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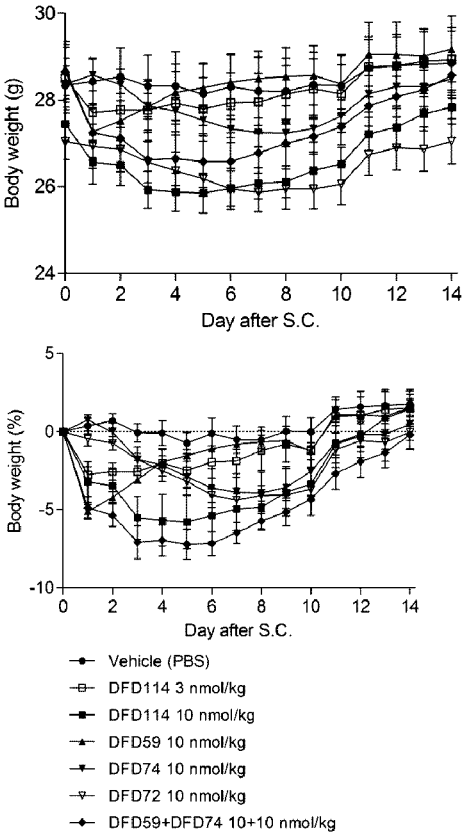

(57) Abstract: The present invention provides a dual function protein prepared by linking a biologically active protein and an FGF mutant protein to an Fc region of an immunoglobulin, which has improved pharmacological efficacy, *in vivo* duration and protein stability. A dual function protein according to the present invention exhibits improved pharmacological efficacy, *in vivo* duration and protein stability, and a pharmaceutical composition containing the dual function protein as an active ingredient may be effectively used as a therapeutic agent for diabetes, obesity, dyslipidemia, metabolic syndrome, non-alcoholic fatty liver diseases, non-alcoholic steatohepatitis or cardiovascular diseases.

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Description

Title of Invention: DUAL FUNCTION PROTEINS AND PHARMACEUTICAL COMPOSITION COMPRISING SAME

Technical Field

[1] The present invention relates to a dual function protein including a biologically active protein and a fibroblast growth factor 21 (FGF21) mutant protein, and a pharmaceutical composition containing same.

Background Art

[2] Glucagon-like peptide-1 (GLP-1) is an incretin hormone consisting of 31 amino acids, which is secreted by L cells in the intestinal tract when stimulated by food, etc. Its biological effects arise via intracellular signaling through the GLP-1 receptor, a G protein-coupled receptor which is expressed in target tissues such as β-cells in the pancreas, brain, etc. GLP-1 secreted in the blood has a very short half-life of less than 2 minutes, which is caused by a loss of activity due to the cleavage of amino acids at the N-terminus by the enzyme dipeptidyl peptidase-4 (DPP-4). Since GLP-1 stimulates the secretion of insulin in β-cells in the pancreas based on blood glucose level, it has a strong effect on lowering blood glucose without inducing hypoglycemia. Further, the administration of GLP-1 results in loss of body weight in various animal models and humans, which is known to be caused by reduced food intake due to its effect on appetite suppression. GLP-1 induces proliferation of β-cells and enhances the viability of β-cells by inhibiting cell death caused by glycolipid toxicity through GLP-1 receptor expressed in β-cells in the pancreas. Excessive secretion of glucagon increases blood glucose, which is known to be one of the causes of hyperglycemia in diabetics. In addition, it is known that GLP-1 acts on α-cells in the pancreas to inhibit fasting blood glucose elevation by inhibiting secretion of protein kinase A (PKA) protein-specific glucagon.

[3] Exendin-4 is a clinically important GLP-1 receptor agonist. Exendin-4 is a polypeptide with 39 amino acid residues, and is normally produced in the salivary glands of the Gila Monster lizard. It is known that exendin-4 an amino acid sequence homology of 52% with GLP-1, and interacts with the GLP-1 receptor in mammals (Thorens et al. (1993) *Diabetes* 42:1678-1682). Exendin-4 has been shown to stimulate the secretion of insulin by insulin-producing cells *in vitro*, and the induction of insulin release by insulin-producing cells is stronger than GLP-1 under equimolar conditions. While exendin-4 strongly stimulates the secretion of insulin to decrease blood glucose levels in both rodents and humans with a duration of action longer than that of GLP-1, exendin-4 has exhibits antigenicity in mammals devoid of GLP-1 as it has unfamiliar

epitopes in such animals.

[4] The ability of GLP-1 and exendin-4 analogs (e.g., liraglutide and byetta) to improve glucose control in humans has been clinically confirmed. It has been reported that GLP-1 increases β-cell mass through the inhibition of apoptosis and induced proliferation. Furthermore, it has been also reported that GLP-1 acts as an intestinal hormone inhibiting gastric acid secretion and gastric emptying while enhancing satiety signals, thereby reducing appetite. Such effects of GLP-1 can explain the weight loss observed when GLP-1 analogs are administered to patients with type 2 diabetes. In addition, GLP-1 exhibits cardioprotective effects following ischemia in rodents.

[5] Various attempts have been made to develop long-acting GLP-1 analogs. Clinically confirmed long-acting GLP-1 analogs include dulaglutide (WO 2005/000892) and albiglutide (WO 2003/059934). Dulaglutide is an Fc-fused GLP-1 analog, and albiglutide is an albumin-fused GLP-1 analog, both of which have pharmacokinetic profiles allowing for once weekly administration. Both drugs have excellent effects on lowering blood glucose and reducing body weight with once weekly administration, and also provide greatly improved convenience in terms of treatment when compared to byetta and liraglutide.

[6] Meanwhile, fibroblast growth factor 21 (FGF21), synthesized in the liver, is a hormone known to play an important role in glucose and lipid homeostasis. FGF21 exhibits pharmacological actions in the liver, adipocytes, β cells of the pancreas, hypothalamus in the brain, and muscle tissues, where both an FGF21-specific receptor, i.e., FGF receptor, and β-klotho complex are expressed. It has been reported that in non-human primate and murine models of various diabetic and metabolic diseases, FGF21 can lower blood glucose levels in an insulin-independent manner, reduce body weight, and lower triglyceride and low-density lipoprotein (LDL) concentrations in the blood. Additionally, due to its effect of improving insulin sensitivity, FGF21 has potential for development as a novel therapeutic agent for diabetes and obesity (*see* WO2003/011213).

[7] Accordingly, in order to develop a novel anti-diabetic drug based on FGF21, attempts have been made to improve its biological activity and *in* vivo stability by constructing FGF21 mutants based on the wild-type FGF21 sequence via substitution, insertion, and deletion of some amino acids (*see* WO2010/065439). However, as FGF21 has a very short half-life, it has proven problematic if used directly as a biotherapeutic agent (Kharitonenkov, A. et al. (2005) *Journal of Clinical Investigation* 115:1627-1635). The *in* vivo half-life of FGF21 is 1 to 2 hours in mice, and 2.5 to 3 hours in monkeys. Therefore, for FGF21 to be used in its current form as a therapeutic agent for diabetes, daily administration is required.

[8]

[9] Various approaches have been reported in attempting to increase the *in vivo* half-life of FGF21 recombinant proteins. One such example is to link polyethylene glycol (PEG), i.e., a polymer material, to FGF21 to increase its molecular weight, thereby inhibiting renal excretion and increasing *in* vivo retention time (*see* WO2012/066075). Another approach attempts to improve the half-life by fusing it with a fatty acid, which binds to human albumin (*see* WO2012/010553). An additional example attempts to increase the half-life while maintaining pharmacological activity equivalent to that of wild-type FGF21 through the generation of an agonist antibody, which specifically binds to the human FGF receptor alone or as a complex with β-klotho (*see* WO2012/170438). In another example, the half-life was improved by preparing long-acting fusion proteins, in which an Fc region of IgG binds to an FGF21 molecule (*see* WO2013/188181).

[10] Among the various technologies available to create long-acting drugs, Fc fusion technology is widely used because it has less of the disadvantages seen with other approaches, such as inducing an immune response or toxicity while increasing *in* vivo half-life. For the development of an Fc-fused FGF21 protein as a long-acting therapeutic drug, the following conditions should be satisfied.

[11] First, the decrease of *in vitro* activity caused by fusion should be minimized. Both the N-terminus and C-terminus of FGF21 are involved in FGF21's activity. In this regard, it is known that the activities of FGF21 fusion proteins greatly vary depending on the location of the fusion. Accordingly, the activities of Fc-fused FGF21 fusion proteins, in which mutations are introduced into FGF21, may be altered depending on the presence/absence or location of the fusion. Second, a pharmacokinetic profile enabling administration at an interval of once per week in humans should be realized by the increase of *in* vivo half-life by the fusion. Third, considering that immunogenicity may be expected in most patients after administration of biopharmaceuticals, the immunogenicity risk due to a fusion linker or mutation should be minimized. Fourth, there should be no stability issues arising from the position of the fusion or the introduction of the mutation. Fifth, since undesired immune responses may occur depending on the isotypes of fused immunoglobulin, a solution to prevent such responses is necessary.

[12] An attempt to develop a long-acting fusion protein by linking the Fc region of an immunoglobulin G (IgG) to an FGF21 molecule has already been reported (*see* WO 2013/188181). In the case of one Fc-FGF21 structure, where the Fc is fused to the N-terminus of the wild-type FGF21, while there is no distinct difference in *in* vitro activity as compared to that of the wild-type FGF21, the half-life is known to be very short due to *in* vivo degradation of the protein. To address this issue, there has been an attempt to improve the *in* vivo half-life by introducing several mutations at specific site locations of FGF21 to resist protein degradation. However, immunogenicity risk may

increase with the introduction of multiple mutations. In contrast, in the case of an FGF21-Fc structure, where the Fc is fused to the C-terminus of the FGF21 molecule, it is known that there is a significant decrease in activity caused by fusion at this site when compared to the Fc-FGF21 structure.

[13] Combined administration of GLP-1 and FGF21 may have a synergistic effect as compared with single administration depending on the action mechanisms and target tissues in the body, and potentially outstanding anti-diabetic efficacy and additional advantages are expected. The effects of combined administration of GLP-1 and FGF21 or a GLP-1/FGF21 dual function protein have been already investigated and reported (*see* WO 2010/142665 and WO 2011/020319).

[14] Various problems must be solved in order to develop a dual function protein comprising GLP-1 and FGF21. Since wild-type GLP-1 and wild-type FGF21 have a very short *in* vivo half-life, they are required to be administered at least once daily, even if developed as therapeutic agents. Accordingly, long-acting technologies such as an Fc fusion are required in order to develop a long-acting dual function protein to improve convenience for patients. In a dual function drug for the two targets of GLP-1 and FGF21, the introduction of mutation(s) is essential to maintain the activity and *in vivo* stability of each drug, and problems associated with changes in activity, structure or stability caused by each mutation should be addressed. Medicinal effects for the two targets of GLP-1 and FGF21 should be well-balanced, and drug designs considering *in vitro* activities, pharmacokinetic profiles, pharmacological efficacy in animal models as well as clinical evaluation of efficacy in humans are required for this purpose. A dual function protein has a structure that cannot exist in a human body, and is structurally complex as compared with a fusion protein for a single target. In addition, since mutation or linker engineering is required to balance the two targets, the possibility of forming aggregate complexes may increase, and further protein engineering to prevent this may be required. Furthermore, potential immunogenicity may increase due to novel mutation sequences or complex structures, which should be addressed or avoided.

[15] The present inventors have endeavored to improve the stability, pharmacokinetic profiles and pharmacological efficacy of dual function proteins including GLP-1 mutant proteins and FGF21 mutant proteins, and discovered that the stability, pharmacokinetic profiles and pharmacological efficacy of dual function proteins may be improved when a GLP-1 mutant protein is fused to an Fc region of an immunoglobulin and a novel FGF21 mutant protein is fused thereto, thereby accomplishing the present invention.

[16]

Disclosure of Invention

Technical Problem

[17] An object of the present invention is to provide a dual function protein including a biologically active protein and an FGF21 mutant protein with improved pharmacokinetic parameters, high stability, low possibility of forming aggregation complexes, and reduced potential immunogenicity.

[18] Another object of the present invention is to provide a pharmaceutical composition including the dual function protein for preventing or treating FGF21-associated disorders.

[19] A further object of the present invention is to provide an isolated nucleic acid molecule encoding the dual function protein, an expression vector including the nucleic acid molecule, and a host cell including the expression vector.

[20]

Solution to Problem

[21] The present invention provides a dual function protein comprising an FGF21 mutant protein; a biologically active protein, or a mutant or fragment thereof; and an Fc region of an immunoglobulin, wherein the FGF21 mutant protein comprises at least one mutation selected from the group consisting of the following mutations (1) to (7):

[22] (1) a substitution of amino acids at positions 98 to 101 from the N-terminus of a wild-type FGF21 protein with an amino acid sequence of EIRP (SEQ ID NO: 68);

[23] (2) a substitution of amino acids at positions 170 to 174 from the N-terminus of a wild-type FGF21 protein with an amino acid sequence of TGLEAV (SEQ ID NO: 69);

[24] (3) a substitution of amino acids at positions 170 to 174 from the N-terminus of a wild-type FGF21 protein with an amino acid sequence of TGLEAN (SEQ ID NO: 70);

[25] (4) a substitution of an amino acid at position 170 from the N-terminus of a wild-type FGF21 protein with an amino acid N;

[26] (5) a substitution of an amino acid at position 174 from the N-terminus of a wild-type FGF21 protein with an amino acid N;

[27] (6) a substitution of an amino acid at position 180 from the N-terminus of a wild-type FGF21 protein with an amino acid E, along with one or more mutations (1) to (5) above; and

[28] (7) a mutation of 1 to 10 amino acids for reducing immunogenicity of a wild-type FGF21 protein.

[29] In addition, the present invention provides a pharmaceutical composition comprising the dual function protein for treating diabetes, obesity, dyslipidemia, metabolic syndrome, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis or cardiovascular diseases.

[30] Further, the present invention provides an isolated nucleic acid molecule encoding the dual function protein, an expression vector comprising the nucleic acid molecule, and a host cell comprising the expression vector.

[31]

Advantageous Effects of Invention

[32] A dual function protein of the present invention, prepared by linking a biologically active protein and an FGF mutant protein to an Fc region of an immunoglobulin, has improved pharmacological efficacy, *in* vivo duration and protein stability. In addition, a pharmaceutical composition including the dual function protein as an active ingredient can be used as a therapeutic agent for diabetes, obesity, dyslipidemia, metabolic syndrome, non-alcoholic fatty liver diseases, non-alcoholic steatohepatitis or cardiovascular diseases.

[33]

Brief Description of Drawings

[34] FIGS. 1A to 1C are graphs showing the *in vitro* activities of fusion proteins including FGF21 mutant proteins (hereinafter, "FGF21 mutant fusion protein") using a HEK293 cell line in which human β-klotho is overexpressed. No FGF21 mutant fusion proteins exhibited a significant decrease in activity due to the introduction of a mutation.

[35] FIGS. 2A and 2B are graphs showing the *in vitro* activities of FGF21 mutant fusion proteins with various linkers connecting the N-terminus of FGF21 to an Fc region, using a HEK293 cell line in which human β-klotho is overexpressed. No FGF21 mutant fusion protein exhibited a significant decrease in activity, although a slight difference was shown in activity depending on the linker sequence.

[36] FIG. 3 is a graph showing the *in vitro* activities of RGE (Amgen), Fc-FGF21 (Lilly) and DFD1 using a HEK293 cell line in which human β-klotho is overexpressed. DFD1 and RGE (Amgen) had similar activities, while Fc-FGF21 (Lilly) had *in vitro* activity two times higher than the other proteins.

[37] FIG. 4 shows the stability of DFD4 and that of DFD13 in order to confirm the effect of the EIRP mutation of FGF21 on the stability of fusion protein. It was confirmed that DFD13 was associated with a lower rate of high molecular weight aggregates (HMW %) at the initial stage and at a time-point of more than 2 weeks later as compared with DFD4, indicating that the introduction of the EIRP mutation improves the stability of the FGF21 mutant fusion protein, thereby reducing HMW % significantly.

[38] FIG. 5 shows the concentration of each protein in the blood over time for 96 hours after subcutaneous administration of FGF21 mutant fusion proteins. Data are indicated as mean values and standard deviation.

[39] FIG. 6 shows blood glucose levels in an *ob/ob* mouse model after single sub-

cutaneous injection of DFD18, DFD72, DFD74 or Fc-FGF21 (Lilly). DFD18, DFD72 and DFD74 all had an effect of lowering blood glucose level continuously. Data are indicated as mean values and standard error of the mean (S.E.M.).

[40] FIG. 7 shows graphs indicating the changes in body weights in the *ob/ob* mouse model from the day of administration to the 14th day after single subcutaneous injection of DFD18, DFD72, DFD74 or Fc-FGF21 (Lilly). DFD18, DFD72 and DFD74 all had an effect of reducing body weight as compared with the PBS-treated group. Data are indicated as mean values and standard error of the mean.

[41] FIG. 8 shows graphs indicating the changes in glycated hemoglobin levels in the *ob/ob* mouse model at the day of administration (1st day) and the 16th day after single subcutaneous injection of DFD18, DFD72, DFD74 or Fc-FGF21 (Lilly). DFD18, DFD72 and DFD74 all reduced glycated hemoglobin levels at the 16th day as compared with those at the day of administration. Data are indicated as mean values and standard error of the mean.

[42] FIG. 9 shows blood glucose levels in an HFD/STZ mouse model after single subcutaneous injection of DFD72 or DFD74. Both DFD72 and DFD74 had the effect of lowering blood glucose level continuously. Data are indicated as mean values and standard error of the mean.

[43] FIG. 10 shows the changes in animal body weights in the HFD/STZ mouse model from the day of administration to the 14th day after single subcutaneous injection of DFD72 or DFD74. Both DFD72 and DFD74 had the effect of reducing body weight as compared with the PBS-treated group. Data are indicated as mean values and standard error of the mean.

[44] FIG. 11 shows graphs indicating the changes in glycated hemoglobin levels in the HFD/STZ mouse model at the 1st day and the 13th day after single subcutaneous injection of DFD72 or DFD74. It was observed that both DFD72 and DFD74 resulted in greater reduction of glycated hemoglobin levels as compared with the PBS-treated group. Data are indicated as mean values and standard error of the mean.

[45] FIG. 12 shows the changes in body weights measured in the diet-induced obesity mouse model from the day of administration to the 14th day after single administration of DFD18. DFD18 had a significant effect on body weight reduction. Data are indicated as mean values and standard error of the mean.

[46] FIG. 13 is a graph showing the *in vitro* GLP-1 activities of dual function proteins depending on the hinges which link the C-terminus of GLP-1 mutants and GLP-1 to the Fc region using a CHO cell line in which human GLP-1 receptor is overexpressed. Generally, the dual function protein including a GLP-1 (A2G) sequence (DFD23) exhibited 2 to 3 times lower activity than those of other dual function proteins including other GLP-1 mutant sequences. No significant difference in GLP-1 activities

was shown between the dual function proteins including mutant sequences except the GLP-1 (A2G) sequence.

[47] FIG. 14 shows graphs indicating the GLP-1 activities of DFD59, DFD69, DFD112 and DFD114 and the FGF21 activities of DFD69, DFD112 and DFD114. *In* vitro GLP-1 activities of three dual function proteins (DFD69, DFD112 and DFD114) and Fc-fused GLP-1 mutant including no FGF21 (DFD59) were measured using a CHO cell line in which human GLP-1 receptor is overexpressed. The three dual function proteins showed similar EC_{50} values, and the Fc-fused GLP-1 mutant (DFD59) showed about 2 times higher activity than those of dual function proteins. *In* vitro activities of dual function proteins depending on FGF21 mutants were measured using a HEK293 cell line in which human β-klotho is overexpressed. It was confirmed that the *in vitro* activities of the FGF21 portion were similar in the three dual function proteins.

[48] FIG. 15 shows the concentrations of proteins in the blood versus time for 240 hours after subcutaneous administration of dual function proteins. Data are indicated as mean values and standard deviation.

[49] FIG. 16 shows the blood glucose levels in a *db/db* mouse model after single subcutaneous injection of DFD114 or DFD59 and single subcutaneous injection of combination of DFD59 and DFD74. The groups treated with dual function proteins showed stronger effects on lowering blood glucose levels than those treated with single function proteins. Data are indicated as mean values and standard error of the mean (S.E.M.).

[50] FIG. 17 shows graphs indicating the changes in body weights in *db/db* mouse model from the day of administration to the 14th day after single subcutaneous injection of DFD114 or DFD59 and single subcutaneous injection of combination of DFD59 and DFD74. The groups treated with dual function proteins showed stronger effects on reducing body weight than those treated with single function proteins. Data are indicated as mean values and standard error of the mean (S.E.M.).

[51] FIG. 18 shows graphs indicating the changes in glycated hemoglobin levels in a *db/db* mouse model at the day of administration (1st day) and the 16th day after single subcutaneous injection of DFD114 or DFD59 and single subcutaneous injection of a combination of DFD59 and DFD74. The groups treated with dual function proteins showed stronger effects on reducing glycated hemoglobin levels than those treated with single function proteins or a combination thereof. Data are indicated as mean values and standard error of the mean.

[52] FIG. 19 shows the blood glucose levels in an HFD/STZ mouse model after single subcutaneous injection of DFD114, DFD59, DFD74 or DFD72 and single subcutaneous injection of combination of DFD59 and DFD74. The groups treated with dual function proteins showed stronger effects on lowering blood glucose levels than

those treated with single function proteins. Data are indicated as mean values and standard error of the mean (S.E.M.).

[53] FIG. 20 shows the changes in body weights in the HFD/STZ mouse model from the day of administration to the 14th day after single subcutaneous injection of DFD59, DFD72, DFD74 or DFD114 and single subcutaneous injection of combination of DFD59 and DFD74. The groups treated with dual function proteins showed stronger effects on reducing body weight than those treated with single function proteins. Data are indicated as mean values and standard error of the mean (S.E.M.).

[54] FIG. 21 shows the changes in glycated hemoglobin levels in the HFD/STZ mouse model at the day of administration (1st day) and the 16th day after single subcutaneous injection of DFD59, DFD72, DFD74 or DFD114 and single subcutaneous injection of combination of DFD59 and DFD74. The groups treated with dual function proteins showed stronger effects on reducing glycated hemoglobin levels than those treated with single function proteins or a combination thereof. Data are indicated as mean values and standard error of the mean.

[55]

Best Mode for Carrying out the Invention

[56] Hereinafter, the present invention will be described in more detail.

[57] In an aspect, the present invention provides a dual function protein comprising a fibroblast growth factor 21 (FGF21) mutant protein; a biologically active protein, or a mutant or fragment thereof; and an Fc region of an immunoglobulin, wherein the FGF21 mutant protein comprises at least one mutation selected from the group consisting of the following mutations (1) to (7):

[58] (1) a substitution of amino acids at positions 98 to 101 from the N-terminus of a wild-type FGF21 protein with an amino acid sequence of EIRP (SEQ ID NO: 68) (hereinafter, "EIRP");

[59] (2) a substitution of amino acids at positions 170 to 174 from the N-terminus of a wild-type FGF21 protein with an amino acid sequence of TGLEAV (SEQ ID NO: 69) (hereinafter, "TGLEAV");

[60] (3) a substitution of amino acids at positions 170 to 174 from the N-terminus of a wild-type FGF21 protein with an amino acid sequence of TGLEAN (SEQ ID NO: 70) (hereinafter, "TGLEAN");

[61] (4) a substitution of an amino acid at position 170 from the N-terminus of a wild-type FGF21 protein with an amino acid N;

[62] (5) a substitution of an amino acid at position 174 from the N-terminus of a wild-type FGF21 protein with an amino acid N;

[63] (6) a substitution of an amino acid at position 180 from the N-terminus of a wild-type

FGF21 protein with an amino acid E, along with one or more mutations (1) to (5) above; and

[64] (7) a mutation of 1 to 10 amino acids for reducing immunogenicity of a wild-type FGF21 protein.

[65] The wild-type FGF21 protein, a hormone known to play an important role in glucose and lipid homeostasis, may be one derived from mammals such as humans, mice, pigs, monkeys, etc., preferably from humans. More preferably, the wild-type FGF21 protein may be the wild-type human FGF21 protein having an amino acid sequence represented by SEQ ID NO: 1.

[66] The mutation included in the FGF21 mutant proteins may be, preferably, any one of the mutations of EIRP, TGLEAV, TGLEAN, G170N and G174N; a combination of any one of the mutations of TGLEAV, TGLEAN, G170N and G174N and the mutation of EIRP; a combination of any one of the mutations of EIRP, TGLEAV, TGLEAN, G170N and G174N and the mutation of A180E; or a combination of any one of the mutations of TGLEAV, TGLEAN, G170N and G174N, the mutation of EIRP and the mutation of A180E. Furthermore, the FGF21 mutant proteins may have a conformation, in which 1 to 10 amino acids at the N-terminus or C-terminus is (are) deleted as compared to the wild-type FGF21 protein. More preferably, the FGF21 mutant proteins may include an amino acid sequence represented by any one of SEQ ID NOs: 6 to 23. Still more preferably, the FGF21 mutant proteins may include an amino acid sequence represented by any one of SEQ ID NOs: 6 to 23 and further have a conformation, in which 1 to 10 amino acids at the N-terminus or C-terminus is (are) deleted as compared to the wild-type FGF21 protein.

[67] In the dual function protein, an amino acid residue N of FGF21 mutant protein introduced by a mutation may be glycosylated.

[68] The biologically active protein may be one selected from the group consisting of insulin, C-peptide, leptin, glucagon, gastrin, gastric inhibitory polypeptide (GIP), amylin, calcitonin, cholecystokinin, peptide YY, neuropeptide Y, bone morphogenetic protein-6 (BMP-6), bone morphogenetic protein-9 (BMP-9), oxyntomodulin, oxytocin, glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), irisin, fibronectin type III domain-containing protein 5 (FNDC5), apelin, adiponectin, C1q and tumor necrosis factor related protein (CTRP family), resistin, visfatin, omentin, retinol binding protein-4 (RBP-4), glicentin, angiopoietin, interleukin-22 (IL-22), exendin-4 and growth hormone. Preferably, the biologically active protein may be one selected from GLP-1, a mutant thereof and exendin-4.

[69] The GLP-1 protein is an incretin hormone consisting of 31 amino acids, which is secreted by L cells in the intestinal tract stimulated by food, etc. For example, the GLP-1 protein may be represented by the amino acid sequence of SEQ ID NO: 42.

[70] A mutant of GLP-1 may be represented, for example, by the amino acid sequence of any one of SEQ ID NOs: 43 to 46.

[71] As used herein, the term "Fc region," "Fc fragment," or "Fc" refers to a protein, which includes a heavy chain constant region 1 (CH1), a heavy chain constant region 2 (CH2) and a heavy chain constant region 3 (CH3) of an immunoglobulin, but does not include variable regions of the heavy and light chains and a light chain constant region 1 (CL1) of an immunoglobulin. Additionally, as used herein, the term "Fc region mutant" refers to one prepared by substituting part of amino acid(s) of an Fc region or by combining Fc regions of different types.

[72] The Fc region of immunoglobulin may be an entire Fc region constituting an antibody, a fragment thereof, or an Fc region mutant. Additionally, the Fc region includes a molecule in the form of a monomer or multimer, and may further include a hinge region of the heavy chain constant region. The Fc region mutant may be modified to prevent cleavage at the hinge region. Furthermore, the hinge sequence of the Fc may have a substitution in some amino acid sequences to reduce antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). In addition, part of the amino acid sequence of the Fc hinge sequence may be substituted to inhibit the rearrangement of the Fab region. A lysine residue at the C-terminus of the Fc may be removed.

[73] Preferably, the Fc region of immunoglobulin may be any one of IgG1, IgG2, IgG3, IgG4 and IgD Fc regions; or a hybrid Fc, which is a combination thereof. Further, the hybrid Fc may include an IgG4 region and an IgD region. Further, the hybrid Fc region may include part of the hinge sequence and CH2 of an IgD Fc, and CH2 and CH3 sequences of IgG4 Fc.

[74] In addition, the Fc fragment of the present invention may be in the form of wild-type glycosylated chain, more glycosylated chain than the wild-type, less glycosylated chain than the wild-type, or deglycosylated chain. The increase, decrease, or removal of glycosylated chain may be performed by a conventional method known in the art, such as a chemical method, an enzymatic method, and a genetic engineering method using microorganisms.

[75] Preferably, the immunoglobulin Fc region may be represented by an amino acid sequence selected from SEQ ID NOs: 24 to 26, 47 and 48.

[76] The dual function protein may include a biologically active protein, an Fc region of an immunoglobulin and an FGF21 mutant protein, linked in this order from the N-terminus to the C-terminus. Further, the dual function protein may include an FGF21 mutant protein, an Fc region of an immunoglobulin and a biologically active protein, linked in this order from the N-terminus to the C-terminus. Preferably, the dual function protein may include a biologically active protein, an Fc region of an im-

munoglobulin and an FGF21 mutant protein, linked in this order from the N-terminus to the C-terminus.

[77] Furthermore, the dual function protein may include a GLP-1 mutant protein, an Fc region of an immunoglobulin and an FGF21 mutant protein, linked in this order from the N-terminus to the C-terminus. Further, the dual function protein may include an FGF21 mutant protein, an Fc region of an immunoglobulin and a GLP-1 mutant protein, linked in this order from the N-terminus to the C-terminus. Preferably, the dual function protein may include a GLP-1 mutant protein, an Fc region of an immunoglobulin and an FGF21 mutant protein, linked in this order from the N-terminus to the C-terminus.

[78] Additionally, the dual function protein may further include a linker.

[79] The dual function protein may be in the form, in which the FGF21 mutant protein is directly connected to the N-terminus or C-terminus of the immunoglobulin Fc region, or the FGF21 mutant protein is connected to the immunoglobulin Fc region via a linker.

[80] In such case, the linker may be connected to the N-terminus, C-terminus, or a free radical of the Fc fragment, and also, may be connected to the N-terminus, C-terminus, or a free radical of the FGF21 mutant protein. When the linker is a peptide linker, the connection may occur in any region. For example, the linker may be connected to the C-terminus of the immunoglobulin Fc region and the N-terminus of the FGF21 mutant protein to form a fusion protein of the immunoglobulin Fc region and the FGF21 mutant protein.

[81] Furthermore, the dual function protein of the present invention may be in the form, in which a biologically active protein is linked to the N-terminus of the Fc region of immunoglobulin of the fusion protein.

[82] When the linker and Fc are separately expressed and then connected, the linker may be a crosslinking agent known in the art. Examples of the crosslinking agent may include 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, imidoesters including N-hydroxysuccinimide ester such as 4-azidosalicylic acid and disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane, but are not limited thereto.

[83] Further, the linker may be a peptide. Preferably, the linker may be a peptide consisting of 10 to 30 amino acid residues.

[84] Furthermore, alanine may additionally be attached to the end of linker. Preferably, the linker may be a peptide having an amino acid sequence represented by any one of SEQ ID NOs: 2 to 5.

[85] The dual function protein may be in a form in which a dimer or multimer of FGF21 mutant proteins, in which one or more FGF21 mutant proteins linked together, is

connected to an immunoglobulin Fc region. Additionally, the dual function protein may be in a form of a dimer or multimer in which two or more immunoglobulin Fc regions are linked, wherein the immunoglobulin Fc regions have the FGF21 mutant protein connected thereto.

[86] Additionally, the dual function protein may be a peptide which preferably has an amino acid sequence represented by any one of SEQ ID NOs: 58 to 67. More preferably, the dual function protein may be a peptide which has an amino acid sequence represented by SEQ ID NO: 65, 66 or 67.

