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(54) Title: CHIMERIC FIBROBLAST GROWTH FACTOR 21 PROTEINS AND METHODS OF USE

(57) Abstract: The present invention relates to a chimeric protein that includes an N-terminus coupled to a C-terminus, where the N-terminus includes a portion of a paracrine fibroblast growth factor ("FGF") and the C-terminus includes a C-terminal portion of an FGF21 molecule. The portion of the paracrine FGF is modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without the modification. The present invention also relates to pharmaceutical compositions including chimeric proteins according to the present invention, methods for treating a subject suffering from diabetes, obesity, or metabolic syndrome, and methods of screening for compounds with enhanced binding affinity for the βKlotho-FGF receptor complex involving the use of chimeric proteins of the present invention.

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CHIMERIC FIBROBLAST GROWTH FACTOR 21 PROTEINS AND METHODS OF USE

[0001] This application claims priority benefit of U.S. Provisional Patent Application No. 61/656,778, filed June 7, 2012, U.S. Provisional Patent Application No. 61/664,081, filed June 25, 2012, and U.S. Patent Application Serial No. 13/837,880, filed March 15, 2013, each of which is hereby incorporated by reference in its entirety.

[0002] This invention was made with government support under grant numbers DE13686, DK077276, AG019712, DK091392, and DK067158 awarded by the U.S. National Institutes of Health. The government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] The present invention relates to chimeric fibroblast growth factor ("FGF") proteins and uses thereof.

BACKGROUND OF THE INVENTION

[0004] Type 2 diabetes is a chronic progressive disorder, which results from end-organ resistance to the action of insulin in combination with insufficient insulin secretion from the pancreas. The metabolic abnormalities associated with insulin resistance and secretory defects, in particular the hyperglycemia, lead over the course of years to extensive irreversible damage to multiple organs including heart, blood vessels, kidney, and eye. Currently, nearly 200 million or 2.9% of the world population have type 2 diabetes (World Health Organization, Diabetes Fact Sheet N° 312, January 2011; Wild et al., "Global Prevalence of Diabetes: Estimates for the Year 2000 and Projections for 2030," *Diabetes Care* 27(5):1047-1053 (2004)), and its prevalence is rising at an alarmingly fast pace in parallel with the rise in the prevalence of overweight and obesity (World Health Organization, Obesity and Overweight Fact Sheet N° 311, January 2011). Until the end of the 20th century, type 2 diabetes was observed only in adults but what was once known as "adult-onset diabetes" is now also diagnosed in children and adolescents, and this growing incidence can be related to the increase in overweight and obesity among children and adolescents. The prevalence of pre-diabetes, an intermediate metabolic stage between normal glucose homeostasis and diabetes, is even greater than that of type 2 diabetes. Currently, nearly 80 million or 26% of the population in the United States alone have pre-diabetes (Center for Disease Control and Prevention, National Diabetes Fact Sheet 2011), and as such are at high risk

for progressing to type 2 diabetes. Type 2 diabetes ranks among the ten leading causes of death worldwide, and the World Health Organization projects that mortality from diabetes (90% of which is type 2) will more than double within the next decade (World Health Organization, Diabetes Fact Sheet N° 312, January 2011). Type 2 diabetes also is a major cause of disability. As a consequence of diabetic retinopathy, about 10% of all patients with diabetes in the world develop severe visual impairment and 2% become blind 15 years into the disease (World Health Organization, Diabetes Fact Sheet N° 312, January 2011). Diabetic neuropathy, which affects up to half of all patients with diabetes worldwide (World Health Organization, Diabetes Fact Sheet N° 312, January 2011), accounts for the majority of nontraumatic lower-limb amputations. Indeed, in its recently published first worldwide report on non-infectious diseases, the World Health Organization considers diabetes, together with other chronic non-infectious diseases like cancer and heart disease, a global economic and social burden, which exceeds that imposed by infectious diseases such as HIV/AIDS.

[0005] The current drug therapy for type 2 diabetes is focused on correcting the hyperglycemia in the patients. Although a number of small molecules and biologics with different mechanisms of anti-hyperglycemic action are available for use as mono-therapy or combination therapy, most, if not all of these have limited efficacy, limited tolerability, and significant adverse effects (Moller, "New Drug Targets for Type 2 Diabetes and the Metabolic Syndrome," *Nature* 414(6865):821-827 (2001)). For example, treatment with sulfonylureas, glinides, thiazolidinediones, or insulin has been associated with weight gain, which is an undesired effect since overweight is considered a driving force in the pathogenesis of type 2 diabetes. Some of these treatments have also been associated with increased risk of hypoglycemia. A limitation specific to the thiazolidinediones is the potential for adverse cardiovascular effects (DeSouza et al., "Therapeutic Targets to Reduce Cardiovascular Disease in Type 2 Diabetes," *Nat Rev Drug Discov* 8(5):361-367 (2009)). A meta-analysis of clinical data on the thiazolidinedione rosiglitazone (Avandia®), which was widely used for the treatment of type 2 diabetes, found that the drug increased the risk of myocardial infarction in patients with type 2 diabetes (Nissen et al., "Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes," *N Engl J Med* 356(24):2457-2471 (2007)). Of all diabetic complications, cardiovascular disease is the main cause of morbidity and mortality in patients with diabetes (World Health Organization, Diabetes Fact Sheet N° 312, January 2011; Center for Disease Control and Prevention, National Diabetes Fact Sheet 2011), and hence an aggravation of cardiovascular risk by drug treatment is absolutely unacceptable. In the wake of the debate

about the cardiovascular safety of thiazolidinediones, the FDA issued a guidance on evaluating cardiovascular risk in new anti-diabetic therapies to treat type 2 diabetes (Opar A, "Diabetes Drugs Pass Cardiovascular Risk Check," *Nat Rev Drug Discov* 8(5):343-344 (2009)). Meanwhile, thiazolidinediones lost their popularity. Even for glucagon-like peptide-1 agonists, one of the latest class of drugs introduced for the treatment of type 2 diabetes, concerns about safety have been raised, namely the potential for carcinogenicity (Opar A, "Diabetes Drugs Pass Cardiovascular Risk Check," *Nat Rev Drug Discov* 8(5):343-344 (2009)). Therefore, novel therapies that are more effective and safer than existing drugs are needed. Since the currently available drugs do not directly target complications of advanced diabetic disease, especially cardiovascular disease, therapies that are not only effective in lowering blood glucose but also reduce cardiovascular risk factors such as dyslipidemia are particularly desired.

[0006] A search conducted by Eli Lilly & Co. for potential novel biotherapeutics to treat type 2 diabetes led to the discovery of fibroblast growth factor (FGF) 21 as a protein that stimulates glucose uptake into adipocytes in an insulin-independent fashion (Kharitonenkov et al., "FGF-21 as a Novel Metabolic Regulator," *J Clin Invest* 115(6):1627-1635 (2005)). FGF21 has since emerged as a key endocrine regulator not only of glucose metabolism but also of lipid metabolism, and has become one of the most promising drug candidates for the treatment of type 2 diabetes, obesity, and metabolic syndrome. In mouse models of diabetes and obesity, pharmacologic doses of FGF21 lower plasma glucose, increase glucose tolerance and insulin sensitivity, and improve pancreatic β-cell function (Kharitonenkov et al., "FGF-21 as a Novel Metabolic Regulator," *J Clin Invest* 115(6):1627-1635 (2005); Coskun et al., "Fibroblast growth factor 21 corrects obesity in mice," *Endocrinology* 149(12):6018-6027 (2008); Wente et al., "Fibroblast Growth Factor-21 Improves Pancreatic Beta-Cell Function and Survival by Activation of Extracellular Signal-Regulated Kinase 1/2 and Akt Signaling Pathways," *Diabetes* 55:2470-2478 (2006)). Concurrently, FGF21 lowers plasma triglyceride and cholesterol, enhances lipolysis and suppresses lipogenesis, accelerates energy expenditure, and reverses fatty liver disease (Kharitonenkov et al., "FGF-21 as a Novel Metabolic Regulator," *J Clin Invest* 115(6):1627-1635 (2005); Coskun et al., "Fibroblast growth factor 21 corrects obesity in mice," *Endocrinology* 149(12):6018-6027 (2008); Xu et al., "Fibroblast Growth Factor 21 Reverses Hepatic Steatosis, Increases Energy Expenditure, and Improves Insulin Sensitivity in Diet-Induced Obese Mice," *Diabetes* 58:250-259 (2009)). In obese mice, FGF21 causes weight loss, in lean mice, it is weight neutral (Kharitonenkov et al., "FGF-21 as a Novel Metabolic Regulator," *J Clin Invest* 115(6):1627-1635 (2005); Coskun et al., "Fibroblast growth factor 21

corrects obesity in mice," *Endocrinology* 149(12):6018-6027 (2008)). Thus, FGF21 has some of the most desired characteristics of a drug for the treatment of type 2 diabetes; not only does it improve glycemic control, but also directly affects cardiovascular risk factors, such as hypertriglyceridemia, and reduces obesity, which is considered the single most important promoter of type 2 diabetes. Importantly, FGF21 does not induce hypoglycemia (Kharitonenkov et al., "FGF-21 as a Novel Metabolic Regulator," *J Clin Invest* 115(6):1627-1635 (2005)), a side effect that can occur with several of the current anti-diabetic therapies, including insulin. Moreover, FGF21 does not exhibit any mitogenic activity in mice (Kharitonenkov et al., "FGF-21 as a Novel Metabolic Regulator," *J Clin Invest* 115(6):1627-1635 (2005)), ruling out the possibility of a carcinogenic risk. The findings on FGF21 therapy in mouse models of diabetes have been reproduced in diabetic rhesus monkeys (Kharitonenkov et al., "The Metabolic State of Diabetic Monkeys is Regulated by Fibroblast Growth Factor-21," *Endocrinology* 148(2):774-781 (2007)), and are currently followed up with clinical trials in humans (Kharitonenkov et al., "FGF21 Reloaded: Challenges of a Rapidly Growing Field," *Trends Endocrinol Metab* 22(3):81-86 (2011)). FGF21 therapy also leads to a decrease in serum levels of pro-inflammatory cytokines, which may contribute to improved insulin sensitivity (Kharitonenkov et al., "The Metabolic State of Diabetic Monkeys is Regulated by Fibroblast Growth Factor-21," *Endocrinology* 148(2):774-781 (2007)) and perhaps also confer anti-atherosclerotic properties on FGF21. However, there is a need for more effective FGF21 therapeutics.

[0007] The present invention overcomes these and other deficiencies in the art.

SUMMARY OF THE INVENTION

[0008] One aspect of the present invention relates to a chimeric protein. The chimeric protein includes an N-terminus coupled to a C-terminus, where the N-terminus includes a portion of a paracrine fibroblast growth factor ("FGF") and the C-terminus includes a C-terminal portion of an FGF21 molecule. The portion of the paracrine FGF is modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without the modification.

[0009] Another aspect of the present invention relates to a method for treating a subject suffering from a disorder. This method involves selecting a subject suffering from the disorder and providing a chimeric FGF protein, where the chimeric FGF protein includes an N-terminus coupled to a C-terminus. The N-terminus includes a portion of a paracrine FGF and the C-terminus includes a C-terminal portion of FGF21. The portion of the paracrine FGF is modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without

the modification. This method also involves administering a therapeutically effective amount of the chimeric FGF protein to the selected subject under conditions effective to treat the disorder.

[0010] Another aspect of the present invention relates to a method of making a chimeric FGF protein possessing enhanced endocrine activity. This method involves introducing one or more modifications to an FGF protein, where the modification decreases the affinity of the FGF protein for heparin and/or heparan sulfate and coupling a Klotho co-receptor binding domain to the modified FGF protein's C-terminus, whereby a chimeric FGF protein possessing enhanced endocrine activity is made.

[0011] Yet another aspect of the present invention relates to a method of facilitating fibroblast growth factor receptor ("FGFR")-βKlotho co-receptor complex formation. This method involves providing a cell that includes a βKlotho co-receptor and an FGFR and providing a chimeric FGF protein. The chimeric FGF protein includes a C-terminal portion of FGF21 and a portion of a paracrine FGF, where the portion of the paracrine FGF is modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without the modification. This method also involves contacting the cell and the chimeric FGF protein under conditions effective to cause FGFR-βKlotho co-receptor complex formation.

[0012] Yet a further aspect of the present invention relates to a method of screening for agents capable of facilitating FGFR-βKlotho complex formation in the treatment of a disorder. This method involves providing a chimeric FGF that includes an N-terminus coupled to a C-terminus, where the N-terminus includes a portion of a paracrine FGF and the C-terminus includes a C-terminal portion of FGF21. The portion of the paracrine FGF is modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without the modification. This method also involves providing a binary βKlotho-FGFR complex and providing one or more candidate agents. This method further involves combining the chimeric FGF, the binary βKlotho-FGFR complex, and the one or more candidate agents under conditions permitting the formation of a ternary complex between the chimeric FGF and the binary βKlotho-FGFR complex in the absence of the one or more candidate agents. This method also involves identifying the one or more candidate agents that decrease ternary complex formation between the chimeric FGF and the binary βKlotho-FGFR complex compared to the ternary complex formation in the absence of the one or more candidate agents as suitable for treating the disorder.

[0013] Fibroblast growth factors (FGFs) 19, 21, and 23 are hormones that regulate in a Klotho co-receptor-dependent fashion major metabolic processes such as glucose and lipid

metabolism (FGF21) and phosphate and vitamin D homeostasis (FGF23). The role of heparan sulfate glycosaminoglycan in the formation of the cell surface signaling complex of endocrine FGFs has remained unclear. To decipher the role of HS in endocrine FGF signaling, we generated FGF19 and FGF23 mutant ligands devoid of HS binding and compared their signaling capacity with that of wild-type ligands. The data presented herein show that the mutated ligands retain full metabolic activity demonstrating that HS does not participate in the formation of the endocrine FGF signaling complex. Here it is shown that heparan sulfate is not a component of the signal transduction unit of FGF19 and FGF23. A paracrine FGF is converted into an endocrine ligand by diminishing heparan sulfate binding affinity of the paracrine FGF and substituting its C-terminal tail for that of an endocrine FGF containing the Klotho co-receptor binding site in order to home the ligand into the target tissue. The ligand conversion provides a novel strategy for engineering endocrine FGF-like molecules for the treatment of metabolic disorders, including global epidemics such as type 2 diabetes and obesity.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIGS. 1A-1D are schematic diagrams showing side-by-side comparison of the HS-binding site of FGF2, FGF19, and FGF23, and two working models for the endocrine FGF signaling complex. FIG. 1A shows interactions of FGF2 (schematic representation) with a heparin hexasaccharide (shown as sticks) as observed in the crystal structure of the 2:2 FGF2-FGFR1c dimer (PDB ID: 1FQ9; (Schlessinger et al., *Mol. Cell* 6:743-750 (2000), which is hereby incorporated by reference in its entirety)). The heparin hexasaccharide consists of three disaccharide units of 1→4 linked N-sulfated-6-O-sulfated D-glucosamine and 2-O-sulfated L-iduronic acid. Note that the heparin hexasaccharide interacts with both side chain and backbone atoms of residues in the HS-binding site of FGF2. Dashed lines denote hydrogen bonds. K128, R129, and K134, which make the majority of hydrogen bonds with the heparin hexasaccharide, are boxed. The β-strand nomenclature follows the original FGF1 and FGF2 crystal structures (Ago et al., *J. Biochem.* 110:360-363 (1991); Eriksson et al., *Proc. Nat'l. Acad. Sci. U.S.A.* 88:3441-3445 (1991); Zhang et al., *Proc. Nat'l. Acad. Sci. U.S.A.* 88:3446-3450 (1991); Zhu et al., *Science* 251:90-93 (1991), which are hereby incorporated by reference in their entirety). Please note that compared to the prototypical β-trefoil fold seen in soybean trypsin inhibitor (PDB ID: 1TIE; (Onesti et al., *J. Mol. Biol.* 217:153-176 (1991), which is hereby incorporated by reference in its entirety)) and interleukin 1β (PDB ID: 1I1B; (Finzel et al., *J. Mol. Biol.* 209:779-

791 (1989), which is hereby incorporated by reference in its entirety)), the β10-β11 strand pairing in FGF2 and other paracrine FGFs is less well defined. FIGS. 1B and 1C show cartoon representation of the crystal structures of FGF19 (PDB ID: 2P23; (Goetz et al., *Mol. Cell Biol.* 27:3417-3428 (2007), which is hereby incorporated by reference in its entirety)) (FIG. 1B) and FGF23 (PDB ID: 2P39; (Goetz et al., *Mol. Cell Biol.* 27:3417-3428 (2007), which is hereby incorporated by reference in its entirety)) (FIG. 1C) shown in the same orientation as the FGF2 structure in FIG. 1A. Side chains of residues that map to the corresponding HS-binding sites of these ligands are shown as sticks. Residues selected for mutagenesis to knock out residual HS binding in FGF19 and FGF23 are boxed. NT and CT indicate N- and C-termini of the FGFs. FIG. 1D is a schematic of two working models for the endocrine FGF-FGFR-Klotho coreceptor signal transduction unit. A recent study on the ternary complex formation between FGF21, FGFR1c, and βKlotho supports the 1:2:1 model rather than the 2:2:2 model (Ming et al., *J. Biol. Chem.* 287:19997-20006 (2012), which is hereby incorporated by reference in its entirety). For comparison, a schematic of the paracrine FGF-FGFR-HS signaling unit is shown, which was made based on the crystal structure of the 2:2:2 FGF2-FGFR1c-HS complex (PDB ID: 1FQ9; (Schlessinger et al., *Mol. Cell* 6:743-750 (2000), which is hereby incorporated by reference in its entirety)). HS engages both paracrine FGF and receptor to enhance binding of FGF to its primary and secondary receptors thus promoting receptor dimerization. A question mark denotes whether or not HS is also a component of the endocrine FGF signaling complex.

[0015] FIG. 2 shows a sequence alignment of the endocrine FGFs, FGF1 and FGF2. The amino acid sequences of the mature human FGF19, FGF21, and FGF23 ligands are aligned. Also included in the alignment are the human sequences of FGF1 and FGF2, prototypical paracrine FGFs, which were used in the experiments described herein, in which FGF1 and FGF2 were converted into endocrine FGF ligands. Residue numbers corresponding to the human sequence of FGF1 (SEQ ID NO:1) (GenBank Accession No. AAH32697, which is hereby incorporated by reference in its entirety), FGF2 (SEQ ID NO: 121) (GenBank Accession No. EAX05222, which is hereby incorporated by reference in its entirety), FGF19 (SEQ ID NO:337) (GenBank Accession No. NP_005108, which is hereby incorporated by reference in its entirety), FGF21 (SEQ ID NO: 233) (GenBank Accession No. NP_061986, which is hereby incorporated by reference in its entirety), and FGF23 (SEQ ID NO:351) (GenBank accession no. AAG09917, which is hereby incorporated by reference in its entirety) are in parenthesis to the left of the alignment. Secondary structure elements are labeled, and residues containing these elements for known secondary structures are boxed. Gaps (dashes) were introduced to optimize the sequence

alignment. The β-trefoil core domain for known FGF crystal structures is shaded gray. Blue bars on top of the alignment indicate the location of the HS-binding regions. HS-binding residues selected for mutagenesis are shaded blue.

[0016] FIGS. 3A-3G show Surface plasmon resonance ("SPR") results relating to knockout of residual heparin binding in FGF19 and FGF23 by site-directed mutagenesis. FIG. 3A shows an overlay of SPR sensorgrams illustrating heparin binding of FGF2, FGF19, FGF21, and FGF23 (left panel) and an exploded view of the binding responses for FGF19-, FGF21-, and FGF23-heparin interactions (right panel). Heparin was immobilized on a biosensor chip, and 400 nM of FGF2, FGF19, FGF21, or FGF23 were passed over the chip. Note that FGF19, FGF21, and FGF23 exhibit measurable, residual heparin binding and that differences in heparin binding exist between these three endocrine FGFs. FIGS. 3B-3D show overlays of SPR sensorgrams illustrating binding of FGF19 to heparin (FIG. 3B) and lack of interaction between the $FGF19^{K149A}$ mutant and heparin (FIG. 3C) and between the $FGF19^{K149A, R157A}$ mutant and heparin (FIG. 3D). Heparin was immobilized on a biosensor chip, and increasing concentrations of FGF19 were passed over the chip. Thereafter, $FGF19^{K149A}$ or $FGF19^{K149A, R157A}$ was injected over the heparin chip at the highest concentration tested for the wild-type ligand. FIGS. 3E-3G show overlays of SPR sensorgrams illustrating binding of FGF23 to heparin (FIG. 3E), poor interaction between the $FGF23^{R48A, N49A}$ mutant and heparin (FIG. 3F), and lack of interaction between the $FGF23^{R140A, R143A}$ mutant and heparin (FIG. 3G). Heparin was immobilized on a biosensor chip, and increasing concentrations of FGF23 were passed over the chip. $FGF23^{R48A, N49A}$ or $FGF23^{R140A, R143A}$ was then injected over the heparin chip at the highest concentration tested for the wild-type ligand.

[0017] FIGS. 4A-4D show results demonstrating that HS is dispensable for the metabolic activity of FGF19 and FGF23. FIG. 4A shows results of an immunoblot analysis of phosphorylation of FRS2α (pFRS2α) and 44/42 MAP kinase (p44/42 MAPK) in H4IIE hepatoma cells following stimulation with the $FGF19^{K149A}$ mutant, the $FGF19^{K149A, R157A}$ mutant, or wild-type FGF19. Numbers above the lanes give the amounts of protein added in ng ml^{-1}. Total 44/42 MAPK protein expression was used as a loading control. FIG. 4B shows results of an immunoblot analysis of phosphorylation of FRS2α (pFRS2α) and 44/42 MAP kinase (p44/42 MAPK) in a HEK293-αKlotho cell line following stimulation with the $FGF23^{R48A, N49A}$ mutant, the $FGF23^{R140A, R143A}$ mutant, or wild-type FGF23. Numbers above the lanes give the amounts of protein added in ng ml^{-1}. Total 44/42 MAPK and αKlotho protein expression were used as loading controls. FIG. 4C shows graphical results of a quantitative analysis of CYP7A1 and

CYP8B1 mRNA expression in liver tissue from mice treated with $FGF19^{K149A}$, $FGF19^{K149A, R157A}$, FGF19, or vehicle. 1 mg of protein per kg of body weight was given. Data are presented as mean ± SEM; ***, $P < 0.001$ by Student's *t* test. FIG. 4D shows graphical results of analysis of serum phosphate concentrations (serum P_i) in mice before and 8 h after intraperitoneal injection of $FGF23^{R48A, N49A}$, $FGF23^{R140A, R143A}$, FGF23, or vehicle. Wild-type mice were given a single dose of protein (0.29 mg kg body $weight^{-1}$), whereas *Fgf23* knockout mice received two doses of 0.71 mg kg body $weight^{-1}$ each. Data are presented as mean ± SEM; *, $P < 0.05$, and **, $P < 0.01$ by ANOVA.

[0018] FIGS. 5A-5G show design and results relating to the conversion of FGF2 into an endocrine ligand. FIG. 5A is a schematic of human FGF2, FGF19, FGF21, FGF23, and engineered FGF2-FGF19, FGF2-FGF21, and FGF2-FGF23 chimeras. Amino acid boundaries of each ligand and of each component of the chimeras are labeled with residue letter and number. The β-trefoil core domain for the known ligand crystal structures is shaded gray. HS-binding residues mutated in the FGF2 portion of chimeras are labeled with residue letter and number. Also labeled are the arginine residues of the proteolytic cleavage site in the C-terminal region of FGF23 that were mutated to glutamine in both FGF23 and the FGF2-FGF23 chimeras. FIGS. 5B and 5C show overlays of SPR sensorgrams illustrating binding of $FGF2^{WTcore}$-$FGF21^{C\text{-}tail}$ (FIG. 5B) and $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ (FIG. 5C) to heparin, and fitted saturation binding curves. Heparin was immobilized on a biosensor chip, and increasing concentrations of $FGF2^{WTcore}$-$FGF21^{C\text{-}tail}$ or $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ were passed over the chip. Dissociation constants (K_Ds) were derived from the saturation binding curves. FIGS. 5D and 5E show overlays of SPR sensorgrams illustrating binding of $FGF2^{WTcore}$-$FGF23^{C\text{-}tail}$ (FIG. 5D) and $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ (FIG. 5E) to heparin. Increasing concentrations of $FGF2^{WTcore}$-$FGF23^{C\text{-}tail}$ or $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ were passed over a chip containing immobilized heparin. FIGS. 5F and 5G show results of immunoblot analysis for Egr1 expression in HEK293 cells following stimulation with chimeras or native FGFs as denoted. Numbers above the lanes give the amounts of protein added in nanomolar. GAPDH protein expression was used as a loading control.

[0019] FIG. 6 is a schematic illustrating the conversion of FGF1 into an endocrine ligand. Shown are schematic drawings of human FGF1, FGF19, FGF21, FGF23, and exemplary FGF1-FGF19, FGF1-FGF21, and FGF1-FGF23 chimeras according to the present invention. Amino acid boundaries of each ligand and of each component of the chimeras are labeled with residue letter and number. The β-trefoil core domain for the known ligand crystal structures is

shaded gray. HS-binding residues mutated in the FGF1 portion of chimeras are labeled with residue letter and number. Also labeled are the arginine residues of the proteolytic cleavage site in the C-terminal region of FGF23 that were mutated to glutamine in both FGF23 and the FGF1-FGF23 chimeras.

[0020] FIGS. 7A-7G show results demonstrating that the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimera exhibits FGF23-like activity. FIGS. 7A and 7B show overlays of SPR sensorgrams illustrating inhibition by $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ (FIG. 7A) or FGF23 (FIG. 7B) of αKlotho-FGFR1c binding to FGF23 immobilized on a biosensor chip. Increasing concentrations of $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ or FGF23 were mixed with a fixed concentration of αKlotho-FGFR1c complex, and the mixtures were passed over a FGF23 chip. FIG. 7C shows an overlay of SPR sensorgrams illustrating failure of FGF2 to inhibit αKlotho-FGFR1c binding to FGF23. FGF2 and αKlotho-FGFR1c complex were mixed at a molar ratio of 15:1, and the mixture was passed over a biosensor chip containing immobilized FGF23. FIGS. 7D and 7E show overlays of SPR sensorgrams illustrating no inhibition by $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ (FIG. 7D) or FGF23 (FIG. 7E) of βKlotho-FGFR1c binding to FGF21. $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ or FGF23 were mixed with βKlotho-FGFR1c complex at a molar ratio of 10:1, and the mixtures were passed over a biosensor chip containing immobilized FGF21. FIG. 7F shows analysis of serum phosphate concentrations (serum P_i) in mice before and 8 h after intraperitoneal injection of $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$, $FGF2^{WTcore}$-$FGF23^{C\text{-}tail}$, FGF23, or vehicle. Wild-type mice and *αKlotho* knockout mice were given 0.21 mg and 0.51 mg of protein, respectively, per kg of body weight. Data are presented as mean ± SEM; **, $P < 0.01$; ***, $P < 0.001$ by ANOVA. FIG. 7G shows quantitative analysis of CYP27B1 mRNA expression in renal tissue from mice injected with $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$, $FGF2^{WTcore}$-$FGF23^{C\text{-}tail}$, FGF23, or vehicle. 0.21 mg of protein per kg of body weight were injected. Data are presented as mean ± SEM; ***, $P < 0.001$ by ANOVA.

[0021] FIGS. 8A-8G show results demonstrating that the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera exhibits FGF21-like activity. FIGS. 8A-8B show overlays of SPR sensorgrams illustrating inhibition by $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ (FIG. 8A) or FGF21 (FIG. 8B) of βKlotho-FGFR1c binding to FGF21 immobilized on a biosensor chip. Increasing concentrations of $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ or FGF21 were mixed with a fixed concentration of βKlotho-FGFR1c complex, and the mixtures were passed over a FGF21 chip. FIG. 8C shows an overlay of SPR sensorgrams illustrating failure of FGF2 to inhibit βKlotho-FGFR1c binding to FGF21. FGF2 and βKlotho-FGFR1c complex were mixed at a molar ratio of 15:1, and the mixture was passed

over a biosensor chip containing immobilized FGF21. FIGS. 8D-8E show overlays of SPR sensorgrams illustrating no inhibition by $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ (FIG. 8D) or FGF21 (FIG. 8E) of αKlotho-FGFR1c binding to FGF23. $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ or FGF21 were mixed with αKlotho-FGFR1c complex at a molar ratio of 10:1, and the mixtures were passed over a biosensor chip containing immobilized FGF23. FIG. 8F shows results of immunoblot analysis for Egr1 expression in HEK293-βKlotho cells stimulated with $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ or FGF21. Numbers above the lanes give the amounts of protein added in ng ml^{-1}. GAPDH protein expression was used as a loading control. Note that the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera is more potent than native FGF21 at inducing Egr1 expression suggesting that the chimera has agonistic property. This is expected since the core domain of FGF2 has inherently greater binding affinity for FGFR than the core domain of FGF21 (see FIGS. 10A and 10C). FIG. 8G shows graphical results of analysis of blood glucose concentrations in mice before and at the indicated time points after intraperitoneal injection of insulin alone, insulin plus $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera, insulin plus FGF21, or vehicle alone. 0.5 units of insulin per kg of body weight and 0.3 mg of FGF21 ligand per kg of body weight were injected. Blood glucose concentrations are expressed as percent of pre-injection values. Data are presented as mean ± SEM.

[0022] FIGS. 9A-9C show the glucose-lowering effects in *ob/ob* mice of FGF1 variants according to the present invention. FIG. 9A shows graphical results of analysis of blood glucose concentrations in *ob/ob* mice before and at the indicated time points after subcutaneous injection of FGF1 or FGF21. FIG. 9B shows graphical results of analysis of blood glucose concentrations in *ob/ob* mice before and at the indicated time points after subcutaneous injection of FGF1, $FGF1^{\Delta NT}$, or $FGF1^{\Delta HBS}$. FIG. 9C shows graphical results of analysis of blood glucose concentrations in *ob/ob* mice before and at the indicated time points after subcutaneous injection of FGF1 or $FGF1^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera. For the experiments shown in FIGS. 9A-9C, *ob/ob* mice were injected with a bolus of 0.5 mg of FGF protein per kg of body weight. Data are presented as mean ± SD.

[0023] FIGS. 10A-10F show results demonstrating that endocrine FGFs have low binding affinity for FGFR1c compared to FGF2. FIGS. 10A-10D show overlays of SPR sensorgrams illustrating binding of FGFR1c to FGF2 (FIG. 10A), FGF19 (FIG. 10B), FGF21 (FIG. 10C), and FGF23 (FIG. 10D), and fitted saturation binding curves. Increasing concentrations of FGFR1c ligand-binding domain were passed over a biosensor chip containing immobilized FGF2, FGF19, FGF21, or FGF23. FIG. 10E shows an overlay of SPR sensorgrams

illustrating binding of αKlotho-FGFR1c complex to FGF23. Increasing concentrations of αKlotho-FGFR1c complex were passed over a biosensor chip containing immobilized FGF23. FIG. 10F shows an overlay of SPR sensorgrams showing lack of interaction between the C-terminal tail peptide of FGF23 and FGFR1c. $FGF2^{3C\text{-}tail}$ was immobilized on a biosensor chip and increasing concentrations of FGFR1c ligand-binding domain were passed over the chip. Dissociation constants (K_Ds) given in FIGS. 10A-10E were derived from the saturation binding curves.

[0024] FIG. 11 shows an alignment of the C-terminal tail sequences of human FGF19 (SEQ ID NO:337) (GenBank Accession No. NP_005108, which is hereby incorporated by reference in its entirety), FGF21 (SEQ ID NO:233) (GenBank Accession No. NP_061986, which is hereby incorporated by reference in its entirety), and FGF23 (SEQ ID NO:351) (GenBank accession no. AAG09917, which is hereby incorporated by reference in its entirety). Residue numbers are in parenthesis to the left of the alignment. Gaps (dashes) were introduced to optimize the alignment. Residues that are identical between FGF19 and FGF21 are shaded gray. Note that 40% of these residues map to the most C-terminal sequence.

[0025] FIG. 12 shows an alignment of the C-terminal tail sequences of human FGF21 (SEQ ID NO:233) (GenBank Accession No. NP_061986, which is hereby incorporated by reference in its entirety), FGF19 (SEQ ID NO:337) (GenBank Accession No. NP_005108, which is hereby incorporated by reference in its entirety), and variants of FGF21 harboring a single amino acid substitution or insertion for a residue unique to FGF19. Residue numbers for the sequences of native FGF21 and FGF19 are in parenthesis to the left of the alignment. Gaps (dashes) were introduced to optimize the alignment. In the sequence of native FGF19, residues unique to FGF19 are bold and boxed, and in the sequences of the variants of the FGF21 C-terminal tail, introduced FGF19 residues are highlighted in the same manner.

[0026] FIG. 13 shows an alignment of the C-terminal tail sequences of human FGF21 (SEQ ID NO:233) (GenBank Accession No. NP_061986, which is hereby incorporated by reference in its entirety), FGF19 (SEQ ID NO:337) (GenBank Accession No. NP_005108, which is hereby incorporated by reference in its entirety), and variants of FGF21 in which residues unique to FGF19 progressively replace the corresponding residues of FGF21 or are inserted into the FGF21 sequence. Residue numbers for the sequences of native FGF21 and FGF19 are in parenthesis to the left of the alignment. Gaps (dashes) were introduced to optimize the alignment. In the sequence of native FGF19, residues unique to FGF19 are bold and boxed, and

in the sequences of variants of the FGF21 C-terminal tail, introduced FGF19 residues are highlighted in the same manner.

DETAILED DESCRIPTION OF THE INVENTION

[0027] One aspect of the present invention relates to a chimeric protein. The chimeric protein includes an N-terminus coupled to a C-terminus, where the N-terminus includes a portion of a paracrine fibroblast growth factor ("FGF") and the C-terminus includes a C-terminal portion of an FGF21 molecule. The portion of the paracrine FGF is modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without the modification.

[0028] As described by Goetz et al. (Goetz et al., "Molecular Insights into the Klotho-Dependent, Endocrine Mode of Action of Fibroblast Growth Factor 19 Subfamily Members," *Mol Cell Biol* 3417-3428 (2007), which is hereby incorporated by reference in its entirety), the mammalian fibroblast growth factor (FGF) family comprises 18 polypeptides (FGF1 to FGF10 and FGF16 to FGF23), which participate in a myriad of biological processes during embryogenesis, including but not limited to gastrulation, body plan formation, somitogenesis, and morphogenesis of essentially every tissue/organ such as limb, lung, brain, and kidney (Bottcher et al., "Fibroblast Growth Factor Signaling During Early Vertebrate Development," *Endocr Rev* 26:63-77 (2005), and Thisse et al., "Functions and Regulations of Fibroblast Growth Factor Signaling During Embryonic Development," *Dev Biol* 287:390-402 (2005), which are hereby incorporated by reference in their entirety).

[0029] FGFs execute their biological actions by binding to, dimerizing, and activating FGFR tyrosine kinases, which are encoded by four distinct genes (Fgfr1 to Fgfr4). Prototypical FGFRs consist of an extracellular domain composed of three immunoglobulin-like domains, a single-pass transmembrane domain, and an intracellular domain responsible for the tyrosine kinase activity (Mohammadi et al., "Structural Basis for Fibroblast Growth Factor Receptor Activation," *Cytokine Growth Factor Rev* 16:107-137 (2005), which is hereby incorporated by reference in its entirety).

[0030] The number of principal FGFRs is increased from four to seven due to a major tissue-specific alternative splicing event in the second half of the immunoglobulin-like domain 3 of FGFR1 to FGFR3, which creates epithelial lineage-specific "b" and mesenchymal lineage-specific "c" isoforms (Mohammadi et al., "Structural Basis for Fibroblast Growth Factor Receptor Activation," *Cytokine Growth Factor Rev* 16:107-137 (2005) and Ornitz et al., "Fibroblast Growth Factors," *Genome Biol* 2(3):reviews3005.1-reviews3005.12 (2001), which

are hereby incorporated by reference in their entirety). Generally, the receptor-binding specificity of FGFs is divided along this major alternative splicing of receptors whereby FGFRb-interacting FGFs are produced by epithelial cells and FGFRc-interacting FGFs are produced by mesenchymal cells (Ornitz et al., "Fibroblast Growth Factors," *Genome Biol* 2(3):reviews3005.1-reviews3005.12 (2001), which is hereby incorporated by reference in its entirety). These reciprocal expression patterns of FGFs and FGFRs result in the establishment of specific paracrine FGF signaling loops between the epithelium and the mesenchyme, which is essential for proper organogenesis and patterning during embryonic development as well as tissue homeostasis in the adult organism.

[0031] Based on sequence homology and phylogenetic and structural considerations, the eighteen mammalian FGFs are grouped into six subfamilies (Itoh et al., "Fibroblast growth factors: from molecular evolution to roles in development, metabolism, and disease," *J Biochem* 149:121-130 (2011); Mohammadi et al., "Structural basis for fibroblast growth factor receptor activation," *Cytokine Growth Factor Rev* 16:107-137 (2005), which are hereby incorporated by reference in its entirety). The FGF core homology domain (approximately 120 amino acids long) is flanked by N- and C-terminal sequences that are highly variable in both length and primary sequence, particularly among different FGF subfamilies. The core region of FGF19 shares the highest sequence identity with FGF21 (38%) and FGF23 (36%), and therefore, these ligands are considered to form a subfamily.

[0032] Based on mode of action, the eighteen mammalian FGFs are grouped into paracrine-acting ligands (five FGF subfamilies) and endocrine-acting ligands (one FGF subfamily) comprising FGF19, FGF21 and FGF23 (Itoh and Ornitz, "Fibroblast Growth Factors: From Molecular Evolution to Roles in Development, Metabolism and Disease," *J. Biochem.* 149:121-130 (2011); Mohammadi et al., "Structural Basis for Fibroblast Growth Factor Receptor Activation," *Cytokine Growth Factor Rev.* 16:107-137 (2005), which are hereby incorporated by reference in their entirety).

[0033] Paracrine FGFs direct multiple processes during embryogenesis, including gastrulation, somitogenesis, organogenesis, and tissue patterning (Itoh and Ornitz, "Fibroblast Growth Factors: From Molecular Evolution to Roles in Development, Metabolism and Disease," *J. Biochem.* 149:121-130 (2011); Bottcher and Niehrs, "Fibroblast Growth Factor Signaling During Early Vertebrate Development," *Endocr. Rev.* 26:63-77 (2005); Thisse et al., "Functions and Regulations of Fibroblast Growth Factor Signaling During Embryonic Development," *Dev. Biol.* 287:390-402 (2005), which are hereby incorporated by reference in their entirety), and also

regulate tissue homeostasis in the adult (Hart et al., "Attenuation of FGF Signalling in Mouse Beta-cells Leads to Diabetes," *Nature* 408:864-868 (2000); Jonker et al., "A PPARγ-FGF1 Axis is Required for Adaptive Adipose Remodelling and Metabolic Homeostasis," *Nature* 485:391-394 (2012), which is hereby incorporated by reference in its entirety).

[0034] Endocrine FGFs control major metabolic processes such as bile acid homeostasis (Inagaki et al., "Fibroblast Growth Factor 15 Functions as an Enterohepatic Signal to Regulate Bile Acid Homeostasis," *Cell Metab.* 2:217-225 (2005), which is hereby incorporated by reference in its entirety), and hepatic glucose and protein metabolism (Kir et al., "FGF19 as a Postprandial, Insulin-Independent Activator of Hepatic Protein and Glycogen Synthesis," *Science* 331:1621-1624 (2011); Potthoff et al., "FGF15/19 Regulates Hepatic Glucose Metabolism by Inhibiting the CREB-PGC-1α Pathway," *Cell Metab.* 13:729-738 (2011), which are hereby incorporated by reference in their entirety) (FGF19), glucose and lipid metabolism (Badman et al., "Hepatic Fibroblast Growth Factor 21 Is Regulated by PPARα and Is a Key Mediator of Hepatic Lipid Metabolism in Ketotic States," *Cell Metab.* 5:426-437 (2007); Inagaki et al., "Endocrine Regulation of the Fasting Response by PPARalpha-mediated Induction of Fibroblast Growth Factor 21," *Cell Metab.* 5:415-425 (2007); Kharitonenkov et al., "FGF-21 as a Novel Metabolic Regulator," *J. Clin. Invest.* 115:1627-1635 (2005); Potthoff et al., "FGF21 Induces PGC-1alpha and Regulates Carbohydrate and Fatty Acid Metabolism During the Adaptive Starvation Response," *Proc. Nat'l. Acad. Sci. U.S.A.* 106:10853-10858 (2009), which are hereby incorporated by reference in their entirety) (FGF21), and phosphate and vitamin D homeostasis (White et al., "Autosomal Dominant Hypophosphataemic Rickets is Associated with Mutations in FGF23," *Nat. Genet.* 26:345-348 (2000); Shimada et al., "Targeted Ablation of Fgf23 Demonstrates an Essential Physiological Role of FGF23 in Phosphate and Vitamin D Metabolism," *J. Clin. Invest.* 113:561-568 (2004), which are hereby incorporated by reference in their entirety) (FGF23). Thus, these ligands have attracted much attention as potential drugs for the treatment of various inherited or acquired metabolic disorders (Beenken and Mohammadi, "The FGF Family: Biology, Pathophysiology and Therapy," *Nat. Rev. Drug Discov.* 8:235-253 (2009); Beenken and Mohammadi, "The Structural Biology of the FGF19 Subfamily," in *Endocrine FGFs and Klothos* (Kuro-o, M. ed.), Landes Bioscience. pp 1-24 (2012), which are hereby incorporated by reference in their entirety).

[0035] FGFs share a core homology region of about one hundred and twenty amino acids that fold into a β-trefoil (Ago et al., *J. Biochem.* 110:360-363 (1991); Eriksson et al., *Proc. Nat'l. Acad. Sci. U.S.A.* 88:3441-3445 (1991); Zhang et al., *Proc. Nat'l. Acad. Sci. U.S.A.* 88:3446-

3450 (1991); Zhu et al., *Science* 251:90-93 (1991), which are hereby incorporated by reference in their entirety) consisting of twelve β strands in paracrine FGFs (β 1- β 12) and eleven β strands in endocrine FGFs (β 1- β 10 and β 12) (Mohammadi et al., "Structural Basis for Fibroblast Growth Factor Receptor Activation," *Cytokine Growth Factor Rev.* 16:107-137 (2005); Goetz et al., *Mol. Cell Biol.* 27:3417-3428 (2007), which are hereby incorporated by reference in their entirety). The conserved core region is flanked by divergent N- and C-termini, which play a critical role in conferring distinct biological activity on FGFs (Mohammadi et al., "Structural Basis for Fibroblast Growth Factor Receptor Activation," *Cytokine Growth Factor Rev.* 16:107-137 (2005); Olsen et al., *Genes Dev.* 20:185-198 (2006), which are hereby incorporated by reference in their entirety).

[0036] All FGFs interact with pericellular heparan sulfate (HS) glycosaminoglycans albeit with different affinities (Asada et al., *Biochim. Biophys. Acta.* 1790:40-48 (2009), which is hereby incorporated by reference in its entirety). The HS-binding site of FGFs is comprised of the β1-β2 loop and the region between β10 and β12 strands (Mohammadi et al., "Structural Basis for Fibroblast Growth Factor Receptor Activation," *Cytokine Growth Factor Rev.* 16:107-137 (2005), which is hereby incorporated by reference in its entirety). HS interacts with both side chain and main chain atoms of the HS-binding site in paracrine FGFs (Schlessinger et al., *Mol. Cell* 6:743-750 (2000), which is hereby incorporated by reference in its entirety). The HS-binding site of endocrine FGFs deviates from the common conformation adopted by paracrine FGFs such that interaction of HS with backbone atoms of the HS-binding site is precluded (Goetz et al., *Mol. Cell Biol.* 27:3417-3428 (2007), which is hereby incorporated by reference in its entirety). As a result, compared to paracrine FGFs, endocrine FGFs exhibit poor affinity for HS (Beenken and Mohammadi, "The FGF Family: Biology, Pathophysiology and Therapy," *Nat. Rev. Drug Discov.* 8:235-253 (2009); Asada et al., *Biochim. Biophys. Acta.* 1790:40-48 (2009), which are hereby incorporated by reference in their entirety). The poor HS affinity enables these ligands to diffuse freely away from the site of their secretion and enter the blood circulation to reach their distant target organs (Goetz et al., *Mol. Cell Biol.* 27:3417-3428 (2007); Asada et al., *Biochim. Biophys. Acta.* 1790:40-48 (2009), which are hereby incorporated by reference in their entirety).

[0037] By contrast, owing to their high HS affinity (Asada et al., *Biochim. Biophys. Acta.* 1790:40-48 (2009), which is hereby incorporated by reference in its entirety), paracrine FGFs are mostly immobilized in the vicinity of the cells secreting these ligands, and hence can only act within the same organ. There is emerging evidence that differences in HS-binding affinity

among paracrine FGFs translate into the formation of ligand-specific gradients in the pericellular matrix (Kalinina et al., *Mol. Cell Biol.* 29:4663-4678 (2009); Makarenkova et al., *Sci. Signal* 2:ra55 (2009), which are hereby incorporated by reference in their entirety), which contribute to the distinct functions of these ligands (Beenken and Mohammadi, "The FGF Family: Biology, Pathophysiology and Therapy," *Nat. Rev. Drug Discov.* 8:235-253 (2009) ; Itoh and Ornitz, "Fibroblast Growth Factors: From Molecular Evolution to Roles in Development, Metabolism and Disease," *J. Biochem.* 149:121-130 (2011), which are hereby incorporated by reference in their entirety).

[0038] Besides controlling ligand diffusion in the extracellular space, HS promotes the formation of the 2:2 paracrine FGF-FGFR signal transduction unit (Schlessinger et al., *Mol. Cell* 6:743-750 (2000); Mohammadi et al., *Curr. Opin. Struct. Biol.* 15:506-516 (2005), which are hereby incorporated by reference in their entirety). HS engages both ligand and receptor to enhance the binding affinity of FGF for receptor and promote dimerization of ligand-bound receptors. Owing to their poor HS-binding affinity, endocrine FGFs rely on Klotho co-receptors to bind their cognate FGFR (Kurosu et al., *J. Biol. Chem.* 282:26687-26695 (2007); Kurosu et al., *J. Biol. Chem.* 281:6120-6123 (2006); Ogawa et al., *Proc. Nat'l. Acad. Sci. U.S.A.* 104:7432-7437 (2007); Urakawa et al., *Nature* 444:770-774 (2006), which are hereby incorporated by reference in their entirety). Klotho co-receptors are single-pass transmembrane proteins with an extracellular domain composed of two type I β -glycosidase domains (Ito et al., *Mech. Dev.* 98:115-119 (2000); Kuro-o et al., *Nature* 390:45-51 (1997), which are hereby incorporated by reference in their entirety). Klotho co-receptors constitutively associate with FGFRs to enhance the binding affinity of endocrine FGFs for their cognate FGFRs in target tissues (Kurosu et al., *J. Biol. Chem.* 282:26687-26695 (2007); Kurosu et al., *J. Biol. Chem.* 281:6120-6123 (2006); Ogawa et al., *Proc. Nat'l. Acad. Sci. U.S.A.* 104:7432-7437 (2007); Urakawa et al., *Nature* 444:770-774 (2006), which are hereby incorporated by reference in their entirety). αKlotho is the co-receptor for FGF23 (Kurosu et al., *J. Biol. Chem.* 281:6120-6123 (2006); Urakawa et al., *Nature* 444:770-774 (2006), which are hereby incorporated by reference in their entirety), and βKlotho is the co-receptor for both FGF19 and FGF21 (Kurosu et al., *J. Biol. Chem.* 282:26687-26695 (2007); Ogawa et al., *Proc. Nat'l. Acad. Sci. U.S.A.* 104:7432-7437 (2007), which are hereby incorporated by reference in their entirety). The C-terminal region of endocrine FGFs mediates binding of these ligands to the FGFR-α/βKlotho co-receptor complex (Goetz et al., *Mol. Cell Biol.* 27:3417-3428 (2007); Goetz et al., *Proc. Nat'l. Acad. Sci. U.S.A* 107:407-412 (2010); Micanovic et al., *J. Cell Physiol.* 219:227-234 (2009); Wu et al., *J. Biol. Chem.*

283:33304-33309 (2008); Yie et al., *FEBS Lett,* 583:19-24 (2009); Goetz et al., *Mol. Cell Biol.* 32:1944-1954 (2012), which are hereby incorporated by reference in their entirety).

[0039] βKlotho promotes binding of FGF21 to its cognate FGFR by engaging ligand and receptor simultaneously through two distinct binding sites (Goetz et al., "Klotho Coreceptors Inhibit Signaling by Paracrine Fibroblast Growth Factor 8 Subfamily Ligands," *Mol Cell Biol* 32:1944-1954 (2012), which is hereby incorporated by reference in its entirety). βKlotho plays the same role in promoting binding of FGF19 to its cognate FGFR (Goetz et al., "Klotho Coreceptors Inhibit Signaling by Paracrine Fibroblast Growth Factor 8 Subfamily Ligands," *Mol Cell Biol* 32:1944-1954 (2012), which is hereby incorporated by reference in its entirety). The binding site for βKlotho was mapped on FGF21 and FGF19 to the C-terminal region of each ligand that follows the β-trefoil core domain (Goetz et al., "Klotho Coreceptors Inhibit Signaling by Paracrine Fibroblast Growth Factor 8 Subfamily Ligands," *Mol Cell Biol* 32:1944-1954 (2012), which is hereby incorporated by reference in its entirety). In the course of these studies, it was found that the C-terminal tail peptides of FGF21 and FGF19 share a common binding site on βKlotho, and that the C-terminal tail of FGF19 binds tighter than the C-terminal tail of FGF21 to this site (Goetz et al., "Klotho Coreceptors Inhibit Signaling by Paracrine Fibroblast Growth Factor 8 Subfamily Ligands," *Mol Cell Biol* 32:1944-1954 (2012), which is hereby incorporated by reference in its entirety).

[0040] Endocrine FGFs still possess residual HS-binding affinity, and moreover, there are differences in this residual binding affinity among the endocrine FGFs (Goetz et al., *Mol. Cell Biol.* 27:3417-3428 (2007), which is hereby incorporated by reference in its entirety). These observations raise the possibility that HS may play a role in endocrine FGF signaling. Indeed, there are several reports showing that HS can promote endocrine FGF signaling in the presence as well as in the absence of Klotho co-receptor. It has been shown that HS augments the mitogenic signal elicited by endocrine FGFs in BaF3 cells over-expressing FGFR and Klotho co-receptor by at least two-fold (Suzuki et al., *Mol. Endocrinol.* 22:1006-1014 (2008), which is hereby incorporated by reference in its entirety). In addition, even in the absence of Klotho co-receptor, HS enables endocrine FGFs to induce proliferation of BaF3 cells over-expressing FGFR (Yu et al., *Endocrinology* 146:4647-4656 (2005); Zhang et al., *J. Biol. Chem.* 281:15694-15700 (2006), which are hereby incorporated by reference in their entirety). Compared to paracrine FGFs, however, significantly higher concentrations of both ligand and HS are needed, and the proliferative response of cells to endocrine FGFs still lags behind that of paracrine FGFs

by about one order of magnitude (Zhang et al., *J. Biol. Chem.* 281:15694-15700 (2006), which is hereby incorporated by reference in its entirety).

[0041] As used herein, the terms "chimeric polypeptide" and "chimeric protein" encompass a polypeptide having a sequence that includes at least a portion of a full-length sequence of first polypeptide sequence and at least a portion of a full-length sequence of a second polypeptide sequence, where the first and second polypeptides are different polypeptides. A chimeric polypeptide also encompasses polypeptides that include two or more non-contiguous portions derived from the same polypeptide. A chimeric polypeptide or protein also encompasses polypeptides having at least one substitution, wherein the chimeric polypeptide includes a first polypeptide sequence in which a portion of the first polypeptide sequence has been substituted by a portion of a second polypeptide sequence.

[0042] As used herein, the term "N-terminal portion" of a given polypeptide sequence is a contiguous stretch of amino acids of the given polypeptide sequence that begins at or near the N-terminal residue of the given polypeptide sequence. An N-terminal portion of the given polypeptide can be defined by a contiguous stretch of amino acids (e.g., a number of amino acid residues). Similarly, the term "C-terminal portion" of a given polypeptide sequence is a contiguous length of the given polypeptide sequence that ends at or near the C-terminal residue of the given polypeptide sequence. A C-terminal portion of the given polypeptide can be defined by a contiguous stretch of amino acids (e.g., a number of amino acid residues).

[0043] The term "portion," when used herein with respect to a given polypeptide sequence, refers to a contiguous stretch of amino acids of the given polypeptide's sequence that is shorter than the given polypeptide's full-length sequence. A portion of a given polypeptide may be defined by its first position and its final position, in which the first and final positions each correspond to a position in the sequence of the given full-length polypeptide. The sequence position corresponding to the first position is situated N-terminal to the sequence position corresponding to the final position. The sequence of the portion is the contiguous amino acid sequence or stretch of amino acids in the given polypeptide that begins at the sequence position corresponding to the first position and ending at the sequence position corresponding to the final position. A portion may also be defined by reference to a position in the given polypeptide sequence and a length of residues relative to the referenced position, whereby the sequence of the portion is a contiguous amino acid sequence in the given full-length polypeptide that has the defined length and that is located in the given polypeptide in reference to the defined position.

[0044] As noted above, a chimeric protein according to the present invention may include an N-terminus coupled to a C-terminus. N-terminus and C-terminus are used herein to refer to the N-terminal region or portion and the C-terminal region or portion, respectively, of the chimeric protein of the present invention. In some embodiments of the present invention, the C-terminal portion and the N-terminal portion of the chimeric protein of the present invention are contiguously joined. In alternative embodiments, the C-terminal portion and the N-terminal portion of the chimeric protein of the present invention are coupled by an intervening spacer. In one embodiment, the spacer may be a polypeptide sequence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more amino acid residues. In some embodiments, the C-terminal portion and/or the N-terminal portion of the chimeric protein of the present invention may include additional portion(s) coupled to the C-terminal residue and/or the N-terminal residue of the chimeric protein of the present invention, respectively. In some embodiments, the additional portion(s) may be a polypeptide sequence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more amino acid residues. In some embodiments, the N-terminal portion and/or the C-terminal portion having such additional portion(s) will maintain the activity of the corresponding naturally occurring N-terminal portion and/or C-terminal portion, respectively. In some embodiments, the N-terminal portion and/or the C-terminal portion having such additional portion(s) will have enhanced and/or prolonged activity compared to the corresponding naturally occurring N-terminal portion and/or C-terminal portion, respectively. In other embodiments, the C-terminal portion and/or the N-terminal portion of the chimeric protein of the present invention do not include any additional portion(s) coupled to the C-terminal residue and/or the N-terminal residue of the chimeric protein of the present invention, respectively.

[0045] The portion of the paracrine FGF may be derived from any suitable paracrine FGF. Suitable paracrine FGFs in accordance with the present invention include FGF1, FGF2, and ligands of the FGF4 and FGF9 subfamilies. Certain embodiments of the present invention may include a full-length amino acid sequence of a paracrine FGF, rather than a portion of a paracrine FGF.

[0046] In one embodiment, the portion of the paracrine FGF is derived from a mammalian FGF. In one embodiment, the portion of the paracrine FGF is derived from a vertebrate FGF. In one embodiment, the portion of the paracrine FGF is derived from a human FGF. In one embodiment, the paracrine FGF is derived from a non-human mammalian FGF. In one embodiment, the portion of the paracrine FGF is derived from a non-human vertebrate FGF. In one embodiment, the paracrine FGF is derived from an ortholog of human FGF, or a

polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species.

[0047] In one embodiment according to the present invention, the portion of the paracrine FGF of the chimeric protein includes an N-terminal portion of the paracrine FGF.

[0048] In one embodiment, the paracrine FGF is FGF1. In one embodiment, the portion of the FGF1 is from human FGF1 having the following amino acid sequence (GenBank Accession No. AAH32697, which is hereby incorporated by reference in its entirety) (SEQ ID NO: 1):

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1   MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ
61  LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYISKKHAEK
121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
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[0049] In one embodiment, the portion of the paracrine FGF includes an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 150 to 155 of SEQ ID NO: 1 (human FGF1). In one embodiment, the portion of the paracrine FGF includes amino acid residues 1-150, 1-151, 1-152, 1-153, 1-154, 1-155, 2-150, 2-151, 2-152, 2-153, 2-154, 2-155, 3-150, 3-151, 3-152, 3-153, 3-154, 3-155, 4-150, 4-151, 4-152, 4-153, 4-154, 4-155, 5-150, 5-151, 5-152, 5-153, 5-154, 5-155, 6-150, 6-151, 6-152, 6-153, 6-154, 6-155, 7-150, 7-151, 7-152, 7-153, 7-154, 7-155, 8-150, 8-151, 8-152, 8-153, 8-154, 8-155, 9-150, 9-151, 9-152, 9-153, 9-154, 9-155, 10-150, 10-151, 10-152, 10-153, 10-154, 10-155, 11-150, 11-151, 11-152, 11-153, 11-154, 11-155, 12-150, 12-151, 12-152, 12-153, 12-154, 12-155, 13-150, 13-151, 13-152, 13-153, 13-154, 13-155, 14-150, 14-151, 14-152, 14-153, 14-154, 14-155, 15-150, 15-151, 15-152, 15-153, 15-154, 15-155, 16-150, 16-151, 16-152, 16-153, 16-154, 16-155, 17-150, 17-151, 17-152, 17-153, 17-154, 17-155, 18-150, 18-151, 18-152, 18-153, 18-154, 18-155, 19-150, 19-151, 19-152, 19-153, 19-154, 19-155, 20-150, 20-151, 20-152, 20-153, 20-154, 20-155, 21-150, 21-151, 21-152, 21-153, 21-154, 21-155, 22-150, 22-151, 22-152, 22-153, 22-154, 22-155, 23-150, 23-151, 23-152, 23-153, 23-154, 23-155, 24-150, 24-151, 24-152, 24-153, 24-154, 24-155, 25-150, 25-151, 25-152, 25-153, 25-154, or 25-155 of FGF1 (SEQ ID NO: 1). In one embodiment, the portion of the paracrine FGF includes amino acid residues 1-150 or 25-150 of SEQ ID NO: 1.

[0050] In one embodiment, the portion of the paracrine FGF includes an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence identity to an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 150 to 155 of SEQ ID NO: 1 (human FGF1). In one embodiment, the portion of the paracrine FGF includes an amino acid sequence that has at least

80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence homology to an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 150 to 155 of SEQ ID NO: 1 (human FGF1).

[0051] Percent (%) amino acid sequence identity with respect to a given polypeptide sequence identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical to the amino acid residues in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Percent (%) amino acid sequence homology with respect to a given polypeptide sequence identified herein is the percentage of amino acid residues in a candidate sequence that are identical to or strongly similar to the amino acid residues in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence homology. Strongly similar amino acid residues may include, for example, conservative amino acid substitutions known in the art. Alignment for purposes of determining percent amino acid sequence identity and/or homology can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared.

[0052] In one embodiment of the present invention, the portion of the paracrine FGF of the chimeric protein is derived from an ortholog of human FGF1. In one embodiment, the portion of FGF1 is derived from Papio Anubis, Pongo abelii, Callithrix jacchus, Equus caballus, Pan troglodytes, Loxodonta Africana, Canis lupus familiaris, Ailuropoda melanoleuca, Saimiri boliviensis boliviensis, Sus scrofa, Otolemur garnettii, Rhinolophus ferrumequinum, Sorex araneus, Oryctolagus cuniculus, Cricetulus griseus, Sarcophilus harrisii, Mus musculus, Cavia porcellus, Monodelphis domestica, Desmodus rotundus, Bos taurus, Ornithorhynchus anatinus, Taeniopygia guttata, Dasypus novemcinctus, Xenopus Silurana tropicalis, Heterocephalus glaber, Pteropus alecto, Tupaia chinensis, Columba livia, Ovis aries, Gallus gallus, Vicugna pacos, Anolis carolinensis, Otolemur garnettii, Felis catus, Pelodiscus sinensis, Latimeria chalumnae, Tursiops truncates, Mustela putorius furo, Nomascus leucogenys, Gorilla gorilla, Erinaceus europaeus, Procavia capensis, Dipodomys ordii, Petromyzon marinus, Echinops telfairi, Macaca mulatta, Pteropus vampyrus, Myotis lucifugus, Microcebus murinus, Ochotona princeps, Rattus norvegicus, Choloepus hoffmanni, Ictidomys tridecemlineatus, Tarsius syrichta,

Tupaia belangeri, Meleagris gallopavo, Macropus eugenii, or Danio rerio. The portions of an ortholog of human paracrine FGF1 include portions corresponding to the above-identified amino acid sequences of human FGF1. Corresponding portions may be determined by, for example, sequence analysis and structural analysis.

[0053] In one embodiment, the portion of the FGF1 of the chimeric protein of the present invention is derived from an ortholog of human FGF1 having the amino acid sequence shown in Table 1.

Table 1:

Amino acid sequence of human FGF1 (SEQ ID NO:1)(GenBank accession no. AAH32697, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYISKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Papio anubis (olive baboon) FGF1(SEQ ID NO:2) (GenBank accession no. NP_001162557, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP ANYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYISKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Pongo abelii (Sumatran orangutan) FGF1(SEQ ID NO:3) (GenBank accession no. NP_001127073, which is hereby incorporated by reference in its entirety) 60 M 61 AEGEITTFTA LTEKFNLPPG NYKKPKLLYC SNGGHFLRIL PDGTVDGTRD RSDQHIQLQL 121 SAESVGEVYI KSTETGQYLA MDTDGLLYGS QTPNEECLFL ERLEENHYNT YISKKHAEKN 181 WFVGLKKNGS CKRGPRTHYG QKAILFLPLP VSSD
Amino acid sequence of Callithrix jacchus (white-tufted-ear marmoset) FGF1(SEQ ID NO:4) (GenBank accession no. XP_002744341, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFDLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYISKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Equus caballus (horse) FGF1(SEQ ID NO:5) (GenBank accession no. NP_001157358, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Pan troglodytes (chimpanzee) FGF1(SEQ ID NO:6) (GenBank accession no. JAA29511, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPS GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYISKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD

Amino acid sequence of Loxodonta africana (elephant) FGF1(SEQ ID NO:7) (GenBank accession no. XP_003404621, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKGTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Canis lupus familiaris (dog) FGF1(SEQ ID NO:8) (GenBank accession no. XP_849274, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYMKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Ailuropoda melanoleuca (giant panda) FGF1(SEQ ID NO:9) (GenBank accession no. XP_002912581, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPA GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Saimiri boliviensis boliviensis (Bolivian squirrel monkey) FGF1(SEQ ID NO:10) (GenBank accession no. XP_003920596, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFDLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDLHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYISKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Sus scrofa (pig) FGF1(SEQ ID NO:11) (GenBank accession no. XP_003124058, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTSGLLYG SQTPSEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Otolemur garnettii (small-eared galago) FGF1(SEQ ID NO:12) (GenBank accession no. XP_003782135, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPL GNYKKPKLLY CSNGGHFLRI LPDGTVDGTQ DRSDQHIQLQ 61 LSAESVGEVY IKSTQTGQYL AMDSDGLLYG SQTPNEECLF LERLEENHYN TYVSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Rhinolophus ferrumequinum (greater horseshoe bat) FGF1(SEQ ID NO:13) (GenBank accession no. ACC62496, which is hereby incorporated by reference in its entirety): 1 MAEGEVTTFT ALTEKFNLPT GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DKSDQHIQLQ 61 LSAESVGEVY IKSTESGQYL AMDSDGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Sorex araneus (European shrew) FGF1(SEQ ID NO:14) (GenBank accession no. ACE75805, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFG ALMEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGHYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD

Amino acid sequence of Oryctolagus cuniculus (rabbit) FGF1(SEQ ID NO:15) (GenBank accession no. NP_001164959, which is hereby incorporated by reference in its entirety): 1 MAEGEVTTFT ALTEKFNLPA GNYKLPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPSEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Cricetulus griseus (Chinese hamster) FGF1(SEQ ID NO:16) (GenBank accession no. XP_003502469, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFS ALTERFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESAGEVY IKGTETGQYR NMDTDGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Sarcophilus harrisii (Tasmanian devil) FGF1(SEQ ID NO:17) (GenBank accession no. XP_003756738, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTERFNLPL GNYKKPKLLY CSNGGHFLRI LPDGKVDGTR DRNDQHIQLQ 61 LSAESVGEVY IKSTESGQYL AMDTDGLLYG SQTPTEECLF LERLEENHYN TYISKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSE
Amino acid sequence of Mus musculus (house mouse) FGF1(SEQ ID NO:18) (GenBank accession no. NP_034327, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFA ALTERFNLPL GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESAGEVY IKGTETGQYL AMDTEGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Cavia porcellus (domestic guinea pig) FGF1(SEQ ID NO:19) (GenBank accession no. XP_003477242, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFA ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAEGVGEVY IQSTETGQYL AMDTDGLLYG SQTPSEECLF LERLEENHYN TYTSKKHVEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSD
Amino acid sequence of Monodelphis domestica (gray short-tailed opossum) FGF1(SEQ ID NO:20) (GenBank accession no. XP_001368921, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTERFNLPL GNYKKPKLLY CSNGGHFLRI LPDGKVDGTR DRNDQHIQLQ 61 LSTESVGEVY IKSTESGQYL AMDTDGLLYG SQTPSEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKKGPRTHY GQKAILFLPL PVSSE
Amino acid sequence of Desmodus rotundus (common vampire bat) FGF1(SEQ ID NO:21) (GenBank accession no. JAA45191, which is hereby incorporated by reference in its entirety): 1 MAEGEVTTFT ALTEKFNLPL ESYKKPKLLY CSNGGHFLRI LPDGTVDGTR DKSDQHIQLQ 61 LSAESVGEVY IKSTGSGQYL AMDSAGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVNSD
Amino acid sequence of Bos taurus (cattle) FGF1(SEQ ID NO:22) (GenBank accession no. NP_776480, which is hereby incorporated by reference in its entirety): 1 MAEGETTTFT ALTEKFNLPL GNYKKPKLLY CSNGGYFLRI LPDGTVDGTK DRSDQHIQLQ 61 LCAESIGEVY IKSTETGQFL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYISKKHAEK 121 HWFVGLKKNG RSKLGPRTHF GQKAILFLPL PVSSD
Amino acid sequence of Ornithorhynchus anatinus (platypus) FGF1(SEQ ID NO:23) (GenBank accession no. XP_001514861, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALMEKFDLPL GNYKKPRLLY CSNGGYFLRI QPDGKVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTESGHYL AMDTEGLLYG SQAPSEDCLF LERLEENHYN TYVSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVASD

Amino acid sequence of Taeniopygia guttata (zebra finch) FGF1(SEQ ID NO:24) (GenBank accession no. XP_002193287, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFS ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGVVH IQSTQSGQYL AMDTNGLLYG SQLPPGECLF LERLEENHYN TYVSKMHADK 121 NWFVGLKKNG TSKLGPRTHY GQKAILFLPL PVAAD
Amino acid sequence of Dasypus novemcinctus (nine-banded armadillo) FGF1(SEQ ID NO:25) (GenBank accession no. ACO06224, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFM ALMEKFNLPL ENYKHPRLLY CRNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSAETGQYL AMDTDGLLYG SETPSEECLF MEKLEENNYN TYISKKHAEK 121 KWFVGLKKDG SSKRGPQTHY GQKAILFLPL PVSSD
Amino acid sequence of Xenopus Silurana tropicalis (western clawed frog) FGF1(SEQ ID NO:26) (GenBank accession no. ACJ50585, which is hereby incorporated by reference in its entirety): 1 MAEGDITTFN PIAESFSLPI GNYKKPKLLY CNNGGYFLRI LPDGVVDGTR DRDDLYITLK 61 LSAQSQGEVH IKSTETGSYL AMDSSGQLYG TLTPNEESLF LETLEENHYN TYKSKKYAEN 121 NWFVGIKKNG ASKKGSRTHY GQKAILFLPL PASPD
Amino acid sequence of Heterocephalus glaber (naked mole-rat) FGF1(SEQ ID NO:27) (GenBank accession no. EHA99379, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGKVDGTR DRSDQHIQLQ 61 LSAEGVGEVY IKSTETGQYL AMDTDGLLYG SQTASEECLF LERLEENHYN TYISKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Pteropus alecto (black flying fox) FGF1(SEQ ID NO:28) (GenBank accession no. ELK02961, which is hereby incorporated by reference in its entirety): 1 MAEGEVTTFT ALTERFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DKSDQHIQLQ 61 LSAESVGEVY IKSTESGQYL AMDSDGLLYG SQTPDEDCLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Tupaia chinensis (Chinese tree shrew) FGF1(SEQ ID NO:29) (GenBank accession no. ELW69091, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFA ALTEKFDLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LTAENVGEVY IKSTETGQYL AMDADGLLYG SQTPNEECLF LERLEENHYN TYISKKHAEK 121 NWFVALKKNG SCKLGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Columba livia (rock pigeon) FGF1(SEQ ID NO:30) (GenBank accession no. EMC79997, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGKVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTQSGQYL AMDPTGLLYG SQLLGEECLF LERIEENHYN TYVSKKHADK 121 NWFVGLKKNG NSKLGPRTHY GQKAILFLPL PVSAD
Amino acid sequence of Ovis aries (sheep) FGF1(SEQ ID NO:31) (GenBank accession no. XP_004008958, which is hereby incorporated by reference in its entirety): 1 MAEGETTTFR ALTEKFNLPL GNYKKPKLLY CSNGGYFLRI LPDGRVDGTK DRSDQHIQLQ 61 LYAESIGEVY IKSTETGQFL AMDTNGLLYG SQTPSEECLF LERLEENHYN TYISKKHAEK 121 NWFIGLKKNG SSKLGPRTHF GQKAILFLPL PVSSD
Amino acid sequence of Gallus gallus (chicken) FGF1(SEQ ID NO:32) (GenBank accession no. NP_990511, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTERFGLPL GNYKKPKLLY CSNGGHFLRI LPDGKVDGTR DRSDQHIQLQ 61 LSAEDVGEVY IKSTASGQYL AMDTNGLLYG SQLPGEECLF LERLEENHYN TYISKKHADK 121 NWFVGLKKNG NSKLGPRTHY GQKAILFLPL PVSAD

Amino acid sequence of Vicugna pacos (alpaca) FGF1(SEQ ID NO:33) (Ensembl accession no. ENSVPAP00000007810; partial sequence corresponding to human FGF1 residues 58 to 155, which is hereby incorporated by reference in its entirety): 1 QLQLSAESVG EVYIKSTETG QYLAMDTDGL LHGSQTPNEE CLFLERLEEN HYNTYTSKKH 61 AEKNWFVGLK KNGSCKRGPR THYGQKAILF LPLPVSSD
Amino acid sequence of Anolis carolinensis (anole lizard) FGF1(SEQ ID NO:34) (Ensembl accession no. ENSACAP00000013203, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTERFALPM ENYKKPKLLY CSNGGHFLRI LPDGKVDGTM DRNDSYIQLL 61 LTAEDVGVVY IKGTETGQYL AMDANGHLYG SQLPTEECLF VETLEENHYN TYTSKMHGDK 121 KWYVGLKKNG KGKLGPRTHR GQKAILFLPL PVSPD
Amino acid sequence of Otolemur garnettii (bushbaby) FGF1(SEQ ID NO:35) (Ensembl accession no. ENSOGAP00000004540, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPL GNYKKPKLLY CSNGGHFLRI LPDGTVDGTQ DRSDQHIQLQ 61 LSAESVGEVY IKSTQTGQYL AMDSDGLLYG SQTPNEECLF LERLEENHYN TYVSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Felis catus (cat) FGF1(SEQ ID NO:36) (Ensembl accession no. ENSFCAP00000008457, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Pelodiscus sinensis (Chinese softshell turtle) FGF1(SEQ ID NO:37) (Ensembl accession no. ENSPSIP00000016356, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPL GNYKNPKLLY CSNGGYFLRI HPDGKVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTESGQFL AMDANGLLYG SLSPSEECLF LERMEENHYN TYISKKHADK 121 NWFVGLKKNG SCKLGPRTHY GQKAVLFLPL PVSAD
Amino acid sequence of Latimeria chalumnae (coelacanth) FGF1(SEQ ID NO:38) (Ensembl accession no. ENSLACP00000015106, which is hereby incorporated by reference in its entirety): 1 MAEDKITTLK ALAEKFNLPM GNYKKAKLLY CSNGGYFLRI PPDGKVEGIR ERSDKYIQLQ 61 MNAESLGMVS IKGVEAGQYL AMNTNGLLYG SQSLTEECLF MEKMEENHYN TYRSKTHADK 121 NWYVGIRKNG SIKPGPRTHI GQKAVLFLPL PASSD
Amino acid sequence of Tursiops truncatus (dolphin) FGF1(SEQ ID NO:39) (Ensembl accession no. ENSTTRP00000004470, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYASKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Mustela putorius furo (ferret) FGF1(SEQ ID NO:40) (Ensembl accession no. ENSMPUP00000007888, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALMEKFNLPA GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Nomascus leucogenys (gibbon) FGF1(SEQ ID NO:41) (Ensembl accession no. ENSNLEP00000011873, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYISKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD

Amino acid sequence of Gorilla gorilla (gorilla) FGF1(SEQ ID NO:42) (Ensembl accession no. ENSGGOP00000017663, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYISKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Erinaceus europaeus (hedgehog) FGF1(SEQ ID NO:43) (Ensembl accession no. ENSEEUP00000005318, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPL GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Procavia capensis (hyrax) FGF1(SEQ ID NO:44) (Ensembl accession no. ENSPCAP00000010969, which is hereby incorporated by reference in its entirety)(partial sequence corresponding to human FGF1 residues 1 to 91): 1 MAEGEITTFT ALTEKFNLPL ENYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKGTETGQYL AMDTDGLLYG S
Amino acid sequence of Dipodomys ordii (kangaroo rat) FGF1(SEQ ID NO:45) (Ensembl accession no. ENSDORP00000006889, which is hereby incorporated by reference in its entirety) (partial sequence corresponding to human FGF1 residues 1 to 16 and 58 to 155): 1 MAEGEITTFT ALTERF---- ---------- ---------- ---------- -------QLQ 61 LSAESVGEVY IKSTETGQYL AMDADGLLYG SQTPDEECLF LERLEENHYN TYIAKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Petromyzon marinus (lamprey) FGF1(SEQ ID NO:46) (Ensembl accession no. ENSPMAP00000010683, which is hereby incorporated by reference in its entirety)(partial sequence corresponding to human FGF1 residues 1 to 93): 1 MEVGHIGTLP VVPAGPVFPG SFKEPRRLYC RSAGHHLQIL GDGTVSGTQD ENEPHAVLQL 61 QAVRRGVVTI RGLCAERFLA MSTEGHLYGA VR
Amino acid sequence of Echinops telfairi (lesser hedgehog tenrec) FGF1(SEQ ID NO:47) (Ensembl accession no. ENSETEP00000014504, which is hereby incorporated by reference in its entirety)(partial sequence corresponding to human FGF1 residues 58 to 155) 1 QLKLVAESVG VVYIKSIKTG QYLAMNPDGL LYGSETPEEE CLFLETLEEN HYTTFKSKKH 61 VEKNWFVGLR KNGRVKIGPR THQGQKAILF LPLPVSSD
Amino acid sequence of Macaca mulatta (rhesus monkey) FGF1(SEQ ID NO:48) (Ensembl accession no. ENSMMUP00000030943, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Pteropus vampyrus (megabat) FGF1(SEQ ID NO:49) (Ensembl accession no. ENSPVAP00000004349, which is hereby incorporated by reference in its entirety): 1 MAEGEVTTFT ALTERFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DKSDQHIQLQ 61 LSAESVGEVY IKSTESGQYL AMDSDGLLYG SQTPDEDCLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD

Amino acid sequence of Myotis lucifugus (microbat) FGF1(SEQ ID NO:50) (Ensembl accession no. ENSMLUP00000006481, which is hereby incorporated by reference in its entirety): 1 MAEGEVTTFT ALTERFNLPL ENYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTESGQYL AMDSDGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Microcebus murinus (mouse lemur) FGF1(SEQ ID NO:51) (Ensembl accession no. ENSMICP00000008602, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESAGEVY IKSTQTGRYL AMDADGLLYG SQTPNEECLF LERLEENHYN TYVSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Ochotona princeps (pika) FGF1(SEQ ID NO:52) (Ensembl accession no. ENSOPRP00000011739, which is hereby incorporated by reference in its entirety): 1 MAEGEVTTFS ALTEKFNLPG GNYKLPKLLY CSNGGHFLRI LPDGTVDGTR DRSDLH---- 61 -------EVF IKSTETGQYL AMDTDGLLYG SQTPSEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGIKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Rattus norvegicus (rat) FGF1(SEQ ID NO:53) (Ensembl accession no. ENSRNOP00000018577, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFA ALTERFNLPL GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESAGEVY IKGTETGQYL AMDTEGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Choloepus hoffmanni (sloth) FGF1(SEQ ID NO:54) (Ensembl accession no. ENSCHOP00000010964, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALMEKFNLPP GNYMKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDLHIQLQ 61 LSAESVGEVY IKSAETGQYL AMDTGGLLYG SQTPSEECLF LERLEENHYN TYVSKKHAEK 121 NWFVGLKKNG SSKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Ictidomys tridecemlineatus (squirrel) FGF1(SEQ ID NO:55) (Ensembl accession no. ENSSTOP00000021782, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYTSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Tarsius syrichta (tarsier) FGF1(SEQ ID NO:56) (Ensembl accession no. ENSTSYP00000006804, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYVSKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Tupaia belangeri (tree shrew) FGF1(SEQ ID NO:57) (Ensembl accession no. ENSTBEP00000010264, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFA ALTEKFDLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ 61 LTAENVGEVY IKSTETGQYL AMDADGLLYG SQTPNEECLF LERLEENHYN TYISKKHAEK 121 NWFVALKKNG SCKLGPRTHY GQKAILFLPL PVSSD
Amino acid sequence of Meleagris gallopavo (turkey) FGF1(SEQ ID NO:58) (Ensembl accession no. ENSMGAP00000016398; partial sequence corresponding to human FGF1 residues 1 to 56, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTERFGLPL GNYKKPKLLY CSNGGHFLRI LPDGKVDGTR DRSDQH

Amino acid sequence of Macropus eugenii (wallaby) FGF1(SEQ ID NO:59) (Ensembl accession no. ENSMEUP00000015084, which is hereby incorporated by reference in its entirety): 1 MAEGEITTFT ALTERFNLPL GNYKKPKLLY CSNGGHFLRI LPDGKVDGTR DRNDQHIQLQ 61 LSAESVGEVY IKSTESGQYL AMDTNGLLYG SQTPSEECLF LERLEENHYN TYISKKHAEK 121 NWFVGLKKNG SCKRGPRTHY GQKAILFLPL PVSSE
Amino acid sequence of Danio rerio (zebrafish) FGF1(SEQ ID NO:60) (Ensembl accession no. ENSDARP00000008825, which is hereby incorporated by reference in its entirety): 1 MTEADIAVKS SPRDYKKLTR LYCMNGGFHL QILADGTVAG AADENTYSIL RIKATSPGVV 61 VIEGSETGLY LSMNEHGKLY ASSLVTDESY FLEKMEENHY NTYQSQKHGE NWYVGIKKNG 121 KMKRGPRTHI GQKAIFFLPR QVEQEED

[0054] As noted above, the portion of the paracrine FGF may be modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without the modification. In one embodiment, the modified portion of the paracrine FGF includes one or more substitutions, additions, or deletions.

