val Gly Lys Phe Phe Lys Phe Asp Thr Trp Arg Gln Ser Ala
            20   25   30
Gly Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala Arg Arg Gly
        35   40   45
Arg Met Leu Ala Lys Glu Leu Glu Ala Phe Arg Glu Ala Lys Arg His
    50   55   60
Arg Pro Leu Ile Val Leu Pro Pro Lys Asp Pro Ala His Gly Gly Ala
65   70   75   80
Ser Ser Glu Met Ser Ser Asn His Gln
                85

<210> 177
<211> 89
<212> PRT
<213> Mus musculus

<220>
<221> PEPTIDE
<222> (1)...(89)
<223> MOUSE NP_034644.1

<400> 177
Arg Asp Val Ser Thr Ser Gln Ala Val Leu Pro Asp Asp Phe Pro Arg
 1   5   10   15
Tyr Pro Val Gly Lys Phe Phe Gln Tyr Asp Thr Trp Arg Gln Ser Ala
            20   25   30
Gly Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala Arg Arg Gly
        35   40   45
Arg Met Leu Ala Lys Glu Leu Lys Glu Phe Arg Glu Ala Lys Arg His
    50   55   60
Arg Pro Leu Ile Val Leu Pro Pro Lys Asp Pro Ala His Gly Gly Ala
65   70   75   80
Ser Ser Glu Met Ser Ser Asn His Gln
                85

<210> 178
<211> 89
<212> PRT
<213> Pan troglodytes

<220>
<221> PEPTIDE
<222> (1)...(89)
<223> CHIMPANZEE XP_001153640.1

<400> 178
Arg His Leu Leu Thr Ser Pro Phe Pro Ser Gln Asp Asn Phe Pro Arg
 1   5   10   15
Tyr Pro Val Gly Lys Phe Phe Gln Tyr Asp Thr Trp Lys Gln Ser Thr
            20   25   30
Gln Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala Arg Arg Gly
        35   40   45
His Met Leu Ala Lys Glu Leu Glu Ala Phe Arg Glu Ala Lys Arg His
    50   55   60

Arg Pro Leu Ile Ala Leu Pro Thr Gln Asp Pro Ala His Gly Gly Ala
65 70 75 80
Pro Pro Glu Met Ala Ser Asn Arg Lys
85

<210> 179
<211> 97
<212> PRT
<213> Danio rerio

<220>
<221> PEPTIDE
<222> (1)...(97)
<223> ZEBRAFISH ISO1 XP_001338042.1

<400> 179
Arg Asp Val Ser Ala Thr Ser Leu Gln Val Ile Pro Val Met Pro Ala
1 5 10 15
Leu Lys Gln Glu Val Pro Arg Lys His Val Thr Val Lys Tyr Ser Lys
20 25 30
Tyr Asp Val Trp Gln Arg Lys Ala Ala Gln Arg Leu Arg Arg Gly Ile
35 40 45
Pro Ala Ile Leu Arg Ala Lys Lys Phe Arg Arg Gln Ala Glu Arg Ile
50 55 60
Lys Ala Gln Glu Gln Leu Leu His His Arg Pro Leu Ile Thr Leu Pro
65 70 75 80
Ser Lys Leu Pro Pro Ile Leu Leu Pro Thr Glu Asn Tyr Val Ser His
85 90 95
Lys

<210> 180
<211> 93
<212> PRT
<213> Danio rerio

<220>
<221> PEPTIDE
<222> (1)...(93)
<223> ZEBRAFISH ISO2B NP_571508.1

<400> 180
Arg Asp Val Ser Ser Thr Ser Leu Gln Val Phe Pro Val Ser Gln Ala
1 5 10 15
Leu His Lys Asp Thr Ile Asn Val Lys Tyr Ser Lys Tyr Glu Val Trp
20 25 30
Gln Gln Lys Ala Ala Gln Arg Leu Arg Arg Gly Val Pro Ser Ile Leu
35 40 45
Leu Ala Arg Lys Phe Arg Arg Gln Met Glu Lys Ile Gln Asp Glu Glu
50 55 60
Gln Thr Ser Phe His Arg Pro Leu Met Thr Leu Pro Asn Arg Gln Pro
65 70 75 80
Ala Ile Val Pro His Val Gln Ile Ser Thr Ser Arg Lys
85 90

<210> 181
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE

<222> (1)...(16)
<223> HIGF-1-EC: P2, E3; R37A; R71, S72

<400> 181

Gly	Thr	Leu	Cys	Gly	Ala	Glu	Leu	Val	Asp	Ala	Leu	Gln	Phe	Val	Cys
1				5				10					15		
Gly	Asp	Arg	Gly	Phe	Tyr	Phe	Asn	Lys	Pro	Thr	Gly	Tyr	Gly	Ser	Ser
			20					25					30		
Ser	Arg	Ala	Ala	Pro	Gln	Thr	Gly	Ile	Val	Asp	Glu	Cys	Cys	Phe	Arg
		35					40					45			
Ser	Cys	Asp	Leu	Arg	Arg	Leu	Glu	Met	Tyr	Cys	Ala	Pro	Leu	Lys	Pro
	50					55					60				
Ala	Lys	Ser	Ala	Val	Arg	Ala	Gln	Arg	His	Thr	Asp	Met	Pro	Lys	Thr
65					70					75					80
Gln	Lys	Tyr	Gln	Pro	Pro	Ser	Thr	Asn	Lys	Asn	Thr	Lys	Ser	Gln	Arg
				85					90					95	
Arg	Lys	Gly	Ser	Thr	Phe	Glu	Glu	Arg	Lys						
			100					105							

21489-11012

CLAIMS:

1. A human IGF-1 precursor protein, wherein:

(i) one or more of the following precursor protein residues is deleted or mutated: G1, P2, E3, R36 and R37; and

(ii) the cleavage of the E-peptide from IGF-1 by a protease is reduced by modification of the precursor protein, wherein the modification is the deletion or mutation of R71 or S72 of the precursor protein, and

wherein the numbering of the amino acids G1, P2, E3, R36, R37, R71 and S72 corresponds to SEQ ID NO: 5.

2. The protein of claim 1, wherein amino acids G1, P2 and E3 are deleted, amino acid R37 is mutated to alanine and amino acids R71 and S72 are deleted.

3. The protein of claim 1, wherein amino acid E3 is deleted, amino acid R37 is mutated to alanine and amino acids R71 and S72 are deleted.

4. The protein of claim 2 comprising SEQ ID NO: 8.

5. The protein of claim 3 comprising SEQ ID NO: 53.

6. The protein of any one of claims 1-3, wherein R36 is mutated to alanine.

7. The protein of any one of claims 1-6, further comprising a poly(ethylene glycol) moiety covalently attached to a side-chain of the precursor protein.

8. Use of the protein of any one of claims 1-7 for the treatment of a musculoskeletal disease, diabetes, neuronal cell death, chronic obstructive pulmonary disease, burn injury, or anemia.

9. Use of the protein of any one of claims 1-7 for the manufacture of a medicament for the treatment of a musculoskeletal disease, diabetes, neuronal cell death, chronic obstructive pulmonary disease, burn injury, or anemia.

10. The protein according to any one of claims 1-7 for use in the treatment of a musculoskeletal disease, diabetes, neuronal cell death, chronic obstructive pulmonary disease, burn injury, or anemia.

11. A nucleic acid encoding the protein of any one of claims 1-7.

12. A vector comprising the nucleic acid according to claim 11.

13. A cell transfected with the vector according to claim 12.

Fig. 1A

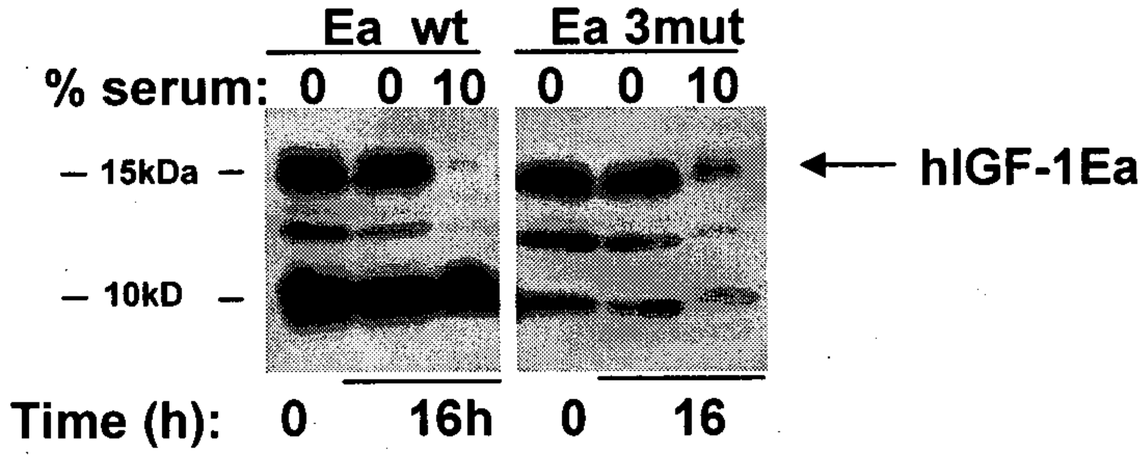


Fig. 1B

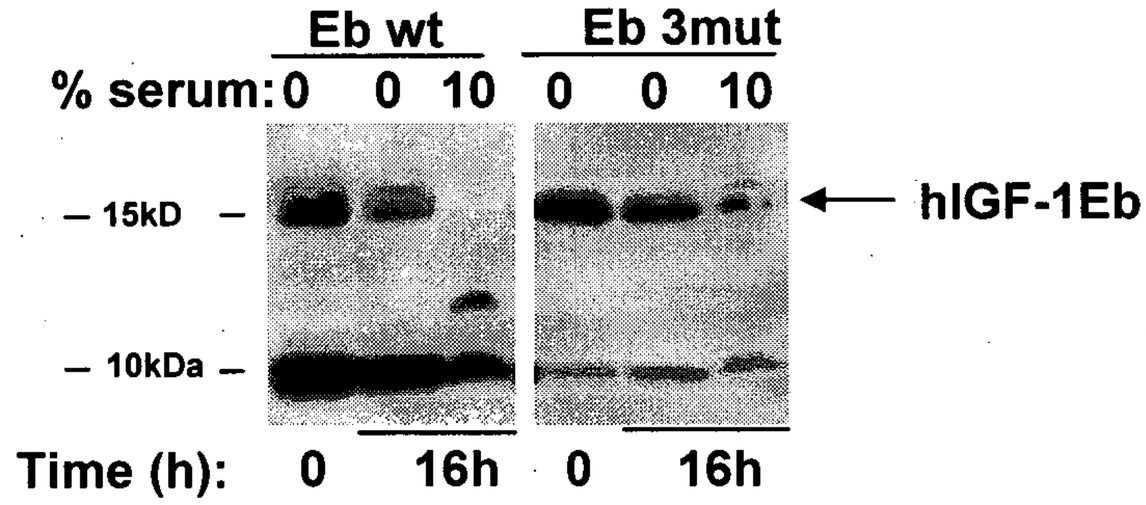


Fig. 1C

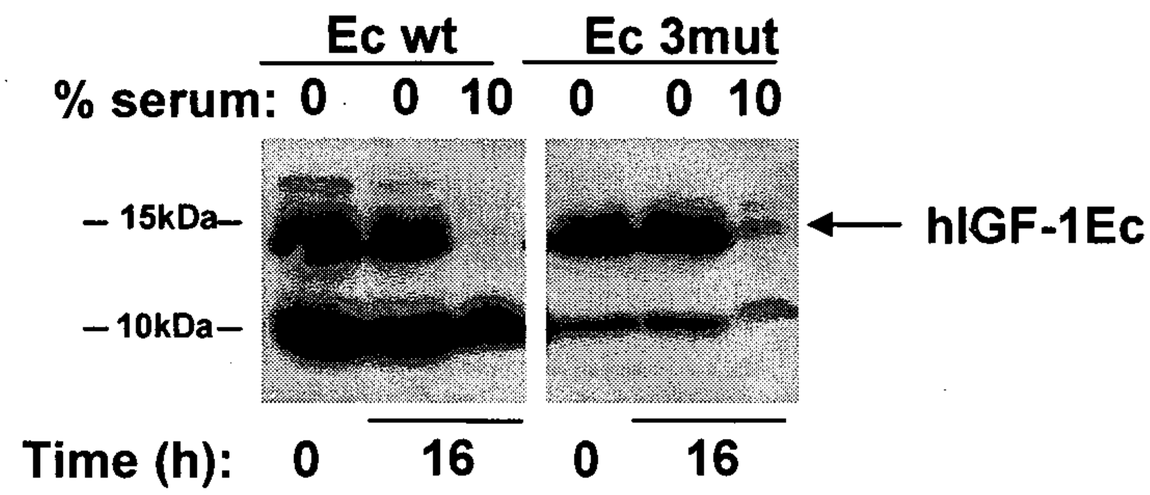


Fig. 2A

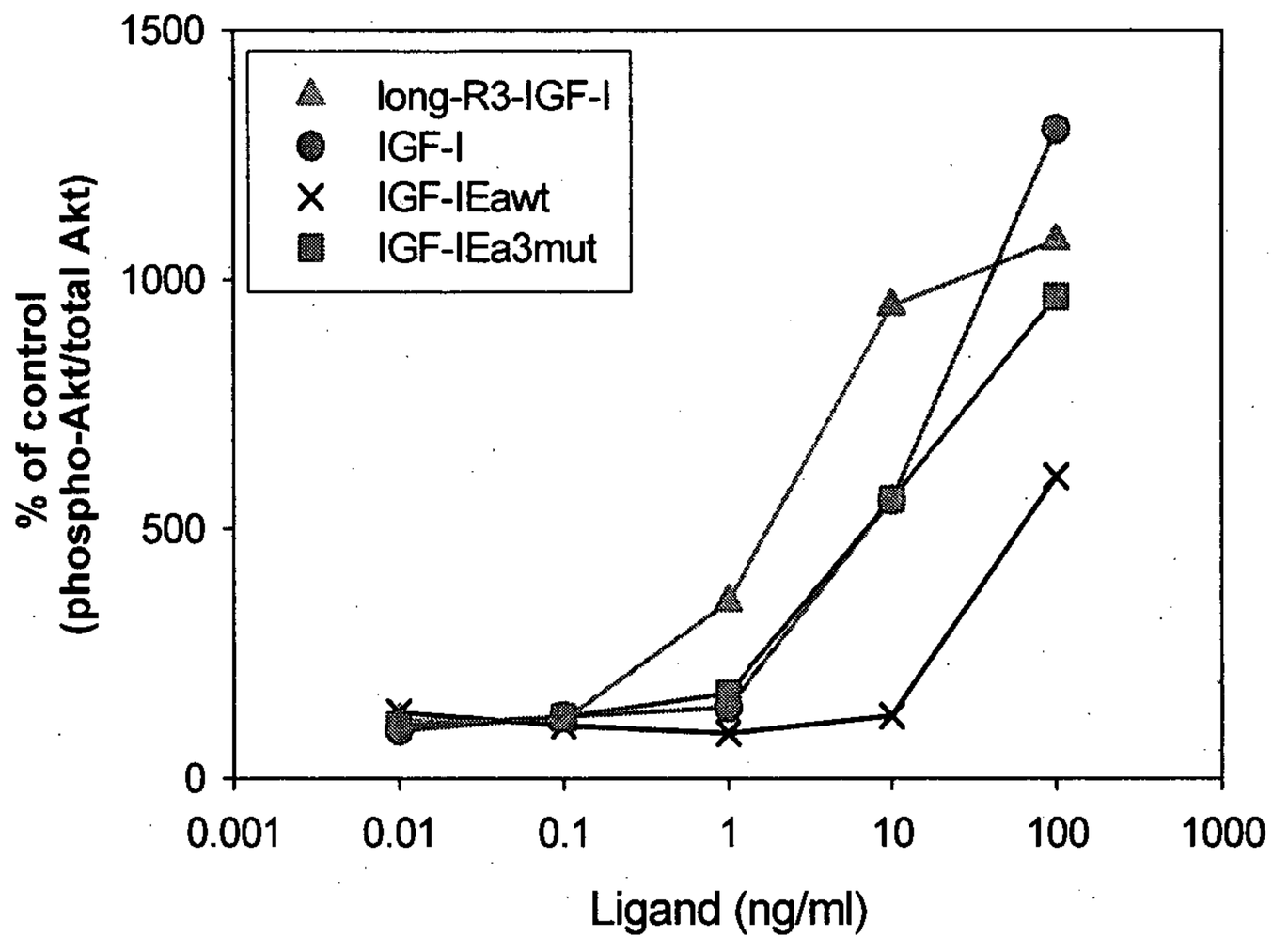

long-R3-IGF-I
IGF-I
IGF-IEawt
IGF-IEa3mut
% of control
(phospho-Akt/total Akt)
1500
1000
500
0
0.001
0.01
0.1
1
10
100
1000
Ligand (ng/ml)