[87] The FGF21 mutant protein may further include a mutation of 1 to 10 amino acids for reducing immunogenicity of the wild-type FGF21 protein. The immunogenicity may be predicted by a conventional method known in the art. For example, the potential immunogenicity of a protein may be screened by using, e.g., iTope™ and TCED™ methods.

[88] Further, the mutation for minimizing the immunogenicity may be designed by a conventional method known in the art. For example, when immunogenicity is observed by performing an EpiScreen™ analysis to evaluate potential immunogenicity, the amino acid sequences inducing the immunogenicity may be identified through T-cell epitope mapping, and the mutants with minimized immunogenicity may be designed via *in silico* prediction.

[89] In another aspect, the present invention provides a pharmaceutical composition containing the dual function protein for treating FGF21-associated disorders.

[90] As used herein, the term "FGF21-associated disorder" may include obesity, type I- and type II diabetes, pancreatitis, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), insulin resistance, hyperinsulinemia, glucose intolerance, hyperglycemia, metabolic syndrome, acute myocardial infarction, hypertension, cardiovascular diseases, atherosclerosis, peripheral arterial disease, apoplexy, heart failure, coronary artery heart disease, renal disease, diabetic complications, neuropathy, gastroparesis, disorder associated with a serious inactivation mutation in insulin receptor, and other metabolic disorders. Preferably, the FGF21-associated disorder may be diabetes, obesity, dyslipidemia, metabolic syndrome, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis or cardiovascular diseases.

[91] Further, the pharmaceutical composition may further include a pharmaceutical carrier. The pharmaceutical carrier may be any carrier as long as it is a non-toxic material suitable for delivering antibodies to patients. For example, distilled water, alcohol, fats, waxes and inactive solids may be included as a carrier. Pharmaceutically acceptable adjuvants (buffering agents, dispersants) may also be included in the pharmaceutical composition. In these formulations, the concentration of the dual function

protein may vary greatly.

[92] Specifically, the pharmaceutical composition may contain a formulation material for altering, maintaining, or conserving the pH, osmolarity, viscosity, transparency, color, isotonicity, odor, sterility, stability, dissolution or release rate, adsorption, or permeability of the composition. Examples of the suitable formulating material may include amino acids (e.g., glycine, glutamine, asparagine, arginine or lysine), antimicroorganism agents, anti-oxidants (e.g., ascorbic acid, sodium sulfite or sodium bisulfite), buffering agents (e.g., borate, bicarbonates, Tris-HCl, citrate, phosphate or other organic acids), bulking agents (e.g., mannitol or glycine), chelating agents (e.g., ethyelenediaminetetraacetic acid (EDTA)), complexing agents (e.g., caffeine, polyvinylpyrrolidione, β-cyclodextrin or hydroxypropyl-β-cyclodextrin), fillers, monosaccharides, disaccharides and other carbohydrates (e.g., glucose, mannose or dextrin), proteins (e.g., serum albumin, gelatin or immunoglobulin), coloring agents, flavoring agents, diluents, emulsifiers, hydrophilic polymers (e.g., polyvinylpyrrolidione), low molecular weight polypeptides, salt-forming counterions (e.g., sodium), preservatives (e.g., benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide), solvents (e.g., glycerin, propylene glycol or polyethylene glycol), sugar alcohols (e.g., mannitol or sorbitol), suspending agents, surfactants or humectants (e.g., pluronics; PEG; sorbitan ester; polysorbate, e.g., polysorbate 20 or polysorbate 80; triton; tromethamine; lecithin; cholesterol or tyloxapol), stability improvers (e.g., sucrose or sorbitol), growth improvers (e.g., alkali metal halides, preferably, sodium chloride or potassium chloride; or mannitol, sorbitol), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants, but are not limited thereto.

[93] In another aspect, the present invention provides a method for preventing or treating FGF21-associated disorders including administering the dual function protein to a subject in need of such prevention or treatment. This method includes, in particular, administering an effective amount of the dual function protein of the present invention to a mammal having a symptom of diabetes, obesity, dyslipidemia, metabolic syndrome, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis or cardiovascular diseases which are FGF21-associated disorders.

[94] The pharmaceutical composition of the present invention may be administered via any route. The composition of the present invention may be provided to an animal directly (e.g., topically, by administering into tissue areas by injection, transplantation, or by topical administration) or systemically (e.g., by oral- or parenteral administration) via any appropriate means. When the composition of the present invention is parenterally provided via intravenous-, subcutaneous-, ophthalmic-, intraperitoneal-,

intramuscular-, oral-, rectal-, intraorbital-, intracerebral-, intracranial-, intraspinal-, intraventricular-, intrathecal-, intracistenal-, intracapsular-, intranasal-, or aerosol administration, the composition is preferably aqueous or may include a portion of a physiologically applicable body liquid suspension or solution. Accordingly, the carrier or vehicle may be added to the composition and be delivered to a patient since it is physiologically applicable. Therefore, a physiologically-appropriate saline solution may generally be included as a carrier like a body fluid for formulations.

[95] Further, the administration frequency may vary depending on the pharmacokinetic parameters of the dual function protein in the formulations to be used. Typically, physicians would administer the composition until an administration dose to achieve a desired effect is reached. Accordingly, the composition may be administered as a unit dose, at least two doses with time intervals (may or may not contain the same amount of a target dual function protein) or administered by a continuous injection via a transplantation device or catheter. The precision of addition of an appropriate administration dose may be routinely performed by those skilled in the art, and corresponds to the scope of work being routinely performed by them.

[96] Additionally, the preferable unit dose of the dual function protein in humans may be in a range from 0.01 μg/kg to 100 mg/kg of body weight, and more preferably from 1 μg/kg to 10 mg/kg of body weight. Although this is the optimal amount, the unit dose may vary depending on the disease to be treated or the presence/absence of adverse effects. Nevertheless, the optimal administration dose may be determined by performing a conventional experiment. The administration of the dual function protein may be performed by a periodic bolus injection, an external reservoir (e.g., an intravenous bag), or a continuous intravenous-, subcutaneous-, or intraperitoneal administration from the internal source (e.g., a bioerodable implant).

[97] In addition, the dual function protein of the present invention may be administered to a subject recipient along with other biologically active molecules. The optimal combination of the dual function protein and other molecule(s), dosage forms, and optimal doses may be determined by a conventional experiment well known in the art.

[98] In still another aspect, the present invention provides an isolated nucleic acid molecule encoding the dual function protein.

[99] As used herein, the term "isolated nucleic acid molecule" refers to a nucleic acid molecule of the present invention, which is isolated from about at least 50% of proteins, lipids, carbohydrates, or other materials, discovered in nature when total nucleic acids are isolated from a source cell; which is operatively linked to a polynucleotide which is not linked in nature; or which is a part of a larger polynucleotide sequence and does not occur in nature. Preferably, in the isolated nucleic acid molecules of the present invention, there are not substantially present any other con-

taminated nucleic acids, or other contaminants which are discovered in the natural environment and inhibit uses of the nucleic acids in the production of polypeptides, or treatment, diagnosis, prevention, or research.

[100] In such case, the isolated nucleic acid molecules encoding the dual function protein may have different sequences with each other due to codon redundancy. Furthermore, as long as the isolated nucleic acid can produce the dual function protein, the isolated nucleic acid may be appropriately modified, or a nucleotide may be added to the N-terminus or C-terminus of the isolated nucleic acid according to desired purposes.

[101] The isolated nucleic acid may include, for example, a nucleotide sequence represented by any one of SEQ ID NOs: 71 to 80.

[102] In still another aspect, the present invention provides an expression vector comprising the isolated nucleic acid molecule.

[103] As used herein, the term "expression vector" refers to a vector containing a nucleic acid sequence, which is suitable for the transformation of a host cell and directs or controls the expression of an inserted heterogenous nucleic acid sequence. The expression vector includes a linear nucleic acid, a plasmid, a phagemid, a cosmid, an RNA vector, a viral vector, and analogs thereof. Examples of the viral vector include a retrovirus, an adenovirus and an adeno-associated virus, but are not limited thereto.

[104] As used herein, the term "expression of a heterogeneous nucleic acid sequence" or "expression" of a target protein refers to transcription of an inserted DNA sequence, translation of an mRNA transcript, and production of an Fc fusion protein product, an antibody or an antibody fragment.

[105] A useful expression vector may be RcCMV (Invitrogen, Carlsbad) or a mutant thereof. The useful expression vector may include a human cytomegalovirus (CMV) promoter for promoting a continuous transcription of a target gene in a mammalian cell, and a bovine growth hormone polyadenylation signal sequence for enhancing the level of post-transcriptional RNA stability. In an exemplary embodiment of the present invention, the expression vector is pAD15, which is a modified vector of RcCMV.

[106] In still another aspect, the present invention provides a host cell comprising the expression vector.

[107] As used herein, the term "host cell" refers to a prokaryotic cell or eukaryotic cell into which a recombinant expression vector may be introduced. As used herein, the term "transformed" or "transfected" refers to introduction of a nucleic acid (e.g., a vector) into a cell by various technologies known in the art.

[108] An appropriate host cell may be transformed or transfected with a DNA sequence of the present invention and may be used for the expression and/or secretion of the target protein. Examples of the appropriate host cell that may be used in the present invention include immortal hybridoma cells, NS/0 myeloma cells, 293 cells, Chinese hamster

ovary (CHO) cells, HeLa cells, CAP cells (human amniotic fluid-derived cells), and COS cells.

[109] Hereinafter, exemplary embodiments of the present invention will be described in detail with reference to the examples. However, these examples according to the present invention can be modified in many different forms and the scope of the present invention should not be construed as limited to the examples set forth herein.

[110]

Mode for the Invention

[111] Preparation Example 1. Preparation and purification of fusion protein containing FGF21 mutant protein

[112]

[113] Preparation Example 1-1. Preparation of expression vectors for expression of FGF21 mutant proteins

[114]

[115] In order to improve the stability, activity and pharmacokinetic profiles of the FGF21 in an Fc-FGF21 structure, mutation studies of FGF21 were performed.

[116] Specifically, mutant proteins were designed for the LLLE region (the amino acids at positions 98 to 101 from the N-terminus of the FGF21 protein) and GPSQG region (the amino acids at positions 170 to 174 from the N-terminus of the FGF21 protein), and A180 site, which were expected to significantly affect protein activities based on 3-dimensional structure analysis of the FGF21 proteins.

[117] The position, sequence information, target and expected effect of each mutation introduced into the FGF21 protein are listed in Table 1 below (in Table 1, N refers to glycosylated asparagine (N)). Further, FGF21 mutant proteins including the mutations described in Table 1 are listed in Table 2 below.

[118] [Table 1]

Sequence	Position	Original sequence	Mutated sequence	Target	Expected effect
EIRP	98-101	LLLE	EIRP	Substitution with FGF19 sequence	Improvement of stability and pharmacokinetics
TGLEAV	170-174	GPSQG	TGLEAV	Substitution with FGF19 sequence	Improvement of pharmacokinetics
TGLEAN	170-174	GPSQG	TGLEAN	Substitution with FGF19 sequence, anddaddition of N-glycosylation	Improvement of pharmacokinetics
G170N	170	G	N	Point mutation, and addition of N-glycosylation	Improvement of pharmacokinetics
G174N	174	G	N	Point mutation, and addition of N-glycosylation	Improvement of pharmacokinetics
A180E	180	A	E	Point mutation	Improvement of pharmacokinetics

[119]

[120] [Table 2]

SEQ ID NO	Sequence of FGF21 mutant protein
6	FGF21 (EIRP)
7	FGF21 (TGLEAV)
8	FGF21 (TGLEAN)
9	FGF21 (G170N)
10	FGF21 (G174N)
11	FGF21 (EIRP, TGLEAV)
12	FGF21 (EIRP, TGLEAN)
13	FGF21 (EIRP, G170N)
14	FGF21 (EIRP, G174N)
15	FGF21 (EIRP, A180E)
16	FGF21 (TGLEAV, A180E)
17	FGF21 (TGLEAN, A180E)
18	FGF21 (G170N, A180E)
19	FGF21 (G174N, A180E)
20	FGF21 (EIRP, TGLEAV, A180E)
21	FGF21 (EIRP, TGLEAN, A180E)
22	FGF21 (EIRP, G170N, A180E)
23	FGF21 (EIRP, G174N, A180E)

[121]

[122] Expression vectors were prepared to express the amino acids of the three components: fusion carrier, linker and FGF21 mutant in this order from the N-terminus to C-terminus. The material code of each FGF21 mutant fusion protein, sequence of mutation introduced into FGF21, sequence of fusion carrier and linker sequence are listed in Table 3 below (in Table 3, N refers to glycosylated asparagine (N)).

[123] [Table 3]

SEQ ID NO	Material code	Sequence of FGF21 mutation	Fusion carrier	Linker sequence
27	DFD1	EIRP, TGLEAV	hyFc (SEQ ID NO: 26)	C (SEQ ID NO: 2)
28	DFD3	TGLEAV	hyFc (SEQ ID NO: 26)	AKA (SEQ ID NO: 3)
29	DFD4	TGLEAV	hyFc (SEQ ID NO: 26)	GS3 (SEQ ID NO: 4)
30	DFD5	TGLEAN	hyFc (SEQ ID NO: 26)	GS3 (SEQ ID NO: 4)
31	DFD6	G170N	hyFc (SEQ ID NO: 26)	GS3 (SEQ ID NO: 4)
32	DFD6 (*E.* coli)	G170N	hyFc (SEQ ID NO: 26)	GS3 (SEQ ID NO: 4)
33	DFD7	G174N	hyFc (SEQ ID NO: 26)	GS3 (SEQ ID NO: 4)
34	DFD9	none	hyFc (SEQ ID NO: 26)	GS3 (SEQ ID NO: 4)
35	DFD13	EIRP, TGLEAV	hyFc (SEQ ID NO: 26)	GS3 (SEQ ID NO: 4)
36	DFD18	EIRP, TGLEAV, A180E	hyFc (SEQ ID NO: 26)	GS3 (SEQ ID NO: 4)
37	DFD72	EIRP, TGLEAN, A180E	hyFc (SEQ ID NO: 26)	GS3 (SEQ ID NO: 4)
38	DFD73	EIRP, G170N	hyFc (SEQ ID NO: 26)	GS3 (SEQ ID NO: 4)
39	DFD74	EIRP, G170N, A180E	hyFc (SEQ ID NO: 26)	GS3 (SEQ ID NO: 4)
40	RGE (Amgen)	L98R, P171G, A180E	IgG1Fc mutant	GS3 (SEQ ID NO: 4)
41	Fc-FGF21(Lilly	X	IgG4Fc	GS3A (SEQ ID NO:

	)		mutant(SEQ ID NO: 25)	5)

[124]

[125] In order to produce the FGF21 mutant fusion proteins, the nucleotide sequences encoding each of the FGF21 mutant proteins were synthesized by consulting with Bioneer Corporation (Korea) based on the amino acid sequence of each protein. *NheI* and *NotI* restriction enzyme sequences were added to the 5' terminus and 3' terminus of the nucleotide sequences encoding each of the FGF21 mutant proteins and an initiation codon for protein translation and a leader sequence (MDAMLRGLCCVLLLCGAVFVSPSHA) capable of secreting the expressed protein to the outside of a cell were inserted next to the restriction enzyme sequence at the 5' terminus. A termination codon was inserted next to the nucleotide sequence, which encodes each of the FGF21 mutant fusion proteins. The nucleotide sequence encoding each of the FGF21 mutant fusion proteins was cloned into a pTrans-empty expression vector by using the two restriction enzymes of *NheI* and *NotI*. The pTrans-empty expression vector, which has a simple structure including a CMV promoter, a pUC-derived replication origin, an SV40-derived replication origin and an ampicillin-resistant gene, was purchased from CEVEC Pharmaceuticals (Germany).

[126] In the case of the fusion proteins of DFD6 (*E.* coli) and RGE (Amgen), the nucleotide sequence encoding each fusion protein was inserted into a pET30a expression vector for expression in *E. coli*.

[127]

[128] Preparation Example 1-2. Construction of plasmid DNA for expression of FGF21 mutant fusion proteins

[129]

[130] *E.* coli was transformed with each of the expression vectors constructed in Preparation Example 1-1 to obtain a large amount of plasmid DNA to be used for expression. *E.* coli cells, whose cell walls were weakened, were transformed with each expression vector through heat shock, and the transformants were plated out on LB plates to obtain colonies. The colonies thus obtained were inoculated into LB media, cultured at 37℃ for 16 hours, and each *E.* coli culture containing each expression vector was obtained in a volume of 100 mL. The *E.* coli thus obtained was centrifuged to remove the culture medium, and then P1, P2, P3 solutions (QIAGEN, Cat No.:12963) were added to break the cell walls, thereby obtaining a DNA suspension in which proteins and DNAs were separated. Plasmid DNA was purified from the DNA suspension thus obtained by using a Qiagen DNA purification column. The eluted plasmid DNA was identified through an agarose gel electrophoresis, and concen-

trations and purities were measured by using a nanodrop device (Thermo scientific, Nanodrop Lite). The DNA thus obtained was used for expression.

[131]

[132] Preparation Example 1-3. Expression of fusion proteins in CAP-T cells

[133]

[134] Human cell lines were transfected with each plasmid DNA type obtained in Preparation Example 1-2. Each plasmid DNA type was transduced into CAP-T cells (CEVEC), which had been cultured in PEM medium (Life technologies), by using PEI solution (Polyplus, Cat. No.:101-10N). The mixed solution of DNA and the PEI solution was mixed with the cell suspension by using a Freestyle293 expression medium (Invitrogen), cultured at 37℃ for 5 hours, and PEM medium was added. After culturing at 37℃ for 5-7 days, the culture was centrifuged to remove cells and a supernatant including FGF21 mutant fusion proteins was obtained.

[135]

[136] Preparation Example 1-4. Expression and purification of FGF21 mutant fusion proteins in *E.* coli

[137]

[138] *E.* coli strain BL21 (DE3) was transformed with each plasmid DNA expressing DFD6 (*E.* coli) and RGE (Amgen) fusion proteins. The transformed *E.* coli expressing each fusion protein was inoculated into 20 mL of LB media, cultured at 37℃ for 15 hours with shaking, and then a portion of the culture media was inoculated into 100 mL of LB media, and cultured at 37℃ for 16 hours with shaking. Upon completion of culturing, the culture was centrifuged to obtain *E.* coli pellets, and then cells were disrupted using a high pressure cell disruptor to obtain inclusion bodies.

[139] he obtained inclusion bodies were purified by washing and elution, followed by a protein refolding process. Specifically, the obtained inclusion bodies were washed 2-3 times with a buffer solution (pH 8.0) containing 0.5% Triton X-100, 50 mM Tris, 1 mM EDTA and 0.1 M NaCl to remove bacterial protein, and then resuspended in 8 M urea buffer containing 8 M urea, 50 mM Tris and 1 mM DTT. Since the proteins in 8 M urea buffer were completely denatured, a protein refolding process was performed as follows.

[140] To begin, 8 M urea buffer was gradually diluted with 20 mM glycine buffer solution (pH 9.0) to remove urea, and from the concentration of 2 M, $CuSO_4$ was added to the concentration of 80 μM to induce stable protein folding. The protein completing the refolding process was suspended in PBS buffer solution (pH 7.4), and the suspension was filtered with a 0.22 μm filter to remove impurities, and then loaded into a Protein A affinity chromatography column. The column was washed with 1X PBS buffer solution (pH 7.4) and then the proteins were eluted using 100 mM glycine buffer

solution (pH 3.0) to prepare DFD6 (*E.* coli) fusion protein.

[141] In the case of RGE (Amgen) fusion protein, the protein completing the refolding process was suspended in 50 mM Tris buffer solution (pH 8.0), the suspension was filtered with a 0.22 μm filter to remove impurities, and then loaded into an anion exchange resin column (POROS® HQ 50 μm, Thermo Fisher Scientific). The column was washed with 50 mM Tris buffer solution (pH 8.0), and then 50 mM Tris buffer solution (pH 8.0) was administered along the concentration gradient to elute RGE (Amgen) fusion protein. The RGE (Amgen) fusion protein obtained by the anion exchange resin was mixed with ammonium sulfate to the concentration of 1 M, and then purified using a hydrophobic interaction chromatography column (Phenyl sepharose FF, GE Healthcare). Specifically, the column was washed with 50 mM Tris buffer solution (pH 8.0) containing 1 M ammonium sulfate, 50 mM Tris buffer solution (pH 8.0) was administered along the concentration gradient, and the eluted fractions were analyzed through 10% Tris-glycine gel electrophoresis. The gel was dyed with coomassie brilliant blue R with mild shaking, and the fractions containing FGF21 mutant fusion protein with high purity were collected and then dialyzed overnight at 4℃ using a final buffer solution (1X PBS, 1 mM EDTA, pH 7.4). Upon completion of the dialysis, the obtained protein stock solution was concentrated at 3,000 rpm by using a 30,000 MW cut-off centrifugation filter at 4℃. The concentration of FGF21 mutant fusion protein was measured via BCA quantitative analysis.

[142]

[143] Preparation Example 1-5. Purification of FGF21 mutant fusion proteins

[144]

[145] otein A affinity chromatography column (GE Healthcare) was equilibrated with 1X PBS buffer solution (pH 7.4). The culture supernatant including each FGF21 mutant fusion protein obtained in Preparation Example 1-3 was filtered with a 0.2 μm filter, and then loaded into a Protein A affinity chromatography column. The column was washed with 1X PBS buffer solution (pH 7.4) and then proteins were eluted using 100 mM glycine buffer solution (pH 3.0). The fusion proteins obtained by affinity chromatography were purified using an anion exchange resin column (POROS® HQ 50 μm, Thermo Fisher Scientific). The anion exchange resin column was equilibrated with 50 mM Tris buffer solution (pH 8.0), before the FGF21 mutant fusion proteins were eluted from the column. Specifically, after washing the column with 50 mM Tris buffer solution (pH 8.0), 50 mM Tris buffer solution (pH 8.0) was dispensed along the concentration gradient and the eluted fractions were analyzed. Each eluted fraction was analyzed using size exclusion chromatography (SEC-HPLC), and the fractions including FGF21 mutant fusion proteins with high purity were collected. The con-

centration and quantitative analysis were performed in accordance with the methods described in Preparation Example 1-4.

[146]

[147] Experimental Example 1. *In vitro* activities of fusion proteins

[148]

[149] Experimental Example 1-1. Effect of FGF21 mutations on protein activity

[150]

[151] The *in vitro* activities of fusion proteins DFD4, DFD5, DFD6, DFD6 (*E. coli*), DFD7, DFD9, DFD13, DFD18, DFD72, DFD73 and DFD74 prepared in Preparation Example 1 were measured.

[152] Specifically, the *in vitro* FGF21 activities of the fusion proteins were evaluated using a HEK293 cell line (Yuhan Corporation, Korea) which was modified to overexpress human β-klotho, a coreceptor of FGF21. For the evaluation of activity, the concentrates containing the fusion proteins prepared in Preparation Examples 1-4 and 1-5 were subjected to a 3-fold serial dilution at a concentration of 3 μM. After having been cultured in a serum-deficient state for 5 hours, the cell line overexpressing human β-klotho was treated with the diluted fusion proteins for 20 minutes, and then was lysed by adding cytolysis buffer (Cisbio/Cat# 64ERKPEG) with stirring at 60 rpm for 30 minutes at room temperature. The cell lysate solution was mixed with antibodies (Cisbio/Cat# 64ERKPEG), which can detect extracellular signal-regulated kinase (ERK) and phosphorylated ERK, and the mixture was maintained at room temperature for 2 hours. Fluorescence was detected using a fluorometric detector (TECAN/GENiosPro). The activities of the fusion proteins were measured by comparing their EC_{50} values.

[153] As shown in FIGS. 1A to 1C, it was confirmed that the *in vitro* activities of the fusion proteins prepared by introducing mutation sequences into the wild-type FGF21 protein were not inhibited, and the activities of each fusion protein were similar to each other. It was also confirmed that through the DFD6 (*E.* coli) sample expressed in *E.* coli and the DFD6 sample expressed in animal cells, the *in vitro* activities of the fusion proteins prepared by introducing N-glycosylation mutation into the wild-type FGF21 protein were not inhibited.

[154]

[155] Experimental Example 1-2. Effect of linker sequence on protein activity

[156]

[157] The *in vitro* activities of fusion proteins DFD1, DFD3, DFD4 and DFD13 prepared in Preparation Example 1 were measured.

[158] Specifically, the FGF21 activities of the fusion proteins were measured by using the concentrates containing the fusion proteins prepared in Preparation Example 1-5 in ac-

cordance with the methods described in Experimental Example 1-1. The results are shown in FIGS. 2A and 2B.

[159] It was confirmed that no FGF21 mutant fusion protein showed a significant decrease in the activity, although a slight difference was shown in the activity depending on the linker sequence, as shown in FIGS. 2A and 2B.

[160]

[161] Experimental Example 1-3. Experimental results for DFD1, RGE (Amgen) and Fc-FGF21 (Lilly)

[162]

[163] The *in vitro* activities of fusion protein DFD1 prepared in Preparation Example 1 and control proteins RGE (Amgen) and Fc-FGF21 (Lilly) were measured.

[164] Specifically, the FGF21 activities of the fusion proteins were measured by using the concentrates containing the fusion proteins prepared in Preparation Example 1-5 and the control proteins in accordance with the methods described in Experimental Example 1-1. The results are shown in FIG. 3.

[165] It was confirmed that DFD1 and RGE (Amgen) had similar *in vitro* activity, while Fc-FGF21 (Lilly) had *in vitro* activity two times higher than those of the other proteins, as shown in FIG. 3.

[166]

[167] Experimental Example 2. Evaluation of stability of fusion proteins

[168]

[169] Experimental Example 2-1. Experimental method for evaluating stability

[170] In order to measure the quantity of protein aggregates at the initial stage of the sample preparation, high molecular weight aggregates (%HMW) were quantified using a size-exclusion chromatography (SEC-HPLC) method. The results are shown in FIG. 4.

[171] Specifically, a TosoHaas model TSK-GEL G3000SW$_{XL}$ column was used for the SEC-HPLC method. The column was equilibrated by flowing a buffer solution (1X PBS, 1 mM EDTA, pH 7.4) at a flow rate of 1 mL/min. The DFD4 and DFD13 protein stock solutions prepared in Preparation Examples 1-5 were concentrated to a target concentration of 20 mg/mL or higher at 3,000 rpm using a 30,000 MW cut-off centrifugation filter at 4℃. After the measurement of the concentration of each sample by BCA quantitative analysis, the samples were diluted with a buffer solution (1X PBS, 1 mM EDTA, pH 7.4) to a final concentration of 20 mg/mL. In order to measure the initial %HMW of DFD4 and DFD13, 20 mg/mL of the samples were diluted with the buffer solution (1X PBS, 1 mM EDTA, pH 7.4) to a final concentration of 1 mg/mL, and each sample in a volume of 100 ㎕ was analyzed by SEC-HPLC column.

[172] For the stability evaluation of each sample, %HMW of the samples was measured

using the SEC-HPLC method on the 4th, the 8th and the 14th days while storing them at 5℃, 25℃ and 37℃ for two weeks.

[173] As shown in FIG. 4, it was confirmed that DFD13 had a lower quantity of high molecular weight aggregates (HMW %) at the initial stage and up to the point of 2 weeks as compared with DFD4, indicating that the introduction of the EIRP mutation improves the stability of the FGF21 mutant fusion protein, thereby reducing HMW % significantly.

[174]

[175] Experimental Example 2-2. Stability results

[176]

[177] In order to investigate the effects of the EIRP mutation introduced into the original sequence LLLE (98-101) of FGF21 on stability, the stability of DFD4 (SEQ ID NO: 29) and DFD13 (SEQ ID NO: 35) was measured in accordance with the methods described in Experimental Example 2-1. The analysis results for the zero-hour sample (initial stage; Day 0) and 4-, 8-, and 14 day-stored samples of DFD4 and DFD13 are summarized in Table 4 below (in Table 4, N.D. means "not detected").

[178] [Table 4]

Stability of DFD4 and DFD13 for 2 weeks at a concentration of 20 mg/mL (%HMW)

	DFD4			DFD13		
Day	5℃	25℃	37℃	5℃	25℃	37℃
0	0.91			0.56		
4	4.25	11.64	5.12	0.36	0.34	0.84
8	6.16	9.99	4.87	N.D.	N.D.	N.D.
14	8.15	8.83	4.71	N.D.	N.D.	0.32

[179]

[180] As shown in Table 4, the quantity of %HMW at the initial stage (Day 0) was 0.91% for DFD4, and 0.56% for DFD13. After 2 weeks, the amount of %HMW increased to 8.83% for DFD4, but it was not observed in DFD13, under the condition of storage at 25℃. DFD13 was shown to have a lower %HMW rate at the initial stage and 2 weeks, as compared with DFD4, which indicates that the %HMW rate of FGF21 mutant fusion protein decreased significantly due to the introduction of the EIRP mutation.

[181]

[182] Experimental Example 3. Pharmacokinetic assessment of fusion proteins

[183]

[184] Experimental Example 3-1. Experimental method for pharmacokinetic assessment

[185]

[186] Six-week old male ICR mice purchased from Orient BIO (Korea) were partitioned into groups (n = 3/blood sampling time) in order to have similar mean values for body weight one day before drug treatment, and subcutaneously administered once with a respective sample at 1 mg/kg (2 mg/kg for RGE). Blood samples were then collected at 1, 4, 8, 12, 24, 48, 72, and 96 hours after the injection, respectively. The concentration of intact full length FGF21 protein in the blood was measured using a Intact human FGF21 ELISA Kit (F1231-K01, Eagle Biosciences, USA), which has immunore-activity to the N-terminus and C-terminus of FGF21 protein. The concentrations of the samples in the blood collected until 96 hours after the subcutaneous injection of each fusion protein into the mice were measured, and pharmacokinetic parameters of each sample were calculated.

[187]

[188] Experimental Example 3-2. Assessment of pharmacokinetic activity

[189] Based on the graph showing the concentrations of each protein in the blood versus time after the subcutaneous administration of fusion proteins in mice (FIG. 5), the pharmacokinetic parameters were calculated. The data are shown in Table 5 below.

[190] [Table 5]

Parameters	DFD4	DFD5	DFD6	DFD7	DFD9	DFD13	DFD18	DFD72	DFD73	DFD74	DFD6 (*E.coli*)	RGE*
T_{max} (hour)	12	12	12	4	4	12	12	8	8	8	8	12
C_{max} (ng/mL)	1288	1732	2868	696	384	1070	3428	2962	3296	3996	1399	9921
AUC_{last} (ng·hr/mL)	258556	40706	100107	14118	4656	28785	104230	115977	123511	206634	37269	325747
Half-life (hour)	5.5	8.0	14.9	19.7	17.4	7.1	11.0	14.4	16.6	26.0	9.1	12.9

[191]

[192] The pharmacokinetic profile of each fusion protein was compared and evaluated based on the value of the area under the curve (AUC) indicating the degree of drug exposure.

[193] As shown in Table 5, upon comparing DFD4 with DFD13, and DFD6 with DFD73, it was determined that the introduction of the EIRP sequence resulted in an approximate 10 to 20% increase in AUC value. Comparing DFD9 with DFD4, the introduction of TGLEAV resulted in an approximate 6-fold increase in AUC value.