[0055] In one embodiment, the one or more substitutions are located at one or more amino acid residues of SEQ ID NO: 1 selected from N33, K127, K128, N129, K133, R134, R137, Q142, K143, and combinations thereof. In one embodiment, the one or more substitutions are selected from N33T, K127D, K128Q, N129T, K133V, R134L, R137H, Q142M, K143T/L/I, and combinations thereof. In one embodiment, the modification is one or more substitutions which are located at one or more amino acid residues corresponding to residues of SEQ ID NO: 1 selected from N33, K127, K128, N129, K133, R134, R137, Q142, K143, and combinations thereof. In one embodiment, the modification is one or more substitutions which are located at one or more amino acid residues corresponding to residues of SEQ ID NO: 1 selected from N33, K127, K128, N129, K133, R134, R137, Q142, K143, and combinations thereof. Amino acid residues corresponding to those of SEQ ID NO:1 may be determined by, for example, sequence analysis and structural analysis.

[0056] Also encompassed within the present invention are portions of paracrine FGFs other than FGF1 (e.g., FGF2, FGF4, FGF5, FGF6, FGF9, FGF16, and FGF20). The portions derived from paracrine FGFs other than FGF1 include portions corresponding to the above-identified amino acid sequences of FGF1. Corresponding portions may be determined by, for example, sequence analysis and structural analysis.

[0057] It will be understood that the portion of the paracrine FGF according to the present invention may be derived from a nucleotide sequence that encodes a paracrine FGF protein. For example, in one embodiment, the nucleotide sequence is the nucleotide sequence that encodes human FGF1 (GenBank Accession No. BC032697, which is hereby incorporated by reference in its entirety) (SEQ ID NO: 61), as follows:

```
91                                    ATGGCTGAAG GGGAAATCAC CACCTTCACA
121 GCCCTGACCG AGAAGTTTAA TCTGCCTCCA GGGAATTACA AGAAGCCCAA ACTCCTCTAC
181 TGTAGCAACG GGGGCCACTT CCTGAGGATC CTTCCGGATG GCACAGTGGA TGGGACAAGG
241 GACAGGAGCG ACCAGCACAT TCAGCTGCAG CTCAGTGCGG AAAGCGTGGG GGAGGTGTAT
301 ATAAAGAGTA CCGAGACTGG CCAGTACTTG GCCATGGACA CCGACGGGCT TTTATACGGC
361 TCACAGACAC CAAATGAGGA ATGTTTGTTC CTGGAAAGGC TGGAGGAGAA CCATTACAAC
421 ACCTATATAT CCAAGAAGCA TGCAGAGAAG AATTGGTTTG TTGGCCTCAA GAAGAATGGG
481 AGCTGCAAAC GCGGTCCTCG GACTCACTAT GGCCAGAAAG CAATCTTGTT TCTCCCCCTG
541 CCAGTCTCTT CTGATTAA
```

[0058] In another embodiment of the present invention, the portion of the paracrine FGF of the chimeric protein may be derived from a nucleotide sequence that encodes an ortholog of human FGF1. Nucleotide sequences that encode FGF1 orthologs are shown in Table 2.

Table 2:

Olive Baboon FGF1 gene coding sequence (1-155) (SEQ ID NO:62) (GenBank accession no. NM_001169086, which is hereby incorporated by reference in its entirety):

```
1   ATGGCTGAAG GGGAAATCAC CACGTTCACA GCCCTGACCG AGAAGTTTAA TCTGCCTCCA
61  GCGAATTACA AGAAGCCCAA ACTGCTCTAC TGTAGCAACG GGGGACACTT CTTGAGGATC
121 CTTCCGGATG GCACAGTGGA TGGGACAAGG GACAGGAGCG ACCAGCACAT TCAGCTGCAG
181 CTCAGTGCGG AAAGCGTGGG GGAGGTGTAT ATAAAGAGTA CCGAGACTGG CCAGTACTTG
241 GCCATGGACA CCGACGGGCT TTTATACGGC TCACAGACAC CAAATGAGGA ATGTTTGTTC
301 CTGGAAAGGC TGGAGGAGAA CCATTACAAC ACCTACATAT CCAAGAAGCA CGCAGAGAAG
361 AATTGGTTTG TTGGCCTCAA GAAGAATGGA AGCTGCAAAC GTGGTCCTCG GACTCACTAT
421 GGCCAGAAAG CAATCTTGTT TCTTCCCCTG CCAGTCTCTT CTGATTAA
```

Sumatran orangutan FGF1 gene coding sequence (60-214) (SEQ ID NO:63) (GenBank accession no. NM_001133601, which is hereby incorporated by reference in its entirety):

```
211                                   ATGGCTGAAG GGGAAATCAC CACCTTCACA
241 GCCCTGACCG AGAAGTTTAA TCTGCCTCCA GGGAATTACA AGAAGCCCAA ACTCCTCTAC
301 TGTAGCAACG GGGGCCACTT CTTGAGGATC CTTCCGGATG GCACAGTGGA TGGGACAAGG
361 GACAGGAGCG ACCAGCACAT TCAGCTGCAG CTCAGTGCGG AAAGCGTGGG GGAGGTGTAT
421 ATAAAGAGTA CCGAGACTGG CCAGTACTTG GCCATGGACA CCGACGGGCT TTTATACGGC
481 TCACAGACAC CAAATGAGGA ATGTTTGTTC CTGGAAAGGC TGGAGGAGAA CCATTACAAC
541 ACCTATATAT CCAAGAAGCA TGCAGAGAAG AATTGGTTTG TTGGCCTCAA GAAGAATGGA
601 AGCTGCAAAC GCGGTCCTCG GACTCACTAT GGCCAGAAAG CAATCTTGTT TCTCCCCCTG
661 CCAGTCTCTT CCGATTAA
```

White-tufted-ear marmoset FGF1 gene coding sequence (1-155) (SEQ ID NO:64) (GenBank accession no. XM_002744295, which is hereby incorporated by reference in its entirety):

```
130           A TGGCTGAAGG GGAAATCACC ACCTTCACAG CCCTGACCGA GAAGTTTGAT
181 CTGCCTCCAG GGAATTACAA GAAGCCCAAA CTCCTCTACT GTAGCAATGG GGGCCACTTC
241 TTGAGGATCC TTCCGGATGG CACAGTGGAT GGGACAAGGG ACAGGAGCGA CCAGCACATT
301 CAGCTGCAGC TCAGTGCGGA AAGCGTGGGG GAGGTGTATA TAAAGAGTAC CGAGACTGGC
361 CAGTACTTGG CCATGGACAC CGACGGGCTT TTATACGGCT CACAGACACC AAATGAGGAA
421 TGTTTGTTCC TGGAGAGGCT GGAGGAGAAC CATTACAACA CCTATATATC CAAGAAACAT
481 GCAGAGAAGA ATTGGTTTGT CGGCCTCAAG AAGAATGGAA GCTGTAAACG TGGTCCTCGG
541 ACTCACTATG GTCAGAAAGC GATCTTGTTT CTCCCCCTGC CAGTTTCTTC TGATTAA
```

Horse FGF1 gene coding sequence (1-155) (SEQ ID NO:65) (GenBank accession no. NM_001163886, which is hereby incorporated by reference in its entirety):

```
34                                      ATGGCTG AAGGAGAAAT CACAACCTTC
61  ACGGCCCTGA CCGAGAAGTT TAATCTGCCT CCAGGGAATT ACAAGAAGCC CAAACTCCTC
121 TACTGTAGCA ATGGGGGCCA CTTCCTGAGG ATCCTTCCAG ATGGCACAGT GGATGGGACA
181 AGGGACAGGA GCGACCAGCA CATTCAGCTG CAGCTCAGTG CGGAAAGCGT GGGGGAGGTG
241 TATATAAAGA GTACCGAGAC TGGCCAGTAC TTGGCCATGG ACACCGACGG GCTGTTGTAC
301 GGCTCACAGA CACCAAACGA GGAATGTTTG TTCCTGGAAA GGCTGGAGGA AAACCATTAC
361 AACACCTACA CATCCAAGAA GCATGCAGAG AAGAACTGGT TCGTTGGTCT CAAGAAGAAT
421 GGGAGCTGCA AACGCGGTCC TCGGACTCAC TATGGGCAGA AAGCAATCTT GTTTCTTCCC
481 CTGCCCGTCT CCTCTGACTA A
```

Chimpanzee FGF1 gene coding sequence (1-155) (SEQ ID NO:66) (GenBank accession no. GABD01003589, which is hereby incorporated by reference in its entirety):

```
80                        A TGGCTGAAGG GGAAATCACC ACCTTCACAG CCCTGACCGA
121 GAAGTTTAAT CTGCCTTCAG GGAATTACAA GAAGCCCAAA CTCCTCTACT GTAGCAACGG
181 GGGCCACTTC CTGAGGATCC TTCCGGATGG CACAGTGGAT GGGACAAGGG ACAGGAGCGA
241 CCAGCACATT CAGCTGCAGC TCAGTGCGGA AAGCGTGGGG GAGGTGTATA TAAAGAGTAC
301 CGAGACTGGC CAGTACTTGG CCATGGACAC CGACGGGCTT TTATACGGCT CACAGACACC
361 AAATGAGGAA TGTTTGTTCC TGGAACGGCT GGAGGAGAAC CATTACAACA CCTATATATC
421 CAAGAAGCAT GCAGAGAAGA ATTGGTTTGT TGGCCTCAAG AAGAATGGAA GCTGCAAACG
481 CGGTCCTCGG ACTCACTATG GCCAGAAAGC AATCTTGTTT CTCCCCCTGC CAGTCTCTTC
541 CGATTAA
```

Elephant FGF1 gene coding sequence (1-155) (SEQ ID NO:67) (GenBank accession no. XM_003404573, which is hereby incorporated by reference in its entirety):

```
1   ATGGCCGAAG GGGAAATCAC AACTTTCACA GCCCTGACAG AGAAGTTCAA CCTGCCTCCA
61  GGGAATTACA AGAAGCCCAA ACTCCTCTAC TGTAGCAATG GAGGTCACTT CTTAAGGATC
121 CTTCCAGATG GCACAGTGGA TGGCACCAGG GACAGGAGTG ACCAGCACAT TCAGCTGCAG
181 CTCAGTGCGG AAAGCGTGGG GGAGGTGTAT ATAAAGGGCA CCGAGACTGG CCAGTACTTG
241 GCCATGGACA CCGACGGGCT TTTATACGGC TCACAGACAC CAAATGAGGA ATGTTTGTTC
301 CTGGAAAGGC TGGAGGAAAA CCATTACAAC ACCTACACAT CCAAGAAGCA CGCAGAGAAG
361 AATTGGTTCG TTGGTCTCAA GAAGAATGGA AGCTGCAAAC GCGGTCCTCG GACTCACTAT
421 GGCCAGAAAG CAATCTTGTT TCTCCCCCTG CCAGTCTCCT CTGATTAA
```

Dog FGF1 gene coding sequence (1-155) (SEQ ID NO:68) (GenBank accession no. XM_844181, which is hereby incorporated by reference in its entirety):

```
164                                                 ATGGCTG AAGGGGAAAT
181 CACAACCTTC ACTGCCCTGA CGGAGAAGTT TAATCTGCCT CCGGGGAATT ACATGAAGCC
241 CAAACTCCTC TACTGTAGCA ACGGGGGCCA CTTCCTGAGG ATCCTTCCAG ATGGCACAGT
301 GGATGGGACA AGGGACAGGA GCGACCAGCA CATTCAGCTG CAGCTCAGCG CGGAAAGCGT
361 GGGGGAGGTG TATATAAAGA GCACCGAGAC TGGCCAGTAC TTGGCCATGG ACACCGATGG
421 GCTTCTGTAC GGCTCACAGA CACCGAATGA GGAATGTTTG TTCCTGGAAA GGCTGGAGGA
481 AAACCATTAC AACACCTACA CATCCAAGAA GCATGCAGAA AAAAATTGGT TTGTTGGTCT
541 CAAGAAGAAT GGAAGCTGCA AACGCGGTCC TCGGACTCAC TATGGTCAAA AAGCAATTTT
601 GTTTCTCCCC CTGCCAGTGT CCTCTGATTA A
```

Giant panda FGF1 gene coding sequence (1-155) (SEQ ID NO:69) (GenBank accession no. XM_002912535, which is hereby incorporated by reference in its entirety):

```
146                             ATGGC TGAAGGGGAG ATCACAACCT TCACCGCCCT
181 GACGGAGAAG TTTAATCTGC CTGCGGGGAA TTACAAGAAG CCCAAACTCC TCTACTGTAG
241 CAACGGGGGC CACTTCCTGA GGATCCTTCC AGATGGCACA GTGGACGGGA CGAGGGACAG
301 GAGCGACCAG CACATTCAAC TGCAGCTCAG CGCGGAAAGC GTAGGGGAGG TGTACATAAA
361 GAGCACCGAG ACCGGCCAGT ACTTGGCCAT GGACACCGAT GGGCTTCTGT ACGGCTCACA
421 GACACCAAAT GAGGAATGTT TGTTCCTGGA AAGGCTGGAG GAAAACCATT ACAACACCTA
481 CACATCCAAG AAGCACGCGG AGAAGAATTG GTTTGTTGGT CTCAAGAAGA ATGGAAGCTG
541 CAAACGTGGT CCTCGGACTC ACTATGGCCA GAAAGCAATT CTGTTTCTCC CCCTGCCAGT
601 CTCCTCTGAT TAA
```

Bolivian squirrel monkey FGF1 gene coding sequence (1-155) (SEQ ID NO:70) (GenBank accession no. XM_003920547, which is hereby incorporated by reference in its entirety):

```
130          A TGGCTGAAGG GGAAATCACC ACCTTTACAG CCCTGACCGA GAAGTTTGAT
181 CTGCCTCCAG GGAATTACAA GAAGCCCAAA CTCCTCTACT GTAGCAACGG GGGCCACTTC
241 TTGAGGATCC TTCCGGATGG CACAGTGGAT GGGACCAGGG ACAGGAGCGA TCTTCACATT
301 CAGCTGCAGC TCAGTGCGGA AAGCGTGGGG GAGGTGTATA TAAAGAGTAC CGAGACTGGC
361 CAGTACTTGG CCATGGACAC CGACGGGCTT TTATACGGCT CACAGACACC AAATGAGGAA
421 TGTTTGTTCC TGGAAAGGCT GGAGGAGAAC CATTACAACA CCTATATATC CAAGAAACAC
481 GCAGAGAAGA ATTGGTTTGT TGGCCTCAAG AAGAATGGAA GCTGCAAGCG CGGTCCTCGG
541 ACTCACTATG GCCAGAAAGC AATCTTGTTT CTCCCCCTGC CAGTCTCTTC TGATTAA
```

Pig FGF1 gene coding sequence (1-155) (SEQ ID NO:71) (GenBank accession no. XM_003124010, which is hereby incorporated by reference in its entirety):

```
35                                      ATGGCT GAAGGCGAAA TCACAACCTT
61  CACGGCCCTG ACCGAGAAGT TTAATCTGCC TCCAGGAAAT TACAAGAAGC CCAAGCTCCT
121 CTACTGCAGC AACGGGGGCC ATTTCCTCAG GATCCTTCCA GATGGCACAG TGGATGGGAC
181 CAGGGACAGG AGCGACCAGC ACATTCAGCT GCAGCTCAGT GCGGAAAGCG TGGGGGAGGT
241 GTATATAAAG AGTACGGAGA CTGGCCAGTA CTTGGCCATG GACACCAGCG GGCTTTTGTA
301 CGGCTCACAG ACACCCAGTG AGGAGTGTTT GTTCCTGGAG AGGCTGGAGG AAAACCATTA
361 CAATACCTAC ACATCCAAGA AGCACGCAGA GAAGAACTGG TTCGTTGGCC TCAAGAAGAA
421 TGGAAGCTGC AAACGCGGTC CTCGGACTCA CTATGGCCAG AAAGCCATCC TGTTTCTCCC
481 CCTGCCAGTA TCCTCGGATT AA
```

Small-eared galago FGF1 gene coding sequence (1-155) (SEQ ID NO:72) (GenBank accession no. XM_003782087, which is hereby incorporated by reference in its entirety):

```
28                                ATG GCTGAAGGGG AAATCACAAC CTTCACAGCC
61  CTCACAGAGA AGTTTAATCT GCCTCTAGGA AATTACAAGA AGCCCAAGCT CCTCTACTGT
121 AGCAACGGGG GTCACTTTCT GAGGATCCTG CCGGATGGCA CCGTGGATGG GACACAAGAC
181 AGGAGCGACC AGCACATTCA GCTGCAGCTC AGTGCGGAAA GCGTGGGGGA GGTGTATATA
241 AAGAGTACCC AGACTGGCCA GTACTTGGCC ATGGACTCCG ACGGGCTTTT ATACGGCTCA
301 CAAACACCAA ATGAGGAATG CCTGTTCCTG GAACGGCTGG AGGAAAACCA TTACAACACC
361 TATGTGTCCA AGAAGCACGC CGAGAAGAAT TGGTTTGTCG GTCTCAAGAA GAACGGAAGT
421 TGCAAACGTG GTCCTCGGAC TCACTACGGC CAGAAAGCAA TCTTGTTTCT CCCCCTGCCA
481 GTCTCCTCTG ATTAA
```

Greater horseshoe bat FGF1 gene coding sequence (1-155) (SEQ ID NO:73) (GenBank accession no. DP000705, which is hereby incorporated by reference in its entirety):

```
190120                                              T TAATCAGAGG AGACTGGCAG
190141 GGGGAGAAAC AGGATTGCTT TCTGGCCATA GTGAGTCCGA GGACCGCGCT TGCAGCTTCC
190201 ATTCTTCTTG AGCCCAACGA ACCAATTCTT TTCTGCGTGC TTCTTGGACG TGTAGGTGTT
190261 GTAATGGTTT TCCTCCAGCC TTTCCAGGAA CAGACATTCC TCATTTGGTG TCTG
194466      TGAGC CGTACAAAAG CCCGTCGGAG TCCATGGCCA AGTACTGGCC ACTCTCGGTG
194521 CTCTTTATAT ACACCTCCCC CACGCTTTCC GCACTGAGCT GCAGCTGAA
208114                                        TGTGCTG GTCACTCTTG TCCCTTGTCC
208141 CATCCACTGT GCCATCTGGA AGGATCCTCA GGAAGTGGCC CCCGTTGCTG CAGTAGAGAA
208201 GTTTGGGTTT CTTGTAATTC CCTGTAGGCA GATTAAACTT CTCAGTAAGG GCTGTGAACG
208261 TGGTGACTTC CCCTTCGGCC AT
```

European shrew FGF1 gene coding sequence (1-155) (SEQ ID NO:74) (GenBank accession no. DP000767, which is hereby incorporated by reference in its entirety):

```
138344                                                  CTAGTCG GAGGAGACGG
138361 GCAGGGGGAG AAACAAGATC GCTTTCTGGC CGTAGTGAGT CCGGGGACCA CGCTTGCAGC
138421 TTCCGTTCTT CTTCAGACCA ACAAACCAAT TCTTCTCGGC ATGCTTCTTG GAGGTATAGG
138481 TGTTGTAATG GTTTTCCTCC AGCCTTTCCA GAAACAGACA TTCCTCATTC GGTGTTTG
143512                                                        TGAGCCGTA
143521 TAAAAGCCCG TCGGTGTCCA TGGCCAAGTA ATGGCCAGTC TCCGTGCTCT TTATATACAC
143581 CTCCCCCACG CTTTCCGCAC TGAGCTGCAG CTGAA
157009                                                    TG TGCTGGTCGC
157021 TGCGGTCCCT GGTCCCATCC ACTGTGCCGT CCGGGAGGAT GCGCAGGAAG TGGCCCCCGT
157081 TGCTGCAGTA CAGGAGTTTG GGCTTCTTGT AGTTCCCTGG TGGCAGGTTA AACTTCTCCA
157141 TGAGGGCCCC AAAGGTGGTG ATCTCCCCCT CGGCCAT
```

Rabbit FGF1 gene coding sequence (1-155) (SEQ ID NO:75) (GenBank accession no. NM_001171488, which is hereby incorporated by reference in its entirety):

```
1   ATGGCTGAGG GGGAGGTCAC CACCTTCACA GCCCTGACCG AGAAGTTCAA CCTGCCTGCA
61  GGGAACTACA AGTTGCCCAA ACTCCTCTAC TGCAGCAACG GGGGCCACTT CCTGAGGATC
121 CTGCCGGACG GCACTGTGGA CGGCACAAGG GACAGGAGCG ACCAGCACAT TCAGCTGCAG
181 CTGAGTGCGG AAAGCGTGGG GGAGGTGTAT ATAAAGAGTA CGGAGACCGG CCAGTACTTG
241 GCCATGGACA CCGACGGCCT TTTATACGGC TCGCAAACGC CCAGTGAGGA GTGTTTGTTC
301 CTGGAACGGC TGGAGGAGAA CCACTACAAC ACCTACACGT CCAAGAAGCA CGCCGAGAAG
361 AACTGGTTCG TGGGGCTGAA GAAAAACGGG AGCTGCAAGC GCGGTCCTCG GACTCACTAC
421 GGCCAGAAAG CCATCTTGTT CCTCCCCCTG CCGGTCTCCT CCGACTAA
```

Chinese hamster FGF1 gene coding sequence (1-155) (SEQ ID NO:76) (GenBank accession no. XM_003502421, which is hereby incorporated by reference in its entirety):

```
1   ATGGCTGAAG GAGAAATCAC CACCTTCTCA GCCCTGACAG AGAGATTTAA TCTGCCTCCA
61  GGAAACTACA AGAAGCCCAA ACTGCTCTAC TGCAGCAACG GGGGCCACTT CTTGAGGATC
121 CTTCCAGATG GCACAGTGGA TGGGACAAGG GACAGGAGTG ACCAGCACAT TCAGCTGCAG
181 CTGAGTGCGG AAAGCGCGGG CGAAGTGTAT ATAAAGGGTA CAGAGACAGG CCAGTACAGG
241 AACATGGACA CGGATGGCCT TTTATACGGC TCACAGACAC CAAATGAAGA ATGCCTGTTC
301 CTGGAAAGGC TGGAAGAAAA CCATTACAAC ACTTATACAT CCAAGAAGCA CGCAGAGAAG
361 AACTGGTTTG TGGGCCTCAA GAAAAACGGG AGCTGCAAGC GTGGTCCTCG GACTCACTAT
421 GGCCAGAAAG CAATCTTGTT TCTCCCCCTG CCTGTATCTT CTGACTAG
```

Tasmanian devil FGF1 gene coding sequence (1-155) (SEQ ID NO:77) (GenBank accession no. XM_003756690, which is hereby incorporated by reference in its entirety):

```
24                          ATGGCCG AAGGGGAGAT CACAACCTTC ACAGCCCTGA
61  CCGAAAGATT TAATCTGCCA CTGGGGAATT ACAAGAAGCC CAAGCTTCTC TACTGTAGCA
121 ATGGGGGCCA CTTTTTGAGG ATTCTTCCTG ATGGTAAAGT GGATGGGACA AGGGACAGAA
181 ATGATCAACA CATTCAACTG CAACTAAGCG CGGAAAGCGT GGGTGAGGTG TATATAAAGA
241 GCACTGAGTC TGGCCAGTAT TTGGCTATGG ACACCGATGG ACTTTTATAC GGCTCACAGA
301 CACCCACTGA AGAATGCTTG TTCCTGGAGA GATTGGAGGA GAATCATTAC AACACCTACA
361 TATCAAAGAA GCATGCGGAG AAAAATTGGT TTGTGGGCCT CAAGAAAAAT GGAAGCTGCA
421 AAAGAGGTCC CAGGACTCAC TATGGCCAGA AAGCCATCCT CTTCCTTCCC CTCCCTGTGT
481 CCTCTGAGTA A
```

House mouse FGF1 gene coding sequence (1-155) (SEQ ID NO:78) (GenBank accession no. NM_010197, which is hereby incorporated by reference in its entirety):

```
188        ATG GCTGAAGGGG AGATCACAAC CTTCGCAGCC CTGACCGAGA GGTTCAACCT
241 GCCTCTAGGA AACTACAAAA AGCCCAAACT GCTCTACTGC AGCAACGGGG GCCACTTCTT
301 GAGGATCCTT CCTGATGGCA CCGTGGATGG GACAAGGGAC AGGAGCGACC AGCACATTCA
361 GCTGCAGCTC AGTGCGGAAA GTGCGGGCGA AGTGTATATA AAGGGTACGG AGACCGGCCA
421 GTACTTGGCC ATGGACACCG AAGGGCTTTT ATACGGCTCG CAGACACCAA ATGAGGAATG
481 TCTGTTCCTG GAAAGGCTGG AAGAAAACCA TTATAACACT TACACCTCCA AGAAGCATGC
541 GGAGAAGAAC TGGTTTGTGG GCCTCAAGAA GAACGGGAGC TGTAAGCGCG GTCCTCGGAC
601 TCACTATGGC CAGAAAGCCA TCTTGTTTCT GCCCCTCCCG GTGTCTTCTG ACTAG
```

Domestic guinea pig FGF1 gene coding sequence (1-154) (SEQ ID NO:79) (GenBank accession no. XM_003477194, which is hereby incorporated by reference in its entirety):

```
1   ATGGCTGAAG GAGAAATCAC AACTTTTGCA GCCCTGACTG AGAAGTTTAA TCTGCCTCCA
61  GGGAATTATA AGAAGCCCAA ACTGCTCTAC TGCAGCAATG GGGGCCACTT CCTGAGGATC
121 CTTCCAGACG GCACAGTGGA CGGCACAAGA GACAGGAGCG ACCAGCACAT TCAGCTGCAG
181 CTCAGTGCGG AAGGCGTGGG GGAGGTGTAT ATACAGAGCA CCGAGACCGG CCAGTACTTG
241 GCCATGGACA CCGACGGGCT TTTATACGGC TCACAGACAC CAAGTGAGGA ATGCTTGTTC
301 CTGGAAAGGC TGGAGGAAAA CCATTACAAC ACCTACACAT CCAAGAAGCA TGTGGAGAAG
361 AATTGGTTTG TTGGCCTCAA GAAGAACGGA AGCTGCAAGC GTGGTCCTCG GACTCACTAT
421 GGCCAGAAAG CAATCTTGTT CCTCCCCTTG CCAGTCTCTG ATTAG
```

Gray short-tailed opossum FGF1 gene coding sequence (1-155) (SEQ ID NO:80) (GenBank accession no. XM_001368884, which is hereby incorporated by reference in its entirety):

```
1   ATGGCCGAAG GGGAGATCAC AACCTTCACA GCCCTGACTG AAAGATTTAA CCTGCCACTG
61  GGGAATTACA AGAAACCCAA GCTTCTCTAC TGTAGCAATG GGGGCCATTT CTTGAGGATC
121 CTTCCTGATG GCAAAGTGGA TGGGACACGG GACAGAAATG ATCAACACAT TCAACTGCAG
181 CTGAGCACGG AAAGTGTGGG TGAGGTGTAT ATAAAGAGCA CTGAGTCTGG CCAGTATTTG
241 GCTATGGACA CCGATGGACT TTTATATGGC TCACAGACAC CCAGTGAAGA ATGCTTGTTT
301 CTGGAGAGGT TGGAGGAGAA TCATTACAAC ACCTACACAT CGAAGAAGCA TGCAGAGAAA
361 AATTGGTTTG TTGGTCTCAA GAAGAATGGA AGCTGCAAAA AGGGTCCCAG GACTCACTAC
421 GGCCAGAAAG CCATCCTGTT CCTTCCCCTC CCTGTGTCCT CTGAGTAA
```

Common vampire bat FGF1 gene coding sequence (1-155) (SEQ ID NO:81) (GenBank accession no. GABZ01008334, which is hereby incorporated by reference in its entirety):

```
1   ATGGCTGAAG GGGAAGTCAC CACGTTCACA GCTCTGACTG AGAAGTTTAA TCTGCCTCTG
61  GAGAGTTACA AGAAGCCCAA ACTTCTCTAC TGCAGCAACG GTGGCCACTT CCTGAGGATC
121 CTTCCAGATG GTACAGTGGA TGGGACAAGG GACAAGAGCG ACCAGCACAT TCAGCTGCAG
181 CTCAGTGCGG AAAGCGTGGG GGAGGTGTAC ATAAAGAGCA CCGGGAGTGG CCAGTACTTG
241 GCCATGGACT CCGCCGGGCT TTTGTATGGC TCACAGACAC CAAATGAGGA ATGTTTGTTC
301 CTGGAAAGGC TGGAGGAAAA CCATTACAAC ACCTACACAT CCAAGAAGCA TGCAGAAAAG
361 AATTGGTTCG TGGGGCTCAA GAAGAATGGA AGCTGCAAGC GTGGCCCCCG GACTCATTAT
421 GGCCAGAAAG CAATCTTGTT TCTCCCCCTG CCAGTCAACT CTGATTAA
```

Cattle FGF1 gene coding sequence (1-155) (SEQ ID NO:82) (GenBank accession no. NM_174055, which is hereby incorporated by reference in its entirety):

```
918                     ATG GCTGAAGGAG AAACCACGAC CTTCACGGCC CTGACTGAGA
961  AGTTTAACCT GCCTCTAGGC AATTACAAGA AGCCCAAGCT CCTCTACTGC AGCAACGGGG
1021 GCTACTTCCT GAGAATCCTC CCAGATGGCA CAGTGGATGG GACGAAGGAC AGGAGCGACC
1081 AGCACATTCA GCTGCAGCTC TGTGCGGAAA GCATAGGGGA GGTGTATATT AAGAGTACGG
1141 AGACTGGCCA GTTCTTGGCC ATGGACACCG ACGGGCTTTT GTACGGCTCA CAGACACCCA
1201 ATGAGGAATG TTTGTTCCTG GAAAGGTTGG AGGAAAACCA TTACAACACC TACATATCCA
1261 AGAAGCATGC AGAGAAGCAT TGGTTCGTTG GTCTCAAGAA GAACGGAAGG TCTAAACTCG
1321 GTCCTCGGAC TCACTTCGGC CAGAAAGCCA TCTTGTTTCT CCCCCTGCCA GTCTCCTCTG
1381 ATTAA
```

Platypus FGF1 gene coding sequence (1-155) (SEQ ID NO:83) (GenBank accession no. XM_001514811, which is hereby incorporated by reference in its entirety):

```
1   ATGGCGGAGG GTGAAATCAC CACGTTCACA GCCCTGATGG AGAAGTTCGA CCTACCCCTG
61  GGCAACTACA AAAAGCCTAG GCTGCTCTAC TGCAGCAATG GCGGCTACTT CCTGCGCATC
121 CAGCCAGACG GTAAAGTGGA CGGGACCAGG GATCGGAGCG ATCAGCACAT TCAACTGCAG
181 CTAAGCGCGG AAAGCGTGGG CGAGGTGTAT ATAAAGAGCA CCGAGTCTGG CCACTATTTG
241 GCTATGGACA CCGAAGGACT TTTATATGGC TCACAGGCAC CCAGTGAAGA CTGCTTGTTC
301 CTGGAGCGGC TGGAGGAGAA CCACTATAAC ACGTACGTGT CCAAGAAGCA CGCTGAGAAG
361 AATTGGTTTG TCGGTCTCAA GAAGAACGGG AGCTGCAAAC GAGGTCCCCG GACTCACTAC
421 GGCCAGAAAG CCATCCTCTT CCTCCCGCTC CCCGTGGCAT CCGACTAG
```

Zebra finch FGF1 gene coding sequence (1-155) (SEQ ID NO:84) (GenBank accession no. XM_002193251, which is hereby incorporated by reference in its entirety):

```
1   ATGGCCGAGG GGGAGATCAC CACCTTCAGC GCCCTGACGG AGAAGTTCAA CCTGCCCCCG
61  GGGAACTACA AGAAGCCCAA ACTGCTGTAC TGCAGCAACG GGGGGCATTT CCTGCGCATC
121 CTCCCGGACG GCACCGTGGA TGGCACCAGG GACCGCAGCG ACCAGCACAT TCAGCTCCAG
181 CTGAGTGCAG AGAGCGTGGG GGTGGTGCAC ATCCAGAGCA CCCAGTCGGG GCAGTACCTG
241 GCCATGGACA CCAACGGGCT GCTCTACGGC TCGCAGCTGC CACCCGGTGA GTGTCTGTTC
301 CTGGAAAGGC TGGAGGAGAA CCATTACAAC ACCTACGTCT CCAAAATGCA CGCGGACAAG
361 AACTGGTTTG TGGGGCTGAA GAAGAACGGG ACAAGCAAGC TGGGCCCGCG GACTCACTAC
421 GGCCAGAAGG CGATCCTGTT CCTGCCGCTG CCCGTGGCGG CCGACTGA
```

Nine-banded armadillo FGF1 gene coding sequence (1-155) (SEQ ID NO:85) (GenBank accession no. DP001080, which is hereby incorporated by reference in its entirety):

```
178389         TT AATCAGAGGA GACTGGCAGG GGAAGAAACA AGATAGCTTT CTGGCCATAG
178441 TGAGTCTGAG GACCACGTTT GCTGCTTCCG TCCTTCTTGA GACCAACAAA CCATTTCTTC
178501 TCTGCATGCT TCTTGGATAT GTAGGTGTTG TAATTGTTTT CTTCCAGCTT TTCCATGAAC
178561 AAGCATTCCT CACTTGGTGT CTC
182873                                                        TGAGCCAT
182881 ATAAAAGCCC GTCGGTGTCC ATGGCTAAGT ACTGGCCGGT CTCTGCACTC TTTATATACA
182941 CCTCCCCCAC GCTTTCCGCA CTGAGCTGCA GCTGAA
197786                            TGTGT TGGTCGCTCC TGTCCCTTGT CCCATCCACC
197821 GTGCCATCTG GAAGGATCCT CAAGAAGTGG CCCCCGTTTC TGCAGTAGAG GAGTCTGGGG
197881 TGCTTGTAAT TTTCTAGGGG CAGGTTGAAC TTCTCCATCA GGGCCATGAA GGTTGTGATC
197941 TCCCCTTCAG CCAT
```

Xenopus Silurana tropicalis FGF1 gene coding sequence (1-155) (SEQ ID NO:86) (GenBank accession no. FJ428265, which is hereby incorporated by reference in its entirety):

```
1   ATGGCAGAGG GAGACATCAC AACATTCAAC CCCATTGCAG AGTCCTTCAG TCTTCCAATT
61  GGCAACTACA AGAAACCAAA ACTTCTGTAC TGTAATAATG GAGGGTATTT TTTGCGCATC
121 CTCCCAGATG GGGTTGTGGA TGGAACAAGA GACAGAGATG ACCTTTACAT TACACTGAAG
181 TTAAGCGCAC AAAGCCAAGG GGAGGTGCAT ATCAAAAGCA CAGAGACAGG GAGTTACTTA
241 GCCATGGACT CCAGTGGACA GTTGTATGGA ACTCTCACAC CAAATGAAGA AAGCCTGTTT
301 CTGGAGACAT TAGAAGAGAA TCACTATAAC ACATACAAGT CAAAGAAGTA TGCAGAAAAT
361 AACTGGTTTG TGGGGATAAA GAAGAACGGG GCAAGCAAAA AGGGATCAAG GACTCACTAT
421 GGACAAAAAG CCATCCTTTT TCTGCCGCTG CCAGCATCAC CTGACTAG
```

Heterocephalus glaber FGF1 gene coding sequence (1-155) (SEQ ID NO:87) (generated using SMS Reverse Translate tool on the ExPASy Bioinformatics Resource website (www.expasy.org):

```
1   ATGGCGGAAG GCGAAATTAC CACCTTTACC GCGCTGACCG AAAAATTTAA CCTGCCGCCG
61  GGCAACTATA AAAAACCGAA ACTGCTGTAT TGCAGCAACG GCGGCCATTT TCTGCGCATT
121 CTGCCGGATG GCAAAGTGGA TGGCACCCGC GATCGCAGCG ATCAGCATAT TCAGCTGCAG
181 CTGAGCGCGG AAGGCGTGGG CGAAGTGTAT ATTAAAAGCA CCGAAACCGG CCAGTATCTG
241 GCGATGGATA CCGATGGCCT GCTGTATGGC AGCCAGACCG CGAGCGAAGA ATGCCTGTTT
301 CTGGAACGCC TGGAAGAAAA CCATTATAAC ACCTATATTA GCAAAAAACA TGCGGAAAAA
361 AACTGGTTTG TGGGCCTGAA AAAAAACGGC AGCTGCAAAC GCGGCCCGCG CACCCATTAT
421 GGCCAGAAAG CGATTCTGTT TCTGCCGCTG CCGGTGAGCA GCGAT
```

Black flying fox FGF1 gene coding sequence (1-155) (SEQ ID NO:88) (generated using SMS Reverse Translate tool on the ExPASy Bioinformatics Resource website (www.expasy.org):

```
1   ATGGCGGAAG GCGAAGTGAC CACCTTTACC GCGCTGACCG AACGCTTTAA CCTGCCGCCG
61  GGCAACTATA AAAAACCGAA ACTGCTGTAT TGCAGCAACG GCGGCCATTT TCTGCGCATT
121 CTGCCGGATG GCACCGTGGA TGGCACCCGC GATAAAAGCG ATCAGCATAT TCAGCTGCAG
181 CTGAGCGCGG AAAGCGTGGG CGAAGTGTAT ATTAAAAGCA CCGAAAGCGG CCAGTATCTG
241 GCGATGGATA GCGATGGCCT GCTGTATGGC AGCCAGACCC CGGATGAAGA TTGCCTGTTT
301 CTGGAACGCC TGGAAGAAAA CCATTATAAC ACCTATACCA GCAAAAAACA TGCGGAAAAA
361 AACTGGTTTG TGGGCCTGAA AAAAAACGGC AGCTGCAAAC GCGGCCCGCG CACCCATTAT
421 GGCCAGAAAG CGATTCTGTT TCTGCCGCTG CCGGTGAGCA GCGAT
```

Chinese tree shrew FGF1 gene coding sequence (1-155) (SEQ ID NO:89) (generated using SMS Reverse Translate tool on the ExPASy Bioinformatics Resource website (www.expasy.org):

```
1   ATGGCGGAAG GCGAAATTAC CACCTTTGCG GCGCTGACCG AAAAATTTGA TCTGCCGCCG
61  GGCAACTATA AAAAACCGAA ACTGCTGTAT TGCAGCAACG GCGGCCATTT TCTGCGCATT
121 CTGCCGGATG GCACCGTGGA TGGCACCCGC GATCGCAGCG ATCAGCATAT TCAGCTGCAG
181 CTGACCGCGG AAAACGTGGG CGAAGTGTAT ATTAAAAGCA CCGAAACCGG CCAGTATCTG
241 GCGATGGATG CGGATGGCCT GCTGTATGGC AGCCAGACCC CGAACGAAGA ATGCCTGTTT
301 CTGGAACGCC TGGAAGAAAA CCATTATAAC ACCTATATTA GCAAAAAACA TGCGGAAAAA
361 AACTGGTTTG TGGCGCTGAA AAAAAACGGC AGCTGCAAAC TGGGCCCGCG CACCCATTAT
421 GGCCAGAAAG CGATTCTGTT TCTGCCGCTG CCGGTGAGCA GCGAT
```

Rock pigeon FGF1 gene coding sequence (1-155) (SEQ ID NO:90) (generated using SMS Reverse Translate tool on the ExPASy Bioinformatics Resource website (www.expasy.org):

```
1   ATGGCGGAAG GCGAAATTAC CACCTTTACC GCGCTGACCG AAAAATTTAA CCTGCCGCCG
61  GGCAACTATA AAAAACCGAA ACTGCTGTAT TGCAGCAACG GCGGCCATTT TCTGCGCATT
121 CTGCCGGATG GCAAAGTGGA TGGCACCCGC GATCGCAGCG ATCAGCATAT TCAGCTGCAG
181 CTGAGCGCGG AAAGCGTGGG CGAAGTGTAT ATTAAAAGCA CCCAGAGCGG CCAGTATCTG
241 GCGATGGATC CGACCGGCCT GCTGTATGGC AGCCAGCTGC TGGGCGAAGA ATGCCTGTTT
301 CTGGAACGCA TTGAAGAAAA CCATTATAAC ACCTATGTGA GCAAAAAACA TGCGGATAAA
361 AACTGGTTTG TGGGCCTGAA AAAAAACGGC AACAGCAAAC TGGGCCCGCG CACCCATTAT
421 GGCCAGAAAG CGATTCTGTT TCTGCCGCTG CCGGTGAGCG CGGAT
```

Sheep FGF1 gene coding sequence (1-155) (SEQ ID NO:91) (GenBank accession no. XM_004008909, which is hereby incorporated by reference in its entirety):

```
361 ATGGCTGAAG GAGAAACCAC AACCTTCAGG GCCCTGACTG AGAAGTTTAA CCTGCCTCTA
421 GGCAATTACA AGAAGCCCAA GCTCCTCTAT TGCAGCAACG GGGGCTACTT CCTGAGAATC
481 CTCCCAGATG GCAGAGTGGA TGGGACGAAG GACAGGAGCG ACCAGCACAT TCAGCTGCAG
541 CTCTATGCGG AAAGCATAGG GGAGGTGTAT ATTAAGAGTA CGGAGACTGG CCAGTTCTTG
601 GCCATGGACA CCAACGGGCT TTTGTACGGC TCACAAACAC CCAGTGAGGA ATGTTTGTTC
661 CTGGAAAGGC TGGAGGAAAA CCATTATAAC ACCTACATAT CCAAGAAGCA TGCAGAGAAG
721 AATTGGTTCA TTGGTCTCAA GAAGAACGGA AGCTCCAAAC TCGGTCCTCG GACTCACTTC
781 GGCCAGAAAG CCATCTTGTT TCTCCCCCTG CCAGTTTCCT CTGATTAA
```

Chicken FGF1 gene coding sequence (1-155) (SEQ ID NO:92) (GenBank accession no. NM_205180, which is hereby incorporated by reference in its entirety):

```
52                                                          ATGGCCGAG
61  GGGGAGATAA CCACCTTCAC CGCCCTGACC GAGCGCTTCG GCCTGCCGCT GGGCAACTAC
121 AAGAAGCCCA AACTCCTGTA CTGCAGCAAC GGGGGCCACT TCCTACGGAT CCTGCCGGAC
181 GGCAAGGTGG ACGGGACGCG GGACCGGAGT GACCAGCACA TTCAGCTGCA GCTCAGCGCG
241 GAAGATGTGG GCGAGGTCTA TATAAAGAGC ACAGCGTCGG GGCAGTACCT GGCAATGGAC
301 ACCAACGGGC TCCTGTATGG CTCGCAGCTA CCAGGCGAGG AGTGCTTGTT CCTTGAGAGG
361 CTCGAGGAGA ACCATTACAA CACATACATC TCCAAAAAGC ACGCAGACAA GAACTGGTTC
421 GTCGGGCTGA AGAAAAACGG GAACAGCAAG CTGGGGCCGC GGACTCACTA TGGGCAAAAG
481 GCGATCCTCT TCCTCCCATT GCCGGTGTCG GCTGACTGA
```

Alpaca FGF1 gene coding sequence (1-155, excluding 1-57) (SEQ ID NO:93) (Ensembl accession no. ENSVPAT00000008395, which is hereby incorporated by reference in its entirety):

```
1   CAGCTGCAGC TCAGTGCGGA AAGCGTGGGG GAGGTGTATA TAAAGAGTAC CGAGACTGGC
61  CAGTACTTGG CCATGGACAC CGACGGGCTT TTGCACGGCT CACAGACACC AAATGAGGAA
121 TGTTTGTTCC TGGAAAGGCT GGAGGAGAAC CATTACAACA CCTACACGTC CAAGAAGCAC
181 GCCGAAAAGA ATTGGTTTGT TGGTCTCAAG AAGAATGGAA GCTGCAAACG CGGTCCTCGG
241 ACTCACTACG GCCAGAAGGC GATCTTGTTT CTCCCCTTGC CAGTCTCCTC TGATTAA
```

Anole lizard FGF1 gene coding sequence (1-155) (SEQ ID NO:94) (Ensembl accession no. ENSACAT00000013467, which is hereby incorporated by reference in its entirety):

1 ATGGCTGAAG GTGAAATAAC AACATTCACA GCCTTGACCG AGAGGTTTGC TCTCCCAATG
61 GAGAATTACA AGAAGCCCAA ACTCCTGTAT TGCAGCAATG GAGGCCACTT CCTGAGGATC
121 CTTCCAGATG GAAAAGTGGA TGGCACCATG GACCGGAATG ACAGCTATAT TCAGTTGCTG
181 TTAACAGCAG AAGATGTGGG TGTGGTATAT ATAAAAGGCA CTGAGACCGG GCAGTACTTG
241 GCCATGGATG CCAATGGACA TTTATATGGC TCGCAGTTGC CAACAGAAGA GTGTTTATTT
301 GTGGAAACGC TGGAAGAAAA CCATTACAAT ACATATACCT CAAAGATGCA TGGCGATAAG
361 AAGTGGTATG TTGGCTTGAA AAAGAATGGG AAAGGCAAAC TGGGGCCACG GACTCATCGC
421 GGCCAAAAGG CAATACTTTT CCTTCCACTG CCAGTATCAC CTGATTAG

Bushbaby FGF1 gene coding sequence (1-155) (SEQ ID NO:95) (Ensembl accession no. ENSOGAT00000005081, which is hereby incorporated by reference in its entirety):

1 ATGGCTGAAG GGGAAATCAC AACCTTCACA GCCCTCACAG AGAAGTTTAA TCTGCCTCTA
61 GGAAATTACA AGAAGCCCAA GCTCCTCTAC TGTAGCAACG GGGGTCACTT TCTGAGGATC
121 CTGCCGGATG GCACCGTGGA TGGGACACAA GACAGGAGCG ACCAGCACAT TCAGCTGCAG
181 CTCAGTGCGG AAAGCGTGGG GGAGGTGTAT ATAAAGAGTA CCCAGACTGG CCAGTACTTG
241 GCCATGGACT CCGACGGGCT TTTATACGGC TCACAAACAC CAAATGAGGA ATGCCTGTTC
301 CTGGAACGGC TGGAGGAAAA CCATTACAAC ACCTATGTGT CCAAGAAGCA CGCCGAGAAG
361 AATTGGTTTG TCGGTCTCAA GAAGAACGGA AGTTGCAAAC GTGGTCCTCG GACTCACTAC
421 GGCCAGAAAG CAATCTTGTT TCTCCCCCTG CCAGTCTCCT CTGATTAA

Cat FGF1 gene coding sequence (1-155) (SEQ ID NO:96) (Ensembl accession no. ENSFCAT00000009123, which is hereby incorporated by reference in its entirety):

1 ATGGCTGAAG GGGAAATCAC AACCTTCACG GCCCTGACGG AGAAGTTCAA TCTGCCTCCA
61 GGGAATTACA AGAAACCCAA ACTCCTCTAC TGTAGCAACG GGGGCCACTT CCTGAGGATC
121 CTTCCAGATG GCACAGTGGA TGGGACGAGG GACAGGAGCG ACCAGCACAT TCAGCTGCAG
181 CTCAGTGCGG AAAGCGTGGG GGAGGTGTAT ATAAAGAGTA CCGAGACTGG CCAGTACTTG
241 GCCATGGACA CCGACGGGCT TTTGTACGGC TCACAGACAC CAAATGAGGA ATGCTTGTTC
301 CTGGAAAGGC TGGAAGAAAA CCATTACAAC ACCTACACAT CCAAGAAGCA CGCAGAAAAG
361 AATTGGTTTG TGGGTCTCAA GAAGAATGGA AGCTGCAAAC GCGGTCCCCG GACTCACTAT
421 GGCCAGAAGG CAATTTTGTT TCTCCCCCTG CCAGTCTCCT CTGATTAA

Chinese softshell turtle FGF1 gene coding sequence (1-155) (SEQ ID NO:97) (Ensembl accession no. ENSPSIT00000016432, which is hereby incorporated by reference in its entirety):

131 ATGGCTGAAG GGGAAATAAC AACGTTCACC GCCCTGACCG AAAAATTCAA
181 CCTTCCCCTG GGGAATTACA AGAATCCCAA ACTCTTATAT TGCAGCAATG GAGGCTACTT
241 CTTGAGGATA CATCCAGATG GCAAAGTAGA TGGGACAAGG GACCGAAGTG ACCAACACAT
301 TCAGCTGCAG CTAAGTGCGG AAAGCGTGGG TGAGGTATAT ATAAAGAGCA CTGAGTCTGG
361 ACAGTTTTTG GCTATGGACG CCAATGGACT TTTATATGGA TCACTGTCAC CGAGTGAGGA
291 ATGCTTATTC TTGGAAAGAA TGGAAGAAAA TCATTATAAC ACCTACATCT CCAAGAAGCA
351 TGCAGACAAG AACTGGTTCG TTGGCTTAAA GAAGAATGGA AGCTGCAAAC TGGGACCGCG
411 GACGCACTAC GGCCAAAAGG CCGTCCTTTT CCTTCCACTG CCAGTGTCAG CTGATTAA

Coelacanth FGF1 gene coding sequence (1-155) (SEQ ID NO:98) (Ensembl accession no. ENSLACT00000015212, which is hereby incorporated by reference in its entirety):

```
1   ATGGCTGAAG ACAAAATAAC AACACTGAAG GCCTTGGCTG AAAAATTTAA CCTTCCTATG
61  GGAAATTACA AGAAAGCAAA ACTCCTCTAC TGCAGCAACG GAGGGTATTT CCTGCGAATA
121 CCCCCAGACG GGAAAGTGGA AGGAATTAGA GAACGAAGCG ACAAGTACAT TCAGCTGCAA
181 ATGAATGCAG AAAGTTTAGG CATGGTGTCT ATAAAGGGTG TGGAGGCAGG GCAATACCTA
241 GCTATGAATA CAAATGGACT CCTGTATGGA TCTCAGTCTC TAACTGAAGA ATGCCTTTTC
301 ATGGAAAAGA TGGAAGAAAA CCACTACAAC ACATACAGGT CTAAGACACA TGCAGATAAA
361 AACTGGTATG TTGGCATTAG AAAGAACGGT AGCATCAAAC CAGGACCAAG GACTCACATT
421 GGCCAAAAGG CTGTTCTTTT TCTCCCTCTG CCTGCCTCGA GTGATTAG
```

Dolphin FGF1 gene coding sequence (1-155) (SEQ ID NO:99) (Ensembl accession no. ENSTTRT00000004742, which is hereby incorporated by reference in its entirety):

```
1   ATGGCTGAAG GGGAAATCAC AACCTTCACA GCCCTGACCG AGAAGTTTAA TCTGCCTCCA
61  GGGAATTACA AGAAGCCCAA ACTCCTCTAC TGTAGCAACG GGGGCCACTT CCTGAGGATC
121 CTTCCAGATG GCACAGTGGA TGGGACAAGG GACAGGAGTG ACCAGCACAT TCAGCTGCAG
181 CTCAGTGCGG AAAGCGTGGG GGAGGTGTAT ATAAAGAGTA CGGAGACTGG CCAGTACTTG
241 GCCATGGACA CCGACGGGCT TTTGTACGGC TCACAGACAC CCAATGAGGA ATGTTTGTTC
301 CTGGAAAGGT TGGAGGAAAA CCATTACAAC ACCTACGCAT CCAAGAAGCA TGCAGAAAAG
361 AATTGGTTCG TTGGTCTCAA GAAGAACGGA AGCTGCAAAC GCGGTCCTCG GACTCACTAC
421 GGCCAGAAAG CAATCTTGTT TCTCCCCCTG CCAGTCTCCT CCGATTAA
```

Ferret FGF1 gene coding sequence (1-155) (SEQ ID NO:100) (Ensembl accession no. ENSMPUT00000008013, which is hereby incorporated by reference in its entirety):

```
1                                       ATGGCT GAAGGGGAAA TCACAACCTT
61  CACAGCCCTG ATGGAGAAGT TTAATCTGCC TGCGGGGAAT TACAAGAAGC CCAAACTCCT
121 CTACTGTAGC AATGGGGGCC ACTTCCTGAG GATCCTTCCA GATGGCACAG TGGACGGCAC
181 AAGGGACAGG AGCGACCAGC ACATTCAGCT GCAGCTCAGT GCGGAAAGCG TGGGGGAGGT
241 GTACATAAAG AGTACCGAGA CTGGCCAGTA CTTGGCCATG GACACCGATG GGCTTTTGTA
301 CGGCTCACAA ACACCAAATG AGGAATGTCT GTTCCTGGAA AGGCTGGAGG AAAACCATTA
361 CAACACCTAC ACATCCAAGA AGCACGCTGA GAAGAATTGG TTTGTAGGTC TCAAGAAGAA
421 CGGAAGCTGC AAACGCGGTC CTCGGACTCA CTATGGCCAG AAAGCAATTC TGTTTCTCCC
481 CCTGCCAGTC TCCTCTGATT AA
```

Gibbon FGF1 gene coding sequence (1-155) (SEQ ID NO:101) (Ensembl accession no. ENSNLET00000012455, which is hereby incorporated by reference in its entirety):

```
241                                                 ATGG CCGAAGGGGA
301 AATCACCACC TTCACAGCCC TGACCGAGAA GTTTAATCTG CCTCCAGGGA ATTACAAGAA
361 GCCCAAACTC CTCTACTGTA GCAACGGGGG CCACTTCTTG AGGATCCTTC CGGATGGCAC
421 AGTGGATGGG ACAAGGGACA GGAGCGACCA GCACATTCAG CTGCAGCTCA GTGCGGAAAG
481 CGTGGGGGAG GTGTATATAA AGAGTACCGA GACTGGCCAG TACTTGGCCA TGGACACCGA
541 CGGGCTTTTA TACGGCTCAC AGACACCAAA TGAGGAATGT TTGTTCCTGG AAAGGCTGGA
601 GGAGAACCAT TACAACACCT ATATATCCAA GAAGCATGCA GAGAAGAATT GGTTTGTTGG
661 CCTCAAGAAG AATGGAAGCT GCAAACGCGG TCCTCGGACT CACTATGGCC AGAAAGCAAT
721 CTTGTTTCTC CCCCTGCCAG TCTCTTCTGA TTAA
```

Gorilla FGF1 gene coding sequence (1-155) (SEQ ID NO:102) (Ensembl accession no. ENSGGOT00000025344, which is hereby incorporated by reference in its entirety):

```
121                                                    ATGG CTGAAGGGGA
181 AATCACCACC TTCACAGCCC TGACCGAGAA GTTTAATCTG CCTCCAGGGA ATTACAAGAA
241 GCCCAAACTC CTCTACTGTA GCAATGGGGG CCACTTCTTG AGGATCCTTC CGGATGGCAC
301 AGTGGATGGG ACAAGGGACA GGAGCGACCA GCACATTCAG CTGCAGCTCA GTGCGGAAAG
361 CGTGGGGGAG GTGTATATAA AGAGTACCGA GACTGGCCAG TACTTGGCCA TGGACACCGA
421 CGGGCTTTTA TACGGCTCAC AGACACCAAA TGAGGAATGT TTGTTCCTGG AAAGGCTGGA
481 GGAGAACCAT TACAACACCT ATATATCCAA GAAGCATGCA GAGAAGAATT GGTTTGTTGG
541 CCTCAAGAAG AATGGAAGCT GCAAACGCGG TCCTCGGACT CACTATGGCC AGAAAGCAAT
601 CTTGTTTCTC CCCCTGCCAG TCTCTTCCGA TTAA
```

Hedgehog FGF1 gene coding sequence (1-155) (SEQ ID NO:103) (Ensembl accession no. ENSEEUT00000005832, which is hereby incorporated by reference in its entirety):

```
1   ATGGCTGAAG GAGAAATCAC CACCTTCACG GCCCTGACTG AGAAGTTTAA TCTGCCACTA
61  GGGAATTACA AGAAGCCCAA GCTCCTCTAC TGTAGCAACG GGGGCCACTT CCTGAGGATC
121 CTTCCAGATG GCACCGTGGA TGGGACAAGG GACAGGAGCG ACCAGCATAT TCAGCTGCAG
181 CTCAGTGCGG AAAGCGTGGG GGAGGTGTAT ATAAAGAGTA CGGAGACTGG CCAGTACTTG
241 GCCATGGACA CCGACGGGCT TTTATACGGC TCACAAACAC CAAATGAGGA ATGTCTGTTC
301 CTTGAAAGGC TGGAAGAGAA CCATTACAAT ACCTACACAT CCAAGAAGCA TGCCGAGAAG
361 AACTGGTTTG TTGGCCTCAA GAAGAATGGA AGCTGCAAGC GTGGTCCTCG GACTCATTAT
421 GGCCAGAAAG CTATTTTGTT TCTCCCCCTG CCAGTTTCCT CTGATTAA
```

Hyrax FGF1 gene coding sequence (1-155, excluding 1-90) (SEQ ID NO:104) (Ensembl accession no. ENSPCAT00000011746, which is hereby incorporated by reference in its entirety):

```
1   ATGGCTGAAG GCGAAATCAC AACCTTCACA GCCCTGACTG AGAAGTTTAA CCTGCCACTA
61  GAGAATTACA AGAAGCCCAA ACTCCTCTAC TGTAGCAACG GAGGCCACTT CCTGAGGATC
121 CTTCCGGACG GCACAGTGGA TGGCACCAGG GACAGGAGTG ACCAGCACAT TCAGCTGCAG
181 CTCAGTGCGG AAAGCGTGGG GGAGGTGTAT ATAAAGGGCA CCGAGACTGG CCAGTACTTG
241 GCCATGGACA CCGACGGGCT TTTATATGGC TCA
```

Kangaroo rat FGF1 gene coding sequence (1-155, excluding 1-16 and 58-155) (SEQ ID NO:105) (Ensembl accession no. ENSDORT00000007345, which is hereby incorporated by reference in its entirety):

```
1   ATGGCTGAAG GGGAAATCAC AACCTTCACA GCCCTGACGG AAAGGTTTAA ----------
    ---------- ---------- ---------- ---------- ---------- ----------
51  ---------- ---------- ---------- ---------- ---------T TCAGCTGCAA
62  CTGAGTGCGG AAAGCGTGGG GGAGGTCTAT ATAAAGAGCA CCGAGACTGG CCAATACTTG
122 GCCATGGATG CCGACGGGCT TTTATACGGC TCACAGACAC CTGATGAAGA ATGCTTGTTC
182 CTGGAGAGGC TGGAAGAAAA TCATTATAAC ACCTACATAG CCAAGAAACA TGCTGAAAAG
242 AATTGGTTTG TCGGCCTCAA AAAGAATGGA AGCTGCAAGC GTGGTCCTCG GACTCACTAT
302 GGCCAGAAAG CAATCCTGTT CCTCCCCCTTG CCTGTCTCCT CTGATTAG
```

Lamprey FGF1 gene coding sequence (1-155, excluding 94-155) (SEQ ID NO:106) (Ensembl accession no. ENSPMAT00000010729, which is hereby incorporated by reference in its entirety):

```
1   ATGGAGGTGG GCCACATCGG CACGCTGCCC GTGGTCCCCG CGGGGCCCGT GTTCCCCGGC
61  AGTTTCAAGG AGCCACGGCG CCTCTACTGC CGCAGCGCGG GCCACCACCT CCAGATCCTG
121 GGGGACGGCA CCGTGAGTGG CACCCAGGAC GAGAACGAGC CCCACGCCGT TCTGCAGCTG
181 CAGGCGGTGC GCCGCGGGGT GGTGACGATC CGTGGGCTCT GCGCCGAGAG GTTCCTCGCC
241 ATGAGCACGG AGGGACACCT GTACGGGGCG GTGAGG
```

Lesser hedgehog tenrec FGF1 gene coding sequence (1-155, excluding 1-57) (SEQ ID NO:107) (Ensembl accession no. ENSETET00000017851, which is hereby incorporated by reference in its entirety):

```
1   CAGCTGAAGC TCGTTGCCGA AAGCGTGGGG GTGGTGTATA TAAAGAGCAT CAAGACCGGC
61  CAGTACTTGG CCATGAACCC CGACGGGCTT TTATACGGCT CCGAGACCCC AGAGGAAGAA
121 TGCTTGTTCC TGGAAACGCT GGAGGAAAAC CACTACACCA CCTTCAAATC TAAGAAGCAC
181 GTAGAGAAGA ATTGGTTCGT TGGTCTCCGG AAGAATGGAA GGGTCAAGAT CGGGCCTCGG
241 ACTCACCAAG GCCAGAAAGC AATCTTGTTC CTGCCCCTCC CGGTGTCCTC TGATTAA
```

Rhesus monkey FGF1 gene coding sequence (1-155) (SEQ ID NO:108) (Ensembl accession no. ENSMMUT00000033070, which is hereby incorporated by reference in its entirety):

```
36                                      ATGGC TGAAGGGGAA ATCACCACGT
61  TCACAGCCCT GACCGAGAAG TTTAATCTGC CTCCAGGGAA TTACAAGAAG CCCAAACTGC
121 TCTACTGTAG CAATGGGGGC CACTTCTTGA GGATCCTTCC GGATGGCACA GTGGATGGGA
181 CAAGGGACAG GAGCGACCAG CACATTCAGC TGCAGCTCAG TGCGGAAAGC GTGGGGGAGG
241 TGTATATAAA GAGTACCGAG ACTGGCCAGT ACTTGGCCAT GGACACCGAC GGGCTTTTAT
301 ACGGCTCACA GACACCAAAT GAGGAATGTT TGTTCCTGGA AAGGCTGGAG GAGAACCATT
361 ACAACACCTA TACATCCAAG AAGCACGCAG AGAAGAATTG GTTTGTTGGC CTCAAGAAGA
421 ATGGAAGCTG CAAACGTGGT CCTCGGACTC ACTATGGCCA GAAAGCAATC TTGTTTCTTC
481 CCCTGCCAGT CTCTTCTGAT TAA
```

Megabat FGF1 gene coding sequence (1-155) (SEQ ID NO:109) (Ensembl accession no. ENSPVAT00000004596, which is hereby incorporated by reference in its entirety):

```
1   ATGGCCGAGG GGGAAGTCAC GACGTTCACG GCCCTGACCG AGAGGTTTAA CCTGCCTCCA
61  GGGAATTACA AGAAGCCCAA ACTTCTCTAC TGCAGCAACG GGGGCCACTT CCTGAGGATC
121 CTCCCAGATG GCACAGTGGA TGGGACAAGG GACAAGAGCG ACCAGCACAT TCAGCTGCAG
181 CTCAGTGCGG AAAGTGTGGG GGAGGTGTAT ATAAAGAGCA CCGAGAGTGG CCAGTACTTG
241 GCCATGGACT CCGACGGGCT TTTGTACGGC TCACAGACAC CAGATGAGGA CTGTTTGTTC
301 CTGGAAAGGC TGGAGGAAAA CCATTACAAC ACCTACACAT CCAAGAAGCA CGCAGAGAAG
361 AATTGGTTTG TTGGGCTCAA GAAGAATGGA AGCTGCAAGC GCGGTCCCCG GACTCACTAC
421 GGCCAGAAAG CGATCCTGTT TCTCCCCCTG CCAGTCTCCT CTGATTAG
```

Microbat FGF1 gene coding sequence (1-155) (SEQ ID NO:110) (Ensembl accession no. ENSMLUT00000007098, which is hereby incorporated by reference in its entirety):

```
66       ATGGC TGAGGGGGAA GTCACCACAT TCACGGCCCT GACCGAGAGG TTCAATCTGC
121 CTCTGGAGAA CTACAAGAAG CCCAAGCTTC TCTACTGCAG CAACGGGGGC CACTTCCTGC
181 GGATCCTCCC AGACGGCACC GTGGACGGGA CGAGGGACAG GAGCGACCAG CACATTCAGC
241 TGCAGCTCAG TGCGGAAAGC GTGGGGGAGG TGTATATAAA GAGCACCGAG AGTGGCCAGT
301 ACTTGGCCAT GGACTCCGAC GGGCTTTTGT ACGGCTCACA AACACCCAAT GAGGAATGTT
361 TGTTCCTGGA AAGGCTGGAG GAGAACCACT ACAACACCTA CACGTCCAAG AAGCACGCAG
421 AAAAGAATTG GTTCGTTGGG CTCAAGAAGA ACGGAAGCTG CAAGCGTGGT CCTCGGACGC
481 ATTATGGCCA GAAAGCAATC TTGTTTCTCC CCCTGCCAGT CTCCTCCGAT TAA
```

Mouse lemur FGF1 gene coding sequence (1-155) (SEQ ID NO:111) (Ensembl accession no. ENSMICT00000009454, which is hereby incorporated by reference in its entirety):

```
1   ATGGCCGAAG GGGAGATCAC AACCTTCACG GCCCTCACCG AGAAGTTTAA CCTGCCTCCG
61  GGGAACTACA AGAAGCCCAA GCTCCTCTAC TGCAGCAACG GCGGCCACTT CCTGCGCATC
121 CTTCCCGACG GCACCGTGGA TGGCACGAGA GACAGGAGCG ACCAGCACAT TCAGCTGCAG
181 CTCAGTGCGG AAAGCGCGGG GGAGGTGTAT ATAAAGAGCA CCCAGACTGG CCGGTACTTG
241 GCCATGGACG CCGACGGGCT TTTATACGGC TCACAAACAC CAAATGAGGA ATGTTTGTTC
301 CTGGAAAGGC TGGAGGAAAA CCATTACAAC ACCTACGTAT CCAAGAAGCA CGCAGAGAAG
361 AATTGGTTTG TTGGCCTCAA GAAGAATGGA AGTTGCAAAC GCGGCCCCCG GACTCACTAT
421 GGCCAGAAAG CAATCTTGTT TCTGCCCCTG CCAGTCTCCT CTGATTAA
```

Pika FGF1 gene coding sequence (1-155, excluding 57-67) (SEQ ID NO:112) (Ensembl accession no. ENSOPRT00000012854, which is hereby incorporated by reference in its entirety):

```
1   ATGGCCGAGG GAGAAGTCAC CACCTTCTCA GCCCTGACGG AGAAGTTCAA TCTGCCTGGA
61  GGAAACTACA AGTTGCCCAA GCTCCTTTAC TGTAGCAACG GAGGCCACTT CCTGAGGATC
121 CTTCCAGATG GCACAGTGGA TGGGACCAGG GACAGGAGCG ACCTGCACA- ----------
170 ---------- ---------- -GAGGTGTTT ATAAAGAGTA CGGAGACTGG CCAGTACTTG
209 GCTATGGACA CCGATGGCCT TTTATATGGC TCGCAGACAC CCAGTGAGGA GTGTTTGTTC
269 CTGGAGCGGC TGGAGGAGAA CCACTACAAC ACCTACACAT CCAAGAAGCA TGCCGAGAAG
329 AACTGGTTTG TGGGCATCAA GAAGAATGGA AGCTGCAAGC GTGGTCCTCG GACTCACTAC
389 GGCCAGAAAG CCATCTTGTT TCTCCCTCTG CCAGTCTCTT CTGACTAA
```

Rat FGF1 gene coding sequence (1-155) (SEQ ID NO:113) (Ensembl accession no. ENSRNOT00000018577, which is hereby incorporated by reference in its entirety):

```
268                             ATG GCCGAAGGGG AGATCACAAC CTTTGCAGCC
301 CTGACCGAGA GGTTCAATCT GCCTCTAGGG AACTACAAAA AACCCAAACT GCTCTACTGC
361 AGCAACGGGG GCCACTTCTT GAGGATTCTT CCCGATGGCA CCGTGGATGG GACCAGGGAC
421 AGGAGCGACC AGCACATTCA GCTGCAGCTC AGTGCGGAAA GCGCGGGCGA AGTGTATATA
481 AAGGGTACAG AGACTGGCCA GTACTTGGCC ATGGACACCG AAGGGCTTTT ATACGGCTCG
541 CAGACACCAA ATGAAGAATG CCTATTCCTG GAAAGGCTAG AAGAAAACCA TTATAACACT
601 TACACATCCA AGAAGCACGC GGAGAAGAAC TGGTTTGTGG GCCTCAAGAA GAACGGGAGT
661 TGTAAGCGCG GTCCTCGGAC TCACTACGGC CAGAAAGCCA TCTTGTTTCT CCCCCTCCCG
721 GTATCTTCTG ACTAA
```

Sloth FGF1 gene coding sequence (1-155) (SEQ ID NO:114) (Ensembl accession no. ENSCHOT00000012416, which is hereby incorporated by reference in its entirety):

```
1   ATGGCTGAAG GGGAAATCAC AACCTTCACA GCTCTGATGG AGAAGTTTAA CCTGCCACCA
61  GGGAATTACA TGAAGCCCAA ACTCCTCTAC TGTAGCAACG GGGGCCACTT CTTGAGGATC
121 CTTCCAGACG GCACAGTGGA TGGGACAAGG GACAGGAGCG ACCTGCACAT TCAGCTGCAG
181 CTCAGTGCGG AAAGCGTGGG GGAGGTGTAT ATAAAGAGTG CGGAGACCGG CCAGTACTTA
241 GCCATGGACA CCGGCGGGCT TTTATACGGC TCACAGACAC CAAGTGAGGA ATGCCTGTTC
301 CTAGAAAGGC TGGAGGAAAA CCATTACAAC ACCTACGTAT CCAAGAAGCA TGCGGAGAAG
361 AACTGGTTCG TTGGCCTAAA GAAGAATGGA AGCAGCAAAC GCGGCCCCCG GACTCACTAT
421 GGCCAGAAAG CCATCTTGTT TCTTCCCCTG CCAGTCTCCT CTGATTAA
```

Squirrel FGF1 gene coding sequence (1-155) (SEQ ID NO:115) (Ensembl accession no. ENSSTOT00000029249, which is hereby incorporated by reference in its entirety):

```
1                                                               ATGG
5   CTGAAGGGGA AATCACAACC TTCACAGCCC TGACCGAGAA GTTCAATCTG CCTCCAGGGA
65  ACTACAAGAA GCCCAAACTG CTCTACTGTA GCAACGGAGG CCACTTCTTG AGGATCCTTC
125 CTGATGGCAC AGTGGATGGG ACAAGAGACA GGAGCGACCA ACACATTCAG CTGCAGCTCA
185 GTGCGGAAAG CGTGGGGGAG GTGTATATAA AGAGTACCGA GACCGGCCAG TACTTGGCCA
245 TGGACACCGA CGGGCTTTTA TATGGCTCAC AGACCCCAAA TGAGGAATGC TTATTCCTGG
305 AAAGGCTGGA GGAAAACCAT TACAACACGT ACACATCCAA GAAGCATGCA GAGAAGAATT
365 GGTTTGTTGG CCTCAAGAAG AACGGAAGCT GCAAGCGCGG TCCCCGGACT CACTATGGCC
425 AGAAAGCGAT CTTGTTTCTC CCACTGCCTG TCTCCTCTGA TTAG
```

Tarsier FGF1 gene coding sequence (1-155) (SEQ ID NO:116) (Ensembl accession no. ENSTSYT00000007425, which is hereby incorporated by reference in its entirety):

```
1   ATGGCCGAAG GGGAAATCAC AACCTTCACA GCCCTGACCG AGAAGTTCAA CCTGCCCCCG
61  GGGAATTACA AGAAGCCCAA ACTCCTCTAC TGCAGCAACG GGGGCCACTT CTTGAGGATC
121 CTTCCGGATG GCACTGTGGA TGGAACGAGG GACAGGAGCG ACCAGCACAT TCAGCTGCAG
181 CTCAGCGCGG AAAGCGTGGG GGAGGTGTAT ATAAAGAGTA CCGAGACCGG CCAGTACTTG
241 GCCATGGACA CCGACGGGCT TTTGTACGGC TCACAGACAC CAAATGAGGA GTGTCTGTTC
301 CTGGAAAGGC TGGAAGAGAA TCATTACAAT ACCTACGTGT CCAAGAAGCA TGCGGAGAAG
361 AATTGGTTTG TCGGCCTCAA GAAGAATGGA AGCTGCAAAC GCGGTCCTCG GACTCACTAT
421 GGCCAGAAAG CAATCTTGTT TCTCCCCCTG CCAGTTTCCT CTGATTAA
```

Tree shrew FGF1 gene coding sequence (1-155) (SEQ ID NO:117) (Ensembl accession no. ENSTBET00000011861, which is hereby incorporated by reference in its entirety):

```
1   ATGGCTGAAG GGGAAATCAC GACCTTCGCA GCCCTGACCG AGAAGTTTGA TCTGCCTCCA
61  GGGAATTACA AGAAGCCCAA ACTTCTCTAC TGTAGCAACG GGGGCCATTT CTTGAGGATT
121 CTTCCAGATG GCACCGTGGA TGGGACAAGA GACAGGAGCG ACCAGCACAT TCAGCTGCAG
181 CTCACTGCGG AAAACGTGGG GGAGGTGTAC ATAAAGAGTA CGGAGACTGG CCAGTACTTG
241 GCCATGGACG CCGACGGGCT TTTATATGGC TCACAGACAC CAAACGAGGA ATGTTTGTTC
301 CTGGAAAGGC TGGAGGAGAA CCATTACAAC ACCTACATAT CCAAGAAGCA CGCAGAGAAG
361 AATTGGTTTG TTGCCCTCAA GAAGAACGGA AGCTGCAAAC TCGGTCCTCG GACTCACTAT
421 GGCCAGAAAG CAATCTTGTT TCTCCCCCTG CCAGTCTCCT CTGATTAA
```

Turkey FGF1 gene coding sequence (1-155, excluding 57-155) (SEQ ID NO:118) (Ensembl accession no. ENSMGAT00000017372, which is hereby incorporated by reference in its entirety):

```
1   ATGGCCGAGG GGGAGATAAC CACCTTCACA GCCCTGACCG AGCGCTTCGG CCTGCCGCTG
61  GGCAACTACA AGAAGCCCAA ACTCCTGTAC TGCAGCAACG GGGGCCACTT CCTACGGATC
121 CTGCCGGACG GCAAGGTGGA CGGGACGCGG GACCGGAGCG ACCAGCAC
```

Wallaby FGF1 gene coding sequence (1-155) (SEQ ID NO:119) (Ensembl accession no. ENSMEUT00000016544, which is hereby incorporated by reference in its entirety):

```
1   ATGGCCGAAG GGGAGATCAC AACCTTCACA GCCCTGACCG AAAGATTTAA CCTGCCACTG
61  GGGAATTACA AGAAGCCCAA GCTTCTCTAC TGTAGCAATG GGGGCCACTT TTTGAGGATC
121 CTTCCTGATG GCAAAGTGGA TGGGACAAGG GACAGAAATG ATCAACACAT TCAACTGCAA
181 CTAAGCGCGG AAAGCGTGGG TGAGGTGTAT ATAAAGAGCA CTGAGTCTGG GCAGTATTTG
241 GCCATGGACA CCAATGGACT TTTATATGGC TCACAGACCC CCAGCGAAGA ATGCTTATTC
301 CTGGAGAGGT TGGAGGAGAA TCATTACAAC ACCTACATAT CAAAGAAGCA TGCGGAGAAA
361 AATTGGTTTG TTGGCCTCAA GAAGAACGGA AGTTGCAAAA GAGGTCCCAG GACTCACTAT
421 GGCCAGAAAG CCATCCTATT CCTTCCCCTC CCTGTGTCCT CTGAGTAA
```

Zebrafish FGF1 gene coding sequence (1-147) (SEQ ID NO:120) (Ensembl accession no. ENSDART00000005842, which is hereby incorporated by reference in its entirety):

```
178                                                             ATG
181 ACCGAGGCCG ATATTGCGGT AAAGTCCAGC CCGCGCGACT ATAAAAAACT GACGCGGCTG
241 TACTGTATGA ATGGAGGATT TCACCTTCAG ATCCTGGCGG ACGGGACAGT GGCTGGAGCA
124 GCAGACGAAA ACACATACAG CATACTGCGC ATAAAAGCAA CAAGTCCAGG AGTGGTGGTG
184 ATCGAAGGAT CAGAAACAGG TCTTTACCTC TCGATGAATG AACATGGCAA GCTGTACGCT
244 TCATCATTAG TGACGGATGA AAGTTATTTC CTGGAGAAGA TGGAGGAAAA CCACTACAAC
304 ACATATCAGT CTCAAAAGCA CGGTGAAAAC TGGTACGTCG GAATAAAAAA GAACGGGAAA
364 ATGAAACGGG GCCCAAGAAC TCACATCGGA CAAAAGGCCA TTTTCTTTCT TCCACGACAG
424 GTGGAGCAGG AAGAGGACTG A
```

[0059] As noted above, also encompassed within the present invention are portions of paracrine FGFs other than FGF1 (e.g., FGF2, FGF4, FGF5, FGF6, FGF9, FGF16, and FGF20). The portions derived from paracrine FGF2 include portions corresponding to the above-identified amino acid sequences of FGF1. Corresponding portions may be determined by, for example, sequence analysis and structural analysis.