Fig. 2B

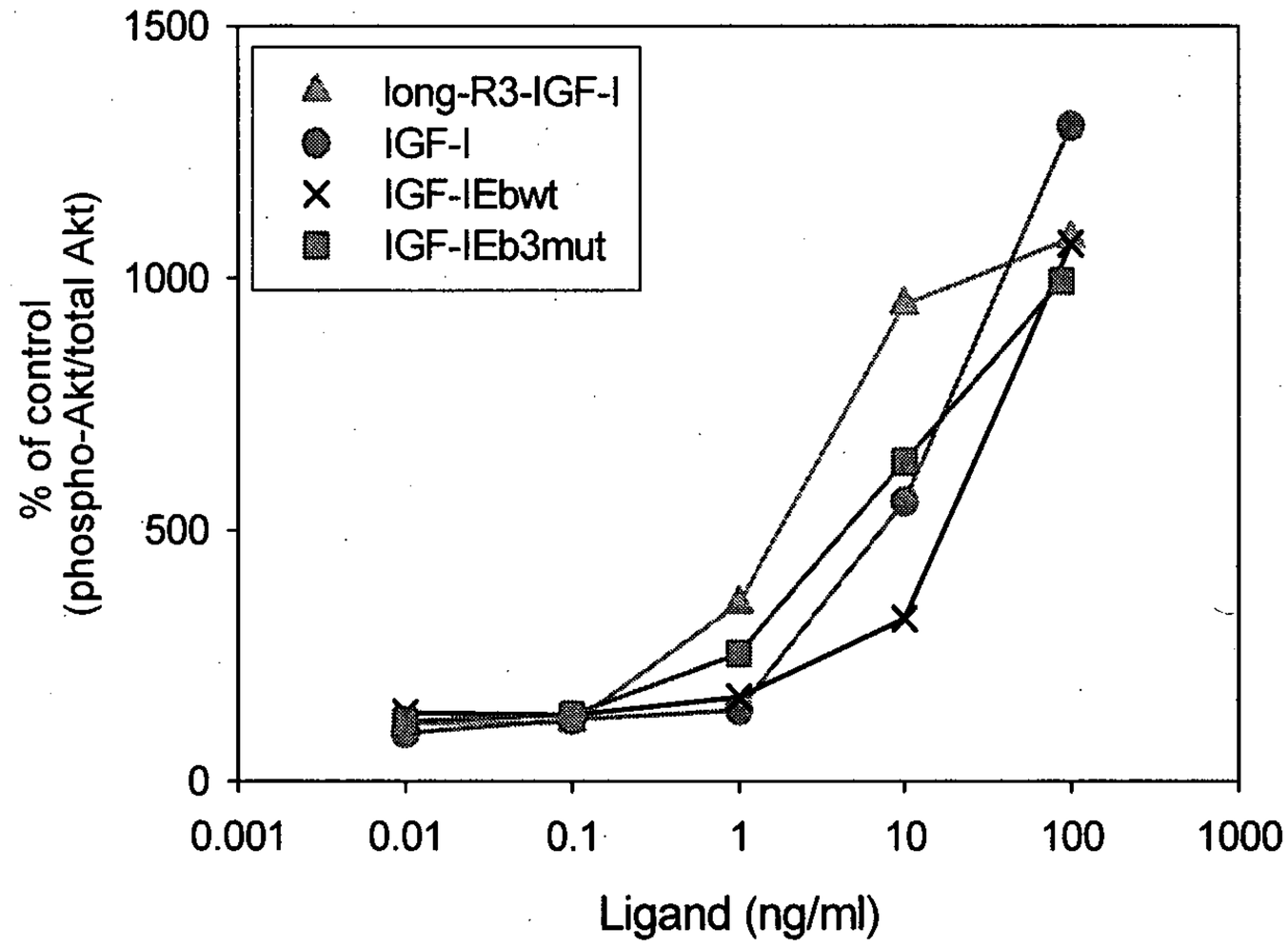

long-R3-IGF-I
IGF-I
IGF-IEbwt
IGF-IEb3mut
% of control
(phospho-Akt/total Akt)
1500
1000
500
0
0.001
0.01
0.1
1
10
100
1000
Ligand (ng/ml)

Fig. 2C

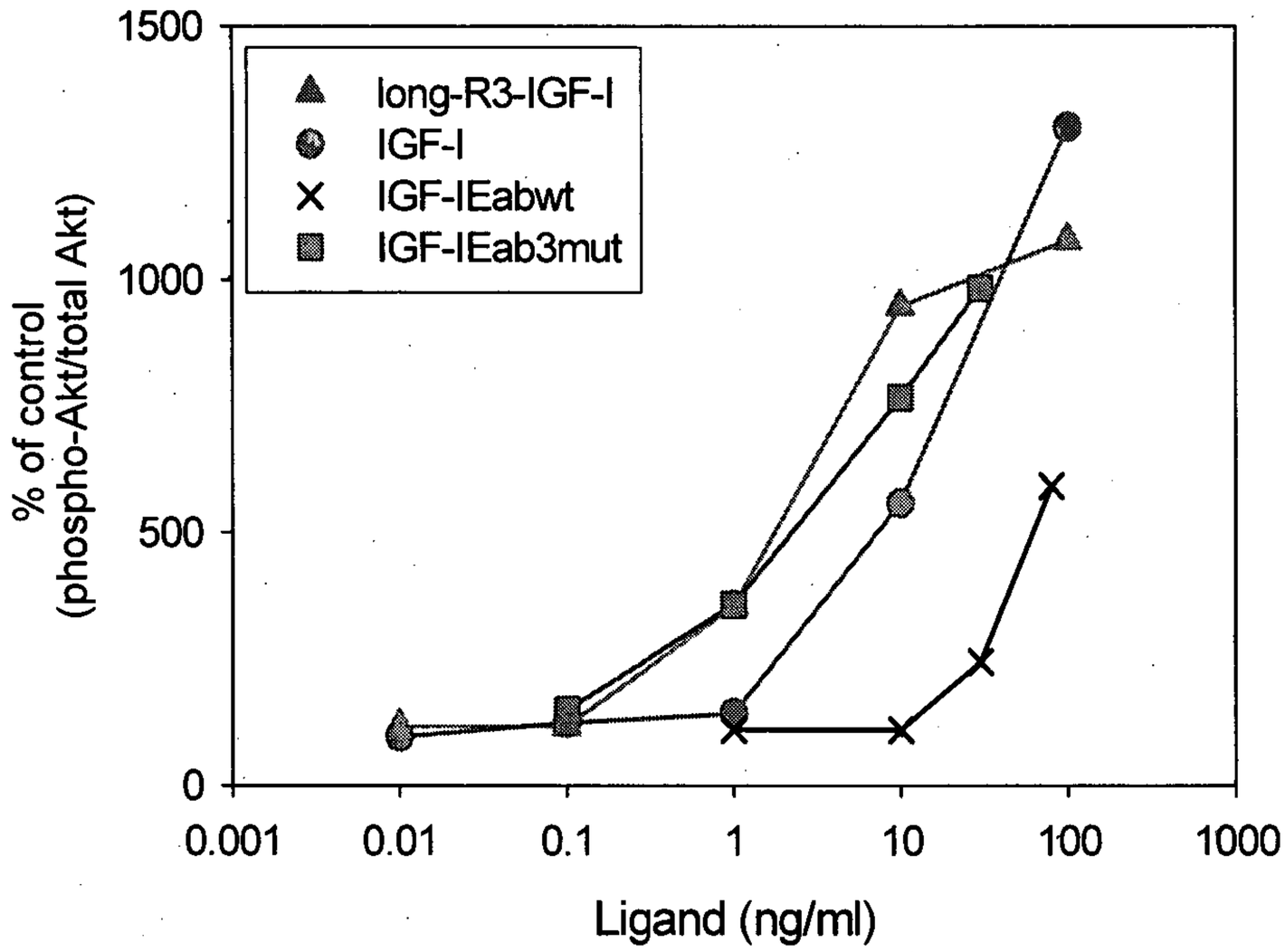

long-R3-IGF-I
IGF-I
IGF-IEabwt
IGF-IEab3mut
% of control
(phospho-Akt/total Akt)
1500
1000
500
0
0.001
0.01
0.1
1
10
100
1000
Ligand (ng/ml)

Fig. 2D

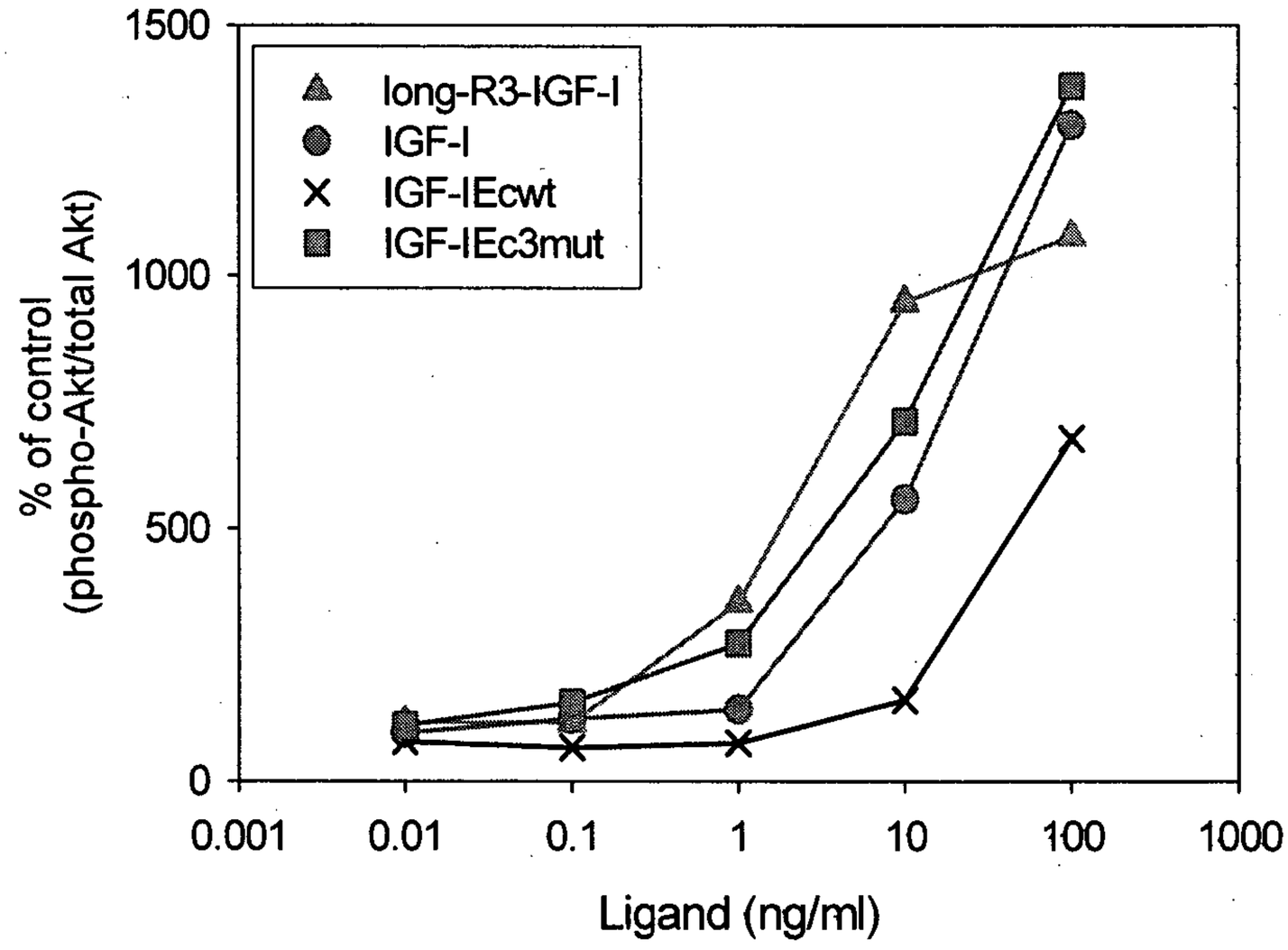

long-R3-IGF-I
IGF-I
IGF-IEcwt
IGF-IEc3mut
% of control
(phospho-Akt/total Akt)
1500
1000
500
0
0.001
0.01
0.1
1
10
100
1000
Ligand (ng/ml)

Fig. 3A

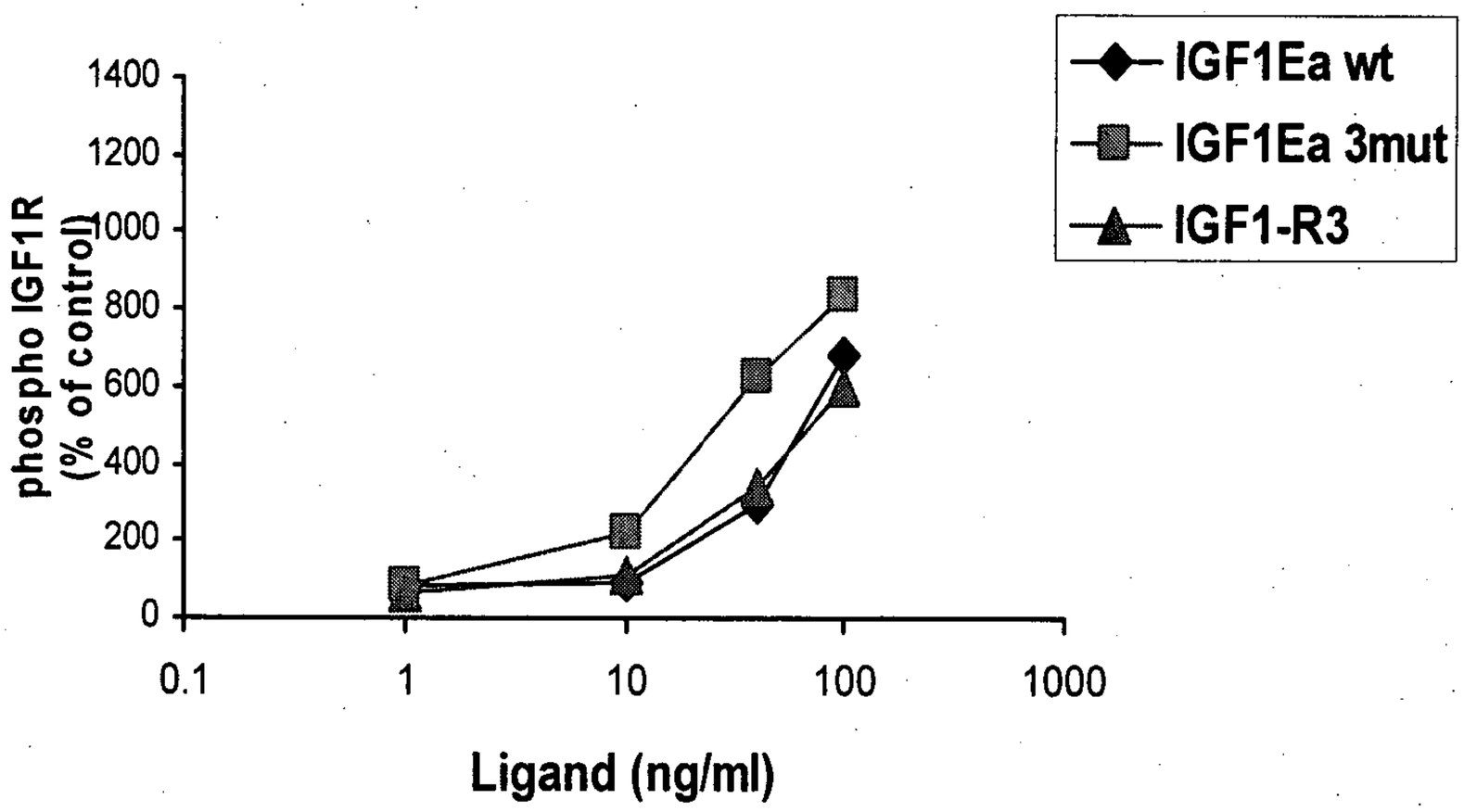

IGF1Ea wt
IGF1Ea 3mut
IGF1-R3
phospho IGF1R (% of control)
1400
1200
1000
800
600
400
200
0
0.1
1
10
100
1000
Ligand (ng/ml)