[194] Furthermore, the mutations of TGLEAN, G170N and G174N are designed to extend the half-life by introducing N-glycosylation into the C-terminus of FGF21, which is known to be proteolyzed *in* vivo. The increase in AUC due to the introduction of N-glycosylation was confirmed by comparing the mutants with each control material. In order to confirm the effect of improvement in AUC due to the introduction of N-glycosylation, the AUC value for DFD6 (*E. coli*) produced by *E.* coli which has no glycosylation was compared with that in DFD6 produced by a human cell line. DFD6 produced by the human cell line showed a 3-fold or higher increase in the AUC value as compared with DFD6 (*E. coli*) produced by *E.* coli, which demonstrated an improvement of pharmacokinetic profile due to glycosylation.

[195] The A180E is a mutation disclosed in WO 2009/149171 owned by Amgen Inc. When the mutation of A180E was further introduced into the mutant DFD13 or DFD73 including the mutation of TGLEAV or G170N, respectively, the resulting mutant DFD18 or DFD74, respectively, showed an approximate 2- to 3-fold additional increase in AUC value.

[196] In summary, it was confirmed that the pharmacokinetic parameters were improved by the introduction of various mutations and combinations thereof, as compared with DFD9, the wild-type FGF21 fusion protein. The fusion protein showing the most improved AUC value was DFD74 containing the mutations of EIRP, G170N and A180E, which showed an approximate 45-fold improvement in AUC value as compared with DFD9. Furthermore, considering RGE (Amgen) at the dose of 2 mg/kg of body weight, DFD74 may have a higher degree of drug exposure as compared with RGE. The overall effects of improvement in pharmacokinetics due to the mutations are summarized in Table 6 below.

[197] [Table 6]

Mutation sequence	Position of mutation	Control material *vs* improved material	Assessment of pharmacokinetic parameters
EIRP	98-101	DFD4 *vs* DFD13	Improvement of AUC
		DFD6 *vs* DFD73	
TGLEAV	170-174	DFD9 *vs* DFD4	Improvement of AUC
TGLEAN	170-174	DFD9 *vs* DFD5	Improvement of AUC
G170N	170	DFD9 *vs* DFD6	Improvement of AUC
		DFD6 (*E.* coli) *vs* DFD6	Improvement of AUC
G174N	174	DFD9 *vs* DFD7	Improvement of AUC
A180E	180	DFD13 *vs* DFD18	Improvement of AUC
		DFD73 *vs* DFD74	Improvement of AUC

[198]

[199] Experimental Example 4. Activity evaluation of fusion proteins in *ob/ob* mice

[200]

[201] Experimental Example 4-1. Experimental method for evaluating activity in *ob/ob* mice

[202]

[203] The *ob/ob* mice, characterized as exhibiting hyperglycemia, insulin resistance, hyperphagia, fatty liver and obesity due to a genetic deficiency in leptin, are widely used for the study of type 2 diabetes. Male *ob/ob* mice (Harlan, USA) were purchased from Raonbio (Korea). These mice were 5 to 6 weeks old at the time of arrival, and 8 to 9 weeks old at the time of drug treatment after 3 weeks of adaptation. The mice were partitioned into groups (n=8/group) in order to have similar mean values for body weight and caudal blood glucose levels one day before the drug treatment (Day 0), and the samples were subcutaneously administered once according to each of their respective dosages. Dulbecco's phosphate buffered saline (DPBS, Gibco, USA) was administered as the vehicle treatment, and the glucose concentration in the blood was measured using a glucose meter, GlucoDr (All Medicus, Korea). The non-fasting glucose levels and body weights were measured every day until the 14th day after administration. Glycated hemoglobin levels were also measured in each group before the administration and after the test. The glycated hemoglobin levels were calculated using a DCA 2000 HbA1c kit (Siemens, 5035C).

[204]

[205] Experimental Example 4-2. Evaluation of activity in *ob/ob* mice

[206]

[207] The changes in non-fasting blood glucose levels and body weights in male *ob/ob* mice were observed after single subcutaneous injection of 30 or 100 nmol/kg of DFD18 and DFD72, or 10, 30 or 100 nmol/kg of DFD74.

[208] It was confirmed that DFD18, DFD72 and DFD74 all had the effect of lowering blood glucose level in a dose-dependent manner. Comparing the three agents at the high dose of 100 nmol/kg, DFD72 and DFD74 showed an improved effect on lowering blood glucose level than DFD18 (FIG. 6). In addition, Fc-FGF21 (Lilly) which was used as a control material in the test, was less effective in lowing blood glucose level as compared with DFD18, DFD72 and DFD74 at the same dose level (30 nmol/kg).

[209] As for the effect on body weight reduction, comparing the three agents at the high dose of 100 nmol/kg, DFD72 was the most effective in *ob/ob* mice resulting in an approximate 6% reduction in body weight, and DFD18 was the next most effective, followed by DFD74 (FIG. 7).

[210] After the termination of the test, the glycated hemoglobin levels indicative of the mean values of blood glucose were measured and the changes in mean blood glucose were analyzed in each test group. All of the treated groups except the control group treated with control protein Fc-FGF21 (Lilly) showed negative values in the differences between before administration and after the test, which confirmed the effectiveness of the test proteins as compared with the control material in lowering blood glucose (FIG. 8).

[211]

[212] Experimental Example 5. Activity evaluation of fusion proteins in HFD/STZ mice

[213]

[214] Experimental Example 5-1. Experimental method for evaluating activity in HFD/STZ mice

[215]

[216] The effects of the FGF21 mutant fusion proteins on lowering blood glucose and body weight were compared and evaluated in another diabetic model, the HFD/STZ mouse model. Conventional dietary-induced obesity mouse models (induced by feeding 60 kcal% high fat diet to C57BL/6 mice for eight weeks or longer) have weak hyperglycemic and diabetic features, although they invoke insulin resistance. The HFD/STZ mice, which may compensate for defects in the conventional dietary-induced obesity mouse models, are capable of producing dysfunctional β cells in the pancreas and decreased secretion of insulin as a result of a high fat diet (HFD) and administration of low level streptozotocin (STZ), and are therefore useful for pharmacological studies of type 2 diabetes.

[217] Specifically, in order to induce the HFD/STZ mouse model, C57BL/6 mice (Japan

SLC) were fed on a 60 kcal% high fat diet for four weeks, and then 50 mg/kg of STZ (Sigma, 85882) was administered intraperitoneally every day for 3 days to induce dysfunction in the β cells of the pancreas. After feeding on the high fat diet for an additional 2 weeks, the mice with non-fasting blood glucose levels of 200 mg/dL or higher were used for the test. The mice were partitioned into groups (n=6/group) in order to have similar mean values of body weight and caudal blood glucose levels one day before the drug treatment (Day 0), and the samples were subcutaneously administered once according to each of their respective dosages. Dulbecco's phosphate buffered saline (DPBS, Gibco, USA) was administered as the vehicle treatment, and the glucose concentration in the blood was measured using a glucose meter, GlucoDr (All Medicus, Korea). The non-fasting glucose levels and body weights were measured every day until the 14th day after administration. Glycated hemoglobin levels were also measured in each group before the administration and after the test. The glycated hemoglobin levels were calculated using a DCA 2000 HbA1c kit (Siemens, 5035C).

[218]

[219] Experimental Example 5-2. Activity evaluation in HFD/STZ mice

[220]

[221] The changes in non-fasting blood glucose levels and body weights over time in male HFD/STZ mice were observed after single subcutaneous injection of 10 nmol/kg of DFD72 or DFD74.

[222] Regarding the changes in non-fasting blood glucose levels, it was confirmed that DFD72 and DFD74 had similar effects on lowering blood glucose levels, and the blood glucose lowering effect was maintained until the 10th day after administration and then lost with metabolism of the drugs after the 10th day (FIG. 9). DFD72 showed a more prolonged effect than DFD74 in terms of changes in non-fasting blood glucose levels after the 10th day after administration.

[223] In terms of the effect on body weight reduction due to the administration of FGF21 mutant proteins, it was confirmed that both DFD72 and DFD74 had similar effects on reducing body weight by approximately 5%, and the effect disappeared after the 10th day after administration (FIG. 10).

[224] After the termination of the test, the glycated hemoglobin levels indicative of the mean value of blood glucose were measured and the changes in mean blood glucose were analyzed in each test group. While the vehicle group had an increase of 0.25 in glycated hemoglobin levels, the group treated with DFD74 had an increase of 0.1 and the group treated with DFD72 had an decrease of 0.27 (FIG. 11).

[225]

[226] Experimental Example 6. Activity of fusion proteins in diet-induced obese mice

[227]

[228] Experimental Example 6-1. Experimental method for evaluating activities in diet-induced obese mice

[229]

[230] The body weight-reduction effect of DFD18, an FGF21 mutant fusion protein, was evaluated in diet-induced obese mice. For the diet-induced obesity model, C57BL/6J mice were purchased from Central Lab. Animal Inc. and fed on a high-fat diet containing 60 kcal % fat (Research diet) for 8 to 12 weeks. The mice were partitioned into groups (n=8/group) in order to have a similar mean value of body weight one day before the drug treatment (Day 0), and then 30 nmol/kg of samples were subcutaneously administered once. The changes in body weights were compared with the group treated with vehicle (PBS).

[231]

[232] Experimental Example 6-2. Protein activity in diet-induced obese mice

[233]

[234] For changes in body weight over time in the diet-induced obesity mouse model following single administration of 30 nmol/kg DFD18, it was confirmed that the weight-reducing effect was continuing by the 10^{th} day after the administration, and the maximum weight reduction (about 18%) was at the 11^{th} day after the administration, which was maintained by the 14^{th} day (FIG. 12).

[235]

[236] Preparation Example 2. Preparation and purification of dual function proteins

[237]

[238] Preparation Example 2-1. Preparation of expression vectors for expression of dual function proteins

[239]

[240] In order to identify the effects of the sequence of the GLP-1 mutant protein and the sequence of the Fc hinge fused thereto on the *in vitro* activity, pharmacokinetic profiles and pharmacological efficacy, various sequences for the Fc-fused GLP-1 mutant proteins were designed. The sequences of the GLP-1 mutant proteins are listed in Table 7 below, and the sequences of Fc-fused GLP-1 mutants are listed in Table 8.

[241] [Table 7]

SEQ ID NO	Sequence of GLP-1 mutant protein
43	GLP-1(A2G)
44	GLP-1(GE)
45	GLP-1(GG)
46	GLP-1(GEG)

[242]

[243] [Table 8]

SEQ ID NO	Fc-fused GLP-1 mutant protein
49	DFD52: GLP1(A2G)-HyFc5
50	DFD53: GLP1(A2G)-HyFc40
51	DFD54: GLP1(GE)-HyFc5
52	DFD55: GLP1(GE)-HyFc40
53	DFD56: GLP1(GG)-HyFc5
54	DFD57: GLP1(GG)-HyFc40
55	DFD58: GLP1(GEG)-HyFc5
56	DFD59: GLP1(GEG)-HyFc40

[244]

[245] In Table 8, HyFc5 refers to SEQ ID NO: 47, and HyFc40 refers to SEQ ID NO: 48.

[246] In order to investigate the effects of the sequences of the GLP-1 mutant proteins and FGF21 mutant proteins, the sequence of the Fc hinge fused to the GLP-1 mutants, the sequence of the linker connected between the FGF21 mutant proteins and Fc on the *in vitro* activity, pharmacokinetic profiles and pharmacological efficacy, various sequences for the dual function proteins were designed. The sequences of the dual function proteins including the GLP-1 mutant proteins and FGF21 mutant proteins are listed in Table 9 below. Each dual function protein contains a GLP-1 mutant protein, an Fc region of an immunoglobulin, a linker and an FGF21 mutant protein connected in this order from the N-terminus to C-terminus.

[247] [Table 9]

SEQ ID NO	Material code	Sequence of GLP-1 mutant protein	Fusion carrier	Linker sequence	Changes in FGF21 sequence
58	DFD23	GLP-1(A2G)	hyFc40(SEQ ID NO: 48)	GS3(SEQ ID NO: 4)	FGF21(EIRP, TGLEAV)
59	DFD24	GLP-1(GE)	hyFc5(SEQ ID NO: 47)	GS3(SEQ ID NO: 4)	FGF21(EIRP, TGLEAV)
60	DFD25	GLP-1(GE)	hyFc40(SEQ ID NO: 48)	GS3(SEQ ID NO: 4)	FGF21(EIRP, TGLEAV)
61	DFD26	GLP-1(GG)	hyFc5(SEQ ID NO: 47)	GS3(SEQ ID NO: 4)	FGF21(EIRP, TGLEAV)
62	DFD27	GLP-1(GG)	hyFc40(SEQ ID NO: 48)	GS3(SEQ ID NO: 4)	FGF21(EIRP, TGLEAV)
63	DFD28	GLP-1(GEG)	hyFc5(SEQ ID NO: 47)	GS3(SEQ ID NO: 4)	FGF21(EIRP, TGLEAV)
64	DFD29	GLP-1(GEG)	hyFc40(SEQ ID NO: 48)	GS3(SEQ ID NO: 4)	FGF21(EIRP, TGLEAV)
65	DFD69	GLP-1(GEG)	hyFc40(SEQ ID NO: 48)	GS3(SEQ ID NO: 4)	FGF21(EIRP, TGLEAV, A180E)
66	DFD112	GLP-1(GEG)	hyFc40(SEQ ID NO: 48)	GS3(SEQ ID NO: 4)	FGF21(EIRP, TGLEAN, A180E)
67	DFD114	GLP-1(GEG)	hyFc40(SEQ ID NO: 48)	GS3(SEQ ID NO: 4)	FGF21(EIRP, G170N, A180E)

[248]

[249] Specifically, the nucleotide sequences encoding each of the dual function proteins were synthesized after consulting with Bioneer Corporation (Korea) based on the amino acid sequence of each protein. *NheI* and *NotI* restriction enzyme sequences were added to the 5' terminus and 3' terminus of the nucleotide sequences encoding each of the dual function proteins and an initiation codon for protein translation and a leader sequence (MDAMLRGLCCVLLLCGAVFVSPSHA) enabling secretion of the expressed protein to the outside of a cell were inserted next to the restriction enzyme

sequence at the 5' terminus. A termination codon was inserted next to the nucleotide sequence, which encodes each of the dual function proteins. The nucleotide sequence encoding each of the dual function proteins was cloned into a pTrans-empty expression vector by using the two restriction enzymes *NheI* and *NotI*. The pTrans-empty expression vector, which has a simple structure including a CMV promoter, a pUC-derived replication origin, an SV40-derived replication origin and an ampicillin-resistance gene, was purchased from CEVEC Pharmaceuticals (Germany).

[250]

[251] Preparation Example 2-2. Construction of plasmid DNA for expression of Fc-fused GLP-1 mutant and dual function proteins

[252]

[253] *E.* coli was transformed with each of the expression vectors constructed in Preparation Example 2-1 to obtain a large quantity of plasmid DNA to be used for expression. *E.* coli cells, with cell walls weakened through heat shock, were transformed with each expression vector, and the transformants were plated out on an LB plate to obtain colonies. The colonies thus obtained were inoculated into LB media, cultured at 37°C for 16 hours, and each *E.* coli culture containing each expression vector was obtained in a volume of 100 mL. The *E.* coli thereafter obtained was centrifuged to remove the culture medium, and then P1, P2, P3 solutions (QIAGEN, Cat No.:12963) were added to break the cell walls, thereby obtaining a DNA suspension in which proteins and DNA were separated. Plasmid DNA was purified from the DNA suspension thus obtained by using a Qiagen DNA purification column. The eluted plasmid DNA was identified by agarose gel electrophoresis, and the concentrations and purities were measured using a nanodrop device (Thermo Scientific, Nanodrop Lite). The DNA thus obtained was used for expression.

[254]

[255] Preparation Example 2-3. Expression of Fc-fused GLP-1 mutants and dual function proteins in CAP-T cells

[256]

[257] Human cell lines were transformed with each plasmid DNA obtained in Preparation Example 2-2. Each plasmid DNA type was transduced into CAP-T cells (CEVEC), which had been cultured in PEM medium (Life Technologies), by using a PEI solution (Polyplus, Cat. No.:101-10N). The mixed solution of DNA and the PEI solution was mixed with the cell suspension using Freestyle293 expression medium (Invitrogen), cultured at 37°C for 5 hours, and PEM medium was added. After culturing at 37°C for 5-7 days, the culture was centrifuged to remove cells and supernatant containing each protein was obtained.

[258]

[259] Preparation Example 2-4. Purification of Fc-fused GLP-1 mutants and dual function proteins

[260]

[261] Protein A affinity chromatography column (GE Healthcare) was equilibrated with 1X PBS buffer solution (pH 7.4). The culture supernatant including each of the Fc-fused GLP-1 mutants and dual function proteins obtained in Preparation Example 2-3 was filtered with a 0.2 μm filter, and then loaded into a Protein A affinity chromatography column. The column was washed with 1X PBS buffer solution (pH 7.4) and then the proteins were eluted using 100 mM glycine buffer solution (pH 3.0). The proteins obtained by affinity chromatography were purified using an anion exchange resin column (POROS® HQ 50 μm, Thermo Fisher Scientific). The anion exchange resin column was equilibrated with 50 mM Tris buffer solution (pH 8.0), before the proteins eluted from the affinity chromatography were loaded thereto.

[262] After washing the column with 50 mM Tris buffer solution (pH 8.0), 50 mM Tris buffer solution (pH 8.0) was dispensed along the concentration gradient and the eluted fractions were analyzed. Each eluted fraction was analyzed by using size exclusion chromatography (SEC-HPLC), and the fractions including the Fc-fused GLP-1 mutants and dual function proteins with high purity were collected and dialyzed overnight at 4℃ using a final buffer solution (1X PBS, 1 mM EDTA, pH 7.4). Upon completion of the dialysis, the obtained protein stock solution was concentrated at 3,000 rpm using a 30,000 MW cut-off centrifugation filter at 4℃. The concentration of each protein was measured via BCA quantitative analysis.

[263]

[264] Experimental Example 7. *In vitro* activity of dual function proteins

[265]

[266] Experimental Example 7-1. Activity of DFD23, DFD24, DFD25, DFD26, DFD27, DFD28 and DFD29

[267]

[268] The *in vitro* GLP-1 activities of the dual function proteins DFD23, DFD24, DFD25, DFD26, DFD27, DFD28 and DFD29 were measured. Specifically, a CHO cell line (Eurofins, HTS163C2), overexpressing the human GLP-1 receptor was purchased and used to evaluate the GLP-1 activities of the dual function proteins. For the evaluation of activity, samples containing the fusion proteins (protein stock solutions prepared in Preparation Example 2-4; hereinafter, "sample") were subjected to a 4-fold serial dilution at a concentration of 25 nM. After the human GLP-1 receptor-overexpressing CHO cell line was treated for 30 minutes, the intracellular cAMP produced was measured (Cisbio, 62AM4PEB). The activity of each protein was evaluated by comparing the EC_{50} values.

[269] As shown in FIG. 13, the dual function protein containing the GLP-1 (A2G) sequence showed activity approximately 2~3 times lower than that for the dual function proteins containing other GLP-1 mutant sequences. No significant difference in GLP-1 activities was observed between the dual function proteins containing the mutation sequences except the GLP-1 (A2G) sequence.

[270]

[271] Experimental Example 7-2. Activities of DFD59, DFD69, DFD112 and DFD114

[272]

[273] The *in vitro* GLP-1 activities of the dual function proteins DFD69, DFD112 and DFD114 prepared in Preparation Example 2 and DFD59 (an Fc-fused GLP-1 mutant) were measured. Specifically, a CHO cell line (Eurofins, HTS163C2) overexpressing the human GLP-1 receptor was purchased and used to evaluate the GLP-1 activities of the dual function proteins. For the evaluation of activity, the sample containing each of the fusion proteins was subjected to a 4-fold serial dilution at a concentration of 25 nM. After the human GLP-1 receptor-overexpressing CHO cell line was treated for 30 minutes, the intracellular cAMP produced was measured (Cisbio, 62AM4PEB).

[274] As shown in FIG. 14, the activity of each protein was evaluated by comparing the EC_{50} value. The three dual function proteins showed similar EC_{50} values, and DFD59 (containing no FGF21 mutant) showed activity approximately 2 times higher than that of the dual function proteins.

[275] Next, the *in vitro* activities of the FGF21 portion in DFD69, DFD112 and DFD114 were measured. Specifically, the *in vitro* activities of the FGF21 portion in the dual function proteins were evaluated using a HEK293 cell line overexpressing human β-klotho (a co-receptor of FGF21). For the evaluation of activity, samples containing each of the dual function proteins were subjected to a 3-fold serial dilution at a concentration of 3 μM. After having been cultured in a serum-deficient state for 5 hours, the human β-klotho-overexpressing HEK293 cell line was treated for 20 minutes, before the cells were lysed by adding cytolysis buffer (Cisbio/Cat# 64ERKPEG) with stirring at 60 rpm for 30 minutes at room temperature. The cell lysate solution was mixed with antibodies which can detect ERK and phosphorylated ERK, and the mixture was maintained at room temperature for 2 hours. Fluorescence was detected using a fluorometric detector (TECAN/GENiosPro). The activities were measured by comparing their EC_{50} values.

[276] It was confirmed that the *in vitro* activities of the FGF21 portion of the dual function proteins DFD69, DFD112 and DFD114 were similar, as shown in FIG. 14.

[277]

[278] Experimental Example 8. Pharmacokinetic assessment of dual function proteins

[279]

[280] Experimental Example 8-1. Experimental method for pharmacokinetic assessment

[281]

[282] Six-week old male ICR mice purchased from Orient BIO (Korea) were partitioned into groups (n = 3/blood sampling time) in order to have a similar mean value of body weight one day before drug treatment, and subcutaneously administered once with a respective sample in a volume of 1 mg/kg. The blood samples were collected at 1, 4, 8, 12, 24, 48, 72, 96, 144, 192 and 240 hours after the injection, respectively. The concentration of each dual function protein in the blood was measured based on the FGF21 portion and the GLP-1-Fc portion separately. The concentration of the intact full length FGF21 portion of the dual function protein in the blood was measured using an Intact human FGF21 ELISA Kit (F1231-K01, Eagle Biosciences, USA), which has immunoreactivity to the N-terminus and C-terminus of FGF21 protein. Further, the concentration of the active GLP-1-Fc portion of the dual function protein in the blood was measured using an antibody, which has immunoreactivity to the N-terminus of GLP-1 and Fc, as determined through ELISA analysis. The concentrations of the FGF21 and GLP-1-Fc portions of each protein in the blood samples collected until 240 hours after single subcutaneous injection of each protein into the mice were measured, and the pharmacokinetic parameters of each protein was calculated.

[283]

[284] Experimental Example 8-2. Pharmacokinetic activity results

[285] Based on the concentration of each active substance in the blood over time after single subcutaneous administration of each protein in mice (FIG. 15), pharmacokinetic parameters for the FGF21 and GLP-1-Fc portions of the dual function proteins were calculated. The data are shown in Table 10 below.

[286] [Table 10]

	FGF21 detection			GLP-1-Fc detection			
Parameter	DFD69	DFD112	DFD114	DFD59	DFD69	DFD112	DFD114
T_{max} (hour)	8	8	24	4	4	8	4
C_{max} (ng/mL)	2715	3619	3711	5202.1	3234	4454	3616
AUC_{last} (ng·hr/mL)	100907	144395	222504	182852	149083	189338	171687
Half-life (hour)	13.4	14.2	39.9	20.7	23.3	24.7	27.2

[287]

[288] The pharmacokinetic profiles of each dual function protein were compared and evaluated based on the value of the area under the curve (AUC), indicating the degree

of drug exposure.

[289] As shown in Table 10, for the pharmacokinetic parameters of the FGF21 portion, DFD114 showed the highest degree of drug exposure (AUC) and half-life, and DFD112 showed the next highest AUC value, followed by DFD69. DFD114 exhibited an approximate 2-fold or higher increase in AUC value as compared with DFD69. For the pharmacokinetics of the GLP-1-Fc portion, the four proteins (DFD59, DFD69, DFD112 and DFD114) containing the same GLP-1 mutant sequence showed similar AUC values.

[290]

[291] Experimental Example 9. Activity evaluation in *db/db* mice

[292]

[293] Experimental Example 9-1. Method for evaluating activities in *db/db* mice

[294]

[295] The *db/db* mice, characterized as having hyperglycemia, insulin resistance, hyperphagia, fatty liver and obesity due to a genetic deficiency for the leptin receptor and exhibiting more serious hyperglycemia and obesity than *ob/ob* mice, are widely used for the study of type 2 diabetes. Male *db/db* mice (Harlan, USA) were purchased from Raonbio (Korea). These mice were 5 to 6 weeks old at the time of arrival, and 8 to 9 weeks old at the time of drug treatment, after 3 weeks of adaptation. The mice were partitioned into groups (n=6/group) in order to have a similar mean value of body weight and caudal blood glucose levels one day before the drug treatment (Day 0), and the samples were subcutaneously administered once according to each of their respective dosages. Dulbecco's phosphate buffered saline (DPBS, Gibco, USA) was administered as the vehicle treatment, and the glucose concentration in the blood was measured using a glucose meter, GlucoDr (All Medicus, Korea). The non-fasting glucose levels and body weights were measured every day until the 14th day after administration. Glycated hemoglobin levels were also measured in each group before the administration and after the test. The glycated hemoglobin levels were calculated using a DCA 2000 HbA1c kit (Siemens, 5035C).

[296]

[297] Experimental Example 9-2. Evaluation of activity in *db/db* mice

[298]

[299] The changes in non-fasting blood glucose levels and body weights in male *db/db* mice were observed after single subcutaneous injection of 10 or 30 nmol/kg of dual function protein DFD114, single subcutaneous injection of 30 nmol/kg of long-acting GLP-1-Fc single function protein DFD59, and combined administration of 30 nmol/kg of DFD59 and DFD74(which are GLP-1-Fc and Fc-FGF21 single function proteins, respectively) to compare the effect of the dual function protein DFD114 with combined

administration of Fc-FGF21 and GLP-1-Fc single function proteins.

[300] The long-acting GLP-1-Fc protein DFD59 caused a sharp reduction in blood glucose levels by the 1st day after administration, but the reduction in blood glucose decreased after the 2nd day and the blood glucose level was similar to that of the vehicle-treated group after the 4th day. Meanwhile, the group treated with DFD114 showed excellent effects on blood glucose reduction by the 3rd day after administration, and the effects on lowering blood glucose level disappeared more rapidly after the 4th day from the administration at the dose of 10 nmol/kg than for 30 nmol/kg, indicating dose-dependent differences in the duration of the blood glucose lowering effect. The groups treated with combined administration of each protein showed the most sustained effects for lowering blood glucose levels as compared with those of the other groups, indicating that the combination of GLP-1 and FGF21 had an excellent effect on controlling blood glucose level (FIG. 16).

[301] As for the effect on body weight reduction, the groups treated with a combination of DFD59 and DFD74 showed the greatest effects on reducing body weight, and the group treated with 30 nmol/kg of DFD114 also showed an outstanding effect on reducing body weight (FIG. 17).

[302] After the termination of the tests, the glycated hemoglobin levels indicative of the mean value of blood glucose were measured and the changes in mean blood glucose were analyzed in each test group. As shown in FIG. 18, the group treated with vehicle showed increased glycated hemoglobin levels after the termination of the tests as compared with the group before the administration, and the group treated with DFD59 showed a similar increase. The group treated with 30 nmol/kg of DFD114 showed the greatest decrease in glycated hemoglobin levels, and the group receiving combined administration showed the next highest effectiveness, followed by the group treated with 10 nmol/kg of DFD114. When evaluating the proteins by comparing them based on the decrease in glycated hemoglobin levels in each group treated, it was confirmed that the dual function protein DFD114 showed a stronger effect on lowering blood glucose level than GLP-1-Fc or Fc-FGF21 single function protein alone.

[303]

[304] Experimental Example 10. Activity of fusion proteins in HFD/STZ mice

[305]

[306] Experimental Example 10-1. Experimental method for evaluating activities in HFD/STZ mice

[307]

[308] The effects of the dual function proteins on lowering blood glucose and body weight were compared and evaluated in another diabetic model, the HFD/STZ mouse model.

[309] The conventional dietary-induced obesity mouse model (induced by feeding 60

kcal% high fat diet to C57BL/6 mice for eight weeks or longer) has weak hyper-glycemic and diabetic features, although invokes insulin resistance. The HFD/STZ mice, which may compensate for the deficiencies of the conventional dietary-induced obesity mouse model, are capable of generating dysfunctional β cells of the pancreas and decreased secretion of insulin following a high fat diet (HFD) and administration of low level streptozotocin (STZ), and are used for pharmacological studies of type 2 diabetes. In order to induce the HFD/STZ mouse model, C57BL/6 mice were fed on a 60 kcal% high fat diet for four weeks, and then 50 mg/kg of STZ (Sigma, 85882) was administered intraperitoneally every day for 3 days to induce dysfunction of the β cells of the pancreas. After feeding on the high fat diet for an additional 2 weeks, the mice with non-fasting blood glucose levels of 200 mg/dL or higher were selected for the test. The mice were partitioned into groups (n=6/group) in order to have a similar mean value of body weight and caudal blood glucose levels one day before the drug treatment (Day 0), and the samples were subcutaneously administered once according to each of their respective dosages. Dulbecco's phosphate buffered saline (DPBS, Gibco, USA) was administered as the vehicle treatment, and the glucose concentration in the blood was measured using a glucose meter, GlucoDr (All Medicus, Korea). The non-fasting glucose levels and body weights were measured every day until the 14th day after administration. Glycated hemoglobin levels were also measured in each group before the administration and after the test. The glycated hemoglobin levels were calculated using a DCA 2000 HbA1c kit (Siemens, 5035C).

[310]

[311] Experimental Example 10-2. Activity in HFD/STZ mice

[312]

[313] The changes in non-fasting blood glucose levels and body weights over time in male HFD/STZ mice were observed after single subcutaneous injection of 3 nmol/kg or 10 nmol/kg of dual function protein DFD114, 10 nmol/kg of Fc-fused GLP-1 mutant DFD59, or 10 nmol/kg of each of the Fc-fused FGF21 mutants DFD72 and DFD74. DFD59 and DFD74 were also subcutaneously injected once at 10 nmol/kg each in order to compare the effect of combined administration of the single function proteins with that of the dual function protein.

[314] As shown in FIG. 19, regarding the changes in blood glucose levels until the 4th day, DFD72 and DFD74 (long-acting FGF21 single function proteins) administration resulted in slower reductions of blood glucose, while DFD114 (long-acting protein including GLP-1), DFD59 and combined administration of DFD59 and DFD74 showed a more rapid reduction of blood glucose from the 1st day of administration. Similar to the results in *db/db* mice, DFD59 showed a sharp reduction in blood glucose at an early stage, but the reduction of blood glucose disappeared slowly after the 4th

day. DFD114 showed a similar pattern at the low dose of 3 nmol/kg. In the groups treated with 10 nmol/kg of DFD114, DFD72, DFD74 and combined administration, similar non-fasting blood glucose profiles were observed.

[315] As for the effect on body weight reduction, the group treated with combined administration of DFD59 and DFD74 showed the greatest effect on body weight reduction (7 to 8%), and the group treated with 10 nmol/kg of DFD114 also showed an outstanding effect on reducing body weight (approximately 6%) (FIG. 20). The group treated with DFD59 exhibited a reduction in body weight by 5% at the 1st day after administration, but the effect disappeared after the 2nd day and became similar to that of the vehicle group after the 7th day. The group treated with each of the long-acting FGF21 single function proteins DFD72 and DFD74 showed a slower reduction in body weight by 4 to 5% until the 7th day after the administration, and the effect disappeared after the 10th day.