[0060] In one embodiment, the paracrine FGF is FGF2. In one embodiment, the portion of the FGF2 is derived from human FGF2 having the amino acid sequence of SEQ ID NO: 121 (GenBank Accession No. EAX05222, which is hereby incorporated by reference in its entirety), as follows:

```
1   MAAGSITTLP ALPEDGGSGA FPPGHFKDPK RLYCKNGGFF LRIHPDGRVD GVREKSDPHI
61  KLQLQAEERG VVSIKGVCAN RYLAMKEDGR LLASKCVTDE CFFFERLESN NYNTYRSRKY
121 TSWYVALKRT GQYKLGSKTG PGQKAILFLP MSAKS
```

[0061] In one embodiment, the portion of the paracrine FGF includes an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 151 to 155 of SEQ ID NO: 121. In one embodiment, the portion of the paracrine FGF includes amino acid residues 1-151, 1-152, 1-153, 1-154, 1-155, 2-151, 2-152, 2-153, 2-154, 2-155, 3-151, 3-152, 3-153, 3-154, 3-155, 4-151, 4-152, 4-153, 4-154, 4-155, 5-151, 5-152, 5-153, 5-154, 5-155, 6-151, 6-152, 6-153, 6-154, 6-155, 7-151, 7-152, 7-153, 7-154, 7-155, 8-151, 8-152, 8-153, 8-154, 8-155, 9-151, 9-152, 9-153, 9-154, 9-155, 10-151, 10-152, 10-153, 10-154, 10-155, 11-151, 11-152, 11-153, 11-154, 11-155, 12-151, 12-152, 12-153, 12-154, 12-155, 13-151, 13-152, 13-153, 13-154, 13-155, 14-151, 14-152, 14-153, 14-154, 14-155, 15-151, 15-152, 15-153, 15-154, 15-155, 16-151, 16-152, 16-153, 16-154, 16-155, 17-151, 17-152, 17-153, 17-154, 17-155, 18-151, 18-152, 18-153, 18-154, 18-155, 19-151, 19-152, 19-153, 19-154, 19-155, 20-151, 20-152, 20-153, 20-154, 21-155, 21-151, 21-152, 21-153, 21-154, 21-155, 22-151, 22-152, 22-153, 22-154, 22-155, 23-151, 23-152, 23-153, 23-154, 23-155, 24-151, 24-152, 24-153, 24-154, 24-155, 25-151, 25-152, 25-153, 25-154, or 25-155 of FGF2 (SEQ ID NO: 121). In one embodiment, the portion of the paracrine FGF includes amino acid residues 1-151 or 1-152 of SEQ ID NO: 121.

[0062] In one embodiment, the portion of the paracrine FGF of the chimeric protein includes an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence identity to the corresponding amino acid sequence of native paracrine FGF (e.g., SEQ ID NO: 121). In one embodiment, the portion of the paracrine FGF includes an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence identity to an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 151 to 155

of SEQ ID NO: 121. In one embodiment, the portion of the paracrine FGF includes an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence homology to the corresponding amino acid sequence of native paracrine FGF (e.g., SEQ ID NO: 121). In one embodiment, the portion of the paracrine FGF includes an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence homology to an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 151 to 155 of SEQ ID NO: 121.

[0063] Also encompassed within the present invention are portions of paracrine FGFs other than FGF2 (e.g., FGF1, FGF4, FGF5, FGF6, FGF9, FGF16, and FGF20). The portions derived from paracrine FGFs other than FGF2 include portions corresponding to the above-identified amino acid sequences of FGF2. Corresponding portions may be determined by, for example, sequence analysis and structural analysis.

[0064] In one embodiment of the present invention, the portion of the paracrine FGF is derived from an ortholog of a human paracrine FGF. In one embodiment of the present invention, the portion of the paracrine FGF of the chimeric protein is derived from an ortholog of human FGF2. In one embodiment, the portion of the FGF2 is derived from Gorilla gorilla, Pongo abelii, Macaca mulatta, Pan troglodytes, Pan paniscus, Saimiri boliviensis boliviensis, Nomascus leucogenys, Equus caballus, Bos taurus, Papio Anubis, Vicugna pacos, Ovis aries, Capreolus capreolus, Loxodonta Africana, Sus scrofa, Ailuropoda melanoleuca, Choloepus hoffmanni, Bubalus bubalis, Canis lupus familiaris, Rattus norvegicus, Heterocephalus glaber, Otolemur garnettii, Mus musculus, Ictidomys tridecemlineatus, Felis catus, Cavia porcellus, Sarcophilus harrisii, Monodelphis domestica, Oryctolagus cuniculus, Meleagris gallopavo, Gallus gallus, Taeniopygia guttata, Cynops pyrrhogaster, Xenopus laevis, Didelphis albiventris, Myotis lucifugus, Anolis carolinensis, Dasypus novemcinctus, Tupaia belangeri, Xenopus silurana tropicalis, Latimeria chalumnae, Tetraodon nigroviridis, Gasterosteus aculeatus, Takifugu rubripes, Oncorhynchus mykiss, Salmo salar, Danio rerio, Oreochromis niloticus, or Oryzias latipes. The portions of an ortholog of human paracrine FGF include portions corresponding to the above-identified amino acid sequences of FGF2. Corresponding portions may be determined by, for example, sequence analysis and structural analysis.

[0065] In one embodiment, the portion of the FGF2 of the chimeric protein of the present invention is derived from an ortholog of human FGF2 having the amino acid sequence shown in Table 3.

Table 3:

Amino acid sequence of Gorilla gorilla (gorilla) FGF2 (SEQ ID NO:122) (Ensembl accession no. ENSGGOP00000004720, which is hereby incorporated by reference in its entirety): 104 MAAGSI TTLPALPEDG 120 GSGAFPPGHF KDPKRLYCKN GGFFLRIHPD GRVDGVREKS DPHIKLQLQA EERGVVSIKG 180 VCANRYLAMK EDGRLLASKC VTDECFFFER LESNNYNTYR SRKYTSWYVA LKRTGQYKLG 240 SKTGPGQKAI LFLPMSAKS
Amino acid sequence of Pongo abelii (sumatran orangutan) FGF2(SEQ ID NO:123) (GenBank accession no. XP_002815172, which is hereby incorporated by reference in its entirety): 168 MAA GSITTLPALP 181 EDGGSGAFPP GHFKDPKRLY CKNGGFFLRI HPDGRVDGVR EKSDPHIKLQ LQAEERGVVS 241 IKGVCANRYL AMKEDGRLLA SKCVTDECFF FERLESNNYN TYRSRKYTSW YVALKRTGQY 301 KLGSKTGPGQ KAILFLPMSA KS
Amino acid sequence of Macaca mulatta (rhesus monkey) FGF2 (SEQ ID NO:124) (GenBank accession no. XP_001099284, which is hereby incorporated by reference in its entirety): 83 MAAGSITT LPALPEDGGS GAFPPGHFKD PKRLYCKNGG 121 FFLRIHPDGR VDGVREKSDP HIKLQLQAEE RGVVSIKGVC ANRYLAMKED GRLLASKCVT 181 DECFFFERLE SNNYNTYRSR KYTSWYVALK RTGQYKLGSK TGPGQKAILF LPMSAKS
Amino acid sequence of Pan troglodytes (chimpanzee) FGF2 (SEQ ID NO:125) (GenBank accession no. NP_001103711, which is hereby incorporated by reference in its entirety): 134 MAAGSIT TLPALPEDGG SGAFPPGHFK DPKRLYCKNG GFFLRIHPDG 181 RVDGVREKSD PHIKLQLQAE ERGVVSIKGV CANRYLAMKE DGRLLASKCV TDECFFFERL 241 ESNNYNTYRS RKYTSWYVAL KRTGQYKLGS KTGPGQKAIL FLPMSAKS
Amino acid sequence of Pan paniscus (Pygmy chimpanzee) FGF2 (SEQ ID NO:126) (GenBank accession no. XP_003816481, which is hereby incorporated by reference in its entirety): 112 MAAGSITTL 121 PALPEDGGSG AFPPGHFKDP KRLYCKNGGF FLRIHPDGRV DGVREKSDPH IKLQLQAEER 181 GVVSIKGVCA NRYLAMKEDG RLLASKCVTD ECFFFERLES NNYNTYRSRK YTSWYVALKR 241 TGQYKLGSKT GPGQKAILFL PMSAKS
Amino acid sequence of Saimiri boliviensis boliviensis (Bolivian squirrel monkey) FGF2 (SEQ ID NO:127) (GenBank accession no. XP_003936290, which is hereby incorporated by reference in its entirety): 1 MAAGSITTLP ALPEDGGSGA FPPGHFKDPK RLYCKNGGFF LRIHPDGRVD GVREKSDPHI 61 KLQLQAEERG VVSIKGVCAN RYLAMKEDGR LLASKCVTDE CFFFERLESN NYNTYRSRKY 121 TSWYVALKRT GQYKLGSKTG PGQKAILFLP MSAKS
Amino acid sequence of Nomascus leucogenys (Northern white-cheeked gibbon) FGF2 (SEQ ID NO:128) (GenBank accession no. XP_003271404, which is hereby incorporated by reference in its entirety): 1 MAAGSITTLP ALPEDGGSGA FPPGHFKDPK RLYCKNGGFF LRIHPDGRVD GVREKSDPHI 61 KLQLQAEERG VVSIKGVCAN RYLAMKEDGR LLASKCVTDE CFFFERLESN NYNTYRSRKY 121 TSWYVALKRT GQYKLGSKTG PGQKAILFLP MSAKS
Amino acid sequence of Equus caballus (horse) FGF2 (SEQ ID NO:129) (GenBank accession no. NP_001182150, which is hereby incorporated by reference in its entirety): 1 MAAGSITTLP ALPEDGGSGA FPPGHFKDPK RLYCKNGGFF LRIHPDGRVD GVREKSDPHI 61 KLQLQAEERG VVSIKGVCAN RYLAMKEDGR LLASKCVTDE CFFFERLESN NYNTYRSRKY 121 SSWYVALKRT GQYKLGPKTG PGQKAILFLP MSAKS

Amino acid sequence of Bos taurus (cattle) FGF2 (SEQ ID NO:130) (GenBank accession no. NP_776481, which is hereby incorporated by reference in its entirety): 1 MAAGSITTLP ALPEDGGSGA FPPGHFKDPK RLYCKNGGFF LRIHPDGRVD GVREKSDPHI 61 KLQLQAEERG VVSIKGVCAN RYLAMKEDGR LLASKCVTDE CFFFERLESN NYNTYRSRKY 121 SSWYVALKRT GQYKLGPKTG PGQKAILFLP MASKS
Amino acid sequence of Papio anubis (Olive baboon) FGF2 (SEQ ID NO:131) (GenBank accession no. XP_003899210, which is hereby incorporated by reference in its entirety): 1 MAAGSITTLP ALPEDGGSGA FPPGHFKDPK RLYCKNGGFF LRIHPDGRVD GVREKSDPHI 61 KLQLQAEERG VVSIKGVCAN RYLAMKEDGR LLASKCVTDE CFFFERLESN NYNTYRSRKY 121 TSWYVALKRT GQYKLGSKTG PGQKAILFLP MSAKS
Amino acid sequence of Vicugna pacos (alpaca) FGF2 (SEQ ID NO:132) (Ensembl accession no. ENSVPAP00000009804, which is hereby incorporated by reference in its entirety): 111 MAAGSITTLP 121 ALPEDGGSGA FPPGHFKDPK RLYCKNGGFF LRIHPDGRVD GVREKSDPHI KLQLQAEERG 181 VVSIKGVCAN RYLAMKEDGR LLASKCVTDE CFFFERLESN NYNTYRSRKY SSWYVALKRT 241 GQYKLGPKTG PGQKAILFLP MSAKS
Amino acid sequence of Ovis aries (sheep) FGF2 (SEQ ID NO:133) (GenBank accession no. NP_001009769, which is hereby incorporated by reference in its entirety): 1 MAAGSITTLP ALPEDGGSSA FPPGHFKDPK RLYCKNGGFF LRIHPDGRVD GVREKSDPHI 61 KLQLQAEERG VVSIKGVCAN RYLAMKEDGR LLASKCVTDE CFFFERLESN NYNTYRSRKY 121 SSWYVALKRT GQYKLGPKTG PGQKAILFLP MSAKS
Amino acid sequence of Capreolus capreolus (Western roe deer) FGF2 (partial amino acid sequence corresponding to human FGF2 residues 42 to 149)(SEQ ID NO:134) (GenBank accession no. AAF73226, which is hereby incorporated by reference in its entirety): 1 RIHPDGRVDG VREKSDPHIK LQLQAEERGV VSIKGVCANR YLAMKEDGRL LASKCVTDEC 61 FFFERLESNN YNTYRSRKYS SWYVALKRTG QYKLGPKTGP GQKAILFL
Amino acid sequence of Loxodonta africana (elephant) FGF2 (partial amino acid sequence corresponding to human FGF2 residues 60 to 155) (SEQ ID NO:135) (Ensembl accession no. ENSLAFP00000008249, which is hereby incorporated by reference in its entirety): 1 VKLQLQAEER GVVSIKGVCA NRYLAMKEDG RLLASRCVTD ECFFFERLES NNYNTYRSRK 61 YTSWYVALKR TGQYKLGSKT GPGQKAILFL PMSAKS
Amino acid sequence of Sus scrofa (pig) FGF2 (partial amino acid sequence corresponding to human FGF2 residues 36 to 155) (SEQ ID NO:136) (GenBank accession no. CAE11791 and Ensembl accession no. ENSSSCP00000009695, which is hereby incorporated by reference in its entirety): 1 NGGFFLRIHP DGRVDGVREK SDPHIKLQLQ AEERGVVSIK GVCANRYLAM KEDGRLLASK 61 CVTDECFFFE RLESNNYNTY RSRKYSSWYV ALKRTGQYKL GPKTGPGQKA ILFLPMSAKS
Amino acid sequence of Ailuropoda melanoleuca (panda) FGF2 (partial amino acid sequence corresponding to human FGF2 residues 60 to 155)(SEQ ID NO:137) (Ensembl accession no. ENSAMEP00000018489, which is hereby incorporated by reference in its entirety): 1 VKLQLQAEER GVVSIKGVCA NRYLAMKEDG RLLASKCVTD ECFFFERLES NNYNTYRSRK 61 YSSWYVALKR TGQYKLGPKT GPGQKAILFL PMSAKS

Amino acid sequence of Choloepus hoffmanni (sloth) FGF2 (SEQ ID NO:138) (Ensembl accession no. ENSCHOP00000010051, which is hereby incorporated by reference in its entirety):

14 MAAGSIT
21 TLPALPEDGG SGALPPGHFK DPKRLYCKNG GFFLRIHPDG RVDGVREKSD PHIKLQLQAE
81 ERGVVSIKGV CANRYLAMKE DGRLQASKCV TDECFFFERL ESNNYNTYRS RKYSSWYVAL
141 KRTGQYKLGP KTGPGQKAIL FLPMSAKS

Amino acid sequence of Bubalus bubalis (water buffalo) FGF2 (SEQ ID NO:139) (GenBank accession no. AFH66795, which is hereby incorporated by reference in its entirety):

1 MAAGSITTLP PLPEDGGSGA FPPGHFKDPK RLYCKNGGFF LRIHPDGRVD GVREKSDPHI
61 KLQLQAEERG VVSIKGVCAN RYLAMKEDGR LLASKCVTDE CFFFERLESS NYNTYRSRKY
121 SSWYVALKRT GQYKLGPKTG PGQKAILFLP MSAKS

Amino acid sequence of Canis lupus familiaris (dog) FGF2 (SEQ ID NO:140) (GenBank accession no. XP_003432529, which is hereby incorporated by reference in its entirety):

40 M AAGSITTLPA LPEDGGSGAF
61 PPGHFKDPKR LYCKKGGFFL RIHPDGRVDG VREKSDPHVK LQLQAEERGV VSIKGVCANR
121 YLAMKEDGRL LASKCVTDEC FFFERLESNN YNTYRSRKYS SWYVALKRTG QYKLGPKTGP
181 GQKAILFLPM SAKS

Amino acid sequence of Rattus norvegicus (Norway rat) FGF2 (SEQ ID NO:141) (GenBank accession no. NP_062178, which is hereby incorporated by reference in its entirety):

1 MAAGSITSLP ALPEDGGGAF PPGHFKDPKR LYCKNGGFFL RIHPDGRVDG VREKSDPHVK
61 LQLQAEERGV VSIKGVCANR YLAMKEDGRL LASKCVTEEC FFFERLESNN YNTYRSRKYS
121 SWYVALKRTG QYKLGSKTGP GQKAILFLPM SAKS

Amino acid sequence of Heterocephalus glaber (naked mole-rat) FGF2 (partial amino acid sequence corresponding to human FGF2 residues 22 to 155) (SEQ ID NO:142) (GenBank accession no. EHB17407, which is hereby incorporated by reference in its entirety):

1 ppghfkdpkr lycknggffl rihpdgrvdg vreksdphvk lqlqaeergv vsikgvcanr
61 ylamkedgrl laskcvtdec ffferlesnn yntyrsrkys swyvalkrtg qyklgsktgp
121 gqkailflpm saks

Amino acid sequence of Otolemur garnettii (bushbaby) FGF2(SEQ ID NO:143) (Ensembl accession no. ENSOGAP00000021960, which is hereby incorporated by reference in its entirety):

52 MAAGSITTL
61 PSLPEDGGSD AFPPGHFKDP KRLYCKNGGF FLRIHPDGRV DGVREKSDPY IKLQLQAEER
121 GVVSIKGVCA NRYLAMKEDG RLLASKLITD ECFFFERLES NNYNTYRSRK YSSWYVALKR
181 TGQYKLGSKT GPGQKAILFL PMSAKS

Amino acid sequence of Mus musculus (house mouse) FGF2 (SEQ ID NO:144) (GenBank accession no. NP_032032, which is hereby incorporated by reference in its entirety):

1 MAASGITSLP ALPEDGGAAF PPGHFKDPKR LYCKNGGFFL RIHPDGRVDG VREKSDPHVK
61 LQLQAEERGV VSIKGVCANR YLAMKEDGRL LASKCVTEEC FFFERLESNN YNTYRSRKYS
121 SWYVALKRTG QYKLGSKTGP GQKAILFLPM SAKS

Amino acid sequence of Ictidomys tridecemlineatus (squirrel) FGF2 (partial amino acid sequence corresponding to human FGF2 residues 12 to 155) (SEQ ID NO:145) (Ensembl accession no. ENSSTOP00000015653, which is hereby incorporated by reference in its entirety):

1 LPEDGGGGAF PPGHFKDPKR LYCKNGGFFL RIHPDGRVDG VREKSDPHIK LQLQAEDRGV
61 VSIKGVCANR YLAMKEDGRL LASKCVTDEC FFFERLESNN YNTYRSRKYS SWYVALKRTG
121 QYKLGSKTGP GQKAILFLPM SAKS

Amino acid sequence of Felis catus (domestic cat) FGF2 (partial amino acid sequence corresponding to human FGF2 residues 25 to 130) (SEQ ID NO:146) (GenBank accession no. ABY47638, which is hereby incorporated by reference in its entirety): 1 HFKDPKRLYC KNGGFFLRIH PDGRVDGVRE KSDPHIKLQL QAEERGVVSI KGVCANRYLA 61 MKEDGRLLAS KCVTDECFFF ERLESNNYNT YRSRKYSSWY VALKRT
Amino acid sequence of Cavia porcellus (guinea pig) FGF2 (partial amino acid sequence corresponding to human FGF2 residues 60 to 155) (SEQ ID NO:147) (Ensembl accession no. ENSCPOP00000004847, which is hereby incorporated by reference in its entirety): 1 VKLQLQAEDR GVVSIKGVCA NRYLAMKEDG RLLASKCVTD ECFFFERLES NNYNTYRSRK 61 YSSWYVALKR TGQYKLGSKT GPGQKAILFL PMSAKS
Amino acid sequence of Sarcophilus harrisii (Tasmanian devil) FGF2 (SEQ ID NO:148) (Ensembl accession no. ENSSHAP00000012215, which is hereby incorporated by reference in its entirety): 48 MAA GSITTLPALA 61 GDGASGGAFP PGHFQDPKRL YCKNGGFFLR IHPDGHVDGI REKSDPHIKL QLQAEERGVV 121 SIKGVCANRY LAMKEDGRLL ALKCVTEECF FFERLESNNY NTYRSRKYSN WYVALKRTGQ 181 YKLGSKTGPG QKAILFLPMS AKS
Amino acid sequence of Monodelphis domestica (gray short-tailed opossum) FGF2 (SEQ ID NO:149) (GenBank accession no. NP_001029148, which is hereby incorporated by reference in its entirety): 1 MAAGSITTLP ALSGDGGGGG AFPPGHFKDP KRLYCKNGGF FLRIHPDGRV DGIREKSDPN 61 IKLQLQAEER GVVSIKGVCA NRYLAMKEDG RLLALKYVTE ECFFFERLES NNYNTYRSRK 121 YSNWYVALKR TGQYKLGSKT GPGQKAILFL PMSAKS
Amino acid sequence of Oryctolagus cuniculus (rabbit) FGF2 (SEQ ID NO:150) (GenBank accession no. XP_002717284, which is hereby incorporated by reference in its entirety): 1 MAAESITTLP ALPEDGGSGA FPPGHFKDPK RLYCKNGGFF LRIHPDGRVD GVREKSDPHI 61 KLQLQAEERG VVSIKGVCAN RYLAMKEDGR LLASKCVTDE CFFFERLESN NYNTYRSRKY 121 SSWYVALKRT GQYKLGSKTG PGQKAILFLP MSAKS
Amino acid sequence of Meleagris gallopavo (turkey) FGF2 (partial amino acid sequence corresponding to human FGF2 residues 31 to 155) (SEQ ID NO:151) (Ensembl accession no. ENSMGAP00000010977, which is hereby incorporated by reference in its entirety): 1 RLYCKNGGFF LRINPDGRVD GVREKSDPHI KLQLQAEERG VVSIKGVSAN RFLAMKEDGR 61 LLALKCATEE CFFFERLESN NYNTYRSRKY SDWYVALKRT GQYKPGPKTG PGQKAILFLP 121 MSAKS
Amino acid sequence of Gallus gallus (chicken) FGF2 (SEQ ID NO:152) (GenBank accession no. NP_990764 1 maagaagsit tlpalpddgg ggafppghfk dpkrlyckng gfflrinpdg rvdgvreksd 61 PHIKLQLQAE ERGVVSIKGV SANRFLAMKE DGRLLALKCA TEECFFFERL ESNNYNTYRS 121 RKYSDWYVAL KRTGQYKPGP KTGPGQKAIL FLPMSAKS
Amino acid sequence of Taeniopygia guttata (zebra finch) FGF2 (SEQ ID NO:153) (GenBank accession no. XP_002188397, which is hereby incorporated by reference in its entirety): 1 MAAAGGIATL PDDGGSGAFP PGHFKDPKRL YCKNGGFFLR INPDGKVDGV REKSDPHIKL 61 QLQAEERGVV SIKGVSANRF LAMKEDGRLL ALKYATEECF FFERLESNNY NTYRSRKYSD 121 WYVALKRTGQ YKPGPKTGPG QKAILFLPMS AKS

Amino acid sequence of Cynops pyrrhogaster (Japanese firebelly newt) FGF2 (SEQ ID NO:154) (GenBank accession no. BAB63249, which is hereby incorporated by reference in its entirety): 1 MAAGSITSLP ALPEDGNGGT FTPGGFKEPK RLYCKNGGFF LRINSDGKVD GAREKSDSYI 61 KLQLQAEERG VVSIKGVCAN RYLAMKDDGR LMALKWITDE CFFFERLESN NYNTYRSRKY 121 SDWYVALKRT GQYKNGSKTG AGQKAILFLP MSAKS
Amino acid sequence of Xenopus laevis (African clawed frog) FGF2 (SEQ ID NO:155) (GenBank accession no. NP_001093341, which is hereby incorporated by reference in its entirety): 1 MAAGSITTLP TESEDGGNTP FSPGSFKDPK RLYCKNGGFF LRINSDGRVD GSRDKSDSHI 61 KLQLQAVERG VVSIKGITAN RYLAMKEDGR LTSLRCITDE CFFFERLEAN NYNTYRSRKY 121 SSWYVALKRT GQYKNGSSTG PGQKAILFLP MSAKS
Amino acid sequence of Didelphis albiventris (white-eared opossum) FGF2 (SEQ ID NO:156) (GenBank accession no. ABL77404, which is hereby incorporated by reference in its entirety): 1 MAAGSITTLP ALSGDGGGGG AFPPGHFKDP KRLYCKNGGF FLRIHPDGRV DGIREKSDPN 61 IKLQLQAEER GVVSIKGVCA NRYLAMKEDG RLLALKYVTE ECFFFERLES NNYNTYRSRK 121 YSNWYVALKR TGQYKLGSKT GPGQKAILFS PCLLRC
Amino acid sequence of Myotis lucifugus (microbat) FGF2 (partial amino acid sequence corresponding to human FGF2 residues 60 to 155) (SEQ ID NO:157) (Ensembl accession no. ENSMLUP00000017859, which is hereby incorporated by reference in its entirety): 1 VKLQLQAEER GVVSIKGVCA NRYLAMKEDG RLQASKCVTD ECFFFERLES NNYNTYRSRK 61 YSSWYVALKR NGQYKLGPKT GPGQKAILFL PMSAKS
Amino acid sequence of Anolis carolinensis (anole lizard) FGF2 (partial amino acid sequence corresponding to human FGF2 residues 16 to 155) (SEQ ID NO:158) (Ensembl accession no. ENSACAP00000011657, which is hereby incorporated by reference in its entirety): 1 AAAASFPPGP FKDPKRLYCK NGGFFLRINP DGGVDGVREK SDPNIKLLLQ AEERGVVSIK 61 GVCANRFLAM NEDGRLLALK YVTDECFFFE RLESNNYNTY RSRKYRDWYI ALKRTGQYKL 121 GPKTGRGQKA ILFLPMSAKS
Amino acid sequence of Dasypus novemcinctus (armadillo) FGF2 (partial amino acid sequence corresponding to human FGF2 residues 1 to 94) (SEQ ID NO:159) (Ensembl accession no. ENSDNOP00000011351, which is hereby incorporated by reference in its entirety): 124 MAAGSIT TLPALPEDGG SGAFPPGHFK DPKRLYCKNG GFFLRIHPDG RVDGVREKSD 181 PNIKLQLQAE ERGVVSIKGV CANRYLAMRE DGRLQAS
Amino acid sequence of Tupaia belangeri (tree shrew) FGF2(SEQ ID NO:160) (Ensembl accession no. ENSTBEP00000000985, which is hereby incorporated by reference in its entirety): 1 AGVRAEREEA PGSGDSRGTD PAARSLIRRP DAAAREALLG ARSRVQGSST SWPASSRTGI 61 KLPDDSGQGM GGYPLDRPSR STGRGLGGAP DPAVKLQLQA EERGVVSIKG VCANRYLAMK 121 EDGRLLASKC VTDECFFFER LESNNYNTYR SRKYSSWYVA LKRTGQYKLG SKTGPGQKAI 181 LFLPMSAKS
Amino acid sequence of Xenopus silurana tropicalis (western clawed frog) FGF2 (SEQ ID NO:161) (GenBank accession no. NP_001017333, which is hereby incorporated by reference in its entirety): 1 MAAGSITTLP TESEDGNTPF PPGNFKDPKR LYCKNGGYFL RINSDGRVDG SRDKSDLHIK 61 LQLQAVERGV VSIKGITANR YLAMKEDGRL TSLKCITDEC FFYERLEANN YNTYRSRKNN 121 SWYVALKRTG QYKNGSTTGP GQKAILFLPM SAKS

Amino acid sequence of Latimeria chalumnae (coelacanth) FGF2 (SEQ ID NO:162) (Ensembl accession no. ENSLACP00000019200, which is hereby incorporated by reference in its entirety): 1 MAAGGITTLP AVPEDGGSST FPPGNFKEPK RLYCKNGGYF LRINPDGRVD GTREKNDPYI 61 KLQLQAESIG VVSIKGVCSN RYLAMNEDCR LFGLKYPTDE CFFHERLESN NYNTYRSKKY 121 SDWYVALKRT GQYKPGPKTG LGQKAILFLP MSAKS
Amino acid sequence of Tetraodon nigroviridis (spotted green pufferfish) FGF2 (SEQ ID NO:163) (GenBank accession no. CAG04681, which is hereby incorporated by reference in its entirety): 34 MATGGIT TLPSTPEDGG SSGFPPGSFK 61 DPKRLYCKNG GFFLRIKSDG VVDGIREKSD PHIKLQLQAT SVGEVVIKGV CANRYLAMNR 121 DGRLFGTKRA TDECHFLERL ESNNYNTYRS RKYPTMFVGL TRTGQYKSGS KTGPGQKAIL 181 FLPMSAKC
Amino acid sequence of Gasterosteus aculeatus (stickleback) FGF2 (SEQ ID NO:164) (Ensembl accession no. ENSGACP00000022078, which is hereby incorporated by reference in its entirety): 1 MATAGFATLP STPEDGGSGG FTPGGFKDPK RLYCKNGGFF LRIRSDGGVD GIREKSDAHI 61 KLQIQATSVG EVVIKGVCAN RYLAMNRDGR LFGVRRATDE CYFLERLESN NYNTYRSRKY 121 PGMYVALKRT GQYKSGSKTG PGQKAILFLP MSAKC
Amino acid sequence of Takifugu rubripes (fugu rubripes) FGF2 (SEQ ID NO:165) (GenBank accession no. CAD19830, which is hereby incorporated by reference in its entirety): 1 MATGGITTLP STPEDGGSGG FPPGSFKDPK RLYCKNGGFF LRIRSDGAVD GTREKTDPHI 61 KLQLQATSVG EVVIKGVCAN RYLAMNRDGR LFGMKRATDE CHFLERLESN NYNTYRSRKY 121 PNMFVGLTRT GNYKSGTKTG PCQKAILFLP MSAKY
Amino acid sequence of Oncorhynchus mykiss (rainbow trout) FGF2 (SEQ ID NO:166) (GenBank accession no. NP_001118008, which is hereby incorporated by reference in its entirety): 1 MATGEITTLP ATPEDGGSGG FLPGNFKEPK RLYCKNGGYF LRINSNGSVD GIRDKNDPHN 61 KLQLQATSVG EVVIKGVSAN RYLAMNADGR LFGPRRTTDE CYFMERLESN NYNTYRSRKY 121 PEMYVALKRT GQYKSGSKTG PGQKAILFLP MSARR
Amino acid sequence of Salmo salar (salmon) FGF2 (SEQ ID NO:167) (GenBank accession no. ACJ02099, which is hereby incorporated by reference in its entirety): 1 MATGEITTLP ATPEDGGSGG FPPGNFKDPK RLYCKNGGYF LRINSNGSVD GIREKNDPHK 61 QPQFVRAWTL QGVKRSTGML AHVDSNASHN CVKVAGCSLG EFGSMSNRPH NRRPRVATPA 121 QDLHIRLLHL RDRLKPATRT ADKTEEYFCL
Amino acid sequence of Danio rerio (zebrafish) FGF2 (SEQ ID NO:168) (GenBank accession no. AAP32155, which is hereby incorporated by reference in its entirety): 1 MATGGITTLP AAPDAENSSF PAGSFRDPKR LYCKNGGFFL RINADGRVDG ARDKSDPHIR 61 LQLQATAVGE VLIKGICTNR FLAMNADGRL FGTKRTTDEC YFLERLESNN YNTYRSRKYP 121 DWYVALKRTG QYKSGSKTSP GQKAILFLPM SAKC
Amino acid sequence of Oreochromis niloticus (Nile tilapia) FGF2 (SEQ ID NO:169) (GenBank accession no. XP_003443412, which is hereby incorporated by reference in its entirety): 1 MATGGITTLP ATPEDGGSSG FPPGNFKDPK RLYCKNGGFF LRIKSDGGVD GIREKNDPHI 61 KLQLQATSVG EVVIKGICAN RYLAMNRDGR LFGARRATDE CYFLERLESN NYNTYRSRKY 121 PNMYVALKRT GQYKSGSKTG PGQKAILFLP MSAKC

Amino acid sequence of Oryzias latipes (medaka) FGF2 (SEQ ID NO:170) (Ensembl accession no. ENSORLP00000025834, which is hereby incorporated by reference in its entirety):

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1   MATGEITTLP SPAENSRSDG FPPGNYKDPK RLYCKNGGLF LRIKPDGGVD GIREKKDPHV
61  KLRLQATSAG EVVIKGVCSN RYLAMHGDGR LFGVRQATEE CYFLERLESN NYNTYRSKKY
121 PNMYVALKRT GQYKPGNKTG PGQKAILFLP MSAKY
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[0066] As noted above, the portion of the paracrine FGF may be modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without the modification. In one embodiment, the modification of the paracrine FGF includes one or more substitutions, additions, or deletions.

[0067] In one embodiment, the modification is one or more substitutions located at one or more amino acid residues of SEQ ID NO: 121 selected from N36, K128, R129, K134, K138, Q143, K144, C78, C96, and combinations thereof. In one embodiment, the one or more substitutions are selected from N36T, K128D, R129Q, K134V, K138H, Q143M, K144T/L/I, C78S, C96S, and combinations thereof. In one embodiment, the modification is one or more substitutions which are located at one or more amino acid residues corresponding to residues of SEQ ID NO: 121 selected from N36, K128, R129, K134, K138, Q143, K144, C78, C96, and combinations thereof. In one embodiment, the modification is one or more substitutions which are located at one or more amino acid residues corresponding to residues of SEQ ID NO: 121 selected from N36, K128, R129, K134, K138, Q143, K144, C78, C96, and combinations thereof. Amino acid residues corresponding to those of SEQ ID NO: 121 may be determined by, for example, sequence analysis and structural analysis.

[0068] It will be understood that the portion of the paracrine FGF according to the present invention may be derived from a nucleotide sequence that encodes a paracrine FGF protein. For example, in one embodiment, nucleotide sequence is the nucleotide sequence that encodes human FGF2 (GenBank Accession No. NM_002006, which is hereby incorporated by reference in its entirety)(SEQ ID NO: 171), as follows:

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468                                                     ATG GCAGCCGGGA
481 GCATCACCAC GCTGCCCGCC TTGCCCGAGG ATGGCGGCAG CGGCGCCTTC CCGCCCGGCC
541 ACTTCAAGGA CCCCAAGCGG CTGTACTGCA AAAACGGGGG CTTCTTCCTG CGCATCCACC
601 CCGACGGCCG AGTTGACGGG GTCCGGGAGA AGAGCGACCC TCACATCAAG CTACAACTTC
661 AAGCAGAAGA GAGAGGAGTT GTGTCTATCA AAGGAGTGTG TGCTAACCGT TACCTGGCTA
721 TGAAGGAAGA TGGAAGATTA CTGGCTTCTA AATGTGTTAC GGATGAGTGT TTCTTTTTTG
781 AACGATTGGA ATCTAATAAC TACAATACTT ACCGGTCAAG GAAATACACC AGTTGGTATG
841 TGGCACTGAA ACGAACTGGG CAGTATAAAC TTGGATCCAA AACAGGACCT GGGCAGAAAG
901 CTATACTTTT TCTTCCAATG TCTGCTAAGA GCTGA
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[0069] In another embodiment of the present invention, the portion of the paracrine FGF of the chimeric protein may be derived from a nucleotide sequence that encodes an ortholog of human FGF2. Nucleotide sequences that encode FGF2 orthologs are shown in Table 4.

Table 4:

Gorilla FGF2 gene coding sequence (amino acids ("aa") 104-258) (SEQ ID NO:172) (Ensembl accession no. ENSGGOT00000004842, which is hereby incorporated by reference in its entirety): 310 ATGGCAGCC GGGAGCATCA CCACGCTGCC CGCCTTGCCC GAGGATGGCG 359 GCAGCGGCGC CTTCCCGCCC GGCCACTTCA AGGACCCCAA GCGGCTGTAC TGCAAAAACG 419 GGGGCTTCTT CCTGCGCATC CACCCCGACG GCCGAGTTGA CGGGGTCCGG GAGAAGAGCG 479 ACCCTCACAT CAAGCTACAA CTTCAAGCAG AAGAGAGAGG AGTTGTGTCT ATCAAAGGAG 539 TGTGTGCTAA CCGTTACCTT GCTATGAAGG AAGATGGAAG ATTACTGGCT TCTAAATGTG 599 TTACGGATGA GTGTTTCTTT TTTGAACGAT TGGAATCTAA TAACTACAAT ACTTACCGGT 659 CAAGGAAATA CACCAGTTGG TATGTGGCAC TGAAACGAAC TGGGCAGTAT AAACTTGGAT 719 CCAAAACAGG ACCTGGGCAG AAAGCTATAC TTTTTCTTCC AATGTCTGCT AAGAGCTGA
Sumatran orangutan FGF2 gene coding sequence (aa 168-322) (SEQ ID NO:173) (GenBank accession no. XM_002815126, which is hereby incorporated by reference in its entirety): 504 ATGGCAG CCGGGAGCAT CACCACGCTG CCCGCCTTGC 541 CCGAGGATGG CGGCAGCGGC GCCTTCCCGC CGGGCCACTT CAAGGACCCC AAGCGGCTGT 601 ACTGCAAAAA CGGGGGCTTC TTCCTGCGCA TCCACCCCGA CGGCCGAGTT GACGGGGTCC 661 GAGAGAAGAG CGACCCTCAC ATCAAACTAC AACTTCAAGC AGAAGAAAGA GGAGTTGTGT 721 CTATCAAAGG AGTGTGTGCT AACCGCTACC TTGCTATGAA GGAAGATGGA AGATTACTGG 781 CTTCTAAATG TGTTACGGAT GAGTGTTTCT TTTTTGAACG ATTGGAATCT AATAACTACA 841 ATACTTACCG GTCAAGGAAA TACACCAGTT GGTATGTGGC ACTGAAACGA ACTGGGCAGT 901 ATAAACTTGG ATCCAAAACA GGACCTGGGC AGAAAGCTAT ACTTTTTCTT CCAATGTCTG 961 CTAAGAGCTG A
Rhesus monkey FGF2 gene coding sequence (aa 83-237) (SEQ ID NO:174) (GenBank accession no. XM_001099284, which is hereby incorporated by reference in its entirety): 247 ATGG CAGCCGGGAG CATCACCACG CTGCCCGCCT TGCCCGAGGA TGGCGGCAGC 301 GGCGCCTTCC CGCCTGGCCA CTTCAAGGAC CCCAAGCGGC TGTACTGCAA AAACGGGGGC 361 TTCTTCCTGC GCATTCACCC CGACGGCCGA GTTGACGGGG TCCGGGAGAA GAGCGACCCT 421 CACATCAAAT TACAACTTCA AGCAGAAGAG AGAGGAGTTG TGTCTATCAA AGGAGTGTGT 481 GCTAACCGTT ACCTTGCTAT GAAGGAAGAT GGAAGATTAC TGGCTTCTAA ATGTGTTACA 541 GATGAGTGTT TCTTTTTTGA ACGATTGGAA TCTAATAACT ACAATACTTA CCGGTCAAGG 601 AAATACACCA GTTGGTATGT GGCACTGAAA CGAACTGGGC AATATAAACT TGGATCCAAA 661 ACAGGACCTG GGCAGAAAGC TATACTTTTT CTTCCAATGT CTGCTAAGAG CTGA
Chimpanzee FGF2 gene coding sequence (aa 134-288) (SEQ ID NO:175) (GenBank accession no. NM_001110241, which is hereby incorporated by reference in its entirety): 400 A TGGCAGCCGG GAGCATCACC 421 ACGCTGCCCG CCTTGCCCGA GGATGGCGGC AGCGGCGCCT TCCCGCCCGG CCACTTCAAG 481 GACCCCAAGC GGCTGTACTG CAAAAACGGG GGCTTCTTCC TGCGCATCCA CCCCGACGGC 541 CGAGTTGACG GGGTCCGGGA GAAGAGCGAC CCTCACATCA AGCTACAACT TCAAGCAGAA 601 GAGAGAGGAG TTGTGTCTAT CAAAGGAGTG TGTGCTAACC GTTACCTTGC TATGAAGGAA 661 GATGGAAGAT TACTGGCTTC TAAATGTGTT ACGGATGAGT GTTTCTTTTT TGAACGATTG 721 GAATCTAATA ACTACAATAC TTACCGGTCA AGGAAATACA CCAGTTGGTA TGTGGCACTG 781 AAACGAACTG GGCAGTATAA ACTTGGATCC AAAACAGGAC CTGGGCAGAA AGCTATACTT 841 TTTCTTCCAA TGTCTGCTAA GAGCTGA

Pygmy chimpanzee FGF2 gene coding sequence (112-266) (SEQ ID NO:176) (GenBank accession no. XM_003816433, which is hereby incorporated by reference in its entirety):

```
334                                     ATGGCAG CCGGGAGCAT CACCACGCTG
361 CCCGCCTTGC CCGAGGATGG CGGCAGCGGC GCCTTCCCGC CCGGCCACTT CAAGGACCCC
421 AAGCGGCTGT ACTGCAAAAA CGGGGGCTTC TTCCTGCGCA TCCACCCCGA CGGCCGAGTT
481 GACGGGGTCC GGGAGAAGAG CGACCCTCAC ATCAAGCTAC AACTTCAAGC AGAAGAGAGA
541 GGAGTTGTGT CTATCAAAGG AGTGTGTGCT AACCGTTACC TTGCTATGAA GGAAGATGGA
601 AGATTACTGG CTTCTAAATG TGTTACGGAT GAGTGTTTCT TTTTTGAACG ATTGGAATCT
661 AATAACTACA ATACTTACCG GTCAAGGAAA TACACCAGTT GGTATGTGGC ACTGAAACGA
721 ACTGGGCAGT ATAAACTTGG ATCCAAAACA GGACCTGGGC AGAAAGCTAT ACTTTTTCTT
781 CCAATGTCTG CTAAGAGCTG A
```

Bolivian squirrel monkey FGF2 gene coding sequence (1-155) (SEQ ID NO:177) (GenBank accession no. XM_003936241, which is hereby incorporated by reference in its entirety):

```
23                            ATGGCAGC CGGGAGCATC ACCACGCTGC CCGCCCTGCC
61  CGAAGACGGC GGCAGCGGCG CCTTCCCGCC CGGCCACTTC AAAGACCCCA AGCGGCTGTA
121 CTGCAAAAAC GGGGGCTTCT TCCTGCGAAT CCACCCCGAC GGCCGAGTGG ACGGGGTCCG
181 GGAGAAGAGC GACCCTCACA TCAAACTACA ACTTCAAGCA GAAGAGAGAG GAGTTGTATC
241 TATCAAAGGA GTGTGTGCTA ACCGTTACCT TGCTATGAAG GAAGATGGAA GATTACTGGC
301 TTCTAAATGT GTTACGGACG AGTGTTTCTT TTTTGAACGA TTGGAATCTA ATAACTACAA
361 TACTTACCGA TCAAGGAAAT ACACCAGTTG GTATGTGGCA CTGAAACGAA CTGGGCAGTA
421 TAAACTTGGA TCCAAAACAG GACCTGGGCA GAAAGCTATA CTTTTTCTTC CAATGTCTGC
481 TAAGAGCTGA
```

Northern white-cheeked gibbon FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:178) (GenBank accession no. XM_003271356, which is hereby incorporated by reference in its entirety):

```
435                                                       ATG GCAGCCGGGA
481 GCATCACCAC GCTGCCCGCC TTGCCGGAGG ATGGCGGCAG CGGCGCCTTC CCGCCCGGCC
541 ACTTCAAGGA CCCCAAGCGG CTGTACTGCA AAAACGGGGG TTTCTTCCTG CGCATCCACC
601 CCGACGGTCG AGTTGACGGG GTCCGGGAGA AGAGCGACCC TCACATCAAA CTACAACTTC
661 AAGCAGAAGA GAGAGGAGTT GTGTCTATCA AAGGAGTGTG TGCTAACCGT TACCTTGCTA
721 TGAAGGAAGA TGGAAGATTA CTGGCTTCTA AATGTGTTAC GGATGAGTGT TTCTTTTTTG
781 AACGATTGGA ATCTAATAAC TACAATACTT ACCGGTCAAG GAAATACACC AGTTGGTATG
841 TGGCACTGAA ACGAACTGGG CAGTATAAAC TTGGATCCAA AACAGGACCT GGGCAGAAAG
901 CTATACTTTT TCTTCCAATG TCTGCTAAGA GCTGA
```

Horse FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:179) (GenBank accession no. NM_001195221, which is hereby incorporated by reference in its entirety):

```
54                                                              ATGGCAG
61  CCGGGAGCAT CACCACGCTG CCCGCCCTGC CCGAGGACGG CGGCAGCGGC GCCTTCCCGC
121 CCGGCCACTT CAAGGACCCC AAGCGGCTCT ACTGCAAAAA CGGGGGCTTC TTCCTGCGCA
181 TCCACCCCGA CGGCCGAGTG GACGGGGTCC GGGAGAAGAG CGACCCTCAC ATCAAACTAC
241 AACTTCAAGC AGAAGAGAGA GGGGTTGTGT CTATCAAAGG AGTGTGTGCG AACCGTTATC
301 TTGCTATGAA GGAAGATGGA AGGTTACTGG CTTCTAAATG TGTTACGGAC GAGTGTTTCT
361 TTTTTGAACG ATTGGAATCT AATAACTACA ATACTTACCG GTCAAGGAAA TACTCCAGTT
421 GGTATGTGGC CCTGAAACGA ACGGGGCAGT ATAAACTTGG ACCCAAAACA GGACCTGGAC
481 AGAAAGCTAT ACTTTTTCTT CCAATGTCTG CTAAGAGCTG A
```

Cattle FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:180) (GenBank accession no. NM_174056, which is hereby incorporated by reference in its entirety):

```
104                                                    ATGGCCG CCGGGAGCAT
121 CACCACGCTG CCAGCCCTGC CGGAGGACGG CGGCAGCGGC GCTTTCCCGC CGGGCCACTT
181 CAAGGACCCC AAGCGGCTGT ACTGCAAGAA CGGGGGCTTC TTCCTGCGCA TCCACCCCGA
241 CGGCCGAGTG GACGGGGTCC GCGAGAAGAG CGACCCACAC ATCAAACTAC AACTTCAAGC
301 AGAAGAGAGA GGGGTTGTGT CTATCAAAGG AGTGTGTGCA AACCGTTACC TTGCTATGAA
361 AGAAGATGGA AGATTACTAG CTTCTAAATG TGTTACAGAC GAGTGTTTCT TTTTTGAACG
421 ATTGGAGTCT AATAACTACA ATACTTACCG GTCAAGGAAA TACTCCAGTT GGTATGTGGC
481 ACTGAAACGA ACTGGGCAGT ATAAACTTGG ACCCAAAACA GGACCTGGGC AGAAAGCTAT
541 ACTTTTTCTT CCAATGTCTG CTAAGAGCTG A
```

Olive baboon FGF2 gene coding sequence (1-155) (SEQ ID NO:181) (GenBank accession no. XM_003899161, which is hereby incorporated by reference in its entirety):

```
467                                                        ATGG CAGCCGGGAG
481 CATCACCACG CTGCCCGCCT TGCCCGAGGA TGGCGGCAGC GGCGCCTTCC CGCCCGGCCA
541 CTTCAAGGAC CCCAAGCGGC TGTACTGCAA AAACGGGGGC TTCTTCCTGC GCATTCACCC
601 CGACGGCCGA GTTGACGGGG TCCGGGAGAA GAGCGACCCT CACATCAAAT TACAACTTCA
661 AGCAGAAGAG AGAGGAGTTG TGTCTATCAA AGGAGTGTGT GCTAACCGTT ACCTTGCTAT
721 GAAGGAAGAT GGAAGATTAC TGGCTTCTAA ATGTGTTACG GATGAGTGTT TCTTTTTTGA
781 ACGATTGGAA TCTAATAACT ACAATACTTA CCGGTCAAGG AAATACACCA GTTGGTATGT
841 GGCACTGAAA CGAACTGGGC AGTATAAACT TGGATCCAAA ACAGGACCTG GGCAGAAAGC
901 TATACTTTTT CTTCCAATGT CTGCTAAGAG CTGA
```

Alpaca FGF2 gene coding sequence (aa 111-265) (SEQ ID NO:182) (Ensembl accession no. ENSVPAT00000010536, which is hereby incorporated by reference in its entirety):

```
341                                    ATGGCAGCTG GGAGCATCAC CACGCTGCCC
361 GCCCTGCCGG AGGACGGCGG CAGCGGCGCC TTCCCGCCCG GCCACTTCAA GGACCCCAAG
421 CGGTTGTACT GCAAAAACGG GGGCTTCTTC CTGCGCATCC ACCCCGACGG CCGAGTGGAC
481 GGGGTCCGGG AGAAGAGCGA CCCTCACATC AAACTACAAC TTCAAGCAGA AGAGAGAGGG
541 GTCGTGTCTA TCAAAGGAGT GTGTGCAAAC CGTTACCTTG CTATGAAGGA AGATGGAAGA
601 TTACTGGCTT CTAAATGTGT CACAGACGAG TGTTTCTTTT TTGAACGATT GGAATCTAAT
661 AACTACAATA CTTACCGGTC AAGGAAATAC TCCAGTTGGT ATGTGGCACT GAAACGAACT
721 GGGCAGTACA AACTTGGACC CAAAACAGGA CCTGGGCAGA AAGCTATACT TTTCCTTCCA
781 ATGTCTGCTA AGAGCTGA
```

Sheep FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:183) (GenBank accession no. NM_001009769, which is hereby incorporated by reference in its entirety):

```
1   ATGGCCGCCG GGAGCATCAC CACGCTGCCA GCCCTGCCGG AGGACGGCGG CAGCAGCGCT
61  TTCCCGCCCG GCCACTTTAA GGACCCCAAG CGGCTGTACT GCAAGAACGG GGGCTTCTTC
121 CTGCGCATCC ACCCCGACGG CCGAGTGGAC GGGGTCCGCG AGAAGAGCGA CCCTCACATC
181 AAACTACAAC TTCAAGCAGA AGAGAGAGGG GTTGTGTCTA TCAAAGGAGT GTGTGCAAAC
241 CGTTACCTTG CTATGAAAGA AGATGGAAGA TTACTAGCTT CTAAATGTGT TACAGACGAG
301 TGTTTCTTTT TTGAACGATT GGAGTCTAAT AACTACAATA CTTACCGGTC AAGGAAATAC
361 TCCAGTTGGT ATGTGGCACT GAAACGAACT GGGCAGTATA AACTTGGACC CAAAACAGGA
421 CCTGGGCAGA AAGCTATACT TTTTCTTCCA ATGTCTGCTA AGAGCTGA
```

Western roe deer FGF2 gene coding sequence (1-108; partial amino acid sequence corresponding to human FGF2 residues 42 to 149) (SEQ ID NO:184) (GenBank accession no. AF152587, which is hereby incorporated by reference in its entirety):

```
1   GCGCATCCAC CCCGACGGCC GAGTGGACGG GGTCCGCGAG AAGAGTGACC CTCACATCAA
61  ACTACAACTT CAAGCAGAAG AGAGAGGGGT TGTGTCTATC AAAGGAGTGT GTGCGAACCG
121 TTATCTTGCT ATGAAAGAAG ACGGAAGATT ATTGGCTTCA AAATGTGTTA CAGACGAATG
181 TTTCTTTTTT GAACGATTGG AGTCTAATAA CTACAATACT TACCGGTCAA GGAAATACTC
241 CAGTTGGTAT GTGGCACTGA AACGAACTGG GCAGTATAAA CTTGGACCCA AAACAGGACC
301 TGGGCAGAAA GCTATACTTT TTCTT
```

Elephant FGF2 gene coding sequence (1-96; partial amino acid sequence corresponding to human FGF2 residues 60 to 155) (SEQ ID NO:185) (Ensembl accession no. ENSLAFT00000008249, which is hereby incorporated by reference in its entirety):

```
1   GTTAAACTAC AGCTTCAAGC AGAAGAGAGA GGTGTTGTGT CTATCAAAGG AGTGTGTGCC
61  AACCGTTATC TGGCTATGAA GGAAGATGGA AGATTGCTGG CTTCTAGATG TGTGACAGAT
121 GAATGTTTCT TCTTTGAACG ACTGGAATCT AATAACTACA ATACTTACCG GTCAAGGAAA
181 TACACCAGTT GGTATGTGGC ACTGAAACGA ACGGGGCAGT ATAAACTTGG ATCCAAAACA
241 GGACCTGGAC AGAAAGCTAT ACTTTTTCTT CCCATGTCTG CTAAGAGC
```

Pig FGF2 gene coding sequence (1-120; partial amino acid sequence corresponding to human FGF2 residues 36 to 155) (SEQ ID NO:186) (GenBank accession no. AJ577089 and Ensembl accession no. ENSSSCT00000009952, which is hereby incorporated by reference in its entirety):

```
1   GAACGGGGGC TTCTTCCTGC GCATCCACCC CGACGGCCGA GTGGATGGGG TCCGGGAGAA
61  GAGCGACCCT CACATCAAAC TACAACTTCA AGCAGAAGAG AGAGGGGTTG TGTCTATCAA
121 AGGAGTGTGT GCAAACCGTT ATCTTGCTAT GAAGGAAGAT GGAAGATTAC TGGCTTCTAA
181 ATGTGTTACA GACGAGTGTT TCTTTTTTGA ACGACTGGAA TCTAATAACT ACAATACTTA
241 CCGGTCGAGG AAATACTCCA GTTGGTATGT GGCACTGAAA CGAACTGGGC AGTATAAACT
301 TGGACCCAAA ACAGGACCTG GGCAGAAAGC TATACTTTTT CTTCCAATGT CTGCTAAGAG
361 C
```

Panda FGF2 gene coding sequence (1-96; partial amino acid sequence corresponding to human FGF2 residues 60 to 155) (SEQ ID NO:187) (Ensembl accession no. ENSAMET00000019232, which is hereby incorporated by reference in its entirety):

```
1   GTCAAACTGC AACTTCAAGC GGAAGAGAGA GGGGTTGTAT CCATCAAAGG AGTATGTGCA
61  AATCGCTATC TTGCCATGAA GGAAGATGGA AGATTACTGG CTTCTAAATG TGTTACCGAT
121 GAGTGTTTCT TTTTTGAGCG ACTGGAATCT AATAACTACA ATACTTACCG GTCAAGGAAA
181 TACTCCAGTT GGTATGTGGC ACTGAAACGA ACTGGGCAGT ATAAACTTGG ACCCAAAACA
241 GGACCTGGGC AGAAAGCTAT ACTTTTTCTT CCAATGTCTG CTAAGAGC
```

Sloth FGF2 gene coding sequence (aa 14-168) (SEQ ID NO:188) (Ensembl accession no. ENSCHOT00000011394, which is hereby incorporated by reference in its entirety):

```
40                                          A TGGCAGCCGG GAGCATCACC
61  ACGCTGCCCG CCCTGCCCGA GGACGGAGGC AGCGGCGCCT TACCGCCCGG CCACTTCAAA
121 GATCCCAAGC GGCTCTACTG CAAAAACGGG GGCTTCTTCC TGCGTATCCA TCCCGACGGC
181 AGAGTGGACG GGGTCCGGGA GAAGAGCGAC CCCCACATCA AACTACAACT TCAAGCAGAA
241 GAGAGAGGGG TTGTGTCTAT CAAAGGTGTG TGTGCAAACC GATATCTTGC TATGAAGGAA
301 GATGGAAGAT TACAGGCTTC TAAATGTGTA ACGGACGAGT GTTTCTTTTT TGAACGATTG
361 GAATCTAATA ACTACAATAC GTACCGATCA AGGAAATACT CCAGTTGGTA TGTGGCACTG
421 AAACGAACTG GGCAATATAA ACTTGGACCC AAAACAGGAC CTGGGCAGAA AGCCATACTT
481 TTTCTTCCAA TGTCTGCTAA GAGCTGA
```

Water buffalo FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:189) (GenBank accession no. JQ326277, which is hereby incorporated by reference in its entirety):

```
1   ATGGCCGCCG GGAGCATCAC CACGCTGCCA CCCCTGCCGG AGGACGGCGG CAGCGGCGCT
61  TTCCCGCCCG GCCACTTCAA GGACCCCAAG CGGCTGTACT GCAAGAACGG GGGCTTCTTC
121 CTGCGCATCC ACCCCGACGG CCGAGTGGAC GGGGTCCGCG AGAAGAGCGA CCCACACATC
181 AAACTACAAC TTCAAGCAGA AGAGAGAGGG GTTGTGTCTA TCAAAGGAGT GTGTGCAAAC
241 CGTTACCTTG CTATGAAAGA AGATGGAAGA TTACTAGCTT CCAAATGTGT TACAGACGAG
301 TGTTTCTTTT TTGAACGATT GGAGTCTAGT AACTACAATA CTTACCGGTC AAGGAAATAC
361 TCCAGTTGGT ATGTGGCACT GAAACGAACT GGGCAGTATA AACTTGGACC CAAAACAGGA
421 CCTGGGCAGA AAGCTATACT TTTTCTTCCA ATGTCTGCTA AGAGCTGA
```

Dog FGF2 gene coding sequence (aa 40-194) (SEQ ID NO:190) (GenBank accession no. XM_003432481, which is hereby incorporated by reference in its entirety):

```
118                                                             ATG
121 GCAGCCGGGA GCATCACCAC GCTGCCCGCC CTGCCGGAGG ACGGCGGCAG CGGCGCCTTC
181 CCGCCCGGCC ACTTCAAGGA CCCCAAGAGG CTGTACTGCA AAAAAGGGGG CTTCTTCCTG
241 CGGATCCACC CCGACGGCCG GGTGGACGGG GTCCGGGAGA AGAGCGATCC CCACGTCAAA
301 TTGCAACTTC AAGCAGAAGA GAGAGGCGTT GTGTCCATCA AAGGAGTATG TGCAAATCGC
361 TATCTTGCTA TGAAGGAAGA TGGAAGATTA CTGGCTTCTA AATGTGTTAC TGACGAGTGC
421 TTCTTTTTTG AACGATTGGA ATCTAATAAC TACAATACTT ACCGGTCAAG GAAATACTCC
481 AGTTGGTATG TGGCACTGAA ACGAACTGGG CAGTATAAAC TTGGACCAAA AACAGGACCT
541 GGGCAGAAAG CTATACTTTT TCTTCCAATG TCTGCTAAGA GCTGA
```

Norway rat FGF2 gene coding sequence (aa 1-154) (SEQ ID NO:191) (GenBank accession no. NM_019305, which is hereby incorporated by reference in its entirety):

```
533                                                         ATGGCTGC
541 CGGCAGCATC ACTTCGCTTC CCGCACTGCC GGAGGACGGC GGCGGCGCCT TCCCACCCGG
601 CCACTTCAAG GATCCCAAGC GGCTCTACTG CAAGAACGGC GGCTTCTTCC TGCGCATCCA
661 TCCAGACGGC CGCGTGGACG GCGTCCGGGA GAAGAGCGAC CCACACGTCA AACTACAGCT
721 CCAAGCAGAA GAGAGAGGAG TTGTGTCCAT CAAGGGAGTG TGTGCGAACC GGTACCTGGC
781 TATGAAGGAA GATGGACGGC TGCTGGCTTC TAAGTGTGTT ACAGAAGAGT GTTTCTTCTT
841 TGAACGCCTG GAGTCCAATA ACTACAACAC TTACCGGTCA CGGAAATACT CCAGTTGGTA
901 TGTGGCACTG AAACGAACTG GGCAGTATAA ACTCGGATCC AAAACGGGGC CTGGACAGAA
961 GGCCATACTG TTTCTTCCAA TGTCTGCTAA GAGCTGA
```

Naked mole-rat FGF2 gene coding sequence (1-134; partial amino acid sequence corresponding to human FGF2 residues 22 to 155) (SEQ ID NO:192) (GenBank accession no. JH173674 , which is hereby incorporated by reference in its entirety):

```
378500                     C CACCCGGCCA CTTCAAGGAC CCAAAGCGGC
378531 TGTACTGCAA AAACGGGGGC TTCTTCCTGC GCATCCACCC CGACGGCCGC
378581 GTGGACGGGG TCCGGGAGAA GAGCGACCCT CACG
418784    TCAAACT ACAACTTCAA GCAGAAGAGA GAGGAGTTGT GTCTATTAAG
418831 GGAGTGTGTG CGAACCGTTA CCTTGCTATG AAGGAAGATG GAAGATTACT
418881 GGCTTCT
433983   AAATGTGT TACAGATGAG TGTTTCTTTT TTGAACGATT GGAATCTAAT
434031 AACTACAATA CTTATCGGTC AAGGAAATAC TCCAGTTGGT ATGTGGCACT
434081 GAAACGAACT GGACAATATA AACTTGGATC CAAAACAGGA CCGGGGCAGA
434131 AAGCTATACT TTTTCTTCCA ATGTCTGCTA AGAGCTGA
```

Bushbaby FGF2 gene coding sequence (aa 52-206) (SEQ ID NO:193) (Ensembl accession no. ENSOGAT00000025228, which is hereby incorporated by reference in its entirety):

```
154                                    ATGGCAG CCGGGAGCAT CACCACGCTG
181 CCCTCCCTGC CCGAGGACGG CGGCAGCGAC GCCTTTCCGC CCGGCCACTT CAAGGACCCC
241 AAGCGACTGT ACTGCAAAAA CGGGGGCTTC TTCCTGCGCA TCCACCCCGA CGGCCGAGTG
301 GACGGGGTCC GGGAGAAGAG CGACCCTTAC ATCAAACTAC AACTTCAAGC AGAAGAGAGA
361 GGAGTTGTGT CTATCAAAGG AGTGTGTGCG AACCGTTACC TTGCTATGAA GGAAGACGGA
421 AGATTGCTGG CTTCTAAATT GATTACAGAC GAGTGCTTCT TTTTTGAACG ACTGGAATCT
481 AATAACTACA ATACTTACCG GTCAAGAAAA TACTCCAGTT GGTATGTGGC ACTGAAACGA
541 ACTGGACAGT ATAAACTTGG ATCCAAAACA GGACCTGGGC AGAAAGCTAT ACTTTTTCTT
601 CCAATGTCTG CTAAGAGCTG A
```

House mouse FGF2 gene coding sequence (aa 1-154) (SEQ ID NO:194) (GenBank accession no. NM_008006, which is hereby incorporated by reference in its entirety):

```
198                    ATG GCTGCCAGCG GCATCACCTC GCTTCCCGCA CTGCCGGAGG
241 ACGGCGGCGC CGCCTTCCCA CCAGGCCACT TCAAGGACCC CAAGCGGCTC TACTGCAAGA
301 ACGGCGGCTT CTTCCTGCGC ATCCATCCCG ACGGCCGCGT GGATGGCGTC CGCGAGAAGA
361 GCGACCCACA CGTCAAACTA CAACTCCAAG CAGAAGAGAG AGGAGTTGTG TCTATCAAGG
421 GAGTGTGTGC CAACCGGTAC CTTGCTATGA AGGAAGATGG ACGGCTGCTG GCTTCTAAGT
481 GTGTTACAGA AGAGTGTTTC TTCTTTGAAC GACTGGAATC TAATAACTAC AATACTTACC
541 GGTCACGGAA ATACTCCAGT TGGTATGTGG CACTGAAACG AACTGGGCAG TATAAACTCG
601 GATCCAAAAC GGGACCTGGA CAGAAGGCCA TACTGTTTCT TCCAATGTCT GCTAAGAGCT
661 GA
```

