(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 December 2010 (23.12.2010)

PCT

(10) International Publication Number
WO 2010/148142 A1

(51) International Patent Classification:
C07K 14/50 (2006.01)

(21) International Application Number:
PCT/US2010/038894

(22) International Filing Date:
16 June 2010 (16.06.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/187,767 17 June 2009 (17.06.2009) US
61/265,548 1 December 2009 (01.12.2009) US

(71) Applicant *(for all designated States except US)*: **AMGEN INC.** [US/US]; One Amgen Center Drive, Thousand Oaks, CA 91320-1799 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **LI, Yang** [US/US]; 1142 Cuesta Drive, Mountain View, CA 94040 (US). **WU, Xinle** [CN/US]; 1910 Bayview Avenue, Belmont, CA 94002 (US). **WANG, Zhulun** [US/US]; 660 Glenbrook Drive, Palo Alto, CA 94306 (US).

(74) Agent: **LAMERDIN, John, A.**; Amgen Inc., One Amgen Center Drive, M/S 28-2-C, Thousand Oaks, CA 91320-1799 (US).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2010/148142 A1

(54) Title: CHIMERIC FGF19 POLYPEPTIDES AND USES THEREOF

(57) Abstract: The disclosure provides nucleic acid molecules encoding chimeric polypeptides, chimeric polypeptides, pharmaceutical compositions comprising chimeric polypeptides, and methods for treating metabolic disorders such as diabetes and obesity using such nucleic acids, polypeptides, or pharmaceutical compositions.

CHIMERIC POLYPEPTIDES AND USES THEREOF

This application claims the benefit of U.S. Provisional Appln. No. 61/187,767 filed June 17, 2009 and U.S. Provisional Appln. No. 61/265,548 filed December 1, 2009, which are incorporated by reference herein.

Field of the Invention

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

Background of the Invention

FGF19, FGF21, and FGF23 form a unique subfamily of fibroblast growth factors (FGFs). Unlike other FGFs, all three have been shown to function as endocrine hormones in the regulation of various metabolic processes (Fukumoto, (2008). *Endocr. J.* 55:23-31). For example, FGF23 originates in bone and regulates phosphate homeostasis in kidney (Fukumoto & Yamashita, (2007) *Bone* 40:1190-1195), FGF21 is expressed predominantly in liver but can signal in adipose tissue (Ogawa *et al.,* (2007) *Proc. Natl. Acad. Sci. U.S.A.* 104:7432-7437), and FGF19 is secreted from ileum and functions as an enterohepatic signal for the regulation of bile acid metabolism (Inagaki *et al.* (2005) *Cell. Metab.* 2:217-225).

FGF19 and FGF21 appear to share many similarities with reported effects on the regulation of glucose, lipid, and energy metabolism. Both FGF19 and FGF21 transgenic mice are resistant to diet induced obesity, have lower body fat mass, and improved insulin sensitivity, glucose disposal, and plasma lipid parameters (Tomlinson *et al.*, (2002) *Endocrinology* 143:1741-1747; Fu *et al.*, (2004) *Endocrinology* 145:2594-2603; Kharitonenkov *et al.* (2005) *J Clin Invest* 115:1627-1635; Xu *et al.*, (2008) *Diabetes* 58:250-59). Injection of recombinant FGF19 or FGF21 proteins in diabetic mouse models resulted in the reduction of serum glucose and insulin levels, improvements in glucose tolerance and liver steatosis, and reduction in body weight (Kharitonenkov *et al.*,

(2005) *J. Clin. Invest.* 115:1627-1635; Xu *et al.* (2008) *Diabetes* 58:250-59). In addition, FGF21 has also been shown to improve glucose, insulin and lipid profiles with reduced body weight in diabetic rhesus monkeys (Kharitonenkov *et al.,* (2007) *Endocrinology* 148:774-781). Taken together, these observations signify the potential utility of these molecules as novel therapies for the treatment of diabetes and obesity.

Although this subfamily displays unique features as compared to other FGF molecules (Kurosu & Kuro-o, (2008) *Curr. Opin. Nephrol. Hypertens.* 17:368-372 (2008); Wu *et al.*, (2008) *J. Biol. Chem.* 283(48):33304-9), FGF19, hepatocellular carcinoma (HCC) formation was observed in transgenic mice overexpressing FGF19 in skeletal muscle (Nicholes *et al.,* (2002) *Am. J. Pathol.* 160:2295-2307). This has been a consideration in developing FGF19 as a therapy for diabetes, obesity and other metabolic disorders.

A chimeric polypeptide that exhibits potential for therapeutic use, while at the same time does not exhibit undesirable properties, such as mitogenicity, that would compromise the use of the polypeptide as a therapeutic, is therefore desirable.

Summary of the Invention

A chimeric polypeptide comprising a wild type mature FGF19 polypeptide scaffold comprising SEQ ID NO:4, further comprising a modification that decreases FGFR4-mediated signaling activity is provided.

In one embodiment, one or more of the residues WGDPI at positions 16-20 of the FGF19 polypeptide scaffold has been substituted with (a) no amino acid; or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence. The tryptophan residue of the WGDPI sequence can be deleted. Additionally, the residues WGDPI can be substituted with 1-5 contiguous residues present in either a wild type FGF21 or a wild type FGF23 amino acid sequence. The 1-5 contiguous residues can be present in a wild type FGF21 amino acid sequence, for example the 1-5 contiguous residues are GQV.

