

TITLE OF THE INVENTION

[0001] GDF15 Fusion Proteins and Uses Thereof

FIELD OF THE INVENTION

[0002] The invention relates to GDF15 fusion proteins. In particular, the invention relates to a fusion protein comprising a half-life extension protein, a linker and a GDF15 protein, nucleic acids and expression vectors encoding the fusion proteins, recombinant cells thereof, and pharmaceutical compositions comprising the fusion proteins. Methods of producing the fusion proteins and using the fusion proteins to treat metabolic disorders are also provided.

BACKGROUND OF THE INVENTION

[0003] GDF15, a member of the TGF β family, is a secreted protein that circulates in plasma as a 25 kDa homodimer. Plasma levels of GDF15 range between 150 and 1150 pg/ml in most individuals (Tsai et al., *J Cachexia Sarcopenia Muscle*. 2012, 3: 239-243). Plasma levels of GDF15 are increased under conditions of injury, cardiovascular disease and certain types of cancer. This upregulation is thought to be a cytoprotective mechanism. High plasma levels of GDF15 are associated with weight loss due to anorexia and cachexia in cancer, and in renal and heart failure. In a clinical trial, GDF15 levels were an independent predictor of insulin resistance in obese, non-diabetic subjects (Kempf et al., *Eur. J. Endo.* 2012, 167: 671-678). A study in twins showed that the differences in levels of GDF15 within twin pairs correlated to the differences in BMI within that pair, suggesting that GDF15 serves as a long-term regulator of energy homeostasis (Tsai et al., *PLoS One*. 2015,10(7):e0133362).

[0004] While GDF15 has been extensively studied as a biomarker for several cardiovascular and other disease states, a protective role for GDF15 has also been described

in myocardial hypertrophy and ischemic injury (Collinson, *Curr. Opin. Cardiol.* 2014, 29: 366-371; Kempf et al., *Nat. Med.* 2011, 17: 581-589; Xu et al., *Circ Res.* 2006, 98:342-50). GDF15 was shown to play an important role in protection from renal tubular and interstitial damage in mouse models of type 1 and type 2 diabetes (Mazagova et al., *Am. J. Physiol. Renal Physiol.* 2013; 305: F1249-F1264). GDF15 is proposed to have a protective effect against age-related sensory and motor neuron loss, and it improves recovery consequent to peripheral nerve damage (Strelau et al., *J. Neurosci.* 2009, 29: 13640-13648; Mensching et al., *Cell Tissue Res.* 2012, 350: 225-238). In fact, GDF15 transgenic mice were shown to have a longer lifespan than their littermate controls, which can indicate that this molecule serves as a long-term survival factor (Wang et al., *Aging.* 2014, 6: 690-700).

[0005] Numerous reports have demonstrated the improvement of glucose tolerance and insulin sensitivity in mouse models upon treatment with GDF15 protein. Two independent strains of transgenic mice overexpressing GDF15 have decreased body weight and fat mass, as well as improved glucose tolerance (Johnen et al., *Nat. Med.* 2007, 13:1333-1340; Macia et al., *PLoS One.* 2012, 7:e34868; Chrysovergis et al., *Int. J. Obesity.* 2014, 38: 1555-1564). Increases in whole-body energy expenditure and oxidative metabolism were reported in GDF15 transgenic mice (Chrysovergis et al., 2014, *Id.*). These were accompanied by an increase in thermogenic gene expression in brown adipose tissue and an increase in lipolytic gene expression in white adipose tissue. Mice lacking the GDF15 gene have increased body weight and fat mass (Tsai et al., *PLoS One.* 2013, 8(2):e55174). An Fc-fusion of GDF15 was shown to decrease body weight and improve glucose tolerance as well as insulin sensitivity in an obese cynomolgus monkey model when administered weekly over a period of six weeks (WO 2013/113008).

[0006] The effects of GDF15 on body weight are thought to be mediated via the reduction of food intake and increased energy expenditure. GDF15 may improve glycemic control via body weight-dependent and independent mechanisms.

[0007] Together, these observations suggest that increasing levels of GDF15 can be
5 beneficial as a therapy for metabolic diseases. There is a need in the art for GDF15-based compositions that can be used to treat or prevent metabolic diseases, disorders, or conditions.

BRIEF SUMMARY OF THE INVENTION

[0008] The invention satisfies this need by providing fusion proteins of GDF15 that demonstrate increased solubility/stability and exhibit features that indicate they can be used
10 to treat or prevent metabolic diseases, disorders, or conditions. Such features include, for example, decreased body weight, increased glucose tolerance, and improved insulin sensitivity of a subject administered with a fusion protein according to an embodiment of the invention.

[0009] In one general aspect, the invention relates to a fusion protein comprising: (a)
15 a half-life extension protein, (b) a linker, and (c) a GDF15 protein, wherein the fusion protein is arranged from N-terminus to C-terminus in the order (a)-(b)-(c).

[0010] In an embodiment of the invention, the GDF15 protein is a human GDF15 protein or a functional variant thereof. In particular embodiments, the GDF15 protein comprises a mature GDF15 protein or functional variant thereof. In more particular
20 embodiments, the GDF15 protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 6-11. In other particular embodiments, the GDF15 protein comprises the amino acid sequence of SEQ ID NO: 11, such as an amino acid sequence selected from the group consisting of SEQ ID NOs: 6-11.

[0011] In an embodiment of the invention, the half life extension protein is human serum albumin (HSA) or a functional variant thereof. In particular embodiments, the half life extension protein comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 1. In other particular embodiments, the half life extension protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3.

[0012] In an embodiment of the invention, the linker is a flexible linker. In particular embodiments, the flexible linker contains the sequence (GGGGS)_n, wherein n is 2 to 20, preferably 4 to 10. In another embodiment of the invention, the linker of the fusion protein is a structured linker. In particular embodiments, the structured linker contains the sequence (AP)_n or (EAAAK)_n, wherein n is 2 to 20, preferably 4 to 10.

[0013] In embodiments of the invention, the fusion protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NOs: 5, 25-30, 36-37, 40, 48, 55-60 or 64-75. In particular embodiments of the invention, the fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 25-30, 40, 55-60, and 70. In more particular embodiment of the invention, the fusion protein comprises the amino acid sequence of SEQ ID NO: 60.

[0014] In another general aspect, the invention relates to an isolated nucleic acid molecule encoding a fusion protein of the invention.

[0015] In another general aspect, the invention relates to an expression vector comprising a nucleic acid molecule encoding a fusion protein of the invention.

[0016] In another general aspect, the invention relates to a recombinant host cell comprising a nucleic acid molecule encoding a fusion protein of the invention.

[0017] In another general aspect, the invention relates to a method of obtaining a fusion protein of the invention. The method comprises: (1) culturing a host cell comprising a

nucleic acid molecule encoding the fusion protein under a condition that the fusion protein is produced, and (2) recovering the fusion protein produced by the host cell.

[0018] In another general aspect, the invention relates to a pharmaceutical composition comprising a fusion protein of the invention and a pharmaceutically acceptable carrier.

[0019] In another general aspect, the invention relates to a pharmaceutical composition comprising a nucleic acid molecule encoding a fusion protein of the invention and a pharmaceutically acceptable carrier.

[0020] In another general aspect, the invention relates to a kit comprising a pharmaceutical composition of the invention.

[0021] In another general aspect, the invention relates to a method of treating or preventing a metabolic disease, disorder or condition, the method comprising administering to a subject in need thereof a therapeutically or prophylactically effective amount of a pharmaceutical composition of the invention. In a particular embodiment, the pharmaceutical composition is administered to the subject subcutaneously or intravenously.

[0022] According to embodiments of the invention, a method of treating a metabolic disorder selected from the group consisting of type 2 diabetes, elevated glucose levels, elevated insulin levels, obesity, dyslipidemia, diabetic nephropathy, myocardial ischemic injury, congestive heart failure, or rheumatoid arthritis, in a subject in need thereof, comprises administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a fusion protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 25-30, 40, 55-60, and 70 and a pharmaceutically acceptable carrier. In a particular embodiment, the method comprises administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a

fusion protein comprising the amino acid sequence of SEQ ID NO: 60 and a pharmaceutically acceptable carrier.

[0023] Other aspects, features and advantages of the invention will be apparent from the following disclosure, including the detailed description of the invention and its preferred
5 embodiments and the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. It should be understood that the invention is not limited to the precise embodiments shown in
10 the drawings.

[0025] In the drawings:

[0026] Figures 1A and 1B show the crystal structure of GDF15, where the disulfide pairing of the first and second Cysteine residues (C1-C2) formed a loop at the N terminus of the protein.

15 [0027] Figure 2 shows the effects of subcutaneous administration of fusion proteins according to embodiments of the invention, e.g., fusion proteins FP1 (SEQ ID NO: 60) and 6xHis-FP1 (SEQ ID NO: 26 with a 6xHis tag attached at the N-terminus), on food intake in C57BL/6 mice, the cumulative food intake at 24 hours post-administration is depicted. Values shown within the bars are % reduction compared to vehicle (PBS) group $\pm$ SEM; N=8
20 animals per group for all groups, except N=9 in FP1 16 nmol/kg group. *-p<0.05, as compared to vehicle; p values were calculated using One-way ANOVA and Tukey's test for multiple comparisons.

[0028] Figure 3 shows the effects of subcutaneous administration of FP1 and 6xHis-FP1 (SEQ ID NO: 26 with a 6xHis tag attached at the N-terminus) on food intake in Sprague-

Dawley rats, the cumulative food intake at 48 hours post-administration is depicted. Values shown within the bars are % reduction compared to vehicle (PBS) group $\pm$ SEM; N=8 animals per group. *-p<0.05, as compared to vehicle; p values were calculated using One-way ANOVA and Tukey's test for multiple comparisons.

5 **[0029]** Figure 4 shows the change in body weight of diet induced obese (DIO) mice during treatment with FP1. Arrows indicate time (days) of subcutaneous administration post initial dose (Day 0); N=8 animals per group. *-p<0.05, for FP1 1 nmol/kg group as compared to vehicle; # - p<0.05, for FP1 10 nmol/kg group as compared to vehicle; p values were calculated using Two-way RM ANOVA and Tukey's test for multiple comparisons.

10 **[0030]** Figures 5A and 5B show the blood glucose levels in DIO mice during an oral glucose tolerance test (OGTT) after 14 days of dosing of FP1 every 3 days (q3d), the levels are expressed as the area under the curve. N=8 animals per group. *-p<0.05, for FP1 1 nmol/kg group as compared to vehicle; p values were calculated using One-way ANOVA and Tukey's test for multiple comparisons.

15 **[0031]** Figure 6 shows the fed blood glucose levels in DIO mice during treatment with FP1. N=8 animals per group. *-p<0.05, as compared to vehicle; p values were calculated using Two-way RM ANOVA and Tukey's test for multiple comparisons.

[0032] Figure 7 shows the 4 hour fasting homeostatic model assessment of insulin resistance (HOMA-IR) in DIO mice after 14 days of treatment with FP1. N=8 animals per
20 group. *-p<0.05, as compared to vehicle; p values were calculated using One-way ANOVA and Tukey's test for multiple comparisons.

[0033] Figure 8 shows the change in body weight in *ob/ob* mice during treatment with FP1 every 3 days (qd3). Arrows indicate time (days) of subcutaneous administration post initial dose (Day 0); N=9 animals per group. *-p<0.05, for FP1 10 nmol/kg group as

compared to vehicle; # - $p < 0.05$, for FP1 1 nmol/kg group as compared to vehicle; p values were calculated using Two-way RM ANOVA and Tukey's test for multiple comparisons.

[0034] Figure 9 shows the blood glucose levels in *ob/ob* mice during treatment with FP1. Arrows indicate time (days) of subcutaneous administration post initial dose (Day 0);

5 N=9 animals per group. * - $p < 0.05$, for FP1 10 nmol/kg group as compared to vehicle; # - $p < 0.05$, for FP1 1 nmol/kg group as compared to vehicle; p values were calculated using Two-way RM ANOVA and Tukey's test for multiple comparisons.

[0035] Figure 10 shows the mean ($\pm$ standard deviation, SD) of the serum drug concentration-time profile of FP1 following 2 mg/kg intravenous (IV) and subcutaneous (SC) administration in C57BI/6 mice.

[0036] Figure 11 shows the mean ($\pm$ SD) of the serum drug concentration-time profile of FP1 following 2 mg/kg IV and SC administration in Sprague-Dawley rats.

[0037] Figure 12 shows the mean ($\pm$ SD) of the serum drug concentration-time profile of FP1 following 1 mg/kg IV and SC administration in cynomolgus monkeys, as determined by immunoassays.

[0038] Figure 13 shows the serum concentration (ng/mL) of FP1 as an intact dimer over time following a single IV administration in cynomolgus monkeys, as determined by immuno-affinity (IA) capture-LCMS analysis.

[0039] Figure 14 shows the serum concentration (ng/mL) of FP1 as an intact dimer over time following a single SC administration in cynomolgus monkeys, as determined by immuno-affinity capture-LCMS analysis.

[0040] Figure 15 shows the concentration of FP1, represented as a % of the starting concentration, after 0, 4, 24 and 48 hours of *ex vivo* incubation in plasma obtained from two human subjects (Sub), as determined by immunoassay.

[0041] Figure 16 shows the average concentration of FP1, represented as a % of time 0, as an intact dimer after 0, 4, 24 and 48 hours of *ex vivo* incubation in plasma obtained from two human subjects (Sub), as determined by intact mass immuno-affinity capture-LCMS analysis.

5 [0042] Figure 17 shows acute food intake in lean C57BL6N male mice before and after the administration of various N-terminal deletion variants of GDF15. (SEQ ID NOs: 92, 111, and 112, compared to wild type fusion with no deletion (SEQ ID 26 with a 6xHis tag attached at the N-terminus). N=8 animals per group; *-p<0.05, as compared to vehicle; p values were calculated using Two-way RM ANOVA and Tukey's test for multiple
10 comparisons.

[0043] Figure 18 shows the effect of a single dose of FP2 on food intake in C57BL/6 mice; specifically, cumulative food intake at 24 hours post administration is shown. Values shown within the bars are % reduction compared to PBS group (mean±SEM); N=8 animals per group for all groups, except N=6 in 6xHis-FP1. **-p<0.01, ***-p<0.001, ****-p<0.0001;
15 p values were calculated using Two-way ANOVA and Dunnetts's test for multiple comparisons.

[0044] Figure 19 shows cumulative food intake measured in Sprague-Dawley rats at 24 hours post administration of a single dose of FP2. Values shown within the bars are % reduction compared to PBS group (mean±SEM); N=8 animals per group. **-p<0.01, p values
20 were calculated using Two-way ANOVA and Tukey's test for multiple comparisons.

[0045] Figure 20 shows the percent change in body weight during treatment with FP2 q3d in DIO mice. Arrows indicate the time of subcutaneous injections of FP2; N=6 animals per group; *-p,0.05, as compared with the vehicle, using Two Way ANOVA and Tukey's test for multiple comparisons;

[0046] Figures 21A and 21B show the area under the curve (AUC) for the blood glucose concentration levels during an OGTT test after 14 days of q3d dosing of FP2 in DIO mice. *- $p < 0.05$, using One Way ANOVA and Tukey's multiple comparisons test, using $n=8$ animals per group.

5 [0047] Figure 22A shows the plasma insulin levels during an OGTT after 8 days of q3d dosing of FP2 in DIO mice. *- $p < 0.05$, Vehicle vs. FP2 (0.3 nmol/kg); FP2 (10 nmol/kg); and Rosiglitazone. # - $p < 0.05$, as compared to Rosiglitazone (10 mg/kg), using Two Way RM ANOVA and Tukey's multiple comparisons test.

[0048] Figures 22B shows the AUC for the plasma insulin levels during an OGTT
10 after 8 days of q3d dosing of FP2 in DIO mice. *- $p < 0.05$, as compared to Vehicle; # - $p < 0.05$, as compared to Rosiglitazone.

[0049] Figure 23 shows the fed blood glucose levels after 8 days of q3d dosing of FP2 in DIO mice. *- $p < 0.05$, as compared to Vehicle, using Two Way RM ANOVA and Tukey's multiple comparisons test, $n=8$ animals per group.

15 [0050] Figure 24 shows fasting HOMA-IR after 14 days of treatment with FP2 q3d, followed by 5-hour fast on Day 14, in DIO mice. *- $p < 0.05$, as compared to Vehicle, using One Way ANOVA and Tukey's multiple comparisons test, for $n=8$ animals per group.

[0051] Figure 25 shows serum concentrations of FP2 following 2 mg/kg intravenous (IV) and 2 mg/kg subcutaneous (SC) administration in C57Bl/6 mice. Values represent
20 mean $\pm$ SD ($n=5$ samples per timepoint).

[0052] Figure 26 shows serum concentrations of FP2 following 2 mg/kg intravenous (IV) and 2 mg/kg subcutaneous (SC) administration in Sprague Dawley rats. $N=5$ samples per time point.

[0053] Figure 27 shows plasma concentrations of FP2 in cynomolgus monkeys analyzed by immunoassay. Values represent mean $\pm$ SD of n=3, except n=2 for IV at day 22 (528 hr). IV -- intravenous, SC -- subcutaneous.

[0054] Figure 28 shows plasma concentrations of FP2 as intact dimer in cynomolgus monkeys analyzed by LCMS. Values represent mean $\pm$ SEM of n=3, except n=2 for subcutaneous (SC) at 168 hours, n=1 for SC at 120 hours and 432 hours, and n=1 for IV -- intravenous at 168 hours and 432 hours.

[0055] Figure 29 shows *ex vivo* stability of FP2 (Normalized Percent Recovery) over 48 hours in human plasma measured by immunoassay.

10 [0056] Figure 30 shows *ex vivo* stability of FP2 (Normalized Percent Recovery) over 48 hours in human plasma measured by intact LC/MS.

[0057] Figure 31 shows concentration response curves for FP2 and HSA-GDF15:GDF15 heterodimer using pAKT Assays in SK-N-AS cells expressing recombinant human GFRAL receptor (N=3).

15 [0058] Figure 32 shows daily food intake (g) prior to and following a single dose of FP1 in cynomolgus monkeys. *- p<0.05 for 10mg/kg of FP1 as compared to vehicle.

[0059] Figure 33 shows percent body weight change prior to and following a single dose of FP1 in cynomolgus monkeys. *- p<0.05 for 10mg/kg FP1 as compared to vehicle; # - p<0.05 for 3mg/kg as compared to vehicle using Two Way RM ANOVA and Tukey's multiple comparisons test, for n=8 animals per group.

20 [0060] Figure 34 shows daily food intake (g) prior to and following a single dose of FP2 in cynomolgus monkeys. *- p<0.05, as compared to Vehicle, using Two Way RM ANOVA and Tukey's multiple comparisons test, for n=8 animals per group.

[0061] Figure 35 shows percent body weight change prior to and following a single dose of FP2 in cynomolgus monkeys. *- p<0.05, for 10 nmol/kg of FP2 as compared to Vehicle, # - p<0.05, for 3 nmol/kg of FP2 as compared to Vehicle, & - p<0.05, for 1 nmol/kg of FP2 as compared to Vehicle, using Two Way RM ANOVA and Tukey's multiple
5 comparisons test, for n=8 animals per group.

DETAILED DESCRIPTION OF THE INVENTION

[0062] Various publications, articles and patents are cited or described in the background and throughout the specification; each of these references is herein incorporated by reference in its entirety. Discussion of documents, acts, materials, devices, articles or the
10 like which has been included in the present specification is for the purpose of providing context for the invention. Such discussion is not an admission that any or all of these matters form part of the prior art with respect to any inventions disclosed or claimed.

[0063] Unless defined otherwise, all technical and scientific terms used herein have the same meaning commonly understood to one of ordinary skill in the art to which this
15 invention pertains. Otherwise, certain terms used herein have the meanings as set in the specification. All patents, published patent applications and publications cited herein are incorporated by reference as if set forth fully herein. It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

20 [0064] The invention relates to a fusion protein comprising (a) a half life-extension protein, (b) a linker, and (c) a GDF15 protein, wherein the fusion protein is arranged from N-terminus to C-terminus in the order (a)-(b)-(c).

[0065] It is found that a fusion protein according to an embodiment of the invention, comprising a half life-extension protein, a linker, and a GDF15 protein, results in an

increased half life of the GDF15 protein, and fusion proteins of the invention exhibit metabolic effects that demonstrate their suitability as therapeutics for treating and preventing metabolic diseases, disorders or conditions. Such effects include, but are not limited to, decreasing body weight, increasing glucose tolerance, and improving insulin sensitivity of animals administered with the fusion proteins.

[0066] As used herein, the term “fusion protein” refers to a protein having two or more portions covalently linked together, where each of the portions is derived from different proteins.

[0067] Fusion proteins according to embodiments of the invention can include any GDF15 protein. As used herein, the term “GDF15 protein” refers to any naturally-occurring wild-type growth differentiation factor 15 protein or a functional variant thereof. The GDF15 protein can be from any mammal, such as a human or another suitable mammal, such as a mouse, rabbit, rat, pig, dog, or a primate. In particular embodiments, the GDF15 protein is a human GDF15 protein or a functional variant thereof. In preferred embodiments, the GDF15 protein is a mature GDF15 protein or a functional variant thereof.

[0068] As used herein, the term “mature GDF15 protein” refers to the portion of the pre-pro-protein of GDF15 that is released from the full-length protein following intracellular cleavage at the RXXR furin-like cleavage site. Mature GDF15 proteins are secreted as homodimers linked by disulfide bonds. In one embodiment of the invention, a mature GDF15 protein, shorthand GDF15(197-308) (SEQ ID NO: 6), contains amino acids 197-308 of a full-length human GDF15 protein.

[0069] As used herein, “functional variant” refers to a variant of a parent protein having substantial or significant sequence identity to the parent protein and retains at least one of the biological activities of the parent protein. A functional variant of a parent protein

can be prepared by means known in the art in view of the present disclosure. A functional variant can include one or more modifications to the amino acid sequence of the parent protein. The modifications can change the physico-chemical properties of the polypeptide, for example, by improving the thermal stability of the polypeptide, altering the substrate
5 specificity, changing the pH optimum, and the like. The modifications can also alter the biological activities of the parent protein, as long as they do not destroy or abolish all of the biological activities of the parent protein.

[0070] According to embodiments of the invention, a functional variant of a parent protein comprises a substitution, preferably a conservative amino acid substitution, to the
10 parent protein that does not significantly affect the biological activity of the parent protein. Conservative substitutions include, but are not limited to, amino acid substitutions within the group of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and
15 tyrosine), and small amino acids (glycine, alanine, serine, threonine and methionine). Non-standard or unnatural amino acids (such as 4-hydroxyproline, 6-N-methyl lysine, 2-aminoisobutyric acid, isovaline, and alpha-methyl serine) can also be used to substitute standard amino acid residues in a parent protein.

[0071] According to other embodiments of the invention, a functional variant of a
20 parent protein comprises a deletion and/or insertion of one or more amino acids to the parent protein. For example, a functional variant of a mature GDF15 protein can include a deletion and/or insertion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more amino acids to the mature GDF15 protein, preferably, a deletion of 1 to 30 amino acids at the N-terminus of the mature GDF15 protein.

[0072] According to yet other embodiments of the invention, a functional variant of a parent protein comprises a substitution, preferably a conservative amino acid substitution, and a deletion and/or insertion, preferably a small deletion and/or insertion of amino acids, to the parent protein.

5 [0073] According to embodiments of the invention, a fusion protein of the invention comprises a GDF15 protein that has an amino acid sequence at least 90% identical to the amino acid sequence of a mature GDF15, such as GDF15(197-308) (SEQ ID NO: 6); or an amino acid sequence at least 90% identical to the amino acid sequence of a mature GDF15 truncated at the N-terminus, such as GDF15(200-308) (SEQ ID NO: 7), GDF15(201-308)
10 (SEQ ID NO: 8), GDF15(202-308) (SEQ ID NO: 9), GDF15(203-308) (SEQ ID NO: 10), or GDF15(211-308) (SEQ ID NO: 11). The GDF15 protein can have at least one of substitutions, insertions and deletions to SEQ ID NO: 6, 7, 8, 9, 10 or 11, as long as it maintains at least one of the biological activities of the GDF15 protein, such as its effects on food intake, blood glucose levels, insulin resistance, and body weight, etc.

15 [0074] In particular embodiments, a fusion protein of the invention comprises a GDF15 protein having the amino acid sequence of SEQ ID NO: 11, including but not limited to, the amino acid sequence of SEQ ID NO: 6, 7, 8, 9, 10 or 11.

[0075] Any suitable half life extension protein can be used in fusion proteins according to embodiments of the invention. As used herein, the term "half life extension
20 protein" can be any protein or fragment thereof that is known to extend the half life of proteins to which it is fused. Examples of such half life extension proteins include, but are not limited to, human serum albumin (HSA), the constant fragment domain (Fc) of an immunoglobulin (Ig), or transferrin (Tf). In embodiments of the invention, the half life extension protein comprises HSA or a functional variant thereof. In particular embodiments

of the invention, the half life extension protein comprises an amino acid sequence that is at least 90% identity to SEQ ID NO: 1. In preferred embodiments of the invention, the half life extension protein comprises HSA or functional variant thereof wherein the cysteine residue at position 34 of the HSA has been replaced by serine or alanine.

5 [0076] In particular embodiments, a fusion protein of the invention comprises a half life extension protein having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3.

[0077] Any suitable linker can be used in fusion proteins according to embodiments of the invention. As used herein, the term “linker” refers to a linking moiety comprising a
10 peptide linker. Preferably, the linker helps insure correct folding, minimizes steric hindrance and does not interfere significantly with the structure of each functional component within the fusion protein. In some embodiments of the invention, the peptide linker comprises 2 to 120 amino acids. For example, the peptide linker comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37,
15 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or 120 amino acids.

[0078] In embodiments of the invention, the linker increases the flexibility of the
20 fusion protein components. In particular embodiments of the invention, the linker can be a flexible linker comprising the sequence (GGGGS)_n, including but not limited to, GS-(GGGGS)_n or AS-(GGGGS)_n-GT, wherein n is 2 to 20, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

[0079] In other embodiments of the invention, the linker is structured. In particular embodiments of the invention, the linker can be a structured linker comprising the sequence (AP)_n or (EAAAK)_n, including but not limited to, AS-(AP)_n-GT or AS-(EAAAK)_n-GT, wherein n is 2 to 20, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15. In other

embodiments of the invention, the linker comprises the sequences (GGGGA)_n, (PGGGS)_n, (AGGGS)_n or GGS-(EGKSSGSGSESKST)_n-GGS wherein n is 2 to 20.

[0080] In embodiments of the invention, the fusion protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NOs: 5, 25-30, 36-37, 40, 48, 55-56, 59-60 or 64-75. In particular embodiments of the invention, the fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 25-30, 36-37, 40, 48, 55-56, 59-60 and 64-75. In more particular embodiments of the invention, the fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 25-30, 40, 55-56, 59-60, and 70. In further more particular embodiments of the invention, the fusion protein comprises the amino acid sequence of SEQ ID NO: 92, SEQ ID NO: 60 or SEQ ID NO: 26. The fusion protein can also include small extension(s) at the amino- or carboxyl-terminal end of the protein, such as a tag that facilitates purification, such as a poly-histidine tag, an antigenic epitope or a binding domain.

[0081] The fusion proteins disclosed herein can be characterized or assessed for GDF15 biological activities including, but not limited to effects on food intake, oral glucose tolerance tests, measurements of blood glucose levels, insulin resistance analysis, changes in body weight, pharmacokinetic analysis, toxicokinetic analysis, immunoassays and mass spec analysis of the level and stability of full-length fusion proteins, and human plasma *ex vivo* stability analysis.

[0082] The invention also provides an isolated nucleic acid molecule encoding a fusion protein of the invention. In embodiments of the invention, the isolated nucleic acid molecule encodes a fusion protein comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NOs: 5, 25-30, 36-37, 40, 48, 55-56, 59-60 or 64-75. In particular embodiments, the isolated nucleic acid molecule encodes a fusion protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 25-31, 36-37, 40, 48, 55-56, 59-60 and 64-75. In more particular embodiments, the isolated nucleic acid molecule encodes a fusion protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 25-30, 40, 55-56, 59-60, and 70. In further more particular embodiments, the isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NOs: 76-91.

[0083] According to other embodiments of the invention, the nucleic acid molecule encoding the fusion protein can be in an expression vector. Expression vectors include, but are not limited to, vectors for recombinant protein expression and vectors for delivery of nucleic acids into a subject for expression in a tissue of the subject, such as viral vectors. Examples of viral vectors suitable for use with the invention include, but are not limited to adenoviral vectors, adeno-associated virus vectors, lentiviral vectors, etc. The vector can also be a non-viral vector. Examples of non-viral vectors include, but are not limited to plasmids, bacterial artificial chromosomes, yeast artificial chromosomes, bacteriophages, etc. The vector can include any element to establish a conventional function of an expression vector, for example, a promoter, ribosome binding element, terminator, enhancer, selection marker, or an origin of replication.

[0084] According to other embodiments of the invention, the nucleic acid molecule encoding the fusion protein can be codon optimized for improved recombinant expression

from a desired host cell, such as Human Embryonic Kidney (HEK) or Chinese hamster ovary (CHO) cells, using methods known in the art in view of the present disclosure.

[0085] The invention also provides a host cell comprising a nucleic acid molecule encoding a fusion protein of the invention. Host cells include, but are not limited to, host
5 cells for recombinant protein expression and host cells for delivery of the nucleic acid into a subject for expression in a tissue of the subject. Examples of host cells suitable for use with the invention include, but are not limited to HEK or CHO cells.

[0086] In another general aspect, the invention relates to a method of obtaining a fusion protein of the invention. In a general aspect, the method comprises: (1) culturing a
10 host cell comprising a nucleic acid molecule encoding a fusion protein under a condition that the fusion protein is produced, and (2) recovering the fusion protein produced by the host cell. The fusion protein can be purified further using methods known in the art.