Fig. 3B

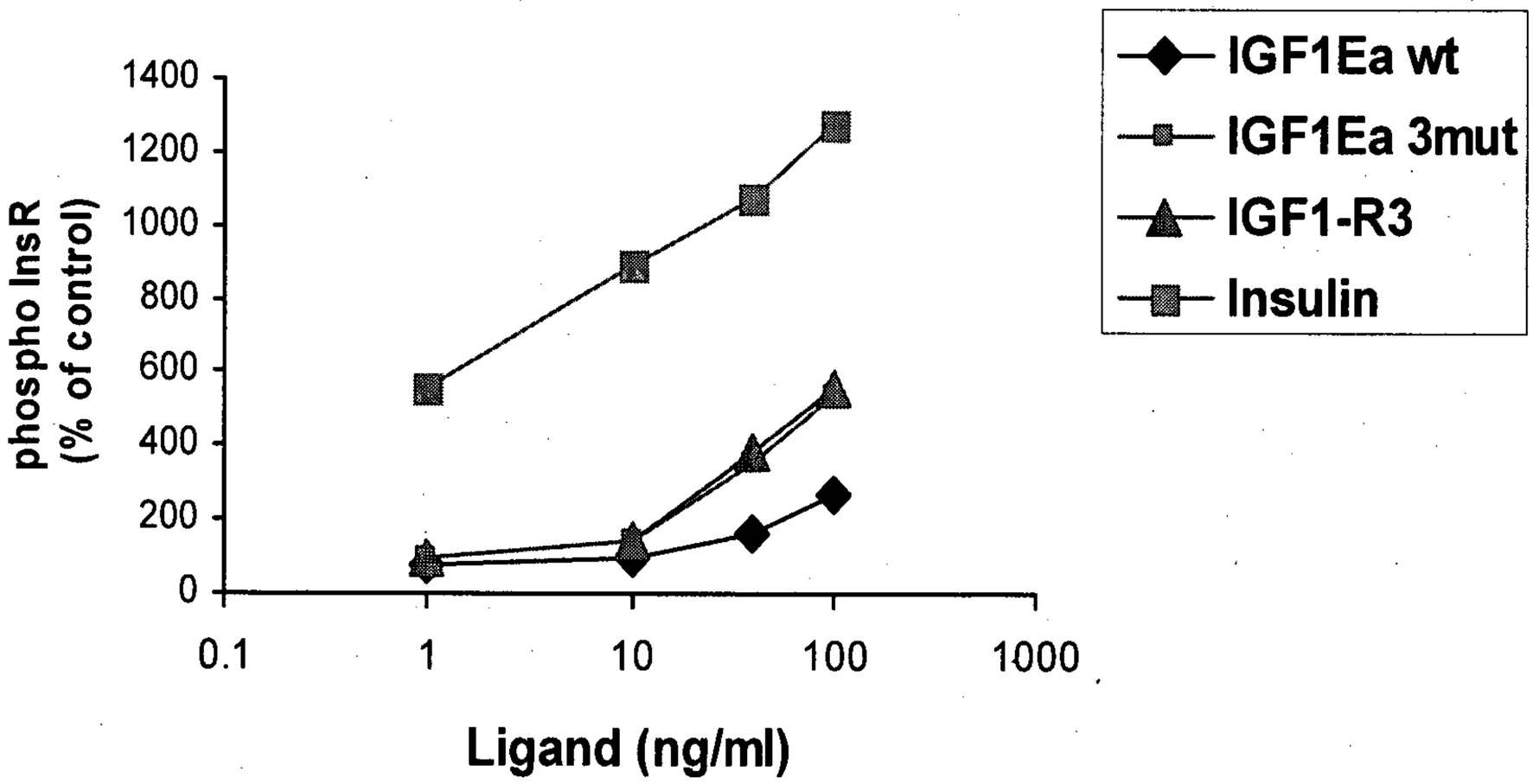

IGF1Ea wt
IGF1Ea 3mut
IGF1-R3
Insulin
phospho InsR (% of control)
1400
1200
1000
800
600
400
200
0
0.1
1
10
100
1000
Ligand (ng/ml)

Fig. 3C

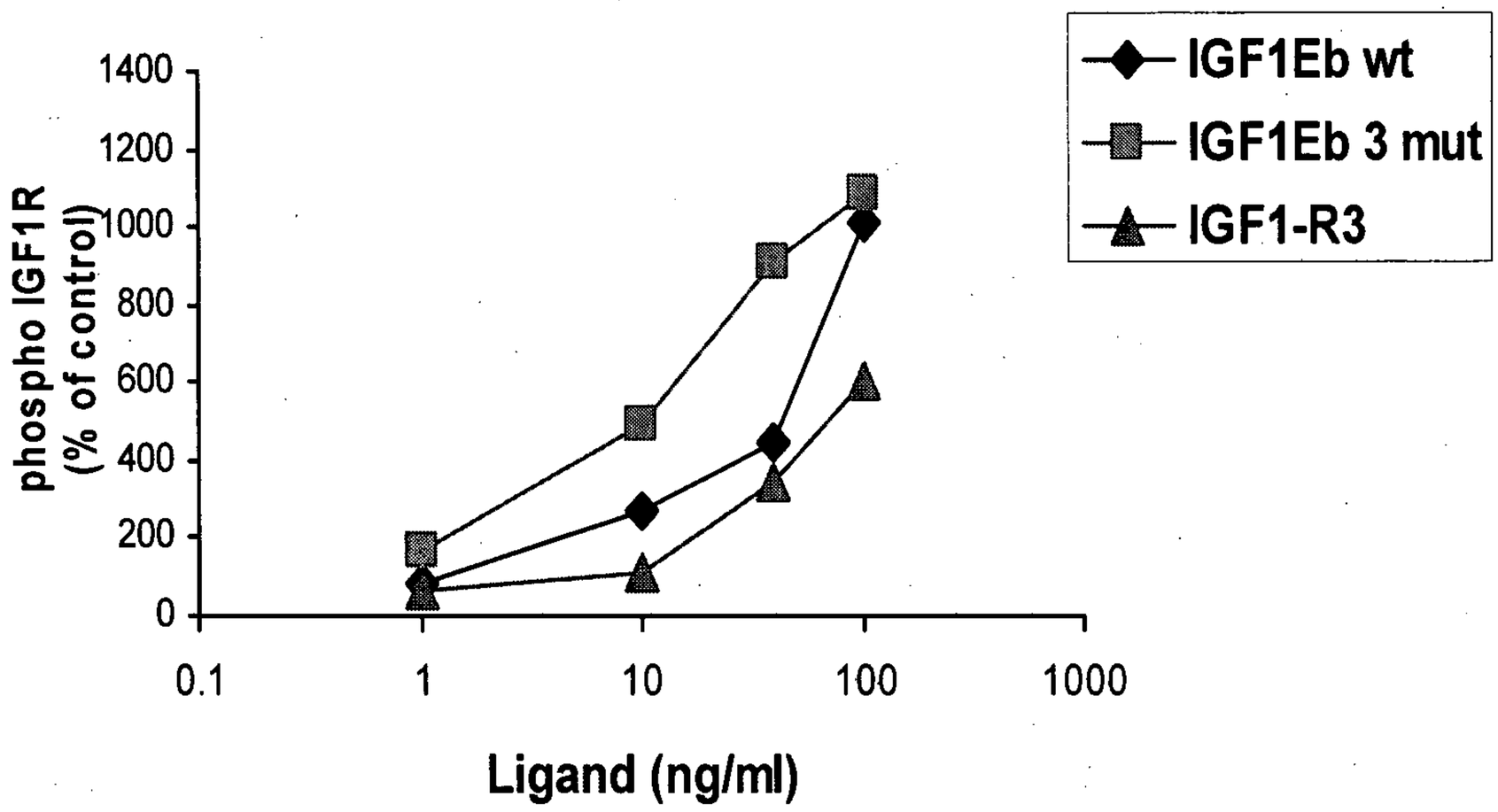

IGF1Eb wt
IGF1Eb 3 mut
IGF1-R3
phospho IGF1R (% of control)
1400
1200
1000
800
600
400
200
0
0.1
1
10
100
1000
Ligand (ng/ml)

Fig. 3D

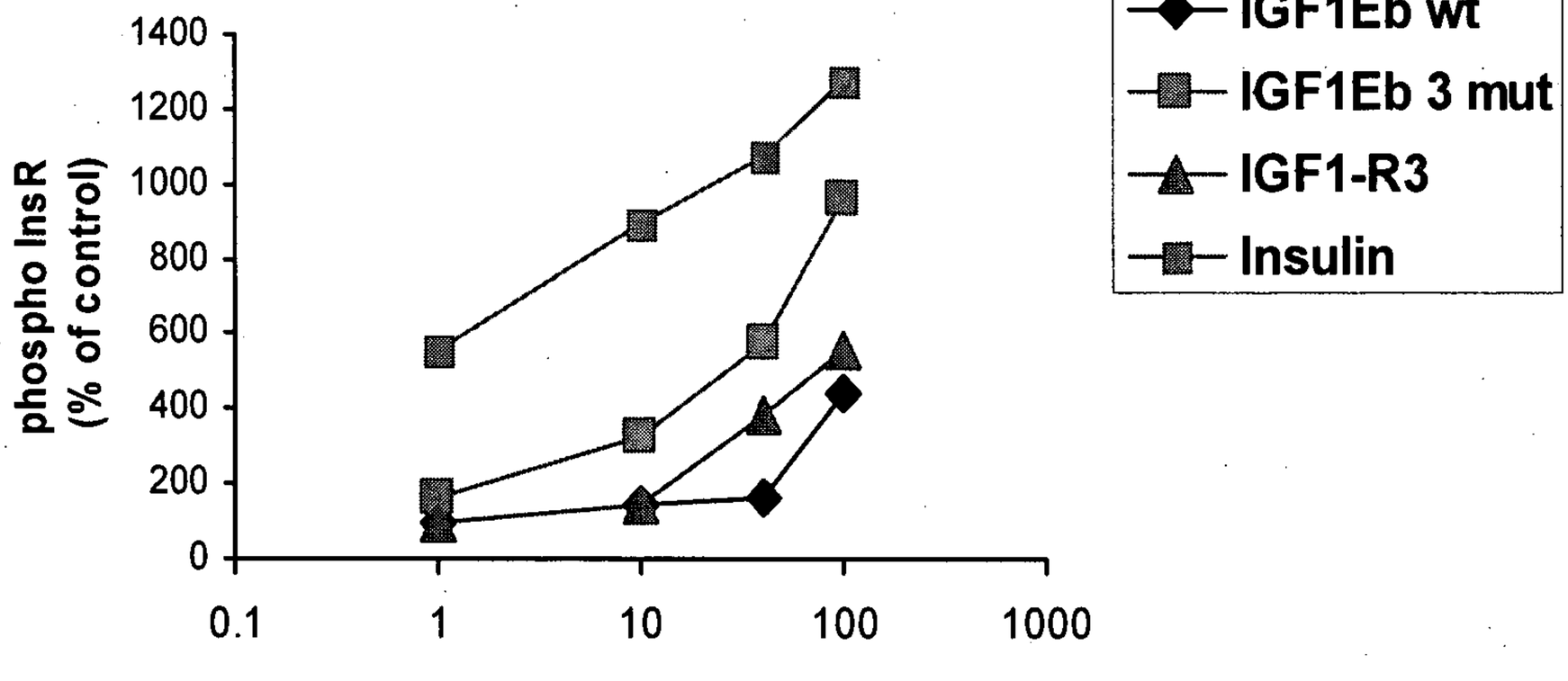

IGF1Eb wt
IGF1Eb 3 mut
IGF1-R3
Insulin
phospho InsR (% of control)
1400
1200
1000
800
600
400
200
0
0.1
1
10
100
1000
Ligand (ng/ml)

Fig. 4A

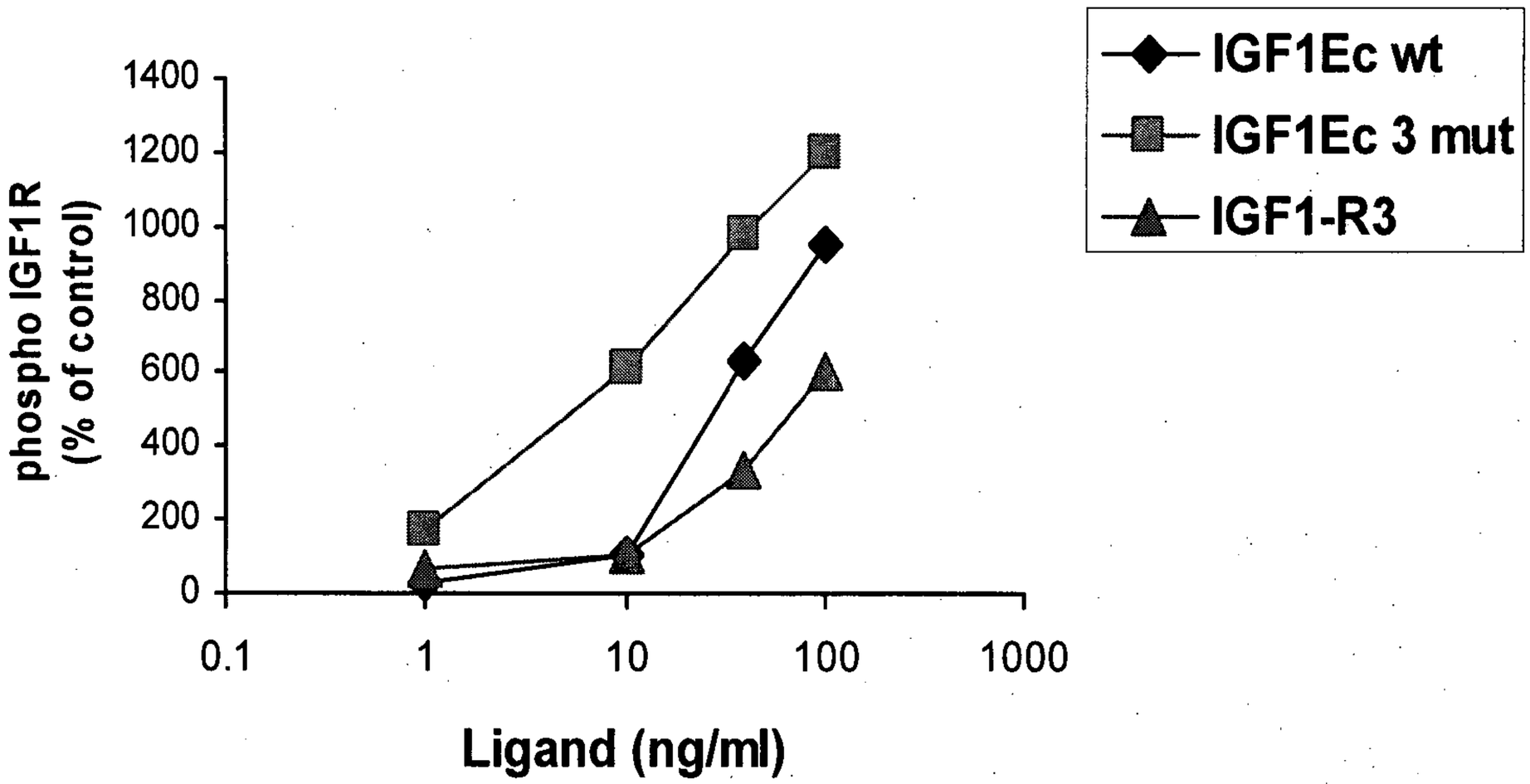

IGF1Ec wt
IGF1Ec 3 mut
IGF1-R3
phospho IGF1R (% of control)
1400
1200
1000
800
600
400
200
0
0.1
1
10
100
1000
Ligand (ng/ml)