In a further embodiment one or more of the residues SGPHGLSS at positions 28-35 of the FGF19 polypeptide scaffold has been substituted with either (a) no amino acid; or (b) an amino acid other than the amino acid located at the position in the wild type

amino acid sequence. The residues SGPHGLSS can be substituted with 1-8 contiguous residues present in either a wild type FGF21 or a wild type FGF23 amino acid sequence. The 1-8 contiguous residues can be present in a wild type FGF21 amino acid sequence, for example the 1-8 contiguous residues can be DDAQQTE.

In another embodiment one or more of the residues SSAKQRQLYKNRGFLPL at positions 124-140 of the FGF19 polypeptide scaffold can be been substituted with either (a) no amino acid; or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence. The residues SSAKQRQLYKNRGFLPL can be substituted with 1-17 contiguous residues present in either a wild type FGF21 or a wild type FGF23 amino acid sequence. When the 1-17 contiguous residues are present in a wild type FGF21 amino acid sequence the 1-17 contiguous residues can be PGNKSPHRDPAPRGP.

Also provided is a chimeric polypeptide comprising a wild type FGF19 polypeptide scaffold comprising SEQ ID NO:4, wherein one or more of the residues WGDPI at positions 16-20 of SEQ ID NO:4 has been substituted with either (a) no amino acid; or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence, and one or both of: (i) one or more of the residues SGPHGLSS at positions 28-35 of SEQ ID NO:4 has been substituted with either (1) no amino acid; or (2) an amino acid other than the amino acid located at the position in the wild type amino acid sequence; and (ii) one or more of the residues SSAKQRQLYKNRGFLPL at positions 124-140 of SEQ ID NO:4 has been substituted with either (1) no amino acid; or (2) an amino acid other than the amino acid located at the position in the wild type amino acid.

The residues WGDPI can be substituted with 1-5 contiguous residues present in either FGF21 or FGF23. The 1-5 contiguous residues can be present in wild type FGF21 amino acid sequence. The 1-5 contiguous residues can be GQV. Further, the tryptophan residue of the WGDPI sequence can be deleted.

The residues SGPHGLSS can be substituted with 1-8 contiguous residues present in either a wild type FGF21 or a wild type FGF23 amino acid sequence. The 1-8 contiguous residues can be present in a wild type FGF21 amino acid sequence. The 1-8 contiguous residues are DDAQQTE.

The residues SSAKQRQLYKNRGFLPL can be substituted with 1-17 contiguous residues present in either a wild type FGF21 or wild type FGF23 amino acid sequence. The 1-17 contiguous residues can be present in a wild type FGF21 amino acid sequence. The 1-17 contiguous residues can be PGNKSPHRDPAPRGP.

In one particular embodiment, the residues WGDPI at positions 16-20 of SEQ ID NO:4 are substituted with GQV, and one or both of: (a) the residues SGPHGLSS at positions 28-35 of SEQ ID NO:4 are substituted with DDAQQTE; and (b) the residues SSAKQRQLYKNRGFLPL at positions 124-140 of SEQ ID NO:4 are substituted with PGNKSPHRDPAPRGP.

In other embodiments of a chimeric polypeptide provided herein, the polypeptide scaffold of SEQ ID NO:4 is truncated on the N terminus by 1-5 amino acids, on the C terminus by 1-15 amino acids or on both N terminus by 1-15 amino acids and on the C terminus by 1-15 amino acids. In yet another embodiment of a chimeric polypeptide except for the modification that decreases FGFR4-mediated signaling activity, the chimeric polypeptide comprises a polypeptide scaffold that is 95% or more identical to SEQ ID NO:4.

Nucleic acid molecules encoding the chimeric polypeptides are also disclosed, as well as vectors and host cells comprising the nucleic acid molecules.

Pharmaceutical compositions comprising the chimeric polypeptides and a pharmaceutically acceptable carrier are also disclosed. In another aspect, methods of treating diabetes and obesity comprising administering to a human patient in need thereof such pharmaceutical compositions are also disclosed.

Antibodies that specifically binds to the disclosed chimeric polypeptides are also disclosed. A kit for detecting the presence of the disclosed chimeric polypeptides comprising such antibodies are also disclosed.

Chimeric fusion polypeptides comprising the chimeric polypeptides fused to a heterogenous moiety, such as a Fc region of an IgG molecule or a PEG molecule are also disclosed.

Brief Description of the Drawings

Figure 1 shows the incorporation of BrdU by FGF19 and FGF21; Figure 1A shows liver sections from FGF19 and FGF21 treated animals and Figure 1B is a bar graph depicting BrdU label incorporation in each test group.

Figure 2 is a series of Western blots depicting FGF21 or FGF19-mediated activation of FGFR1c (Figure 2A), FGFR2c (Figure 2B), FGFR3c (Figure 2C), and FGFR4 (Figure 2D).

Figure 3 depicts the structure and activity a C-terminally truncated form of FGF19; Figure 3A is a diagram graphically depicting the structure of the C-terminally truncated form of FGF19; Figure 3B is a series of Western blots depicting FGF19 or truncated FGF19-mediated activation of FGFR4 or FGFR1c; Figure 3C is a bar graph depicting BrdU incorporation by FGF19 or the truncated form of FGF19.