[0087] In some embodiments, the fusion protein is expressed in host cells and purified therefrom using a combination of one or more standard purification techniques,
15 including, but not limited to, affinity chromatography, size exclusion chromatography, ultrafiltration, and dialysis. Preferably, the fusion protein is purified to be free of any proteases.

[0088] The invention also provides a pharmaceutical composition comprising a fusion protein of the invention and a pharmaceutically acceptable carrier.

20 [0089] The invention further provides a composition comprising a nucleic acid molecule encoding a fusion protein of the invention and a pharmaceutically acceptable carrier. Compositions comprising a nucleic acid molecule encoding a fusion protein of the invention can comprise a delivery vehicle for introduction of the nucleic acid molecule into a cell for expression of the fusion protein. Examples of nucleic acid delivery vehicles include

liposomes, biocompatible polymers, including natural polymers and synthetic polymers, lipoproteins, polypeptides, polysaccharides, lipopolysaccharides, artificial viral envelopes, metal particles, and bacteria, viruses, such as baculoviruses, adenoviruses and retroviruses, bacteriophages, cosmid, plasmids, fungal vectors and other recombination vehicles typically
5 used in the art which have been described for expression in a variety of eukaryotic hosts.

[0090] The pharmaceutically acceptable carrier can include one or more of pharmaceutically acceptable excipient, buffer, stabilizer or other materials known to those skilled in the art. Examples of pharmaceutically acceptable carriers include, but are not limited to, one or more of water, saline, buffer, isotonic agents such as sugars, polyalcohols,
10 auxiliary substances such as wetting or emulsifying agents, preservatives, as well as combinations thereof. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient at the dosages and concentrations employed. The precise nature of the carrier or other material can depend on the route of administration, e.g., intramuscular, subcutaneous, oral, intravenous, cutaneous, intramucosal (e.g., gut), intranasal
15 or intraperitoneal routes. For example, liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol can also be included. Compositions for parenteral administration can be stored in lyophilized form or in a solution, and are
20 generally placed into a container having a sterile access port, such as an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0091] According to embodiments of the invention, a pharmaceutical composition can comprise one or more additional components, such as another active ingredient.

[0092] The invention also relates to kits comprising a pharmaceutical composition of the invention. The kits can contain a first container having a dried fusion protein of the invention and a second container having an aqueous solution to be mixed with the dried fusion protein prior to administration to a subject, or a single container containing a liquid pharmaceutical composition of the invention. The kit can contain a single-dose administration unit or multiple dose administration units of a pharmaceutical composition of the invention. The kit can also include one or more pre-filled syringes (e.g., liquid syringes and lyosyringes). A kit can also comprise instructions for the use thereof. The instructions can describe the use and nature of the materials provided in the kit, and can be tailored to the precise metabolic disorder being treated.

[0093] The invention also relates to use of the pharmaceutical compositions described herein to treat or prevent a metabolic disease, disorder or condition, such as type 2 diabetes, elevated glucose levels, elevated insulin levels, obesity, dyslipidemia, diabetic nephropathy, myocardial ischemic injury, congestive heart failure, or rheumatoid arthritis. According to embodiments of the invention, a method of treating or preventing a metabolic disease, disorder or condition in a subject in need of the treatment comprises administering to the subject a therapeutically or prophylactically effective amount of a pharmaceutical composition of the invention. Any of the pharmaceutical compositions described herein can be used in a method of the invention, including pharmaceutical compositions comprising a fusion protein of the invention or pharmaceutical compositions comprising a nucleic acid encoding the fusion protein.

[0094] As used herein, “subject” means any animal, particularly a mammal, most particularly a human, who will be or has been treated by a method according to an embodiment of the invention. The term “mammal” as used herein, encompasses any

mammal. Examples of mammals include, but are not limited to, cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, non-human primates (NHPs) such as monkeys or apes, humans, etc., more particularly a human.

[0095] A “metabolic disease, disorder or condition” refers to any disorder related to abnormal metabolism. Examples of metabolic diseases, disorders or conditions that can be treated according to a method of the invention include, but are not limited to, type 2 diabetes, elevated glucose levels, elevated insulin levels, obesity, dyslipidemia, diabetic nephropathy, myocardial ischemic injury, congestive heart failure, or rheumatoid arthritis.

[0096] The terms “treat,” “treating,” and “treatment” as used herein refer to administering a composition to a subject to achieve a desired therapeutic or clinical outcome in the subject. In one embodiment, the terms “treat,” “treating,” and “treatment” refer to administering a pharmaceutical composition of the invention to reduce, alleviate or slow the progression or development of a metabolic disorder, such as type 2 diabetes, elevated glucose levels, elevated insulin levels, obesity, dyslipidemia, diabetic nephropathy, myocardial ischemic injury, congestive heart failure, or rheumatoid arthritis.

[0097] The term “therapeutically effective amount” means an amount of a therapeutically active compound needed to elicit the desired biological or clinical effect. According to embodiments of the invention, “a therapeutically effective amount” is an amount sufficient to effect beneficial or desired results, including clinical results. A therapeutically effective amount can be administered in one or more administrations. In terms of a disease state, an effective amount is an amount sufficient to ameliorate, stabilize, or delay development of a disease. According to specific embodiments of the invention, a therapeutically effective amount is an amount of a fusion protein needed to treat or prevent a metabolic disease, disorder or condition, such as type 2 diabetes, elevated glucose levels,

elevated insulin levels, obesity, dyslipidemia, diabetic nephropathy, myocardial ischemic injury, congestive heart failure, or rheumatoid arthritis.

[0098] According to embodiments of the invention, a pharmaceutical composition of the invention can be administered to a subject by any method known to those skilled in the art in view of the present disclosure, such as by intramuscular, subcutaneous, oral, intravenous, cutaneous, intramucosal (e.g., gut), intranasal or intraperitoneal route of administration. In particular embodiments, a pharmaceutical composition of the invention is administered to a subject by intravenous injection or subcutaneous injection.

[0099] Parameters such as the dosage amount, frequency of administration, and duration of administration of a pharmaceutical composition to a subject according to an embodiment of the invention are not limited in any particular way. The optimum values of such parameters can depend on a variety of factors, such as the subject to be treated, the particular metabolic disease to be treated, the severity of the disease, the route of administration, etc., and one of ordinary skill in the art will be able to determine the optimum values for such parameters in order to achieve the desired therapeutic or clinical outcome. For example, a pharmaceutical composition can be administered once per day, or more than once per day, such as twice, three times, four times, etc. A typical dosage can range from about 0.1 µg/kg to up to about 100 mg/kg or more of the fusion protein, depending on the factors such as those mentioned above.

EMBODIMENTS

[00100] Embodiment 1 is a fusion protein comprising: (a) a half-life extension protein, (b) a linker, and (c) a GDF15 protein; wherein the fusion protein is arranged from N-terminus to C-terminus in the order (a)-(b)-(c).

[00101] Embodiment 2 is a fusion protein according to Embodiment 1, wherein the GDF15 protein is a human GDF15 protein or a functional variant thereof.

[00102] Embodiment 3 is a fusion protein according to Embodiment 1, wherein the GDF15 protein comprises an amino acid sequence having at least 90% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 6-11.

[00103] Embodiment 4 is a fusion protein according to Embodiment 1, wherein the GDF15 protein comprises the amino acid sequence of SEQ ID NO: 11.

[00104] Embodiment 5 is a fusion protein according to Embodiment 4, wherein the GDF15 protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6-11.

[00105] Embodiment 6 is a fusion protein according to any of Embodiments 1 to 5, wherein the half-life extension protein comprises human serum albumin (HSA) or a functional variant thereof.

[00106] Embodiment 7 is a fusion protein according to Embodiment 6, wherein the half-life extension protein comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 1.

[00107] Embodiment 8 is a fusion protein according to Embodiment 7, wherein the half-life extension protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-3.

[00108] Embodiment 9 is a fusion protein according to any of Embodiments 1 to 8, wherein the linker is a flexible linker.

[00109] Embodiment 10 is a fusion protein according to Embodiment 9, wherein the linker comprises the sequence (GGGGS)_n, wherein n is 2 to 20, such as GS-(GGGGS)_{x8} or AS-(GGGGS)_{x8}-GT.

[00110] Embodiment 11 is a fusion protein according to any of Embodiments 1 to 9, wherein the linker is a structured linker.

[00111] Embodiment 12 is a fusion protein according to Embodiment 11, wherein the linker comprises the sequence (AP)_n or (EAAAK)_n, wherein n is 2 to 20, such as AS-(AP)_n-GT or AS-(EAAAK)_n-GT.

[00112] Embodiment 13 is a fusion protein comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NOs: 5, 25-31, 36-37, 40, 48, 55-60 or 64-75.

[00113] Embodiment 14 is a fusion protein according to Embodiment 13, comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5, 25-31, 36-37, 40, 48, 55-60 and 64-75.

[00114] Embodiment 15 is a fusion protein according to Embodiment 14, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 25-30, 40, 55-60, and 70.

[00115] Embodiment 16 is an isolated nucleic acid molecule encoding the fusion protein of any one of Embodiments 1 to 15.

[00116] Embodiment 17 is an isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOs: 76-91.

[00117] Embodiment 18 is an expression vector comprising the nucleic acid molecule of Embodiment 16 or 17.

[00118] Embodiment 19 is a host cell comprising the nucleic acid molecule of Embodiment 16 or 17.

[00119] Embodiment 20 is a method of producing the fusion protein of any one of Embodiments 1 to 15, comprising: (1) culturing a host cell comprising a nucleic acid

molecule encoding the fusion protein under a condition that the fusion protein is produced;
and (2) recovering the fusion protein produced by the host cell.

[00120] Embodiment 21 is a method according to Embodiment 20, wherein the
recovering step comprises purifying the fusion protein to remove proteases.

5 [00121] Embodiment 22 is a pharmaceutical composition comprising a therapeutically
effective amount of the fusion protein of any one of Embodiments 1 to 15 and a
pharmaceutically acceptable carrier.

[00122] Embodiment 23 is a pharmaceutical composition comprising a therapeutically
effective amount of a nucleic acid molecule encoding the fusion protein of any one of
10 Embodiments 1 to 15 and a pharmaceutically acceptable carrier.

[00123] Embodiment 24 is a kit comprising a pharmaceutical composition according to
Embodiment 22 or 23.

[00124] Embodiment 25 is a method of treating or preventing a metabolic disorder,
comprising administering to a subject in need thereof an effective amount of the
15 pharmaceutical composition according to any of Embodiments 22 and 23.

[00125] Embodiment 26 is a method according to Embodiment 25, wherein the
metabolic disorder is selected from the group consisting of type 2 diabetes, elevated glucose
levels, elevated insulin levels, obesity, dyslipidemia, diabetic nephropathy, myocardial
ischemic injury, congestive heart failure, or rheumatoid arthritis.

20 [00126] Embodiment 27 is a method according to Embodiment 25 or 26, wherein the
pharmaceutical composition is administered to the subject subcutaneously or intravenously.

[00127] Embodiment 28 is a method of treating a metabolic disorder selected from the
group consisting of type 2 diabetes, elevated glucose levels, elevated insulin levels, obesity,
dyslipidemia, diabetic nephropathy, myocardial ischemic injury, congestive heart failure, or

rheumatoid arthritis, in a subject in need thereof, the method comprising subcutaneously or intravenously administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a fusion protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 25-30, 40, 55-60, and 70 and a pharmaceutically acceptable carrier.

[00128] Embodiment 29 is a method of treating a metabolic disorder selected from the group consisting of type 2 diabetes, elevated glucose levels, elevated insulin levels, obesity, dyslipidemia, diabetic nephropathy, myocardial ischemic injury, congestive heart failure, or rheumatoid arthritis, in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a fusion protein comprising the amino acid sequence of SEQ ID NO: 60 and a pharmaceutically acceptable carrier.

[00129] Embodiment 30 is a method of any one of Embodiments 25 to 29, wherein the pharmaceutical composition is administered to the subject intravenously or subcutaneously.

[00130] Embodiment 31 is a fusion protein of any one of Embodiments 1 to 15 for use in treating or preventing a metabolic disorder selected from the group consisting of type 2 diabetes, elevated glucose levels, elevated insulin levels, obesity, dyslipidemia, diabetic nephropathy, myocardial ischemic injury, congestive heart failure, or rheumatoid arthritis.

EXAMPLES

[00131] The following examples of the invention are to further illustrate the nature of the invention. It should be understood that the following examples do not limit the invention and that the scope of the invention is to be determined by the appended claims.

[00132] **Example 1: Design of fusion molecules comprising GDF15 – Effect of GDF15 truncations**

[00133] Like other TGF β family members, GDF15 is synthesized as a pre-pro-protein that forms a dimer in the endoplasmic reticulum and undergoes furin cleavage to produce
5 secreted mature GDF15 (amino acids 197-308). The secreted mature GDF15 homodimer is about 25k Daltons, and each monomer has the potential to form up to 4 intramolecular disulfide bonds with a single intermolecular disulfide linking the homodimer components.

[00134] The crystal structure of GDF15 was determined in the invention and is depicted in Figures 1A and 1B. The crystal structure shows that the C-terminus of the mature
10 GDF15 is buried in the dimer interface, while the N-terminus is exposed. This exposed terminus allows for the linkage of fusion proteins, such as half life extension proteins, to the N-terminus of GDF15.

[00135] The crystal structure also depicts the novel disulfide pairing pattern of GDF15 cysteine residues. While TGF β 1 has C1-C3 and C2-C7 pairing (i.e., pairing between its first
15 and third cysteine residues as well as between its second and seventh cysteine residues), GDF15 has C1-C2 and C3-C7 pairing (see Figures 1A and 1B). This unique disulfide pairing results in a loop formed by the C1-C2 pairing that is located at the N-terminus of the protein and away from the cysteine knot that contains other disulfide bonds. The structure predicts that the N-terminus of GDF15 may not be critical for dimer formation or overall protein
20 folding, and that GDF15 and N-terminal fusion molecules thereof may be tolerable to N-terminal deletions that delete C1 and C2, residues within the C1-C2 loop, or even residues C-terminal to C2.

[00136] **Example 2: Design of fusion molecules comprising GDF15 – Effect of the linker**

[00137] Different linkers between the HSA molecule and the GDF15 molecule were evaluated. Both flexible linkers, containing the sequence (GGGGS)_n, and structured linkers,
5 containing the sequence (AP)_n or (EAAAK)_n, wherein n is 2 to 20, were evaluated.

[00138] Fusion proteins comprising the different linkers were compared for their biophysical properties, their effect on the efficacy of food intake in lean mice, their mouse pharmacokinetic (PK) values, and their *ex vivo* stability in human blood. The results of tested linker variants are shown in Table 1. The molecule comprising SEQ ID NO: 31, which
10 contained the (EAAAK)₈ linker, showed aggregation by HPLC. The remaining seven linker variants in Table 1 demonstrated no aggregation.

[00139] Table 1: Summary of linker variant analysis

SEQ ID NO*	Linker of	Aggregation	Good Mouse PK (WT)	<i>Ex vivo</i> stability in human blood
25	AS(GGGGS) ₂ GT	No	Yes	Yes
5	GS(GGGGS) ₄	No	Yes	Yes
26	AS(GGGGS) ₈ GT	No	Yes	Yes
27	AS(AP) ₅ GT	No	Yes	Yes
28	AS(AP) ₁₀ GT	No	Yes	Yes

SEQ ID NO*	Linker of	Aggregation	Good Mouse PK (WT)	<i>Ex vivo</i> stability in human blood
29	AS(AP) ₂₀ GT	No	Yes	Yes
30	AS(EAAAK) ₄ GT	No	Yes	Yes
31	AS(EAAAK) ₈ GT	YES	Not tested	Not tested

*- 6xHis tag was attached at the N-terminus for purification purpose

[00140] Linker stability was also evaluated for these variants by *in vivo* studies in mice and by *ex vivo* stability studies in human whole blood and plasma samples. Two forms of detection were used to analyze the results from these studies. An immunoassay with anti-
5 GDF15 capture and anti-HSA detection antibody pairs was used to evaluate how intact the linker was by measuring the presence of both molecules on either side of the linker. A broader picture of the whole-molecule integrity was analyzed by liquid chromatography-mass spectrometry (LC-MS) analysis using different surrogate peptide sequences from both HSA and GDF15. The immunoassay demonstrated a stable PK profile for all of the linker variants
10 and no loss of spiked plasma sample concentration for any of the linker variants observed over 48 hours. The LC-MS results were consistent with the immunoassay showing that the surrogate peptides from different parts of the HSA and GDF15 molecules were intact. The PK profile of the linker variants analyzed by LC-MS using surrogate peptides showed a similar trend for different linker variants, where they all had detectable levels at day 7. All
15 the variants in Table 1 except for SEQ ID 31 had desirable biophysical properties and PK values.

[00141] The linker variants were evaluated for their *in vivo* activity by carrying out food intake studies in lean mice. Table 2 shows the influence of the linker variants on the efficacy of the fusion protein in decreasing food intake. There was a clear influence of the linker on the efficacy. With regard to the flexible (GGGGS)_n linkers, an increase in the linker length from 2 to 4 to 8 dramatically increased the fusion protein efficacy. For the more rigid (AP)_n linkers, the trend was less obvious, suggesting that the degree of freedom of the GDF15 molecule within the fusion protein plays a critical role in its efficacy.

[00142] Table 2: Effect of the linker on the *in vivo* efficacy of HSA-GDF15 fusion proteins in lean mice

SEQ ID NO*	Linker	% Decrease in food intake (mean)
25	AS(GGGGS) ₂ GT	28.8
5	GS(GGGGS) ₄	40.5
26	AS(GGGGS) ₈ GT	60.7
27	AS(AP) ₅ GT	48.2
28	AS(AP) ₁₀ GT	66.2
29	AS(AP) ₂₀ GT	55.1
30	AS(EAAAK) ₄ GT	51.9

10 *- 6xHis tag was attached at the N-terminus for purification purpose

[00143] **Example 3: Design of fusion molecules comprising GDF15 – Effect of HSA mutations**

[00144] Recombinant proteins with the half life extension protein human serum albumin fused to the N-terminus of GDF15 through a linker were designed. This design
5 should allow for the GDF15 dimerization interface to remain unperturbed and allow for the formation of the native inter-chain disulfide linkages, resulting in a GDF15 homodimer with HSA fusion extended from each GDF15 arm. With this approach, only a single gene is required to generate the HSA-GDF15 homodimer.

[00145] Native human serum albumin protein contains 35 cysteine (Cys, C) residues
10 that form 17 disulfide bonds, with the Cys-34 residue being the only free cysteine in the molecule. This free Cys-34 has been shown to function as a free radical scavenger, by trapping multiple reactive oxygen species (ROS) and reactive nitrogen species (RNS). This free Cys was thus mutated to minimize the risk of heterogeneity due to oxidation.

[00146] The free cysteine at position 34 of HSA was mutated to either serine or alanine,
15 and the GDF15 fusion molecules with either a HSA(C34S) or a HSA(C34A) mutation were analyzed. Both of the molecules were purified using a three-step purification method: (i) ion-exchange chromatography, (ii) hydrophobic interaction chromatography, and (iii) size-exclusion chromatography. When they were first generated, HPLC analysis showed that both molecules were pure and aggregation-free (Table 3).

20 [00147] However, two weeks after its generation, the fusion protein containing the HSA(C34A) mutation (comprising SEQ ID NO: 48) showed aggregation by HPLC, while the fusion protein containing the HSA(C34S) mutation (SEQ ID NO: 40) remained aggregation-free after four weeks.

[00148] Table 3: The influence of mutating HSA C34 on fusion protein aggregation

SEQ ID NO	HSA mutation	% aggregation when purified	% aggregation 2 weeks post purification
40	C34S	0	0
48	C34A	0	33.29

[00149] Example 4: Protease cleavage propensity on GDF15

[00150] It was observed by the inventors that the arginine residue at amino acid

5 position 198 of GDF15 (R198) is susceptible to protease degradation within the HSA-GDF15 fusion molecules. Such degradation results in a heterogeneous population and is undesirable for therapeutic compositions. The cleavage can be prevented by a protease inhibitor cocktail. Purification methods were investigated for the removal of the protease. Table 4 lists the two

10 types of HSA affinity columns that were tested for purification of HSA-GDF15 fusion proteins, as measured by HPLC. At the time of purification, the HSA-GDF15 fusion proteins purified by both methods were 100% pure and intact. At low concentrations (2-5mg/ml), proteins purified by both methods remained intact for the entire test period of 4 weeks. However, at high concentrations (40-50 mg/ml), only the antibody-based HSA resin (CaptureSelect) produced protease-free proteins that remain intact for the entire 4 week test

15 period. The HSA-ligand-based resin (Albupure) generated proteins that were intact initially but demonstrated degradation over time when stored at high concentrations. Adding a protease inhibitor cocktail (PI) and EDTA completely arrested the degradation of the high concentration HSA-GDF15 fusion protein batch purified using the Albupure resin. Thus, the

purification method plays a critical role in generating a stable therapeutic composition.

Corresponding degradation was not observed *in vivo* or *ex vivo*, suggesting that once the therapeutic composition has been made protease-free, degradation of the fusion proteins is not an issue *in vivo*. Therefore, purification methods that can effectively remove potential

- 5 proteases during production, such as those using the CaptureSelect resin, are key to successfully manufacturing GDF15 therapeutics that are homogenous, intact and stable.

[00151] Table 4: Protease cleavage of the HSA-GDF15 fusion proteins can be eliminated by sample purification methods

Condition			% degraded population of HSA-GDF15 (SEQ ID 60)				
Concentration	Resin	+PI /EDTA	WEEK 0	WEEK 1	WEEK 2	WEEK 3	WEEK 4
Low	Capture Select	No	0	0	0	0	0
Low	Albupure	No	0	0	0	0	0
High	Capture Select	No	0	0	0	0	0
High	Albupure	No	0	3.04	9.47	11.57	13.89
High	Capture Select	Yes	0	0	0	0	0
High	Albupure	Yes	0	0	0	0	0

[00152] **Example 5: N-terminal deletion variants of GDF15**

[00153] The GDF15 crystal structure depicted in Figures 1A and 1B predicts that the N-terminus of GDF15 involved in the deletion variants is not critical for dimer formation and overall protein folding. It also predicts that such N-terminal deletions should not affect any potential receptor interaction. HSA-GDF15 fusion proteins comprising various deletions of the N terminal of GDF15 were tested for *in vivo* activity.

[00154] GDF15 N-terminal deletion variants were designed that removed the protease cleavage site at GDF15 (R198). Immediately following the R198 residue, there is a potential deamidation site at residues N199- G200, and substrate deamidation is also not favored in therapeutic compositions. GDF15 N-terminal deletions can remove both the proteolytic cleavage site and the deamidation sites simultaneously. The resulting GDF15 deletion variants that were incorporated into fusion proteins with HSA included GDF15 (201-308; SEQ ID NO: 8), GDF15 (202-308; SEQ ID NO: 9), and GDF15 (211-308; SEQ ID NO: 11). *In vivo* studies in mice showed that the N-terminal deletion variants of GDF15 are still active in reducing food intake (Figure 17). The experimental results confirmed that such GDF15 N-terminal deletion variants express properly, form appropriate dimers, and are active *in vivo*.

[00155] **Example 6: Inactive mutants of GDF15**

[00156] Table 5 lists twelve mutants of GDF15 that were made to eliminate GDF15 *in vivo* activity and identify the functional epitope of GDF15. The mutants include five single mutants, two double mutants, and five triple mutants. HSA-GDF15 fusion proteins comprising these mutations were characterized for their biophysical properties and activities (Table 5). Out of the 12 mutants, one did not express and four formed aggregates over time, indicating that the mutations interrupt protein folding and biophysical properties. Of the remaining seven mutants, four of them contained a single mutation of GDF15, and these

mutants were tested in mice for food intake reduction compared to wild type. Three of the single mutants (I89R, I89W and W32A) lost *in vivo* activity, while the remaining mutant (Q60W) is as active as the wild type. These results indicated that the I89R, I89W or W32A mutation interrupts the interaction of the receptor/co-receptor with GDF15, suggesting that the functional epitopes of GDF15 are around residues I89 and W32. The numbering of the mutation is based on the mature GDF15 present in fusion protein, e.g., “1” refers to the 1st amino acid of the mature GDF15 (SEQ ID NO: 6) and “89” refers to the 89th amino acid of the mature GDF15 protein.

[00157] Table 5: Summary of the biophysical properties and activities of fusion proteins comprising GDF15 mutants

SEQ ID NO	Mutations in GDF15	Biophysical properties	Activities
5	Wild type	Expresses well, stable	Wild type activity
64	I89R	Expresses well, stable	Complete loss of activity
65	I89W	Expresses well, stable	Complete loss of activity
66	L34A, S35A, R37A	Expresses well, stable	
67	V87A, I89A, L98A	Expresses well, unstable	
68	L34A, S35A, I89A	Expresses well, stable	
69	V87A, I89A	Expresses well, stable	
70	Q60W	Expresses well, stable	As active as wild type
71	W32A	Expresses well, stable	Complete loss of activity
72	W29A	Expresses well, unstable	
73	Q60A, S64A, R67A	Expresses well, unstable	
74	W29A, Q60A, I61A	Does not express	

SEQ ID NO	Mutations in GDF15	Biophysical properties	Activities
75	W29A, W32A	Expresses well, unstable	

*- 6xHis tag was attached at the N-terminus for purification purpose

[00158] Example 7: Expression and purification methods

[00159] Expression

[00160] For expression of 20ml and greater, the expression was done using HEK

- 5 Expi293™ cells grown in Expi293™ Expression media. The cells were grown at 37°C while shaking at 125RPM with 8% CO₂. The cells were transfected at 2.5 x 10⁶ cells per ml using the Expi293™ Expression Kit. For each liter of cells transfected, 1mg of total DNA was diluted in 25ml of Opti-MEM, and 2.6ml of Expi293™ reagent was diluted in 25ml of Opti-MEM and incubated for 5 minutes at room temperature. The diluted DNA and diluted
- 10 Expi293 reagent were combined and incubated for 20 minutes at room temperature. The DNA complex was then added to the cells. The cells were placed in the shaking incubator overnight. The day after transfection, 5ml of Enhancer 1 from the kit was diluted into 50ml of Enhancer 2 from the kit, and the total volume of the two Enhancers was added to the cells. The transfected cells were placed back into the incubator for 4 days until they were harvested.
- 15 The cells were concentrated by centrifugation at 6,000g for 30 minutes and then filtered with a 0.2um filter before the purification step.

[00161] The expression was also done in CHO cells. The plasmid was purified and characterized. Prior to transfection, 1 aliquot of 200 µg of plasmid DNA containing the coding region of HSA-GDF15 was linearized by restriction enzyme digestion with Acl I.

- 20 The digestion with this restriction endonuclease ensures the removal of the ampicillin resistance gene. Two linearized 15 µg DNA aliquots were transfected into two 1 x 10⁷ CHO

cells (designated transfection pool A and B) using the BTX ECM 830 Electro Cell Manipulator (Harvard Apparatus, Holliston, MA). Cells were electroporated 3 times at 250 volts with 15 millisecond pulse lengths and 5 second pulse intervals in a 4 mm gap cuvette. Transfected cells were transferred to MACH-1 + L-glutamine in a shake flask and incubated for 1 day. Transfection pool A and transfection pool B were centrifuged, resuspended in MACH-1 + MSX, and transferred to shake flasks to incubate for 6 days. Transfected HSA-protein fusion-producing cells from transfection pool A and transfection pool B were pooled and plated in methylcellulose on day 8 post-electroporation.

[00162] Purification

10 [00163] Two-step purification using CaptureSelect resin and size exclusion chromatography was used. Cell supernatants from transiently transfected Expi293TM cells were loaded onto a pre-equilibrated (PBS, pH 7.2) HSA CaptureSelect column (CaptureSelect Human Albumin Affinity Matrix from ThermoFisher Scientific) at an approximate capacity of 10 mg protein per ml of resin. After loading, unbound proteins were removed by washing the column with 10 column volumes (CV) of PBS pH7.2. The HSA-GDF15 that was bound to the column was eluted with 10 CV of 2M MgCl₂ in 20mM Tris, pH 7.0. Peak fractions were pooled, filtered (0.2 μ), and dialyzed against PBS pH 7.2 at 4°C. After dialysis, the protein was filtered (0.2 μ) again and concentrated to an appropriate volume before loading onto a 26/60 superdex 200 column (GE Healthcare). Protein fractions that eluted from the size exclusion chromatography (SEC) column with high purity (determined by SDS-PAGE) were pooled. The concentration of protein was determined by the absorbance at 280nm on a BioTek Synergy HTTM spectrophotometer. The quality of the purified proteins was assessed by SDS-PAGE and analytical size exclusion HPLC (SE-HPLC,

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Dionex HPLC system). Endotoxin levels were measured using a LAL assay (Pyrotell®-T, Associates of Cape Cod).

[00164] Two-step purification using Albupure resin and SEC was also used. HSA-GDF15 fusion proteins were purified at room temperature using AlbuPure resin (ProMetic BioSciences Ltd) which utilizes an immobilized synthetic triazine ligand to selectively bind HSA. The expression supernatants were applied to the AlbuPure resin. The resin was then washed, first with 4 CV PBS pH 7.2 followed by 4 CV of 50 mM Tris pH 8.0, 150 mM NaCl buffer. The HSA-GDF15 that was bound to the column was eluted with 4 CV of PBS pH 7.2 buffer containing 100 mM Na Octanoate. The protein-containing fractions were concentrated to a 10 mL volume using a 30,000 kDa molecular weight cutoff spin concentrator (Amicon) and then applied to a 26/60 Superdex S200pg column (GE) that was equilibrated in PBS pH 7.2 buffer. SEC fractions containing HSA-GDF15 homodimer were identified via SDS-PAGE and pooled for analysis. The protein purities were assessed by SDS-PAGE and SE-HPLC.