Squirrel FGF2 gene coding sequence (1-144; partial amino acid sequence corresponding to human FGF2 residues 12 to 155) (SEQ ID NO:195) (Ensembl accession no. ENSSTOT00000022105 , which is hereby incorporated by reference in its entirety):

```
1   CTGCCCGAGG ACGGCGGCGG CGGCGCCTTC CCGCCCGGCC ACTTTAAGGA CCCCAAGCGG
61  CTCTACTGCA AAAACGGAGG CTTCTTCCTG CGCATCCACC CCGACGGCCG AGTGGACGGG
121 GTCCGGGAGA AGAGCGACCC CCACATCAAG CTCCAGCTTC AAGCCGAAGA CCGAGGGGTT
181 GTGTCCATCA AGGGAGTGTG TGCAAACCGA TACCTGGCCA TGAAGGAGGA CGGGAGGCTC
241 CTGGCTTCTA AATGTGTTAC GGACGAGTGT TTCTTTTTTG AACGACTGGA ATCAAATAAC
301 TACAATACTT ACCGGTCAAG GAAATACTCC AGTTGGTATG TGGCCCTGAA ACGAACAGGG
361 CAGTATAAAC TTGGATCCAA AACAGGACCT GGGCAGAAAG CTATACTTTT TCTTCCAATG
421 TCTGCTAAGA GC
```

Domestic cat FGF2 gene coding sequence (1-106; partial amino acid sequence corresponding to human FGF2 residues 25 to 130) (SEQ ID NO:196) (GenBank accession no. EU314952 , which is hereby incorporated by reference in its entirety):

```
1   CCACTTCAAG GACCCCAAGC GTCTGTACTG CAAAAACGGG GGCTTCTTCC TGCGCATCCA
61  CCCCGACGGC CGAGTGGATG GGGTCCGGGA GAAGAGCGAC CCTCACATCA AACTGCAACT
121 TCAGGCAGAA GAGAGAGGGG TTGTGTCCAT CAAAGGAGTC TGTGCAAACC GCTATCTTGC
181 CATGAAGGAA GATGGAAGAT TACTGGCTTC TAAATGTGTT ACGGACGAGT GTTTCTTTTT
241 TGAACGATTG GAATCTAATA ACTACAATAC TTATCGGTCA AGGAAATACT CCAGCTGGTA
301 TGTGGCACTG AAACGAAC
```

Guinea pig FGF2 gene coding sequence (1-96; partial amino acid sequence corresponding to human FGF2 residues 60 to 155) (SEQ ID NO:197) (Ensembl accession no. ENSCPOT00000005443 , which is hereby incorporated by reference in its entirety):

1 GTTAAACTAC AACTTCAAGC CGAAGACAGA GGAGTTGTGT CTATCAAGGG AGTCTGTGCG
61 AACCGTTACC TTGCTATGAA GGAAGACGGA AGATTATTGG CTTCCAAATG TGTTACAGAT
121 GAATGTTTCT TTTTTGAACG ACTGGAATCT AATAACTACA ACACTTACCG GTCAAGGAAA
181 TACTCCAGTT GGTATGTGGC ACTGAAACGA ACTGGACAAT ATAAACTTGG GTCCAAAACA
241 GGACCAGGGC AGAAAGCCAT ACTTTTTCTT CCAATGTCTG CGAAGAGC

Tasmanian devil FGF2 gene coding sequence (aa 48-203) (SEQ ID NO:198) (Ensembl accession no. ENSSHAP00000012215, which is hereby incorporated by reference in its entirety):

142 ATGGCCGCG GGCAGCATCA CCACGTTGCC GGCCCTGGCC
181 GGGGATGGAG CCAGCGGGGG CGCCTTTCCC CCGGGCCACT TCCAGGACCC CAAGCGGCTG
241 TACTGCAAGA ACGGAGGCTT CTTCTTGCGC ATCCATCCCG ACGGTCACGT GGACGGCATC
301 CGCGAGAAGA GCGATCCGCA CATTAAACTT CAGCTTCAGG CAGAAGAGAG AGGAGTAGTG
361 TCTATTAAAG GAGTTTGTGC CAACCGCTAT CTTGCCATGA AAGAGGATGG CAGATTACTG
421 GCTCTGAAAT GTGTGACTGA AGAGTGTTTC TTCTTTGAAC GTCTAGAGTC CAACAATTAC
481 AACACTTATC GCTCAAGGAA ATACTCCAAT TGGTATGTGG CATTGAAACG CACAGGCCAG
541 TATAAGCTTG GATCCAAGAC TGGACCAGGG CAGAAAGCCA TCCTTTTCCT TCCCATGTCT
601 GCTAAGAGCT GA

Gray short-tailed opossum FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:199) (GenBank accession no. NM_001033976, which is hereby incorporated by reference in its entirety):

29 AT GGCCGCAGGC AGCATCACCA CGCTGCCAGC
61 CCTGTCCGGG GACGGAGGCG GCGGGGGCGC CTTTCCCCCG GGCCACTTCA AGGACCCCAA
121 GCGGCTGTAC TGCAAGAACG GAGGCTTCTT CCTGCGCATC CACCCCGACG GCCGTGTGGA
181 CGGCATCCGC GAGAAGAGCG ACCCGAACAT TAAACTACAA CTTCAGGCAG AAGAGAGAGG
241 AGTGGTGTCT ATTAAAGGAG TATGTGCCAA TCGCTATCTT GCCATGAAGG AAGATGGAAG
301 ATTATTGGCT TTGAAATATG TGACCGAAGA GTGTTTCTTT TTCGAACGCT TGGAGTCCAA
361 CAACTACAAC ACTTATCGCT CGAGGAAATA TTCCAATTGG TACGTGGCAC TGAAACGAAC
421 GGGGCAGTAC AAGCTTGGAT CCAAGACTGG CCCGGGGCAG AAAGCCATCC TTTTCCTCCC
481 CATGTCTGCT AAGAGCTGA

Rabbit FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:200) (GenBank accession no. XM_002717238, which is hereby incorporated by reference in its entirety):

1 ATGGCAGCCG AGAGCATCAC CACGCTGCCC GCCCTGCCGG AGGATGGAGG CAGCGGCGCC
61 TTCCCGCCCG GCCACTTCAA GGACCCCAAG CGGCTGTACT GCAAAAACGG GGGTTTCTTC
121 CTGCGTATCC ACCCCGACGG CCGCGTGGAC GGGGTCCGGG AGAAGAGCGA CCCACACATC
181 AAATTACAAC TTCAAGCAGA AGAGAGAGGA GTTGTATCCA TCAAAGGTGT GTGTGCAAAC
241 CGTTACCTTG CTATGAAGGA AGATGGAAGA CTGCTGGCTT CTAAATGTGT TACAGACGAG
301 TGCTTCTTTT TTGAACGACT GGAGTCTAAT AACTACAATA CTTACCGGTC AAGGAAATAT
361 TCCAGCTGGT ATGTGGCACT GAAACGAACT GGGCAGTATA AACTTGGATC CAAAACAGGA
421 CCTGGGCAGA AGGCTATACT TTTTCTTCCA ATGTCTGCTA AGAGCTGA

Turkey FGF2 gene coding sequence (1-125; partial amino acid sequence corresponding to human FGF2 residues 31 to 155) (SEQ ID NO:201) (Ensembl accession no. ENSMGAT00000011845 , which is hereby incorporated by reference in its entirety):

```
1   CGGCTCTACT GTAAGAACGG CGGCTTCTTC CTGCGCATCA ATCCCGACGG CAGAGTGGAC
61  GGCGTCCGCG AGAAGAGCGA TCCGCACATC AAACTGCAGC TTCAGGCAGA AGAAAGAGGA
121 GTGGTATCAA TCAAAGGTGT AAGTGCAAAC CGCTTTCTGG CTATGAAGGA GGATGGCAGA
181 TTGCTGGCAC TGAAATGTGC AACAGAAGAA TGTTTCTTTT TTGAGCGTTT GGAATCTAAT
241 AATTATAACA CTTACCGGTC ACGGAAGTAC TCTGATTGGT ATGTGGCACT GAAAAGAACT
301 GGACAGTACA AGCCCGGACC AAAAACTGGA CCTGGACAGA AAGCTATCCT TTTTCTTCCA
361 ATGTCTGCTA AAAGC
```

Gallus gallus FGF2 gene coding sequence (aa 1-158) (SEQ ID NO:202) (GenBank accession no. NM_205433, which is hereby incorporated by reference in its entirety):

```
98                                          ATG GCGGCGGGGG CGGCGGGGAG
121 CATCACCACG CTGCCGGCGC TGCCCGACGA CGGGGGCGGC GGCGCTTTTC CCCCCGGGCA
181 CTTCAAGGAC CCCAAGCGGC TCTACTGCAA GAACGGCGGC TTCTTCCTGC GCATCAACCC
241 CGACGGCAGG GTGGACGGCG TCCGCGAGAA GAGCGATCCG CACATCAAAC TGCAGCTTCA
301 AGCAGAAGAA AGAGGAGTAG TATCAATCAA AGGCGTAAGT GCAAACCGCT TTCTGGCTAT
361 GAAGGAGGAT GGCAGATTGC TGGCACTGAA ATGTGCAACA GAGGAATGTT TCTTTTTCGA
421 GCGCTTGGAA TCTAATAACT ATAACACTTA CCGGTCACGG AAGTACTCTG ATTGGTATGT
481 GGCACTGAAA AGGACTGGAC AGTACAAGCC CGGACCAAAA ACTGGACCTG GACAGAAAGC
541 TATCCTTTTT CTTCCAATGT CTGCTAAAAG CTGA
```

Zebra finch FGF2 gene coding sequence (aa 1-153) (SEQ ID NO:203) (GenBank accession no. XM_002188361, which is hereby incorporated by reference in its entirety):

```
1   ATGGCGGCGG CGGGGGGCAT CGCTACGCTG CCCGACGACG GCGGCAGCGG CGCCTTTCCC
61  CCGGGGCACT TCAAGGACCC CAAGCGCCTG TACTGCAAGA ACGGCGGCTT CTTCCTGCGC
121 ATCAACCCCG ACGGGAAGGT GGACGGCGTC CGCGAGAAGA GCGACCCGCA CATCAAGCTG
181 CAGCTTCAGG CGGAGGAACG AGGAGTGGTG TCCATCAAAG GTGTCAGTGC CAATCGCTTC
241 CTGGCCATGA AAGAGGATGG CAGATTGCTG GCCTTGAAAT ATGCAACAGA AGAATGTTTC
301 TTTTTTGAAC GTTTGGAATC CAATAACTAT AACACTTACC GGTCACGGAA ATACTCGGAT
361 TGGTATGTGG CACTGAAAAG AACTGGACAG TACAAACCTG GACCAAAAAC TGGACCTGGA
421 CAGAAAGCTA TCCTTTTCCT TCCTATGTCT GCTAAAAGCT GA
```

Japanese firebelly newt FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:204) (GenBank accession no. AB064664, which is hereby incorporated by reference in its entirety):

```
384                           ATGGCTG CTGGGAGCAT CACCAGTCTC CCTGCCCTAC
421 CCGAGGACGG GAATGGCGGC ACCTTCACAC CCGGCGGATT CAAAGAGCCG AAGAGGCTGT
481 ACTGCAAGAA CGGGGGCTTC TTTCTCCGGA TCAACTCCGA CGGCAAGGTG GACGGAGCCC
541 GGGAGAAGAG CGACTCCTAC ATTAAACTGC AGCTTCAAGC AGAAGAGCGC GGTGTGGTGT
601 CCATCAAGGG AGTATGTGCA AACCGCTATC TCGCTATGAA GGATGATGGC AGGCTGATGG
661 CGCTGAAATG GATAACCGAT GAATGCTTCT TTTTCGAGCG ACTGGAGTCC AACAACTATA
721 ACACGTATCG ATCACGGAAA TATTCCGATT GGTATGTGGC GCTGAAAAGA ACTGGGCAAT
781 ACAAAAATGG ATCAAAAACC GGAGCAGGAC AGAAAGCAAT CCTTTTTCTA CCCATGTCGG
841 CCAAGAGTTG A
```

African clawed frog FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:205) (GenBank accession no. NM_001099871, which is hereby incorporated by reference in its entirety):

```
335                                     ATGGCG GCAGGGAGCA TCACAACTCT
361 GCCAACTGAA TCCGAGGATG GGGGAAACAC TCCTTTTTCA CCAGGGAGTT TTAAAGACCC
421 CAAGAGGCTC TACTGCAAGA ACGGGGGCTT CTTCCTCAGG ATAAACTCAG ACGGGAGAGT
481 GGACGGGTCA AGGGACAAAA GTGACTCGCA CATAAAATTA CAGCTACAAG CTGTAGAGCG
541 GGGAGTGGTA TCAATAAAGG GAATCACTGC AAATCGCTAC CTTGCCATGA AGGAAGATGG
601 GAGATTAACA TCGCTGAGGT GTATAACAGA TGAATGCTTC TTTTTTGAAC GACTGGAAGC
661 TAATAACTAC AACACTTACC GGTCTCGGAA ATACAGCAGC TGGTATGTGG CACTAAAGCG
721 AACCGGGCAG TACAAAAATG GATCGAGCAC TGGACCGGGA CAAAAAGCTA TTTTATTTCT
781 CCCAATGTCC GCAAAGAGCT GA
```

White-eared opossum FGF2 gene coding sequence (aa 1-156) (SEQ ID NO:206) (GenBank accession no. EF057322, which is hereby incorporated by reference in its entirety):

```
1   ATGGCAGCAG GCAGCATCAC CACATTGCCG GCCCTGTCCG GGGACGGAGG CGGCGGGGGA
61  GCCTTTCCTC CAGGCCACTT CAAGGACCCC AAGCGGCTGT ACTGCAAGAA CGGAGGCTTC
121 TTCCTGCGCA TCCACCCCGA CGGCCGCGTG GACGGCATCC GCGAGAAGAG CGACCCGAAC
181 ATTAAACTAC AACTTCAGGC AGAAGAGAGA GGAGTAGTGT CTATTAAAGG AGTATGTGCC
241 AACCGATATC TTGCCATGAA GGAGGATGGC AGATTATTGG CTTTGAAATA TGTGACCGAA
301 GAGTGTTTCT TTTTTGAACG TTTGGAGTCC AACAACTACA ACACTTATCG CTCAAGAAAA
361 TATTCCAATT GGTATGTGGC ACTGAAACGA ACGGGGCAGT ATAAGCTTGG ATCCAAGACT
421 GGCCCGGGGC AGAAAGCCAT CCTTTTCTCC CCATGTCTGC TAAGATGCTG A
```

Microbat FGF2 gene coding sequence (1-96; partial amino acid sequence corresponding to human FGF2 residues 60 to 155) (SEQ ID NO:207) (Ensembl accession no. ENSMLUT00000027717 , which is hereby incorporated by reference in its entirety):

```
1   GTCAAACTCC AACTTCAAGC AGAAGAGAGA GGGGTCGTGT CTATCAAAGG AGTGTGTGCC
61  AACCGCTATC TCGCTATGAA GGAGGACGGC CGGTTACAGG CTTCTAAATG TGTTACGGAT
121 GAGTGTTTCT TTTTTGAACG GTTGGAATCC AATAACTACA ACACTTACCG GTCAAGAAAG
181 TACTCCAGTT GGTATGTGGC ATTGAAGCGG AATGGGCAGT ATAAACTTGG ACCCAAAACA
241 GGACCTGGCC AGAAAGCCAT ACTTTTTCTT CCCATGTCTG CTAAGAGC
```

Anole lizard FGF2 gene coding sequence (1-140; partial amino acid sequence corresponding to human FGF2 residues 16 to 155) (SEQ ID NO:208) (Ensembl accession no. ENSACAT00000011897, which is hereby incorporated by reference in its entirety):

```
1   GCGGCGGCGG CCTCTTTCCC CCCGGGCCCC TTCAAGGACC CCAAGCGCCT CTACTGCAAG
61  AACGGGGGCT TCTTCCTGCG GATCAACCCC GACGGCGGCG TGGACGGCGT CCGAGAGAAG
121 AGCGACCCCA ACATCAAATT GCTGCTCCAG GCAGAGGAGA GAGGTGTAGT GTCCATCAAA
181 GGTGTATGCG CAAACCGTTT CCTGGCTATG AATGAAGACG GTCGATTGTT AGCACTGAAA
241 TACGTAACAG ATGAATGCTT CTTTTTTGAA CGCTTGGAAT CTAATAATTA CAATACTTAT
301 CGGTCTCGTA AATACCGTGA TTGGTACATT GCACTGAAAC GAACTGGTCA GTACAAACTT
361 GGACCAAAAA CTGGACGAGG CCAGAAAGCT ATCCTTTTCC TTCCAATGTC TGCCAAAAGT
```

Armadillo FGF2 gene coding sequence (124-217; partial amino acid sequence corresponding to human FGF2 residues 1 to 94) (SEQ ID NO:209) (Ensembl accession no. ENSDNOT00000014647, which is hereby incorporated by reference in its entirety):

```
361           A TGGCAGCCGG GAGCATCACC ACGCTGCCCG CTCTGCCCGA GGACGGCGGC
421 AGCGGCGCCT TCCCGCCGGG CCACTTCAAG GACCCCAAGC GGCTGTACTG CAAAAACGGG
481 GGCTTCTTCC TGCGCATCCA TCCCGACGGC CGAGTGGACG GGGTCCGGGA GAAGAGCGAC
541 CCTAACATCA AACTACAACT TCAAGCAGAA GAGAGAGGGG TCGTGTCTAT CAAAGGCGTG
601 TGTGCGAACC GTTACCTTGC TATGCGGGAA GACGGAAGAC TCCAGGCGTC T
```

Tree shrew FGF2 gene coding sequence (1-189) (SEQ ID NO:210) (Ensembl accession no. ENSTBET00000001143, which is hereby incorporated by reference in its entirety):

```
1   GCGGGGGTTA GAGCTGAGAG GGAGGAGGCA CCGGGGAGCG GTGACAGCCG GGGGACCGAT
61  CCCGCCGCGC GTTCGCTCAT CAGGAGGCCG GATGCTGCAG CGCGAGAGGC GCTTCTTGGA
121 GCCAGGAGCC GGGTTCAGGG CAGCTCCACC TCCTGGCCAG CCTCGTCACG AACCGGGATC
181 AAGTTGCCGG ACGACTCAGG TCAAGGAATG GGCGGCTATC CTCTGGACCG CCCGAGCCGG
241 AGCACAGGGC GAGGGCTGGG CGGTGCCCCG GACCCTGCCG TAAAACTACA GCTTCAAGCG
301 GAAGAGAGAG GGGTCGTGTC TATCAAAGGA GTGTGTGCAA ACCGTTACCT GGCCATGAAG
361 GAGGATGGGC GACTGCTGGC TTCTAAATGT GTTACAGATG AGTGTTTCTT TTTTGAACGA
421 CTGGAATCTA ATAACTACAA TACTTACCGG TCCCGAAAGT ACTCCAGCTG GTATGTGGCA
481 CTGAAACGAA CTGGGCAGTA TAAACTTGGA TCCAAAACAG GACCTGGGCA GAAAGCTATA
541 CTTTTTCTTC CAATGTCTGC TAAAAGC
```

Western clawed frog FGF2 gene coding sequence (aa 1-154) (SEQ ID NO:211) (GenBank accession no. NM_001017333, which is hereby incorporated by reference in its entirety):

```
171                                                     ATGGCAGCAG
181 GAAGCATCAC AACCCTACCA ACCGAATCTG AGGATGGAAA CACTCCTTTC CCACCGGGGA
241 ACTTTAAGGA CCCCAAGAGG CTCTACTGCA AGAATGGGGG CTACTTCCTC AGGATTAACT
301 CAGACGGGAG AGTGGACGGA TCAAGGGATA AAAGTGACTT ACACATAAAA TTACAGCTAC
361 AAGCAGTAGA GCGGGGAGTG GTATCAATAA AGGGAATCAC TGCAAATCGC TACCTTGCCA
421 TGAAGGAAGA TGGGAGATTA ACATCGCTGA AGTGTATAAC AGATGAATGC TTCTTTTATG
481 AACGATTGGA AGCTAATAAC TACAACACTT ACCGGTCTCG GAAAAACAAC AGCTGGTATG
541 TGGCACTAAA GCGAACTGGG CAGTATAAAA ATGGATCGAC CACTGGACCA GGACAAAAAG
601 CTATTTTGTT TCTCCCAATG TCAGCAAAAA GCTGA
```

Coelacanth FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:212) (Ensembl accession no. ENSLACT00000019333, which is hereby incorporated by reference in its entirety):

```
1                       ATGGCTGCGG GAGGAATCAC TACCCTGCCG GCGGTACCTG
41  AGGATGGAGG CAGCAGCACC TTCCCTCCAG GAAACTTCAA GGAGCCCAAG AGACTTTACT
101 GTAAGAATGG AGGCTATTTC TTAAGGATAA ACCCCGATGG AAGAGTGGAT GGAACAAGGG
161 AGAAAAATGA TCCTTATATA AAATTACAAC TGCAAGCTGA ATCTATAGGA GTGGTGTCGA
221 TAAAGGGAGT TTGTTCAAAC CGTTACCTAG CGATGAATGA AGACTGTAGA CTTTTTGGAT
281 TGAAATATCC AACGGATGAA TGTTTCTTCC ATGAGAGGCT GGAGTCCAAC AACTACAATA
341 CTTATCGTTC AAAGAAGTAT TCGGATTGGT ATGTGGCGCT GAAACGGACT GGTCAGTACA
401 AACCTGGGCC AAAAACTGGA CTGGGACAAA AAGCAATCCT TTTCCTTCCG ATGTCTGCCA
461 AGAGTTGA
```

Spotted green pufferfish FGF2 gene coding sequence (aa 34-188) (SEQ ID NO:213) (Ensembl accession no. ENSTNIT00000016254, which is hereby incorporated by reference in its entirety):

```
1   ATGGCCACGG GAGGGATCAC GACGCTTCCA TCCACACCTG AAGACGGCGG CAGCAGCGGC
61  TTTCCTCCCG GCAGCTTCAA GGATCCCAAA AGGCTCTACT GTAAAAACGG AGGTTTCTTC
121 CTGAGGATCA AGTCCGACGG GGTCGTGGAC GGAATCCGGG AGAAGAGTGA CCCCCACATA
181 AAGCTTCAGC TCCAGGCGAC CTCTGTGGGG GAGGTGGTCA TCAAGGGGGT GTGCGCTAAC
241 CGCTATCTGG CCATGAACAG AGATGGACGG CTGTTCGGAA CGAAACGAGC CACGGACGAA
301 TGCCATTTCT TAGAGCGGCT TGAGAGCAAC AACTACAACA CTTACCGCTC CAGGAAGTAC
361 CCAACCATGT TTGTGGGACT GACGCGGACG GGCCAGTACA AGTCTGGGAG CAAAACTGGA
421 CCGGGCCAAA AGGCCATCCT TTTTCTTCCG ATGTCCGCCA AATGCTAA
```

Stickleback FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:214) (Ensembl accession no. ENSGACT00000022120, which is hereby incorporated by reference in its entirety):

```
1                     AT GGCCACGGCA GGCTTCGCGA CGCTTCCCTC CACGCCCGAA
43  GACGGCGGCA GCGGCGGCTT CACCCCCGGG GGATTCAAGG ATCCCAAGAG GCTGTACTGC
103 AAAAACGGGG GCTTCTTCTT GAGGATCAGG TCCGACGGAG GTGTAGATGG AATCAGGGAG
163 AAGAGCGACG CCCACATAAA GCTCCAAATC CAGGCGACGT CGGTGGGGGA GGTGGTCATC
223 AAAGGAGTCT GTGCCAACCG CTATCTGGCC ATGAACAGAG ACGGCCGGCT GTTCGGAGTG
283 AGACGGGCGA CGGACGAATG CTACTTCCTG GAGCGGCTGG AGAGTAACAA CTACAACACC
343 TACCGCTCCA GGAAGTACCC CGGCATGTAC GTGGCTCTGA AGCGGACCGG CCAGTACAAG
403 TCCGGGAGCA AAACCGGACC CGGTCAAAAG GCCATTCTGT TCCTCCCCAT GTCGGCTAAG
463 TGCTAA
```

Fugu rubripes FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:215) (Ensembl accession no. ENSTRUT00000022363, which is hereby incorporated by reference in its entirety):

```
127       ATGG CCACGGGAGG GATCACAACA CTTCCATCCA CACCTGAAGA CGGCGGCAGC
181 GGCGGTTTTC CTCCCGGGAG CTTCAAGGAT CCCAAAAGGC TGTACTGTAA AAACGGCGGC
241 TTCTTCCTGA GGATCAGGTC CGACGGGGCC GTGGACGGAA CCCGGGAGAA GACTGACCCC
301 CACATAAAGC TTCAGCTCCA GGCGACCTCT GTGGGGGAGG TGGTCATCAA GGGGGTTTGT
361 GCTAATCGTT ATCTGGCCAT GAACAGAGAT GGACGACTGT TTGGAATGAA ACGAGCGACG
421 GATGAATGCC ACTTCTTAGA GCGGCTCGAG AGCAACAACT ACAACACCTA CCGCTCCAGG
481 AAGTACCCCA ACATGTTTGT GGGACTGACG CGAACTGGCA ACTACAAGTC TGGGACTAAA
541 ACTGGACCGG GCCAAAAGGC CATCCTCTTT CTTCCGATGT CGGCCAAATA CTAA
```

Rainbow trout FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:216) (GenBank accession no. NM_001124536, which is hereby incorporated by reference in its entirety):

```
390                               A TGGCCACAGG AGAAATCACC ACTCTACCCG
421 CCACACCTGA AGATGGAGGC AGTGGCGGCT TCCTTCCAGG AAACTTTAAG GAGCCCAAGA
481 GGTTGTACTG TAAAAATGGA GGCTACTTCT TGAGGATAAA CTCTAACGGA AGCGTGGACG
541 GGATCAGAGA TAAGAACGAC CCCCACAATA AGCTTCAACT CCAGGCGACC TCAGTGGGGG
601 AAGTAGTAAT CAAAGGGGTC TCAGCCAACC GCTATCTGGC CATGAATGCA GATGGAAGAC
661 TGTTTGGACC GAGACGGACA ACAGATGAAT GCTACTTCAT GGAGAGGCTG GAGAGTAACA
721 ACTACAACAC CTACCGCTCT CGAAAGTACC CTGAAATGTA TGTGGCACTG AAAAGGACTG
781 GCCAGTACAA GTCAGGATCC AAAACTGGAC CCGGCCAAAA AGCCATCCTC TTCCTCCCCA
841 TGTCAGCCAG ACGCTGA
```

Salmon FGF2 gene coding sequence (1-150) (SEQ ID NO:217) (GenBank accession no. EU816603, which is hereby incorporated by reference in its entirety):

```
99402                                                 ATGGCCACA GGAGAAATCA
99421  CCACTCTACC CGCCACACCT GAAGATGGAG GCAGTGGCGG CTTCCCTCCA GGAAACTTTA
99481  AGGATCCCAA GAGGCTGTAC TGTAAAAACG GGGGCTACTT CTTGAGAATA AACTCTAATG
99541  GAAGCGTGGA CGGGATCCGA GAGAAGAACG ACCCCCACA
100968                                                      AAC AGCCTCAATT
100981 TGTCAGGGCA TGGACTCTTC AAGGTGTCAA ACGTTCCACA GGGATGCTGG CCCATGTTGA
101041 CTCCAACGCT TCCCACAATT GTGTCAAGGT GGCTGGATGT TCTTTGGGAG
101845                            AATTTG GCAGTATGTC CAACCGGCCT CATAACCGCA
101881 GACCACGTGT AGCTACACCA GCCCAGGACC TCCACATCCG GCTTCTTCAT CTACGGGATC
101941 GTCTGAAACC AGCCACCCGA ACAGCTGATA AAACTGAGGA GTATTTCTGT CTGTAA
```

Zebrafish FGF2 gene coding sequence (aa 1-154) (SEQ ID NO:218) (GenBank accession no. AY269790, which is hereby incorporated by reference in its entirety):

```
43                                                  ATGGCCAC CGGAGGGATC
61  ACCACACTCC CGGCCGCTCC GGACGCCGAA AACAGCAGCT TTCCCGCGGG CAGCTTCAGG
121 GATCCCAAGC GCCTGTACTG CAAAAACGGA GGATTCTTCC TGCGGATCAA CGCGGACGGC
181 CGAGTGGACG GAGCCCGAGA CAAGAGCGAC CCGCACATTC GTCTGCAGCT GCAGGCGACG
241 GCAGTGGGTG AAGTACTCAT TAAAGGCATC TGTACCAACC GTTTCCTTGC CATGAACGCA
301 GACGGACGAC TGTTCGGGAC GAAAAGGACC ACAGATGAAT GTTATTTCCT GGAGCGCCTG
361 GAGTCCAACA ACTACAACAC ATACAGATCC CGCAAGTATC CCGACTGGTA CGTGGCTCTG
421 AAGAGAACCG GCCAGTATAA AAGCGGCTCT AAAACCAGCC CGGGACAGAA GGCCATCCTG
481 TTTCTGCCCA TGTCGGCCAA ATGCTGA
```

Nile tilapia FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:219) (GenBank accession no. XM_003443364, which is hereby incorporated by reference in its entirety):

```
1   ATGGCCACGG GAGGAATCAC AACACTTCCC GCTACACCTG AAGACGGCGG CAGCAGCGGC
61  TTTCCTCCTG GGAACTTCAA GGACCCTAAA AGGCTGTACT GTAAAAATGG TGGCTTCTTC
121 TTGAGGATAA AATCTGATGG AGGAGTGGAT GGAATACGAG AGAAAAACGA CCCCCACATA
181 AAGCTTCAAC TCCAGGCGAC CTCAGTGGGA GAAGTGGTCA TCAAAGGGAT TTGTGCAAAC
241 CGATATCTGG CAATGAACAG AGATGGACGA CTGTTTGGAG CGAGAAGAGC AACAGATGAG
301 TGCTACTTCT TAGAGCGGCT CGAGAGCAAC AACTACAACA CCTACCGCTC CAGGAAGTAC
361 CCAAACATGT ACGTGGCGCT GAAGCGGACT GGCCAGTACA AGTCTGGAAG CAAAACTGGA
421 CCGGGTCAAA AGGCAATTCT CTTTCTCCCA ATGTCTGCTA AATGCTAA
```

Medaka FGF2 gene coding sequence (aa 1-155) (SEQ ID NO:220) (Ensembl accession no. ENSORLT00000025835, which is hereby incorporated by reference in its entirety):

```
1   ATGGCTACGG GAGAAATCAC AACACTTCCC TCCCCAGCTG AAAACAGCAG AAGCGATGGC
61  TTTCCTCCAG GGAACTACAA GGATCCTAAG AGGCTCTACT GTAAAAATGG AGGTTTGTTT
121 TTGAGGATTA AACCTGATGG AGGAGTGGAT GGAATCCGGG AAAAAAAAGA TCCCCACGTT
181 AAGCTTCGCC TTCAGGCTAC CTCAGCGGGA GAGGTGGTGA TCAAAGGAGT TTGTTCAAAC
241 AGATATCTGG CGATGCATGG AGATGGACGT CTATTTGGAG TGAGACAAGC AACAGAGGAA
301 TGCTACTTCT TGGAGCGACT AGAGAGCAAC AACTATAACA CCTATCGCTC TAAAAAGTAC
361 CCAAACATGT ACGTGGCACT GAAGCGGACA GGCCAGTACA AACCTGGAAA CAAAACTGGA
421 CCAGGTCAAA AGGCCATTCT CTTTCTGCCT ATGTCTGCCA AGTACTAA
```

[0070] As noted above, also encompassed within the present invention are portions of paracrine FGFs other than FGF1 and/or FGF2 (e.g., FGF4, FGF5, FGF6, FGF9, FGF16, and FGF20). The portion of the paracrine FGF may be from human FGF4, FGF5, FGF6, FGF9, FGF16, and/or FGF20 having the amino acid sequences shown in Table 5, or orthologs thereof.

Table 5:

Amino acid sequence of human FGF4(SEQ ID NO:221) (GenBank accession no. NP_001998, which is hereby incorporated by reference in its entirety):

```
1   MSGPGTAAVA LLPAVLLALL APWAGRGGAA APTAPNGTLE AELERRWESL VALSLARLPV
61  AAQPKEAAVQ SGAGDYLLGI KRLRRLYCNV GIGFHLQALP DGRIGGAHAD TRDSLLELSP
121 VERGVVSIFG VASRFFVAMS SKGKLYGSPF FTDECTFKEI LLPNNYNAYE SYKYPGMFIA
181 LSKNGKTKKG NRVSPTMKVT HFLPRL
```

Amino acid sequence of human FGF5(SEQ ID NO:222) (GenBank Accession No. NP_004455, which is hereby incorporated by reference in its entirety): 1 MSLSFLLLLF FSHLILSAWA HGEKRLAPKG QPGPAATDRN PRGSSSRQSS SSAMSSSSAS 61 SSPAASLGSQ GSGLEQSSFQ WSPSGRRTGS LYCRVGIGFH LQIYPDGKVN GSHEANMLSV 121 LEIFAVSQGI VGIRGVFSNK FLAMSKKGKL HASAKFTDDC KFRERFQENS YNTYASAIHR 181 TEKTGREWYV ALNKRGKAKR GCSPRVKPQH ISTHFLPRFK QSEQPELSFT VTVPEKKKPP 241 SPIKPKIPLS APRKNTNSVK YRLKFRFG
Amino acid sequence of human FGF6(SEQ ID NO:223) (NP_066276, which is hereby incorporated by reference in its entirety): 1 MALGQKLFIT MSRGAGRLQG TLWALVFLGI LVGMVVPSPA GTRANNTLLD SRGWGTLLSR 61 SRAGLAGEIA GVNWESGYLV GIKRQRRLYC NVGIGFHLQV LPDGRISGTH EENPYSLLEI 121 STVERGVVSL FGVRSALFVA MNSKGRLYAT PSFQEECKFR ETLLPNNYNA YESDLYQGTY 181 IALSKYGRVK RGSKVSPIMT VTHFLPRI
Amino acid sequence of human FGF9(SEQ ID NO:224) (GenBank accession no. NP_002001, which is hereby incorporated by reference in its entirety): 1 MAPLGEVGNY FGVQDAVPFG NVPVLPVDSP VLLSDHLGQS EAGGLPRGPA VTDLDHLKGI 61 LRRRQLYCRT GFHLEIFPNG TIQGTRKDHS RFGILEFISI AVGLVSIRGV DSGLYLGMNE 121 KGELYGSEKL TQECVFREQF EENWYNTYSS NLYKHVDTGR RYYVALNKDG TPREGTRTKR 181 HQKFTHFLPR PVDPDKVPEL YKDILSQS
Amino acid sequence of human FGF16(SEQ ID NO:225) (GenBank accession no. NP_003859, which is hereby incorporated by reference in its entirety): 1 MAEVGGVFAS LDWDLHGFSS SLGNVPLADS PGFLNERLGQ IEGKLQRGSP TDFAHLKGIL 61 RRRQLYCRTG FHLEIFPNGT VHGTRHDHSR FGILEFISLA VGLISIRGVD SGLYLGMNER 121 GELYGSKKLT RECVFREQFE ENWYNTYAST LYKHSDSERQ YYVALNKDGS PREGYRTKRH 181 QKFTHFLPRP VDPSKLPSMS RDLFHYR
Amino acid sequence of human FGF20(SEQ ID NO:226) (GenBank accession no. NP_062825, which is hereby incorporated by reference in its entirety): 1 MAPLAEVGGF LGGLEGLGQQ VGSHFLLPPA GERPPLLGER RSAAERSARG GPGAAQLAHL 61 HGILRRRQLY CRTGFHLQIL PDGSVQGTRQ DHSLFGILEF ISVAVGLVSI RGVDSGLYLG 121 MNDKGELYGS EKLTSECIFR EQFEENWYNT YSSNIYKHGD TGRRYFVALN KDGTPRDGAR 181 SKRHQKFTHF LPRPVDPERV PELYKDLLMY T

[0071] It will be understood that the portion of the paracrine FGF according to the present invention may be derived from a nucleotide sequence that encodes human FGF4, FGF5, FGF6, FGF9, FGF16, and/or FGF20 having the nucleotide sequences shown in Table 6, or orthologs thereof.

Table 6:

Human FGF4 gene coding sequence (1-206) (SEQ ID NO:227) (GenBank accession no. NM_002007, which is hereby incorporated by reference in its entirety): 320 A TGTCGGGGCC CGGGACGGCC GCGGTAGCGC TGCTCCCGGC 361 GGTCCTGCTG GCCTTGCTGG CGCCCTGGGC GGGCCGAGGG GGCGCCGCCG CACCCACTGC 421 ACCCAACGGC ACGCTGGAGG CCGAGCTGGA GCGCCGCTGG GAGAGCCTGG TGGCGCTCTC 481 GTTGGCGCGC CTGCCGGTGG CAGCGCAGCC CAAGGAGGCG GCCGTCCAGA GCGGCGCCGG 541 CGACTACCTG CTGGGCATCA AGCGGCTGCG GCGGCTCTAC TGCAACGTGG GCATCGGCTT 601 CCACCTCCAG GCGCTCCCCG ACGGCCGCAT CGGCGGCGCG CACGCGGACA CCCGCGACAG 661 CCTGCTGGAG CTCTCGCCCG TGGAGCGGGG CGTGGTGAGC ATCTTCGGCG TGGCCAGCCG 721 GTTCTTCGTG GCCATGAGCA GCAAGGGCAA GCTCTATGGC TCGCCCTTCT TCACCGATGA 781 GTGCACGTTC AAGGAGATTC TCCTTCCCAA CAACTACAAC GCCTACGAGT CCTACAAGTA 841 CCCCGGCATG TTCATCGCCC TGAGCAAGAA TGGGAAGACC AAGAAGGGGA ACCGAGTGTC 901 GCCCACCATG AAGGTCACCC ACTTCCTCCC CAGGCTGTGA
Human FGF5 gene coding sequence (1-268) (SEQ ID NO:228) (GenBank Accession No. NM_004464, which is hereby incorporated by reference in its entirety): 238 ATG 241 AGCTTGTCCT TCCTCCTCCT CCTCTTCTTC AGCCACCTGA TCCTCAGCGC CTGGGCTCAC 301 GGGGAGAAGC GTCTCGCCCC CAAAGGGCAA CCCGGACCCG CTGCCACTGA TAGGAACCCT 361 AGAGGCTCCA GCAGCAGACA GAGCAGCAGT AGCGCTATGT CTTCCTCTTC TGCCTCCTCC 421 TCCCCCGCAG CTTCTCTGGG CAGCCAAGGA AGTGGCTTGG AGCAGAGCAG TTTCCAGTGG 481 AGCCCCTCGG GGCGCCGGAC CGGCAGCCTC TACTGCAGAG TGGGCATCGG TTTCCATCTG 541 CAGATCTACC CGGATGGCAA AGTCAATGGA TCCCACGAAG CCAATATGTT AAGTGTTTTG 601 GAAATATTTG CTGTGTCTCA GGGGATTGTA GGAATACGAG GAGTTTTCAG CAACAAATTT 661 TTAGCGATGT CAAAAAAAGG AAAACTCCAT GCAAGTGCCA AGTTCACAGA TGACTGCAAG 721 TTCAGGGAGC GTTTTCAAGA AAATAGCTAT AATACCTATG CCTCAGCAAT ACATAGAACT 781 GAAAAAACAG GGCGGGAGTG GTATGTGGCC CTGAATAAAA GAGGAAAAGC CAAACGAGGG 841 TGCAGCCCCC GGGTTAAACC CCAGCATATC TCTACCCATT TTCTGCCAAG ATTCAAGCAG 901 TCGGAGCAGC CAGAACTTTC TTTCACGGTT ACTGTTCCTG AAAAGAAAAA GCCACCTAGC 961 CCTATCAAGC CAAAGATTCC CCTTTCTGCA CCTCGGAAAA ATACCAACTC AGTGAAATAC 1021 AGACTCAAGT TTCGCTTTGG ATAA
Human FGF6 gene coding sequence (1-208) (SEQ ID NO:229) (NM_020996, which is hereby incorporated by reference in its entirety): 45 ATGGCC CTGGGACAGA 61 AACTGTTCAT CACTATGTCC CGGGGAGCAG GACGTCTGCA GGGCACGCTG TGGGCTCTCG 121 TCTTCCTAGG CATCCTAGTG GGCATGGTGG TGCCCTCGCC TGCAGGCACC CGTGCCAACA 181 ACACGCTGCT GGACTCGAGG GGCTGGGGCA CCCTGCTGTC CAGGTCTCGC GCGGGGCTAG 241 CTGGAGAGAT TGCCGGGGTG AACTGGGAAA GTGGCTATTT GGTGGGGATC AAGCGGCAGC 301 GGAGGCTCTA CTGCAACGTG GGCATCGGCT TTCACCTCCA GGTGCTCCCC GACGGCCGGA 361 TCAGCGGGAC CCACGAGGAG AACCCCTACA GCCTGCTGGA AATTTCCACT GTGGAGCGAG 421 GCGTGGTGAG TCTCTTTGGA GTGAGAAGTG CCCTCTTCGT TGCCATGAAC AGTAAAGGAA 481 GATTGTACGC AACGCCCAGC TTCCAAGAAG AATGCAAGTT CAGAGAAACC CTCCTGCCCA 541 ACAATTACAA TGCCTACGAG TCAGACTTGT ACCAAGGGAC CTACATTGCC CTGAGCAAAT 601 ACGGACGGGT AAAGCGGGGC AGCAAGGTGT CCCCGATCAT GACTGTCACT CATTTCCTTC 661 CCAGGATCTA A

Human FGF9 gene coding sequence (1-208)(SEQ ID NO:230) (GenBank accession no. NM_002010, which is hereby incorporated by reference in its entirety):

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838                                                                 ATG
841  GCTCCCTTAG GTGAAGTTGG GAACTATTTC GGTGTGCAGG ATGCGGTACC GTTTGGGAAT
901  GTGCCCGTGT TGCCGGTGGA CAGCCCGGTT TTGTTAAGTG ACCACCTGGG TCAGTCCGAA
961  GCAGGGGGGC TCCCCAGGGG ACCCGCAGTC ACGGACTTGG ATCATTTAAA GGGGATTCTC
1021 AGGCGGAGGC AGCTATACTG CAGGACTGGA TTTCACTTAG AAATCTTCCC CAATGGTACT
1081 ATCCAGGGAA CCAGGAAAGA CCACAGCCGA TTTGGCATTC TGGAATTTAT CAGTATAGCA
1141 GTGGGCCTGG TCAGCATTCG AGGCGTGGAC AGTGGACTCT ACCTCGGGAT GAATGAGAAG
1201 GGGGAGCTGT ATGGATCAGA AAAACTAACC CAAGAGTGTG TATTCAGAGA ACAGTTCGAA
1261 GAAAACTGGT ATAATACGTA CTCATCAAAC CTATATAAGC ACGTGGACAC TGGAAGGCGA
1321 TACTATGTTG CATTAAATAA AGATGGGACC CCGAGAGAAG GGACTAGGAC TAAACGGCAC
1381 CAGAAATTCA CACATTTTTT ACCTAGACCA GTGGACCCCG ACAAAGTACC TGAACTGTAT
1441 AAGGATATTC TAAGCCAAAG TTGA
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Human FGF16 gene coding sequence (1-207) (SEQ ID NO:231) (GenBank accession no. NM_003868, which is hereby incorporated by reference in its entirety):

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1   ATGGCAGAGG TGGGGGGCGT CTTCGCCTCC TTGGACTGGG ATCTACACGG CTTCTCCTCG
61  TCTCTGGGGA ACGTGCCCTT AGCTGACTCC CCAGGTTTCC TGAACGAGCG CCTGGGCCAA
121 ATCGAGGGGA AGCTGCAGCG TGGCTCACCC ACAGACTTCG CCCACCTGAA GGGGATCCTG
181 CGGCGCCGCC AGCTCTACTG CCGCACCGGC TTCCACCTGG AGATCTTCCC CAACGGCACG
241 GTGCACGGGA CCCGCCACGA CCACAGCCGC TTCGGAATCC TGGAGTTTAT CAGCCTGGCT
301 GTGGGGCTGA TCAGCATCCG GGGAGTGGAC TCTGGCCTGT ACCTAGGAAT GAATGAGCGA
361 GGAGAACTCT ATGGGTCGAA GAAACTCACA CGTGAATGTG TTTTCCGGGA ACAGTTTGAA
421 GAAAACTGGT ACAACACCTA TGCCTCAACC TTGTACAAAC ATTCGGACTC AGAGAGACAG
481 TATTACGTGG CCCTGAACAA AGATGGCTCA CCCCGGGAGG GATACAGGAC TAAACGACAC
541 CAGAAATTCA CTCACTTTTT ACCCAGGCCT GTAGATCCTT CTAAGTTGCC CTCCATGTCC
601 AGAGACCTCT TTCACTATAG GTAA
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Human FGF20 gene coding sequence (1-211) (SEQ ID NO:232) (GenBank accession no. NM_019851, which is hereby incorporated by reference in its entirety):

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134                ATGGCTC CCTTAGCCGA AGTCGGGGGC TTTCTGGGCG GCCTGGAGGG
181 CTTGGGCCAG CAGGTGGGTT CGCATTTCCT GTTGCCTCCT GCCGGGGAGC GGCCGCCGCT
241 GCTGGGCGAG CGCAGGAGCG CGGCGGAGCG GAGCGCGCGC GGCGGGCCGG GGGCTGCGCA
301 GCTGGCGCAC CTGCACGGCA TCCTGCGCCG CCGGCAGCTC TATTGCCGCA CCGGCTTCCA
361 CCTGCAGATC CTGCCCGACG GCAGCGTGCA GGGCACCCGG CAGGACCACA GCCTCTTCGG
421 TATCTTGGAA TTCATCAGTG TGGCAGTGGG ACTGGTCAGT ATTAGAGGTG TGGACAGTGG
481 TCTCTATCTT GGAATGAATG ACAAAGGAGA ACTCTATGGA TCAGAGAAAC TTACTTCCGA
541 ATGCATCTTT AGGGAGCAGT TTGAAGAGAA CTGGTATAAC ACCTATTCAT CTAACATATA
601 TAAACATGGA GACACTGGCC GCAGGTATTT TGTGGCACTT AACAAAGACG GAACTCCAAG
661 AGATGGCGCC AGGTCCAAGA GGCATCAGAA ATTTACACAT TTCTTACCTA GACCAGTGGA
721 TCCAGAAAGA GTTCCAGAAT TGTACAAGGA CCTACTGATG TACACTTGA
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[0072] As noted above, the chimeric protein includes a portion of a paracrine FGF coupled to a C-terminal region derived from an FGF21 molecule. FGF21 is an endocrine FGF expressed primarily by the pancreas (Fon Tacer et al., "Research Resource: Comprehensive Expression Atlas of the Fibroblast Growth Factor System in Adult Mouse," *Mol Endocrinol* 24(10):2050-2063 (2010), which is hereby incorporated by reference in its entirety) and has

metabolic effects similar to that of FGF19, such as increased energy metabolism, weight loss, lowered blood glucose levels, and resistance to obesity and diabetes (Kharitonenkov et al., "FGF-21 as a Novel Metabolic Regulator," *J Clin Invest* 115(6), 1627-1635 (2005); Coskun et al., "Fibroblast growth factor 21 corrects obesity in mice," *Endocrinology* 149(12):6018-6027 (2008), which are hereby incorporated by reference in their entirety). Transgenic mice overexpressing FGF21 are also resistant to diet-induced obesity (Kharitonenkov et al., "FGF-21 as a Novel Metabolic Regulator," *J Clin Invest* 115(6), 1627-1635 (2005), which is hereby incorporated by reference in its entirety). Moreover, in diabetic rodent models, FGF21 administration lowers blood glucose and triglyceride levels (Kharitonenkov et al., "FGF-21 as a Novel Metabolic Regulator," *J Clin Invest* 115(6), 1627-1635 (2005), which is hereby incorporated by reference in its entirety).