Figure 4 depicts the structure of several FGF19/FGF21 chimeric polypeptides and the effect of each on FGFR4-mediated activity and BrdU incorporation; Figure 4A is a graphical depiction of the FGF19/21-1, FGF19/21-2, FGF19/21-3, FGF19/21-4 and FGF19/21-5 chimeric proteins, with FGF19 shown in white and FGF21 shown as gray; Figure 4B is a series of Western blots showing the effect of the FGF19/21-1, FGF19/21-2, FGF19/21-3, FGF19/21-4 and FGF19/21-5 chimeric polypeptides on FGFR1c (top panel) and FGFR4 (lower panel) mediated activity; Figure 4C is a plot showing the effect of the FGF19/21-1, FGF19/21-2, FGF19/21-3, FGF19/21-4 and FGF19/21-5 chimeric polypeptides on glucose uptake; Figure 4D is a bar graph depicting incorporation of BrdU by the FGF19/21-1, FGF19/21-2, FGF19/21-3, FGF19/21-4 and FGF19/21-5 chimeric polypeptides.

Figure 5 depicts the structure and activity of the FGF21/19$^{38-42}$ chimeric polypeptide in which residues 42-44 of FGF21 were replaced by residues 38-42 of FGF19; Figure 5A is a graphical depiction of the chimeric polypeptide with FGF21 shown in gray and FGF19 in white; Figure 5B is a series of Western blots showing FGFR1c and FGFR4-mediated activity of the chimeric FGF21/19$^{38-42}$ polypeptide; Figure 5C is a bar graph depicting incorporation of BrdU by the chimeric FGF21/19$^{38-42}$ polypeptide.

Figure 6 depicts the structure and activity of two chimeric polypeptides, the FGF21/19$^{38-42}$ chimeric polypeptide in which either residues 42-44 of FGF21 were replaced by residues 38-42 of FGF19, and the FGF19/21$^{42-44}$ chimeric polypeptide in which residues 38-42 of FGF19 were replaced with residues 42-44 of FGF21; Figure 6A is a graphical depiction of the chimeric polypeptides and indicates FGFR4 activity and BrdU incorporation; Figure 6B depicts regions of FGF19 (SEQ ID NO:47) and FGF21 (SEQ ID NO:48), subsequences of which (SEQ ID NO:49 for FGF19) were exchanged in two chimeric polypeptides, FGF21/19$^{38-42}$ and FGF19/21$^{42-44}$, and analogous regions of FGF23 (SEQ ID NO:50) are also shown; Figure 6C is a series of Western blots showing FGFR1c and FGFR4-mediated activity of the FGF21/19$^{38-42}$ and FGF19/21$^{42-44}$chimeric polypeptides; Figure 6D is a series of plots showing the results of a solid-phase binding assay measuring the interaction between FGFR4 and FGF21/19$^{38-42}$ or FGF19/21$^{42-44}$ in the presence and absence of heparin; Figure 6E is bar graph depicting the results of a semiquantitative analysis of BrdU immunostaining of livers from female FVB mice treated for 6 days with PBS, 2mg/kg/day recombinant FGF19, FGF21/19$^{38-42}$ or FGF19/21$^{42-44}$.

Figure 7 depicts the β1−β2 loop region (SEQ ID NO:51) and the loop itself (underlined, SEQ ID NO:52), and β10-β12 segment region (SEQ ID NO:57) and the segment itself (underlined, SEQ ID NO:58) in FGF19 and analogous sequences in FGF21 (β1−β2 loop region, SEQ ID NO:53, loop underlined, SEQ ID NO:54; β10−β12 segment region, SEQ ID NO:57, segment underlined , SEQ ID NO:60) and FGF23 (β1−β2 loop region SEQ ID NO:55, loop underlined SEQ ID NO:56; β10−β12 segment region, SEQ ID NO:61, segment underlined, SEQ ID NO:62).

Figure 8 depicts the structure and activity of the three chimeric polypeptides, FGF19-1, FGF19-2 and FGF19-3, in which residues 50-57 of FGF19 were replaced with residues 52-58 of FGF21 (FGF19-1), residues 146-162 of FGF19 were replaced with residues 147-161 of FGF21 (FGF-2), or residues 50-57 of FGF19 were replaced with residues 52-58 of FGF21 and residues 146-162 of FGF19 were replaced with residues 147-161 of FGF21 (FGF19-3); Figure 8A is a graphical depiction of the three chimeric polypeptides FGF19-1, FGF19-2 and FGF19-3; Figure 8B is a series of plots showing the glucose lowering effect of the FGF19-1, FGF19-2 and FGF19-3 constructs in the

presence and absence of heparin; Figure 8C is a series of plots showing the glucose lowering effect of the FGF19-1, FGF19-2 and FGF19-3 constructs in the presence and absence of βKlotho; Figure 8D is a series of Western blots showing FGFR1c and FGFR4-mediated activity of the FGF19-1, FGF19-2 and FGF19-3 chimeric polypeptides in the presence and absence of βKlotho; Figure 8E is a bar graph showing BrdU incorporation mediated by FGF19 and FGF19-1.

Figure 9 depicts the structure and activity of the three chimeric polypeptides FGF19-4, FGF19-5 and FGF19-6 in which residues 38-42 of FGF19 were replaced with residues 42-44 of FGF21 and residues 50-57 of FGF19 were replaced with residues 52-58 of FGF21 (FGF19-4), residues 38-42 of FGF19 were replaced with residues 42-44 of FGF21 and residues 146-162 of FGF19 were replaced with residues 147-161 of FGF21 (FGF19-5), and residues 38-42 of FGF19 were replaced with residues 42-44 of FGF21 , residues 50-57 of FGF19 were replaced with residues 52-58 of FGF21, and residues 146-162 of FGF19 were replaced with residues 147-161 of FGF21 (FGF19-6); Figure 9A is a graphical depiction of the three chimeric polypeptides FGF19-4, FGF19-5 and FGF19-6; Figure 9B is a series of Western blots showing FGFR1c and FGFR4-mediated activity of the FGF19-4, FGF19-5 and FGF19-6 chimeric polypeptides in the presence and absence of βKlotho; Figure 9C is a bar graph showing BrdU incorporation mediated by FGF19 and FGF19-4, FGF19-5 and FGF19-6.