[00165] The Examples 8-14, and 19 involve characterization of an exemplary fusion protein of the invention, which has the amino acid sequence of SEQ ID NO: 60. This fusion protein is a fully recombinant protein that exists as a homodimer of a fusion of HSA with the mature human GDF15 through a 42-amino acid linker consisting of glycine and serine residues, GS-(GGGGS)₈. The predicted molecular weight of this fusion protein is 162,696 Daltons, and the single native free cysteine at position 34 of HSA has been mutated to serine. This particular HSA-GDF15 fusion protein will be referred to simply as "FP1" in the following examples, for simplicity. A 6xHis-tagged variant of FP1 (6xHis-FP1, SEQ ID NO: 26), containing an AS-(GGGGS)₈-GT linker, was used for comparison in some of the following examples.

[00166] **Example 8: Effects of FP1 on the food intake of C57Bl/6 mice**

[00167] The purpose of this experiment was to demonstrate the dose-responsive effect of FP1 on the inhibition of food intake in C57Bl/6 mice.

[00168] Male C57Bl/6 mice were acclimated for a minimum of 72 hours in BioDAQ cages. Mice were then grouped based on food intake in the previous 24 hours into six groups of eight. Between 4:00 and 5:00 pm, animals were weighed and dosed with vehicle or a composition comprising FP1 via subcutaneous injection. The change in food weight for each cage was recorded continuously by the BioDAQ system for a period of 48 hours after the injections. 6xHis-FP1 was used for comparison in this study.

[00169] The results (Figure 2 and Table 6) were expressed as an average of cumulative food intake for a given time interval. The results indicated that subcutaneous administration of FP1 to C57BL/6 mice significantly inhibited food intake relative to vehicle-treated animals at all doses and time points tested. 6xHis-FP1 reduced food intake at the 8 nmol/kg dose.

[00170] Table 6: Effects of subcutaneous administration of FP1 on food intake in C57BL/6 mice; Cumulative food intake at 12, 24 and 48 hours post administration is shown

Cumulative Food Intake (g)			
Treatment	12 hours	24 hours	48 hours
PBS	3.7 ± 0.2	4.3 ± 0.1	8.4 ± 0.3
FP1, 1 nmol/kg	$2.9 \pm 0.1^*$	$3.4 \pm 0.2^{**}$	$7.0 \pm 0.3^{**}$
FP1, 4 nmol/kg	$2.3 \pm 0.2^{****}$	$3.1 \pm 0.1^{****}$	$6.6 \pm 0.3^{***}$
FP1, 8 nmol/kg	$2.1 \pm 0.2^{****}$	$3.2 \pm 0.1^{***}$	$6.4 \pm 0.2^{****}$
FP1, 16 nmol/kg	$1.7 \pm 0.2^{****}$	$2.7 \pm 0.2^{****}$	$6.2 \pm 0.3^{****}$

6xHis-FP1, 8 nmol/kg	1.8 ± 0.2****	2.6 ± 0.2****	6.0 ± 0.2****
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Data are expressed as Mean ± SEM.

*p≤0.05, versus PBS; **p≤0.01, versus PBS; ***p≤0.001, versus PBS; ****p≤0.0001, versus PBS

One-Way ANOVA-Tukey's multiple comparisons test; n=8/group

5 [00171] **Example 9: Effects of FP1 on food intake in Sprague Dawley rats**

[00172] The purpose of this experiment was to demonstrate the dose-responsive effect of FP1 on the inhibition of food intake in Sprague Dawley rats.

[00173] Male Sprague-Dawley rats were acclimated for a minimum of 72 hours in the BioDAQ cages. Rats were then grouped based on food intake in the previous 24 hours into
 10 six groups of eight. Between 4:00 and 5:00 pm, animals were weighed and dosed with vehicle or a composition comprising the fusion protein via subcutaneous injection. The change in food weight for each cage was recorded continuously by the BioDAQ system, for a period of 48 hours after the injections. 6xHis-FP1 was used for comparison in this study.

[00174] The results are shown in Figure 3 and Table 7. Subcutaneous administration
 15 of FP1 inhibited food intake at doses of 2.5 nmol/kg and 10 nmol/kg compared to vehicle-treated animals. The inhibition reached statistical significance only with the highest dose tested (10 nmol/kg) at 24 and 48 hours post-administration. FP1 reduced food intake at the 8 nmol/kg dose, and the effect was significant at 24 and 48 hours.

[00175] Table 7: Effects of subcutaneous administration of FP1 on food intake in
 20 Sprague-Dawley rats; cumulative food intake at 12, 24 and 48 hours post administration is shown

Cumulative Food Intake (g)			
Treatment	12 hours	24 hours	48 hours
PBS	20.6 ± 1.3	25.3 ± 1.3	49.1 ± 2.1
FP1, 0.1 nmol/kg	23.3 ± 1.4	26.9 ± 0.8	52.8 ± 1.3
FP1, 0.5 nmol/kg	22.6 ± 1.7	25.1 ± 1.0	48.3 ± 1.8
FP1, 2.5 nmol/kg	20.0 ± 1.4	22.0 ± 1.0	44.6 ± 1.4
FP1, 10 nmol/kg	18.7 ± 0.9	19.9 ± 1.0*	39.9 ± 2.3*
6xHis-FP1, 8 nmol/kg	17.0 ± 1.5	18.8 ± 1.4**	38.4 ± 2.5**

Data are expressed as Mean ± SEM.

*p≤0.05, versus PBS; **p≤0.01, versus PBS

One-Way ANOVA-Tukey's multiple comparisons test; n=8/group

[00176] **Example 10: Effects of FP1 on glucose homeostasis and body weight in**

5 **diet-induced obese (DIO) mice**

[00177] The purpose of this experiment was to evaluate the effects of FP1 on food intake, body weight, and glucose homeostasis throughout two weeks of treatment in DIO C57Bl/6 mice.

[00178] Male DIO mice were weighed, and FP1 was dosed subcutaneously at 2mL/kg
 10 every three days (q3d) at Day 0, 3, 6, 9, and 12. The vehicle and rosiglitazone treatment groups were dosed with PBS on a similar regimen. The control rosiglitazone was provided in the diet at 0.015% ad libitum. Mouse and food weights were recorded daily. Glucose was measured using a glucometer (One Touch®Ultra®, Lifescan, Milpitas, CA). Fat and lean

mass was quantitated in conscious mice by time-domain NMR (TD-NMR) using the Bruker Mini-Spec LF110. For an oral glucose tolerance test (OGTT), mice were fasted for 4 hours. Blood glucose was measured via tail snip at 0, 30, 60, 90, and 120 minutes post oral gavage administration of 2 g/kg glucose at 10mL/kg. Insulin was measured at 0, 30, and 90 minutes post glucose administration.

[00179] At the end of the study, the mice were euthanized via CO₂ inhalation, and a terminal blood sample was collected. Serum was placed into a 96 well plate on wet ice and then stored at -80°C. The liver was removed, and the fat content relative to the total mass of liver sections was assessed using TD-NMR with the Bruker MiniSpec mq60 according to the manufacturer's instructions.

[00180] The fasted homeostatic model assessment of insulin resistance (HOMA-IR) was calculated based on the product of fasted glucose (in mg/dL) and insulin (in mU/L) divided by a factor of 405.

[00181] Treatment of DIO mice with Fp1 q3d at 1 nmol/kg and 10 nmol/kg reduced body weight (Table 8) and food intake (Table 9). The reductions reached statistical significance only at certain time points, as described below.

[00182] Fp1 decreased body weight at doses of 1 (from day 2 to 14) and 10 nmol/kg (from day 1 to 14) in DIO mice (Table 8 and Figure 4). A significant reduction in food intake was seen at days 1 and 2 of the study at the dose of 1 nmol/kg and at days 1, 8 and 9 at the 10 nmol/kg dose (Table 9).

[00183] Table 8: Body weight change (% of starting) during treatment with FP1 in DIO mice

Treatment	Vehicle	FP1 (nmol/kg)			Rosiglitazone
Day	n/a	0.1	1	10	10mpk/day
-2	0.1 ± 0.2	-0.1 ± 0.4	0.6 ± 0.4	0.2 ± 0.3	0.1 ± 0.2
-1	-0.8 ± 0.3	-0.2 ± 0.3	0.4 ± 0.3	-0.3 ± 0.4	-0.9 ± 0.2
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1	0.1 ± 0.2	-0.1 ± 0.4	-1.5 ± 0.5	-2.8 ± 0.4*	1.9 ± 0.2
2	0.0 ± 0.3	-0.8 ± 0.5	-3.2 ± 0.4*	-3.1 ± 0.5*	2.2 ± 0.5
3	-0.2 ± 0.4	-0.4 ± 0.4	-3.2 ± 0.6*	-4.0 ± 0.7*	2.6 ± 0.5
4	-0.4 ± 0.5	-0.7 ± 0.5	-3.8 ± 0.6*	-4.4 ± 0.8*	3.0 ± 0.5*
5	-0.5 ± 0.4	-1.0 ± 0.3	-4.0 ± 0.5*	-4.6 ± 0.9*	3.8 ± 0.6*
6	-0.4 ± 0.6	-0.5 ± 0.5	-3.8 ± 0.4*	-5.6 ± 0.9*	4.0 ± 0.7*
7	-0.3 ± 0.5	-0.5 ± 0.5	-4.3 ± 0.6*	-6.1 ± 0.9*	5.5 ± 0.8*
8	-0.6 ± 0.5	-0.5 ± 0.5	-4.2 ± 0.6*	-6.7 ± 1.1*	6.1 ± 0.9*
9	-0.4 ± 0.5	-0.2 ± 0.5	-4.5 ± 0.6*	-7.2 ± 1.2*	7.0 ± 1.0*
10	-0.3 ± 0.5	0.3 ± 0.5	-4.4 ± 0.8*	-7.8 ± 1.2*	7.8 ± 1.1*
11	-0.5 ± 0.5	0.5 ± 0.4	-4.4 ± 0.8*	-7.9 ± 1.4*	8.2 ± 1.2*

12	-0.8 ± 0.6	0.8 ± 0.5	$-4.3 \pm 1.0^*$	$-7.9 \pm 1.5^*$	$8.5 \pm 1.2^*$
13	-0.8 ± 0.6	0.5 ± 0.5	$-3.9 \pm 0.9^*$	$-8.7 \pm 1.6^*$	$8.6 \pm 1.3^*$
14	-1.7 ± 0.6	-0.4 ± 0.5	$-4.6 \pm 1.0^*$	$-9.0 \pm 1.7^*$	$7.6 \pm 1.3^*$
Data are expressed as Mean $\pm$ SEM. n = 8 per group.					
* = p<0.05, compared to that of the vehicle treated group.					

[00184] Table 9: Daily food intake (gm) during treatment with FP1 in DIO mice

Treatment	Vehicle	FP1 (nmol/kg)			Rosiglitazone
Day	n/a	0.1	1	10	10mpk/day
-2	2.9 ± 0.1	3.0 ± 0.2	2.9 ± 0.1	2.8 ± 0.1	2.8 ± 0.1
0	3.0 ± 0.1	3.0 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	3.0 ± 0.1
1	2.9 ± 0.1	3.1 ± 0.1	$2.1 \pm 0.2^*$	$1.5 \pm 0.1^*$	$3.6 \pm 0.1^*$
2	3.0 ± 0.1	3.0 ± 0.1	$2.3 \pm 0.1^*$	2.6 ± 0.2	$3.9 \pm 0.2^*$
3	2.8 ± 0.1	3.0 ± 0.1	2.6 ± 0.1	2.4 ± 0.2	$3.5 \pm 0.1^*$
4	2.3 ± 0.1	2.6 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	$3.3 \pm 0.1^*$
5	2.8 ± 0.1	3.0 ± 0.1	2.8 ± 0.1	2.6 ± 0.1	$3.7 \pm 0.2^*$
6	2.8 ± 0.1	3.1 ± 0.1	2.9 ± 0.1	2.4 ± 0.2	$3.7 \pm 0.2^*$
7	2.7 ± 0.1	2.9 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	$3.8 \pm 0.2^*$

8	2.7 ± 0.1	2.9 ± 0.1	2.6 ± 0.1	2.1 ± 0.1*	3.5 ± 0.2*
9	3.0 ± 0.1	3.3 ± 0.1	2.8 ± 0.1	2.5 ± 0.2*	4.3 ± 0.2*
10	2.6 ± 0.1	3.0 ± 0.1	2.6 ± 0.1	2.2 ± 0.1	3.6 ± 0.1*
11	2.9 ± 0.1	3.1 ± 0.1	2.7 ± 0.2	2.6 ± 0.2	3.4 ± 0.1*
12	2.7 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	3.0 ± 0.2	3.6 ± 0.2*
13	2.6 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.2 ± 0.2	3.2 ± 0.1*
Data are expressed as Mean ± SEM. n = 8 per group.					
* = p<0.05, compared to that of the vehicle treated group.					

[00185] In an OGTT performed on day 14 of the study, FP1 significantly lowered glucose levels compared to vehicle-treated animals at all time points after time 0 at all three doses tested (Table 10). This was further quantitated as total area under the curve (AUC) and delta AUC, which were significantly lower compared to vehicle for all three doses tested

5 (Table 10 and Figures 5A and 5B).

[00186] Table 10: Blood glucose (mg/dL) levels during an OGTT after fourteen days of q3d dosing of FP1 in DIO mice

Treatment	Dose (nmol/kg)	Time after Glucose Challenge (min)					Total AUC (mg/dL/120min)	Delta AUC (mg/dL/120min)
		0	30	60	90	120		
Vehicle	NA	209.6 ± 6	399.4 ± 4	338.5 ± 5	297.5 ± 5	269.3 ± 3	38244.4 ± 4308.2	13089.4 ± 3573.0

Treatment	Dose (nmol/kg)	Time after Glucose Challenge (min)					Total AUC (mg/dL/120min)	Delta AUC (mg/dL/120min)
		0	30	60	90	120		
		16.5	46.4	46.3	38.0	36.6		
FP1	0.1	196.6 ± 7.6	319.0 ± 21.5 *	241.6 ± 14.4 *	196.0 ± 12.8 *	184.0 ± 6.3 *	28408.1 ± 1236.1 *	4885.5 ± 955.1 *
	1	164.0 ± 7.2	251.4 ± 13.0 *	227.6 ± 12.6 *	191.9 ± 9.4 *	180.6 ± 13.1 *	25295.6 ± 1026.7 *	5615.6 ± 647.6 *
	10	146.6 ± 5.9	195.6 ± 14.5 *	197.1 ± 6.9 *	172.1 ± 6.3 *	174.9 ± 13.8 *	21768.8 ± 603.0 *	4211.4 ± 425.8 *
Rosi-glitazone	10 mpk/day	130.8 ± 6.0	199.1 ± 12.9 *	204.4 ± 8.5 *	184.1 ± 10.9 *	169.1 ± 5.7 *	22115.6 ± 671.9 *	6425.6 ± 599.6
Data are expressed as Mean ± SEM. n = 8 per group.								
* = p<0.05, compared to that of the vehicle treated group.								

[00187] Fed blood glucose levels were measured at the start (day 0), at day 7 and at day 13 of the study (Table 11 and Figure 6). FP1 decreased blood glucose in a statistically significant manner at doses of 1 nmol/kg and 10 nmol/kg on day 13 of the study.

[00188] Table 11: Fed blood glucose during treatment of DIO mice with q3d treatment of FP1

Treatment	Dose (nmol/kg)	Time after start of treatment (days)		
		0	7	13
Vehicle	NA	177.6 ± 10.9	173.6 ± 10.6	225.3 ± 23.0
FP1	0.1	174.1 ± 8.5	171.8 ± 16.3	196.4 ± 8.1
	1	167.5 ± 7.8	135.1 ±	165.3 ± 10.3 *

			11.2	
	10	165.3 ± 13.7	145.3 ± 4.9	153.8 ± 7.5*
Rosiglitazone	10mpk/day	189.6 ± 17.3	122.6 ± 8.1*	154.8 ± 6.5*
Data are expressed as Mean ± SEM. n = 8 per group.				
* = p<0.05, compared to that of the vehicle treated group.				

[00189] Plasma insulin levels during the OGTT were significantly higher for FP1 than for the corresponding vehicle group for a 0.1 nmol/kg dose at 30 minutes, and lower at the 1 and 10 nmol/kg doses at the same time point (Table 12). The insulin excursion during the OGTT, as measured by total AUC, was higher than the vehicle group for the 0.1 nmol/kg dose of FP1 (Table 12), and lower at the 1 and 10 nmol/kg dose. In both cases, statistical significance was reached only at the lowest dose. At the 90 minute time point, mice treated with 1 and 10 nmol/kg of FP1 had lower insulin levels; however, this effect did not achieve statistical significance. HOMA-IR, used as a measure of insulin sensitivity, was measured on day 14 of the study. At this time point, FP1 decreased HOMA-IR, or improved insulin sensitivity, at 10 nmol/kg (Table 13 and Figure 7).

[00190] Table 12: Plasma insulin (pg/mL) levels during an OGTT after fourteen days of q3d dosing of FP1 in DIO mice

Treatment	Dose (nmol/kg)	Time after Glucose Challenge (min)			Total AUC (pg/mL/90min)
		0	30	90	
Vehicle	NA	6096.0 ± 774.3	14660.0 ± 3031.2	5034.4 ± 405.1	902151.9 ± 143123.2
FP1	0.1	7861.0 ± 779.5	33825.8 ± 7902.0*	6494.4 ± 797.0	1834808.8 ± 381276.1*
	1	4808.1 ± 795.8	13061.8 ± 2226.3	3443.5 ± 342.5	763218.1 ± 119766.8
	10	3478.3 ± 634.6	7147.0 ± 823.8	2958.0 ± 414.0	462528.8 ± 44653.1
Rosiglitazone	10mpk/day	2965.6 ±	2203.1 ±	1180.0 ±	179025.0 ±

		Time after Glucose Challenge (min)			
		524.2	193.5*	57.1	9970.7

Data are expressed as Mean $\pm$ SEM. n = 8 per group.

* = p<0.05, compared to that of the vehicle treated group.

[00191] Table 13: Fasted HOMA-IR in DIO mice after fourteen days of q3d treatment of FP1

Treatment	Dose (nmol/kg)	HOMA-IR
Vehicle	NA	93.5 $\pm$ 15.3
FP1	0.1	112.4 $\pm$ 14.6
	1	56.7 $\pm$ 9.7
	10	37.7 $\pm$ 8.2*
Rosiglitazone	10mpk/day	27.3 $\pm$ 4.7*

Data are expressed as Mean $\pm$ SEM.

n = 8 per group.

* = p<0.05, compared to that of the vehicle treated group.

[00192] The magnitude of weight loss achieved by day 13 did not result in measurable changes in absolute fat mass or percent fat mass at any dose (Table 14). At the 10 nmol/kg dose, there was a significant decrease in absolute lean mass. This decrease was not observed when expressed as percent lean mass. Liver weights were measured during terminal necropsy on day 15 of the study (Table 15). FP1 decreased absolute liver weight and liver weight as a percentage of body weight at the 10 nmol/kg dose. A decrease was observed at the 1 nmol/kg dose, but this did not reach statistical significance for either parameter. Liver fat was measured on a biopsy by NMR (Table 16). FP1 fusion protein decreased hepatic fat content, expressed as a percentage of liver biopsy weight, at 1 and 10 nmol/kg doses. The reduction was significant at the higher dose.

[00193] Table 14: Body composition after thirteen days of treatment with FP1 q3d in DIO mice

Treatment	Dose (nmol/kg)	Fat Mass (g)	Lean Mass (g)	Fat Mass (% of body)	Lean Mass (% of body)
Vehicle	NA	11.6 ± 0.5	29.6 ± 0.4	23.4 ± 0.8	59.6 ± 0.9
FP1	0.1	12.2 ± 0.4	29.7 ± 0.4	24.1 ± 0.6	58.5 ± 0.6
	1	12.1 ± 0.3	28.0 ± 0.4	25.1 ± 0.3	58.3 ± 0.6
	10	10.6 ± 0.3	27.7 ± 0.4*	23.1 ± 0.3	60.6 ± 0.6
Rosiglitazone	10mpk/day	14.9 ± 0.6*	30.0 ± 0.5	27.2 ± 0.4*	55.0 ± 0.6*

Data are expressed as Mean ± SEM. n = 8 per group.

* = p<0.05, compared to that of the vehicle treated group.

[00194] Table 15: Liver weight after fifteen days of treatment with FP1 q3d in DIO mice

Treatment	Dose (nmol/kg)	Liver weight (g)	Liver Weight (% of body)
Vehicle	NA	2.6 ± 0.1	5.4 ± 0.2
FP1	0.1	2.9 ± 0.2	5.8 ± 0.2
	1	2.2 ± 0.1	4.6 ± 0.2
	10	1.9 ± 0.1*	4.2 ± 0.1*

Treatment	Dose (nmol/kg)	Liver weight (g)	Liver Weight (% of body)
Rosiglitazone	10mpk/day	2.5 ± 0.2	4.6 ± 0.2

Data are expressed as Mean ± SEM. n = 8 per group.

* = p<0.05, compared to that of the vehicle treated group.

[00195] Table 16: Liver fat content measured after fifteen days of treatment with FP1 q3d in DIO mice

Treatment	Dose (nmol/kg)	Fat (%)
Vehicle	NA	27.3 ± 2.1
FP1	0.1	26.0 ± 1.4
	1	22.5 ± 1.4
	10	17.8 ± 1.6*
Rosiglitazone	10mpk/day	25.9 ± 0.8

Data are expressed as Mean ± SEM. n = 8 per group.

* = p<0.05, compared to that of the vehicle treated group.

[00196] **Example 11: Effects of FP1 on blood glucose levels and body weight in *ob/ob* mice**

[00197] The purpose of this experiment was to evaluate the effects of FP1 on body weight and blood glucose levels over eight days of treatment in obese, hyperglycemic, leptin-
5 deficient *ob/ob* mice.

[00198] Male *ob/ob* mice were weighed and FP1 was administered subcutaneously at 2mL/kg every three days (q3d) at Day 0, 3 and 6. Mouse and food weights were recorded daily. Glucose was measured daily using a glucometer. At the end of the study, mice were euthanized, and a terminal blood sample was collected.

10 [00199] FP1, at the 1 nmol/kg dose, significantly decreased body weight (expressed as a percentage of starting body weight) in *ob/ob* mice starting at day 2 until day 8, relative to vehicle-treated mice. FP1, at the 10 nmol/kg dose, decreased body weight (expressed as a percentage of starting body weight) in *ob/ob* mice starting at day 1 until day 8 relative to vehicle-treated mice (Table 17 and Figure 8).

15 [00200] Table 17: Body weight change (% of starting) during treatment with FP1 q3d in *ob/ob* mice

Treatment	Vehicle	FP1	
Day	n/a	1 nmol/kg	10 nmol/kg
-6	-2.5 ± 0.3	-2.8 ± 0.4	-3.6 ± 0.5
-5	-2.4 ± 0.3	-3.0 ± 0.5	-3.5 ± 0.5
-4	-1.9 ± 0.2	-2.5 ± 0.4	-2.8 ± 0.3
-1	-0.3 ± 0.3	0.1 ± 0.2	-0.3 ± 0.2

0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1	0.5 ± 0.2	-1.6 ± 0.2	-2.3 ± 0.5*
2	1.1 ± 0.2	-1.8 ± 0.4*	-2.0 ± 0.9*
3	1.3 ± 0.3	-2.5 ± 0.4*	-3.1 ± 1.1*
4	1.8 ± 0.3	-3.3 ± 0.5*	-4.2 ± 1.3*
5	1.8 ± 0.4	-3.5 ± 0.5*	-4.7 ± 1.5*
6	2.0 ± 0.5	-4.0 ± 0.7*	-5.7 ± 1.6*
7	2.8 ± 0.6	-4.8 ± 0.8*	-6.3 ± 1.7*
8	3.5 ± 0.8	-4.5 ± 1.0*	-6.1 ± 1.9*
Data are expressed as Mean ± SEM. n = 8 per group.			
* = p<0.05, compared to that of the vehicle treated group.			

[00201] FP1, at the 10 nmol/kg dose, decreased fed blood glucose values in *ob/ob* mice on study day 1 and 2 and from day 4 until day 8 relative to vehicle-treated mice. A reduction in in blood glucose was observed at 1 nmol/kg; however, this effect did not reach statistical

5 significance (Table 18 and Figure 9).

[00202] Table 18: Fed blood glucose during treatment of *ob/ob* mice with FP1 q3d

Treatment	Vehicle	FP1	
Day	n/a	1 nmol/kg	10 nmol/kg
-6	463.6 ± 31.2	395.7 ± 52.0	463.6 ± 41.0
-5	554.9 ± 57.5	609.6 ± 53.6	552.9 ± 53.0
-4	502.9 ± 41.6	517.9 ± 71.6	490.3 ± 54.4
-1	552.1 ± 45.1	567.2 ± 51.2	586.0 ± 42.6
0	468.8 ± 57.3	479.7 ± 61.8	437.0 ± 42.7
1	537.0 ± 42.8	439.0 ± 80.4	336.2 ± 51.0*
2	511.7 ± 43.5	440.0 ± 74.3	293.7 ± 37.6*
3	447.8 ± 54.2	369.6 ± 75.9	279.4 ± 38.8
4	516.3 ± 47.0	384.6 ± 84.0	261.2 ± 43.7*
5	531.0 ± 47.1	410.9 ± 92.2	286.9 ± 55.8*
6	596.1 ± 45.1	451.1 ± 82.6	301.9 ± 49.3*
7	566.7 ± 44.3	425.3 ± 86.9	246.7 ± 43.5*
8	509.9 ± 37.6	337.8 ± 75.1	223.4 ± 34.1*
Data are expressed as Mean ± SEM. n = 8 per group.			

[00203] Example 12: Multispecies Pharmacokinetics

[00204] Mouse Pharmacokinetics

[00205] FP1 was administered to female C57Bl/6 mice at a dose of 2 mg/kg IV and SC in PBS, pH 7. Blood samples were collected, serum was processed and drug concentrations were measured up to 7 days following both routes of administration. The concentration of FP1 was determined using an immunoassay method. The serum drug concentration-time profile is summarized in Tables 19 and 20 and illustrated in Figure 10.

[00206] Table 19: Serum concentration (nM) of FP1 over time following a single SC administration in C57Bl/6 female mice

	FP1 - SC Dose						
Timepoint	Animal 56 Result (nM)	Animal 57 Result (nM)	Animal 58 Result (nM)	Animal 60 Result (nM)	Animal 63 Result (nM)	Average Result (nM)	Std Dev
4 hr	32.299	50.735	42.766	32.407	23.018	36.245	10.698
24 hr	88.822	106.418	88.648	103.841	80.346	93.615	11.093
72 hr	33.563	38.473	32.473	33.625	32.769	34.181	2.451
96 hr	20.639	24.988	21.247	19.356	20.771	21.400	2.124
Day 7	5.399	6.919	7.234	5.994	5.637	6.237	0.803

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[00207] Table 20: Serum concentration (nM) of FP1 over time following a single IV administration in C57Bl/6 female mice

	FP1 - IV Dose						
Timepoint	Animal 52 Result (nM)	Animal 53 Result (nM)	Animal 65 Result (nM)	Animal 66 Result (nM)	Animal 70 Result (nM)	Average Result (nM)	Std Dev
1 hr	240.419	233.318	232.484	276.913	272.727	251.172	21.857

24 hr	86.823	95.774	80.201	93.153	88.853	88.961	6.027
72 hr	33.634	37.447	33.108	41.680	34.034	35.981	3.612
96 hr	22.666	20.588	19.458	33.718	20.361	23.358	5.909
Day 7	7.401	5.606	4.896	8.556	4.205	6.133	1.803

[00208] Pharmacokinetic analysis revealed a terminal half-life of 1.67 and 1.57 days for FP1 in C57Bl/6 mice following SC and IV administration, respectively (Table 21). FP1 demonstrated a mean bioavailability of ~71% following SC administration.

- 5 [00209] Table 21: Mean ($\pm$ SD) pharmacokinetic parameters of FP1 following 2 mg/kg IV and SC administration in female C57Bl/6 mice

Route		$t_{1/2}$ (day)	CL or CL/F (ml/day/kg)	V _{ss} (ml/kg)	C _{max} (ng/ml)	T _{max} * (day)	AUC _{0-last} (day*ng/ml)	AUC _{0-inf} (day*ng/ml)
SC	Mean	1.67	49.48		14994	1	38315	40734
	(SD)	(0.14)	(4.791)		(1776)		(3851)	(4072)
IV	Mean	1.57	35.00	69.03	40231	0.04	55263	57531
	(SD)	(0.19)	(3.13)	(5.8)	(3500)		(4853)	(5416)

Note: *T_{max} (median)

[00210] Rat Pharmacokinetics

- [00211] FP1 was administered to female Sprague Dawley rats at a dose of 2 mg/kg IV and SC in PBS, pH 7. Blood samples were collected, serum was processed and drug concentrations were measured up to 7 days following both routes of administration. The
- 10

concentration of FP1 was determined using an immunoassay method. The serum drug concentration-time profile is summarized in Tables 22 and 23 and illustrated in Figure 11.

[00212] Table 22: Serum concentration (nM) of FP1 over time following a single SC administration in female Sprague Dawley rats.

FP1 - Group 3 (SC Dose)							
Timepoint	Animal 53 Result (nM)	Animal 55 Result (nM)	Animal 67 Result (nM)	Animal 68 Result (nM)	Animal 69 Result (nM)	Average Result (nM)	Std Dev
4 hr	4.766	3.500	3.932	3.546	3.250	3.799	0.593
24 hr	45.118	53.192	39.196	39.823	40.804	43.627	5.826
72 hr	18.900	24.102	23.124	23.718	18.933	21.755	2.615
96 hr	12.193	14.333	14.185	14.669	11.256	13.327	1.511
Day 7	2.805	2.821	2.447	3.438	2.358	2.774	0.426

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[00213] Table 23: Serum concentration (nM) of FP1 over time following a single IV administration in female Sprague Dawley rats

FP1 - Group 4 (IV Dose)							
Timepoint	Animal 51 Result (nM)	Animal 52 Result (nM)	Animal 57 Result (nM)	Animal 64 Result (nM)	Animal 66 Result (nM)	Average Result (nM)	Std Dev
1 hr	43.620*	382.676	403.255	443.080	510.105	356.547	181.560
24 hr	102.665*	142.661	139.066	124.528	126.425	127.069	15.728
72 hr	46.720	60.105	67.090	59.257	70.423	60.719	9.127
96 hr	29.409	39.897	41.225	42.258	48.074	40.173	6.779
Day 7	6.976	11.251	8.913	9.540	13.006	9.937	2.298

* Repeat analysis confirmed results

[00214] Pharmacokinetic analysis revealed a terminal half-life of 1.34 and 1.51 days for FP1 in Sprague Dawley rats following SC and IV administration, respectively (Table 24). FP1 demonstrated a mean bioavailability of ~23% following SC administration.

