FGF21 MUTANTS AND USES THEREOF

This application claims the benefit of U.S. Provisional Application No. 61/175,736 filed May 5, 2009 and U.S. Provisional Application No. 61/285,118 filed December 9, 2009, all of which are incorporated by reference herein.

FIELD OF THE INVENTION

The invention relates to nucleic acid molecules encoding FGF21 mutant polypeptides, FGF21 mutant polypeptides, pharmaceutical compositions comprising FGF21 mutant polypeptides, and methods for treating metabolic disorders using such nucleic acids, polypeptides, or pharmaceutical compositions.

BACKGROUND OF THE INVENTION

FGF21 is a secreted polypeptide that belongs to a subfamily of fibroblast growth factors (FGFs) that includes FGF19, FGF21, and FGF23 (Itoh *et al.*, 2004, *Trend Genet.* 20: 563-69). FGF21 is an atypical FGF in that it is heparin independent and functions as a hormone in the regulation of glucose, lipid, and energy metabolism.

FGF21 was isolated from a liver cDNA library as a hepatic secreted factor. It is highly expressed in liver and pancreas and is the only member of the FGF family to be primarily expressed in liver. Transgenic mice overexpressing FGF21 exhibit metabolic phenotypes of slow growth rate, low plasma glucose and triglyceride levels, and an absence of age-associated type 2 diabetes, islet hyperplasia, and obesity. Pharmacological administration of recombinant FGF21 protein in rodent and primate models results in normalized levels of plasma glucose, reduced triglyceride and cholesterol levels, and improved glucose tolerance and insulin sensitivity. In addition, FGF21 reduces body weight and body fat by increasing energy expenditure, physical activity, and metabolic rate. Experimental research provides support for the pharmacological administration of FGF21 for the treatment of type 2 diabetes, obesity, dyslipidemia, and other metabolic conditions or disorders in humans.

Human FGF21 has a short half-life *in vivo*. In mice, the half-life of human FGF21 is 1 to 2 hours, and in *cynomolgus* monkeys, the half-life is 2.5 to 3 hours. In developing an FGF21 protein for use as a therapeutic in the treatment of type 2 diabetes, an increase in half-life would be desirable. FGF21 proteins having an

enhanced half-life would allow for less frequent dosing of patients being administered the protein. Such proteins are described herein.

SUMMARY OF THE INVENTION

An isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 4 and further comprising: (a) at least one amino acid substitution at position 180; and (b) at least one amino acid substitution selected from the group consisting of: (i) a substitution at position 171; and (ii) a substitution at position 98; and (c) combinations thereof is disclosed.

In one embodiment the isolated polypeptide comprises a substitution at position 98 and a substitution at position 180 and wherein: (a) the substitution at position 98 is selected from the group consisting of an arginine, cysteine, glutamic acid, glutamine, lysine, and threonine; and (b) the substitution at position 180 is selected from the group consisting of glycine, proline, serine or glutamic acid; and (c) combinations thereof. In one embodiment the residue at position 98 is arginine and the residue at position 180 is glutamic acid. Also provided is a fusion polypeptide comprising the isolated polypeptide fused to a heterologous amino acid sequence. In one embodiment the heterologous amino acid sequence is an Fc domain or fragment thereof, and can comprise the amino acid sequence of SEQ ID NO:11. In another embodiment the polypeptide is fused to the Fc domain via a linker and in yet another embodiment the linker comprises GGGGSGGGGSGGGGS (SEQ ID NO:31). In one specific embodiment the polypeptide comprises SEQ ID NO:47. Also disclosed is a multimer comprising two or more of the fusion polypeptides. A pharmaceutical composition comprising the isolated polypeptide and a pharmaceutically acceptable formulation agent, such as a hydrogel, is also disclosed. In another aspect, a method of treating a metabolic disorder is provided and in one embodiment comprises administering to a human patient in need thereof a pharmaceutical composition provided herein. In one embodiment the metabolic disorder is diabetes and in another the metabolic disorder is obesity. Nucleic acids encoding the isolated polypeptide are also provided and in one embodiment the nucleic acid comprises SEQ ID NO:46. The nucleic acid can be present in a vector, which can itself be present in a host cell. In still another embodiment the polypeptide comprises: (a) an amino-terminal truncation of no more than 8 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; (b) a carboxyl-terminal truncation of no

more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; or (c) an amino-terminal truncation of no more than 8 amino acid residues and a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal. In still other embodiments the polypeptide is covalently linked to one or more polymers, such as PEG.

In another embodiment the isolated polypeptide comprises a substitution at position 171 and a substitution at position 180 and wherein: (a) the substitution at position 180 is selected from the group consisting of glycine, proline, serine and glutamic acid; (b) the substitution at position 171 is selected from the group consisting of an alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, lysine, serine, threonine, tryptophan, and tyrosine; and (c) combinations thereof. In one embodiment the residue at position 171 is glycine and the residue at position 180 is glutamic acid. Also provided is a fusion polypeptide comprising the isolated polypeptide fused to a heterologous amino acid sequence. In one embodiment the heterologous amino acid sequence is an Fc domain or fragment thereof, and can comprise the amino acid sequence of SEQ ID NO:11. In another embodiment the polypeptide is fused to the Fc domain via a linker and in yet another embodiment the linker comprises GGGGSGGGGSGGGGS (SEQ ID NO:31). In one specific embodiment the polypeptide comprises SEQ ID NO:47. Also disclosed is a multimer comprising two or more of the fusion polypeptides. A pharmaceutical composition comprising the isolated polypeptide and a pharmaceutically acceptable formulation agent, such as a hydrogel, is also disclosed. In another aspect, a method of treating a metabolic disorder is provided and in one embodiment comprises administering to a human patient in need thereof a pharmaceutical composition provided herein. In one embodiment the metabolic disorder is diabetes and in another the metabolic disorder is obesity. Nucleic acids encoding the isolated polypeptide are also provided and in one embodiment the nucleic acid comprises SEQ ID NO:46. The nucleic acid can be present in a vector, which can itself be present in a host cell. In still another embodiment the polypeptide comprises: (a) an amino-terminal truncation of no more than 8 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; (b) a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; or (c) an amino-

terminal truncation of no more than 8 amino acid residues and a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal. In still other embodiments the polypeptide is covalently linked to one or more polymers, such as PEG.

In yet another embodiment the polypeptide comprises a substitution at position 98, a substitution at position 171 and a substitution at position 180 of SEQ ID NO:4 and wherein: (a) the substitution at position 171 is selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, lysine, serine, threonine, tryptophan, or tyrosine; (b) the substitution at position 98 is selected from the group consisting of arginine, cysteine, glutamic acid, glutamine, lysine, or threonine; and (c) the substitution at position 180 is selected from the group consisting of glycine, proline, serine or glutamic acid; and combinations thereof. In a further embodiment the residue at position 98 is arginine, the residue at position 171 is glycine and the residue at position 180 is a glutamic acid. Also provided is a fusion polypeptide comprising the isolated polypeptide fused to a heterologous amino acid sequence. In one embodiment the heterologous amino acid sequence is an Fc domain or fragment thereof, and can comprise the amino acid sequence of SEQ ID NO:11. In another embodiment the polypeptide is fused to the Fc domain via a linker and in yet another embodiment the linker comprises GGGGSGGGGSGGGGS (SEQ ID NO:31). In one specific embodiment the polypeptide comprises SEQ ID NO:47. Also disclosed is a multimer comprising two or more of the fusion polypeptides. A pharmaceutical composition comprising the isolated polypeptide and a pharmaceutically acceptable formulation agent, such as a hydrogel, is also disclosed. In another aspect, a method of treating a metabolic disorder is provided and in one embodiment comprises administering to a human patient in need thereof a pharmaceutical composition provided herein. In one embodiment the metabolic disorder is diabetes and in another the metabolic disorder is obesity. Nucleic acids encoding the isolated polypeptide are also provided and in one embodiment the nucleic acid comprises SEQ ID NO:46. The nucleic acid can be present in a vector, which can itself be present in a host cell. In still another embodiment the polypeptide comprises: (a) an amino-terminal truncation of no more than 8 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; (b) a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a

mammal; or (c) an amino-terminal truncation of no more than 8 amino acid residues and a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal. In still other embodiments the polypeptide is covalently linked to one or more polymers, such as PEG.

Specific embodiments of the invention will become evident from the following more detailed description of certain embodiments and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1B show the results of an ELK-luciferase activity assay performed on the FGF21 truncation mutants 7-181 and 8-181 (Figure 1A) and the FGF21 truncation mutants 1-172, 1-171, 1-169, and 1-164 (Figure 1B); each panel shows the results obtained for a human FGF21 control.

Figure 2 shows the results of an ELK-luciferase activity assay performed on a human FGF21 control and the FGF21 truncation mutants 3-181, 4-181, 5-181, 7-181, 8-181, 1-180, 1-178, 1-177, 1-176, 1-175, 1-174, 1-173, 1-172, 9-181, and 1-149.

Figure 3 shows the blood glucose levels measured in mice injected with PBS (solid bar), human FGF21 control (open bar), or the FGF21 truncation mutants 8-181 (gray bar) and 9-181 (stippled bar).

Figure 4 shows the percent change in blood glucose levels measured in mice injected with PBS (solid circles), an Fc-FGF21 control (WT) (open circles), or truncated Fc-FGF21 fusion proteins comprising amino acid residues 5-181 (solid triangles) or 7-181 (open triangles).

Figure 5 shows the percent change in blood glucose levels measured in mice injected with PBS (solid circles), an FGF21-Fc control (WT) (open circles), a truncated FGF21-Fc fusion protein comprising residues 1-175 (solid triangles), or a truncated Fc-FGF21 protein comprising amino acid residues 1-171 (open triangles).

Figures 6A-6D show the results of liquid chromatography-mass spectrometry (LC-MS) analysis of a human Fc-(G5)-FGF21 (SEQ ID NO:107) control sample (Figure 6A) and samples of Fc-(G5)-FGF21 drawn from mice at 6 hours (Sample D6; Figure 6B), 24 hours (Sample D24; Figure 6C), and 48 hours (Sample D48; Figure 6D) after injection.

Figures 7A-7D show the results if LC-MS analysis of a mammalian-derived human FGF21-(G3)-Fc (SEQ ID NO:105) control sample (Figure 7A) and samples of

FGF21-(G3)-Fc drawn from mice at 6 hours (Sample E6; Figure 7B), 24 hours (Sample E24; Figure 7C), and 48 hours (Sample E48; Figure 7D) after injection.

Figures 8A-8D show the results of LC-MS analysis of an Fc-(L15)-FGF21 (SEQ ID NO:49) control sample (Figure 8A) and samples of Fc-(L15)-FGF21 drawn from mice at 6 hours (Figure 8B), 24 hours (Figure 8C), and 48 hours (Figure 8D) after injection.

Figures 9A-9D show the results of LC-MS analysis of an FGF21-(L15)-Fc (SEQ ID NO:41) control sample (Figure 9A) and samples of FGF21-(L15)-Fc drawn from mice at 6 hours (Figure 9B), 24 hours (Figure 9C), and 48 hours (Figure 9D) after injection.

Figures 10A-10B show the cleavage sites identified by LC-MS analysis of Fc-(L15)-FGF21 (Figure 10A, SEQ ID NO:49) and FGF21-(L15)-Fc (Figure 10B, SEQ ID NO:41) fusion proteins injected into mice.

Figure 11 shows the blood glucose levels measured in mice injected with PBS (solid bar), Fc-(L15)-FGF21 (SEQ ID NO:49) (open bar), or the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 G170E (SEQ ID NO:51) (gray bar), Fc-(L15)-FGF21 P171A (SEQ ID NO:53) (stippled bar), Fc-(L15)-FGF21 S172L (SEQ ID NO:55) (open diagonally crosshatched bar), Fc-(L15)-FGF21(G170E, P171A, S172L) (SEQ ID NO:59) (solid horizontally crosshatched bar), or Fc-(L15)-FGF21 G151A (SEQ ID NO:61) (open diagonally crosshatched bar).

Figure 12 shows the percent change in blood glucose levels measured in mice injected with PBS (solid circles), Fc-(L15)-FGF21 (SEQ ID NO:49) (open circles), or the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 G170E (SEQ ID NO:51) (solid triangles), Fc-(L15)-FGF21 P171A (SEQ ID NO:53) (open triangles), Fc-(L15)-FGF21 S172L (SEQ ID NO:55) (solid diamonds), Fc-(L15)-FGF21(G170E, P171A, S172L) (SEQ ID NO:59) (open diamonds), or Fc-(L15)-FGF21 G151A (SEQ ID NO:61) (solid squares).

Figure 13 shows the blood glucose levels measured in mice injected with PBS (solid bar), Fc-(L15)-FGF21 (SEQ ID NO:49) (open bar), or the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21(P150A, G151A, I152V) (gray bar), Fc-(L15)-FGF21 G170E (SEQ ID NO:51) (open diagonally crosshatched bar), Fc-(L15)-FGF21(G170E, P171A) (SEQ ID NO:63) (gray diagonally crosshatched bar), or Fc-(L15)-FGF21(G170E, S172L) (SEQ ID NO:67) (open diagonally crosshatched bar).

Figure 14 shows the percent change in blood glucose levels measured in mice

injected with PBS (solid squares), Fc-(L15)-FGF21 (SEQ ID NO:49) (open squares), or the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21(P150A, G151A, I152V) (SEQ ID NO:65) (solid inverted triangles), Fc-(L15)-FGF21 G170E (SEQ ID NO:51) (open inverted triangles), Fc-(L15)-FGF21(G170E, P171A) (SEQ ID NO:63) (solid circles), or Fc-(L15)-FGF21(G170E, S172L) (SEQ ID NO:67) (open circles).

Figure 15 shows the blood glucose levels measured in mice injected with PBS (solid bar) or the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 G170E (SEQ ID NO:51) (open bar), Fc-(L15)-FGF21 G170A (SEQ ID NO:69) (gray bar), Fc-(L15)-FGF21 G170C (SEQ ID NO:71) (open crosshatched bar), Fc-(L15)-FGF21 G170D (SEQ ID NO:73) (gray and white bar), Fc-(L15)-FGF21 G170N (SEQ ID NO:75) (solid crosshatched bar), or Fc-(L15)-FGF21 G170S (SEQ ID NO:77) (open crosshatched bar).

Figure 16 shows the percent change in blood glucose levels measured in mice injected with PBS (solid circles) or the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 G170E (SEQ ID NO:51) (open circles), Fc-(L15)-FGF21 G170A (SEQ ID NO:69) (solid triangles), Fc-(L15)-FGF21 G170C (SEQ ID NO:71) (open triangles), Fc-(L15)-FGF21 G170D (SEQ ID NO:73) (solid diamonds), Fc-(L15)-FGF21 G170N (SEQ ID NO:75) (open diamonds), or Fc-(L15)-FGF21 G170S (SEQ ID NO:77) (inverted solid triangles).

Figure 17 shows the blood glucose levels measured in mice injected with PBS (solid bar) or the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 G170E (SEQ ID NO:51) (open bar), Fc-(L15)-FGF21 P171E (SEQ ID NO:79) (gray bar), Fc-(L15)-FGF21 P171H (SEQ ID NO:81) (solid crosshatched bar), Fc-(L15)-FGF21 P171Q (SEQ ID NO:83) (open crosshatched bar), Fc-(L15)-FGF21 P171T (SEQ ID NO:85) (stippled bar), or Fc-(L15)-FGF21 P171Y (SEQ ID NO:87) (gray crosshatched bar).

Figure 18 shows the percent change in blood glucose levels measured in mice injected with PBS (solid circles) or the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 G170E (SEQ ID NO:51) (open circles), Fc-(L15)-FGF21 P171E (SEQ ID NO:79) (solid triangles), Fc-(L15)-FGF21 P171H (SEQ ID NO:81) (open triangles), Fc-(L15)-FGF21 P171Q (SEQ ID NO:83) (solid diamonds), Fc-(L15)-FGF21 P171T (SEQ ID NO:85) (open diamonds), or Fc-(L15)-FGF21 P171Y (SEQ ID NO:87) (solid squares).

Figures 19A-19D show the results of LC-MS analysis of an Fc-(L15)-FGF21 control sample (Figure 19A, SEQ ID NO:49) and samples drawn from mice at time 6

hours (Figure 19B), 24 hours (Figure 19C), and 48 hours (Figure 19D) after injection.

Figures 20A-20D show the results of LC-MS analysis of an Fc-(L15)-FGF21 G170E control sample (Figure 20A, SEQ ID NO:51) and samples of Fc-(L15)-FGF21 G170E drawn from mice at 6 hours (Figure 20B), 24 hours (Figure 20C), and 48 hours (Figure 20D) after injection.

Figures 21A-21D show the results of LC-MS analysis of an Fc-(L15)-FGF21 P171A control sample (Figure 21A, SEQ ID NO:53) and samples of Fc-(L15)-FGF21 P171A drawn from mice at 6 hours (Figure 21B), 24 (Figure 21C), and 48 hours (Figure 21D) after injection.

Figures 22A-22D show the results of LC-MS analysis of an Fc-(L15)-FGF21 S172L control sample (Figure 22A, SEQ ID NO:55) and samples of Fc-(L15)-FGF21 S172L drawn from mice at 6 hours (Figure 22B), 24 hours (Figure 22C), and 48 hours (Figure 22D) after injection.

Figures 23A-23D show the cleavage sites identified by LC-MS analysis of Fc-(L15)-FGF21 (Figure 23A, SEQ ID NO:49), Fc-(L15)-FGF21 G170E (Figure 23B, SEQ ID NO:51), Fc-(L15)-FGF21 P171A (Figure 23C, SEQ ID NO:53), and Fc-(L15)-FGF21 S172L (Figure 23D, SEQ ID NO:55) fusion proteins injected in mice.

Figures 24A-24C show the results of an ELK-luciferase activity assay performed on the FGF21 mutants FGF21 L99R (SEQ ID NO:109), FGF21 L99D (SEQ ID NO:111), and FGF21 A111T (SEQ ID NO:113) (Figure 24A); the FGF21 mutants FGF21 A129D (SEQ ID NO:115), FGF21 A129Q (SEQ ID NO:117), and FGF21 A134K (SEQ ID NO:119) (Figure 24B); and the FGF21 mutants FGF21 A134Y(SEQ ID NO:121), FGF21 A134E (SEQ ID NO:123), and FGF21 A129K (SEQ ID NO:125) (Figure 24C); each panel shows the results obtained for a human FGF21 control.

Figures 25A-25D show the results of an ELK-luciferase activity assay performed on the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 P171G (SEQ ID NO:89), Fc-(L15)-FGF21 P171S (SEQ ID NO:91), and Fc-(L15)-FGF21 P171T (SEQ ID NO:85) (Figure 25A); the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 P171Y (SEQ ID NO:87), Fc-(L15)-FGF21 P171W (SEQ ID NO:93), and Fc-(L15)-FGF21 P171C (SEQ ID NO:95) (Figure 25B); Fc-(L15)-FGF21 (SEQ ID NO:49), Fc-(L15)-FGF21 (A45K, G170E) (SEQ ID NO:97), and FGF21 A45K (SEQ ID NO:99) (Figure 25C); and Fc-(L15)-FGF21 (SEQ ID NO:49), Fc-(L15)-FGF21 P171E (SEQ ID NO:79), and Fc-(L15)-FGF21 (A45K, G170E) (SEQ ID NO:97) (Figure 25D); each

panel shows the results obtained for a human FGF21 control.

Figures 26A-B show the aggregation as a function of time for wild type mature FGF21 and various FGF21 mutants; Figure 26A shows the change in percent aggregation for an FGF21 control (WT, solid diamonds) and FGF21 A45K (solid circles) following incubation of 65 mg/mL protein at 4°C for 1, 2, and 4 days, while Figure 26B shows the change in percent aggregation for an FGF21 control (WT) (SEQ ID NO:4) and FGF21 P78C (SEQ ID NO:127), FGF21 P78R (SEQ ID NO:129), FGF21 L86T (SEQ ID NO:131), FGF21 L86C (SEQ ID NO:133), FGF21 L98C (SEQ ID NO:135), FGF21 L98R (SEQ ID NO:137), FGF21 A111T (SEQ ID NO:113), FGF21 A129D (SEQ ID NO:115), FGF21 A129Q (SEQ ID NO:117), FGF21 A129K (SEQ ID NO:125), FGF21 A134K (SEQ ID NO:119), FGF21 A134Y (SEQ ID NO:121), and FGF21 A134E (SEQ ID NO:123) (all labeled on the plot) following incubation of 65 mg/mL protein at 4°C for 1, 6, and 10 days.

Figure 27 shows the results of an ELK-luciferase activity assay performed on a human FGF21 control and the FGF21 mutants FGF21 A45K (SEQ ID NO:99), FGF21 L52T (SEQ ID NO:139), and FGF21 L58E (SEQ ID NO:141).

Figure 28A is a plot showing the change in aggregation levels for the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21(6-181, G170E) (SEQ ID NO:101) (solid diamonds), Fc-(L15)-FGF21 (A45K, G170E) (SEQ ID NO:97) (open squares), Fc-(L15)-FGF21 P171E (SEQ ID NO:79) (solid triangles), Fc-(L15)-FGF21 P171A (SEQ ID NO:53) (crosses), Fc-(L15)-FGF21 G170E (SEQ ID NO:51) (open triangles) , and an FGF21 control (solid circles) following incubation at 4°C for 1, 4, and 8 days, and Figure 28B is a bar graph also showing the results of the incubation.

Figure 29 shows the blood glucose levels measured in mice injected with PBS (vehicle) (solid circles) or the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21(A45K, G170E) (SEQ ID NO:97) (open circles), Fc-(L15)-FGF21 (A45K, P171G) (SEQ ID NO:103) (solid triangles), or Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) (open triangles).

Figure 30 is a plot showing the results of an ELK-luciferase activity assay performed on human FGF21 (solid circles, solid line), Fc-(L15)- FGF21 (SEQ ID NO:49) (open circles, solid line) and Fc-(L15) FGF21 (L98R, P171G) (SEQ ID NO:43) (solid triangles, dotted line).

Figure 31 is a plot showing the percent high molecular weight aggregates observed after nine days at room temperature (Figure 31A) and at 4°C (Figure 31B)

for FGF21 (SEQ ID NO:4) (solid circles, solid line), Fc-(L15)-FGF21 (SEQ ID NO:49) (open circle, solid line) and Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) (solid triangles, dotted line).

Figure 32 is a series of MALDI mass spectrometry traces showing observed changes in Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) at various points over a 168 hour time period.

Figure 33 is a plot showing the percent change in blood glucose levels in ob/ob mice for each of a PBS vehicle control (open circles), wild-type mature FGF21 (solid squares), and the FGF21 mutants Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) (inverted solid triangles); Fc-(L15)-FGF21 (L98R, P171G, 182P) (SEQ ID NO:143) (open diamonds), and Fc-(L15)-FGF21 (L98R, P171G, 182G) (SEQ ID NO:145) (solid circles).

Figure 34 is a plot showing the percent change in blood glucose levels in ob/ob mice for each of a PBS vehicle control (solid circles), and the FGF21 mutants Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) (solid triangles); Fc-(L15)-FGF21 (L98R, P171G, 182G, 183G) (SEQ ID NO:147) (open triangles), Fc-(L15)-FGF21 (L98R, P171G, 182G) (SEQ ID NO:145) (solid diamonds) and Fc-(L15)-FGF21 (L98R, P171G, 182P) (SEQ ID NO:143) (open diamonds).

Figure 35 is a plot showing the percent change in blood glucose levels in ob/ob mice for each of a PBS vehicle control (open circles), and the FGF21 mutants Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) (solid squares); Fc-(L15)-FGF21 (L98R, P171G, Y179S) (SEQ ID NO:149) (open triangles), Fc-(L15)-FGF21 (L98R, P171G, Y179A) (SEQ ID NO:153) (inverted solid triangles), Fc-(L15)-FGF21 (L98R, P171G, A180S) (SEQ ID NO:155) (open diamonds) and Fc-(L15)-FGF21 (L98R, P171G, A180G) (SEQ ID NO:157) (solid circles).

Figure 36 is a plot showing the percent change in blood glucose levels in ob/ob mice for each of a PBS vehicle control (solid circles), and the FGF21 mutants Fc-(L15)-FGF21(L98R, P171G) (SEQ ID NO:43) (open squares); Fc-(L15)-FGF21 (L98R, P171G, Y179F) (SEQ ID NO:151) (solid triangles), and Fc-(L15)-FGF21 (L98R, P171G, A180E) (SEQ ID NO:57) (open diamonds).

Figure 37 is a diagram graphically depicting the study design for a six-week dose escalation study performed in *Rhesus* monkeys. In the figure, shaded symbols indicate blood draws in the fasted state and stippled symbols indicated blood draws in the fed state.

Figures 38A-D is a series of plots depicting how the rhesus monkeys were randomized on OGTT profiles, OGTT AUCs and body weight; Figure 38A depicts baseline glucose levels in OGTT1, solid square corresponds to group A, solid circle, solid line corresponds to group B and open circle, dashed line corresponds to group C before compounds or vehicle were assigned to each group; Figure 38B depicts baseline glucose levels in OGTT2, solid square corresponds to group A, solid circle, solid line corresponds to group B and open circle, solid line corresponds to group C before compounds or vehicle were assigned to each group; Figure 38C shows baseline glucose levels for OGTTs 1 and 2 shown in terms of AUC, the stippled bar corresponds to group A, the shaded bar corresponds to group B and the open bar corresponds to group C; and Figure 38D shows baseline body weight, the stippled bar corresponds to group A, the shaded bar corresponds to group B and the open bar corresponds to group C.

Figure 39 is a plot showing the percent change in body weight relative to baseline of vehicle, FGF21 (SEQ ID NO:4) and Fc-(L15)-FGF21(L98R, P171G) (SEQ ID NO:43) in *Rhesus* monkeys; shaded bars 1 and 2 correspond to weeks 1 and 2 at the low dose, open bars 3 and 4 correspond to weeks 3 and 4 at the mid dose, solid bars 5 and 6 correspond to weeks 5 and 6 at the high dose and stippled bars 7, 8 and 9 correspond to weeks 7-9 during the washout period.

Figure 40 is a plot showing the percent change in fasted insulin relative to baseline of vehicle, FGF21 (SEQ ID NO:4) and Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) on fasted insulin levels in *Rhesus* monkeys; shaded bars 1 and 2 correspond to weeks 1 and 2 at the low dose, open bars 3 and 4 correspond to weeks 3 and 4 at the mid dose, solid bars 5 and 6 correspond to weeks 5 and 6 at the high dose and stippled bars 7 and 8 correspond to weeks 7 and 8 during the washout period.

Figure 41 is a plot showing the effects of vehicle, FGF21 (SEQ ID NO:4) and Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43), given at the high dose, on fed insulin levels of *Rhesus* monkeys acquired during weeks 5 and 6 of the study; solid bars correspond to week 5 and shaded bars correspond to week 6.

Figure 42 is a plot showing the glucose profiles of OGTT5 performed at the end of the two week high-dose treatment with Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43); solid circle, solid line corresponds to vehicle, open square, dotted line corresponds to FGF21 and solid triangle, solid line corresponds to Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43).

Figure 43 is a plot showing the insulin profiles of OGTT5 performed at the end of the two week high-dose treatment with Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43); solid circle, solid line corresponds to vehicle, open square, dotted line corresponds to FGF21 and solid triangle, solid line corresponds to Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43).

Figure 44 is a plot showing the percent change from baseline of glucose OGTT AUC3-5 determined at the end of each dose period (low, mid and high dose) of the *Rhesus* monkeys; open bars correspond to AUC3 calculated from glucose measurements during OGTT3, solid bars correspond to AUC4 calculated from glucose measurements during OGTT4 and shaded bars correspond to AUC5 calculated from glucose measurements during OGTT5.

Figure 45 is a graph showing the effects of vehicle, FGF21 and Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) on percent change from baseline of the fasted plasma triglyceride levels from each group of *Rhesus* monkeys; shaded bars 1 and 2 correspond to weeks 1 and 2 at the low dose, open bars 3 and 4 correspond to weeks 3 and 4 at the mid dose, solid bars 5 and 6 correspond to weeks 5 and 6 at the high dose and stippled bars 7, 8 and 9 correspond to weeks 7-9 during the washout period..

Figure 46 is a graph showing fed plasma triglyceride levels from each group of the *Rhesus* monkeys; as measured during the fifth and sixth weeks of treatment with vehicle, FGF21 or Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) at the high dose; shaded bars correspond to week 5 and solid bars correspond to week 6.

Figure 47 is a plot showing human FGF21 levels in individual monkeys measured at pre-dose, and 5, 12, 19, and 26 days, with samples acquired at approximately 21 hours after each injection.

Figure 48 is a plot showing individual monkey Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) levels measured at pre-dose, and 5, 12, 19, and 26 days, with samples acquired approximately 5 days after each injection.

Figure 49 is a plot showing mean concentrations of FGF21 and Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) levels measured from the three OGTTs performed following each of the low, mid and high doses; shaded bars correspond to OGTT3 at the low dose, solid bars correspond to OGTT4 at the mid dose and open bars correspond to OGTT5 at the high dose.

Figure 50 is the amino acid sequence of the Fc-(G4S)3-FGF21 (L98R, P171G,

A180E) fusion protein (SEQ ID NO:47); IgG1 Fc residues (SEQ ID NO:11) are in bold, the (G4S)3 linker (SEQ ID NO:31) is in italics and the point mutations in the FGF21 sequence (SEQ ID NO:39) are in bold and underlined.

Figure 51 is a plot showing the dose-response of the tested compounds in Erk-luciferase assays; Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47), Fc-(L15)-FGF21 (L98R, P171G, A180E) (SEQ ID NO:57), wild-type FGF21, and an Fc fusion with wild-type FGF21 were tested.

Figure 52 is a plot showing the results from a Biacore solution equilibrium binding assay of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) and Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) to human (right) and cyno β-Klotho (left).

Figure 53 is a pair of plots showing the dose response of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) in db/db mice after a single injection; Figure 53A shows the blood glucose levels in db/db mice at various time points following vehicle or Fc-(G4S)3-FGF21 (L98R, P171G, A180E) injection, while Figure 53B shows the effect of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) on body weight after a single injection into db/db mice.

Figure 54 is a schematic graphically presenting a dose frequency study of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) and Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) in DIO mice.

Figure 55 is a plot showing the GTT profiles of mice treated with vehicle, Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) or Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) at different dosing frequencies.

Figure 56 is a plot showing change of body weight from baseline (day 0) in mice treated vehicle, Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) or Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) at different dosing frequencies.

Figure 57 is a plot showing the results of an in vitro study of hydrogels comprising the FGF21 (L98R, P171G)) (SEQ ID NO:37) FGF21 mutant.

Figure 58 is a plot showing the effect on blood glucose level of 8 week old db/db mice dosed with various hydrogel formulations.

Figure 59 is a plot showing the effect on blood glucose level of 8 week old db/db mice dosed with various hydrogel formulations.

Figure 60 is a plot showing the effect on the blood glucose level of 8 week old

db/B6 mice dosed with various hydrogel formulations; solid circles represent a mice dosed with a hydrogel control, solid squares represent mice dosed with FGF21 (L98R, P171G) (SEQ ID NO:37) in a hydrogel at 10 mg/kg, solid triangles represent mice dosed with FGF21 (L98R, P171G) in a hydrogel at 30 mg/kg, and inverted triangles represent mice dosed with Fc-(L15)-FGF21 (L98R, P171G, A180E) (SEQ ID NO:57) alone.

Figure 61 is a plot showing the percent change in blood glucose level of 8 week old db/B6 mice dosed with various hydrogel formulations; solid circles represent a mice dosed with a hydrogel control, solid squares represent mice dosed with FGF21 (L98R, P171G) (SEQ ID NO:37) in a hydrogel at 10 mg/kg, solid triangles represent mice dosed with FGF21 (L98R, P171G) in a hydrogel at 30 mg/kg, and inverted triangles represent mice dosed with Fc-(L15)-FGF21 (L98R, P171G, A180E) (SEQ ID NO:57) alone.

Figure 62 is a plot showing the effect on body weight of 8 week old db/B6 mice dosed with various hydrogel formulations; solid circles represent a mice dosed with a hydrogel control, solid squares represent mice dosed with FGF21 (L98R, P171G) (SEQ ID NO:37) in a hydrogel at 10 mg/kg, solid triangles represent mice dosed with FGF21 (L98R, P171G) in a hydrogel at 30 mg/kg, and inverted triangles represent mice dosed with Fc-(L15)-FGF21 (L98R, P171G, A180E) (SEQ ID NO:57) alone.

Figure 63 is a plot showing the percent change in weight of 8 week old db/B6 mice dosed with various hydrogel formulations; solid circles represent a mice dosed with a hydrogel control, solid squares represent mice dosed with FGF21 (L98R, P171G) (SEQ ID NO:37) in a hydrogel at 10 mg/kg, solid triangles represent mice dosed with FGF21 (L98R, P171G) in a hydrogel at 30 mg/kg, and inverted triangles represent mice dosed with Fc-(L15)-FGF21 (L98R, P171G, A180E) (SEQ ID NO:57) alone.

Figure 64 is a diagram graphically depicting the study design for a nine-week dose escalation study performed in *cynomolgus* monkeys with impaired glucose tolerance (IGT).

Figure 65 is a plot depicting the effects of vehicle, Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) on AM meal food intake of the IGT *cynomolgus* monkeys studied.

Figure 66 is a plot depicting the effects of vehicle, Fc-(L15)-FGF21 (L98R,

P171G) (SEQ ID NO:43) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) on fruit intake of the IGT *cynomolgus* monkeys studied.

Figure 67 is a plot depicting the effects of vehicle, Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) on PM meal food intake of the IGT *cynomolgus* monkeys studied.

Figure 68 is a plot depicting the effects of vehicle, Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) on body weight of the IGT *cynomolgus* monkeys studied.

Figure 69 is a plot showing the effects of vehicle, Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) on body mass index of the IGT *cynomolgus* monkeys studied.

Figure 70 is a plot showing the effects of vehicle, Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) on skin fold thickness of the IGT *cynomolgus* monkeys studied.

Figure 71 is a plot showing the effects of vehicle, Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) on abdominal circumference of the IGT *cynomolgus* monkeys studied.

Figure 72 is a plot showing the effects of vehicle, Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) on plasma glucose levels of the IGT *cynomolgus* monkeys studied.

Figure 73 is a plot showing the effects of vehicle, Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) on glucose tolerance of the IGT *cynomolgus* monkeys studied.

Figure 74 is a plot showing the effects of vehicle, Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) on plasma triglyceride levels of the IGT *cynomolgus* monkeys studied.

Figure 75 is a plot showing the effects of vehicle, Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) on plasma total cholesterol levels of the IGT *cynomolgus* monkeys studied.

Figure 76 is a plot showing the effects of vehicle, Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (SEQ ID NO:47) on plasma HDL-cholesterol levels of the IGT *cynomolgus* monkeys studied.

Figure 77 is a series of MALDI mass spectrometry traces showing observed changes in Fc-(L15)-FGF21 (L98R, P171G) (left panel, SEQ ID NO:43) and Fc-

(L15)-FGF21 (L98R, P171G, A180E) (right panel, SEQ ID NO:57) at various points over a 168 hour time period.

Figure 78 is a plot showing the relative abundance (%) of full-length C-terminal peptide over the total C-terminal peptide fragments derived from both Fc-(L15)-FGF21 (L98R, P171G) and Fc-(L15)-FGF21 (L98R, P171G, A180E) as analyzed by MRM LC/MS/MS following Asp-N digestion.

Figure 79 is a plot showing the results of an ELISA assay for the plasma concentration of intact full-length Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) and Fc-(L15)-FGF21 (L98R, P171G, A180E) (SEQ ID NO:57) over a period of 240 hours following intravenous injection in mice.

Figure 80 is a plot showing the results of an ELK-luciferase activity assay performed on a negative control, human FGF21 (SEQ ID NO:4) and the FGF21 glycosylation mutants FGF21 (Y179N, S181T) (SEQ ID NO:161), FGF21 Y179N (SEQ ID NO:163) and FGF21 P124S (SEQ ID NO:165).

DETAILED DESCRIPTION OF THE INVENTION

A human FGF21 protein having enhanced properties such as an increased half-life and/or decreased aggregation can be prepared using the methods disclosed herein and standard molecular biology methods. Optionally, the half-life can be further extended by fusing an antibody, or portion thereof, to the N-terminal or C-terminal end of the wild-type FGF21 sequence. It is also possible to further extend the half-life or decrease aggregation of the wild-type FGF21 protein by introducing amino acid substitutions into the protein. Such modified proteins are referred to herein as mutants, or FGF21 mutants, and form embodiments of the present invention.

Recombinant nucleic acid methods used herein, including in the Examples, are generally those set forth in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., Green Publishers Inc. and Wiley and Sons 1994), both of which are incorporated herein by reference for any purpose.

1. General Definitions

The term "isolated nucleic acid molecule" refers to a nucleic acid molecule provided herein that (1) has been separated from at least about 50 percent of proteins,

lipids, carbohydrates, or other materials with which it is naturally found when total nucleic acid is isolated from the source cells, (2) is not linked to all or a portion of a polynucleotide to which the "isolated nucleic acid molecule" is linked in nature, (3) is operably linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature as part of a larger polynucleotide sequence. Preferably, an isolated nucleic acid molecule is substantially free from any other contaminating nucleic acid molecules or other contaminants that are found in its natural environment that would interfere with its use in polypeptide production or its therapeutic, diagnostic, prophylactic or research use.

The term "vector" is used to refer to any molecule (*e.g.*, nucleic acid, plasmid, or virus) used to transfer coding information to a host cell.

The term "expression vector" refers to a vector that is suitable for transformation of a host cell and contains nucleic acid sequences that direct and/or control the expression of inserted heterologous nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns are present.