Figure 10 is a series of plots depicting the results of several assays performed on FGF19, FGF19-1 and FGF19-4; Figure 10A depicts the effect of FGF19 and the FGF19-4 chimeric polypeptide on glucose uptake in a 3T3L1 cell-based assay; Figure 10B shows the effect of FGF19 and FGF19-4 on plasma glucose in a ob/ob mouse model; Figure 10C depicts the effect of FGF19 and the FGF19-1 chimeric polypeptide on glucose uptate in a 3T3L1 cell-based assay; Figure 10D shows the effect of FGF19 and FGF19-1 on plasma glucose in a ob/ob mouse model.

Figure 11 is a plot depicting the pharmacokinetic properties of various FGF19/21 chimeric proteins.

Figure 12 is a table showing the binding response of various FGF19 mutants having one or more mutations or deletions in the WGDPI region to FGFR1c and FGFR4.

Figure 13 is a series of bar graphs showing FGFR1c-induced activity of several FGF19 mutants having one or more mutations or deletions in the WGDPI region; each construct was tested at concentrations of 0, 2.5, 16 and 100 nM.

Figure 14 is a series of bar graphs showing FGFR1c-mediated activity of several FGF19 mutants having one or more mutations or deletions in the WGDPI region; each construct was tested at concentrations of 0, 2.5, 7.4, 33, 67 and 200 nM.

Figure 15 is a series of bar graphs showing FGFR4-mediated activity of several FGF19 mutants having one or more mutations or deletions in the WGDPI region; each construct was tested at concentrations of 0, 2.5, 16 and 100 nM.

Figure 16 is a series of bar graphs showing FGFR4-mediated activity of several FGF19 mutants having one or more mutations or deletions in the WGDPI region; each construct was tested at concentrations of 0, 2.5, 7.4, 22, 67 and 200 nM.

Detailed Description of the Invention

It has been suggested that constitutive hepatocellular proliferation is a prerequisite for transformation (Fausto, (1999) *Seminars in Liver Disease* 19:243-252). Accordingly, it is noted that dramatic increases in the proliferation of pericentral hepatocytes, as measured by enhanced BrdU labeling, was observed as early as 2 to 4 months of age in FGF19 transgenic animals as well as in normal mice subjected to six daily injections of recombinant FGF19 (Nicholes *et al.,* (2002) *Am. J. Pathol.* 160:2295-2307). Cell lineage analysis of FGF19 induced tumors suggest that dysplastic and neoplastic hepatocytes originated from around the central veins, coincident with the increased pericentral proliferation observed by BrdU labeling (Nicholes *et al.,* (2002) *Am. J. Pathol.* 160:2295-2307). Thus the relatively shorter BrdU labeling assay could serve as a marker to study mitogenic potential of these molecules *in vivo*. FGF21 has been shown to lack potential for cell proliferation *in vitro* (Kharitonenkov *et al.,* (2005) *J. Clin. Invest.* 115:1627-1635).

The receptors for the FGF19, FGF21, FGF23 subfamily have been elucidated in recent years. Both FGF19 and FGF21 utilize βKlotho, a single transmembrane protein, as a co-receptor required for signaling mediated through FGFRs 1c, 2c, and 3c (Kurosu & Kuro-o, (2009) *Mol. Cell. Endocrinol.* 299:72-78). Because FGFR1c and 2c are the

predominant receptors expressed in adipose tissue, induction of ERK phosphorylation and increased glucose uptake in adipocytes *in vitro* and *in vivo* upon treatment with either FGF19 or FGF21 are likely mediated through these receptors complexed with βKlotho in adipocytes (Kurosu *et al.,* (2007) *J. Biol. Chem.* 282:26687-26695). Despite these similarities, a major difference between FGF19 and FGF21 exists with respect to FGFR4. Although both FGF19 and FGF21 appear to be able to bind to βKlotho/FGFR4 complexes, only FGF19 signals efficiently through FGFR4 (Kurosu *et al.,* (2007) *J. Biol. Chem.* 282:26687-26695). Consistent with these *in vitro* observations, FGF19, but not FGF21, activates liver ERK phosphorylation, which is likely mediated through FGFR4, the predominant receptor expressed in liver (Kurosu *et al.,* (2007) *J. Biol. Chem.* 282:26687-26695). Additionally, it has been suggested that FGFR4 could contribute to hepatocellular carcinoma progression, and increased production of alpha-fetoprotein, a hepatocellular carcinoma biomarker, has been observed with FGF19 stimulated liver cancer cell lines (Ho *et al.,* (2009) *J. Hepatol.* 50:118-127).