[00215] Table 24: Mean ($\pm$ SD) pharmacokinetic parameters of FP1 following 2

5 mg/kg IV and SC administration in Sprague Dawley rats

Route		$t_{1/2}$ (day)	CL or CL/F (ml/day/kg)	V _{ss} (ml/kg)	C _{max} (ng/ml)	T _{max} * (day)	AUC _{0-last} (day*ng/ml)	AUC _{0-inf} (day*ng/ml)
SC	Mean	1.34	100.09		6987	1	19250	20112
	(SD)	(0.04)	(3.97)		(417)		(794)	(820)
IV	Mean	1.51	24.75	53.41	59000	0.04	83028	86525
	(SD)	(0.12)	(8.53)	(17.15)	(25031)		(20126)	(20881)

Note: *Tmax (median)

[00216] Monkey Pharmacokinetics

[00217] FP1 was administered to naïve male cynomolgus monkeys (*Macaca*

fascicularis) at a dose of 1 mg/kg IV and SC in PBS, pH 7. Blood samples were collected,

10 serum was processed and drug concentrations were measured up to 21 days following both routes of administration, using immunoassay bioanalysis. The serum drug concentration-time profile is summarized in Tables 25 and 26 and illustrated in Figure 12.

[00218] Table 25: Serum concentration (nM) of FP1 over time following a single SC administration in cynomolgus monkeys as determined by immunoassay

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	FP1 (SC Dose)				
Timepoint	Animal 110 (nM)	Animal 111 (nM)	Animal 112 (nM)	Average Result (nM)	Std Dev
Predose	< LLOQ	< LLOQ	< LLOQ	< LLOQ	N/A
6 hr	49.304	43.784	72.110	55.066	15.017
24 hr	93.368	71.958	96.863	87.396	13.483
48 hr	107.689	97.509	115.144	106.781	8.853
72 hr	113.601	104.190	104.449	107.414	5.360
120 hr	101.490	95.049	91.717	96.085	4.968
168 hr	82.167	75.435	81.569	79.724	3.726
240 hr	71.033	56.732	59.266	62.344	7.631
336 hr	44.380	42.758	42.571	43.236	0.995
432 hr	30.911	29.445	32.839	31.065	1.702
528 hr	21.277	20.404	26.427	22.703	3.255

[00219] Table 26: Serum concentration (nM) of FP1 over time following a single IV administration in cynomolgus monkeys as determined by immunoassay

	FP1 (IV Dose)				
Timepoint	Animal 104 (nM)	Animal 105 (nM)	Animal 106 (nM)	Average Result (nM)	Std Dev
Predose	< LLOQ	< LLOQ	< LLOQ	< LLOQ	N/A
1 hr	212.661	235.168	189.000	212.276	23.087
6 hr	190.315	185.331	183.575	186.407	3.497
24 hr	141.743	155.943	146.487	148.058	7.229
48 hr	111.765	126.105	120.744	119.538	7.246
72 hr	105.076	106.955	106.441	106.157	0.971
120 hr	92.591	103.882	103.757	100.077	6.483
168 hr	71.368	93.055	87.706	84.043	11.298
240 hr	71.554	65.093	65.685	67.444	3.572
336 hr	46.184	38.961	41.696	42.280	3.647
432 hr	34.589	31.266	19.492	28.449	7.933
528 hr	26.885	24.154	22.422	24.487	2.250

[00220] Pharmacokinetic analysis revealed a terminal half-life between 8.5 and 9.2 days for FP1 in cynomolgus monkeys following SC and IV administration, respectively with a mean bioavailability of ~88% following SC administration (Table 27).

[00221] Table 27: Mean ($\pm$ SD) pharmacokinetic parameters of FP1 following 1 mg/kg

5 IV and SC administration in cynomolgus monkeys.

Route		$t_{1/2}$ (day)	CL or CL/F (ml/day/ kg)	V _{ss} (ml/kg)	C _{max} (ng/ml)	T _{max} [*] (day)	AUC _{0-last} (day*ng/ml)	AUC _{0-inf} (day*ng/ ml)
SC	Mean	8.5	3.9		17776	3	211030	256202
	(SD)	(1.5)	(0.3)		(950)		(11332)	(20192)
IV	Mean	9.2	3.4	45.6	34001	0.04	239758	292206
	(SD)	(0.5)	(0.1)	(1.6)	(3698)		(6095)	(11516)

Note: *T_{max} (median)

[00222] Immuno-affinity capture-LCMS analysis was used to quantitate the concentration of intact dimer present in the serum of cynomolgus monkeys after IV and SC administration (Tables 28 and 29 and Figures 13 and 14). Concentrations determined by this
 10 method were similar to concentrations determined by the immunoassay (IA), demonstrating that FP1 circulates as an intact dimer, with no detectable metabolic liability in cynomolgus monkeys.

[00223] Table 28: Serum concentration (ng/mL) of FP1 as an intact dimer over time following a single IV administration in cynomolgus monkeys as determined by immuno-

15 affinity capture-LCMS analysis

Day	Dimer Intact MS Data (Pooled Samples)	IA Data (Average)
0.00	0	0
0.04	33347	34537
0.25	29686	30328
1.00	28787	24089
2.00	17249	19449
3.00	16827	17272
5.00	16159	16282
7.00	11124	13674
10.00	8746	10973
14.00	5328	6879
18.00	3857	4629
22.00	2252	3984

[00224] Table 29: Serum concentration (ng/mL) of FP1 as an intact dimer over time following a single SC administration in cynomolgus monkeys as determined by immuno-affinity capture-LCMS analysis.

Day	Dimer Intact MS Data (Pooled Samples)	IA Data (Average)
0.00	0	0
0.25	9625	8959
1.00	15799	14219
2.00	17671	17373
3.00	19130	17476
5.00	12284	15633
7.00	10808	12971
10.00	8910	10143

Day	Dimer Intact MS Data (Pooled Samples)	IA Data (Average)
14.00	5814	7034
18.00	4074	5054
22.00	2967	3694

[00225] The concentration of analytes in cynomolgus monkey serum after IV and SC administration was also measured by immuno-affinity capture-trypsin digestion-LC-MS/MS analysis (Tables 30 and 31). Selected tryptic peptides, namely, ALV

- 5 (ALVLIAFAQYLQQSPFEDHVK), ASL (ASLEDLGWADWVLSPR), and TDT (TDTGVSLQTYDDLAK), which are located within FP1 near the N-terminus of the HSA region, the N-terminus of GDF15, and the C-terminal of GDF15, respectively. The peptides were monitored as surrogate peptides of FP1. The concentrations of all of the surrogate peptides were similar to each other and the concentrations measured by immunoassay,
- 10 demonstrating that the GDF15 sequence in FP1 remains intact and linked to the full HSA sequence *in vivo*.

[00226] Table 30: Serum concentration (ng/mL) of surrogate peptides representing various regions of FP1 over time following a single IV administration in cynomolgus monkeys as determined by immuno-affinity capture-trypsin digestion-LC-MS/MS analysis

Time point		FP1 Average (ng/mL)			Std Dev			IA Data (ng/mL)
Day	hour	ALV	TDT	ASL	ALV	TDT	ASL	
0.00	0	<LLOQ	<LLOQ	<LLOQ	N/A	N/A	N/A	0.0
0.04	1	32400.0	39766.7	33333.3	2623.0	3162.8	2722.7	34536.9
0.25	6	29100.0	27166.7	30600.0	3439.5	1006.6	2095.2	30328.1
1.00	24	24366.7	23300.0	23800.0	4215.8	3996.2	4100.0	24088.7
2.00	48	19433.3	17733.3	18700.0	1457.2	1193.0	854.4	19448.6
3.00	72	18100.0	17200.0	17166.7	360.6	871.8	1001.7	17271.6
5.00	120	15966.7	14033.3	14233.3	1001.7	642.9	1011.6	16282.3
7.00	168	13733.3	11800.0	12100.0	1115.0	600.0	300.0	13673.6
10.00	240	9303.3	8570.0	8570.0	2290.1	1682.2	1685.9	10973.0

Time point		FP1 Average (ng/mL)			Std Dev			IA Data (ng/mL)
14.00	336	5860.0	5890.0	6056.7	415.8	312.2	388.0	6878.9
18.00	432	4143.3	4400.0	4226.7	374.3	52.9	571.2	4628.6
22.00	528	2830.0	3256.7	2753.3	355.4	420.0	319.0	3984.0

[00227] Table 31: Serum concentration (ng/mL) of surrogate peptides representing various regions of FP1 over time following a single SC administration in cynomolgus monkeys as determined by immuno-affinity capture-trypsin digestion-LC-MS/MS analysis

Time point		FP1 Average (ng/mL)			Std Dev			IA Data (ng/mL)
Day	hour	ALV	TDT	ASL	ALV	TDT	ASL	
0.00	0	<LLOQ	<LLOQ	<LLOQ	N/A	N/A	N/A	0.0
0.25	6	9323.3	7430.0	8123.3	1900.3	2471.6	1954.1	8959.1
1.00	24	15233.3	13390.0	14533.3	2926.3	3222.0	2683.9	14219.2
2.00	48	15366.7	14000.0	14166.7	2579.4	1646.2	2311.6	17373.0
3.00	72	17300.0	16033.3	15333.3	1571.6	1001.7	986.6	17476.0
5.00	120	15333.3	13366.7	13666.7	1222.0	1692.1	1501.1	15632.9
7.00	168	13333.3	11633.3	11700.0	709.5	472.6	781.0	12970.9
10.00	240	8496.7	7376.7	8343.3	1475.5	189.0	1039.1	10143.2
14.00	336	6046.7	6116.7	6253.3	90.2	118.5	110.6	7034.5
18.00	432	4593.3	5250.0	4636.7	802.6	1157.5	621.7	5054.2
22.00	528	3056.7	3490.0	3116.7	424.5	687.7	220.5	3693.7

[00228] Human plasma stability assay

[00229] The purpose of this study was to analyze the *ex vivo* stability of FP1 in human plasma. Fresh, non-frozen human plasma was generated from heparinized blood from two subjects (one male and one female) by centrifugation. FP1 was incubated in this matrix at 37°C with gentle mixing, for 0, 4, 24 and 48 hours. The concentration of FP1 was determined using an immunoassay method. The average percent difference from the starting concentration (0 hours) ranged from -4.1 to -12.9 and did not increase over time, demonstrating that FP1 is stable in human plasma for up to 48 hours *ex vivo* (Table 32 and Figure 15).

10 [00230] Table 32: FP1 concentration (µg/mL) after 0, 4, 24, and 48 hours (hr) of *ex vivo* incubation in plasma obtained from two human subjects (Sub) as determined by immunoassay

<i>Ex Vivo</i> Sample	Sub 1 Male Conc. (ug/mL)	% Diff T0	Sub 2 Female Conc. (ug/mL)	% Diff T0	Average Conc. (ug/mL)	% Diff T0
Plasma - T0hr_ FP1	11.503	N/A	12.649	N/A	12.076	N/A
Plasma - T4hr_ FP1	10.524	-8.5	10.521	-16.8	10.523	-12.9
Plasma - T24hr_ FP1	9.934	-13.6	12.402	-2.0	11.168	-7.5
Plasma - T48hr_ FP1	10.582	-8.0	12.575	-0.6	11.578	-4.1

[00231] Immuno-affinity capture-LCMS was used to quantitate the concentration of intact dimer present after incubation in human plasma. Concentrations determined by this method were stable over time (0, 4, 24, and 48 hours), demonstrating that FP1 remains an intact dimer in human plasma *ex vivo* up to 48 hours (Table 33 and Figure 16).

[00232] Table 33: Average FP1 concentration ($\mu\text{g/mL}$) and % difference from starting concentration as an intact dimer after 0, 4, 24, and 48 hours (hr) of *ex vivo* incubation in plasma obtained from two human subjects as determined by immuno-affinity capture-LCMS analysis

	Dimer Conc. ($\mu\text{g/mL}$)	% Difference
0 hr	15.8	100.0
4 hr	15.8	100.1
24 hr	15.9	100.9
48 hr	15.2	96.0

5

[00233] **Example 13: IR Strategy**

[00234] Immune response (IR) assays will be developed for anti-drug antibody (ADA) detection in animals and clinical samples. The IR assay will identify ADA-positive samples for comparison of ADA status with pharmacokinetic/toxicokinetic (PK/TK) results, enabling
 10 assessment of FP1 exposure and pharmacokinetics. The clinical IR assay will be used to screen serum samples, confirm specificity of ADA-positive samples, and determine the ADA titer for confirmed positive samples. Neutralizing antibody (NAb) assay development will follow for use in confirmed positive samples from ADA-positive subjects in Phase 1 of the program. Additionally, determination of ADA cross-reactivity to endogenous GDF15 will
 15 follow for use in Phase 2 of the program. An immunogenicity risk assessment will be conducted prior to the first in human (FIH) study, and additional immune response characterization assays can be implemented if they are warranted.

[00235] **Example 14: Toxicology Plan**

[00236] Since the endogenous target receptor for GDF15 has not been identified, there
 20 is a lack of *in vitro* binding and functional data for FP1. However, single and multiple dose pharmacology and efficacy studies in rats, mice and cynomolgus monkeys have demonstrated

activity of FP1 in these species, showing its effect of reducing food intake, decreasing body weight and modulating oral glucose tolerance. The rat and monkey will be the rodent and non-rodent toxicology testing species, respectively, based on the efficacy results, with the understanding that the intrinsic potency of FP1 on the receptor in these species (versus that of humans) has not been fully characterized.

[00237] The Examples 15-19 involve characterization of another exemplary fusion protein of the invention, described in Example 5, which has the amino acid sequence of SEQ ID NO: 92 (encoded by nucleotide sequences SEQ ID NOs: 92 (codon optimization 1) and 110 (codon optimization 2)). This fusion protein is a fully recombinant protein that exists as a homodimer of a fusion of HSA (C34S) with the deletion variant of the mature human GDF15 (201-308; SEQ ID NO: 8) through a 42-amino acid linker consisting of glycine and serine residues, GS-(GGGGS)₈. The single native free cysteine at position 34 of HSA has been mutated to serine. This particular HSA-GDF15 fusion protein will be referred to as “FP2” in the following examples, for simplicity.

[00238] **Example 15: Effects of FP2 on the food intake of C57Bl/6 mice**

[00239] FP2 was evaluated for its ability to reduce food intake in male C57Bl/6 mice after a single dose. Male C57Bl/6N mice (age 10-12 weeks) obtained from Taconic Biosciences (Hudson, NY) were used in the study. Mice were singly housed in a temperature-controlled room with 12-hour light/dark cycle (6am/6pm) and allowed *ad libitum* access to water and chow. Male C57Bl/6 mice were acclimated for a minimum of 72 hours in the BioDAQ cages; mice were then grouped based on food intake in the last 24 hours into six groups of eight each. Between 4:00-5:00 pm, animals were weighed and dosed with vehicle or compounds via subcutaneous injection. Change in food weight for each cage was

recorded continuously by the BioDAQ system, for a period of 48 hours after compound administration. 6xHis-FP1 was used as a comparator in this study.

[00240] FP2 had significant effects on reducing food intake at 12, 24 and 48 hours after administration at all dose levels tested (Table 34). There was a reduction in percent change in food intake relative to PBS at all time points and all dose levels (Table 35) in mice.

Table 34: Effect of a Single Dose of FP2 on Food Intake over 48 hours in C57Bl/6 Mice

Cumulative Food Intake (g)			
Treatment	12 hours	24 hours	48 hours
PBS	3.7±0.2	4.4±0.2	8.8±0.4
FP2, 1 nmol/kg	2.8±0.4*	3.0±0.4**	6.9±0.7*
FP2, 4 nmol/kg	2.4±0.2***	3.1±0.2**	7.0±0.3*
FP2, 8 nmol/kg	1.9±0.2****	2.4±0.1****	6.2±0.2***
FP2, 16 nmol/kg	1.8±0.1****	2.7±0.1***	6.3±0.4**
6xHis-FP1, 8 nmol/kg	2.3±0.3***	2.8±0.4**	6.6±0.7**

Data are expressed as Mean ± SEM.

*p≤0.05, versus PBS

**p≤0.01, versus PBS

10 ***p≤0.001, versus PBS

****p≤0.0001, versus PBS, respectively

Statistical analyses used: ANOVA and Dunnett's multiple comparisons test.

n=8/group, except for 6xHis-FP1 8nmol/kg (n=6).

Table 35: Effect of a Single Dose of FP2 on Percent Reduction in Food Intake (Relative to Vehicle) over 48 hours in C57Bl/6 Mice

Percentage of inhibition relative to PBS			
Treatment	12 hours	24 hours	48 hours
PBS	0.0±7.9	0.0±7.7	0.0±5.9
FP2, 1 nmol/kg	22.4±12.5*	31.8±10.5**	21.8±8.1*

FP2, 4 nmol/kg	36.6±7.2***	30.4±5.8**	20.2±4.7*
FP2, 8 nmol/kg	47.0±6.2****	45.7±3.8****	29.7±3.9****
FP2, 16 nmol/kg	49.2±4.1****	38.1±4.1***	27.8±5.1**
6xHis-FP1, 8 nmol/kg	36.9±9.9***	36.5±8.8**	24.6±8.2**

The anorectic effect of FP2 is expressed as the relative reduction in food intake compared with the respective PBS controls.

Data are expressed as Mean ± SEM.

*p≤0.05, versus PBS

5 **p≤0.01, versus PBS

***p≤0.001, versus PBS

****p≤0.0001, versus PBS, respectively

Statistical analyses used: ANOVA and Dunnett's multiple comparisons test.

n=8/group, except for 6xHis-FP1 8nmol/kg (n=6).

10 [00241] **Example 16: Effects of FP2 on food intake in Sprague Dawley rats**

[00242] FP2 was evaluated for its ability to reduce food intake and body weight gain in male Sprague-Dawley rats after a single dose. The animals were obtained from Charles River Labs (Wilmington, MA) at 200-225 g body weight and used within one week of delivery. They were housed one per cage on alpha dry bedding and a plastic tube for enrichment in a temperature-controlled room with 12-hour light/dark cycle. They were allowed *ad libitum* access to water and were fed laboratory rodent diet; Irradiated Certified PicoLab® Rodent Diet 20, 5K75* (supplied from Purina Mills, St. Louis, MO. via ASAP Quakertown, PA). Animal weights were taken and recorded for each rat prior to dosing.

[00243] Animals were acclimated for a minimum of 72 hours in the BioDAQ cages; rats were then grouped based on food intake in the last 24 hours into six groups of eight each. Between 4:00-5:00 pm, animals were weighed and dosed with vehicle or compounds via subcutaneous injection. Change in food weight for each cage was recorded continuously by

the BioDAQ system, for a period of 48 hours after compound administration. 6XHis-FP1 was used as a comparator in this study.

[00244] Dose-dependent reductions of food intake were tested after a single dose of FP2. No significant differences in food intake were observed at the dose of 0.3 nmol/kg.

- 5 Significant effects in reduction of food intake were observed 12 hours but not 24 or 48 hours at 1 nmol/kg. Significant reductions in food intake were observed at all time points for the 3 and 10 nmol/kg dose levels (Table 36, Figure 19). There was a reduction in percent change in food intake relative to PBS at all time points and all dose levels (Table 37).

Table 36: Effect of a single dose of FP2 on food intake over 48 hours in Sprague Dawley rats.

Cumulative Food Intake (g)			
Treatment	12 hours	24 hours	48 hours
PBS	21.7±0.6	25.0±0.8	53.1±1.6
FP2, 0.3 nmol/Kg	19.5±0.9	22.9±0.7	48.4±1.5
FP2, 1 nmol/Kg	17.5±0.9*	19.8±0.8	47.0±2.6
FP2, 3 nmol/Kg	16.1±1.2**	17.0±1.0**	39.2±3.2**
FP2, 10 nmol/Kg	15.8±0.9***	16.5±0.9**	36.5±3.2**
6XHis-FP1 8 nmol/Kg	15.0±1.4***	15.7±1.2**	37.2±4.5**

- 10 Data are expressed as Mean ± SEM.

*p≤0.05, versus PBS

**p≤0.01, versus PBS

***p≤0.001, versus PBS, respectively

Statistical analyses used: ANOVA and Dunnett's multiple comparisons test.

- 15 n=8/group

Table 37: Effect of a single dose of FP2 on percent reduction in food intake (relative to vehicle) over 48 hours in Sprague Dawley rats.

Percentage of inhibition relative to PBS			
Treatment	12 hours	24 hours	48 hours

PBS	0.0±4.0	0.0±4.6	0.0±4.3
FP2 0.3 nmol/kg	10.0±4.7	8.5±4.1	8.8±3.9
FP2 1 nmol/kg	19.3±4.7*	20.8±4.1	11.5±5.5
FP2 3 nmol/kg	25.9±6.1**	32.0±4.7**	26.2±6.5**
FP2 10 nmol/kg	27.4±4.8***	33.8±4.1**	31.2±6.4**
6XHis-FP1 8 nmol/kg	30.8±6.6***	37.0±5.4**	29.9±8.8**

The anorectic effect of FP2 is expressed as the relative reduction in food intake compared with the respective PBS controls.

Data are expressed as Mean ± SEM.

*p≤0.05, versus PBS

5 **p≤0.01, versus PBS

***p≤0.001, versus PBS, versus PBS, respectively

Statistical analyses used: ANOVA and Dunnett's multiple comparisons test.

n=8/group

[00245] **Example 17: Effects of FP2 on Food Intake, Body Weight and Glucose**

10 **Homeostasis in Diet-induced Obese (DIO) C57Bl/6 Mice**

[00246] FP2 was evaluated for its ability to reduce food intake and body weight and improve glucose homeostasis on repeat dosing in male DIO C57Bl/6 mice over a period of 8 days. Male DIO C57Bl/6 mice (age 21 weeks, high fat-fed for 15 weeks) obtained from Taconic Biosciences (Hudson, NY) were used in the study. Mice were singly housed in a temperature-controlled room with 12-hour light/dark cycle (6am/6pm) and allowed *ad libitum* access to water and fed with Research Diet D12492 (Research Diets, New Brunswick, NJ). Mice were acclimated >1 week in the mouse housing room prior to the experiment. The endpoints of the study were measurements of food intake, body weight, body composition and glycemic endpoints (OGTT, blood glucose). One day prior to dosing, animals were weighed and grouped by body weight (BW). Mice were dosed by subcutaneous injection. Animals dosed with FP2 received this compound on Day 0, Day 3, and Day 6, Day 9 and

Day 12. The vehicle group and rosiglitazone group received sterile PBS s.c. on these days as well. Rosiglitazone was provided in the diet at 0.015% w/w *ad libitum*. BW and food intake were recorded daily, over a period of fifteen days. Blood glucose was measured on Days 0, 7 and 13. An oral glucose tolerance test (OGTT) was performed on Day 14. Insulin levels were measured at selected time points during the OGTT. Mice were euthanized with CO₂ and terminal blood samples were collected for exposure via cardiac puncture on day 15. A separate PK arm was run with three mice per dose group with a total of 15 mice.

[00247] *Exposure-response (E-R) Analysis for FP2 in DIO Mouse*

[00248] Most animals in the pharmacodynamics (PD) (efficacy) arms had undetectable drug concentrations on the last study day when the pharmacokinetics (PK) samples were obtained, potentially due to immunogenicity. Therefore, the mean PK profiles from the PK arms, instead of individual PK from the PD arms, were used to conduct exposure-response (from day 3, 6 and 9, respectively) for the %weight change from baseline in the PD arms at the corresponding dose level. This method assumes that the PK arms behave similarly to the PD arms in terms of drug exposure.

[00249] The E_{max} model (GraphPad Prism 6, log(agonist) vs. response) was used to correlate exposure with response data (log transformed drug concentrations). Hill Slope was set to be 1. Note that the model fitted EC₁₀ to EC₅₀ values were within two fold amongst day 3, 6 and 9, despite that the E_{max} estimates were different (E_{max} = -4.26%, -8.18% and -9.85%, respectively). Some animals on day 9 also showed the loss of drug exposure, due to potential ADA formation and therefore, the E-R parameter estimates based on day 9 data should be interpreted with caution.

[00250] The effects of two weeks of exposure of FP2 on food intake, body weight, glucose homeostasis, and liver fat content was assessed in diet induced obese male C57Bl/6

mice. Trough exposure between 1.7 and 3.3 nM FP2 for the 0.3 nmol/kg treatment group, between 7.1 and 14 nM for the 1.0 nmol/kg treatment group, between 20.8 and 41.6 nM for the 3.0 nmol/kg treatment group, and between 28.5 and 112.9 nM FP2 for the 10 nmol/kg treatment group was maintained until day 9 in the PK arm of the study (n = 2 or 3, Table 49).

5 After day 9, a decrease in circulating levels was observed in the majority of animals despite continued q3d dosing (Table 49). Consistent with this accelerated clearance, the majority of animals in the PD arm of the study had undetectable circulating levels of FP2 on day 15 (Table 50).

[00251] Treatment of DIO mice with FP2 q3d reduced food intake (Table 38), body
10 weight (Table 39, 40 and Figure 20) and fed blood glucose compared to vehicle treatment (Table 43 and Figure 23). A significant reduction in food intake was seen on day 2, day 5, and day 8 for 0.3 nmol/kg, from day 1 through day 7 for 1.0 nmol/kg, on day 1, day 2, day 4 through day 6, and day 8 for 3.0 nmol/kg, and on day 1, day 3 through day 6, day 8 and day 9 for 10.0 nmol/kg. Percent body weight changes were significant from day 5 through day 13
15 for 0.3 nmol/kg, from day 3 through day 13 for 1.0 nmol/kg and 10.0 nmol/kg, and from day 4 through day 13 for 3.0 nmol/kg. Changes in grams of body weight were significant from day 8 for 0.3 nmol/kg, from day 6 for 1.0 nmol/kg, from day 7 for 3.0 nmol/kg and from day 5 for 10.0 nmol/kg. Decreases in fed blood glucose levels were significant on day 7 for the animals in the 3.0 nmol/kg dose level and were significant on day 13 for the animals in the
20 3.0 and 10.0 nmol/kg dose levels.

[00252] DIO mice treated with FP2 q3d had improved glucose tolerance on day 14 compared to vehicle treatment during an oral glucose challenge (Table 41; Figure 21A and 21B). Glucose was significantly lower at 30 minutes for the 0.3 nmol/kg group, at 60 minutes and 120 minutes for the 1.0 nmol/kg group, at 120 minutes for the 3.0 nmol/kg group, and at

30, 90, and 120 minutes for the 10.0 nmol/kg group. Total area under the curve was significant for all dose groups. Insulin levels during the glucose challenge were significantly lower for 0.3 and 10.0 nmol/kg groups at 30 minutes (Table 42; Figures 22A and 22B). In addition, compared to vehicle treated animals, there was a significant reduction in the calculated fasted HOMA-IR in DIO mice after 14 days of treatment with FP2 q3d at 10.0 nmol/kg indicative of improved insulin sensitivity (Table 44 and Figure 24).

[00253] Body composition was measured by MRI on day -1 before the start of the study and on day 13 (Table 47 and Table 48). DIO mice treated with FP2 at 1.0 nmol/kg and 10.0 nmol/kg had significant reductions in fat mass on day 13; whereas there were no changes in lean mass for any treatment groups. On day 13, the 10.0 nmol/kg treatment group had a significant increase in percent lean mass and a significant reduction in percent fat mass compared to the vehicle treated group. Changes from day -1 to day 13 were significant for lean mass in the 0.3 nmol/kg, 1.0 nmol/kg, and 10.0 nmol/kg treatment groups and were significant for percent lean mass in the 1.0, 3.0, and 10.0 nmol/kg treatment groups. Changes from day -1 to day 13 were significant for fat mass and percent lean mass in all treatment groups compared to vehicle.

[00254] There was no significant difference in endogenous mouse GDF15 serum levels between vehicle treated animals and mice treated with FP2 q3d for 15 days (Table 46).

[00255] Conclusion: the results suggest that the higher drug exposure is generally associated with greater % weight change from baseline on a population level across the studied dose groups on day 3, 6 and 9.

[00256] Exposure to FP2 over two weeks led to reduced food intake, decreased body weight, decreased blood glucose, improved glucose tolerance and insulin sensitivity in DIO mice. Significant decreases in food intake over multiple days were achieved at 1.0, 3.0, and

10.0 nmol/kg q3d. Body weight was decreased significantly starting three to five days after the initiation of the study. Fed blood glucose on day 13 was significantly decreased after q3d administration of FP2 at 3.0 and 10.0 nmol/kg. Insulin sensitivity represented by significantly decreased fasting HOMA-IR was achieved 14 days after 10.0 nmol/kg FP2 administered q3d.

- 5 On day 13, a significant increase in percent lean mass and a significant reduction in percent fat mass was observed in DIO mice treated q3d with 10.0 nmol/kg FP2.

Table 38: Effect of FP2 on daily food intake (g) over 13 days of treatment.