The term "operably linked" is used herein to refer to an arrangement of flanking sequences wherein the flanking sequences so described are configured or assembled so as to perform their usual function. Thus, a flanking sequence operably linked to a coding sequence may be capable of effecting the replication, transcription and/or translation of the coding sequence. For example, a coding sequence is operably linked to a promoter when the promoter is capable of directing transcription of that coding sequence. A flanking sequence need not be contiguous with the coding sequence, so long as it functions correctly. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

The term "host cell" is used to refer to a cell which has been transformed, or is capable of being transformed with a nucleic acid sequence, such as a nucleic acid provided herein, and then of expressing a selected gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present.

The term "isolated polypeptide" refers to a polypeptide provided herein that (1) has been separated from at least about 50 percent of polynucleotides, lipids,

carbohydrates, or other materials with which it is naturally found when isolated from the source cell, (2) is not linked (by covalent or noncovalent interaction) to all or a portion of a polypeptide to which the "isolated polypeptide" is linked in nature, (3) is operably linked (by covalent or noncovalent interaction) to a polypeptide with which it is not linked in nature, or (4) does not occur in nature. Preferably, the isolated polypeptide is substantially free from any other contaminating polypeptides or other contaminants that are found in its natural environment that would interfere with its therapeutic, diagnostic, prophylactic or research use.

The term "naturally occurring" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by man. Similarly, "non-naturally occurring" as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by man. When used in connection with nucleotides, the term "naturally occurring" refers to the bases adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U). When used in connection with amino acids, the term "naturally occurring" refers to the 20 amino acids alanine (A), cysteine (C), aspartic acid (D), glutamic acid (E), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), lysine (K), leucine (L), methionine (M), asparagine (N), proline (P), glutamine (Q), arginine (R), serine (S), threonine (T), valine (V), tryptophan (W), and tyrosine (Y).

The term "FGF21 polypeptide" refers to a naturally-occurring wild-type polypeptide expressed in humans. For purposes of this disclosure, the term "FGF21 polypeptide" can be used interchangeably to refer to any full-length FGF21 polypeptide, *e.g.*, SEQ ID NOs:2 and 6, which consist of 209 amino acid residues and which are encoded by the nucleotide sequences of SEQ ID NOs:1 and 5, respectively; any mature form of the polypeptide, *e.g.*, SEQ ID NOs:4 and 8, which consist of 181 amino acid residues and which are encoded by the nucleotide sequences of SEQ ID NOs:3 and 7, respectively, and in which the 28 amino acid residues at the amino-terminal end of the full-length FGF21 polypeptide (*i.e.*, which constitute the signal peptide) have been removed. Full-length and mature FGF21 polypeptides can but need not comprise an amino-terminal methionine, which may be introduced by engineering or as a result of a bacterial expression process.

The terms "FGF21 polypeptide mutant" and "FGF21 mutant" can be used interchangeably and refer to an FGF21 polypeptide in which a naturally occurring

FGF21 amino acid sequence (e.g., SEQ ID NOs:2, 4, 6, or 8) has been modified. Such modifications include, but are not limited to, one or more amino acid substitutions, including substitutions with non-naturally occurring amino acids and non-naturally-occurring amino acid analogs, and truncations. Thus, FGF21 polypeptide mutants include, but are not limited to, site-directed FGF21 mutants, truncated FGF21 polypeptides, proteolysis-resistant FGF21 mutants, aggregation-reducing FGF21 mutants, FGF21 combination mutants, and FGF21 fusion proteins, as described herein. For the purpose of identifying the specific truncations and amino acid substitutions of the FGF21 mutants of the present invention, the numbering of the amino acid residues truncated or mutated corresponds to that of the mature 181-residue FGF21 polypeptide. FGF21 mutants can but need not comprise an amino-terminal methionine, which may be introduced by engineering or as a result of a bacterial expression process. In other embodiments of the present invention, an FGF21 polypeptide mutant comprises an amino acid sequence that is at least about 85 percent identical to the mutant FGF21's amino acid sequence, but wherein specific residues conferring a desirable property to the FGF21 polypeptide mutant, e.g., proteolysis-resistance, increased half life or aggregation-reducing properties and combinations thereof, have not been further modified. In other words, with the exception of residues in the FGF21 mutant sequence that have been modified in order to confer proteolysis-resistance, aggregation-reducing, or other properties, about 15 percent of all other amino acid residues in the FGF21 mutant sequence can be modified. For example, in the FGF21 mutant Q173E, up to 15 percent of all amino acid residues other than the glutamic acid residue, which was substituted for glutamine at position 173, could be modified. In still other embodiments, an FGF21 polypeptide mutant comprises an amino acid sequence that is at least about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the mutant FGF21's amino acid sequence, but wherein the specific residues conferring the FGF21 polypeptide mutant's proteolysis-resistance or aggregation-reducing properties have not been further modified. Such FGF21 polypeptide mutants possess at least one activity of the wild-type FGF21 polypeptide.

The present invention also encompasses a nucleic acid molecule encoding an FGF21 polypeptide mutant comprising an amino acid sequence that is at least about 85 percent identical to the mutant FGF21's amino acid sequence but wherein specific residues conferring a desirable property to the FGF21 polypeptide mutant, *e.g.*,

proteolysis-resistance, increased half life or aggregation-reducing properties and combinations thereof have not been further modified.

In other words, with the exception of residues in the FGF21 mutant sequence that have been modified in order to confer proteolysis-resistance, aggregation-reducing, or other properties, about 15 percent of all other amino acid residues in the FGF21 mutant sequence can be modified. For example, in the FGF21 mutant Q173E, up to 15 percent of all amino acid residues other than the glutamic acid residue, which was substituted for glutamine at position 173, could be modified. The present invention further encompasses a nucleic acid molecule comprising a nucleotide sequence that is at least about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the nucleotide sequence encoding an FGF21 mutant, but wherein the nucleotides encoding amino acid residues conferring the encoded FGF21 polypeptide mutant's proteolysis-resistance or aggregation-reducing properties have not been further modified. Such nucleic acid molecules encode FGF21 mutant polypeptides possessing at least one activity of the wild-type FGF21 polypeptide.

The term "biologically active FGF21 polypeptide mutant" refers to any FGF21 polypeptide mutant described herein that possesses an activity of the wild-type FGF21 polypeptide, such as the ability to lower blood glucose, insulin, triglyceride, or cholesterol; reduce body weight; and improve glucose tolerance, energy expenditure, or insulin sensitivity, regardless of the type or number of modifications that have been introduced into the FGF21 polypeptide mutant. FGF21 polypeptide mutants possessing a somewhat decreased level of FGF21 activity relative to the wild-type FGF21 polypeptide can nonetheless be considered to be biologically active FGF21 polypeptide mutants.

The terms "effective amount" and "therapeutically effective amount" each refer to the amount of an FGF21 polypeptide mutant used to support an observable level of one or more biological activities of the wild-type FGF21 polypeptide, such as the ability to lower blood glucose, insulin, triglyceride, or cholesterol levels; reduce body weight; or improve glucose tolerance, energy expenditure, or insulin sensitivity.

The term "pharmaceutically acceptable carrier" or "physiologically acceptable carrier" as used herein refers to one or more formulation agents suitable for accomplishing or enhancing the delivery of an FGF21 polypeptide mutant into the body of a human or non-human subject. The term includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and

absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In some cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in a pharmaceutical composition. Pharmaceutically acceptable substances such as wetting or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the FGF21 polypeptide mutant can also act as, or form a component of, a carrier.

The term "antigen" refers to a molecule or a portion of a molecule that is capable of being bound by an antibody, and additionally that is capable of being used in an animal to produce antibodies that are capable of binding to an epitope of that antigen. An antigen may have one or more epitopes.

The term "native Fc" refers to molecule or sequence comprising the sequence of a non-antigen-binding fragment resulting from digestion of whole antibody or produced by other means, whether in monomeric or multimeric form, and can contain the hinge region. The original immunoglobulin source of the native Fc is preferably, but not necessarily, of human origin and can be any of the immunoglobulins, although IgG1 and IgG2 are preferred. IgG4 can also be employed. Native Fc molecules are made up of monomeric polypeptides that can be linked into dimeric or multimeric forms by covalent (*i.e.*, disulfide bonds) and non-covalent association. The number of intermolecular disulfide bonds between monomeric subunits of native Fc molecules ranges from 1 to 4 depending on class (*e.g.*, IgG, IgA, and IgE) or subclass (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgGA2). One example of a native Fc is a disulfide-bonded dimer resulting from papain digestion of an IgG (*see* Ellison *et al.*, 1982, *Nucleic Acids Res.* 10: 4071-9). The term "native Fc" as used herein is generic to the monomeric, dimeric, and multimeric forms. An example of an Fc polypeptide sequence is presented in SEQ ID NO:11, which is derived from a human IgG1 molecule. A native Fc can, but need not, comprise an amino-terminal methionine, which may be introduced by engineering or as a result of a bacterial expression process; such Fc molecules are still considered to be "native Fc" molecules.

The term "Fc variant" refers to a molecule or sequence that is modified from a native Fc but still comprises a binding site for the salvage receptor, FcRn (neonatal Fc receptor). International Publication Nos. WO 97/34631 and WO 96/32478 describe

exemplary Fc variants, as well as interaction with the salvage receptor, and are hereby incorporated by reference. Thus, the term "Fc variant" can comprise a molecule or sequence that is humanized from a non-human native Fc. Furthermore, a native Fc comprises regions that can be removed because they provide structural features or biological activity that are not required for the fusion molecules of the FGF21 mutants of the present invention. Thus, the term "Fc variant" comprises a molecule or sequence that lacks one or more native Fc sites or residues, or in which one or more Fc sites or residues has be modified, that affect or are involved in: (1) disulfide bond formation, (2) incompatibility with a selected host cell, (3) N-terminal heterogeneity upon expression in a selected host cell, (4) glycosylation, (5) interaction with complement, (6) binding to an Fc receptor other than a salvage receptor, or (7) antibody-dependent cellular cytotoxicity (ADCC). Fc variants are described in further detail hereinafter. An Fc variant can, but need not, comprise an amino-terminal methionine, which may be introduced by engineering or as a result of a bacterial expression process; such Fc molecules are still considered to be "Fc variants."

The term "Fc domain" encompasses native Fc and Fc variants and sequences as defined above. As with Fc variants and native Fc molecules, the term "Fc domain" includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means. In some embodiments of the present invention, an Fc domain can be fused to FGF21 or a FGF21 mutant (including a truncated form of FGF21 or a FGF21 mutant) via, for example, a covalent bond between the Fc domain and the FGF21 sequence. Such fusion proteins can form multimers via the association of the Fc domains and both these fusion proteins and their multimers are an aspect of the present invention. An Fc domain can, but need not, comprise an amino-terminal methionine, which may be introduced by engineering or as a result of a bacterial expression process.

2. FGF21 Mutants

The term "FGF21 mutant" refers to an FGF21 mutant polypeptide having an amino acid sequence that differs from the amino acid sequence of a naturally occurring FGF21 polypeptide sequence, *e.g.*, SEQ ID NOs:2, 4, 6 or 8,by one or more amino acids. FGF21 mutants can be generated by introducing one or more amino

acid substitutions, either conservative or non-conservative and using naturally or non-naturally occurring amino acids, at particular positions of the FGF21 polypeptide.

A "Conservative amino acid substitution" can involve a substitution of a native amino acid residue (*i.e.*, a residue found in a given position of the wild-type FGF21 polypeptide sequence) with a nonnative residue (*i.e.*, a residue that is not found in a given position of the wild-type FGF21 polypeptide sequence) such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Conservative amino acid substitutions also encompass non-naturally occurring amino acid residues that are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics, and other reversed or inverted forms of amino acid moieties.

Naturally occurring residues can be divided into classes based on common side chain properties:

(1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr;

(3) acidic: Asp, Glu;

(4) basic: Asn, Gln, His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro; and

(6) aromatic: Trp, Tyr, Phe.

Conservative substitutions can involve the exchange of a member of one of these classes for another member of the same class. Non-conservative substitutions can involve the exchange of a member of one of these classes for a member from another class.

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. An exemplary (but not limiting) list of amino acid substitutions is set forth in Table 1.

Table 1

Amino Acid Substitutions

Original Residue	Exemplary Substitutions
Ala	Val, Leu, Ile
Arg	Lys, Gln, Asn
Asn	Gln
Asp	Glu
Cys	Ser, Ala

Original Residue	Exemplary Substitutions
Gln	Asn
Glu	Asp
Gly	Pro, Ala
His	Asn, Gln, Lys, Arg
Ile	Leu, Val, Met, Ala, Phe
Leu	Ile, Val, Met, Ala, Phe
Lys	Arg, Gln, Asn
Met	Leu, Phe, Ile
Phe	Leu, Val, Ile, Ala, Tyr
Pro	Ala
Ser	Thr, Ala, Cys
Thr	Ser
Trp	Tyr, Phe
Tyr	Trp, Phe, Thr, Ser
Val	Ile, Met, Leu, Phe, Ala

3. Truncated FGF21 Polypeptides

One embodiment of the present invention is directed to truncated forms of a mature FGF21 polypeptide or FGF21 mutant. This embodiment of the present invention arose from an effort to identify truncated FGF21 polypeptides that are capable of providing an activity that is similar, and in some instances superior, to untruncated forms of the mature FGF21 polypeptide.

As used herein, the term "truncated FGF21 polypeptide" refers to an FGF21 polypeptide in which amino acid residues have been removed from the amino-terminal (or N-terminal) end of the FGF21 polypeptide, amino acid residues have been removed from the carboxyl-terminal (or C-terminal) end of the FGF21 polypeptide, or amino acid residues have been removed from both the amino-terminal and carboxyl-terminal ends of the FGF21 polypeptide. The various truncations disclosed herein were prepared as described herein Examples 3 and 6.

The activity of N-terminally truncated FGF21 polypeptides and C-terminally truncated FGF21 polypeptides can be assayed using an *in vitro* ELK-luciferase assay as described in Example 4. Specific details of the *in vitro* assays that can be used to examine the activity of truncated FGF21 polypeptides can be found in Example 4.

The activity of the truncated FGF21 polypeptides of the present invention can also be assessed in an *in vivo* assay, such as db/db mice, or ob/ob mice as shown in Examples 5 and 7. Generally, to assess the *in vivo* activity of a truncated FGF21 polypeptide, the truncated FGF21 polypeptide can be administered to a test animal

intraperitoneally. After a desired incubation period (e.g., one hour or more), a blood sample can be drawn, and blood glucose levels can be measured. Specific details of the *in vivo* assays that can be used to examine the activity of truncated FGF21 polypeptides can be found in Examples 5 and 7.

a. N-terminal Truncations

In some embodiments of the present invention, N-terminal truncations comprise 1, 2, 3, 4, 5, 6, 7, or 8 amino acid residues from the N-terminal end of the mature FGF21 polypeptide or FGF21 mutant. As demonstrated in, for example, Example 5 and Figure 3, truncated FGF21 polypeptides having N-terminal truncations of fewer than 9 amino acid residues retain the ability of the mature FGF21 polypeptide to lower blood glucose in an individual. Accordingly, in particular embodiments, the present invention encompasses truncated forms of the mature FGF21 polypeptide or FGF21 polypeptide mutants having N-terminal truncations of 1, 2, 3, 4, 5, 6, 7, or 8 amino acid residues.

b. C-terminal Truncations

In some embodiments of the present invention, C-terminal truncations comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 amino acid residues from the C-terminal end of the mature FGF21 polypeptide. As demonstrated in, for example, Example 4 and Figure 1B, truncated FGF21 polypeptides having C-terminal truncations of fewer than 13 amino acid residues exhibited an efficacy of at least 50% of the efficacy of wild-type FGF21 in an *in vitro* ELK-luciferase assay, indicating that these FGF21 mutants retain the ability of the mature FGF21 polypeptide to lower blood glucose in an individual. Accordingly, in particular embodiments, the present invention encompasses truncated forms of the mature FGF21 polypeptide or FGF21 polypeptide mutants having C-terminal truncations of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 amino acid residues.

c. N-terminal and C-terminal Truncations

In some embodiments of the present invention, truncated FGF21 polypeptides can have a combination of N-terminal and C-terminal truncations. Truncated FGF21 polypeptides having a combination of N-terminal and C-terminal truncations share the activity of corresponding truncated FGF21 polypeptides having either the N-terminal

or C-terminal truncations alone. In other words, truncated FGF21 polypeptides having both N-terminal truncations of fewer than 9 amino acid residues and C-terminal truncations of fewer than 13 amino acid residues possess similar or greater biological activity, e.g, blood glucose-lowering activity, as truncated FGF21 polypeptides having N-terminal truncations of fewer than 9 amino acid residues or truncated FGF21 polypeptides having C-terminal truncations of fewer than 13 amino acid residues. Accordingly, in particular embodiments, the present invention encompasses truncated forms of the mature FGF21 polypeptide or FGF21 polypeptide mutants having both N-terminal truncations of 1, 2, 3, 4, 5, 6, 7, or 8 amino acid residues and C-terminal truncations of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 amino acid residues.

As with all FGF21 mutants of the present invention, truncated FGF21 polypeptides and FGF21 mutants can optionally comprise an amino-terminal methionine residue, which can be introduced by directed mutation or as a result of a bacterial expression process.

The truncated FGF21 polypeptides of the present invention can be prepared as described in Examples 3 and 6. Those of ordinary skill in the art, familiar with standard molecular biology techniques, can employ that knowledge, coupled with the instant disclosure, to make and use the truncated FGF21 polypeptides of the present invention. Standard techniques can be used for recombinant DNA, oligonucleotide synthesis, tissue culture, and transformation (*e.g.*, electroporation, lipofection). *See, e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, *supra*, which is incorporated herein by reference for any purpose. Enzymatic reactions and purification techniques can be performed according to manufacturer's specifications, as commonly accomplished in the art, or as described herein. Unless specific definitions are provided, the nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical syntheses; chemical analyses; pharmaceutical preparation, formulation, and delivery; and treatment of patients.

The truncated FGF21 polypeptides of the present invention can also be fused to another entity, which can impart additional properties to the truncated FGF21 polypeptide. In one embodiment of the present invention, a truncated FGF21

polypeptide can be fused to an Fc sequence. Such fusion can be accomplished using known molecular biological methods and/or the guidance provided herein. The benefits of such fusion polypeptides, as well as methods for making such fusion polypeptides, are discussed in more detail herein.

4. Proteolysis-resistant FGF21 Mutants

As described in Example 8, mature FGF21 was found to be undergoing *in vivo* degradation, which was ultimately determined to arise from proteolytic attack. The *in vivo* degradation of mature FGF21 was found to lead to shorter effective half-life, which can adversely affect the therapeutic potential of a molecule. Accordingly, a directed study was performed to identify FGF21 mutants that exhibit a resistance to proteolysis. As a result of this investigation, the sites in the mature FGF21 polypeptide that were determined to be particularly susceptible to proteolysis include the peptide bond between the amino acid residues at positions 4-5, 20-21, 151-152, 171-172 and 178-181.

A broad but focused and directed study was performed to identify particular substitutions that eliminate the observed proteolytic effect while not affecting the activity of the protein to an unacceptable degree. Tables 8 and 11 highlight some of the mutants that were prepared and tested. As described in, for example, Examples 13 and 14, not all FGF21 mutants exhibited an ideal profile; some mutants conferred proteolysis resistance but at the cost of compromised FGF21 activity. Other mutations retained FGF21 activity but did not confer proteolysis resistance. Several mutants, including, for example, FGF21 P171G, retained a similar level of activity as wild-type FGF21 while also exhibiting resistance to proteolytic degradation.

One selection criteria for identifying desirable proteolysis-resistant FGF21 mutants was that the activity of the FGF21 mutant be essentially the same as, or greater than, the activity of wild-type FGF21. Therefore, another embodiment of the present invention is directed to FGF21 mutants that are resistant to proteolysis and still retain activity that is essentially the same as, or greater than, wild-type FGF21. Although less desirable in some cases, FGF21 mutants that are resistant to proteolysis but exhibit somewhat decreased activity form another embodiment of the present invention. In some cases it can be desirable to maintain a degree of proteolysis, and consequently, FGF21 mutants that allow some degree of proteolysis to occur also form another embodiment of the present invention.

As with all FGF21 mutants provided herein, the proteolysis-resistant FGF21 mutants of the present invention can be prepared as described herein. Those of ordinary skill in the art, for example, those familiar with standard molecular biology techniques, can employ that knowledge, coupled with the instant disclosure, to make and use the proteolysis-resistant FGF21 mutants disclosed herein. Standard techniques can be used for recombinant DNA, oligonucleotide synthesis, tissue culture, and transformation (*e.g.*, electroporation, lipofection). See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, *supra*, which is incorporated herein by reference for any purpose. Enzymatic reactions and purification techniques can be performed according to manufacturer's specifications, as commonly accomplished in the art, or as described herein. Unless specific definitions are provided, the nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical syntheses; chemical analyses; pharmaceutical preparation, formulation, and delivery; and treatment of patients.

The proteolysis-resistant FGF21 mutants of the present invention can be fused to another entity, which can impart additional properties to the proteolysis-resistant FGF21 mutant. In one embodiment of the present invention, a proteolysis-resistant FGF21 mutant can be fused to an IgG Fc sequence, *e.g.*, SEQ ID NO:11. Such fusion can be accomplished using known molecular biological methods and/or the guidance provided herein. The benefits of such fusion polypeptides, as well as methods for making such fusion polypeptides, are known and are discussed in more detail herein.

5. Aggregation-reducing FGF21 Mutants

As described in Example 15, one property of the wild-type FGF21 polypeptide is its propensity to aggregate. At concentrations over about 5 mg/mL, the aggregation rate is high at room temperature. As shown and described herein, the aggregation rate for the wild-type FGF21 polypeptide is both concentration and temperature dependent.

Aggregation can prove to be a challenge when working with wild-type FGF21 at these concentrations, such as in the context of a therapeutic formulation. Accordingly, a directed study was performed to identify FGF21 mutants that exhibit

reduced FGF21 aggregation. The resulting FGF21 mutants were then tested for the propensity to aggregate at various concentrations.

A broad but focused and directed study was performed to identify particular substitutions that eliminate or reduce the observed aggregation effect of wild-type FGF21 while not affecting the activity of the protein to an unacceptable degree. The approach for identifying suitable aggregation-reducing mutants is described in Example 15. Table 16 highlights some of the mutants that were prepared and tested. As described in, for example, Example 17, not all FGF21 mutants exhibited an ideal profile. Some mutants, such as FGF21 L58E had compromised FGF21 activity and were not studied further. Other mutations, such as FGF21 A134E, retained FGF21 activity but did not confer reduced aggregation properties. Several mutants, such as FGF21 L98R, retained FGF21 activity and also exhibited reduced aggregation. One mutant, FGF21 A45K, surprisingly exhibited increased FGF21 activity while also exhibiting reduced aggregation properties.

One selection criteria for identifying desirable aggregation-reducing FGF21 mutants was that the activity of the FGF21 mutant be essentially similar to, or greater than, the activity of wild-type FGF21. Therefore, another embodiment of the present invention is directed to FGF21 mutants having reduced aggregation properties while still retaining an FGF21 activity that is similar to, or greater than, wild-type FGF21. Although less desirable in some cases, FGF21 mutants having reduced aggregation properties but exhibiting somewhat decreased FGF21 activity form another embodiment of the present invention. In some cases it may be desirable to maintain a degree of aggregation, and consequently, FGF21 mutants that allow some degree of aggregation to occur also form another embodiment of the present invention.

As with all FGF21 mutants provided herein, the aggregation-reducing FGF21 mutants provided herein can be prepared as described herein. Those of ordinary skill in the art, familiar with standard molecular biology techniques, can employ that knowledge, coupled with the instant disclosure, to make and use the aggregation-reducing FGF21 mutants of the present invention. Standard techniques can be used for recombinant DNA, oligonucleotide synthesis, tissue culture, and transformation (*e.g.*, electroporation, lipofection). See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, *supra*, which is incorporated herein by reference for any purpose. Enzymatic reactions and purification techniques can be performed according to manufacturer's specifications, as commonly accomplished in the art, or as described

herein. Unless specific definitions are provided, the nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical syntheses; chemical analyses; pharmaceutical preparation, formulation, and delivery; and treatment of patients.

The aggregation-reducing FGF21 mutants of the present invention can be fused to another entity, which can impart additional properties to the aggregation-reducing FGF21 mutant. In one embodiment of the present invention, an aggregation-reducing FGF21 mutant can be fused to an IgG Fc sequence, e.g., SEQ ID NO:11. Such fusion can be accomplished using known molecular biological methods and/or the guidance provided herein. The benefits of such fusion polypeptides, as well as methods for making such fusion polypeptides, are discussed in more detail herein.

6. FGF21 Combination Mutants

As described herein, the wild-type FGF21 sequence possesses several properties that can pose significant challenges when FGF21 is used as a therapeutic molecule. Among these challenges are the protein's susceptibility to degradation and its propensity for aggregation at high concentration. After an exhaustive effort to identify FGF21 polypeptides that overcome each of these challenges, a directed study was performed to determine whether the amino acid substitutions conferring proteolysis-resistance and those conferring aggregation-reducing properties could be combined in an additive or synergistic fashion in a single polypeptide sequence while maintaining activity levels that are equal to or greater than the activity of wild-type FGF21. This represented a significant challenge, as it is known in the art that the introduction of multiple mutations in a given polypeptide can sometimes adversely affect the expression, activity, and subsequent manufacturing of the protein.

Surprisingly, as demonstrated in, for example, Examples 19 and 20, it was found that the desirable properties of several FGF21 mutants could indeed be combined in an additive or synergistic fashion to generate an FGF21 mutant having enhanced pharmaceutical properties. FGF21 mutants that are resistant to proteolysis, have a reduced rate of aggregation, and which still retain activity that is the same as, or greater than, wild-type FGF21, are disclosed herein.

One selection criteria for identifying desirable FGF21 combination mutants was that the activity of the FGF21 mutant be similar to, or greater than, the activity of wild-type FGF21. Therefore, another embodiment of the present invention is directed to FGF21 mutants that are proteolysis-resistant and have reduced aggregation properties while still retaining an FGF21 activity that is similar to, or greater than, wild-type FGF21. Although less desirable in some cases, FGF21 mutants that are proteolysis-resistant and have reduced aggregation properties but exhibit somewhat decreased FGF21 activity form another embodiment of the present invention. In some cases it may be desirable to maintain a degree of proteolysis and/or aggregation, and consequently, FGF21 mutants that allow some degree of proteolysis and/or aggregation also form another embodiment of the present invention.

As with all FGF21 mutants of the present invention, the FGF21 combination mutants of the present invention can be prepared as described herein. Those of ordinary skill in the art, familiar with standard molecular biology techniques, can employ that knowledge, coupled with the instant disclosure, to make and use the FGF21 combination mutants of the present invention. Standard techniques can be used for recombinant DNA, oligonucleotide synthesis, tissue culture, and transformation (*e.g.*, electroporation, lipofection). See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, *supra*, which is incorporated herein by reference for any purpose. Enzymatic reactions and purification techniques can be performed according to manufacturer's specifications, as commonly accomplished in the art, or as described herein. Unless specific definitions are provided, the nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical syntheses; chemical analyses; pharmaceutical preparation, formulation, and delivery; and treatment of patients.

The FGF21 combination mutants of the present invention can be fused to another entity, which can impart additional properties to the FGF21 combination mutant. In one embodiment of the present invention, an FGF21 combination mutant can be fused to an IgG Fc sequence, e.g., SEQ ID NO:11. Such fusion can be accomplished using known molecular biological methods and/or the guidance provided herein. The benefits of such fusion polypeptides, as well as methods for

making such fusion polypeptides, are discussed in more detail herein.

7. FGF21 Fusion Proteins

As used herein, the term "FGF21 fusion polypeptide" or "FGF21 fusion protein" refers to a fusion of one or more amino acid residues (such as a heterologous protein or peptide) at the N-terminus or C-terminus of any FGF21 polypeptide mutant described herein.

Heterologous peptides and polypeptides include, but are not limited to, an epitope to allow for the detection and/or isolation of an FGF21 polypeptide mutant; a transmembrane receptor protein or a portion thereof, such as an extracellular domain or a transmembrane and intracellular domain; a ligand or a portion thereof which binds to a transmembrane receptor protein; an enzyme or portion thereof which is catalytically active; a polypeptide or peptide which promotes oligomerization, such as a leucine zipper domain; a polypeptide or peptide which increases stability, such as an immunoglobulin constant region (e.g., an Fc domain); a half life-extending sequence comprising a combination of two or more (e.g., 2, 5, 10, 15, 20, 25, etc) naturally occurring or non-naturally occurring charged and/or uncharged amino acids (e.g., Serine, Glycine, Glutamic or Aspartic Acid) designed to form a predominantly hydrophilic or predominantly hydrophobic fusion partner for an FGF21 mutant; a functional or non-functional antibody, or a heavy or light chain thereof; and a polypeptide which has an activity, such as a therapeutic activity, different from the FGF21 polypeptide mutants of the present invention. Also encompassed by the present invention are FGF21 mutants fused to human serum albumin (HSA).

FGF21 fusion proteins can be made by fusing heterologous sequences at either the N-terminus or at the C-terminus of an FGF21 polypeptide mutant. As described herein, a heterologous sequence can be an amino acid sequence or a non-amino acid-containing polymer. Heterologous sequences can be fused either directly to the FGF21 polypeptide mutant or via a linker or adapter molecule. A linker or adapter molecule can be one or more amino acid residues (or -mers), *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, or 9 residues (or -mers), preferably from 10 to 50 amino acid residues (or -mers), *e.g.*, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50 residues (or -mers), and more preferably from 15 to 35 amino acid residues (or -mers). A linker or adapter molecule can also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of the fused moieties.

a. Fc Fusions

In one embodiment of the present invention, an FGF21 polypeptide mutant is fused to an Fc domain, e.g., one or more domains of an Fc region of a human IgG. Antibodies comprise two functionally independent parts, a variable domain known as "Fab," that binds an antigen, and a constant domain known as "Fc," that is involved in effector functions such as complement activation and attack by phagocytic cells. An Fc has a long serum half-life, whereas a Fab is short-lived (Capon *et al.*, 1989, *Nature* 337: 525-31). When joined together with a therapeutic protein, an Fc domain can provide longer half-life or incorporate such functions as Fc receptor binding, protein A binding, complement fixation, and perhaps even placental transfer (Capon *et al.*, 1989).

In vivo pharmacokinetic analysis indicated that human FGF21 has a short half-life of about 1 hour in mice due to rapid clearance and *in vivo* degradation. Therefore, to extend the half-life of FGF21 a native Fc sequence was fused to the N- or C-terminal end of the FGF21 polypeptide. The fusion of the Fc sequence to wild type FGF21, in particularly Fc fused to the N-terminus of wild type FGF21, did not extend the half-life as expected, however this observationled to an investigation of the proteolytic degradation of FGF21 *in vivo* and the identification of FGF21 mutants that were resistant to such degradation. Such mutants are described in, for example, Examples 9 and 11, and exhibit longer half-lives than wild-type FGF21. These and other FGF21 fusion proteins form embodiments of the present invention.

Throughout the instant disclosure, Fc-FGF21 refers to a fusion protein in which the Fc sequence is fused to the N-terminus of FGF21. Similarly, throughout the disclosure, FGF21-Fc refers to a fusion protein in which the Fc sequence is fused to the C-terminus of FGF21.

The resulting FGF21 fusion protein can be purified, for example, by the use of a Protein A affinity column. Peptides and proteins fused to an Fc region have been found to exhibit a substantially greater half-life *in vivo* than the unfused counterpart. Also, a fusion to an Fc region allows for dimerization/multimerization of the fusion polypeptide. The Fc region can be a naturally occurring Fc region, or can be altered to improve certain qualities, such as therapeutic qualities, circulation time, or reduced aggregation.

Useful modifications of protein therapeutic agents by fusion with the "Fc"

domain of an antibody are discussed in detail in International Publication No. WO 00/024782, which is hereby incorporated by reference in its entirety.

b. Fusion Protein Linkers

When forming the fusion proteins of the present invention, a linker can, but need not, be employed. When present, the linker's chemical structure may not be critical, since it serves primarily as a spacer. The linker can be made up of amino acids linked together by peptide bonds. In some embodiments of the present invention, the linker is made up of from 1 to 20 amino acids linked by peptide bonds, wherein the amino acids are selected from the 20 naturally occurring amino acids. In various embodiments, the 1 to 20 amino acids are selected from the amino acids glycine, serine, alanine, proline, asparagine, glutamine, and lysine. In some embodiments, a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. In some embodiments, linkers are polyglycines (such as $(Gly)_4$ (SEQ ID NO:29) and $(Gly)_5$ (SEQ ID NO:30)), polyalanines, combinations of glycine and alanine (such as poly(Gly-Ala)), or combinations of glycine and serine (such as poly(Gly-Ser)). Other suitable linkers include: $(Gly)_5$-Ser-$(Gly)_3$-Ser-$(Gly)_4$-Ser (SEQ ID NO:28), $(Gly)_4$-Ser-$(Gly)_4$-Ser-$(Gly)_4$-Ser (SEQ ID NO:31), $(Gly)_3$-Lys-$(Gly)_4$ (SEQ ID NO:32), $(Gly)_3$-Asn-Gly-Ser-$(Gly)_2$ (SEQ ID NO:33), $(Gly)_3$-Cys-$(Gly)_4$ (SEQ ID NO:34), and Gly-Pro-Asn-Gly-Gly (SEQ ID NO:35). While a linker of 15 amino acid residues has been found to work particularly well for FGF21 fusion proteins, the present invention contemplates linkers of any length or composition. When a linker was employed to join a heterologous sequence, such as an Fc domain, and an FGF21 polypeptide or FGF21 mutant, the linker is expressed in parentheses.

The linkers described herein are exemplary, and linkers that are much longer and which include other residues are contemplated by the present invention. Non-peptide linkers are also contemplated by the present invention. For example, alkyl linkers such as $-NH-(CH_2)_S-C(O)-$, wherein s = 2 to 20, could be used. These alkyl linkers can further be substituted by any non-sterically hindering group, including, but not limited to, a lower alkyl (*e.g.*, C1–C6), lower acyl, halogen (*e.g.*, Cl, Br), CN, NH_2, or phenyl. An exemplary non-peptide linker is a polyethylene glycol linker, wherein the linker has a molecular weight of 100 to 5000 kD, for example, 100 to 500 kD.

8. Chemically-modified FGF21 Mutants

Chemically modified forms of the FGF21 polypeptide mutants described herein, including the truncated forms of FGF21 described herein, can be prepared by one skilled in the art, given the disclosures described herein. Such chemically modified FGF21 mutants are altered such that the chemically modified FGF21 mutant is different from the unmodified FGF21 mutant, either in the type or location of the molecules naturally attached to the FGF21 mutant. Chemically modified FGF21 mutants can include molecules formed by the deletion of one or more naturally-attached chemical groups.

In one embodiment, FGF21 polypeptide mutants of the present invention can be modified by the covalent attachment of one or more polymers. For example, the polymer selected is typically water-soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. Included within the scope of suitable polymers is a mixture of polymers. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable. Non-water soluble polymers conjugated to FGF21 polypeptide mutants of the present invention also form an aspect of the invention.

Exemplary polymers each can be of any molecular weight and can be branched or unbranched. The polymers each typically have an average molecular weight of between about 2 kDa to about 100 kDa (the term "about" indicating that in preparations of a water-soluble polymer, some molecules will weigh more and some less than the stated molecular weight). The average molecular weight of each polymer is preferably between about 5 kDa and about 50 kDa, more preferably between about 12 kDa and about 40 kDa, and most preferably between about 20 kDa and about 35 kDa.

Suitable water-soluble polymers or mixtures thereof include, but are not limited to, N-linked or O-linked carbohydrates, sugars, phosphates, polyethylene glycol (PEG) (including the forms of PEG that have been used to derivatize proteins, including mono-(C_1-C_{10}), alkoxy-, or aryloxy-polyethylene glycol), monomethoxy-polyethylene glycol, dextran (such as low molecular weight dextran of, for example, about 6 kD), cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), and

polyvinyl alcohol. Also encompassed by the present invention are bifunctional crosslinking molecules that can be used to prepare covalently attached FGF21 polypeptide mutant multimers. Also encompassed by the present invention are FGF21 mutants covalently attached to polysialic acid.

In some embodiments of the present invention, an FGF21 mutant is covalently, or chemically, modified to include one or more water-soluble polymers, including, but not limited to, polyethylene glycol (PEG), polyoxyethylene glycol, or polypropylene glycol. *See, e.g.*, U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; and 4,179,337. In some embodiments of the present invention, an FGF21 mutant comprises one or more polymers, including, but not limited to, monomethoxy-polyethylene glycol, dextran, cellulose, another carbohydrate-based polymer, poly-(N-vinyl pyrrolidone)-polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, or mixtures of such polymers.

In some embodiments of the present invention, an FGF21 mutant is covalently-modified with PEG subunits. In some embodiments, one or more water-soluble polymers are bonded at one or more specific positions (for example, at the N-terminus) of the FGF21 mutant. In some embodiments, one or more water-soluble polymers are randomly attached to one or more side chains of an FGF21 mutant. In some embodiments, PEG is used to improve the therapeutic capacity of an FGF21 mutant. Certain such methods are discussed, for example, in U.S. Patent No. 6,133,426, which is hereby incorporated by reference for any purpose.