As disclosed herein, a C-terminally truncated FGF19 and a series of FGF19 and FGF21 chimeric proteins was prepared and it was possible to identify three regions in FGF19 that are responsible for FGFR4 activation. A correlation between FGFR4 activation and hepatocellular proliferation, as indicated by BrdU incorporation, is disclosed. These results provide a direct link between liver FGFR4 activity and hepatocyte proliferation *in vivo.* Furthermore, it is disclosed that in contrast to FGF19, FGF21 does not activate FGFR4 and does not induce hepatocyte proliferation *in vivo*, making the various forms of FGF19 disclosed herein a unique potential therapeutic approach for the treatment metabolic diseases, such as obesity, diabetes and dyslipidemia.

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

I. Definitions

As used herein, the terms "a" and "an" mean one or more unless specifically indicated otherwise.

As used herein, the term "chimeric polypeptide" refers to a polypeptide scaffold in which at least one amino acid of one or more regions comprising between 1 to 20 contiguous amino acids has been replaced with either no amino acid or an amino acid that is not found at the replaced amino acid's position in the region in a wild type polypeptide scaffold. In one example of a chimeric polypeptide, residues 38-42 of an FGF19 polypeptide scaffold are replaced with three residues from an FGF21 polypeptide, for example GQV, which is found at positions 42-44 of FGF21 or three residues from a FGF23 polypeptide, for example WGG which is found at positions 36-38 of FGF23. In another example the tryptophan residue at position 38 of a FGF19 polypeptide scaffold is replaced by an amino acid other than a tryptophan.

As used herein, the term "isolated nucleic acid molecule" refers to a nucleic acid molecule of the present disclosure that (1) has been separated from at least about 50, 60, 70, 80, 90, 95 or more percent of proteins, lipids, carbohydrates, or other materials with which it is naturally found when total nucleic acid is isolated from the source cells, (2) is not linked to all or a portion of a polynucleotide to which the "isolated nucleic acid molecule" is linked in nature, (3) is operably linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature as part of a larger polynucleotide sequence. Preferably, the isolated nucleic acid molecule of the present invention is substantially free from any other contaminating nucleic acid molecules or other contaminants that are found in its natural environment that would interfere with its use in polypeptide production or its therapeutic, diagnostic, prophylactic or research use.

The terms "polynucleotide" and "nucleic acid" are generally used interchangeably herein and refer to a polymeric molecule having a backbone that supports bases capable of hydrogen bonding to typical polynucleotides, where the polymer backbone presents the bases in a manner to permit such hydrogen bonding in a sequence specific fashion between the polymeric molecule and a typical polynucleotide (*e.g.*, single-stranded DNA). Common bases include inosine, adenosine, guanosine, cytosine, uracil and thymidine.

As used herein, the term "isolated polypeptide" refers to a polypeptide of the present invention that (1) has been separated from at least about 50, 60, 70, 80, 90, 95 or more percent of polynucleotides, lipids, carbohydrates, or other materials with which it is naturally found when isolated from the source cell, (2) is not linked (by covalent or noncovalent interaction) to all or a portion of a polypeptide to which the "isolated polypeptide" is linked in nature, (3) is operably linked (by covalent or noncovalent interaction) to a polypeptide with which it is not linked in nature, or (4) does not occur in nature. Preferably, the isolated polypeptide is substantially free from any other contaminating polypeptides or other contaminants that are found in its natural environment that would interfere with its therapeutic, diagnostic, prophylactic or research use.

The terms "polypeptide" and "protein" are used interchangeably and refer to a compound made up of a single chain of amino acid residues linked by peptide bonds. A polypeptide or protein can, but need not, comprise non-naturally occurring amino acids and amino acid derivatives. A non-limiting lists of examples of non-naturally occurring amino acids that can be inserted into a protein or polypeptide (including chimeric polypeptides disclosed herein) include β-amino acids, homoamino acids, cyclic amino acids and amino acids with derivatized side chains. Examples include (in the L-form or D-form; abbreviated as in parentheses): para-acetyl-phenylalanine, para-azido-phenylalanine, para-bromo-phenylalanine, para-iodo-phenylalanine and para-ethynyl-phenylalanine, citrulline (Cit), homocitrulline (hCit), Nα-methylcitrulline (NMeCit), Nα-methylhomocitrulline (Nα-MeHoCit), ornithine (Orn), Nα-Methylornithine (Nα-MeOrn or NMeOrn), sarcosine (Sar), homolysine (hLys or hK), homoarginine (hArg or hR), homoglutamine (hQ), Nα-methylarginine (NMeR), Nα-methylleucine (Nα-MeL or NMeL), N-methylhomolysine (NMeHoK), Nα-methylglutamine (NMeQ), norleucine (Nle), norvaline (Nva), 1,2,3,4-tetrahydroisoquinoline (Tic), Octahydroindole-2-carboxylic acid (Oic), 3-(1-naphthyl)alanine (1-Nal), 3-(2-naphthyl)alanine (2-Nal), 1,2,3,4-tetrahydroisoquinoline (Tic), 2-indanylglycine (IgI), para-iodophenylalanine (pI-Phe), para-aminophenylalanine (4AmP or 4-Amino-Phe), 4-guanidino phenylalanine (Guf), glycyllysine (abbreviated "K(Nε-glycyl)" or "K(glycyl)" or "K(gly)"), nitrophenylalanine (nitrophe), aminophenylalanine (aminophe or Amino-Phe),