Treatment	Vehicle	FP2 (nmol/kg)				Rosiglitazone
Day	N/A	0.3	1.0	3.0	10.0	10mpk/day
0	2.0±0.1	1.9±0.1	2.2±0.2	1.9±0.2	1.9±0.2	2.2±0.1
1	2.4±0.2	2.1±0.1	1.6±0.1*	1.5±0.1*	1.3±0.1*	2.7±0.1
2	2.5±0.1	1.8±0.1*	1.7±0.2*	1.9±0.1*	2.0±0.1	3.0±0.2
3	2.5±0.1	2.1±0.1	1.7±0.1*	1.9±0.1	1.8±0.1*	2.8±0.2
4	2.6±0.1	2.0±0.1	1.8±0.1*	1.9±0.1*	1.9±0.1*	2.9±0.2
5	2.8±0.1	2.1±0.2*	2.2±0.1*	2.2±0.0*	1.9±0.1*	2.7±0.2
6	2.8±0.1	2.3±0.2	2.1±0.1*	2.2±0.1*	2.0±0.1*	2.8±0.2
7	2.6±0.2	2.3±0.1	2.0±0.1*	2.0±0.2	2.1±0.1	2.9±0.2
8	2.7±0.2	2.1±0.1*	2.2±0.1	2.0±0.1*	2.0±0.1*	3.1±0.2
9	2.8±0.1	2.3±0.1	2.3±0.2	2.3±0.2^	2.1±0.2*	3.2±0.1
10	2.6±0.1	2.5±0.1	2.4±0.2	2.3±0.2^	2.1±0.2	3.0±0.2
11	2.9±0.1	2.5±0.1	2.8±0.2	2.6±0.4	2.6±0.1	3.3±0.1
12	2.8±0.1	2.7±0.2	2.8±0.1	2.8±0.2	2.9±0.1	3.1±0.2
13	2.7±0.1	2.5±0.2	2.6±0.1	2.6±0.1	2.2±0.1	3.1±0.2

Values represent mean±SEM for data from 8 animals per time per group, except n=7 when noted by ^

- 10 *- p<0.05, versus vehicle

Statistical analyses used: Two-Way ANOVA RM, Tukey's multiple comparison test

Table 39: Effect of FP2 on Percent Body Weight Change Over 13 days of Treatment

Treatment Day	Vehicle N/A	FP2 (nmol/kg)				Rosiglitazone 10mpk/day
		0.3	1.0	3.0	10.0	
-1	-0.1±0.4	0.5±0.2	-0.1±0.1	-0.4±0.2	0.0±0.6	0.1±0.2
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
1	-0.4±0.5	-1.0±0.3	-2.2±0.4	-1.8±0.6	-2.4±0.5	1.3±0.4
2	-0.4±0.3	-2.2±0.4	-3.4±0.6	-3.0±0.6	-3.1±0.4	1.3±0.5
3	-0.3±0.4	-2.3±0.4	-4.2±0.6*	-3.5±0.6	-4.3±0.5*	1.9±0.5
4	-0.3±0.5	-2.9±0.5	-5.3±0.6*	-4.9±0.7*	-5.8±0.7*	1.8±0.7
5	-0.2±0.4	-4.2±0.7*	-6.2±0.6*	-5.6±0.7*	-6.8±0.7*	1.7±0.8
6	-0.4±0.6	-5.1±0.7*	-7.4±0.8*	-6.9±0.7*	-8.5±0.9*	1.1±0.9
7	0.2±0.8	-5.1±0.7*	-7.7±0.9*	-7.4±0.7*	-8.7±0.9*	2.0±1.0
8	0.2±1.0	-6.1±0.8*	-7.9±0.9*	-8.0±0.8*	-9.7±0.8*	2.4±1.1
9	0.5±1.1	-6.0±0.9*	-8.4±0.9*	-8.8±0.9*	-10.1±1.0*	3.1±1.0
10	1.1±1.2	-5.7±0.8*	-8.1±0.9*	-8.9±0.9*	-10.7±1.2*	3.5±1.2
11	1.2±1.3	-6.1±0.9*	-8.2±0.8*	-8.6±1.3*	-11.1±1.4*	3.7±1.2
12	1.4±1.3	-6.3±1.2*	-7.7±0.8*	-8.2±1.4*	-10.9±1.4*	4.1±1.3
13	1.7±0.9	-5.8±1.4*	-7.1±1.0*	-7.2±1.4*	-10.9±1.4*	4.7±1.3

Values represent mean±SEM for data from 8 animals per time per group

*p<0.05, versus Vehicle

5 Statistical analyses used: Two-Way ANOVA RM, Tukey's multiple comparison test

Table 40: Effect of FP2 on body weight change (g) over 13 days of treatment

Treatment	Vehicle	FP2 (nmol/kg)				Rosiglitazone
Day	N/A	0.3	1.0	3.0	10.0	10mpk/day
-1	44.6±0.6	44.5±0.6	44.5±0.6	44.5±0.6	44.5±0.6	44.5±0.6
0	44.6±0.6	44.3±0.6	44.6±0.6	44.7±0.6	44.6±0.7	44.4±0.6
1	44.5±0.7	43.8±0.6	43.6±0.6	43.9±0.7	43.5±0.7	45.0±0.7
2	44.5±0.7	43.3±0.7	43.1±0.6	43.3±0.7	43.2±0.7	45.0±0.7
3	44.5±0.7	43.2±0.7	42.7±0.6	43.1±0.7	42.6±0.6	45.3±0.7
4	44.5±0.7	43.0±0.7	42.2±0.6	42.5±0.7	42.0±0.7	45.2±0.7
5	44.5±0.7	42.4±0.7	41.8±0.5	42.2±0.7	41.5±0.6*	45.2±0.7
6	44.5±0.7	42.0±0.7	41.3±0.6*	41.6±0.7	40.7±0.5*	44.9±0.8
7	44.7±0.7	42.0±0.7	41.1±0.6*	41.4±0.7*	40.6±0.5*	45.3±0.8
8	44.7±0.7	41.6±0.7*	41.1±0.7*	41.1±0.7*	40.2±0.6*	45.5±0.8
9	44.8±0.7	41.6±0.8*	40.8±0.7*	40.8±0.8*	40.0±0.8*	45.8±0.7
10	45.1±0.7	41.8±0.8*	41.0±0.8*	40.8±0.8*	39.8±0.8*	46.0±0.8
11	45.2±0.8	41.6±0.8*	40.9±0.7*	40.8±0.9*	39.6±0.9*	46.1±0.8
12	45.3±0.7	41.5±0.9*	41.2±0.7*	41.0±0.9*	39.7±0.9*	46.3±0.9
13	45.4±0.7	41.7±0.9*	41.4±0.8*	41.5±0.9*	39.7±0.9*	46.5±0.9

Values represent mean±SEM for data from 8 animals per time per group

*- p<0.05, versus Vehicle

Statistical analyses: Two-Way ANOVA RM, Tukey's multiple comparison test

Table 41: Effect of FP2 on blood glucose (mg/dL) levels during an OGTT after 14 days of treatment

Treatment	Dose (nmol/ kg)	Time after Glucose Challenge (min)					Total AUC (mg/dL/ 120min)	Δ AUC (mg/dL/ 120min)
		0	30	60	90	120		
Vehicle	NA	161 $\pm$ 10	225 $\pm$ 17	228 $\pm$ 14	208 $\pm$ 18	213 $\pm$ 19	25429 $\pm$ 1228	6094 $\pm$ 1430
FP2	0.3	144 $\pm$ 4	163 $\pm$ 14*	209 $\pm$ 11	180 $\pm$ 12	171 $\pm$ 7	21270 $\pm$ 399*	3985 $\pm$ 521
	1.0	151 $\pm$ 9	179 $\pm$ 18	188 $\pm$ 7	179 $\pm$ 13	163 $\pm$ 8*	21096 $\pm$ 754*	2972 $\pm$ 907
	3.0	149 $\pm$ 8	180 $\pm$ 18	179 $\pm$ 11*	164 $\pm$ 6	163 $\pm$ 9*	20338 $\pm$ 876*	2426 $\pm$ 1058
	10.0	134 $\pm$ 6	163 $\pm$ 7*	190 $\pm$ 13	152 $\pm$ 7*	163 $\pm$ 8*	19599 $\pm$ 614*	3539 $\pm$ 1198
Rosiglitazone	10mpk/ day	132 $\pm$ 9	152 $\pm$ 10*	162 $\pm$ 13*	138 $\pm$ 9*	159 $\pm$ 9*	17904 $\pm$ 632*	2139 $\pm$ 1179

Values represent mean $\pm$ SEM for data from 8 animals per time per group

*- p<0.05, versus Vehicle

5 Statistical analyses: Two-Way ANOVA RM, Tukey's multiple comparison test for Glucose Values;

One-Way ANOVA, Tukey's multiple comparison test for AUC

Table 42: Effect of FP2 on insulin (ng/mL) levels during an OGTT after 14 days of treatment

Treatment	Dose (nmol/kg)	Time after Glucose Challenge (min)			Total AUC (ng/mL/90min)
		0	30	90	
Vehicle	NA	4.2±0.7	12.2±2.3	3.6±0.5	716.1±122.0
FP2	0.3	2.9±0.6	5.8±1.8*	2.4±0.4	377.1±97.3
	1.0	2.9±0.4	8.2±2.6	2.6±0.4	491.8±118.2
	3.0	3.0±0.3	8.4±1.5	2.5±0.3	498.6±77.0
	10.0	2.4±0.4	4.3±0.5*	2.1±0.4	295.5±32.0*
Rosiglitazone	10mpk/day	0.9±0.1	1.8±0.2*	0.8±0.1	118.5±9.6*

Values represent mean±SEM for data from 8 animals per time per group

*- p<0.05, versus Vehicle

Statistical analyses: Two-Way ANOVA RM, Tukey's multiple comparison test for insulin

5 Values;

One-Way ANOVA, Tukey's multiple comparison test for AUC

Table 43: Effect of FP2 on fed blood glucose (mg/dL) levels

Treatment	Dose (nmol/kg)	Time after start of treatment (days)		
		0	7	13
Vehicle	NA	162±5	159±6	180±9
FP2	0.3	143±5	150±9	168±9
	1.0	153±12	135±7	159±7
	3.0	148±3	127±6*	151±8*

	10.0	146±6	136±4	148±3*
Rosiglitazone	10mpk/day	137±7	117±4*	134±6*

Values represent mean±SEM for data from 8 animals per time per group

*- p<0.05, versus Vehicle

Statistical analyses: Two-Way ANOVA RM, Tukey's multiple comparison test

5 **Table 44:** Fasted HOMA-IR in DIO mice after 14 days of q3d treatment with FP2

Treatment	Dose (nmol/kg)	HOMA-IR
Vehicle	NA	48.7±9.2
FP2	0.1	30.1±6.6
	1.0	30.5±3.8
	3.0	31.3±3.2
	10.0	22.7±3.1*
Rosiglitazone	10mpk/day	8.7±1.0*

Values represent mean±SEM for data from 8 animals per time per group

*- p<0.05, versus Vehicle

Statistical analyses: One-Way ANOVA, Tukey's multiple comparison test

10 **Table 45:** Liver weight after 15 days of treatment with FP2 q3d in DIO mice

Treatment	Dose (nmol/kg)	Liver Weight, (g)	Liver Weight (% of body)
Vehicle	NA	1.9±0.1	4.3±0.3
FP2	0.1	1.7±0.1	4.0±0.1
	1.0	1.7±0.0	4.2±0.1
	3.0	1.8±0.1	4.2±0.1

	10.0	1.7±0.1	4.2±0.1
Rosiglitazone	10mpk/day	1.9±0.1	4.1±0.2

Values represent mean±SEM for data from 8 animals per time per group

*- p<0.05, versus Vehicle

Statistical analyses: One-Way ANOVA, Tukey's multiple comparison test

- 5 **Table 46:** Serum mouse GDF15 (pg/mL) levels after 15 days of treatment with FP2 q3d in DIO mice

Treatment	Dose (nmol/kg)	mGDF15
Vehicle	NA	258.3±21.4
FP2	0.1	214.1±10.3
	1.0	191.6±12.7
	3.0	254.5±28.6
	10.0	202.0±10.0
Rosiglitazone	10mpk/day	509.6±26.2*

Values represent mean±SEM for data from 8 animals per time per group

*- p<0.05, versus Vehicle

Statistical analyses: One-Way ANOVA, Tukey's multiple comparison test

10

Table 47: Effect of JNJ-64739090 q3d in DIO mice on body composition (g) measured by MRI

Treat-ment	Dose (nmol/kg)	Fat (g) Day -1	Fat (g) Day 13	Δ Fat (g)	Lean (g) Day -1	Lean (g) Day 13	Δ Lean (g)
Vehicle	NA	17.4±0.6	18.2±0.6	0.9±0.3	25.4±0.3	25.1±0.3	-0.4±0.2
FP2	0.3	17.0±0.6	15.2±0.8	-1.9±0.6*	25.8±0.7	24.5±0.5	-1.2±0.2*

	1.0	17.0±0.6	14.9±0.6*	-2.1±0.4*	25.8±0.5	24.6±0.4	-1.2±0.2*
	3.0	17.4±0.7	15.1±1.1	-2.3±0.5*	25.3±0.4	24.2±0.4	-1.1±0.1
	10.0	16.7±0.6	13.2±0.8*	-3.6±0.5*	26.1±0.6	24.5±0.6	-1.7±0.2*
Rosi-glitazone	10mpk/ day	17.2±0.6	19.1±0.5	1.9±0.6	25.5±0.7	25.1±0.6	-0.4±0.2

Values represent mean±SEM for data from 8 animals per time per group

*p<0.05, versus Vehicle

Statistical analyses: One-Way ANOVA, Tukey's multiple comparison test

5 **Table 48:** Effect of FP2 q3d in DIO mice on body composition (%) measured by MRI

Treat-ment	Dose (nmol/kg)	Fat (%) Day -1	Fat (%) Day 13	Δ Fat (%)	Lean (%) Day -1	Lean (%) Day 13	Δ Lean (%)
Vehicle	NA	38.1±1.0	40.1±1.0	2.1±0.3	55.9±0.9	55.3±0.9	-0.6±0.3
FP2	0.3	37.6±1.2	36.3±1.3	-1.3±0.9*	56.8±1.3	59.0±1.4	2.2±0.8
	1.0	37.2±1.1	35.8±0.9	-1.4±0.5*	56.7±1.1	59.5±1.0	2.8±0.6*
	3.0	38.3±1.4	36.1±2.0	-2.3±0.7*	55.9±1.2	58.7±2.0	2.8±0.8*
	10.0	36.9±1.1	33.1±1.5*	-3.8±0.8*	57.5±1.0	61.7±1.4*	4.2±0.7*
Rosi-glitazone	10mpk/ day	38.0±1.2	41.1±0.9	3.1±0.9	56.2±1.2	53.9±0.8	-2.3±0.8

Values represent mean±SEM for data from 8 animals per time per group

*p<0.05, versus Vehicle

Statistical analyses: One-Way ANOVA, Tukey's multiple comparison test

Table 49: FP2 serum exposures (nM) of the PK arm during q3d treatment in DIO mice

Treatment Group	Subject ID	Time Post Initial Dose (Time post last dose)						
		Day 3	Day 6	Day 9	Day 12	Day 14 (48hr)	Day 15 (72hr)	Day 17 (120hr)
0.1 nmol/ kg	9	1.930	2.993	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	10	1.853	3.369	2.817	< LOQ	< LOQ	< LOQ	< LOQ
	11	1.715	2.637	2.568	2.709	3.441	1.313	< LOQ
1.0 nmol/ kg	9	8.198	9.660	9.645	< LOQ	< LOQ	< LOQ	< LOQ
	10	7.073	10.595	8.966	< LOQ	< LOQ	< LOQ	< LOQ
	11	6.689	13.967	10.802	< LOQ	< LOQ	< LOQ	< LOQ
3.0 nmol/ kg	9	20.802	26.329	27.863	< LOQ	< LOQ	< LOQ	< LOQ
	10	23.563	32.020	41.576	1.168	< LOQ	< LOQ	< LOQ
	11	21.704	30.101	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
10.0 nmol/ kg	9	28.495	33.050	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	10	70.779	112.898	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	11	70.404	111.767	55.117	< LOQ	< LOQ	< LOQ	< LOQ

Data are expressed as concentration for each animal.

< LOQ = below limit of quantitation; LOQ is 0.494 nM

** Values at Day 3, 6, 9 and 12 are immediately prior to next dose

Table 50: Terminal serum exposures (nM) of FP2 after 15 days of q3d treatment in DIO mice

Animal ID	Treatment Group (nmol/kg)			
	0.1	1.0	3.0	10.0
1	2.567	< LOQ	< LOQ	< LOQ
2	< LOQ	< LOQ	< LOQ	44.149
3	< LOQ	< LOQ	< LOQ	< LOQ
4	1.144	0.678	< LOQ	< LOQ
5	< LOQ	< LOQ	< LOQ	< LOQ
6	3.440	< LOQ	< LOQ	< LOQ
7	2.727	< LOQ	< LOQ	< LOQ
8	< LOQ	< LOQ	< LOQ	< LOQ

Data are expressed as concentration for each animal.

< LOQ = below limit of quantitation; LOQ is 0.494 nM

5

[00257] **Example 18: FP2 Multispecies Pharmacokinetics and Immune Response**

[00258] Mouse pharmacokinetics

[00259] The pharmacokinetic properties of FP2 were evaluated when administered subcutaneously to female C57Bl/6 mice. FP2 was administered subcutaneously (n = 5 samples per time point) and intravenously (n = 5 samples per time point) to female C57Bl/6 mice (Sage Laboratories, St Louis, MO) at a dose level of 2.0 mg/kg in PBS, (pH 7.3-7.5). The collection of sample at the last time point was via a terminal bleed. Blood samples were collected, serum processed and drug concentrations were measured up to 168 hours. The levels of FP2 were measured using an immunoassay method. The drug concentration profiles in plasma are summarized in Table 51 and 52 and illustrated in Figure 25.

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[00260] Pharmacokinetic analysis of FP2 in C57Bl/6 mice demonstrated a terminal half-life of ~1.51 and ~1.76 days following IV and SC dosing respectively, with a mean bioavailability of ~61% following SC administration.

Table 51: Serum concentration (ng/mL) of FP2 following a single subcutaneous (SC) dose in

5 C57Bl/6 mice

	FP2 - SC Dose						
Timepoint	Animal 31 Result (ng/mL)	Animal 32 Result (ng/mL)	Animal 33 Result (ng/mL)	Animal 35 Result (ng/mL)	Animal 36 Result (ng/mL)	Average Result (ng/mL)	Std Dev (ng/mL)
4 hr	11310.11	6323.44	23489.21	4357.74	4835.54	10063.21	7995.0
24 hr	12378.58	10896.80	17769.03	15928.14	15404.08	14475.33	2785.0
72 hr	5127.95	5569.27	6727.37	7909.66	7550.32	6576.91	1210.5
96 hr	3059.83	3350.25	4042.04	4095.15	4431.94	3795.84	569.0
168 hr	784.61	1202.66	1364.68	1557.60	1678.02	1317.51	348.9

Table 52: Serum concentration (ng/mL) of FP2 following a single intravenous (IV) dose in C57Bl/6 mice.

	FP2 - IV Dose						
Timepoint	Animal 37 Result (ng/mL)	Animal 40 Result (ng/mL)	Animal 41 Result (ng/mL)	Animal 45 Result (ng/mL)	Animal 48 Result (ng/mL)	Average Result (ng/mL)	Std Dev (ng/mL)
1 hr	49573.59	42382.59	40001.18	43085.75	46443.59	44297.34	3742.9
24 hr	22173.85	19120.39	19393.78	19798.29	17459.96	19589.25	1696.7
72 hr	8334.33	7989.10	8573.44	9086.37	8186.68	8433.98	422.5
96 hr	4478.83	4699.80	4749.93	5331.39	4579.08	4767.81	332.3

168 hr	1044.24	1419.64	1393.44	1835.63	979.49	1334.49	343.6
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Table 53: Pharmacokinetic parameters of FP2 following 2 mg/kg IV and 2 mg/kg SC administration in C57Bl/6 mice.

Route		t _{1/2} (day)	CL or CL/F (ml/day/ kg)	V _z or V _z /F (ml/kg)	C _{max} (ng/ml)	T _{max} * (day)	AUC _{0-last} (day* ng/ml)	AUC _{0-inf} (day* ng/ml)
SC	Mean	1.76	43	108	15619	1	44972	48379
	SD	0.16	8	20	4870		8563	9147
IV	Mean	1.51	25	55	44297	0.042	76269	79253
	SD	0.18	1	6	3743		3788	4051

5 [00261] Rat Pharmacokinetics

[00262] FP2 was administered subcutaneously (n = 5 samples per time point) and intravenously (n = 5 samples per time point) to female Sprague-Dawley rats (Sage Laboratories, St. Louis, MO) at a dose level of 2.0 mg/kg in PBS, (pH 7.3-7.5). The collection of sample at the last time point was via a terminal bleed. Blood samples were collected, serum processed and drug concentrations were measured up to 168 hours. The levels of FP2 were measured using an immunoassay method. The drug concentration profiles in plasma are summarized in Table 54 and 55, and illustrated in Figure 26. Pharmacokinetic parameters calculated from these data are summarized in Table 56.

[00263] Pharmacokinetic analysis of FP2 in Sprague Dawley rats demonstrated a terminal half-life of ~1.46 and ~1.37 days following IV and SC dosing respectively, with a mean bioavailability of ~28% following SC administration.

Table 54: Serum concentration (ng/mL) of FP2 following a single subcutaneous (SC) dose in Sprague-Dawley rats.

Timepoint	FP2 - SC Dose						
	Animal 01 Result (ng/ml)	Animal 02 Result (ng/ml)	Animal 03 Result (ng/ml)	Animal 04 Result (ng/ml)	Animal 05 Result (ng/ml)	Average Result (ng/ml)	Std Dev (ng/ml)
4 hr	730.95	257.94	469.95	496.34	400.65	471.16	172.2
24 hr	9236.27	6658.79	7728.92	7449.56	6502.60	7515.23	1092.1
72 hr	4559.04	4464.72	5619.82	4287.25	3844.15	4555.00	655.6
96 hr	2791.97	2452.35	3387.55	2316.06	2176.00	2624.79	483.8
168 hr	622.32	606.36	< 80.00	514.19	476.49	554.84	70.7

Table 55: Serum concentration (ng/mL) of FP2 following a single intravenous (IV) dose in Sprague-Dawley rats.

Timepoint	FP2 - IV Dose						
	Animal 06 Result (ng/ml)	Animal 07 Result (ng/ml)	Animal 08 Result (ng/ml)	Animal 09 Result (ng/ml)	Animal 10 Result (ng/ml)	Average Result (ng/ml)	Std Dev (ng/ml)
1 hr	60785.80	47392.80	43046.54	46014.25	44547.65	48357.41	7134.5
24 hr	25470.36	24729.88	21157.29	20497.32	21459.71	22662.91	2267.1
72 hr	8208.46	9262.13	8866.76	8635.93	8843.83	8763.42	384.1
96 hr	4433.18	4833.22	4995.63	4630.60	4604.13	4699.35	218.1
168 hr	1083.14	1469.35	1614.61	1394.17	1053.89	1323.03	245.7

Table 56: Pharmacokinetic parameters of FP2 following 2 mg/kg IV and 2 mg/kg SC administration in Sprague-Dawley Rats.

Route		$t_{1/2}$ (day)	CL or CL/F (ml/day/kg)	V_z or V_z/F (ml/kg)	C_{max} (ng/ml)	T_{max}^* (day)	AUC _{0-last} (day* ng/ml)	AUC _{0-inf} (day* ng/ml)
SC	Mean	1.37	84	165	7515	1	22614	24036
	SD	0.04	10	17	1092		2509	2893
IV	Mean	1.46	23	49	48357	0.042	83271	86089
	SD	0.12	2	6	7135		6081	5820

[00264] Monkey Pharmacokinetics

5 **[00265]** FP2 was administered subcutaneously at 1 mg/kg and intravenously at 1 mg/kg to three male cynomolgus monkeys each in PBS, (pH 7.0-7.6). Blood samples were collected, plasma processed and drug concentrations were measured up to 21 days.

[00266] The pharmacokinetics (PK) of FP2 was characterized following administration of a single dose IV (1.0 mg/kg) and SC (1.0 mg/kg) in cynomolgus monkeys.

10 The plasma drug concentration-time profile after SC administration is summarized in Tables 57 and 58 for immunoassay and LCMS analyses respectively and after IV administration in Tables 59 and 60 for immunoassay and LCMS analyses respectively. The immunoassay data is graphed in Figure 27, and the LCMS data is represented in Figure 28.

[00267] Using results from the immunoassay analysis, the mean NCA-based terminal half-life ($t_{1/2}$) for FP2 was ~7.05 and ~8.51 days following IV and SC dosing, respectively. The mean PK parameters following IV and SC administration are summarized in Table 61. Using results from the immunoassay bioanalysis, the mean non-compartment model estimated terminal half-life ($t_{1/2}$) for FP2 was 7.05 and 8.51 days following IV and SC

dosing, respectively. The mean bioavailability (F%) of FP2 was estimated to be ~98.5% based on AUC_{0-last} and estimated to be ~109.2% based on AUC_{0-inf} in cynomolgus monkeys following SC administration.

Table 57: Plasma concentration (ng/mL) of FP2 measured by immunoassay following a

5 single SC dose in cynomolgus monkeys.

Time (hr)	Immunoassay			SC Ave	Std Dev
	Animal 704	Animal 705	Animal 706		
0	< 80.0	< 80.0	< 80.0	< 80.0	N/A
6	7365.3	6128.5	6056.9	6516.9	735.6
24	19716.8	10903.8	10554.7	13725.1	5191.9
48	18191.7	14353.7	14464.7	15670.0	2184.5
72	17813.9	14207.5	12684.0	14901.8	2634.5
120	13823.9	11445.8	10590.8	11953.5	1675.3
168	12359.6	10103.8	9467.8	10643.8	1519.7
240	9457.9	7642.6	8109.1	8403.2	942.7
336	6796.8	5679.8	5235.7	5904.1	804.3
432	5581.3	3830.6	3746.5	4386.1	1035.9
528	4126.2	2613.4	2879.3	3206.3	807.7

N/A = not applicable

Table 58: Plasma concentration (ng/mL) of FP2 measured by LCMS following a single SC dose in cynomolgus monkeys.

Time (hr)	LC/MS			SC Ave	SEM
	Animal 704	Animal 705	Animal 706		
0	< 1000	< 1000	< 1000	< 1000	N/A
6	6110.0	5910	6200.0	6073.3	85.7
24	#	10420.0	11300.0	10860.0	359.3
48	16560.0	13510.0	14960.0	15010.0	880.8
72	13690.0	13450.0	13380.0	13506.7	93.9
120	11040.0	#	#	11040.0	N/A
168	#	8680.0	9400.0	9040.0	293.9
240	6940.0	7070.0	7140.0	7050.0	58.6
336	4400.0	4580.0	4430.0	4470.0	55.7
432	-	2910.0	-	2910.0	N/A
528	< 1000	< 1000	< 1000	< 1000	N/A

- = initial run failed, not enough sample for repeat analysis

= mislabeled tube, sample excluded from the analysis

5 N/A = not applicable

Table 59: Plasma concentration (ng/mL) of FP2 measured by immunoassay following a single IV dose in cynomolgus monkeys.

Time (hr)	Immunoassay			IV Ave	Std Dev
	Animal 701	Animal 702	Animal 703		
0	< 80.0	< 80.0	< 80.0	< 80.0	N/A
1	29938.3	31139.0	23545.3	28207.6	4082.0
6	24711.9	21173.0	20434.4	22106.4	2286.5
24	19648.4	21420.9	8662.7	16577.3	6911.3
48	17790.1	17107.0	12983.1	15960.0	2600.7
72	17889.4	13871.1	13692.4	15151.0	2373.3
120	15298.6	11982.5	11361.5	12880.9	2116.7
168	11752.0	10982.8	9933.8	10889.5	912.7
240	8921.1	7465.0	6733.4	7706.5	1113.6
336	6572.8	5750.5	4687.6	5670.3	945.1
432	1099.6	3425.0	3458.0	2660.9	1352.2
528	< 80.0	2069.0	2416.2	2242.6	N/A

N/A = not applicable

5 **Table 60:** Plasma concentration (ng/mL) of FP2 measured by LCMS following a single IV dose in cynomolgus monkeys

Time (hr)	LC/MS			IV Ave	SEM
	Animal 701	Animal 702	Animal 703		
0	< 1000	< 1000	< 1000	< 1000	N/A
1	25340.0	28820	26220.0	26793.3	1044.7

6	24610.0	26340.0	23810.0	24920.0	746.6
24	18410.0	18680.0	9810.0	15633.3	2912.7
48	16290.0	17370.0	13840.0	15833.3	1044.3
72	15430.0	14920.0	14280.0	14876.7	332.7
120	11180.0	11480.0	11440.0	11366.7	94.0
168	9170.0	#	#	9170.0	N/A
240	6800.0	7380.0	7600.0	7260.0	238.6
336	2870.0	3860.0	3480.0	3403.3	288.3
432	-	-	3030.0	3030.0	N/A
528	1150.0	1680.0	1400.0	1410.0	153.1

- = initial run failed, not enough sample for repeat analysis

N/A = not applicable

= mislabeled tube, sample excluded from the analysis

- 5 **Table 61:** Mean ($\pm$ SD) pharmacokinetic parameters of FP2 following 1 mg/kg IV and SC administration in cynomolgus monkey.

Route		t _{1/2} (day)	CL or CL/F (ml/day/kg)	V _z or V _z /F (ml/kg)	C _{max} (ng/ml)	T _{max} * (day)	AUC _{0-last} (day*ng/ml)	AUC _{0-inf} (day*ng/ml)
SC	Mean	8.51	4.6	56	16178	1.67	180792	221032
	SD	1.28	0.8	7	3065		29990	44931
IV	Mean	7.05	4.9	51	28208	0.042	183468	202380
	SD	1.45	0.2	12	4082		18268	9617

PK parameters are mean values based on NCA of immunoassay PK data.

*T_{max} (median)

[00268] Human Plasma Stability Assay

[00269] The *ex vivo* stability of FP2 was examined in fresh heparinized plasma at 37°C for up to 48 hours. Fresh, non-frozen human plasma was generated from heparinized blood from two subjects (one male and one female) by centrifugation. FP2 was incubated in this matrix at 37°C with gentle mixing at 0, 4, 24 and 48 hours. The concentration of FP2 was determined using an immunoassay method. An independent immunoaffinity capture followed by LCMS method was used to quantitate the concentration of the intact dimer present in this matrix under the assay conditions.