In embodiments of the present invention wherein the polymer is PEG, the PEG group can be of any convenient molecular weight, and can be linear or branched. The average molecular weight of the PEG group will preferably range from about 2 kD to about 100 kDa, and more preferably from about 5 kDa to about 50 kDa, *e.g.*, 10, 20, 30, 40, or 50 kDa. The PEG groups will generally be attached to the FGF21 mutant via acylation or reductive alkylation through a reactive group on the PEG moiety (*e.g.*, an aldehyde, amino, thiol, or ester group) to a reactive group on the FGF21 mutant (*e.g.*, an aldehyde, amino, or ester group).

The PEGylation of a polypeptide, including the FGF21 mutants of the present invention, can be specifically carried out using any of the PEGylation reactions known in the art. Such reactions are described, for example, in the following references: Francis *et al.*, 1992, *Focus on Growth Factors* 3: 4-10; European Patent

Nos. 0 154 316 and 0 401 384; and U.S. Patent No. 4,179,337. For example, PEGylation can be carried out via an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule (or an analogous reactive water-soluble polymer) as described herein. For the acylation reactions, a selected polymer should have a single reactive ester group. For reductive alkylation, a selected polymer should have a single reactive aldehyde group. A reactive aldehyde is, for example, polyethylene glycol propionaldehyde, which is water stable, or mono C_1-C_{10} alkoxy or aryloxy derivatives thereof (*see, e.g.*, U.S. Patent No. 5,252,714).

In some embodiments of the present invention, a useful strategy for the attachment of the PEG group to a polypeptide involves combining, through the formation of a conjugate linkage in solution, a peptide and a PEG moiety, each bearing a special functionality that is mutually reactive toward the other. The peptides can be easily prepared with conventional solid phase synthesis. The peptides are "preactivated" with an appropriate functional group at a specific site. The precursors are purified and fully characterized prior to reacting with the PEG moiety. Ligation of the peptide with PEG usually takes place in aqueous phase and can be easily monitored by reverse phase analytical HPLC. The PEGylated peptides can be easily purified by preparative HPLC and characterized by analytical HPLC, amino acid analysis and laser desorption mass spectrometry.

Polysaccharide polymers are another type of water-soluble polymer that can be used for protein modification. Therefore, the FGF21 mutants of the present invention fused to a polysaccharide polymer form embodiments of the present invention. Dextrans are polysaccharide polymers comprised of individual subunits of glucose predominantly linked by alpha 1-6 linkages. The dextran itself is available in many molecular weight ranges, and is readily available in molecular weights from about 1 kD to about 70 kD. Dextran is a suitable water-soluble polymer for use as a vehicle by itself or in combination with another vehicle (*e.g.*, Fc). *See, e.g.*, International Publication No. WO 96/11953. The use of dextran conjugated to therapeutic or diagnostic immunoglobulins has been reported. *See, e.g.*, European Patent Publication No. 0 315 456, which is hereby incorporated by reference. The present invention also encompasses the use of dextran of about 1 kD to about 20 kD.

In general, chemical modification can be performed under any suitable condition used to react a protein with an activated polymer molecule. Methods for preparing chemically modified polypeptides will generally comprise the steps of: (a)

reacting the polypeptide with the activated polymer molecule (such as a reactive ester or aldehyde derivative of the polymer molecule) under conditions whereby an FGF21 polypeptide mutant becomes attached to one or more polymer molecules, and (b) obtaining the reaction products. The optimal reaction conditions will be determined based on known parameters and the desired result. An example of this is, the larger the ratio of polymer molecules to protein, the greater the percentage of attached polymer molecule. In one embodiment of the present invention, chemically modified FGF21 mutants can have a single polymer molecule moiety at the amino-terminus (*see, e.g.*, U.S. Patent No. 5,234,784)

In another embodiment of the present invention, FGF21 polypeptide mutants can be chemically coupled to biotin. The biotin/FGF21 polypeptide mutants are then allowed to bind to avidin, resulting in tetravalent avidin/biotin/FGF21 polypeptide mutants. FGF21 polypeptide mutants can also be covalently coupled to dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting conjugates precipitated with anti-DNP or anti-TNP-IgM to form decameric conjugates with a valency of 10.

Generally, conditions that can be alleviated or modulated by the administration of the disclosed chemically modified FGF21 mutants include those conditions described herein for FGF21 polypeptide mutants. However, the chemically modified FGF21 mutants disclosed herein can have additional activities, enhanced or reduced biological activity, or other characteristics, such as increased or decreased half-life, as compared to unmodified FGF21 mutants.

9. Pharmaceutical Compositions of FGF21 Mutants and Administration Thereof

Pharmaceutical compositions comprising FGF21 mutants are within the scope of the present invention, and are specifically contemplated in light of the identification of several mutant FGF21 sequences exhibiting enhanced properties. Such FGF21 mutant pharmaceutical compositions can comprise a therapeutically effective amount of an FGF21 polypeptide mutant in admixture with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration.

Acceptable formulation agents preferably are nontoxic to recipients at the dosages and concentrations employed.

The pharmaceutical composition can contain formulation agent(s) for modifying, maintaining, or preserving, for example, the pH, osmolarity, viscosity,

clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. Suitable formulation agents include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine, or lysine), antimicrobials, antioxidants (such as ascorbic acid, sodium sulfite, or sodium hydrogen-sulfite), buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, or other organic acids), bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)), complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin, or hydroxypropyl-beta-cyclodextrin), fillers, monosaccharides, disaccharides, and other carbohydrates (such as glucose, mannose, or dextrins), proteins (such as serum albumin, gelatin, or immunoglobulins), coloring, flavoring and diluting agents, emulsifying agents, hydrophilic polymers (such as polyvinylpyrrolidone), low molecular weight polypeptides, salt-forming counterions (such as sodium), preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, or hydrogen peroxide), solvents (such as glycerin, propylene glycol, or polyethylene glycol), sugar alcohols (such as mannitol or sorbitol), suspending agents, surfactants or wetting agents (such as pluronics; PEG; sorbitan esters; polysorbates such as polysorbate 20 or polysorbate 80; triton; tromethamine; lecithin; cholesterol or tyloxapal), stability enhancing agents (such as sucrose or sorbitol), tonicity enhancing agents (such as alkali metal halides – preferably sodium or potassium chloride – or mannitol sorbitol), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants (*see, e.g., Remington's Pharmaceutical Sciences* (18th Ed., A.R. Gennaro, ed., Mack Publishing Company 1990), and subsequent editions of the same, incorporated herein by reference for any purpose).

The optimal pharmaceutical composition will be determined by a skilled artisan depending upon, for example, the intended route of administration, delivery format, and desired dosage (*see, e.g., Remington's Pharmaceutical Sciences, supra*). Such compositions can influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the FGF21 polypeptide mutant.

The primary vehicle or carrier in a pharmaceutical composition can be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier for injection can be water, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral

administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which can further include sorbitol or a suitable substitute. In one embodiment of the present invention, FGF21 polypeptide mutant compositions can be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (*Remington's Pharmaceutical Sciences, supra*) in the form of a lyophilized cake or an aqueous solution. Furthermore, the FGF21 polypeptide mutant product can be formulated as a lyophilizate using appropriate excipients such as sucrose.

The FGF21 polypeptide mutant pharmaceutical compositions can be selected for parenteral delivery. Alternatively, the compositions can be selected for inhalation or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the skill of the art.

The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8.

When parenteral administration is contemplated, the therapeutic compositions for use in this invention can be in the form of a pyrogen-free, parenterally acceptable, aqueous solution comprising the desired FGF21 polypeptide mutant in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which an FGF21 polypeptide mutant is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads, or liposomes, that provides for the controlled or sustained release of the product which can then be delivered via a depot injection. Hyaluronic acid can also be used, and this can have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

In one embodiment, a pharmaceutical composition can be formulated for inhalation. For example, an FGF21 polypeptide mutant can be formulated as a dry powder for inhalation. FGF21 polypeptide mutant inhalation solutions can also be

formulated with a propellant for aerosol delivery. In yet another embodiment, solutions can be nebulized. Pulmonary administration is further described in International Publication No. WO 94/20069, which describes the pulmonary delivery of chemically modified proteins.

It is also contemplated that certain formulations can be administered orally. In one embodiment of the present invention, FGF21 polypeptide mutants that are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule can be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the FGF21 polypeptide mutant. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders can also be employed.

Another pharmaceutical composition can involve an effective quantity of FGF21 polypeptide mutants in a mixture with non-toxic excipients that are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or another appropriate vehicle, solutions can be prepared in unit-dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

Additional FGF21 polypeptide mutant pharmaceutical compositions will be evident to those skilled in the art, including formulations involving FGF21 polypeptide mutants in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art (*see*, *e.g.*, International Publication No. WO 93/15722, which describes the controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions, and Wischke & Schwendeman, 2008, *Int. J. Pharm.* 364: 298-327, and Freiberg & Zhu, 2004, *Int. J. Pharm.* 282: 1-18, which discuss microsphere/microparticle preparation and use). As described herein, a hydrogel is an example of a sustained- or controlled-delivery formulation.

Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, *e.g.* films, or microcapsules. Sustained release matrices can include polyesters, hydrogels, polylactides (U.S. Patent No. 3,773,919 and European Patent No. 0 058 481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, 1983, *Biopolymers* 22: 547-56), poly(2-hydroxyethyl-methacrylate) (Langer *et al.*, 1981, *J. Biomed. Mater. Res.* 15: 167-277 and Langer, 1982, *Chem. Tech.* 12: 98-105), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D(-)-3-hydroxybutyric acid (European Patent No. 0 133 988). Sustained-release compositions can also include liposomes, which can be prepared by any of several methods known in the art. *See, e.g.*, Epstein *et al.*, 1985, *Proc. Natl. Acad. Sci. U.S.A.* 82: 3688-92; and European Patent Nos. 0 036 676, 0 088 046, and 0 143 949.

The FGF21 polypeptide mutant pharmaceutical composition to be used for *in vivo* administration typically should be sterile. This can be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method can be conducted either prior to, or following, lyophilization and reconstitution. The composition for parenteral administration can be stored in lyophilized form or in a solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

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

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

The effective amount of an FGF21 polypeptide mutant pharmaceutical composition to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the

molecule delivered, the indication for which the FGF21 polypeptide mutant is being used, the route of administration, and the size (body weight, body surface, or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician can titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage can range from about 0.1 μg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage can range from 0.1 μg/kg up to about 100 mg/kg; or 1 μg/kg up to about 100 mg/kg; or 5 μg/kg, 10 μg/kg, 15 μg/kg, 20 μg/kg, 25 μg/kg, 30 μg/kg, 35 μg/kg, 40 μg/kg, 45 μg/kg, 50 μg/kg, 55 μg/kg, 60 μg/kg, 65 μg/kg, 70 μg/kg, 75 μg/kg, up to about 100 mg/kg. In yet other embodiments, the dosage can be 50 μg/kg, 100 μg/kg, 150 μg/kg, 200 μg/kg, 250 μg/kg, 300 μg/kg, 350 μg/kg, 400 μg/kg, 450 μg/kg, 500 μg/kg, 550 μg/kg, 600 μg/kg, 650 μg/kg, 700 μg/kg, 750 μg/kg, 800 μg/kg, 850 μg/kg, 900 μg/kg, 950 μg/kg, 100 μg/kg, 200 μg/kg, 300 μg/kg, 400 μg/kg, 500 μg/kg, 600 μg/kg, 700 μg/kg, 800 μg/kg, 900 μg/kg, 1000 μg/kg, 2000 μg/kg, 3000 μg/kg, 4000 μg/kg, 5000 μg/kg, 6000 μg/kg, 7000 μg/kg, 8000 μg/kg, 9000 μg/kg or 10 mg/kg.

The frequency of dosing will depend upon the pharmacokinetic parameters of the FGF21 polypeptide mutant in the formulation being used. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition can therefore be administered as a single dose, as two or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via an implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages can be ascertained through use of appropriate dose-response data.

The route of administration of the pharmaceutical composition is in accord with known methods, *e.g.*, orally; through injection by intravenous, intraperitoneal, intracerebral (intraparenchymal), intracerebroventricular, intramuscular, intraocular, intraarterial, intraportal, or intralesional routes; by sustained release systems (which may also be injected); or by implantation devices. Where desired, the compositions can be administered by bolus injection or continuously by infusion, or by implantation device.

Alternatively or additionally, the composition can be administered locally via implantation of a membrane, sponge, or other appropriate material onto which the desired molecule has been absorbed or encapsulated. Where an implantation device is used, the device can be implanted into any suitable tissue or organ, and delivery of the desired molecule can be via diffusion, timed-release bolus, or continuous administration.

In order to deliver drug, *e.g.*, an FGF21 mutant disclosed herein, at a predetermined rate such that the drug concentration can be maintained at a desired therapeutically effective level over an extended period, a variety of different approaches can be employed. In one example, a hydrogel comprising a polymer such as a gelatin (*e.g.*, bovine gelatin, human gelatin, or gelatin from another source) or a naturally-occurring or a synthetically generated polymer can be employed. Any percentage of polymer (*e.g.*, gelatin) can be employed in a hydrogel, such as 5, 10, 15 or 20%. The selection of an appropriate concentration can depend on a variety of factors, such as the therapeutic profile desired and the pharmacokinetic profile of the therapeutic molecule.

Examples of polymers that can be incorporated into a hydrogel include polyethylene glycol ("PEG"), polyethylene oxide, polyethylene oxide-co-polypropylene oxide, co-polyethylene oxide block or random copolymers, polyvinyl alcohol, poly(vinyl pyrrolidinone), poly(amino acids), dextran, heparin, polysaccharides, polyethers and the like.

Another factor that can be considered when generating a hydrogel formulation is the degree of crosslinking in the hydrogel and the crosslinking agent. In one embodiment, cross-linking can be achieved via a methacrylation reaction involving methacrylic anhydride. In some situations, a high degree of cross-linking may be desirable while in other situations a lower degree of crosslinking is preferred. In some cases a higher degree of crosslinking provides a longer sustained release. A higher degree of crosslinking may provide a firmer hydrogel and a longer period over which drug is delivered.

Any ratio of polymer to crosslinking agent (e.g., methacrylic anhydride) can be employed to generate a hydrogel with desired properties. For example, the ratio of polymer to crosslinker can be, *e.g.*, 8:1, 16:1, 24:1, or 32:1. For example, when the hydrogel polymer is gelatin and the crosslinker is methacrylate, ratios of 8:1, 16:1, 24:1, or 32:1 methyacrylic anhydride:gelatin can be employed.

10. Therapeutic Uses of FGF21 Polypeptide Mutants

FGF21 polypeptide mutants can be used to treat, diagnose, ameliorate, or prevent a number of diseases, disorders, or conditions, including, but not limited to metabolic disorders. In one embodiment, the metabolic disorder to be treated is diabetes, *e.g.*, type 2 diabetes. In another embodiment, the metabolic disorder is obesity. Other embodiments include metabolic conditions or disorders such as dyslipidimia; hypertension; hepatosteaotosis, such as non-alcoholic steatohepatitis (NASH); cardiovascular disease, such as atherosclerosis; and aging.

In application, a disorder or condition such as diabetes or obesity can be treated by administering an FGF21 polypeptide mutant as described herein to a patient in need thereof in the amount of a therapeutically effective dose. The administration can be performed as described herein, such as by IV injection, intraperitoneal injection, intramuscular injection, or orally in the form of a tablet or liquid formation. In most situations, a desired dosage can be determined by a clinician, as described herein, and can represent a therapeutically effective dose of the FGF21 mutant polypeptide. It will be apparent to those of skill in the art that a therapeutically effective dose of FGF21 mutant polypeptide will depend, *inter alia*, upon the administration schedule, the unit dose of agent administered, whether the nucleic acid molecule or polypeptide is administered in combination with other therapeutic agents, the immune status and the health of the recipient. The term "therapeutically effective dose," as used herein, means that amount of FGF21 mutant polypeptide that elicits the biological or medicinal response in a tissue system, animal, or human being sought by a researcher, medical doctor, or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated.

11. Antibodies

Antibodies and antibody fragments that specifically bind to the FGF21 mutant polypeptides of the present invention but do not specifically bind to wild-type FGF21 polypeptides are contemplated and are within the scope of the present invention. The antibodies can be polyclonal, including monospecific polyclonal; monoclonal (MAbs); recombinant; chimeric; humanized, such as complementarity-determining region (CDR)-grafted; human; single chain; and/or bispecific; as well as fragments; variants; or chemically modified molecules thereof. Antibody fragments include

those portions of the antibody that specifically bind to an epitope on an FGF21 mutant polypeptide. Examples of such fragments include Fab and F(ab') fragments generated by enzymatic cleavage of full-length antibodies. Other binding fragments include those generated by recombinant DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions.

The term "specifically binding," when used in the context of an antibody, means that the antibody binds its target in the presence of a heterogeneous population of proteins and/or other biologic material. More particularly, when an antibody specifically binds its target, this means that under predetermined immunoassay conditions, the antibody binds to its target and does not bind in a significant amount to other proteins present in the sample. Any convenient immunoassay format can be employed to identify an antibody that specifically binds its target, e.g., solid-phase ELISA immunoassays. See, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York.Polyclonal antibodies directed toward an FGF21 mutant polypeptide generally are produced in animals (*e.g.*, rabbits or mice) by means of multiple subcutaneous or intraperitoneal injections of the FGF21 mutant polypeptide and an adjuvant. It can be useful to conjugate an FGF21 mutant polypeptide to a carrier protein that is immunogenic in the species to be immunized, such as keyhole limpet hemocyanin, serum, albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents such as alum are used to enhance the immune response. After immunization, the animals are bled and the serum is assayed for anti-FGF21 mutant antibody titer.

Monoclonal antibodies directed toward FGF21 mutant polypeptides can be produced using any method that provides for the production of antibody molecules by continuous cell lines in culture. Examples of suitable methods for preparing monoclonal antibodies include the hybridoma methods of Kohler *et al.*, 1975, *Nature* 256: 495-97 and the human B-cell hybridoma method (Kozbor, 1984, *J. Immunol.* 133: 3001; Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications* 51-63 (Marcel Dekker, Inc., 1987). Also provided by the invention are hybridoma cell lines that produce monoclonal antibodies reactive with FGF21 mutant polypeptides.

Monoclonal antibodies of the invention can be modified for use as therapeutics. In one embodiment, the monoclonal antibody is a "chimeric" antibody

in which a portion of the heavy (H) and/or light (L) chain is identical with or homologous to a corresponding sequence in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies, so long as they exhibit the desired biological activity. *See, e.g.*, U.S. Patent No. 4,816,567; Morrison *et al.*, 1985, *Proc. Natl. Acad. Sci. U.S.A.* 81: 6851-55.

In another embodiment, a monoclonal antibody of the invention is a "humanized" antibody. Methods for humanizing non-human antibodies are well known in the art. *See, e.g.*, U.S. Patent Nos. 5,585,089 and 5,693,762. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. Humanization can be performed, for example, using methods described in the art (*see, e.g.*, Jones *et al.*, 1986, *Nature* 321: 522-25; Riechmann *et al.*, 1998, *Nature* 332: 323-27; Verhoeyen *et al.*, 1988, *Science* 239: 1534-36), by substituting at least a portion of a rodent complementarity-determining region for the corresponding regions of a human antibody.

Also encompassed by the invention are human antibodies that bind the FGF21 mutant polypeptides of the present invention. Using transgenic animals (*e.g.*, mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production such antibodies are produced by immunization with an antigen derived from an FGF21 mutant (*i.e.*, having at least 6 contiguous amino acids), optionally conjugated to a carrier. *See, e.g.*, Jakobovits *et al.*, 1993, *Proc. Natl. Acad. Sci. U.S.A.* 90: 2551-55; Jakobovits *et al.*, 1993, *Nature* 362: 255-58; Bruggermann *et al.*, 1993, *Year in Immuno.* 7: 33. In one method, such transgenic animals are produced by incapacitating the endogenous loci encoding the heavy and light immunoglobulin chains therein, and inserting loci encoding human heavy and light chain proteins into the genome thereof. Partially modified animals, *i.e.*, animals having less than the full complement of modifications, are then cross-bred to obtain an animal having all of the desired immune system modifications. When administered an immunogen, these transgenic animals produce antibodies with human (rather than, *e.g.*, murine) amino acid sequences, including variable regions that are immunospecific for these antigens. *See, e.g.*, International Publication Nos. WO 96/33735 and WO 94/02602. Additional methods are described in U.S. Patent

No. 5,545,807, International Publication Nos. WO 91/10741 and WO 90/04036, and in European Patent No. 0 546 073. Human antibodies can also be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

In an alternative embodiment, human antibodies can also be produced from phage-display libraries (*see*, *e.g.*, Hoogenboom *et al.*, 1991, *J. Mol. Biol.* 227: 381; Marks *et al.*, 1991, *J. Mol. Biol.* 222: 581). These processes mimic immune selection through the display of antibody repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to an antigen of choice.

Chimeric, CDR grafted, and humanized antibodies are typically produced by recombinant methods. Nucleic acids encoding the antibodies are introduced into host cells and expressed using materials and procedures described herein. In one embodiment, the antibodies are produced in mammalian host cells, such as CHO cells. Monoclonal (*e.g.*, human) antibodies can be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

The anti-FGF21 mutant antibodies of the invention can be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays (*see*, *e.g.*, Sola, *Monoclonal Antibodies: A Manual of Techniques* 147-158 (CRC Press, Inc., 1987), incorporated herein by reference in its entirety) for the detection and quantitation of FGF21 mutant polypeptides. The antibodies will bind FGF21 mutant polypeptides with an affinity that is appropriate for the assay method being employed.

For diagnostic applications, in certain embodiments, anti-FGF21 mutant antibodies can be labeled with a detectable moiety. The detectable moiety can be any one that is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{125}I, ^{99}Tc, ^{111}In, or ^{67}Ga; a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; or an enzyme, such as alkaline phosphatase, β-galactosidase, or horseradish peroxidase (Bayer *et al.*, 1990, *Meth. Enz.* 184: 138-63).

Competitive binding assays rely on the ability of a labeled standard (*e.g.*, an FGF21 mutant polypeptide, or an immunologically reactive portion thereof) to

compete with the test sample analyte (*e.g.*, an FGF21 mutant polypeptide) for binding with a limited amount of anti-FGF21 mutant antibody. The amount of an FGF21 mutant polypeptide in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies typically are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies can conveniently be separated from the standard and analyte that remain unbound.

Sandwich assays typically involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected and/or quantitated. In a sandwich assay, the test sample analyte is typically bound by a first antibody that is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. *See, e.g.*, U.S. Patent No. 4,376,110. The second antibody can itself be labeled with a detectable moiety (direct sandwich assays) or can be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assays). For example, one type of sandwich assay is an enzyme-linked immunosorbent assay (ELISA), in which case the detectable moiety is an enzyme.

The anti-FGF21 mutant antibodies of the present invention are also useful for *in vivo* imaging. An antibody labeled with a detectable moiety can be administered to an animal, preferably into the bloodstream, and the presence and location of the labeled antibody in the host assayed. The antibody can be labeled with any moiety that is detectable in an animal, whether by nuclear magnetic resonance, radiology, or other detection means known in the art.

The FGF21 mutant antibodies of the invention can be used as therapeutics. These therapeutic agents are generally agonists or antagonists, in that they either enhance or reduce, respectively, at least one of the biological activities of an FGF21 mutant polypeptide. In one embodiment, antagonist antibodies of the invention are antibodies or binding fragments thereof which are capable of specifically binding to an FGF21 mutant polypeptide and which are capable of inhibiting or eliminating the functional activity of an FGF21 mutant polypeptide *in vivo* or *in vitro*. In some embodiments, the antagonist antibody will inhibit the functional activity of an FGF21 mutant polypeptide by at least about 50%, and preferably by at least about 80%. In another embodiment, the anti-FGF21 mutant antibody is capable of interfering with the interaction between an FGF21 mutant polypeptide and an FGF receptor thereby

inhibiting or eliminating FGF21 mutant polypeptide activity *in vitro* or *in vivo*. Agonist and antagonist anti-FGF21 mutant antibodies are identified by screening assays that are well known in the art.

The invention also relates to a kit comprising FGF21 mutant antibodies and other reagents useful for detecting FGF21 mutant polypeptide levels in biological samples. Such reagents can include a detectable label, blocking serum, positive and negative control samples, and detection reagents.

EXAMPLES

The Examples that follow are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and should not be construed as limiting the scope of the invention in any way.

EXAMPLE 1

Preparation of FGF21 Expression Constructs

A nucleic acid sequence encoding the mature FGF21 polypeptide was obtained by polymerase chain reaction (PCR) amplification using primers having nucleotide sequences corresponding to the 5' and 3' ends of the mature FGF21 sequence. Table 2 lists the primers that were used to amplify the mature FGF21 sequence.

Table 2

PCR Primers for Preparing FGF21 Construct

Primer	Sequence	SEQ ID NO:
Sense	5'-AGGAGGAATAACATATGCATCCAATTCCAGATTCTTCTCC-3'	12
Antisense	5'-TAGTGAGCTCGAATTCTTAGGAAGCGTAGCTGG-3'	13

The primers used to prepare the mature FGF21 expression construct incorporated restriction endonuclease sites (the NdeI site also comprises an N-terminal methionine for bacterial expression) for directional cloning of the sequence into a suitable expression vector (*e.g.*, pET30 (Novagen/EMD Biosciences; San Diego, CA) or pAMG33 (Amgen; Thousand Oaks, CA)). The expression vector pAMG33 contains a low-copy number R-100 origin of replication, a modified *lac* promoter, and a kanamycin-resistance gene. The expression vector pET30 contains a

pBR322-derived origin of replication, an inducible T7 promoter, and a kanamycin-resistance gene. While expression from pAMG33 was found to be higher, pET30 was found to be a more reliable cloning vector. Thus, the majority of the constructs described in the instant disclosure were first generated in pET30 and then screened for efficacy. Selected sequences were then transferred to pAMG33 for further amplification.

The FGF21 sequence was amplified in a reaction mixture containing 40.65 µL dH_2O, 5µL PfuUltra II Reaction Buffer (10x), 1.25 µL dNTP Mix (40 mM – 4 x 10mM), 0.1 µL Template (100 ng/mL), 1 µL Primer1 (10 µM), 1 µL Primer2 (10 µM), and 1 µL PfuUltra II fusion HS DNA Polymerase (Stratagene; La Jolla, CA). Amplification reactions were performed by heating for 2 minutes at 95°C; followed by ten cycles at 95°C for 20 seconds, 60°C for 20 seconds (with an additional 1°C subtracted per cycle), and 72°C for 15 seconds/kilobase of desired product; followed by 20 cycles at 94°C for 20 seconds, 55°C for 20 seconds, and 72°C for 15 seconds/kilobase of desired product; followed by 72°C for 3 minutes. Amplification products were digested with the restriction endonucleases NdeI, DpnI, and EcoRI; ligated into a suitable vector; and then transformed into competent cells.

EXAMPLE 2

Purification of FGF21 Proteins from Bacteria

In the Examples that follow, various FGF21 proteins, including the wild-type FGF21 polypeptide, truncated FGF21 polypeptides, FGF21 mutants, and FGF21 fusion proteins, were expressed in a bacterial expression system. After expression, which is described below, the FGF21 proteins were purified as described in this Example, unless otherwise indicated.

To purify the wild-type FGF21 polypeptide, truncated FGF21 polypeptides, and FGF21 mutants from bacterial inclusion bodies, double-washed inclusion bodies (DWIBs) were solubilized in a solubilization buffer containing guanidine hydrochloride and DTT in Tris buffer at pH 8.5. They were then mixed for one hour at room temperature, and the solubilization mixture was added to a refold buffer containing urea, arginine, cysteine, and cystamine hydrochloride at pH 9.5 and then mixed for 24 hours at 5°C (*see, e.g.*, Clarke, 1998, *Curr. Opin. Biotechnol.* 9: 157-63; Mannall *et al.*, 2007, *Biotechnol. Bioeng.* 97: 1523-34; Rudolph *et al.*, 1997, "Folding proteins," Protein Function: A Practical Approach (Creighton, ed., New York, IRL

Press) 57-99; and Ishibashi *et al.*, 2005, *Protein Expr. Purif.* 42: 1-6).

Following solubilization and refolding, the mixture was filtered through a 0.45 micron filter. The refold pool was then concentrated approximately 10-fold with a 10 kD molecular weight cut-off Pall Omega cassette at a transmembrane pressure (TMP) of 20 psi, and dialfiltered with 3 column volumes of 20 mM Tris, pH 8.0 at a TMP of 20 psi.

The clarified sample was then subjected to anion exchange (AEX) chromatography using a Q Sepharose HP resin. A linear salt gradient of 0 to 250 mM NaCl in 20 mM Tris was run at pH 8.0 at 5°C. Peak fractions were analyzed by SDS-PAGE and pooled.

The AEX eluate pool was then subjected to hydrophobic interaction chromatography (HIC) using a Phenyl Sepharose HP resin. Protein was eluted using a decreasing linear gradient of 0.7 M to 0 M ammonium sulfate at pH 8.0 and ambient temperature. Peak fractions were analyzed by SDS-PAGE (Laemmli, 1970, *Nature* 227: 680-85) and pooled.

The HIC pool was concentrated with a 10 kD molecular weight cut-off Pall Omega 0.2 m^2 cassette to 7 mg/mL at a TMP of 20 psi. The concentrate was dialfiltered with 5 column volumes of formulation buffer at a TMP of 20 psi, and the recovered concentrate was diluted to 5 mg/mL. Finally, the solution was filtered through a Pall mini-Kleenpac 0.2 μM Posidyne membrane.

To purify FGF21 fusion proteins and FGF21 fusion mutant proteins from bacterial inclusion bodies, double-washed inclusion bodies (DWIBs) were solubilized in a solubilization buffer containing guanidine hydrochloride and DTT in Tris buffer at pH 8.5 and then mixed for one hour at room temperature. Then the solubilization mixture was added to a refold buffer containing urea, arginine, cysteine, and cystamine hydrochloride at pH 9.5 and then mixed for 24 hours at 5°C (*see, e.g.*, Clarke, 1998, *Curr. Opin. Biotechnol.* 9: 157-63; Mannall *et al.*, 2007, *Biotechnol. Bioeng.* 97: 1523-34; Rudolph *et al.*, 1997, "Folding proteins," Protein Function: A Practical Approach (Creighton, ed., New York, IRL Press) 57-99; and Ishibashi *et al.*, 2005, *Protein Expr. Purif.* 42: 1-6).

Following solubilization and refolding, the mixture was dialyzed against 5 volumes of 20 mM Tris, pH 8.0 using 10 kD dialysis tubing. The pH of the dialyzed refold was adjusted to 5.0 with 50% acetic acid, and then clarified by centrifugation for 30 minutes at 4K.

The clarified sample was then subjected to anion exchange (AEX) chromatography using a Q Sepharose HP resin. A linear salt gradient of 0 to 250 mM NaCl in 20 mM Tris was run at pH 8.0 at 5°C. Peak fractions were analyzed by SDS-PAGE (Laemmli, 1970, *Nature* 227: 680-85) and pooled.

The AEX eluate pool was then subjected to hydrophobic interaction chromatography (HIC) using a Phenyl Sepharose HP resin. Protein was eluted using a decreasing linear gradient of 0.6 M to 0 M ammonium sulfate at pH 8.0 at ambient temperature. Peak fractions were analyzed by SDS-PAGE and pooled.

Following the HIC step, the pool was then dialyzed 60 volumes of formulation buffer. The dialyzed pool was concentrated to 5 mg/mL using a Pall Jumbosep. Finally, the solution was filtered through a Pall mini-Kleenpac 0.2 μM Posidyne membrane.

EXAMPLE 3

Preparation and Expression of Truncated FGF21 Proteins

Constructs encoding the truncated FGF21 proteins listed in Table 3 were prepared by PCR amplification of the wild-type FGF21 expression vector as described below (the construction of the wild-type FGF21 expression vector is described in Example 1).

Table 3

FGF21 Truncations

Amino Acid Residues	Number of Residues Truncated*
C-terminus Truncations	
1 – 180	1
1 – 179	2
1 – 178	3
1 – 177	4
1 – 176	5
1 – 175	6
1 – 174	7
1 – 173	8
1 – 172	9
1 – 171	10
1 – 169	12
1 – 168	13

Amino Acid Residues	Number of Residues Truncated*
1 – 167	14
1 – 166	15
1 – 165	16
1 – 164	17
1 – 160	21
1 – 156	25
1 – 152	29
1 – 149	32
1 – 113	68
N-terminus Truncations	
2 – 181	1
3 – 181	2
4 – 181	3
5 – 181	4
6 – 181	5
7 – 181	6
8 – 181	7
9 – 181	8
C- and N-terminus Truncations	
5 – 174	11
7 – 172	17
9 – 169	20
9 – 149	40
15 – 169	26
15 – 149	46
15 – 113	82

* relative to mature FGF21 polypeptide

Truncated FGF21 protein constructs were prepared using primers having sequences that are homologous to regions upstream and downstream of a codon (or codons) to be deleted (resulting in the truncation). The primers used in such amplification reactions also provided approximately 15 nucleotides of overlapping sequence to allow for recircularization of the amplified product, namely the entire vector now having the desired mutant or truncation.

An exemplary truncated FGF21 construct, encoding an FGF21 protein lacking the histidine residue at position 1 of the mature FGF21 sequence (*i.e.*, the 2-181 truncation mutant), was prepared using the primers shown in Table 4.

Table 4

PCR Primers for Preparing Exemplary Truncation FGF21 Mutant

Primer	Sequence	SEQ ID NO:
Sense	5'-GGAGATATACATATGCCAATTCCAGATTCTTCTCCATTATT-3'	14
Antisense	5'-CATATGTATATCTCCTTCTTAAAGTTAAACAAAA-3'	15

The primers shown in Table 4 allow for the deletion of the histidine residue as shown below, wherein the upper sequence (SEQ ID NO:9) is a portion of a mature FGF21 polypeptide comprising a N-terminal methionine, the second sequence is the sense primer (SEQ ID NO:14), the third and fourth sequences (SEQ ID NOs:17 and 18) are portions of an FGF21 expression construct, and the fifth sequence is the antisense primer (SEQ ID NO:16):

MetHisProIleProAspSerSerProLeu

5'-GGAGATATACATATG---

CCAATTCCAGATTCTTCTCCATTATT

TTTTGTTTAACTTTAAGAAGGAGATATACATATG**CAT**CCAATTCCAGATTCTTCTCCATTAT

T

AAAACAAATTGAAATTCTTCCTCTATATGTATAC**GTA**GGTTAAGGTCTAAGAAGAGGTAATA

A

AAAACAAATTGAAATTCTTCCTCTATATGTATAC-5'

Truncated FGF21 protein constructs were prepared using essentially the PCR conditions described in Example 1. Amplification products were digested with the restriction endonuclease DpnI, and then transformed into competent cells. The resulting clones were sequenced to confirm the absence of polymerase-generated errors.

Truncated FGF21 proteins were expressed by transforming competent BL21 (DE3) or BL21 Star (Invitrogen; Carlsbad, CA) cells with the construct encoding a particular truncated FGF21 protein. Transformants were grown overnight with limited aeration in TB media supplemented with 40 μg/mL kanamycin, were aerated the next morning, and after a short recovery period, were induced in 0.4 mM IPTG. FGF21 mutants were harvested by centrifugation 18-20 hours after induction.

EXAMPLE 4

In vitro Activity of Truncated FGF21 Proteins

Experiments were performed to identify truncated FGF21 proteins that retain wild-type FGF21 activity in an ELK-luciferase *in vitro* assay. Table 5 summarizes the results obtained for FGF21 proteins having truncations at the N-terminus, the C-terminus, or at both the N-terminus and C-terminus. ELK-luciferase assays were performed using a recombinant human 293T kidney cell system, in which the 293T cells overexpress β-Klotho and luciferase reporter constructs. These constructs also contain sequences encoding GAL4-ELK1 and 5xUAS-Luc, a luciferase reporter driven by a promoter containing five tandem copies of the Gal4 binding site. β-Klotho is a co-receptor that is required by FGF21 for activation of its FGF receptors and induction of intracellular signal transduction, which in turn leads to Erk and ELK phosphorylation. Luciferase activity is regulated by the level of phosphorylated Erk/ELK1, and is used to indirectly monitor and quantify FGF21 activity.

ELK-luciferase assays were performed by culturing the 293T cells in the presence of different concentrations of wild-type FGF21 or FGF21 mutant polypeptide for 6 hours, and then assaying the cell lysates for luciferase activity. Figures 1A-1B show the results of an ELK-luciferase activity assay performed on the FGF21 truncation mutants 7-181 and 8-181 (Figure 1A) and the FGF21 truncation mutants 1-172, 1-171, 1-169, and 1-164 (Figure 1B). The luminescence obtained in ELK-luciferase assays for each of the FGF21 truncation mutants 3-181, 4-181, 5-181, 7-181, 8-181, 1-180, 1-178, 1-177, 1-176, 1-175, 1-174, 1-173, 1-172, 9-181, and 1-149 is shown in Figure 2.

FGF21 mutant polypeptides were compared with a wild-type FGF21 standard and mutants showing an efficacy of at least 50% of the efficacy of wild-type FGF21 were considered as having not lost FGF21 activity and were assigned a "+" in Table 5.