Fig. 4B

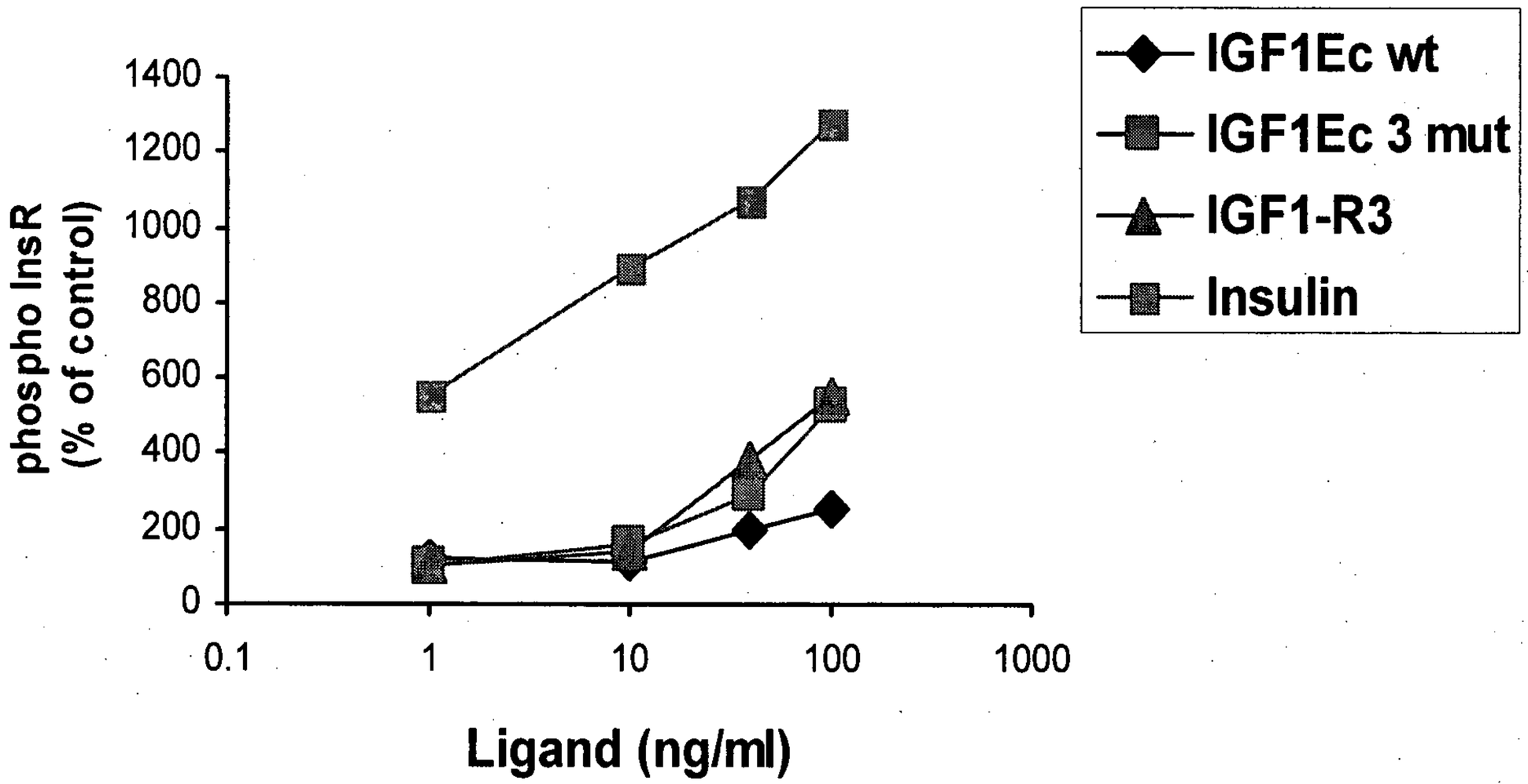

IGF1Ec wt
IGF1Ec 3 mut
IGF1-R3
Insulin
phospho InsR (% of control)
1400
1200
1000
800
600
400
200
0
0.1
1
10
100
1000
Ligand (ng/ml)

Fig. 4C

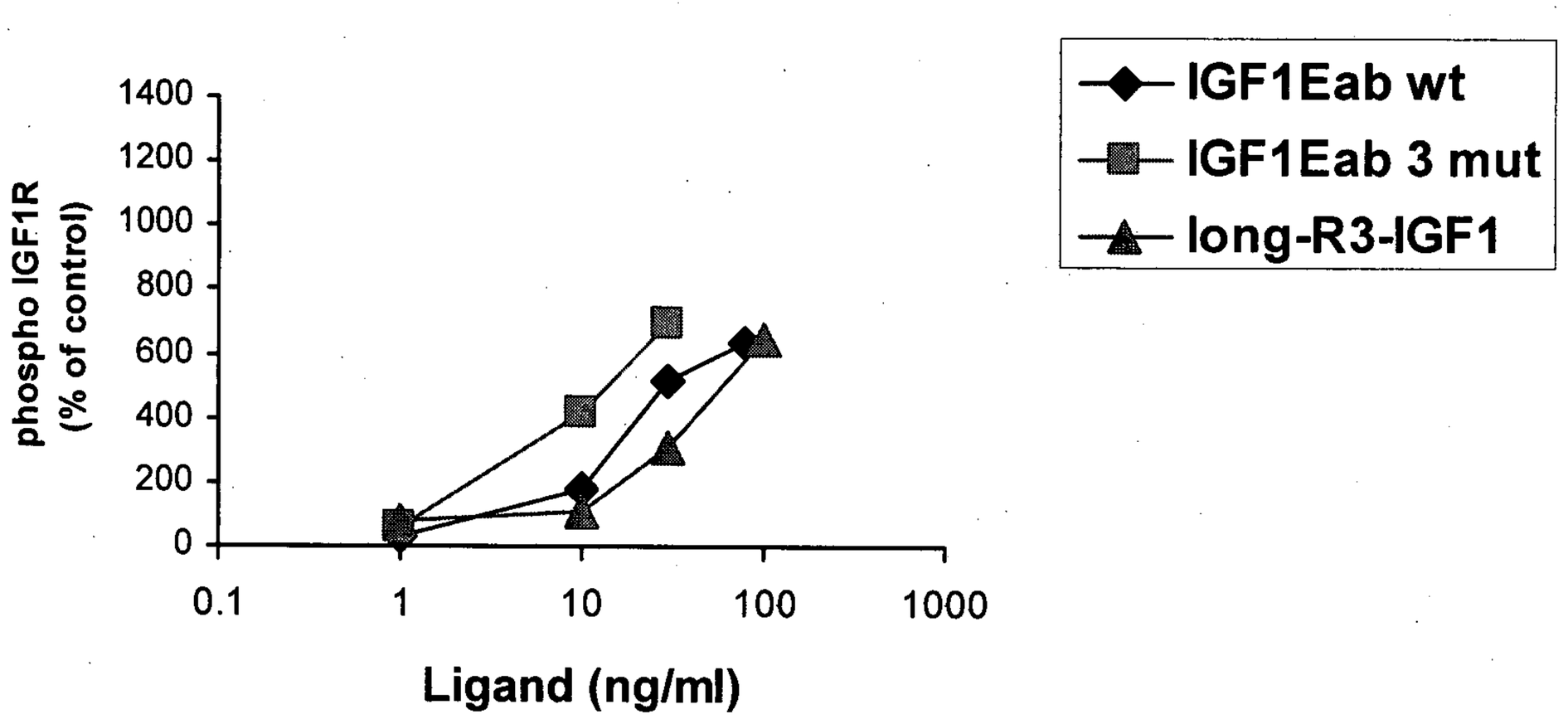

IGF1Eab wt
IGF1Eab 3 mut
long-R3-IGF1
phospho IGF1R (% of control)
1400
1200
1000
800
600
400
200
0
0.1
1
10
100
1000
Ligand (ng/ml)

Fig. 4D

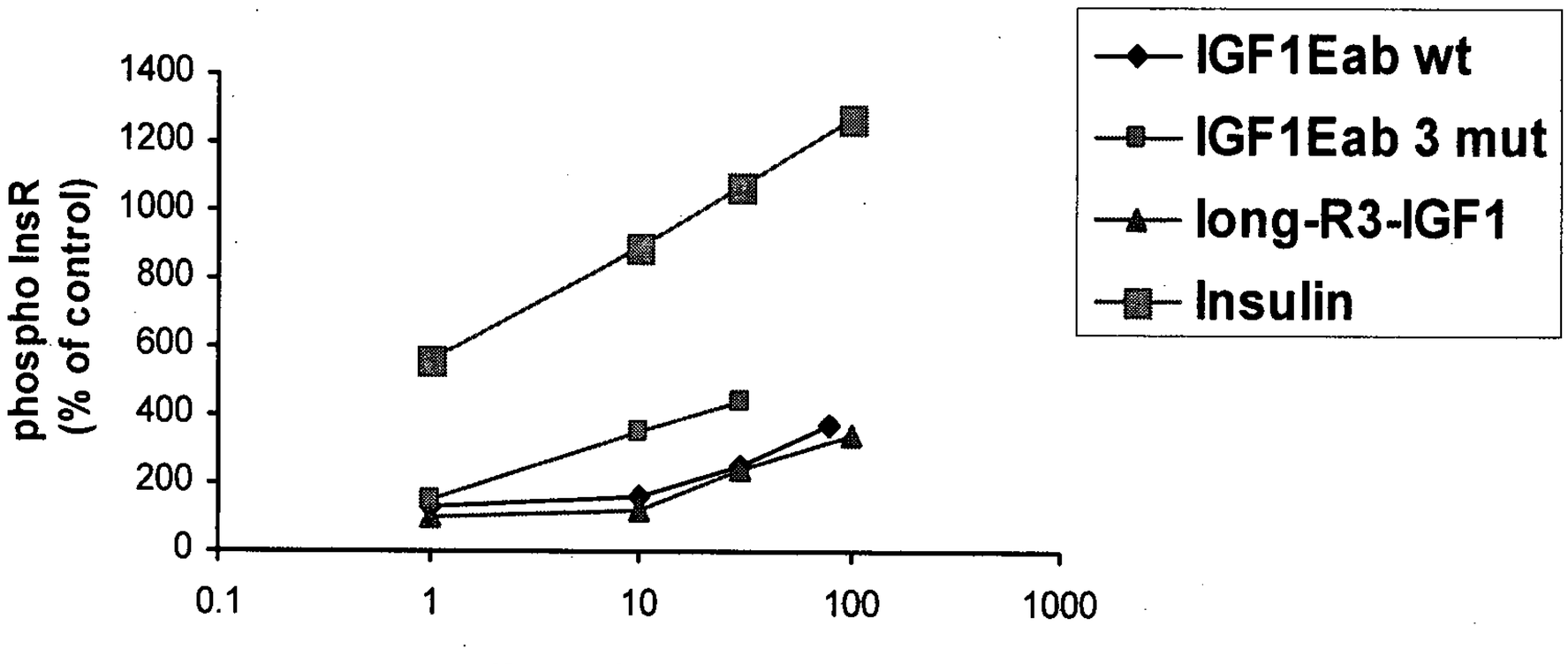

IGF1Eab wt
IGF1Eab 3 mut
long-R3-IGF1
Insulin
phospho InsR (% of control)
1400
1200
1000
800
600
400
200
0
0.1
1
10
100
1000
ligand (ng/ml)