benzylphenylalanine (benzylphe), γ-carboxyglutamic acid (γ-carboxyglu), hydroxyproline (hydroxypro), p-carboxyl-phenylalanine (Cpa), α-aminoadipic acid (Aad), Nα-methyl valine (NMeVal), N-α-methyl leucine (NMeLeu), Nα-methylnorleucine (NMeNle), cyclopentylglycine (Cpg), cyclohexylglycine (Chg), acetylarginine (acetylarg), α, β-diaminopropionoic acid (Dpr), α, γ-diaminobutyric acid (Dab), diaminopropionic acid (Dap), cyclohexylalanine (Cha), 4-methyl-phenylalanine (MePhe), β, β-diphenyl-alanine (BiPhA), aminobutyric acid (Abu), 4-phenyl-phenylalanine (or biphenylalanine; 4Bip), α-amino-isobutyric acid (Aib), beta-alanine, beta-aminopropionic acid, piperidinic acid, aminocaprioic acid, aminoheptanoic acid, aminopimelic acid, desmosine, diaminopimelic acid, N-ethylglycine, N-ethylaspargine, hydroxylysine, allo-hydroxylysine, isodesmosine, allo-isoleucine, N-methylglycine, N-methylisoleucine, N-methylvaline, 4-hydroxyproline (Hyp), γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, ω-methylarginine, 4-Amino-O-Phthalic Acid (4APA), and other similar amino acids, and derivatized forms of any of those specifically listed.

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

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

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

As used herein, the term "naturally occurring" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by man. Similarly,

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

As used herein, the term "FGF19 polypeptide" refers to a polypeptide expressed in any species, including humans. For purposes of this disclosure, the term "FGF19 polypeptide" can be used interchangeably to refer to any full-length FGF19 polypeptide, *e.g.*, SEQ ID NO:2, which consists of 216 amino acid residues and which is encoded by the nucleotide sequence of SEQ ID NO: 1, and any mature form of the polypeptide, *e.g.*, SEQ ID NO:4, which consists of 194 amino acid residues and which is encoded by the nucleotide sequence of SEQ ID NO:3, and in which the 22 amino acid residues at the amino-terminal end of the full-length FGF19 polypeptide (*i.e.*, those residues which constitute the signal peptide) have been removed. A bacterially expressed form of a mature FGF19 polypeptide can be produced from the nucleotide of SEQ ID NO:5 and have the amino acid sequence of SEQ ID NO:6, and which will comprise an N-terminal methionine residue. A "FGF19 polypeptide" can be encoded by SEQ ID NOs:1, 3 and 5, for example, as well as any polynucleotide sequence that, due to the degeneracy of the genetic code, has a polynucleotide sequence that is altered by one or more bases from the polynucleotide sequences of SEQ ID NOs:1, 3 and 5, as well as allelic variants of SEQ ID NOs:1, 3 and 5. The term "FGF19 polypeptide" also encompasses naturally-occurring FGF19 variants. A "FGF19" polypeptide can but need not incorporate one or more non-naturally occurring amino acids.

As used herein, the term "FGF21 polypeptide" refers to a polypeptide expressed in any species, including humans. For purposes of this disclosure, the term "FGF21 polypeptide" can be used interchangeably to refer to any full-length FGF21 polypeptide, *e.g.*, SEQ ID NO:8, which consists of 209 amino acid residues and which is encoded by

the nucleotide sequence of SEQ ID NO:7; any mature form of the polypeptide, *e.g.*, SEQ ID NO:10, which consists of 181 amino acid residues and which is encoded by the nucleotide sequence of SEQ ID NO:9, and in which the 28 amino acid residues at the amino-terminal end of the full-length FGF21 polypeptide (*i.e.*, those residues which constitute the signal peptide) have been removed. A bacterially expressed form of a mature FGF21 polypeptide can be produced from the nucleotide of SEQ ID NO:11 and have the amino acid sequence of SEQ ID NO:12 and will comprise an N-terminal methionine residue. A "FGF21 polypeptide" can be encoded by SEQ ID NOs:7, 9 and 11, for example, as well as any polynucleotide sequence that, due to the degeneracy of the genetic code, has a polynucleotide sequence that is altered by one or more bases from the polynucleotide sequence of SEQ ID NOs:7, 9 and 11, as well as allelic variants of SEQ ID NOs:7, 9 and 11. The term "FGF21 polypeptide" also encompasses naturally-occurring variants. A "FGF21" polypeptide can but need not incorporate one or more non-naturally occurring amino acids.

As used herein, the term "FGF23 polypeptide" refers to a polypeptide expressed in any species, including humans. For purposes of this disclosure, the term "FGF23 polypeptide" can be used interchangeably to refer to any full-length FGF23 polypeptide, *e.g.*, SEQ ID NO:14, which consists of 251 amino acid residues and which is encoded by the nucleotide sequence of SEQ ID NO:13; any mature form of the polypeptide, *e.g.*, SEQ ID NO:16, which consists of 227 amino acid residues and which is encoded by the nucleotide sequence of SEQ ID NO:15, and in which the 24 amino acid residues at the amino-terminal end of the full-length FGF23 polypeptide (*i.e.*, those residues which constitute the signal peptide) have been removed. A bacterially expressed form of a mature FGF23 polypeptide can be produced from the nucleotide of SEQ ID NO:18 and have the amino acid sequence of SEQ ID NO:17, and will comprise an N-terminal methionine residue. A "FGF23 polypeptide" can be encoded by SEQ ID NOs: 13, 15 and 17, for example, as well as any polynucleotide sequence that, due to the degeneracy of the genetic code, has a polynucleotide sequence that is altered by one or more bases from the polynucleotide sequence of SEQ ID NOs:13, 15 and 17, as well as allelic variants of SEQ ID NOs:13, 15 and 17. The term "FGF23 polypeptide" also encompasses naturally-

occurring variants. A "FGF23" polypeptide can but need not incorporate one or more non-naturally occurring amino acids.