Table 5

Truncated FGF21 Proteins: *in vitro* Assay

C-terminus Truncations		
Amino Acid Residues	**Efficacy**	**Activity (+/–)**
1 – 180	93.2%	+

C-terminus Truncations		
Amino Acid Residues	**Efficacy**	**Activity (+/–)**
1 – 178	95.0%	+
1 – 177	112.0%	+
1 – 176	104.8%	+
1 – 174	104.6%	+
1 – 173	96.1%	+
1 – 172	97.5%	+
1 – 171	113.0%	+
1 – 169	84.9%	+
1 – 167	20%	–
1 – 166	20%	–
1 – 165	10%	–
N-terminus Truncations		
Amino Acid Residues	**Efficacy**	**Activity (+/–)**
2 – 181	112.5%	+
3 – 181	130.3%	+
4 – 181	117.0%	+
5 – 181	119.6%	+
7 – 181	74.2%	+
8 – 181	24.9%	–
9 – 181	12.5%	–

Collectively, the results presented in Table 5 indicate that C-terminal deletions of 14 or more amino acid residues (*i.e.*, a C-terminally truncated FGF21 protein consisting of amino acid residues 1-167 and shorter proteins) eliminate the activity of FGF21. In addition, Table 5 indicates that N-terminal deletions of 7 or more amino acid residues (*i.e.*, an N-terminally truncated FGF21 protein consisting of amino acid residues 8-181 and shorter proteins) eliminate the activity of FGF21. Not surprisingly, truncated FGF21 proteins possessing both an N-terminal truncation of 8 to 14 residues and a C-terminal truncation of 12 or 32 residues were found to lack activity in ELK-luciferase assays.

Consistent with the data presented in Table 5, truncated FGF21 polypeptides having N-terminal truncations of fewer than 7 amino acid residues constitute embodiments of the present invention. Similarly, truncated FGF21 polypeptides having C-terminal truncations of fewer than 13 amino acid residues constitute embodiments of the present invention.

EXAMPLE 5

In vivo Activity of Truncated FGF21 Proteins

FGF21 possesses a number of biological activities, including the ability to lower blood glucose, insulin, triglyceride, or cholesterol levels; reduce body weight; or improve glucose tolerance, energy expenditure, or insulin sensitivity. Truncated FGF21 polypeptides were further analyzed for *in vivo* FGF21 activity, by introducing the truncated FGF21 polypeptides into insulin resistant ob/ob mice, and measuring the ability of a particular truncated FGF21 polypeptide to lower blood glucose. The truncated FGF21 polypeptide to be tested was injected intraperitoneally into an 8 week old ob/ob mouse (Jackson Laboratory), and blood samples were obtained at various time points following a single injection, *e.g.*, 0, 6, 24, 72, 120, and 168 hours after injection. Blood glucose levels were measured with a OneTouch Glucometer (LifeScan, Inc. Milpitas, CA), and the results expressed as a percent change of blood glucose relative to the baseline level of blood glucose (*i.e.*, at time 0).

The results of one experiment are provided in Figure 3, which shows the amount of blood glucose detected in mice injected with the FGF21 truncation mutants 8-181 and 9-181. This experiment demonstrated that truncated FGF21 fusion proteins comprising amino acid residues 8-181 exhibit blood glucose lowering activity *in vivo* however the activity is slightly less than the activity of wild-type FGF21 at 3 and 6 hours after injection, but that truncated FGF21 fusion proteins comprising amino acid residues 9-181 do not exhibit such activity. Thus, the *in vivo* analysis of truncated FGF21 polypeptides indicated that the deletion of up to 7 amino acids from the N-terminus of mature FGF21 does not abolish the molecule's biological activity (in contrast with the *in vitro* analysis, which suggested that the deletion of 7 amino acids from the N-terminus of mature FGF21 would abolish activity).

The differing results obtained with particular N-terminally truncated FGF21 polypeptides (*e.g.*, FGF21 8-181) in *in vitro* and *in vivo* assays can be explained by the interaction of FGF21 with β-Klotho and FGF receptor in effecting signal transduction. In particular, FGF21 activates a dual receptor complex comprising the co-receptor β-Klotho and FGF receptor (FGFR), which initiates a signaling cascade involving tyrosine kinase. The N-terminus of FGF21 has been shown to be involved in binding and activation of FGFR while the C-terminus of FGF21 is required for β-Klotho interaction (Yie et al., 2009 *FEBS Lett.* 583:19-24). The ELK-luciferase *in*

vitro assay is performed in 293 kidney cells in which the co-receptor β-Klotho is overexpressed and FGFR is expressed at normal levels. The amount of FGFR is low relative to that of β-Klotho and the ratio of β-Klotho to FGFR in 293 cells is therefore non-physiological, which may affect receptor complex formation and ultimately ligand binding and activation of FGFR. The 293 *in vitro* system appears to be more vulnerable to N-terminally truncated FGF21 polypeptides and therefore may have produced loss of activity results for a few of the N-terminally truncated mutants tested, such as FGF21 8-181. Thus, in determining whether a particular N-terminally truncated FGF21 mutant retained wild-type FGF21 activity, the activity of that FGF21 mutant in the *in vivo* assay was considered to be dispositive. Accordingly, truncated FGF21 polypeptides having N-terminal truncations of fewer than 8 amino acid residues are encompassed by the invention.

EXAMPLE 6

Preparation and Expression of Truncated FGF21 Fusion Proteins

Because the half-life of a protein can be increased by fusing the protein to an Fc sequence, fusion proteins comprising truncated FGF21 polypeptides were prepared and analyzed. The truncated FGF21 fusion proteins listed in Table 6 were prepared from amplified FGF21 sequences by SOEing (gene splicing by overlap extension) PCR. FGF21 fusion proteins were prepared such that the Fc portion of the human immunoglobulin IgG1 gene (SEQ ID NO:11) was fused to either the N-terminus or the C-terminus of the FGF21 protein.

Table 6

Truncated FGF21 Fusion Proteins

Amino Acid Residues	Fc Position	Linker
C-terminus Truncations		
1 – 178	-NH_2	15
1 – 175	-NH_2	14
1 – 175	-COOH	15
1 – 171	-NH_2	15
1 – 171	-COOH	15
1 – 170	-COOH	15
N-terminus Truncations		
5 – 181	-NH_2	15
5 – 181	-COOH	15
7 – 181	-NH_2	15

Amino Acid Residues	Fc Position	Linker
7 – 181	-COOH	15
C- and N-terminus Truncations		
5 – 175	$-NH_2$	15
5 – 175	-COOH	15
5 – 171	$-NH_2$	15
5 – 171	-COOH	15
6 – 170	-COOH	15
7 – 178	-COOH	35
7 – 175	$-NH_2$	15
7 – 175	-COOH	15
7 – 174	-COOH	35
7 – 172	-COOH	35
7 – 171	$-NH_2$	15
7 – 171	-COOH	35
7 – 171	-COOH	15

In particular, FGF21 fusion protein constructs (including those encoding truncated FGF21 fusion proteins) were prepared in a series of three amplification reactions using essentially the reaction conditions described in Example 1. In the first reaction, a pair of primers was designed to produce a sequence containing an NdeI cloning site (including an N-teriminal methionine for bacterial expression), Fc region, and linker sequence. In the second reaction, a pair of primers was designed to produce a sequence containing an overlapping portion of the linker, a portion of the FGF21 coding sequence, and an EcoRI cloning site. Finally, in the third reaction, a pair of primers was designed for the purpose of linking the products of the first two reactions. An exemplary set of primers for the construction of Fc-FGF21 1-181 is listed in Table 7.

Table 7

PCR Primers for Preparing Exemplary FGF21 Fusion Protein Construct

Primer	Sequence	SEQ ID NO:
Reaction 1		
Sense	5'-AGGAGGAATAACATATGGACAAAACTCACACATG-3'	19
Antisense	5'-GGATCCACCACCACCGCTACCAC-3'	20
Reaction 2		
Sense	5'-GGTGGTGGTGGATCCCATCCAATTCCAGATTCTTCTCCA-3'	21
Antisense	5'-TAGTGAGCTCGAATTCTTAGGAAGCGTAGCTGG-3'	22
Reaction 3		
Sense	5'-AGGAGGAATAACATATGGACAAAACTCACACATG-3'	19
Antisense	5'-TAGTGAGCTCGAATTCTTAGGAAGCGTAGCTGG-3'	22

The product of the final reaction was digested with the restriction endonucleases NdeI and EcoRI, ligated into the pET30 vector, and then transformed into competent cells. The resulting clones were sequenced to confirm the absence of polymerase-generated errors.

EXAMPLE 7

In vivo Activity of Truncated FGF21 Fusion Proteins

Fusion proteins comprising a truncated FGF21 sequence fused to an Fc sequence were generated and assayed for *in vivo* activity. Truncated FGF21 fusion proteins were prepared by fusing an IgG1 Fc molecule to either the N-terminal or C-terminal end of a truncated FGF21 protein to form a single contiguous sequence. To distinguish between N-terminal and C-terminal fusions, FGF21 fusion proteins in which the Fc molecule was fused to the N-terminal end of the FGF21 protein are designated as Fc-FGF21, and fusion proteins in which the Fc molecule was fused to the C-terminal end of the FGF21 protein are designated as FGF21-Fc.

FGF21 possesses a number of biological activities, including the ability to lower blood glucose, insulin, triglyceride, or cholesterol levels; reduce body weight; or improve glucose tolerance, energy expenditure, or insulin sensitivity. To assess *in vivo* FGF21 activity, FGF21 polypeptides, FGF21 mutant polypeptides, and FGF21 fusion polypeptides were introduced into insulin resistant ob/ob mice, and the ability of a particular FGF21 protein to lower blood glucose levels was measured. The FGF21 polypeptide, FGF21 mutant polypeptide, or FGF21 fusion polypeptide to be tested was injected intraperitoneally into 8 week old ob/ob mice (Jackson Laboratory), and blood samples were obtained at various time points following a single injection, *e.g.*, 0, 6, 24, 72, 120, and 168 hours after injection. Blood glucose levels were measured with a OneTouch Glucometer (LifeScan, Inc. Milpitas, CA), and the results expressed as a percent change of blood glucose relative to the baseline level of blood glucose (*i.e.*, at time 0).

The results of one experiment are provided in Figure 4, which shows the percent change in blood glucose levels observed in mice injected with a PBS control, a wild-type Fc-FGF21 control comprising amino acid residues 1-181, or truncated Fc-FGF21 fusion proteins comprising amino acid residues 5-181 or 7-181. This experiment demonstrated that truncated Fc-FGF21 fusion proteins comprising amino

acid residues 5-181 or 7-181 exhibit blood glucose lowering activity that is similar to the activity of wild-type Fc-FGF21 at 6 hours after injection. Thus, the *in vivo* analysis of truncated FGF21 polypeptides indicated that the deletion of up to 6 amino acids from the N-terminus of mature FGF21 does not affect the molecule's biological activity. *In vivo* analysis also indicated, however, that the ability of truncated FGF21 polypeptides to lower blood glucose was reduced and that blood glucose levels returned to baseline at 24 hours after injection (similar results were obtained with wild-type FGF21). The short *in vivo* activity was found to be a result of the proteolytic degradation of FGF21, as described in Example 8.

The results of another experiment are provided in Figure 5, which shows the percent change in blood glucose levels observed in mice injected with a PBS control, a wild-type FGF21-Fc control comprising amino acid residues 1-181, a truncated FGF21-Fc fusion protein comprising residues 1-175, or a truncated Fc-FGF21 protein comprising amino acid residues 1-171. This experiment demonstrates that the wild-type FGF21-Fc comprising amino acid residues 1-181 has a sustained glucose-lowering activity resulting in a reduction of blood glucose levels of approximately 30% over the time period of 24 hours to 120 hours following injection. The truncated Fc-FGF21 protein comprising amino acid residues 1-171 exhibits delayed blood glucose lowering activity evident only at 72 hours after injection. However, the activity observed is the same as the activity of wild-type FGF21-Fc. The truncated FGF21-Fc fusion protein comprising residues 1-175 is not active *in vivo* in lowering blood glucose.

Collectively, the truncation experiments described herein demonstrate that truncated FGF21 fusion proteins having an N-terminal truncation exhibit blood glucose lowering activity that is similar to that of the wild-type FGF21 fusion protein, and further, that truncated FGF21 fusion proteins in which the Fc molecule has been fused to the N-terminal end of the truncated FGF21 protein exhibit more activity than fusion proteins in which the Fc molecule has been fused to the C-terminal end of the truncated FGF21 protein.

EXAMPLE 8

Observed *in vivo* Degradation of FGF21

FGF21 degradation was first observed with FGF21 Fc fusion protein constructs as described in Example 7. *In vivo* pharmacokinetic analysis indicated that

human FGF21 has a short half-life of about 1 hour in mice due to rapid clearance and *in vivo* degradation. Therefore, to extend the half-life of FGF21 an Fc sequence was fused to the N- or C-terminal end of the FGF21 polypeptide. However, the fusion of an Fc region did not completely resolve the half-life issue since fusion proteins in which an Fc sequence was fused to the N- or C-terminal end of the FGF21 polypeptide (and in particular Fc-FGF21 fusions, *i.e.*, in which the Fc sequence is fused to the N-terminus of mature FGF21), did not exhibit the expected *in vivo* efficacy, and instead were found to maintain blood glucose lowering activity for no more than 24 hours in ob/ob mice. As described in Figure 4, Fc-FGF21 fusion proteins reduced blood glucose levels by about 30-40% at 6 hours after injection, while the blood glucose levels returned to baseline levels at 24 hours.

The proteolytic degradation of wild-type FGF21 was subsequently investigated, and the rapid loss of *in vivo* activity with Fc-FGF21 fusion proteins was found to be the result of *in vivo* degradation of FGF21. Proteolytic degradation leads to decreased biological activity of the molecule *in vivo* and thus a shorter effective half-life, and such degradation adversely impacts the therapeutic use of that molecule. Accordingly, the observed degradation of FGF21 Fc fusion proteins led to the investigation of the proteolytic degradation of FGF21 *in vivo* and to identify FGF21 mutants that were resistant to such degradation.

To determine the sites of degradation, LC-MS analysis and Edman sequencing was performed on wild-type human FGF21 and FGF21 Fc fusion proteins obtained at various time points after injection into male C57B6 mice. The Edman sequencing helped confirm whether the N-terminal or C-terminal end of the protein was undergoing degradation. When an Fc sequence was fused to the N-terminus of human FGF21, degradation was found to occur at the peptide bond between amino acid residues 151 and 152 and between amino acid residues 171 and 172 of the human FGF21 portion of the fusion molecule (the residue numbering above is based on the mature FGF21 sequence and does not include the Fc portion of the fusion protein). The degradation at 171-172 was found to occur first, and was followed by degradation at 151-152. Degradation at 171-172 appears to be the rate-limiting step and plays a role in the half-life of the molecule. When an Fc sequence was fused to the C-terminus of FGF21, degradation was found to occur at the peptide bond between amino acid residues 4 and 5 and between amino acid residues 20 and 21. As a result of these experiments, it was determined that the Fc sequence appears to

protect the portion of the FGF21 sequence that is adjacent to the Fc sequence from degradation. An analysis of the *in vivo* degradation of wild-type FGF21 and Fc-FGF21 fusion proteins was further conducted in *cynomolgus* monkeys. These studies confirmed that the cleavage site of FGF21 at amino acid residues 171-172 is the major site of degradation in monkeys and that this site of degradation is conserved between murine and primate.

EXAMPLE 9

Identification of FGF21 Proteolysis-Resistant Mutants

Suitable FGF21 mutants were identified by experimentally determining the positions of the wild-type FGF21 sequence that are sites of major proteolytic activity, and specific amino acid substitutions were introduced at these sites. Amino acid substitutions were based on FGF21 sequence conservation with other species (as described in Example 8) and biochemical conservation with other amino acid residues. A list of amino acid substitutions that were or can be introduced into the wild-type FGF21 protein is provided in Table 8, although Table 8 is only exemplary and other substitutions can be made. The numbers of the positions given in Table 8 correspond to the residue position in the mature FGF21 protein, which consists of 181 amino acid residues.

Table 8

FGF21 Residues Mutated

Amino Acid Position	Native Residue	Mutations
19	Arg	Gln, Ile, Lys
20	Tyr	His, Leu, Phe
21	Leu	Ile, Phe, Tyr, Val
22	Tyr	Ile, Phe, Val
150	Pro	Ala, Arg
151	Gly	Ala, Val
152	Ile	His, Leu, Phe, Val
170	Gly	Ala, Asn, Asp, Cys, Gln, Glu, Pro, Ser
171	Pro	Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Lys, Ser, Thr, Trp, Tyr
172	Ser	Leu, Thr
173	Gln	Arg, Glu

EXAMPLE 10

In vivo Analysis of Fc-FGF21 and FGF21-Fc Degradation

The stability of FGF21 Fc fusion proteins *in vivo* was determined by injecting mice with a fusion protein, drawing blood from the mice at various time points, and analyzing the serum by liquid chromatography-mass spectrometry (LC-MS). In particular, mice were intraperitoneally injected with 10 mg/kg of Fc-(G5)-FGF21 (SEQ ID NO:107) (expressed in *E. coli* and purified as described in Example 2) or FGF21-(G3)-Fc (SEQ ID NO:105) (expressed in mammalian cells and purified according to standard procedures). Blood was drawn from the mice at 6, 24, and 48 hours after injection (Table 9) and collected into EDTA tubes pretreated with protease inhibitor cocktails (Roche Diagnostics). Plasma was separated by centrifuging the samples at 12,000x*g* for 10 minutes. FGF21 proteins were affinity purified from blood using an anti-human-Fc agarose resin.

Table 9

FGF21 Samples

Sample	Protein Administered	Blood Withdrawn
D6	Fc-(G5)-FGF21	6 hours
D24	Fc-(G5)-FGF21	24 hours
D48	Fc-(G5)-FGF21	48 hours
E6	FGF21-(G3)-Fc	6 hours
E24	FGF21-(G3)-Fc	24 hours
E48	FGF21-(G3)-Fc	48 hours

Prior to analyzing the affinity purified samples by LC-MS, Fc-(G5)-FGF21 and FGF21-(G3)-Fc protein standards were analyzed as a reference. Protein standards were either reduced with tris[2-carboxyethyl] phosphine (TCEP) or not reduced. Reduced and non-reduced standards were analyzed by LC-MS using an ACE cyano 0.3 mm x 30 cm column with the column effluent spraying into an LCQ Classic ion-trap mass spectrometer. Since the deconvoluted spectra of the reduced samples were cleaner, the affinity purified samples were reduced prior to LC-MS analysis.

The observed masses for the reduced Fc-(G5)-FGF21 standard and samples D6, D24, and D48 are shown in Figures 6A-6D. The observed masses for the reduced FGF21-(G3)-Fc standard and samples E6, E24, and E48 are shown in Figures 7A-7D. Some of the standard and sample eluates were subjected to Edman sequencing in

order to confirm the N-terminus of the proteins and the fragments as determined by LC-MS. Results of the LC-MS analysis of the standards and samples are provided in Table 10.

Table 10

Results of LC-MS Analysis and Predicted Fragments

FGF21 Sample	Major Observed Masses	Fragment	Intact N-terminus?
Fc-(G5)-FGF21 standard	45,339 Da	1-414	Yes
D6	45,338 Da 44,317 Da	1-414 1-404	Yes
D24	44,321 Da	1-404	Yes
D48	44,327 Da 42,356 Da	1-404 ?	Yes
FGF21-(G3)-Fc standard	46,408 Da (glycosylated, G0F) 44,964 Da (non-glycosylated)	1-410 1-410	Yes
E6	45,963 Da (glycosylated, G0F) 44,516 Da (non-glycoylated)	5-410 5-410	No
E24	45,963 Da (glycosylated, G0F) 44,526 Da (non-glycosylated) 44,130 Da (glycosylated, G0F)	5-410 5-410 21-410	No
E48	45,984 Da 44,130 Da 44,022 Da	5-410? 21-410 ?	No

As indicated in Table 10, all of the affinity purified samples showed some degree of degradation after only 6 hours of circulation. After 24 hours of circulation, the major product of Fc-(G5)-FGF21 was a fragment consisting of amino acid residues 1-404, which was seen in both the D and E samples. In the E samples, however, the major product of FGF21-(G3)-Fc was a fragment consisting of amino acid residues 5-410. For both of the fusion proteins tested, the FGF21 portion of the fusion protein was more susceptible to degradation than the Fc portion of the protein.

EXAMPLE 11

Preparation and Expression of Proteolysis-Resistant FGF21 Mutants and Fusion Proteins

Constructs encoding the FGF21 mutants listed in Table 11 were prepared by PCR amplification of the wild-type FGF21 expression vector as described below (the construction of the wild-type FGF21 expression vector is described in Example 1). When a linker was included in the construct, the linker used was GGGGGSGGGSGGGGS ("L15," SEQ ID NO: 28), The goal of these experiments was to generate FGF21 mutants that are resistant to proteolysis and exhibit longer half-lives.

Table 11

Proteolysis-Resistant FGF21 Mutants

Mutation(s)	Fc	Linker
R19I		
R19I	-COOH	L15
R19K		
R19K	-COOH	L15
R19Q		
R19Q	-COOH	L15
R19K, Y20H		
R19K, Y20H	-COOH	L15
R19K, L21I		
R19K, L21I	-COOH	L15
R19K, Y20H, L21I		
R19K, Y20H, L21I	-COOH	L15
Y20F		
Y20F	-COOH	L15
Y20H		
Y20H	-COOH	L15
Y20L		
Y20L	-COOH	L15
Y20H, L21I		
Y20H, L21I	-COOH	L15
L21I		
L21I	-COOH	L15
L21F		
L21F	-COOH	L15
L21V		
L21V	-COOH	L15
L21Y		
L21Y	-COOH	L15

Mutation(s)	Fc	Linker
Y22F		
Y22F	-COOH	L15
Y22I		
Y22I	-COOH	L15
Y22V		
Y22V	-COOH	L15
P150A		
P150A	$-NH_2$	L15
P150R	$-NH_2$	L15
P150A, G151A		
P150A, G151A	$-NH_2$	L15
P150A, I152V		
P150A, I152V	$-NH_2$	L15
P150A, G151A, I152V		
P150A, G151A, I152V	$-NH_2$	L15
G151A		
G151A	$-NH_2$	L15
G151V		
G151V	$-NH_2$	L15
G151A, I152V		
G151A, I152V	$-NH_2$	L15
I152F		
I152F	$-NH_2$	L15
I152H		
I152H	$-NH_2$	L15
I152L		
I152L	$-NH_2$	L15
I152V		
G170A		
G170A	$-NH_2$	L15
G170C		
G170C	$-NH_2$	L15
G170D		
G170D	$-NH_2$	L15
G170E		
G170E	$-NH_2$	L15
G170N		
G170N	$-NH_2$	L15
G170P		
G170P	$-NH_2$	L15
G170Q		
G170Q	$-NH_2$	L15
G170S		
G170S	$-NH_2$	L15
G170E, P171A		
G170E, P171A	$-NH_2$	L15
G170E, S172L		

Mutation(s)	Fc	Linker
G170E, S172L	-NH_2	L15
G170E, P171A, S172L		
G170E, P171A, S172L	-NH_2	L15
P171A		
P171A	-NH_2	L15
P171C	-NH_2	L15
P171D	-NH_2	L15
P171E	-NH_2	L15
P171G	-NH_2	L15
P171H	-NH_2	L15
P171K	-NH_2	L15
P171N	-NH_2	L15
P171Q	-NH_2	L15
P171S	-NH_2	L15
P171T	-NH_2	L15
P171W	-NH_2	L15
P171Y	-NH_2	L15
P171A, S172L		
P171A, S172L	-NH_2	L15
S172L	-NH_2	L15
S172T		
S172T	-NH_2	L15
Q173E		
Q173E	-NH_2	L15
Q173R		
Q173R	-NH_2	L15

FGF21 mutant constructs were prepared using primers having sequences that are homologous to regions upstream and downstream of a codon (or codons) to be mutated. The primers used in such amplification reactions also provided approximately 15 nucleotides of overlapping sequence to allow for recircularization of the amplified product, namely the entire vector now having the desired mutant.

An exemplary FGF21 mutant construct, encoding an FGF21 mutant having a glutamic acid residue at position 170 instead of the native glycine residue (*i.e.*, the G170E mutant), was prepared using the primers shown in Table 12.

Table 12

PCR Primers for Preparing Exemplary FGF21 Mutant

Primer	Sequence	SEQ ID NO:
Sense	5'-ATGGTGGAACCTTCCCAGGGCCGAAGC-3'	23
Antisense	5'-GGAAGGTTCCACCATGCTCAGAGGGTCCGA-3'	24

The primers shown in Table 12 allow for the substitution of the glycine residue with a glutamic acid residue as shown below, wherein the upper sequence is the sense primer (SEQ ID NO:23), the second and third sequences (SEQ ID NOs: 25 and 27) are portions of an FGF21 expression construct, and the fourth sequence is the antisense primer (SEQ ID NO:26):

5'-ATGGTGGAACCTTCCCAGGGCCGAAGC
CTCCTCGGACCCTCTGAGCATGGTGGGACCTTCCCAGGGCCGAAGCCCCA
GAGGAGCCTGGGAGACTCGTACCACCCTGGAAGGGTCCCGGCTTCGGGGT
AGCCTGGGAGACTCGTACCACCTTGGAAGG-5'

FGF21 mutant constructs were prepared using essentially the PCR conditions described in Example 1. Amplification products were digested with the restriction endonuclease DpnI, and then transformed into competent cells. The resulting clones were sequenced to confirm the absence of polymerase-generated errors. Fc-(L15)-FGF21 and FGF21-(L15)-Fc fusion proteins were generated as described herein, *e.g.*, in Example 6.

FGF21 mutants were expressed by transforming competent BL21 (DE3) or BL21 Star (Invitrogen; Carlsbad, CA) cells with the construct encoding a particular mutant. Transformants were grown overnight with limited aeration in TB media supplemented with 40 μg/mL kanamycin, were aerated the next morning, and after a short recovery period, were induced in 0.4 mM IPTG. FGF21 mutant polypeptides were harvested by centrifugation 18-20 hours after induction.

FGF21 mutants were also analyzed for predicted immunogenicity. Immune responses against proteins are enhanced by antigen processing and presentation in the major histocompatability complex (MHC) class II binding site. This interaction is required for T cell help in maturation of antibodies that recognize the protein. Since the binding sites of MHC class II molecules have been characterized, it is possible to predict whether proteins have specific sequences that can bind to a series of common human alleles. Computer algorithms have been created based on literature references and MHC class II crystal structures to determine whether linear amino acid peptide sequences have the potential to break immune tolerance. The TEPITOPE computer program was used to determine if point mutations in particular FGF21 mutants would

increase antigen specific T cells in a majority of humans. Based on an analysis of the linear protein sequence of each FGF21 mutant, none of the mutants was predicted to enhance immunogenicity.

EXAMPLE 12

Impact of Linker Sequence on FGF21 Degradation

To determine whether the presence of a longer amino acid linker between the Fc sequence and the FGF21 sequence affects FGF21 degradation, mice were injected with FGF21 fusion proteins in which the Fc region was separated from the FGF21 sequence by a 15 amino acid linker having the sequence GGGGGSGGGSGGGGS (designated "L15," SEQ ID NO:28), blood was withdrawn from the mice at various time points, and the serum was analyzed by LC-MS. In particular, mice were injected with Fc-(L15)-FGF21 or FGF21-(L15)-Fc (obtained from *E. coli*) at 23 mg/kg, blood was drawn at 6, 24, and 48 hours, and drawn blood was affinity purified using an anti-human-Fc agarose resin.

Prior to analyzing the purified samples by LC-MS, Fc-(L15)-FGF21 and FGF21-(L15)-Fc protein standards were analyzed as a reference. Protein standards were either reduced with TCEP or not reduced. Both reduced and non-reduced standards were analyzed by LC-MS using an ACE cyano 0.3 mm x 30 cm column with the column effluent spraying into an LCQ Classic ion-trap mass spectrometer. Since the deconvoluted spectra of the reduced samples were cleaner, the affinity purified samples were reduced prior to LC-MS analysis.

The observed masses for the reduced Fc-(L15)-FGF21 standard and corresponding affinity purified samples withdrawn at various time points are shown in Figures 8A-8D. The observed masses for the reduced FGF21-(L15)-Fc standard and corresponding affinity purified samples withdrawn at various time points are shown in Figures 9A-9D. Some of the standard and sample eluates were subjected to Edman sequencing in order to confirm the N-terminus of the proteins and assist in predicting the identity of the fragments observed by LC-MS. Results of the LC-MS analysis of the standards and samples and an indication of predicted fragments are provided in Table 13.

Table 13

Results of LC-MS Analysis and Predicted Fragments

FGF21 Sample	Major Observed Masses	Percent of Total	Fragment	Intact N-terminus?
Fc-(L15)-FGF21 Standard	46,002 Da	100%	1-424	Yes
Fc-(L15)-FGF21 6 hours	46,000 Da 44,978 Da	65% 35%	1-424 1-414	Yes
Fc-(L15)-FGF21 24 hours	44,978 Da 43,022 Da	85% 15%	1-414 1-394	Yes
Fc-(L15)-FGF21 48 hours	44,976 Da 43,019 Da	60% 40%	1-414 1-394	Yes
FGF21-(L15)-Fc Standard	45,999 Da	100%	1-424	Yes
FGF21-(L15)-Fc 6 hours	45,870 Da	100%	1-423	Yes
FGF21-(L15)-Fc 24 hours	45,869 Da 45,301 Da 43,460 Da	40% 35% 25%	1-423 6-423 22-423	Some
FGF21-(L15)-Fc 48 hours	45,870 Da 45,297 Da 43,461 Da	15% 20% 65%	1-423 6-423 22-423	Some

As indicated in Table 13, all of the affinity purified samples showed some degree of degradation after only 6 hours of circulation. After 24 hours of circulation, the major products of Fc-(L15)-FGF21 were fragments consisting of amino acid residues 1-414 (85% of sample) and 1-394 (15% of sample), and the major products of FGF21(15)Fc were fragments consisting of amino acid residues 1-423 (40% of sample), 6-423 (35% of sample), and 22-423 (25% of sample). Identified cleavage points for the Fc-(L15)-FGF21 and FGF21-(L15)-Fc proteins are shown in Figures 10A and 10B, respectively.

EXAMPLE 13

In vivo Activity of Proteolysis-resistant Fc-(L15)-FGF21 Mutants at 1-7 Days after Injection

As described herein, proteolytic cleavage of FGF21 Fc fusion proteins depends upon the orientation of the Fc sequence, with the Fc end of the fusion protein being more stable than the FGF21 end of the fusion protein (*i.e.*, the N-terminal portion of Fc-(L15)-FGF21 fusion proteins and the C-terminal portion of FGF21-(L15)-Fc fusion proteins were found to be more stable). For example, cleavage was

identified at positions 5 and 21 of FGF21-(L15)Fc and positions 151 and 171 of Fc-(L15)-FGF21.

As a result of these observations, an investigation was performed to identify proteolysis-resistant FGF21 mutants. LC-MS analysis of Fc-(L15)-FGF21 demonstrates that *in vivo* proteolytic degradation first occurs between amino acid residues 171-172, followed by degradation between amino acid residues 151-152. By blocking proteolytic degradation at position 171, the cleavage at position 151 can be prevented, effectively extending the half-life of the molecule. However, proteolysis-resistant mutants in which cleavage is prevented at position 151 can still possess residues at position 171 that are susceptible to protease attack, thereby resulting in a molecule missing the last 10 amino acids, which are known to be involved in the binding of the co-receptor β-Klotho, which is a determinant of ligand receptor affinity and *in vitro* and *in vivo* potency. Therefore, the mutagenesis of amino acid residues surrounding position 171 in mature FGF21 appear to be more critical for improving the *in vivo* stability, potency, and efficacy of the molecule.

The *in vivo* activity of particular proteolysis-resistant Fc-(L15)-FGF21 mutants was assayed by intraperitoneally injecting ob/ob mice with an FGF21 mutant, drawing blood samples from injected mice at 0, 0.25, 1, 3, 5, and 7 days after injection, and then measuring blood glucose levels in the samples. The results of one experiment are provided in Figure 11, which shows the blood glucose levels measured in mice injected with a PBS control, an Fc-(L15)-FGF21 (SEQ ID NO:49) control, or the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 G170E (SEQ ID NO:51), Fc-(L15)-FGF21 P171A (SEQ ID NO:53), Fc-(L15)-FGF21 S172L (SEQ ID NO:55), Fc-(L15)-FGF21 (G170E, P171A, S172L) (SEQ ID NO:59), or Fc-(L15)-FGF21 G151A (SEQ ID NO:61). Figure 12 shows the percent change in blood glucose levels as determined in this experiment. This experiment demonstrates that the Fc-(L15)-FGF21 G170E, Fc-(L15)-FGF21 P171A, Fc-(L15)-FGF21 S172L, and Fc-(L15)-FGF21 (G170E, P171A, S172L) mutants exhibit sustained blood glucose lowering activity for up to 5 days, which is superior to the activity of wild-type Fc-(L15)-FGF21. The Fc-(L15)-FGF21 G151A mutant only partially improved the duration of blood glucose lowering activity as compared with wild-type Fc-(L15)-FGF21 fusion protein. Surprisingly, although the Fc-(L15)-FGF21 S172L mutant is not a proteolysis-resistant mutant, and therefore has similar degradation profile as the wild-

type Fc-(L15)-FGF21 polypeptide, this mutant was found to exhibit improved *in vivo* efficacy as compared with the wild-type Fc-(L15)-FGF21 polypeptide.

The results of another experiment are provided in Figure 13, which shows the blood glucose levels measured in mice injected with a PBS control, an Fc-(L15)-FGF21 control, or the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 (P150A, G151A, I152V) (SEQ ID NO:65), Fc-(L15)-FGF21 G170E (SEQ ID NO:51), Fc-(L15)-FGF21 (G170E, P171A) (SEQ ID NO:63), or Fc-(L15)-FGF21 (G170E, S172L) (SEQ ID NO:67). Figure 14 shows the percent change in blood glucose levels as determined in this experiment. As in the experiment described above, the wild-type Fc-FGF21 fusion protein and the Fc-(L15)-FGF21 (P150A, G151A, I152V) mutant do not exhibit sustained blood glucose lowering activity, possibly because the degradation at 171 site could still occur, and blood glucose levels in animals injected with these proteins returned to baseline at 24 hours after injection. However, the Fc-(L15)-FGF21 G170E, Fc-(L15)-FGF21 (G170E, P171A), or Fc-(L15)-FGF21 (G170E, S172L) exhibit maximal blood glucose lowering activity up to 5 days after injection, which is superior to the wild-type Fc-(L15)-FGF21 fusion protein and the Fc-(L15)-FGF21 (P150A, G151A, I152V) mutant.

The results of another experiment are provided in Figure 15, which shows the blood glucose levels measured in mice injected with a PBS control or the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 G170E (SEQ ID NO:51), Fc-(L15)-FGF21 G170A (SEQ ID NO:69), Fc-(L15)-FGF21 G170C (SEQ ID NO:71), Fc-(L15)-FGF21 G170D (SEQ ID NO:73), Fc-(L15)-FGF21 G170N (SEQ ID NO:75), or Fc-(L15)-FGF21 G170S (SEQ ID NO:77). Figure 16 shows the percent change in blood glucose levels as determined in this experiment. All of the FGF21 mutants tested in this experiment exhibited sustained blood glucose lowering activity for up to 5 days after injection.

The results of another experiment are provided in Figure 17, which shows the blood glucose levels measured in mice injected with PBS or the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 G170E (SEQ ID NO:51), Fc-(L15)-FGF21 P171E (SEQ ID NO:79), Fc-(L15)-FGF21 P171H (SEQ ID NO:81), Fc-(L15)-FGF21 P171Q (SEQ ID NO:83), Fc-(L15)-FGF21 P171T (SEQ ID NO:85), or Fc-(L15)-FGF21 P171Y (SEQ ID NO:87). Figure 18 shows the percent change in blood glucose levels as determined in this experiment. All of the FGF21 mutants tested in this experiment exhibited improved blood glucose lowering activity when compared with wild-type

Fc-FGF21.

EXAMPLE 14

In vivo Degradation of Proteolysis-resistant Fc-(L15)-FGF21 Mutants at 6 to 120 Hours after Injection

The *in vivo* stability of selected FGF21 mutants was analyzed by injecting mice with an FGF21 mutant, drawing blood from the mice at various time points, and analyzing the serum by LC-MS. In particular, mice were injected with either the Fc-(L15)-FGF21 G170E, Fc-(L15)-FGF21 P171A, or Fc-(L15)-FGF21 S172L mutants (obtained from *E. coli* as described in Example 2), each of which were diluted in approximately 180 µL of 10 mM HCl prior to injection, and blood was drawn at 6, 24, 48, 72, and 120 hours. FGF21 proteins were affinity purified from the drawn blood using an anti-human-Fc agarose resin column. Samples were eluted from the column using 10 mM HCl. All of the FGF21 constructs comprise an Fc region and 15 amino acid linker at the amino-terminal end of the FGF21 protein. Mice were also injected with a wild-type FGF21 control.

Prior to analyzing the affinity purified samples by LC-MS, unprocessed wild-type FGF21 and unprocessed FGF21 mutants were analyzed as a reference. All standards and time point samples were reduced with TCEP, and then analyzed by LC-MS using an ACE cyano 0.3 mm x 30 cm column with the column effluent spraying into an LCQ Classic ion-trap mass spectrometer. Affinity purified samples were diluted with ammonium acetate, reduced with TCEP, and then analyzed by LC-MS as described above.

The observed masses for wild-type Fc-(L15)-FGF21 at 0, 6, 24, and 48 hours after injection are shown in Figures 19A-19D, respectively. The observed masses for Fc-(L15)-FGF21 G170E at 0, 6, 24, and 48 hours after injection are shown in Figures 20A-20D, respectively. The observed masses for Fc-(L15)-FGF21 P171A at 0, 6, 24, and 48 hours after injection are shown in Figures 21A-21D, respectively. The observed masses for Fc-(L15)-FGF21 S172L at 0, 6, 24, and 48 hours after injection are shown in Figures 22A-22D, respectively.