Fig. 5

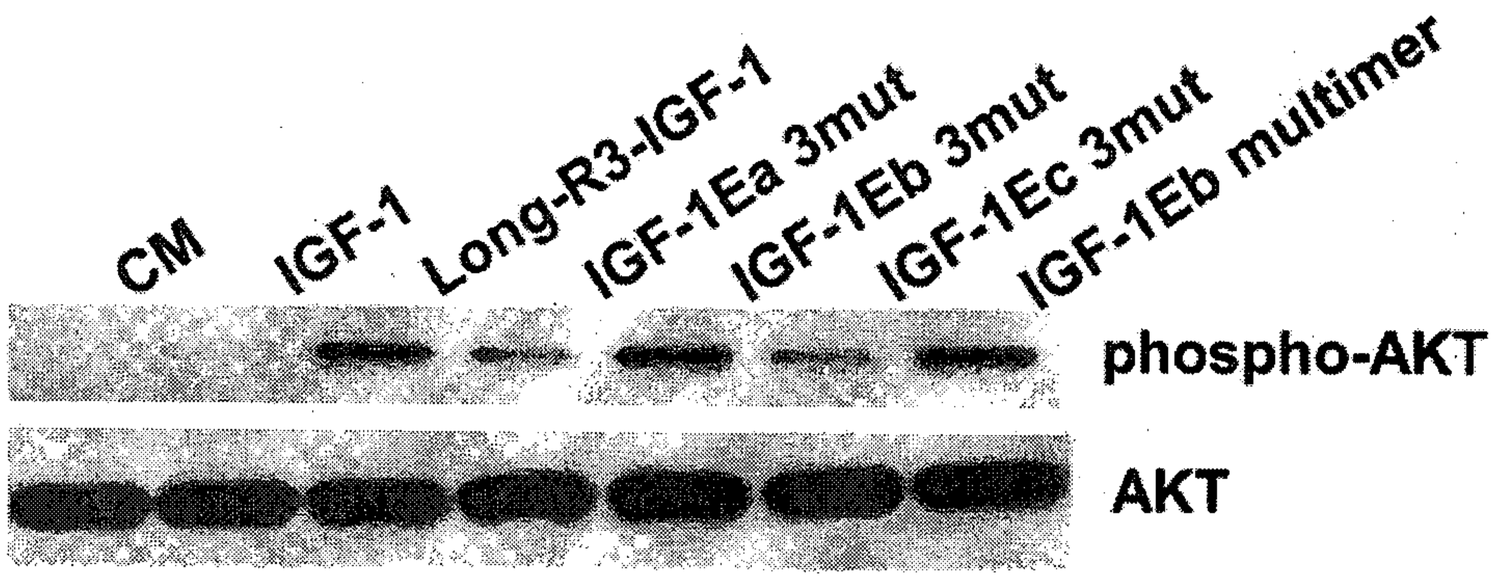


Fig. 6A

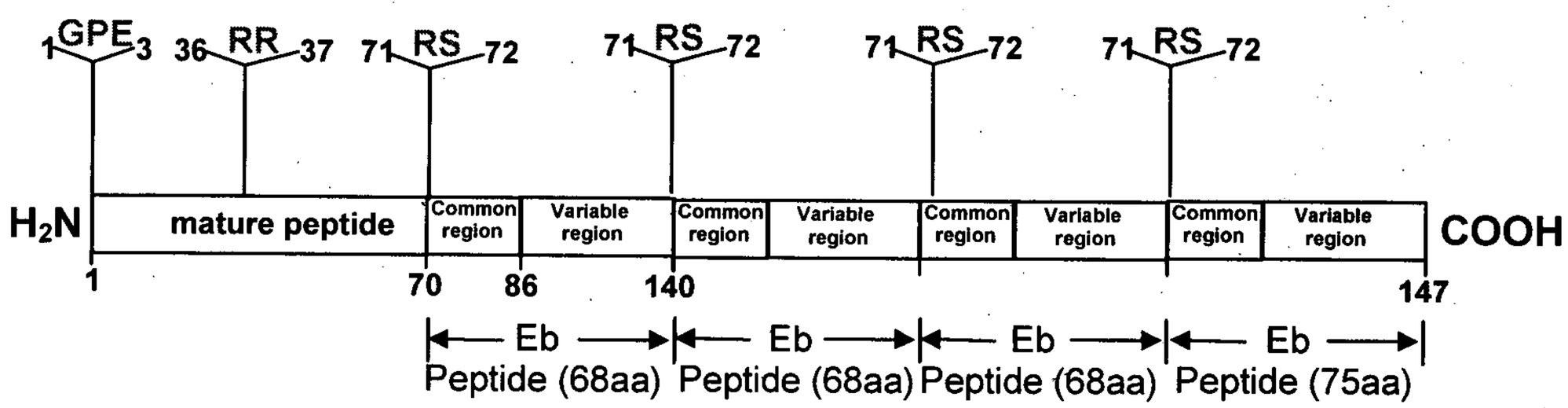


Fig. 6B

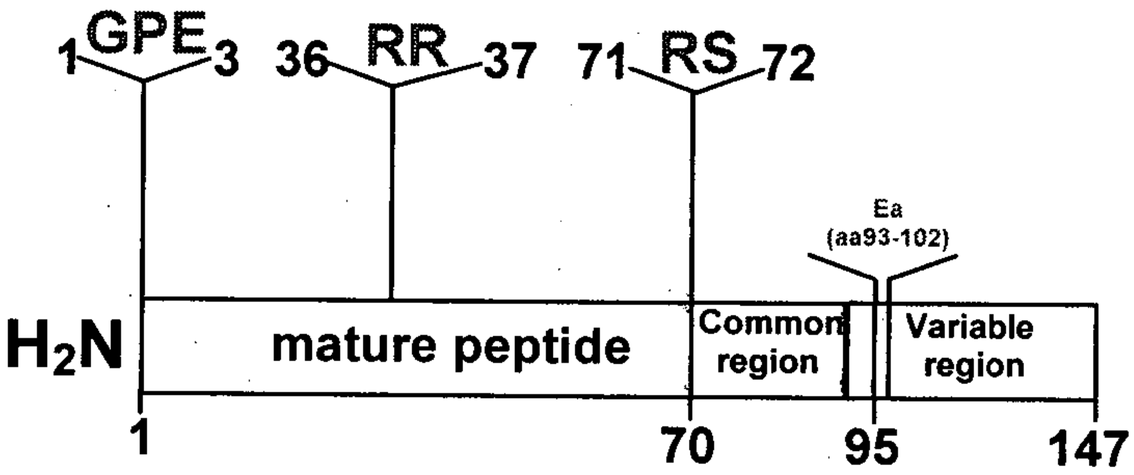
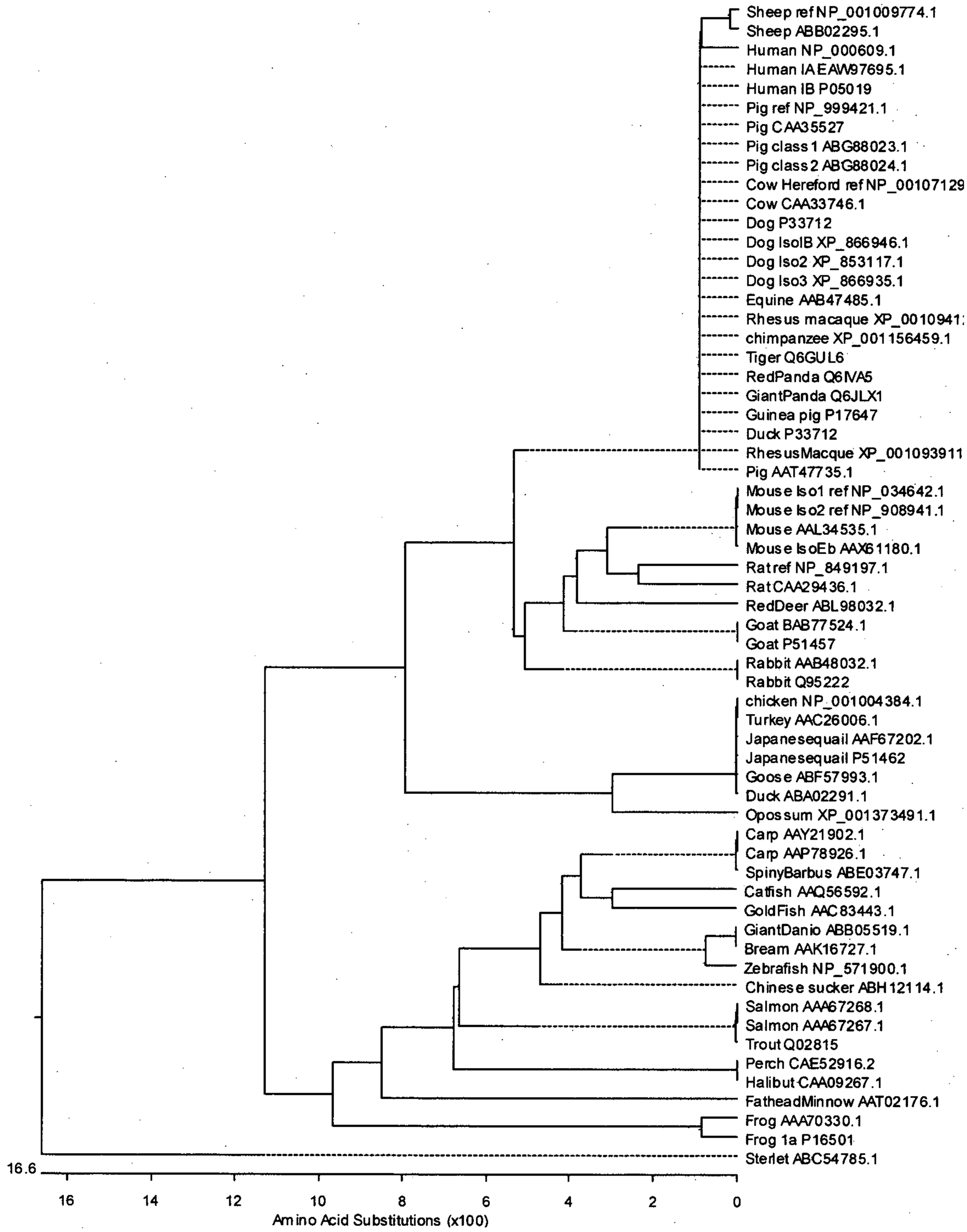


Fig. 7A

		SEQ ID NO
Majority	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
	--+---------+---------+---------+---------+---------+---------+--------	
	50 60 70 80 90 100 110	
	--+---------+---------+---------+---------+---------+---------+--------	
Human IGF-1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Human IA EAW97695.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Human IB P05019	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Pig ref NP_999421.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Pig CAA35527	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Pig class1 ABG88023.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Pig class2 ABG88024.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Cow Hereford ref NP_001071296.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Cow CAA33746.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Sheep ref NP_001009774.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKAAKSA	85
Sheep ABB02295.1	---------LVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKAAKSA	86
Goat BAB77524.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPTKSA	87
Goat P51457	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPTKSA	87
Dog P33712	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Dog IsoIB XP_866946.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Dog Iso2 XP_853117.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Dog Iso3 XP_866935.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Equine AAB47485.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Rhesus macaque XP_001094129.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
chimpanzee XP_001156459.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Tiger Q6GUL6	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
RedPanda Q6IVA5	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
GiantPanda Q6JLX1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	88
RedDeer ABL98032.1	GPETLCGAELVDALQFVCGDRGSYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPTKAA	89
Rabbit AAB48032.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKAA	90
Rabbit Q95222	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKAA	90
Guinea pig P17647	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Rat ref NP_849197.1	GPETLCGAELVDALQFVCGPRGFYFNKPTGYGSSIRRAPQTGIVDECCFRSCDLRRLEMYCVRCKPTKSA	91
Rat CAA29436.1	GPETLCGAELVDALQFVCGPRGFYFNKPTGYGSSIRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPTKSA	92
Mouse Iso1 ref NP_034642.1	GPETLCGAELVDALQFVCGPRGFYFNKPTGYGSSIRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPTKAA	93
Mouse Iso2 ref NP_908941.1	GPETLCGAELVDALQFVCGPRGFYFNKPTGYGSSIRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPTKAA	93
Mouse AAL34535.1	GPETLCGAELVDALQFVCGPRGFYFNKPTGYGSSIRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPTKAA	93
Mouse IsoEb AAX61180.1	GPETLCGAELVDALQFVCGPRGFYFNKPTGYGSSIRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPTKAA	93
chicken NP_001004384.1	GPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLEMYCAPIKPPKSA	94
Turkey AAC26006.1	GPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLEMYCAPIKPPKSA	94
Japanesequail AAF67202.1	GPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLEMYCAPIKPPKSA	94
Japanesequail P51462	GPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLEMYCAPIKPPKSA	94
Goose ABF57993.1	GPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLEMYCAPIKPPKSA	94
Duck P33712	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Duck ABA02291.1	GPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLEMYCAPIKPPKSA	94
Salmon AAA67268.1	GPETLCGAELVDTLQFVCGERGFYFSKPTGYGPSSRRSHNRGIVDECCFQSCELRRLEMYCAPVKSGKAA	95
Salmon AAA67267.1	GPETLCGAELVDTLQFVCGERGFYFSKPTGYGPSSRRSHNRGIVDECCFQSCELRRLEMYCAPVKSGKAA	95
Sterlet ABC54785.1	GSETLCGAELVDTLQFVCGERGFYFNKPTGYGASSRRPHHRGIVNECCFQSCDLRRLEMYCAPVKPAKAS	96
Perch CAE52916.2	GPETLCGAELVDTLQFVCGERGFYFSKPTGYGPNARRS--RGIVDECCFQSCELRRLEMYCAPAKTSKAA	97
Trout Q02815	GPETLCGAELVDTLQFVCGERGFYFSKPTGYGPSSRRSHNRGIVDECCFQSCELRRLEMYCAPVKSGKAA	98
Halibut CAA09267.1	GPETLCGAELVDTLQFVCGERGFYFSKPTGYGPNARRS--RGIVDECCFQSCELRRLEMYCAPAKTSKAA	97
Catfish AAQ56592.1	GPETLCGAELVDTLQFVCGDRGFYFSKPTGYGPNSRRLHNRGIVDECCFQSCELKRLEMYCAPVKSGKAP	99
Carp AAY21902.1	GPETLCGAELVDTLQFVCGDRGFYFSKPTGYGPSSRRSHNRGIVDECCFQSCELRRLEMYCAPVKPGKTP	100
Carp AAP78926.1	GPETLCGAELVDTLQFVCGDRGFYFSKPTGYGPSSRRSHNRGIVDECCFQSCELRRLEMYCAPVKPGKTP	100
GiantDanio ABB05519.1	GPETLCGAELVDTLQFVCGDRGFYFSKPTGYGPSSRRSHNRGIVDECCFQSCELRRLEMYCAPVKTGKTP	101
Zebrafish NP_571900.1	GPETLCGAELVDTLQFVCGDRGFYFSKPTGYGPSSRRSHNRGIVDECCFQSCELRRLEMYCAPVKTGKSP	102
Chinese sucker ABH12114.1	GPETLCGAELVDTLQFVCGDRGFYFSKPTGYGPSSRRSHNRGIVDECCFQSCELRRLEMYCAPVKPGKAP	103
SpinyBarbus ABE03747.1	GPETLCGAELVDTLQFVCGDRGFYFSKPTGYGPSSRRSHNRGIVDECCFQSCELRRLEMYCAPVKPGKTP	100
FatheadMinnow AAT02176.1	GPETLCGAELVDTLQFVCGDRGFYFNKPAGYGSNSRRSNNYGIVDECCFQSCELRRLEMYCAPVKTGKTP	104
GoldFish AAC83443.1	GPETLCGAELVDTLQFVCGDRGFYFSKPTGYGPNSRRSHNRGIVDECCFQSCELRRLEMYCAPVKPGKTP	105
Frog AAA70330.1	GPETLCGAELVDTLQFVCGDRGFYFSKPTGYGSNNRRSHHRGIVDECCFQSCDFRRLEMYCAPAKPAKSA	106
Bream AAK16727.1	GPETLCGAELVDTLQFVCGDRGFYFSKPTGYGPSSRRSHNRGIVDECCFQSCELRRLEMYCAPVKTGKTP	101
Frog 1a P16501	GPETLCGAELVDTLQFVCGDRGFYFSKPTGYGSNNRRSHHRGIVDECCFQSCDFRRLEMYCAPAKQAKSA	107
RhesusMacque XP_001093911.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1
Opossum XP_001373491.1	GPETLCGAELVDALQFVCGERGFYFSKPTGYGSSSRRLHHTGIVDECCFRSCDLRRLEMYCAPIKPAKSA	108
Pig AAT47735.1	GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA	1

Fig. 7B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Sheep ABB02295.1
Human NP_000609.1
Human IAEAW97695.1
Human IB P05019
Pig ref NP_999421.1
Pig CAA35527
Pig class 1 ABG88023.1
Pig class 2 ABG88024.1
Cow Hereford ref NP_00107129
Cow CAA33746.1
Dog P33712
Dog IsoIB XP_866946.1
Dog Iso2 XP_853117.1
Dog Iso3 XP_866935.1
Equine AAB47485.1
Rhesus macaque XP_0010941
chimpanzee XP_001156459.1
Tiger Q6GUL6
RedPanda Q6IVA5
GiantPanda Q6JLX1
Guinea pig P17647
Duck P33712
RhesusMacque XP_001093911
Pig AAT47735.1
Mouse Iso1 ref NP_034642.1
Mouse Iso2 ref NP_908941.1
Mouse AAL34535.1
Mouse IsoEb AAX61180.1
Rat ref NP_849197.1
Rat CAA29436.1
RedDeer ABL98032.1
Goat BAB77524.1
Goat P51457
Rabbit AAB48032.1
Rabbit Q95222
chicken NP_001004384.1
Turkey AAC26006.1
Japanesequail AAF67202.1
Japanesequail P51462
Goose ABF57993.1
Duck ABA02291.1
Opossum XP_001373491.1
Carp AAY21902.1
Carp AAP78926.1
SpinyBarbus ABE03747.1
Catfish AAQ56592.1
GoldFish AAC83443.1
GiantDanio ABB05519.1
Bream AAK16727.1
Zebrafish NP_571900.1
Chinese sucker ABH12114.1
Salmon AAA67268.1
Salmon AAA67267.1
Trout Q02815
Perch CAE52916.2
Halibut CAA09267.1
FatheadMinnow AAT02176.1
Frog AAA70330.1
Frog 1a P16501
Sterlet ABC54785.1
16.6
16
14
12
10
8
6
4
2
0
Amino Acid Substitutions (x100)

Fig. 8A

		SEQ ID NO
Majority	RSVRAQRHTDMPKAQKEVHLKNTSRGSTGNKNYRM	109
	10 20 30	
Human Ea	RSVRAQRHTDMPKTQKEVHLKNASRGSAGNKNYRM	2
Pig ref NP_999421.1	RSVRAQRHTDMPKAQKEVHLKNTSRGSSGNKNYRM	109
Pig CAA35527	RSVRAQRHTDMPKAQKEVHLKNTSRGSSGNKNYRM	109
Pig class1 ABG88023.1	RSVRAQRHTDMPKARKEVHLKNTSRGSSGNKNYRM	110
Pig class2 ABG88024.1	RSVRAQRHTDMPKARKEVHLKNTSRGSSGNKNYRM	110
Cow Hereford ref NP_001071296.1	RSVRAQRHTDMPKAQKEVHLKNTSRGSAGNKNYRM	111
Cow CAA33746.1	RSVRAQRHTDMPKAQKEVHLKNTSRGSAGNKNYRM	111
Sheep ref NP_001009774.1	RSVRAQRHTDMPKAQKEVHLKNTSRGSAGNKNYRM	111
Sheep ABB02295.1	RSVRAQRHTDMPKAQKEVHLKNTSRGSAGNKNYRM	111
Goat BAB77524.1	RSVRAQRHTDMPKAQKEVHLKNTSRGSAGNKNYRM	111
Goat P51457	RSVRAQRHTDMPKAQKEVHLKNTSRGSAGNKNYRM	111
Dog P33712	RSVRAQRHTDMPKAQKEVHLKNASRGSAGNKTY	112
Dog Iso2 XP_853117.1	RSVRAQRHTDMPKAQKEVHLKNASRGSAGNKNYRM	113
Dog Iso3 XP_866935.1	RSVRAQRHTDMPKAQKEVHLKNASRGSAGNKNYRM	113
Equine AAB47485.1	RSVRAQRHTDMPKAQKEVHLKNTSRGSAGNKNYRM	111
Rhesus macaque XP_001094129.1	RSVRAQRHTDMPKTQKEVHLKNASRGSAGNKNYRM	2
Tiger Q6GUL6	RSVRAQRHTDMPKAQKEVHLKNASRGSAGNKNYRM	113
RedPanda Q6IVA5	RSVRAQRHTDMPKAQKEVHLKNASRGSAGNKNYRM	113
GiantPanda Q6JLX1	RSVRAQRHTDMPKAQKEVHLKNASRGSAGNKNYRM	113
RedDeer ABL98032.1	RSVRAQRHTDMPKAQKEVHLKNTSRGSAGNKNYRM	111
Rabbit AAB48032.1	RSVRAQRHTDMPKTQKEVHLKNTSRGSAGNKNYRM	114
Guinea pig P17647	RSVRAQRHTDMPKTQKEVHLKNASRGSAGNKNYRM	2
Rat ref NP_849197.1	RSIRAQRHTDMPKTQKEVHLKNTSRGSAGNKTYRM	115
Rat CAA29436.1	RSIRAQRHTDMPKTQKEVHLKNTSRGSAGNKTYRM	115
Mouse AAL34535.1	RSIRAQRHTDMPKTQKEVHLKNTSRGSAGNKTYRM	115
chicken NP_001004384.1	RSVRAQRHTDMPKAQKEVHLKNTSRGNTGNRNYRM	116
Turkey AAC26006.1	RSVRAQRHTDMPKAQKELHLKNTSRGNTGNRNYRM	117
Japanesequail AAF67202.1	RSVRAQRHTDMPKAQKEVHLKNTSRGNTGNRNYRM	116
Japanesequail P51462	RSVRAQRHTDMPKAQKEVHLKNTSRGNTGNRNYRM	116
Goose ABF57993.1	RSVRAQRHTDMPKAQKEVHLKNTSRGNTEN	118
Duck P33712	RSVRAQRHTDMPKAQKEVHLKNASRGSAGNKTY	112
Salmon AAA67268.1	RSVRAQRHTDMPRTPKEVHQKNSSRGNTGGRNYRM	119
Salmon AAA67267.1	RSVRAQRHTDMPRTPKEVHQKNSSRGNTGGRNYRM	119
Sterlet ABC54785.1	RSVRAQRHTDMPKAQKEVHSKNSSRGNTGNRNYRI	120
Perch CAE52916.2	RSVRAQRHTDMPRAPKEVHQKNSSRGNTGGRNYRM	121
Trout Q02815	RSVRAQRHTDMPRTPKEVHQKNSSRGNTGGRNYRM	119
Halibut CAA09267.1	RSVRAQRHTDMPRAPKEVHQKNSSRGTTGGRNYRM	122
Catfish AAQ56592.1	RSVREQRHTDTPKTPKEVHQKNSSRGNTGGRNYRM	123
Carp AAY21902.1	RSIRAQRHTDSPKTAKEVHQKNSSRGNTGGRNYRM	124
Carp AAP78926.1	RSVRAQRHTDSPRTAKEVHQKNSSRGNTGGRNYRI	125
GiantDanio ABB05519.1	RSLRAQRHTDIPRTAKEVHQKNSSRGNTGGRNYRM	126
Zebrafish NP_571900.1	RSLRAQRHTDIPRTPKEVHQKNSSRGNTGGRNYRM	127
Chinese sucker ABH12114.1	RSLRAQRHTDIPRTPKDVHQKNSSRGNTGGRNYRM	128
SpinyBarbus ABE03747.1	RSLRAQRHTDSPRTAKEVHQKNSSRGNTGGRNYRI	129
FatheadMinnow AAT02176.1	RSLRAQRHTDITRTAKEVHQKNSSRGITGGRNYRM	130
GoldFish AAC83443.1	RSLRAQRHTDGTRTAKEVHQKNSSRGNTGGRNYRM	131
Frog AAA70330.1	RSVRAQRHTDMPKAQKEVHPKNTSRGNTGSRGFRM	132
Bream AAK16727.1	RSLRAQRHTDITRTAKEVHQKNSSRGNTGGRNYRI	133
Frog 1a P16501	RSVRTQRHTDMPKAQKEVHPKNTSRGNTGSRGFRM	134