As used herein, the terms "effective amount" and "therapeutically effective amount" each refer to the amount of a chimeric polypeptide disclosed herein used to support an observable level of one or more biological activities of the wild-type polypeptide scaffold, such as the ability to lower blood glucose, insulin, triglyceride, or cholesterol levels; reduce body weight; or improve glucose tolerance, energy expenditure, or insulin sensitivity in a human or non-human subject.

As used herein, the term "pharmaceutically acceptable carrier" or "physiologically acceptable carrier" as used herein refers to one or more formulation materials suitable for accomplishing or enhancing the delivery of a chimeric polypeptide disclosed herein.

As used herein, the terms "biological activity" and "biologically active" when used in connection with a polypeptide scaffold or a chimeric polypeptide of the instant disclosure mean that the polypeptide scaffold or chimeric polypeptide possesses an activity of the polypeptide scaffold, such as the ability to lower blood glucose, insulin, triglyceride, or cholesterol; to reduce body weight; or to improve glucose tolerance, energy expenditure, or enhance insulin sensitivity when assayed in an appropriate assay such as those provided herein, regardless of the type or number of modifications that have been introduced into the chimeric polypeptide. Chimeric polypeptides possessing a somewhat decreased level of activity relative to the polypeptide scaffold can nonetheless be considered to be biologically active chimeric polypeptides. One particular example of a biological activity is the ability to increase glucose uptake in 3T3L1 cells by 1.2 fold or higher over basal levels in an *in vitro* glucose uptake assay as shown in Example 10.

As used herein, the term "polypeptide scaffold" means a polypeptide which has been modified to form a chimeric polypeptide as described herein. Examples of polypeptide scaffolds that can form the basis of a chimeric polypeptide include wild type FGF19, FGF21 and FGF23 polypeptide sequences.

As used herein, the term "conservative amino acid substitution" means a substitution of a native amino acid residue (*i.e.*, a residue found in a given position of a wild-type polypeptide scaffold sequence) with a nonnative residue (*i.e.*, a residue that is

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

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

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

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

(3) acidic: Asp, Glu;

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

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

(6) aromatic: Trp, Tyr, Phe.

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

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

Table 1

Amino Acid Substitutions

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

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

II. Chimeric Polypeptides

In one aspect of the present disclosure, a series of chimeric polypeptides are described. These chimeric polypeptides are based on a wild type FGF19, FGF21 or FGF23 polypeptide scaffold wherein one or more of the residues in a contiguous region of 1-20 amino acids of the polypeptide scaffold, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids, have been replaced with (a) no amino acid (a deletion); or (b) an amino acid other than the amino acid located at the position in the wild type sequence (*i.e.*, a substitution or a mutation).

In addition to having a modulating effect on particular biological activities of a polypeptide scaffold, the chimeric polypeptides described herein can have other retained or enhanced biological activities of the polypeptide scaffold, such as the ability to lower blood glucose *in vivo*. For example, the chimeric polypeptides disclosed herein can exhibit an enhanced or decreased degree of FGFR4 activation and yet still retain any glucose lowering effects inherent to the polypeptide scaffold, possibly making such a molecule more therapeutically attractive than the unmodified polypeptide scaffold. In the case of a FGF19 polypeptide scaffold, for example, a chimeric polypeptide built on the FGF19 scaffold can have decreased ability to activate FGFR4 and/or decreased hepatocyte mitogenicity, while at the same time still showing the glucose lowering effects inherent to FGF19. In another example, a chimeric polypeptide built on a FGF21 scaffold can have an increased ability to activate FGFR4 and/or enhanced hepatocyte mitogenicity, while at the same time showing the glucose lowering effects inherent in FGF21. Thus, in some embodiments the chimeric polypeptides disclosed herein, comprise molecules in which properties deemed desirable in a given circumstance have

been maintained or augmented, while properties deemed undesirable in the same circumstance have been decreased or eliminated.

In any of the disclosed chimeric polypeptides, a substitution can comprise any naturally or non-naturally occurring amino acid. Such a substitution can be a conservative substitution, as described herein, or non-conservative. In some cases, in which it is desired to disrupt a particular effect it may be desirable to make a non-conservative substitution at a given position or insert a non-naturally occurring amino acid. In other cases, a conservative substitution may mitigate or enhance a particular effect to a desired degree. In still other cases, a substitution of no amino acid at one or more positions (a deletion) may mitigate or enhance a particular effect to a desired degree.

Over the span of a contiguous region of a polypeptide scaffold in which two or more amino acid residues are being replaced with amino acids or no amino acid, any combination can be employed without restriction. That is, a two or more amino acids in a contiguous region can be replaced with no amino acid, a naturally occurring amino acid, a non-naturally occurring amino acid, a conservative substitution, a non-conservative substitution or any combination thereof. In fact, in some cases an omission at a given position in a region of a polypeptide scaffold coupled with a conservative and/or non-conservative substitution at one or more other positions in the same region of the scaffold may mitigate or enhance a particular effect to a desired degree, allowing a level of control over the activity of a chimeric polypeptide.