[0073] In one embodiment, the C-terminal portion of FGF21 of the chimeric protein of the present invention is from human FGF21 having the amino acid sequence of SEQ ID NO: 233 (GenBank Accession No. NP_061986, which is hereby incorporated by reference in its entirety), as follows:

```
1   MDSDETGFEH SGLWVSVLAG LLLGACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQQTEAH
61  LEIREDGTVG GAADQSPESL LQLKALKPGV IQILGVKTSR FLCQRPDGAL YGSLHFDPEA
121 CSFRELLLED GYNVYQSEAH GLPLHLPGNK SPHRDPAPRG PARFLPLPGL PPALPEPPGI
181 LAPQPPDVGS SDPLSMVGPS QGRSPSYAS.
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[0074] In one embodiment, the C-terminal portion of FGF21 of the chimeric protein of the present invention includes a β-Klotho co-receptor binding domain.

[0075] In one embodiment, the C-terminal portion of FGF21 of the chimeric protein of the present invention includes amino acid residues 168-209 of SEQ ID NO: 233.

[0076] In one embodiment, the C-terminal portion of FGF21 of the chimeric protein further includes one or more substitutions, deletions, or additions. In one embodiment, the C-terminal portion of FGF21 of the chimeric protein further includes one or more substitutions, deletions, or additions while retaining the ability to bind β-Klotho. In one embodiment, the C-terminal portion of FGF21 of the chimeric protein further includes one or more substitutions, deletions, or additions while retaining the ability to selectively bind β-Klotho. In one embodiment, the C-terminal portion of FGF21 of the chimeric protein further includes one or more substitutions, additions, or deletions to enhance binding affinity for β-Klotho.

[0077] In one embodiment of the present invention, the C-terminal portion of the chimeric protein according to the present invention is or is derived from a mammalian FGF21. In one embodiment of the present invention, the C-terminal portion of the chimeric protein

according to the present invention is or is derived from a vertebrate FGF21. In one embodiment, the C-terminal portion of the chimeric protein according to the present invention is derived from a non-human vertebrate FGF21. It will be understood that this includes orthologs of human FGF21, or a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. In one embodiment of the present invention, the C-terminal portion of FGF21 of the chimeric protein according to the present invention is derived from human, pongo abelii, pan troglodytes, canis lupus familiaris, bos taurus, equus caballus, ailuropoda melanoleuca, oryctolagus cuniculus, gorilla gorilla, nomascus leucogenys, procavia capensis, cavia porcellus, tupaia belangeri, sorex araneus, ictidomys tridecemlineatus, loxodonta africana, sus scrofa, felis catus, otolemur garnettii, rattus norvegicus, mus musculus, vicugna pacos, anolis carolinensis, gadus morhua, latimeria chalumnae, tursiops truncatus, mustela putorius furo, takifugu rubripes, dipodomys ordii, echinops telfairi, macaca mulatta, microcebus murinus, ochotona princeps, xiphophorus maculatus, gasterosteus aculeatus, sarcophilus harrisii, macropus eugenii, xenopus tropicalis, danio rerio, bos grunniens mutus, saimiri boliviensis boliviensis, callithrix jacchus, tupaia chinensis, papio anubis, pteropus alecto, heterocephalus glaber, cricetulus griseus, ovies aries, pan paniscus, macaca fascicularis, mesocricetus auratus, or oreochromis niloticus.

[0078] In one embodiment of the present invention, the portion of FGF21 of the chimeric protein of the present invention is from an ortholog of human FGF21 having an amino acid sequence as shown in Table 7. The portions of an ortholog of human FGF21 of a chimeric protein according to the present invention include portions corresponding to the above-identified amino acid sequences of human FGF21. Corresponding portions may be determined by, for example, sequence analysis and structural analysis.

Table 7:

Pongo abelii (Sumatran orangutan) FGF21 (GenBank Accession No. XP_002829565, which is hereby incorporated by reference in its entirety) (SEQ ID NO:234) 1 MDSDETGFEH SGLWVPVLAG LLLGACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQQTEAH 61 LEIREDGTVG GAADQSPESL LQLKALKPGV IQILGVKTSR FLCQRPDGAL YGSLHFDPEA 121 CSFRELLLED GYNVYQSEAH GLPLHLPGNK SPHRDPAPRG PARFLPLPGL PPAPPEPPGI 181 LAPQPPDVGS SDPLSMVGPS QGRSPSYAS
Pan troglodytes (chimpanzee) FGF21 (GenBank Accession No. XP_524333, which is hereby incorporated by reference in its entirety) (SEQ ID NO:235) 1 MDSDETGFEH SGLWVSVLAG LLLGACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQQTEAH 61 LEIREDGTVG GAADQSPESL LQLKALKPGV IQILGVKTSR FLCQRPDGAL YGSLHFDPEA 121 CSFRELLLED GYNVYQSEAH GLPLHLPGNK SPHRDPAPRG PARFLPLPGL PPAPPEPPGI 181 LAPQPPDVGS SDPLSMVGPS QGRSPSYTS

Canis lupus familiaris (dog) FGF21 (GenBank Accession No. XP_541510, which is hereby incorporated by reference in its entirety) (SEQ ID NO:236) 1 MGWAEAGFEH LGLWVPVLAV LLLEACRAHP IPDSSPLLQF GGQVRQRYLY TDDAQETEAH 61 LEIRADGTVV GAARQSPESL LELKALKPGV IQILGVKTSR FLCQGPDGTL YGSLHFDPVA 121 CSFRELLLED GYNIYHSETL GLPLRLRPHN SAYRDLAPRG PARFLPLPGL LPAPPEPPGI 181 LAPEPPDVGS SDPLSMVGPS QGRSPSYAS
Bos taurus (bovine) FGF21 (GenBank Accession No. XP_001789639, which is hereby incorporated by reference in its entirety) (SEQ ID NO:237) 1 MGWDEAKFKH LGLWVPVLAV LLLGTCRAHP IPDSSPLLQF GGQVRQRYLY TDDAQETEAH 61 LEIRADGTVV GAARQSPESL LELKALKPGV IQILGVKTSR FLCQGPDGKL YGSLHFDPKA 121 CSFRELLLED GYNVYQSETL GLPLRLPPQR SSNRDPAPRG PARFLPLPGL PAAPPDPPGI 181 LAPEPPDVGS SDPLSMVGPS YGRSPSYTS
Equus caballus (horse) FGF21 (GenBank Accession No. XP_001489202, which is hereby incorporated by reference in its entirety) (SEQ ID NO:238) 1 MDWDKTGFKY QGLWVPVLAV LLLGACQSHP IPDSSPLLQF GGQVRQRHLY TDDAQETEAH 61 LEIRADGTVA GAVHRSPESL LELKALKPGV IQILGVKTSR FLCQGPDGTL YGSLHFDPVA 121 CSFRELLLED GYNVYQSETL GLPLRLPHHS SPYQDPAPRA PARFLPLPGF PPAPPEPPGI 181 PAPEPPDVGS SDPLSMVGPS RSRSPSYTS
Ailuropoda melanoleuca (giant panda) FGF21 (GenBank Accession No. XP_002917910, which is hereby incorporated by reference in its entirety) (SEQ ID NO:239) 1 MGWDEARSEQ LGLWVPVLAV LLLEACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQETEAH 61 LAIRADGTVV GAASRSPESL LELKALKPGV IQILGVKTSR FLCQGPDGTL YGSVRFDPVA 121 CSFRELLLED GYNIYHSETL GLPLRLPAHN SPYRDSAPRG PARFLPLPGL LPVPPDPPGI 181 LGPEPPDVGS SDPLSMVGPS QGRSPSYAS
Oryctolagus cuniculus (rabbit) FGF21 (GenBank Accession No. XP_002723745, which is hereby incorporated by reference in its entirety) (SEQ ID NO:240) 1 MDWGKAKCRP PGLWVPALAA LLLGACQAHP IPDSSPLLQF GDQVRQQHLY TDDAQETEAH 61 LEIRADGTVV GAARRSPESL LQMKALQPGI IQILGVQTSR FLCQRPDGTL YGSLHFDREA 121 CSFRELLRED GYNVYLSEAL GLPLRLSPGS SPRRAPAPRG PARFLPLPGL PPDLPEPPGL 181 LAAAPPDVDS PDPLSMVQPA LDQSPSYTS
Gorilla gorilla (gorilla) FGF21 (Ensembl Accession No. ENSGGOP00000001229, which is hereby incorporated by reference in its entirety) (SEQ ID NO:241) 1 MDSDETGFEH SGLWVSVLAG LLLGACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQQTEAH 61 LEIREDGTVG GAADQSPESL LQLKALKPGV IQILGVKTSR FLCQRPDGAL YGSLHFDPEA 121 CSFRELLLED GYNVYQSEAH GLPLHLPGNK SPHRDPAPRG PARFLPLPGL PPAPPEPPGI 181 LAPQPPDVGS SDPLSMVGPS QGRSPSYAS
Nomascus leucogenys (Northern white-cheeked gibbon) FGF21 (Ensembl Accession No. ENSNLEP00000005639, which is hereby incorporated by reference in its entirety) (SEQ ID NO:242) 1 MDSDETGFEH SGLWVPVLAG LLLGACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQQTEAH 61 LEIREDGTVG GAADQSPESL LQLKALKPGV IQILGVKTSR FLCQRPDGAL YGSLHFDPEA 121 CSFRELLLED GYNVYQSEAH GLPLHLPGNK SPHRDPAPRG PARFLPLPGL PPAPPEPPGI 181 LAPQPPDVGS SDPLSMVGPS QGRSPSYAS

Procavia capensis (hyrax) FGF21 (Ensembl Accession No. ENSOGAG00000001210, which is hereby incorporated by reference in its entirety) (SEQ ID NO:243) 1 MDWAKFGIEH PGLWVPVMAV LLLGACQGYP IPDSSPLLQF GGQVRQRYLY TDDAQETEAH 61 LEIRADGTVV GAAHRSPESL LELKALKPGI IQILGVKTSR FLCQGPDGVL YGSLRFDPVA 121 CSFRELLLED GYNVYQSEAH GLPLRLPSHN SPQRDLASRV PARFLPLPGR LTVLPEPSGV 181 LGPEPPDVDS SDPLSMVGPS QGRSPSYAS
Cavia porcellus (guinea pig) FGF21 (Ensembl Accession No. ENSCPOP00000000237, which is hereby incorporated by reference in its entirety) (SEQ ID NO:244) 1 MDWARTECER PRLWVSMLAI LLVGACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQDTEVH 61 LEIRADGSVR GIAHRSPESL LELKALKPGV IQILGIRTSR FLCQRPDGSL YGSLHFDPEA 121 CSFRELLLAD GYNVYKSEAH GLPLHLLRGD SLSQEPAPPG PARFLPLPGL PATPPEPPRM 181 LPPGPPDVGS SDPLSMVGPL WDRSPSYTS
Tupaia belangeri (tree shrew) FGF21 (Ensembl Accession No. ENSTBEP00000013946, which is hereby incorporated by reference in its entirety) (SEQ ID NO:245) 1 MGWDKARFEH LGAWAPVLAV LLLGACQAYP IPDSSPLLQF GGQVRQRYLY TDDTQDTEAH 61 LEIRADGTVV GAAHQSPESL LELKALKPGV IQILGVKTSR FLCQRPDGAL YGSLHFDPEA 121 CSFRELLLED GYNIYQSEAR GLPLRLPPHD SPHRDRTPRG PARFLPLPGL PLVPPELPGV 181 LALEPPDVGS SDPLSMMGPS QGQSPSYAS
Sorex araneus (shrew) FGF21 (Ensembl Accession No. ENSSARP00000002784, which is hereby incorporated by reference in its entirety) (SEQ ID NO:246) 1 MVWDKARGQQ LGLWAPMLLG LLLGACQAHP LPDSSPLLQF GGQVRLRFLY TDDAQRTGAH 61 LEIRADGTVQ GAAHRTPECL LELKALKPGV IQILGVSTSR FLCQRPDGVL YGSLRFDPEA 121 CSFRELLLQD GYNVYQSEAL GLPLYLHPPS APVSQEPASR GAVRFLPLPG LPPASLEPPR 181 PPAPVPPDVG SSDPLSMVGP PERHSPSYTS
Ictidomys tridecemlineatus (squirrel) FGF21 (SEQ ID NO:247) 1 MDWVKAKLEP LGLWVLVLAA LVLGACQAYP IPDSSPLLQF GGQVRQRYLY TDDAQETEAH 61 LEIRADGTVV GAAHQSPESL LELKALKPGV IQILGVKTSR FLCQRPDGVL YGSLHFDPEA 121 CSFREQLLED GYNVYQSESH GLPVRLPPNS PYRDPAPPGP ARFLPLPGLP PAALEPPGIL 181 GPEPPDVGSS DPLSMVGPLQ GRSPSYAS
Loxodonta africana (elephant) FGF21 (Ensembl Accession No. ENSLAFP00000016854, which is hereby incorporated by reference in its entirety) (SEQ ID NO:248) 1 MDWAKFGLE HPGLWVPVMA VLLLGACQGH PIPDSSPLLQ FGGQVRQRYL YTDDQETEAH 60 LEIRADGTVA GAAHRSSESL LELKALKPGI IQILGVKTSR FLCQGPDGVL YGSLHFDPAA 120 CSFRELLLED GYNVYWSEAH GLPIRLPSHN SPYRDPASRV PARFLPLPGL LPMLQEPPGV 180 LAPEPPDVDS SDPLSMVGPS QGRSPSYAS
Sus scrofa (pig) FGF21 (GenBank Accession No. NP_001156882, which is hereby incorporated by reference in its entirety) (SEQ ID NO:249) 1 MGWAEAKFER LGLWVPVLAV LLGACQARPI PDSSPLLQFG GQVRQRYLYT DDAQETEAHL 61 EIRADGTVAG VARQSPESLL ELKALKPGVI QILGVQTSRF LCQGPDGRLY GSLHFDPEAC 121 SFRELLLEDG YNVYQSEALG LPLRLPPHRS SNRDLAPRGP ARFLPLPGLP PAPPEPPGIL 181 APEPPDVGSS DPLSMVGPSH GRSPSYTS
Felis catus (cat) FGF21 (Ensembl Accession No. ENSFCAP00000006832, which is hereby incorporated by reference in its entirety) (SEQ ID NO:250) 1 MDWDEAGSQ RLGLWVVLGV LLPEACQAHP IPDSSPLLQF GGQVRQRFLY TDDAQETEVH 60 LEIKADGTVV GTARRSPESL LELKALKPGV IQILGVKTSR FLCQGPDGTL YGSLRFDPAA 120 CSFRELLLED GYNIYHSETL GLPLRLPPHN SPYRDLAPRA PARFLPLPGL LPAPPEPPGI 180 LAPEPPDVGS SDPLSMVGPS QGRSPSYAS

Otolemur garnettii (bushbaby) FGF21 (Ensembl Accession No. ENSOGAG00000003581, which is hereby incorporated by reference in its entirety) (SEQ ID NO:251) 1 DKARTGFKH PGPWFPLLAV LLLGACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQETEAH 60 LEIREDGTVV GAAQQSPESL LELKALKPGV IQILGVKTSR FLCQRPDGGL YGSLYFDPKA 120 CSFRELLLED GYNVYWSETY GLPLHLPPAN SPYWGPSLRS PARFLPLPGP PAASPELPGI 180 LALEPPDVGS SDPLSMVGPS QGRSPSYAS
Rattus norvegicus (Norway rat) FGF21 (GenBank Accession No. NP_570108, which is hereby incorporated by reference in its entirety) (SEQ ID NO:252) 1 MDWMKSRVGA PGLWVCLLLP VFLLGVCEAY PISDSSPLLQ FGGQVRQRYL YTDDDQDTEA 61 HLEIREDGTV VGTAHRSPES LLELKALKPG VIQILGVKAS RFLCQQPDGT LYGSPHFDPE 121 ACSFRELLLK DGYNVYQSEA HGLPLRLPQK DSQDPATRGP VRFLPMPGLP HEPQEQPGVL 181 PPEPPDVGSS DPLSMVEPLQ GRSPSYAS
Mus musculus (house mouse) FGF21 (GenBank Accession No. NP_064397, which is hereby incorporated by reference in its entirety) (SEQ ID NO:253) 1 MEWMRSRVGT LGLWVRLLLA VFLLGVYQAY PIPDSSPLLQ FGGQVRQRYL YTDDDQDTEA 61 HLEIREDGTV VGAAHRSPES LLELKALKPG VIQILGVKAS RFLCQQPDGA LYGSPHFDPE 121 ACSFRELLLE DGYNVYQSEA HGLPLRLPQK DSPNQDATSW GPVRFLPMPG LLHEPQDQAG 181 FLPPEPPDVG SSDPLSMVEP LQGRSPSYAS
Vicugna pacos (alpaca) FGF21 (Ensembl Accession No. ENSVPAP00000005562, which is hereby incorporated by reference in its entirety) (SEQ ID NO:254); partial sequence corresponding to human FGF21 residues 1 to 78, 169 to 171, and 183 to 209 1 MDWDEAKFEH RGLWVPVLTV LLLGACQARP IPDSSPLLQF GGQVRQRYLY TDDAQETEAH 61 LEIRADGTVV GVARQPE--- ---------- ---------- ---------- ---------- 121 ---------- ---------- ---------- ---------- --------GI P--------- 181 --PEPPDVGS SDPLSMVGPS YSRSPSYTS
Anolis carolinensis (anole lizard) FGF21 (Ensembl Accession No. ENSACAP00000016895, which is hereby incorporated by reference in its entirety) (SEQ ID NO:255) 1 CKSKGGGKGG ERMWVDLVFW AALLRTAPAL PLRNSNPIYQ FDGQVRLRHL YTADEQTHLH 61 LEILPDGTVG GSRFQNPFSL MEIKAVKPGV IRMQAKKTSR FLCMKPNGRL YGSLFYSEEA 121 CNFHEKVLSD GYNLYYSENY NIPVSLSSAG NLGQSRQLPP FSQFLPLVNK IPLEPVLEDF 181 DFYGHQLDVE SADPLSILGQ NPGFMSPSYV F
Gadus morhua (cod) FGF21 (Ensembl Accession No. ENSGMOP00000013789, which is hereby incorporated by reference in its entirety) (SEQ ID NO:256) 1 LLLATLLHIG LSFYVPDSGP LLWLGDQVRE RHLYTAESHR RGLFLEMSPD GQVTGSAAQT 61 PLSVLELRSV RAGDTVIRAR LSSLYLCVDR AGHLTGQRQY TESDCTFREV ILEDGYTHFL 121 SVHHGLPISL APRHSPGRQG LRFSRFLPLR SSLSEDRVAE PPDSPLNLDS EDPLGMGLGS 181 LLSPAFSM
Latimeria chalumnae (coelacanth) FGF21 (Ensembl Accession No. ENSLACP00000003781, which is hereby incorporated by reference in its entirety) (SEQ ID NO:257) 1 MLCQSFVILS QKFIFGLFLT GLGLTGLAWT RPFQDSNPIL QYSDSIRLRH LYTASESRHL 61 HLQINSDGQV GGTTKQSPYS LLEMKAVKTG FVVIRGKKSA RYLCMERSGR LYGSLQYTEK 121 DCTFKEVVLA DGYNLYVSEE HQATVTLSPM RARIAQGKKI PPFSHFLPMV NKVPVEDVAA 181 EMEFVQVLRE MTADVDSPDP FGMTWEESVH SPSFFA

Tursiops truncatus (dolphin) FGF21 (Ensembl Accession No. ENSTTRP00000013808, which is hereby incorporated by reference in its entirety) (SEQ ID NO:258) 1 MGWDKTKLEH LGLWVPVLAV LLGPCQAHPI PDSSPLLQFG GQVRQRYLYT DDAQETEAHL 61 EIRADGTVVG TARRSPEGVK TSRFLCQGPE GRLYGSLHFN PQACSFRELL LEDGYNVYQS 121 EALGIPLRLP PHRSSNWDLA PRGPARFLPL PGFLPPPLEP PGILAPEPPN VGSSDPLSMV 181 GPSHGRSPSY TS
Mustela putorius furo (ferret) FGF21 (Ensembl Accession No. ENSMPUP00000003687, which is hereby incorporated by reference in its entirety) (SEQ ID NO:259) 1 MGWEEARSEH LGLWVPVLAV LLLGACQAYP IPDSSPLLQF GGQVRQRYLY TDDAQETEAH 61 LEIRADGTVV GAARRSPESL LELKALKPGV IQILGVKTSR FLCQGPNGTL YGSFHFDPVA 121 CSFREVLLED GYNIYHSETL GLPLRLPPHN SPHRDLAPRG PARFLPLPGL LPATPESRGI 181 PAPEPPNVGS SDPLSMVGPL QGQSPSYTS
Takifugu rubripes (fugu) FGF21 (Ensembl Accession No. ENSTRUP00000033950, which is hereby incorporated by reference in its entirety) (SEQ ID NO:260) 1 FIYLFIQTAL FSPSKWFNFY LPDSNPLLSF DSHGRGIHLY TDNQRRGMYL QMSTDGSVSG 61 SDVQTANSVL ELKSVRNGHV VIRGKSSSLF LCMDSRGRLW GQRHPTEADC TFREVLLADG 121 YTRFLSLHNG TPVSLAPKQS PDQHTVPFTR FLPLRNTLAE ESMSEPPSNQ QRYFNIDSDD 181 LLGMDLNAMV SPQFSGDK
Dipodomys ordii (kangaroo rat) FGF21 (Ensembl Accession No. ENSDORP00000001155, which is hereby incorporated by reference in its entirety) (SEQ ID NO:261) 1 MDQAKTRVGA RGLGGLVLAV IILGACKARP IPDSSPLLQF GGQVRLRHLY TDDTQETEAH 61 LEIRADGTVV GTAHRSPESL LELKALKPGV IQILGIKTSR FLCQRPDGTL YGSLHFDPEV 121 CSFQELLLED GYNIYRSEAL GLPLRLSPDP APWGPARFLP LPGVPPAPPE PPGILAPEPP 181 DVGSSDPLSM VGLLQGRSPS YAS
Echinops telfairi (lesser hedgehog tenrec) FGF21 (Ensembl Accession No. ENSETEP00000008707, which is hereby incorporated by reference in its entirety) (SEQ ID NO:262) 1 MGCTKSGWKS PGLWVPVLAS LLLGGCGAHP IPDSSPLLQF GGQVRQRYLY TDDAQTTEAH 61 LEIRADGTVG GVAHQSPEKF LSQWREKPLR SLHFDPAACS FREKLLEDGY NLYHSETHGL 121 PLRLPPRGGD PSSQPGARFP PLPGQLPQLQ ETPGVLAPEP PDVGSSDPLS MVGPWRGQSP 181 SYAS
Macaca mulatta (rhesus monkey) FGF21 (Ensembl Accession No. ENSMMUP00000031540, which is hereby incorporated by reference in its entirety) (SEQ ID NO:263) 1 MDSDETGFEH SGLWVPVLAG LLLGACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQQTEAH 61 LEIREDGTVG GAAHQSPESE CGPEPGSEGG GAVGGAEGPG LLGLREAGLG PGSWLHFDPE 121 ACSFRELLLE NGYNVYQSEA HGLPLHLPGN KSPHRDPASQ GPARFLPLPG LPPAPPEPPG 181 ILAPQPPDVG SSDPLSMVGP SQARSPSYAS
Microcebus murinus (mouse lemur) FGF21 (Ensembl Accession No. ENSMICP00000012089, which is hereby incorporated by reference in its entirety) (SEQ ID NO:264) 1 MGWDEAGAGF EHPGLWFPML GVLLLGACQA YPIPDSSPLL QFGGQVRQRH LYTDDIQETE 61 AHLEIRADGT VVGAARQSPE LELKALKPGV IQILGVKTSR FLCQRPDGAL YGSLHFDPEC 121 SFRELLLEDG YNVYCPYLPL HLSPRIELAG SRSALPLPPA PERRILAPEP PDGSSDPLSM 181 VGPSQGRSPS YAS

Ochotona princeps (pika) FGF21 (Ensembl Accession No. ENSOPRP00000006754, which is hereby incorporated by reference in its entirety) (SEQ ID NO:265) 1 KDMDGLQPPG LRVPVLAALL LGVGQARPIP DSSPLLQFGG QVRQRHLYTD DAQESEVHLE 61 IRADGTVAGT ARRSPESLLE MKALKPGVIQ ILGVHTSRFL CQRPDGTLYG SLHFDHKACS 121 FREQLLEDGY NVYHSETHGL PLRLSPDRAP RGPARFLPLP GPPPDLLVPP LPPDVLAPEP 181 PDVDSPDPLS MVGPLQGQSP SYTS
Xiphophorus maculatus (platyfish) FGF21 (Ensembl Accession No. ENSXMAP00000001576, which is hereby incorporated by reference in its entirety) (SEQ ID NO:266) 1 CPFPFLFLIL SLPFFSSSFY IPESNPIFAF RNQLREVHLY TENHRRGLYV EIHLDGRVTG 61 SDAQSPYSVL QIKSVKPGHV VIKGQTSSLF LCMDDSGNLR GQTTYDEADC SFRELLLADG 121 YTRFLNSQHG VPLSLASRNS PDRHSVPFTR FLPLRNTLTV SEESTKTQRD FNLDSDDLLG 181 MG
Gasterosteus aculeatus (stickleback) FGF21 (Ensembl Accession No. ENSGACP00000010703, which is hereby incorporated by reference in its entirety) (SEQ ID NO:267) 1 SLLLMVPLPF CSSFYLTDSS PLLPFNNQVK EVHLYTAENH RRAMYLQIAL DGSVSGSDAR 61 STYSVLQLKS IQPGHVVIRG KASSMFLCVD SGGRLRGQGP YSEADCSFRE LLLGDGYTRF 121 LSSQHGSPLS LASRPSPDPN SVPFTRFLPI RTAPEAESVI EEPPSNQRYV NVDSEDLLGM 181 GLNTVVSPQF SA
Sarcophilus harrisii (tasmanian devil) FGF21 (Ensembl Accession No. ENSSHAP00000005963, which is hereby incorporated by reference in its entirety) (SEQ ID NO:268); partial sequence corresponding to human FGF21 residues 3 to 172 1 VSAMGLRERA PRYLAPLLSL LLACRASGHP LPDSSPMLLF GGQVRLRHLY TDVGQEAEAH 61 VELASDGTVR AAARRSPNSL LELKAVKPGI VRILAVHSSR FLCMRPNGEL YGAIHYDPSA 121 CNFRERLLGD GYNVYESEAH GRTLRLPPKA APGPAGPSRF LPLPG
Macropus eugenii (wallaby) FGF21 (Ensembl Accession No. ENSMEUP00000013936, which is hereby incorporated by reference in its entirety) (SEQ ID NO:269) 1 TEEPSTGSRH LGQWAPGLPG PLLSLLLAYR GWGSPIPDSS PMLLFGGQVR LRHLYTDDGQ 61 DTEAHVELGP DGVVRAVAER SPNSLLELKA VKPGVIRILA VQSSRFLCMR PNGELYGAVH 121 YDPSACNFRE HLLGDGYNVY ESETHRRTLR LSPSLGQAGP SRFLPLPGDW LPGPDPPWAQ 181 GPEPPDVGSA DPLSMVGAVQ GLSPSYSS
Xenopus tropicalis (Western clawed frog) FGF21 (Ensembl Accession No. ENSXETP00000009917, which is hereby incorporated by reference in its entirety) (SEQ ID NO:270); partial sequence corresponding to human FGF21 residues 1 to 169 1 RGGRTKKKTL LRKWLCLLAI MLSRSRFSLA NPIQNSNPIL SNDNQVRTQY LYTDNNNMHL 61 YLQITHNGVV TGTEEKNDYG VLEIKAVKAG VVVIKGIRSN LYLCMDSRHQ LYASAYDKDD 121 CHFHEKITPD NYNMYSSEKH SEYVSLAPLK GSQMARFLPI
Danio rerio (zebrafish) FGF21 (Ensembl Accession No. ENSDARP00000094287, which is hereby incorporated by reference in its entirety) (SEQ ID NO:271) 1 MLLACFFIFF ALFPHLRWCM YVPAQNVLLQ FGTQVRERLL YTDGLFLEMN PDGSVKGSPE 61 KNLNCVLELR SVKAGETVIQ SAATSLYLCV DDQDKLKGQH HYSALDCTFQ ELLLDGYSFF 121 LSPHTNLPVS LLSKRQKHGN PLSRFLPVSR AEDSRTQEVK QYIQDINLDS DDPLGMGHRS 181 HLQTVFSPSL HTKK

Bos grunniens mutus (yak) FGF21 (GenBank Accession No. ELR56628, which is hereby incorporated by reference in its entirety) (SEQ ID NO:272) 1 MGWDEAKFKH LGLWVPVLAV LLLGTCRAHP IPDSSPLLQF GGQVRQRYLY TDDAQETEAH 61 LEIRADGTVV GAARQSPESL LELKALKPGV IQILGVKTSR FLCQGPDGKL YGSLHFDPKA 121 CSFRELLLED GYNVYQSETL GLPLRLPPQR SSNRDPAPRG PARFLPLPGL PAEPPDPPGI 181 LAPEPPDVGS SDPLSMVGPS YGRSPSYTS
Saimiri boliviensis boliviensis (Bolivian squirrel monkey) FGF21 (GenBank Accession No. XP_003940375, which is hereby incorporated by reference in its entirety) (SEQ ID NO:273) 1 MGSEEVALER PALWVSVLAG LLLGTCQAYP IPDSSPLLQF GGQVRQRYLY TDDAQQTEAH 61 LEIREDGTVA GAAHQSPESL LQLKALKPGV IQILGVKTSR FLCQRPDGAL YGSLYFDPEA 121 CSFRELLLED GYNVYQSVAH SLPLHLPGGR SPPWDPAPRG PARFLPLPGL PPEPPEAPGI 181 LAPEPPDVGS SDPLSMVGPS QGQSPSYTS
Callithrix jacchus (white-tufted-ear marmoset) FGF21 (GenBank Accession No. XP_003735669, which is hereby incorporated by reference in its entirety) (SEQ ID NO:274) 1 MGSEEVGLEH PALWVSVLAG LLLGTCQAHP IPDSSPLLQF GGQVRQRYLY TDDAQQKEAH 61 LEIXEDGTVA GAATKVPKVS LLQLKALKPG VIQILGVKTS RFLCQRPDGA LYGSLHFDPE 121 ACSFRELLLE DGYNVYQSVA HGLPLHLPES RSPPRDPAPR GPARFLPLPG LPPEPPEPPG 181 ILAPEPPDVG SSDPLSMVGP SQGQSPSYAS
Tupaia chinensis (Chinese tree shrew) FGF21 (GenBank Accession No. ELW47159, which is hereby incorporated by reference in its entirety) (SEQ ID NO:275) 1 MGWDKARFEH LGAWAPVLAV LLLGACQAYP IPDSSPLLQF GGQVRQRYLY TDDTQDTEAH 61 LEIRADGTVV GAAHQSPESL LELKALKPGV IQILGVKTSR FLCQRPDGAL YGSLHFDPEA 121 CSFRELLLED GYNIYQSEAR GLPLRLPPHD SPHRDRTPQG PARFLPLPGL PLVPPELPGV 181 LALEPPDVGS SDPLSMMGPS QGQSPSYAS
Papio anubis (olive baboon) FGF21 (GenBank Accession No. XP_003915900, which is hereby incorporated by reference in its entirety) (SEQ ID NO:276) 1 MDSDETGFEH SGLWVPVLAG LLLGACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQQTEAH 61 LEIREDGTVG GAAHQSPESK CGPEPGSEGG GALHFDPEAC SFRELLLENG YNVYQSEAHG 121 LPLHLPGNKS PHRDPASRGP ARFLPLPGLP PAPPEPPGIL APQPPDVGSS DPLSMVGPSQ 181 ARSPSYAS
Pteropus alecto (black flying fox) FGF21 (GenBank Accession No. ELK18566, which is hereby incorporated by reference in its entirety) (SEQ ID NO:277) 1 MGWGKARLQH PGLWGPVLAV LLGACQAHPI LDSSPLFQFG SQVRRRYLYT DDAQDTEAHL 61 EIRADGTVAG AARRSPESLL ELKALKPGVI QVLGVKTSRF LCQRPDGTLY GSLHFDPAAC 121 SFRELLLKDG YNVYQSEALA RPLRLPPYSS PSSDPARRGP ARFLPLPGPP PEPPQPPGRL 181 APEPPDVGSS DPLSMVWPSR GRSPSYTS
Heterocephalus glaber (naked mole-rat) FGF21 (GenBank Accession No. EHB06286, which is hereby incorporated by reference in its entirety) (SEQ ID NO:278) 1 MDWARAESER PGLWVPAVLA VLLLGACQAH PIPDSSPLLQ FGGQVRQRHL YTDDAQDTEV 61 HLEIRADGSV GGAAHRSPES LLELKALKPG VIQILGVRTS RFLCQRPDGT LYGSLHFDPE 121 ACSFRELLLA DGYNIYQSEA YGLPLRMLPS DSASRDPVPP GPARFLPLPG LHPPPLEPPG 181 MLPPEPPDVG SSDPLSMVGP LQGRSPSYAF

Cricetulus griseus (Chinese hamster) FGF21 (GenBank Accession No. XP_003508726, which is hereby incorporated by reference in its entirety) (SEQ ID NO:279) 1 MDWMKSGVGV PGLWVPLLPI FLLGVSQAHP IPDSSPLLQF GGQVRHRHLY TDDNQETEVH 61 LEIRQDGTVI GTTHRSPESL LELKALKPEV IPVLGVKASR FLCQQPDGTL YGSPHFDPEA 121 CSFRELLLED GYNVYQSEVH GLPLRLPQRD SPNQAPASWG PVPPLPVPGL LHQPQELPGF 181 LAPEPPDVGS SDPLSMVGPL QGRSPSYAS
Ovis aries (sheep) FGF21 (GenBank Accession No. XP_004015845, which is hereby incorporated by reference in its entirety) (SEQ ID NO:280) 1 MGWDEAKFKH LGLWVPVLAV LLLGTCRAHP IPDSSPLLQF GGQVRQRYLY TDDAQETEAH 61 LEIRADGTVV GAARQSPESL LELKALKPGV IQIFGVKTSR FLCQGPDGKL YGSLHFDPKA 121 CSFRELLLED GYNVYQSETL GLPLRLPPQR SSNRDPAPRG PPKPQLHFLK TSAVQYWPRY 181 EKVPAFLHPF PG
Pan paniscus (pygmy chimpanzee) FGF21 (GenBank Accession No. XP_003814163, which is hereby incorporated by reference in its entirety) (SEQ ID NO:281); partial sequence corresponding to human FGF21 residues 1 to 116 and 195 to 201 1 MDSDETGFEH SGLWVSVLAG LLLGACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQQTEAH 61 LEIREDGTVG GAADQSPESL LQLKALKPGV IQILGVKTSR FLCQRPDGAL YGSVSF---- 121 ---------- ---------- ---------- ----Q----- ---------- -----DPP-- 181 --HHPP---C S---SYMSPS Q---PG---
Macaca fascicularis (crab-eating macaque) FGF21 (GenBank Accession No. EHH59757, which is hereby incorporated by reference in its entirety) (SEQ ID NO:282); partial sequence corresponding to human FGF21 residues 1 to 116 1 MDSDETGFEH SGLWVPVLAG LLLGACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQQTEAH 61 LEIREDGTVG GAAHQSPESL LQLKALKPGV IQILGVKTSR FLCQKPDGAL YGSVSF
Mesocricetus auratus (golden hamster) FGF21 (GenBank Accession No. ACB30542, which is hereby incorporated by reference in its entirety) (SEQ ID NO:283); partial sequence corresponding to human FGF21 residues 90 to 193 1 VIQILGVKAA RFPCQQPDGS LYGSPHFDPE ACSFRELLLE DGYNVYQSEA HGLPLRLPQR 61 DAPSQPPASW GPVRFLPVPG LFQPPHDLPG RPAPEPPDVG SSDP
Oreochromis niloticus (Nile tilapia) FGF21 (GenBank Accession No. XP_003438516, which is hereby incorporated by reference in its entirety) (SEQ ID NO:284); partial sequence corresponding to human FGF21 residues 59 to 209 1 MYLQMNMDGR VTGSDAQTPY SLMQLKSVKP GHVIIKGPSS SLFLCVDSEG NLRGQSHYSE 61 TSCTFREMLL ADGYTRFISS QYGFPMSLAS RHSPDRHALP FTRFLPLRNN LKTDSVSEQL 121 PNNQRLFNVD SDDLLGMGLN SMGSPQFSMD K

[0079] In certain embodiments according to the present invention, the C-terminal portion of FGF21 of the chimeric protein of the present invention includes a polypeptide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% amino acid sequence identity to amino acid residues 168-209 of SEQ ID NO: 233. In certain embodiments according to the present invention, the C-terminal portion of FGF21 of the chimeric protein of the present invention includes a polypeptide sequence that has at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% amino acid sequence homology to amino acid residues 168-209 of SEQ ID NO: 233.

[0080] It will be understood that the portion of FGF21 of the chimeric protein of the present invention may be derived from a nucleotide sequence that encodes a vertebrate or a non-vertebrate FGF21 protein. In one embodiment, the portion of FGF21 of the chimeric protein of the present invention may be derived from a nucleotide sequence that encodes a mammalian FGF21 protein. Nucleotide sequences encoding a vertebrate FGF21 protein according to the present invention may include, but are not limited to, those shown in Table 8. The portion of FGF21 of the chimeric protein of the present invention derived from an ortholog of human FGF21 include portions corresponding to the above-identified amino acid sequences of FGF21. Corresponding portions may be determined by, for example, sequence analysis and structural analysis.

Table 8:

Human FGF21 gene coding sequence (SEQ ID NO:285) (GenBank Accession No. NM_019113, which is hereby incorporated by reference in its entirety) 151 ATGGACTCGG ACGAGACCGG GTTCGAGCAC TCAGGACTGT GGGTTTCTGT GCTGGCTGGT 211 CTTCTGCTGG GAGCCTGCCA GGCACACCCC ATCCCTGACT CCAGTCCTCT CCTGCAATTC 271 GGGGGCCAAG TCCGGCAGCG GTACCTCTAC ACAGATGATG CCCAGCAGAC AGAAGCCCAC 331 CTGGAGATCA GGGAGGATGG GACGGTGGGG GGCGCTGCTG ACCAGAGCCC CGAAAGTCTC 391 CTGCAGCTGA AAGCCTTGAA GCCGGGAGTT ATTCAAATCT TGGGAGTCAA GACATCCAGG 451 TTCCTGTGCC AGCGGCCAGA TGGGGCCCTG TATGGATCGC TCCACTTTGA CCCTGAGGCC 511 TGCAGCTTCC GGGAGCTGCT TCTTGAGGAC GGATACAATG TTTACCAGTC CGAAGCCCAC 571 GGCCTCCCGC TGCACCTGCC AGGGAACAAG TCCCCACACC GGGACCCTGC ACCCCGAGGA 631 CCAGCTCGCT TCCTGCCACT ACCAGGCCTG CCCCCCGCAC TCCCGGAGCC ACCCGGAATC 691 CTGGCCCCCC AGCCCCCCGA TGTGGGCTCC TCGGACCCTC TGAGCATGGT GGGACCTTCC 751 CAGGGCCGAA GCCCCAGCTA CGCTTCCTGA
Pongo abelii (Sumatran orangutan) FGF21 gene coding sequence (SEQ ID NO:286) (GenBank Accession No. XM_002829519, which is hereby incorporated by reference in its entirety) 165 ATGGAC TCGGACGAGA CCGGGTTCGA GCACTCAGGA CTGTGGGTTC CTGTGCTGGC 221 TGGTCTTCTG CTGGGAGCCT GCCAGGCACA CCCCATCCCT GACTCCAGTC CTCTCCTGCA 281 ATTCGGGGGC CAAGTCCGGC AGCGGTACCT CTACACAGAT GATGCCCAGC AGACAGAAGC 341 CCACCTGGAG ATCAGGGAGG ATGGGACGGT GGGGGGCGCT GCTGACCAGA GCCCCGAAAG 401 TCTCCTGCAG CTGAAAGCCT TGAAGCCGGG AGTTATTCAA ATCTTGGGAG TCAAGACATC 461 CAGGTTCCTG TGCCAGAGGC CAGATGGGGC CCTGTATGGA TCGCTCCACT TTGACCCTGA 521 GGCCTGCAGC TTCCGGGAGC TGCTTCTTGA GGACGGATAC AATGTTTATC AGTCCGAGGC 581 CCATGGCCTC CCGCTGCACC TGCCGGGAAA CAAGTCCCCA CACCGGGACC CTGCACCCCG 641 AGGACCAGCT CGCTTCCTGC CACTACCAGG CCTGCCCCCC GCACCCCCAG AGCCGCCCGG 701 AATCCTGGCC CCCCAGCCCC CCGATGTGGG CTCCTCGGAC CCTCTGAGCA TGGTGGGACC 761 TTCCCAGGGC CGAAGCCCCA GCTATGCTTC CTGA

Pan troglodytes (chimpanzee) FGF21 gene coding sequence (SEQ ID NO:287) (GenBank Accession No. XM_524333, which is hereby incorporated by reference in its entirety)

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573    ATGGACTC GGACGAGACC GGGTTCGAGC ACTCAGGACT GTGGGTTTCT GTGCTGGCTG
631  GTCTTCTGCT AGGAGCCTGC CAGGCACACC CCATCCCTGA CTCCAGTCCT CTCCTGCAAT
691  TCGGGGGCCA AGTCCGGCAG CGGTACCTCT ACACAGATGA TGCCCAGCAG ACAGAAGCCC
751  ACCTGGAGAT CAGGGAGGAT GGGACGGTGG GGGGCGCTGC TGACCAGAGC CCCGAAAGTC
811  TCCTGCAGCT GAAAGCCTTG AAGCCGGGAG TTATTCAAAT CTTGGGAGTC AAGACATCCA
871  GGTTCCTGTG CCAGAGGCCA GATGGGGCCC TGTATGGATC GCTCCACTTT GACCCTGAGG
931  CCTGCAGCTT CCGGGAGCTG CTTCTTGAGG ACGGATACAA TGTTTACCAG TCCGAGGCCC
991  ACGGCCTCCC GCTGCACCTG CCGGGGAACA AGTCCCCACA CCGGGACCCT GCACCCCGAG
1051 GACCAGCTCG CTTCCTGCCA CTACCAGGCC TGCCCCCCGC ACCCCCGGAG CCACCCGGAA
1111 TCCTGGCCCC CCAGCCCCCC GATGTGGGCT CCTCAGACCC TCTGAGCATG GTGGGACCTT
1171 CCCAGGGCCG AAGCCCCAGC TACACTTCCT GA
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Canis lupus familiaris (dog) FGF21 gene coding sequence (SEQ ID NO:288) (GenBank Accession No. XM_541510, which is hereby incorporated by reference in its entirety)

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1   ATGGGCTGGG CCGAGGCCGG GTTCGAGCAC CTGGGACTGT GGGTCCCTGT GCTGGCTGTG
61  CTTTTGCTGG AAGCCTGCCG GGCACATCCG ATCCCTGACT CCAGCCCCCT CCTACAATTT
121 GGAGGTCAAG TTCGACAGCG GTACCTCTAC ACCGACGATG CCCAGGAGAC AGAGGCCCAC
181 CTAGAGATCA GGGCCGATGG CACAGTGGTG GGGGCTGCCC GCCAGAGCCC TGAAAGTCTC
241 CTGGAGCTGA AAGCCCTAAA GCCAGGGGTC ATTCAAATCT TGGGAGTCAA AACATCCAGG
301 TTCCTGTGCC AGGGCCCAGA TGGGACACTA TATGGCTCGC TCCATTTCGA CCCTGTGGCC
361 TGCAGTTTCC GAGAACTGCT TCTTGAGGAT GGGTACAACA TCTACCACTC CGAGACCCTT
421 GGTCTCCCGC TTCGCCTGCG CCCCCACAAC TCCGCATACC GGGACTTGGC ACCCCGCGGG
481 CCTGCCCGCT TCCTGCCACT GCCAGGCCTG CTTCCAGCAC CCCCAGAGCC TCCAGGGATC
541 CTGGCCCCGG AGCCTCCTGA CGTGGGCTCC TCGGACCCTC TGAGCATGGT GGGGCCTTCA
601 CAGGGCCGGA GTCCCAGCTA TGCTTCCTAA
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Bos taurus (bovine) FGF21 gene coding sequence (SEQ ID NO:289) (GenBank Accession No. XP_001789587, which is hereby incorporated by reference in its entirety)

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1   ATGGGCTGGG ACGAGGCCAA GTTCAAGCAC TTGGGACTGT GGGTCCCTGT GCTGGCTGTC
61  CTCCTGCTAG GAACCTGCCG GGCGCATCCC ATTCCAGACT CCAGCCCCCT CCTCCAGTTT
121 GGGGGCCAAG TCCGCCAGCG GTACCTCTAC ACGGATGATG CCCAGGAGAC AGAGGCCCAC
181 CTGGAGATCA GGGCCGATGG CACAGTGGTG GGGGCAGCCC GCCAGAGCCC CGAAAGTCTC
241 TTGGAGCTGA AAGCCCTGAA GCCAGGCGTC ATTCAGATCT TGGGAGTTAA AACATCCAGG
301 TTTCTCTGCC AGGGGCCAGA TGGGAAGCTG TACGGATCGC TGCACTTTGA CCCCAAAGCC
361 TGCAGCTTTC GGGAGCTGCT TCTTGAAGAT GGATACAACG TCTACCAGTC GGAGACCCTG
421 GGCCTTCCAC TCCGCCTGCC CCCCCAGCGC TCGTCCAACC GGGACCCGGC CCCGCGGGGA
481 CCTGCTCGCT TCCTTCCACT GCCGGGCCTG CCCGCGGCGC CCCCGGATCC TCCAGGGATC
541 TTGGCCCCCG AGCCTCCCGA CGTGGGCTCC TCGGATCCCC TGAGTATGGT GGGACCCTCG
601 TATGGCCGAA GCCCCAGCTA CACTTCTTGA
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Equus caballus (horse) FGF21 gene coding sequence (SEQ ID NO:290) (GenBank Accession No. XM_001489152, which is hereby incorporated by reference in its entirety)

1 ATGGACTGGG ACAAGACGGG GTTCAAGTAC CAGGGACTGT GGGTCCCTGT GCTGGCTGTC
61 CTTCTGCTGG GAGCCTGCCA GTCACACCCC ATCCCTGACT CCAGTCCCCT CCTCCAATTC
121 GGGGGCCAAG TCAGGCAGCG CCACCTCTAC ACAGATGATG CCCAGGAGAC AGAGGCGCAC
181 CTGGAGATCA GGGCTGACGG CACTGTGGCA GGGGCTGTCC ACCGGAGCCC AGAAAGTCTC
241 TTGGAGCTGA AAGCCCTGAA GCCAGGGGTA ATTCAAATCT TGGGAGTCAA GACATCCAGG
301 TTTCTGTGCC AGGGGCCAGA CGGGACGCTG TACGGATCGC TCCACTTCGA CCCCGTGGCC
361 TGCAGCTTCC GGGAGCTGCT TCTCGAAGAC GGCTACAACG TTTACCAGTC TGAGACCCTT
421 GGCCTCCCAC TCCGCCTGCC CCACCACAGC TCCCCATACC AGGATCCGGC CCCTCGGGCA
481 CCCGCCCGCT TCCTGCCGCT GCCAGGCTTT CCCCCAGCAC CCCCGGAGCC TCCAGGGATC
541 CCGGCCCCCG AGCCCCCGGA CGTGGGCTCC TCGGACCCCC TGAGCATGGT GGGGCCTTCA
601 CGCAGCCGGA GCCCCAGCTA CACTTCCTGA

Ailuropoda melanoleuca (giant panda) FGF21 gene coding sequence (SEQ ID NO:291) (GenBank Accession No. XM_002917864, which is hereby incorporated by reference in its entirety)

1 ATGGGCTGGG ACGAGGCCAG GTCCGAGCAG CTGGGGCTGT GGGTCCCTGT GCTGGCTGTC
61 CTTTTGCTGG AAGCTTGCCA GGCACACCCT ATCCCTGACT CCAGCCCCCT CCTCCAATTC
121 GGAGGCCAAG TTCGACAGCG GTACCTCTAC ACGGACGATG CCCAGGAGAC AGAGGCCCAC
181 CTAGCGATCA GGGCTGATGG CACAGTGGTG GGGGCTGCCA GCCGGAGCCC AGAAAGTCTC
241 TTGGAGCTGA AAGCCCTGAA ACCGGGGGTC ATTCAAATCC TGGGAGTGAA AACATCTAGG
301 TTCCTGTGCC AGGGCCCAGA TGGGACACTG TACGGATCGG TCCGCTTCGA CCCCGTAGCC
361 TGCAGCTTCC GGGAACTGCT CCTGGAGGAT GGGTACAACA TCTACCACTC TGAGACCCTC
421 GGCCTCCCAC TTCGCCTGCC CGCCCACAAC TCTCCATACC GGGACTCGGC GCCCCGGGGG
481 CCTGCCCGCT TCCTGCCCCT GCCAGGCCTG CTTCCGGTCC CCCCGGACCC CCCAGGGATC
541 CTGGGCCCCG AGCCTCCCGA CGTGGGCTCC TCGGACCCCC TGAGCATGGT GGGGCCTTCA
601 CAGGGCCGAA GTCCCAGCTA CGCTTCCTGA

Oryctolagus cuniculus (rabbit) FGF21 gene coding sequence (SEQ ID NO: 292) (GenBank Accession No. XM_002723699, which is hereby incorporated by reference in its entirety)

1 ATGGACTGGG GCAAGGCCAA GTGCCGGCCC CCGGGGCTGT GGGTCCCCGC GCTCGCTGCC
61 CTGCTGCTGG GGGCCTGCCA GGCACACCCC ATCCCCGACT CCAGCCCCCT CCTCCAGTTT
121 GGGGACCAAG TGCGGCAGCA GCACCTGTAC ACGGACGATG CGCAGGAAAC AGAAGCCCAC
181 CTGGAGATCA GGGCGGATGG CACGGTGGTG GGGGCTGCCC GGAGGAGCCC AGAAAGTCTC
241 TTGCAGATGA AAGCCTTACA ACCGGGGATC ATTCAGATCT TGGGGGTCCA GACGTCCAGG
301 TTCCTCTGCC AGAGGCCGGA TGGCACGCTC TACGGCTCGC TCCACTTCGA CCGCGAGGCC
361 TGCAGCTTCC GGGAGCTGCT GCGTGAGGAT GGGTACAACG TTTACCTCTC GGAGGCCCTG
421 GGCCTGCCCC TGCGCCTGTC CCCCGGCAGC TCCCCACGCA GGGCGCCGGC CCCCCGGGGA
481 CCAGCCCGCT TCCTGCCGCT GCCCGGCCTG CCGCCAGACC TTCCGGAACC GCCAGGCCTC
541 CTGGCCGCCG CGCCCCCCGA TGTCGACTCC CCGGACCCCC TGAGCATGGT GCAGCCTGCG
601 CTGGACCAGA GCCCCAGCTA CACCTCCTGA

Gorilla gorilla (gorilla) FGF21 gene coding sequence (SEQ ID NO:293) (Ensembl Accession No. ENSGGOT00000001253, which is hereby incorporated by reference in its entirety)

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151 ATGGACTCGG ACGAGACCGG GTTCGAGCAC TCAGGACTGT GGGTTTCTGT GCTGGCTGGT
211 CTTCTGCTGG GAGCCTGCCA GGCACACCCC ATCCCTGACT CCAGTCCTCT CCTGCAATTC
271 GGGGGCCAAG TCCGGCAGCG GTACCTCTAC ACAGATGATG CCCAGCAGAC AGAAGCCCAC
331 CTGGAGATCA GGGAGGATGG GACGGTGGGG GGTGCTGCTG ACCAGAGCCC TGAAAGTCTC
391 CTGCAGCTGA AAGCCTTGAA GCCGGGAGTT ATTCAAATCT TGGGAGTCAA GACATCCAGG
451 TTCCTGTGCC AGAGGCCAGA TGGGGCCCTG TATGGATCGC TCCACTTTGA CCCTGAGGCC
511 TGCAGCTTCC GGGAGCTGCT TCTTGAGGAC GGATACAATG TTTACCAGTC CGAGGCCCAC
571 GGCCTCCCGC TGCACCTGCC GGGGAACAAG TCCCCACACC GGGACCCTGC ACCCCGAGGA
631 CCAGCTCGCT TCCTGCCACT ACCAGGCCTG CCCCCCGCAC CCCCGGAGCC ACCCGGAATC
691 CTGGCCCCCC AGCCCCCCGA TGTGGGCTCC TCGGACCCTC TGAGCATGGT GGGACCTTCC
751 CAGGGCCGAA GCCCCAGCTA CGCTTCCTGA
```

Nomascus leucogenys (Northern white-cheeked gibbon) FGF21 gene coding sequence (SEQ ID NO:294) (Ensembl Accession No. ENSNLET00000005931, which is hereby incorporated by reference in its entirety)

```
587        ATGG ACTCGGACGA GACCGGGTTC GAGCACTCAG GACTGTGGGT TCCTGTGCTG
647  GCTGGTCTTC TGCTGGGAGC CTGCCAGGCA CACCCCATCC CTGACTCCAG TCCTCTCCTG
707  CAATTCGGGG GCCAAGTCCG GCAGCGGTAC CTCTACACAG ATGATGCCCA GCAGACAGAA
767  GCCCACCTGG AGATCAGGGA GGATGGGACG GTGGGGGGCG CTGCTGACCA GAGCCCTGAA
831  AGTCTCCTGC AGCTGAAAGC CTTGAAGCCG GGAGTTATTC AAATCTTGGG AGTCAAGACA
891  TCCAGGTTCC TATGCCAGAG GCCAGATGGG GCCCTGTATG GATCGCTCCA CTTTGACCCT
951  GAGGCCTGCA GCTTCCGGGA GCTGCTTCTT GAGGACGGAT ACAATGTTTA CCAGTCCGAG
1011 GCCCATGGCC TCCCGCTGCA CCTGCCGGGG AACAAGTCCC CACACCGGGA CCCTGCACCC
1071 CGAGGACCAG CTCGCTTCCT GCCACTACCA GGCCTGCCCC CTGCACCCCC AGAGCCGCCC
1131 GGAATCCTGG CCCCCCAGCC CCCCGATGTG GGCTCCTCGG ACCCTCTGAG CATGGTGGGA
1191 CCTTCCCAGG GCCGAAGCCC CAGCTACGCT TCCTGA
```

Procavia capensis (hyrax) FGF21 gene coding sequence (SEQ ID NO:295) (Ensembl Accession No. ENSPCAT00000001288, which is hereby incorporated by reference in its entirety)

```
1   ATGGACTGGG CCAAGTTTGG GATCGAGCAC CCGGGACTGT GGGTCCCGGT GATGGCAGTA
61  CTTCTGCTGG GAGCCTGCCA AGGATACCCT ATTCCTGACT CCAGCCCCCT TCTCCAATTC
121 GGAGGCCAGG TCCGGCAACG TTACCTCTAC ACAGATGACG CGCAGGAGAC CGAGGCCCAC
181 CTGGAGATCC GAGCAGACGG CACGGTGGTG GGGGCTGCCC ACCGGAGCCC CGAGAGTCTC
241 TTGGAGCTGA AAGCTTTGAA GCCCGGCATA ATTCAGATCT TGGGAGTCAA GACATCCAGA
301 TTCCTCTGCC AGGGTCCTGA TGGGGTGCTG TATGGATCGC TCCGTTTTGA CCCAGTGGCC
361 TGCAGCTTCC GGGAGCTGCT TCTTGAAGAT GGATACAATG TTTACCAGTC TGAGGCCCAC
421 GGCCTCCCGC TTCGCCTACC ATCCCACAAT TCCCCACAGA GGGACCTGGC GTCCCGGGTG
481 CCAGCCCGCT TCCTGCCACT GCCAGGCCGG CTCACGGTGC TCCCAGAACC TTCGGGGGTC
541 CTGGGCCCTG AGCCCCCCGA TGTGGACTCC TCAGACCCCC TGAGCATGGT GGGGCCTTCG
601 CAGGGCCGAA GCCCCAGTTA CGCCTCCTGA
```

Cavia porcellus (guinea pig) FGF21 gene coding sequence (SEQ ID NO:296) (Ensembl Accession No. ENSCPOT00000000273, which is hereby incorporated by reference in its entirety)

```
1   ATGGACTGGG CCCGGACTGA GTGTGAGCGC CCAAGGCTGT GGGTCTCCAT GCTGGCCATC
61  CTTCTGGTGG GAGCCTGCCA GGCACACCCT ATCCCTGACT CCAGCCCCCT CCTCCAGTTT
121 GGGGGCCAGG TCCGGCAGCG GTACCTCTAC ACAGATGATG CTCAGGACAC TGAAGTGCAC
181 CTGGAGATCA GGGCCGATGG CTCAGTACGG GGCATTGCCC ACAGGAGCCC TGAAAGTCTC
241 CTGGAGCTGA AAGCCTTGAA GCCAGGAGTC ATTCAGATCT TGGGAATCAG GACTTCCAGG
301 TTCCTGTGCC AGAGGCCCGA TGGGAGTCTG TATGGATCAC TCCACTTTGA TCCTGAGGCC
361 TGCAGCTTCC GGGAGCTGCT GCTTGCTGAT GGCTACAATG TCTACAAGTC TGAAGCCCAC
421 GGCCTCCCTC TGCACCTGCT GCGCGGTGAC TCTCTATCGC AGGAACCAGC ACCCCCAGGA
481 CCAGCCCGAT TTCTGCCACT ACCAGGCCTG CCCGCAACAC CCCCGGAGCC ACCCAGGATG
541 CTGCCCCCAG GGCCCCCAGA TGTGGGCTCC TCGGACCCTT TGAGCATGGT GGGGCCTTTA
601 TGGGACCGAA GCCCCAGCTA TACTTCCTGA
```

Tupaia belangeri (tree shrew) FGF21 gene coding sequence (SEQ ID NO:297) (Ensembl Accession No. ENSTBET00000016056, which is hereby incorporated by reference in its entirety)

```
1   ATGGGCTGGG ACAAGGCCCG GTTCGAGCAC CTGGGAGCGT GGGCTCCTGT GCTGGCTGTC
61  CTCCTCCTGG GAGCCTGCCA GGCATACCCC ATCCCTGACT CCAGCCCCCT CCTACAATTC
121 GGGGGCCAGG TCCGGCAGCG GTACCTCTAC ACGGACGACA CGCAGGACAC AGAAGCCCAC
181 CTTGAGATCA GGGCCGACGG CACCGTGGTG GGGGCCGCCC ACCAAAGCCC GGAAAGTCTC
241 CTGGAGCTGA AAGCCTTGAA GCCGGGGGTC ATTCAAATCC TGGGAGTCAA GACCTCCAGG
301 TTCCTGTGCC AGAGGCCAGA CGGGGCCCTG TACGGGTCGC TTCACTTCGA CCCCGAGGCC
361 TGCAGCTTCC GGGAGCTGCT TCTCGAGGAT GGATACAACA TTTACCAGTC TGAGGCTCGT
421 GGCCTCCCCC TGCGCCTGCC GCCCCACGAC TCCCCACATC GGGACCGGAC CCCTCGGGGA
481 CCAGCTCGTT TCCTGCCGCT GCCTGGCCTG CCCCTGGTTC CTCCAGAGCT GCCAGGGGTC
541 CTGGCCCTTG AGCCCCCCGA CGTGGGCTCC TCAGACCCGC TGA
```

Sorex araneus (shrew) FGF21 gene coding sequence (SEQ ID NO:298) (Ensembl Accession No. ENSSART00000003074, which is hereby incorporated by reference in its entirety)

```
1   ATGGTCTGGG ACAAGGCCAG GGGGCAGCAG TTGGGACTGT GGGCCCCCAT GCTGCTGGGC
61  TTGCTGCTGG GTGCCTGCCA GGCACACCCC CTCCCTGACT CCAGCCCCCT CCTCCAATTT
121 GGGGGCCAAG TCCGACTGAG GTTCCTGTAC ACCGACGATG CCCAGAGGAC AGGGGCGCAC
181 CTGGAGATCA GGGCCGACGG CACAGTGCAG GGTGCGGCCC ACAGGACCCC AGAATGTCTC
241 CTGGAGCTGA AAGCCTTGAA GCCAGGCGTA ATTCAAATCC TTGGGGTCAG CACATCCAGA
301 TTCCTGTGCC AGCGGCCCGA TGGGGTCCTG TATGGATCGC TTCGCTTTGA CCCAGAGGCC
361 TGCAGTTTCC GGGAACTTCT TCTCCAGGAT GGATATAACG TTTACCAGTC TGAGGCCCTG
421 GGTCTCCCGC TCTACCTACA CCCGCCCAGT GCCCCAGTGT CCCAGGAACC AGCCTCACGG
481 GGCGCCGTCC GCTTCCTGCC ACTGCCAGGA CTGCCACCTG CCTCCCTGGA GCCCCCCAGG
541 CCCCCCGCCC CGGTGCCTCC AGACGTGGGT TCCTCAGACC CCCTGA
```

Ictidomys tridecemlineatus (squirrel) FGF21 gene coding sequence (SEQ ID NO:299)

```
1   ATGTACCCCA TCCCTGACTC AAGCCCCCTC CTCCAATTTG GGGGCCAAGT CCGGCAGCGG
61  TACCTGTACA CAGATGATGC CCAGGAGACT GAGGCCCACC TGGAGATCAG GGCTGATGGC
121 ACCGTGGTGG GGGCTGCCCA TCAAAGCCCG GAAAGTCTCT TGGAACTGAA AGCCTTGAAG
181 CCTGGGGGTCA TTCAAATCTT GGGGGTCAAA ACATCCAGGT TCCTGTGCCA GAGGCCAGAT
241 GGAGTGCTGT ATGGATCGCT CCACTTTGAC CCTGAGGCCT GCAGCTTCCG GGAGCAGCTT
301 CTGGAGGACG GGTACAACGT TTACCAGTCA GAATCCCACG GCCTCCCCGT GCGCCTGCCC
361 CCTAACTCAC CATACCGGGA CCCAGCGCCG CCAGGACCAG CCCGCTTCCT TCCACTGCCA
421 GGCCTGCCCC CAGCAGCCCT GGAGCCGCCA GGGATCCTGG GCCCTGAGCC CCCTGATGTG
481 GGCTCCTCCG ACCCACTCAG CATGGTGGGG CCTTTGCAGG GCCGAAGCCC CAGTTACGCT
541 TCCTGA
```

Loxodonta africana (elephant) FGF21 gene coding sequence (SEQ ID NO:300) (Ensembl Accession No. ENSLAFT00000022429, which is hereby incorporated by reference in its entirety)

```
1   ATGGACTGGG CCAAGTTTGG GTTGGAGCAC CCAGGACTGT GGGTCCCTGT GATGGCTGTC
61  CTTCTGCTGG GAGCCTGCCA GGGACACCCC ATCCCTGACT CCAGCCCCCT CCTCCAATTC
121 GGGGGCCAGG TCCGGCAACG TTACCTCTAC ACAGATGATC AGGAGACCGA GGCCCACCTG
181 GAGATCAGAG CAGATGGCAC AGTGGCGGGA GCCGCTCACC GGAGCTCTGA GAGTCTCTTG
241 GAGCTGAAAG CTTTGAAGCC TGGAATAATT CAGATCTTGG GGGTCAAGAC ATCCCGGTTC
301 CTGTGCCAGG GGCCTGATGG GGTGCTGTAC GGATCGCTCC ATTTCGACCC AGCCGCCTGC
361 AGCTTCCGGG AGCTGCTTCT TGAAGATGGA TACAATGTTT ACTGGTCCGA GGCCCATGGA
421 CTCCCAATCC GCCTGCCCTC CCACAACTCC CCATATAGGG ACCCAGCATC CCGGGTACCA
481 GCCCGCTTCC TGCCACTGCC AGGCCTGCTC CCAATGCTCC AAGAACCTCC AGGGGTCCTG
541 GCCCCTGAGC CCCCTGATGT GGACTCCTCA GACCCCCTGA GCATGGTGGG GCCTTCACAG
601 GGCCGAAGCC CCAGCTATGC CTCCTGA
```

Sus scrofa (pig) FGF21 gene coding sequence (SEQ ID NO:301) (GenBank Accession No. NM_001163410, which is hereby incorporated by reference in its entirety

```
131 ATGGGCTGGG CCGAGGCCAA GTTCGAGCGC TTGGGACTGT GGGTCCCTGT GCTGGCTGTC
191 CTGCTGGGAG CCTGCCAGGC ACGTCCCATT CCTGACTCCA GCCCCCTCCT CCAATTTGGG
251 GGCCAAGTGC GCCAACGATA CCTCTACACG GATGATGCCC AGGAAACTGA AGCCCACCTG
311 GAGATCAGAG CTGATGGCAC CGTGGCAGGG GTAGCCCGCC AGAGCCCTGA AAGTCTCTTG
371 GAGCTGAAAG CCCTGAAGCC AGGGGTCATT CAAATTTTGG GAGTCCAGAC ATCCCGGTTC
431 CTGTGCCAGG GGCCAGACGG GAGACTGTAC GGATCGCTCC ACTTCGACCC TGAGGCCTGC
491 AGCTTCCGGG AGCTGCTTCT TGAGGATGGC TACAACGTTT ACCAGTCTGA GGCCCTTGGC
551 CTCCCACTCC GGCTGCCTCC GCACCGCTCC TCCAACCGGG ACCTGGCCCC CCGGGGACCT
611 GCTCGCTTCC TGCCACTGCC AGGCCTGCCC CCGGCACCCC CGGAGCCGCC AGGGATCTTG
671 GCCCCTGAAC CTCCCGACGT GGGCTCCTCG GACCCCCTGA GCATGGTGGG GCCTTCACAC
731 GGCCGGAGCC CCAGCTACAC TTCTTGA
```

Felis catus (cat) FGF21 gene coding sequence (SEQ ID NO:302) (Ensembl Accession No. ENSFCAT00000007367, which is hereby incorporated by reference in its entirety)

```
1   ATGGGCTGGG ACGAGGCCGG GTCCCAGCGC CTGGGACTGT GGGTCGTGCT GGGGGTCCTT
61  TTGCCGGAAG CCTGCCAGGC ACACCCTATC CCTGACTCCA GCCCCCTCCT CCAATTCGGG
121 GGCCAAGTTC GACAGCGGTT CCTCTACACG GACGACGCCC AGGAGACAGA GGTCCACCTC
181 GAGATCAAGG CTGATGGCAC AGTGGTGGGG ACCGCTCGCC GGAGCCCTGA GAGTCTCTTG
241 GAGCTAAAAG CCCTGAAGCC GGGGGTAATT CAAATCTTGG GGGTCAAAAC GTCCAGGTTC
301 CTGTGCCAGG GCCCAGATGG GACACTGTAT GGATCGCTCC GCTTTGACCC CGCAGCCTGC
361 AGCTTCCGGG AACTGCTCCT GGAGGACGGA TACAACATCT ACCACTCGGA GACCCTCGGG
421 CTCCCACTCC GCCTGCCCCC CCACAACTCC CCATACCGGG ACTTGGCCCC CCGGGCACCT
481 GCCCGCTTCC TGCCGCTGCC AGGCCTGCTT CCGGCACCCC CGGAGCCTCC AGGGATCCTG
541 GCCCCCGAGC CCCCGGACGT GGGCTCCTCG GACCCTCTGA GCATGGTGGG GCCTTCCCAG
601 GGCCGAAGTC CCAGCTACGC TTCCTGA
```

Otolemur garnettii (bushbaby) FGF21 gene coding sequence (SEQ ID NO: 303) (Ensembl Accession No. ENSOGAT00000003585, which is hereby incorporated by reference in its entirety)

```
1   GACAAGGCCA GGACTGGGTT CAAGCACCCA GGACCATGGT TTCCCCTGCT GGCTGTACTT
61  TTGTTGGGAG CCTGCCAGGC ACACCCTATC CCTGACTCCA GCCCCCTACT CCAGTTTGGT
121 GGCCAAGTCC GGCAGCGGTA CCTCTACACA GATGATGCCC AGGAGACAGA AGCCCACCTG
181 GAGATCAGGG AAGATGGCAC AGTGGTGGGG GCTGCACAAC AGAGCCCTGA AAGTCTCTTG
241 GAGCTGAAAG CTTTAAAGCC AGGGGTCATT CAAATCTTGG GAGTCAAGAC ATCCAGGTTC
301 CTGTGCCAGA GGCCAGATGG GGGCCTATAT GGATCGCTCT ACTTTGACCC CAAGGCCTGC
361 AGTTTCCGGG AGCTGCTTCT TGAGGATGGA TACAACGTTT ACTGGTCTGA GACCTATGGC
421 CTCCCACTGC ACCTGCCTCC TGCCAATTCC CCATACTGGG GCCCATCCCT TCGGAGCCCA
481 GCCCGCTTCC TGCCACTGCC AGGCCCTCCT GCAGCATCCC CAGAGCTGCC GGGGATCTTG
541 GCCCTGGAAC CCCCCGATGT GGGCTCCTCG GACCCTCTGA GCATGGTGGG GCCTTCGCAG
601 GGCCGAAGCC CCAGCTATGC TTCCTGA
```

Rattus norvegicus (Norway rat) FGF21 gene coding sequence (SEQ ID NO: 304) (GenBank Accession No. NM_130752, which is hereby incorporated by reference in its entirety)

```
1   ATGGACTGGA TGAAATCTAG AGTTGGGGCC CCGGGACTGT GGGTCTGTCT CCTGCTGCCT
61  GTCTTCCTGC TGGGGGGTGTG CGAGGCATAC CCCATCTCTG ACTCCAGCCC CCTCCTCCAG
121 TTTGGGGGTC AAGTCCGACA GAGGTATCTC TACACAGATG ACGACCAGGA CACCGAAGCC
181 CACCTGGAGA TCAGGGAGGA CGGAACAGTG GTGGGCACAG CACACCGCAG TCCAGAAAGT
241 CTCCTGGAGC TCAAAGCCTT GAAGCCAGGG GTCATTCAAA TCCTGGGTGT CAAAGCCTCT
301 AGGTTTCTTT GCCAACAACC AGATGGAACT CTCTATGGAT CGCCTCACTT TGATCCTGAG
361 GCCTGCAGTT TCAGAGAGCT GCTGCTTAAG GACGGATACA ATGTGTACCA GTCTGAGGCC
421 CATGGCCTGC CCCTGCGTCT GCCCCAGAAG GACTCCCAGG ATCCAGCAAC CCGGGGACCT
481 GTGCGCTTCC TGCCCATGCC AGGCCTGCCC CACGAGCCCC AAGAGCAACC AGGAGTCCTT
541 CCCCCAGAGC CCCCAGATGT GGGTTCCTCC GACCCCCTGA GCATGGTAGA GCCTTTGCAA
601 GGCCGAAGCC CCAGCTATGC ATCTTGA
```

Mus musculus (house mouse) FGF21 gene coding sequence (SEQ ID NO:305) (GenBank Accession No. NM_020013, which is hereby incorporated by reference in its entirety)

```
185     ATGGAA TGGATGAGAT CTAGAGTTGG GACCCTGGGA CTGTGGGTCC GACTGCTGCT
241 GGCTGTCTTC CTGCTGGGGG TCTACCAAGC ATACCCCATC CCTGACTCCA GCCCCCTCCT
301 CCAGTTTGGG GGTCAAGTCC GGCAGAGGTA CCTCTACACA GATGACGACC AAGACACTGA
361 AGCCCACCTG GAGATCAGGG AGGATGGAAC AGTGGTAGGC GCAGCACACC GCAGTCCAGA
421 AAGTCTCCTG GAGCTCAAAG CCTTGAAGCC AGGGGTCATT CAAATCCTGG GTGTCAAAGC
481 CTCTAGGTTT CTTTGCCAAC AGCCAGATGG AGCTCTCTAT GGATCGCCTC ACTTTGATCC
541 TGAGGCCTGC AGCTTCAGAG AACTGCTGCT GGAGGACGGT TACAATGTGT ACCAGTCTGA
601 AGCCCATGGC CTGCCCCTGC GTCTGCCTCA GAAGGACTCC CCAAACCAGG ATGCAACATC
661 CTGGGGACCT GTGCGCTTCC TGCCCATGCC AGGCCTGCTC CACGAGCCCC AAGACCAAGC
721 AGGATTCCTG CCCCCAGAGC CCCCAGATGT GGGCTCCTCT GACCCCCTGA GCATGGTAGA
781 GCCTTTACAG GGCCGAAGCC CCAGCTATGC GTCCTGA
```

Vicugna pacos (alpaca) FGF21 gene coding sequence (SEQ ID NO:306) (Ensembl accession no. ENSVPAT00000005993, which is hereby incorporated by reference in its entirety) (1-209, excluding 79-168 and 172-182)

```
1   ATGGACTGGG ACGAGGCCAA GTTCGAGCAT CGGGGACTGT GGGTCCCAGT GCTCACTGTC
61  CTTCTGCTGG GAGCCTGCCA GGCACGCCCC ATTCCTGACT CCAGCCCCCT CCTCCAATTC
121 GGGGGCCAAG TCCGGCAGCG GTACCTCTAC ACGGATGACG CCCAGGAGAC AGAAGCCCAC
181 CTGGAGATCA GGGCTGATGG CACAGTGGTG GGGGTGGCCC GCCAG---CC CGAA------
241 ---------- ---------- ---------- ---------- ---------- ----------
301 ---------- ---------- ---------- ---------- ---------- ----------
361 ---------- ---------- ---------- ---------- ---------- ----------
421 ---------- ---------- ---------- ---------- ---------- ----------
481 ---------- ---------- ----GGAATT CCT------- ---------- ----------
541 ------CCCG AGCCTCCTGA CGTGGGCTCC TCAGACCCCC TGAGCATGGT GGGGCCTTCA
601 TACAGCAGAA GCCCCAGCTA CACTTCCTGA
```

Anolis carolinensis (anole lizard) FGF21 gene coding sequence (SEQ ID NO:307) (Ensembl accession no. ENSACAT00000017230, which is hereby incorporated by reference in its entirety)

```
1   TGTAAAAGCA AGGGAGGAGG GAAGGGGGGA GAGAGGATGT GGGTAGACCT AGTTTTCTGG
61  GCTGCCTTGC TCCGCACAGC TCCTGCTCTT CCCTTGCGGA ATTCCAACCC CATCTACCAA
121 TTTGATGGGC AGGTCCGGCT TCGGCACCTC TACACAGCAG ATGAACAGAC GCACCTCCAC
181 TTGGAGATCT TGCCAGACGG TACCGTGGGT GGATCCAGGT TTCAGAATCC CTTCAGTTTG
241 ATGGAGATCA AAGCTGTGAA GCCAGGAGTC ATTCGCATGC AGGCCAAGAA GACCTCTAGA
301 TTTCTCTGTA TGAAACCCAA TGGACGACTG TATGGCTCGC TGTTCTACTC TGAGGAGGCA
361 TGCAACTTCC ATGAGAAGGT TCTCAGCGAT GGCTACAACC TCTACTATTC TGAAAACTAC
421 AACATACCTG TCAGCCTCAG CTCGGCAGGG AACCTGGGTC AGAGCCGTCA GTTGCCTCCC
481 TTCTCCCAAT TCCTGCCGTT AGTCAACAAA ATTCCTCTTG AGCCTGTGCT TGAAGACTTT
541 GACTTCTATG GACATCAATT GGATGTTGAA TCAGCTGATC CTTTGAGCAT TTTAGGACAA
601 AACCCTGGTT TCATGAGTCC GAGCTATGTC TTC
```

Gadus morhua (cod) FGF21 gene coding sequence (SEQ ID NO:308) (Ensembl accession no. ENSGMOT00000014151, which is hereby incorporated by reference in its entirety)

```
1   CTCCTCCTCG CCACCCTCCT CCACATCGGC CTCTCCTTCT ACGTCCCCGA CTCCGGCCCC
61  CTGCTGTGGC TGGGCGACCA GGTCAGGGAG AGACACCTCT ACACAGCAGA GAGCCACCGG
121 AGGGGGCTGT TCCTGGAGAT GAGCCCGGAC GGTCAGGTGA CAGGAAGTGC TGCTCAGACG
181 CCGCTCAGTG TTCTGGAGCT GAGGTCGGTC AGAGCAGGAG ATACGGTCAT CAGAGCGCGC
241 CTCTCCTCTC TCTACCTGTG TGTGGACAGG GCAGGTCACC TGACAGGACA GAGACAGTAC
301 ACAGAGTCCG ACTGCACCTT CAGAGAGGTC ATCCTTGAGG ACGGCTACAC CCACTTCCTG
361 TCCGTGCACC ACGGACTTCC TATTTCGCTG GCGCCGAGAC ACTCCCCAGG GAGACAGGGG
421 CTGCGCTTCA GCAGGTTCCT CCCGCTGAGG AGCAGTCTGT CAGAGGATAG GGTCGCCGAG
481 CCCCCAGACA GCCCACTGAA CCTGGACTCT GAAGACCCCC TGGGGATGGG TCTGGGTTCG
541 CTCCTCAGCC CGGCCTTCTC CATG
```

Latimeria chalumnae (coelacanth) FGF21 gene coding sequence (SEQ ID NO:309) (Ensembl accession no. ENSLACT00000003815, which is hereby incorporated by reference in its entirety)

```
1   ATGTTATGCC AGAGTTTTGT GATATTAAGT CAGAAATTCA TTTTTGGGCT CTTTTTGACT
61  GGATTGGGGC TAACAGGATT GGCTTGGACA AGGCCCTTCC AGGATTCCAA TCCCATCCTG
121 CAGTATTCCG ATTCCATCCG GCTCCGACAT CTGTACACTG CCAGTGAGAG TCGGCACCTT
181 CACCTACAAA TCAACTCGGA TGGACAGGTG GGAGGGACAA CCAAGCAAAG CCCTTACAGT
241 CTGTTGGAGA TGAAGGCGGT GAAGACAGGT TTTGTGGTCA TCAGGGGCAA GAAAAGCGCC
301 CGTTACCTCT GTATGGAACG TAGTGGACGG CTCTATGGAT CGCTGCAGTA TACAGAAAAA
361 GACTGCACCT TCAAAGAGGT TGTGTTGGCA GATGGATACA ACCTGTATGT CTCAGAGGAA
421 CACCAGGCCA CAGTGACGCT GAGCCCCATG AGGGCGAGGA TAGCGCAAGG GAAAAAGATC
481 CCACCCTTTT CCCATTTCCT TCCAATGGTG AACAAGGTGC CTGTGGAGGA TGTTGCCGCT
541 GAGATGGAGT TTGTCCAGGT GCTGCGGGAA ATGACGGCCG ACGTGGACTC TCCGGATCCC
601 TTTGGAATGA CCTGGGAAGA ATCGGTTCAC AGTCCGAGCT TTTTTGCC
```

Tursiops truncatus (dolphin) FGF21 gene coding sequence (SEQ ID NO:310) (Ensembl accession no. ENSTTRT00000014561, which is hereby incorporated by reference in its entirety)

```
1   ATGGGCTGGG ACAAGACCAA ACTCGAGCAC CTGGGACTGT GGGTCCCTGT GCTAGCTGTC
61  CTGCTGGGAC CCTGCCAGGC ACATCCCATT CCTGACTCCA GCCCCCTCCT CCAATTTGGG
121 GGCCAAGTCC GCCAGCGATA CCTCTACACG GATGACGCCC AGGAGACGGA GGCCCACCTG
181 GAGATCAGGG CTGATGGCAC AGTGGTGGGG ACGGCCCGCC GGAGCCCCGA AGGAGTTAAA
241 ACATCCAGGT TCCTGTGCCA GGGGCCAGAG GGGAGGCTGT ATGGATCGCT CCACTTCAAC
301 CCCCAGGCCT GCAGCTTCCG GGAGCTGCTT CTTGAGGATG GATACAACGT TTACCAGTCT
361 GAGGCTCTTG GCATTCCCCT CCGCCTGCCC CCGCACCGCT CCTCCAACTG GGACCTGGCC
421 CCCCGGGGAC CTGCTCGCTT CCTGCCGCTG CCAGGCTTCC TCCCGCCACC CCTGGAGCCT
481 CCAGGGATCT TGGCCCCCGA GCCTCCCAAC GTAGGTTCCT CGGACCCCTT GAGCATGGTG
541 GGACCTTCAC ATGGCCGAAG CCCCAGCTAC ACTTCCTGA
```

Mustela putorius furo (ferret) FGF21 gene coding sequence (SEQ ID NO:311) (Ensembl accession no. ENSMPUT00000003755, which is hereby incorporated by reference in its entirety)

```
188        ATG GGCTGGGAAG AGGCCAGGTC CGAGCACCTG GGGCTGTGGG TCCCTGTGCT
241 GGCGGTCCTT TTGCTGGGAG CCTGCCAGGC ATACCCTATT CCTGACTCCA GCCCCCTCCT
301 CCAATTTGGA GGCCAAGTTC GACAGCGGTA CCTCTACACA GACGACGCTC AGGAGACGGA
361 GGCCCACCTA GAGATCAGGG CTGATGGCAC GGTGGTGGGG GCTGCCCGCC GGAGCCCCGA
421 AAGTCTCTTG GAGCTGAAAG CCCTGAAGCC AGGGGTCATT CAGATCTTGG GAGTGAAAAC
481 ATCCAGGTTC CTGTGCCAGG GCCCGAATGG GACACTGTAC GGATCGTTCC ACTTCGACCC
541 CGTAGCCTGC AGCTTCCGGG AAGTGCTTCT GGAAGATGGA TACAACATCT ACCACTCTGA
601 GACCCTGGGC CTCCCACTGC GCCTGCCCCC CCACAACTCC CCACACAGGG ACCTGGCGCC
661 CCGGGGGCCT GCCCGCTTCC TGCCCCTGCC AGGCCTGCTT CCGGCCACCC CGGAGTCCCG
721 GGGGATCCCA GCCCCCGAGC CTCCCAACGT GGGCTCCTCA GACCCCCTGA GCATGGTGGG
781 GCCTTTGCAG GGTCAAAGTC CCAGCTACAC TTCCTGA
```

Takifugu rubripes (fugu) FGF21 gene coding sequence (SEQ ID NO:312) (Ensembl accession no. ENSTRUT00000034076, which is hereby incorporated by reference in its entirety)

```
1   TTTATTTATT TATTTATTCA AACTGCACTT TTTTCCCCTT CCAAATGGTT CAACTTTTAT
61  CTCCCTGACT CCAACCCGCT CTTATCCTTT GACAGTCATG GCAGAGGCAT CCACCTCTAC
121 ACAGATAATC AAAGGCGAGG GATGTATCTG CAGATGAGCA CAGATGGAAG CGTTTCCGGG
181 AGTGATGTCC AGACGGCGAA CAGTGTGCTG GAACTGAAGT CAGTCAGAAA CGGCCACGTC
241 GTCATCCGAG GAAAATCGTC TTCTCTGTTT CTCTGTATGG ACAGCAGAGG CCGTTTATGG
301 GGGCAGAGGC ACCCCACTGA GGCCGACTGC ACTTTCAGGG AAGTGTTGCT GGCAGATGGA
361 TACACTCGCT TCCTGTCCCT GCACAACGGA ACTCCTGTGT CTCTGGCACC TAAACAATCT
421 CCAGACCAGC ACACAGTCCC CTTCACTCGT TTCCTGCCGC TCAGGAATAC ACTGGCAGAG
481 GAGAGCATGT CTGAACCACC ATCAAACCAA CAGAGATATT TTAACATTGA CTCTGATGAT
541 CTTCTTGGAA TGGATTTAAA TGCGATGGTC AGTCCTCAGT TTTCAGGGGA CAAGTGA
```

Dipodomys ordii (kangaroo rat) FGF21 gene coding sequence (SEQ ID NO: 313) (Ensembl accession no. ENSDORT00000001234, which is hereby incorporated by reference in its entirety)

```
1   ATGGACCAGG CAAAGACCAG GGTTGGGGCC CGGGGGCTGG GGGGCCTTGT GCTGGCTGTC
61  ATAATTCTGG GAGCATGCAA GGCACGGCCT ATCCCTGACT CCAGCCCCCT CCTCCAATTT
121 GGGGGTCAAG TTCGGCTTCG GCACCTCTAC ACAGATGACA CTCAGGAGAC GGAAGCCCAT
181 CTGGAGATCA GGGCAGATGG CACGGTAGTG GGGACTGCCC ACCGGAGCCC TGAAAGTCTC
241 TTGGAGCTGA AAGCCTTGAA GCCAGGAGTC ATTCAAATCT TAGGGATCAA GACATCCAGA
301 TTCTTATGCC AGAGACCAGA CGGGACACTG TATGGATCAC TCCACTTTGA CCCTGAGGTT
361 TGCAGCTTCC AGGAGCTGCT TCTGGAAGAT GGATACAACA TTTACCGTTC TGAAGCCCTG
421 GGTCTCCCCC TGCGCCTGTC CCCAGATCCA GCACCCTGGG GGCCAGCCCG CTTCCTGCCC
481 CTGCCTGGTG TGCCCCCCGC ACCGCCGGAG CCCCCCGGGA TCCTGGCTCC CGAACCCCCT
541 GATGTCGGCT CCTCCGACCC TCTGAGTATG GTGGGACTGT TGCAGGGCCG AAGCCCCAGC
601 TATGCATCCT GA
```

Echinops telfairi (lesser hedgehog tenrec) FGF21 gene coding sequence (SEQ ID NO:314) (Ensembl accession no. ENSETET00000010721, which is hereby incorporated by reference in its entirety)

```
1   ATGGGTTGCA CCAAATCTGG GTGGAAGTCC CCGGGACTGT GGGTCCCTGT GCTGGCCAGC
61  CTTCTGCTGG GAGGCTGCGG AGCACACCCC ATCCCTGACT CCAGCCCCCT CCTCCAATTC
121 GGGGGCCAAG TCCGGCAGCG ATACCTCTAT ACGGATGACG CCCAGACCAC CGAGGCCCAC
181 CTGGAGATCA GAGCGGATGG CACAGTGGGG GGCGTCGCCC ACCAGAGCCC AGAGAAGTTC
241 CTGAGTCAAT GGCGTGAAAA GCCCCTGAGA TCACTCCATT TCGACCCAGC CGCCTGCAGC
301 TTCCGGGAGA AGCTTCTAGA AGACGGATAC AACTTGTACC ACTCTGAGAC CCACGGCCTC
361 CCCCTCCGCC TCCCACCCCG TGGGGGCGAC CCCTCTTCTC AGCCTGGGGC CCGCTTCCCA
421 CCGCTGCCGG GCCAGCTCCC ACAACTCCAA GAGACGCCAG GGGTCCTCGC CCCCGAACCC
481 CCCGACGTGG GCTCTTCAGA CCCCCTGAGC ATGGTGGGGC CTTGGCGAGG GCAAAGTCCC
541 AGTTATGCCT CCTGA
```

Macaca mulatta (rhesus monkey) FGF21 gene coding sequence (SEQ ID NO:315) (Ensembl accession no. ENSMMUT00000038440, which is hereby incorporated by reference in its entirety)

```
1   ATGGACTCGG ACGAGACCGG GTTCGAGCAC TCAGGACTGT GGGTTCCTGT GCTGGCTGGT
61  CTTCTGCTGG GAGCCTGCCA GGCACACCCC ATCCCTGACT CCAGTCCTCT CCTGCAATTC
121 GGGGGCCAAG TCCGGCAACG GTACCTCTAC ACAGATGATG CCCAGCAGAC AGAAGCCCAC
181 CTGGAGATCA GGGAGGATGG GACAGTGGGG GGCGCTGCTC ACCAGAGCCC CGAAAGTGAG
241 TGTGGGCCAG AGCCTGGGTC TGAGGGAGGA GGGGCTGTGG GAGGTGCTGA GGGACCTGGA
301 CTCCTGGGTC TGAGGGAGGC AGGGCTGGGG CCTGGATCCT GGCTCCACTT TGACCCTGAG
361 GCCTGCAGCT TCCGGGAGCT GCTTCTTGAG AACGGATACA ATGTTTACCA GTCCGAGGCC
421 CACGGCCTCC CACTGCACCT GCCGGGAAAC AAGTCCCCAC ACCGGGACCC TGCATCCCAA
481 GGACCAGCTC GCTTCCTGCC ACTACCAGGC CTGCCCCCCG CACCCCCGGA GCCGCCAGGA
541 ATCCTCGCCC CCCAGCCCCC CGATGTGGGC TCCTCGGACC CTCTGAGCAT GGTGGGACCT
601 TCCCAGGCCC GAAGCCCCAG CTATGCTTCC TGA
```

Microcebus murinus (mouse lemur) FGF21 gene coding sequence (SEQ ID NO:316) (Ensembl accession no. ENSMICT00000013258, which is hereby incorporated by reference in its entirety)

```
1   ATGGGCTGGG ACGAGGCCGG CGCCGGGTTC GAGCACCCAG GACTGTGGTT TCCCATGCTG
61  GGTGTCCTGC TGCTGGGAGC CTGCCAGGCG TACCCCATCC CTGACTCCAG CCCCCTCCTC
121 CAATTTGGCG GCCAAGTCCG GCAGCGGCAC CTCTACACAG ACGATATCCA GGAGACAGAA
181 GCCCACCTGG AGATCAGGGC GGACGGCACA GTGGTGGGGG CCGCCCGACA GAGCCCTGAG
241 TTGGAGCTGA AAGCCTTAAA GCCAGGGGTC ATTCAAATCT TGGGAGTCAA GACCTCCAGG
301 TTCCTGTGCC AGAGGCCAGA CGGGGCCCTG TACGGATCGC TCCACTTTGA CCCCGAGTGC
361 AGCTTCCGGG AGCTGCTTCT TGAGGATGGA TACAACGTCT ACTGTCCCTA CCTCCCGCTG
421 CACCTGTCCC CACGCATCGA ACTGGCCGGA TCACGCTCTG CGCTGCCACT GCCCCCAGCA
481 CCTGAACGCA GGATTTTGGC CCCGGAGCCC CCGGATGGCT CCTCGGACCC TCTGAGCATG
541 GTGGGGCCTT CGCAGGGCCG AAGTCCCAGC TATGCTTCCT GA
```

Ochotona princeps (pika) FGF21 gene coding sequence (SEQ ID NO:317) (Ensembl accession no. ENSOPRT00000007373, which is hereby incorporated by reference in its entirety)

```
1   AAAGACATGG ACGGGCTCCA GCCTCCGGGG CTGCGGGTTC CTGTGCTGGC TGCCCTGCTT
61  TTGGGAGTTG GCCAGGCACG CCCCATCCCT GATTCTAGCC CTCTCCTCCA ATTCGGGGGC
121 CAGGTCCGGC AGAGGCACCT CTACACGGAT GACGCCCAGG AATCGGAAGT ACACCTGGAG
181 ATCCGGGCAG ACGGCACCGT GGCAGGGACT GCCCGCCGGA GCCCTGAAAG TCTCTTAGAA
241 ATGAAAGCGT TGAAGCCAGG CGTCATTCAG ATCCTGGGGG TCCACACATC CAGGTTCCTG
301 TGCCAGAGAC CAGACGGGAC GCTGTACGGC TCGCTCCACT TCGACCACAA GGCCTGCAGC
361 TTCCGGGAGC AGCTGCTGGA GGATGGGTAC AACGTGTACC ACTCAGAGAC ACACGGCCTC
421 CCGCTGCGCC TGTCTCCAGA CCGAGCCCCC CGGGGCCCAG CCCGCTTCCT GCCACTGCCA
481 GGCCCTCCTC CTGACCTCCT GGTGCCACCC CTGCCACCGG ACGTCCTAGC CCCTGAGCCC
541 CCCGACGTGG ACTCCCCAGA CCCCCTGAGC ATGGTGGGGC CCTTGCAGGG CCAAAGCCCC
601 AGCTACACTT CCTGA
```

Xiphophorus maculatus (platyfish) FGF21 gene coding sequence (SEQ ID NO:318) (Ensembl accession no. ENSXMAT00000001579, which is hereby incorporated by reference in its entirety)

```
1   TGCCCGTTCC CCTTCCTTTT CTTAATCCTC TCTCTTCCCT TTTTCTCTTC CTCGTTTTAC
61  ATCCCAGAAT CCAACCCAAT CTTTGCCTTC AGGAATCAGC TCAGAGAGGT GCATCTCTAC
121 ACAGAAAATC ACAGACGGGG TTTGTATGTG GAGATACATC TGGATGGGAG AGTGACTGGA
181 AGTGATGCTC AGAGTCCTTA TAGTGTGTTG CAGATAAAGT CTGTTAAACC GGGTCATGTG
241 GTCATAAAGG GACAGACATC GTCCCTGTTC CTCTGCATGG ACGACTCCGG GAATCTAAGA
301 GGACAGACAA CCTATGACGA GGCTGACTGC TCCTTCAGGG AACTGCTGCT GGCCGATGGC
361 TACACCCGTT TCCTGAACTC ACAACATGGC GTTCCTTTAT CACTGGCATC CAGAAACTCT
421 CCAGATCGAC ACTCCGTTCC TTTCACAAGA TTTTTACCTC TCAGGAATAC TTTAACGGTT
481 TCAGAAGAAT CAACAAAAAC TCAGAGGGAC TTCAACCTGG ACTCGGACGA CCTTCTCGGG
541 ATGGGA
```

Gasterosteus aculeatus (stickleback) FGF21 gene coding sequence (SEQ ID NO:319) (Ensembl accession no. ENSGACT00000010725, which is hereby incorporated by reference in its entirety)

```
1   TCTCTCCTCC TCATGGTCCC ACTTCCTTTC TGTTCATCCT TTTATCTCAC TGACTCCAGC
61  CCACTTCTAC CCTTCAATAA TCAAGTCAAA GAGGTGCACC TCTACACAGC AGAGAATCAC
121 AGAAGAGCGA TGTACCTGCA GATCGCTCTG GACGGGAGCG TGTCGGGAAG CGACGCTCGG
181 TCCACTTACA GTGTGCTGCA GCTGAAATCT ATCCAGCCGG GCCACGTGGT CATCAGAGGG
241 AAGGCCTCCT CCATGTTCCT CTGCGTGGAC AGCGGGGGCC GTTTGAGAGG ACAGGGGCCG
301 TACTCAGAGG CCGACTGCAG CTTCAGGGAG CTGCTGCTGG GGGATGGCTA CACCCGGTTC
361 CTGTCCTCGC AGCACGGGTC CCCGCTGTCT CTGGCGTCGA GGCCTTCCCC GGATCCCAAC
421 TCGGTGCCCT TCACTCGATT CCTACCCATC CGGACCGCCC CCGAGGCTGA GAGCGTGATC
481 GAAGAGCCAC CGAGCAATCA GAGATACGTC AACGTGGACT CCGAGGATCT TCTTGGAATG
541 GGCCTGAACA CTGTGGTCAG TCCTCAGTTC TCGGCG
```

Sarcophilus harrisii (Tasmanian devil) FGF21 gene coding sequence (SEQ ID NO:320) (Ensembl accession no. ENSSHAT00000006017, which is hereby incorporated by reference in its entirety) (1-209, excluding 1-2 and 173-209)

```
132             GTGTCTGCC ATGGGCCTGA GGGAGCGAGC TCCCAGGTAC CTGGCCCCGC
181 TGCTGTCCTT GCTCTTGGCC TGCAGGGCCT CGGGTCACCC CCTCCCGGAT TCCAGCCCCA
241 TGCTCCTGTT TGGGGGGCAG GTCCGCCTCC GGCACCTCTA CACGGATGTG GGCCAGGAGG
301 CCGAGGCCCA CGTGGAACTG GCGTCCGACG GCACAGTCCG GGCGGCAGCG CGGAGGAGTC
361 CCAACAGTCT CCTGGAGCTG AAGGCTGTGA AGCCGGGCAT CGTCCGAATC CTGGCCGTCC
421 ACAGCTCTCG GTTTCTGTGT ATGAGGCCCA ACGGGGAGCT GTACGGAGCG ATACACTACG
481 ACCCTTCCGC CTGCAACTTT CGGGAGCGCC TGCTGGGGGA CGGCTACAAC GTGTACGAGT
541 CCGAGGCTCA CGGGAGGACC CTCCGCCTGC CCCCCAAGGC CGCACCGGGA CCCGCCGGAC
601 CTTCTCGCTT CCTGCCGCTC CCCGGC
```

Macropus eugenii (wallaby) FGF21 gene coding sequence (SEQ ID NO:321) (Ensembl accession no. ENSMEUT00000015309, which is hereby incorporated by reference in its entirety)

```
1   ACAGAGGAGC CTTCTACTGG GTCCAGGCAC CTGGGACAAT GGGCTCCCGG GCTGCCTGGT
61  CCTCTGCTGT CCTTGCTCCT GGCCTACAGG GGCTGGGGCT CCCCCATCCC TGATTCCAGC
121 CCCATGCTCC TGTTTGGTGG CCAGGTCCGC CTCCGACACC TGTACACAGA TGATGGCCAG
181 GACACGGAGG CCCATGTGGA GCTGGGGCCA GATGGAGTGG TTCGAGCTGT GGCTGAGAGG
241 AGCCCCAACA GTCTTCTGGA ACTGAAGGCG GTGAAGCCTG GAGTCATCCG AATCCTCGCT
301 GTCCAGAGCT CTCGGTTTCT GTGTATGAGG CCCAACGGGG AACTGTATGG AGCGGTACAC
361 TATGACCCTT CTGCCTGCAA CTTTCGGGAA CATCTGCTGG GGGATGGTTA TAATGTGTAT
421 GAATCAGAGA CTCACAGAAG GACCCTCCGT CTGTCCCCAT CCCTGGGTCA GGCTGGCCCC
481 TCTCGCTTCC TGCCACTTCC AGGCGACTGG CTGCCCGGCC CTGATCCACC TTGGGCACAG
541 GGCCCTGAGC CCCCAGACGT GGGCTCTGCA GACCCCCTGA GCATGGTGGG GGCCGTGCAG
601 GGCCTCAGCC CCAGCTACTC CTCCTGA
```

Xenopus tropicalis (Western clawed frog) FGF21 gene coding sequence (SEQ ID NO:322) (Ensembl accession no. ENSXETT00000009917, which is hereby incorporated by reference in its entirety) (1-209, excluding 170-209)

```
1   AGAGGGGGTA GGACCAAAAA AAAGACGTTA CTCAGGAAAT GGCTTTGCCT TTTAGCCATT
61  ATGTTGAGTA GGTCAAGGTT TTCTTTAGCA AATCCTATCC AGAATTCGAA CCCAATCTTA
121 TCCAACGACA ACCAAGTACG GACTCAGTAT TTATACACAG ATAACAATAA CATGCACCTG
181 TATCTTCAGA TCACCCACAA TGGAGTAGTA ACTGGTACCG AAGAAAAGAA TGACTATGGT
241 GTGCTGGAAA TAAAGGCAGT AAAAGCTGGG GTTGTAGTTA TAAAAGGAAT TCGAAGCAAT
301 CTCTACCTAT GCATGGATTC TAGACACCAA TTGTATGCGT CGGCATATGA TAAAGATGAC
361 TGCCATTTCC ATGAAAAGAT CACACCAGAT AATTACAACA TGTATAGCTC AGAGAAGCAT
421 TCAGAATACG TGTCCTTAGC TCCATTAAAA GGAAGCCAGA TGGCTCGTTT TCTACCTATA
```