Fig. 8B

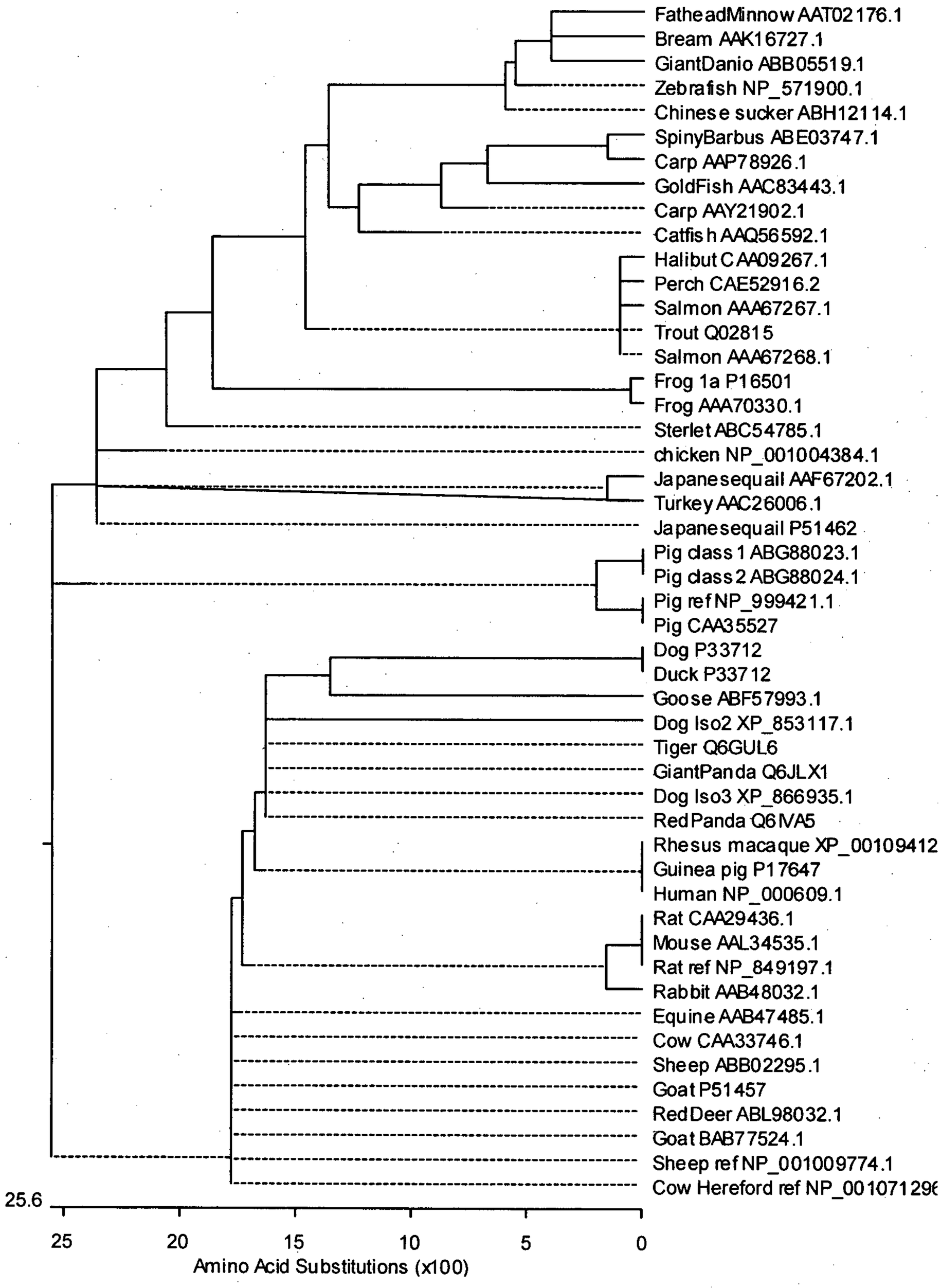
FatheadMinnow AAT02176.1
Bream AAK16727.1
GiantDanio ABB05519.1
Zebrafish NP_571900.1
Chinese sucker ABH12114.1
SpinyBarbus ABE03747.1
Carp AAP78926.1
GoldFish AAC83443.1
Carp AAY21902.1
Catfish AAQ56592.1
Halibut CAA09267.1
Perch CAE52916.2
Salmon AAA67267.1
Trout Q02815
Salmon AAA67268.1
Frog 1a P16501
Frog AAA70330.1
Sterlet ABC54785.1
chicken NP_001004384.1
Japanesequail AAF67202.1
Turkey AAC26006.1
Japanesequail P51462
Pig class 1 ABG88023.1
Pig class 2 ABG88024.1
Pig ref NP_999421.1
Pig CAA35527
Dog P33712
Duck P33712
Goose ABF57993.1
Dog Iso2 XP_853117.1
Tiger Q6GUL6
GiantPanda Q6JLX1
Dog Iso3 XP_866935.1
RedPanda Q6IVA5
Rhesus macaque XP_00109412
Guinea pig P17647
Human NP_000609.1
Rat CAA29436.1
Mouse AAL34535.1
Rat ref NP_849197.1
Rabbit AAB48032.1
Equine AAB47485.1
Cow CAA33746.1
Sheep ABB02295.1
Goat P51457
RedDeer ABL98032.1
Goat BAB77524.1
Sheep ref NP_001009774.1
Cow Hereford ref NP_001071296
25.6
25
20
15
10
5
0
Amino Acid Substitutions (x100)

Fig. 9A

```
Majority                          ----RSVRAQRHTDMPKTQKYQPPSTNKKTKSQRRRKGGPKTHPGGEQKE----------
                                  ---------+---------+---------+- --------+---------+---------+---------+-----
                                           10        20        30         40        50        60        70
                                  ---------+---------+---------+- --------+---------+---------+---------+-----
human Eb                          ----RSVRAQRHTDMPKTQKYQPPSTNKNTKSQRR-KGWPKTHPGGEQKEGTEASLQIRGKKKEQRREIGSRNAECRGKK
Human P05019                      ----RSVRAQRHTDMPKTQKYQPPSTNKNTKSQRR-KGWPKTHPGGEQKEGTEASLQIRGKKKEQRREIGSRNAECRGKK
Pig AAT47735.1                    ----RSVRAQRHTDMPKAQKYQPPSTNKKTKSQRRRKGS--TFEEHK
Cow India AAU93628.1              ----------YQPPSTNKKMKSQRRRKGGPKKRPGGEQKE
Cattle AAA03497.1                 HAQGSEGKPARGGGEGRPSSYQPPSTNKKMKSQRRRKGGPKKRPGGEQKE
Water Buffalo AAU93630.1          ----------YQPPSTNKKMKSQRRRKGGPKKHPGGEQKE
Sheep AAU93626.1                  ----------YQLPSTNKKMKSQRRRKGGPKKHPGGEQKE
Dog XP_866946.1                   ----RSVRAQRHTDMPKAQKYHPPSTTKRMKSQRRRKGS--TFEECK
Rabbit Q95222                     ----RSVRAQRHTDMPKTQKYQPPSTNKKMKSQRRRKGS--TFEEHK
Chimpanzee XP_001156459.1         ----RSVRAQRHTDMPKTQKYQPPSTNKNTKSQRRRKGGPKTHPGGEQKEGTEASLQIRGKKKEQRREIGSRNAECRGKK
Rhesus monkey XP_001093911.1      ----RSVRAQRHTDMPKTQKYQPPSTNKNTKSQRRRKGGPKTHPGGEQKEGTEASLQIRGKKKEQRREIGSRNAECRGKK
Mouse NP_908941.1                 ----RSIRAQRHTDMPKTQKSPSLSTNKKTKLQRRRKGEPKTHPEGEQEEVTEATRKIRGPREKRLG
Rat AAA41214.1                    ----RSIRAQRHTDMPKTQKSQPLSTHKKRKLQRRRKGESKAHPGGEQEEGAEATQKIRGDRERRPS
Mouse AAX61180.1                  ----RSIRAQRHTDMPKTQKSPSLSTNKKTKLQRRRKGS--TFEEHK
```

SEQ ID NO

		135
Majority	----------	
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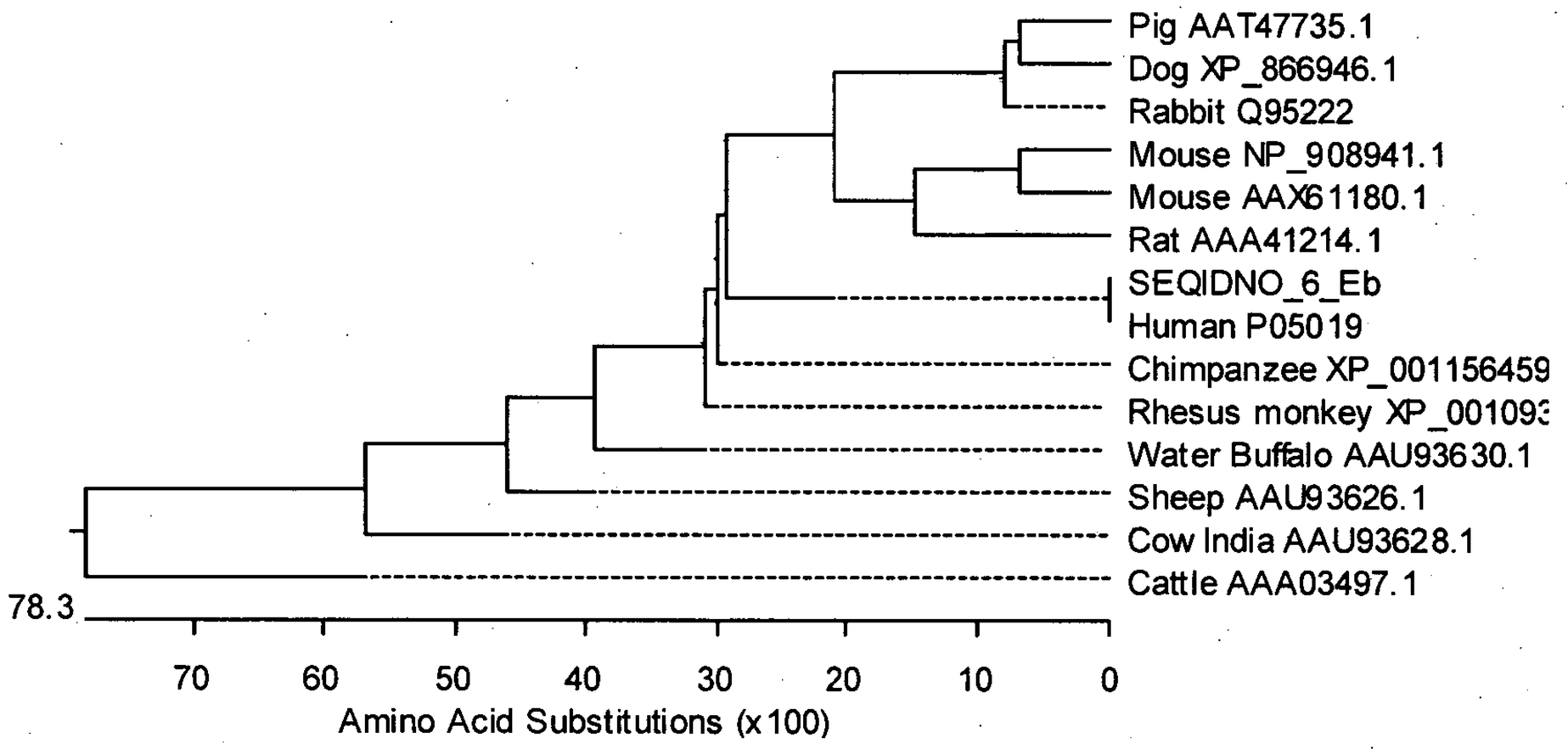
	--	
Human Eb	GK	3
Human P05019	GK	3
Pig AAT47735.1		136
Cow India AAU93628.1		137
Cattle AAA03497.1		138
Water Buffalo AAU93630.1		139
Sheep AAU93626.1		140
Dog XP_866946.1		141
Rabbit Q95222		142
Chimpanzee XP_001156459.1	GK	143
Rhesus monkey XP_001093911.1	GKWRTGGLSRQRQG	144
Mouse NP_908941.1		145
Rat AAA41214.1		146
Mouse AAX61180.1		147

Fig. 9B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Dog XP_866946.1
Rabbit Q95222
Mouse NP_908941.1
Mouse AAX61180.1
Rat AAA41214.1
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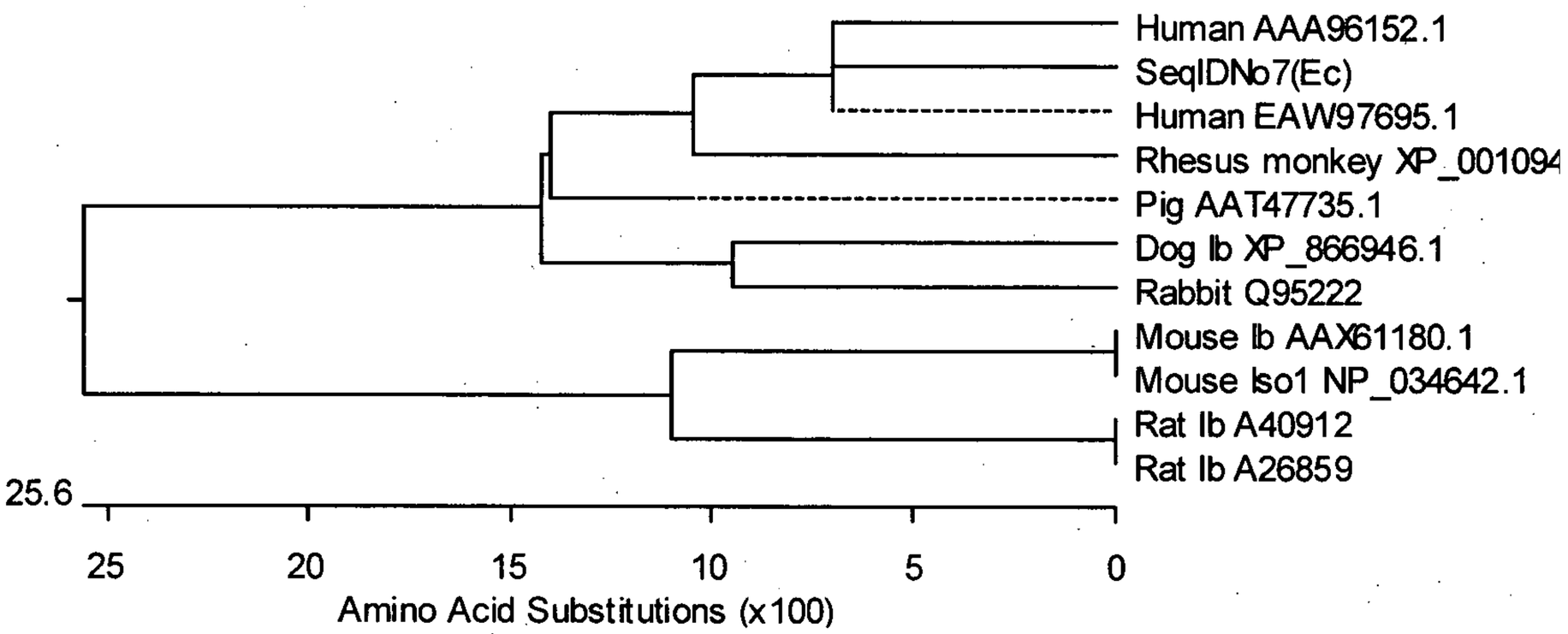
Human P05019
Chimpanzee XP_001156459
Rhesus monkey XP_001093
Water Buffalo AAU93630.1
Sheep AAU93626.1
Cow India AAU93628.1
Cattle AAA03497.1
78.3
70
60
50
40
30
20
10
0
Amino Acid Substitutions (x100)