[316] After the termination of the tests, the glycated hemoglobin levels indicative of the mean value of blood glucose were measured and the changes in mean blood glucose were analyzed in each test group (FIG. 21). The vehicle group had an increase in glycated hemoglobin levels after the termination of the test as compared with before administration, and the group treated with DFD59 showed a similar increase. In contrast, the group treated with DFD114 showed reductions in glycated hemoglobin levels in a dose-dependent manner, and the group treated with 10 nmol/kg of DFD114 had the greatest effect in terms of reduced glycated hemoglobin levels (-0.42%). The group treated with combined administration of DFD59 and DFD74 showed reduced glycated hemoglobin levels (-0.38%) similar to that of DFD114. For the long-acting FGF21 single function proteins, it was observed that DFD72 was superior to DFD74. Comparing the proteins based on the reduced levels of glycated hemoglobin in each group, it was confirmed that the dual function protein DFD114 was superior to both GLP-1-Fc and Fc-FGF21 single function proteins.

[317]

[318] Experimental Example 11. Prediction and evaluation of immunogenicity

[319]

[320] Experimental Example 11-1. Prediction method for immunogenicity and results

[321]

[322] In order to predict the potential immunogenicity of dual function proteins, *in* silico analysis of immunogenicity was performed for each protein.

[323] Specifically, the potential immunogenicity of dual function proteins was rapidly screened by using iTope™ and TCED™ methods (Prediction of immunogenicity of therapeutic proteins: validity of computational tools, BioDrugs, 2010). According to the two methods, the T-cell epitope may be more accurately predicted as compared

with the *in* silico analytical method which depends on MHC class II binding analysis only.

[324]

[325] Experimental Example 11-2. *Ex* vivo evaluation method for immunogenicity and results

[326]

[327] In order to evaluate the potential immunogenicity of dual function proteins, EpiScreen™ analysis (Increased brain bio-distribution and chemical stability and decreased immunogenicity of an engineered variant of GDNF, Exp Neurol, 2015) was performed. When immunogenicity is detected, the amino acid sequences inducing immunogenicity may be identified through T-cell epitope mapping, and deimmunized mutants with minimized immunogenicity may be designed and prepared via *in silico* prediction to reevaluate immunogenicity.

Claims

[Claim 1] A dual function protein comprising a fibroblast growth factor 21 (FGF21) mutant protein; a biologically active protein, or a mutant or fragment thereof; and an Fc region of an immunoglobulin,
wherein the FGF21 mutant protein comprises at least one mutation selected from the group consisting of the following mutations (1) to (7):
(1) a substitution of amino acids at positions 98 to 101 from the N-terminus of a wild-type FGF21 protein with an amino acid sequence of EIRP (SEQ ID NO: 68);
(2) a substitution of amino acids at positions 170 to 174 from the N-terminus of a wild-type FGF21 protein with an amino acid sequence of TGLEAV (SEQ ID NO: 69);
(3) a substitution of amino acids at positions 170 to 174 from the N-terminus of a wild-type FGF21 protein with an amino acid sequence of TGLEAN (SEQ ID NO: 70);
(4) a substitution of an amino acid at position 170 from the N-terminus of a wild-type FGF21 protein with an amino acid N;
(5) a substitution of an amino acid at position 174 from the N-terminus of a wild-type FGF21 protein with an amino acid N;
(6) a substitution of an amino acid at position 180 from the N-terminus of a wild-type FGF21 protein with an amino acid E, along with one or more mutations (1) to (5) above; and
(7) a mutation of 1 to 10 amino acids for reducing immunogenicity of a wild-type FGF21 protein.

[Claim 2] The dual function protein of claim 1, wherein an amino acid residue N of the FGF21 mutant protein introduced by a mutation is glycosylated.

[Claim 3] The dual function protein of claim 1, wherein the biologically active protein is one selected from the group consisting of insulin, C-peptide, leptin, glucagon, gastrin, gastric inhibitory polypeptide (GIP), amylin, calcitonin, cholecystokinin, peptide YY, neuropeptide Y, bone morphogenetic protein-6 (BMP-6), bone morphogenetic protein-9 (BMP-9), oxyntomodulin, oxytocin, glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), irisin, fibronectin type III domain-containing protein 5 (FNDC5), apelin, adiponectin, C1q and tumor necrosis factor related protein (CTRP family), resistin, visfatin, omentin, retinol binding protein-4 (RBP-4), glicentin, angiopoietin, interleukin-22 (IL-22), exendin-4 and growth hormone.

[Claim 4] The dual function protein of claim 3, wherein the biologically active protein is one selected from GLP-1, a mutant thereof and exendin-4.

[Claim 5] The dual function protein of claim 4, wherein the mutant of GLP-1 has an amino acid sequence represented by any one of SEQ ID NOs: 43 to 46.

[Claim 6] The dual function protein of claim 1, wherein the wild-type FGF21 protein has an amino acid sequence represented by SEQ ID NO: 1.

[Claim 7] The dual function protein of claim 1, wherein the FGF21 mutant protein has an amino acid sequence represented by any one of SEQ ID NOs: 6 to 23.

[Claim 8] The dual function protein of claim 1, wherein the dual function protein further comprises a linker.

[Claim 9] The dual function protein of claim 8, wherein the linker connects the FGF21 mutant protein to the Fc region of the immunoglobulin.

[Claim 10] The dual function protein of claim 9, wherein the linker is connected to the C-terminus of the Fc region of the immunoglobulin and the N-terminus of the FGF21 mutant protein.

[Claim 11] The dual function protein of claim 9, wherein the linker is a peptide consisting of 10 to 30 amino acid residues.

[Claim 12] The dual function protein of claim 11, wherein the linker has an amino acid sequence represented by any one of SEQ ID NOs: 2 to 5.

[Claim 13] The dual function protein of claim 1, wherein the Fc region of the immunoglobulin is any one of the Fc region of IgG1, IgG2, IgG3, IgG4 and IgD, or a hybrid Fc containing a combination thereof.

[Claim 14] The dual function protein of claim 13, wherein the hybrid Fc comprises an IgG4 region and an IgD region.

[Claim 15] The dual function protein of claim 1, wherein the dual function protein comprises the biologically active protein, the Fc region of the immunoglobulin and the FGF21 mutant protein, connected in this order from the N-terminus to the C-terminus.

[Claim 16] The dual function protein of claim 15, wherein a linker is additionally connected between the Fc region of the immunoglobulin and the FGF21 mutant protein.

[Claim 17] The dual function protein of claim 16, wherein the linker is connected to the C-terminus of the Fc region of the immunoglobulin and the N-terminus of the FGF21 mutant protein.

[Claim 18] The dual function protein of claim 16, wherein the linker is a peptide consisting of 10 to 30 amino acid residues.

[Claim 19] The dual function protein of claim 16, wherein the linker has an amino acid sequence represented by any one of SEQ ID NOs: 2 to 5.

[Claim 20] The dual function protein of claim 1, wherein the dual function protein has an amino acid sequence represented by SEQ ID NO: 65.

[Claim 21] The dual function protein of claim 1, wherein the dual function protein has an amino acid sequence represented by SEQ ID NO: 66.

[Claim 22] The dual function protein of claim 1, wherein the dual function protein has an amino acid sequence represented by SEQ ID NO: 67.

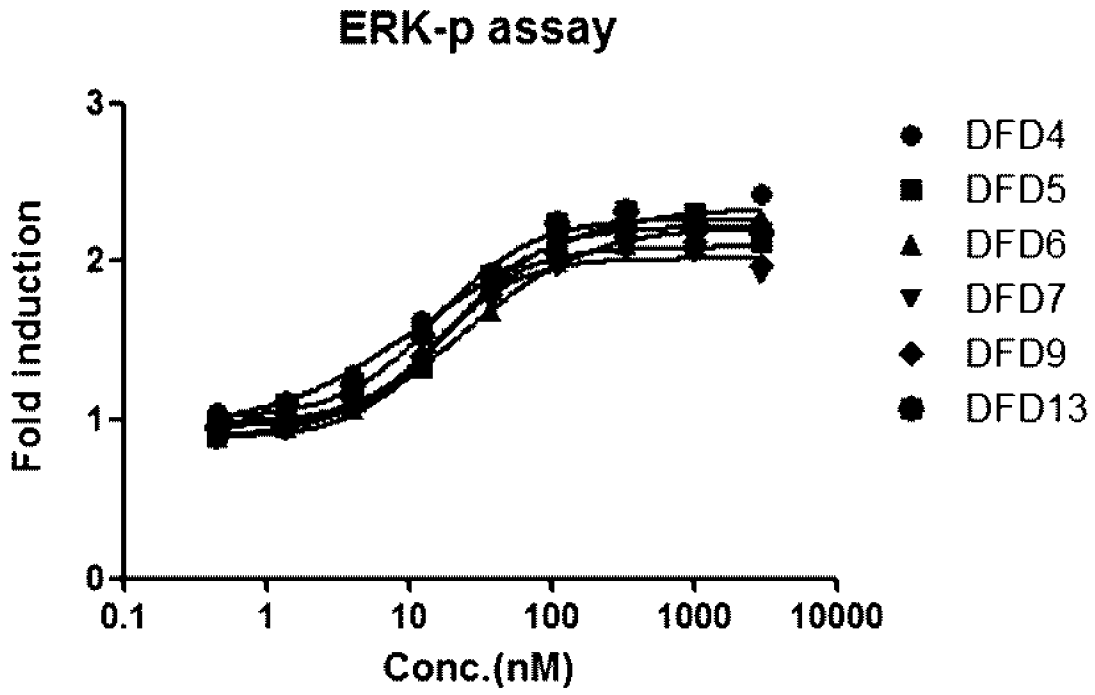
[Claim 23] A pharmaceutical composition comprising the dual function protein according to any one of claims 1 to 22 for treating diabetes, obesity, dyslipidemia, metabolic syndrome, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis or cardiovascular diseases.

[Claim 24] An isolated nucleic acid molecule encoding the dual function protein according to any one of claims 1 to 22.

[Claim 25] An expression vector comprising the nucleic acid molecule of claim 24.

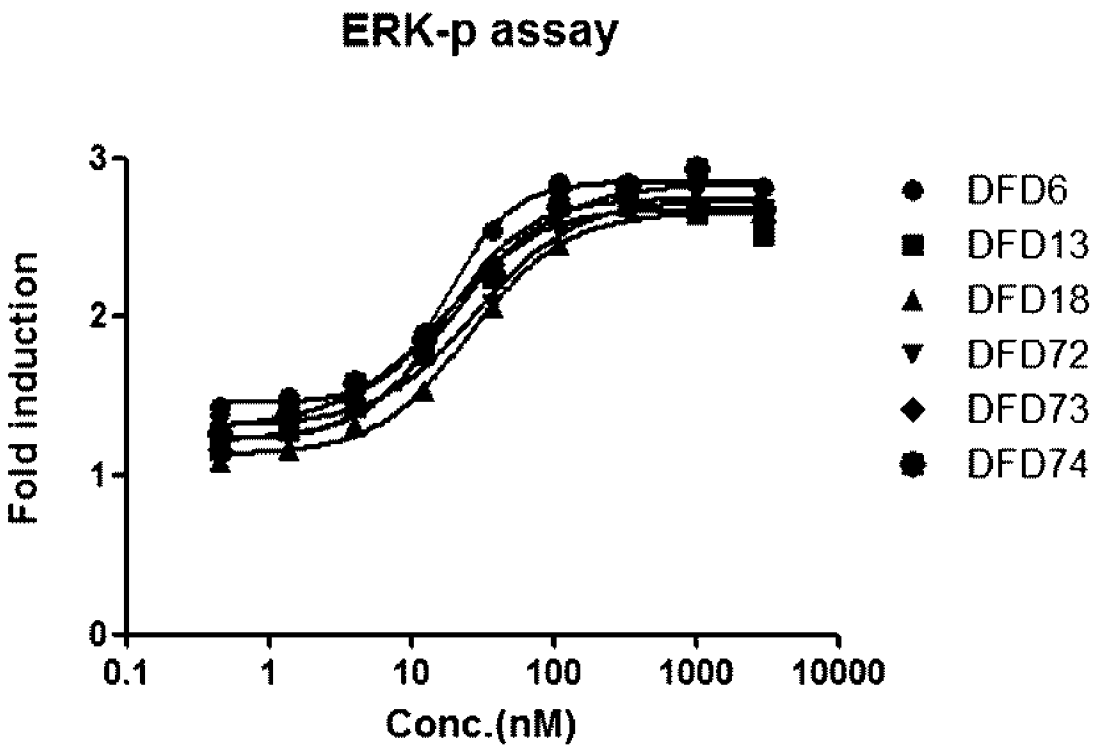
[Claim 26] A host cell comprising the expression vector of claim 25.

[Fig. 1a]



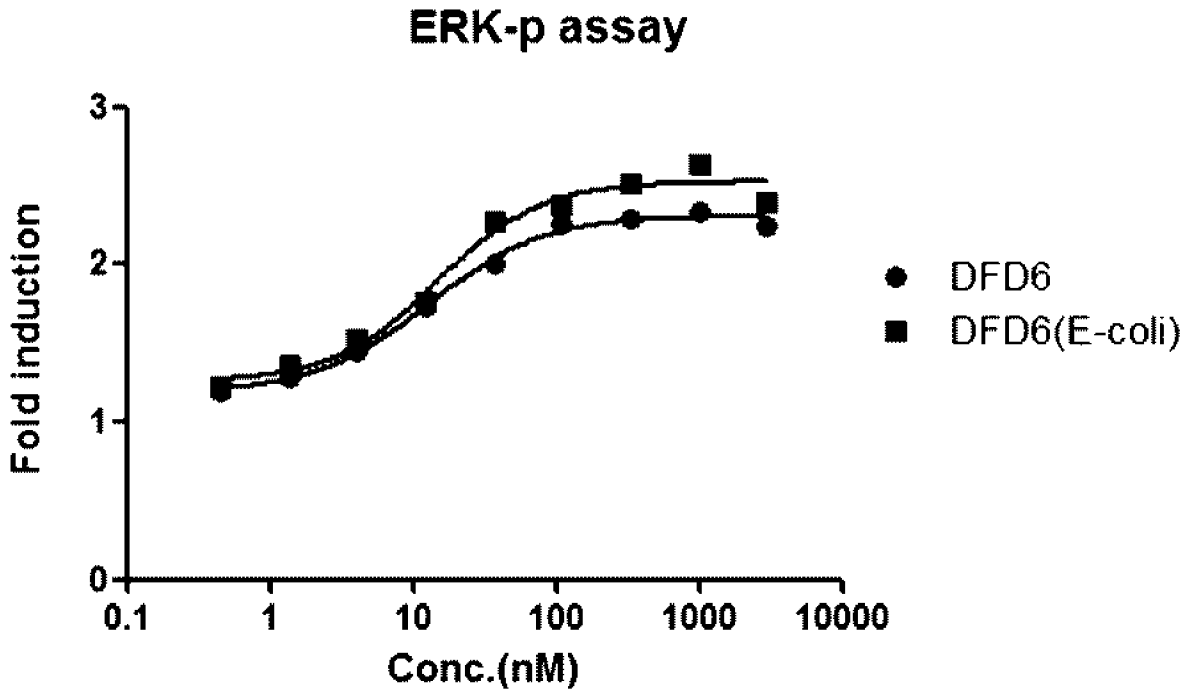
	EC_{50} (nM)
DFD4	13.97
DFD5	20.19
DFD6	23.73
DFD7	14.73
DFD9	17.13
DFD13	15.31

[Fig. 1b]



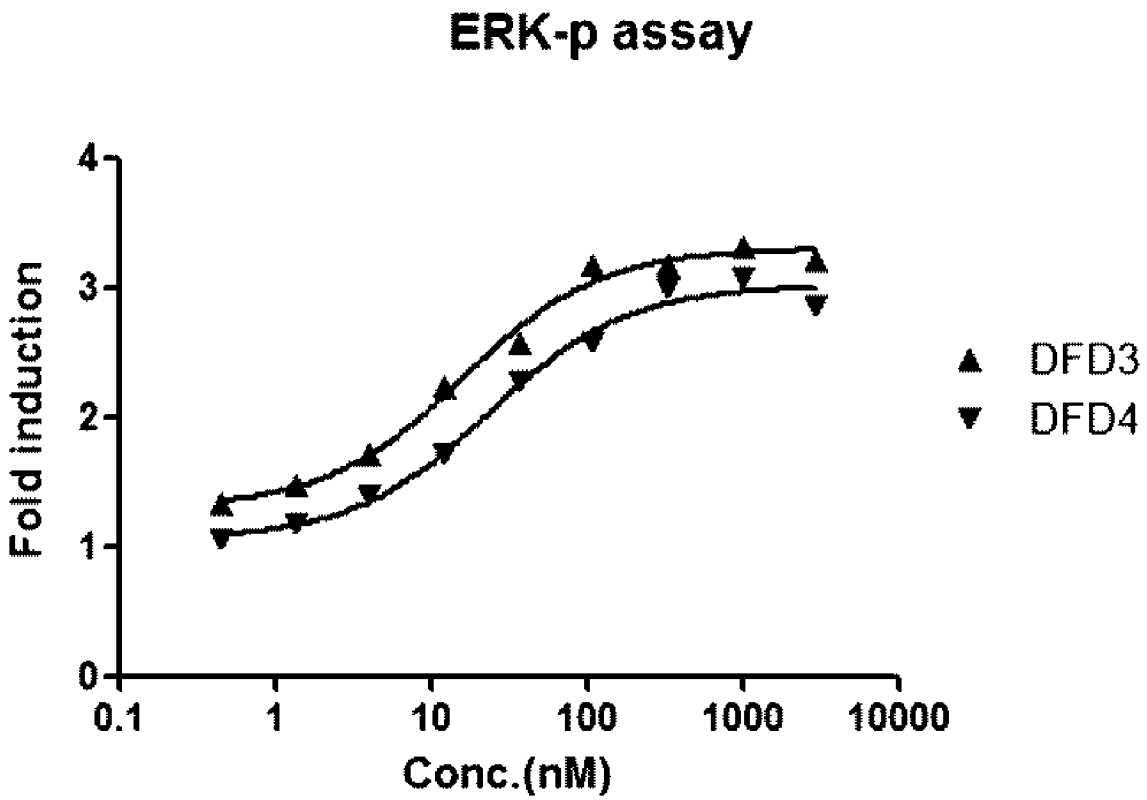
	EC_{50} (nM)
DFD6	18.41
DFD13	17.28
DFD18	25.52
DFD72	28.13
DFD73	16.96
DFD74	19.45

[Fig. 1c]



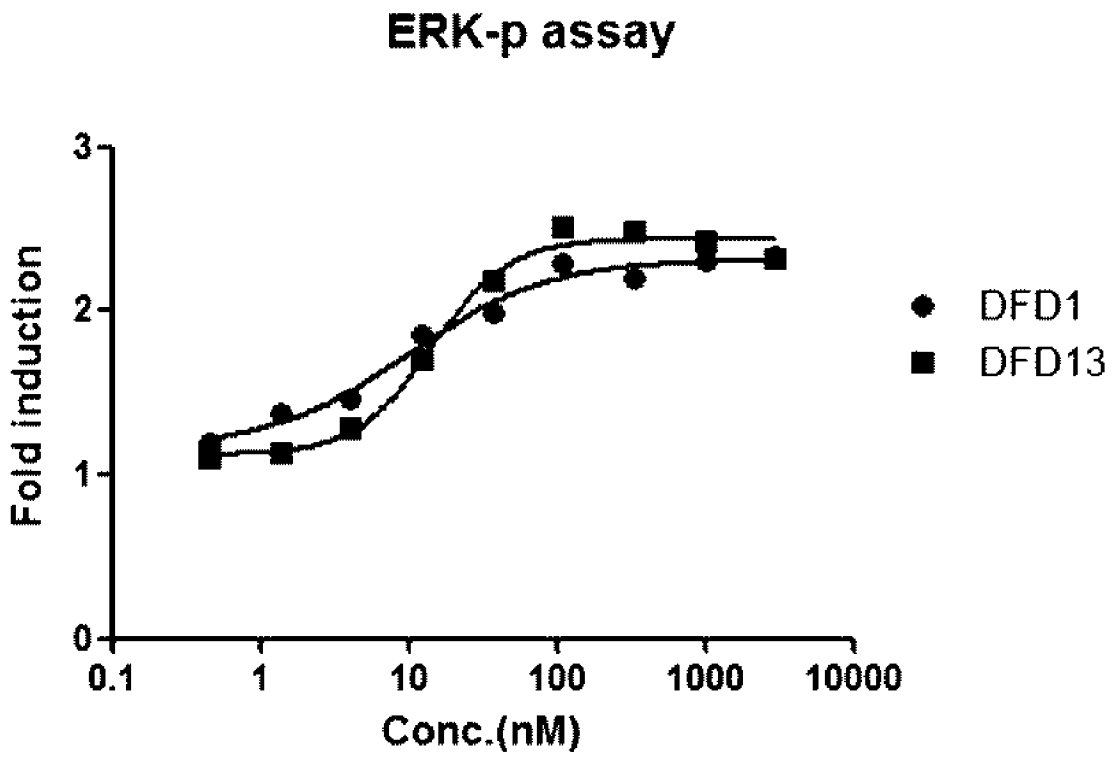
	EC_{50} (nM)
DFD6	12.61
DFD6(E-coli)	14.49

[Fig. 2a]



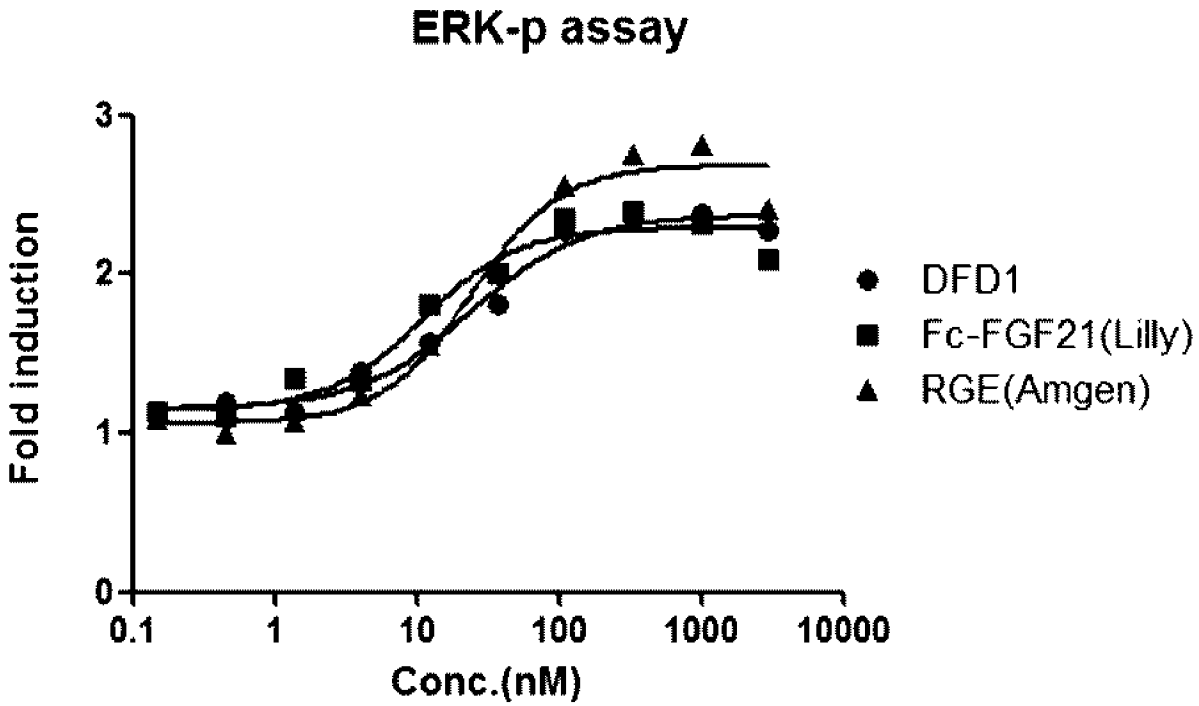
	EC_{50} (nM)
DFD3	15.69
DFD4	23.37

[Fig. 2b]



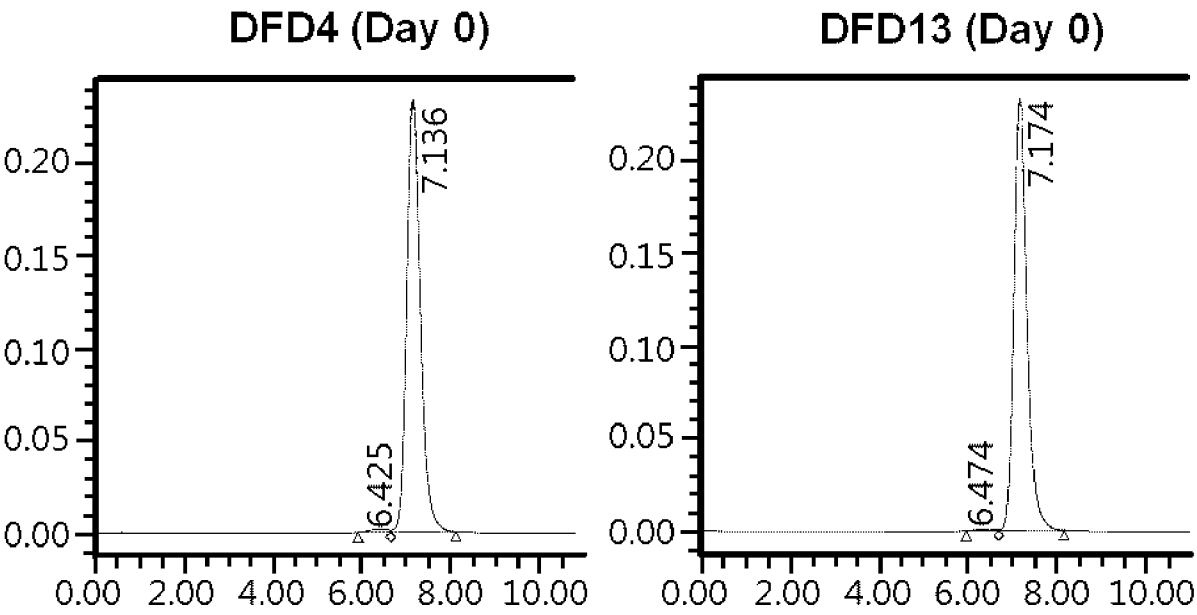
	EC_{50} (nM)
DFD1	9.77
DFD13	14.18

[Fig. 3]



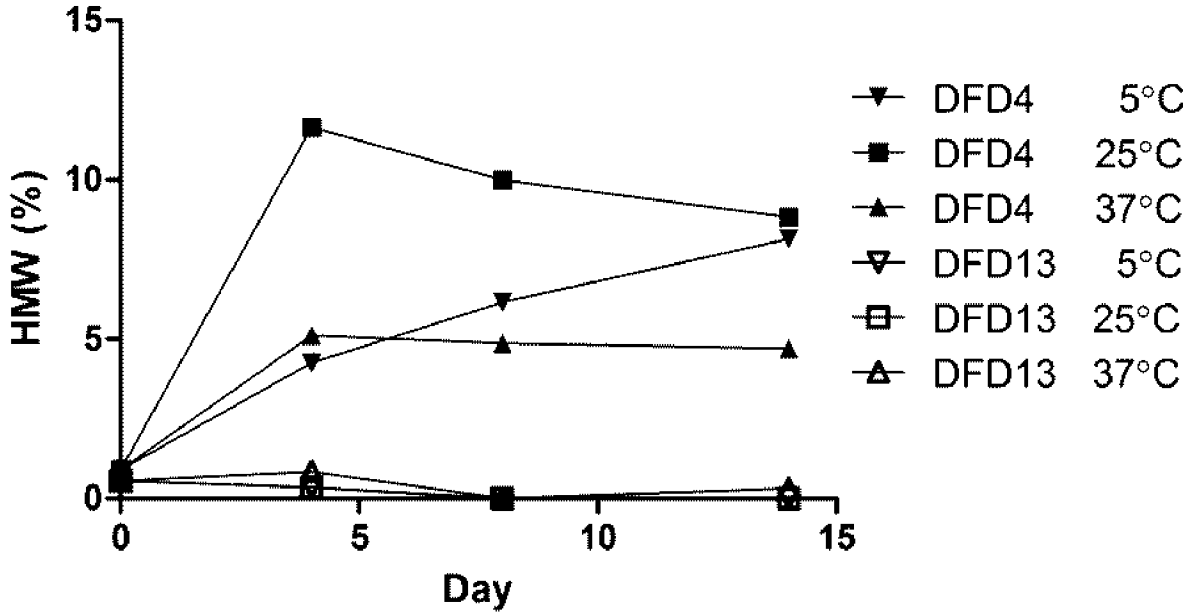
	EC_{50} (nM)
DFD1	23.44
Fc-FGF21(Lilly)	10.95
RGE(Amgen)	23.81

[Fig. 4]