Danio rerio (zebrafish) FGF21 gene coding sequence (SEQ ID NO:323) (Ensembl accession no. ENSDART00000103511, which is hereby incorporated by reference in its entirety)

```
30                                  A TGCTTCTTGC CTGCTTTTTT ATATTTTTTG
61  CTCTTTTTCC TCATCTTCGG TGGTGTATGT ATGTTCCTGC ACAGAACGTG CTTCTGCAGT
121 TTGGCACACA AGTCAGGGAA CGCCTGCTTT ACACAGATGG GTTGTTTCTT GAAATGAATC
181 CAGATGGCTC CGTCAAAGGC TCTCCTGAAA AGAATCTAAA TTGTGTGCTG GAGCTGCGTT
241 CAGTCAAAGC GGGTGAAACC GTCATCCAGA GTGCAGCTAC ATCTCTCTAC CTCTGCGTCG
301 ATGATCAAGA CAAGCTGAAA GGACAGCATC ATTACTCTGC ACTAGACTGC ACCTTTCAGG
361 AATTGCTACT GGATGGATAT TCGTTTTTCC TTTCTCCACA CACTAATCTT CCCGTATCGC
421 TCCTCTCGAA ACGTCAGAAA CACGGCAATC CTCTTTCTCG CTTCCTCCCT GTTAGCAGAG
481 CAGAGGACAG CCGGACACAG GAGGTGAAAC AGTATATTCA GGATATAAAC CTGGACTCTG
541 ACGACCCACT AGGAATGGGA CATCGGTCAC ACTTACAGAC CGTCTTCAGT CCCAGTCTGC
572 ATACTAAAAA ATGA
```

Bos grunniens mutus (yak) FGF21 gene coding sequence (SEQ ID NO:324) (generated using SMS Reverse Translate tool on the ExPASy Bioinformatics Resource website (www.expasy.org))

```
1   ATGGGCTGGG ATGAAGCGAA ATTTAAACAT CTGGGCCTGT GGGTGCCGGT GCTGGCGGTG
61  CTGCTGCTGG GCACCTGCCG CGCGCATCCG ATTCCGGATA GCAGCCCGCT GCTGCAGTTT
121 GGCGGCCAGG TGCGCCAGCG CTATCTGTAT ACCGATGATG CGCAGGAAAC CGAAGCGCAT
181 CTGGAAATTC GCGCGGATGG CACCGTGGTG GGCGCGGCGC GCCAGAGCCC GGAAAGCCTG
241 CTGGAACTGA AAGCGCTGAA ACCGGGCGTG ATTCAGATTC TGGGCGTGAA AACCAGCCGC
301 TTTCTGTGCC AGGGCCCGGA TGGCAAACTG TATGGCAGCC TGCATTTTGA TCCGAAAGCG
361 TGCAGCTTTC GCGAACTGCT GCTGGAAGAT GGCTATAACG TGTATCAGAG CGAAACCCTG
421 GGCCTGCCGC TGCGCCTGCC GCCGCAGCGC AGCAGCAACC GCGATCCGGC GCCGCGCGGC
481 CCGGCGCGCT TTCTGCCGCT GCCGGGCCTG CCGGCGGAAC CGCCGGATCC GCCGGGCATT
541 CTGGCGCCGG AACCGCCGGA TGTGGGCAGC AGCGATCCGC TGAGCATGGT GGGCCCGAGC
601 TATGGCCGCA GCCCGAGCTA TACCAGCTAA
```

Saimiri boliviensis boliviensis (Bolivian squirrel monkey) FGF21 gene coding sequence (SEQ ID NO:325) (GenBank accession no. XM_003940326, which is hereby incorporated by reference in its entirety)

```
163                                                 atgggctc ggaggaggtc
181 GCGTTGGAGC GCCCTGCACT GTGGGTCTCT GTGTTGGCTG GTCTCCTGCT GGGAACCTGC
241 CAGGCATACC CCATCCCTGA CTCTAGTCCC CTCCTGCAAT TTGGAGGCCA AGTCCGGCAG
301 CGGTACCTCT ACACAGATGA CGCTCAGCAG ACAGAAGCCC ACCTGGAGAT CAGGGAAGAT
361 GGCACGGTGG CGGGGGCTGC CCACCAGAGC CCCGAAAGTC TCTTGCAGCT GAAAGCCTTA
421 AAGCCAGGGG TTATTCAAAT CTTGGGAGTC AAGACCTCCA GGTTCCTGTG CCAGAGGCCG
481 GACGGGGCCC TGTACGGATC GCTCTACTTT GACCCCGAGG CCTGCAGCTT CCGGGAGCTG
541 CTTCTTGAGG ACGGATACAA TGTGTACCAG TCCGTGGCCC ACAGCCTCCC GCTGCACCTG
601 CCAGGGGGCA GGTCCCCACC CTGGGACCCT GCACCTCGAG GACCAGCTCG CTTCCTGCCG
661 CTACCAGGCC TGCCCCCCGA ACCCCCCGAG GCGCCAGGAA TCCTGGCCCC CGAGCCCCCC
721 GATGTGGGCT CCTCAGACCC TCTGAGCATG GTGGGGCCTT CCCAAGGCCA AAGCCCCAGC
781 TACACTTCCT GA
```

Callithrix jacchus (white-tufted-ear marmoset) FGF21 gene coding sequence (SEQ ID NO:326) (GenBank accession no. XM_003735621, which is hereby incorporated by reference in its entirety)

```
1   ATGGGCTCGG AGGAGGTCGG GTTGGAGCAC CCTGCACTGT GGGTTTCTGT GCTGGCTGGT
61  CTCCTGCTGG GAACCTGCCA GGCGCACCCC ATCCCTGACT CCAGTCCCCT CCTGCAATTT
121 GGAGGCCAAG TCCGGCAGCG GTACCTCTAC ACAGATGACG CCCAGCAGAA AGAAGCCCAC
181 CTGGAGATCN AGGAAGATGG CACAGTGGCC GGGGCTGCCA CCAAAGTCCC GAAAGTGAGT
241 CTCTTGCAGC TGAAAGCCTT AAAGCCAGGG GTTATTCAAA TCTTGGGAGT CAAGACATCC
301 AGGTTCCTGT GCCAGAGGCC AGACGGGGCG CTGTATGGAT CGCTCCACTT TGACCCCGAG
361 GCCTGCAGCT TCCGGGAGCT GCTTCTTGAG GACGGATACA ATGTGTACCA GTCTGTGGCC
421 CACGGCCTCC CGCTGCACCT GCCAGAGAGC AGGTCACCAC CCCGGGACCC TGCACCCCGA
481 GGACCAGCTC GCTTCCTGCC ACTACCAGGC CTGCCCCCTG AACCCCCAGA GCCGCCAGGA
541 ATCCTGGCCC CTGAGCCCCC CGACGTGGGC TCCTCAGACC CTCTGAGCAT GGTGGGGCCT
601 TCCCAAGGCC AAAGCCCCAG CTACGCTTCC TGA
```

Tupaia chinensis (Chinese tree shrew) FGF21 gene coding sequence (SEQ ID NO:327)(generated using SMS Reverse Translate tool on the ExPASy Bioinformatics Resource website (www.expasy.org))

```
1   ATGGGCTGGG ATAAAGCGCG CTTTGAACAT CTGGGCGCGT GGGCGCCGGT GCTGGCGGTG
61  CTGCTGCTGG GCGCGTGCCA GGCGTATCCG ATTCCGGATA GCAGCCCGCT GCTGCAGTTT
121 GGCGGCCAGG TGCGCCAGCG CTATCTGTAT ACCGATGATA CCCAGGATAC CGAAGCGCAT
181 CTGGAAATTC GCGCGGATGG CACCGTGGTG GGCGCGGCGC ATCAGAGCCC GGAAAGCCTG
241 CTGGAACTGA AAGCGCTGAA ACCGGGCGTG ATTCAGATTC TGGGCGTGAA AACCAGCCGC
301 TTTCTGTGCC AGCGCCCGGA TGGCGCGCTG TATGGCAGCC TGCATTTTGA TCCGGAAGCG
361 TGCAGCTTTC GCGAACTGCT GCTGGAAGAT GGCTATAACA TTTATCAGAG CGAAGCGCGC
421 GGCCTGCCGC TGCGCCTGCC GCCGCATGAT AGCCCGCATC GCGATCGCAC CCCGCAGGGC
481 CCGGCGCGCT TTCTGCCGCT GCCGGGCCTG CCGCTGGTGC CGCCGGAACT GCCGGGCGTG
541 CTGGCGCTGG AACCGCCGGA TGTGGGCAGC AGCGATCCGC TGAGCATGAT GGGCCCGAGC
601 CAGGGCCAGA GCCCGAGCTA TGCGAGCTAA
```

Papio anubis (olive baboon) FGF21 gene coding sequence (SEQ ID NO:328) (GenBank accession no. XM_003915851, which is hereby incorporated by reference in its entirety)

```
1   ATGGACTCGG ACGAGACCGG GTTCGAGCAC TCAGGACTGT GGGTTCCTGT GCTGGCTGGT
61  CTTCTGCTGG GAGCCTGCCA GGCACACCCC ATCCCTGACT CCAGTCCTCT CCTGCAATTC
121 GGGGGCCAAG TCCGGCAACG GTACCTCTAC ACAGATGATG CCCAGCAGAC AGAAGCCCAC
181 CTGGAGATCA GGGAGGATGG GACAGTGGGG GGCGCTGCTC ACCAGAGCCC CGAAAGTAAG
241 TGTGGGCCAG AGCCTGGGTC TGAGGGAGGA GGGGCTCTCC ACTTTGACCC TGAGGCCTGC
301 AGCTTCCGCG AGCTGCTTCT TGAGAACGGA TACAATGTTT ACCAGTCCGA GGCCCACGGC
361 CTCCCACTGC ACCTGCCGGG AAACAAGTCC CCACACCGGG ACCCTGCATC CCGAGGACCA
421 GCTCGCTTCC TGCCACTACC AGGCCTGCCC CCCGCACCCC CAGAGCCACC AGGAATCCTC
481 GCCCCCCAGC CCCCCGATGT GGGCTCCTCG GACCCTCTGA GCATGGTGGG ACCTTCCCAG
541 GCCCGAAGCC CTAGCTACGC TTCCTGA
```

Pteropus alecto (black flying fox) FGF21 gene coding sequence (SEQ ID NO:329) (generated using SMS Reverse Translate tool on the ExPASy Bioinformatics Resource website (www.expasy.org))

```
1   ATGGGCTGGG GCAAAGCGCG CCTGCAGCAT CCGGGCCTGT GGGGCCCGGT GCTGGCGGTG
61  CTGCTGGGCG CGTGCCAGGC GCATCCGATT CTGGATAGCA GCCCGCTGTT TCAGTTTGGC
121 AGCCAGGTGC GCCGCCGCTA TCTGTATACC GATGATGCGC AGGATACCGA AGCGCATCTG
181 GAAATTCGCG CGGATGGCAC CGTGGCGGGC GCGGCGCGCC GCAGCCCGGA AAGCCTGCTG
241 GAACTGAAAG CGCTGAAACC GGGCGTGATT CAGGTGCTGG GCGTGAAAAC CAGCCGCTTT
301 CTGTGCCAGC GCCCGGATGG CACCCTGTAT GGCAGCCTGC ATTTTGATCC GGCGGCGTGC
361 AGCTTTCGCG AACTGCTGCT GAAAGATGGC TATAACGTGT ATCAGAGCGA AGCGCTGGCG
421 CGCCCGCTGC GCCTGCCGCC GTATAGCAGC CCGAGCAGCG ATCCGGCGCG CCGCGGCCCG
481 GCGCGCTTTC TGCCGCTGCC GGGCCCGCCG CCGGAACCGC CGCAGCCGCC GGGCCGCCTG
541 GCGCCGGAAC CGCCGGATGT GGGCAGCAGC GATCCGCTGA GCATGGTGTG GCCGAGCCGC
601 GGCCGCAGCC CGAGCTATAC CAGCTAA
```

Heterocephalus glaber (naked mole-rat) FGF21 gene coding sequence (SEQ ID NO:330) (generated using SMS Reverse Translate tool on the ExPASy Bioinformatics Resource website (www.expasy.org))

```
1   ATGGATTGGG CGCGCGCGGA AAGCGAACGC CCGGGCCTGT GGGTGCCGGC GGTGCTGGCG
61  GTGCTGCTGC TGGGCGCGTG CCAGGCGCAT CCGATTCCGG ATAGCAGCCC GCTGCTGCAG
121 TTTGGCGGCC AGGTGCGCCA GCGCCATCTG TATACCGATG ATGCGCAGGA TACCGAAGTG
181 CATCTGGAAA TTCGCGCGGA TGGCAGCGTG GGCGGCGCGG CGCATCGCAG CCCGGAAAGC
241 CTGCTGGAAC TGAAAGCGCT GAAACCGGGC GTGATTCAGA TTCTGGGCGT GCGCACCAGC
301 CGCTTTCTGT GCCAGCGCCC GGATGGCACC CTGTATGGCA GCCTGCATTT TGATCCGGAA
361 GCGTGCAGCT TTCGCGAACT GCTGCTGGCG GATGGCTATA ACATTTATCA GAGCGAAGCG
421 TATGGCCTGC CGCTGCGCAT GCTGCCGAGC GATAGCGCGA GCCGCGATCC GGTGCCGCCG
481 GGCCCGGCGC GCTTTCTGCC GCTGCCGGGC CTGCATCCGC CGCCGCTGGA ACCGCCGGGC
541 ATGCTGCCGC CGGAACCGCC GGATGTGGGC AGCAGCGATC CGCTGAGCAT GGTGGGCCCG
601 CTGCAGGGCC GCAGCCCGAG CTATGCGTTT TAA
```

Cricetulus griseus (Chinese hamster) FGF21 gene coding sequence (SEQ ID NO:331) (GenBank accession no. XM_003508678, which is hereby incorporated by reference in its entirety)

```
1   ATGGACTGGA TGAAATCTGG AGTTGGGGTC CCGGGACTGT GGGTCCCTCT GCTGCCTATC
61  TTCCTGCTGG GGGTCTCCCA GGCACACCCC ATCCCTGACT CCAGCCCCCT CCTCCAGTTT
121 GGGGGTCAAG TCCGGCACAG GCACCTCTAC ACAGATGACA ACCAGGAAAC TGAAGTCCAC
181 CTGGAGATTA GGCAGGATGG CACGGTGATA GGGACCACAC ACCGCAGCCC AGAAAGTCTC
241 CTGGAGCTCA AAGCCTTGAA GCCAGAGGTC ATCCCAGTGC TGGGTGTCAA GGCCTCCAGG
301 TTTCTTTGCC AACAACCAGA CGGAACCCTG TATGGATCGC CTCACTTTGA TCCTGAGGCC
361 TGCAGTTTCA GGGAGCTCTT GCTTGAGGAT GGATACAATG TGTACCAATC TGAAGTCCAT
421 GGCCTGCCCC TGCGCCTGCC CCAGAGGGAC TCTCCAAACC AGGCCCCAGC ATCCTGGGGA
481 CCTGTGCCCC CCCTGCCAGT GCCAGGACTG CTCCACCAGC CCCAGGAGCT ACCAGGGTTC
541 CTGGCCCCAG AACCTCCAGA TGTGGGCTCC TCTGACCCAC TGAGCATGGT GGGACCTTTG
601 CAGGGCCGAA GCCCCAGCTA TGCTTCCTGA
```

Ovis aries (sheep) FGF21 gene coding sequence (SEQ ID NO:332) (GenBank accession no. XM_004015796, which is hereby incorporated by reference in its entirety)

```
1   ATGGGCTGGG ACGAGGCCAA GTTCAAGCAC TTGGGACTGT GGGTCCCTGT GCTGGCTGTC
61  CTCCTGCTAG GAACCTGCCG GGCGCATCCA ATTCCAGACT CCAGCCCCCT CCTCCAGTTT
121 GGGGGCCAAG TCCGCCAGCG GTACCTCTAC ACGGATGATG CCCAGGAGAC AGAGGCCCAC
181 CTGGAGATCA GGGCCGATGG CACAGTGGTG GGGGCGGCCC GCCAGAGTCC CGAAAGTCTC
241 TTGGAGCTGA AAGCCCTGAA GCCAGGAGTC ATTCAGATCT TTGGAGTTAA AACATCCAGG
301 TTCCTGTGCC AGGGGCCAGA TGGGAAGCTG TATGGATCGC TGCACTTTGA CCCCAAAGCC
361 TGCAGCTTCC GGGAGCTGCT TCTTGAAGAT GGGTACAATG TCTACCAGTC GGAGACCCTG
421 GGCCTTCCAC TCCGCCTGCC GCCGCAGCGC TCATCCAACC GGGACCCGGC CCCGCGGGGA
481 CCTCCGAAGC CCCAGCTACA CTTCTTGAAG ACGTCCGCTG TGCAGTACTG GCCACGTTAT
541 GAGAAGGTCC CAGCTTTTCT GCACCCCTTC CCCGGCTGA
```

Pan paniscus (pygmy chimpanzee) FGF21 gene coding sequence (SEQ ID NO: 333) (GenBank accession no. XM_003814115, which is hereby incorporated by reference in its entirety) (1-209, excluding 117-194 and 202-209)

```
573                                 ATGGACTC GGACGAGACC GGGTTCGAGC
601 ACTCAGGACT GTGGGTTTCT GTGCTGGCTG GTCTTCTGCT GGGAGCCTGC CAGGCACACC
661 CCATCCCTGA CTCCAGTCCT CTCCTGCAAT TCGGGGGCCA AGTCCGGCAG CGGTACCTCT
721 ACACAGATGA TGCCCAGCAG ACAGAAGCCC ACCTGGAGAT CAGGGAGGAT GGGACGGTGG
781 GGGGCGCTGC TGACCAGAGC CCCGAAAGTC TCCTGCAGCT GAAAGCCTTG AAGCCGGGAG
841 TTATTCAAAT CTTGGGAGTC AAGACATCCA GGTTCCTGTG CCAGAGGCCA GATGGGGCCC
901 TGTATGGATC GGTGAGTTTC ---------- ---------- ---------- ----------
    ---------- ---------- ---------- ---------- ---------- ----------
921 ---------- ----CAG--- ---------- ---------- ---------- ----------
924 ---------- -------GAC CCTCCT---- --------CA CCACCCACCA ---------T
946 GCTCC----- ----TCCTAT ATGTCGCCCTCACAG------ ---CCTGGG
```

Macaca fascicularis (crab-eating macaque) FGF21 gene coding sequence (SEQ ID NO:334) (generated using SMS Reverse Translate tool on the ExPASy Bioinformatics Resource website (www.expasy.org)) (1-209, excluding 117-209)

```
1   ATGGATAGCG ATGAAACCGG CTTTGAACAT AGCGGCCTGT GGGTGCCGGT GCTGGCGGGC
61  CTGCTGCTGG GCGCGTGCCA GGCGCATCCG ATTCCGGATA GCAGCCCGCT GCTGCAGTTT
121 GGCGGCCAGG TGCGCCAGCG CTATCTGTAT ACCGATGATG CGCAGCAGAC CGAAGCGCAT
181 CTGGAAATTC GCGAAGATGG CACCGTGGGC GGCGCGGCGC ATCAGAGCCC GGAAAGCCTG
241 CTGCAGCTGA AAGCGCTGAA ACCGGGCGTG ATTCAGATTC TGGGCGTGAA AACCAGCCGC
301 TTTCTGTGCC AGAAACCGGA TGGCGCGCTG TATGGCAGCG TGAGCTTTTA A
```

Mesocricetus auratus (golden hamster) FGF21 gene coding sequence (SEQ ID NO:335) (GenBank accession no. EU497769, which is hereby incorporated by reference in its entirety) (1-209, excluding 1-89 and 194-209) 1 GGTCATCCAA ATCCTGGGTG TCAAGGCTGC TAGGTTTCCT TGCCAGCAAC CAGACGGAAG 61 CCTGTACGGA TCGCCTCACT TCGATCCCGA GGCCTGCAGT TTCCGGGAGC TCCTGCTTGA 121 GGATGGATAC AATGTGTACC AGTCGGAAGC CCACGGCCTG CCCCTGCGCC TGCCCCAGAG 181 GGACGCTCCG AGCCAGCCCC CAGCATCCTG GGGACCGGTG CGCTTCCTGC CAGTGCCCGG 241 ACTGTTCCAG CCGCCCCACG ACCTCCCAGG GCGCCCGGCC CCAGAGCCTC CGGACGTGGG 301 CTCCTCCGAC CCAC
Nile tilapia FGF21 gene coding sequence (SEQ ID NO:336) (GenBank accession no. XM_003438468, which is hereby incorporated by reference in its entirety) (1-209, excluding 1-58) 1 ATGTATTTGC AGATGAACAT GGATGGGAGA GTCACAGGAA GTGATGCTCA GACACCTTAC 61 AGTTTGATGC AGCTGAAATC AGTTAAACCA GGCCATGTAA TCATTAAAGG ACCATCATCA 121 TCTCTTTTTC TCTGTGTGGA CAGCGAAGGC AATCTGAGAG GGCAGAGTCA CTACTCAGAA 181 ACCAGCTGCA CCTTCAGAGA AATGCTGCTG GCTGACGGAT ACACCCGTTT CATTTCCTCA 241 CAATATGGAT TTCCCATGTC ACTGGCATCA AGACATTCCC CAGATCGACA CGCGCTTCCC 301 TTTACGCGGT TCCTACCACT GAGGAATAAC TTGAAAACGG ATAGCGTATC AGAGCAGCTG 361 CCAAACAATC AGAGACTCTT CAACGTGGAC TCTGATGACC TTCTTGGAAT GGGTCTAAAT 421 TCTATGGGCA GTCCTCAGTT TTCTATGGAC AAATAA

[0081] In one embodiment of the present invention, the chimeric protein may include one or more substitutions for or additions of amino acids from another FGF. In one embodiment, the C-terminal portion from FGF21 includes a modification that includes a substitution for or addition of amino acid residues from an FGF19 (including a human FGF19 and orthologs of human FGF19). In one embodiment the FGF19 is a human FGF19 protein having an amino acid sequence of SEQ ID NO: 337 (GenBank Accession No. NP_005108, which is hereby incorporated by reference in its entirety) or a portion or ortholog thereof, as follows:

1 MRSGCVVVHV WILAGLWLAV AGRPLAFSDA GPHVHYGWGD PIRLRHLYTS GPHGLSSCFL

61 RIRADGVVDC ARGQSAHSLL EIKAVALRTV AIKGVHSVRY LCMGADGKMQ GLLQYSEEDC

121 AFEEEIRPDG YNVYRSEKHR LPVSLSSAKQ RQLYKNRGFL PLSHFLPMLP MVPEEPEDLR

181 GHLESDMFSS PLETDSMDPF GLVTGLEAVR SPSFEK

Exemplary substitutions and additions of such residues are shown in Figures 12 and 13.

[0082] In one embodiment, the C-terminal portion from FGF21 includes a modification that includes a substitution of amino acid residues from an FGF19 molecule. In one embodiment, the modification includes a substitution for or addition of amino acid residues 169 to 216 of SEQ ID NO: 337 (FGF19). In one embodiment, the modification is a substitution of amino acid residues from SEQ ID NO: 337 (FGF19) for corresponding amino acid residues of SEQ ID NO: 233 (FGF21). The corresponding residues of FGFs may be identified by sequence analysis and/or structural analysis. *See* FIGS. 2, 11, 12, and 13. In one embodiment, the modification includes a substitution of a contiguous stretch of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,

12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48 amino acid residues 169 to 216 of SEQ ID NO: 337 (FGF19) for the corresponding contiguous stretch of amino acid residues of SEQ ID NO: 233 (FGF21). In one embodiment, amino acid residues 168 to 209, 191 to 209, or 198 to 209 of SEQ ID NO: 233 (FGF21) are substituted with the corresponding amino acid residues selected from the sequence including amino acid residues 169 to 216 of SEQ ID NO: 337 (FGF19).

[0083] In one embodiment, the modification includes a substitution of one or more individual amino acid residues from residues 169 to 216 of SEQ ID NO: 337 (FGF19) for the corresponding amino acid residues of SEQ ID NO: 233 (FGF21). In one embodiment, the C-terminal portion includes substitutions of one or more of amino acid residues 168, 169, 170, 171, 173, 174, 177, 178, 179, 180, 181, 182, 183, 184, 186, 187, 188, 189, 191, 194, 195, 196, 199, 200, 201, 202, 207, 208, or 209 of SEQ ID NO: 233 (FGF21) for the corresponding amino acid residues of SEQ ID NO:337 (FGF19).

[0084] In one embodiment of the present invention, the C-terminal portion from FGF21 includes a modification that includes an addition of amino acid residues that are present in the corresponding C-terminal portion from FGF19. As shown in Figures 11, 12, and 13, FGF19 residues that are absent in the corresponding C-terminal portion of FGF21 may be identified by sequence analysis and/or structural analysis. In one embodiment, the modification includes an addition of amino acid residues selected from residues 204 to 216, 197 to 216, 174 to 216, or 169 to 216 of SEQ ID NO: 337 (FGF19). In one embodiment, the modification includes an addition of amino acid residue 204 of SEQ ID NO: 337 (FGF19). In one embodiment, the modification includes an addition of amino acid residues 178, 179, 180, 181, and/or 182 of SEQ ID NO: 337 (FGF19) individually or in combination.

[0085] It will be understood that the C-terminal portion from FGF21 that includes a substitution of amino acid residues from an FGF19 molecule may be derived using a nucleotide sequence that encodes a human FGF19 protein having a nucleotide sequence of SEQ ID NO: 338 (Human FGF19 gene coding sequence (1-216); GenBank Accession No. NM_005117, which is hereby incorporated by reference in its entirety) or a portion or ortholog thereof, as follows:

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464     ATGCGGA GCGGGTGTGT GGTGGTCCAC GTATGGATCC TGGCCGGCCT CTGGCTGGCC
521  GTGGCCGGGC GCCCCCTCGC CTTCTCGGAC GCGGGGCCCC ACGTGCACTA CGGCTGGGGC
581  GACCCCATCC GCCTGCGGCA CCTGTACACC TCCGGCCCCC ACGGGCTCTC CAGCTGCTTC
641  CTGCGCATCC GTGCCGACGG CGTCGTGGAC TGCGCGCGGG GCCAGAGCGC GCACAGTTTG
701  CTGGAGATCA AGGCAGTCGC TCTGCGGACC GTGGCCATCA AGGGCGTGCA CAGCGTGCGG
761  TACCTCTGCA TGGGCGCCGA CGGCAAGATG CAGGGGCTGC TTCAGTACTC GGAGGAAGAC
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821  TGTGCTTTCG AGGAGGAGAT CCGCCCAGAT GGCTACAATG TGTACCGATC CGAGAAGCAC
881  CGCCTCCCGG TCTCCCTGAG CAGTGCCAAA CAGCGGCAGC TGTACAAGAA CAGAGGCTTT
941  CTTCCACTCT CTCATTTCCT GCCCATGCTG CCCATGGTCC CAGAGGAGCC TGAGGACCTC
1001 AGGGGCCACT TGGAATCTGA CATGTTCTCT TCGCCCCTGG AGACCGACAG CATGGACCCA
1061 TTTGGGCTTG TCACCGGACT GGAGGCCGTG AGGAGTCCCA GCTTTGAGAA GTAA
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[0086] In one embodiment, the chimeric protein of the present invention includes the amino acid sequence of SEQ ID NO: 339, SEQ ID NO: 340, SEQ ID NO: 341, or SEQ ID NO: 342, as shown in Table 9.

Table 9:

Description of Chimeric Protein	Sequence
Amino acid sequence of a FGF1/FGF21 chimera composed of residues M1 to L150 of human FGF1 harboring K127D/K128Q/K133V triple mutation (bold) and residues P168 to S209 of human FGF21 (bold)	SEQ ID NO: 339 MAEGEITTFT ALTEKFNLPP GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYISKKHAEK NWFVGL**DQ**NG SC**V**RGPRTHY GQKAILFLPL **PGLPPALPEP PGILAPQPPD VGSSDPLSMV** **GPSQGRSPSY AS**
Amino acid sequence of a FGF1/FGF21 chimera composed of residues K25 to L150 of human FGF1 harboring K127D/K128Q/K133V triple mutation (bold) and residues P168 to S209 of human FGF21 (bold)	SEQ ID NO: 340 KPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ LSAESVGEVY IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYISKKHAEK NWFVGL**DQ**NG SC**V**RGPRTHY GQKAILFLPL **PGLPPALPEP PGILAPQPPD VGSSDPLSMV** **GPSQGRSPSY AS**
Amino acid sequence of a FGF2/FGF21 chimera composed of residues M1 to M151 of human FGF2 harboring K128D/R129Q/K134V triple mutation (bold) and residues P168 to S209 of human FGF21 (bold)	SEQ ID NO: 341 MAAGSITTLP ALPEDGGSGA FPPGHFKDPK RLYCKNGGFF LRIHPDGRVD GVREKSDPHI KLQLQAEERG VVSIKGVCAN RYLAMKEDGR LLASKCVTDE CFFFERLESN NYNTYRSRKY TSWYVAL**DQ**T GQY**V**LGSKTG PGQKAILFLP M**PGLPPALPE PPGILAPQPP DVGSSDPLSM** **VGPSQGRSPS YAS**
Amino acid sequence of a FGF2/FGF21 chimera composed of residues H25 to M151 of human FGF2 harboring K128D/R129Q/K134V triple mutation (bold) and residues P168 to S209 of human FGF21 (bold)	SEQ ID NO: 342 HFKDPK RLYCKNGGFF LRIHPDGRVD GVREKSDPHI KLQLQAEERG VVSIKGVCAN RYLAMKEDGR LLASKCVTDE CFFFERLESN NYNTYRSRKY TSWYVAL**DQ**T GQY**V**LGSKTG PGQKAILFLP M**PGLPPALPE PPGILAPQPP DVGSSDPLSM** **VGPSQGRSPS YAS**

[0087] Chimeric proteins according to the present invention may be isolated proteins or polypeptides. The isolated chimeric proteins of the present invention may be prepared for use in

accordance with the present invention using standard methods of synthesis known in the art, including solid phase peptide synthesis (Fmoc or Boc strategies) or solution phase peptide synthesis. Alternatively, peptides of the present invention may be prepared using recombinant expression systems.

[0088] Accordingly, another aspect of the present invention relates to an isolated nucleic acid molecule encoding a chimeric protein according to the present invention. In one embodiment, the nucleic acid molecule includes the nucleotide sequence of SEQ ID NO: 343, SEQ ID NO: 344, SEQ ID NO: 345, or SEQ ID NO: 346 (as shown in Table 10).

Table 10:

Description of Chimeric Protein	**Sequence**
Nucleotide sequence of a FGF1/FGF21 chimera composed of residues M1 to L150 of human FGF1 harboring K127D/K128Q/K133V triple mutation (bold) and residues P168 to S209 of human FGF21 (bold)	SEQ ID NO: 343 ATGGCTGAAG GGGAAATCAC CACCTTCACA GCCCTGACCG AGAAGTTTAA TCTGCCTCCA GGGAATTACA AGAAGCCCAA ACTCCTCTAC TGTAGCAACG GGGGCCACTT CCTGAGGATC CTTCCGGATG GCACAGTGGA TGGGACAAGG GACAGGAGCG ACCAGCACAT TCAGCTGCAG CTCAGTGCGG AAAGCGTGGG GGAGGTGTAT ATAAAGAGTA CCGAGACTGG CCAGTACTTG GCCATGGACA CCGACGGGCT TTTATACGGC TCACAGACAC CAAATGAGGA ATGTTTGTTC CTGGAAAGGC TGGAGGAGAA CCATTACAAC ACCTATATAT CCAAGAAGCA TGCAGAGAAG AATTGGTTTG TTGGCCTC**GA TCAG**AATGGG AGCTGC**GTT**C GCGGTCCTCG GACTCACTAT GGCCAGAAAG CAATCTTGTT TCTCCCCCTG **CCAGGCCTGC CCCCCGCACT CCCGGAGCCA** **CCCGGAATCC TGGCCCCCCA GCCCCCCGAT** **GTGGGCTCCT CGGACCCTCT GAGCATGGTG** **GGACCTTCCC AGGGCCGAAG CCCCAGCTAC** **GCTTCC**
Nucleotide sequence of a FGF1/FGF21 chimera composed of residues K25 to L150 of human FGF1 harboring K127D/K128Q/K133V triple mutation (bold) and residues P168 to S209 of human FGF21 (bold)	SEQ ID NO: 344 AAGCCCAA ACTCCTCTAC TGTAGCAACG GGGGCCACTT CCTGAGGATC CTTCCGGATG GCACAGTGGA TGGGACAAGG GACAGGAGCG ACCAGCACAT TCAGCTGCAG CTCAGTGCGG AAAGCGTGGG GGAGGTGTAT ATAAAGAGTA CCGAGACTGG CCAGTACTTG GCCATGGACA CCGACGGGCT TTTATACGGC TCACAGACAC CAAATGAGGA ATGTTTGTTC CTGGAAAGGC TGGAGGAGAA CCATTACAAC ACCTATATAT CCAAGAAGCA TGCAGAGAAG AATTGGTTTG TTGGCCTC**GA TCAG**AATGGG AGCTGC**GTT**C GCGGTCCTCG GACTCACTAT GGCCAGAAAG CAATCTTGTT TCTCCCCCTG **CCAGGCCTGC CCCCCGCACT CCCGGAGCCA** **CCCGGAATCC TGGCCCCCCA GCCCCCCGAT** **GTGGGCTCCT CGGACCCTCT GAGCATGGTG** **GGACCTTCCC AGGGCCGAAG CCCCAGCTAC** **GCTTCC**

Description of Chimeric Protein	Sequence
Nucleotide sequence of a FGF2/FGF21 chimera composed of residues M1 to M151 of human FGF2 harboring K128D/R129Q/K134V triple mutation (bold) and residues P168 to S209 of human FGF21 (bold)	SEQ ID NO: 345 ATG GCAGCCGGGA GCATCACCAC GCTGCCCGCC TTGCCCGAGG ATGGCGGCAG CGGCGCCTTC CCGCCCGGCC ACTTCAAGGA CCCCAAGCGG CTGTACTGCA AAAACGGGGG CTTCTTCCTG CGCATCCACC CCGACGGCCG AGTTGACGGG GTCCGGGAGA AGAGCGACCC TCACATCAAG CTACAACTTC AAGCAGAAGA GAGAGGAGTT GTGTCTATCA AAGGAGTGTG TGCTAACCGT TACCTGGCTA TGAAGGAAGA TGGAAGATTA CTGGCTTCTA AATGTGTTAC GGATGAGTGT TTCTTTTTTG AACGATTGGA ATCTAATAAC TACAATACTT ACCGGTCAAG GAAATACACC AGTTGGTATG TGGCACTG**GA TCAG**ACTGGG CAGTAT**GTT**C TTGGATCCAA AACAGGACCT GGGCAGAAAG CTATACTTTT TCTTCCAATG **CCAGGCCTGC** **CCCCCGCACT CCCGGAGCCA CCCGGAATCC** **TGGCCCCCCA GCCCCCCGAT GTGGGCTCCT** **CGGACCCTCT GAGCATGGTG GGACCTTCCC** **AGGGCCGAAG CCCCAGCTAC GCTTCC**
Nucleotide sequence of a FGF2/FGF21 chimera composed of residues H25 to M151 of human FGF2 harboring K128D/R129Q/K134V triple mutation (bold) and residues P168 to S209 of human FGF21 (bold)	SEQ ID NO: 346 C ACTTCAAGGA CCCCAAGCGG CTGTACTGCA AAAACGGGGG CTTCTTCCTG CGCATCCACC CCGACGGCCG AGTTGACGGG GTCCGGGAGA AGAGCGACCC TCACATCAAG CTACAACTTC AAGCAGAAGA GAGAGGAGTT GTGTCTATCA AAGGAGTGTG TGCTAACCGT TACCTGGCTA TGAAGGAAGA TGGAAGATTA CTGGCTTCTA AATGTGTTAC GGATGAGTGT TTCTTTTTTG AACGATTGGA ATCTAATAAC TACAATACTT ACCGGTCAAG GAAATACACC AGTTGGTATG TGGCACTG**GA TCAG**ACTGGG CAGTAT**GTT**C TTGGATCCAA AACAGGACCT GGGCAGAAAG CTATACTTTT TCTTCCAATG **CCAGGCCTGC** **CCCCCGCACT CCCGGAGCCA CCCGGAATCC** **TGGCCCCCCA GCCCCCCGAT GTGGGCTCCT** **CGGACCCTCT GAGCATGGTG GGACCTTCCC** **AGGGCCGAAG CCCCAGCTAC GCTTCC**

[0089] Another aspect of the present invention relates to a nucleic acid construct including a nucleic acid molecule encoding a chimeric protein according to the present invention, a 5' DNA promoter sequence, and a 3' terminator sequence. The nucleic acid molecule, the promoter, and the terminator are operatively coupled to permit transcription of the nucleic acid molecule.

[0090] Also encompassed are vectors or expression vectors including such nucleic acid molecules and host cells including such nucleic acid molecules. Nucleic acid molecules

according to the present invention can be expressed in a host cell, and the encoded polynucleotides isolated, according to techniques that are known in the art.

[0091] Generally, the use of recombinant expression systems involves inserting the nucleic acid molecule encoding the amino acid sequence of the desired peptide into an expression system to which the molecule is heterologous (*i.e.*, not normally present). One or more desired nucleic acid molecules encoding a peptide of the invention may be inserted into the vector. When multiple nucleic acid molecules are inserted, the multiple nucleic acid molecules may encode the same or different peptides. The heterologous nucleic acid molecule is inserted into the expression system or vector in proper sense (5'→3') orientation relative to the promoter and any other 5' regulatory molecules, and correct reading frame.

[0092] The preparation of the nucleic acid constructs can be carried out using standard cloning procedures well known in the art as described by Joseph Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL (Cold Springs Harbor 1989). U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference in its entirety, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in a suitable host cell.

[0093] A variety of genetic signals and processing events that control many levels of gene expression (e.g., DNA transcription and messenger RNA ("mRNA") translation) can be incorporated into the nucleic acid construct to maximize protein production. For the purposes of expressing a cloned nucleic acid sequence encoding a desired protein, it is advantageous to use strong promoters to obtain a high level of transcription. Depending upon the host system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *rec*A promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lac*UV5, *omp*F, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lac*UV5 (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene. Common promoters suitable for directing expression in mammalian cells include, without limitation, SV40, MMTV, metallothionein-1, adenovirus Ela, CMV, immediate early, immunoglobulin heavy chain promoter and enhancer, and RSV-LTR.

[0094] There are other specific initiation signals required for efficient gene transcription and translation in prokaryotic cells that can be included in the nucleic acid construct to maximize protein production. Depending on the vector system and host utilized, any number of suitable transcription and/or translation elements, including constitutive, inducible, and repressible promoters, as well as minimal 5' promoter elements, enhancers or leader sequences may be used. For a review on maximizing gene expression see Roberts and Lauer, "Maximizing Gene Expression On a Plasmid Using Recombination *In Vitro*," *Methods in Enzymology* 68:473–82 (1979), which is hereby incorporated by reference in its entirety.

[0095] A nucleic acid molecule encoding an isolated protein of the present invention, a promoter molecule of choice, including, without limitation, enhancers, and leader sequences; a suitable 3' regulatory region to allow transcription in the host, and any additional desired components, such as reporter or marker genes, are cloned into the vector of choice using standard cloning procedures in the art, such as described in Joseph Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL (Cold Springs Harbor 1989); Frederick M. Ausubel, SHORT PROTOCOLS IN MOLECULAR BIOLOGY (Wiley 1999); and U.S. Patent No. 4,237,224 to Cohen and Boyer, which are hereby incorporated by reference in their entirety.

[0096] Once the nucleic acid molecule encoding the protein has been cloned into an expression vector, it is ready to be incorporated into a host. Recombinant molecules can be introduced into cells, without limitation, via transfection (if the host is a eukaryote), transduction, conjugation, mobilization, or electroporation, lipofection, protoplast fusion, mobilization, or particle bombardment, using standard cloning procedures known in the art, as described by JOSEPH SAMBROOK et al., MOLECULAR CLONING: A LABORATORY MANUAL (Cold Springs Harbor 1989), which is hereby incorporated by reference in its entirety.

[0097] A variety of suitable host-vector systems may be utilized to express the recombinant protein or polypeptide. Primarily, the vector system must be compatible with the host used. Host-vector systems include, without limitation, the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria.

[0098] Purified proteins may be obtained by several methods readily known in the art, including ion exchange chromatography, hydrophobic interaction chromatography, affinity chromatography, gel filtration, and reverse phase chromatography. The protein is preferably

produced in purified form (preferably at least about 80% or 85% pure, more preferably at least about 90% or 95% pure) by conventional techniques. Depending on whether the recombinant host cell is made to secrete the protein into growth medium (*see* U.S. Patent No. 6,596,509 to Bauer et al., which is hereby incorporated by reference in its entirety), the protein can be isolated and purified by centrifugation (to separate cellular components from supernatant containing the secreted protein) followed by sequential ammonium sulfate precipitation of the supernatant. The fraction containing the protein is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the protein of interest from other proteins. If necessary, the protein fraction may be further purified by HPLC.

[0099] Another aspect of the present invention relates to a pharmaceutical composition that includes a chimeric protein according to the present invention and a pharmaceutically acceptable carrier.

[0100] "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0101] The term "pharmaceutically acceptable" means it is, within the scope of sound medical judgment, suitable for use in contact with the cells of humans and lower animals without undue toxicity, irritation, allergic response, and the like, and is commensurate with a reasonable benefit/risk ratio.

[0102] In one embodiment, the pharmaceutical composition includes an organotropic targeting agent. In one embodiment, the targeting agent is covalently linked to the chimeric protein via a linker that is cleaved under physiological conditions.

[0103] Chimeric and/or modified proteins according to the present invention may also be modified using one or more additional or alternative strategies for prolonging the *in vivo* half-life of the protein. One such strategy involves the generation of D-peptide chimeric proteins, which

consist of unnatural amino acids that are not cleaved by endogenous proteases. Alternatively, the chimeric and/or modified proteins may be fused to a protein partner that confers a longer half-life to the protein upon *in vivo* administration. Suitable fusion partners include, without limitation, immunoglobulins (*e.g.*, the Fc portion of an IgG), human serum albumin (HAS) (linked directly or by addition of the albumin binding domain of streptococcal protein G), fetuin, or a fragment of any of these. The chimeric and/or modified proteins may also be fused to a macromolecule other than protein that confers a longer half-life to the protein upon *in vivo* administration. Suitable macromolecules include, without limitation, polyethylene glycols (PEGs). Methods of conjugating proteins or peptides to polymers to enhance stability for therapeutic administration are described in U.S. Patent No. 5,681,811 to Ekwuribe, which is hereby incorporated by reference in its entirety. Nucleic acid conjugates are described in U.S. Patent No. 6,528,631 to Cook et al., U.S. Patent No. 6,335,434 to Guzaev et al., U.S. Patent No. 6,235,886 to Manoharan et al., U.S. Patent No. 6,153,737 to Manoharan et al., U.S. Patent No. 5,214,136 to Lin et al., or U.S. Patent No. 5,138,045 to Cook et al., which are hereby incorporated by reference in their entirety.

[0104] The pharmaceutical composition according to the present invention can be formulated for administration orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, by implantation, by intracavitary or intravesical instillation, intraocularly, intraarterially, intralesionally, transdermally, or by application to mucous membranes. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.

[0105] Another aspect of the present invention relates to a method for treating a subject suffering from a disorder. This method involves selecting a subject suffering from the disorder and administering the pharmaceutical composition according to the present invention to the selected subject under conditions effective to treat the disorder. In one embodiment the disorder is diabetes, obesity, or metabolic syndrome.

[0106] Another aspect of the present invention relates to a method for treating a subject suffering from a disorder. This method involves selecting a subject suffering from the disorder and providing a chimeric FGF protein, where the chimeric FGF protein includes an N-terminus coupled to a C-terminus. The N-terminus includes a portion of a paracrine FGF and the C-terminus includes a C-terminal portion of FGF21. The portion of the paracrine FGF is modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without

the modification. This method also involves administering a therapeutically effective amount of the chimeric FGF protein to the selected subject under conditions effective to treat the disorder.

[0107] Suitable chimeric proteins for use in accordance with this aspect of the present invention are described above and throughout the present application.

[0108] In one embodiment, the selected subject is a mammal. In one embodiment, the selected subject is a human. In another embodiment, the selected subject is a rodent.

[0109] In one embodiment, the selected subject is in need of increased FGF21-βKlotho-FGF receptor ("FGFR") complex formation.

[0110] In one embodiment, the disorder is a selected from diabetes, obesity, and metabolic syndrome. As used herein, diabetes includes type I diabetes, type II diabetes, gestational diabetes, and drug-induced diabetes. In yet another embodiment, the subject has obesity. In yet another embodiment, the subject has metabolic syndrome.

[0111] The chimeric protein of the present invention or pharmaceutical composition thereof can be used to treat a number of conditions. In one embodiment, the condition is one which the therapeutic outcome includes a decrease in blood glucose, a decrease in blood fructosamine, an increase in energy expenditure, an increase in fat utilization, a decrease in body weight, a decrease in body fat, a decrease in triglycerides, a decrease in free fatty acids, an increase in fat excretion, an improvement, or even a preservation, of pancreatic β-cell function and mass, a decrease in total blood cholesterol, a decrease in blood low-density lipoprotein cholesterol, an increase in blood high-density lipoprotein cholesterol, an increase in blood adiponectin, an increase in insulin sensitivity, an increase in leptin sensitivity, a decrease in blood insulin, a decrease in blood leptin, a decrease in blood glucagon, an increase in glucose uptake by adipocytes, a decrease in fat accumulation in hepatocytes, and/or an increase in fat oxidation in hepatocytes. Each of these parameters can be measured by standard methods, for example, by measuring oxygen consumption to determine metabolic rate, using scales to determine weight, and measuring lean body mass composition or mass to determine fat. Moreover, the presence and amount of triglycerides, free fatty acids, glucose and leptin can be determined by standard methods (e.g., blood test).

[0112] Additional conditions that are treatable in accordance with the present invention include one or more of type 1 diabetes, type 2 diabetes, gestational diabetes, drug-induced diabetes, high blood glucose, metabolic syndrome, lipodystrophy syndrome, dyslipidemia, insulin resistance, leptin resistance, atherosclerosis, vascular disease, inflammatory disease,

fibrotic disease, hypercholesterolemia, hypertriglyceridemia, non-alcoholic fatty liver disease, overweight, and obesity.

[0113] In one embodiment, the chimeric protein of the present invention or pharmaceutical composition thereof is administered with a pharmaceutically-acceptable carrier.

[0114] The chimeric protein according to the present invention or pharmaceutical composition thereof can be administered orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, by implantation, by intracavitary or intravesical instillation, intraocularly, intraarterially, intralesionally, transdermally, or by application to mucous membranes. The most suitable route may depend on the condition and disorder of the recipient. Formulations including chimeric proteins according to the present invention may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.

[0115] Dosages and desired drug concentrations of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary physician. Those skilled in the art can readily optimize pharmaceutically effective dosages and administration regimens for therapeutic compositions including the chimeric protein according to the present invention, as determined by good medical practice and the clinical condition of the individual patient.

[0116] When *in vivo* administration of a chimeric protein of the present invention or is employed, normal dosage amounts may vary from, for example, about 10 ng/kg to up to 100 mg/kg of mammal body weight or more per day. In one embodiment, the dosage may be from about 1 μg/kg/day to 10 mg/kg/day, depending upon the route of administration. In one embodiment, the chimeric protein according to the present invention is administered at a dose of about 0.1 to 10 mg/kg once or twice daily. In one embodiment, the chimeric protein according to the present invention is administered at a dose of about 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2 mg/kg. In one embodiment, the dosage is the same as that of a native FGF21 therapeutic. In one embodiment, the dosage is less than that of a native FGF21 therapeutic, but has the same effect as a higher dosage of a native FGF21 therapeutic. Guidance as to particular dosages and methods of delivery of proteins is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760; 5,206,344; or 5,225,212, which are hereby incorporated by reference in their entirety. It is anticipated that different formulations will be effective for different treatment

compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different from that to another organ or tissue.

[0117] Where sustained-release administration of a chimeric protein of the present invention is desired in a formulation with release characteristics suitable for the treatment of any disease or disorder requiring administration of the chimeric protein of the present invention, microencapsulation is contemplated. Microencapsulation of recombinant proteins for sustained release has been successfully performed with human growth hormone (rhGH), interferon-(rhIFN-), interleukin-2, and MN rgp120. Johnson et al., "Preparation and Characterization of Poly(D,L-lactide-co-glycolide) Microspheres for Controlled Release of Human Growth Hormone," *Nat. Med.* 2:795-799 (1996); Yasuda, "Sustained Release Formulation of Interferon," *Biomed. Ther.* 27:1221-1223 (1993); Hora et al., "Controlled Release of Interleukin-2 from Biodegradable Microspheres," *Nat. Biotechnol.* 8:755-758 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems," in VACCINE DESIGN: THE SUBUNIT AND ADJUVANT APPROACH 439-462 (Powell and Newman, eds. 1995); WO 97/03692; WO 96/40072; WO 96/07399; and U.S. Pat. No. 5,654,010, which are hereby incorporated by reference in their entirety. The sustained-release formulations of these proteins were developed using poly-lactic-coglycolic acid (PLGA) polymer due to its biocompatibility and wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids, can be cleared quickly within the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. Lewis, "Controlled release of bioactive agents from lactide/glycolide polymer," in: BIODEGRADABLE POLYMERS AS DRUG DELIVERY SYSTEMS 1-41 (M. Chasin and R. Langer eds. 1990), which is hereby incorporated by reference in its entirety.

[0118] The chimeric protein of the present invention or pharmaceutical composition thereof may be administered as frequently as necessary in order to obtain the desired therapeutic effect. Some patients may respond rapidly to a higher or lower dose and may find much weaker maintenance doses adequate. For other patients, it may be necessary to have long-term treatments at the rate of 1 to 4 doses per day, in accordance with the physiological requirements of each particular patient. For other patients, it will be necessary to prescribe not more than one or two doses per day.

[0119] In some embodiments, the chimeric protein of the present invention or a pharmaceutical composition thereof is administered in a therapeutically effective amount in combination with a therapeutically effective amount of a second agent. In one embodiment, the

chimeric protein of the present invention or pharmaceutical composition thereof is administered in conjunction with the second agent, i.e., the respective periods of administration are part of a single administrative regimen. In one embodiment, the chimeric protein of the present invention or pharmaceutical composition thereof and the second agent are administered concurrently, i.e., the respective periods of administration overlap each other. In one embodiment, the chimeric protein of the present invention or pharmaceutical composition thereof and the second agent are administered non-concurrently, i.e., the respective periods of administration do not overlap each other. In one embodiment, the chimeric protein of the present invention or pharmaceutical composition thereof and the second agent are administered sequentially, i.e., the chimeric protein of the present invention or pharmaceutical composition thereof is administered prior to and/or after the administration of the second agent. In one embodiment, the chimeric protein of the present invention or pharmaceutical composition thereof and the second agent are administered simultaneously as separate compositions. In one embodiment, the chimeric protein of the present invention or pharmaceutical composition thereof and the second agent are administered simultaneously as part of the same compositions.

[0120] In one embodiment, the second agent is an anti-inflammatory agent, an anti-fibrotic agent, an antihypertensive agent, an anti-diabetic agent, a triglyceride-lowering agent, and/or cholesterol-lowering drug such as a drug of the "statin" class. In one embodiment, the second agent is insulin. In one embodiment, the insulin is rapid acting, short acting, regular acting, intermediate acting, or long acting insulin. In one embodiment, the insulin is and/or comprises Humalog®, Lispro, Novolog®, Apidra®, Humulin®, Aspart, regular insulin, NPH, Lente, Ultralente, Lantus®, Glargine, Levemir®, or Detemir. In one embodiment, the second agent is a statin. In one embodiment, the statin is and/or comprises Atorvastatin (e.g., Lipitor® or Torvast®), Cerivastatin (e.g., Lipobay® or Baycol®), Fluvastatin (e.g., Lescol® or LescolXL®), Lovastatin (e.g., Mevacor®, Altocor®, or Altoprev®) Mevastatin, Pitavastatin (e.g., Livalo® or Pitava®), Pravastatin (e.g., Pravachol®, Selektine, or Lipostat®) Rosuvastatin (e.g., Crestor®), Simvastatin (e.g., Zocor® or Lipex®), Vytorin®, Advicor®, Besylate Caduet® or Simcor®.

[0121] In one embodiment of the present invention, the chimeric protein according to the present invention or the pharmaceutical composition thereof is administered with an anti-inflammatory agent, an antifibrotic agent, an antihypertensive agent, an antidiabetic agent, a triglyceride-lowering agent, and/or a cholesterol-lowering agent.

[0122] Another aspect of the present invention relates to a method of making a chimeric FGF protein possessing enhanced endocrine activity. This method involves introducing one or

more modifications to an FGF protein, where the modification decreases the affinity of the FGF protein for heparin and/or heparan sulfate and coupling a Klotho co-receptor binding domain to the modified FGF protein's C-terminus, whereby a chimeric FGF protein possessing enhanced endocrine activity is made.

[0123] In one embodiment, the method includes selecting a Klotho co-receptor binding domain, where the Klotho co-receptor binding domain is selected to target an endocrine FGF target tissue. In one embodiment, the Klotho co-receptor binding domain is selected to home the chimeric FGF protein into a target tissue of endocrine FGF. In one embodiment, the Klotho co-receptor binding domain is selected to target white adipose tissue, brown adipose tissue, skeletal muscle, pancreas, and/or liver.

[0124] In one embodiment, the Klotho co-receptor binding domain includes a β-Klotho co-receptor binding domain. In one embodiment, the β-Klotho co-receptor binding domain includes a C-terminal portion from FGF21. In one embodiment, the C-terminal portion from the FGF21 includes amino acid residues 168-209 of SEQ ID NO: 233. In one embodiment, the C-terminal portion derived from FGF21 further includes one or more substitutions while retaining the ability to bind β-Klotho. In one embodiment, the C-terminal portion derived from FGF21 further includes one or more substitutions to enhance its binding affinity for β-Klotho. In one embodiment, the C-terminal portion from FGF21 is derived from a mammalian FGF21. In one embodiment, the C-terminal portion derived from FGF21 is from a vertebrate FGF21. Suitable FGF21 molecules, C-terminal portions therof, and modifications thereto, are described above.

[0125] In one embodiment, the chimeric FGF protein has greater binding affinity for FGFR than native endocrine FGF ligand having the Klotho co-receptor binding domain. In one embodiment, the chimeric FGF protein possesses enhanced endocrine activity compared to the chimeric FGF protein in the absence of the modification or the Klotho co-receptor binding domain. In one embodiment, the native endocrine FGF ligand having the Klotho co-receptor binding domain is native FGF21. In one embodiment, the FGFR is FGFR1c, FGFR2c, or FGFR4.

[0126] In one embodiment, the chimeric FGF protein has greater stability than a native endocrine FGF ligand possessing the Klotho co-receptor binding domain. In one embodiment, increasing the stability includes an increase in thermal stability of the protein as compared to either wild type protein or native endocrine FGF ligand. In one embodiment, increasing the stability includes increasing the half-life of the protein in the blood circulation as compared to wild type protein or native endocrine FGF ligand.

[0127] In one embodiment, the method involves introducing one or more modifications to the FGF protein, where the modification alters the receptor-binding specificity of the FGF protein. In one embodiment, the method involves introducing one or more modifications to the FGF protein, where the modification alters the receptor-binding affinity of the FGF protein.

[0128] In one embodiment, the FGF is derived from a mammalian FGF. In one embodiment, the FGF is derived from a vertebrate FGF. In one embodiment, the FGF protein is a paracrine FGF molecule. In one embodiment the FGF molecule is FGF1 or FGF2. In one embodiment, the FGF protein is an FGF protein that possesses intrinsically greater binding affinity for FGF receptor than a native endocrine FGF ligand. In one embodiment, the FGF protein is an FGF protein that possesses intrinsically greater thermal stability than a native endocrine FGF ligand. In one embodiment, the method involves introducing one or more modifications to the FGF protein, where the modification alters receptor-binding specificity and/or receptor-binding affinity of the FGF protein. In one embodiment, the method involves introducing one or more modifications to the FGF protein, where the modification alters the stability of the FGF protein. For example, receptor-binding specificity of FGF1, which by nature binds to all the seven principal FGFRs, may be altered to, for example, reduce any risk for adverse effects (e.g., mitogenicity). Paracrine FGFs, portions of paracrine FGFs, and modifications thereto are described above.

[0129] In one embodiment, the chimeric FGF protein is effective to treat diabetes, obesity, and/or metabolic syndrome.

[0130] Suitable methods of generating chimeric proteins according to the present invention include standard methods of synthesis known in the art, as described above.

[0131] Yet another aspect of the present invention relates to a method of facilitating fibroblast growth factor receptor ("FGFR")-βKlotho co-receptor complex formation. This method involves providing a cell that includes a βKlotho co-receptor and an FGFR and providing a chimeric FGF protein. The chimeric FGF protein includes a C-terminal portion of FGF21 and a portion of a paracrine FGF, where the portion of the paracrine FGF is modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without the modification. This method also involves contacting the cell and the chimeric FGF protein under conditions effective to cause FGFR-βKlotho co-receptor complex formation.

[0132] The portion of the paracrine FGF may also be modified to alter receptor-binding specificity and/or receptor-binding affinity of the FGF, as noted above. Suitable portions of the paracrine FGFs for use in accordance with the present invention, as well as modifications to alter

receptor-binding specificity and/or receptor-binding affinity of the FGF, are described above. Suitable modifications to the paracrine FGFs for use in accordance with the present invention are also described above. Suitable C-terminal portions from FGF21 are described above and throughout the present application.

[0133] In one embodiment according to the present invention, βKlotho is mammalian βKlotho. In one embodiment, βKlotho is human or mouse βKlotho. In one particular embodiment of the present invention, βKlotho is human or mouse βKlotho having the amino acid sequence of SEQ ID NO: 347 (i.e., GenBank Accession No. NP_783864, which is hereby incorporated by reference in its entirety) or SEQ ID NO: 348 (i.e., GenBank Accession No. NP_112457, which is hereby incorporated by reference in its entirety), respectively, as follows:

SEQ ID NO: 347:

```
1    MKPGCAAGSP GNEWIFFSTD EITTRYRNTM SNGGLQRSVI LSALILLRAV TGFSGDGRAI
61   WSKNPNFTPV NESQLFLYDT FPKNFFWGIG TGALQVEGSW KKDGKGPSIW DHFIHTHLKN
121  VSSTNGSSDS YIFLEKDLSA LDFIGVSFYQ FSISWPRLFP DGIVTVANAK GLQYYSTLLD
181  ALVLRNIEPI VTLYHWDLPL ALQEKYGGWK NDTIIDIFND YATYCFQMFG DRVKYWITIH
241  NPYLVAWHGY GTGMHAPGEK GNLAAVYTVG HNLIKAHSKV WHNYNTHFRP HQKGWLSITL
301  GSHWIEPNRS ENTMDIFKCQ QSMVSVLGWF ANPIHGDGDY PEGMRKKLFS VLPIFSEAEK
361  HEMRGTADFF AFSFGPNNFK PLNTMAKMGQ NVSLNLREAL NWIKLEYNNP RILIAENGWF
421  TDSRVKTEDT TAIYMMKNFL SQVLQAIRLD EIRVFGYTAW SLLDGFEWQD AYTIRRGLFY
481  VDFNSKQKER KPKSSAHYYK QIIRENGFSL KESTPDVQGQ FPCDFSWGVT ESVLKPESVA
541  SSPQFSDPHL YVWNATGNRL LHRVEGVRLK TRPAQCTDFV NIKKQLEMLA RMKVTHYRFA
601  LDWASVLPTG NLSAVNRQAL RYYRCVVSEG LKLGISAMVT LYYPTHAHLG LPEPLLHADG
661  WLNPSTAEAF QAYAGLCFQE LGDLVKLWIT INEPNRLSDI YNRSGNDTYG AAHNLLVAHA
721  LAWRLYDRQF RPSQRGAVSL SLHADWAEPA NPYADSHWRA AERFLQFEIA WFAEPLFKTG
781  DYPAAMREYI ASKHRRGLSS SALPRLTEAE RRLLKGTVDF CALNHFTTRF VMHEQLAGSR
841  YDSDRDIQFL QDITRLSSPT RLAVIPWGVR KLLRWVRRNY GDMDIYITAS GIDDQALEDD
901  RLRKYYLGKY LQEVLKAYLI DKVRIKGYYA FKLAEEKSKP RFGFFTSDFK AKSSIQFYNK
961  VISSRGFPFE NSSSRCSQTQ ENTECTVCLF LVQKKPLIFL GCCFFSTLVL LLSIAIFQRQ
1021 KRRKFWKAKN LQHIPLKKGK RVVS
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SEQ ID NO: 348:

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1    MKTGCAAGSP GNEWIFFSSD ERNTRSRKTM SNRALQRSAV LSAFVLLRAV TGFSGDGKAI
61   WDKKQYVSPV NPSQLFLYDT FPKNFSWGVG TGAFQVEGSW KTDGRGPSIW DRYVYSHLRG
121  VNGTDRSTDS YIFLEKDLLA LDFLGVSFYQ FSISWPRLFP NGTVAAVNAQ GLRYYRALLD
181  SLVLRNIEPI VTLYHWDLPL TLQEEYGGWK NATMIDLFND YATYCFQTFG DRVKYWITIH
241  NPYLVAWHGF GTGMHAPGEK GNLTAVYTVG HNLIKAHSKV WHNYDKNFRP HQKGWLSITL
301  GSHWIEPNRT DNMEDVINCQ HSMSSVLGWF ANPIHGDGDY PEFMKTGAMI PEFSEAEKEE
361  VRGTADFFAF SFGPNNFRPS NTVVKMGQNV SLNLRQVLNW IKLEYDDPQI LISENGWFTD
```

421 SYIKTEDTTA IYMMKNFLNQ VLQAIKFDEI RVFGYTAWTL LDGFEWQDAY TTRRGLFYVD
481 FNSEQKERKP KSSAHYYKQI IQDNGFPLKE STPDMKGRFP CDFSWGVTES VLKPEFTVSS
541 PQFTDPHLYV WNVTGNRLLY RVEGVRLKTR PSQCTDYVSI KKRVEMLAKM KVTHYQFALD
601 WTSILPTGNL SKVNRQVLRY YRCVVSEGLK LGVFPMVTLY HPTHSHLGLP LPLLSSGGWL
661 NMNTAKAFQD YAELCFRELG DLVKLWITIN EPNRLSDMYN RTSNDTYRAA HNLMIAHAQV
721 WHLYDRQYRP VQHGAVSLSL HCDWAEPANP FVDSHWKAAE RFLQFEIAWF ADPLFKTGDY
781 PSVMKEYIAS KNQRGLSSSV LPRFTAKESR LVKGTVDFYA LNHFTTRFVI HKQLNTNRSV
841 ADRDVQFLQD ITRLSSPSRL AVTPWGVRKL LAWIRRNYRD RDIYITANGI DDLALEDDQI
901 RKYYLEKYVQ EALKAYLIDK VKIKGYYAFK LTEEKSKPRF GFFTSDFRAK SSVQFYSKLI
961 SSSGLPAENR SPACGQPAED TDCTICSFLV EKKPLIFFGC CFISTLAVLL SITVFHHQKR
1021 RKFQKARNLQ NIPLKKGHSR VFS

[0134] In one particular embodiment of the present invention, βKlotho is human or mouse βKlotho encoded by a nucleotide sequence having the nucleotide sequences of SEQ ID NO: 349 (GenBank Accession No. NM_175737, which is hereby incorporated by reference in its entirety) and SEQ ID NO: 350 (GenBank Accession No. NM_031180, which is hereby incorporated by reference in its entirety), as follows:

SEQ ID NO: 349 (Human βKlotho gene coding sequence):

98 ATG AAGCCAGGCT GTGCGGCAGG ATCTCCAGGG AATGAATGGA TTTTCTTCAG
151 CACTGATGAA ATAACCACAC GCTATAGGAA TACAATGTCC AACGGGGGAT TGCAAAGATC
211 TGTCATCCTG TCAGCACTTA TTCTGCTACG AGCTGTTACT GGATTCTCTG GAGATGGAAG
271 AGCTATATGG TCTAAAAATC CTAATTTTAC TCCGGTAAAT GAAAGTCAGC TGTTTCTCTA
331 TGACACTTTC CCTAAAAACT TTTTCTGGGG TATTGGGACT GGAGCATTGC AAGTGGAAGG
391 GAGTTGGAAG AAGGATGGAA AAGGACCTTC TATATGGGAT CATTTCATCC ACACACACCT
451 TAAAAATGTC AGCAGCACGA ATGGTTCCAG TGACAGTTAT ATTTTTCTGG AAAAAGACTT
511 ATCAGCCCTG GATTTTATAG GAGTTTCTTT TTATCAATTT TCAATTTCCT GGCCAAGGCT
571 TTTCCCCGAT GGAATAGTAA CAGTTGCCAA CGCAAAAGGT CTGCAGTACT ACAGTACTCT
631 TCTGGACGCT CTAGTGCTTA GAAACATTGA ACCTATAGTT ACTTTATACC ACTGGGATTT
691 GCCTTTGGCA CTACAAGAAA AATATGGGGG GTGGAAAAAT GATACCATAA TAGATATCTT
751 CAATGACTAT GCCACATACT GTTTCCAGAT GTTTGGGGAC CGTGTCAAAT ATTGGATTAC
811 AATTCACAAC CCATATCTAG TGGCTTGGCA TGGGTATGGG ACAGGTATGC ATGCCCCTGG
871 AGAGAAGGGA AATTTAGCAG CTGTCTACAC TGTGGGACAC AACTTGATCA AGGCTCACTC
931 GAAAGTTTGG CATAACTACA ACACACATTT CCGCCCACAT CAGAAGGGTT GGTTATCGAT
991 CACGTTGGGA TCTCATTGGA TCGAGCCAAA CCGGTCGGAA AACACGATGG ATATATTCAA
1051 ATGTCAACAA TCCATGGTTT CTGTGCTTGG ATGGTTTGCC AACCCTATCC ATGGGGATGG
1111 CGACTATCCA GAGGGGATGA GAAAGAAGTT GTTCTCCGTT CTACCCATTT TCTCTGAAGC
1171 AGAGAAGCAT GAGATGAGAG GCACAGCTGA TTTCTTTGCC TTTTCTTTTG GACCCAACAA
1231 CTTCAAGCCC CTAAACACCA TGGCTAAAAT GGGACAAAAT GTTTCACTTA ATTTAAGAGA
1291 AGCGCTGAAC TGGATTAAAC TGGAATACAA CAACCCTCGA ATCTTGATTG CTGAGAATGG
1351 CTGGTTCACA GACAGTCGTG TGAAAACAGA AGACACCACG GCCATCTACA TGATGAAGAA

1411 TTTCCTCAGC CAGGTGCTTC AAGCAATAAG GTTAGATGAA ATACGAGTGT TTGGTTATAC
1471 TGCCTGGTCT CTCCTGGATG GCTTTGAATG GCAGGATGCT TACACCATCC GCCGAGGATT
1531 ATTTTATGTG GATTTTAACA GTAAACAGAA AGAGCGGAAA CCTAAGTCTT CAGCACACTA
1591 CTACAAACAG ATCATACGAG AAAATGGTTT TTCTTTAAAA GAGTCCACGC CAGATGTGCA
1651 GGGCCAGTTT CCCTGTGACT TCTCCTGGGG TGTCACTGAA TCTGTTCTTA AGCCCGAGTC
1711 TGTGGCTTCG TCCCCACAGT TCAGCGATCC TCATCTGTAC GTGTGGAACG CCACTGGCAA
1771 CAGACTGTTG CACCGAGTGG AAGGGGTGAG GCTGAAAACA CGACCCGCTC AATGCACAGA
1831 TTTTGTAAAC ATCAAAAAAC AACTTGAGAT GTTGGCAAGA ATGAAAGTCA CCCACTACCG
1891 GTTTGCTCTG GATTGGGCCT CGGTCCTTCC CACTGGCAAC CTGTCCGCGG TGAACCGACA
1951 GGCCCTGAGG TACTACAGGT GCGTGGTCAG TGAGGGGCTG AAGCTTGGCA TCTCCGCGAT
2011 GGTCACCCTG TATTATCCGA CCCACGCCCA CCTAGGCCTC CCCGAGCCTC TGTTGCATGC
2071 CGACGGGTGG CTGAACCCAT CGACGGCCGA GGCCTTCCAG GCCTACGCTG GGCTGTGCTT
2131 CCAGGAGCTG GGGGACCTGG TGAAGCTCTG GATCACCATC AACGAGCCTA ACCGGCTAAG
2191 TGACATCTAC AACCGCTCTG GCAACGACAC CTACGGGGCG GCGCACAACC TGCTGGTGGC
2251 CCACGCCCTG GCCTGGCGCC TCTACGACCG GCAGTTCAGG CCCTCACAGC GCGGGGCCGT
2311 GTCGCTGTCG CTGCACGCGG ACTGGGCGGA ACCCGCCAAC CCCTATGCTG ACTCGCACTG
2371 GAGGGCGGCC GAGCGCTTCC TGCAGTTCGA GATCGCCTGG TTCGCCGAGC CGCTCTTCAA
2431 GACCGGGGAC TACCCCGCGG CCATGAGGGA ATACATTGCC TCCAAGCACC GACGGGGGCT
2491 TTCCAGCTCG GCCCTGCCGC GCCTCACCGA GGCCGAAAGG AGGCTGCTCA AGGGCACGGT
2551 CGACTTCTGC GCGCTCAACC ACTTCACCAC TAGGTTCGTG ATGCACGAGC AGCTGGCCGG
2611 CAGCCGCTAC GACTCGGACA GGGACATCCA GTTTCTGCAG GACATCACCC GCCTGAGCTC
2671 CCCCACGCGC CTGGCTGTGA TTCCCTGGGG GGTGCGCAAG CTGCTGCGGT GGGTCCGGAG
2731 GAACTACGGC GACATGGACA TTTACATCAC CGCCAGTGGC ATCGACGACC AGGCTCTGGA
2791 GGATGACCGG CTCCGGAAGT ACTACCTAGG GAAGTACCTT CAGGAGGTGC TGAAAGCATA
2851 CCTGATTGAT AAAGTCAGAA TCAAAGGCTA TTATGCATTC AAACTGGCTG AAGAGAAATC
2911 TAAACCCAGA TTTGGATTCT TCACATCTGA TTTTAAAGCT AAATCCTCAA TACAATTTTA
2971 CAACAAAGTG ATCAGCAGCA GGGGCTTCCC TTTTGAGAAC AGTAGTTCTA GATGCAGTCA
3031 GACCCAAGAA AATACAGAGT GCACTGTCTG CTTATTCCTT GTGCAGAAGA AACCACTGAT
3091 ATTCCTGGGT TGTTGCTTCT TCTCCACCCT GGTTCTACTC TTATCAATTG CCATTTTTCA
3151 AAGGCAGAAG AGAAGAAAGT TTTGGAAAGC AAAAAACTTA CAACACATAC CATTAAAGAA
3211 AGGCAAGAGA GTTGTTAGCT AA

SEQ ID NO: 350 (House mouse βKlotho gene coding sequence):

2 ATGAAGACA GGCTGTGCAG CAGGGTCTCC GGGGAATGAA TGGATTTTCT TCAGCTCTGA
61 TGAAAGAAAC ACACGCTCTA GGAAAACAAT GTCCAACAGG GCACTGCAAA GATCTGCCGT
121 GCTGTCTGCG TTTGTTCTGC TGCGAGCTGT TACCGGCTTC TCCGGAGACG GGAAAGCAAT
181 ATGGGATAAA AAACAGTACG TGAGTCCGGT AAACCCAAGT CAGCTGTTCC TCTATGACAC
241 TTTCCCTAAA AACTTTTCCT GGGGCGTTGG GACCGGAGCA TTTCAAGTGG AAGGGAGTTG
301 GAAGACAGAT GGAAGAGGAC CCTCGATCTG GGATCGGTAC GTCTACTCAC ACCTGAGAGG
361 TGTCAACGGC ACAGACAGAT CCACTGACAG TTACATCTTT CTGGAAAAAG ACTTGTTGGC

421 TCTGGATTTT TTAGGAGTTT CTTTTTATCA GTTCTCAATC TCCTGGCCAC GGTTGTTTCC
481 CAATGGAACA GTAGCAGCAG TGAATGCGCA AGGTCTCCGG TACTACCGTG CACTTCTGGA
541 CTCGCTGGTA CTTAGGAATA TCGAGCCCAT TGTTACCTTG TACCATTGGG ATTTGCCTCT
601 GACGCTCCAG GAAGAATATG GGGGCTGGAA AAATGCAACT ATGATAGATC TCTTCAACGA
661 CTATGCCACA TACTGCTTCC AGACCTTTGG AGACCGTGTC AAATATTGGA TTACAATTCA
721 CAACCCTTAC CTTGTTGCTT GGCATGGGTT TGGCACAGGT ATGCATGCAC CAGGAGAGAA
781 GGGAAATTTA ACAGCTGTCT ACACTGTGGG ACACAACCTG ATCAAGGCAC ATTCGAAAGT
841 GTGGCATAAC TACGACAAAA ACTTCCGCCC TCATCAGAAG GGTTGGCTCT CCATCACCTT
901 GGGGTCCCAT TGGATAGAGC CAAACAGAAC AGACAACATG GAGGACGTGA TCAACTGCCA
961 GCACTCCATG TCCTCTGTGC TTGGATGGTT CGCCAACCCC ATCCACGGGG ACGGCGACTA
1021 CCCTGAGTTC ATGAAGACGG GCGCCATGAT CCCCGAGTTC TCTGAGGCAG AGAAGGAGGA
1081 GGTGAGGGGC ACGGCTGATT TCTTTGCCTT TTCCTTCGGG CCCAACAACT TCAGGCCCTC
1141 AAACACCGTG GTGAAAATGG GACAAAATGT ATCACTCAAC TTAAGGCAGG TGCTGAACTG
1201 GATTAAACTG GAATACGATG ACCCTCAAAT CTTGATTTCG GAGAACGGCT GGTTCACAGA
1261 TAGCTATATA AAGACAGAGG ACACCACGGC CATCTACATG ATGAAGAATT TCCTAAACCA
1321 GGTTCTTCAA GCAATAAAAT TTGATGAAAT CCGCGTGTTT GGTTATACGG CCTGGACTCT
1381 CCTGGATGGC TTTGAGTGGC AGGATGCCTA TACGACCCGA CGAGGGCTGT TTTATGTGGA
1441 CTTTAACAGT GAGCAGAAAG AGAGGAAACC CAAGTCCTCG GCTCATTACT ACAAGCAGAT
1501 CATACAAGAC AACGGCTTCC CTTTGAAAGA GTCCACGCCA GACATGAAGG GTCGGTTCCC
1561 CTGTGATTTC TCTTGGGGAG TCACTGAGTC TGTTCTTAAG CCCGAGTTTA CGGTCTCCTC
1621 CCCGCAGTTT ACCGATCCTC ACCTGTATGT GTGGAATGTC ACTGGCAACA GATTGCTCTA
1681 CCGAGTGGAA GGGGTAAGGC TGAAAACAAG ACCATCCCAG TGCACAGATT ATGTGAGCAT
1741 CAAAAAACGA GTTGAAATGT TGGCAAAAAT GAAAGTCACC CACTACCAGT TTGCTCTGGA
1801 CTGGACCTCT ATCCTTCCCA CTGGCAATCT GTCCAAAGTT AACAGACAAG TGTTAAGGTA
1861 CTATAGGTGT GTGGTGAGCG AAGGACTGAA GCTGGGCGTC TTCCCCATGG TGACGTTGTA
1921 CCACCCAACC CACTCCCATC TCGGCCTCCC CCTGCCACTT CTGAGCAGTG GGGGGTGGCT
1981 AAACATGAAC ACAGCCAAGG CCTTCCAGGA CTACGCTGAG CTGTGCTTCC GGGAGTTGGG
2041 GGACTTGGTG AAGCTCTGGA TCACCATCAA TGAGCCTAAC AGGCTGAGTG ACATGTACAA
2101 CCGCACGAGT AATGACACCT ACCGTGCAGC CCACAACCTG ATGATCGCCC ATGCCCAGGT
2161 CTGGCACCTC TATGATAGGC AGTATAGGCC GGTCCAGCAT GGGGCTGTGT CGCTGTCCTT
2221 ACATTGCGAC TGGGCAGAAC CTGCCAACCC CTTTGTGGAT TCACACTGGA AGGCAGCCGA
2281 GCGCTTCCTC CAGTTTGAGA TCGCCTGGTT TGCAGATCCG CTCTTCAAGA CTGGCGACTA
2341 TCCATCGGTT ATGAAGGAAT ACATCGCCTC CAAGAACCAG CGAGGGCTGT CTAGCTCAGT
2401 CCTGCCGCGC TTCACCGCGA AGGAGAGCAG GCTGGTGAAG GGTACCGTCG ACTTCTACGC
2461 ACTGAACCAC TTCACTACGA GGTTCGTGAT ACACAAGCAG CTGAACACCA ACCGCTCAGT
2521 TGCAGACAGG GACGTCCAGT TCCTGCAGGA CATCACCCGC CTAAGCTCGC CCAGCCGCCT
2581 GGCTGTAACA CCCTGGGGAG TGCGCAAGCT CCTTGCGTGG ATCCGGAGGA ACTACAGAGA
2641 CAGGGATATC TACATCACAG CCAATGGCAT CGATGACCTG GCTCTAGAGG ATGATCAGAT
2701 CCGAAAGTAC TACTTGGAGA AGTATGTCCA GGAGGCTCTG AAAGCATATC TCATTGACAA
2761 GGTCAAAATC AAAGGCTACT ATGCATTCAA ACTGACTGAA GAGAAATCTA AGCCTAGATT

2821 TGGATTTTTC ACCTCTGACT TCAGAGCTAA GTCCTCTGTC CAGTTTTACA GCAAGCTGAT
2881 CAGCAGCAGT GGCCTCCCCG CTGAGAACAG AAGTCCTGCG TGTGGTCAGC CTGCGGAAGA
2941 CACAGACTGC ACCATTTGCT CATTTCTCGT GGAGAAGAAA CCACTCATCT TCTTCGGTTG
3001 CTGCTTCATC TCCACTCTGG CTGTACTGCT ATCCATCACC GTTTTTCATC ATCAAAAGAG
3061 AAGAAAATTC CAGAAAGCAA GGAACTTACA AAATATACCA TTGAAGAAAG GCCACAGCAG
3121 AGTTTTCAGC TAA

[0135] In one embodiment, the FGFR is FGFR1c, FGFR2c, or FGFR4. In one embodiment of the present invention, the FGF receptor is FGFR1c receptor. In one particular embodiment, the FGFR1c receptor is the human FGFR1c receptor (GenBank Accession No. NP_075598, which is hereby incorporated by reference in its entirety). In another embodiment, the FGF receptor is FGFR2c receptor. In one particular embodiment, the FGFR2c receptor is the human FGFR2c receptor (GenBank Accession No. NP_000132, which is hereby incorporated by reference in its entirety). In another embodiment, the FGF receptor is FGFR4 receptor. In one particular embodiment, the FGFR4 receptor is the human FGFR4 receptor (GenBank Accession No. NP_002002, which is hereby incorporated by reference in its entirety).