Fig. 10A

		SEQ ID NO
Majority	RSVRAQRHTDMPKTQKYQPPSTNKKTKSQRRRKGSTFEEH-	148
	---------+---------+---------+- --------+	
	10 20 30 40	
	---------+---------+---------+- --------+	
human Ec	RSVRAQRHTDMPKTQKYQPPSTNKNTKSQRR-KGSTFEERK	4
Human EAW97695.1	RSVRAQRHTDMPKTQKYQPPSTNKNTKSQRR-KGSTFEER	149
Human AAA96152.1	RSVRAQRHTDMPKTQKYQPPSTNKNTKSQRR-KGSTFEER	149
Pig AAT47735.1	RSVRAQRHTDMPKAQKYQPPSTNKKTKSQRRRKGSTFEEH	150
Dog Ib XP_866946.1	RSVRAQRHTDMPKAQKYHPPSTTKRMKSQRRRKGSTFEEC	151
Rabbit Q95222	RSVRAQRHTDMPKTQKYQPPSTNKKMKSQRRRKGSTFEEH	152
Rhesus monkey XP_001094016.1	RSVRAQRHTDMPKTQKYQPPSTNKNTKSQRRRKGSTFEER	153
Mouse Ib AAX61180.1	RSIRAQRHTDMPKTQKSPSLSTNKKTKLQRRRKGSTFEEH	154
Mouse Iso1 NP_034642.1	RSIRAQRHTDMPKTQKSPSLSTNKKTKLQRRRKGSTFEEH	154
Rat Ib A40912	RSIRAQRHTDMPKTQKSQPLSTHKKRKLQRRRKGSTLEEH	155
Rat Ib A26859	RSIRAQRHTDMPKTQKSQPLSTHKKRKLQRRRKGSTLEEH	155

Fig. 10B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SeqIDNo7(Ec)
Human EAW97695.1
Rhesus monkey XP_001094
Pig AAT47735.1
Dog Ib XP_866946.1
Rabbit Q95222
Mouse Ib AAX61180.1
Mouse Iso1 NP_034642.1
Rat Ib A40912
Rat Ib A26859
25.6
25
20
15
10
5
0
Amino Acid Substitutions (x100)

Fig. 11A

```
Majority                        AYRPSETLCGGELVDTLQFVCGDRGFYFS----------RPAS-RVNRRS--RGIVEECCFRSCDLALLETYCATPAK
                                ---------+---------+---------+-          --    ------  +---------+---------+-----
                                         10        20        30                        40        50        60
                                ---------+---------+---------+-          --    ------  +---------+---------+-----
Human IGF-2                     AYRPSETLCGGELVDTLQFVCGDRGFYFSRP---------AS----RVSRRS--RGIVEECCFRSCDLALLETYCATPAK
Human NP_000603.1               AYRPSETLCGGELVDTLQFVCGDRGFYFSRP---------AS----RVSRRS--RGIVEECCFRSCDLALLETYCATPAK
Pig NP_999048.1                 AYRPSETLCGGELVDTLQFVCGDRGFYFS----------RPAS-RVNRRS--RGIVEECCFRSCDLALLETYCATPAK
Cattle NP_776512.2              AYRPSETLCGGELVDTLQFVCGDRGFYFS----------RPSS-RINRRS--RGIVEECCFRSCDLALLETYCATPAK
Sheep NP_001009311.1            AYRPSETLCGGELVDTLQFVCGDRGFYFS----------RPSS-RINRRS--RGIVEECCFRSCDLALLETYCAAPAK
Dog Iso1 XP_540785.2            AYRPSETLCGGELVDTLQFVCGDRGFYFS----------RPAS-RVTRRSS-RGIVEECCFRSCDLALLETYCATPAK
Dog Iso2 XP_851137.1            AYRPSETLCGGELVDTLQFVCGDRGFYFSDL---------SRPAS-RVTRRSS-RGIVEECCFRSCDLALLETYCATPAK
Dog Iso3 XP_863200.1            AYRPSETLCGGELVDTLQFVCGDRGFYFSDAALLPPVGLPGRPAS-RVTRRSS-RGIVEECCFRSCDLALLETYCATPAK
RedJungleFowl XP_421026.2       AYGTAETLCGGELVDTLQFVCGDRGFYFS----------RPVG-RNNRRIN-RGIVEECCFRSCDLALLETYCAKSVK
Rat NP_113699.1                 AYRPSETLCGGELVDTLQFVCSDRGFYFS----------RPSS-RANRRS--RGIVEECCFRSCDLALLETYCATPAK
Mouse NP_034644.1               AYGPGETLCGGELVDTLQFVCSDRGFYFS----------RPSS-RANRRS--RGIVEECCFRSCDLALLETYCATPAK
Chimpanzee XP_001153640.1       AYRPSETLCGGELVDTLQFVCGDRGFYFSKA---------STPAAFPITRPL--RRVGQRCCRGGCPPADLRDASAFPRR
Zebrafish Iso1 XP_001338042.1   EVASAETLCGGELVDALQFVCEDRGFYFS----------RPTSRSNSRRSQNRGIVEECCFSSCNLALLEQYCAKPAK
Zebrafish Iso2a NP_001001815.1  EVASAETLCGGELVDALQFVCEDRGFYFS----------RPTSRSNSRRSQNRGIVEECCFSSCNLALLEQYCAKPAK
Zebrafish Iso2b NP_571508.1     NVTAGETLCGGELVDTLQFVCGEDGFYIS----------RPN-RSNSRRPQ-RGIVEECCFRSCELHLLQQYCAKPVK

Majority                        SERDVSTP--------PTVLPDNFPRYPVGKFFQYDTW-KQSAQRLRRGLPALLRARRGRMLAKELEAFREAKR-HRPLI
                                ----+---         ------+---------+----- ----+---------+---------+---------+ -----
                                    70                80        90        100       110       120       130
                                ----+---         ------+---------+----- ----+---------+---------+---------+ -----
Human IGF-2                SERDVSTP--------PTVLPDNFPRYPVGKFFQYDTW-KQSTQRLRRGLPALLRARRGHVLAKELEAFREAKR-HRPLI
Human NP_000603.1               SERDVSTP--------PTVLPDNFPRYPVGKFFQYDTW-KQSTQRLRRGLPALLRARRGHVLAKELEAFREAKR-HRPLI
Pig NP_999048.1                 SERDVSTP--------PTVLPDNFPRYPVGKFFRYDTW-KQSAQRLRRGLPALLRARRGRTLAKELEAVREAKR-HRPLT
Cattle NP_776512.2              SERDVSAS--------TTVLPDDVTAYPVGKFFQYDIW-KQSTQRLRRGLPAFLRARRGRTLAKELEALREAKS-HRPLI
Sheep NP_001009311.1            SERDVSAS--------TTVLPDDFTAYPVGKFFQSDTW-KQSTQRLRRGLPAFLRARRGRTLAKELEALREAKS-HRPLI
Dog Iso1 XP_540785.2            SERDVSTP--------PTVLPDNFPRYPVGKFFQYDTW-KQSAQRLRRGLPALLRARRGRMLAKELEAFREAKR-HRPLI
Dog Iso2 XP_851137.1            SERDVSTP--------PTVLPDNFPRYPVGKFFQYDTW-KQSAQRLRRGLPALLRARRGRMLAKELEAFREAKR-HRPLI
Dog Iso3 XP_863200.1            SERDVSTP--------PTVLPDNFPRYPVGKFFQYDTW-KQSAQRLRRGLPALLRARRGRMLAKELEAFREAKR-HRPLI
RedJungleFowl XP_421026.2       SERDLSATSLAGL---PALNKESFQKPSHAKYSKYNVWQKKSSQRLQREVPGILRARRYRWQAEGLQAAEEARAMHRPLI
Rat NP_113699.1                 SERDVSTS--------QAVLPDDFPRYPVGKFFKFDTW-RQSAGRLRRGLPALLRARRGRMLAKELEAFREAKR-HRPLI
Mouse NP_034644.1               SERDVSTS--------QAVLPDDFPRYPVGKFFQYDTW-RQSAGRLRRGLPALLRARRGRMLAKELKEFREAKR-HRPLI
Chimpanzee XP_001153640.1       ESRHLLTS--------PFPSQDNFPRYPVGKFFQYDTW-KQSTQRLRRGLPALLRARRGHMLAKELEAFREAKR-HRPLI
Zebrafish Iso1 XP_001338042.1   SERDVSATSLQVIPVMPALKQEVPRKHVTVKYSKYDVWQRKAAQRLRRGIPAILRAKKFRRQAERIKAQEQLLH-HRPLI
Zebrafish Iso2a NP_001001815.1  SERDVSATSLQVIPVMPALKQEVPRKHVTVKYSKYDVWQRKAAQRLRRGIPAILRAKKFRRQAERIKAQEQLLH-HRPLI
Zebrafish Iso2b NP_571508.1     SERDVSSTSLQVFPVSQALHKDT----INVKYSKYEVWQQKAAQRLRRGVPSILLARKFRRQMEKIQDEEQTSF-HRPLM
```

SEQ ID NO

```
Majority                        ALPTQDPA-HGGASPEASSNRK---  156
                                ----+--- ------+---------
                                    140       150
                                ----+--- ------+---------
Human IGF-2                     ALPTQDPA-HGGAPPEMASNRKAYR  7
Human NP_000603.1               ALPTQDPA-HGGAPPEMASNRK     157
Pig NP_999048.1                 ARPTRDPAAHGGASPEASGHRK     158
Cattle NP_776512.2              ALPTQDPATHGGASSKASSD       159
Sheep NP_001009311.1            ALPTQDPATHGGASSEASSD       160
Dog Iso1 XP_540785.2            ALPTHDPATHGGASPEASGNQK     161
Dog Iso2 XP_851137.1            ALPTHDPATHGGASPEASGNQK     162
Dog Iso3 XP_863200.1            ALPTHDPATHGGASPEASGNQK     163
RedJungleFowl XP_421026.2       SLPSQRPP-APRASPEATGPQE     164
Rat NP_113699.1                 VLPPKDPA-HGGASSEMSSNHQ     165
Mouse NP_034644.1               VLPPKDPA-HGGASSEMSSNHQ     166
Chimpanzee XP_001153640.1       ALPTQDPA-HGGAPPEMASNRK     167
Zebrafish Iso1 XP_001338042.1   TLPSKLPP--ILLPTENYVSHK     168
Zebrafish Iso2a NP_001001815.1  TLPSKLPP--ILLPTENYVSHK     168
Zebrafish Iso2b NP_571508.1     TLPNRQPA--IVPHVQISTSRK     169
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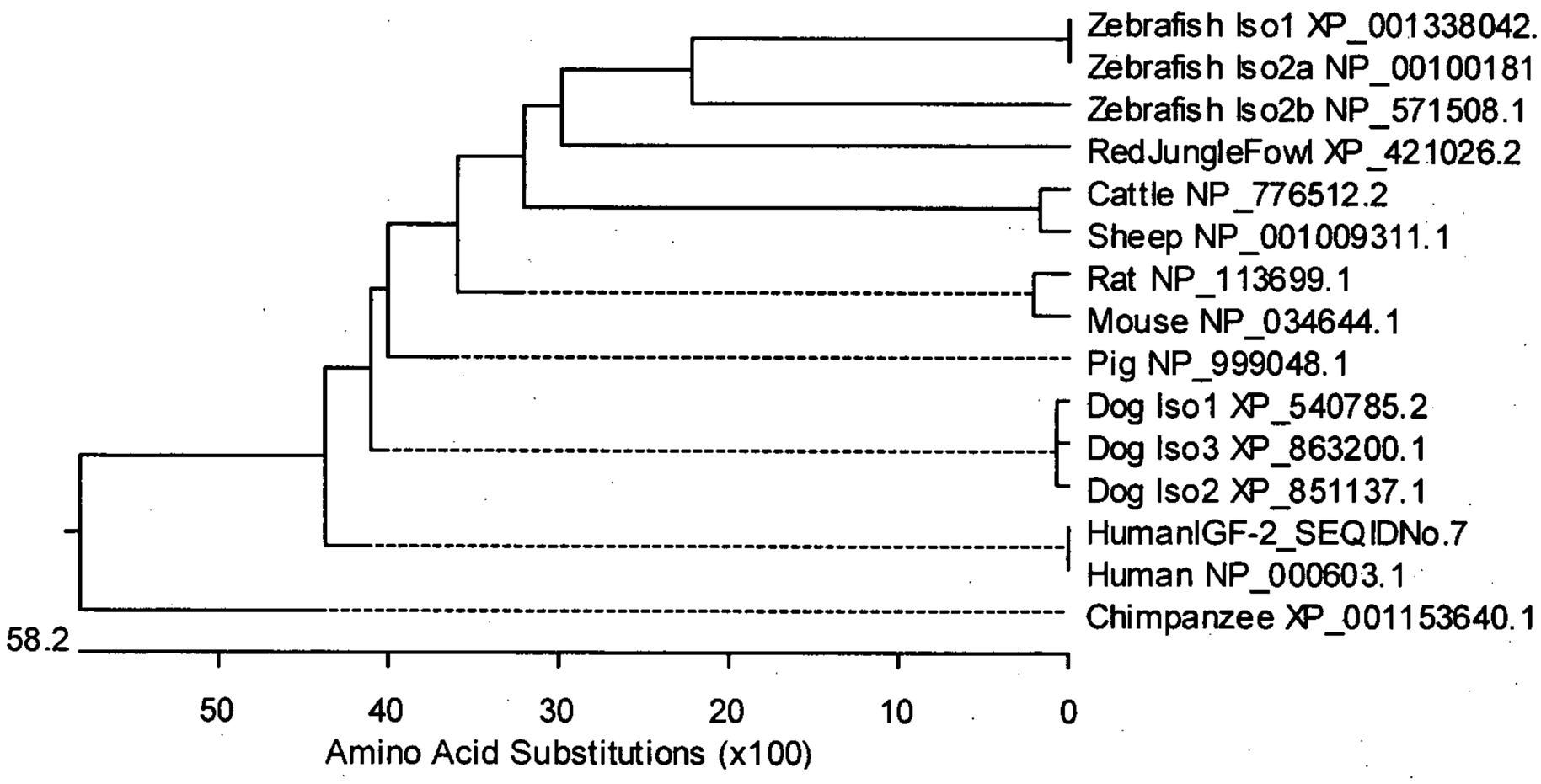
Fig. 11B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Zebrafish Iso2a NP_00100181
Zebrafish Iso2b NP_571508.1
RedJungleFowl XP_421026.2
Cattle NP_776512.2
Sheep NP_001009311.1
Rat NP_113699.1
Mouse NP_034644.1
Pig NP_999048.1
Dog Iso1 XP_540785.2
Dog Iso3 XP_863200.1
Dog Iso2 XP_851137.1
HumanIGF-2_SEQIDNo.7
Human NP_000603.1
Chimpanzee XP_001153640.1
58.2
50
40
30
20
10
0
Amino Acid Substitutions (x100)

Fig. 12A

```
Majority                        RDVSTP--------PTVLPDNFPRYPVGKFFQYDTW-KQSAQRLRRGLPALLRARRGRMLAKELEAFREAKR-HRPLIAL
                                --+---        ------+---------+----- ----+---------+---------+---------+ -------
                                  70                80        90        100       110       120       130
                                --+---        ------+---------+----- ----+---------+---------+---------+ -------
Human IGF-2                     RDVSTP--------PTVLPDNFPRYPVGKFFQYDTW-KQSTQRLRRGLPALLRARRGHVLAKELEAFREAKR-HRPLIAL
Human NP_000603.1               RDVSTP--------PTVLPDNFPRYPVGKFFQYDTW-KQSTQRLRRGLPALLRARRGHVLAKELEAFREAKR-HRPLIAL
Pig NP_999048.1                 RDVSTP--------PTVLPDNFPRYPVGKFFRYDTW-KQSAQRLRRGLPALLRARRGRTLAKELEAVREAKR-HRPLTAR
Cattle NP_776512.2              RDVSAS--------TTVLPDDVTAYPVGKFFQYDIW-KQSTQRLRRGLPAFLRARRGRTLAKELEALREAKS-HRPLIAL
Sheep NP_001009311.1            RDVSAS--------TTVLPDDFTAYPVGKFFQSDTW-KQSTQRLRRGLPAFLRARRGRTLAKELEALREAKS-HRPLIAL
Dog Iso1 XP_540785.2            RDVSTP--------PTVLPDNFPRYPVGKFFQYDTW-KQSAQRLRRGLPALLRARRGRMLAKELEAFREAKR-HRPLIAL
Dog Iso2 XP_851137.1            RDVSTP--------PTVLPDNFPRYPVGKFFQYDTW-KQSAQRLRRGLPALLRARRGRMLAKELEAFREAKR-HRPLIAL
Dog Iso3 XP_863200.1            RDVSTP--------PTVLPDNFPRYPVGKFFQYDTW-KQSAQRLRRGLPALLRARRGRMLAKELEAFREAKR-HRPLIAL
RedJungleFowl XP_421026.2       RDLSATSLAGL---PALNKESFQKPSHAKYSKYNVWQKKSSQRLQREVPGILRARRYRWQAEGLQAAEEARAMHRPLISL
Rat NP_113699.1                 RDVSTS--------QAVLPDDFPRYPVGKFFKFDTW-RQSAGRLRRGLPALLRARRGRMLAKELEAFREAKR-HRPLIVL
Mouse NP_034644.1               RDVSTS--------QAVLPDDFPRYPVGKFFQYDTW-RQSAGRLRRGLPALLRARRGRMLAKELKEFREAKR-HRPLIVL
Chimpanzee XP_001153640.1       RHLLTS--------PFPSQDNFPRYPVGKFFQYDTW-KQSTQRLRRGLPALLRARRGHMLAKELEAFREAKR-HRPLIAL
Zebrafish Iso1 XP_001338042.1   RDVSATSLQVIPVMPALKQEVPRKHVTVKYSKYDVWQRKAAQRLRRGIPAILRAKKFRRQAERIKAQEQLLH-HRPLITL
Zebrafish Iso2a NP_001001815.1  RDVSATSLQVIPVMPALKQEVPRKHVTVKYSKYDVWQRKAAQRLRRGIPAILRAKKFRRQAERIKAQEQLLH-HRPLITL
Zebrafish Iso2b NP_571508.1     RDVSSTSLQVFPVSQALHKDT----INVKYSKYEVWQQKAAQRLRRGVPSILLARKFRRQMEKIQDEEQTSF-HRPLMTL
```

SEQ ID NO