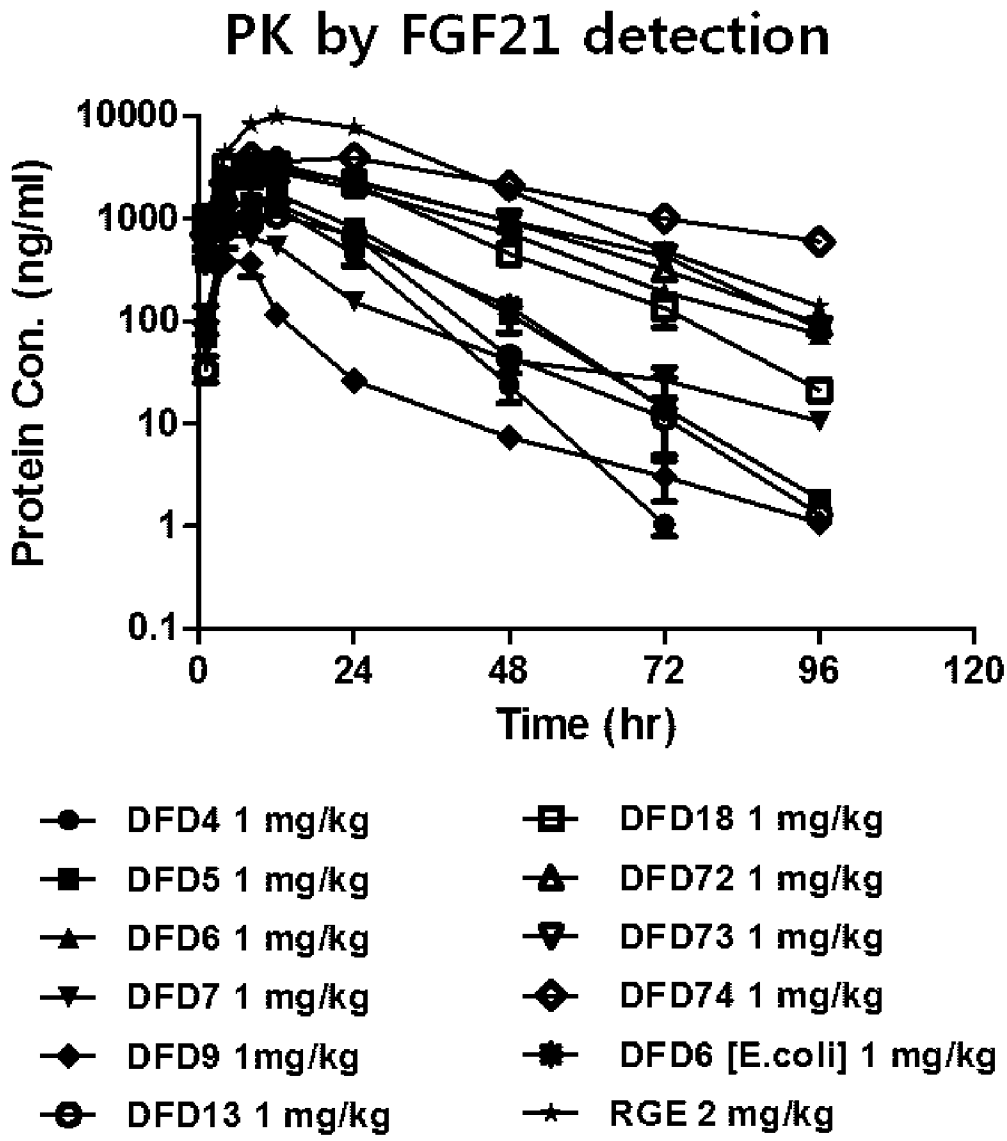


	RT	Area	% Area	Height
1	6.425	46359	0.93	1520
2	7.136	4940917	99.07	234651

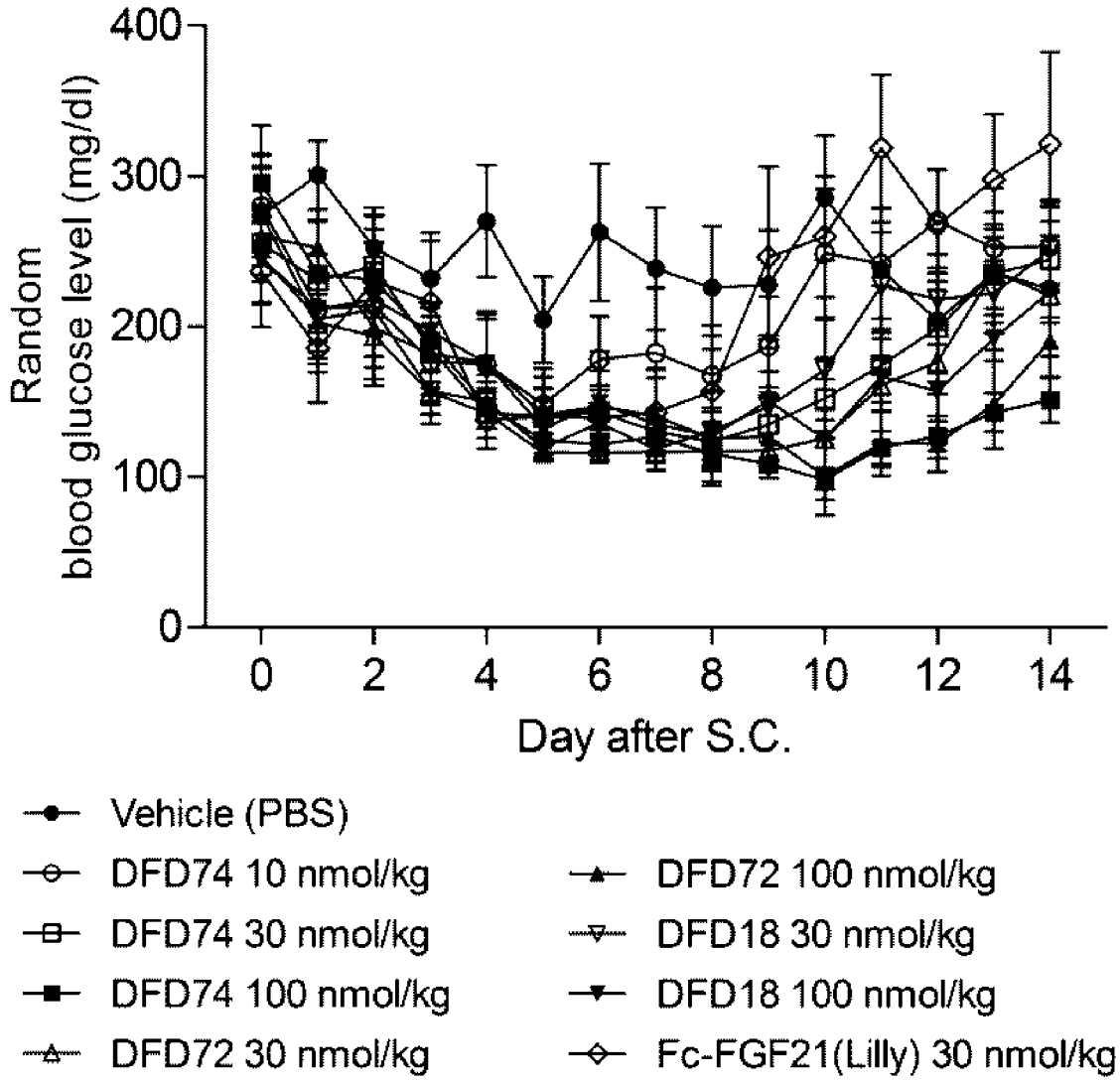
	RT	Area	% Area	Height
1	6.474	28735	0.60	984
2	7.174	4789139	99.40	232719



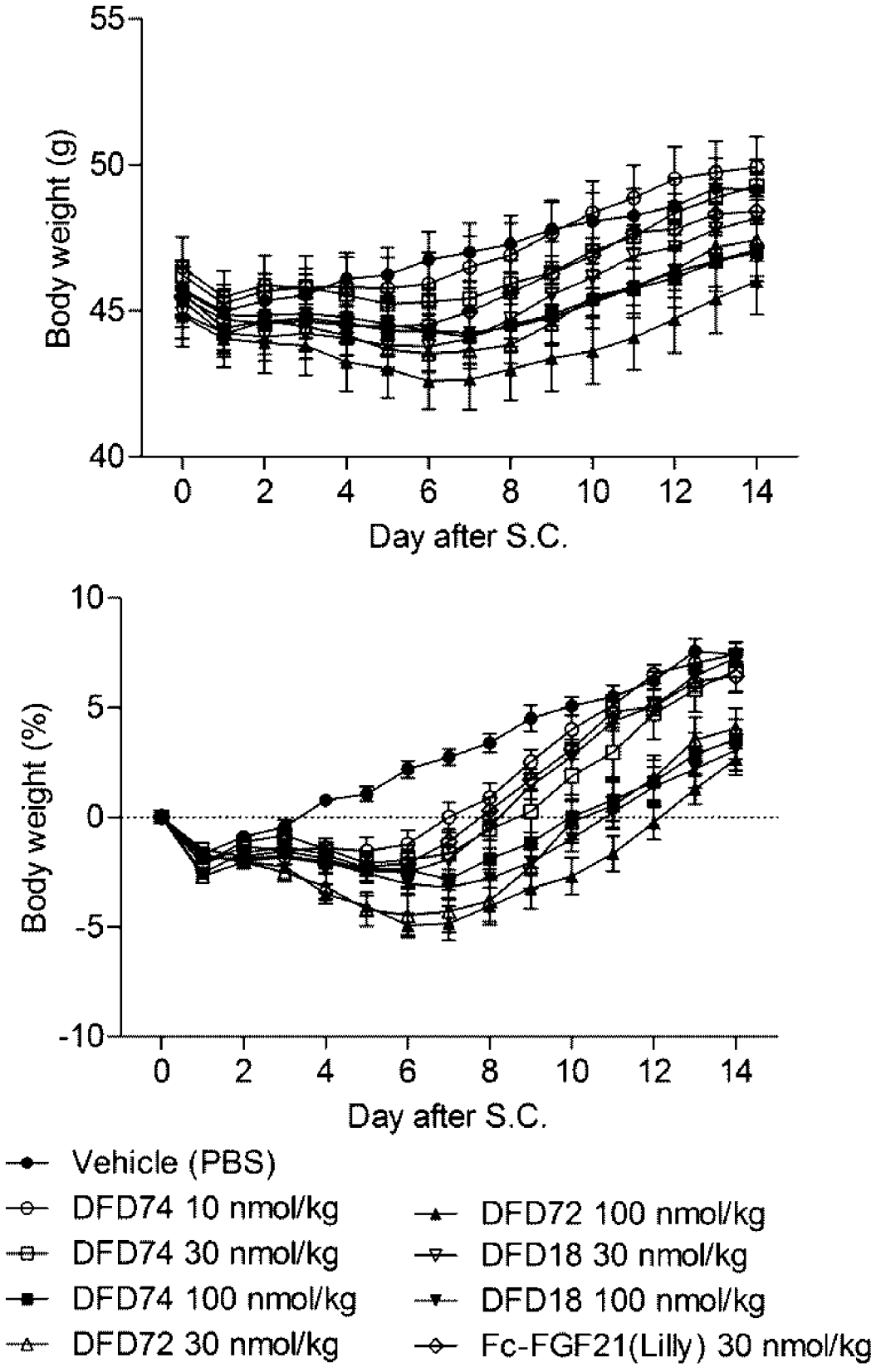
[Fig. 5]


PK by FGF21 detection
Protein Con. (ng/ml)
10000
1000
100
10
1
0.1
0
24
48
72
96
120
Time (hr)
DFD4 1 mg/kg
DFD5 1 mg/kg
DFD6 1 mg/kg
DFD7 1 mg/kg
DFD9 1mg/kg
DFD13 1 mg/kg
DFD18 1 mg/kg
DFD72 1 mg/kg
DFD73 1 mg/kg
DFD74 1 mg/kg
DFD6 [E.coli] 1 mg/kg
RGE 2 mg/kg

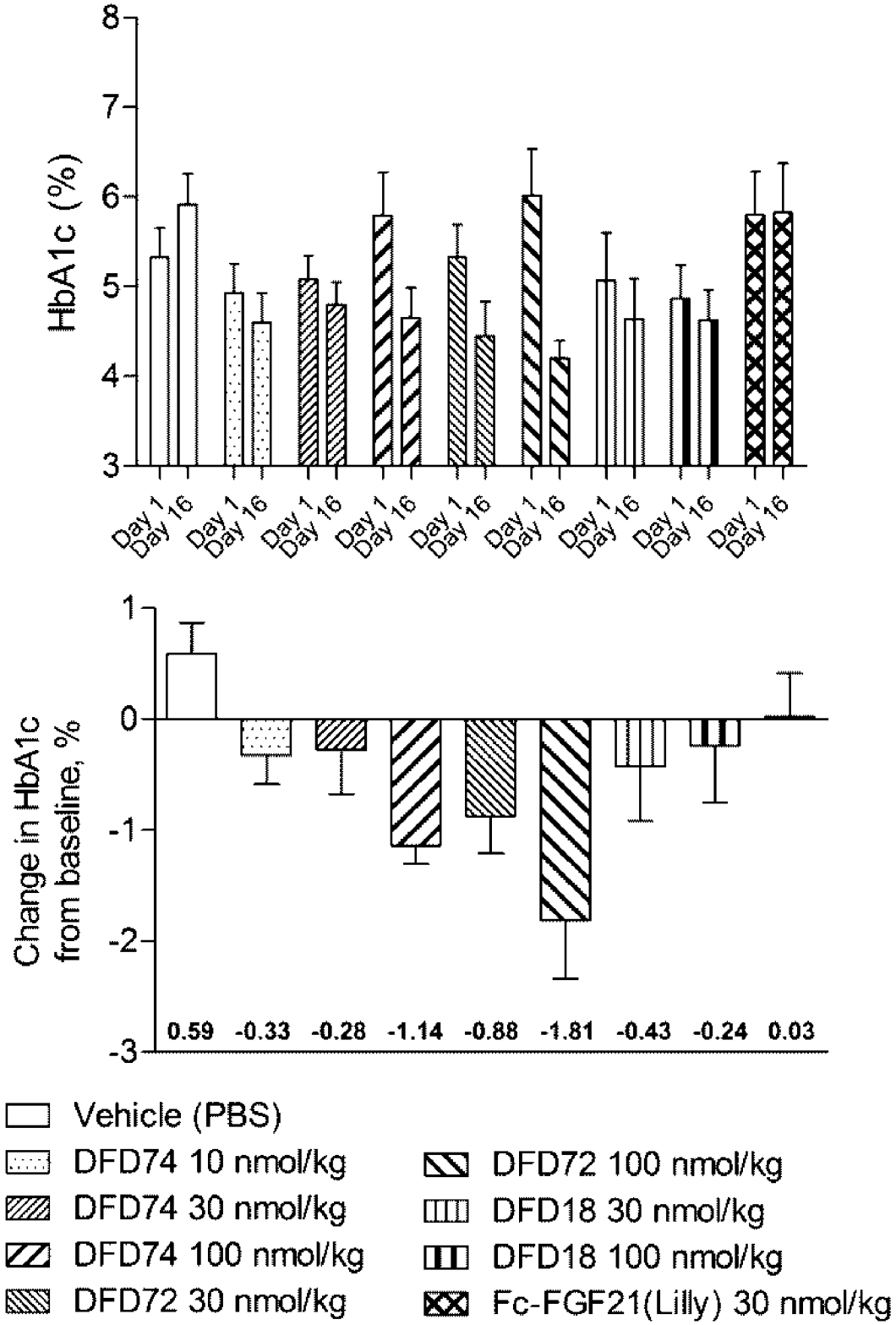
[Fig. 6]


Random blood glucose level (mg/dl)
400
300
200
100
0
0
2
4
6
8
10
12
14
Day after S.C.
Vehicle (PBS)
DFD74 10 nmol/kg
DFD74 30 nmol/kg
DFD74 100 nmol/kg
DFD72 30 nmol/kg
DFD72 100 nmol/kg
DFD18 30 nmol/kg
DFD18 100 nmol/kg
Fc-FGF21(Lilly) 30 nmol/kg

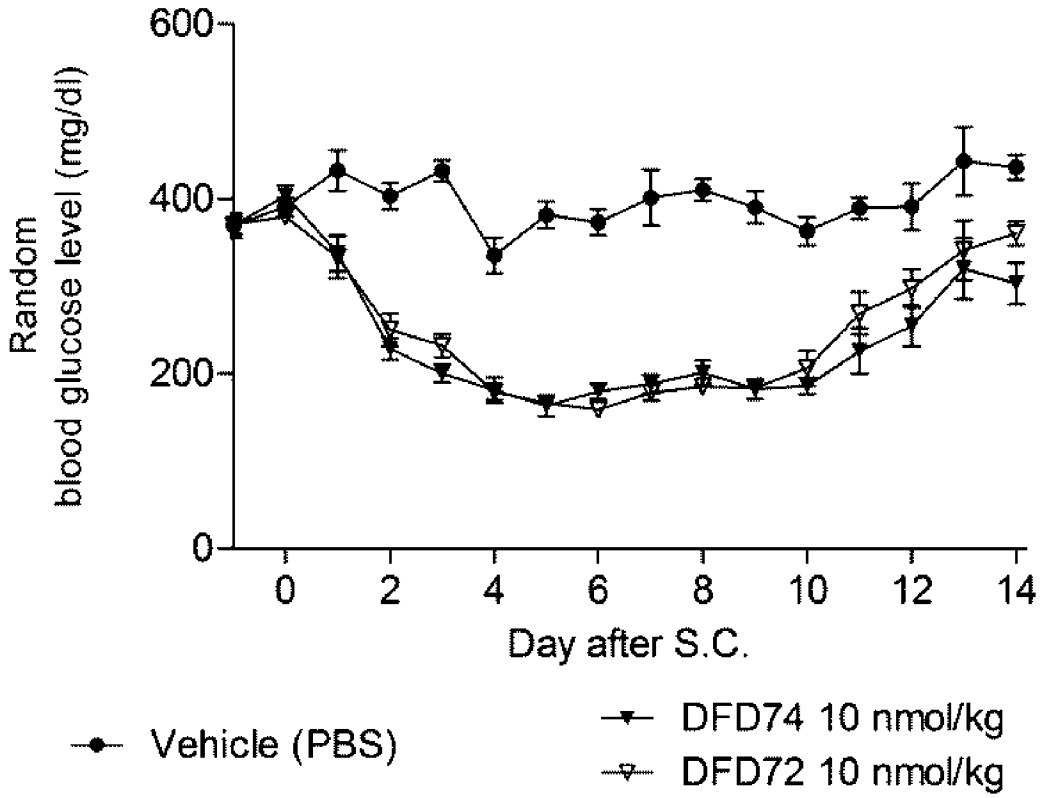
[Fig. 7]


55
50
45
40
Body weight (g)
0
2
4
6
8
10
12
14
Day after S.C.
10
5
0
-5
-10
Body weight (%)
0
2
4
6
8
10
12
14
Day after S.C.
Vehicle (PBS)
DFD74 10 nmol/kg
DFD74 30 nmol/kg
DFD74 100 nmol/kg
DFD72 30 nmol/kg
DFD72 100 nmol/kg
DFD18 30 nmol/kg
DFD18 100 nmol/kg
Fc-FGF21(Lilly) 30 nmol/kg

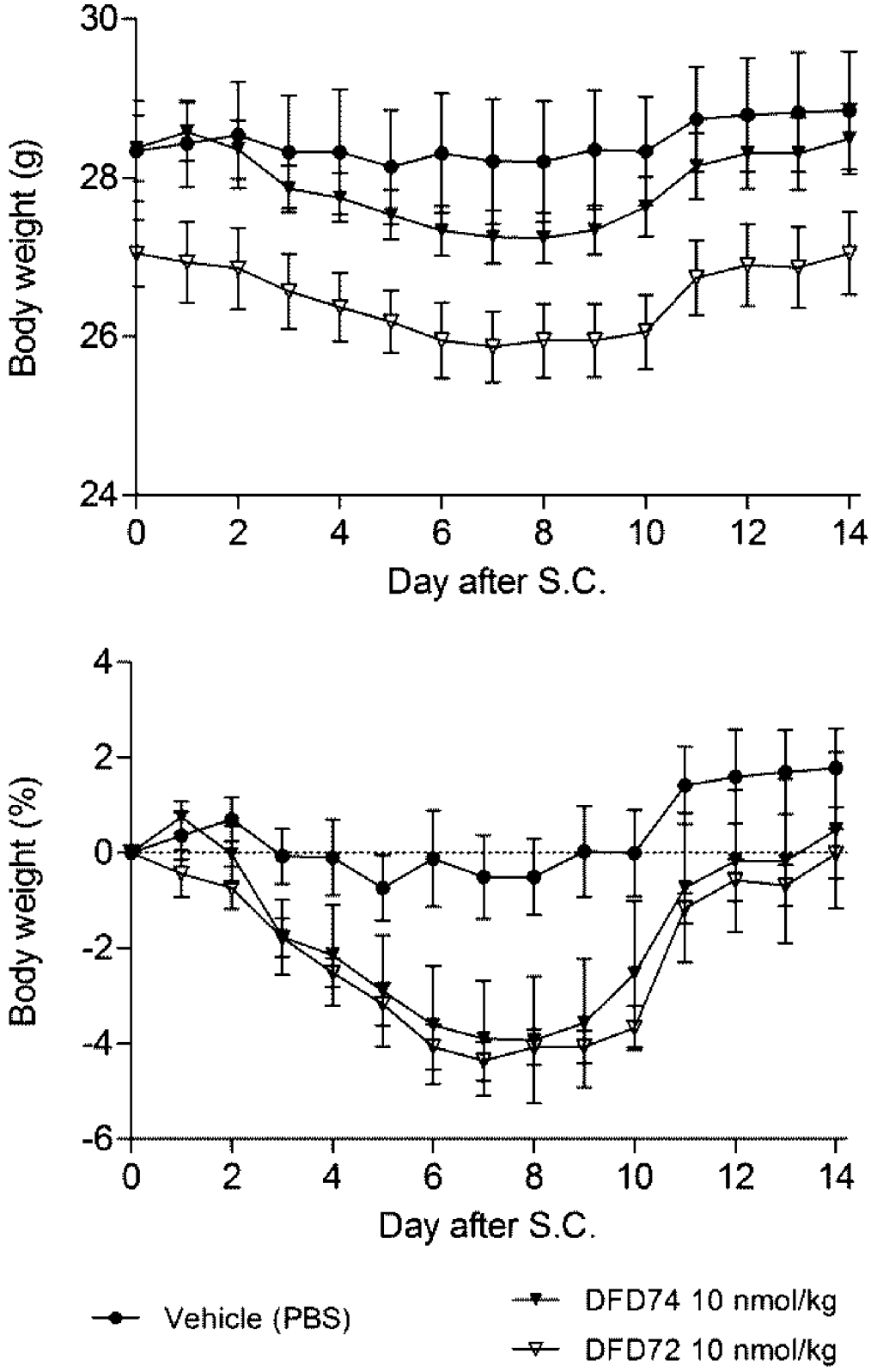
[Fig. 8]


HbA1c (%)
8
7
6
5
4
3
Day 1
Day 16
Change in HbA1c from baseline, %
1
0
-1
-2
-3
0.59
-0.33
-0.28
-1.14
-0.88
-1.81
-0.43
-0.24
0.03
Vehicle (PBS)
DFD74 10 nmol/kg
DFD74 30 nmol/kg
DFD74 100 nmol/kg
DFD72 30 nmol/kg
DFD72 100 nmol/kg
DFD18 30 nmol/kg
DFD18 100 nmol/kg
Fc-FGF21(Lilly) 30 nmol/kg

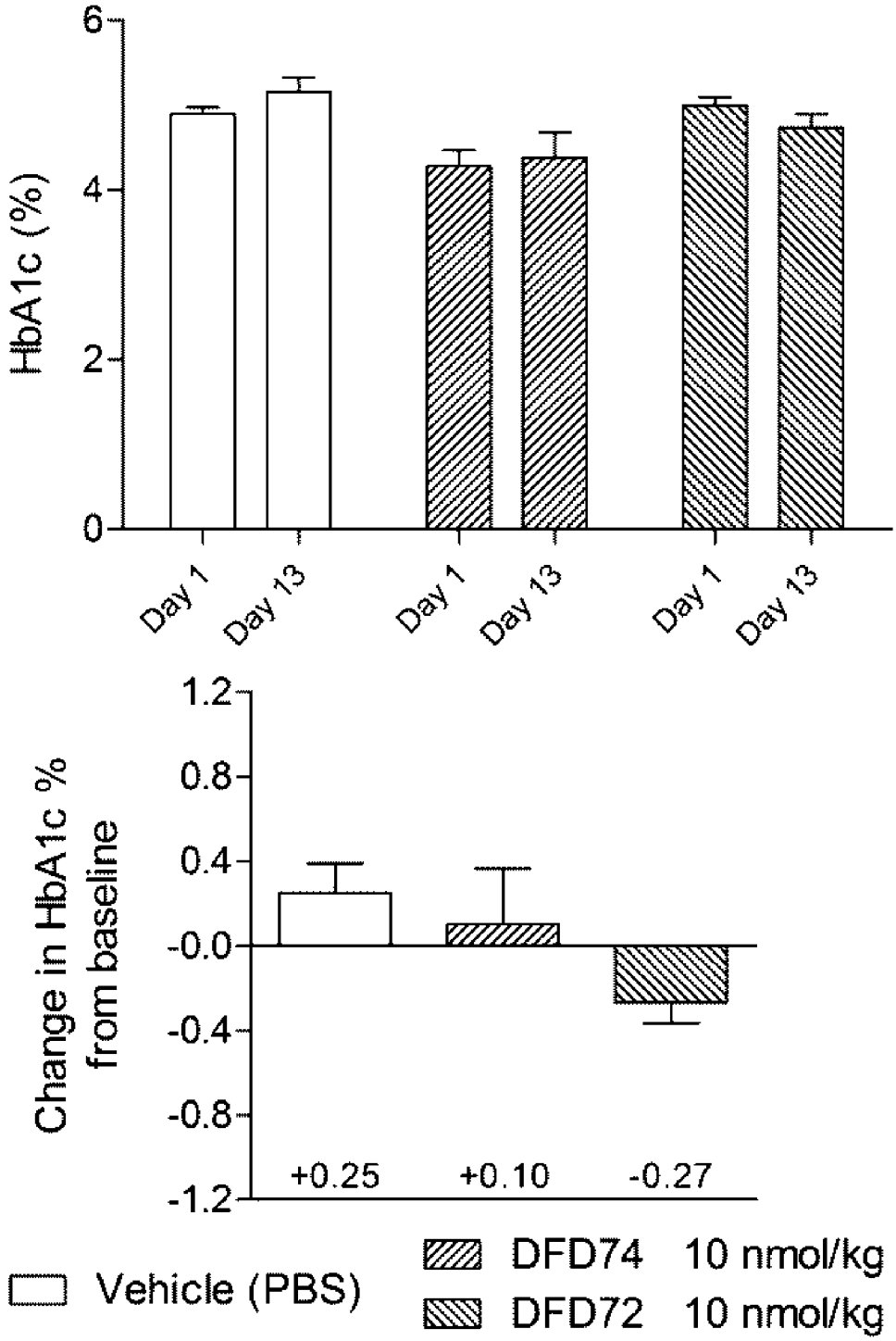
[Fig. 9]


600
400
200
0
Random blood glucose level (mg/dl)
0
2
4
6
8
10
12
14
Day after S.C.
Vehicle (PBS)
DFD74 10 nmol/kg
DFD72 10 nmol/kg

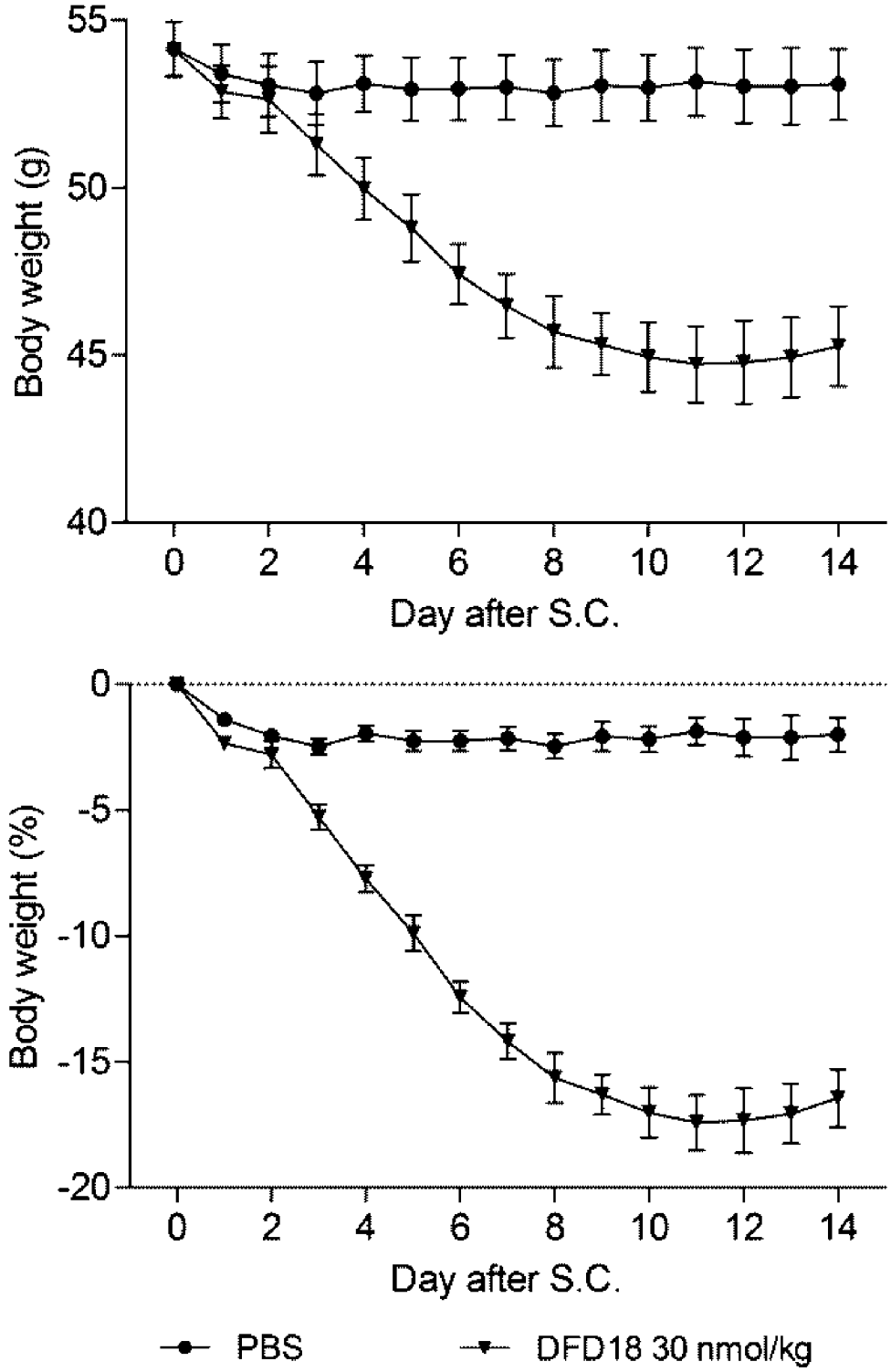
[Fig. 10]


Body weight (g)
30
28
26
24
0
2
4
6
8
10
12
14
Day after S.C.
Body weight (%)
4
2
0
-2
-4
-6
0
2
4
6
8
10
12
14
Day after S.C.
Vehicle (PBS)
DFD74 10 nmol/kg
DFD72 10 nmol/kg

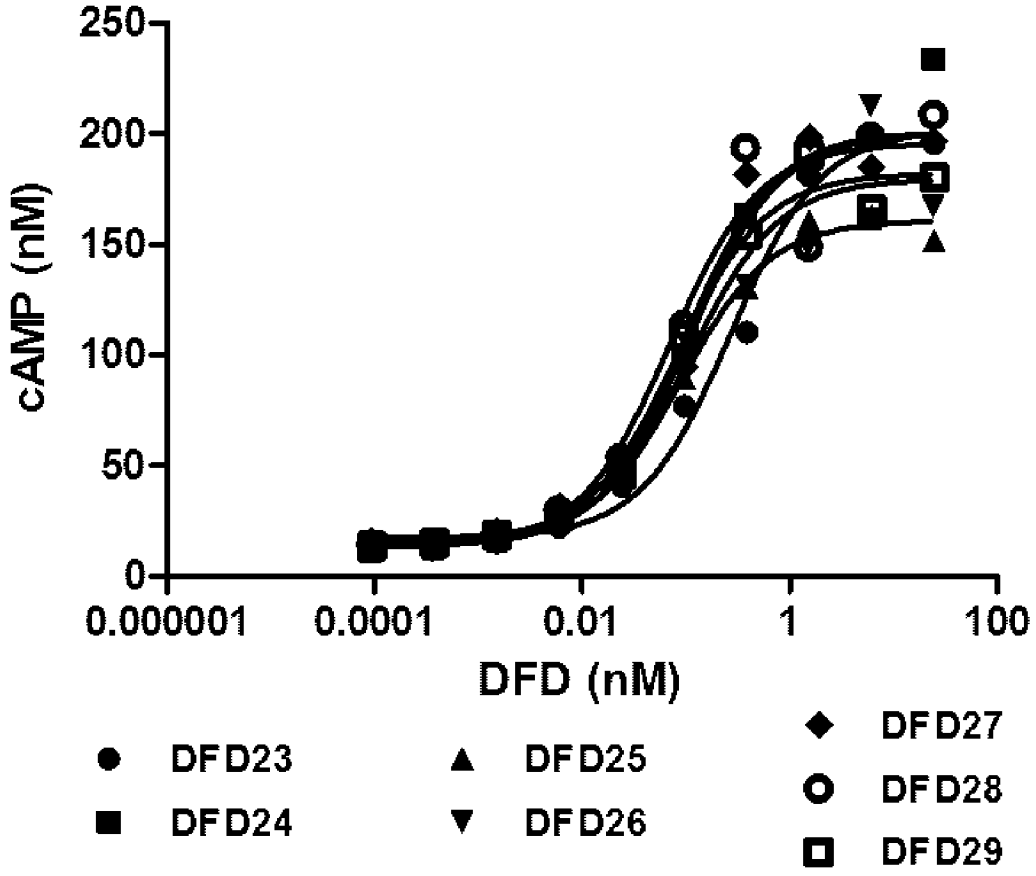
[Fig. 11]