[0136] In one embodiment, the method of facilitating FGFR-βKlotho co-receptor complex formation is carried out *in vitro*. In one embodiment, the method is carried out in an adipocyte. In another embodiment, the method is carried out in a skeletal muscle cell, a pancreatic β cell, or a hepatocyte.

[0137] In one embodiment, the method of facilitating FGFR-Klotho co-receptor complex formation is carried out *in vivo*. In one embodiment, the method is carried out in a mammal. In one particular embodiment, the mammal is a mouse.

[0138] Yet a further aspect of the present invention relates to a method of screening for agents capable of facilitating FGFR-βKlotho complex formation in the treatment of a disorder. This method involves providing a chimeric FGF that includes an N-terminus coupled to a C-terminus, where the N-terminus includes a portion of a paracrine FGF and the C-terminus includes a C-terminal portion of FGF21. The portion of the paracrine FGF is modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without the modification. This method also involves providing binary βKlotho-FGFR complex and providing one or more candidate agents. This method further involves combining the chimeric FGF, the binary βKlotho-FGFR complex, and the one or more candidate agents under conditions permitting the formation of a ternary complex between the chimeric FGF and the binary βKlotho-FGFR complex in the absence of the one or more candidate agents. This method also involves identifying the one or more candidate agents that decrease ternary complex formation

between the chimeric FGF and the binary βKlotho-FGFR complex compared to the ternary complex formation in the absence of the one or more candidate agents as suitable for treating the disorder.

[0139] The portion of the paracrine FGF may also be modified to alter receptor-binding specificity and/or reduce receptor-binding affinity compared to the portion without the modification.

[0140] Suitable chimeric proteins for use in accordance with this aspect of the present invention are described above and throughout the present application. Suitable paracrine FGFs, as well as suitable modifications to decrease binding affinity for heparin and/or heparan sulfate, to alter receptor-binding specificity and/or to reduce receptor-binding affinity compared to the portion without the modification, are also described above.

[0141] In one embodiment, the modulation is a competitive interaction between the chimeric FGF molecule and the one or more candidate agents for binding to the binary βKlotho-FGFR complex.

[0142] In one embodiment, the FGFR is FGFR1c, FGFR2c, or FGFR4.

[0143] In one embodiment, the disorder is a selected from diabetes, obesity, and metabolic syndrome. In one embodiment, the disorder is diabetes selected from type II diabetes, gestational diabetes, or drug-induced diabetes. In one embodiment, the disorder is type I diabetes. In one embodiment, the disorder is obesity. In one embodiment, the disorder is metabolic syndrome.

[0144] In one embodiment of the screening aspects of the present invention, a plurality of compounds or agents is tested. Candidate agents may include small molecule compounds or larger molecules (e.g., proteins or fragments thereof). In one embodiment, the candidate compounds are biomolecules. In one embodiment, the biomolecules are proteins. In one embodiment, the biomolecules are peptides. In one embodiment, the candidates are peptides or peptide mimetics having similar structural features to native FGF ligand. In one embodiment, the candidate agent is a second chimeric FGF molecule. In one particular embodiment, the peptides are synthetic peptides. In one embodiment, the compounds are small organic molecules.

[0145] In one embodiment of the screening aspects of the present invention, the method is carried out using a cell-based assay. In one embodiment, the identifying is carried out using a cell-based assay.

[0146] In one embodiment of the screening aspects of the present invention, the method is carried out using a binding assay. In one embodiment, the binding assay is a direct binding assay. In one embodiment, the binding assay is a competition-binding assay. In one embodiment, the modulation stabilizes the ternary complex between the chimeric FGF molecule and the binary βKlotho-FGFR complex. In one embodiment, the stabilization is compared to the native ternary complex.

[0147] In one embodiment, the modulation is an allosteric or kinetic modulation. In one embodiment, the allosteric or kinetic modulation is compared to the native ternary complex. Such stabilization or allosteric or kinetic modulation can be measured using methods known in the art (e.g., by use of surface plasmon resonance (SPR) spectroscopy experiments as described in the Examples *infra*).

[0148] In one embodiment, the binding assay is carried out using surface plasmon resonance spectroscopy. In one embodiment, the identifying is carried out using a binding assay. In one embodiment, the identifying is carried out using surface plasmon resonance spectroscopy.

[0149] In one embodiment of the screening aspects of the present invention, the cell-based assay is carried out with adipocytes. In one embodiment, the cell-based assay is carried out with skeletal muscle cells. In one embodiment, the cell-based assay is carried out with pancreatic β cells. In one embodiment, the cell-based assay is carried out with hepatocytes. In one embodiment, stimulation of glucose uptake is the assay readout. In one embodiment, induction of glucose transporter 1 gene expression is the assay readout. In one embodiment, a dose-response curve is generated for the stimulation of glucose uptake by a candidate compound to determine potency and efficacy of the candidate compound. In one embodiment, a dose-response curve is generated for the induction of glucose transporter 1 gene expression by a candidate compound to determine potency and efficacy of the candidate compound. For example, if the dose-response curve is shifted to the left compared to that obtained for the chimeric FGF protein, the candidate compound has greater potency than the chimeric FGF protein and/or native FGF21. In one embodiment, an IC_{50} value is derived from the dose-response curve of a candidate compound to determine potency of the candidate compound. An IC_{50} value smaller than that obtained for the chimeric FGF protein identifies a candidate compound as more potent than the chimeric FGF protein and/or native FGF21.

[0150] In one embodiment of the screening aspects of the present invention, the cell-based assay is carried out with mammalian cells ectopically expressing βKlotho. In one particular embodiment, the cells are HEK293 cells. In one embodiment, activation of FGF

receptor is the assay readout. In one embodiment, tyrosine phosphorylation of an FGF receptor substrate is used as readout for FGF receptor activation. In one particular embodiment, the FGF receptor substrate is FGF receptor substrate 2α. In one embodiment, activation of downstream mediators of FGF signaling is used as readout for (or an indicator of) FGF receptor activation. In one particular embodiment, the downstream mediator of FGF signaling is 44/42 mitogen-activated protein kinase. In one embodiment, the downstream mediator of FGF signaling is a transcription factor. In one particular embodiment, the transcription factor is early growth response 1. In one embodiment, a dose-response curve is generated for βKlotho-dependent activation of FGF receptor by a candidate compound to determine potency and efficacy of the candidate compound. For example, if the dose-response curve is shifted to the left compared to that obtained for the chimeric FGF protein, the candidate compound is more potent than the chimeric FGF protein and/or native FGF21. In one embodiment, an IC_{50} value is derived from the dose-response curve of a candidate compound to determine potency of the candidate compound. An IC_{50} value smaller than that obtained for the chimeric FGF protein identifies a candidate compound as more potent than the chimeric FGF protein and/or native FGF21.

[0151] In one embodiment of the screening aspects of the present invention, the surface plasmon resonance spectroscopy-based assay is carried out using the chimeric FGF protein as ligand coupled to a biosensor chip. In one embodiment, mixtures of βKlotho ectodomain with increasing concentrations of a candidate compound are passed over a biosensor chip containing chimeric FGF protein. In one embodiment, mixtures of the binary complex of FGFR ligand-binding domain and βKlotho ectodomain with increasing concentrations of a candidate compound are passed over a biosensor chip containing chimeric FGF protein. In one particular embodiment, the FGFR ligand-binding domain is the FGFR1c ligand-binding domain. In one embodiment, an inhibition-binding curve is plotted for a candidate compound to determine potency of the candidate compound. For example, if the inhibition-binding curve is shifted to the left compared to that obtained for the chimeric FGF protein, the candidate compound has greater potency than the chimeric FGF protein and/or native FGF21. In one embodiment, an IC_{50} value is derived from the inhibition-binding curve of a candidate compound to determine potency of the candidate compound. An IC_{50} value smaller than that obtained for containing chimeric FGF protein identifies a candidate compound as more potent than the chimeric FGF protein and/or native FGF21. In one embodiment, the inhibition constant K_i is determined for a candidate compound to determine potency of the candidate compound. A K_i value smaller than

that obtained for native FGF21 identifies a candidate compound as more potent than the chimeric FGF protein and/or native FGF21.

[0152] In one embodiment of the screening aspects of the present invention, the method is carried out *in vivo*. In one embodiment, the method is carried out in a mammal. In one particular embodiment, the mammal is a mouse. In one embodiment, the mammal has obesity, diabetes, or a related metabolic disorder. In one embodiment, the ability of a candidate compound to potentiate the hypoglycemic effect of insulin is used as readout for FGF21-like metabolic activity. This involves fasting the mammal for a period of time prior to insulin injection and measuring fasting blood glucose levels. The mammal is then injected with insulin alone or co-injected with insulin plus a candidate compound. Blood glucose levels are measured at several time points after the injection. If a candidate compound potentiates the hypoglycemic effect of insulin to a greater degree than the chimeric FGF protein and/or native FGF21 does, the candidate compound exhibits enhanced efficacy. Likewise, if a candidate compound potentiates the hypoglycemic effect of insulin to a similar degree than the chimeric FGF protein and/or native FGF21 does but at a lower dose compared to that of the chimeric FGF protein and/or native FGF21 and/or for a longer period of time compared to the chimeric FGF protein and/or native FGF21, the candidate compound has enhanced agonistic properties. In one embodiment, the ability of a candidate compound to elicit a hypoglycemic effect in a mammal with diabetes, obesity, or a related metabolic disorder is used as readout for FGF21-like metabolic activity. This involves injecting a mammal suffering from diabetes, obesity, or a related metabolic disorder with the candidate compound. Blood glucose levels are measured before the injection and at several time points thereafter. If a candidate compound has a greater hypoglycemic effect than the chimeric FGF protein and/or native FGF21 does, the candidate compound exhibits enhanced efficacy. Likewise, if a candidate compound shows a similar hypoglycemic effect than the chimeric FGF protein and/or native FGF21 does but at a lower dose compared to that of the chimeric FGF protein and/or native FGF21 and/or for a longer period of time compared to the chimeric FGF protein and/or native FGF21, the candidate compound has enhanced agonistic properties.

EXAMPLES

Example 1 - Purification of FGF, FGFR, and Klotho Proteins

[0153] The N-terminally hexahistidine-tagged, mature form of human FGF19 (SEQ ID NO: 337) (R23 to K216), human FGF21 (SEQ ID NO:233) (H29 to S209; FIG. 5A), and human

FGF23 (SEQ ID NO: 351) (Y25 to I251; FIG. 5A) was refolded *in vitro* from bacterial inclusion bodies, and purified by published protocols (Ibrahimi et al., *Hum. Mol. Genet.* 13:2313-2324 (2004); Plotnikov et al., *Cell* 101:413-424 (2000), which is hereby incorporated by reference in its entirety). The amino acid sequence of human FGF23 (SEQ ID NO:351) (GenBank accession no. AAG09917, which is hereby incorporated by reference in its entirety) is as follows:

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1   MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS YHLQIHKNGH
61  VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG NIFGSHYFDP ENCRFQHQTL
121 ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN PPPYSQFLSR RNEIPLIHFN TPIPRRHTRS
181 AEDDSERDPL NVLKPRARMT PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG
241 PEGCRPFAKF I
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[0154] HS-binding site mutants of FGF19 (K149A) and FGF23 (R140A/R143A) were purified from bacterial inclusion bodies by similar protocols as the wild-type proteins. In order to minimize proteolysis of FGF23 wild-type and mutant proteins, arginine residues 176 and 179 of the proteolytic cleavage site ^{176}RXXR^{179} were replaced with glutamine as it occurs in the phosphate wasting disorder "autosomal dominant hypophosphatemic rickets" (ADHR) (White et al., *Nat. Genet.* 26:345-348 (2000); White et al., *Kidney Int.* 60:2079-2086 (2001), which are hereby incorporated by reference in their entirety). Human FGF1 (SEQ ID NO:1) (M1 to D155; FIG. 6), N-terminally truncated human FGF1 (K25 to D155, termed FGF1$^{\Delta NT}$; FIG. 6), human FGF2 (SEQ ID NO: 121) (M1 to S155; FIG. 5A), and human FGF homologous factor 1B (FHF1B; M1 to T181) were purified by published protocols (Plotnikov et al., *Cell* 101:413-424 (2000); Olsen et al., *J. Biol. Chem.* 278:34226-34236 (2003), which are hereby incorporated by reference in their entirety).

[0155] Chimeras composed of the core domain of FGF2 (M1 to M151) and the C-terminal region of either FGF21 (P168 to S209) or FGF23 (R161 to I251) (termed FGF2^{WTcore}-FGF21$^{C\text{-}tail}$ and FGF2^{WTcore}-FGF23$^{C\text{-}tail}$, respectively; FIG. 5A) were purified by the same protocol as that for native FGF2 (Plotnikov et al., *Cell* 101:413-424 (2000), which is hereby incorporated by reference in its entirety). Analogous chimeras containing three mutations in the HS-binding site of the FGF2 core (K128D/R129Q/K134V) (termed FGF2$^{\Delta HBScore}$-FGF21$^{C\text{-}tail}$ and FGF2$^{\Delta HBScore}$-FGF23$^{C\text{-}tail}$, respectively, FIG. 5A) were purified from the soluble bacterial cell lysate fraction by ion-exchange and size-exclusion chromatographies. In order to minimize proteolysis of the chimeras containing the C-terminal sequence from R161 to I251 of FGF23, arginine residues 176 and 179 of the proteolytic cleavage site ^{176}RXXR^{179} located within this sequence were replaced with glutamine as it occurs in ADHR (White et al., *Nat. Genet.* 26:345-348 (2000); White et al., *Kidney Int.* 60:2079-2086 (2001), which are hereby incorporated by

reference in their entirety). In addition, in order to prevent disulfide-mediated dimerization of FGF2 and chimeric FGF2 proteins, cysteine residues 78 and 96 were mutated to serine. An HS-binding site mutant of FGF1 (K127D/K128Q/K133V) (termed $FGF1^{\Delta HBScore}$; FIG. 6) and chimeras composed of the core domain of the HS-binding site mutant of FGF1 (M1 to L150, K127D/K128Q/K133V) and the C-terminal region of either FGF19 (L169 to K216) or FGF21 (P168 to S209) (termed $FGF1^{\Delta HBScore}$-$FGF19^{C\text{-}tail}$ and $FGF1^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$, respectively; FIG. 6) were purified from the soluble bacterial cell lysate fraction by ion-exchange and size-exclusion chromatographies. The N-terminally hexahistidine-tagged C-terminal tail peptide of FGF23 (S180 to I251, termed $FGF23^{C\text{-}tail}$) was purified by a published protocol (Goetz et al., *Proc. Nat'l. Acad. Sci. U.S.A* 107:407-412 (2010), which is hereby incorporated by reference in its entirety). The ligand-binding domain of human FGFR1c (D142 to R365) was refolded *in vitro* from bacterial inclusion bodies, and purified by published protocols (Ibrahimi et al., *Hum. Mol. Genet.* 13:2313-2324 (2004); Plotnikov et al., *Cell* 101:413-424 (2000), which are hereby incorporated by reference in their entirety). The ectodomain of murine αKlotho (A35 to K982) and the ectodomain of murine βKlotho (F53 to L995) were expressed in HEK293 cells as fusion proteins with a C-terminal FLAG tag (Kurosu et al., *J. Biol. Chem.* 281:6120-6123 (2006); Kurosu et al., *Science* 309:1829-1833 (2005), which are hereby incorporated by reference in their entirety). The binary complex of FGFR1c ligand-binding domain with αKlotho ectodomain (referred to as αKlotho-FGFR1c complex) was prepared by a published protocol (Goetz et al., *Proc. Nat'l. Acad. Sci. U.S.A* 107:407-412 (2010), which is hereby incorporated by reference in its entirety). The binary complex of FGFR1c ligand-binding domain with βKlotho ectodomain (referred to as βKlotho-FGFR1c complex) was prepared in the same fashion as the αKlotho-FGFR1c complex.

Example 2 - Analysis of FGF-heparin and FGF-FGFR-α/βKlotho Interactions by Surface Plasmon Resonance Spectroscopy

[0156] Surface plasmon resonance (SPR) experiments were performed on a Biacore 2000 instrument (Biacore AB), and the interactions were studied at 25 °C in HBS-EP buffer (10 mM HEPES-NaOH, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% (v/v) polysorbate 20). To study endocrine FGF-heparin interactions, a heparin chip was prepared by immobilizing biotinylated heparin (Sigma-Aldrich) on flow channels of a research-grade streptavidin chip (Biacore AB). The coupling density was ~ 5 fmol mm^{-2} of flow channel. To measure binding of chimeric FGF2 proteins to heparin, biotinylated heparin was coupled to a streptavidin chip at an approximately 4-fold lower density as judged based on the binding responses obtained for FGF1. To study

FGF-FGFR-α/βKlotho interactions, FGF chips were prepared by covalent coupling of FGF proteins through their free amino groups on flow channels of research grade CM5 chips (Biacore AB). Proteins were injected over a chip at a flow rate of 50 μl min^{-1}, and at the end of each protein injection (180 and 300 s, respectively), HBS-EP buffer (50 μl min^{-1}) was flowed over the chip to monitor dissociation for 180 or 240 s. The heparin chip surface was regenerated by injecting 50 μl of 2.0 M NaCl in 10 mM sodium acetate, pH 4.5. For FGF chips, regeneration was achieved by injecting 2.0 M NaCl in 10 mM sodium/potassium phosphate, pH 6.5. To control for nonspecific binding in experiments where an FGF ligand was immobilized on the chip, FHF1B, which shares structural similarity with FGFs but does not exhibit any FGFR binding (Olsen et al., *J. Biol. Chem.* 278:34226-34236 (2003), which is hereby incorporated by reference in its entirety), was coupled to the control flow channel of the chip (~ 15-30 fmol mm^{-2}). In experiments where heparin was immobilized on the chip, the control flow channel was left blank. The data were processed with BiaEvaluation software (Biacore AB). For each protein injection over the heparin chip, the nonspecific responses from the control flow channel were subtracted from the responses recorded for the heparin flow channel. Similarly, for each protein injection over a FGF chip, the nonspecific responses from the FHF1B control flow channel were subtracted from the responses recorded for the FGF flow channel. Where possible, equilibrium dissociation constants (K_Ds) were calculated from fitted saturation binding curves. Fitted binding curves were judged to be accurate based on the distribution of the residuals (even and near zero) and χ^2 (<10% of R_{max}).

[0157] To examine whether the K149A mutation abrogates residual heparin binding of FGF19, increasing concentrations of wild-type FGF19 were passed over a heparin chip. Thereafter, the $FGF19^{K149A}$ mutant was injected over the heparin chip at the highest concentration tested for the wild-type ligand. The effect of the R140A/R143A double mutation in the HS-binding site of FGF23 on residual heparin binding of FGF23 was examined in the same fashion as was the effect of the HS-binding site mutation in FGF19.

[0158] To verify that the K128D/R129Q/K134V triple mutation in the HS-binding site of the FGF2 core domain diminishes heparin-binding affinity of the FGF2 core, increasing concentrations of $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ and $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ were passed over a heparin chip. As a control, binding of $FGF2^{WTcore}$-$FGF21^{C\text{-}tail}$ and $FGF2^{WTcore}$-$FGF23^{C\text{-}tail}$ to heparin was studied.

[0159] To examine whether the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimera can compete with FGF23 for binding to the αKlotho-FGFR1c complex, FGF23 was immobilized on a chip (~ 16

fmol mm^{-2} of flow channel). Increasing concentrations of $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ were mixed with a fixed concentration of αKlotho-FGFR1c complex in HBS-EP buffer, and the mixtures were injected over the FGF23 chip. As controls, the binding competition was carried out with FGF23 or FGF2 as the competitor in solution. As an additional specificity control, competition of the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimera with FGF21 for binding to the αKlotho-FGFR1c complex was studied. αKlotho-FGFR1c complex was mixed with $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ or FGF23 at a molar ratio of 1:10, and the mixture was injected over a chip containing immobilized FGF21 (~ 12 fmol mm^{-2} of flow channel).

[0160] To test whether the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera can compete with FGF21 for binding to the βKlotho-FGFR1c complex, increasing concentrations of $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ were mixed with a fixed concentration of βKlotho-FGFR1c complex in HBS-EP buffer, and the mixtures were passed over a chip containing immobilized FGF21 (~ 19 fmol mm^{-2} of flow channel). As controls, the binding competition was carried out with FGF21 or FGF2 as the competitor in solution. As an additional specificity control, competition of the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera with FGF23 for binding to the αKlotho-FGFR1c complex was studied. αKlotho-FGFR1c complex was mixed with $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ or FGF21 at a molar ratio of 1:10, and the mixture was injected over a chip containing immobilized FGF23 (~ 12 fmol mm^{-2} of flow channel).

[0161] To measure binding of FGFR1c to each of the three endocrine FGFs, increasing concentrations of FGFR1c ligand-binding domain were injected over a chip containing immobilized FGF19, FGF21, and FGF23 (~ 30 fmol mm^{-2} of flow channel). As a control, binding of FGFR1c to FGF2 immobilized on a chip was studied. As additional controls, binding of the αKlotho-FGFR1c complex to FGF23 and binding of FGFR1c to the C-terminal tail peptide of FGF23 was measured.

Example 3 - Analysis of Phosphorylation of FRS2α and 44/42 MAP Kinase in Hepatoma and Epithelial Cell Lines

[0162] To examine whether the $FGF19^{K149A}$ and $FGF23^{R140A/R143A}$ mutants can activate FGFR in a α/βKlotho-dependent fashion, induction of tyrosine phosphorylation of FGFR substrate 2α (FRS2α) and downstream activation of MAP kinase cascade was used as readout for FGFR activation. Subconfluent cells of the H4IIE rat hepatoma cell line, which endogenously expresses βKlotho (Kurosu et al., *J. Biol. Chem.* 282:26687-26695 (2007), which is hereby incorporated by reference in its entirety), were serum starved for 16 h and then stimulated for 10 min with the $FGF19^{K149A}$ mutant or wild-type FGF19 (0.2 ng ml^{-1} to 2.0 μg ml^{-1}). Similarly,

subconfluent cells of a HEK293 cell line ectopically expressing the transmembrane isoform of murine αKlotho (Kurosu et al., *J. Biol. Chem.* 281:6120-6123 (2006), which is hereby incorporated by reference in its entirety) were treated with the $FGF23^{R140A/R143A}$ mutant or wild-type FGF23 (0.1 to 100 ng ml^{-1}). After stimulation, the cells were lysed (Kurosu et al., *Science* 309:1829-1833 (2005), which is hereby incorporated by reference in its entirety), and cellular proteins were resolved on SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The protein blots were probed with antibodies to phosphorylated FRS2α, phosphorylated 44/42 MAP kinase, total (phosphorylated and nonphosphorylated) 44/42 MAP kinase, and αKlotho. Except for the anti-αKlotho antibody (KM2119) (Kato et al., *Biochem. Biophys. Res. Commun.* 267:597-602 (2000), which is hereby incorporated by reference in its entirety), all antibodies were from Cell Signaling Technology.

Example 4 - Analysis of Egr1 Protein Expression in an Epithelial Cell Line

[0163] To examine whether the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ and $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimeras can activate FGFR in a HS-dependent fashion, induction of protein expression of the transcription factor early growth response 1 (Egr1), a known downstream mediator of FGF signaling, was used as readout for FGFR activation. HEK293 cells were serum starved overnight and then stimulated for 90 min with $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ or $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ (0.1 and 0.3 nM). Cell stimulation with $FGF2^{WTcore}$-$FGF21^{C\text{-}tail}$, $FGF2^{WTcore}$-$FGF23^{C\text{-}tail}$, FGF21, and FGF23 served as controls. To test whether the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera can activate FGFR in a βKlotho-dependent fashion, HEK293 cells transfected with murine βKlotho were serum starved overnight and then stimulated for 90 min with $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ or FGF21 (3 to 300 ng ml^{-1}). After stimulation, the cells were lysed (Kurosu et al., *Science* 309:1829-1833 (2005), which is hereby incorporated by reference in its entirety), and cellular proteins were resolved on SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The protein blots were probed with antibodies to Egr1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The anti-Egr1 antibody was from Cell Signaling Technology and the anti-GAPDH antibody was from Abcam.

Example 5 - Analysis of CYP7A1 and CYP8B1 mRNA Expression in Murine Liver Tissue

[0164] To examine the metabolic activity of the $FGF19^{K149A}$ mutant *in vivo*, 6- to 8-week old C57BL/6 mice were fasted overnight and then given intraperitoneally a single dose (1 mg kg body $weight^{-1}$) of $FGF19^{K149A}$ or FGF19 as a control. 6 h after the injection, the mice were sacrificed, and liver tissue was excised and frozen. Total RNA was isolated from liver tissue,

and mRNA levels of cholesterol 7α-hydroxylase (CYP7A1) and sterol 12α-hydroxylase (CYP8B1) were measured using quantitative real time RT-PCR as described previously (Inagaki et al., *Cell Metab.* 2:217-225 (2005); Kim et al., *J. Lipid Res.* 48:2664-2672 (2007), which are hereby incorporated by reference in their entirety). The Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center at Dallas had approved the experiments.

Example 6 - Measurement of Serum Phosphate in Mice

[0165] The metabolic activity of the $FGF23^{R140A/R143A}$ mutant was examined both in normal mice and in *Fgf23* knockout mice. 4- to 5-week old C57BL/6 mice were given intraperitoneally a single dose (0.29 mg kg body $weight^{-1}$) of $FGF23^{R140A/R143A}$ or FGF23 as a control. Before the injection and 8 h after the injection, blood was drawn from the cheek pouch and spun at 3,000xg for 10 min to obtain serum. Phosphate concentration in serum was measured using the Phosphorus Liqui-UV Test (Stanbio Laboratory). 6- to 8-week old *Fgf23* knockout mice (Sitara et al., *Matrix Biol.* 23:421-432 (2004), which is hereby incorporated by reference in its entirety) (56) were given two injections of $FGF23^{R140A/R143A}$ or FGF23 at 8 h intervals (0.71 mg kg body $weight^{-1}$ each), and blood samples were collected for phosphate analysis before the first injection and 8 h after the second injection.

[0166] To test whether the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimera exhibits FGF23-like metabolic activity, 5- to 6-week old C57BL/6 mice were given a single injection of $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ (0.21 mg kg body $weight^{-1}$). As controls, mice were injected with $FGF2^{WTcore}$-$FGF23^{C\text{-}tail}$ or FGF23. Before the injection and 8 h after the injection, blood samples were collected for measurement of serum phosphate. To confirm that αKlotho is required for the metabolic activity of the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimera, 7- to 8-week old *αKlotho* knockout mice (Lexicon Genetics) were injected once with $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ or FGF23 as a control (0.51 mg kg body $weight^{-1}$). Before the injection and 8 h after the injection, blood samples were collected for phosphate analysis. The Harvard University Animal Care and Research committee board had approved all the experiments.

Example 7 - Analysis of CYP27B1 mRNA Expression in Murine Renal Tissue

[0167] The ability of the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimera to reduce renal expression of 25-hydroxyvitamin D_3 1α-hydroxylase (CYP27B1) was used as another readout for FGF23-like metabolic activity. C57BL/6 mice injected with $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$, $FGF2^{WTcore}$-$FGF23^{C\text{-}tail}$, or FGF23 were sacrificed 8 h after the protein injection, and renal tissue was excised and

frozen. CYP27B1 mRNA levels in total renal tissue RNA were measured using real time quantitative PCR as described previously (Nakatani et al., *FASEB J.* 23:3702-3711 (2009); Ohnishi et al., *Kidney Int.* 75:1166-1172 (2009), which are hereby incorporated by reference in their entirety). The Harvard University Animal Care and Research committee board had approved the experiments.

Example 8 - Insulin Tolerance Test in Mice

[0168] The ability of the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera to potentiate the hypoglycemic effect of insulin was used as readout for FGF21-like metabolic activity (Ohnishi et al., *FASEB J.* 25:2031-2039 (2011), which is hereby incorporated by reference in its entirety). 8- to 12-week old C57BL/6 mice were kept on normal chow. On the day of the insulin tolerance test, mice were fasted for 4 h and then bled from the cheek pouch for measuring fasting blood glucose levels. Thereafter, mice were administered intraperitoneally insulin (0.5 units kg body $weight^{-1}$) alone or insulin (0.5 units·kg body $weight^{-1}$) plus $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera (0.3 mg kg body $weight^{-1}$). As a control, mice were co-injected with insulin plus FGF21. At the indicated time points after the injection (FIG. 7G), blood was drawn from the tail vein. Glucose concentrations in the blood samples were determined using Bayer Contour® blood glucose test strips (Bayer Corp.). The Harvard University Animal Care and Research committee board had approved the experiments.

Example 9 - Analysis of Blood Glucose in *ob/ob* Mice

[0169] *ob/ob* mice were injected subcutaneously with $FGF1^{\Delta NT}$, $FGF1^{\Delta HBS}$, or $FGF1^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera. Injection of native FGF1 or native FGF21 served as controls. A single bolus of 0.5 mg of protein per kg of body weight was injected. This dose was chosen on the basis that maximal efficacy of the hypoglycemic effect of native FGF1 is seen at this dose. Before the protein injection and at the indicated time points after the injection (FIGS. 9A-9C), blood glucose concentrations were measured using an OneTouch Ultra glucometer (Lifescan). The Institutional Animal Care and Use Committee at the Salk Institute for Biological Sciences at La Jolla had approved the experiments.

Example 10 - Statistical Analysis

[0170] Data are expressed as mean $\pm$ SEM. A Student's *t* test or analysis of variance (ANOVA) was used as appropriate to make statistical comparisons. A value of $P < 0.05$ was considered significant.

Example 11 - HS is Dispensable for the Metabolic Activity of FGF19 and FGF23

[0171] In order to engineer endocrine FGFs devoid of HS binding, the FGF19 crystal structure (PDB ID: 2P23; (Goetz et al., *Mol. Cell Biol.* 27:3417-3428 (2007), which is hereby incorporated by reference in its entirety) was compared with that of FGF2 bound to a heparin hexasaccharide (PDB ID: 1FQ9; (Schlessinger et al., *Mol. Cell* 6:743-750 (2000), which is hereby incorporated by reference in its entirety)). This analysis shows that solvent-exposed residues K149, Q150, Q152, and R157 of FGF19 lie at the corresponding HS-binding site of this ligand, and hence could account for the residual HS binding of FGF19 (FIGS. 1A, 1B, and 2). Likewise, comparative analysis of the FGF23 crystal structure (PDB ID: 2P39; (Goetz et al., *Mol. Cell Biol.* 27:3417-3428 (2007), which is hereby incorporated by reference in its entirety) (29)) with that of heparin-bound FGF2 (PDB ID: 1FQ9; (Schlessinger et al., *Mol. Cell* 6:743-750 (2000), which is hereby incorporated by reference in its entirety)) points to R48, N49, R140, and R143 as candidates mediating the residual HS binding of this ligand (FIGS. 1*A*, 1C, and 2). In agreement with the structural predictions, replacement of K149 alone in FGF19 with alanine and combined substitution of R140 and R143 in FGF23 for alanine were sufficient to abolish residual HS binding of these ligands (FIGS. 3B–3G).

[0172] To test the impact of knocking out residual HS binding of FGF19 on the signaling by this ligand, H4IIE hepatoma cells were stimulated with the $FGF19^{K149A}$ mutant or wild-type FGF19. H4IIE cells endogenously express FGFR4 and βKlotho (Kurosu et al., *J. Biol. Chem.* 282:26687-26695 (2007), which is hereby incorporated by reference in its entirety), the cognate receptor and co-receptor, respectively, for FGF19. The $FGF19^{K149A}$ mutant was as effective as wild-type FGF19 in inducing tyrosine phosphorylation of FRS2α and downstream activation of MAP kinase cascade (FIG. 4A). These data show that elimination of residual HS binding has no impact on the ability of FGF19 to signal in cultured cells. To test whether the same holds true for FGF23 signaling, HEK293 cells, which naturally express two of the three cognate receptors of FGF23, namely FGFR1c and FGFR3c (Kurosu et al., *J. Biol. Chem.* 281:6120-6123 (2006), which is hereby incorporated by reference in its entirety) were transfected with the transmembrane isoform of αKlotho, the co-receptor of FGF23. These cells were treated with the $FGF23^{R140A/R143A}$ double mutant or wild-type FGF23. The $FGF23^{R140A/R143A}$ mutant had the same capacity as wild-type FGF23 in inducing phosphorylation of FRS2α and downstream activation of MAP kinase cascade (FIG. 4B). These data show that similar to FGF19, FGF23 does not need to bind HS in order to activate FGFR in cultured cells.

[0173] To substantiate the findings in cells, the metabolic activity of wild-type and mutated ligands *in vivo* were compared. Mice were injected with the $FGF19^{K149A}$ mutant or wild-type FGF19 and liver gene expression of CYP7A1 and CYP8B1, which are key enzymes in the major bile acid biosynthetic pathway (Russell, D. W., *Annu. Rev. Biochem.* 72:137-174 (2003), which is hereby incorporated by reference in its entirety), was analyzed. Like wild-type FGF19, the $FGF19^{K149A}$ mutant markedly decreased CYP7A1 and CYP8B1 mRNA levels (FIG. 4C), demonstrating that knockout of residual HS binding does not affect the metabolic activity of FGF19. To examine whether residual HS binding is also dispensable for the metabolic activity of FGF23, mice were injected with the $FGF23^{R140A/R143A}$ mutant or wild-type FGF23 and serum phosphate concentrations were measured. The $FGF23^{R140A/R143A}$ mutant reduced serum phosphate as effectively as wild-type FGF23 (FIG. 4D). Moreover, when injected into *Fgf23* knockout mice, the $FGF23^{R140A/R143A}$ mutant exhibited as much of phosphate-lowering activity as wild-type FGF23 (FIG. 4D). These data show that, as in the case of FGF19, abolishment of residual HS binding does not impact the metabolic activity of FGF23 leading to the conclusion that HS is not a component of the endocrine FGF signal transduction unit (FIG. 1D).

Example 12 - Conversion of a Paracrine FGF Into an Endocrine Ligand Confirms that HS is Dispensable for the Metabolic Activity of Endocrine FGFs

[0174] If HS is dispensable for the metabolic activity of endocrine FGFs, then it should be feasible to convert a paracrine FGF into an endocrine FGF by eliminating HS-binding affinity of the paracrine FGF and substituting its C-terminal tail for that of an endocrine FGF containing the Klotho co-receptor binding site. Reducing HS-binding affinity will allow the ligand to freely diffuse and enter the blood circulation while attaching the C-terminal tail of an endocrine FGF will home the ligand into its target tissues. FGF2, a prototypical paracrine FGF, was chosen for conversion into FGF23-like and FGF21-like ligands, respectively. FGF2 was selected as paracrine ligand for this protein engineering exercise because it preferentially binds to the "c" isoform of FGFR1, the principal receptor mediating the metabolic activity of FGF23 (Gattineni et al., *Am. J. Physiol. Renal Physiol.* 297:F282-291 (2009); Liu et al., *J. Am. Soc. Nephrol.* 19:2342-2350 (2008), which are hereby incorporated by reference in their entirety) and FGF21 (Kurosu et al., *J. Biol. Chem.* 282:26687-26695 (2007), which is hereby incorporated by reference in its entirety), respectively. In the crystal structure of heparin-bound FGF2 (PDB ID: 1FQ9; (Schlessinger et al., *Mol. Cell* 6:743-750 (2000), which is hereby incorporated by reference in its entirety)), K128, R129, and K134 mediate the majority of hydrogen bonds with heparin and hence mutation of these residues was predicted to cause a major reduction in HS-

binding affinity of FGF2 (FIGS. 1A, 2, and 5A). Accordingly, these three residues were mutated and then the short C-terminal tail of the mutated FGF2 was replaced with the C-terminal tail of FGF23 (R161 to I251) or the C-terminal tail of FGF21 (P168 to S209) (FIG. 5A). The resulting chimeras were termed $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ and $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ (FIG. 5A). To demonstrate that reduction in HS-binding affinity is required for converting FGF2 into an endocrine ligand, two control chimeras were made in which the HS-binding site of the FGF2 core was left intact ($FGF2^{WTcore}$-$FGF23^{C\text{-}tail}$ and $FGF2^{WTcore}$-$FGF21^{C\text{-}tail}$; FIG. 5A).

[0175] Consistent with the structural prediction, $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ and $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ exhibited poor binding affinity for HS compared to the corresponding control chimeras with intact HS-binding site (FIGS. 5B–5E). Since HS is an obligatory cofactor in paracrine FGF signaling, the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ and $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimeras were predicted to lose the ability to activate FGFR1c in an HS-dependent fashion. To test this, HEK293 cells, which endogenously express FGFR1c, were stimulated with $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ or $FGF2^{WTcore}$-$FGF23^{C\text{-}tail}$. Induction of protein expression of the transcription factor Egr1, a known downstream mediator of FGF signaling, was used as readout for FGFR activation. As shown in FIG. 5G, the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimera, like native FGF23, was ineffective in inducing Egr1 expression at concentrations at which the $FGF2^{WTcore}$-$FGF23^{C\text{-}tail}$ chimera elicited a near maximal effect. The same observations were made for the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera (FIG. 5F). These data show that, similar to native FGF23 and FGF21, the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ and $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimeras lost the ability to activate FGFR in an HS-dependent, paracrine fashion.

[0176] To determine whether the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ and $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimeras gained the ability to signal in a Klotho co-receptor-dependent, endocrine fashion, it was first analyzed whether these chimeras can form ternary complexes with FGFR1c and Klotho co-receptor. To this end, a SPR-based binding competition assay was employed. FGF23 was immobilized onto a SPR biosensor chip, and mixtures of a fixed concentration of binary αKlotho-FGFR1c complex with increasing concentrations of $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimera were passed over the chip. $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ competed, in a dose-dependent fashion, with immobilized FGF23 for binding to the αKlotho-FGFR1c complex (FIG. 7A), demonstrating that the chimera, like native FGF23 (FIG. 7B), is able to form a ternary complex with FGFR1c and αKlotho. To test whether the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera can likewise form a ternary complex with FGFR1c and βKlotho, FGF21 was coupled to a SPR biosensor chip, and mixtures

of the binary βKlotho-FGFR1c complex with $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ were passed over the chip. $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ effectively competed with immobilized FGF21 for binding to the βKlotho-FGFR1c complex (FIG. 8A), demonstrating that the chimera, like native FGF21 (FIG. 8B), is capable of binding to the binary complex of FGFR1c and βKlotho. Notably, native FGF2 failed to compete with FGF23 for binding to the αKlotho-FGFR1c complex (FIG. 7C), and with FGF21 for binding to the βKlotho-FGFR1c complex (FIG. 8C) since it lacks the Klotho co-receptor binding domain. To further confirm the binding specificity of the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimera for the αKlotho-FGFR1c complex, $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ and βKlotho-FGFR1c complex were mixed at a molar ratio of 10:1, and the mixture was injected over a chip containing immobilized FGF21. $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$, like native FGF23, failed to compete with FGF21 for binding to the βKlotho-FGFR1c complex (FIGS. 7D and 7E). Similarly, the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera, like native FGF21, failed to compete with FGF23 for binding to the αKlotho-FGFR1c complex (FIGS. 8D and 8E). For the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera, we investigated whether it is able to activate FGFR1c in a βKlotho-dependent fashion in cells. HEK293 cells were transfected with βKlotho and then stimulated with $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ or FGF21. Similar to native FGF21, the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera induced Egr1 protein expression in HEK293-βKlotho cells (FIG. 8F), indicating that the chimera is capable of activating FGFR1c in the presence of βKlotho.

[0177] To provide definite proof for the ligand conversion, the metabolic activity of the chimeras *in vivo* was tested. Specifically, to determine whether the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimera exhibits FGF23-like metabolic activity, its ability to lower serum phosphate and to reduce renal gene expression of CYP27B1, which catalyzes the conversion of vitamin D into its bioactive form, was examined (Shimada et al., "Cloning and Characterization of FGF23 as a Causative Factor of Tumor-Induced Osteomalacia," *Proc Natl Acad Sci USA* 98:6500-6505 (2001); Saito et al., "Human Fibroblast Growth Factor-23 Mutants Suppress Na^+-Dependent Phosphate Co-Transport Activity and 1alpha,25-Dihydroxyvitamin D3 Production," *J Biol Chem* 278:2206-2211 (2003); Shimada et al., "Targeted Ablation of FGF23 Demonstrates an Essential Physiological Role of FGF23 in Phosphate and Vitamin D Metabolism," *J Clin Invest* 113:561-568 (2004), which are hereby incorporated by reference in their entirety). Mice were injected with $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ or as controls, FGF23 or $FGF2^{WTcore}$-$FGF23^{C\text{-}tail}$, and serum phosphate concentrations and renal CYP27B1 mRNA levels were measured. Similar to native FGF23, the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimera caused a decrease in serum phosphate in wild-type

mice (FIG. 7F). The chimera also induced a marked decrease in CYP27B1 mRNA levels, just like the native FGF23 ligand (FIG. 7G). These data show that the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimera acts as an FGF23-like hormone. Importantly, the $FGF2^{WTcore}$-$FGF23^{C\text{-}tail}$ chimera failed to decrease serum phosphate or CYP27B1 mRNA levels (FIGS. 7F and 7G). This is expected because, owing to its high affinity for HS, this chimera should be trapped in the vicinity of the injection site and hence not be able to enter the blood circulation. Moreover, these data show that adding the Klotho co-receptor binding site is not sufficient to convert a paracrine FGF into an endocrine ligand. To confirm that the metabolic activity of the $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ chimera is dependent on αKlotho (Kurosu et al., "Regulation of Fibroblast Growth Factor-23 Signaling by Klotho," *J Biol Chem* 281(10):6120-6123 (2006); Urakawa et al., "Klotho Converts Canonical FGF Receptor into a Specific Receptor for FGF23," *Nature* 444(7120):770-774 (2006); Nakatani et al., "Inactivation of Klotho Function Induces Hyperphosphatemia Even in Presence of High Serum Fibroblast Growth Factor 23 Levels in a Genetically Engineered Hypophosphatemic (*Hyp*) Mouse Model," *FASEB J* 23:3702-3711 (2009), which are hereby incorporated by reference in their entirety), *αKlotho* knockout mice were injected with $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ or FGF23 as a control, and serum concentrations of phosphate were measured. As shown in FIG. 7F, $FGF2^{\Delta HBScore}$-$FGF23^{C\text{-}tail}$ failed to lower serum phosphate, demonstrating that the chimera, like native FGF23 (FIG. 7F), requires αKlotho for metabolic activity.

[0178] To determine whether the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera exhibits FGF21-like metabolic activity, its ability to potentiate the hypoglycemic effect of insulin was examined (Ohnishi et al., *FASEB J.* 25:2031-2039 (2011), which is hereby incorporated by reference in its entirety). Mice were injected with insulin plus $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$, insulin plus FGF21, or insulin alone, and blood glucose concentrations were monitored for up to one hour after the injection. Similar to FGF21, the $FGF2^{\Delta HBScore}$-$FGF21^{C\text{-}tail}$ chimera enhanced the hypoglycemic effect of insulin (FIG. 8G), demonstrating that the chimera acts as an FGF21-like hormone.

[0179] To substantiate further the concept of FGF ligand conversion, another FGF21-like ligand was engineered using FGF1 as paracrine FGF, and the metabolic activity of the engineered protein was tested *in vivo* in a mouse model of diabetes and obesity. Besides serving as an additional proof-of-concept, the use of FGF1 for this particular ligand conversion was appealing because FGF1 on its own plays an essential role in glucose metabolism (Jonker et al., "A PPARγ-FGF1 Axis is Required for Adaptive Adipose Remodelling and Metabolic Homeostasis," *Nature* 485:391-394 (2012), which is hereby incorporated by reference in its

entirety). Notably, similar to FGF21, FGF1 is induced postprandially in gonadal white adipose tissue by the nuclear hormone receptor PPARγ (peroxisome proliferator activated receptor-γ) (Jonker et al., "A PPARγ-FGF1 Axis is Required for Adaptive Adipose Remodelling and Metabolic Homeostasis," *Nature* 485:391-394 (2012); Dutchak et al., "Fibroblast Growth Factor-21 Regulates PPARγ Activity and the Antidiabetic Actions of Thiazolidinediones," *Cell* 148:556-567 (2012), which are hereby incorporated by reference in their entirety). FGF1 is required for the remodeling of adipose tissue to adjust to fluctuations in nutrient availability (Jonker et al., "A PPARγ-FGF1 Axis is Required for Adaptive Adipose Remodelling and Metabolic Homeostasis," *Nature* 485:391-394 (2012), which is hereby incorporated by reference in its entirety), and this process is influenced by FGF21 (Hotta et al., "Fibroblast Growth Factor 21 Regulates Lipolysis in White Adipose Tissue But is Not Required for Ketogenesis and Triglyceride Clearance in Liver," *Endocrinology* 150:4625-4633 (2009); Dutchak et al., "Fibroblast Growth Factor-21 Regulates PPARγ Activity and the Antidiabetic Actions of Thiazolidinediones," *Cell* 148:556-567 (2012), which are hereby incorporated by reference in their entirety). As part of a positive feedback loop, FGF21 stimulates PPARγ activity in adipocytes (Dutchak et al., "Fibroblast Growth Factor-21 Regulates PPARγ Activity and the Antidiabetic Actions of Thiazolidinediones," *Cell* 148:556-567 (2012), which is hereby incorporated by reference in its entirety), raising the intriguing possibility that FGF21 regulates FGF1 signaling in adipose tissue through PPARγ. An FGF1$^{\Delta HBScore}$-FGF21$^{C\text{-}tail}$ chimera was generated in the same manner as the FGF2$^{\Delta HBScore}$-FGF21$^{C\text{-}tail}$ chimera (FIGS. 5 and 6). Specifically, K127, K128, and K133 of FGF1, which correspond to the key HS-binding residues identified in the crystal structure of heparin-bound FGF2 (PDB ID: 1FQ9; (Schlessinger et al., *Mol. Cell* 6:743-750 (2000), which is hereby incorporated by reference in its entirety)), were mutated and then the short C-terminal tail of the mutated FGF1 was replaced with the C-terminal tail of FGF21 (P168 to S209) (FIG. 6). A full-length FGF1 protein harboring the HS-binding site mutations was used as a control (FIG. 6). Consistent with the structural prediction, this protein exhibited poor binding affinity for HS compared to wild-type FGF1 as evidenced by the fact that, unlike the wild-type ligand, the mutant protein did not bind to a Heparin sepharose column. A subcutaneous bolus injection of the FGF1$^{\Delta HBScore}$-FGF21$^{C\text{-}tail}$ chimera elicited a hypoglycemic effect in *ob/ob* mice (FIG. 9C), demonstrating that the chimera has metabolic activity. The effect was of similar magnitude as that observed for native FGF1 (FIG. 9C), which itself has a much greater hypoglycemic effect in *ob/ob* mice than native FGF21 (FIG. 9*A*). The HS-binding site mutant of FGF1, which was included as a control in these experiments, showed

a similar hypoglycemic effect as the wild-type ligand (FIG. 9B), indicating that the loss in HS-binding affinity had no impact on the metabolic activity of FGF1. To alter the receptor-binding specificity of FGF1 such that FGF1 selectively binds to the "c" splice isoform of FGFR1, the principal receptor mediating the metabolic activity of FGF21, an N-terminally truncated FGF1 protein was made (FIG. 6). The truncated FGF1 ligand lacked twenty four residues from the N-terminus including the nine residues that are critical for the promiscuous binding of FGF1 to both splice isoforms of FGFR1-3 (Beenken et al., "Plasticity in Interactions of Fibroblast Growth Factor 1 (FGF1) N Terminus with FGF Receptors Underlies Promiscuity of FGF1," *J Biol Chem* 287(5):3067-3078 (2012), which is hereby incorporated by reference in its entirety). Based on the crystal structures of FGF1-FGFR complexes, the truncation was also predicted to reduce the receptor-binding affinity of FGF1, and hence the ligand's mitogenicity. The truncated FGF1 protein induced a similar hypoglycemic effect in *ob/ob* mice as native FGF1 did, indicating that the metabolic activity of FGF1 is mediated through the "c" splice isoform of FGFR. Together, these findings provide a starting point for engineering FGF1 ligands that have no mitogenicity but the same or enhanced metabolic activity compared to native FGF1.

[0180] The demonstrated ability to convert a paracrine FGF into an endocrine ligand by means of reducing HS-binding affinity of the paracrine FGF and adding the Klotho co-receptor binding site substantiates that HS does not participate in the formation of the endocrine FGF signal transduction unit. The dispensability of HS for the metabolic activity of endocrine FGFs has an intriguing implication as to how these FGFs have evolved to become hormones. It appears that these ligands have lost the requirement to bind HS in order to signal, while acquiring the ability to bind Klotho co-receptors, which is necessary to direct these ligands to their target organs.

[0181] In the target tissue, Klotho co-receptors constitutively associate with cognate receptors of endocrine FGFs to offset the inherently low receptor-binding affinity of endocrine FGFs (FIGS. 10B–10D; Kurosu et al., *J. Biol. Chem.* 282:26687-26695 (2007); Kurosu et al., *J. Biol. Chem.* 281:6120-6123 (2006); Ogawa et al., *Proc. Nat'l. Acad. Sci. U.S.A.* 104:7432-7437 (2007); Urakawa et al., *Nature* 444:770-774 (2006), which are hereby incorporated by reference in their entirety). This low binding affinity is due to the fact that key receptor-binding residues in the β-trefoil core of endocrine FGFs are replaced by residues that are suboptimal for receptor binding (Goetz et al., *Mol. Cell Biol.* 27:3417-3428 (2007), which is hereby incorporated by reference in its entirety). To measure the degree to which Klotho co-receptors enhance the receptor-binding affinity of endocrine FGFs, SPR experiments were conducted using FGF23 and

FGFR1c and αKlotho co-receptor as an example (see FIGS. 10A-10F). The SPR data show that αKlotho enhances the affinity of FGF23 for FGFR1c by over 20-fold (FIGS. 10D and 10E). The affinity of FGF23 for FGFR1c in the presence of αKlotho is comparable to that of FGF2 for FGFR1c in the absence of its HS cofactor (FIGS. 10A and 10E). It should be noted, however, that HS further increases the binding affinity of FGF2 for FGFR1c by at least an order of magnitude (Pantoliano et al., *Biochemistry* 33:10229-10248 (1994); Roghani et al., *J. Biol. Chem.* 269:3976-3984 (1994), which are hereby incorporated by reference in their entirety). Hence, the receptor-binding affinity of FGF23 in the presence of αKlotho co-receptor still is lower than that of FGF2 in the presence of HS cofactor. These observations imply that the signaling capacity of the endocrine FGF signal transduction unit should be weaker than that of the paracrine FGF signaling unit. Indeed, cell-based studies show that even in the presence of their Klotho co-receptor, endocrine FGFs are inferior to paracrine FGFs at activating FGFR-induced intracellular signaling pathways (Kurosu et al., *J. Biol. Chem.* 282:26687-26695 (2007); Urakawa et al., *Nature* 444:770-774 (2006), which are hereby incorporated by reference in their entirety).

[0182] The finding that endocrine FGFs do not need to rely on HS for signaling has another important implication in regard to the role of Klotho co-receptors. Since FGFR dimerization is a prerequisite for FGF signaling in general, it is proposed that Klotho co-receptors not only enhance the binding affinity of endocrine ligand for receptor but also promote receptor dimerization upon ligand binding. In other words, Klotho co-receptors must fulfill the same dual role that HS plays in signaling by paracrine FGFs (FIG. 1D). The ligand conversion also provides the framework for the rational design of endocrine FGF-like molecules for the treatment of metabolic disorders. An FGF23-like molecule, for example, will be useful for the treatment of inherited or acquired hyperphosphatemia, and an FGF21-like molecule, for example, for the treatment of type 2 diabetes, obesity, and related metabolic disorders.

[0183] Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

WHAT IS CLAIMED:

1. A chimeric protein comprising:

an N-terminus coupled to a C-terminus, wherein the N-terminus comprises a portion of a paracrine fibroblast growth factor ("FGF") and the C-terminus comprises a C-terminal portion of an FGF21 molecule and wherein the portion of the paracrine FGF is modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without the modification.

2. The chimeric protein according to claim 1, wherein the paracrine FGF is FGF1 or FGF2.

3. The chimeric protein according to claim 1, wherein the portion of the paracrine FGF comprises an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 150 to 155 of SEQ ID NO: 1.

4. The chimeric protein according to claim 1, wherein the portion of the paracrine FGF comprises an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 151 to 155 of SEQ ID NO: 121.

5. The chimeric protein according to claim 3, wherein the portion of the paracrine FGF comprises amino acid residues 1-150, 1-151, 1-152, 1-153, 1-154, 1-155, 2-150, 2-151, 2-152, 2-153, 2-154, 2-155, 3-150, 3-151, 3-152, 3-153, 3-154, 3-155, 4-150, 4-151, 4-152, 4-153, 4-154, 4-155, 5-150, 5-151, 5-152, 5-153, 5-154, 5-155, 6-150, 6-151, 6-152, 6-153, 6-154, 6-155, 7-150, 7-151, 7-152, 7-153, 7-154, 7-155, 8-150, 8-151, 8-152, 8-153, 8-154, 8-155, 9-150, 9-151, 9-152, 9-153, 9-154, 9-155, 10-150, 10-151, 10-152, 10-153, 10-154, 10-155, 11-150, 11-151, 11-152, 11-153, 11-154, 11-155, 12-150, 12-151, 12-152, 12-153, 12-154, 12-155, 13-150, 13-151, 13-152, 13-153, 13-154, 13-155, 14-150, 14-151, 14-152, 14-153, 14-154, 14-155, 15-150, 15-151, 15-152, 15-153, 15-154, 15-155, 16-150, 16-151, 16-152, 16-153, 16-154, 16-155, 17-150, 17-151, 17-152, 17-153, 17-154, 17-155, 18-150, 18-151, 18-152, 18-153, 18-154, 18-155, 19-150, 19-151, 19-152, 19-153, 19-154, 19-155, 20-150, 20-151, 20-152, 20-153, 20-154, 21-150, 21-155, 21-151, 21-152, 21-153, 21-154, 21-155, 22-150, 22-151, 22-152, 22-153, 22-154, 22-155, 23-150, 23-151, 23-152, 23-153, 23-154, 23-155, 24-150, 24-151, 24-

152, 24-153, 24-154, 24-155, 25-150, 25-151, 25-152, 25-153, 25-154, or 25-155 of SEQ ID NO: 1.

6. The chimeric protein according to claim 4, wherein the portion of the paracrine FGF comprises amino acid residues 1-151, 1-152, 1-153, 1-154, 1-155, 2-151, 2-152, 2-153, 2-154, 2-155, 3-151, 3-152, 3-153, 3-154, 3-155, 4-151, 4-152, 4-153, 4-154, 4-155, 5-151, 5-152, 5-153, 5-154, 5-155, 6-151, 6-152, 6-153, 6-154, 6-155, 7-151, 7-152, 7-153, 7-154, 7-155, 8-151, 8-152, 8-153, 8-154, 8-155, 9-151, 9-152, 9-153, 9-154, 9-155, 10-151, 10-152, 10-153, 10-154, 10-155, 11-151, 11-152, 11-153, 11-154, 11-155, 12-151, 12-152, 12-153, 12-154, 12-155, 13-151, 13-152, 13-153, 13-154, 13-155, 14-151, 14-152, 14-153, 14-154, 14-155, 15-151, 15-152, 15-153, 15-154, 15-155, 16-151, 16-152, 16-153, 16-154, 16-155, 17-151, 17-152, 17-153, 17-154, 17-155, 18-151, 18-152, 18-153, 18-154, 18-155, 19-151, 19-152, 19-153, 19-154, 19-155, 20-151, 20-152, 20-153, 20-154, 21-155, 21-151, 21-152, 21-153, 21-154, 21-155, 22-151, 22-152, 22-153, 22-154, 22-155, 23-151, 23-152, 23-153, 23-154, 23-155, 24-151, 24-152, 24-153, 24-154, 24-155, 25-151, 25-152, 25-153, 25-154, or 25-155 of SEQ ID NO: 121.

7. The chimeric protein according to claim 3, wherein the modification comprises one or more substitutions located at one or more amino acid residues of SEQ ID NO: 1 selected from the group consisting of N33, K127, K128, N129, K133, R134, R137, Q142, K143, and combinations thereof.

8. The chimeric protein according to claim 7, wherein the one or more substitutions are selected from the group consisting of N33T, K127D, K128Q, N129T, K133V, R134L, R137H, Q142M, K143T/L/I, and combinations thereof.

9. The chimeric protein according to claim 4, wherein the modification comprises one or more substitutions located at one or more amino acid residues of SEQ ID NO: 121 selected from the group consisting of N36, K128, R129, K134, K138, Q143, K144, C78, C96, and combinations thereof.

10. The chimeric protein according to claim 9, wherein the one or more substitutions are selected from the group consisting of N36T, K128D, R129Q, K134V, K138H, Q143M, K144T/L/I, C78S, C96S, and combinations thereof.

11. The chimeric protein according to claim 1, wherein the C-terminal portion comprises a β-Klotho co-receptor binding domain.

12. The chimeric protein according to claim 1, wherein the C-terminal portion comprises amino acid residues 168-209 of SEQ ID NO: 233.

13. The chimeric protein according to claim 12, wherein the C-terminal portion further comprises one or more deletions or substitutions while retaining the ability to bind β-Klotho.

14. The chimeric protein according to claim 12, wherein the C-terminal portion of FGF21 further comprises one or more substitutions, additions, or deletions to enhance binding affinity for β-Klotho compared to the C-terminal portion without the modification.

15. A pharmaceutical composition comprising the chimeric protein of claim 1 and a pharmaceutically-acceptable carrier.

16. The pharmaceutical composition according to claim 15 further comprising:

one or more agents selected from the group consisting of an anti-inflammatory agent, an antifibrotic agent, an antihypertensive agent, an antidiabetic agent, a triglyceride-lowering agent, and a cholesterol-lowering agent.

17. The pharmaceutical composition according to claim 15 further comprising an organotropic targeting agent.

18. A method for treating a subject suffering from a disorder, the method comprising:

selecting a subject suffering from the disorder;

providing a chimeric fibroblast growth factor ("FGF") protein, wherein the chimeric FGF protein comprises an N-terminus coupled to a C-terminus, wherein the N-terminus comprises a portion of a paracrine FGF and the C-terminus comprises a C-terminal portion of FGF21, and wherein the portion of the paracrine FGF is modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without the modification; and

administering a therapeutically effective amount of the chimeric FGF protein to the selected subject under conditions effective to treat the disorder.

19. The method according to claim 18, wherein the paracrine FGF is FGF1 or FGF2.

20. The method according to claim 18, wherein the portion of the paracrine FGF comprises an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 150 to 155 of SEQ ID NO: 1.

21. The method according to claim 18, wherein the portion of the paracrine FGF comprises an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 151 to 155 of SEQ ID NO: 121.

22. The method according to claim 20, wherein the portion of the paracrine FGF comprises amino acid residues 1-150, 1-151, 1-152, 1-153, 1-154, 1-155, 2-150, 2-151, 2-152, 2-153, 2-154, 2-155, 3-150, 3-151, 3-152, 3-153, 3-154, 3-155, 4-150, 4-151, 4-152, 4-153, 4-154, 4-155, 5-150, 5-151, 5-152, 5-153, 5-154, 5-155, 6-150, 6-151, 6-152, 6-153, 6-154, 6-155, 7-150, 7-151, 7-152, 7-153, 7-154, 7-155, 8-150, 8-151, 8-152, 8-153, 8-154, 8-155, 9-150, 9-151, 9-152, 9-153, 9-154, 9-155, 10-150, 10-151, 10-152, 10-153, 10-154, 10-155, 11-150, 11-151, 11-152, 11-153, 11-154, 11-155, 12-150, 12-151, 12-152, 12-153, 12-154, 12-155, 13-150, 13-151, 13-152, 13-153, 13-154, 13-155, 14-150, 14-151, 14-152, 14-153, 14-154, 14-155, 15-150, 15-151, 15-152, 15-153, 15-154, 15-155, 16-150, 16-151, 16-152, 16-153, 16-154, 16-155, 17-150, 17-151, 17-152, 17-153, 17-154, 17-155, 18-150, 18-151, 18-152, 18-153, 18-154, 18-155, 19-150, 19-151, 19-152, 19-153, 19-154, 19-155, 20-150, 20-151, 20-152, 20-153, 20-154, 21-150, 21-155, 21-151, 21-152, 21-153, 21-154, 21-155, 22-150, 22-151, 22-152, 22-153, 22-154, 22-155, 23-150, 23-151, 23-152, 23-153, 23-154, 23-155, 24-150, 24-151, 24-152, 24-153, 24-154, 24-155, 25-150, 25-151, 25-152, 25-153, 25-154, or 25-155 of SEQ ID NO: 1.

23. The method according to claim 21, wherein the portion of the paracrine FGF comprises amino acid residues 1-151, 1-152, 1-153, 1-154, 1-155, 2-151, 2-152, 2-153, 2-154, 2-155, 3-151, 3-152, 3-153, 3-154, 3-155, 4-151, 4-152, 4-153, 4-154, 4-155, 5-151, 5-152, 5-153, 5-154, 5-155, 6-151, 6-152, 6-153, 6-154, 6-155, 7-151, 7-152, 7-153, 7-154, 7-155, 8-151, 8-152, 8-153, 8-154, 8-155, 9-151, 9-152, 9-153, 9-154, 9-155, 10-151, 10-152, 10-153, 10-154, 10-155, 11-151, 11-152, 11-153, 11-154, 11-155, 12-151, 12-152, 12-153, 12-154, 12-155, 13-151, 13-152, 13-153, 13-154, 13-155, 14-151, 14-152, 14-153, 14-154, 14-155, 15-151, 15-152, 15-153, 15-154, 15-155, 16-151, 16-152, 16-153, 16-154, 16-155, 17-151, 17-152, 17-153, 17-154, 17-155, 18-151, 18-152, 18-153, 18-154, 18-155, 19-151, 19-152, 19-153, 19-154, 19-155, 20-151, 20-152, 20-153, 20-154, 21-155, 21-151, 21-152, 21-153, 21-154, 21-155, 22-151, 22-152, 22-153, 22-154, 22-155, 23-151, 23-152, 23-153, 23-154, 23-155, 24-151, 24-152, 24-153, 24-154, 24-155, 25-151, 25-152, 25-153, 25-154, or 25-155 of SEQ ID NO: 121.

24. The method according to claim 20, wherein the modification comprises one or more substitutions located at one or more amino acid residues of SEQ ID NO: 1 selected from the group consisting of N33, K127, K128, N129, K133, R134, R137, Q142, K143, and combinations thereof.

25. The method according to claim 24, wherein the one or more substitutions are selected from the group consisting of N33T, K127D, K128Q, N129T, K133V, R134L, R137H, Q142M, K143T/L/I, and combinations thereof.

26. The method according to claim 21, wherein the modification comprises one or more substitutions located at one or more amino acid residues of SEQ ID NO: 121 selected from the group consisting of N36, K128, R129, K134, K138, Q143, K144, C78, C96, and combinations thereof.

27. The method according to claim 26, wherein the one or more substitutions are selected from the group consisting of N36T, K128D, R129Q, K134V, K138H, Q143M, K144T/L/I, C78S, C96S, and combinations thereof.

28. The method according to claim 18, wherein the C-terminal portion comprises a β-Klotho co-receptor binding domain.

29. The method according to claim 18, wherein the C-terminal portion comprises amino acid residues 168-209 of SEQ ID NO: 233.

30. The method according to claim 29, wherein the C-terminal portion of FGF21 further comprises one or more substitutions, additions, or deletions while retaining the ability to bind β-Klotho.

31. The method according to claim 29, wherein the C-terminal portion of FGF21 further comprises one or more substitutions, additions, or deletions to enhance binding affinity for β-Klotho compared to the C-terminal portion without the modification.

32. The method according to claim 18, wherein the disorder is a selected from the group consisting of diabetes, obesity, and metabolic syndrome.

33. The method according to claim 18, wherein the disorder is type II diabetes, gestational diabetes, or drug-induced diabetes.

34. The method according to claim 18, wherein the disorder is type I diabetes.

35. The method according to claim 18, wherein the disorder is obesity.

36. The method according to claim 18, wherein the disorder is metabolic syndrome.

37. The method according to claim 18, wherein said administering is carried out parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, by implantation, by intracavitary or intravesical instillation, intraocularly, intraarterially, intralesionally, transdermally, or by application to mucous membranes.

38. The method according to claim 18, wherein the chimeric FGF protein is administered with a pharmaceutically-acceptable carrier.

39. The method according to claim 18, wherein the selected subject is a mammal.

40. The method according to claim 18, wherein the selected subject is a human.

41. The method according to claim 18, wherein the chimeric FGF is co-administered with one or more agents selected from the group consisting of an anti-inflammatory agent, an antifibrotic agent, an antihypertensive agent, an antidiabetic agent, a triglyceride-lowering agent, and a cholesterol-lowering agent.

42. A method of making a chimeric fibroblast growth factor ("FGF") protein possessing enhanced endocrine activity, the method comprising:

introducing one or more modifications to a FGF protein, wherein the modification decreases the affinity of the FGF protein for heparin and/or heparan sulfate; and

coupling a Klotho co-receptor binding domain to the modified FGF protein's C-terminus, whereby a chimeric FGF protein possessing enhanced endocrine activity is made.

43. The method according to claim 42 further comprising:

selecting, prior to said coupling, the Klotho co-receptor binding domain, wherein the Klotho co-receptor binding domain is selected to target an endocrine FGF target tissue.

44. The method according to claim 42, wherein the chimeric FGF protein has greater binding affinity for FGF receptor ("FGFR") than the native endocrine FGF ligand having the Klotho co-receptor binding domain.

45. The method according to claim 42, wherein the FGF protein is a paracrine FGF.

46. The method according to claim 45, wherein the FGF protein is FGF1 or FGF2.

47. The method according to claim 42, wherein the FGF protein comprises an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 150 to 155 of SEQ ID NO: 1.

48. The method according to claim 42, wherein the FGF protein comprises an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 151 to 155 of SEQ ID NO: 121.

49. The method according to claim 47, wherein the FGF protein comprises amino acid residues 1-150, 1-151, 1-152, 1-153, 1-154, 1-155, 2-150, 2-151, 2-152, 2-153, 2-154, 2-155, 3-150, 3-151, 3-152, 3-153, 3-154, 3-155, 4-150, 4-151, 4-152, 4-153, 4-154, 4-155, 5-150, 5-151, 5-152, 5-153, 5-154, 5-155, 6-150, 6-151, 6-152, 6-153, 6-154, 6-155, 7-150, 7-151, 7-152, 7-153, 7-154, 7-155, 8-150, 8-151, 8-152, 8-153, 8-154, 8-155, 9-150, 9-151, 9-152, 9-153, 9-154, 9-155, 10-150, 10-151, 10-152, 10-153, 10-154, 10-155, 11-150, 11-151, 11-152, 11-153, 11-154, 11-155, 12-150, 12-151, 12-152, 12-153, 12-154, 12-155, 13-150, 13-151, 13-152, 13-153, 13-154, 13-155, 14-150, 14-151, 14-152, 14-153, 14-154, 14-155, 15-150, 15-151, 15-152, 15-153, 15-154, 15-155, 16-150, 16-151, 16-152, 16-153, 16-154, 16-155, 17-150, 17-151, 17-152, 17-153, 17-154, 17-155, 18-150, 18-151, 18-152, 18-153, 18-154, 18-155, 19-150, 19-151, 19-152, 19-153, 19-154, 19-155, 20-150, 20-151, 20-152, 20-153, 20-154, 21-150, 21-155, 21-151, 21-152, 21-153, 21-154, 21-155, 22-150, 22-151, 22-152, 22-153, 22-154, 22-155, 23-150, 23-151, 23-152, 23-153, 23-154, 23-155, 24-150, 24-151, 24-152, 24-153, 24-154, 24-155, 25-150, 25-151, 25-152, 25-153, 25-154, or 25-155 of SEQ ID NO: 1.