[00270] In the immunoassay method, the percent recovery from the starting concentration ranged from 104.8 to 94.1 and did not decrease over time, demonstrating that FP2 is stable in human plasma up to 48 hours *ex vivo* (Figure 29 and Table 62). The LCMS method showed that concentrations were stable over time demonstrating that JNJ-64739090 remains an intact dimer in human plasma up to 48 hours *ex vivo* (Figure 30 and Table 63).

Table 62: Ex vivo stability of FP2 (Normalized Percent Recovery) over 48 hours in human plasma (ng/ml) measured by immunoassay.

Compound	Gender	Time (hr)	Concentration (ng/mL)	Normalized % Recovery
FP2	female	0	9104	100.0
		4	9056	99.5
		24	9332	102.5
		48	9374	103.0
	male	0	9473	100.0
		4	9929	104.8
		24	9081	95.9
		48	8912	94.1

Table 63: Ex vivo stability of FP2 (Normalized Percent Recovery) over 48 hours in human plasma (ng/ml) measured by intact LC/MS.

Compound	Gender	Time (hr)	Concentration (ng/mL)	Normalized % Recovery
FP2	female	0	11090	100.0
		4	10830	97.7
		24	10500	94.7
		48	10030	90.4
	male	0	10760	100.0
		4	9640	89.6
		24	10190	94.7
		48	8500	79.0

5 [00271] **Example 19: Efficacy of FP1 and FP2 in cynomolgus monkeys**

[00272] The effects of FP1 and FP2 on food intake and body weight after a single dose in naïve cynomolgus monkeys were evaluated.

[00273] FP1 was administered subcutaneously to a cohort of naïve cynomolgus monkeys at three dose levels; 1, 3 and 10 nmol/kg. A vehicle treated group was also
10 included. The animals were treated in a blinded manner. The study lasted a total of 6 weeks: 2 weeks of baseline food intake measurement and data collection, 4 weeks of data collection after single administration of compound. Plasma drug exposures were measured on days 1, 7, 14, 21, and 28 following dosing.

[00274] Treatment of cynomolgus monkeys with a single dose of FP1 reduced food
15 intake and body weight compared to vehicle treatment (Figures 32-33). A significant reduction in daily food intake was seen on days 4, 5, 6, and 8 through 12 for the 10 nmol/kg dose level (Figure 32). The weekly average of daily food intake was significantly reduced for during week 2 post administration for the 10 nmol/kg dose level. The 3 nmol/kg dose level

had a significant percent reduction from the average weekly food intake prior to dosing on week 2 post administration and the 10 nmol/kg dose level had a significant percent reduction from the average weekly food intake prior to dosing in weeks 1 and 2 post administration. A significant reduction in percent body weight change from day 0 was seen at day 28 for the 3 nmol/kg dose level, and on day 14, 21, and 28 for the 10 nmol/kg dose level (Figure 33).

[00275] FP2 was administered subcutaneously to a cohort of naïve cynomolgus monkeys at three dose levels; 1, 3 and 10 nmol/kg. A vehicle treated group was also included. The animals were treated in a blinded manner. The study lasted a total of 11 weeks: 5 weeks of baseline food intake measurement and data collection, 1 week of treatment and 5 weeks of wash-out phase data collection. Plasma drug exposures were measured on days 1, 7, 14, 21, 28, 35, and 42 following dosing.

[00276] Treatment of cynomolgus monkeys with a single dose of FP2 reduced food intake and body weight compared to vehicle treatment (Figures 34-35). A significant reduction in daily food intake was seen on days 3, 5 through 8, 10 and 12 for the 3 nmol/kg dose level and from days 3 through 38 and day 40 for the 10 nmol/kg dose level (Figure 34). The weekly average of daily food intake was significantly reduced for week 1 post administration for the 3 nmol/kg dose level and significantly reduced for weeks 1 through 6 for the 10 nmol/kg dose level. The 3 nmol/kg dose level had a significant percent reduction from the week prior to dosing in weekly average daily food intake on week 2 post administration and the 10 nmol/kg dose level had a significant percent reduction from the week prior to dosing in weekly average daily food intake on weeks 1 through 6 post administration. A significant reduction in percent body weight change from day 0 was seen from days 21 through 42 for the 1 nmol/kg dose level, from days 14 through 42 for the 3 nmol/kg dose level and from days 7 through 42 for the 10 nmol/kg dose level (Figure 34).

[00277] **Example 20: HSA-GDF15:GDF15 heterodimer**

[00278] Bioactivity of HSA-GDF15:GDF15 heterodimer was investigated.

[00279] To generate an HSA-GDF15:GDF15 heterodimer two constructs were designed. The first construct contained HSA fused to the N-terminus of mature GDF15 (AA 203-308) via a glycine-serine linker (SEQ ID NO: 93). The second construct contained a 6x histidine tagged HSA fused to the N-terminus of mature GDF15 (AA 197-308) via a glycine-serine linker and an HRV3C protease cleavage site (SEQ ID NO: 94). The plasmids were co-transfected at a 1:1 ratio using the Expi293™ Expression System (Thermo Fisher Scientific) according to the manufacturer's protocol. Peptides were secreted as HSA-GDF15 proteins, including both heterodimer and homodimer forms, wherein monomers were linked by disulfide bonds.

[00280] Cell supernatants from transiently transfected Expi293™ cells were harvested 5 days after transfection, clarified by centrifugation and sterile filtered (0.2µ PES membrane, Corning). Clarified supernatants were loaded onto a HisTrap HP column (GE Healthcare) equilibrated with 20 mM sodium phosphate, 500 mM NaCl, pH 7.4. After loading, unbound protein was removed by washing the column with equilibration buffer. HSA-GDF15 proteins, including both heterodimer and homodimer forms, bound to the column and were eluted with 20 mM sodium phosphate, 150 mM imidazole, pH 7.4. Eluate fractions were pooled and incubated overnight at 4 °C in the presence of 6x histidine tagged HRV3C enzyme (Janssen) to generate the HSA-GDF15:GDF15 heterodimer. Following incubation, the protein solution was dialyzed into equilibration buffer to remove imidazole before being applied to a HisTrap HP column one more time. The HSA-GDF15:GDF15 heterodimer eluted in the 20mM sodium phosphate, 50mM imidazole, pH 7.4 wash step, while histidine tagged proteins were retained. The heterodimer was further polished by size exclusion chromatography (SEC)

using a HiLoad 26/60 Superdex 200 pg column (GE Healthcare) equilibrated in 1x DPBS, pH 7.2. Eluate fractions from the SEC with high purity (determined by SDS-PAGE) of the HSA-GDF15:GDF15 heterodimer were pooled and filtered. The protein concentration was determined by absorbance at 280 nm on a BioTek SynergyHTTM spectrophotometer. The quality of the purified protein was assessed by SDS-PAGE and analytical size exclusion HPLC (Ultimate3000 HPLC system). Endotoxin levels were measured using an LAL assay (Pyrotell®-T, Associates of Cape Cod). The purified protein was stored at 4 °C.

[00281] SK-N-AS cells (ATCC) stably expressing GDF15 receptor (GFRAL) were seeded in growth medium (10% FBS) in a 96-well plate 24 hours prior to the assay. After 24 hrs the cells were starved by replacing the culture medium with 200 µl of DMEM medium supplemented with 1% HI horse serum for 3 hours in a 37°C incubator. The 1% HI horse serum supplemented medium was then replaced with 200 µl of AB1 and incubated for an additional 2 hours in a 37°C incubator. To perform the assay, the AB1 was aspirated from all wells and 100 µl of variable concentrations of the testing compound in AB2 was added and the plate was incubated for 15 min in a 37°C incubator. After 15 minutes, the testing solution was removed and 30 µl of lysis buffer (provided in the detection kit) was added and the plate was shaken on a plate shaker at room temperature for 30 min. For detection, 16 µl of the lysed sample was transferred to a 384-well assay plate and 4 µl of HTRF pAKT detection antibodies were added. The plate was incubated overnight at room temperature and then the HTRF signal read on the Envision (Perkin Elmer).

[00282] EC₅₀ values were calculated using GraphPad Prism® Nonlinear Regression (Curve fit). Data are expressed as the Mean ± Standard Error (SE) from three separate experiments with three replicates per data point. Molecular identity of the HSA-GDF15:GDF15 heterodimer was confirmed by mass spectrometry. The left-shift of the

heterodimer curve suggested that the HSA-GDF15:GDF15 heterodimer is more potent in inducing pAKT than the relevant homodimer molecule with an additional albumin.

[00283] Example 21: Linker thermal stability

[00284] Thermal stability was investigated for various linkers that connect HSA and GDF15. To evaluate the potential to fragment and aggregate, HSA-GDF15 fusion proteins with various linkers were diluted to 10mg/ml. After addition of EDTA and Methionine, the samples were incubated under 40°C for 14 days. Then samples were diluted to the concentration of 1mg/ml and evaluated under size-exclusion high-performance liquid chromatography (SE-HPLC). Percent of intact protein as well as aggregate and fragment were quantified for these proteins. Table 64 shows that the HSA-GDF15 proteins with linkers that consist of AP repeats are most stable against fragment under thermal stress.

[00285] To evaluate the whether these linker affects GDF15 interaction with its receptor, an immunoassay with GFRAL-Fc fusion protein coated on plate and anti-GDF15 or anti-HSA detection was performed, using monoclonal antibodies for GDF15 (Janssen) and HSA (Kerafast, Inc., Boston, MA). The assay showed all these linker variants in Table 66 has similar binding to receptor.

Table 64. SE-HPLC results after thermal stress for 14 days.

SEQ ID NO	Linker	aggregate (%)	intact (%)	fragment (%)
113	GS(GGGGS) ₈	3.33	84.44	12.22
115	GA(GGGGA) ₈	3.51	87.98	8.5
117	(AP) ₁₀	1.64	98.36	0
119	(AP) ₁₂	2.36	97.64	0
121	GGs-(EGKSSGSGSESKST) ₃ -GGs	1.67	85.24	13.09
123	GS(PGGGS) ₈	2.96	88.12	8.91
125	GS(AGGGS) ₈	3.44	86.22	10.34
127	GGs-(EGKSSGSGSESKST) ₂ -GGs	1.71	91.17	7.12

[00286] While the invention has been described in detail, and with reference to specific embodiments thereof, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made therein without departing from the spirit and scope of the invention.

5 [00287] The sequences referenced in this application are provided in the table below:

WT – wild type

SEQ ID NO	Description	Sequence
1	Human Serum Albumin, WT (HSA)	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQCPFEDHVKLVNE VTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADC CAKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFL KKYLYEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPK LDELRDEGKASSAKQRLK CASLQKFGERAFAKAWAVARLSQRFPKA EFAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSIS SKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKN YAEAKDVFLGMFLY EYARRHPDYSVVL LRLAKTYETTLEKCCAA ADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQGEYKFQNALLV RYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLS VVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPK EFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKA VMDDFAAFVEKCKCKADDKETCFAEEGKKLVAASQAALGL
2	HSA variant, C34S	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DEL RDEGKASSAKQRLK CASLQKFGERAFAKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVL LRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQGEYKFQNALLV YTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKCKADDKETCFAEEGKKLVAASQAALGL

3	HSA variant, C34A	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQAPFEDHVKLVNE VTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADC CAKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFL KKYLYEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPK LDEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKA EFAEVS KLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSIS SKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKN YAEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAA ADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNALLV RYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLS VVLNQLCVLHEKTPVSDRVT KCCTESLVNRRPCFSALEVDETYVPK EFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKA VMDDFAAFVEKCKADDKETCF AE EGKKLVAASQAALGL
5	HSA (C34S)- GS(GGGGS) ₄ - GDF15 (WT) Fusion	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVS KLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNALLV YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVT KCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCF AE EGKKLVAASQAALGLSGGGG SGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGW ADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVP APCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI
6	Mature GDF15 (197-308)	ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI
7	Truncated mature GDF15 (200-308)	GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGAC PSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI
8	Truncated mature GDF15 (201-308)	DHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI
9	Truncated mature GDF15 (202-308)	HCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI

10	Truncated mature GDF15 (203-308)	CPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTCIGACPSQ FRAANMHAQIKTSLHRLKPDTPAPCCVPASYNPMVLIQKTDGVS LQTYDDLLAKDCHCI
11	Truncated mature GDF15 (211-308)	CRLHTVRASLEDLGWADWVLSPREVQVTCIGACPSQFRAANMH AQIKTSLHRLKPDTPAPCCVPASYNPMVLIQKTDGVS LQTYDDL LAKDCHCI
25	HSA (C34S)- AS(GGGGS) ₂ G T - -GDF15 Fusion	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKL VNEV TEFAKTCVADESAENCDSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNCLEFEQLGEYKFQNAL VR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLASGGGG SGGGGSGGTARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPR EVQVTCIGACPSQFRAANMHAQIKTSLHRLKPDTPAPCCVPASY NPMVLIQKTDGVS LQTYDDLLAKDCHCI
26	HSA(C34S)- AS(GGGGS) ₈ G T - GDF15(WT)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKL VNEV TEFAKTCVADESAENCDSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNCLEFEQLGEYKFQNAL VR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLASGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGTARNGDH CPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTCIGACPSQ FRAANMHAQIKTSLHRLKPDTPAPCCVPASYNPMVLIQKTDGVS LQTYDDLLAKDCHCI

27	HSA (C34S)- AS(AP) ₅ GT- GDF15 Fusion	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNAL LVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVT KCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGLASAPAPA PAPAPGTARNGDHCPLGPGRCRLHTVRASLEDLGWADWVLSPRE VQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPAPCCVPASYN PMVLIQKTDGTGVSLQTYDDLLAKDCHCI
28	HSA (C34S) AS(AP) ₁₀ GT - GDF15 Fusion	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNAL LVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVT KCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGLASAPAPA PAPAPAPAPAPAPGTARNGDHCPLGPGRCRLHTVRASLEDLGW ADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTP APCCVPASYNPMVLIQKTDGTGVSLQTYDDLLAKDCHCI
29	HSA (C34S) – AS(AP) ₂₀ GT- GDF15 Fusion	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNAL LVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVT KCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGLASAPAPA PAPAPAPAPAPAPAPAPAPAPAPAPAPAPAPAPGTARNGDHCPLG PGRCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAA NMHAQIKTSLHRLKPDTPAPCCVPASYNPMVLIQKTDGTGVSLQTY DDLLAKDCHCI

30	HSA (C34S) – AS(EAAAK) ₄ G T – GDF15 Fusion	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNAL LVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLASEAAA KEAAAKEAAAKEAAAKGTARNGDHCPLGPGRCRLHTVRASLEDL GWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDT VPAPCCVPASYNPMVLIQKTD TGVSLOTYDDLLAKDCHCI
31	HSA (C34S)- AS(EAAAK) ₈ G T- GDF15 Fusion	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNAL LVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLASEAAA KEAAAKEAAAKEAAAKEAAAKEAAAKEAAAKEAAAKGTARNGD HCPLGPGRCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPS QFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTD TGVS SLQTYDDLLAKDCHCI
36	HSA (C34S)- GS(GGGGS) ₄ - GDF15 Fusion (deletion 13 mutant)	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNAL LVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSCSRLHTVRASLEDLGWADWVLSPREVQVT MCIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVL IQKTD TGVSLOTYDDLLAKDCHCI

37	HSA (C34S) – GS(GGGGS) ₄ – GDF15 Fusion (deletion 14 mutant)	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNALLV YTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSCRLHTVRASLEDLGWADWVLSPREVQVT MCIGACPSQFRAANMHAQIKTSLHRLKPDTPVAPCCVPASYNPMVL IQKTD TGVS LQTYDDLLAKDCHCI
40	HSA (C34S) – GS(GGGGS) ₄ – GDF15 Fusion	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNALLV YTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSARNGDHCPLGPGRCRLHTVRASLEDLGW ADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPV APCCVPASYNPMVLIQKTD TGVS LQTYDDLLAKDCHCI
48	HSA(C34A)– GS(GGGGS) ₄ – GDF15 Fusion	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQAPFEDHVKLVNE VTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADC CAKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFL KKYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPK LDEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKA EFAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSIS SKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKN YAEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAA ADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNALLV RYTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLS VVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPK EFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKA VMDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGLGSGGG GSGGGGSGGGGSGGGGSARNGDHCPLGPGRCRLHTVRASLEDLG WADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPV PAPCCVPASYNPMVLIQKTD TGVS LQTYDDLLAKDCHCI

55	HSA(C34A)- GS(GGGGS) ₈ - GDF15	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQAPFEDHVKLVNE VTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADC CAKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFL KKYLYEIAARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPK LDELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKA EFAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSIS SKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKN YAEAKDVFLGMFLYEYARRHPDYSV VLLLRLAKTYETTLEKCCAA ADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQLEGEYKFQNALLV RYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLS VVLNQLCVLHEKTPVSDRVT KCCTESLVNRRPCFSALEVDETYVPK EFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKA VMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGG GSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGGSARNGDHC PLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQF RAANMHAQIKTSLHRLKPDTPAPCCVPASYNPMVLIQKTDGTGVS LQTYDDLLAKDCHCI
56	HSA(C34A)- (AP) ₁₀ - GDF15	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQAPFEDHVKLVNE VTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADC CAKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFL KKYLYEIAARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPK LDELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKA EFAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSIS SKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKN YAEAKDVFLGMFLYEYARRHPDYSV VLLLRLAKTYETTLEKCCAA ADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQLEGEYKFQNALLV RYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLS VVLNQLCVLHEKTPVSDRVT KCCTESLVNRRPCFSALEVDETYVPK EFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKA VMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLAPAPAP APAPAPAPAPAPAPARNGDHCPLGPGRCCRLHTVRASLEDLGWAD WVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPAPC CVPASYNPMVLIQKTDGTGVS LQTYDDLLAKDCHCI
59	HSA(C34S)- (AP) ₁₀ - GDF15	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNE TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYLEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLYEYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQLEGEYKFQNALLV YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVT KCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLAPAPAPA PAPAPAPAPAPAPARNGDHCPLGPGRCCRLHTVRASLEDLGWADW VLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPAPCC VPASYNPMVLIQKTDGTGVS LQTYDDLLAKDCHCI

60	HSA (C34S)- GS-(GGGGS) ₈ - GDF15(WT)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFY APELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLKQNCLEFEQLGEYKFQNALVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG LGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFR AANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDGTGVSLO TYDDLAKDCHCI
64	HSA (C34S) – GS(GGGGS) ₄ - GDF15 (I89R)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFY APELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLKQNCLEFEQLGEYKFQNALVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG ADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVP APCCVPASYNPMVLRQKTDGTGVSLOTYDDLAKDCHCI
65	HSA (C34S) – GS(GGGGS) ₄ - GDF15 (I89W)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFY APELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLKQNCLEFEQLGEYKFQNALVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG ADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTVP APCCVPASYNPMVLRQKTDGTGVSLOTYDDLAKDCHCI

66	HSA (C34S) – GS(GGGGS) ₄ – GDF15 (L34A, S35A, R37A)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLKASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLKQNCLEFEQLGEYKFQNALVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEYDLSV VLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGW ADWVAAPAEVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPV APCCVPASYNPMVLIQKTDGTGVSLOTYDDLLAKDCHCI
67	HSA (C34S) – GS(GGGGS) ₄ – GDF15 (V87A, I89A, L98A)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLKASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLKQNCLEFEQLGEYKFQNALVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEYDLSV VLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGW ADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPV APCCVPASYNPMALAQKTDGTGVSLOTYDDLLAKDCHCI
68	HSA (C34S) – GS(GGGGS) ₄ – GDF15 (L34A, S35A, I89A)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLKASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLKQNCLEFEQLGEYKFQNALVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEYDLSV VLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGW ADWVAAPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPV APCCVPASYNPMVLAQKTDGTGVSLOTYDDLLAKDCHCI

69	HSA (C34S) – GS(GGGGS) ₄ – GDF15 (V87A, I89A)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLKCSLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLKQNCLEFQELGEYKFQNALVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGW ADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPV APCCVPASYNPMALAOKTDTGVSLQTYDDLLAKDCHCI
70	HSA (C34S) – GS(GGGGS) ₄ – GDF15 (Q60W)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLKCSLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLKQNCLEFQELGEYKFQNALVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGW ADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPV APCCVPASYNPMVLIQKTDGTGVSLQTYDDLLAKDCHCI
71	HSA (C34S) – GS(GGGGS) ₄ – GDF15 (W32A)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLKCSLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLKQNCLEFQELGEYKFQNALVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGW ADAVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPV PCCVPASYNPMVLIQKTDGTGVSLQTYDDLLAKDCHCI

72	HSA (C34S) – GS(GGGGS) ₄ – GDF15 (W29A)	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNL IKQNC ELFQ LGEYKFQNAL LVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGA ADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPV APCCVPASYNPMVLIQKTD TGVS LQTYDDLLAKDCHCI
73	HSA (C34S) – GS(GGGGS) ₄ – GDF15 (Q60A, S64A, R67A)	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNL IKQNC ELFQ LGEYKFQNAL LVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGA ADWVLSPREVQVTMCIGACPSQFRAANMHAAIKTALHALKPDTPV APCCVPASYNPMVLIQKTD TGVS LQTYDDLLAKDCHCI
74	HSA (C34S) – GS(GGGGS) ₄ – GDF15 (W29A, Q60A, I61A)	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAAACLLPKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNL IKQNC ELFQ LGEYKFQNAL LVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGA ADWVLSPREVQVTMCIGACPSQFRAANMHAAAKTSLHRLKPDTPV APCCVPASYNPMVLIQKTD TGVS LQTYDDLLAKDCHCI

75	HSA (C34S) -- GS(GGGGS) ₄ -- GDF15 (W29A, W32A)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLPLKL DELRDEGKASSAKQRLKASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLLLR LAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVR YTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSARNGDHCPLGPGRCCRLHTVRASLEDLGA ADAVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPVA PCCVPASYNPMVLIQKTD TGVS LQTYDDLLAKDCHCI
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89	nucleic acid encoding SEQ ID NO:59	gatgcacacaagagtgaagggtgctcatcggtttaagattgggagaagaaaattcaaagccttg gtgttgattgcctttgctcagtatcttcagcagtcceccattgaagatcatgtaaaattagtgaaatgaag taactgaatttgcacaaaacatgtgttgctgatgagtcagctgaaaattgtgacaaatcacttcatacc cttttggagacaaaattatgcacagttgcaactcttcgtgaaacctatgggtaaatggctgactgctgt gcaaaacaagaacctgagagaaatgaatgcttctgcaacacaaagatgacaacccaacctccc ccgattggtagagaccagagggtgatgtgatgtgcactgctttcatgacaatgaagagacattttga aaaaatacttatatgaattgccagaagacatccttacttttatgcccggaactccttttttctaa aaggatataaagctgctttacagaatgttgccaagctgctgataaagctgcctgctgttgccaag ctcgtatgaacttcgggatgaagggaaggcttcgtctgccaacagagactcaagtgtgccagctc ccaaaatttggagaagagcttcaagcatgggcagtagctgcctgagccagagatttccca aagctgagttgcagaagtgtccaagtttagtgacagatcttaccaaagtcacacgggaatgctgcca tgagatctgctggaatgtgctgatgacaggcgacctgccaagtatatctgtgaaaatcaagat tcgatctccagtaaaactgaaggaaatgctgtgaaaaacctgtgtgaaaaatcccactgcattgccg aagtggaaaatgatgagatgctgctgactgcttctcattagctgctgattttgtgaaagtaaggat gtttgcaaaaactatgctgaggcaaggatgtcttctgggcatgtttttgtatgaatatgaagaag gcatcctgattactctgctgctgctgctgagactgccaagacatatgaaccactctagagaag tgctgtgcccgtgcagatcctcatgaatgctatgccaagtgttcgatgaattaaacctctgtggga agagcctcagaatttaatacaacaaaattgtgagcttttgagcagcttgagaggtacaattccag aatgctgctattagttcgttacaccaagaagaaagtacccaagtgtcaactccaactctgtagaggctc aagaaacctaggaaggtgggcagcaaatgtgttaacatcctgaagcaaaaagaatgcctgtg cagaagactatctatcgtgtgctgaaccagttatgtgtgtgcatgagaaaacgccagtaagtga cagagtcaccaaattctgcacagaatccttggtgaacaggcgaccatgctttcagctctggaagt cgatgaaacatacgttccaaagagtttaattgctgaaacattcacttccatgcagatatatgcacac tttctgagaaggagagacaaatcaagaacaaactgcactgttgagctcgtgaaacacaagccc aaggcaacaaaagagcaactgaaagctgttatggatgatttcgcagctttttagagaagtgtctgc aaggctgacgataaggagacctgctttgcccaggagggtaaaaaactgtgtgcaagtcaagc tgcttaggcttagcacctgccccgctccagcaccgccccagccccctgctcccgaccagctc ctgcaccagcgcgcaacggggaccactgtccgctcggggcccgggcggtgctgcccgtctgcaca cgggtccgctgctgctggaagacctgggctgggcccgaattgggtgctgtccgacgggagggtgc aagtgacctgtgcatcgcgctgcccagccagttccggcgcgcaaacatgcacgcgcagaga tcaagacgagcctgcacgcctgaagcccacacgggtgccagcgccctgctgctgcccgccca gctacaatcccaggtgctcattcaaaagaccgacacgggggtgctgctccagacctatgatgact gttagccaaagactgccactgcata
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90	nucleic acid encoding SEQ ID NO:60 (Codon optimization 1)	gatgcacacaagagtgaagggtgctcatcggtttaagattgggagaagaaaatttcaaagccttg gtgttgattgcccttgcagtatcttcagcagtccecatitgaagatcatgtaaattagtgaaatgaag taactgaatttgcacaaacatgtgttgcgtgatgagtcagctgaaaattgtgacaaatcacttcatacc cttttggagacaaattatgcacagttgcaactcttcgtgaaacctatgggtgaaatggctgactgctgt gcaaaacaagaacctgagagaaatgaatgcttctgcaacacaaagatgacaaccaaacctccc ccgattgggtgagaccagagggtgatgtgatgtgcactgctttcatgacaatgaagagacattttga aaaaatacttatatgaattgccagaagacatccttactttatgccccggaactccttttttctaa aaggatataaagctgctttacagaatgttgccaagctgctgataaagctgcctgcctgttgccaag ctcgatgaacttcgggatgaagggaaggcttcgtctgccaacagagactcaagtgtgccagctc ccaaaatttggagaagagcttcaagcatgggcagtagctgcctgagccagagatttccca aagctgagttgcagaagtttcaagtttagtgacagatcttaccaaagtcacacgggaatgctgcc tgagatctgctgaatgtgctgatgacaggcgacctgccaagtatatctgtgaaaatcaagat tcgatctccagtaactgaaggaaatgctgtgaaaaacctgtgtgaaaaatcccactgcattgccg aagtggaaaatgatgagatgctgctgactgcttctattagctgctgattttgtgaaagtaaggat gtttgcaaaaactatgctgaggcaaggatgtcttctgggcatgtttttgtatgaatatgaagaag gcatcctgattactctgctgctgctgctgagactgccaagacatatgaaaccactctagagaag tgctgtgcccgtgcagatcctcatgaatgctatgccaagtgttcgatgaattaaacctctgtgga agagcctcagaatttaatacaaaaaattgtgagctttttagcagcttggagagtacaattccag aatgcgctattagttcgttacaccaagaagaatgcccaagtgcaactccaactctgtagaggctc aagaaacctaggaagaagggtggcagcaaatgtgttaacatcctgaagcaaaaagaatgcctgtg cagaagactatctatcgtgtctgaaccagttatgtgtgtgcatgagaaaacgccagtaagtga cagagtcaccaaattctgcacagaatccttggtgaacaggcgaccatgctttcagctctggaagt cgatgaaacatacgttccaaagagtttaattgctgaacattcacttccatgcagatatatgcacac tttctgagaaggagagacaaatcaagaacaaactgcactgttgagctcgtgaaacacaagccc aaggcaacaaaagagcaactgaaagctgttatggatgatttcgcagctttttagagaagtgtctgc aaggctgacgataaggagacctgcttgcaggaggaggtaaaaaactgtgtgctgcaagtcaagc tgcccttaggcttaggcagcggcgggcgggcggcagcggcgggcgggcgatctgttgagggtggca gtggaggagggggatccggcgggcgggcggcagcggcgggcgggcgatctgttgagggtggca gtggaggagggggatccggcgcaacggggaccactgtccgctcggggccggggcgtgtctgc cgtctgcacacgggtccggcgctgctggaagacctgggctgggcccattgggtgctgtgccac gggagggtgcaagtaccatgtgcatcggcgcgtgcccagccagttccggggcggaacatgc acgcgcagatcaagacgagcctgcaccgcctgaagcccacacgggtgccagcgcctgtgc gtgcccgcagctacaatcccatgggtctcattcaaaagaccgacacgggggtgtcgtccagac ctatgatgactgttagcacaagactgccactgcata
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[illegible]

92	HSA (C34S) – GS-(GGGGS) ₈ – GDF15 (deletion 4)	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFY APELLFFAKRYKAAFTTECCQAADKAAACLLPKL DEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNAL LVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVD ETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSDHCPLGPG RCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANM HAQIKTSLHRLKPDTPAPCCVPASYNPMVLIQKTD TGVS LQTYDD LLAKDCHCI
93	HSA (C34S)- GS-(GGGGS) ₈ - GDF15 (deletion 5)	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFY APELLFFAKRYKAAFTTECCQAADKAAACLLPKL DEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNAL LVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVD ETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSHCPLGPG RCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANM HAQIKTSLHRLKPDTPAPCCVPASYNPMVLIQKTD TGVS LQTYDD LLAKDCHCI
94	6x histidine tag- HSA (C34S)- GS-(GGGGS) ₈ - HRV3C site GDF15	EFHHHHHHDAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFED HVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRET YGEMADCCAKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFH DNEETFLKKYL YEIARRHPYFY APELLFFAKRYKAAFTTECCQAADK AACLLPKLDEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARL SQRFPKAEFAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYIC ENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVES KDVCKNYAEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLE KCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKF QNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPC AEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVD ETYPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATK EQLKAVMDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGL GSGGGGSGGGGSGGGGSGGGGSGGGGSLVLFQGPARNGDHCPLGPG RCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHA QIKTSLHRLKPDTPAPCCVPASYNPMVLIQKTD TGVS LQTYDDLL AKDCHCI