All of the samples drawn at 72 and 120 hours were found to contain a high molecular weight (>200 kDa by non-reducing SDS-PAGE) component of fibrinogen that is much more abundant than the remaining Fc-(L15)-FGF21 fusion protein.

Results of the LC-MS analysis of the other standards and samples are provided in Table 14.

Table 14

Results of LC-MS Analysis and Predicted Fragments

FGF21 Sample	Major Observed Masses	Percent of Total	Fragment	Edman
Fc-(L15)-FGF21 WT Standard	45,994 Da	100%	1-424	–
Fc-(L15)-FGF21 WT 6 hours	46,001 Da 44,987 Da	80% 20%	1-424 1-414	No
Fc-(L15)-FGF21 WT 24 hours	44,979 Da	~100%	1-414	No
Fc-(L15)-FGF21 WT 48 hours	44,980 Da	~100%	1-414	–
Fc-(L15)-FGF21 G170E Standard	46,068 Da	100%	1-424	–
Fc-(L15)-FGF21 G170E 6 hours	46,078 Da	100%	1-424	No
Fc-(L15)-FGF21 G170E 24 hours	46,074 Da 45,761 Da	80% 20%	1-424 1-421	No
Fc-(L15)-FGF21 G170E 48 hours	46,072 Da 45,760 Da	~60% ~40%	1-424 1-421	No
Fc-(L15)-FGF21 P171A Standard	45,970 Da	100%	1-424	–
Fc-(L15)-FGF21 P171A 6 hours	45,980 Da	100%	1-424	No
Fc-(L15)-FGF21 P171A 24 hours	45,973 Da 45,657 Da	~70% ~30%	1-424 1-421	No
Fc-(L15)-FGF21 P171A 48 hours	45,992 Da 45,673 Da	~50% ~50%	1-424 1-421	No
Fc-(L15)-FGF21 S172L Standard	46,022 Da	100%	1-424	–
Fc-(L15)-FGF21 S172L 6 hours	46,027 Da	100%	1-424	No
Fc-(L15)-FGF21 S172L 24 hours	44,984 Da	100%	1-414	No
Fc-(L15)-FGF21 S172L 48 hours	44,985 Da	100%	1-414	No

As indicated in Table 14, the degradation of wild-type Fc-(L15)-FGF21 and the S172L mutant look similar, in that after 24 hours of circulation, the major product of the fusion protein was a fragment consisting of amino acid residues 1-414. The degradation products of the Fc-(L15)-FGF21 G170E and Fc-(L15)-FGF21 P171A

mutants also look similar in that the samples drawn after 24 hours of circulation contain 70-80% intact protein (amino acids 1-424) and 20-30% of a fragment consisting of amino acid residues 1-421. Even after 48 hours, the Fc-(L15)-FGF21 G170E and Fc-(L15)-FGF21 P171A mutants still retain intact protein while showing an increase in the amount of the fragment consisting of amino acid residues 1-421. As observed in prior analyses of Fc-FGF21 constructs, degradation of the FGF21 portion of the fusion protein was detected and the Fc portion was found to remain stable. The cleavage sites identified for wild-type, Fc-(L15)-FGF21 G170E, Fc-(L15)-FGF21 P171A, and Fc-(L15)-FGF21 S172L are shown in Figures 23A-23D, respectively.

EXAMPLE 15

Identification of Aggregation-reducing FGF21 Mutants

One property of wild-type FGF21 is its propensity to aggregate. In view of this property, it was desired to generate aggregation-reducing FGF21 mutants. Aggregation-reducing FGF21 mutants were identified on the basis of two hypotheses. The first hypothesis is that, with respect to FGF21, aggregation (or dimerization) is triggered by hydrophobic interactions and van der Waals interactions between FGF21 molecules caused by hydrophobic residues that are exposed to hydrophilic water-based solvent environment. The second hypothesis is that these exposed hydrophobic residues can be substituted to create aggregation-reducing point-mutations in the FGF21 amino acid sequence without compromising FGF21 activity.

A systematic rational protein engineering approach was used to identify exposed hydrophobic residues in FGF21. As there were no known X-ray or NMR structures of FGF21 that could be used to identify exposed hydrophobic residues, a high resolution (1.3 Å) X-ray crystal structure of FGF19 (1PWA) obtained from the Protein Databank (PDB) was used to create a 3D homology model of FGF21 using MOE (Molecular Operating Environment; Chemical Computing Group; Montreal, Quebec, Canada) modeling software. FGF19 was chosen as a template, since among the proteins deposited in the PDB, FGF19 is the most closely related protein to FGF21 in terms of the amino acid sequence homology.

Solvent accessibility was calculated by the following method using MOE. A first measure of surface area (SA1) is defined as the area of the residue's accessible surface in $Å^2$. While a particular amino acid residue appears in a protein's primary

sequence multiple times, each occurrence of the residue can have a different surface area due to differences in, *inter alia*, the residue's proximity to the protein surface, the orientation of the residue's side-chain, and the spatial position of adjacent amino acid residues. Therefore, a second measure of surface area (SA2) is made wherein the residue of interest is extracted from the protein structure along with that residue's neighboring, or adjacent, residues. These spatially adjacent residues are mutated *in silico* to glycines to remove their side-chains, and then the SA2 for the residue of interest is calculated, giving a measure of the total possible surface area for that residue in its particular conformation. A ratio of SA1 to SA2 (SA1/SA2) can then give a measure of the percentage of the possible surface area for that residue that is actually exposed.

Several hydrophobic residues that are highly exposed to the solvent were selected for further analysis, and *in silico* point mutations were made to these residues to replace the selected residue with the other naturally occurring amino acid residues. The changes in protein thermal stability resulting from different substitutions were calculated using the FGF21 model and the interactive web-based program CUPSAT (Cologne University Protein Stability Analysis Tools) according to instructions provided at the CUPSAT website. *See* Parthiban *et al.*, 2006, *Nucleic Acids Res.* 34: W239-42; Parthiban *et al.*, 2007, *BMC Struct. Biol.* 7:54. Significantly destabilizing or hydrophobic mutations were excluded in the design of aggregation-reducing point-mutation FGF21 mutants. Stabilizing (or, in rare cases, slightly destabilizing) substitutions that introduce improved hydrophilic and/or ionic characteristics were considered as candidates for aggregation-reducing FGF21 mutants.

A summary of the data generated through this rational protein engineering approach is provided in Table 15, which also lists exemplary FGF21 mutants expected to have reduced protein aggregation and improved stability.

Table 15

Calculated Effect of FGF21 Mutants on Stability

Residue #	WT Residue	Mutation	Stabilization (Kcal/mol)
26	A	K	1.25
		E	1.54
		R	2.016
45	A	T	0.66

Residue #	WT Residue	Mutation	Stabilization (Kcal/mol)
		Q	0.71
		K	1.8
		E	2.34
		R	1.59
52	L	T	-0.33
58	L	G	0.16
		S	-0.15
		C	1.0
		E	0.08
60	P	A	1.3
		K	1.51
		E	0.66
		R	1.31
78	P	A	0.14
		C	2.48
		R	0.08
		H	0.13
86	L	T	0.18
		C	4.1
88	F	A	2.52
		S	3.08
		K	2.88
		E	1.48
98	L	T	0.49
		Q	0.17
		K	-0.19
		C	3.08
		E	0.84
		R	3.4
99	L	C	7.34
		E	2.0
		D	1.01
		R	1.61
111	A	T	0.47
		K	-0.12
129	A	Q	3.93
		K	1.02
		N	3.76
		E	3.01
		D	3.76
		R	1.68
		H	2.9
134	A	K	5.37
		Y	4.32
		E	5.13
		R	6.18
		H	2.86

EXAMPLE 16

Preparation and Expression of Aggregation-reducing FGF21 Mutants and Fusion Proteins

Constructs encoding the FGF21 mutants listed in Table 16 were prepared by PCR amplification of the wild-type FGF21 expression vector as described in Example 11 (the construction of the wild-type FGF21 expression vector is described in Example 1). Fusion proteins were generated as described herein, *e.g.*, in Example 6. When a linker was employed it was GGGGGSGGGSGGGGS ("L15," SEQ ID NO:28)

Table 16

Aggregation-reducing FGF21 Mutants

Mutation(s)	Fc	Linker
A26E		
A26K		
A26R		
A45E		
A45K		
A45K	$-NH_2$	L15
A45R	$-NH_2$	L15
A45Q	$-NH_2$	L15
A45T	$-NH_2$	L15
A45K, L98R	$-NH_2$	L15
L52T		
L58C		
L58E		
L58G		
L58S		
P60A		
P60E		
P60K		
P60R		
P78A		
P78C		
P78H		
P78R		
L86C		
L86T		
F88A		
F88E		
F88K		
F88R		
F88S		

Mutation(s)	Fc	Linker
L98C		
L98E	-NH_2	L15
L98K	-NH_2	L15
L98Q	-NH_2	L15
L98R		
L98R	-NH_2	L15
L99C		
L99D		
L99E		
L99R		
A111K	-NH_2	L15
A111T		
A129D		
A129E	-NH_2	L15
A129H	-NH_2	L15
A129K		
A129N	-NH_2	L15
A129R	-NH_2	L15
A129Q		
A134E		
A134H	-NH_2	L15
A134K		
A134Y		

The aggregation of various FGF21 proteins, including wild-type FGF21, truncated FGF21 polypeptides, FGF21 mutants, and FGF21 fusion proteins was assayed by Size Exclusion Chromatography (SEC). Samples to be analyzed were incubated at 4°C, room temperature, or 37°C for various time points, and then subjected to SEC analysis. Experiments were performed on a Beckman HPLC system equipped with a SEC column. For wild-type FGF21, a TOSOHAAS TSK-Gel G2000 SEC column was used with 2x PBS containing 2% isopropyl alcohol as the mobile phase. For FGF21 Fc fusion proteins and FGF21 mutant polypeptides, a TOSOHAAS TSK-Gel G3000 SEC column was used with 2x PBS as the mobile phase.

EXAMPLE 17

In vitro Activity of Aggregation-reducing FGF21 Mutants

Experiments were performed to identify aggregation-reducing mutants that retain wild-type FGF21 activity in an ELK-luciferase *in vitro* assay. ELK-luciferase assays were performed as described in Example 4. Figures 24A-24C show the results

of an ELK-luciferase activity assay performed on the FGF21 mutants FGF21 L99R (SEQ ID NO:109), FGF21 L99D (SEQ ID NO:111), and FGF21 A111T (SEQ ID NO:113) (Figure 24A); the FGF21 mutants FGF21 A129D (SEQ ID NO:115), FGF21 A129Q (SEQ ID NO:117), and FGF21 A134K (SEQ ID NO:119) (Figure 24B); and the FGF21 mutants FGF21 A134Y (SEQ ID NO:121), FGF21 A134E (SEQ ID NO:123), and FGF21 A129K (SEQ ID NO:125) (Figure 24C). The results of these experiments demonstrate that some of the aggregation-reducing mutations did not adversely impact FGF21 activity as assayed in ELK-luciferase assays.

EXAMPLE 18

Preparation and Expression of Fc-(L15)-FGF21 Combination Mutants Showing Longer Half-life and Lower Levels of Aggregation

A number of FGF21 combination mutants, containing mutations shown to reduce aggregation as well as to increase half-life by disrupting proteolytic degradation, were prepared and conjugated to IgG1 Fc molecules (SEQ ID NO:11). These FGF21 mutants were prepared essentially as described in Example 11.

EXAMPLE 19

In vitro Studies of Fc-(L15)-FGF21 Mutants Showing Longer Half-life and Lower Levels of Aggregation

Experiments were performed to identify FGF21 combination mutants that retain wild-type FGF21 activity in an ELK-luciferase *in vitro* assay. ELK-luciferase assays were performed as described in Example 4.

Figures 25A-25D show the results of an ELK-luciferase activity assay performed on the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 P171G, Fc-(L15)-FGF21 P171S, and Fc-(L15)-FGF21 P171T (Figure 25A); the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 P171Y, Fc-(L15)-FGF21 P171W, and Fc-(L15)-FGF21 P171C (Figure 25B); Fc-(L15)-FGF21, Fc-(L15)-FGF21 (A45K, G170E), and FGF21 A45K (Figure 25C); and Fc-(L15)-FGF21, Fc-(L15)-FGF21 P171E, and Fc-(L15)-FGF21 (A45K, G170E) (Figure 25D). The results of these experiments demonstrate that mutations aimed at improving stability, or both stability and solubility, did not compromise the *in vitro* activity as compared with wild-type Fc-(L15)-FGF21. Interestingly, the FGF21 A45K mutant showed improved potency relative to wild-type Fc-(L15)-FGF21.

Figure 26A shows the change in percent aggregation for an FGF21 control (WT) and FGF21 A45K following incubation of 65 mg/mL protein at 4°C for 1, 2, and 4 days. The data indicated that the A45K mutation leads to a decrease in aggregation of the protein, compared to the wild-type protein.

Figure 26B shows the change in percent aggregation for an FGF21 control (WT) and FGF21 P78C, FGF21 P78R, FGF21 L86T, FGF21 L86C, FGF21 L98C, FGF21 L98R, FGF21 A111T, FGF21 A129D, FGF21 A129Q, FGF21 A129K, FGF21 A134K, FGF21 A134Y, and FGF21 A134E following incubation of 65 mg/mL protein at 4°C for 1, 6, and 10 days. The data indicated that the FGF21 L86C, FGF21 L98C, FGF21 L98R, FGF21 A111T, FGF21 A129Q, and FGF21 A129K lead to a decrease in aggregation of the protein, compared to the wild-type protein.

Figure 27 shows the results of an ELK-luciferase activity assay performed on a human FGF21 control and the FGF21 mutants FGF21 A45K, FGF21 L52T, and FGF21 L58E. This experiment demonstrates that the FGF21 A45K mutant retains the full efficacy of wild-type FGF21 and exhibits a potency that is even greater than wild-type FGF21. However, the FGF21 L52T, and FGF21 L58E mutants show reduced potency and efficacy as compared with wild-type FGF21.

Figures 28A-28B show the change in aggregation levels for the Fc-(L15)-FGF21 mutants Fc-(L15)-FGF21 (6-181, G170E), Fc-(L15)-FGF21 (A45K, G170E), Fc-(L15)-FGF21 P171E, Fc-(L15)-FGF21 P171A, Fc-(L15)-FGF21 G170E, and an FGF21 control following incubation at 4°C for 1, 4, and 8 days. This experiment demonstrates that over the 8 day period, the Fc-(L15)-FGF21 (A45K, G170E) mutant showed less aggregation than did the Fc-(L15)-FGF21 G170E or Fc-(L15)-FGF21 P171E mutants, but all three mutants showed less aggregation than did the Fc-(L15)-FGF21 control. Table 17 shows the percent aggregation obtained for an Fc-(L15)-FGF21 control and the Fc-(L15)-FGF21 (A45K, G170E) mutant following incubation at 4°C or room temperature for 0, 2, 3, 4, or 7 days.

Table 17

Percent Aggregation for Fc-FGF21 and Fc-FGF21 Mutant

Sample		Day 0	Day 2	Day 3	Day 4	Day 7
Fc-(L15)-FGF21 WT	4°C	1.12	1.71	1.89	2.14	2.32
32 mg/mL	RT	1.12	6.09	7.94	9.57	12.59

Sample		Day 0	Day 2	Day 3	Day 4	Day 7
Fc-(L15)-FGF21 (A45K, G170E)	4°C	0.45	0.77	0.88	1.03	1.24
33 mg/mL	RT	0.45	3.86	5.22	6.62	8.60

EXAMPLE 20

Preparation and Expression of Fc-FGF21 Fusion Combination Mutants

As described above, the stability and solubility of FGF21 can be modulated through the introduction of specific truncations and amino acid substitutions. In addition, FGF21 stability can be further enhanced by fusing such modified FGF21 proteins with the Fc portion of the human immunoglobulin IgG1 gene. Moreover, by introducing combinations of the above modifications, FGF21 molecules having both enhanced stability and solubility can be generated. Nucleic acid sequences encoding the FGF21 combination mutants listed in Table 18 were prepared using the techniques described above. The linker employed was the L15 linker, GGGGGSGGGSGGGGS (SEQ ID NO:28).

Table 18

FGF21 Combination Mutants

Amino Acid Residues	Proteolysis Mutation	Aggregation Mutation	Fc	Linker
1-181	G170E	A45K	-NH_2	L15
1-181	G170E	L98R	-NH_2	L15
1-181	G170E	A45K, L98R	-NH_2	L15
1-181	P171G	A45K	-NH_2	L15
1-181	P171S	A45K	-NH_2	L15
1-181	P171G	L98R	-NH_2	L15
1-181	P171S	L98R	-NH_2	L15
1-181	P171G	A45K, L98R	-NH_2	L15
1-178	G170E		-NH_2	L15
6-181	G170E		-NH_2	L15
6-181	G170E	A45K	-NH_2	L15
6-181	G170E	L98R	-NH_2	L15
6-181	P171G		-NH_2	L15
6-181	P171G	L98R	-NH_2	L15
7-181	G170E		-NH_2	L15

Figure 29 shows the blood glucose levels measured in mice injected with the Fc-(L15)-FGF21 combination mutants Fc-(L15)-FGF21 (A45K, G170E), Fc-(L15)-FGF21 (A45K, P171G), or Fc-(L15)-FGF21 (L98R, P171G).

In another experiment the FGF21 mutant Fc-(L15)-FGF21 (L98R, P171G) was studied side-by-side with wild-type mature FGF21 and Fc-FGF21. In one experiment, a recombinant 293T cell line was cultured in the presence of different concentrations of FGF21, Fc-(L15)-FGF21, or Fc-(L15) FGF21 (L98R, P171G) for 6 hours. Cell lysates were then assayed for luciferase activity. As shown in Figure 30, Fc-(L15)-FGF21 (L98R, P171G) had similar activity to Fc-(L15)-FGF21, indicating that the introduction of the two point mutations didn't alter the molecule's *in vitro* activity.

In yet another experiment, the stability of the Fc-(L15)-FGF21 (L98R, P171G) at 65 mg/mL was evaluated for nine days at two different temperatures, namely room temperature and 4°C, side-by-side with FGF21 and Fc-(L15)-FGF21. After the incubation period cell lysates were then analyzed with SEC-HPLC to determine an aggregation versus time profile at various temperatures. The data shown in Figure 31A and 31B indicate that the rate of aggregation formation was significantly reduced in the Fc-(L15)-FGF21 (L98R, P171G) at room temperature (solid triangles, dotted line in Figure 31A) and at 4°C (solid triangles, dotted line in Figure 31B).

EXAMPLE 21

Proteolysis-resistant FGF21 Mutants Comprising C-terminal Mutations

The *in vivo* stability of combination mutants was also studied. Specifically, the *in vivo* stability of Fc-(L15)-FGF21 (L98R, P171G) was compared with the stability of Fc-(L15)-FGF21 in murine and *cynomolgus* models. The results were found to be similar in both species. In the *cynomolgus* study, Fc-(L15)-FGF21 (L98R, P171G) and Fc-(L15)-FGF21 were injected IV at 23.5 mg/kg and aliquots of serum and plasma were collected at time points out to 840 hours post dose. Time points out to 168 hours were analyzed. Time point samples were affinity-purified using anti-Fc reagents, then analyzed using MALDI mass spectrometry. The results correlated well between the two analyses.

Analyzing data generated using immunoaffinity-MALDI, clipping at the P171 site was seen to be eliminated in the Fc-(L15)-FGF21 (L98R, P171G) molecule as a result of the mutation of P171 to P171G. However, a minor and slow degradation resulting in a loss of up to 3 C-terminal residues was observed for Fc-(L15)-FGF21 (L98R, P171G) (Figure 32). The minor cleavages at the three C-terminal residues

were also observed with other FGF21 mutants after the more susceptible cleavage site between amino acid residues 171 and 172 was blocked as shown in Figures 20 and 21. The 3 C-terminal residue cleavage may represent the cessation of cleavage from the C-terminal end of the molecule by a carboxypeptidase in a sequential, residue-by-residue fashion or a specific protease attack at amino acid residues 178 and 179 with non-specific clipping at amino acid residues 179-180 and 180-181. The loss of 2-3 amino acids at the C-terminus could cause reduced β-Klotho binding and ultimately decreased potency and in vivo activity of the molecule *See, e.g.*, Yie et al., 2009, *FEBS Lett.* 583:19-24. To address the apparent carboxypeptidase degradation of the C-terminus, the impact of adding an amino acid residue "cap" to various FGF21 mutant polypeptides were studied. A variety of constructs, including those presented in Table 19, were made and assayed using the techniques described herein. Table 19 summarizes the results of the *in vitro* ELK luciferase assay.

Suitable amino acid caps can be between 1 and 15 amino acids in length, for example 1, 2, 3, 4, 5, 10 or 15 amino acids in length. Any number and type of amino acid(s) can be employed as a cap, for example, a single proline residue, and single glycine residue, two glycine residues, five glycine residues, as well as other combinations. Additional examples of caps are provided in the instant Example and in Table 19.

Additionally, to address the apparent protease attack at amino acid residues 178 and 179, mutation of amino acid residues at positions 179, 180 and 181 was studied. Again, a variety of constructs, including those presented in Table 19, were made and assayed using the techniques described herein. The impact of combinations of cap and mutations at these sites was also explored. Table 19 summarizes exemplary constructs that were made and studied in the *in vitro* ELK-luciferase assay, which was performed as described herein. Consistent with the terminology used herein, hFc means a human Fc sequence (*i.e.*, SEQ ID NO:11), L15 refers to the L15 linker (*i.e.*, GGGGGSGGGSGGGGS, SEQ ID NO:28).

Table 19

Efficacy and EC50 Values for FGF21 Polypeptides Comprising C-terminal Modifications

Constructs	EC50(nM)	Efficacy
huFGF21	0.4	100.0%

Constructs	EC50(nM)	Efficacy
hFc-(L15)-hFGF21(L98R, P171G)	2.5	76.1%
hFc-(L15)-hFGF21(L98R, P171G, Y179F)	2.6	78.3%
hFc-(L15)-hFGF21(L98R, P171G, 1-180)		
hFc-(L15)-hFGF21(L98R, P171G, 1-179)	7.8	77.4%
hFc-(L15)-hFGF21(L98R, P171G, A180E)	1.9	79.6%
hFc-(L15)-hFGF21(L98R, P171G, S181K)	130	87.9%
GSGSGSGSGS-hFGF21 -(L15)-hFc		
MKEDD-hFGF21-(L15)-hFc	834	83.1%
hFc-(L15)-hFGF21(L98R, P171G, S181P, P182)	272	69.9%
hFc-(L15)-hFGF21(L98R, P171G, A180G)	3.25	76.9%
hFc-(L15)-hFGF21(L98R, P171G, S181G)	3.43	77.3%
hFc-(L15)-hFGF21(L98R, P171G, L182)		
hFGF21(L98R, P171G, G182)		
hFc-(L15)-hFGF21(L98R, P171G, Y179P)	428	44.4%
hFc-(L15)-hFGF21(L98R, P171G, Y179G)	61	82.6%
hFc-(L15)-hFGF21(L98R, P171G, Y179S)	25.3	74.8%
hFc-(L15)-hFGF21(L98R, P171G, Y179A)	43.2	79.6%
hFc-(L15)-hFGF21(L98R, P171G, S181T)	3.07	77.6%
hFc-(L15)-hFGF21(L98R, P171G, S181A)	2.66	73.5%
hFc-(L15)-hFGF21(L98R, P171G, S181L)	3.46	72.6%
hFc-(L15)-hFGF21(L98R, P171G, S181P)	33.8	79.5%
hFc-(L15)-hFGF21(L98R, P171G, A180P)	617	77.1%
hFc-(L15)-hFGF21(L98R, P171G, A180S)	2.18	84.7%
hFGF21(L98R, P171G, GGGGG182-6)		
hFc-(L15)-hFGF21(L98R, P171G, P182)	6.1	85.9%
hFc-(L15)-hFGF21(L98R, P171G, G182)	6.5	71.1%
hFc-(L15)-hFGF21(1-178, L98R, P171G)	167	63.9%
hFc-(L15)-hFGF21(L98R, P171G, GG182-3)	1941	84.2%
hFc-(L15)-hFGF21(L98R, P171G, GGGGG182-6)	4307	99.7%

Figure 33 shows the percent change in blood glucose levels observed in diabetic db/db mice (C57B6 background) injected with a PBS control, wild type native FGF21, Fc-(L15)-FGF21 (L98R, P171G) and two capped molecules to which either a proline or glycine residue was added at the C-terminal end, *i.e.*, Fc-(L15)-FGF21 (L98R, P171G, 182P) and Fc-(L15)-FGF21 (L98R, P171G, 182G). When a residue was added to the C-terminus of a wild-type or mutant FGF21 polypeptide, the residue is referred to by its position in the resultant protein. Thus, "182G" indicates that a glycine residue was added to the C-terminus of the mature 181 residue wild-type or mutant protein. Figure 33 shows that native FGF21 lowered blood glucose

levels for 6 hours while all three Fc-FGF21 mutants studied showed sustained blood glucose-lowering activity for at least 120 hours. Fc-(L15)-FGF21 (L98R, P171G, 182P), a molecule comprising the addition of a proline residue at the C-terminus of the FGF21 component of the fusion molecule, appeared most potent and resulted in lowest blood glucose levels compared with Fc-(L15)-FGF21 (L98R, P171G) and Fc-(L15)-FGF21 (L98R, P171G, 182G).

In a subsequent experiment, the *in vivo* activity of Fc-(L15)-FGF21 (L98R, P171G, 182G) and Fc-(L15)-FGF21 (L98R, P171G, 182P) was studied and compared to the *in vivo* activity of a capped molecule comprising a two glycine addition at the C-terminus, namely Fc-(L15)-FGF21 (L98R, P171G, 182G, 183G). Figure 34 shows the percent change in blood glucose levels observed in ob/ob mice injected with PBS control, Fc-(L15)-FGF21 (L98R, P171G), Fc-(L15)-FGF21 (L98R, P171G, 182G, 183G), Fc-(L15)-FGF21 (L98R, P171G, 182G) and Fc-(L15)-FGF21 (L98R, P171G, 182P).

As shown in Figure 34, all of the molecules studied showed sustained glucose-lowering activity compared with the PBS control. This experiment confirmed the previous results (Figure 33) that Fc-(L15)-FGF21 (L98R, P171G, 182P) with a proline addition at the C-terminus showed slightly enhanced glucose-lowering efficacy compared with the molecule without a proline cap, e.g. Fc-(L15)-FGF21 (L98R, P171G). However, the addition of two glycine residues at the C-terminus, e.g. Fc-(L15)-FGF21 (L98R, P171G, 182G 183G), appeared to reduce the molecule's *in vivo* potency and shortened the duration of *in vivo* glucose-lowering effect.

Figure 35 shows the percent change in blood glucose levels observed in diabetic db/db mice (C57B6 background) injected with PBS control or the FGF21 mutant polypeptides Fc-(L15)-FGF21 (L98R, P171G), Fc-(L15)-FGF21 (L98R, P171G, Y179S), Fc-(L15)-FGF21 (L98R, P171G, Y179A), Fc-(L15)-FGF21 (L98R, P171G, A180S), and Fc-(L15)-FGF21 (L98R, P171G, A180G). All mutants showed similar glucose-lowering activity with similar duration of action.

Figure 36 shows the percent change in blood glucose levels observed in diabetic db/db mice (C57B6 background) injected with vehicle control, Fc-(L15)-FGF21 (L98R, P171G), Fc-(L15)-FGF21 (L98R, P171G, Y179F), and Fc-(L15)-FGF21 (L98R, P171G, A180E). Compared with Fc-(L15)-FGF21 (L98R, P171G), Fc-(L15)-FGF21 (L98R, P171G, Y179F) was less efficacious in lowering blood glucose. However, Fc-(L15)-FGF21 (L98R, P171G, A180E), in which alanine at

amino acid position of 180 was mutated to glutamic acid, was more efficacious than Fc-(L15)-FGF21 (L98R, P171G) and caused additional 20% reduction of blood glucose levels compared with Fc-(L15)-FGF21 (L98R, P171G). These data suggest that A180E mutation may have reduced the C-terminal degradation *in vivo* and thereby improved *in vivo* potency and efficacy of the molecule.

EXAMPLE 22

Rhesus Monkey Study

An Fc-Linker-FGF21 construct was generated using methodology described herein. The construct comprised an IgG1 Fc sequence (SEQ ID NO:11) fused at the C-terminus to the L15 $(Gly)_5$-Ser-$(Gly)_3$-Ser-$(Gly)_4$-Ser linker sequence (SEQ ID NO:28) which was then fused at the C-terminus to the N terminus of a mature FGF21 sequence (SEQ ID NO:4), into which two mutations, L98R and P171G, had been introduced. This construct was then expressed and purified as described herein. A dimeric form of the protein was isolated, which was linked via intermolecular disulfide bonds between the Fc region of each monomer. This molecule is referred to in the instant Example 22 as "Fc-(L15)-FGF21 (L98R, P171G)" and has the amino acid sequence of SEQ ID NO:43 and is encoded by SEQ ID NO:42. In this Example, FGF21 refers to the mature form of FGF21, namely SEQ ID NO:4.

22.1 Study Design

The Fc-(L15)-FGF21 (L98R, P171G) construct was administered chronically and subcutaneously ("SC") into non-diabetic male *Rhesus* monkeys with a BMI > 35. Two other groups of monkeys (n=10 per group) were treated with either mature FGF21 (i.e., SEQ ID NO:4) or a vehicle control.

Animals were acclimated for 42 days prior to administration of any test compound and were then divided into groups of 10 and administered multiple SC injections of test compounds or control article in a blinded fashion, as depicted graphically in Figure 37. In brief, each animal was injected once a day with compound or vehicle. FGF21 was administered daily, whereas Fc-(L15)-FGF21 (L98R, P171G) was administered weekly. Fc-(L15)-FGF21 (L98R, P171G) and FGF21 doses were escalated every 2 weeks, as shown in Figure 37. Body weight and food intake were monitored throughout the study. The CRO was blinded to the treatment.

Two oral glucose tolerance tests (OGTTs) were performed prior to the start of the treatment. OGTT1 was used to sort the animals into three equivalent groups having a similar distribution of animals based on area under the curve (AUC) and body weight. The results of the second OGTT (OGTT2) were used to confirm the sorting of the first OGTT (OGTT1). Monkeys with OGTT profiles that were inconsistent from one test (OGTT1) to the next (OGTT2) were excluded. The results of OGTTs 1 and 2 are shown in Figures 38A and 38B, with AUC measurements shown in Figure 38C. Baseline body weight is shown in Figure 38D and Table 20.

OGTTs 3, 4, and 5 were performed every 2 weeks at the end of each dose treatment of low, mid and high doses. Blood samples were collected from fasted animals weekly and were used to measure glucose, insulin, triglyceride levels, as well as the levels of test compound. Blood samples were also collected weekly during the 3-week washout period.

Baseline OGTT1 and OGTT2 showed an expected glucose profile as seen in normal animals, with a maximum plasma glucose obtained at 30 minutes, and demonstrated stable AUCs for the 3 different groups.

Fasting baselines values for plasma chemistry are shown in Table 20. Plasma chemistry measurements were performed on blood samples collected prior to the start of the treatment.

Table 20

Baseline Values for Body Weight, Fasting Plasma Glucose, Insulin, and Triglyceride Levels of the Three Groups of *Rhesus* Monkeys

	Vehicle	FGF21	Fc-(L15)-FGF21 (L98R, P171G)
N	10	10	10
Body weight (kg)	8.5 ± 0.5	8.7 ± 0.4	8.5 ± 0.4
Plasma glucose (mg/dL)	91.9 ± 4.8	94.8 ± 5.3	82.2 ± 3.7
Insulin (pg/mL)	942.6 ± 121.4	976.1 ±107.7	1023.4 ± 205.1
Triglycerides (mg/dL)	44.4 ± 4.8	58.6 ± 5.2	71.7 ± 9.8

Three different dose levels were selected, the low dose was 0.1 and 0.3 mg/kg, the mid dose was 0.3 and 1 mg/kg and the high dose was 1 and 5 mg/kg for FGF21 and Fc-(L15)-FGF21(L98R, P171G), respectively. Dose levels were chosen based on the observed dose-response in mice, with a dosing regimen based on the anticipated frequency of injection in humans. Equimolar doses of FGF21 were used for the low and mid doses, and the Fc-(L15)-FGF21(L98R, P171G) high dose was raised to 5 mg/kg (i.e., instead of 3 mg/kg, which would have been equimolar to the 1 mg/kg FGF21 dose).

22.2 Effect of Test Compounds on Body Weight

In this experiment, in order to measure effect of the test compounds on body weight measured weekly, the percent body weight change from baseline was calculated weekly in the three different groups of *Rhesus* monkeys. Body weight was also measured during the three week of wash out period. Baseline body weight values for each group are included in Table 20.

Body weight was followed throughout the study, both pre- and post-administration of test compounds. Body weight percent change from baseline of the vehicle animals increased with time, whereas body weight of animals treated with Fc-(L15)-FGF21 (L98R, P171G) and FGF21 decreased in a dose-dependent fashion over the course of the 6 week treatment period, as shown in Figure 39. As observed previously in rodents (Xu et al., *Diabetes* 58(1):250-9 (2009)), treatment with FGF21 statistically significantly decreased body weight. Fc-(L15)-FGF21 (L98R, P171G) had a greater exposure than did FGF21 (Figure 48 and Figure 47, respectively), offering a possible explanation for the observation that Fc-(L15)-FGF21 (L98R, P171G) showed a more pronounced body weight decrease than FGF21.

22.3. Effect of Test Compounds on Insulin Levels

Insulin levels were measured in blood samples that had been collected after an overnight fast or after an afternoon meal.

Fasting plasma insulin levels were measured in *Rhesus* monkeys every week in animals treated with either vehicle, FGF21 or Fc-(L15)-FGF21 (L98R, P171G) and during the 3-week washout period. Fasted blood samples were drawn approximately five days after the last Fc-(L15)-FGF21 (L98R, P171G) injection and approximately 21 hours after the last FGF21 injection.

Fed plasma insulin levels were measured in *Rhesus* monkeys during the fifth and sixth week of treatment with either vehicle or FGF21 during the high dose treatment. Fed blood samples were drawn approximately three days after Fc-(L15)-FGF21 (L98R, P171G) injection and approximately 2 hours after last FGF21 injection. Figure 40 shows the effect of vehicle, FGF21 and Fc-(L15)-FGF21 (L98R, P171G) on fasted insulin levels over the full nine week study, while Figure 41 depicts fed insulin levels determined from samples taken during weeks 5 and 6.

Summarily, at the two highest doses, both FGF21 and Fc-(L15)-FGF21(L98R, P171G) statistically significantly decreased fasted and fed plasma insulin levels. The observation that insulin levels of animals treated with FGF21 and Fc-(L15)-FGF21 (L98R, P171G) were decreased without observing increased glucose levels is indicative of increased insulin sensitivity.

22.4 Effect of Test Compounds on OGTT (Glucose and Insulin)

Three OGTTs (OGTTs 3, 4 and 5) were performed after treatment was initiated. OGTT5 glucose and insulin level profiles were measured in animals treated for 6 weeks with vehicle, FGF21 or Fc-FGF21 (L98R, P171G), corresponding to the last two weeks of the high dose escalation regimen. OGTT5 was conducted approximately 7 days after the last Fc-(L15)-FGF21 (L98R, P171G) injection, and approximately 21 hours after the last FGF21 injection. The OGTT5 glucose and insulin profiles are shown in Figure 42 and Figure 43, respectively. Animals treated with Fc-(L15)-FGF21(L98R, P171G) showed an improved glucose clearance compared to vehicle-treated animals only at the highest dose and at the last time point measured, as shown in Figure 42. At the end of the last dose, Fc-(L15)-FGF21(L98R, P171G) showed the strongest improvement in glucose clearance. FGF21 showed no improvement in glucose clearance. Fc-(L15)-FGF21 (L98R, P171G) had a greater exposure than did FGF21 (Figure 48 and Figure 47, respectively), offering a possible explanation for the observation that Fc-(L15)-FGF21 (L98R, P171G) showed a more pronounced effect in glucose clearance than FGF21. Insulin levels during OGTT5 were statistically significantly lowered at the last time point measured in animals treated with Fc-(L15)-FGF21 (L98R, P171G) compared to animals treated with vehicle.

Glucose AUC percent change from baseline was calculated for the three OGTT (OGTTs 3, 4 and 5) performed at the end of each of the low, mid and high

doses in the three groups different groups of *Rhesus* monkeys as shown in Figure 44. OGTT5 was conducted approximately seven days after the last Fc-(L15)-FGF21 (L98R, P171G) injection and 21 hours after last FGF21 injection and showed that Fc-(L15)-FGF21 (L98R, P171G) statistically significantly reduced AUC5. Baseline OGTT values for each group are shown on Figure 38C.

Fasted plasma glucose levels were measured on days when no OGTTs were performed. There were no meaningful statistical differences observed in fasted plasma glucose levels measured among the three groups of animals.

22.5 Effect of Test Compounds on Triglyceride Levels

Percent change of fasting plasma triglyceride levels was calculated in *Rhesus* monkeys every week in animals treated with either vehicle, FGF21 or Fc-(L15)-FGF21 (L98R, P171G) and during the 3-week washout period. Fasted blood samples were drawn approximately five days after last Fc-(L15)-FGF21 (L98R, P171G) injection and approximately 21 hours after last FGF21 injection. Triglyceride levels were measured every week after the treatment was initiated and percent changes from baseline are shown in Figure 45, fasting baseline values are shown in Table 20.

As depicted in Figure 45, animals treated with either Fc-(L15)-FGF21 (L98R, P171G) or FGF21 showed a dose-dependent decrease in triglyceride levels, with Fc-(L15)-FGF21 (L98R, P171G) having the greatest lowering effect compared to FGF21.