50. The method according to claim 48, wherein the FGF protein comprises amino acid residues 1-151, 1-152, 1-153, 1-154, 1-155, 2-151, 2-152, 2-153, 2-154, 2-155, 3-151, 3-152, 3-153, 3-154, 3-155, 4-151, 4-152, 4-153, 4-154, 4-155, 5-151, 5-152, 5-153, 5-154, 5-155, 6-151, 6-152, 6-153, 6-154, 6-155, 7-151, 7-152, 7-153, 7-154, 7-155, 8-151, 8-152, 8-153, 8-154, 8-155, 9-151, 9-152, 9-153, 9-154, 9-155, 10-151, 10-152, 10-153, 10-154, 10-155, 11-151, 11-152, 11-153, 11-154, 11-155, 12-151, 12-152, 12-153, 12-154, 12-155, 13-151, 13-152, 13-153, 13-154, 13-155, 14-151, 14-152, 14-153, 14-154, 14-155, 15-151, 15-152, 15-153, 15-154, 15-155, 16-151, 16-152, 16-153, 16-154, 16-155, 17-151, 17-152, 17-153, 17-154, 17-155, 18-151, 18-152, 18-153, 18-154, 18-155, 19-151, 19-152, 19-153, 19-154, 19-155, 20-151, 20-152, 20-153, 20-154, 21-155, 21-151, 21-152, 21-153, 21-154, 21-155, 22-151, 22-152, 22-

153, 22-154, 22-155, 23-151, 23-152, 23-153, 23-154, 23-155, 24-151, 24-152, 24-153, 24-154, 24-155, 25-151, 25-152, 25-153, 25-154, or 25-155 of SEQ ID NO: 121.

51. The method according to claim 47, wherein the modification is one or more substitutions located at one or more amino acid residues of SEQ ID NO: 1 selected from the group consisting of N33, K127, K128, N129, K133, R134, R137, Q142, K143, and combinations thereof.

52. The method according to claim 51, wherein the one or more substitutions are selected from the group consisting of N33T, K127D, K128Q, N129T, K133V, R134L, R137H, Q142M, K143T/L/I, and combinations thereof.

53. The method according to claim 48, wherein the modification is one or more substitutions located at one or more amino acid residues of SEQ ID NO: 121 selected from the group consisting of N36, K128, R129, K134, K138, Q143, K144, C78, C96, and combinations thereof.

54. The method according to claim 53, wherein the one or more substitutions are selected from the group consisting of N36T, K128D, R129Q, K134V, K138H, Q143M, K144T/L/I, C78S, C96S, and combinations thereof.

55. The method according to claim 42, wherein the Klotho co-receptor binding domain comprises a C-terminal portion of FGF21.

56. The method according to claim 39, wherein the Klotho co-receptor binding domain comprises a β-Klotho co-receptor binding domain.

57. The method according to claim 39, wherein the C-terminal portion of FGF21 comprises amino acid residues 168-209 of SEQ ID NO: 233.

58. The method according to claim 55, wherein the C-terminal portion of FGF21 further comprises one or more substitutions, additions, or deletions while retaining the ability to bind β-Klotho.

59. The method according to claim 55, wherein the C-terminal portion of FGF21 further comprises one or more substitutions, additions, or deletions to enhance binding affinity for β-Klotho compared to the C-terminal portion without the modification.

60. A method of facilitating fibroblast growth factor receptor ("FGFR")-βKlotho co-receptor complex formation, the method comprising:

providing a cell comprising a βKlotho co-receptor and an FGFR;

providing a chimeric fibroblast growth factor ("FGF") protein comprising a C-terminal portion of FGF21 and a portion of a paracrine FGF, wherein the portion of the paracrine FGF is modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without the modification; and

contacting the cell with the chimeric FGF protein under conditions effective to cause FGFR-βKlotho co-receptor complex formation.

61. The method according to claim 60, wherein the paracrine FGF is FGF1 or FGF2.

62. The method according to claim 60, wherein the portion of the paracrine FGF protein comprises an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 150 to 155 of SEQ ID NO: 1.

63. The method according to claim 60, wherein the portion of the paracrine FGF protein comprises an amino acid sequence beginning at any one of residues 1 to 25 and ending at any one of residues 151 to 155 of SEQ ID NO: 121.

64. The method according to claim 62, wherein the portion of the paracrine FGF protein comprises amino acid residues 1-150, 1-151, 1-152, 1-153, 1-154, 1-155, 2-150, 2-151, 2-152, 2-153, 2-154, 2-155, 3-150, 3-151, 3-152, 3-153, 3-154, 3-155, 4-150, 4-151, 4-152, 4-153, 4-154, 4-155, 5-150, 5-151, 5-152, 5-153, 5-154, 5-155, 6-150, 6-151, 6-152, 6-153, 6-154, 6-155, 7-150, 7-151, 7-152, 7-153, 7-154, 7-155, 8-150, 8-151, 8-152, 8-153, 8-154, 8-155, 9-150, 9-151, 9-152, 9-153, 9-154, 9-155, 10-150, 10-151, 10-152, 10-153, 10-154, 10-155, 11-150, 11-151, 11-152, 11-153, 11-154, 11-155, 12-150, 12-151, 12-152, 12-153, 12-154, 12-155, 13-150, 13-151, 13-152, 13-153, 13-154, 13-155, 14-150, 14-151, 14-152, 14-153, 14-154, 14-

155, 15-150, 15-151, 15-152, 15-153, 15-154, 15-155, 16-150, 16-151, 16-152, 16-153, 16-154, 16-155, 17-150, 17-151, 17-152, 17-153, 17-154, 17-155, 18-150, 18-151, 18-152, 18-153, 18-154, 18-155, 19-150, 19-151, 19-152, 19-153, 19-154, 19-155, 20-150, 20-151, 20-152, 20-153, 20-154, 21-150, 21-155, 21-151, 21-152, 21-153, 21-154, 21-155, 22-150, 22-151, 22-152, 22-153, 22-154, 22-155, 23-150, 23-151, 23-152, 23-153, 23-154, 23-155, 24-150, 24-151, 24-152, 24-153, 24-154, 24-155, 25-150, 25-151, 25-152, 25-153, 25-154, or 25-155 of SEQ ID NO: 1.

65. The method according to claim 63, wherein the portion of the paracrine FGF protein comprises amino acid residues 1-151, 1-152, 1-153, 1-154, 1-155, 2-151, 2-152, 2-153, 2-154, 2-155, 3-151, 3-152, 3-153, 3-154, 3-155, 4-151, 4-152, 4-153, 4-154, 4-155, 5-151, 5-152, 5-153, 5-154, 5-155, 6-151, 6-152, 6-153, 6-154, 6-155, 7-151, 7-152, 7-153, 7-154, 7-155, 8-151, 8-152, 8-153, 8-154, 8-155, 9-151, 9-152, 9-153, 9-154, 9-155, 10-151, 10-152, 10-153, 10-154, 10-155, 11-151, 11-152, 11-153, 11-154, 11-155, 12-151, 12-152, 12-153, 12-154, 12-155, 13-151, 13-152, 13-153, 13-154, 13-155, 14-151, 14-152, 14-153, 14-154, 14-155, 15-151, 15-152, 15-153, 15-154, 15-155, 16-151, 16-152, 16-153, 16-154, 16-155, 17-151, 17-152, 17-153, 17-154, 17-155, 18-151, 18-152, 18-153, 18-154, 18-155, 19-151, 19-152, 19-153, 19-154, 19-155, 20-151, 20-152, 20-153, 20-154, 21-155, 21-151, 21-152, 21-153, 21-154, 21-155, 22-151, 22-152, 22-153, 22-154, 22-155, 23-151, 23-152, 23-153, 23-154, 23-155, 24-151, 24-152, 24-153, 24-154, 24-155, 25-151, 25-152, 25-153, 25-154, or 25-155 of SEQ ID NO: 121.

66. The method according to claim 62, wherein the modification is one or more substitutions located at one or more amino acid residues of SEQ ID NO: 1 selected from the group consisting of N33, K127, K128, N129, K133, R134, R137, Q142, K143, and combinations thereof.

67. The method according to claim 66, wherein the one or more substitutions are selected from the group consisting of N33T, K127D, K128Q, N129T, K133V, R134L, R137H, Q142M, K143T/L/I, and combinations thereof.

68. The method according to claim 63, wherein the modification is one or more substitutions located at one or more amino acid residues of SEQ ID NO: 121 selected from the group consisting of N36, K128, R129, K134, K138, Q143, K144, C78, C96, and combinations thereof.

69. The method according to claim 68, wherein the one or more substitutions are selected from the group consisting of N36T, K128D, R129Q, K134V, K138H, Q143M, K144T/L/I, C78S, C96S, and combinations thereof.

70. The method according to claim 60, wherein the Klotho is a β-Klotho co-receptor protein.

71. The method according to claim 60, wherein the FGFR is FGFR1c, FGFR2c, or FGFR4.

72. The method according to claim 60, wherein the C-terminal portion comprises a β-Klotho co-receptor binding domain.

73. The method according to claim 60, wherein the C-terminal portion from the FGF21 comprises amino acid residues 168-209 of SEQ ID NO: 233.

74. The method according to claim 73, wherein the C-terminal portion from the FGF21 comprises one or more substitutions, additions, or deletions while retaining the ability to bind β-Klotho.

75. The method according to claim 73, wherein the C-terminal portion derived from the FGF21 comprises one or more substitutions, additions, or deletions to enhance binding affinity for β-Klotho compared to the portion without the substitutions, additions, or deletions.

76. A method of screening for agents capable of facilitating fibroblast growth factor receptor ("FGFR")-βKlotho complex formation in the treatment of a disorder, the method comprising:

providing a chimeric fibroblast growth factor ("FGF") comprising an N-terminus coupled to a C-terminus, wherein the N-terminus comprises a portion of a paracrine FGF and the C-terminus comprises a C-terminal portion of FGF21, and wherein the portion of the paracrine FGF is modified to decrease binding affinity for heparin and/or heparan sulfate compared to the portion without the modification;

providing binary βKlotho-FGFR complex;

providing one or more candidate agents;

combining the chimeric FGF, the binary βKlotho-FGFR complex, and the one or more candidate agents under conditions permitting the formation of a ternary complex between the chimeric FGF and the binary βKlotho-FGFR complex in the absence of the one or more candidate agents; and

identifying the one or more candidate agents that decrease ternary complex formation between the chimeric FGF and the binary βKlotho-FGFR complex compared to the ternary complex formation in the absence of the one or more candidate agents as suitable for treating the disorder.

77. The method according to claim 76, wherein the paracrine FGF is FGF1 or FGF2.

78. The method according to claim 76, wherein the modulation is a competitive interaction between the chimeric FGF and the one or more candidate agents for binding to the binary βKlotho-FGFR complex.

79. The method according to claim 76, wherein the modulation stabilizes the ternary complex.

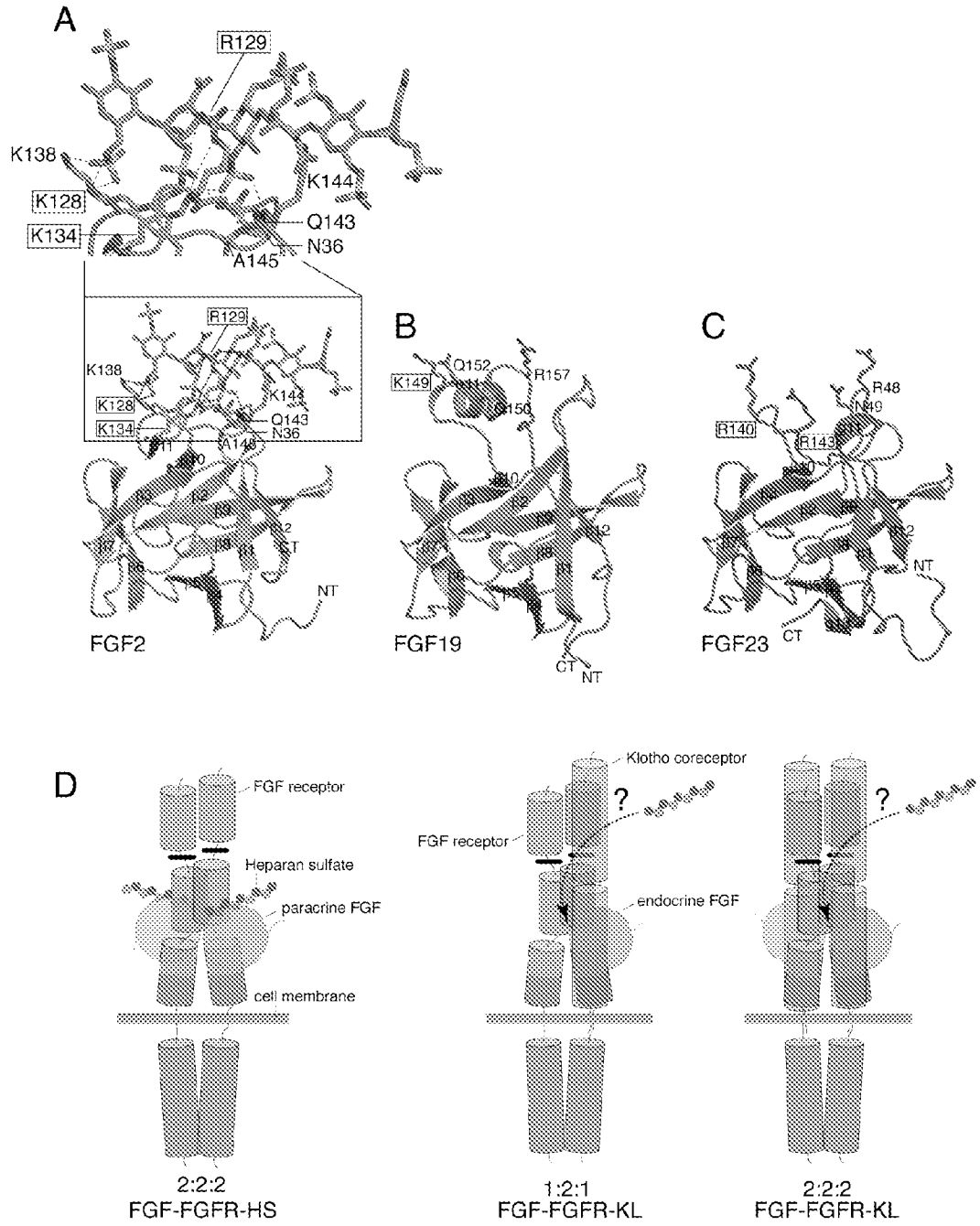
80. The method according to claim 76, wherein the modulation is an allosteric or kinetic modulation.

81. The method according to claim 76, wherein the FGFR is FGFR1c, FGFR2c, or FGFR4.

82. The method according to claim 76, wherein a plurality of candidate agents are provided.

83. The method according to claim 76, wherein said identifying is carried out using surface plasmon resonance spectroscopy.

84. The method according to claim 76, wherein the disorder is selected from the group consisting of diabetes, obesity, and metabolic syndrome.


A
R129
K138
K144
K128
Q143
K134
N36
A145
FGF2
B
Q152
K149
R157
FGF19
C
R48
R140
R143
NT
CT
FGF23
D
FGF receptor
Heparan sulfate
paracrine FGF
cell membrane
2:2:2
FGF-FGFR-HS
Klotho coreceptor
?
endocrine FGF
1:2:1
FGF-FGFR-KL
2:2:2
FGF-FGFR-KL

FIGS. 1A-1D

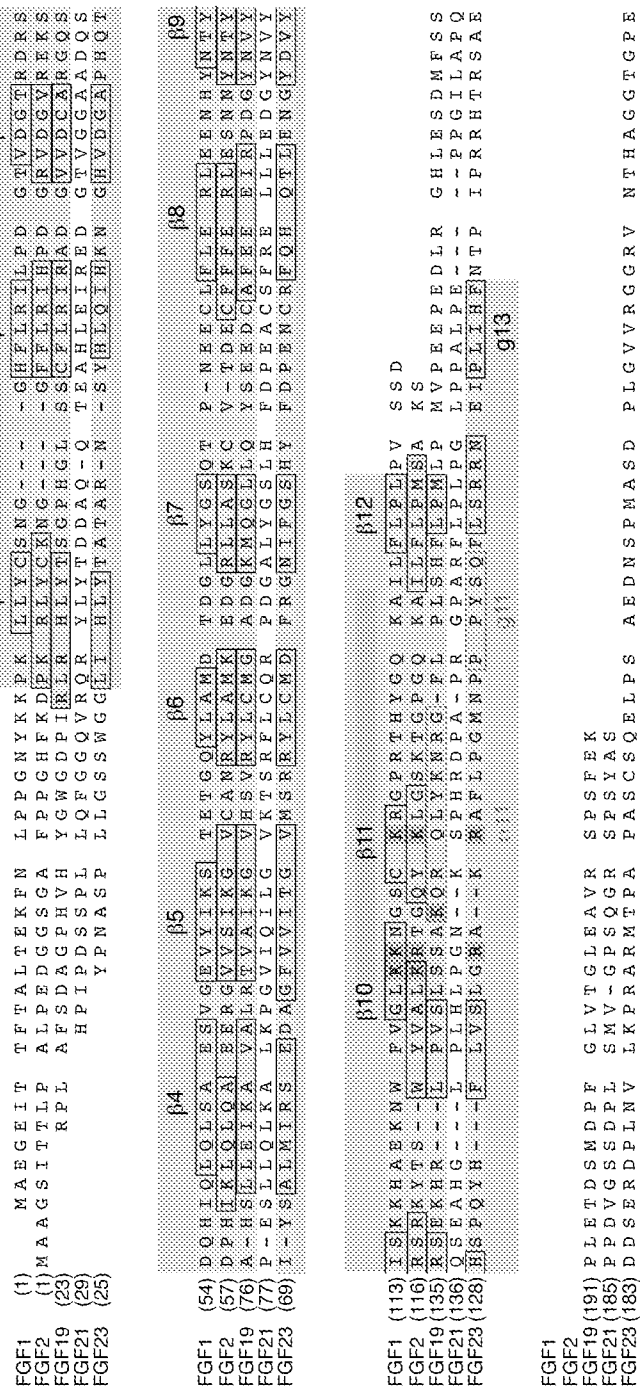
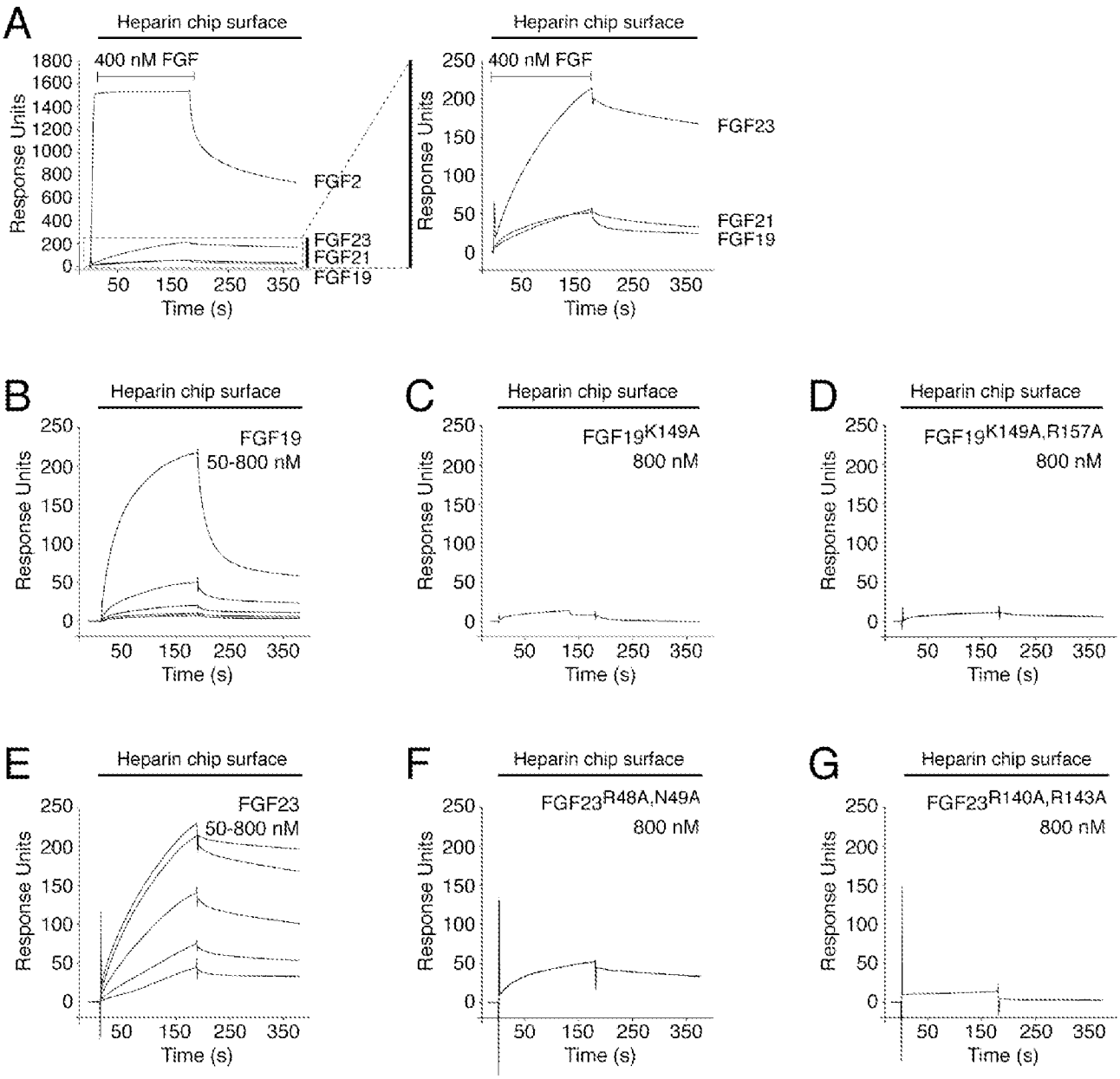
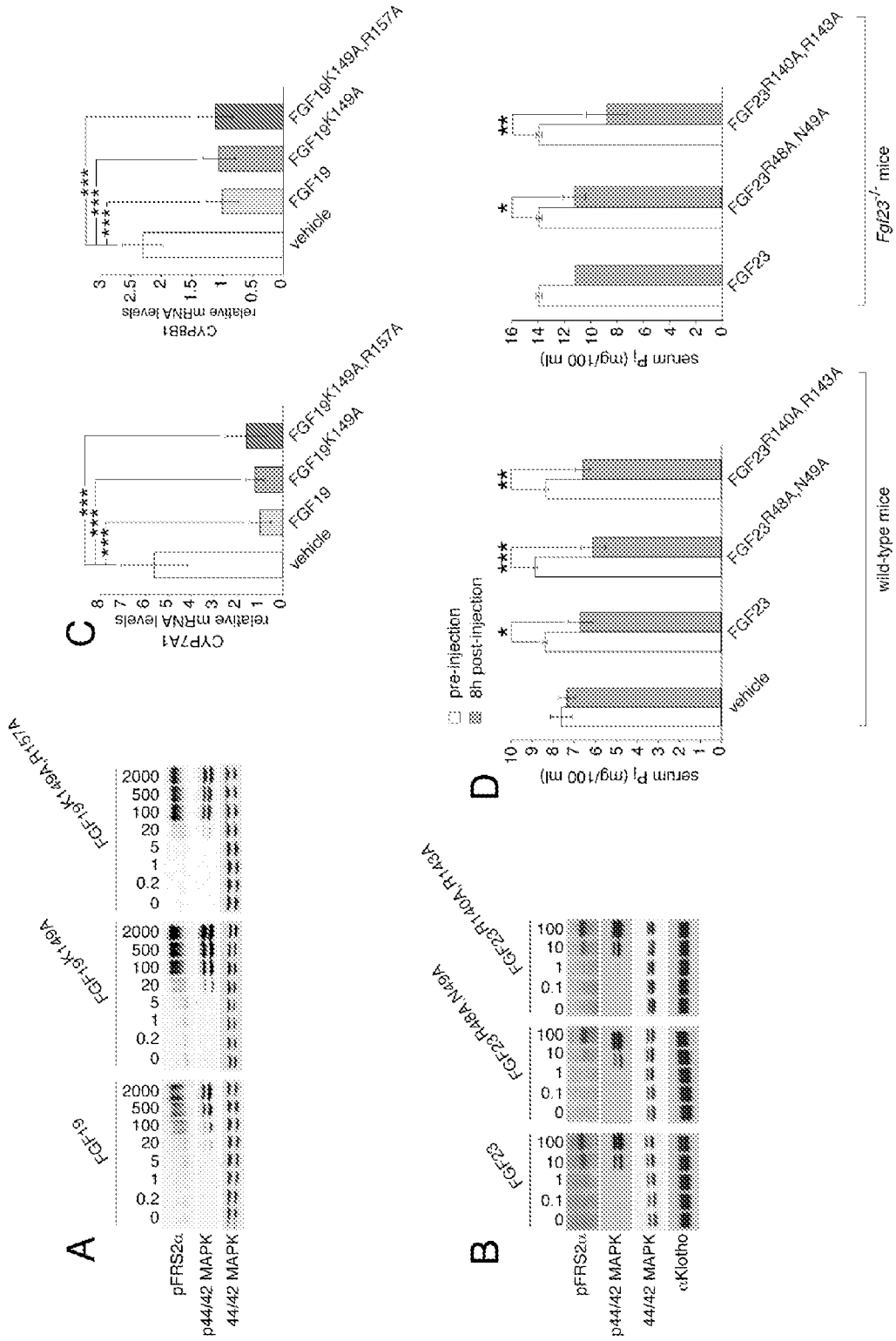


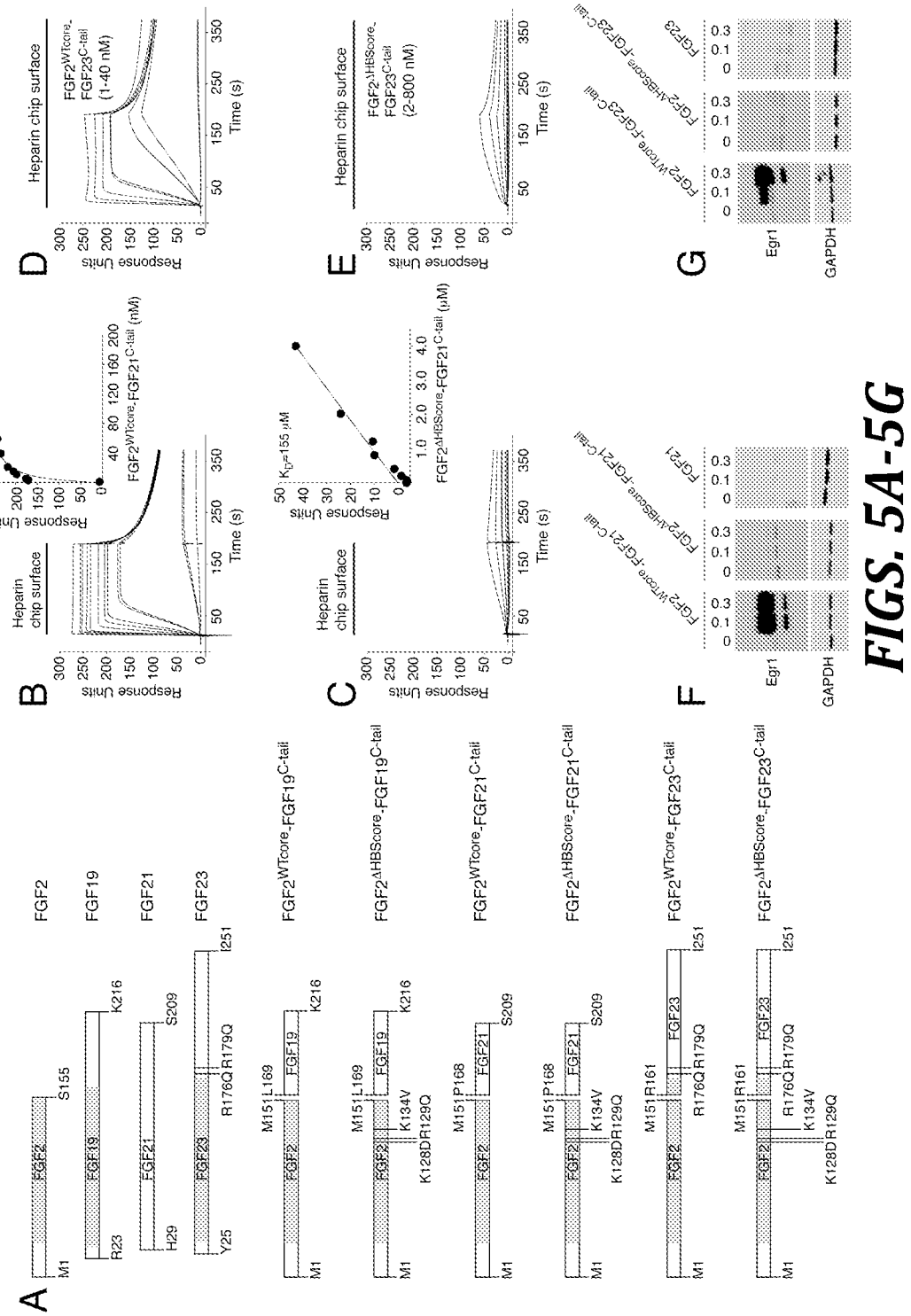
FIG. 2


A
Heparin chip surface
400 nM FGF
Response Units
Time (s)
FGF2
FGF23
FGF21
FGF19
Heparin chip surface
400 nM FGF
FGF23
FGF21
FGF19
B
Heparin chip surface
FGF19
50-800 nM
C
Heparin chip surface
FGF19K149A
800 nM
D
Heparin chip surface
FGF19K149A,R157A
800 nM
E
Heparin chip surface
FGF23
50-800 nM
F
Heparin chip surface
FGF23R48A,N49A
800 nM
G
Heparin chip surface
FGF23R140A,R143A
800 nM

FIGS. 3A-3G


A
FGF19
FGF19K149A
FGF19K149A,R157A
2000
500
100
20
5
1
0.2
0
pFRS2α
p44/42 MAPK
44/42 MAPK
B
FGF23
FGF23R48A,N49A
FGF23R140A,R143A
100
10
1
0.1
0
pFRS2α
p44/42 MAPK
44/42 MAPK
αKlotho
C
CYP7A1
relative mRNA levels
CYP8B1
relative mRNA levels
vehicle
FGF19
FGF19K149A
FGF19K149A,R157A
***
D
pre-injection
8h post-injection
serum Pi (mg/100 ml)
vehicle
FGF23
FGF23R48A,N49A
FGF23R140A,R143A
*
**
***
wild-type mice
Fgf23-/- mice

FIGS. 4A-4D


A
FGF2
FGF19
FGF21
FGF23
M1
S155
R23
K216
H29
S209
Y25
R176Q R179Q
I251
M151L169
K134V
K128D R129Q
M151P168
M151R161
B
Heparin chip surface
Response Units
Time (s)
K_D=4.0 nM
FGF2^{WTcore}-FGF21^{C-tail} (nM)
C
K_D=155 μM
FGF2^{ΔHBScore}-FGF21^{C-tail} (μM)
D
FGF2^{WTcore}-FGF23^{C-tail} (1-40 nM)
E
FGF2^{ΔHBScore}-FGF23^{C-tail} (2-800 nM)
F
FGF2-WTcore-FGF21 C-tail
FGF2-ΔHBScore-FGF21 C-tail
FGF21
G
FGF2-WTcore-FGF23 C-tail
FGF2-ΔHBScore-FGF23 C-tail
FGF23
Egr1
GAPDH

FIGS. 5A-5G

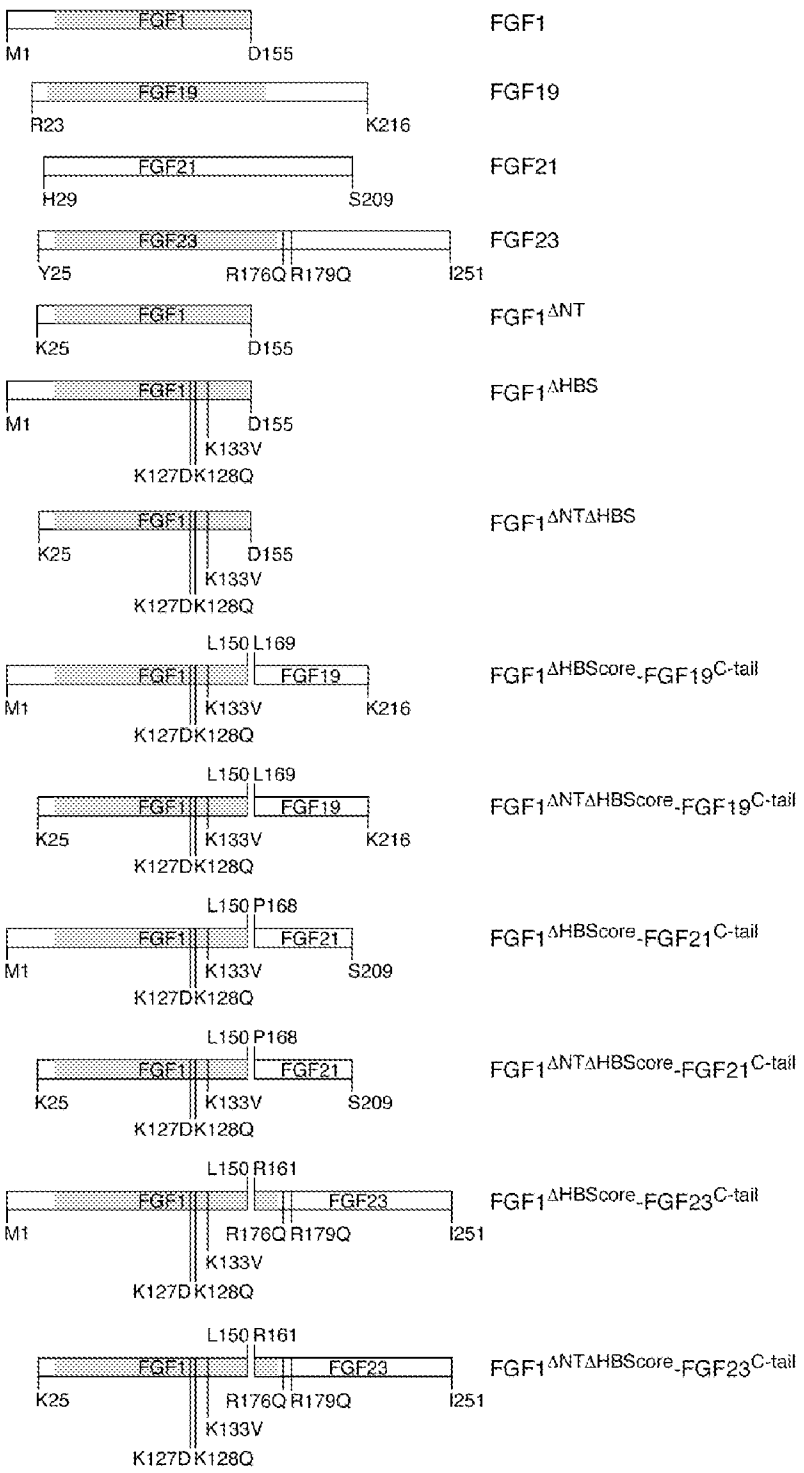
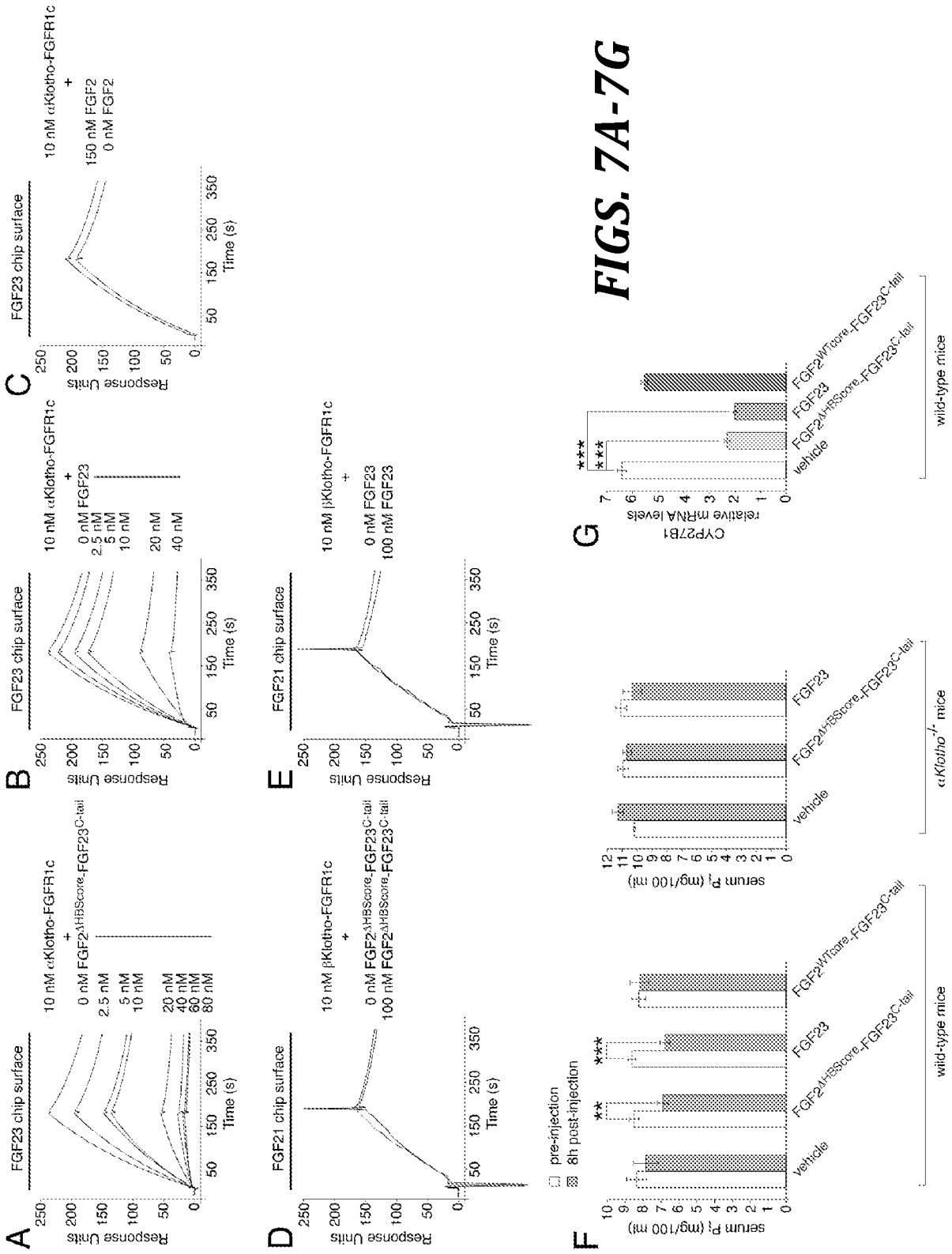
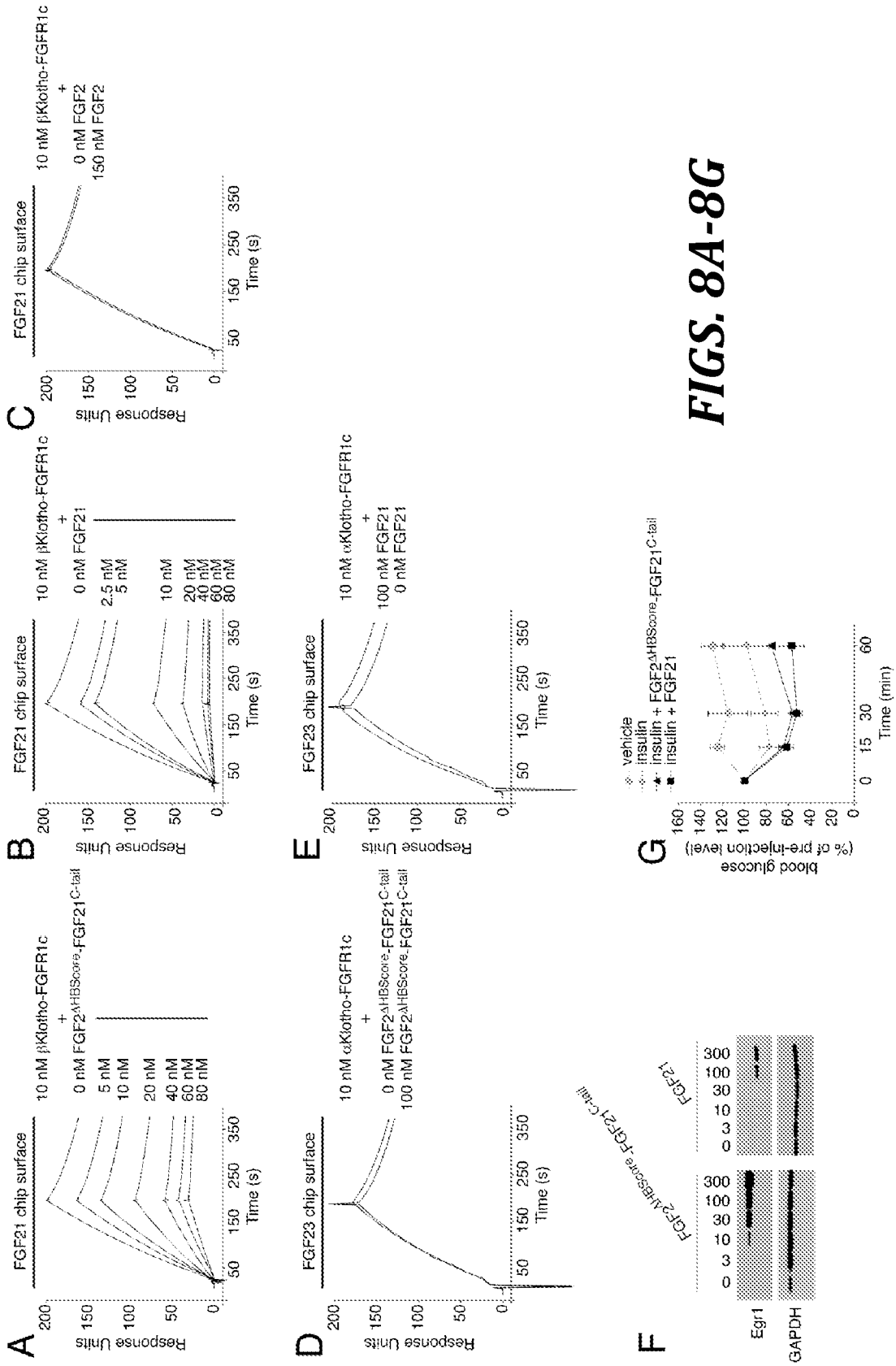

FGF1
M1
D155
FGF1
FGF19
R23
K216
FGF19
FGF21
H29
S209
FGF21
FGF23
Y25
R176Q R179Q
I251
FGF23
FGF1
K25
D155
FGF1ΔNT
FGF1
M1
D155
K133V
K127DK128Q
FGF1ΔHBS
FGF1
K25
D155
K133V
K127DK128Q
FGF1ΔNTΔHBS
L150 L169
FGF1
FGF19
M1
K133V
K216
K127DK128Q
FGF1ΔHBScore-FGF19C-tail
L150 L169
FGF1
FGF19
K25
K133V
K216
K127DK128Q
FGF1ΔNTΔHBScore-FGF19C-tail
L150 P168
FGF1
FGF21
M1
K133V
S209
K127DK128Q
FGF1ΔHBScore-FGF21C-tail
L150 P168
FGF1
FGF21
K25
K133V
S209
K127DK128Q
FGF1ΔNTΔHBScore-FGF21C-tail
L150 R161
FGF1
FGF23
M1
R176Q R179Q
I251
K133V
K127D K128Q
FGF1ΔHBScore-FGF23C-tail
L150 R161
FGF1
FGF23
K25
R176Q R179Q
I251
K133V
K127D K128Q
FGF1ΔNTΔHBScore-FGF23C-tail

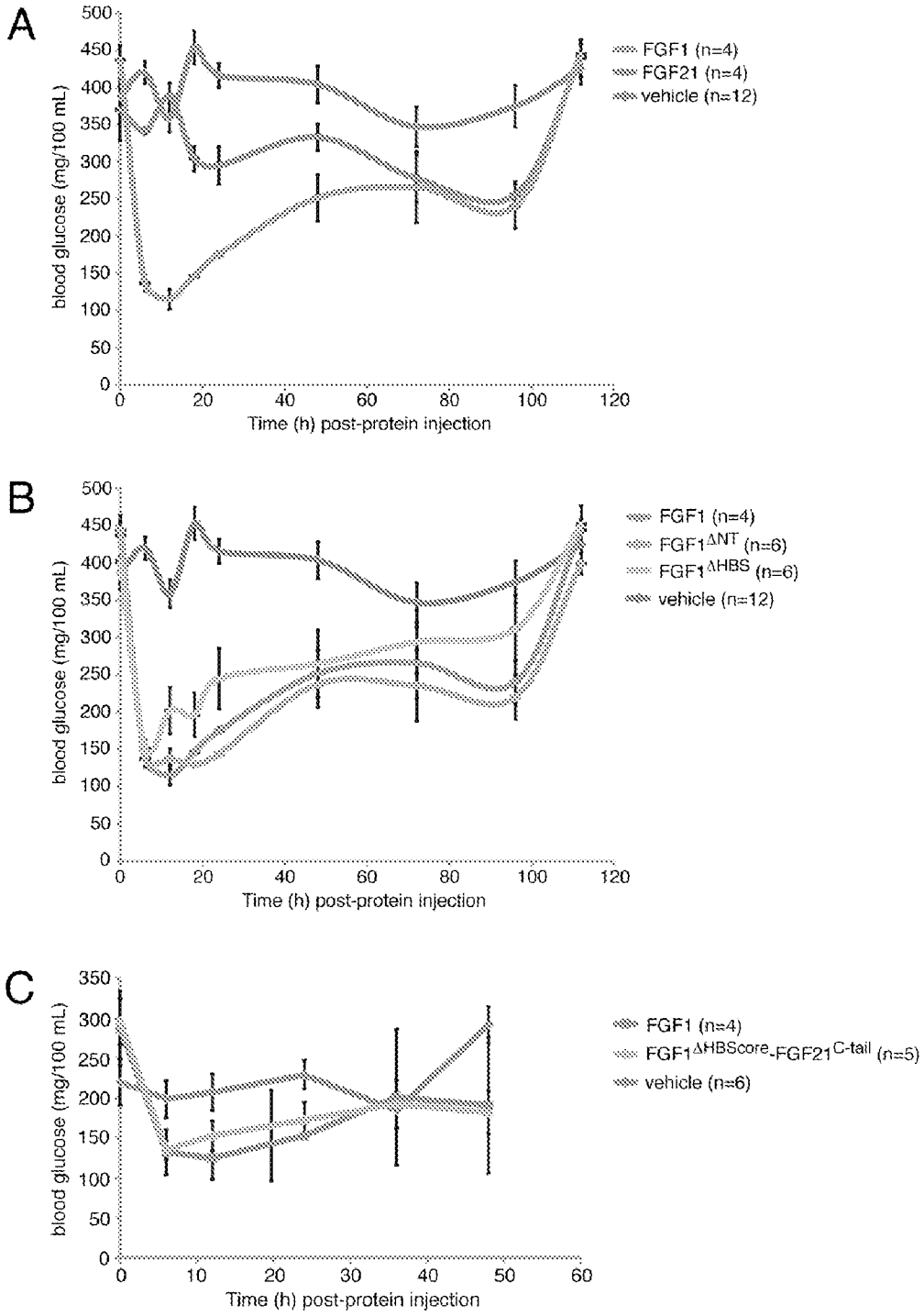
FIG. 6


A
FGF23 chip surface
10 nM αKlotho-FGFR1c
+
0 nM FGF2ΔHBScore-FGF23C-tail
2.5 nM
5 nM
10 nM
20 nM
40 nM
60 nM
80 nM
Response Units
Time (s)
B
FGF23 chip surface
10 nM αKlotho-FGFR1c
+
0 nM FGF23
2.5 nM
5 nM
10 nM
20 nM
40 nM
Response Units
Time (s)
C
FGF23 chip surface
10 nM αKlotho-FGFR1c
+
150 nM FGF2
0 nM FGF2
Response Units
Time (s)
D
FGF21 chip surface
10 nM βKlotho-FGFR1c
+
0 nM FGF2ΔHBScore-FGF23C-tail
100 nM FGF2ΔHBScore-FGF23C-tail
Response Units
Time (s)
E
FGF21 chip surface
10 nM βKlotho-FGFR1c
+
0 nM FGF23
100 nM FGF23
Response Units
Time (s)
F
pre-injection
8h post-injection
**
***
serum P$_i$ (mg/100 ml)
Vehicle
FGF23
FGF2ΔHBScore-FGF23C-tail
FGF2WTcore-FGF23C-tail
wild-type mice
αKlotho$^{-/-}$ mice
G
***
***
CYP27B1
relative mRNA levels
Vehicle
FGF2ΔHBScore-FGF23C-tail
FGF23
FGF2WTcore-FGF23C-tail
wild-type mice

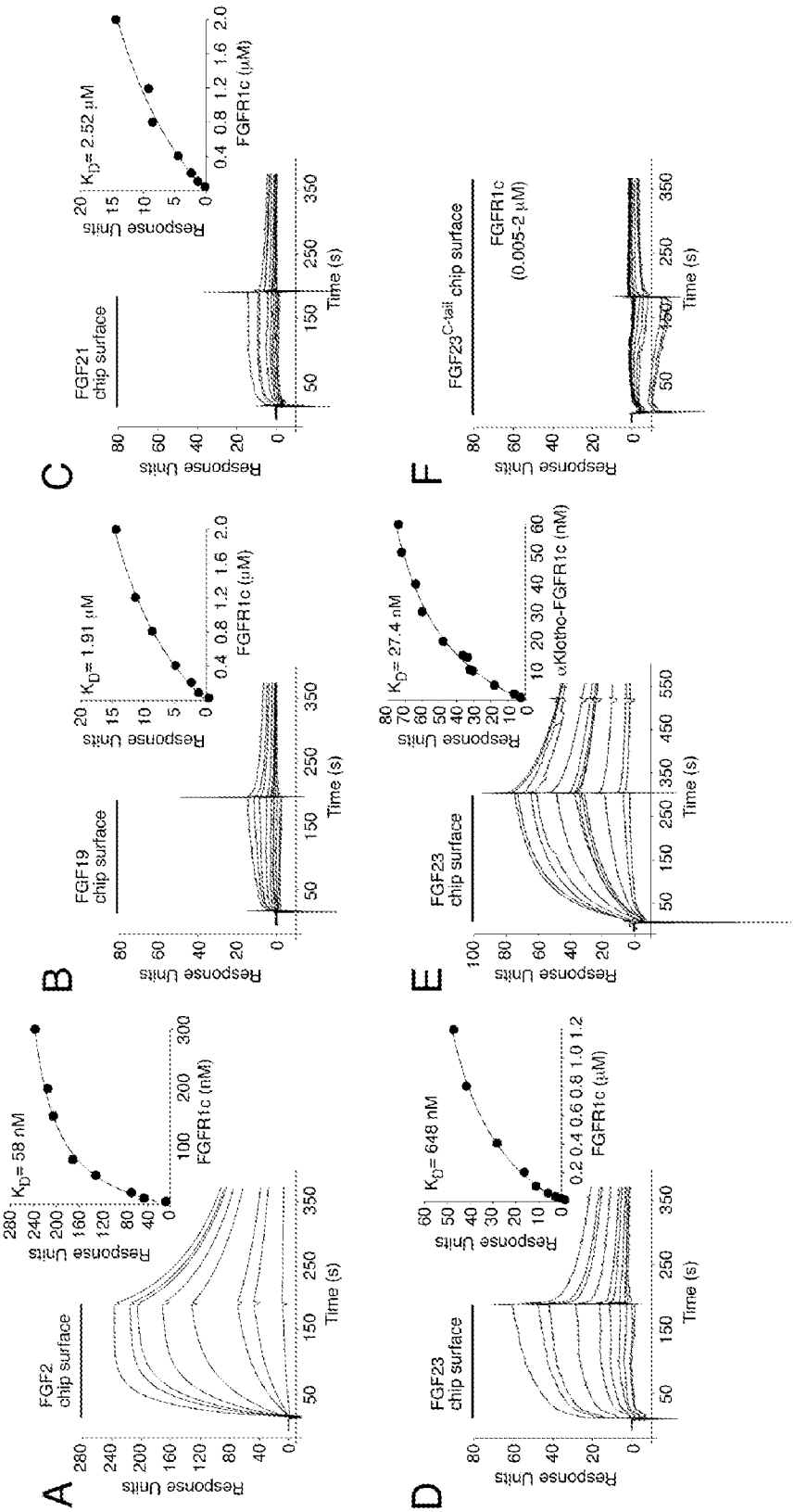
FIGS. 7A-7G


A
FGF21 chip surface
10 nM βKlotho-FGFR1c
+
0 nM FGF2^ΔHBScore-FGF21C-tail
5 nM
10 nM
20 nM
40 nM
60 nM
80 nM
Response Units
Time (s)
B
FGF21 chip surface
10 nM βKlotho-FGFR1c
+
0 nM FGF21
2.5 nM
5 nM
10 nM
20 nM
40 nM
60 nM
80 nM
Response Units
Time (s)
C
FGF21 chip surface
10 nM βKlotho-FGFR1c
+
0 nM FGF2
150 nM FGF2
Response Units
Time (s)
D
FGF23 chip surface
10 nM αKlotho-FGFR1c
+
0 nM FGF2^ΔHBScore-FGF21C-tail
100 nM FGF2^ΔHBScore-FGF21C-tail
Response Units
Time (s)
E
FGF23 chip surface
10 nM αKlotho-FGFR1c
+
100 nM FGF21
0 nM FGF21
Response Units
Time (s)
F
FGF2^ΔHBScore-FGF21C-tail
FGF21
300
100
30
10
3
0
Egr1
GAPDH
G
vehicle
insulin
insulin + FGF2^ΔHBScore-FGF21C-tail
insulin + FGF21
blood glucose
(% of pre-injection level)
Time (min)

FIGS. 8A-8G


A
blood glucose (mg/100 mL)
Time (h) post-protein injection
FGF1 (n=4)
FGF21 (n=4)
vehicle (n=12)
B
blood glucose (mg/100 mL)
Time (h) post-protein injection
FGF1 (n=4)
FGF1ΔNT (n=6)
FGF1ΔHBS (n=6)
vehicle (n=12)
C
blood glucose (mg/100 mL)
Time (h) post-protein injection
FGF1 (n=4)
FGF1ΔHBScore-FGF21C-tail (n=5)
vehicle (n=6)

FIGS. 9A-9C


A
FGF2
chip surface
Response Units
Time (s)
K_D= 58 nM
FGFR1c (nM)
B
FGF19
chip surface
K_D= 1.91 μM
FGFR1c (μM)
C
FGF21
chip surface
K_D= 2.52 μM
FGFR1c (μM)
D
FGF23
chip surface
K_D= 648 nM
FGFR1c (μM)
E
FGF23
chip surface
K_D= 27.4 nM
αKlotho-FGFR1c (nM)
F
$FGF23^{C\text{-}tail}$ chip surface
FGFR1c
(0.005-2 μM)

FIGS. 10A-10F

```
FGF19 (169)  LPMV  PEEPEDLRGH  LESDMFSSPL  ETDSMDPFGL  VTGLEAVRSP  SFEK-----
FGF21 (168)  PGLP  PALPE-----  PPGILAPQPP  DVGSSDPLSM  V-GPSQGRSP  SYAS-----
FGF23 (163)  --EI  PLI--HFNTP  IPRRHTRSAE  DDSERDPLN-  VLKPRARMTP  APASCSQELP

FGF19        ----------  ----------  ----------  ----------
FGF21        ----------  ----------  ----------  ----------
FGF23 (212)  SAEDNSPMAS  DPLGVVRGGR  VNTHAGGTGP  EGCRPFAKFI
```

FIG. 11

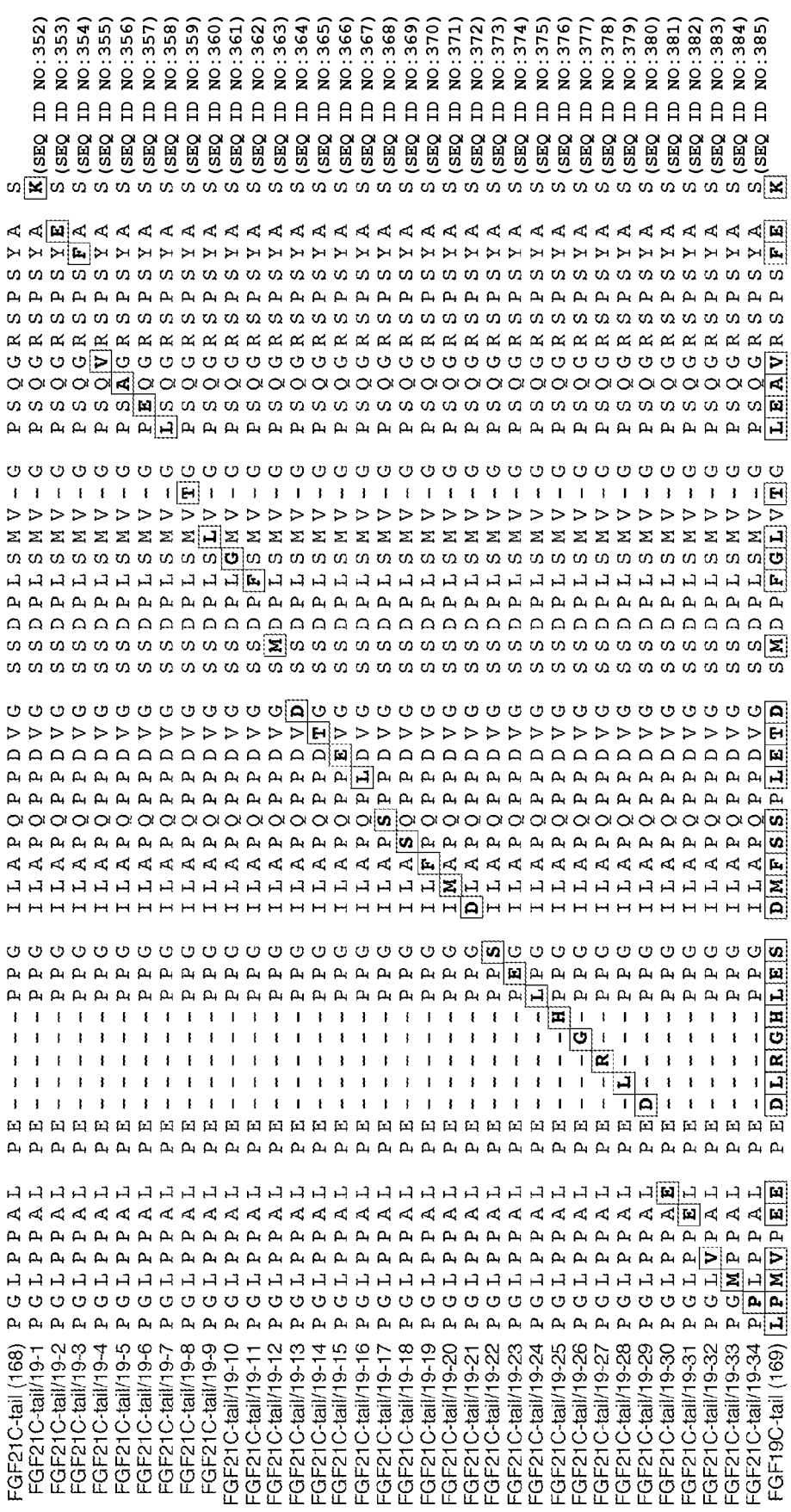

FGF21C-tail (168) PGLPPAALPE---PPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAS
FGF21C-tail/19-1 PGLPPAALPE---PPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAK(SEQ ID NO:352)
FGF21C-tail/19-2 PGLPPAALPE---PPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAE(SEQ ID NO:353)
FGF21C-tail/19-3 PGLPPAALPE---PPGILAPQPPDVGSSDPLSMV-GPSQGRSPSFAS(SEQ ID NO:354)
FGF21C-tail/19-4 PGLPPAALPE---PPGILAPQPPDVGSSDPLSMV-GPSQVRSPSYAS(SEQ ID NO:355)
FGF21C-tail/19-5 PGLPPAALPE---PPGILAPQPPDVGSSDPLSMV-GPSAGRSPSYAS(SEQ ID NO:356)
FGF21C-tail/19-6 PGLPPAALPE---PPGILAPQPPDVGSSDPLSMV-GPEQGRSPSYAS(SEQ ID NO:357)
FGF21C-tail/19-7 PGLPPAALPE---PPGILAPQPPDVGSSDPLSMV-GLSQGRSPSYAS(SEQ ID NO:358)
FGF21C-tail/19-8 PGLPPAALPE---PPGILAPQPPDVGSSDPLSMVTGPSQGRSPSYAS(SEQ ID NO:359)
FGF21C-tail/19-9 PGLPPAALPE---PPGILAPQPPDVGSSDPLSLV-GPSQGRSPSYAS(SEQ ID NO:360)
FGF21C-tail/19-10 PGLPPAALPE---PPGILAPQPPDVGSSDPLGMV-GPSQGRSPSYAS(SEQ ID NO:361)
FGF21C-tail/19-11 PGLPPAALPE---PPGILAPQPPDVGSSDPFSMV-GPSQGRSPSYAS(SEQ ID NO:362)
FGF21C-tail/19-12 PGLPPAALPE---PPGILAPQPPDVGSSMDPLSMV-GPSQGRSPSYAS(SEQ ID NO:363)
FGF21C-tail/19-13 PGLPPAALPE---PPGILAPQPPDVDSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:364)
FGF21C-tail/19-14 PGLPPAALPE---PPGILAPQPPDTGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:365)
FGF21C-tail/19-15 PGLPPAALPE---PPGILAPQPPEVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:366)
FGF21C-tail/19-16 PGLPPAALPE---PPGILAPQPLDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:367)
FGF21C-tail/19-17 PGLPPAALPE---PPGILAPSPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:368)
FGF21C-tail/19-18 PGLPPAALPE---PPGILASQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:369)
FGF21C-tail/19-19 PGLPPAALPE---PPGILFPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:370)
FGF21C-tail/19-20 PGLPPAALPE---PPGIMAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:371)
FGF21C-tail/19-21 PGLPPAALPE---PPGDLAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:372)
FGF21C-tail/19-22 PGLPPAALPE---PPSTLAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:373)
FGF21C-tail/19-23 PGLPPAALPE---PPEGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:374)
FGF21C-tail/19-24 PGLPPAALPE---LPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:375)
FGF21C-tail/19-25 PGLPPAALPE---HPPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:376)
FGF21C-tail/19-26 PGLPPAALPE---GPPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:377)
FGF21C-tail/19-27 PGLPPAALPE---RPPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:378)
FGF21C-tail/19-28 PGLPPAALPE---LPPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:379)
FGF21C-tail/19-29 PGLPPAALPED---PPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:380)
FGF21C-tail/19-30 PGLPPAAEPE---PPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:381)
FGF21C-tail/19-31 PGLPPELPE---PPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:382)
FGF21C-tail/19-32 PGLVPAALPE---PPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:383)
FGF21C-tail/19-33 PGMPPAALPE---PPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:384)
FGF21C-tail/19-34 PPLPPAALPE---PPGILAPQPPDVGSSDPLSMV-GPSQGRSPSYAS(SEQ ID NO:385)
FGF19C-tail (169) LPMVPEEPEDLRGHLESDMFSPLETDSMDPFGLVTGLEAVRSPSFEK

FIG. 12

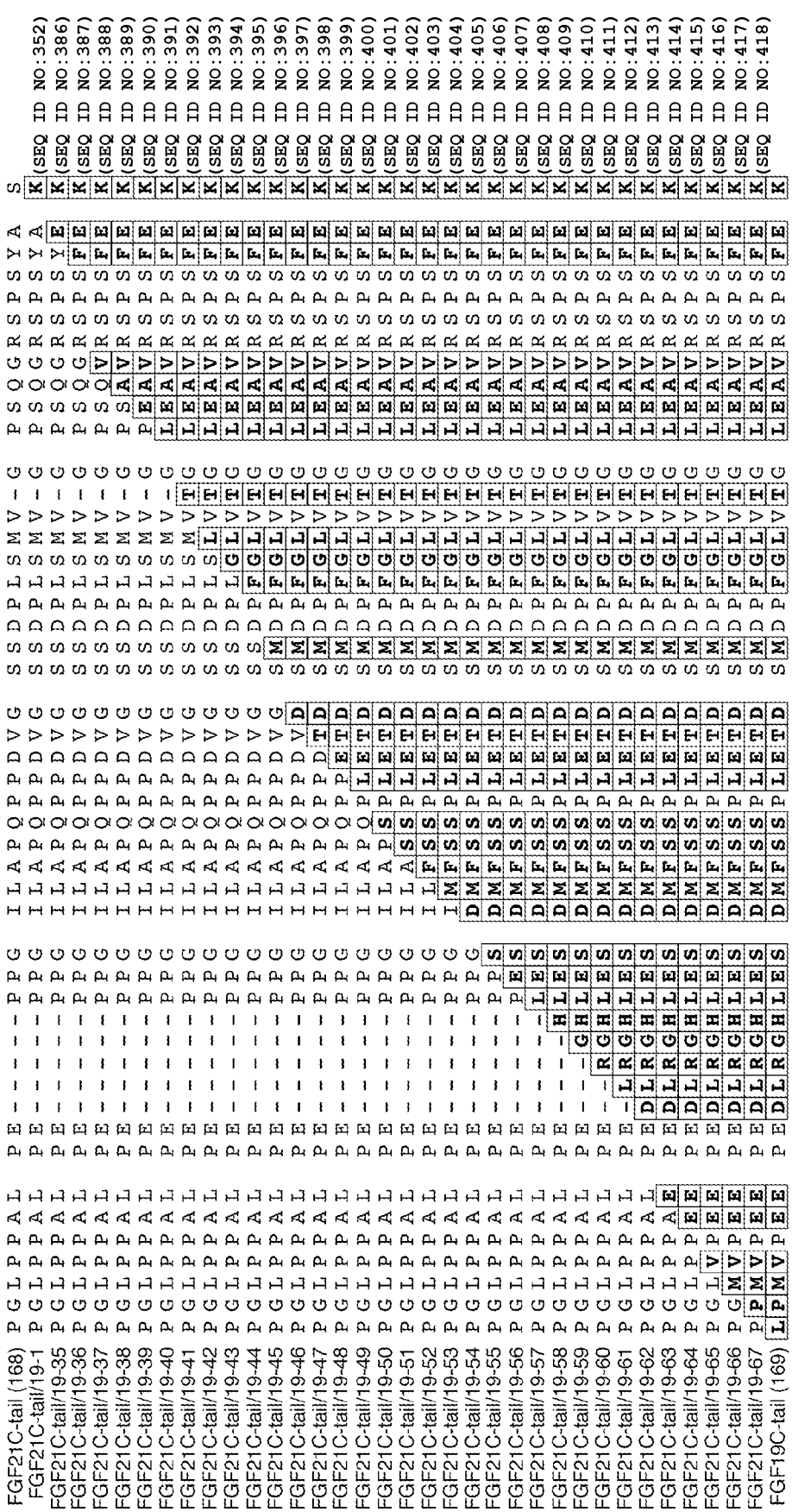

FGF21C-tail (168)
FGF21C-tail/19-1
FGF21C-tail/19-35
FGF21C-tail/19-36
FGF21C-tail/19-37
FGF21C-tail/19-38
FGF21C-tail/19-39
FGF21C-tail/19-40
FGF21C-tail/19-41
FGF21C-tail/19-42
FGF21C-tail/19-43
FGF21C-tail/19-44
FGF21C-tail/19-45
FGF21C-tail/19-46
FGF21C-tail/19-47
FGF21C-tail/19-48
FGF21C-tail/19-49
FGF21C-tail/19-50
FGF21C-tail/19-51
FGF21C-tail/19-52
FGF21C-tail/19-53
FGF21C-tail/19-54
FGF21C-tail/19-55
FGF21C-tail/19-56
FGF21C-tail/19-57
FGF21C-tail/19-58
FGF21C-tail/19-59
FGF21C-tail/19-60
FGF21C-tail/19-61
FGF21C-tail/19-62
FGF21C-tail/19-63
FGF21C-tail/19-64
FGF21C-tail/19-65
FGF21C-tail/19-66
FGF21C-tail/19-67
FGF19C-tail (169)
S
(SEQ ID NO:352)
(SEQ ID NO:386)
(SEQ ID NO:387)
(SEQ ID NO:388)
(SEQ ID NO:389)
(SEQ ID NO:390)
(SEQ ID NO:391)
(SEQ ID NO:392)
(SEQ ID NO:393)
(SEQ ID NO:394)
(SEQ ID NO:395)
(SEQ ID NO:396)
(SEQ ID NO:397)
(SEQ ID NO:398)
(SEQ ID NO:399)
(SEQ ID NO:400)
(SEQ ID NO:401)
(SEQ ID NO:402)
(SEQ ID NO:403)
(SEQ ID NO:404)
(SEQ ID NO:405)
(SEQ ID NO:406)
(SEQ ID NO:407)
(SEQ ID NO:408)
(SEQ ID NO:409)
(SEQ ID NO:410)
(SEQ ID NO:411)
(SEQ ID NO:412)
(SEQ ID NO:413)
(SEQ ID NO:414)
(SEQ ID NO:415)
(SEQ ID NO:416)
(SEQ ID NO:417)
(SEQ ID NO:418)

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/44589

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/18, C12P 21/04, A61K 38/00, A61K 38/24, C07K 14/50, C07K 5/00 (2013.01)
USPC - 514/9.1, 435/69.7, 530/324, 530/399
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 514/9.1, 435/69.7, 530/324, 530/399

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 435/69.1, 435/69.6, 514/4.8, 514/6.9, 514/7.3, 436/501

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); Google/Patents/Scholar, PatBase, Dialog ProQuest: FGF ligands, paracrine, endocrine, chimera, chimeric, fusion, basic FGF, bFGF, FGF-beta, FGF2, FGF19, FGF21, FGF23, heparan sulfate, heparin binding, fibroblast growth factor 1 (acidic) isoform 1, FGF1; AFGF; ECGF; FGFA. GenCore 6.4.1:SEQ ID NO: 1, 121, 233

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Goetz, et al. Klotho coreceptors inhibit signaling by paracrine fibroblast growth factor 8 subfamily ligands. Mol Cell Biol. Epub March 2012, 32(10):1944-1954; Abstract	1-84
A	Beenken, et al. The structural biology of the FGF19 subfamily. 01 March 2012 [The date is according to the properties of the posted document]. [Retrieved from the Internet 30 October 2013: <http://www.med.nyu.edu/mohammadi/adv_exp_med_biol_2012_728.pdf>]. Adv Exp Med Biol. 728:1-24; in entirety	1-84
A	Beenken et al. Plasticity in Interactions of Fibroblast Growth Factor 1 (FGF1) N Terminus with FGF Receptors Underlies Promiscuity of FGF1. J Biol Chem. January 2012, 287(5):3067-78; Abstract	1-84
A	Wu, et al. C-terminal tail of FGF19 determines its specificity toward Klotho co-receptors. J Biol Chem. 28 November 2008, 283(48):33304-33309; Abstract, pg 2, 2nd col, last para to pg 3, 1st col, 1st para of the posted document	1-84
A	Presta, et al. Structure-function relationship of basic fibroblast growth factor: site-directed mutagenesis of a putative heparin-binding and receptor-binding region. Biochem Biophys Res Commun. 1992, 185(3):1098-1107; Abstract only	1-84
A	Zakrzewska, et al. Increased protein stability of FGF1 can compensate for its reduced affinity for heparin. J Biol Chem. 2009, 284(37):25388-403; Abstract; pg 25401, col 1, 1st para; Fig 2	1-84
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	Kharitonenkov, et al. The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. Endocrinology 2007, 148(2):774-81; Abstract	1-84
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A	US 2011/0104152 A1(Sonoda) 05 May 2011 (05.05.2011) Abstract, para [0012]-[0020], [0035]-0045], [0051]-[0065], [0071]-[0073], [0076]-[0077]	1-84
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A	US 2010/0286042 A1 (Imamura, et al.) 11 November 2010 (11.10.2010) Abstract	1-84
A	US 2011/0195077 A1 (Gass, et al.) 11 August 2011 (11.08.2011) Abstract, para [0013]-[0027], [0075]-[0081]	1-84
A	US 2010/0184665 A1 (Suzuki, et al.) 22 July 2010 (22.07.2010) Abstract, para [0005]-[0007], [0022]-[0031], [0038]-[0039], [0179],	1-84
A	US 2010/0062984 A1 (Kumar, et al.) 11 March 2010 (11.03.2010) Abstract, para [0021]	1-84
A	US 2010/0158914 A1 (Desnoyers) 24 June 2010 (24.06.2010) Abstract, para [0036], [0089]	1-84
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