HbA1c (%)
6
4
2
0
Day 1
Day 13
Day 1
Day 13
Day 1
Day 13
Change in HbA1c % from baseline
1.2
0.8
0.4
-0.0
-0.4
-0.8
-1.2
+0.25
+0.10
-0.27
Vehicle (PBS)
DFD74 10 nmol/kg
DFD72 10 nmol/kg

[Fig. 12]

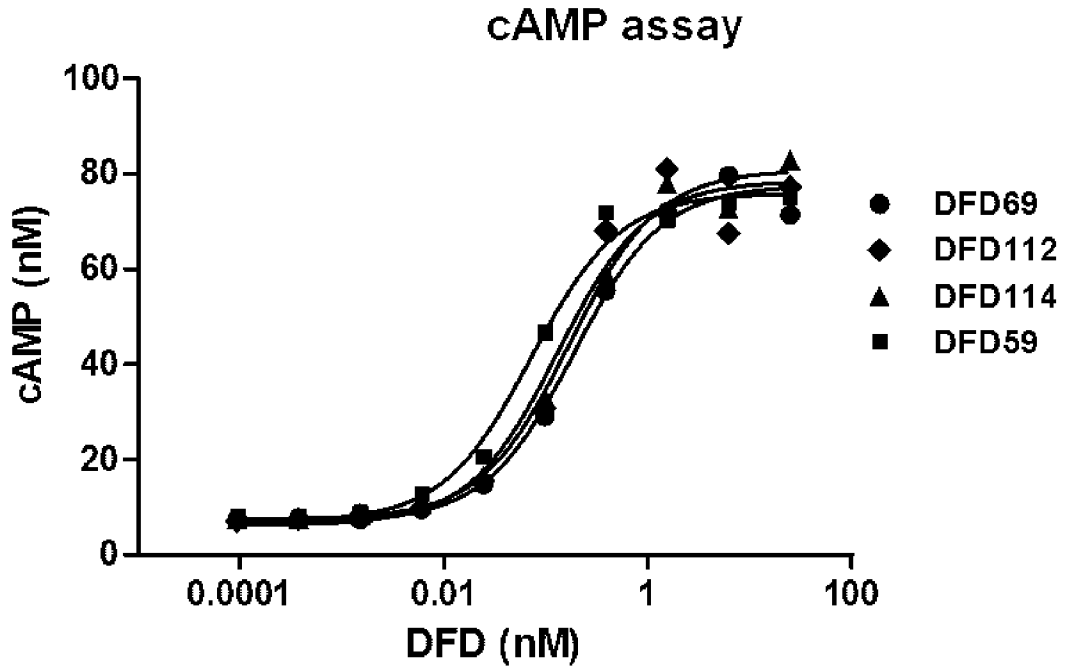

55
50
45
40
Body weight (g)
0
2
4
6
8
10
12
14
Day after S.C.
0
-5
-10
-15
-20
Body weight (%)
0
2
4
6
8
10
12
14
Day after S.C.
PBS
DFD18 30 nmol/kg

[Fig. 13]

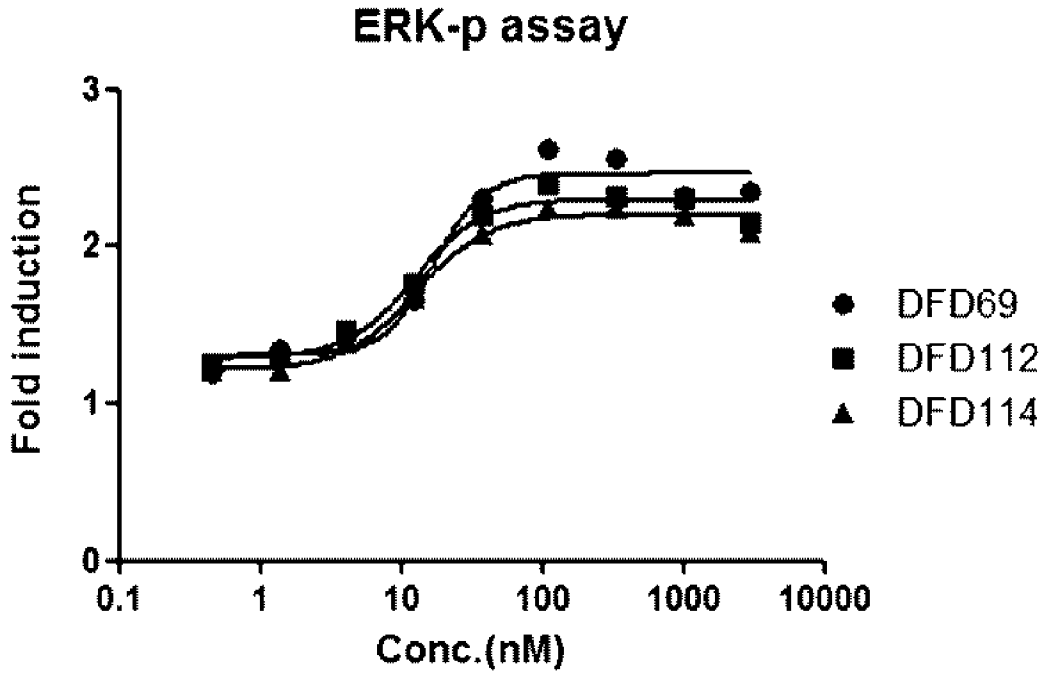


	EC_{50}(pM)
DFD23	272.8
DFD24	107.4
DFD25	88.6
DFD26	104.9
DFD27	99.0
DFD28	69.7
DFD29	78.6

[Fig. 14]

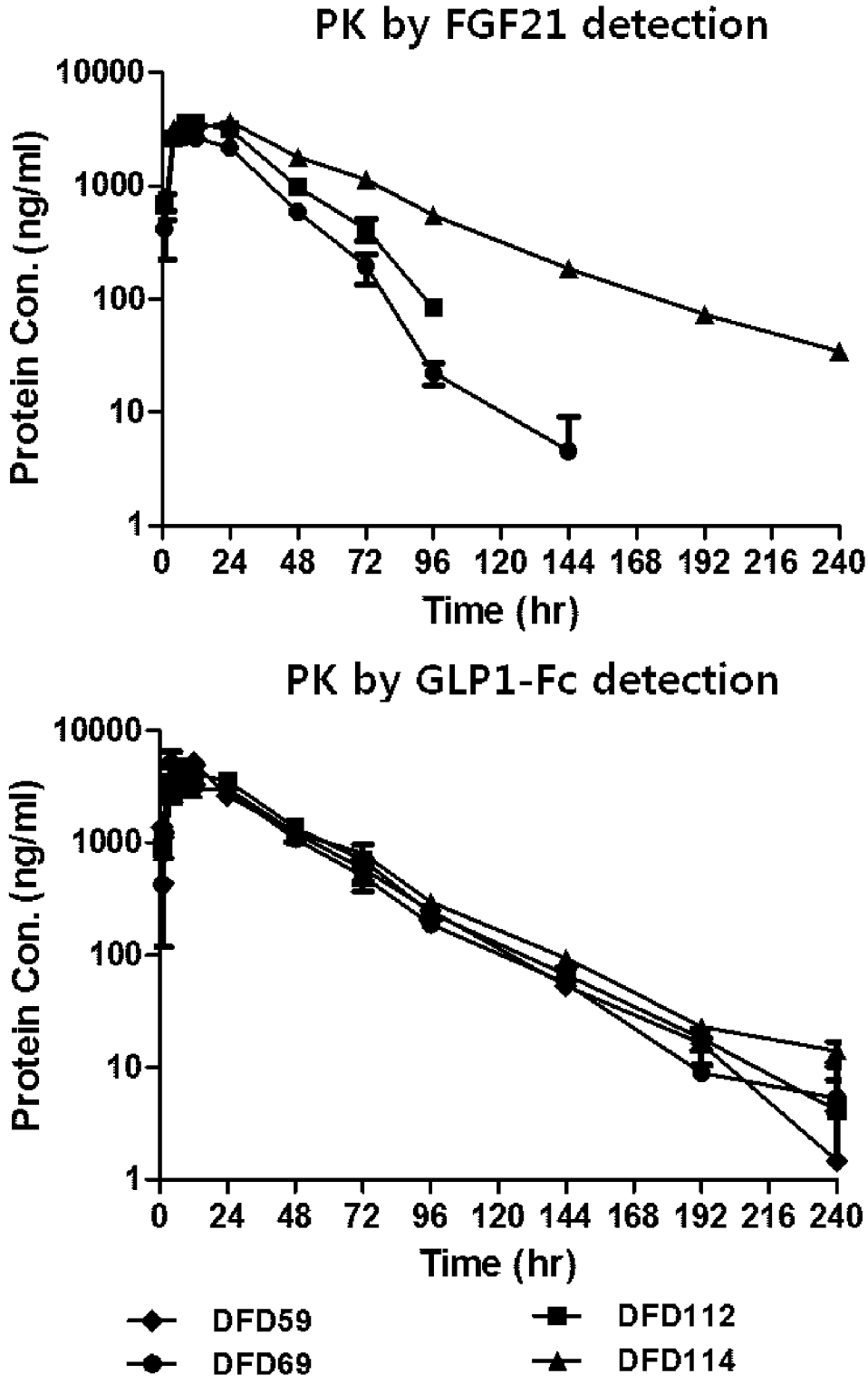


	DFD69	DFD112	DFD114	DFD59
EC_{50}(pM)	187.5	129.5	168.8	72.1

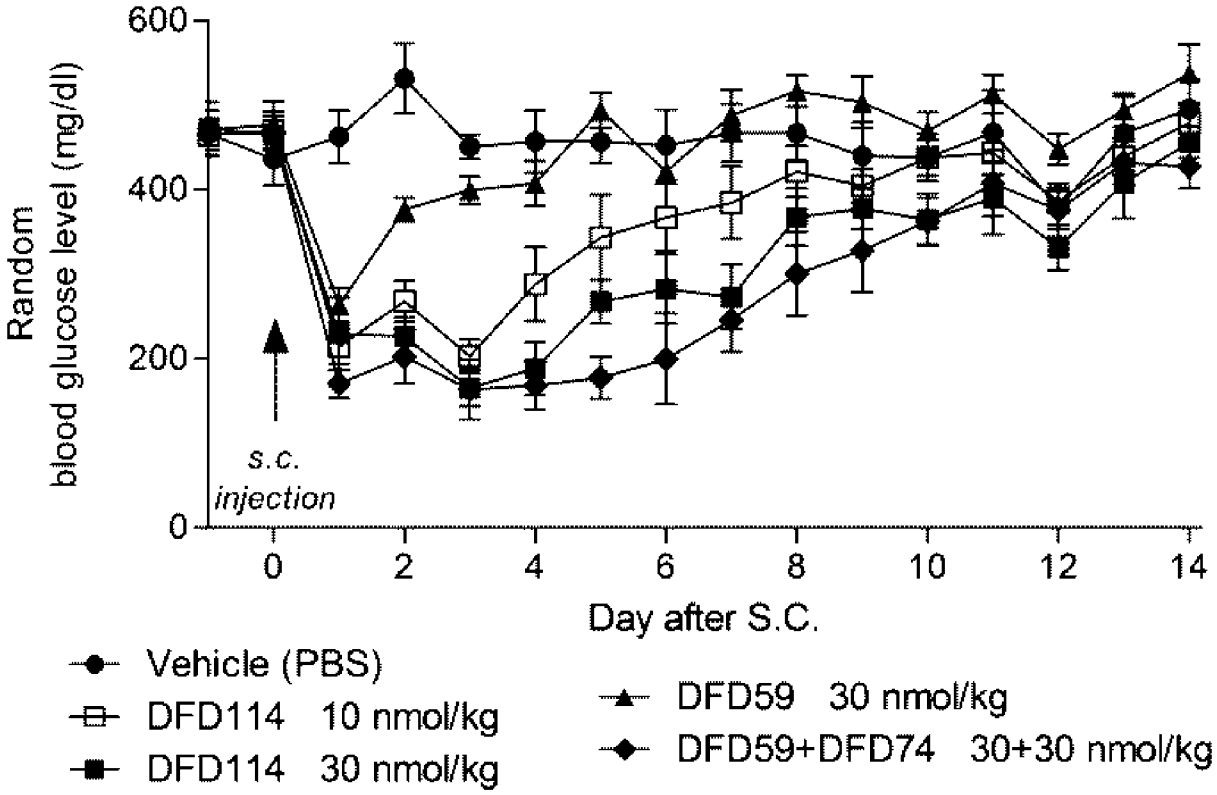


	DFD69	DFD112	DFD114
EC_{50}(pM)	16.84	12.16	15.52

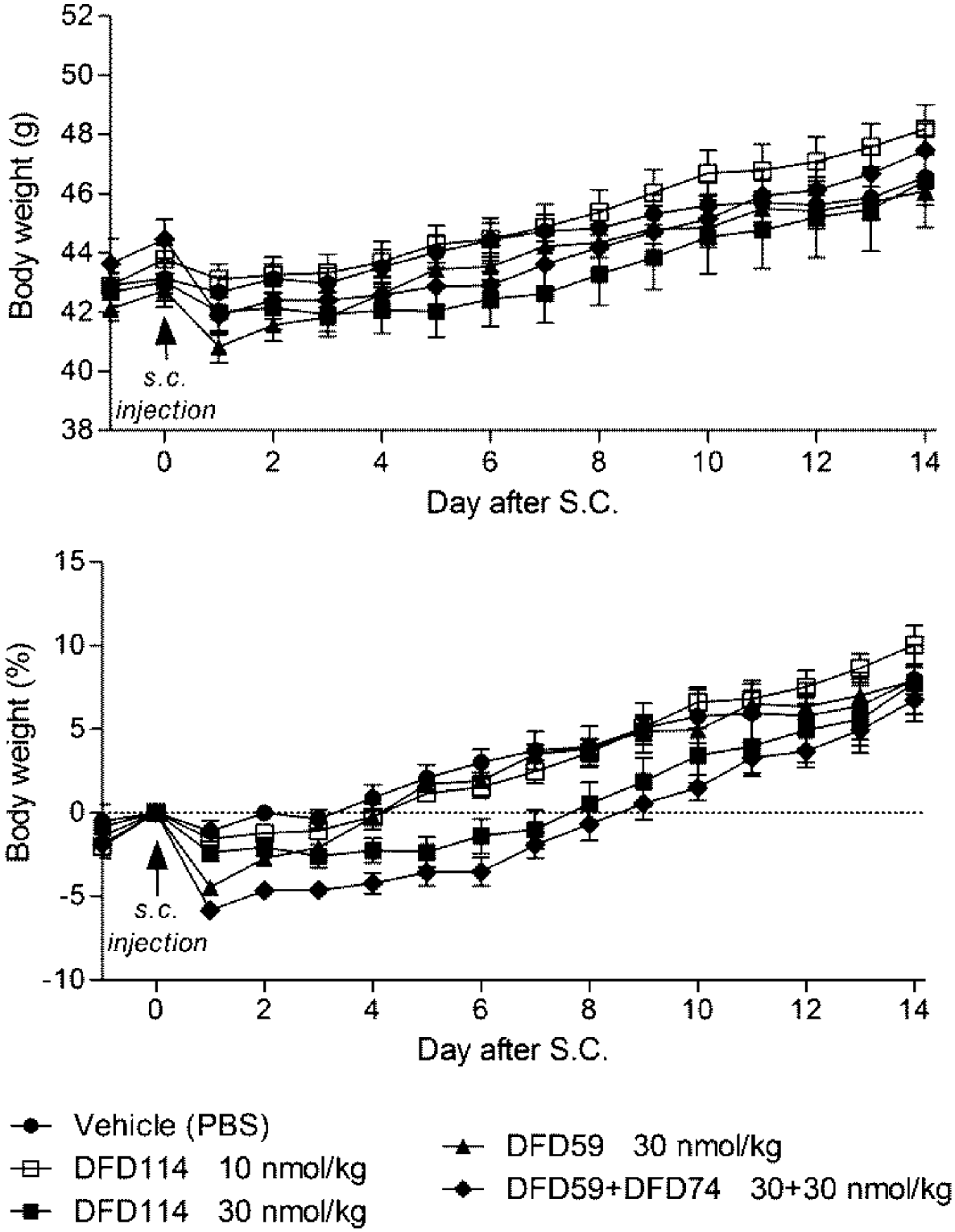
[Fig. 15]


PK by FGF21 detection
Protein Con. (ng/ml)
10000
1000
100
10
1
0 24 48 72 96 120 144 168 192 216 240
Time (hr)
PK by GLP1-Fc detection
Protein Con. (ng/ml)
10000
1000
100
10
1
0 24 48 72 96 120 144 168 192 216 240
Time (hr)
DFD59
DFD69
DFD112
DFD114

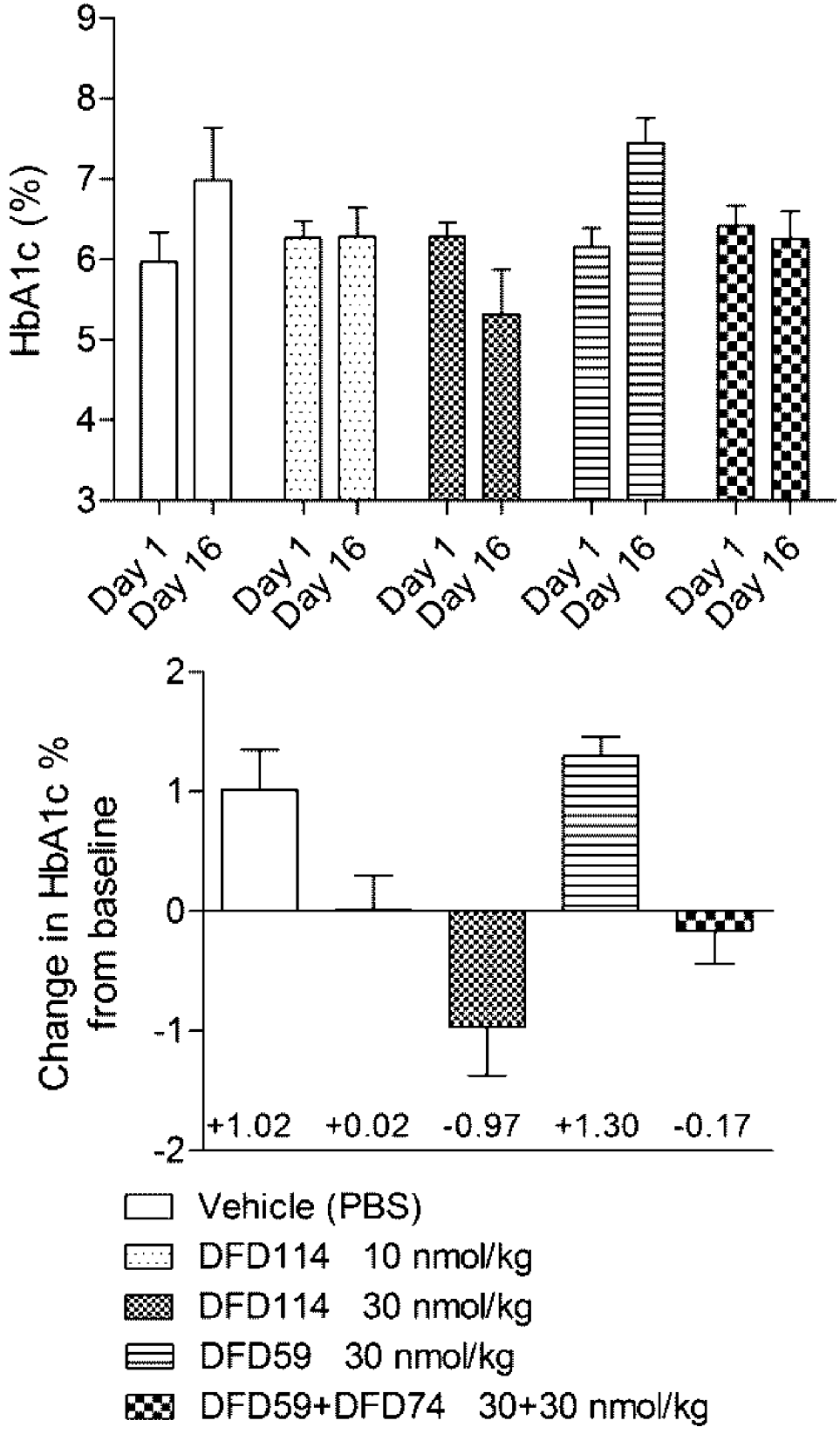
[Fig. 16]


600
400
200
0
Random blood glucose level (mg/dl)
s.c. injection
0
2
4
6
8
10
12
14
Day after S.C.
Vehicle (PBS)
DFD114 10 nmol/kg
DFD114 30 nmol/kg
DFD59 30 nmol/kg
DFD59+DFD74 30+30 nmol/kg

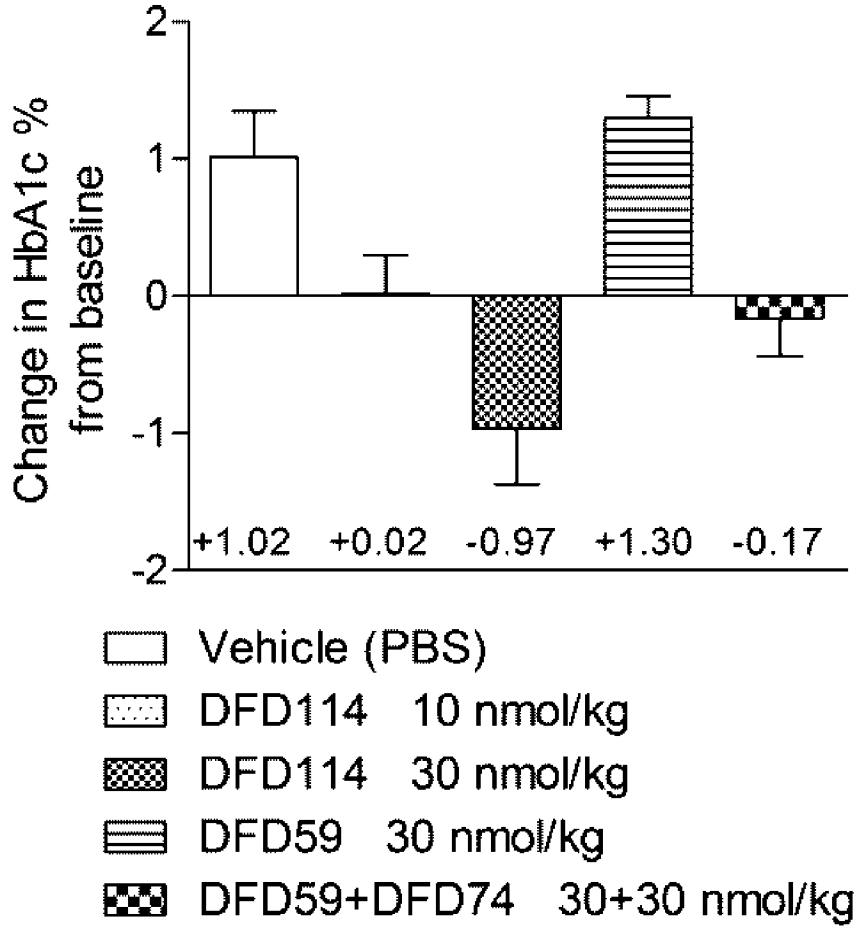
[Fig. 17]


Body weight (g)
52
50
48
46
44
42
40
38
s.c.
injection
0
2
4
6
8
10
12
14
Day after S.C.
Body weight (%)
15
10
5
0
-5
-10
s.c.
injection
0
2
4
6
8
10
12
14
Day after S.C.
Vehicle (PBS)
DFD114 10 nmol/kg
DFD114 30 nmol/kg
DFD59 30 nmol/kg
DFD59+DFD74 30+30 nmol/kg

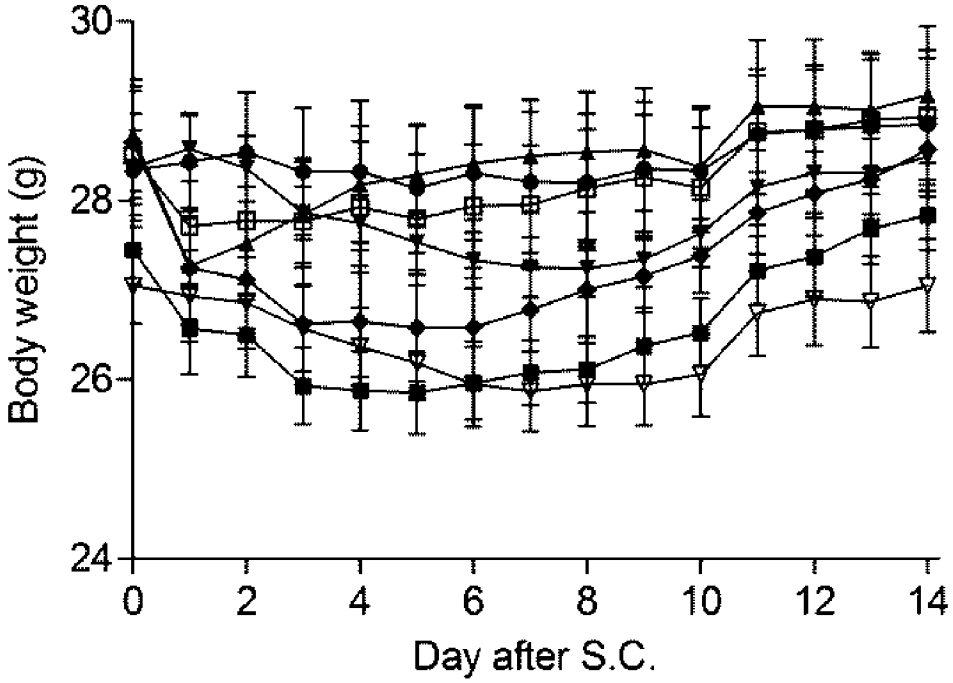
[Fig. 18]

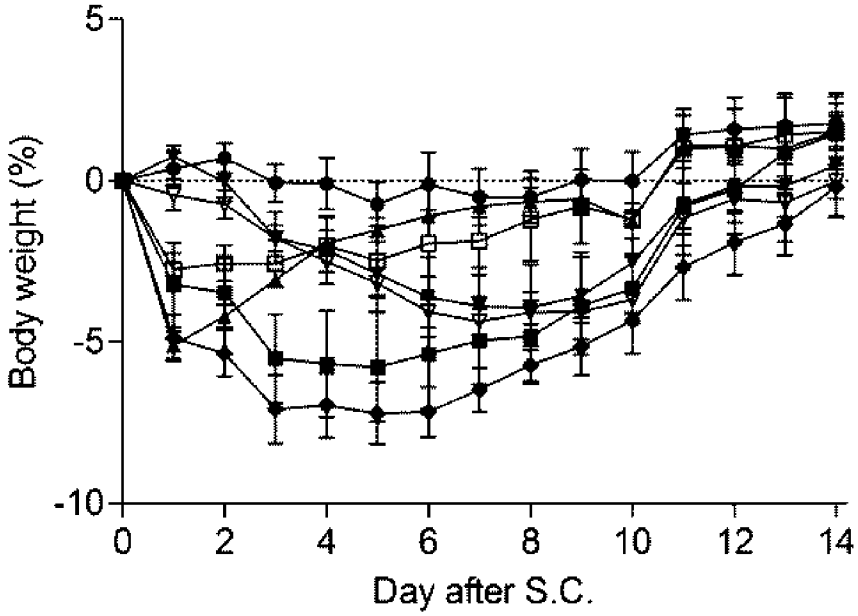

HbA1c (%)
9
8
7
6
5
4
3
Day 1
Day 16
Change in HbA1c %
from baseline
2
1
0
-1
-2
+1.02
+0.02
-0.97
+1.30
-0.17
Vehicle (PBS)
DFD114 10 nmol/kg
DFD114 30 nmol/kg
DFD59 30 nmol/kg
DFD59+DFD74 30+30 nmol/kg

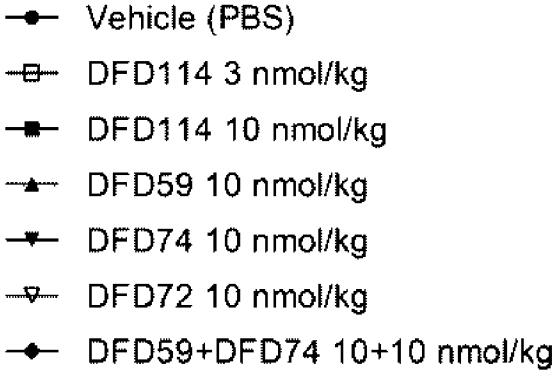
[Fig. 19]


Change in HbA1c % from baseline
2
1
0
-1
-2
+1.02
+0.02
-0.97
+1.30
-0.17
Vehicle (PBS)
DFD114 10 nmol/kg
DFD114 30 nmol/kg
DFD59 30 nmol/kg
DFD59+DFD74 30+30 nmol/kg

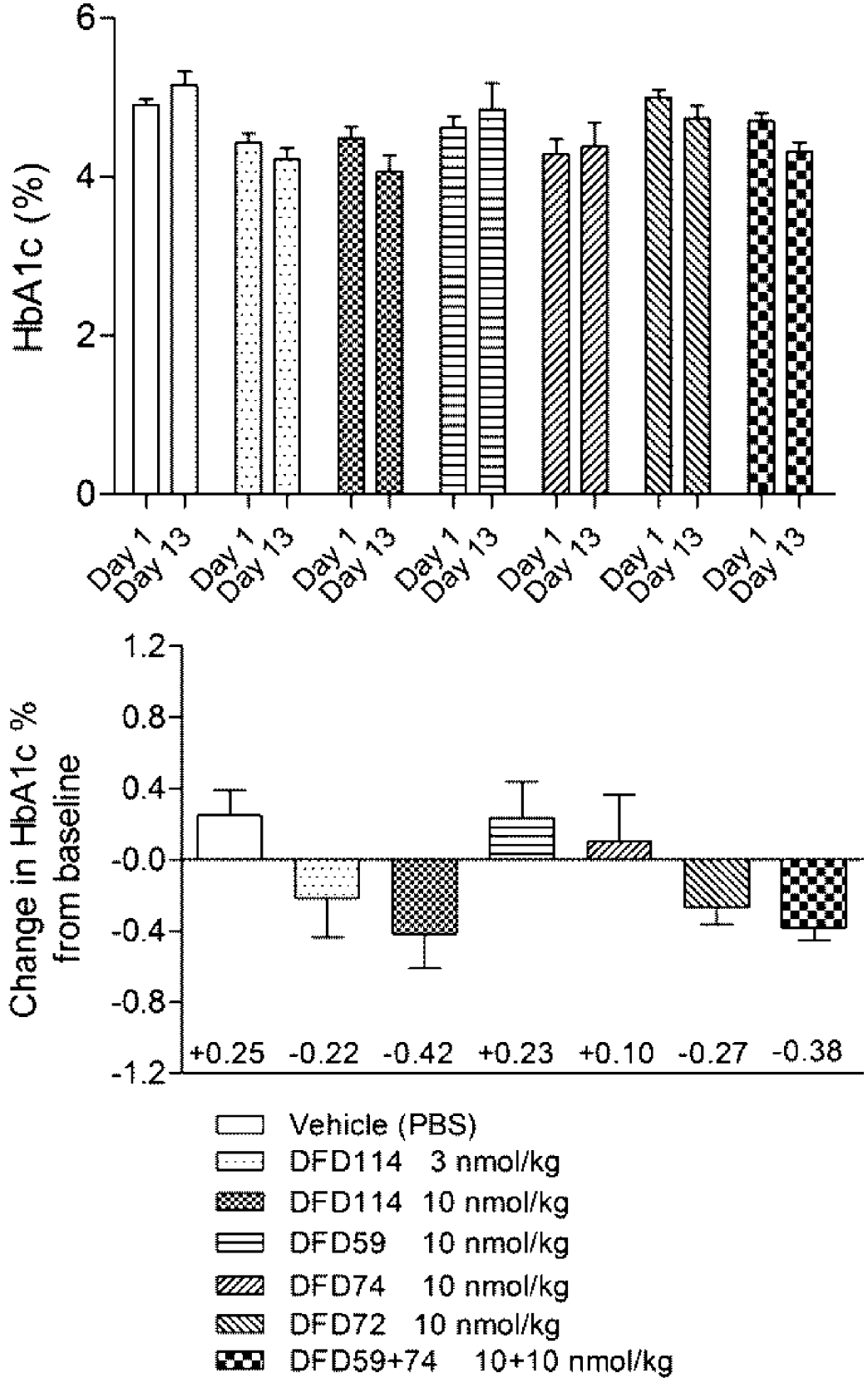
[Fig. 20]


30
28
26
24
Body weight (g)
0
2
4
6
8
10
12
14
Day after S.C.