Figure 46 shows the plasma triglyceride levels in samples acquire from *Rhesus* monkeys in a fed state, during the fifth and sixth week of treatment with vehicle or Fc-(L15)-FGF21 (L98R, P171G) or FGF21. Fed blood samples were drawn approximately 3 days after Fc-(L15)-FGF21 (L98R, P171G) injection and approximately 2 hours after last FGF21 injection. Fed plasma triglyceride levels of animals treated with FGF21 and Fc-(L15)-FGF21 (L98R, P171G) were statistically significantly reduced, compared to the triglyceride levels of animals treated with vehicle (Figure 46).

22.6 Concentration of Test Compounds

The exposure of the tested compounds administered at approximately equivalent molar dose levels was assessed throughout the study period. The concentration of Fc-(L15)-FGF21 (L98R, P171G) was measured at pre-dose, and approximately 5 days after the last injection. FGF21 levels were measured at pre-

dose, and at 5, 12, 19, and 26 days. Blood samples were drawn at approximately 21 hours after the last injection.

The individual concentration of the tested compounds in each monkeys are shown in Figures 47 and 48. As shown in Figure 47, the majority of the animals in the FGF21-treated group had concentrations below the quantitation limit. Figure 48 shows that animals in the Fc-(L15)-FGF21 (L98R, P171G)-treated group had detectable levels of Fc-(L15)-FGF21 (L98R, P171G) during each dosing phase (two weekly doses at the same dose strength). The average concentration from each dosing phase increased approximately dose-proportionally from 0.3 to 5 mg/kg for Fc-(L15)-FGF21 (L98R, P171G). There is minimal accumulation as demonstrated by the steady concentrations after the first and second weekly dose within each dose escalation phase for both compounds. During the treatment-free phase (washout period) Fc-(L15)-FGF21 (L98R, P171G) levels were detectable up to approximately day 47 (12 days post last dose) and were below lower limit of quantification (LLOQ) afterwards.

Exposure of the test compounds was also monitored during each OGTT. FGF21 was not detectable during OGTTs 3 and 4, following low- and mid-dose FGF21 treatment. However, measurable levels were observed during OGTT5, following high-dose treatment. A dose proportional increase in Fc-(L15)-FGF21 (L98R, P171G) levels was observed across the third to fifth OGTT with escalating dose levels, as shown in Figure 49.

Compound levels data confirm that the animals were exposed to the expected amount of each compound, namely FGF21 and Fc-(L15)-FGF21 (L98R, P171G), in a dose escalation manner. A large variability was observed in the amount of FGF21 measured, which was an expected result considering the sampling was performed approximately 21 hours post the last dose and the half life of FGF21 is approximately 1 hour.

22.7 Conclusions

FGF21 decreased fasted and fed plasma triglyceride and insulin levels and decreased body weight at the highest doses. Fc-(L15)-FGF21 (L98R, P171G) improved OGTT and decreased insulin levels at the highest dose, and dose dependently decreased fasted and fed plasma triglyceride levels as well as body weight. Both FGF21 and Fc-(L15)-FGF21(L98R, P171G) decreased a number of metabolic

parameters in the non diabetic *Rhesus* monkeys. Insulin and triglyceride level decreases were identical between FGF21 and Fc-(L15)-FGF21 (L98R, P171G) when circulating compound levels were in a similar range, in the fed condition. Due to its improved properties, Fc-(L15)-FGF21 (L98R, P171G) was superior to FGF21 in most of the parameters measured and could be administered once-a-week to observe efficacy on metabolic parameters.

EXAMPLE 23

Fc-(G4S)3-FGF21(L98R, P171G, A180E) Fc fusion Molecule

An Fc fusion comprising an FGF21 mutant, which was joined to an IgG1 Fc component by a linker, was generated. The FGF21 component of the Fc fusion comprised three point mutations engineered in the polypeptide sequence of FGF21, namely L98R, P171G, A180E (numbering based on the mature form of FGF21, provided as SEQ ID NO:4). This molecule was constructed by conjugating a human Fc (SEQ ID NO:11) to the N-terminus of the L98R, P171G, A180E mutant FGF21 (SEQ ID NO:39) via a 15 amino acid linker comprising the sequence of GGGGSGGGGSGGGGS (SEQ ID NO:31). This molecule was designated as "Fc-(G4S)3-FGF21(L98R, P171G, A180E)" and its full length amino acid sequence is shown in Figure 50 and in SEQ ID NO:47; it is encoded by the nucleic acid of SEQ ID NO:46. *In vitro* testing of Fc-(G4S)3-FGF21(L98R, P171G, A180E) showed it is a potent stimulator of Erk phosphorylation in a recombinant cell line overexpressing β-Klotho. Fc-(G4S)3-FGF21 (L98R, P171G, A180E) also showed enhanced β-Klotho binding affinity compared with Fc fusion of wild type FGF21 or Fc fusion of FGF21-(G4S)3-FGF21 (L98R, P171G) (SEQ ID NO:45). When injected into diabetic animal models, Fc-(G4S)3-FGF21(L98R, P171G, A180E) reduced blood glucose levels, decreased body weight, and was suitable for biweekly injection.

23.1

In vitro Activity of Fc-(G4S)3-FGF21(L98R, P171G, A180E)

Experiments were performed to examine whether Fc-(G4S)3-FGF21 (L98R, P171G, A180E) retains similar activity to Fc fusion of wild type FGF21 or wild type native FGF21 in an ELK-luciferase *in vitro* assay.

ELK-luciferase assays were performed using a recombinant human 293T kidney cell system, in which the 293T cells overexpress β-Klotho and luciferase

reporter constructs. β-klotho is a co-receptor required for FGF21 to activate FGF receptors and induce intracellular signal transduction, including Erk phosphorylation. The Erk- luciferase reporter constructs contain sequences encoding GAL4-ELK1 and 5xUAS-Luciferase reporter. The 5xUAS-Luciferase reporter is driven by a promoter containing five tandem copies of the Gal4 binding site. The reporter activity is regulated by the level of phosphorylated Erk, and is used to indirectly monitor and quantify FGF21 activity.

ELK-luciferase assays were performed by culturing the 293T cells in the presence of different concentrations of wild-type FGF21, Fc fusion of wild type FGF21, Fc-L15-FGF21 (L98R, P171G, A180E) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) for 6 hours, and then assaying the cell lysates for luciferase activity. The luminescences obtained in ELK-luciferase assays for each of the FGF21 constructs were expressed in y-axis and the compound concentrations were expressed in x-axis.

Figure 51 shows the dose-response of the tested compounds in Erk-luciferase assays. Fc-(G4S)3-FGF21 (L98R, P171G, A180E) retained similarly activity compared with Fc fusion of FGF21 wild type, suggesting that a combination of mutations with L98R, P171G and A180E didn't change the bioactivity of FGF21. Compared with native wild type FGF21, Fc fusion constructs showed slightly reduced potency and the maximal activity in this cell-based assay with the co-receptor β-Klotho overexpressed.

23.2

***In vitro* Activity of Fc-FGF21(L98R, P171G, A180E) Fusions Comprising Different Linker Sequences**

A similar Fc fusion analog was generated by fusing the human IgG1 Fc to FGF21 (L98R, P171G, A180E) via a different linker sequence, GGGGGSGGGSGGGGS (SEQ ID NO:28). This linker was designated L15 and the resulting fusion molecule was designated Fc-L15-FGF21 (L98R, P171G, A180E) (SEQ ID NO:57). In this experiment, the effect of different linker sequences on the activity of Fc-FGF21 (L98R, P171G, A180E) fusions was studied.

ELK-luciferase assays were performed by culturing the 293T cells in the presence of different concentrations of Fc-(G4S)3-FGF21 (L98R, P171G, A180E)

and Fc-L15-FGF21 (L98R, P171G, A180E) for 6 hours, and then assaying the cell lysates for luciferase activity. Fc-(G4S)3-FGF21 (L98R, P171G, A180E) showed similar activity to Fc-L15-FGF21 (L98R, P171G, A180E), indicating that different linker sequences, *e.g.*, (G4S)3 or L15 linkers, had no significant impact on the bioactivity of Fc-FGF21 fusions.

23.3

***In vitro* Binding Affinity of Fc-(G4S)3-FGF21(L98R, P171G, A180E) for β-Klotho in Binding Assays**

The binding of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) to human and cyno β-Klotho was tested in a Biacore solution equilibrium binding assay. The affinity of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) was also compared with an Fc- fusion FGF21 analog with only L98R and P171G mutations, namely Fc-L15-FGF21 (L98R, P171G) (SEQ ID NO:43).

Neutravidin was immobilized on a CM5 chip using amine coupling. Biotin-FGF21 was captured on the second flow cell to ~1500RU. The first flow cell was used as a background control. FGF21 mutants at 5x dilutions (0.03~2000 nM) were incubated with 10nM human or 25nM cyno β-Klotho in PBS plus 0.1mg/ml BSA, 0.005% P20 at room temperature for 1 hour. Binding of the free β-Klotho in the mixed solutions was measured by injecting over the biotin-FGF21 surface. 100% β-Klotho binding signal was determined in the absence of FGF21 mutants in the solution. A decreased β-Klotho binding response with increasing concentrations of FGF21 mutants indicated that β-Klotho was binding to FGF21 mutants in solution, which blocked β-Klotho from binding to the immobilized biotin-FGF21 surface. Relative binding of the mixture versus molar concentration of FGF21 was plotted using GraphPad Prizm 5. EC_{50} was calculated using one site competition nonlinear fit in the same software.

Figure 52 showed the results from a Biacore solution equilibrium binding assay of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) and Fc-L15-FGF21 (L98R, P171G) to human (right) and cyno β-Klotho (left). Fc-(G4S)3-FGF21 (L98R, P171G, A180E) showed at least 2x improved binding activity to both human and cyno β-Klotho compared to Fc-L15-FGF21 (L98R, P171G).

23.4

In vivo Efficacy of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) in Diabetic *db/db* Mice

The question of whether Fc-(G4S)3-FGF21 (L98R, P171G, A180E) could exert metabolic beneficial effects, such as lowering blood glucose and reducing body weight, in diabetic db/db mice was studied. The study was also intended to examine the duration and dose response of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) after a single injection. Fc-(G4S)3-FGF21 (L98R, P171G, A180E) at doses of 0.1, 0.3, 1 and 3 mg/kg was intraperitoneally injected into diabetic db/db mice. A vehicle (10mM Tris, 2.2% sucrose, 3.3% Sorbitol, pH8.5)-treated group was also included in the study. Blood samples were obtained from each animal (n=10 per group) at baseline (before injection), and 6, 24, 72, 120, and 168 hours after injection. Blood glucose levels were measured with a OneTouch Glucometer (LifeScan, Inc. Milpitas, CA). Body weight was measured at baseline (time 0), 24, 72, 120, and 168 hours after injection.

Figure 53A shows the blood glucose levels in db/db mice at various time points following vehicle or Fc-(G4S)3-FGF21 (L98R, P171G, A180E) injection. Fc-(G4S)3-FGF21 (L98R, P171G, A180E) resulted in a dose-dependent decrease in blood glucose levels in db/db mice. The maximum glucose reduction was about 50% from baseline or compared with the vehicle-treated group. The maximum effect was reached within 6 hours after injection and sustained for 120 hours post injection. The blood glucose levels started to return to baseline in about 168 hours. The estimated ED50, the dose required to achieve the half maximum effect, for Fc-(G4S)3-FGF21 (L98R, P171G, A180E) was approximately 1 mg/kg in db/db mice.

Figure 53B shows the effect of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) on body weight after a single injection into db/db mice. Results are expressed as change of body weight from time 0 (before injection). Vehicle-treated mice showed progressive and stable body weight gain during the 7 days study period. However, the rate of body weight growth was inhibited in mice treated with Fc-(G4S)3-FGF21 (L98R, P171G, A180E) in a dose-dependent manner. The higher the dose, the longer the growth inhibition was. In one example, Fc-(G4S)3-FGF21 (L98R, P171G, A180E) blunted body weight gain for 5 days at 3 mg/kg, 3 days at 1 mg/kg, or 1 day

at 0.3 mg/kg. The growth rates were recovered afterwards. The estimated ED50 of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) on body weight reduction was approximately 1 mg/kg in this experiment.

23.5

Efficacy Comparison of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) and Fc-(L15)-FGF21(L98R, P171G) in DIO Mice with Different Injection Frequencies

The study was to determine whether Fc-(G4S)3-FGF21 (L98R, P171G, A180E) could be injected less frequently than Fc-(L15)-FGF21 (L98R, P171G) but achieve similar efficacy. The study was conducted in DIO mice with Fc-(G4S)3-FGF21 (L98R, P171G, A180E) given once a week (Q7D) or once every two weeks (Q14D), or Fc-L15-FGF21 (L98R, P171G) administered at twice a week (BIW), Q7D or Q14D.

DIO mice were prepared by feeding 4-week old male C57BL/6 mice a high fat-diet that contained 60% of energy from fat enriched with saturated fatty acids (D12492, Research Diets, Inc., New Brunswick, NJ). After 12 weeks of high fat diet feeding, body weight and blood glucose levels were measured. DIO mice were then randomized into vehicle or treatment groups to achieve similar baseline average blood glucose levels and body weight. A total of 7 groups were included in the study: vehicle administered at Q7D; Fc-(G4S)3-FGF21 (L98R, P171G, A180E) administered at Q7D or Q14D; or Fc-(L15)-FGF21 (L98R, P171G) administered at BIW, Q7D or Q14D. The injection was intraperitoneal and the study was carried out for 31 days. Body weight was measured weekly. A GTT was performed at study day 28 and the study was terminated at day 31. The study design is shown graphically in Figure 54.

Figure 55 shows the GTT profiles in mice treated with vehicle, Fc-(G4S)3-FGF21 (L98R, P171G, A180E) or Fc-(L15)-FGF21(L98R, P171G) at different dosing frequencies. Glucose tolerance was statistically significantly improved in mice treated with Fc-(G4S)3-FGF21 (L98R, P171G, A180E) at either Q7D or Q14D dosing when compared with vehicle, suggesting that Fc-(G4S)3-FGF21(L98R, P171G, A180E) is efficacious and suitable for Q14D administration in DIO mice. Fc-(L15)-FGF21(L98R, P171G) improved glucose tolerance when administered at BIW or Q7D, but not Q14D, suggesting that Fc-(L15)-FGF21(L98R, P171G) may be less

suitable for Q14D injection in mice. The efficacy of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) at Q7D or Q14D was comparable to that of Fc-(L15)-FGF21(L98R, P171G) at BIW or Q7D respectively, suggesting that Fc-(G4S)3-FGF21(L98R, P171G, A180E) could be given 2 fold less frequently than Fc-(L15)-FGF21(L98R, P171G).

Figure 56 shows changes of body weight from baseline (day 0) in mice treated vehicle, Fc-(G4S)3-FGF21 (L98R, P171G, A180E) or Fc-(L15)-FGF21 (L98R, P171G) at different dosing frequencies. Mice treated Q7D with Fc-(G4S)3-FGF21 (L98R, P171G, A180E) lost significant body weight as did mice treated BIW with Fc-(L15)-FGF21 (L98R, P171G). The body weight was moderately reduced in mice treated Q14D with Fc-(G4S)3-FGF21 (L98R, P171G, A180E) or Q7D with Fc-(L15)-FGF21 (L98R, P171G). No significant body weight effect was observed in mice treated Q14D with Fc-(L15)-FGF21 (L98R, P171G). The effect on body weight was consistent with that on GTT as described above, suggesting that Fc-(G4S)4-FGF21 (L98R, P171G, A180E) was efficacious at Q14D dosing and required about 2 fold less frequent injections than Fc-(L15)-FGF21 (L98R, P171G) to achieve the same effect.

EXAMPLE 24

Hydrogel Formulations Comprising FGF21 Mutants

As a formulation agent for protein-based therapeutics, hydrogels offer a number of desired properties. For example, hydrogels preserve the native structure and function of the protein incorporated in the hydrogel. Moreover, they are well tolerated and depending on the polymer and type of crosslinking, they may be biodegradable. Additionally, hydrogels have previously been used successfully for sustained release of proteins. Accordingly, hydrogels were investigated as a possible delivery method for the FGF21 mutants disclosed herein.

For all experiments described in this example, hydrogels were prepared as follows. A 1.25% bovine gelatin (Sigma) solution in PBS was prepared. The cross-linking agent methacrylic anhydride was added to a molar ratio (MA to gelatin) of 16:1, 24:1 or 32:1. The resulting solution was dialyzed against water to remove any un-polymerized methacrylamide to create a hydrogel vehicle. Finally, the hydrogel vehicle was lyophilized and stored at 4°C until ready to make hydrogels comprising an FGF21 mutant. This prep can be used to prepare gelatin-based hydrogel vehicles

that can subsequently be adapted to comprise any of the FGF21 mutants disclosed herein.

Hydrogels with specific FGF21 mutants were then prepared. Starting with a 10% lyophilized, methacrylic gelatin hydrogel vehicle, solutions were prepared. The hydrogel vehicles were warmed and then centrifuged to dissolve and liquify the lyophilized MA gelatin hydrogel vehicle. The selected FGF21 protein, (FGF21 (L98R, P171G), (SEQ ID NO:37)) in the instant Example), was then added to the liquefied gelatin solution to a pre-determined concentration. A TEMED stock solution was then added. A KPS stock solution was then added and the solution was mixed gently. 1 ml syringes were filled to 200 µl and allowed to set for 1.5 to 2 hours at room temperature. The syringes were stored at -20°C and thawed at 4°C overnight prior to use. Hydrogel vehicle comprising 10mM Tris, 9% sucrose, pH8.5 with no FGF21 mutant added was used as a control.

For the *in vivo* experiments, the syringes were put on heating pad at 37°C for approximately 10 minutes before injection into the animals.

24.1

In vitro Activity of FGF21 (L98R, P171G) Released from 10% Hydrogels with Various Crosslink Ratios

A goal of this experiment was to test whether FGF21 (L98R, P171G) released from hydrogel is biologically active compared with the native form of FGF21 (L98R, P171G) in an ELK-luciferase *in vitro* assay.

FGF21 (L98R, P171G) was prepared and incorporated into 10% methacrylic gelatin solutions with methacrylic gelatin crosslink ratios at 16:1, 24:1 and 32:1 as described. The hydrogels were then dispersed into an *in vitro* buffer solution to allow release of FGF21 (L98R, P171G). The medium were collected after 100 or 150 hours and subject to *in vitro* assays for FGF21 (L98R, P171G) activity. Analytical assays (e.g., SDS-PAGE, size exclusion HPLC, and reverse phase HPLC) show the FGF21 (L98R, P171G) released was intact at all time points.

ELK-luciferase assays were performed using a recombinant human 293T kidney cell system, in which the 293T cells overexpress β-Klotho and luciferase reporter constructs. β-Klotho is a co-receptor that is required by FGF21 for activation of its FGF receptors. The FGF receptors used in this assay are endogenous FGF

receptors expressed in 293T kidney cell. The luciferase reporter constructs contain sequences encoding GAL4-ELK1 and a luciferase reporter driven by a promoter containing five tandem copies of the Gal4 binding site (5xUAS-Luc). Luciferase activity is regulated by the level of phosphorylated Erk/ELK1, and is used to indirectly monitor and quantify FGF21 activity.

ELK-luciferase assays were performed by culturing the 293T cells in the presence of different concentrations of the native form of FGF21 (L98R, P171G) or hydrogel-released FGF21 (L98R, P171G) for 6 hours, and then assaying the cell lysates for luciferase activity. Figure 57 shows the *in vitro* results from the ELK-luciferase assay. FGF21 (L98R, P171G) released from hydrogels with methacrylic gelatin crosslink ratios at 16:1, 24:1 and 32:1 was biologically active with equivalent activity to the native form of FGF21 (L98R, P171G). The data demonstrated that the hydrogel preserved the structure and function of the incorporated protein and the released FGF21 (L98R, P171G) is active and stable even after 10-15 hours in the medium.

24.2

***In vivo efficacy* of Hydrogel FGF21 (L98R, P171G) at Different Crosslink Ratios in ob/ob Mice**

The goal of this experiment was to determine whether an FGF21 (L98R, P171G) hydrogel prepared with methacrylic gelatin crosslink ratios at 24:1 and 32:1 provided a sustained release of biologically active FGF21 (L98R, P171G) *in vivo* and ultimately resulting in longer *in vivo* efficacy as compared with native form of FGF21 (L98R, P171G). In addition, based on the assessment of *in vitro* release rates, it was determined that higher cross-linking ratios of methacrylic gelatin gives better sustained release of the incorporated FGF21 (L98R, P171G). Therefore, another goal of this experiment was to compare two FGF21 (L98R, P171G) hydrogels prepared with methacrylic gelatin crosslink ratios at 24:1 and 32:1.

FGF21 possesses a number of biological activities, including the ability to lower blood glucose, insulin, triglyceride, or cholesterol levels; reduce body weight; or improve glucose tolerance, energy expenditure, or insulin sensitivity. FGF21 (L98R, P171G) hydrogels were introduced into insulin resistant ob/ob mice, and the abilities of FGF21 (L98R, P171G) hydrogel to lower blood glucose and reduce body weight were measured. The procedure for the *in vivo* work was as follows.

Hydrogels were prepared using the procedure described above. 8 week old ob/ob mice (Jackson Laboratory) were hair-shaved at the injection site and were anesthetized with isoflurane and O_2 just before injection. Hydrogels (0.2 ml) were slowly injected under the skin and Vetbond was applied at the injection site after injection. Vehicle (10mM Tris, 9% sucrose pH8.5) or native FGF21 (L98R, P171G) were also included in the experiment and injected similarly as hydrogels. Animals were returned to their cage after awaking from anesthesia. Blood samples were obtained before injection and at various time points following injection, *e.g.*, 0, 3, 6, 24, 72, 120, 192 and 264 hours after injection. Blood glucose levels were measured with a OneTouch Glucometer (LifeScan, Inc. Milpitas, CA). Body weight was also monitored.

Figures 58 and 59 summarize the results of the experiment. Compared with vehicle or control hydrogel, the native form of FGF21 (L98R, P171G) rapidly reduced blood glucose levels at 3 and 6 hr post injection. However, the *in vivo* activity of the native form of FGF21 (L98R, P171G) waned and the blood glucose levels returned to baseline 24 hours after injection. FGF21 (L98R, P171G) hydrogels resulted in blood glucose reduction as early as 3 hr post injection and the activity was sustained up to 8 days. There was no significant difference between the crosslink ratios at 24:1 or 32:1. FGF21 (L98R, P171G) hydrogel groups also showed slower body weight gain than mice treated with vehicle or hydrogel alone. These results demonstrated that FGF21 (L98R, P171G) hydrogels prepared with methacrylic gelatin crosslink ratios at 24:1 and 32:1 are capable of providing a sustained release of biologically active FGF21 (L98R, P171G) *in vivo* and ultimately resulting in longer *in vivo* efficacy as compared with the native form of FGF21 (L98R, P171G).

24.3

***In vivo* Efficacy of Hydrogel FGF21 (L98R, P171G) and FGF21 (L98R, P171G, A180E) at Different Crosslink Ratios in db/B6 Mice**

Hydrogel formulations were prepared using bovine gelatin (Sigma) and several FGF21 mutants and constructs, namely FGF21 (L98R, P171G) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E). A hydrogel control was also prepared. The 0.2 mL of hydrogel was placed in a 1 ml syringe having a 21G needle.

Hydrogel controls and hydrogels comprising an FGF21 mutant were prepared as described above. 8 week old db/B6 mice (Jackson Laboratory) were hair-shaved at

the injection site and were anesthetized with isoflurane and O_2 just before injection. Hydrogels (0.2 ml) were slowly injected under the skin and Vetbond was applied at the injection site after injection. Vehicle (10mM Tris, 9% sucrose pH8.5) or native FGF21 (L98R, P171G) were also included in the experiment and injected similarly as hydrogels. Animals were returned to their cage after awaking from anesthesia. Blood samples were obtained before injection and at various time points following injection, *e.g.*, 0, 24, 96, 168, 240, 312 hours after injection. Blood glucose levels were measured with a OneTouch Glucometer (LifeScan, Inc. Milpitas, CA). Body weight was also monitored.

The experimental design was as follows:

Group of animals (n=9 per group)

A.	Control hydrogel 32:1 (10%) MA:HU4	200µl
B.	FGF21 (L98R, P171G) hydrogel 32:1 (10%) MA:HU4	0.5 mg/mouse (200µl, ~ 10 mg/kg)
C.	FGF21 (L98R, P171G) hydrogel 32:1 (10%) MA:HU4	1.5 mg/mouse (200µl ~ 30 mg/kg)
D.	Fc-(G4S)3-FGF21 (L98R, P171G, A180E)	(no hydrogel) 3 mg/kg

Various parameters were measured and the results of the experiments are shown in Figures 60-63. Figure 60 shows the change in blood glucose over the course of the 14 day experiment, while Figure 61 shows the percent change in blood glucose over the same period. Figure 62 shows the change in body weight over the course of the 14 day experiment, while Figure 63 shows the percent change in body weight over the same period.

The results of the experiment shown graphically in Figures 60-63 can be summarized as follows:

FGF21 (L98R, P171G) 32:1(10%)MA:HU4 at 10 mg/kg was efficacious in reducing blood glucose at 24 hours post injection and the blood glucose level returned to baseline between 4-7 days.

FGF21 (L98R, P171G) 32:1(10%)MA:HU4 at 30 mg/kg was more efficacious in reducing blood glucose than the 10 mg/kg dosage, and the blood glucose level was seen to return to baseline between 7-10 days.

Fc-(G4S)3-FGF21(L98R, P171G, A180E) at 3 mg/kg itself reduced blood glucose at 24 hours post injection to a degree that was similar to FGF21 (L98R, P171G) at 30mg/kg from hydrogels.

The hydrogel control (which did not comprise an FGF21 mutant) did not have any effect in reducing blood glucose.

The FGF21 (L98R, P171G) hydrogel groups and Fc-(G4S)3-FGF21(L98R, P171G, A180E) (which was not presented in a hydrogel) showed reduced body weight gain compared to mice treated with a hydrogel control.

EXAMPLE 25

Cynomolgus Monkey Study

Two Fc-Linker-FGF21 constructs were generated using methodology described herein. One construct comprised an IgG1 Fc sequence (SEQ ID NO:11) fused at the C-terminus to a $(Gly)_5$-Ser-$(Gly)_3$-Ser-$(Gly)_4$-Ser linker sequence (SEQ ID NO:28) which was then fused to the N terminus of a mature FGF21 sequence (SEQ ID NO:4), in which two mutations, L98R and P171G, were introduced. This molecule is referred to in the instant Example as "Fc-(L15)-FGF21 (L98R, P171G)" (SEQ ID NO:43). A second construct comprised an IgG1 Fc sequence (SEQ ID NO:11) fused at the C-terminus to a $(Gly)_4$-Ser-$(Gly)_4$-Ser-$(Gly)_4$-Ser linker sequence (SEQ ID NO:31) which was then fused to the N terminus of a mature FGF21 sequence (SEQ ID NO:4), in which three mutations, L98R, P171G, and A180E, were introduced. This molecule is referred to in the instant Example as "Fc-(G4S)3-FGF21 (L98R, P171G, A180E)" (SEQ ID NO:47). These constructs were then expressed and purified as described herein, and were isolated as a dimeric form of the protein, each monomer of which was linked via intermolecular disulfide bonds between the Fc region of each monomer.

25.1 Study Design

The study was conducted in *cynomolgus* monkeys with characteristics of impaired glucose tolerance (IGT). The monkeys were 8-18 years old. Their body weights ranged from 5-15 kg and BMI ranged from 32-70 kg/m2. 44 monkeys were acclimated for 6 weeks prior to the initiation of compound administration. During the acclimation period, monkeys were trained 4 times a week for 4 weeks to familiarize the procedures including chair-restrain, subcutaneous injection (PBS, 0.1 ml/kg),

gavage (Water, 10 ml/kg), blood drawn for non OGTT and OGTT samples. After 4 weeks of training, baseline OGTT and plasma metabolic parameters were measured. 40 out of 44 monkeys were selected and randomized into three treatment groups to achieve similar baseline levels of body weight, OGTT AUC response, and plasma glucose and triglyceride levels.

The study was conducted in a blind fashion. Vehicle (n=14), Fc-(L15)-FGF21 (L98R, P171G) (n=13) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) (n=13) were labeled as compound A, B and C and administered once a week via subcutaneous injection. Compounds were given in a dose-escalation fashion from low (0.3 mg/kg), medium (1 mg/kg) to high (3 mg/kg) levels and the dose was escalated every three weeks. After 9 weeks of compound treatments, animals were monitored for additional 3 weeks for compound washout and recovery from treatments. Food intake, body weight, clinical chemistry and OGTT were monitored throughout the study. Food intake was measured every meal. Body weight was measured weekly. Blood samples were collected weekly 5 days post each injection to measure glucose, triglyceride, total cholesterol, HDL- and LDL-cholesterol levels. OGTTs were conducted every three weeks after the initiation of treatments (at the end of each dose level). The day starting the treatment is designated as 0 and the detailed study plan is shown in Figure 64.

The results shown in this example are data collected at the end of 9 weeks treatment.

25.2 Effect of Test Compounds on Food Intake

Animals were fed twice a day, with each animal receiving 120 g of formulated food established during the acclimation period. The remaining food was removed and weighed after each meal to calculate food intake. The feeding time were from 8:00 AM to 8:30 AM (±30 minutes) and then from 4:30PM to 5:00PM (±30 minutes). To produce treats, apple (150 g) was supplied to each animal at 11:30 to 12:30 PM (±30 minutes) every day.

Compared with vehicle, both Fc-(L15)-FGF21 (L98R, P171G) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) reduced food intake in monkeys (Figures 65, 66 and 67). Fc-(G4S)3-FGF21 (L98R, P171G, A180E) inhibited food intake on every meal including AM, fruit and PM meals at 0.3 mg/kg dose. However, the effect

diminished and the food intake returned close to baseline or control levels after about 30 days of treatment when the dose was escalated to 1 mg/kg. Fc-(L15)-FGF21 (L98R, P171G) didn't have a significant effect on AM food intake and only modestly reduced food intake on PM meal when the dose was escalated to 1 and 3 mg/kg. However, Fc-(L15)-FGF21 (L98R, P171G) reduced fruit intake similarly as Fc-(G4S)3-FGF21 (L98R, P171G, A180E). Overall, Fc-(G4S)3-FGF21 (L98R, P171G, A180E) showed a stronger effect on inhibiting food intake than Fc-(L15)-FGF21 (L98R, P171G). The effect on food intake appeared to be short term and food intake was recovered after approximately 30 days of treatment.

25.3. Effect of Test Compounds on Body Weight

Body weight was monitored weekly throughout the study. Over the course of the 9 week treatments, the body weight of animals treated with vehicle remained constant while body weight of animals treated with Fc-(L15)-FGF21 (L98R, P171G) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) progressively decreased. Fc-(G4S)3-FGF21 (L98R, P171G, A180E) resulted in a more pronounced body weight decrease than Fc-(L15)-FGF21 (L98R, P171G) as shown in Figure 68.

25.4. Effect of Test Compounds on Body Mass Index (BMI), Skin Fold Thickness (SFT) and Abdominal Circumference (AC)

BMI, SFT and AC were monitored weekly throughout the study, both pre- and post-administration of test compounds when the body weight was taken. BMI is defined as the individual's body weight divided by the square of his or her height. SFT is the thickness of a double layer of skin and the fat beneath it with a special caliber that exerts a constant tension on the site. BMI, SFT and AC are relatively accurate, simple, and inexpensive measurements of body composition particularly indicative of subcutaneous fat. Animals treated with vehicle showed relatively stable BMI, SFT and AC throughout the study. Animals treated with Fc-(L15)-FGF21 (L98R, P171G) and Fc-(G4S)3-FGF21 (L98R, P171G, A180E) showed decreased levels of BMI, SFT and AC over the course of the 9 week study, suggesting both compounds resulted in reduction of fat mass. Fc-(G4S)3-FGF21 (L98R, P171G, A180E) was more effective and resulted in more pronounced reductions in BMI, SFT and AC than Fc-(L15)-FGF21 (L98R, P171G). Results are shown in Figures 69, 70 and 71, respectively.

25.5 Effect of Test Compounds on Fasting Blood Glucose Levels

Blood was collected from overnight fasted animals. The blood drawn was conducted weekly at 5 days post each injection. Both Fc-(G4S)3-FGF21 (L98R, P171G, A180E) and Fc-(L15)-FGF21 (L98R, P171G) reduced fasting blood glucose levels. Fc-(G4S)3-FGF21 (L98R, P171G, A180E) reduced fasting blood glucose levels at the dose of 0.3 mg/kg and the maximum glucose reduction was achieved when the dose was escalated to 1 mg/kg. However, Fc-(L15)-FGF21 (L98R, P171G) only resulted in a modest reduction of blood glucose levels at the highest dose tested (3 mg/kg). Therefore, Fc-(G4S)3-FGF21 (L98R, P171G, A180E) was more efficacious and produced more pronounced blood glucose reduction than Fc-(L15)-FGF21 (L98R, P171G). No hypoglycemia was observed in any of the monkeys treated with Fc-(G4S)3-FGF21 (L98R, P171G, A180E) or Fc-(L15)-FGF21 (L98R, P171G). Figure 72 shows the levels of fasting plasma glucose during the course of study.

25.6 Effect of Test Compounds on Oral Glucose Tolerance Test (OGTT)

OGTTs were conducted before and after initiation of treatments. Post-dose OGTTs were performed every three weeks to test compound effect at each dose level. Fc-(G4S)3-FGF21 (L98R, P171G, A180E) improved glucose tolerance at all tested doses from 0.3 to 3 mg/kg. Glucose levels were reduced and the glucose excursion following a bolus of glucose challenge increased in response to Fc-(G4S)3-FGF21 (L98R, P171G, A180E) treatment. There was no dose-response observed suggesting that Fc-(G4S)3-FGF21 (L98R, P171G, A180E) achieved its maximal effect at the dose of 0.3 mg/kg. Fc-(L15)-FGF21 (L98R, P171G) only resulted in an improvement of glucose tolerance at 1 mg/kg dose and it was not clear why the effect diminished when the dose was escalated to 3 mg/kg. Figure 73 shows pre- and post-OGTT curve profiles and the area under the OGTT curve.

25.7 Effect of Test Compounds on Triglyceride Levels

Blood was collected from overnight fasted animals. The blood drawn was conducted weekly at 5 days post each injection. Triglyceride levels were significantly reduced in animals treated with Fc-(G4S)3-FGF21 (L98R, P171G, A180E) or Fc-(L15)-FGF21 (L98R, P171G). However, Fc-(G4S)3-FGF21 (L98R, P171G, A180E)

was more effective than Fc-(L15)-FGF21 (L98R, P171G). Fc-(G4S)3-FGF21 (L98R, P171G, A180E) resulted in a maximal reduction of plasma triglyceride levels at 0.3 mg/kg while Fc-(L15)-FGF21 (L98R, P171G) only resulted in an intermediate reduction of triglyceride levels with the highest tested dose (3 mg/kg). Figure 74 shows the levels of fasting plasma triglycerides during the course of study.

25.8 Effect of Test Compounds on Total Cholesterol and HDL-Cholesterol Levels

Blood was collected from overnight fasted animals. The blood drawn was conducted weekly at 5 days post each injection. Plasma total cholesterol and HDL-cholesterol levels tended to increase following Fc-(G4S)3-FGF21 (L98R, P171G, A180E) or Fc-(L15)-FGF21 (L98R, P171G) treatment. Figures 75 and 76 show the levels of total cholesterol and HDL-cholesterol over the course of study.

25.9 Conclusions

In a dose-escalation study conducted in male IGT *cynomolgus* monkeys, animals treated with Fc conjugated FGF21 mutants, namely Fc-(G4S)3-FGF21 (L98R, P171G, A180E) and Fc-(L15)-FGF21 (L98R, P171G), showed improved metabolic parameters. Body weight was reduced and body composition was improved. Short-term reduction of food intake was observed and and the food intake recovered to baseline or control levels mid study. Fasting blood glucose and triglyceride levels were also reduced by both compounds, Fc-(G4S)3-FGF21 (L98R, P171G, A180E) or Fc-(L15)-FGF21(L98R, P171G). OGTT was improved and HDL-cholesterol levels were slightly elevated. Compared with Fc-(L15)-FGF21 (L98R, P171G), Fc-(G4S)3-FGF21 (L98R, P171G, A180E) appeared to be superior to Fc-(L15)-FGF21 (L98R, P171G) in all parameters measured at any tested dose. Fc-(G4S)3-FGF21 (L98R, P171G, A180E) achieved its maximal effects for most of the parameters measured when administered at 0.3 mg/kg. Therefore the therapeutic effective dose of Fc-(G4S)3-FGF21 (L98R, P171G, A180E) in higher species may be lower than 0.3 mg/kg.

EXAMPLE 26

Stability Study in *Cynomolgus* Monkeys

This study was designed to determine whether Fc-(L15)-FGF21 (L98R,

P171G, A180E) (SEQ ID NO:57) was more protease-resistant than Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43). Carboxy terminal processing were observed following Fc-(L15)-FGF21 (L98R, P171G) injection into mice or monkeys as shown in Example 21. The degradation resulted in a successive loss of 1 to 3 amino acid residues from the C-terminus. Efforts by capping the C-terminus or introducing additional mutations into the C-terminus of Fc-(L15)-FGF21 (L98R, P171G) yielded a superior molecule Fc-(L15)-FGF21 (L98R, P171G, A180E). This study was designed to assess whether Fc-(L15)-FGF21 (L98R, P171G, A180E) had improved *in vivo* stability compared with Fc-(L15)-FGF21 (L98R, P171G).