95	nucleic acid encoding SEQ ID NO:92 (Codon optimization 1)	GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGG AGAAGAAAATTTCAAAGCCTTGGTGTTGATTGCCTTTGCTCAGTA TCTTCAGCAGTCCCCATTTGAAGATCATGTAAAATTAGTGAATGA AGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAGTCAGCTG AAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTAT GCACAGTTGCAACTCTTCGTGAAACCTATGGTGAAATGGCTGAC TGCTGTGCAAAACAAGAACCTGAGAGAAATGAATGCTTCTTGCA ACACAAAGATGACAACCCAAACCTCCCCGATTGGTGAGACCAG AGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACA TTTTTGAAAAAATACTTATATGAAATTGCCAGAAGACATCCTTAC TTTTATGCCCGGAACCTCTTTTCTTTGCTAAAAGGTATAAAGCT GCTTTTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTG TTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTC TGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAG AAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGA TTTCCCAAAGCTGAGTTTGCAGAAAGTTTCCAAGTTAGTGACAGAT CTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGA ATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAA ATCAAGATTCGATCTCCAGTAAACTGAAGGAATGCTGTGAAAAA CCTCTGTTGAAAAATCCCACTGCATTGCCGAAGTGGAAAAATGA TGAGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTGTA AAGTAAGGATGTTTGCAAAACTATGCTGAGGCAAAGGATGTCT TCCTGGGCATGTTTTTGTATGAATATGCAAGAAGGCATCCTGATT ACTCTGTCGTGCTGCTGCTGAGACTTGCCAAGACATATGAAACC ACTCTAGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTA TGCCAAAGTGTTTCGATGAATTTAAACCTCTTGTGGAAGAGCCTC AGAATTTAATCAAACAAAATTGTGAGCTTTTTTGAGCAGCTTGGA GAGTACAAATTCCAGAATGCGCTATTAGTTTCGTTACACCAAGAA AGTACCCCAAGTGTCAACTCCAACCTCTTGTAGAGGTCTCAAGAA ACCTAGGAAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCA AAAAGAATGCCCTGTGCAGAAAGACTATCTATCCGTGGTCCTGAA CCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAAGTGACAGAG TCACCAAATGCTGCACAGAATCCTTGGTGAACAGGCGACCATGC TTTTAGCTCTGGAAGTTCGATGAAACATACGTTCCCAAAGAGTTT AATGCTGAAACATTACCTTCCATGCAGATATATGCACACTTTCT GAGAAGGAGAGACAAATCAAGAAACAACTGCACTTGTGAGC TCGTGAAACACAAGCCCAAGGCAACAAAAGAGCAACTGAAAGC TGTTATGGATGATTTTCGACAGCTTTTGTAGAGAAGTGCTGCAAGGC TGACGATAAGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTG TTGCTGCAAGTCAAGCTGCCTTAGGGCTTGGAAGCGGCGGAGGG GGGAGTGGCGGCGGTGGCTCCGGGGGGGGCGGATCCGGCGGAG GGGGCAGCGGGGGTGGAGGGAGTGGCGGGGGAGGATCAGGGGG AGGAGGATCAGGAGGGGGCGGAAGTGATCATTGCCCTCTCGGGC CCGGACGGTGTGCGCGCTCCACACTGTGAGGGCTTCACTTGAA GACCTTGATGGGCCGACTGGGTGCTGTCCCAAGAGAGGTACA AGTCACAATGTGTATTGGCGCCTGCCCCAGCCAGTTTCGCGCCGC TAACATGCACGCCAGATAAAAACAGCCTGCACCGCCTGAAGC CCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAAT CCCATGGTGCTCATTCAAAGACCGACACCGGGGTGTCGCTCCA GACCTATGATGACTTGTTAGCCAAAGACTGCCACTGCATA
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96	nucleic acid encoding SEQ ID NO:7	GGGGACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCA CACGGTCCGCGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGG TGCTGTGCGCCACGGGAGGTGCAAGTGACCATGTGCATCGGCGCG TGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAA GACGAGCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCT GCTGCGTGCCCGCCAGCTACAATCCCATGGTGCTCATTCAAAAG ACCGACACCGGGGTGTGCTCCAGACCTATGATGACTTGTTAGC CAAAGACTGCCACTGCATA
97	nucleic acid encoding SEQ ID NO:8 (Codon optimization 1)	GACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACAC GGTCCGCGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGC TGTCGCCACGGGAGGTGCAAGTGACCATGTGCATCGGCGCGTGC CCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGAC GAGCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCT GCGTGCCCGCCAGCTACAATCCCATGGTGCTCATTCAAAAGACC GACACCGGGGTGTGCTCCAGACCTATGATGACTTGTTAGCCAA AGACTGCCACTGCATA
98	nucleic acid encoding SEQ ID NO:8 (Codon optimization 2)	GATCATTGCCCTCTCGGGCCCGGACGGTGTTGCCGCCTCCACACT GTGAGGGCTTCACTTGAAGACCTTGGATGGGCCGACTGGGTGCT GTCCCCAAGAGAGGTACAAGTCACAATGTGTATTGGCGCCTGCC CCAGCCAGTTTCGCGCCGCTAACATGCACGCCCAGATAAAAAACC AGCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTG CGTGCCCGCCAGCTACAATCCCATGGTGCTCATTCAAAAGACCG ACACCGGGGTGTGCTCCAGACCTATGATGACTTGTTAGCCAAA GACTGCCACTGCATA
99	nucleic acid encoding SEQ ID NO:8 (Codon optimization 3)	GATCATTGTCCCTTGGACCGGGTAGATGCTGTGCGCTGCACACT GTGCGGGCTTCACTGGAGGACCTCGGCTGGGCTGACTGGGTGCT GTCCCCACGGGAGGTGCAAGTGACCATGTGCATCGGCGCCTGTC CTTCGCAATTCCGGGCGCGAATATGCACGCCCAGATCAAGACC TCCCTGCATCGCCTCAAGCCCGACACTGTGCCTGCTCCATGCTGT GTGCGGGCTCCTATAACCCCATGGTGCTGATCCAGAAAACCGA TACCGGCGTCAGCCTGCAGACGTATGATGATCTGCTGGCCAAGG ACTGCCATTGCATC
100	nucleic acid encoding SEQ ID NO:9 (Codon optimization 1)	CACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTC CGCGCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTC GCCACGGGAGGTGCAAGTGACCATGTGCATCGGCGCGTGCCCGA GCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGAG CCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCG TGCCCGCCAGCTACAATCCCATGGTGCTCATTCAAAAGACCGAC ACCGGGGTGTGCTCCAGACCTATGATGACTTGTTAGCCAAAGA CTGCCACTGCATA

101	nucleic acid encoding SEQ ID NO:9 (Codon optimization 2)	CATTGCCCTCTCGGGCCCGGACGGTGTTGCCGCCTCCACACTGTG AGGGCTTCACTTGAAGACCTTGGATGGGCCGACTGGGTGCTGTC CCCAAGAGAGGTACAAGTCACAATGTGTATTGGCGCCTGCCCCA GCCAGTTTCGCGCCGCTAACATGCACGCCCAGATAAAAACCAGC CTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGT GCCCCGCCAGCTACAATCCCATGGTGCTCATTCAAAAGACCGACA CCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCCAAAGAC TGCCACTGCATA
102	nucleic acid encoding SEQ ID NO:9 (Codon optimization 3)	CATTGTCCCCTTGGACCGGGTAGATGCTGTGCGCTGCACACTGTG CGGGCTTCACTGGAGGACCTCGGCTGGGCTGACTGGGTGCTGTC CCCACGGGAGGTGCAAGTGACCATGTGCATCGGCGCCTGTCCTT CGCAATTCGGGGCCGCGAATATGCACGCCCAGATCAAGACCTCC CTGCATCGCCTCAAGCCCGACACTGTGCCTGCTCCATGCTGTGTG CCGGCCTCCTATAACCCCATGGTGCTGATCCAGAAAACCGATAC CGGCGTCAGCCTGCAGACGTATGATGATCTGCTGGCCAAGGACT GCCATTGCATC
103	nucleic acid encoding SEQ ID NO:10 (Codon optimization 1)	TGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCGC GCGTCGCTGGAAGACCTGGGCTGGGCCGATTGGGTGCTGTGCGC ACGGGAGGTGCAAGTGACCATGTGCATCGGCGCGTGCCCGAGCC AGTTCCGGGCGGCAACATGCACGCGCAGATCAAGACGAGCCTG CACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCC CGCCAGCTACAATCCCATGGTGCTCATTCAAAAGACCGACACCG GGGTGTCGCTCCAGACCTATGATGACTTGTTAGCCAAAGACTGC CACTGCATA
104	nucleic acid encoding SEQ ID NO:10 (Codon optimization 2)	TGCCCTCTCGGGCCCGGACGGTGTTGCCGCCTCCACACTGTGAG GGCTTCACTTGAAGACCTTGGATGGGCCGACTGGGTGCTGTCCC CAAGAGAGGTACAAGTCACAATGTGTATTGGCGCCTGCCCCAGC CAGTTTCGCGCCGCTAACATGCACGCCCAGATAAAAACCAGCCT GCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGC CCGCCAGCTACAATCCCATGGTGCTCATTCAAAAGACCGACACC GGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCCAAAGACTG CCACTGCATA

105	nucleic acid encoding SEQ ID NO:10 (Codon optimization 3)	TGTCCTTGGACCGGGTAGATGCTGTCGCCTGCACACTGTGCGG GCTTCACTGGAGGACCTCGGCTGGGCTGACTGGGTGCTGTCCCC ACGGGAGGTGCAAGTGACCATGTGCATCGGCGCCTGTCCTTCGC AATTCCGGGCCGCGAATATGCACGCCCAGATCAAGACCTCCCTG CATCGCCTCAAGCCCGACACTGTGCCTGCTCCATGCTGTGTGCCG GCCTCCTATAACCCCATGGTGCTGATCCAGAAAACCGATACCGG CGTCAGCCTGCAGACGTATGATGATCTGCTGGCCAAGGACTGCC ATTGCATC
106	nucleic acid encoding SEQ ID NO:11 (Codon optimization 1)	TGCCGTCTGCACACGGTCCGCGCGTCGCTGGAAGACCTGGGCTG GGCCGATTGGGTGCTGTGCCACGGGAGGTGCAAGTGACCATGT GCATCGGCGCGTGCCCCAGCCAGTTCCGGGCGGCAACATGCAC GCGCAGATCAAGACGAGCCTGCACCGCCTGAAGCCCGACACGGT GCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCCCATGGTGC TCATTCAAAAGACCGACACCGGGGTGTGCTCCAGACCTATGAT GACTTGTTAGCCAAAGACTGCCACTGCATA
107	nucleic acid encoding SEQ ID NO:11 (Codon optimization 2)	TGCCGCTCCACACTGTGAGGGCTTCACTTGAAGACCTTGATG GGCCGACTGGGTGCTGTCCCCAAGAGAGGTACAAGTCACAATGT GTATTGGCGCCTGCCCCAGCCAGTTTCGCGCCGCTAACATGCAC GCCCAGATAAAAACCAGCCTGCACCGCCTGAAGCCCGACACGGT GCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCCCATGGTGC TCATTCAAAAGACCGACACCGGGGTGTGCTCCAGACCTATGAT GACTTGTTAGCCAAAGACTGCCACTGCATA
108	nucleic acid encoding SEQ ID NO:11 (Codon optimization 3)	TGTCGCCTGCACACTGTGCGGGCTTCACTGGAGGACCTCGGCTG GGCTGACTGGGTGCTGTCCCCACGGGAGGTGCAAGTGACCATGT GCATCGGCGCCTGTCCTTCGCAATTCCGGGCCGCGAATATGCAC GCCCAGATCAAGACCTCCCTGCATCGCCTCAAGCCCGACACTGT GCCTGCTCCATGCTGTGTGCGGCGCTCCTATAACCCCATGGTGCT GATCCAGAAAACCGATACCGGCGTCAGCCTGCAGACGTATGATG ATCTGCTGGCCAAGGACTGCCATTGCATC

109	nucleic acid encoding SEQ ID NO:60 (Codon optimization 2)	GATGCGCACAAAGTCGGAAGTGGCCCATCGCTTTAAGGACCTGGG AGAAGAGAACTTCAAGGCCCTGGTCCTGATCGCGTTTCGCCAGT ACCTCCAGCAGTCCCCGTTTGAGGACCACGTCAAGCTTGTGAAC GAAGTGACCGAGTTCGCAAAGACTTGTGTGGCCGATGAGTCCGC CGAAAACCTGCGACAAGTCCCTGCACACCTTGTTCGGAGACAAGC TGTGCACCGTCGCGACTTTGCGGGAGACTTACGGCGAAATGGCG GACTGCTGCGCAAAGCAGGAGCCCGAAAGGAACGAGTGCTTCCT GCAACACAAGGACGACAACCCGAACCTTCCGAGACTCGTGCGGC CTGAGGTCGACGTGATGTGCACTGCATTCCATGATAACGAAGAA ACATTCCTGAAGAAGTACCTGTATGAAATTGCCAGACGCCACCC GTACTTCTACGCCCCCGAACTGCTGTTCTTCGCCAAGAGATACAA GGCCGCCTTTACCGAATGTTGTCAAGCCGCCGATAAGGCAGCGT GCCTGCTGCCGAAGTTGGACGAGCTCAGGGACGAAGGAAAGGC CTCGTCCGCCAAGCAGAGGCTGAAGTGCGCGTTCGCTCCAGAAGT TTGGAGAGCGGGCTTTTAAGGCCTGGGCAGTGGCTAGGTTGAGC CAGAGGTTCCCCAAGGCGGAGTTTGCCGAAGTGTCCAAGCTCGT GACTGACCTGACTAAAGTCCATACCGAATGCTGCCACGGCGATC TGCTCGAATGCGCAGATGACCGGGCGGATTTGGCCAAGTACATT TGCGAAAACCAAGACTCCATAAGCTCCAAGCTGAAGGAGTGCTG TGAAAAGCCTCTGCTCGAGAAGTCCCACTGTATCGCCGAGGTGG AGAACGACGAAATGCCGGCAGACCTCCCTAGCCTGGCAGCCGAC TTCGTGCAATCCAAGGACGTGTGCAAGAACTACGCCGAAGCGAA GGACGTGTTCTTGGGAATGTTCTGTACGAGTACGCCAGACGGC ATCCAGACTACTCCGTGGTGCTTCTCTTGCGGCTGGCCAAGACTT ATGAAACGACCCTGGAGAAATGTTGCGCTGCTGCTGACCCACAC GAGTGCTACGCCAAAGTGTTTCGACGAGTTTAAGCCTCTCGTGGA GGAACCCCAAGACCTCATCAAGCAGAACTGCGAACTTTTCGAGC AGCTCGGGGAGTACAAGTTCCAAAACGCGCTGCTTGTCCGCTAC ACCAAGAAAGTGCCGCAAGTGTCACACCGACCTCGTGGAAGT GTCCAGGAACCTGGGCAAAGTCGGAAGCAAATGTTGCAAGCACC CCGAAGCCAAGCGCATGCCGTGCGCAGAGGACTACCTTTTCGGTG GTGTTGAACCAGCTCTGCGTCTGACGAAAAGACCCCGGTGTC AGACCGCGTGACCAAGTGCTGTACCGAAAGCCTCGTGAATCGGC GCCCCTGCTTCTCGGCCCTGGAGGTGGACGAAACTTACGTGCCG AAAGAGTTCAACGCGGAAACCTTCACCTTTCATGCCGATATCTG CACCTGTCCGAGAAGGAGCGGCAGATCAAGAAGCAGACCGCC CTGGTGGAGCTTGTGAAACACAAGCCGAAGGCCACTAAGGAAC AGCTGAAGGCCGTCATGGACGATTTTCGCTGCCCTCGTCGAGAAG TGCTGCAAGGCCGACGACAAGGAGACTTGCTTCGCTGAAGAAGG GAAGAAGCTTGTGGCCGCTAGCCAGGCTGCACTGGGACTGGGTA GCGGTGGAGGGGGATCAGGGGGTGGTGGATCGGGAGGAGGAGG ATCAGGAGGTGGCGGCTCAGGAGGAGGCGGATCAGGCGGTGGA GGATCCGGAGGCGGAGGATCGGGTGGAGGAGGCTCAGCGAGGA ACGGGGATCATTGTCCCTTGGACCGGGTAGATGCTGTGCCTG CACACTGTGCGGGCTTCACTGGAGGACCTCGGCTGGGCTGACTG GGTGCTGTCCCAACGGGAGGTGCAAGTGACCATGTGCATCGGCG CCTGTCTTCGCAATTCCGGGGCCGCAATATGCACGCCAGATC AAGACCTCCCTGCATCGCCTCAAGCCCGACACTGTGCCTGCTCCA TGCTGTGTGCCGGCCTCCTATAACCCCATGGTGCTGATCCAGAAA ACCGATACCGGCGTCAGCCTGCAGACGTATGATGATCTGCTGGC CAAGGACTGCCATTGCATC
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110	nucleic acid encoding SEQ ID NO:92 (Codon optimization 2)	GATGCGCACAAAGTCGGAAGTGGCCCATCGCTTTAAGGACCTGGG AGAAGAGAACTTCAAGGCCCTGGTCCTGATCGCGTTTCGCCAGT ACCTCCAGCAGTCCCCGTTTGAGGACCACGTCAAGCTTGTGAAC GAAGTGACCGAGTTCGCAAAGACTTGTGTGGCCGATGAGTCCGC CGAAAAGTGCACAAAGTCCCTGCACACCTTGTTCGGAGACAAGC TGTGCACCGTCGCGACTTTGCGGGAGACTTACGGCGAAATGGCG GACTGCTGCGCAAAGCAGGAGCCCGAAAGGAACGAGTGCTTCCT GCAACACAAGGACGACAACCCGAACCTTCCGAGACTCGTGCGGC CTGAGGTCGACGTGATGTGCACTGCATTCCATGATAACGAAGAA ACATTCTGAAGAAGTACCTGTATGAAATTGCCAGACGCCACCC GTACTTCTACGCCCCCGAACTGCTGTTCTTCGCCAAGAGATACAA GGCCGCCTTTACCGAATGTTGTCAAGCCGCCGATAAGGCAGCGT GCCTGCTGCCGAAGTTGGACGAGCTCAGGGACGAAGGAAAGGC CTCGTCCGCCAAGCAGAGGCTGAAGTGCGCGTTCGCTCCAGAAGT TTGGAGAGCGGGCTTTTAAAGGCCTGGGCAGTGGCTAGGTTGAGC CAGAGGTTCCCCAAGGCGGAGTTTGCCGAAGTGTCCAAGCTCGT GACTGACCTGACTAAAGTCCATACCGAATGCTGCCACGGCGATC TGCTCGAATGCGCAGATGACCGGGCGGATTTGGCCAAGTACATT TGCGAAAACCAAGACTCCATAAGCTCCAAGCTGAAGGAGTGCTG TGAAAAGCCTCTGCTCGAGAAGTCCCACTGTATCGCCGAGGTGG AGAACGACGAAATGCCGGCAGACCTCCCTAGCCTGGCAGCCGAC TTCGTGCAATCCAAGGACGTGTGCAAGAACTACGCCGAAGCGAA GGACGTGTTCTGGGAATGTTCTGTACGAGTACGCCAGACGGC ATCCAGACTACTCCGTGGTGCTTCTCTTGCGGCTGGCCAAGACTT ATGAAACGACCCTGGAGAAATGTTGCGCTGCTGCTGACCCACAC GAGTGCTACGCCAAAGTGTTTCGACGAGTTTAAAGCCTCTCGTGGA GGAACCCCAAGACCTCATCAAGCAGAACTGCGAACTTTTCGAGC AGCTCGGGGAGTACAAGTTCCAAAACGCGCTGCTTGTCCGCTAC ACCAAGAAAGTGCCGCAAGTGTCACACCGACCTCGTGGAAGT GTCCAGGAACCTGGGCAAAGTCGGAAGCAAATGTTGCAAGCACC CCGAAGCCAAGCGCATGCCGTGCGCAGAGGACTACCTTTTCGGTG GTGTTGAACCAGCTCTGCGTCTGTCACGAAAAGACCCCGGTGTC AGACCGCGTGACCAAGTGCTGTACCGAAAGCCTCGTGAATCGGC GCCCCTGCTTCTCGGCCCTGGAGGTGGACGAAACTTACGTGCCG AAAGAGTTCAACGCGGAAACCTTCACCTTTCATGCCGATATCTG CACCTGTCCGAGAAGGAGCGGCAGATCAAGAAGCAGACCGCC CTGGTGGAGCTTGTGAAACACAAGCCGAAGGCCACTAAGGAAC AGCTGAAGGCCGTCATGGACGATTTTCGCTGCCCTCGTCGAGAAG TGCTGCAAGGCCGACGACAAGGAGACTTGCTTCGCTGAAGAAGG GAAGAAGCTTGTGGCCGCTAGCCAGGCTGCACTGGGACTGGGTA GCGGTGGAGGGGGATCAGGGGGTGGTGGATCGGGAGGAGGAGG ATCAGGAGGTGGCGGCTCAGGAGGAGGCGGATCAGGCGGTGGA GGATCCGGAGGCGGAGGATCGGGTGGAGGAGGCTCAGATCATT GTCCCCTTGGACCGGGTAGATGCTGTGCGCTGCACACTGTGCGG GCTTCACTGGAGGACCTCGGCTGGGCTGACTGGGTGCTGTCCCC ACGGGAGGTGCAAGTGACCATGTGCATCGGCGCCTGTCCTTCGC AATTCCGGGCCGCGAATATGCACGCCCAGATCAAGACCTCCCTG CATCGCCTCAAGCCCGACACTGTGCCTGCTCCATGCTGTGTGCCG GCCTCCTATAACCCCATGGTGCTGATCCAGAAAACCGATACCGG CGTCAGCCTGCAGACGTATGATGATCTGCTGGCCAAGGACTGCC ATTGCATC
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111	HSA(C34S)-GS- (GGGGS)- (Deletion 5) GDF15	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFY APELLFFAKRYKAAFTTECCQAADKAACLLPKL DEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLRLAKTYETTTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNCLEFEQLGEYKFQNALLVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSHCPLGPGR CCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANM HAQIKTSLHRLKPDTPAPCCVPASYNPMVLIQKTDGTGVS LQTYDD LLAKDCHCI
112	HSA(C34S)-GS- (GGGGS)- (deletion 14) GDF15	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFY APELLFFAKRYKAAFTTECCQAADKAACLLPKL DEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLRLAKTYETTTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNCLEFEQLGEYKFQNALLVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSCRLHTVRA SLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHR LKPDTPAPCCVPASYNPMVLIQKTDGTGVS LQTYDDLLAKDCHCI
113	(Deletion 2) HSA(C34S)-GS- (GGGGS)- (deletion 4) GDF15	HKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTE FAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAK QEPERNECFLQHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLKKYL YEIARRHPYFY APELLFFAKRYKAAFTTECCQAADKAACLLPKLDEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAEFAE VSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISSKLK ECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEA KDVFLGMFLY EYARRHPDYSVVLRLAKTYETTTLEKCCAAADPH ECYAKVFDEFKPLVEEPQNLIKQNCLEFEQLGEYKFQNALLVRYTK KVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLN QLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNA ETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDD FAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSGGGGSGGG GSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSDHCPLGPGRCCR LHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQI KTS LHLRLKPDTPAPCCVPASYNPMVLIQKTDGTGVS LQTYDDLLAK DCHCI