5
0
-5
-10
Body weight (%)
0
2
4
6
8
10
12
14
Day after S.C.


Vehicle (PBS)
DFD114 3 nmol/kg
DFD114 10 nmol/kg
DFD59 10 nmol/kg
DFD74 10 nmol/kg
DFD72 10 nmol/kg
DFD59+DFD74 10+10 nmol/kg

[Fig. 21]


HbA1c (%)
6
4
2
0
Day 1
Day 13
Change in HbA1c % from baseline
1.2
0.8
0.4
-0.0
-0.4
-0.8
-1.2
+0.25
-0.22
-0.42
+0.23
+0.10
-0.27
-0.38
Vehicle (PBS)
DFD114 3 nmol/kg
DFD114 10 nmol/kg
DFD59 10 nmol/kg
DFD74 10 nmol/kg
DFD72 10 nmol/kg
DFD59+74 10+10 nmol/kg

<110> YUHAN CORPORATION

<120> DUAL FUNCTION PROTEINS AND PHARMACEUTICAL COMPOSITION COMPRISING SAME

<130> PCB608054YUH

<150> KR 2015-0150576
<151> 2015-10-28

<160> 80

<170> KopatentIn 2.0

<210> 1
<211> 181
<212> PRT
<213> human FGF21

<400> 1

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His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
  1               5                  10                  15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
             20                  25                  30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
         35                  40                  45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
     50                  55                  60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
 65                  70                  75                  80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                 85                  90                  95

Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100                 105                 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115                 120                 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130                 135                 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145                 150                 155                 160

Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser
                165                 170                 175

Pro Ser Tyr Ala Ser
            180
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<210> 2
<211> 30
<212> PRT
<213> Artificial Sequence

<220>

<223> linker

<400> 2
Ala Lys Ala Thr Thr Ala Pro Ala Thr Thr Arg Asn Thr Gly Arg Gly
  1   5   10   15

Gly Glu Glu Lys Lys Lys Glu Lys Glu Lys Glu Glu Gln Glu
            20   25   30

<210> 3
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 3
Ala Lys Ala Thr Thr Ala Pro Ala Thr Thr Arg Asn Thr Gly Arg Gly
  1   5   10   15

Gly

<210> 4
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 4
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
  1   5   10   15

<210> 5
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 5
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala
  1   5   10   15

<210> 6
<211> 181
<212> PRT
<213> Artificial Sequence

<220>
<223> FGF21 variant

<400> 6
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
  1   5   10   15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
             20   25   30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
         35   40   45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
     50   55   60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
 65   70   75   80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                 85   90   95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100   105   110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115   120   125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130   135   140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145   150   155   160

Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser
                165   170   175

Pro Ser Tyr Ala Ser
            180

<210> 7
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> FGF21 variant

<400> 7
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
  1   5   10   15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
             20   25   30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
         35   40   45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
     50   55   60

```
Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
 65                  70                  75                  80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                 85                  90                  95

Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100                 105                 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115                 120                 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130                 135                 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145                 150                 155                 160

Gly Ser Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Val Arg
                165                 170                 175

Ser Pro Ser Tyr Ala Ser
            180


<210>    8
<211>    182
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    FGF21 variant


<400>    8
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
  1                 5                  10                  15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
             20                  25                  30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
         35                  40                  45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
     50                  55                  60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
 65                  70                  75                  80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                 85                  90                  95

Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100                 105                 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115                 120                 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130                 135                 140
```

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145 150 155 160

Gly Ser Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Asn Arg
165 170 175

Ser Pro Ser Tyr Ala Ser
180

<210> 9
<211> 181
<212> PRT
<213> Artificial Sequence

<220>
<223> FGF21 variant

<400> 9
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
35 40 45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
50 55 60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
65 70 75 80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
85 90 95

Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
100 105 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
115 120 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
130 135 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145 150 155 160

Gly Ser Ser Asp Pro Leu Ser Met Val Asn Pro Ser Gln Gly Arg Ser
165 170 175

Pro Ser Tyr Ala Ser
180

<210> 10
<211> 181
<212> PRT
<213> Artificial Sequence

<220>

<223> FGF21 variant

<400> 10

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His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
  1               5                  10                  15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
             20                  25                  30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
         35                  40                  45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
     50                  55                  60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
 65                  70                  75                  80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                 85                  90                  95

Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100                 105                 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115                 120                 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130                 135                 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145                 150                 155                 160

Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Asn Arg Ser
                165                 170                 175

Pro Ser Tyr Ala Ser
            180
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<210> 11
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> FGF21 variant

<400> 11

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His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
  1               5                  10                  15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
             20                  25                  30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
         35                  40                  45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
     50                  55                  60
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```
Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
 65                  70                  75                  80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                 85                  90                  95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100                 105                 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115                 120                 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130                 135                 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145                 150                 155                 160

Gly Ser Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Val Arg
                165                 170                 175

Ser Pro Ser Tyr Ala Ser
            180


<210>    12
<211>    182
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    FGF21 variant


<400>    12
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
  1               5                  10                  15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
             20                  25                  30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
         35                  40                  45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
     50                  55                  60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
 65                  70                  75                  80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                 85                  90                  95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100                 105                 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115                 120                 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130                 135                 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
```

145 150 155 160

Gly Ser Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Asn Arg
165 170 175

Ser Pro Ser Tyr Ala Ser
180

<210> 13
<211> 181
<212> PRT
<213> Artificial Sequence

<220>
<223> FGF21 variant

<400> 13
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
35 40 45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
50 55 60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
65 70 75 80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
85 90 95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
100 105 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
115 120 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
130 135 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145 150 155 160

Gly Ser Ser Asp Pro Leu Ser Met Val Asn Pro Ser Gln Gly Arg Ser
165 170 175

Pro Ser Tyr Ala Ser
180

<210> 14
<211> 181
<212> PRT
<213> Artificial Sequence

<220>
<223> FGF21 variant

<400> 14
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
35 40 45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
50 55 60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
65 70 75 80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
85 90 95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
100 105 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
115 120 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
130 135 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145 150 155 160

Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Asn Arg Ser
165 170 175

Pro Ser Tyr Ala Ser
180

<210> 15
<211> 181
<212> PRT
<213> Artificial Sequence

<220>
<223> FGF21 variant

<400> 15
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
35 40 45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
50 55 60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly

65 70 75 80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
85 90 95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
100 105 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
115 120 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
130 135 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145 150 155 160

Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser
165 170 175

Pro Ser Tyr Glu Ser
180

<210> 16
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> FGF21 variant

<400> 16
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
35 40 45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
50 55 60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
65 70 75 80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
85 90 95

Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
100 105 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
115 120 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
130 135 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145 150 155 160

```
Gly Ser Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Val Arg
                165                 170                 175

Ser Pro Ser Tyr Glu Ser
            180


<210>    17
<211>    182
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    FGF21 variant


<400>    17
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
  1               5                  10                  15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
             20                  25                  30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
         35                  40                  45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
     50                  55                  60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
 65                  70                  75                  80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                 85                  90                  95

Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100                 105                 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115                 120                 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130                 135                 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145                 150                 155                 160

Gly Ser Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Asn Arg
                165                 170                 175

Ser Pro Ser Tyr Glu Ser
            180


<210>    18
<211>    181
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    FGF21 variant
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<400> 18
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
35 40 45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
50 55 60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
65 70 75 80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
85 90 95

Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
100 105 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
115 120 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
130 135 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145 150 155 160

Gly Ser Ser Asp Pro Leu Ser Met Val Asn Pro Ser Gln Gly Arg Ser
165 170 175

Pro Ser Tyr Glu Ser
180

<210> 19
<211> 181
<212> PRT
<213> Artificial Sequence

<220>
<223> FGF21 variant

<400> 19
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
35 40 45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
50 55 60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
65 70 75 80

```
Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                85                  90                  95

Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100                 105                 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115                 120                 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130                 135                 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145                 150                 155                 160

Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Asn Arg Ser
                165                 170                 175

Pro Ser Tyr Glu Ser
            180


<210>    20
<211>    182
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    FGF21 variant


<400>    20
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
  1               5                  10                  15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
             20                  25                  30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
         35                  40                  45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
     50                  55                  60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
 65                  70                  75                  80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                85                  90                  95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100                 105                 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115                 120                 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130                 135                 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145                 150                 155                 160
```

```
Gly Ser Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Val Arg
                165                 170                 175

Ser Pro Ser Tyr Glu Ser
            180


<210>    21
<211>    182
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    FGF21 variant


<400>    21
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
  1               5                  10                  15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
             20                  25                  30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
         35                  40                  45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
     50                  55                  60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
 65                  70                  75                  80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                 85                  90                  95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100                 105                 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115                 120                 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130                 135                 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145                 150                 155                 160

Gly Ser Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Asn Arg
                165                 170                 175

Ser Pro Ser Tyr Glu Ser
            180


<210>    22
<211>    181
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    FGF21 variant
```

<400> 22
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
35 40 45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
50 55 60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
65 70 75 80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
85 90 95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
100 105 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
115 120 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
130 135 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145 150 155 160

Gly Ser Ser Asp Pro Leu Ser Met Val Asn Pro Ser Gln Gly Arg Ser
165 170 175

Pro Ser Tyr Glu Ser
180

<210> 23
<211> 181
<212> PRT
<213> Artificial Sequence

<220>
<223> FGF21 variant

<400> 23
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
35 40 45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
50 55 60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
65 70 75 80

```
Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                 85                  90                  95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100                 105                 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115                 120                 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130                 135                 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145                 150                 155                 160

Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Asn Arg Ser
                165                 170                 175

Pro Ser Tyr Glu Ser
            180


<210>    24
<211>    229
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    Human IgG4 Fc


<400>    24
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe
  1               5                  10                  15

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
             20                  25                  30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
         35                  40                  45

Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
     50                  55                  60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
 65                  70                  75                  80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
                 85                  90                  95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
            100                 105                 110

Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
        115                 120                 125

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
    130                 135                 140

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

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
```

165 170 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
180 185 190

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
195 200 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
210 215 220

Leu Ser Leu Gly Lys
225

<210> 25
<211> 228
<212> PRT
<213> Artificial Sequence

<220>
<223> Human IgG4 Fc variant

<400> 25
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Ala
1 5 10 15

Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
20 25 30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
35 40 45

Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
50 55 60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
65 70 75 80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
85 90 95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
100 105 110

Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
115 120 125

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
130 135 140

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

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
165 170 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
180 185 190

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
195 200 205

```
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    210                 215                 220

Leu Ser Leu Gly
225


<210>    26
<211>    223
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    Hybrid Fc variant


<400>    26
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
  1               5                  10                  15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
             20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
         35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
     50                  55                  60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
 65                  70                  75                  80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                 85                  90                  95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
            100                 105                 110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140

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

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
            180                 185                 190

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
    210                 215                 220
```

<210> 27
<211> 435
<212> PRT
<213> Artificial Sequence

<220>
<223> modified FGF21 variant connected to hybrid Fc

<400> 27

```
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
  1               5                  10                  15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
             20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
         35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
     50                  55                  60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
 65                  70                  75                  80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                 85                  90                  95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
            100                 105                 110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140

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

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
            180                 185                 190

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Ala
    210                 215                 220

Lys Ala Thr Thr Ala Pro Ala Thr Thr Arg Asn Thr Gly Arg Gly Gly
225                 230                 235                 240

Glu Glu Lys Lys Lys Glu Lys Glu Lys Glu Glu Gln Glu His Pro Ile
                245                 250                 255

Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg
            260                 265                 270

Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile
        275                 280                 285
```

```
Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser
    290                 295                 300

Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly
305                 310                 315                 320

Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr
                325                 330                 335

Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Glu Ile
            340                 345                 350

Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro
        355                 360                 365

Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro Arg
    370                 375                 380

Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu Pro
385                 390                 395                 400

Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser Ser
                405                 410                 415

Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Val Arg Ser Pro Ser
            420                 425                 430

Tyr Ala Ser
        435


<210>    28
<211>    422
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    modified FGF21 variant connected to hybrid Fc


<400>    28
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
  1               5                  10                  15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
             20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
         35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
     50                  55                  60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
 65                  70                  75                  80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                 85                  90                  95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
            100                 105                 110
```

```
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140

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

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
            180                 185                 190

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Ala
    210                 215                 220

Lys Ala Thr Thr Ala Pro Ala Thr Thr Arg Asn Thr Gly Arg Gly Gly
225                 230                 235                 240

His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
                245                 250                 255

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
            260                 265                 270

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
        275                 280                 285

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
    290                 295                 300

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
305                 310                 315                 320

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                325                 330                 335

Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            340                 345                 350

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        355                 360                 365

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    370                 375                 380

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
385                 390                 395                 400

Gly Ser Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Val Arg
                405                 410                 415

Ser Pro Ser Tyr Ala Ser
            420


<210>    29
<211>    420
```

<212> PRT
<213> Artificial Sequence

<220>
<223> modified FGF21 variant connected to hybrid Fc

<400> 29

```
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
  1               5                  10                  15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
             20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
         35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
     50                  55                  60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
 65                  70                  75                  80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                 85                  90                  95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
            100                 105                 110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140

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

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
            180                 185                 190

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Gly
    210                 215                 220

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His Pro
225                 230                 235                 240

Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln
                245                 250                 255

Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu
            260                 265                 270

Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu
        275                 280                 285

Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu
```

```
    290                 295                 300

Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu
305                 310                 315                 320

Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu
                325                 330                 335

Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu
            340                 345                 350

Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro
        355                 360                 365

Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu
    370                 375                 380

Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser
385                 390                 395                 400

Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Val Arg Ser Pro
                405                 410                 415

Ser Tyr Ala Ser
            420


<210>    30
<211>    420
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    modified FGF21 variant connected to hybrid Fc


<400>    30
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
  1               5                  10                  15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
             20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
         35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
     50                  55                  60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
 65                  70                  75                  80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                 85                  90                  95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
            100                 105                 110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140
```

```
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
145                 150                 155                 160

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
            180                 185                 190

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Gly
    210                 215                 220

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His Pro
225                 230                 235                 240

Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln
                245                 250                 255

Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu
            260                 265                 270

Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu
        275                 280                 285

Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu
    290                 295                 300

Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu
305                 310                 315                 320

Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu
                325                 330                 335

Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu
            340                 345                 350

Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro
        355                 360                 365

Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu
    370                 375                 380

Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser
385                 390                 395                 400

Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Asn Arg Ser Pro
                405                 410                 415

Ser Tyr Ala Ser
            420


<210>    31
<211>    419
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    modified FGF21 variant connected to hybrid Fc
```

<400> 31

```
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
  1               5                  10                  15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
         35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
     50                  55                  60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
 65                  70                  75                  80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                85                  90                  95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
            100                 105                 110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140

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

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
            180                 185                 190

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Gly
    210                 215                 220

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His Pro
225                 230                 235                 240

Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln
                245                 250                 255

Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu
            260                 265                 270

Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu
        275                 280                 285

Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu
    290                 295                 300

Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu
305                 310                 315                 320
```

```
Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu
                325                 330                 335

Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu
            340                 345                 350

Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro
        355                 360                 365

Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu
    370                 375                 380

Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser
385                 390                 395                 400

Ser Asp Pro Leu Ser Met Val Asn Pro Ser Gln Gly Arg Ser Pro Ser
                405                 410                 415

Tyr Ala Ser



<210>    32
<211>    419
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    modified FGF21 variant connected to hybrid Fc


<400>    32
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
  1               5                  10                  15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
             20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
         35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
     50                  55                  60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
 65                  70                  75                  80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                 85                  90                  95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
            100                 105                 110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140

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

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
```

```
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
            180                 185                 190

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Gly
    210                 215                 220

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His Pro
225                 230                 235                 240

Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln
                245                 250                 255

Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu
            260                 265                 270

Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu
        275                 280                 285

Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu
    290                 295                 300

Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu
305                 310                 315                 320

Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu
                325                 330                 335

Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu
            340                 345                 350

Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro
        355                 360                 365

Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu
    370                 375                 380

Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser
385                 390                 395                 400

Ser Asp Pro Leu Ser Met Val Asn Pro Ser Gln Gly Arg Ser Pro Ser
                405                 410                 415

Tyr Ala Ser


<210>    33
<211>    419
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    modified FGF21 variant connected to hybrid Fc


<400>    33
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
  1               5                  10                  15
```

```
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
        35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    50                  55                  60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
65                  70                  75                  80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                85                  90                  95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
            100                 105                 110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140

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

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
            180                 185                 190

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Gly
    210                 215                 220

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His Pro
225                 230                 235                 240

Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln
                245                 250                 255

Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu
            260                 265                 270

Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu
        275                 280                 285

Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu
    290                 295                 300

Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu
305                 310                 315                 320

Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu
                325                 330                 335

Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu
            340                 345                 350
```

```
Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro
        355                 360                 365

Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu
    370                 375                 380

Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser
385                 390                 395                 400

Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Asn Arg Ser Pro Ser
                405                 410                 415

Tyr Ala Ser
```

<210> 34
<211> 419
<212> PRT
<213> Artificial Sequence

<220>
<223> modified FGF21 variant connected to hybrid Fc

<400> 34

```
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
  1               5                  10                  15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
             20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
         35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
     50                  55                  60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
 65                  70                  75                  80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                 85                  90                  95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
            100                 105                 110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140

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

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
            180                 185                 190
```

```
Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Gly
    210                 215                 220

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His Pro
225                 230                 235                 240

Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln
                245                 250                 255

Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu
            260                 265                 270

Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu
        275                 280                 285

Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu
    290                 295                 300

Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu
305                 310                 315                 320

Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu
                325                 330                 335

Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu
            340                 345                 350

Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro
        355                 360                 365

Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu
    370                 375                 380

Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser
385                 390                 395                 400

Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser
                405                 410                 415

Tyr Ala Ser



<210>    35
<211>    420
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    modified FGF21 variant connected to hybrid Fc


<400>    35
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
  1               5                  10                  15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
             20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
```

```
         35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
     50                  55                  60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
 65                  70                  75                  80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                 85                  90                  95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
            100                 105                 110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140

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

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
            180                 185                 190

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Gly
    210                 215                 220

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His Pro
225                 230                 235                 240

Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln
                245                 250                 255

Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu
            260                 265                 270

Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu
        275                 280                 285

Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu
    290                 295                 300

Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu
305                 310                 315                 320

Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Glu
                325                 330                 335

Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu
            340                 345                 350

Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro
        355                 360                 365

Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu
```

370 375 380

Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser
385 390 395 400

Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Val Arg Ser Pro
405 410 415

Ser Tyr Ala Ser
420

<210> 36
<211> 420
<212> PRT
<213> Artificial Sequence

<220>
<223> modified FGF21 variant connected to hybrid Fc

<400> 36
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
1 5 10 15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
20 25 30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
35 40 45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
50 55 60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
65 70 75 80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
85 90 95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
100 105 110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
115 120 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
130 135 140

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

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
165 170 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
180 185 190

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
195 200 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Gly
210 215 220

```
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His Pro
225                 230                 235                 240

Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln
                245                 250                 255

Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu
            260                 265                 270

Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu
        275                 280                 285

Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu
    290                 295                 300

Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu
305                 310                 315                 320

Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Glu
                325                 330                 335

Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu
            340                 345                 350

Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro
        355                 360                 365

Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu
    370                 375                 380

Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser
385                 390                 395                 400

Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Val Arg Ser Pro
                405                 410                 415

Ser Tyr Glu Ser
            420


<210>    37
<211>    420
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    modified FGF21 variant connected to hybrid Fc


<400>    37
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
  1               5                  10                  15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
             20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
         35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
     50                  55                  60
```

```
Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
 65                  70                  75                  80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                 85                  90                  95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
            100                 105                 110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140

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

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
            180                 185                 190

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Gly
    210                 215                 220

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His Pro
225                 230                 235                 240

Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln
                245                 250                 255

Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu
            260                 265                 270

Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu
        275                 280                 285

Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu
    290                 295                 300

Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu
305                 310                 315                 320

Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Glu
                325                 330                 335

Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu
            340                 345                 350

Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro
        355                 360                 365

Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu
    370                 375                 380

Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser
385                 390                 395                 400
```

Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu Ala Asn Arg Ser Pro
405 410 415

Ser Tyr Glu Ser
420

<210> 38
<211> 419
<212> PRT
<213> Artificial Sequence

<220>
<223> modified FGF21 variant connected to hybrid Fc

<400> 38
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
1 5 10 15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
20 25 30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
35 40 45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
50 55 60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
65 70 75 80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
85 90 95

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
100 105 110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
115 120 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
130 135 140

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

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
165 170 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
180 185 190

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
195 200 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Gly
210 215 220

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His Pro
225 230 235 240

Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln

```
                245                 250                 255

Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu
            260                 265                 270

Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu
        275                 280                 285

Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu
    290                 295                 300

Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu
305                 310                 315                 320

Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Glu
                325                 330                 335

Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu
            340                 345                 350

Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro
        355                 360                 365

Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu
    370                 375                 380

Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser
385                 390                 395                 400

Ser Asp Pro Leu Ser Met Val Asn Pro Ser Gln Gly Arg Ser Pro Ser
                405                 410                 415

Tyr Ala Ser



<210>    39
<211>    419
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    modified FGF21 variant connected to hybrid Fc


<400>    39
Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr Gln Pro Leu Gly Val
  1               5                  10                  15

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
             20                  25                  30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
         35                  40                  45

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
     50                  55                  60

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
 65                  70                  75                  80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                 85                  90                  95
```

```
Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
            100                 105                 110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        115                 120                 125

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    130                 135                 140

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

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                165                 170                 175

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
            180                 185                 190

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        195                 200                 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Gly
    210                 215                 220

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His Pro
225                 230                 235                 240

Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln
                245                 250                 255

Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu
            260                 265                 270

Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu
        275                 280                 285

Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu
    290                 295                 300

Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu
305                 310                 315                 320

Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Glu
                325                 330                 335

Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu
            340                 345                 350

Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro
        355                 360                 365

Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu
    370                 375                 380

Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser
385                 390                 395                 400

Ser Asp Pro Leu Ser Met Val Asn Pro Ser Gln Gly Arg Ser Pro Ser
                405                 410                 415

Tyr Glu Ser
```

<210> 40
<211> 423
<212> PRT
<213> Artificial Sequence

<220>
<223> REG(Amgen)

<400> 40
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1 5 10 15

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
20 25 30

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
35 40 45

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
50 55 60

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65 70 75 80

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
85 90 95

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
100 105 110

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
115 120 125

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
130 135 140

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145 150 155 160

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
165 170 175

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
180 185 190

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
195 200 205

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
210 215 220

Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
225 230 235 240

Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly
245 250 255

Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu
260 265 270

```
Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp
        275                 280                 285

Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val
    290                 295                 300

Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro
305                 310                 315                 320

Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser
                325                 330                 335

Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu
            340                 345                 350

Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg
        355                 360                 365

Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu
    370                 375                 380

Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro
385                 390                 395                 400

Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln Gly
                405                 410                 415

Arg Ser Pro Ser Tyr Glu Ser
            420


<210>    41
<211>    424
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    FGF21 connected to Fc(lilly)


<400>    41
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Ala
  1               5                  10                  15

Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
             20                  25                  30

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
         35                  40                  45

Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
     50                  55                  60

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
 65                  70                  75                  80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
                 85                  90                  95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
            100                 105                 110

Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
```

```
        115                 120                 125

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
    130                 135                 140

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

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                165                 170                 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
            180                 185                 190

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
        195                 200                 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    210                 215                 220

Leu Ser Leu Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

Gly Gly Ser Ala His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe
                245                 250                 255

Gly Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln
            260                 265                 270

Thr Glu Cys His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Cys Ala
        275                 280                 285

Ala Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro
    290                 295                 300

Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln
305                 310                 315                 320

Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala
                325                 330                 335

Cys Ser Phe Arg Glu Asp Leu Lys Glu Asp Gly Tyr Asn Val Tyr Gln
            340                 345                 350

Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asp Lys Ser Pro
        355                 360                 365

His Arg Lys Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro
    370                 375                 380

Gly Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln
385                 390                 395                 400

Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Arg Leu Val Glu Pro Ser
                405                 410                 415

Gln Leu Arg Ser Pro Ser Phe Glu
            420


<210>    42
<211>    31
<212>    PRT
```

<213> Artificial Sequence

<220>
<223> GLP-1

<400> 42
His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
20 25 30

<210> 43
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> GLP-1 variant

<400> 43
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
20 25 30

<210> 44
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> GLP-1 variant

<400> 44
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
20 25 30

<210> 45
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> GLP-1 variant

<400> 45
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly
20 25 30

<210> 46
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> GLP-1 variant

<400> 46
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly
20 25 30

<210> 47
<211> 245
<212> PRT
<213> Artificial Sequence

<220>
<223> hybrid Fc5

<400> 47
Arg Asn Thr Gly Arg Gly Gly Glu Glu Lys Lys Lys Glu Lys Glu Lys
1 5 10 15

Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys Pro Ser His
20 25 30

Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
35 40 45

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
50 55 60

Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
65 70 75 80

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
85 90 95

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
100 105 110

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
115 120 125

```
Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
    130                 135                 140

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
145                 150                 155                 160

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

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
            180                 185                 190

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
        195                 200                 205

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
    210                 215                 220

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
225                 230                 235                 240

Leu Ser Leu Gly Lys
                245


<210>    48
<211>    233
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    hybrid Fc40


<400>    48
Glu Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu
  1               5                  10                  15

Cys Pro Ser His Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys
             20                  25                  30

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
         35                  40                  45

Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
     50                  55                  60

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
 65                  70                  75                  80

Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
                 85                  90                  95

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
            100                 105                 110

Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
        115                 120                 125

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met
    130                 135                 140

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
```

145 150 155 160

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
165 170 175

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
180 185 190

Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val
195 200 205

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
210 215 220

Lys Ser Leu Ser Leu Ser Leu Gly Lys
225 230

<210> 49
<211> 276
<212> PRT
<213> Artificial Sequence

<220>
<223> GLP-1 variant connected to hybrid Fc5

<400> 49
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly Arg
20 25 30

Asn Thr Gly Arg Gly Gly Glu Glu Lys Lys Lys Glu Lys Glu Lys Glu
35 40 45

Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr
50 55 60

Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
65 70 75 80

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
85 90 95

Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
100 105 110

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
115 120 125

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
130 135 140

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser
145 150 155 160

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
165 170 175

Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
180 185 190

```
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
        195                 200                 205

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

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
225                 230                 235                 240

Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
                245                 250                 255

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
            260                 265                 270

Ser Leu Gly Lys
        275


<210>    50
<211>    264
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    GLP-1 variant connected to hybrid Fc40


<400>    50
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly Glu
             20                  25                  30

Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys
         35                  40                  45

Pro Ser His Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro
     50                  55                  60

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 65                  70                  75                  80

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
                85                  90                  95

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            100                 105                 110

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        115                 120                 125

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
    130                 135                 140

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
145                 150                 155                 160

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
                165                 170                 175
```

```
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            180                 185                 190

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        195                 200                 205

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    210                 215                 220

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
225                 230                 235                 240

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                245                 250                 255

Ser Leu Ser Leu Ser Leu Gly Lys
            260


<210>    51
<211>    276
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    GLP-1 variant connected to hybrid Fc5


<400>    51
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly Arg
             20                  25                  30

Asn Thr Gly Arg Gly Gly Glu Glu Lys Lys Lys Glu Lys Glu Lys Glu
         35                  40                  45

Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr
     50                  55                  60

Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 65                  70                  75                  80

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
                 85                  90                  95

Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
            100                 105                 110

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
        115                 120                 125

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
    130                 135                 140

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser
145                 150                 155                 160

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
                165                 170                 175

Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
```

180 185 190

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
195 200 205

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

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
225 230 235 240

Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
245 250 255

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
260 265 270

Ser Leu Gly Lys
275

<210> 52
<211> 264
<212> PRT
<213> Artificial Sequence

<220>
<223> GLP-1 variant connected to hybrid Fc40

<400> 52
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly Glu
20 25 30

Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys
35 40 45

Pro Ser His Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro
50 55 60

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
65 70 75 80

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
85 90 95

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
100 105 110

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
115 120 125

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
130 135 140

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
145 150 155 160

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
165 170 175

```
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            180                 185                 190

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        195                 200                 205

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    210                 215                 220

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
225                 230                 235                 240

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                245                 250                 255

Ser Leu Ser Leu Ser Leu Gly Lys
            260


<210>    53
<211>    276
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    GLP-1 variant connected to hybrid Fc5


<400>    53
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly Arg
             20                  25                  30

Asn Thr Gly Arg Gly Gly Glu Glu Lys Lys Lys Glu Lys Glu Lys Glu
         35                  40                  45

Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr
     50                  55                  60

Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 65                  70                  75                  80

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
                 85                  90                  95

Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
            100                 105                 110

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
        115                 120                 125

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
    130                 135                 140

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser
145                 150                 155                 160

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
                165                 170                 175
```

```
Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
            180                 185                 190

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
        195                 200                 205

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

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
225                 230                 235                 240

Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
                245                 250                 255

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
            260                 265                 270

Ser Leu Gly Lys
        275


<210>    54
<211>    264
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    GLP-1 variant connected to hybrid Fc40


<400>    54
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly Glu
             20                  25                  30

Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys
         35                  40                  45

Pro Ser His Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro
     50                  55                  60

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 65                  70                  75                  80

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
                 85                  90                  95

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            100                 105                 110

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        115                 120                 125

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
    130                 135                 140

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
145                 150                 155                 160

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
```