```
Majority                        PTQDPA-HGGASPEASSNRK  170
                                --+--- ------+------
                                  140        150
                                --+--- ------+------
Human IGF-2                     PTQDPA-HGGAPPEMASNRK  6
Human NP_000603.1               PTQDPA-HGGAPPEMASNRK  6
Pig NP_999048.1                 PTRDPAAHGGASPEASGHRK  171
Cattle NP_776512.2              PTQDPATHGGASSKASSD    172
Sheep NP_001009311.1            PTQDPATHGGASSEASSD    173
Dog Iso1 XP_540785.2            PTHDPATHGGASPEASGNQK  174
Dog Iso2 XP_851137.1            PTHDPATHGGASPEASGNQK  174
Dog Iso3 XP_863200.1            PTHDPATHGGASPEASGNQK  174
RedJungleFowl XP_421026.2       PSQRPP-APRASPEATGPQE  175
Rat NP_113699.1                 PPKDPA-HGGASSEMSSNHQ  176
Mouse NP_034644.1               PPKDPA-HGGASSEMSSNHQ  177
Chimpanzee XP_001153640.1       PTQDPA-HGGAPPEMASNRK  178
Zebrafish Iso1 XP_001338042.1   PSKLPP--ILLPTENYVSHK  179
Zebrafish Iso2a NP_001001815.1  PSKLPP--ILLPTENYVSHK  179
Zebrafish Iso2b NP_571508.1     PNRQPA--IVPHVQISTSRK  180
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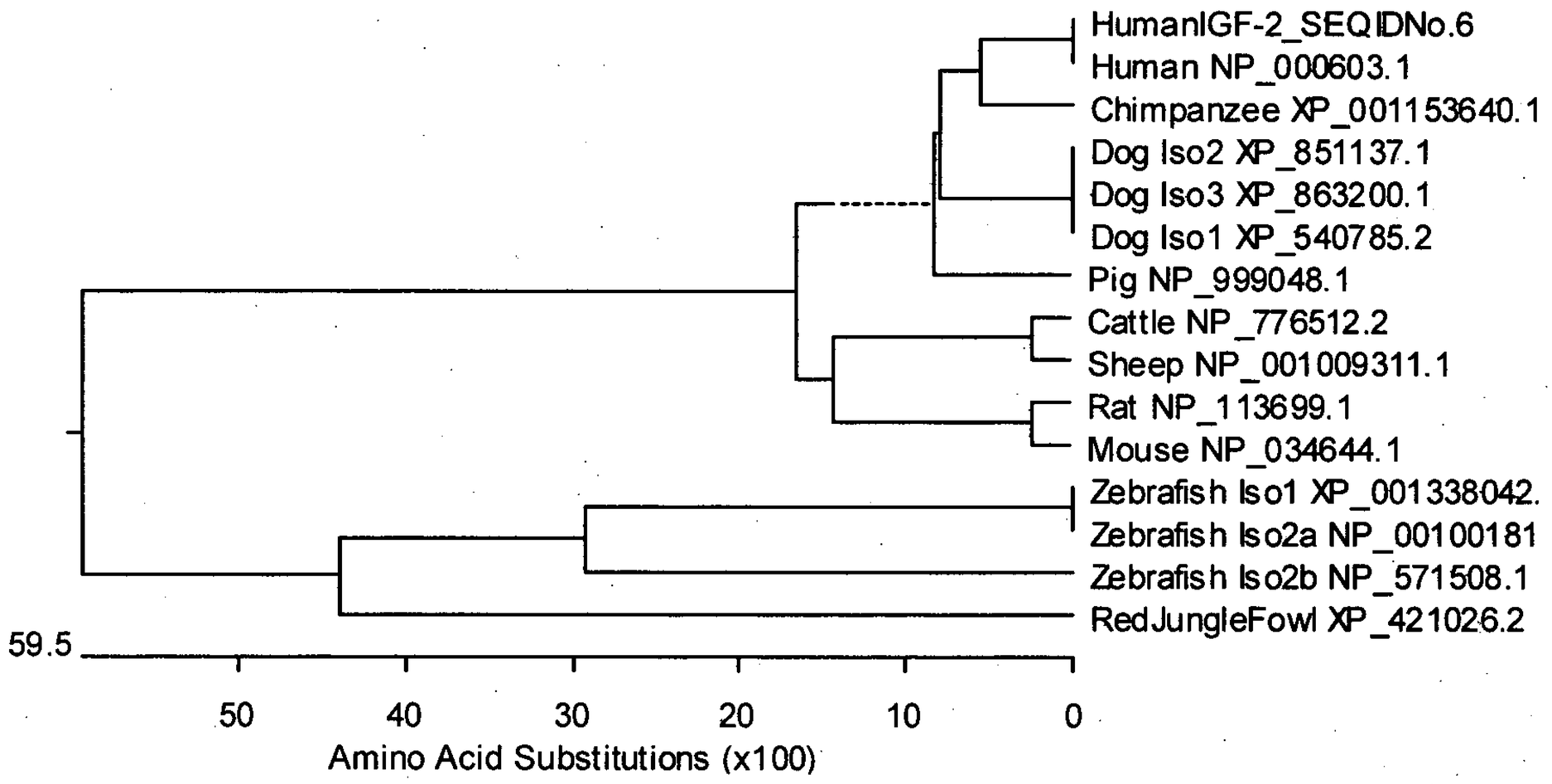
Fig. 12B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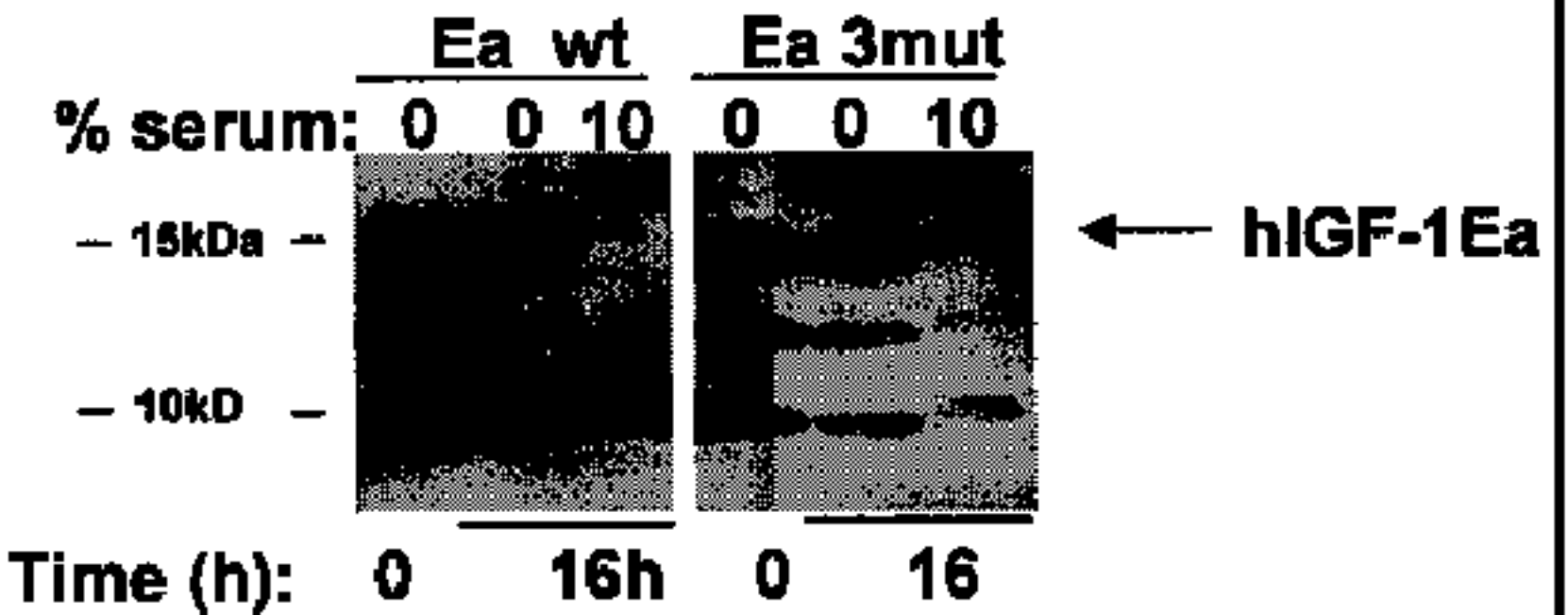
Dog Iso2 XP_851137.1
Dog Iso3 XP_863200.1
Dog Iso1 XP_540785.2
Pig NP_999048.1
Cattle NP_776512.2
Sheep NP_001009311.1
Rat NP_113699.1
Mouse NP_034644.1
Zebrafish Iso1 XP_001338042.
Zebrafish Iso2a NP_00100181
Zebrafish Iso2b NP_571508.1
RedJungleFowl XP_421026.2
59.5
50
40
30
20
10
0
Amino Acid Substitutions (x100)

A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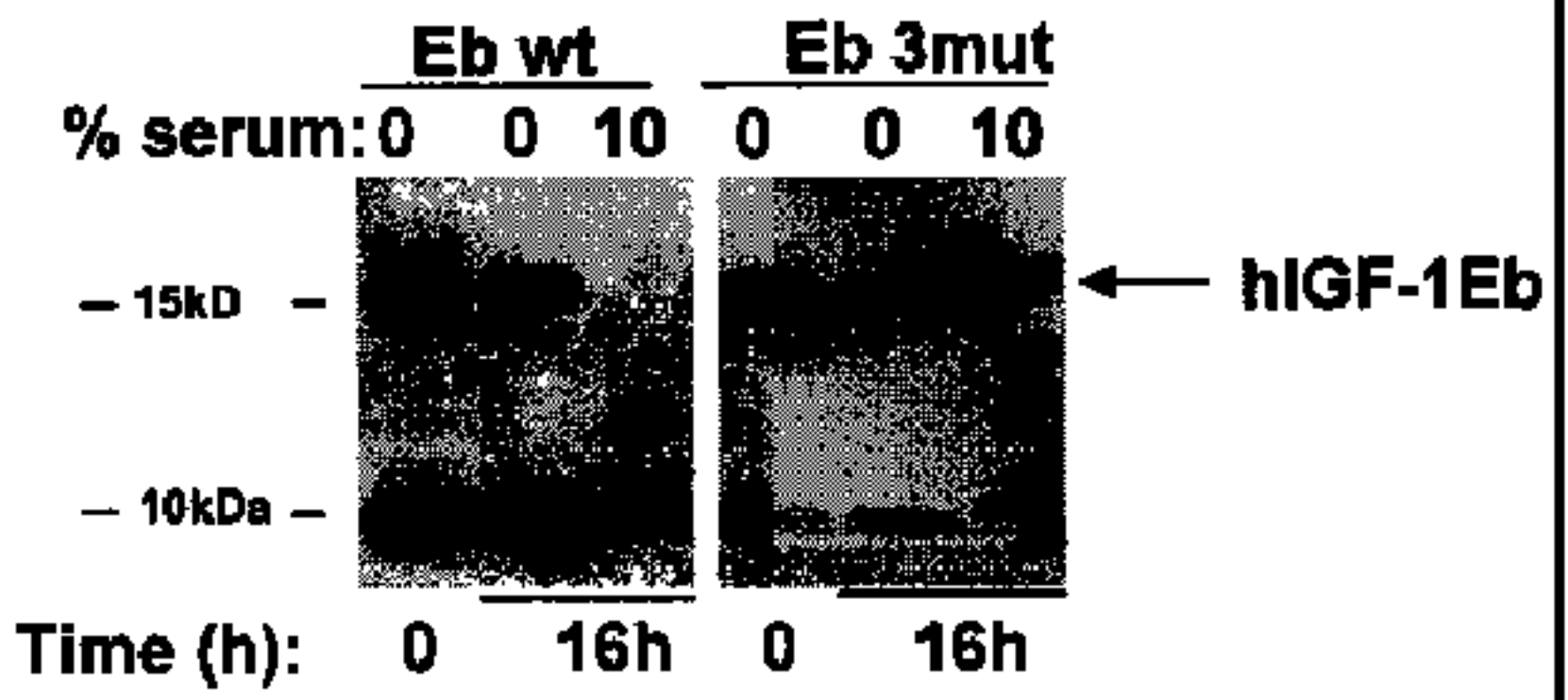
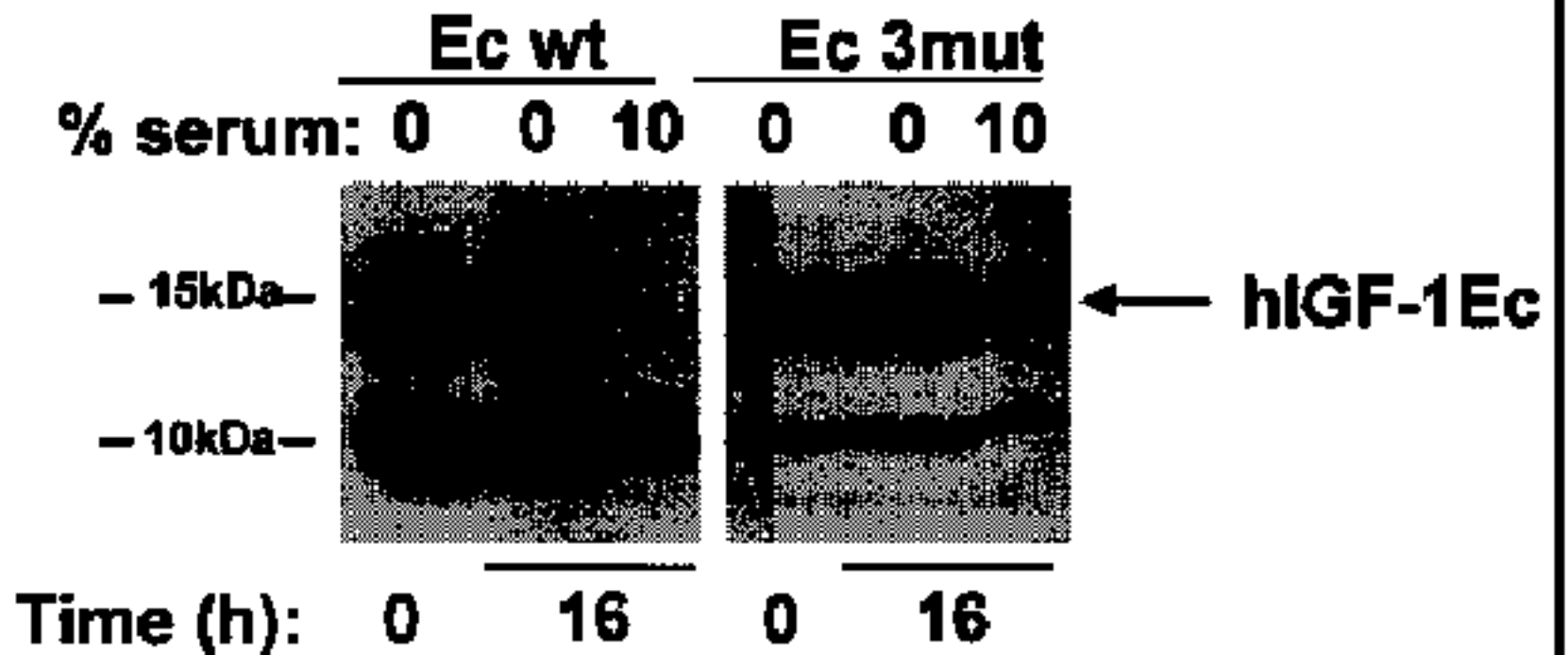
Ea 3mut
% serum: 0 0 10 0 0 10
– 15kDa –
– 10kD –
hIGF-1Ea
Time (h): 0 16h 0 16

B


Eb wt
Eb 3mut
% serum: 0 0 10 0 0 10
– 15kD –
– 10kDa –
hIGF-1Eb
Time (h): 0 16h 0 16h

C


Ec wt
Ec 3mut
% serum: 0 0 10 0 0 10
– 15kDa–
–10kDa–
hIGF-1Ec
Time (h): 0 16 0 16