Fc-(L15)-FGF21 (L98R, P171G, A180E) and Fc-(L15)-FGF21 (L98R, P171G) constructs were generated. These constructs comprised an IgG1 Fc sequence (SEQ ID NO:11) fused at the C-terminus to a (Gly)5-Ser-(Gly)3-Ser-(Gly)4-Ser linker sequence (SEQ ID NO:28) which was then fused to the N terminus of a mature FGF21 sequence (SEQ ID NO:4), in which either two mutations, L98R, P171G, or three mutations, L98R, P171G, and A180E, were introduced. These constructs were then expressed and purified as described herein, and were isolated as a dimeric form of the protein, each monomer of which was linked via intermolecular disulfide bonds between the Fc region of each monomer.

The *in vivo* stability of Fc-(L15)-FGF21 (L98R, P171G) and Fc-(L15)-FGF21 (L98R, P171G, A180E) was compared in male *cynomolgus* monkeys. Fc-(L15)-FGF21 (L98R, P171G) and Fc-(L15)-FGF21 (L98R, P171G, A180E) were intravenously injected into cynomolgus monkeys at 23.5 mg/kg. Blood samples were collected at various time points following a single iv injection. Immunoaffinity-MALDI-TOF mass spectrometry was used to monitor metabolites at each time point following injection. Results are shown in Figure 77.

Compared with Fc-(L15)-FGF21(L98R, P171G), Fc-(L15)-FGF21 (L98R, P171G, A180E) showed significantly reduced C-terminal degradation with fewer detectable mass peaks adjacent to the parental peak of the intact molecule, suggesting that the A180E mutation slowed down C-terminal peptidase degradation. Larger truncations with mass losses estimated at [1-376], [1-394] and [1-401] were also observed and the sites corresponded to 133-134, 153-154 and 158-159 in the FGF21 polypeptide sequence. The internal endopeptidase clipping contributed to the overall metabolism of both Fc-(L15)-FGF21 (L98R, P171G) and Fc-(L15)-FGF21 (L98R, P171G, A180E), and the A180E mutation did not appear to significantly impact the

rate of internal endopeptidase degradation.

In order to increase the resolution and provide details of the degradation mixture, MRM (multiple-reaction-monitoring) LC-MS mass spectrometry was also performed to monitor various forms of the C-terminal degradation fragments. Monkey samples were affinity-purified and then subjected to Asp-N digestion. The C-terminal digested peptides were then monitored by MRM. Results for various forms of the C-terminal degradation fragments are expressed as relative amount to full length peptide species (%) shown in Figure 78. Consistent with MALDI spectra, the MRM semi-quantitative analysis of the C-terminal fragments also showed reduced relative abundance of the peptide fragments missing 1-3 amino acids from the C-terminus and the increased relative abundance of the intact molecule in monkeys administered with Fc-(L15)-FGF21 (L98R, P171G, A180E) compared with Fc-(L15)-FGF21 (L98R, P171G).

In summary, Fc-(L15)-FGF21 (L98R, P171G, A180E) showed reduced C-terminal degradation and enhanced *in vivo* stability compared with Fc-(L15)-FGF21 (L98R, P171G) in *cynomolgus* monkeys.

EXAMPLE 27

Pharmacokinetics of Fc-(L15)-FGF21 (L98R, P171G, A180E) and Fc-(L15)-FGF21 (L98R, P171G) in Mice

This study was designed to assess the pharmacokinetics of Fc-(L15)-FGF21 (L98R, P171G, A180E) (SEQ ID NO:57) and Fc-(L15)-FGF21 (L98R, P171G) (SEQ ID NO:43) following a single intravenous dose to male C57BL/6 mice.

Fc-(L15)-FGF21 (L98R, P171G, A180E) and Fc-(L15)-FGF21 (L98R, P171G) were given at 20 mg/kg through intravenous injection. Blood samples were collected at 0.083 (5 minutes), 1, 4, 8, 16, 24, 48, 72, 96, 168, and 240 hours post-dosing. In order to determine plasma concentrations of intact full-length molecule, an ELISA assay with the immunoreactivity directed to the N-terminal and C-terminal FGF21 was developed. The assay tracks the full length intact molecule with negligible contaminations from other degradation products. The plasma concentrations of intact Fc-(L15)-FGF21 (L98R, P171G, A180E) and Fc-(L15)-FGF21 (L98R, P171G) over a period of 240 hours following intravenous injection in mice are shown in Figure 79.

The plasma concentrations of Fc-(L15)-FGF21 (L98R, P171G, A180E) were

significantly higher than those of Fc-(L15)-FGF21 (L98R, P171G) administered at the same dose level from 24 to 168 hours post injection. A significant amount of Fc-(L15)-FGF21 (L98R, P171G, A180E) was measurable at 168 hours post injection in mice. As a result, Fc-(L15)-FGF21 (L98R, P171G, A180E) showed increased AUC coverage and plasma circulating half-life by 2 fold compared with Fc-(L15)-FGF21 (L98R, P171G) in mice. The half-life of Fc-(L15)-FGF21 (L98R, P171G, A180E) was 16.6 hours and that of Fc-(L15)-FGF21 (L98R, P171G) was 9.4 hours. Both compounds were below detectable level at 240 hours post dose.

EXAMPLE 28

Generation of N-linked Glycosylation Mutants to Improve Solubility or Decrease C-terminal Clipping to Increase Half-life

FGF21 mutants were designed and generated to create potential N-linked glycosylation sites for mammalian expression with minimal disruption to the native amino acid sequence. The mutants constructed include FGF21 (Y179N, S181T) (SEQ ID NO:161), FGF21 Y179N (SEQ ID NO:163) and FGF21 P124S (SEQ ID NO:165).

Expression of the mutants was performed transiently in 293-6E cells and conditioned media was tested for activity in an ELK-luciferase *in vitro* assay. ELK-luciferase assays were performed as described in Example 4, with the exception that serial dilutions of conditioned media were used rather than different concentrations of purified proteins.

Analysis of the conditioned media revealed that increased glycosylation compared to wild type was not achieved in the transient expression system. Figure 80 shows the results of an ELK-luciferase activity assay. The results shown in Figure 80 demonstrate that the FGF21 P124S mutant did not adversely impact FGF21 activity but the FGF21 Y179N and FGF21 (Y179N, S181T) mutants, in the absence of glycosylation, resulted in reduced activity as assayed in the ELK-luciferase assay.

While the present invention has been described in terms of various embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations that come within the scope of the invention as claimed. In

addition, the section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

All references cited in this application are expressly incorporated by reference herein for any purpose.

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CLAIMS

What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 4 and further comprising:

(a) at least one amino acid substitution at position 180; and

(b) at least one amino acid substitution selected from the group consisting of:

(i) a substitution at position 171; and

(ii) a substitution at position 98; and

(c) combinations thereof.

2. The isolated polypeptide of claim 1, wherein the polypeptide comprises a substitution at position 98 and a substitution at position 180 and wherein:

(a) the substitution at position 98 is selected from the group consisting of arginine, cysteine, glutamic acid, glutamine, lysine, and threonine; and

(b) the substitution at position 180 is selected from the group consisting of glycine, proline, serine or glutamic acid; and

(c) combinations thereof.

3. The isolated polypeptide of claim 2, wherein the residue at position 98 is arginine and the residue at position 180 is glutamic acid.

4. A fusion polypeptide comprising the isolated polypeptide of claim 3, fused to a heterologous amino acid sequence.

5. The fusion polypeptide of claim 4, wherein the heterologous amino acid sequence is an Fc domain or fragment thereof.

6. The fusion polypeptide of claim 5, wherein the Fc domain comprises the amino acid sequence of SEQ ID NO:11.

7. The fusion polypeptide of claim 6, wherein the polypeptide is fused to the Fc domain via a linker.

8. The fusion polypeptide of claim 7, wherein the linker comprises GGGGSGGGGSGGGGS (SEQ ID NO:31).

9. The fusion polypeptide of claim 8, wherein the polypeptide comprises SEQ ID NO:47.

10. A multimer comprising two or more of the fusion polypeptide of claim 9.

11. A pharmaceutical composition comprising the isolated polypeptide of claim 2 and a pharmaceutically acceptable formulation agent.

12. The pharmaceutical composition of claim 11, wherein the pharmaceutically acceptable formulation agent is a hydrogel.

13. Use of a pharmaceutical composition of claim 12 in the manufacture of a medicament for treating a metabolic disorder, wherein the use comprises administration to a human patient in need thereof the medicament.

14. The use of claim 13, wherein the metabolic disorder is diabetes.

15. The use of claim 13, wherein the metabolic disorder is obesity.

16. An isolated nucleic acid encoding the polypeptide of claim 2.

17. The isolated nucleic acid of claim 16, wherein the nucleic acid comprises SEQ ID NO:46.

18. A vector comprising the nucleic acid molecule claim 17.

19. A host cell comprising the nucleic acid molecule of claim 18.

20. The isolated polypeptide of claim 2, wherein the polypeptide comprises:

(a) an amino-terminal truncation of no more than 8 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal;

(b) a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; or

(c) an amino-terminal truncation of no more than 8 amino acid residues and a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal.

21. The isolated polypeptide of claim 2, wherein the polypeptide is covalently linked to one or more polymers.

22. The isolated polypeptide of claim 21, wherein the polymer is PEG.

23. The isolated polypeptide of claim 1, wherein the polypeptide comprises a substitution at position 171 and a substitution at position 180 and wherein:

(a) the substitution at position 180 is selected from the group consisting of glycine, proline, serine and glutamic acid;

(b) the substitution at position 171 is selected from the group consisting of an alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, lysine, serine, threonine, tryptophan, and tyrosine; and

(c) combinations thereof.

24. The isolated polypeptide of claim 23, wherein the residue at position 171 is glycine and the residue at position 180 is glutamic acid.

25. A fusion polypeptide comprising the isolated polypeptide of claim 24, fused to a heterologous amino acid sequence.

26. The fusion polypeptide of claim 25, wherein the heterologous amino acid sequence is an Fc domain or fragment thereof.

27. The fusion polypeptide of claim 26, wherein the Fc domain comprises the amino acid sequence of SEQ ID NO:11.

28. The fusion polypeptide of claim 27, wherein the polypeptide is fused to the Fc domain via a linker.

29. The fusion polypeptide of claim 28, wherein the linker comprises GGGGSGGGGSGGGGS (SEQ ID NO:31).

30. The fusion polypeptide of claim 29, wherein the polypeptide comprises SEQ ID NO:47.

31. A multimer comprising two or more fusion polypeptides of claim 30.

32. A pharmaceutical composition comprising the isolated polypeptide of claim 30 and a pharmaceutically acceptable formulation agent.

33. The pharmaceutical composition of claim 32, wherein the pharmaceutically acceptable formulation agent is a hydrogel.

34. Use of a pharmaceutical composition of claim 32 in the manufacture of a medicament for treating a metabolic disorder, wherein the use comprises administration to a human patient in need thereof the medicament.

35. The use of claim 34, wherein the metabolic disorder is diabetes.

36. The use of claim 34, wherein the metabolic disorder is obesity.

37. An isolated nucleic acid encoding the polypeptide of claim 23.

38. The isolated nucleic acid of claim 37, wherein the nucleic acid

comprises SEQ ID NO:46.

39. A vector comprising the nucleic acid molecule claim 38.

40. A host cell comprising the nucleic acid molecule of claim 39.

41. The isolated polypeptide of claim 23, wherein the polypeptide comprises:

(a) an amino-terminal truncation of no more than 8 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal;

(b) a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; or

(c) an amino-terminal truncation of no more than 8 amino acid residues and a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal.

42. The isolated polypeptide of claim 23, wherein the polypeptide is covalently linked to one or more polymers.

43. The isolated polypeptide of claim 42, wherein the polymer is PEG.

44. The isolated polypeptide of claim 1, wherein the polypeptide comprises a substitution at position 98, a substitution at position 171 and a substitution at position 180 of SEQ ID NO:4 and wherein:

(a) the substitution at position 171 is selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, lysine, serine, threonine, tryptophan, or tyrosine;

(b) the substitution at position 98 is selected from the group consisting of arginine, cysteine, glutamic acid, glutamine, lysine, or threonine; and

(c) the substitution at position 180 is selected from the group consisting of glycine, proline, serine or glutamic acid;

and combinations thereof.

45. The isolated polypeptide of claim 44, wherein the residue at position 98 is arginine, the residue at position 171 is glycine and the residue at position 180 is glutamic acid.

46. A fusion polypeptide comprising the isolated polypeptide of claim 45 fused to a heterologous amino acid sequence.

47. The fusion polypeptide of claim 46, wherein the heterologous amino acid sequence is an Fc domain or fragment thereof.

48. The fusion polypeptide of claim 47, wherein the IgG constant domain comprises the amino acid sequence of SEQ ID NO:11.

49. The fusion polypeptide of claim 48, wherein the polypeptide is fused to the Fc domain via a linker.

50. The fusion polypeptide of claim 49, wherein the linker comprises GGGGSGGGGSGGGGS (SEQ ID NO:31).

51. The fusion polypeptide of claim 50, wherein the polypeptide comprises SEQ ID NO:47.

52. A multimer comprising two or more fusion polypeptides of claim 51.

53. A polypeptide comprising:

(a) the polypeptide of SEQ ID NO:4, wherein

(i) the leucine at position 98 is substituted with an arginine;

(ii) the proline at position 171 is substituted with glycine; and

(iii) the alanine at position 180 is substituted with glutamic acid;

(b) a linker sequence comprising SEQ ID NO:31; and

(c) an Fc domain comprising SEQ ID NO:11.

54. A polypeptide comprising the sequence of SEQ ID NO:47.

55. A polypeptide encoded by the nucleic acid sequence of SEQ ID NO:46.

56. A pharmaceutical composition comprising the isolated polypeptide of claims 44 or 53 and a pharmaceutically acceptable formulation agent.

57. The pharmaceutical composition of claim 56, wherein the pharmaceutically acceptable formulation agent is a hydrogel.

58. Use of a pharmaceutical composition of claim 56 or 57 in the manufacture of a medicament for treating a metabolic disorder, wherein the use comprises administration to a human patient in need thereof the medicament.

59. The use of claim 58, wherein the metabolic disorder is diabetes.

60. The use of claim 58, wherein the metabolic disorder is obesity.

61. An isolated nucleic acid encoding the polypeptide of claims 44 or 53.

62. The isolated nucleic acid of claim 61, wherein the nucleic acid comprises SEQ ID NO:46.

63. A vector comprising the nucleic acid molecule claim 62.

64. A host cell comprising the nucleic acid molecule of claim 63.

65. The isolated polypeptide of claims 44 or 53, wherein the polypeptide comprises:

(a) an amino-terminal truncation of no more than 8 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal;

(b) a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; or

(c) an amino-terminal truncation of no more than 8 amino acid residues and a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal.

66. The isolated polypeptide of claim 44 or 53, wherein the polypeptide is covalently linked to one or more polymers.

67. The isolated polypeptide of claim 66, wherein the polymer is PEG.

SEQUENCE LISTING

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Hecht, Randy I.
Li, Yue-Sheng
Michaels, Mark L.
Sun, Jeonghoon
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Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu
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            100                 105                 110

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        115                 120                 125

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gac tcc agt cct ctc ctg caa ttc ggg ggc caa gtc cgg cag cgg tac         144
Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr
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ctc tac aca gat gat gcc cag cag aca gaa gcc cac ctg gag atc agg         192
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Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu
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gag gac gga tac aat gtt tac cag tcc gaa gcc cac ggc ctc ccg ctg         432
Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu
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cac ctg cca ggg aac aag tcc cca cac cgg gac cct gca ccc cga gga         480
His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly
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            180                 185                 190

cct ctg agc atg gtg gga cct tcc cag ggc cga agc ccc agc tac gct         624
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Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg
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Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu
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Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val
                85                  90                  95

Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly
            100                 105                 110

Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu
        115                 120                 125

Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu
    130                 135                 140

His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly
145                 150                 155                 160

Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu Pro Glu
                165                 170                 175

Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp
            180                 185                 190

Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser Tyr Ala
        195                 200                 205

Ser
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<210> 7
<211> 546
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(546)

<400> 7
cac ccc atc cct gac tcc agt cct ctc ctg caa ttc ggg ggc caa gtc 48

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

cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc cac          96
Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
            20                  25                  30

ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag agc         144
Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
        35                  40                  45

ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att caa         192
Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
    50                  55                  60

atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat ggg         240
Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
65                  70                  75                  80

gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc cgg         288
Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                85                  90                  95

gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc cac         336
Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100                 105                 110

ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac cct         384
Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115                 120                 125

gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc ccc         432
Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130                 135                 140

gca ctc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat gtg         480
Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145                 150                 155                 160

ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga agc         528
Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser
                165                 170                 175

ccc agc tac gct tcc tga                                                 546
Pro Ser Tyr Ala Ser
            180


<210>  8
<211>  181
<212>  PRT
<213>  Homo sapiens

<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 Gly Pro Ser Gln Gly Arg Ser
165 170 175

Pro Ser Tyr Ala Ser
180

<210> 9
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> portion of a mature human FGF21 polypeptide

<400> 9

Met His Pro Ile Pro Asp Ser Ser Pro Leu
1 5 10

<210> 10
<211> 681
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(681)

<400> 10
gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg ggg 48
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1 5 10 15

gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg 96
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
20 25 30

```
atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac        144
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        35                  40                  45

gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg        192
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    50                  55                  60

cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg tac        240
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65                  70                  75                  80

cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc        288
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                85                  90                  95

aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc        336
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            100                 105                 110

gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg        384
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        115                 120                 125

tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc agc        432
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
    130                 135                 140

ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag        480
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145                 150                 155                 160

tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc        528
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                165                 170                 175

gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg        576
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            180                 185                 190

gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg        624
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        195                 200                 205

cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct        672
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    210                 215                 220

ccg ggt aaa                                                            681
Pro Gly Lys
225
```

<210> 11
<211> 227
<212> PRT
<213> Homo sapiens

<400> 11

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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
225

<210> 12
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 12
aggaggaata acatatgcat ccaattccag attcttctcc 40

<210> 13
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 13
tagtgagctc gaattcttag gaagcgtagc tgg 33

<210> 14
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 14
ggagatatac atatgccaat tccagattct tctccattat t 41

<210> 15
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 15
catatgtata tctccttctt aaagttaaac aaaa 34

<210> 16
<211> 34
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<400> 16
aaaacaaatt gaaattcttc ctctatatgt atac 34

<210> 17
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<223> sense strand from a portion of an FGF21 expression construct

<400> 17
ttttgtttaa ctttaagaag gagatataca tatgcatcca attccagatt cttctccatt 60

att 63

<210> 18
<211> 63
<212> DNA
<213> Artificial

<220>
<223> antisense strand from a portion of an FGF21 expression construct

<400> 18
aaaacaaatt gaaattcttc ctctatatgt atacgtaggt taaggtctaa gaagaggtaa 60

taa 63

<210> 19
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 19
aggaggaata acatatggac aaaactcaca catg 34

<210> 20
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 20
ggatccacca ccaccgctac cac 23

<210> 21
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 21
ggtggtggtg gatcccatcc aattccagat tcttctcca 39

<210> 22
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 22
tagtgagctc gaattcttag gaagcgtagc tgg 33

<210> 23
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 23
atggtggaac cttcccaggg ccgaagc 27

<210> 24
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 24
ggaaggttcc accatgctca gagggtccga 30

<210> 25
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> sense strand from a portion of an FGF21 expression construct

<400> 25
ctcctcggac cctctgagca tggtgggacc ttcccagggc cgaagcccca 50

<210> 26
<211> 30
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<400> 26
agcctgggag actcgtacca ccttggaagg 30

<210> 27
<211> 50
<212> DNA
<213> artificial

<220>
<223> antisense strand from a portion of an FGF21 expression construct

<400> 27
gaggagcctg ggagactcgt accaccctgg aagggtcccg gcttcggggt 50

<210> 28
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> G5SG3SG4S (L15) Linker

<400> 28

Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 29
<211> 4
<212> PRT
<213> Artificial

<220>
<223> G4 Linker

<400> 29

Gly Gly Gly Gly
1

<210> 30
<211> 5
<212> PRT
<213> Artificial

<220>
<223> G5 Linker

<400> 30

Gly Gly Gly Gly Gly
1 5

<210> 31
<211> 15
<212> PRT
<213> Artificial

<220>
<223> (G4S)3 Linker

<400> 31

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 32
<211> 8
<212> PRT
<213> Artificial

<220>
<223> G3KG4 linker

<400> 32

Gly Gly Gly Lys Gly Gly Gly Gly
1 5

<210> 33
<211> 8
<212> PRT
<213> Artificial

<220>
<223> G3NGSG2 Linker

<400> 33

Gly Gly Gly Asn Gly Ser Gly Gly
1 5

<210> 34
<211> 8
<212> PRT
<213> Artificial

<220>
<223> G3CG4 linker

<400> 34

Gly Gly Gly Cys Gly Gly Gly Gly
1 5

<210> 35
<211> 5
<212> PRT
<213> Artificial

<220>
<223> GPNG2 Linker

<400> 35

Gly Pro Asn Gly Gly
1 5

<210> 36
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 RG

<220>
<221> CDS
<222> (1)..(549)

<400> 36
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa 48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1 5 10 15

gtc cgg cag cgt tac ctc tac aca gat gat gcc cag cag aca gaa gcc 96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
20 25 30

cac ctg gag atc agg gag gac ggg acg gtg ggg ggt gct gct gac cag 144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
35 40 45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg ggt gtt att 192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
50 55 60

caa atc ttg ggt gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat 240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65 70 75 80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc 288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
85 90 95

cgt gag cgt ctt ctt gag gac ggt tac aat gtt tac cag tcc gaa gcc 336
Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
100 105 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgt gac 384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
115 120 125

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc 432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro

130 135 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat 480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145 150 155 160

gtg ggc tcc tcg gac cct ctg agc atg gtg ggt ggt tcc cag ggc cga 528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln Gly Arg
165 170 175

agc ccc agc tac gct tcc taa 549
Ser Pro Ser Tyr Ala Ser
180

<210> 37
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 37

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

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

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

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

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

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

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

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

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

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

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

```
Ser Pro Ser Tyr Ala Ser
            180


<210>  38
<211>  546
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  FGF21 RGE


<220>
<221>  CDS
<222>  (1)..(546)

<400>  38
cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa gtc          48
His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
1               5                   10                  15

cgg cag cgt tac ctc tac aca gat gat gcc cag cag aca gaa gcc cac          96
Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
            20                  25                  30

ctg gag atc agg gag gac ggg acg gtg ggg ggt gct gct gac cag agc         144
Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
        35                  40                  45

ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg ggt gtt att caa         192
Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
    50                  55                  60

atc ttg ggt gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat ggg         240
Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
65                  70                  75                  80

gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc cgt         288
Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
                85                  90                  95

gag cgt ctt ctt gag gac ggt tac aat gtt tac cag tcc gaa gcc cac         336
Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
            100                 105                 110

ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgt gac cct         384
Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
        115                 120                 125

gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc ccc         432
Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
    130                 135                 140

gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat gtg         480
Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145                 150                 155                 160

ggc tcc tcg gac cct ctg agc atg gtg ggt ggt tcc cag ggc cga agc         528
Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln Gly Arg Ser
                165                 170                 175

ccc agc tac gaa tcc taa                                                 546
Pro Ser Tyr Glu Ser
            180
```

<210> 39
<211> 181
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 39

```
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 Arg 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 Pro 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 Gly Ser Gln Gly Arg Ser
                165                 170                 175

Pro Ser Tyr Glu Ser
            180
```

<210> 40
<211> 1272
<212> DNA
<213> Artificial Sequence

<220>
<223> WT21-L15-Fc

<220>

<221> CDS
<222> (1)..(1272)

<400> 40

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atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa          48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1               5                   10                  15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc          96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag         144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att         192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat         240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc         288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc         336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac         384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg cca         432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat         480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga         528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc ggt gga ggt ggt ggt tct ggt ggt ggt agc         576
Ser Pro Ser Tyr Ala Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser
            180                 185                 190

ggt ggt ggt gga tcc gac aaa act cac aca tgc cca ccg tgc cca gca         624
Gly Gly Gly Gly Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
        195                 200                 205

cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc         672
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
    210                 215                 220

aag gac acc ctc atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg         720
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
225                 230                 235                 240

gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg         768
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
                245                 250                 255
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gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgt gag gag cag        816
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            260                 265                 270

tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag        864
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        275                 280                 285

gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc        912
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
    290                 295                 300

ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc        960
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
305                 310                 315                 320

cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc       1008
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
                325                 330                 335

aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc       1056
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            340                 345                 350

gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac       1104
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        355                 360                 365

aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tat       1152
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    370                 375                 380

agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc       1200
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
385                 390                 395                 400

tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag       1248
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                405                 410                 415

agc ctc tcc ctg tct ccg ggt aaa                                      1272
Ser Leu Ser Leu Ser Pro Gly Lys
            420
```

<210> 41
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 41

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

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

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

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

```
50                  55                  60

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

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

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

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

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

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

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

Ser Pro Ser Tyr Ala Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser
            180                 185                 190

Gly Gly Gly Gly Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
        195                 200                 205

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
    210                 215                 220

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
225                 230                 235                 240

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
                245                 250                 255

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            260                 265                 270

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        275                 280                 285

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
    290                 295                 300

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
305                 310                 315                 320

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
```

325 330 335

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
340 345 350

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
355 360 365

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
370 375 380

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
385 390 395 400

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
405 410 415

Ser Leu Ser Leu Ser Pro Gly Lys
420

<210> 42
<211> 1275
<212> DNA
<213> Artificial

<220>
<223> Fc L15 RG

<220>
<221> CDS
<222> (1)..(1275)

<400> 42
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg 48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1 5 10 15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc 96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
20 25 30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc 144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
35 40 45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag 192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
50 55 60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg 240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65 70 75 80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat 288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
85 90 95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc 336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
100 105 110

```
atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag cgt ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380
```

```
ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga ggt tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 43
<211> 424
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 43

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

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

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 44
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-(G4S)3-RG

<220>
<221> CDS
<222> (1)..(1275)

<400> 44

```
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg         672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt ggt ggt ggt tcc ggt ggc ggc ggc tct ggt ggt         720
Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
```

```
225                 230                 235                 240

ggt ggc agc cat ccg atc ccg gac tct tct ccg ctg ctg cag ttc ggt        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggt cag gtt cgt cag cgt tac ctg tac acc gac gac gcg cag cag acc        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gag gcg cac ctg gag atc cgt gaa gac ggt acc gtt ggt ggt gcg gcc        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag tct ccg gaa tct ctg ctg cag ctg aaa gcc ctg aaa ccg ggt        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt atc cag atc ctg ggc gtt aaa acc tct cgt ttc ctg tgc cag cgt        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

ccg gac ggc gcc ctg tac ggt tct ctg cac ttc gac ccg gag gcg tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

tct ttt cgt gaa cgt ctg ctc gaa gac ggt tac aac gtt tac cag tct       1056
Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gag gcg cac ggt ctg ccg ctg cac ctg ccg ggt aac aaa tct ccg cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgt gac ccg gcg cca cgt ggt cct gcg cgt ttc ctg cca ctg ccg ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccg cct gcg cct cct gaa ccg cct ggt atc ctg gct ccg cag ccg       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

cca gac gtt ggt tct tct gac ccg ctg tct atg gtt ggt ggc tct cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

ggt cgt tct ccg tct tac gcc tct taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 45
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 45

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300
```

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

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

Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

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

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 46
<211> 1275
<212> DNA
<213> Artificial

<220>
<223> Fc-(G4S)3-RGE

<220>
<221> CDS
<222> (1)..(1275)

<400> 46

```
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
```

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

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc  336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag  384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc  432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg  480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct  528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc  576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg  624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg  672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt ggt ggt ggt tct ggt ggt ggt ggt agc ggt ggt  720
Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt ggt tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg  768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgt tac ctc tac aca gat gat gcc cag cag aca  816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gac ggg acg gtg ggg ggt gct gct  864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg ggt  912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg ggt gtc aag aca tcc agg ttc ctg tgc cag cgg  960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc 1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgt gag cgt ctt ctt gag gac ggt tac aat gtt tac cag tcc 1056
Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac 1104
```

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

cgt gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg ggt ggt tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gaa tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Glu Ser
            420
```

<210> 47
<211> 424
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 47

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

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

Gly Arg Ser Pro Ser Tyr Glu Ser
            420
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<210> 48
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-WT21

<220>
<221> CDS
<222> (1)..(1275)

<400> 48

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205
```

```
atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 49
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 49

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Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
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```
1               5                   10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
```

275 280 285

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
290 295 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
340 345 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
355 360 365

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

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

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

Gly Arg Ser Pro Ser Tyr Ala Ser
420

<210> 50
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-G170E

<220>
<221> CDS
<222> (1)..(1275)

<400> 50
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg 48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1 5 10 15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc 96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
20 25 30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc 144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
35 40 45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag 192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
50 55 60

```
gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg        240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat        288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc        336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335
```

```
agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gaa cct tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Glu Pro Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 51
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 51

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Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

```
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400
```

```
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Glu Pro Ser Gln
                405                 410                 415


Gly Arg Ser Pro Ser Tyr Ala Ser
            420


<210>  52
<211>  1275
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Fc-L15-P171A


<220>
<221>  CDS
<222>  (1)..(1275)

<400>  52
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
```

```
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga gct tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Ala Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 53
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 53

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

```
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Ala Ser Gln
                405                 410                 415

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 54
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-S172L

<220>
<221> CDS
<222> (1)..(1275)

<400> 54

```
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
```

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

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag        192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg        240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat        288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc        336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
```

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

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct ctg cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Leu Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 55
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 55

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

```
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

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

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 56
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-RGE

<220>
<221> CDS
<222> (1)..(1275)

<400> 56

```
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160
```

```
gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt ggt ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt ggt tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgt tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gac ggg acg gtg ggg ggt gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg ggt        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg ggt gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgt gag cgt ctt ctt gag gac ggt tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgt gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg ggt ggt tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gaa tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Glu Ser
            420
```

<210> 57
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 57

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1 5 10 15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly

225 230 235 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
260 265 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
290 295 300

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

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

Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
340 345 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
355 360 365

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

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

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

Gly Arg Ser Pro Ser Tyr Glu Ser
420

<210> 58
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-G170E P171A S172L

<220>
<221> CDS
<222> (1)..(1275)

<400> 58
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg 48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1 5 10 15

```
ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg         672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt         720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg         768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca         816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct         864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285
```

```
gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gaa gct ctg cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Glu Ala Leu Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 59
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 59

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350
```

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

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

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

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

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
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<210> 60
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-G151A

<220>
<221> CDS
<222> (1)..(1275)

<400> 60

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
```

```
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gca atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Ala Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln
```

405 410 415

ggc cga agc ccc agc tac gct tcc taa 1275
Gly Arg Ser Pro Ser Tyr Ala Ser
420

<210> 61
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 61

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1 5 10 15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

Leu Pro Pro Ala Pro Pro Glu Pro Pro Ala Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

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

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 62
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-G170E P171A

<220>
<221> CDS

<222> (1)..(1275)

<400> 62

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg         672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt         720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg         768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca         816
```

```
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gaa gct tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Glu Ala Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 63
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 63

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60
```

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

```
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

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

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 64
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-P150A G151A I152V

<220>
<221> CDS
<222> (1)..(1275)

<400> 64

```
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110
```

```
atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380
```

```
ctg ccc ccc gca ccc ccg gag cca gcc gca gtt ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Ala Ala Val Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                   1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 65
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 65

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Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

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

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

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

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
```

180 185 190

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225 230 235 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
260 265 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
290 295 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
340 345 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
355 360 365

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

Leu Pro Pro Ala Pro Pro Glu Pro Ala Ala Val Leu Ala Pro Gln Pro
385 390 395 400

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

Gly Arg Ser Pro Ser Tyr Ala Ser
420

<210> 66
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-G170E S172L

<220>
<221> CDS
<222> (1)..(1275)

<400> 66

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg         672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt         720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240
```

```
ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gaa cct ctg cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Glu Pro Leu Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 67
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 67

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Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300
```

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Glu Pro Leu Gln
                405                 410                 415

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
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<210> 68
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-G170A

<220>
<221> CDS
<222> (1)..(1275)

<400> 68

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
```

```
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc        336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
```

```
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gct cct tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Ala Pro Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420


<210>  69
<211>  424
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  69

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Ala Pro Ser Gln
                405                 410                 415

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
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<210> 70
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-G170C

<220>
<221> CDS
<222> (1)..(1275)

<400> 70
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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg         672
```

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

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg tgc cct tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Cys Pro Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 71
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 71

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Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15
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```
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285
```

```
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Cys Pro Ser Gln
                405                 410                 415

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 72
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-G170D

<220>
<221> CDS
<222> (1)..(1275)

<400> 72

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60
```

```
gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg        240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat        288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc        336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335
```

```
agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gac cct tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Asp Pro Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 73
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 73

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

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

130 135 140

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225 230 235 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
260 265 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
290 295 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
340 345 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
355 360 365

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

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

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Asp Pro Ser Gln

405 410 415

Gly Arg Ser Pro Ser Tyr Ala Ser
420

<210> 74
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-G170N

<220>
<221> CDS
<222> (1)..(1275)

<400> 74

```
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190
```

```
gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg aac cct tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Asn Pro Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 75
<211> 424
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 75

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

```
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

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

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 76
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-G170S

<220>
<221> CDS
<222> (1)..(1275)

<400> 76

```
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
```

```
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag        192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg        240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat        288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc        336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
```

```
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg tcc cct tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Ser Pro Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 77
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 77

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

```
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

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

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 78
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-P171E

<220>
<221> CDS
<222> (1)..(1275)

<400> 78

```
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
```

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

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga gaa tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Glu Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 79
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 79

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240
```

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Glu Ser Gln
                405                 410                 415

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 80
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-P171H

<220>
<221> CDS
<222> (1)..(1275)

<400> 80

```
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg        48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15
```

```
ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg         672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt         720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg         768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca         816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct         864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285
```

```
gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga cac tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly His Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 81
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 81

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
```

85 90 95

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225 230 235 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
260 265 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
290 295 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
340 345 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His

```
        355                 360                 365

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

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

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly His Ser Gln
                405                 410                 415

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 82
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-P171Q

<220>
<221> CDS
<222> (1)..(1275)

<400> 82

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140
```

```
agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga cag tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gln Ser Gln
                405                 410                 415
```

ggc cga agc ccc agc tac gct tcc taa 1275
Gly Arg Ser Pro Ser Tyr Ala Ser
420

<210> 83
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 83

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1 5 10 15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

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

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 84
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-P171T

<220>
<221> CDS
<222> (1)..(1275)

<400> 84

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg         672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt         720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg         768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca         816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
```

```
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga act tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Thr Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420


<210>  85
<211>  424
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  85

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15


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


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


His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60
```

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
340 345 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
355 360 365

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

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

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Thr Ser Gln
405 410 415

Gly Arg Ser Pro Ser Tyr Ala Ser
420

<210> 86
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-P171Y

<220>
<221> CDS
<222> (1)..(1275)

<400> 86
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg 48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1 5 10 15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc 96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
20 25 30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc 144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
35 40 45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag 192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
50 55 60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg 240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65 70 75 80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat 288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
85 90 95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc 336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
100 105 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag 384

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

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
```

```
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga tac tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Tyr Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                   1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 87
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 87

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Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Tyr Ser Gln
                405                 410                 415

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 88
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>

<223> Fc-L15-P171G

<220>
<221> CDS
<222> (1)..(1275)

<400> 88

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg         672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt         720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240
```

```
ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga ggt tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 89
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 89

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
```

```
        35                  40                  45

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
```

305 310 315 320

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
340 345 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
355 360 365

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

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

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

Gly Arg Ser Pro Ser Tyr Ala Ser
420

<210> 90
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-P171S

<220>
<221> CDS
<222> (1)..(1275)

<400> 90
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg 48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1 5 10 15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc 96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
20 25 30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc 144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
35 40 45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag 192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
50 55 60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg 240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65 70 75 80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat 288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
85 90 95

```
ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc        336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365
```

```
cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga tct tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Ser Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 91
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 91

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

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

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 92
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-P171W

<220>
<221> CDS
<222> (1)..(1275)

<400> 92

```
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg         672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
```

```
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga tgg tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Trp Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 93
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 93

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15
```

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285
```

```
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Trp Ser Gln
                405                 410                 415

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 94
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-P171C

<220>
<221> CDS
<222> (1)..(1275)

<400> 94

```
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
```

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

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat        288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc        336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
```

```
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga tgc tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Cys Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420


<210>  95
<211>  424
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  95

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15


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


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


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


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


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


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


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


Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140
```

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225 230 235 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
260 265 270

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

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
290 295 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
340 345 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
355 360 365

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

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

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Cys Ser Gln
405 410 415

```
Gly Arg Ser Pro Ser Tyr Ala Ser
            420


<210>  96
<211>  1275
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Fc-L15-A45K G170E


<220>
<221>  CDS
<222>  (1)..(1275)

<400>  96
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190
```

```
gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct aaa        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Lys
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gaa cct tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Glu Pro Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 97
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 97

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Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
```

260 265 270

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Lys
275 280 285

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
290 295 300

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
340 345 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
355 360 365

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

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

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Glu Pro Ser Gln
405 410 415

Gly Arg Ser Pro Ser Tyr Ala Ser
420

<210> 98
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 A45K

<220>
<221> CDS
<222> (1)..(549)

<400> 98
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa 48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1 5 10 15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc 96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
20 25 30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct aaa gac cag 144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Lys Asp Gln
35 40 45

```
agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att        192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat        240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc        288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc        336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac        384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc        432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat        480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga        528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                          549
Ser Pro Ser Tyr Ala Ser
            180
```