165 170 175

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
180 185 190

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
195 200 205

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
210 215 220

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
225 230 235 240

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
245 250 255

Ser Leu Ser Leu Ser Leu Gly Lys
260

<210> 55
<211> 276
<212> PRT
<213> Artificial Sequence

<220>
<223> GLP-1 variant connected to hybrid Fc5

<400> 55
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly Arg
20 25 30

Asn Thr Gly Arg Gly Gly Glu Glu Lys Lys Lys Glu Lys Glu Lys Glu
35 40 45

Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr
50 55 60

Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
65 70 75 80

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
85 90 95

Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
100 105 110

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
115 120 125

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
130 135 140

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser
145 150 155 160

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
165 170 175

```
Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
            180                 185                 190

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
        195                 200                 205

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

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
225                 230                 235                 240

Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
                245                 250                 255

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
            260                 265                 270

Ser Leu Gly Lys
        275


<210>    56
<211>    264
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    GLP-1 variant connected to hybrid Fc40


<400>    56
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly Glu
             20                  25                  30

Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys
         35                  40                  45

Pro Ser His Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro
     50                  55                  60

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 65                  70                  75                  80

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
                 85                  90                  95

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            100                 105                 110

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        115                 120                 125

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
    130                 135                 140

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
145                 150                 155                 160
```

```
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
                165                 170                 175

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            180                 185                 190

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        195                 200                 205

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    210                 215                 220

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
225                 230                 235                 240

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                245                 250                 255

Ser Leu Ser Leu Ser Leu Gly Lys
            260


<210>    57
<211>    275
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    Dulaglutide


<400>    57
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly Gly
             20                  25                  30

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Glu
         35                  40                  45

Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala
     50                  55                  60

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 65                  70                  75                  80

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
                 85                  90                  95

Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
            100                 105                 110

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
        115                 120                 125

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
    130                 135                 140

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser
145                 150                 155                 160

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
```

```
                165                 170                 175

Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
            180                 185                 190

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
        195                 200                 205

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

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
225                 230                 235                 240

Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
                245                 250                 255

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
            260                 265                 270

Ser Leu Gly
        275


<210>    58
<211>    461
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    GLP1(A2G)-HyFc40-GS3-FGF21(EIRP, TGLEAV)


<400>    58
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly Glu
             20                  25                  30

Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys
         35                  40                  45

Pro Ser His Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro
     50                  55                  60

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 65                  70                  75                  80

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
                 85                  90                  95

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            100                 105                 110

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        115                 120                 125

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
    130                 135                 140

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
145                 150                 155                 160
```

```
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
                165                 170                 175

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            180                 185                 190

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        195                 200                 205

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    210                 215                 220

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
225                 230                 235                 240

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                245                 250                 255

Ser Leu Ser Leu Ser Leu Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly
            260                 265                 270

Gly Ser Gly Gly Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu
        275                 280                 285

Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp
    290                 295                 300

Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val
305                 310                 315                 320

Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala
                325                 330                 335

Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe
            340                 345                 350

Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp
        355                 360                 365

Pro Glu Ala Cys Ser Phe Arg Glu Glu Ile Arg Pro Asp Gly Tyr Asn
    370                 375                 380

Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn
385                 390                 395                 400

Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu
                405                 410                 415

Pro Leu Pro Gly Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu
            420                 425                 430

Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val
        435                 440                 445

Thr Gly Leu Glu Ala Val Arg Ser Pro Ser Tyr Ala Ser
    450                 455                 460
```

<210> 59
<211> 473

<212> PRT
<213> Artificial Sequence

<220>
<223> GLP1(GE)-HyFc5-GS3-FGF21(EIRP, TGLEAV)

<400> 59

```
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly Arg
             20                  25                  30

Asn Thr Gly Arg Gly Gly Glu Glu Lys Lys Lys Glu Lys Glu Lys Glu
         35                  40                  45

Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr
     50                  55                  60

Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 65                  70                  75                  80

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
                 85                  90                  95

Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
            100                 105                 110

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
        115                 120                 125

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
    130                 135                 140

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser
145                 150                 155                 160

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
                165                 170                 175

Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
            180                 185                 190

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
        195                 200                 205

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

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
225                 230                 235                 240

Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
                245                 250                 255

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
            260                 265                 270

Ser Leu Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
        275                 280                 285

Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
```

290 295 300

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
305 310 315 320

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
325 330 335

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
340 345 350

Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
355 360 365

Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
370 375 380

Ser Phe Arg Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser
385 390 395 400

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
405 410 415

Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
420 425 430

Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
435 440 445

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu
450 455 460

Ala Val Arg Ser Pro Ser Tyr Ala Ser
465 470

<210> 60
<211> 461
<212> PRT
<213> Artificial Sequence

<220>
<223> GLP1(GE)-HyFc40-GS3-FGF21(EIRP, TGLEAV)

<400> 60
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly Glu
20 25 30

Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys
35 40 45

Pro Ser His Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro
50 55 60

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
65 70 75 80

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
85 90 95

```
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            100                 105                 110

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        115                 120                 125

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
    130                 135                 140

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
145                 150                 155                 160

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
                165                 170                 175

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            180                 185                 190

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        195                 200                 205

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    210                 215                 220

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
225                 230                 235                 240

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                245                 250                 255

Ser Leu Ser Leu Ser Leu Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly
            260                 265                 270

Gly Ser Gly Gly Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu
        275                 280                 285

Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp
    290                 295                 300

Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val
305                 310                 315                 320

Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala
                325                 330                 335

Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe
            340                 345                 350

Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp
        355                 360                 365

Pro Glu Ala Cys Ser Phe Arg Glu Glu Ile Arg Pro Asp Gly Tyr Asn
    370                 375                 380

Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn
385                 390                 395                 400

Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu
                405                 410                 415

Pro Leu Pro Gly Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu
            420                 425                 430
```

```
Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val
        435                 440                 445

Thr Gly Leu Glu Ala Val Arg Ser Pro Ser Tyr Ala Ser
    450                 455                 460
```

<210> 61
<211> 473
<212> PRT
<213> Artificial Sequence

<220>
<223> GLP1(GG)-HyFc5-GS3-FGF21(EIRP, TGLEAV)

<400> 61

```
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly Arg
             20                  25                  30

Asn Thr Gly Arg Gly Gly Glu Glu Lys Lys Lys Glu Lys Glu Lys Glu
         35                  40                  45

Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr
     50                  55                  60

Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 65                  70                  75                  80

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
                 85                  90                  95

Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
            100                 105                 110

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
        115                 120                 125

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
    130                 135                 140

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser
145                 150                 155                 160

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
                165                 170                 175

Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
            180                 185                 190

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
        195                 200                 205

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

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
```

```
225                 230                 235                 240

Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
                245                 250                 255

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
            260                 265                 270

Ser Leu Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
        275                 280                 285

Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
    290                 295                 300

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
305                 310                 315                 320

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
                325                 330                 335

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
            340                 345                 350

Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
        355                 360                 365

Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
    370                 375                 380

Ser Phe Arg Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser
385                 390                 395                 400

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
                405                 410                 415

Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
            420                 425                 430

Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
        435                 440                 445

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu
    450                 455                 460

Ala Val Arg Ser Pro Ser Tyr Ala Ser
465                 470


<210>    62
<211>    461
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    GLP1(GG)-HyFc40-GS3-FGF21(EIRP, TGLEAV)


<400>    62
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly Glu
             20                  25                  30
```

```
Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys
         35                  40                  45

Pro Ser His Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro
     50                  55                  60

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 65                  70                  75                  80

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
                 85                  90                  95

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            100                 105                 110

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        115                 120                 125

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
    130                 135                 140

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
145                 150                 155                 160

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
                165                 170                 175

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            180                 185                 190

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        195                 200                 205

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    210                 215                 220

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
225                 230                 235                 240

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                245                 250                 255

Ser Leu Ser Leu Ser Leu Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly
            260                 265                 270

Gly Ser Gly Gly Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu
        275                 280                 285

Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp
    290                 295                 300

Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val
305                 310                 315                 320

Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala
                325                 330                 335

Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe
            340                 345                 350

Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp
        355                 360                 365
```

```
Pro Glu Ala Cys Ser Phe Arg Glu Glu Ile Arg Pro Asp Gly Tyr Asn
    370                 375                 380

Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn
385                 390                 395                 400

Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu
                405                 410                 415

Pro Leu Pro Gly Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu
            420                 425                 430

Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val
        435                 440                 445

Thr Gly Leu Glu Ala Val Arg Ser Pro Ser Tyr Ala Ser
    450                 455                 460



<210>    63
<211>    473
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    GLP1(GEG)-HyFc5-GS3-FGF21(EIRP, TGLEAV)


<400>    63
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly Arg
             20                  25                  30

Asn Thr Gly Arg Gly Gly Glu Glu Lys Lys Lys Glu Lys Glu Lys Glu
         35                  40                  45

Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys Pro Ser His Thr
     50                  55                  60

Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 65                  70                  75                  80

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
                 85                  90                  95

Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
            100                 105                 110

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
        115                 120                 125

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
    130                 135                 140

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser
145                 150                 155                 160

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
```

```
                165                 170                 175

Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
            180                 185                 190

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
        195                 200                 205

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

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
225                 230                 235                 240

Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
                245                 250                 255

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
            260                 265                 270

Ser Leu Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
        275                 280                 285

Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
    290                 295                 300

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
305                 310                 315                 320

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
                325                 330                 335

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
            340                 345                 350

Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
        355                 360                 365

Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
    370                 375                 380

Ser Phe Arg Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Gln Ser
385                 390                 395                 400

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
                405                 410                 415

Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
            420                 425                 430

Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
        435                 440                 445

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Thr Gly Leu Glu
    450                 455                 460

Ala Val Arg Ser Pro Ser Tyr Ala Ser
465                 470


<210>    64
<211>    461
<212>    PRT
```

<213> Artificial Sequence

<220>
<223> GLP1(GEG)-HyFc40-GS3-FGF21(EIRP, TGLEAV)

<400> 64

```
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly Glu
             20                  25                  30

Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys
         35                  40                  45

Pro Ser His Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro
     50                  55                  60

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 65                  70                  75                  80

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
                 85                  90                  95

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            100                 105                 110

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        115                 120                 125

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
    130                 135                 140

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
145                 150                 155                 160

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
                165                 170                 175

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            180                 185                 190

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        195                 200                 205

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    210                 215                 220

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
225                 230                 235                 240

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                245                 250                 255

Ser Leu Ser Leu Ser Leu Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly
            260                 265                 270

Gly Ser Gly Gly Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu
        275                 280                 285

Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp
    290                 295                 300
```

```
Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val
305                 310                 315                 320

Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala
                325                 330                 335

Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe
            340                 345                 350

Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp
        355                 360                 365

Pro Glu Ala Cys Ser Phe Arg Glu Glu Ile Arg Pro Asp Gly Tyr Asn
    370                 375                 380

Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn
385                 390                 395                 400

Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu
                405                 410                 415

Pro Leu Pro Gly Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu
            420                 425                 430

Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val
        435                 440                 445

Thr Gly Leu Glu Ala Val Arg Ser Pro Ser Tyr Ala Ser
    450                 455                 460
```

<210> 65
<211> 461
<212> PRT
<213> Artificial Sequence

<220>
<223> GLP1(GEG)-HyFc40-GS3-FGF21(EIRP, TGLEAV, A180E)

<400> 65

```
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly Glu
             20                  25                  30

Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys
         35                  40                  45

Pro Ser His Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro
     50                  55                  60

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 65                  70                  75                  80

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
                 85                  90                  95

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
```

```
            100                 105                 110

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        115                 120                 125

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
    130                 135                 140

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
145                 150                 155                 160

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
                165                 170                 175

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            180                 185                 190

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        195                 200                 205

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    210                 215                 220

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
225                 230                 235                 240

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                245                 250                 255

Ser Leu Ser Leu Ser Leu Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly
            260                 265                 270

Gly Ser Gly Gly Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu
        275                 280                 285

Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp
    290                 295                 300

Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val
305                 310                 315                 320

Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala
                325                 330                 335

Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe
            340                 345                 350

Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp
        355                 360                 365

Pro Glu Ala Cys Ser Phe Arg Glu Glu Ile Arg Pro Asp Gly Tyr Asn
    370                 375                 380

Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn
385                 390                 395                 400

Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu
                405                 410                 415

Pro Leu Pro Gly Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu
            420                 425                 430

Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val
```

435 440 445

Thr Gly Leu Glu Ala Val Arg Ser Pro Ser Tyr Glu Ser
450 455 460

<210> 66
<211> 461
<212> PRT
<213> Artificial Sequence

<220>
<223> GLP1(GEG)-HyFc40-GS3-FGF21(EIRP, TGLEAN, A180E)

<400> 66
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly Glu
20 25 30

Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys
35 40 45

Pro Ser His Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro
50 55 60

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
65 70 75 80

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
85 90 95

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
100 105 110

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
115 120 125

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
130 135 140

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
145 150 155 160

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
165 170 175

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
180 185 190

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
195 200 205

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
210 215 220

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
225 230 235 240

```
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                245                 250                 255

Ser Leu Ser Leu Ser Leu Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly
            260                 265                 270

Gly Ser Gly Gly Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu
        275                 280                 285

Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp
    290                 295                 300

Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val
305                 310                 315                 320

Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala
                325                 330                 335

Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe
            340                 345                 350

Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp
        355                 360                 365

Pro Glu Ala Cys Ser Phe Arg Glu Glu Ile Arg Pro Asp Gly Tyr Asn
    370                 375                 380

Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn
385                 390                 395                 400

Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu
                405                 410                 415

Pro Leu Pro Gly Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu
            420                 425                 430

Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val
        435                 440                 445

Thr Gly Leu Glu Ala Asn Arg Ser Pro Ser Tyr Glu Ser
    450                 455                 460
```

<210> 67
<211> 460
<212> PRT
<213> Artificial Sequence

<220>
<223> GLP1(GEG)-HyFc40-GS3-FGF21(EIRP, G170N, A180E)

<400> 67

```
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
  1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Gly Glu
             20                  25                  30

Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys
         35                  40                  45
```

```
Pro Ser His Thr Gln Pro Leu Gly Val Phe Leu Phe Pro Pro Lys Pro
     50                  55                  60

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 65                  70                  75                  80

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
                 85                  90                  95

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            100                 105                 110

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        115                 120                 125

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
    130                 135                 140

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
145                 150                 155                 160

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
                165                 170                 175

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            180                 185                 190

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        195                 200                 205

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    210                 215                 220

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
225                 230                 235                 240

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                245                 250                 255

Ser Leu Ser Leu Ser Leu Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly
            260                 265                 270

Gly Ser Gly Gly Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu
        275                 280                 285

Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp
    290                 295                 300

Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val
305                 310                 315                 320

Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala
                325                 330                 335

Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe
            340                 345                 350

Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp
        355                 360                 365

Pro Glu Ala Cys Ser Phe Arg Glu Glu Ile Arg Pro Asp Gly Tyr Asn
    370                 375                 380
```

```
Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn
385                 390                 395                 400

Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu
                405                 410                 415

Pro Leu Pro Gly Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu
            420                 425                 430

Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val
        435                 440                 445

Asn Pro Ser Gln Gly Arg Ser Pro Ser Tyr Glu Ser
    450                 455                 460


<210>    68
<211>    4
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    FGF21 variant


<400>    68
Glu Ile Arg Pro
  1


<210>    69
<211>    6
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    FGF21 variant


<400>    69
Thr Gly Leu Glu Ala Val
  1               5


<210>    70
<211>    6
<212>    PRT
<213>    Artificial Sequence

<220>
<223>    FGF21 variant


<400>    70
Thr Gly Leu Glu Ala Asn
  1               5


<210>    71
<211>    1383
<212>    DNA
<213>    Artificial Sequence
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<220>
<223> nucleic acid molecule coding for DFD23

<400> 71
cacggcgagg ggaccttcac aagcgacgtg tcctcttatc tggaaggaca ggccgctaag 60

gagtttatcg catggctcgt caaaggcaga ggcgaaaagg agaaggaaga gcaggaggag 120

agagaaacca aaacacccga gtgtcccagt cacactcagc ctctgggagt gtttctcttc 180

ccacctaagc ccaaggatac ccttatgatt tctaggacac ctgaggtgac ctgcgtcgtt 240

gtggacgtga gtcaagagga cccagaggtc cagtttaact ggtatgttga cggcgtggaa 300

gtgcataatg caaaaactaa accccgcgag gaacaattca attcaaccta ccgggtcgtt 360

tctgtgttga cagtgctgca tcaagattgg ctgaacggga aggagtataa gtgtaaagtc 420

agtaataagg gactcccctc tagtatcgaa aaaactattt caaaggccaa aggccagcct 480

agagagccac aggtgtacac ccttcctcca tcccaagagg agatgacaaa gaaccaggtg 540

tctctgactt gtctcgtgaa ggggttctac cctagtgaca tcgctgtcga atgggagtca 600

aacggacagc cagagaataa ttataagaca actcctcccg ttctggattc tgacggcagc 660

ttctttctgt actctaggct tactgtggac aaaagtcgct ggcaagaagg gaacgtcttt 720

tcatgttctg ttatgcacga ggccttgcac aatcattata cacagaagtc tctgagtctc 780

tcactgggca aaggcggggg aggcagcggg ggaggcgggt ccggaggcgg gggatctcat 840

cccatccctg actccagtcc tctcctgcaa ttcgggggcc aagtccggca gcggtacctc 900

tacacagatg atgctcagca gacagaagcc cacctggaga tcagggagga tgggaccgtg 960

gggggcgctg ctgaccagag ccccgaaagt ctcctgcagc tgaaagcctt gaagcctgga 1020

gttattcaaa tcttgggagt caagactagt aggttcctgt gccagcggcc agatggggcc 1080

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ctgccccccg cattgcctga gccacccgga atcctggccc cccagccccc tgatgtggga 1320

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tcc 1383

<210> 72
<211> 1419
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleic acid molecule coding for DFD24

<400> 72
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gaatttatcg catggctcgt caaaggaaga gggaggaaca ccggacgggg cggggaagag 120

aagaagaaag aaaaggagaa ggaagagcag gaggagagag aaaccaaaac acccgagtgt 180

cccagtcaca ctcagcctct gggagtgttt ctcttcccac ctaagcccaa ggataccctt 240

atgatttcta ggacacctga ggtgacctgc gtcgttgtgg acgtgagtca agaggaccca 300

gaggtccagt ttaactggta tgttgacggc gtggaagtgc ataatgcaaa aactaaaccc 360

cgcgaggaac aattcaattc aacctaccgg gtcgtttctg tgttgacagt gctgcatcaa 420

gattggctga acgggaagga gtataagtgt aaagtcagta ataagggact cccctctagt 480

atcgaaaaaa ctatttcaaa ggccaaaggc cagcctagag agccacaggt gtacaccctt 540

cctccatccc aagaggagat gacaaagaac caggtgtctc tgacttgtct cgtgaagggg 600

ttctacccta gtgacatcgc tgtcgaatgg gagtcaaacg gacagccaga gaataattat 660

aagacaactc ctcccgttct ggattctgac ggcagcttct ttctgtactc taggcttact 720

gtggacaaaa gtcgctggca agaagggaac gtcttttcat gttctgttat gcacgaggcc 780

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cccggaatcc tggcccccca gccccctgat gtgggatcct ctgaccctct gagcatggtg 1380

acaggcctgg aggccgtgag aagccccagc tacgcttcc 1419

<210> 73
<211> 1383
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleic acid molecule coding for DFD25

<400> 73
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gaatttatcg catggctcgt caaaggaaga ggggaaaagg agaaggaaga gcaggaggag 120

agagaaacca aaacacccga gtgtcccagt cacactcagc ctctgggagt gtttctcttc 180

ccacctaagc ccaaggatac ccttatgatt tctaggacac ctgaggtgac ctgcgtcgtt 240

gtggacgtga gtcaagagga cccagaggtc cagtttaact ggtatgttga cggcgtggaa 300

gtgcataatg caaaaactaa accccgcgag gaacaattca attcaaccta ccgggtcgtt 360

tctgtgttga cagtgctgca tcaagattgg ctgaacggga aggagtataa gtgtaaagtc 420

agtaataagg gactcccctc tagtatcgaa aaaactattt caaaggccaa aggccagcct 480

agagagccac aggtgtacac ccttcctcca tcccaagagg agatgacaaa gaaccaggtg 540

tctctgactt gtctcgtgaa ggggttctac cctagtgaca tcgctgtcga atgggagtca 600

aacggacagc cagagaataa ttataagaca actcctcccg ttctggattc tgacggcagc 660

ttctttctgt actctaggct tactgtggac aaaagtcgct ggcaagaagg gaacgtcttt 720

tcatgttctg ttatgcacga ggccttgcac aatcattata cacagaagtc tctgagtctc 780

tcactgggca aaggcggggg aggcagcggg ggaggcgggt ccggaggcgg gggatctcat 840

cccatccctg actccagtcc tctcctgcaa ttcgggggcc aagtccggca gcggtacctc 900

tacacagatg atgctcagca gacagaagcc cacctggaga tcagggagga tgggaccgtg 960

gggggcgctg ctgaccagag ccccgaaagt ctcctgcagc tgaaagcctt gaagcctgga 1020

gttattcaaa tcttgggagt caagactagt aggttcctgt gccagcggcc agatggggcc 1080

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tcc 1383

<210> 74
<211> 1419
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleic acid molecule coding for DFD26

<400> 74
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gagtttatcg catggctcgt caaaggcggc ggcaggaaca ccggacgggg cggggaagag 120

aagaagaaag aaaaggagaa ggaagagcag gaggagagag aaaccaaaac acccgagtgt 180

cccagtcaca ctcagcctct gggagtgttt ctcttcccac ctaagcccaa ggataccctt 240

atgatttcta ggacacctga ggtgacctgc gtcgttgtgg acgtgagtca agaggaccca 300

gaggtccagt ttaactggta tgttgacggc gtggaagtgc ataatgcaaa aactaaaccc 360

cgcgaggaac aattcaattc aacctaccgg gtcgtttctg tgttgacagt gctgcatcaa 420

gattggctga acgggaagga gtataagtgt aaagtcagta ataagggact cccctctagt 480

atcgaaaaaa ctatttcaaa ggccaaaggc cagcctagag agccacaggt gtacaccctt 540

cctccatccc aagaggagat gacaaagaac caggtgtctc tgacttgtct cgtgaagggg 600

ttctacccta gtgacatcgc tgtcgaatgg gagtcaaacg gacagccaga gaataattat 660

aagacaactc ctcccgttct ggattctgac ggcagcttct ttctgtactc taggcttact 720

gtggacaaaa gtcgctggca agaagggaac gtcttttcat gttctgttat gcacgaggcc 780

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cccggaatcc tggcccccca gccccctgat gtgggatcct ctgaccctct gagcatggtg 1380

acaggcctgg aggccgtgag aagccccagc tacgcttcc 1419

<210> 75
<211> 1383
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleic acid molecule coding for DFD27

<400> 75
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gagtttatcg catggctcgt caaaggcggc ggcgaaaagg agaaggaaga gcaggaggag 120

agagaaacca aaacacccga gtgtcccagt cacactcagc ctctgggagt gtttctcttc 180

ccacctaagc ccaaggatac ccttatgatt tctaggacac ctgaggtgac ctgcgtcgtt 240

gtggacgtga gtcaagagga cccagaggtc agtttaact ggtatgttga cggcgtggaa 300

gtgcataatg caaaaactaa accccgcgag gaacaattca attcaaccta ccgggtcgtt 360

tctgtgttga cagtgctgca tcaagattgg ctgaacggga aggagtataa gtgtaaagtc 420

agtaataagg gactcccctc tagtatcgaa aaaactattt caaaggccaa aggccagcct 480

agagagccac aggtgtacac ccttcctcca tcccaagagg agatgacaaa gaaccaggtg 540

tctctgactt gtctcgtgaa ggggttctac cctagtgaca tcgctgtcga atgggagtca 600

aacggacagc cagagaataa ttataagaca actcctcccg ttctggattc tgacggcagc 660

ttctttctgt actctaggct tactgtggac aaaagtcgct ggcaagaagg gaacgtcttt 720

tcatgttctg ttatgcacga ggccttgcac aatcattata cacagaagtc tctgagtctc 780

tcactgggca aaggcggggg aggcagcggg ggaggcgggt ccggaggcgg gggatctcat 840

cccatccctg actccagtcc tctcctgcaa ttcgggggcc aagtccggca gcggtacctc 900

tacacagatg atgctcagca gacagaagcc cacctggaga tcagggagga tgggaccgtg 960

gggggcgctg ctgaccagag ccccgaaagt ctcctgcagc tgaaagcctt gaagcctgga 1020

gttattcaaa tcttgggagt caagactagt aggttcctgt gccagcggcc agatggggcc 1080

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tcc 1383

<210> 76
<211> 1419
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleic acid molecule coding for DFD28

<400> 76
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gaatttatcg catggctcgt caaaggaggc gggaggaaca ccggacgggg cggggaagag 120

aagaagaaag aaaaggagaa ggaagagcag gaggagagag aaaccaaaac acccgagtgt 180

cccagtcaca ctcagcctct gggagtgttt ctcttcccac ctaagcccaa ggataccctt 240

atgatttcta ggacacctga ggtgacctgc gtcgttgtgg acgtgagtca agaggaccca 300

gaggtccagt ttaactggta tgttgacggc gtggaagtgc ataatgcaaa aactaaaccc 360

cgcgaggaac aattcaattc aacctaccgg gtcgtttctg tgttgacagt gctgcatcaa 420

gattggctga acgggaagga gtataagtgt aaagtcagta ataagggact cccctctagt 480

atcgaaaaaa ctatttcaaa ggccaaaggc cagcctagag agccacaggt gtacaccctt 540

cctccatccc aagaggagat gacaaagaac caggtgtctc tgacttgtct cgtgaagggg 600

ttctacccta gtgacatcgc tgtcgaatgg gagtcaaacg gacagccaga gaataattat 660

aagacaactc ctcccgttct ggattctgac ggcagcttct ttctgtactc taggcttact 720

gtggacaaaa gtcgctggca agaagggaac gtcttttcat gttctgttat gcacgaggcc 780

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cccggaatcc tggcccccca gccccctgat gtgggatcct ctgaccctct gagcatggtg 1380

acaggcctgg aggccgtgag aagccccagc tacgcttcc 1419

<210> 77
<211> 1383
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleic acid molecule coding for DFD29

<400> 77
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gaatttatcg catggctcgt caaaggaggc ggggaaaagg agaaggaaga gcaggaggag 120

agagaaacca aaacacccga gtgtcccagt cacactcagc ctctgggagt gtttctcttc 180

ccacctaagc ccaaggatac ccttatgatt tctaggacac ctgaggtgac ctgcgtcgtt 240

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gtgcataatg caaaaactaa accccgcgag gaacaattca attcaaccta ccgggtcgtt 360

tctgtgttga cagtgctgca tcaagattgg ctgaacggga aggagtataa gtgtaaagtc 420

agtaataagg gactcccctc tagtatcgaa aaaactattt caaaggccaa aggccagcct 480

agagagccac aggtgtacac ccttcctcca tcccaagagg agatgacaaa gaaccaggtg 540

tctctgactt gtctcgtgaa ggggttctac cctagtgaca tcgctgtcga atgggagtca 600

aacggacagc cagagaataa ttataagaca actcctcccg ttctggattc tgacggcagc 660

ttctttctgt actctaggct tactgtggac aaaagtcgct ggcaagaagg gaacgtcttt 720

tcatgttctg ttatgcacga ggccttgcac aatcattata cacagaagtc tctgagtctc 780

tcactgggca aaggcggggg aggcagcggg ggaggcgggt ccggaggcgg gggatctcat 840

cccatccctg actccagtcc tctcctgcaa ttcgggggcc aagtccggca gcggtacctc 900

tacacagatg atgctcagca gacagaagcc cacctggaga tcagggagga tgggaccgtg 960

gggggcgctg ctgaccagag ccccgaaagt ctcctgcagc tgaaagcctt gaagcctgga 1020

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tcc 1383

<210> 78
<211> 1383
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleic acid molecule coding for DFD69

<400> 78
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gaatttatcg catggctcgt caaaggaggc ggggaaaagg agaaggaaga gcaggaggag 120

agagaaacca aaacacccga gtgtcccagt cacactcagc ctctgggagt gtttctcttc 180

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tctgtgttga cagtgctgca tcaagattgg ctgaacggga aggagtataa gtgtaaagtc 420

agtaataagg gactcccctc tagtatcgaa aaaactattt caaaggccaa aggccagcct 480

agagagccac aggtgtacac ccttcctcca tcccaagagg agatgacaaa gaaccaggtg 540

tctctgactt gtctcgtgaa ggggttctac cctagtgaca tcgctgtcga atgggagtca 600

aacggacagc cagagaataa ttataagaca actcctcccg ttctggattc tgacggcagc 660

ttctttctgt actctaggct tactgtggac aaaagtcgct ggcaagaagg gaacgtcttt 720

tcatgttctg ttatgcacga ggccttgcac aatcattata cacagaagtc tctgagtctc 780

tcactgggca aaggcggggg aggcagcggg ggaggcgggt ccggaggcgg gggatctcat 840

cccatccctg actccagtcc tctcctgcaa ttcgggggcc aagtccggca gcggtacctc 900

tacacagatg atgctcagca gacagaagcc cacctggaga tcagggagga tgggaccgtg 960

gggggcgctg ctgaccagag ccccgaaagt ctcctgcagc tgaaagcctt gaagcctgga 1020

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tcc 1383

<210> 79
<211> 1383
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleic acid molecule coding for DFD112

<400> 79
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gaatttatcg catggctcgt caaaggaggc ggggaaaagg agaaggaaga gcaggaggag 120

agagaaacca aaacacccga gtgtcccagt cacactcagc ctctgggagt gtttctcttc 180

ccacctaagc ccaaggatac ccttatgatt tctaggacac ctgaggtgac ctgcgtcgtt 240

gtggacgtga gtcaagagga cccagaggtc cagtttaact ggtatgttga cggcgtggaa 300

gtgcataatg caaaaactaa accccgcgag gaacaattca attcaaccta ccgggtcgtt 360

tctgtgttga cagtgctgca tcaagattgg ctgaacggga aggagtataa gtgtaaagtc 420

agtaataagg gactcccctc tagtatcgaa aaaactattt caaaggccaa aggccagcct 480

agagagccac aggtgtacac ccttcctcca tcccaagagg agatgacaaa gaaccaggtg 540

tctctgactt gtctcgtgaa ggggttctac cctagtgaca tcgctgtcga atgggagtca 600

aacggacagc cagagaataa ttataagaca actcctcccg ttctggattc tgacggcagc 660

ttctttctgt actctaggct tactgtggac aaaagtcgct ggcaagaagg gaacgtcttt 720

tcatgttctg ttatgcacga ggccttgcac aatcattata cacagaagtc tctgagtctc 780

tcactgggca aaggcggggg aggcagcggg ggaggcgggt ccggaggcgg gggatctcat 840

cccatccctg actccagtcc tctcctgcaa ttcgggggcc aagtccggca gcggtacctc 900

tacacagatg atgctcagca gacagaagcc cacctggaga tcagggagga tgggaccgtg 960

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tcc 1383

<210> 80
<211> 1380
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleic acid molecule coding for DFD114

<400> 80
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gaatttatcg catggctcgt caaaggaggc ggggaaaagg agaaggaaga gcaggaggag 120

agagaaacca aaacacccga gtgtcccagt cacactcagc ctctgggagt gtttctcttc 180

ccacctaagc ccaaggatac ccttatgatt tctaggacac ctgaggtgac ctgcgtcgtt 240

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gtgcataatg caaaaactaa accccgcgag gaacaattca attcaaccta ccgggtcgtt 360

tctgtgttga cagtgctgca tcaagattgg ctgaacggga aggagtataa gtgtaaagtc 420

agtaataagg gactcccctc tagtatcgaa aaaactattt caaaggccaa aggccagcct 480

agagagccac aggtgtacac ccttcctcca tcccaagagg agatgacaaa gaaccaggtg 540

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aacggacagc cagagaataa ttataagaca actcctcccg ttctggattc tgacggcagc 660

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cccatccctg actccagtcc tctcctgcaa ttcgggggcc aagtccggca gcggtacctc 900

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ctgccccccg cattgcctga gccacccgga atcctggccc cccagccccc tgatgtggga      1320

tcctctgacc ctctgagcat ggtgaaccct tcccagggca gaagccccag ctacgagtcc      1380

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