114	DNA for SEQ ID NO: 113 (Deletion 2) HSA(C34S)-GS- (GGGGS) ₈ - (deletion 4) GDF15	CACAAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGGAGAAGA AAATTTCAAAGCCTTGGTGTTGATTGCCTTTGCTCAGTATCTTCA GCAGTCCCCATTTGAAGATCATGTAAAATTAGTGAATGAAGTAA CTGAATTTGCAAAAACATGTGTTGCTGATGAGTCAGCTGAAAAT TGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTATGCACA GTTGCAACTCTTCGTGAAACCTATGGTGAAATGGCTGACTGCTGT GCAAAACAAGAACCTGAGAGAAATGAATGCTTCTTGCAACACAA AGATGACAACCCAAACCTCCCCCGATTGGTGAGACCAGAGGTTG ATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACATTTTTGA AAAAATACTTATATGAAATTGCCAGAAGACATCCTTACTTTTATG CCCCGGAACCTCTTTCTTTGCTAAAAGGTATAAAGCTGCTTTTA CAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTGTTGCCA AAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTCTGCCAA ACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAGAAAGAG CTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGATTTCCC AAAGCTGAGTTTGCAGAAAGTTTCCAAGTTAGTGACAGATCTTAC CAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGAATGTG CTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAAATCAA GATTCGATCTCCAGTAAACTGAAGGAATGCTGTGAAAAACCTCT GTTGGAAAAATCCCACTGCATTGCCGAAGTGGAATGATGAGA TGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTGAAAGTA AGGATGTTTGCAAAACTATGCTGAGGCAAAGGATGTCTTCCTG GGCATGTTTTGTATGAATATGCAAGAAGGCATCCTGATTACTCT GTCGTGCTGCTGCTGAGACTTGCCAAGACATATGAAACCACTCT AGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTATGCCA AAGTGTTGATGAATTTAAACCTCTTGTGGAAGAGCCTCAGAAT TTAATCAAACAAAATTGTGAGCTTTTTGAGCAGCTTGGAGAGTA CAAATTCAGAATGCGCTATTAGTTCGTTACACCAAGAAAGTAC CCCAAGTGTCAACTCCAACCTCTTGTAGAGGTCTCAAGAAACCTA GGAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCAAAAA GAATGCCCTGTGCAGAAGACTATCTATCCGTGGTCTGAACCAG TTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAGTCAC CAAATGCTGCACAGAATCCTTGGTGAACAGGCGACCATGCTTTT CAGCTCTGGAAGTCGATGAAACATACGTTCCCAAAGAGTTTAAT GCTGAAACATTACCTTCCATGCAGATATATGCACACTTTCTGAG AAGGAGAGACAAATCAAGAAACAAACTGCACTTGTGAGCTCGT GAAACACAAGCCCAAGGCAACAAAAGAGCAACTGAAAGCTGTT ATGGATGATTTCCGAGCTTTTGTAGAGAAGTGCTGCAAGGCTGA CGATAAGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTGTG CTGCAAGTCAAGCTGCCTTAGGGCTTGGAAGCGGCGGAGGGGGG AGTGCGGCGGTGGCTCCGGGGGGGGCGGATCCGGCGGAGGGG GCAGCGGGGGTGGAGGGAGTGGCGGGGGAGGATCAGGGGGAGG AGGATCAGGAGGGGGCGGAAGTGATCATTGCCCTCTCGGGCCCG GACGGTGTGCGGCCTCCACACTGTGAGGGGCTTCACTTGAAGAC CTTGATGGGCGGACTGGGTGCTGTCCCAAGAGAGGTACAAGT CACAAATGTGTATTGGCGCCTGCCCCAGCCAGTTTCGCGCCGCTAA CATGCACGCCAGATAAAAACCGCCTGCACCGCCTGAAGCCCG ACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAATCCC ATGGTGCTCATTCAAAAGACCGACACCGGGGTGTGCTCCAGAC CTATGATGACTTGTAGCCAAAGACTGCCACTGCATA
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115	HSA(C34S)- GA-(GGGGA) ₈ - (deletion 4) GDF15	DAHKSEVAHRFKDLGEENFKALVLIQFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLK KYLVEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLKASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLTQVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLYFYARRHPDYSVVLLLRLLAKTYETTLKCCAAA DPHECYAKVFDEFKPLVEEPQNLKQNCLEFEQLGEYKFQNALLVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEYDLSV VLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFEEGKKLVAASQAALGLGAGGGG AGGGGAGGGGAGGGGAGGGGAGGGGAGGGGAGGGGAGGGGADHCPLG PGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAA NMHAQIKTSLHRLKPDTPAPCCVPASYNPMVLIQKTDGTGVSQTY DDLLAKDCHCI
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116	DNA for SEQ ID NO: 115 HSA(C34S)- GA-(GGGGA) ₈ - (deletion 4) GDF15	GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGG AGAAGAAAATTTCAAAGCCTTGGTGTTGATTGCCTTTGCTCAGTA TCTTCAGCAGTCCCCATTTGAAGATCATGTAAAATTAGTGAATGA AGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAGTCAGCTG AAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTAT GCACAGTTGCAACTCTTCGTGAAACCTATGGTGAATGGCTGAC TGCTGTGCAAAACAAGAACCTGAGAGAAATGAATGCTTCTTGCA ACACAAAGATGACAACCCAAACCTCCCCGATTGGTGAGACCAG AGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACA TTTTTGAAAAAATACTTATATGAAATTGCCAGAAGACATCCTTAC TTTTATGCCCCGGAACCTCTTTCTTTGCTAAAAGGTATAAAGCT GCTTTTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTG TTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTC TGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAG AAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGA TTTCCCAAAGCTGAGTTTGCAGAAAGTTTCCAAGTTAGTGACAGAT CTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGA ATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAA ATCAAGATTCGATCTCCAGTAAACTGAAGGAATGCTGTGAAAAA CCTCTGTTGGAAAAATCCCACTGCATTGCCGAAGTGGAAAAATGA TGAGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTGTA AAGTAAGGATGTTTGCAAAACTATGCTGAGGCAAAGGATGTCT TCCTGGGCATGTTTTTGTATGAATATGCAAGAAGGCATCCTGATT ACTCTGTCGTGCTGCTGCTGAGACTTGCCAAGACATATGAAACC ACTCTAGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTA TGCCAAAGTGTTTCGATGAATTTAAACCTCTTGTGGAAGAGCCTC AGAATTTAATCAAACAAAATTGTGAGCTTTTTTGAGCAGCTTGGA GAGTACAAATTCCAGAATGCGCTATTAGTTTCGTTACACCAAGAA AGTACCCCAAGTGTCAACTCCAACCTCTTGTAGAGGTCTCAAGAA ACCTAGGAAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCA AAAAGAATGCCCTGTGCAGAAAGACTATCTATCCGTGGTCCTGAA CCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAAGTGACAGAG TCACCAAATGCTGCACAGAATCCTTGGTGAACAGGCGACCATGC TTTTCACTCTGGAAGTCGATGAAACATACGTTCCCAAAGAGTTT AATGCTGAAACATTACCTTCCATGCAGATATATGCACACTTTCT GAGAAGGAGAGACAAATCAAGAAACAACTGCACTTGTGAGC TCGTGAAACACAAGCCCAAGGCAACAAAAGAGCAACTGAAAGC TGTTATGGATGATTTGCGCAGCTTTTGTAGAGAAGTGCTGCAAGGC TGACGATAAGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTG TTGCTGCAAGTCAAGCTGCCTTAGGGCTTGGTGCTGGAGGAGGC GGGGCGGGCGGGCGGGGTGCCGGTGGGGGTGGCGCAGGGGGAG GTGGTGCGGGTGGTGGTGGGGCTGGTGGGGGAGGTGCAGGCGG TGGCGGTGCCGGGGGGGGTGGCGCGGATCATTGCCCTCTCGGGC CCGGACGGTGTGCGCGCTCCACACTGTGAGGGCTTCACTTGAA GACCTTGGATGGGCCGACTGGGTGCTGTCCCAAGAGAGGTACA AGTCACAATGTGTATTGGCGCCTGCCCCAGCCAGTTTCGCGCCGC TAACATGCACGCCAGATAAAAACAGCCTGCACCGCCTGAAGC CCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAAT CCCATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCA GACCTATGATGACTTGTTAGCCAAAGACTGCCACTGCATA
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117	HSA(C34S)- (AP) ₁₀ -(deletion 4) GDF15	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DEL RDEGKASSAKQRLKASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNALLVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGLAPAPAPA PAPAPAPAPAPDPHCPLGPGRCRLHTVRASLEDLGWADWVLS REVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPAPCCVPAS YNPMVLIQKTD TGVS LQTYDDLLAKDCHCI
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118	DNA for SEQ ID NO: 117 HSA(C34S)- (AP) ₁₀ -(deletion 4) GDF15	GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGG AGAAGAAAATTTCAAAGCCTTGGTGTTGATTGCCCTTGCTCAGTA TCTTCAGCAGTCCCCATTTGAAGATCATGTAAAATTAGTGAATGA AGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAGTCAGCTG AAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTAT GCACAGTTGCAACTCTTCGTGAAACCTATGGTGAAATGGCTGAC TGCTGTGCAAAACAAGAACCTGAGAGAAATGAATGCTTCTTGCA ACACAAAGATGACAACCCAAACCTCCCCGATTGGTGAGACCAG AGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACA TTTTTGAAAAAATACTTATATGAAATTGCCAGAAGACATCCTTAC TTTTATGCCCCGGAACCTCTTTCTTTGCTAAAAGGTATAAAGCT GCTTTTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTG TTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTC TGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAG AAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGA TTTCCCAAAGCTGAGTTTGCAGAAAGTTTCCAAGTTAGTGACAGAT CTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGA ATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAA ATCAAGATTCGATCTCCAGTAAACTGAAGGAATGCTGTGAAAAA CCTCTGTTGGAAAAATCCCACTGCATTGCCGAAGTGGAAAAATGA TGAGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTGTA AAGTAAGGATGTTTGCAAAACTATGCTGAGGCAAAGGATGTCT TCCTGGGCATGTTTTTGTATGAATATGCAAGAAGGCATCCTGATT ACTCTGTCGTGCTGCTGCTGATGCAAGACTTGCCAAGACATATGAACC ACTCTAGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTA TGCCAAAGTGTTTCGATGAATTTAAACCTCTTGTGGAAGAGCCTC AGAATTTAATCAAACAAAATTGTGAGCTTTTTTGAGCAGCTTGGA GAGTACAAATTCCAGAATGCGCTATTAGTTTCGTTACACCAAGAA AGTACCCCAAGTGTCAACTCCAACCTCTTGTAGAGGTCTCAAGAA ACCTAGGAAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCA AAAAGAATGCCCTGTGCAGAAAGACTATCTATCCGTGGTCCTGAA CCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAAGTGACAGAG TCACCAAATGCTGCACAGAATCCTTGGTGAACAGGCGACCATGC TTTTCAGCTCTGGAAGTTCGATGAAACATACGTTCCCAAAGAGTTT AATGCTGAAACATTTCACCTTCCATGCAGATATATGCACACTTTCT GAGAAGGAGAGACAAATCAAGAAACAACTGCACTTGTGAGC TCGTGAAACACAAGCCCAAGGCAACAAAAGAGCAACTGAAAGC TGTTATGGATGATTTTCGACAGCTTTTGTAGAGAAGTGCTGCAAGGC TGACGATAAGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTG TTGCTGCAAGTCAAGCTGCCCTTAGGGCTTGCAACCAGCCCCCTGCC CTGCACCTGCACCTGCTCCCCGACCGGCTCCAGCCCCAGCTCCG GATCATTGCCCTCTCGGGCCCGGACGGTGTTGCCGCCTCCACACT GTGAGGGCTTCACTTGAAGACCTTGGATGGGCCGACTGGGTGCT GTCCCCAAGAGAGGTACAAGTACAATGTGTATTGGCGCCTGCC CCAGCCAGTTTCGCGCCGCTAACATGCACGCCAGATAAAAACC AGCCTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTG CGTGCCCGCCAGCTACAATCCCATGGTGCTCATTCAAAGACCG ACACCGGGGTGTCGCTCCAGACCTATGATGACTTGTTAGCCAAA GACTGCCACTGCATA
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119	HSA(C34S)- (AP) ₁₂ -(deletion 4) GDF15	DAHKSEVAHRFKDLGEENFKALVLIQFAQYLQQSPFEDHVKL VNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLKASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLLLR LAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNALLVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDR VTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGLAPAPAPA PAPAPAPAPAPAPAPADHCP LGPGRCCRLHTVRASLEDLGWADW VLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKPDTPVAPCC VPASYNPMVLIQKTD TGVS LQTYDDLLAKDCHCI
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120	DNA for SEQ ID NO: 119 HSA(C34S)- (AP) ₁₂ -(deletion 4) GDF15	GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGG AGAAGAAAATTTCAAAGCCTTGGTGTGATTGCCTTTGCTCAGTA TCTTCAGCAGTCCCCATTTGAAGATCATGTAAAATTAGTGAATGA AGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAGTCAGCTG AAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTAT GCACAGTTGCAACTCTTCGTGAAACCTATGGTGAATGGCTGAC TGCTGTGCAAAACAAGAACCTGAGAGAAATGAATGCTTCTTGCA ACACAAAGATGACAACCCAAACCTCCCCGATTGGTGAGACCAG AGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACA TTTTTGAAAAAATACTTATATGAAATTGCCAGAAGACATCCTTAC TTTTATGCCCCGGAACCTCTTTCTTTGCTAAAAGGTATAAAGCT GCTTTTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTG TTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTC TGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAG AAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGA TTTCCCAAAGCTGAGTTTGCAGAAAGTTTCCAAGTTAGTGACAGAT CTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGA ATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAA ATCAAGATTCGATCTCCAGTAAACTGAAGGAATGCTGTGAAAAA CCTCTGTTGGAAAAATCCCACTGCATTGCCGAAGTGGAAAAATGA TGAGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTGTA AAGTAAGGATGTTTGCAAAACTATGCTGAGGCCAAAGGATGTCT TCCTGGGCATGTTTTTGTATGAATATGCAAGAAGGCATCCTGATT ACTCTGTCGTGCTGCTGCTGAGACTTGCCAAGACATATGAAACC ACTCTAGAGAAGTGTGCTGTGCCGCTGCAGATCCTCATGAATGCTA TGCCAAAGTGTTTCGATGAATTTAAACCTCTTGTGGAAGAGCCTC AGAATTTAATCAAACAAAATTGTGAGCTTTTTTGAGCAGCTTGGA GAGTACAAATTCCAGAATGCGCTATTAGTTTCGTTACACCAAGAA AGTACCCCAAGTGTCAACTCCAACCTCTTGTAGAGGTCTCAAGAA ACCTAGGAAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCA AAAAGAATGCCCTGTGCAGAAAGACTATCTATCCGTGGTCCTGAA CCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAG TCACCAAATGCTGCACAGAATCCTTGGTGAACAGGCGACCATGC TTTTCAGCTCTGGAAGTTCGATGAAACATACGTTCCCAAAGAGTTT AATGCTGAAACATTTCACCTTCCATGCAGATATATGCACACTTTCT GAGAAGGAGAGACAAATCAAGAAACAACTGCACTTGTGAGC TCGTGAAACACAAGCCCAAGGCAACAAAAGAGCAACTGAAAGC TGTTATGGATGATTTTCGACAGCTTTTGTAGAGAAGTGCTGCAAGGC TGACGATAAGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTG TTGCTGCAAGTCAAGCTGCCCTTAGGGCTTGCAACCAGCCCCCTGCCC CTGCACCTGCACCTGCTCCCCGACCGGCTCCAGCCCCAGCTCCG GCTCCAGCTCCTGATCATTGCCCTCTCGGGCCCCGGACGGTGTGTC CGCCTCCACACTGTGAGGGCTTCACTTGAAGACCTTGGATGGGC CGACTGGGTGCTGTCCCCAAGAGAGGTACAAGTCACAATGTGTA TTGGCGCCTGCCCCAGCCAGTTTCGCGCCGCTAACATGCACGCCC AGATAAAAACCAGCCTGCACCGCCTGAAGCCCCGACACGGTGCCA GCGCCCTGCTGCGTGCCCGCCAGCTACAATCCCATGGTGCTCATT CAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTT GTTAGCCAAAGACTGCCACTGCATA
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121	HSA(C34S)- GGG- (EGKSSGSGSE SKST) ₃ -GGG- (deletion4) GDF15	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLK KYLVEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLKASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLTQVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLYFYARRHPDYSVVLLLRLLAKTYETTTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLKQNCLEFEQLGEYKFQNALLVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEYDLSV VLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGLGGSEKKS SGSGSESKSTEGKSSGSGSESKSTEGKSSGSGSESKSTGGSDHCPLGP GRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAAN MHAQIKTSLHRLKPDTPAPCCVPASYNPMVLIQKTDGTGVSQTQYD DLLAKDCHCI
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122	DNA for SEQ ID NO: 121 HSA(C34S)- GGS- (EGKSSGSGSE SKST)3-GGS- (deletion4) GDF15	GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGG AGAAGAAAATTTCAAAGCCTTGGTGTGATTGCCCTTTGCTCAGTA TCTTCAGCAGTCCCCATTTGAAGATCATGTAAAATTAGTGAATGA AGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAGTCAGCTG AAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTAT GCACAGTTGCAACTCTTCGTGAAACCTATGGTGAATGGCTGAC TGCTGTGCAAAACAAGAACCTGAGAGAAATGAATGCTTCTTGCA ACACAAAGATGACAACCCAAACCTCCCCGATTGGTGAGACCAG AGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACA TTTTTGAAAAAATACTTATATGAAATTGCCAGAAGACATCCTTAC TTTTATGCCCCGGAACCTCTTTCTTTGCTAAAAGGTATAAAGCT GCTTTTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTG TTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTC TGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAG AAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGA TTTCCCAAAGCTGAGTTTGCAGAAAGTTTCCAAGTTAGTGACAGAT CTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGA ATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAA ATCAAGATTCGATCTCCAGTAAACTGAAGGAATGCTGTGAAAAA CCTCTGTTGAAAAATCCCACTGCATTGCCGAAGTGGAAAATGA TGAGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTGTA AAGTAAGGATGTTTGCAAAAACATATGCTGAGGCAAAGGATGTCT TCCTGGGCATGTTTTTGTATGAATATGCAAGAAGGCATCCTGATT ACTCTGTCGTGCTGCTGCTGAGACTTGCCAAGACATATGAAACC ACTCTAGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTA TGCCAAAGTGTTTCGATGAATTTAAACCTCTTGTGGAAGAGCCTC AGAATTTAATCAAACAAAATTGTGAGCTTTTTTGAGCAGCTTGGA GAGTACAAATTCCAGAATGCGCTATTAGTTTCGTTACACCAAGAA AGTACCCCAAGTGTCAACTCCAACCTCTTGTAGAGGTCTCAAGAA ACCTAGGAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCA AAAAGAATGCCCTGTGCAGAAAGACTATCTATCCGTGGTCCTGAA CCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAG TCACAAAATGCTGTACTGAGAGCTTGGTCAACAGGCGGCCGTGC TTCAGCGCCCTCGAGGTGGATGAGACTTATGTCCCAAAGGAGTT TAATGCGGAAACTTTTACTTTCCACGCAGACATTTGCACCTTGTC TGAAAAGGAAAAGACAGATTAAGAAAACAGACTGCTCTTGTGGAA CTGGTAAAACATAAACCAAAAGCTACGAAGGAGCAGCTTAAGG CTGTTATGGATGATTTTCGCCGCGTTTGTGCGAGAAGTGCTGCAAAG CGGACGATAAGGAAAACCTTGCTTTGCAGAGGAAGGTAAGAACTC GTAGCGGCAAGTCAGGCTGCGCTTGGCCTTGGAGGCAGTGAAGG CAAATCCTCTGGGAGTGGCTCTGAAAGTAAATCCACCGAGGGCA AATCCAGTGGATCTGGGTCTGAATCTAAGTCTACCGAGGGGAAG TCTTCTGGCAGTGGGTCTGAAATCTAAATCTACAGGCGGCTCTGA CCATTGCCCGTTGGGACCAGGACGCTGCTGTGCGCTTCATACAGT GCGAGCGAGTTTGGAAAGACCTGGGCTGGGCTGACTGGGTGCTTA GCCCTCGGGAGGTCCAGGTCACAATGTGCATTGGCGCGTGTCCC AGTCAATTTAGAGCAGCAAATATGCACGCCCAAATAAAAACCTC CCTGCATAGGCTTAAGCCAGATACTGTCCCCGCACCATGCTGTGT GCCTGCTTCTTACAATCCTATGGTACTCATCCAGAAGACCGACAC GGGAGTTAGCCTCCAGACTTATGACGACCTCTTGGCTAAAGATT GCCATTGTATT
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123	HSA(C34S)-GS- (PGGGS) ₈ - (deletion4)GDF1 5	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPNLPRLVREVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL DELRDEGKASSAKQRLKASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSVVLLLR LAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVR YTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGSPGGGS PGGGSPGGGSPGGGSPGGGSPGGGSPGGGSPGGGSDHCPLGPGRCC RLHTVSRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHA QIKTSLHRLKPDTPAPCCVPASYNPMVLIQKTD TGVS LQTYDDLL AKDCHCI
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124	DNA for SEQ ID NO: 123 HSA(C34S)-GS- (PGGS) ₈ - (deletion4)GDF1 5	GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGG AGAAGAAAATTTCAAAGCCTTGGTGTGATTGCCCTTGCTCAGTA TCTTCAGCAGTCCCCATTTGAAGATCATGTAAAATTAGTGAATGA AGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAGTCAGCTG AAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTAT GCACAGTTGCAACTCTTCGTGAAACCTATGGTGAAATGGCTGAC TGCTGTGCAAAACAAGAACCTGAGAGAAATGAATGCTTCTTGCA ACACAAAGATGACAACCCAAACCTCCCCGATTGGTGAGACCAG AGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACA TTTTTGAAAAAATACTTATATGAAATTGCCAGAAGACATCCTTAC TTTTATGCCCCGGAACCTCTTTTCTTTGCTAAAAGGTATAAAGCT GCTTTTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTG TTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTC TGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAG AAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGA TTTCCCAAAGCTGAGTTTGCAGAAAGTTTCCAAGTTAGTGACAGAT CTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGA ATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAA ATCAAGATTCGATCTCCAGTAAACTGAAGGAATGCTGTGAAAAA CCTCTGTTGGAAAAATCCCACTGCATTGCCGAAGTGGAAAAATGA TGAGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTGTA AAGTAAGGATGTTTGCAAAACTATGCTGAGGCCAAAGGATGTCT TCCTGGGCATGTTTTTGTATGAATATGCAAGAAGGCATCCTGATT ACTCTGTCGTGCTGCTGCTGCTGAGACTTGCCAAGACATATGAAACC ACTCTAGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTA TGCCAAAGTGTTTCGATGAATTTAAACCTCTTGTGGAAGAGCCTC AGAATTTAATCAAACAAAATTGTGAGCTTTTTTGAGCAGCTTGGA GAGTACAAATTCCAGAATGCGCTATTAGTTCGTTACACCAAGAA AGTACCCCAAGTGTCAACTCCAACCTCTTGTAGAGGTCTCAAGAA ACCTAGGAAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCA AAAAGAATGCCCTGTGCAGAAAGACTATCTATCCGTGGTCCTGAA CCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAG TCACGAAATGTTGCACAGAGTCACTGGTCAACAGGAGACCTTGC TTCTCCGCTCTTGAGGTTGACGAAACGTATGTCCCAAAGAGTTT AACGCCGAAACGTTTACGTTTCATGCGGACATATGCACTCTCAGT GAGAAGGAGCGACAAATCAAAAAACAGACTGCTCTTGTAGAGTT GGTAAAACACAAACCTAAAGCAACAAAAGAGCAATTGAAAGCT GTGATGGACGATTTTGCAGCTTTCGTAGAAAAGTGCTGCAAGGC CGACGATAAAGAAACCTGTTTCGCTGAAGAAGGCCAAAAAACTTG TTGCGGCATCTCAGGCCGCTCTTGGACTTGGGAGCCCGGGTGGC GGGTCTCCAGGCGGAGGCTCTCCGGGCGGAGGTAGTCCCGGAGG GGGTAGTCCGGGCGGCGGTTCTCCAGGTGGAGGTTCTCCTGGTG GTGGCAGTCTTGCGGAGGATCTGATCACTGTCCCCTTGGGCCC GGGAGGTGCTGCCGACTTCATACAGTTTCGCGCCAGCCTTGAAGA TTTGGGGTGGGCCGACTGGGTGTTGAGCCCAGAGAGGTCCAAG TCACGATGTGTATTGGAGCCTGTCCCTCTCAATTCGAGCCGCAA ATATGCATGCGCAAATAAAGACGAGTCTCCATCGGTTGAAGCCT GATACTGTCCCAGCTCCGTGCTGCGTCCCCGCGAGTTATAATCCC ATGGTCCTTATACAGAAAAACAGACACTGGTGTCAGCCTTCAGAC GTATGACGATTTGCTTGCTAAAGACTGTCATTGTATT
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125	HSA(C34S)-GS- (AGGGS) ₈ - (deletion 4) GDF15	DAHKSEVAHRFKDLGEEFNKALVLIAFAYLQQSPFEDHVKLVNEV TEFAKTCVADESAENCDSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNPNLPRLVPRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAAACLLPKL DEL RDEGKASSAKQRLK CASLQKFGERAFAKAWAVARLSQRFPKAE FAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLYEYARRHPDYSVVLRLAKTYETTLKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNCLEFEQLGEYKFQNALLV YTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKADDKETCF AE EGKKLVAASQAALGLGSAGGG SAGGGSAGGGSAGGGSAGGGSAGGGSAGGGSAGGGSAGGGSAGGGS RCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANM HAQIKTSLHRLKPDTPAPCCVPASYNPMVLIQKTDGTGVS LQTYDD LLAKDCHCI
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126	DNA for SEQ ID NO: 125	GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGG AGAAGAAAATTTCAAAGCCTTGGTGTTGATTGCCCTTGCTCAGTA TCTTCAGCAGTCCCCATTTGAAGATCATGTAAAATTAGTGAATGA AGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAGTCAGCTG AAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTAT GCACAGTTGCAACTCTTCGTGAAACCTATGGTGAAATGGCTGAC TGCTGTGCAAAACAAGAACCTGAGAGAAATGAATGCTTCTTGCA ACACAAAGATGACAACCCAAACCTCCCCGATTGGTGAGACCAG AGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACA TTTTTGAAAAAATACTTATATGAAATTGCCAGAAGACATCCTTAC TTTTATGCCCCGGAACCTCTTTCTTTGCTAAAAGGTATAAAGCT GCTTTTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTG TTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTC TGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAG AAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGA TTTCCCAAAGCTGAGTTTGCAGAAAGTTTCCAAGTTAGTGACAGAT CTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGA ATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAA ATCAAGATTCGATCTCCAGTAAACTGAAGGAATGCTGTGAAAAA CCTCTGTTGAAAAATCCCACTGCATTGCCGAAGTGGAAAAATGA TGAGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTGTA AAGTAAGGATGTTTGCAAAACTATGCTGAGGCAAAGGATGTCT TCCTGGGCATGTTTTGTATGAATATGCAAGAAGGCATCCTGATT ACTCTGTCGTGCTGCTGCTGAGACTTGCCAAGACATATGAAACC ACTCTAGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTA TGCCAAAGTGTTTCGATGAATTTAAACCTCTTGTGGAAGAGCCTC AGAATTTAATCAAACAAAATTGTGAGCTTTTTGAGCAGCTTGGA GAGTACAAATTCCAGAATGCGCTATTAGTTTCGTTACACCAAGAA AGTACCCCAAGTGTCAACTCCAACCTCTTGTAGAGGTCTCAAGAA ACCTAGGAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCA AAAAGAATGCCCTGTGCAGAAAGACTATCTATCCGTGGTCCTGAA CCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAG TCACTAAATGTTGTACCGAGTCTCTTGTAAATAGGCGGCCATGCT TCAGTGCATTGGAAGTCGACGAAACCTATGTACCAAAGGAGTTC AACGCAGAAACATTTACATTCCATGCTGATATCTGCACATTGAG CGAGAAAGAGAGACAGATTAAGAAACAGACAGCGCTTGTGAA CTGGTTAAACACAAACCAAAGCTACCAAGGAGCAGCTTAAGGC AGTAATGGATGACTTCGCGGCCTTTGTGAGAAATGTTGTAAAG CGGATGATAAAGAGACATGCTTCGCCGAAGAGGGCAAAAAACT TGTAAGCGGCAAGCCAGGCCGCACTGGGTCTCGGTAGTGCGGGCG GTGGTTCAGCGGGGGGAGGATCTGCAGGTGGTGGCTCAGCGGGT GGCGGTAGCGCTGGGGGGGGCTCCGCAGGTGGGGGATCAGCAG GCGGCGGATCAGCCGGCGGTGGATCCGACCACTGTCTCTCGGG CCTGGTCGGTGTGCGCCTCCATACTGTGCGCGCTCTCTTGAG GATCTGGGGTGGGCTGATTGGGTCTCTCTCCCCGCGAAGTGCA GGTGACCATGTGTATTGGTGCTTGCCCAAGTCAATTCCGAGCAG CTAACATGCACGCCCAGATCAAGACTAGCCTGCATCGGCTTAAG CCCGACACTGTTCTGCCCCTTGCTGTGTTCTGTCATCTTATAATC CAATGGTCCTGATCCAGAAAACCGATACGGGTGTATCATTGCAA ACATACGACGACTTGCTTGCCAAAGATTGCCATTGCATT
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127	HSA(C34S)- GGs- (EGKSSGSGSE SKST) ₂ -GGs- (deletion4) GDF15	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHVKL VNEV TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLK KYL YEIARRHPYFY APELLFFAKRYKAAFT ECCCQAADKAACL PKL DELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAE FAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY AEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKCCAAA DPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNALLVR YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDR VTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAV MDDFAAFVEKCKKADDKETCFAEEGKKLVAASQAALGLGGSEGKS SGSGSESKSTEGKSSGSGSESKSTGGSDHCPLGPGRCCRLHTVRASL EDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLK PDTVPAPCCVPASYNPMVLIQKTD TGVS LQTYDDLLAKDCHCI
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128	DNA for SEQ ID NO: 127	GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGG AGAAGAAAATTTCAAAGCCTTGGTGTTGATTGCCTTTGCTCAGTA TCTTCAGCAGTCCCCATTTGAAGATCATGTAAAATTAGTGAATGA AGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAGTCAGCTG AAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTAT GCACAGTTGCAACTCTTCGTGAAACCTATGGTGAAATGGCTGAC TGCTGTGCAAAACAAGAACCTGAGAGAAATGAATGCTTCTTGCA ACACAAAGATGACAACCCAAACCTCCCCGATTGGTGAGACCAG AGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACA TTTTTGAAAAAATACTTATATGAAATTGCCAGAAGACATCCTTAC TTTTATGCCCCGGAACCTTTTTCTTTGCTAAAAGGTATAAAGCT GCTTTTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTG TTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTC TGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAG AAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGA TTTCCCAAAGCTGAGTTTGCAGAAAGTTTCCAAGTTAGTGACAGAT CTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGA ATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAA ATCAAGATTGATCTCCAGTAAACTGAAGGAATGCTGTGAAAAA CCTCTGTTGAAAAATCCCACTGCATTGCCGAAGTGGAAAATGA TGAGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTGTA AAGTAAGGATGTTTGTGAAAACTATGCTGAGGCAAAGGATGTCT TCCTGGGCATGTTTTTGTATGAATATGCAAGAAGGCATCCTGATT ACTCTGTCGTGCTGCTGCTGAGACTTGCCAAAGACATATGAAACC ACTCTAGAGAAGTGCTGTGCGCTGCAGATCCTCATGAATGCTA TGCCAAAGTGTTTCGATGAATTTAAACCTCTTGTGGAAGAGCCTC AGAATTTAATCAAACAAAATTGTGAGCTTTTTTGAGCAGCTTGGA GAGTACAAATTCCAGAATGCGCTATTAGTTCGTTACACCAAGAA AGTACCCCAAGTGTCAACTCCAACCTCTTGTAGAGGTCTCAAGAA ACCTAGGAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCA AAAAGAATGCCCTGTGCAGAAAGACTATCTATCCGTGGTCCTGAA CCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAG TCACGAAATGCTGTACAGAATCCCTCGTGAATAGAAGGCCCTGC TTCTCTGCCCTTGAGGTGGACGAGACTTACGTCCCTAAGGAGTTT AACGCCGAGACCTTTACTTTTCATGCTGATATTTGCACCCTTTCC GAAAAGGAGCGGCAGATCAAGAAACAAACAGCCTTGGTGGAAC TCGTAAAACATAAACCCAAAGCCACCAAGGAACAACCTTAAAGCT GTTATGGATGACTTCGCAGCCTTCGTGAGAAAATGTTGCAAGGC GGATGATAAGGAAACGTGTTTTGCTGAGGAAGGGAAGAAGTTG GTTGCTGCCTCTCAAGCGGCTCTGGGGCTTGGCGGATCAGAGGG GAAGTCCTCCGGGTCCGGTAGCGAGTCCAAATCTACGGAAGGGA AGTCATCCGGTTCTGGGTGAGAGTCCAAATCCACAGGAGGATCA GACCATTTGCCATTGGGACCAGGACGATGTTGTGCGCTGCATAC GGTAAGAGCGTCTCTGGAGGATCTCGGCTGGGCGGATTGGGTTT TCTACCCACGAGAAGTACAGGTACAAATGTGCATAGGAGCTTGT CCGAGCCAATTCCGGGCGGCTAATATGCACGCACAGATCAAGAC CTCTTTGCACCGCTTGAAGCCCGATACCGTGCCAGCACCGTGTTG CGTCCCAGCATCTTACAACCTATGGTTTTGATACAGAAAACCTGA CACAGGTGTGAGCCTCCAGACATATGATGATTTGCTGGCTAAGG ATTGCCACTGTATA
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Claims

1. A fusion protein comprising: a. the amino acid sequence of SEQ ID NO: 2; b. a linker having a length of 35-48 amino acids and comprising the amino acid sequence of (GGGGS)_n, wherein n is 7 or 8 (SEQ ID NO: 42 or 43); and c. a truncated GDF15 consisting of the amino acid sequence of SEQ ID NO: 8; wherein the fusion protein is arranged from N-terminus to C-terminus in the order (a)-(b)-(c), and the C-terminus of the amino acid sequence of SEQ ID NO: 2 is fused to the N-terminus of the truncated GDF15 through the linker.
2. The fusion protein of claim 1, wherein the linker comprises the sequence (GGGGS)_n, wherein n is 8 (SEQ ID NO: 43).
3. A fusion protein of claim 1, wherein the linker consists of the amino acid sequence of SEQ ID NO: 12.
4. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the fusion protein of claim 1.
5. The isolated nucleic acid molecule of claim 4 comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 97-99.
6. A vector comprising a nucleic acid molecule encoding the fusion protein of claim 1.
7. The vector of claim 6 comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 97-99.
8. A host cell comprising a nucleic acid molecule encoding the fusion protein of claim 1.
9. A method of producing the fusion protein of claim 1, comprising: (1) culturing a host cell comprising a nucleic acid molecule encoding the fusion protein under a condition that the fusion protein is produced; and (2) recovering the fusion protein produced by the host cell.
10. A pharmaceutical composition comprising the fusion protein of claim 1 and a pharmaceutically acceptable carrier.
11. A fusion protein comprising the amino acid sequence of SEQ ID NO: 92.
12. A pharmaceutical composition comprising the fusion protein of claim 11 and a pharmaceutically acceptable carrier.
13. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the fusion protein of claim 11.
14. The isolated nucleic acid molecule of claim 13, comprising the nucleotide sequence of SEQ ID NO: 95.
15. A vector comprising a nucleic acid molecule encoding the fusion protein of claim 11.

16. A host cell comprising a nucleic acid molecule encoding the fusion protein of claim 11.
17. A method of producing the fusion protein of claim 11, comprising: (1) culturing a host cell comprising a nucleic acid molecule encoding the fusion protein under a condition that the fusion protein is produced; and (2) recovering the fusion protein produced by the host cell.
18. The isolated nucleic acid molecule of claim 13, comprising the nucleotide sequence of SEQ ID NO: 110.