<210> 99
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 99

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Ser Tyr Ala Ser
            180
```

<210> 100
<211> 1260
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-FGF21 6-181 G170E

<220>
<221> CDS
<222> (1)..(1260)

<400> 100

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
```

```
                100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc tct tct cca tta tta caa ttc ggt ggt caa gtc cgg cag        768
Gly Gly Ser Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln
                245                 250                 255

cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc cac ctg gag        816
Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu
            260                 265                 270

atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag agc ccc gaa        864
Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu
        275                 280                 285

agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att caa atc ttg        912
Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu
    290                 295                 300

gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat ggg gcc ctg        960
Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu
305                 310                 315                 320

tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc cgg gag ctg       1008
Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu
                325                 330                 335

ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc cac ggc ctc       1056
Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu
            340                 345                 350

ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac cct gca ccc       1104
Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro
        355                 360                 365

cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc ccc gca ccc       1152
Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Pro
```

370 375 380

ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat gtg ggc tcc 1200
Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser
385 390 395 400

tcg gac cct ctg agc atg gtg gaa cct tcc cag ggc cga agc ccc agc 1248
Ser Asp Pro Leu Ser Met Val Glu Pro Ser Gln Gly Arg Ser Pro Ser
405 410 415

tac gct tcc taa 1260
Tyr Ala Ser

<210> 101
<211> 419
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 101

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1 5 10 15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

Gly Gly Ser 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 Pro
    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 Glu Pro Ser Gln Gly Arg Ser Pro Ser
                405                 410                 415

Tyr Ala Ser
```

<210> 102
<211> 1275
<212> DNA

<213> Artificial Sequence

<220>
<223> Fc-L15-A45K P171G

<220>
<221> CDS
<222> (1)..(1275)

<400> 102

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg         672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt         720
```

```
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct aaa        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Lys
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga ggt tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 103
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 103

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

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

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Lys
        275                 280                 285

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300
```

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

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

Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

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

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

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

Gly Arg Ser Pro Ser Tyr Ala Ser
            420
```

<210> 104
<211> 1320
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21-(G3)-Fc; 28 AA signal sequence removed during processing

<220>
<221> CDS
<222> (1)..(1320)

<400> 104

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atg gac tcg gac gag acc ggg ttc gag cac tca gga ctg tgg gtt tct         48
Met Asp Ser Asp Glu Thr Gly Phe Glu His Ser Gly Leu Trp Val Ser
1               5                   10                  15

gtg ctg gct ggt ctt ctg ctg gga gcc tgc cag gca cac ccc atc cct         96
Val Leu Ala Gly Leu Leu Leu Gly Ala Cys Gln Ala His Pro Ile Pro
            20                  25                  30

gac tcc agt cct ctc ctg caa ttc ggg ggc caa gtc cgg cag cgg tac        144
Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr
        35                  40                  45

ctc tac aca gat gat gcc cag cag aca gaa gcc cac ctg gag atc agg        192
Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg
    50                  55                  60

gag gat ggg acg gtg ggg ggc gct gct gac cag agc ccc gaa agt ctc        240
Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu
65                  70                  75                  80
```

```
ctg cag ctg aaa gcc ttg aag ccg gga gtt att caa atc ttg gga gtc        288
Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val
                85                  90                  95

aag aca tcc agg ttc ctg tgc cag cgg cca gat ggg gcc ctg tat gga        336
Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly
            100                 105                 110

tcg ctc cac ttt gac cct gag gcc tgc agc ttc cgg gag ctg ctt ctt        384
Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu
        115                 120                 125

gag gac gga tac aat gtt tac cag tcc gaa gcc cac ggc ctc ccg ctg        432
Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu
    130                 135                 140

cac ctg cca ggg aac aag tcc cca cac cgg gac cct gca ccc cga gga        480
His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly
145                 150                 155                 160

cca gct cgc ttc ctg cca cta cca ggc ctg cca ccc gca ccc ccg gag        528
Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Pro Pro Glu
                165                 170                 175

cca ccc gga atc ctg gcc ccc cag ccc ccc gat gtg ggc tcc tcg gac        576
Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp
            180                 185                 190

cct ctg agc atg gtg gga cct tcc cag ggc cga agc ccc agc tac gct        624
Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser Tyr Ala
        195                 200                 205

tcc ggt gga ggt gac aaa act cac aca tgc cca ccg tgc cca gca cct        672
Ser Gly Gly Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
    210                 215                 220

gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag        720
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
225                 230                 235                 240

gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg        768
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
                245                 250                 255

gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac        816
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
            260                 265                 270

ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac        864
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
        275                 280                 285

aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac        912
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
    290                 295                 300

tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc        960
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
305                 310                 315                 320

cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga       1008
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
                325                 330                 335

gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag       1056
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
            340                 345                 350
```

```
aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac       1104
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
        355                 360                 365

atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag       1152
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
    370                 375                 380

acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tat agc       1200
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
385                 390                 395                 400

aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca       1248
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
                405                 410                 415

tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc       1296
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
            420                 425                 430

ctc tcc ctg tct ccg ggt aaa tga                                      1320
Leu Ser Leu Ser Pro Gly Lys
        435
```

<210> 105
<211> 439
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 105

```
Met Asp Ser Asp Glu Thr Gly Phe Glu His Ser Gly Leu Trp Val Ser
1               5                   10                  15

Val Leu Ala Gly Leu Leu Leu Gly Ala Cys Gln Ala His Pro Ile Pro
            20                  25                  30

Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr
        35                  40                  45

Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg
    50                  55                  60

Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu
65                  70                  75                  80

Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val
                85                  90                  95

Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly
            100                 105                 110

Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu
        115                 120                 125

Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu
```

130 135 140

His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly
145 150 155 160

Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Pro Pro Glu
165 170 175

Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp
180 185 190

Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser Tyr Ala
195 200 205

Ser Gly Gly Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
210 215 220

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
225 230 235 240

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
245 250 255

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
260 265 270

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
275 280 285

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
290 295 300

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

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
325 330 335

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
340 345 350

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
355 360 365

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
370 375 380

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
385 390 395 400

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

405 410 415

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
420 425 430

Leu Ser Leu Ser Pro Gly Lys
435

<210> 106
<211> 1245
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-G5-FGF21

<220>
<221> CDS
<222> (1)..(1245)

<400> 106

```
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175
```

```
ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt ggc gga ggg ggt cat cca att cca gat tct tct        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly His Pro Ile Pro Asp Ser Ser
225                 230                 235                 240

cca tta tta caa ttc ggg ggc caa gtc cgg cag cgg tac ctc tac aca        768
Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr
                245                 250                 255

gat gat gcc cag cag aca gaa gcc cac ctg gag atc agg gag gat ggg        816
Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg Glu Asp Gly
            260                 265                 270

acg gtg ggg ggc gct gct gac cag agc ccc gaa agt ctc ctg cag ctg        864
Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu
        275                 280                 285

aaa gcc ttg aag ccg gga gtt att caa atc ttg gga gtc aag aca tcc        912
Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser
    290                 295                 300

agg ttc ctg tgc cag cgg cca gat ggg gcc ctg tat gga tcg ctc cac        960
Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His
305                 310                 315                 320

ttt gac cct gag gcc tgc agc ttc cgg gag ctg ctt ctt gag gac gga       1008
Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly
                325                 330                 335

tac aat gtt tac cag tcc gaa gcc cac ggc ctc ccg ctg cac ctg cca       1056
Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro
            340                 345                 350

ggg aac aag tcc cca cac cgg gac cct gca ccc cga gga cca gct cgc       1104
Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg
        355                 360                 365

ttc ctg cca cta cca ggc ctg ccc ccc gca ccc ccg gag cca ccc gga       1152
Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly
    370                 375                 380

atc ctg gcc ccc cag ccc ccc gat gtg ggc tcc tcg gac cct ctg agc       1200
Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser
385                 390                 395                 400

atg gtg gga cct tcc cag ggc cga agc ccc agc tac gct tcc taa           1245
Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser Tyr Ala Ser
                405                 410
```

<210> 107
<211> 414
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 107

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

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

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

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Gly Lys Gly Gly Gly Gly Gly His Pro Ile Pro Asp Ser Ser
225                 230                 235                 240

Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr
                245                 250                 255
```

```
Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg Glu Asp Gly
            260                 265                 270

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

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

Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His
305                 310                 315                 320

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

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

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

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

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

Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser Tyr Ala Ser
                405                 410
```

<210> 108
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 L99R

<220>
<221> CDS
<222> (1)..(549)

<400> 108

```
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa          48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1               5                   10                  15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc          96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag         144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att         192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
```

```
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat        240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc        288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag ctg cgt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc        336
Arg Glu Leu Arg Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac        384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc        432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat        480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga        528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                           549
Ser Pro Ser Tyr Ala Ser
            180
```

<210> 109
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 109

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Ser Tyr Ala Ser
            180
```

<210> 110
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 L99D

<220>
<221> CDS
<222> (1)..(549)

<400> 110

```
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa          48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1               5                   10                  15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc          96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag         144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att         192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat         240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc         288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag ctg gac ctt gag gac gga tac aat gtt tac cag tcc gaa gcc         336
Arg Glu Leu Asp Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac         384
```

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

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc        432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat        480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga        528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                           549
Ser Pro Ser Tyr Ala Ser
            180


<210>  111
<211>  182
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  111

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


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


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


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


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


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


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


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


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


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

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

Ser Pro Ser Tyr Ala Ser
            180


<210>  112
<211>  549
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  FGF21 A111T


<220>
<221>  CDS
<222>  (1)..(549)

<400>  112
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa          48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1               5                   10                  15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc          96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag         144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att         192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat         240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc         288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa acc         336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Thr
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac         384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc         432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat         480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga         528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175
```

agc ccc agc tac gct tcc taa 549
Ser Pro Ser Tyr Ala Ser
180

<210> 113
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 113

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

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

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

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

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

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

Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Thr
100 105 110

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

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

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

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

Ser Pro Ser Tyr Ala Ser
180

<210> 114
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 A129D

<220>
<221> CDS
<222> (1)..(549)

<400> 114

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atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa          48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1               5                   10                  15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc          96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag         144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att         192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat         240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc         288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc         336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac         384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct gac ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc         432
Pro Asp Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat         480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga         528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                             549
Ser Pro Ser Tyr Ala Ser
            180
```

<210> 115
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 115

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Ser Tyr Ala Ser
180

<210> 116
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 A129Q

<220>
<221> CDS
<222> (1)..(549)

<400> 116
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa 48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1 5 10 15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc 96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala

```
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag        144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att        192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat        240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc        288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc        336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac        384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct cag ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc        432
Pro Gln Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat        480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga        528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                           549
Ser Pro Ser Tyr Ala Ser
            180
```

<210> 117
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 117

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Ser Tyr Ala Ser
            180
```

<210> 118
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 A134K

<220>
<221> CDS
<222> (1)..(549)

<400> 118

```
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa          48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1               5                   10                  15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc          96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag         144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att         192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat         240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc         288
```

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

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc        336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac        384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct gca ccc cga gga cca aaa cgc ttc ctg cca cta cca ggc ctg ccc        432
Pro Ala Pro Arg Gly Pro Lys Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat        480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga        528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                           549
Ser Pro Ser Tyr Ala Ser
            180
```

<210> 119
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 119

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Ser Tyr Ala Ser
            180


<210>  120
<211>  549
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  FGF21 A134Y


<220>
<221>  CDS
<222>  (1)..(549)

<400>  120
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa          48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1               5                   10                  15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc          96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag         144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att         192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat         240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc         288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc         336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac         384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct gca ccc cga gga cca tat cgc ttc ctg cca cta cca ggc ctg ccc         432
Pro Ala Pro Arg Gly Pro Tyr Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140
```

```
ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat        480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga        528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                        549
Ser Pro Ser Tyr Ala Ser
            180


<210>  121
<211>  182
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  121

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Ser Tyr Ala Ser
```

180

<210> 122
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 A134E

<220>
<221> CDS
<222> (1)..(549)

<400> 122

```
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa          48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1               5                  10                  15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc          96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag         144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att         192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat         240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc         288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc         336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac         384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct gca ccc cga gga cca gaa cgc ttc ctg cca cta cca ggc ctg ccc         432
Pro Ala Pro Arg Gly Pro Glu Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat         480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga         528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                             549
Ser Pro Ser Tyr Ala Ser
            180
```

<210> 123
<211> 182

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 123

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Ser Tyr Ala Ser
180

<210> 124
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 A129K

<220>
<221> CDS
<222> (1)..(549)

<400> 124
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa 48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1 5 10 15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc 96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
20 25 30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag 144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
35 40 45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att 192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
50 55 60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat 240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65 70 75 80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc 288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
85 90 95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc 336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
100 105 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac 384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
115 120 125

cct aaa ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc 432
Pro Lys Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
130 135 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat 480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145 150 155 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga 528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
165 170 175

agc ccc agc tac gct tcc taa 549
Ser Pro Ser Tyr Ala Ser
180

<210> 125
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 125

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Ser Tyr Ala Ser
            180
```

<210> 126
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 P78C

<220>
<221> CDS
<222> (1)..(549)

<400> 126

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atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa          48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1               5                   10                  15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc          96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag         144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att         192
```

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

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg tgc gat        240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Cys Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc        288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc        336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac        384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc        432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat        480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga        528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                           549
Ser Pro Ser Tyr Ala Ser
            180
```

<210> 127
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 127

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Ser Tyr Ala Ser
            180
```

<210> 128
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 P78R

<220>
<221> CDS
<222> (1)..(549)

<400> 128

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atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa          48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1               5                   10                  15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc          96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag         144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att         192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cgt gat         240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Arg Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc         288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc         336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110
```

```
cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac        384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc        432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat        480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga        528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                           549
Ser Pro Ser Tyr Ala Ser
            180
```

<210> 129
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 129

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

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

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

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

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

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

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

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

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

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

145 150 155 160

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

Ser Pro Ser Tyr Ala Ser
180

<210> 130
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 L86T

<220>
<221> CDS
<222> (1)..(549)

<400> 130
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa 48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1 5 10 15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc 96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
20 25 30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag 144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
35 40 45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att 192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
50 55 60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat 240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65 70 75 80

ggg gcc ctg tat gga tcg acc cac ttt gac cct gag gcc tgc agc ttc 288
Gly Ala Leu Tyr Gly Ser Thr His Phe Asp Pro Glu Ala Cys Ser Phe
85 90 95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc 336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
100 105 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac 384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
115 120 125

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc 432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
130 135 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat 480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145 150 155 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga 528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
165 170 175

agc ccc agc tac gct tcc taa 549
Ser Pro Ser Tyr Ala Ser
180

<210> 131
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 131

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Ser Tyr Ala Ser
180

<210> 132
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 L86C

<220>
<221> CDS
<222> (1)..(549)

<400> 132
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa 48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1 5 10 15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc 96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
20 25 30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag 144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
35 40 45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att 192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
50 55 60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat 240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65 70 75 80

ggg gcc ctg tat gga tcg tgc cac ttt gac cct gag gcc tgc agc ttc 288
Gly Ala Leu Tyr Gly Ser Cys His Phe Asp Pro Glu Ala Cys Ser Phe
85 90 95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc 336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
100 105 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac 384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
115 120 125

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc 432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
130 135 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat 480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145 150 155 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga 528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
165 170 175

agc ccc agc tac gct tcc taa 549
Ser Pro Ser Tyr Ala Ser
180

<210> 133
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 133

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

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

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

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

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

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

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

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

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

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

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

Ser Pro Ser Tyr Ala Ser
180

<210> 134
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 L98C

<220>
<221> CDS
<222> (1)..(549)

<400> 134
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa 48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1 5 10 15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc 96

```
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag        144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att        192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat        240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc        288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag tgc ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc        336
Arg Glu Cys Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac        384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc        432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat        480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga        528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                           549
Ser Pro Ser Tyr Ala Ser
            180


<210>  135
<211>  182
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  135

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


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


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


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

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

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

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

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

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

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

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

Ser Pro Ser Tyr Ala Ser
            180
```

<210> 136
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 L98R

<220>
<221> CDS
<222> (1)..(549)

<400> 136

```
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa          48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1               5                   10                  15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc          96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag         144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga gtt att         192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat         240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65                  70                  75                  80
```

```
ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc        288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag cgt ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc        336
Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac        384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc        432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat        480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga        528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                           549
Ser Pro Ser Tyr Ala Ser
            180
```

<210> 137
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 137

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

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

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

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

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

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

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

His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
```

115 120 125

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

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

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

Ser Pro Ser Tyr Ala Ser
180

<210> 138
<211> 549
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 L52T

<220>
<221> CDS
<222> (1)..(549)

<400> 138
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa 48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1 5 10 15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc 96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
20 25 30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag 144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
35 40 45

agc ccc gaa agt acc ctg cag ctg aaa gcc ttg aag ccg gga gtt att 192
Ser Pro Glu Ser Thr Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile
50 55 60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat 240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65 70 75 80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc 288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
85 90 95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc 336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
100 105 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac 384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
115 120 125

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc 432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
130 135 140

```
ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat        480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga        528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                           549
Ser Pro Ser Tyr Ala Ser
            180
```

<210> 139
<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 139

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

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

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

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

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

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

Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175
```

```
Ser Pro Ser Tyr Ala Ser
            180


<210>  140
<211>  549
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  FGF21 L58E


<220>
<221>  CDS
<222>  (1)..(549)

<400>  140
atg cat cca att cca gat tct tct cca tta tta caa ttc ggg ggc caa          48
Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1               5                   10                  15

gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca gaa gcc          96
Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
            20                  25                  30

cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct gac cag         144
His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
        35                  40                  45

agc ccc gaa agt ctc ctg cag ctg aaa gcc gaa aag ccg gga gtt att         192
Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Glu Lys Pro Gly Val Ile
    50                  55                  60

caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg cca gat         240
Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65                  70                  75                  80

ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc agc ttc         288
Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
                85                  90                  95

cgg gag ctg ctt ctt gag gac gga tac aat gtt tac cag tcc gaa gcc         336
Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
            100                 105                 110

cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac cgg gac         384
His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
        115                 120                 125

cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc ctg ccc         432
Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
    130                 135                 140

ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc ccc gat         480
Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145                 150                 155                 160

gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct tcc cag ggc cga         528
Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
                165                 170                 175

agc ccc agc tac gct tcc taa                                             549
Ser Pro Ser Tyr Ala Ser
            180


<210>  141
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<211> 182
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 141

Met His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln
1 5 10 15

Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala
20 25 30

His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln
35 40 45

Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Glu Lys Pro Gly Val Ile
50 55 60

Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp
65 70 75 80

Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe
85 90 95

Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala
100 105 110

His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp
115 120 125

Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro
130 135 140

Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp
145 150 155 160

Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg
165 170 175

Ser Pro Ser Tyr Ala Ser
180

<210> 142
<211> 1278
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-L98R P171G 182P

<220>
<221> CDS

<222> (1)..(1278)

<400> 142

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg         672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt         720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg         768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca         816
```

```
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag cgt ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga ggt tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc ccg taa                              1278
Gly Arg Ser Pro Ser Tyr Ala Ser Pro
            420                 425


<210>  143
<211>  425
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  143

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15


Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30


Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45


His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60
```

```
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335
```

```
Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

Gly Arg Ser Pro Ser Tyr Ala Ser Pro
            420                 425


<210>  144
<211>  1278
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Fc-L15-L98R P171G 182G


<220>
<221>  CDS
<222>  (1)..(1278)

<400>  144
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110
```

```
atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag          384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc          432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg          480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct          528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc          576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg          624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg          672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt          720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg          768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca          816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct          864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga          912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg          960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc         1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag cgt ctt ctt gag gac gga tac aat gtt tac cag tcc         1056
Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac         1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc         1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380
```

```
ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga ggt tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc ggc taa                               1278
Gly Arg Ser Pro Ser Tyr Ala Ser Gly
            420                 425
```

<210> 145
<211> 425
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 145

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Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
```

180 185 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
195 200 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
210 215 220

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225 230 235 240

Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
245 250 255

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
260 265 270

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
275 280 285

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
290 295 300

Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305 310 315 320

Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
325 330 335

Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
340 345 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
355 360 365

Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
370 375 380

Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385 390 395 400

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
405 410 415

Gly Arg Ser Pro Ser Tyr Ala Ser Gly
420 425

<210> 146
<211> 1281
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-L98R P171G 182G 183G

<220>
<221> CDS
<222> (1)..(1281)

<400> 146

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg         672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt         720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240
```

```
ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag cgt ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga ggt tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac gct tcc ggt ggc taa                          1281
Gly Arg Ser Pro Ser Tyr Ala Ser Gly Gly
            420                 425
```

<210> 147
<211> 426
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 147

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Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30
```

```
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300
```

```
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

Gly Arg Ser Pro Ser Tyr Ala Ser Gly Gly
            420                 425


<210>  148
<211>  1275
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Fc-L15-L98R P171G Y179S


<220>
<221>  CDS
<222>  (1)..(1275)

<400>  148
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
```

```
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc        336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag cgt ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
```

355 360 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc 1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
370 375 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc 1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385 390 395 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga ggt tcc cag 1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
405 410 415

ggc cga agc ccc agc tcc gct tcc taa 1275
Gly Arg Ser Pro Ser Ser Ala Ser
420

<210> 149
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 149

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1 5 10 15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
20 25 30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
35 40 45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
50 55 60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65 70 75 80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
85 90 95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
100 105 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
115 120 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
130 135 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145 150 155 160

```
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

Gly Arg Ser Pro Ser Ser Ala Ser
            420
```

<210> 150
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-L98R P171G Y179F

<220>
<221> CDS
<222> (1)..(1275)

<400> 150

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg         624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg         672
```

```
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag cgt ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga ggt tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

ggc cga agc ccc agc ttc gct tcc taa                                  1275
Gly Arg Ser Pro Ser Phe Ala Ser
            420
```

<210> 151
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 151

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Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15
```

```
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285
```

```
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

Gly Arg Ser Pro Ser Phe Ala Ser
            420
```

<210> 152
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-L98R P171G Y179A

<220>
<221> CDS
<222> (1)..(1275)

<400> 152

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60
```

```
gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg        240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat        288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc        336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335
```

```
agc ttc cgg gag cgt ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga ggt tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

ggc cga agc ccc agc gct gct tcc taa                                  1275
Gly Arg Ser Pro Ser Ala Ala Ser
            420
```

<210> 153
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 153

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
```

130 135 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145 150 155 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
165 170 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
180 185 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
195 200 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
210 215 220

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225 230 235 240

Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
245 250 255

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
260 265 270

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
275 280 285

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
290 295 300

Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305 310 315 320

Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
325 330 335

Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
340 345 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
355 360 365

Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
370 375 380

Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385 390 395 400

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln

405 410 415

Gly Arg Ser Pro Ser Ala Ala Ser
420

<210> 154
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-L98R P171G A180S

<220>
<221> CDS
<222> (1)..(1275)

<400> 154

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag         192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg         240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat         288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc         336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag         384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc         432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg         480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct         528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc         576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190
```

```
gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag cgt ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga ggt tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac tcc tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Ser Ser
            420
```

<210> 155
<211> 424
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 155

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Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255
```

```
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

Gly Arg Ser Pro Ser Tyr Ser Ser
            420
```

<210> 156
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Fc-L15-L98R P171G A180G

<220>
<221> CDS
<222> (1)..(1275)

<400> 156

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atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg          48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc          96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgt acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc         144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
```

```
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag        192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgt gag gag cag tac aac agc acg        240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat        288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc        336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgt gat gag ctg acc aag aac cag gtc        432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc cgt tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa ggt gga ggt ggt ggt tct ggt ggt ggt agc ggt ggt        720
Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

ggt gga tcc cat cca att cca gat tct tct cca tta tta caa ttc ggg        768
Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag cag aca        816
Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc gct gct        864
Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag ccg gga        912
Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc cag cgg        960
Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
```

```
305                 310                 315                 320

cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag gcc tgc       1008
Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

agc ttc cgg gag cgt ctt ctt gag gac gga tac aat gtt tac cag tcc       1056
Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc cca cac       1104
Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta cca ggc       1152
Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380

ctg ccc ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc cag ccc       1200
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga ggt tcc cag       1248
Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

ggc cga agc ccc agc tac ggt tcc taa                                  1275
Gly Arg Ser Pro Ser Tyr Gly Ser
            420
```

<210> 157
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 157

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Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110
```

```
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

Ser Pro Gly Lys Gly Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

Gly Gly Ser His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly
                245                 250                 255

Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr
            260                 265                 270

Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala
        275                 280                 285

Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly
    290                 295                 300

Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg
305                 310                 315                 320

Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys
                325                 330                 335

Ser Phe Arg Glu Arg Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser
            340                 345                 350

Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His
        355                 360                 365

Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly
    370                 375                 380
```

```
Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro
385                 390                 395                 400

Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Gly Ser Gln
                405                 410                 415

Gly Arg Ser Pro Ser Tyr Gly Ser
            420
```

<210> 158
<211> 1287
<212> DNA
<213> Artificial Sequence

<220>
<223> MKEDD-FGF21-(L15)-Fc

<220>
<221> CDS
<222> (1)..(1287)

<400> 158

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atg aaa gaa gat gat cac cca att cca gat tct tct cca tta tta caa          48
Met Lys Glu Asp Asp His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln
1               5                   10                  15

ttc ggg ggc caa gtc cgg cag cgg tac ctc tac aca gat gat gcc cag          96
Phe Gly Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln
            20                  25                  30

cag aca gaa gcc cac ctg gag atc agg gag gat ggg acg gtg ggg ggc         144
Gln Thr Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly
        35                  40                  45

gct gct gac cag agc ccc gaa agt ctc ctg cag ctg aaa gcc ttg aag         192
Ala Ala Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys
    50                  55                  60

ccg gga gtt att caa atc ttg gga gtc aag aca tcc agg ttc ctg tgc         240
Pro Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys
65                  70                  75                  80

cag cgg cca gat ggg gcc ctg tat gga tcg ctc cac ttt gac cct gag         288
Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu
                85                  90                  95

gcc tgc agc ttc cgg gag ctg ctt ctt gag gac gga tac aat gtt tac         336
Ala Cys Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr
            100                 105                 110

cag tcc gaa gcc cac ggc ctc ccg ctg cac ctg cca ggg aac aag tcc         384
Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser
        115                 120                 125

cca cac cgg gac cct gca ccc cga gga cca gct cgc ttc ctg cca cta         432
Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu
    130                 135                 140

cca ggc ctg cca ccc gca ccc ccg gag cca ccc gga atc ctg gcc ccc         480
Pro Gly Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro
145                 150                 155                 160

cag ccc ccc gat gtg ggc tcc tcg gac cct ctg agc atg gtg gga cct         528
```

```
Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro
                165                 170                 175

tcc cag ggc cga agc ccc agc tac gct tcc ggt gga ggt ggt ggt tct        576
Ser Gln Gly Arg Ser Pro Ser Tyr Ala Ser Gly Gly Gly Gly Gly Ser
            180                 185                 190

ggt ggt ggt agc ggt ggt ggt gga tcc gac aaa act cac aca tgc cca        624
Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys Thr His Thr Cys Pro
        195                 200                 205

ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc        672
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
    210                 215                 220

ccc cca aaa ccc aag gac acc ctc atg atc tcc cgt acc cct gag gtc        720
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
225                 230                 235                 240

aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc        768
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
                245                 250                 255

aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg        816
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
            260                 265                 270

cgt gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc        864
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
        275                 280                 285

gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc        912
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
    290                 295                 300

tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc        960
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
305                 310                 315                 320

aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg       1008
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
                325                 330                 335

gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc       1056
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
            340                 345                 350

ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg       1104
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
        355                 360                 365

gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc       1152
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
    370                 375                 380

ttc ttc ctc tat agc aag ctc acc gtg gac aag agc agg tgg cag cag       1200
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
385                 390                 395                 400

ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac       1248
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
                405                 410                 415

tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa taa                  1287
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
            420                 425
```

<210> 159
<211> 428
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 159

Met Lys Glu Asp Asp His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln
1 5 10 15

Phe Gly Gly Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln
20 25 30

Gln Thr Glu Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly
35 40 45

Ala Ala Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys
50 55 60

Pro Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys
65 70 75 80

Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu
85 90 95

Ala Cys Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr
100 105 110

Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser
115 120 125

Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu
130 135 140

Pro Gly Leu Pro Pro Ala Pro Pro Glu Pro Pro Gly Ile Leu Ala Pro
145 150 155 160

Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro
165 170 175

Ser Gln Gly Arg Ser Pro Ser Tyr Ala Ser Gly Gly Gly Gly Gly Ser
180 185 190

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys Thr His Thr Cys Pro
195 200 205

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
210 215 220

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
225 230 235 240

```
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
                245                 250                 255

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
            260                 265                 270

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
        275                 280                 285

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
    290                 295                 300

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
305                 310                 315                 320

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
                325                 330                 335

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
            340                 345                 350

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
        355                 360                 365

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
    370                 375                 380

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
385                 390                 395                 400

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
                405                 410                 415

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
            420                 425
```

<210> 160
<211> 630
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 Y179N S181T, 28AA signal sequence removed during processing

<220>
<221> CDS
<222> (1)..(630)

<400> 160

```
atg gac tcg gac gag acc ggg ttc gag cac tca gga ctg tgg gtt tct        48
Met Asp Ser Asp Glu Thr Gly Phe Glu His Ser Gly Leu Trp Val Ser
1               5                   10                  15
```

```
gtg ctg gct ggt ctt ctg ctg gga gcc tgc cag gca cac ccc atc cct          96
Val Leu Ala Gly Leu Leu Leu Gly Ala Cys Gln Ala His Pro Ile Pro
            20                  25                  30

gac tcc agt cct ctc ctg caa ttc ggg ggc caa gtc cgg cag cgg tac         144
Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr
        35                  40                  45

ctc tac aca gat gat gcc cag cag aca gaa gcc cac ctg gag atc agg         192
Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg
    50                  55                  60

gag gat ggg acg gtg ggg ggc gct gct gac cag agc ccc gaa agt ctc         240
Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu
65                  70                  75                  80

ctg cag ctg aaa gcc ttg aag ccg gga gtt att caa atc ttg gga gtc         288
Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val
                85                  90                  95

aag aca tcc agg ttc ctg tgc cag cgg cca gat ggg gcc ctg tat gga         336
Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly
            100                 105                 110

tcg ctc cac ttt gac cct gag gcc tgc agc ttc cgg gag ctg ctt ctt         384
Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu
        115                 120                 125

gag gac gga tac aat gtt tac cag tcc gaa gcc cac ggc ctc ccg ctg         432
Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu
    130                 135                 140

cac ctg cca ggg aac aag tcc cca cac cgg gac cct gca ccc cga gga         480
His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly
145                 150                 155                 160

cca gct cgc ttc ctg cca cta cca ggc ctg cca ccc gca ccc ccg gag         528
Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Pro Pro Glu
                165                 170                 175

cca ccc gga atc ctg gcc ccc cag ccc ccc gat gtg ggc tcc tcg gac         576
Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp
            180                 185                 190

cct ctg agc atg gtg gga cct tcc cag ggc cga agc ccc agc aac gct         624
Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser Asn Ala
        195                 200                 205

acc tga                                                                 630
Thr
```

<210> 161
<211> 209
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 161

```
Met Asp Ser Asp Glu Thr Gly Phe Glu His Ser Gly Leu Trp Val Ser
1               5                   10                  15

Val Leu Ala Gly Leu Leu Leu Gly Ala Cys Gln Ala His Pro Ile Pro
```

20 25 30

Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr
35 40 45

Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg
50 55 60

Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu
65 70 75 80

Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val
85 90 95

Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly
100 105 110

Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu
115 120 125

Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu
130 135 140

His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly
145 150 155 160

Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Pro Pro Glu
165 170 175

Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp
180 185 190

Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser Asn Ala
195 200 205

Thr

<210> 162
<211> 630
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 Y179N, 28 AA signal sequence removed during processing

<220>
<221> CDS
<222> (1)..(630)

<400> 162
atg gac tcg gac gag acc ggg ttc gag cac tca gga ctg tgg gtt tct 48
Met Asp Ser Asp Glu Thr Gly Phe Glu His Ser Gly Leu Trp Val Ser
1 5 10 15

```
gtg ctg gct ggt ctt ctg ctg gga gcc tgc cag gca cac ccc atc cct          96
Val Leu Ala Gly Leu Leu Leu Gly Ala Cys Gln Ala His Pro Ile Pro
            20                  25                  30

gac tcc agt cct ctc ctg caa ttc ggg ggc caa gtc cgg cag cgg tac         144
Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr
        35                  40                  45

ctc tac aca gat gat gcc cag cag aca gaa gcc cac ctg gag atc agg         192
Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg
    50                  55                  60

gag gat ggg acg gtg ggg ggc gct gct gac cag agc ccc gaa agt ctc         240
Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu
65                  70                  75                  80

ctg cag ctg aaa gcc ttg aag ccg gga gtt att caa atc ttg gga gtc         288
Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val
                85                  90                  95

aag aca tcc agg ttc ctg tgc cag cgg cca gat ggg gcc ctg tat gga         336
Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly
            100                 105                 110

tcg ctc cac ttt gac cct gag gcc tgc agc ttc cgg gag ctg ctt ctt         384
Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu
        115                 120                 125

gag gac gga tac aat gtt tac cag tcc gaa gcc cac ggc ctc ccg ctg         432
Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu
    130                 135                 140

cac ctg cca ggg aac aag tcc cca cac cgg gac cct gca ccc cga gga         480
His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly
145                 150                 155                 160

cca gct cgc ttc ctg cca cta cca ggc ctg cca ccc gca ccc ccg gag         528
Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Pro Pro Glu
                165                 170                 175

cca ccc gga atc ctg gcc ccc cag ccc ccc gat gtg ggc tcc tcg gac         576
Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp
            180                 185                 190

cct ctg agc atg gtg gga cct tcc cag ggc cga agc ccc agc aac gct         624
Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser Asn Ala
        195                 200                 205

tcc tga                                                                 630
Ser
```

<210> 163
<211> 209
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 163

```
Met Asp Ser Asp Glu Thr Gly Phe Glu His Ser Gly Leu Trp Val Ser
1               5                   10                  15
```

Val Leu Ala Gly Leu Leu Leu Gly Ala Cys Gln Ala His Pro Ile Pro
20 25 30

Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr
35 40 45

Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg
50 55 60

Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu
65 70 75 80

Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val
85 90 95

Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly
100 105 110

Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu
115 120 125

Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu
130 135 140

His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly
145 150 155 160

Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Pro Pro Glu
165 170 175

Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp
180 185 190

Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser Asn Ala
195 200 205

Ser

<210> 164
<211> 630
<212> DNA
<213> Artificial Sequence

<220>
<223> FGF21 P124S, 28 AA signal sequence removed during processing

<220>
<221> CDS
<222> (1)..(630)

<400> 164
atg gac tcg gac gag acc ggg ttc gag cac tca gga ctg tgg gtt tct 48
Met Asp Ser Asp Glu Thr Gly Phe Glu His Ser Gly Leu Trp Val Ser

```
1               5                   10                  15

gtg ctg gct ggt ctt ctg ctg gga gcc tgc cag gca cac ccc atc cct          96
Val Leu Ala Gly Leu Leu Leu Gly Ala Cys Gln Ala His Pro Ile Pro
            20                  25                  30

gac tcc agt cct ctc ctg caa ttc ggg ggc caa gtc cgg cag cgg tac         144
Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr
        35                  40                  45

ctc tac aca gat gat gcc cag cag aca gaa gcc cac ctg gag atc agg         192
Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg
    50                  55                  60

gag gat ggg acg gtg ggg ggc gct gct gac cag agc ccc gaa agt ctc         240
Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu
65                  70                  75                  80

ctg cag ctg aaa gcc ttg aag ccg gga gtt att caa atc ttg gga gtc         288
Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val
                85                  90                  95

aag aca tcc agg ttc ctg tgc cag cgg cca gat ggg gcc ctg tat gga         336
Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly
            100                 105                 110

tcg ctc cac ttt gac cct gag gcc tgc agc ttc cgg gag ctg ctt ctt         384
Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu
        115                 120                 125

gag gac gga tac aat gtt tac cag tcc gaa gcc cac ggc ctc ccg ctg         432
Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu
    130                 135                 140

cac ctg cca ggg aac aag tcc tca cac cgg gac cct gca ccc cga gga         480
His Leu Pro Gly Asn Lys Ser Ser His Arg Asp Pro Ala Pro Arg Gly
145                 150                 155                 160

cca gct cgc ttc ctg cca cta cca ggc ctg cca ccc gca ccc ccg gag         528
Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Pro Pro Glu
                165                 170                 175

cca ccc gga atc ctg gcc ccc cag ccc ccc gat gtg ggc tcc tcg gac         576
Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp
            180                 185                 190

cct ctg agc atg gtg gga cct tcc cag ggc cga agc ccc agc tac gct         624
Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser Tyr Ala
        195                 200                 205

tcc tga                                                                 630
Ser
```

<210> 165
<211> 209
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 165

```
Met Asp Ser Asp Glu Thr Gly Phe Glu His Ser Gly Leu Trp Val Ser
1               5                   10                  15
```

```
Val Leu Ala Gly Leu Leu Leu Gly Ala Cys Gln Ala His Pro Ile Pro
            20                  25                  30

Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr
        35                  40                  45

Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg
    50                  55                  60

Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu
65                  70                  75                  80

Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val
                85                  90                  95

Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly
            100                 105                 110

Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu
        115                 120                 125

Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu
    130                 135                 140

His Leu Pro Gly Asn Lys Ser Ser His Arg Asp Pro Ala Pro Arg Gly
145                 150                 155                 160

Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Pro Pro Glu
                165                 170                 175

Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp
            180                 185                 190

Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser Tyr Ala
        195                 200                 205

Ser
```