The present disclosure, therefore, encompasses not only chimeric polypeptides that fully impart a desired effect or property, but also that that partially impart a desired effect or property. By way of example, the present disclosure encompasses both chimeric polypeptides that completely eliminate an effect such as the mitogenicity or FGFR4 activation normally associated with a FGF19 polypeptide scaffold, and chimeric polypeptides that partially eliminate the same effects, which can be desirable in some circumstances. Also encompassed are chimeric polypeptides that enhance an effect such as mitogenicity or FGFR4 activation, which can be desirable in some circumstances.

The biological activity of the chimeric polypeptides disclosed herein can be assayed in any assay appropriate to the metric desired. For example, a binding assay

such as an *in vitro* or *in vivo* ERK or FRS2 assay can be employed to examine FGFR activity, target gene expression analysis can be performed *in vitro* or *in vivo*, glucose uptake can be studied in adipocytes, and glucose lowering ability, as shown herein in Examples 2.3 and 10, effects on body weight, plasma lipid profiles, energy expenditures, can be studied in other *in vitro* or *in vivo* functional assays. Various *in vivo* assays can also be employed to study the biological activity of polypeptide scaffolds and chimeric polypeptides, including histopathological analysis to examine BrdU incorporation in the livers of test animals. This assay is demonstrated in Example 2.4 and exemplary results are shown in Figure 1A.

II.A. Chimeric Polypeptides That Do Not Signal Through FGFR4

The present disclosure relates to chimeric polypeptides that do not signal through FGFR4. In one embodiment, an isolated chimeric polypeptide comprises the amino acid sequence of a wild type FGF19 (*e.g.*, SEQ ID NOs:2, 4 or 6) polypeptide scaffold, which has been modified such that it does not signal through FGFR4. As shown in the Examples presented herein, three regions or a subset thereof, are sufficient for FGFR4 signaling in a FGF19 polypeptide scaffold, namely residues 38-42, 50-57 and 146-162 of SEQ ID NO:2 (residues 16-20, 28-35 and 124-140 of SEQ ID NO:4 and residues 17-21, 29-36 and 125-141 of SEQ ID NO:6). Thus, a chimeric polypeptide based on a FGF19 polypeptide scaffold that signals through FGFR1c but not FGFR4 will have at least one of these regions modified. In one example, at least one of these regions of the FGF19 polypeptide scaffold can be modified by replacing at least one amino acid in the region with either no amino acid or an amino acid not found at the position in the region of the scaffold. Thus, in this example at least one residue of residues 38-42 of the FG19 scaffold will be replaced with either no amino acid or an amino acid not found at the position in the scaffold. In one particular embodiment, the tryptophan residue at position 38 is mutated to a residue other than tryptophan or is deleted (see Example 12).

Similarly, if it is desired to impart the ability to signal through FGFR4 but not FGFR1c, residues 38-42, 50-57 and 146-162 of SEQ ID NO:2 (residues 16-20, 28-35 and 124-140 of SEQ ID NO:4 and residues 17-21, 29-36 and 125-141 of SEQ ID NO:6) can

be used to replace the analogous regions of a non-FGF19 polypeptide scaffold, such as a FGF21 or FGF23 polypeptide scaffold.

It is noted that although the disclosed regions of FGF19 are sufficient for FGFR4-mediated signaling, additional regions of FGF19 may also contribute to FGFR4-mediated signaling. The present disclosure, therefore, contemplates that additional residues and/or regions of a scaffold polypeptide may play a role in FGFR4-mediated signaling, in conjunction with the disclosed regions. Accordingly, chimeric polypeptides comprising substitutions and/or deletions at those regions form an embodiment of the present disclosure.

II.B. Chimeric Polypeptides Lacking or Incorporating FGF19 Residues 38-42

In one aspect, the present disclosure relates to chimeric polypeptides that are based on an FGF21 polypeptide scaffold. In one embodiment, such a chimeric polypeptide comprises the amino acid sequence of a wild type FGF21 (*e.g.*, SEQ ID NOs:8, 10 and 12) polypeptide scaffold, wherein one or more of the residues of GQV at positions 42-44 of SEQ ID NO:8 (positions 14-16 of SEQ ID NO:10 and 15-17 of SEQ ID NO:12) has been replaced with (a) no amino acid (a deletion); or (b) an amino acid other than the amino acid located at the position in the wild type sequence. In another example, the residues at positions 42-44 of a wild type FGF21 polypeptide (*i.e.*, GQV) are replaced by residues at position 38-42 of a wild type FGF19 sequence (*i.e.*, WGDPI) (SEQ ID NO:49). In this particular chimeric polypeptide, it is noted that three residues in a region of a FGF21 polypeptide scaffold are replaced by five residues from a FGF19 polypeptide, highlighting the option of replacing a particular residue at a particular position in a polypeptide scaffold with more than one amino acid, *i.e.*, replacing three amino acids in a region with five amino acids.

The present disclosure also relates to chimeric polypeptides that are based on a FGF19 polypeptide scaffold. In one embodiment, such a chimeric polypeptide comprises the amino acid sequence of a wild type FGF19 (SEQ ID NOs:2, 4 or 6) polypeptide scaffold, wherein one or more of the residues of WGDPI (SEQ ID NO:49) at positions 38-42 of SEQ ID NO:2 (positions 16-20 of SEQ ID NO:4 and 17-21 of SEQ ID NO:6) has been replaced with (a) no amino acid (a deletion); or (b) an amino acid other than the

amino acid located at the position in the wild type sequence. In another example, the residues of FGF21 at positions 42-44 of SEQ ID NO:8 (positions 14-16 of SEQ ID NO:10 and 15-17 of SEQ ID NO:12), *i.e.*, the residues GQV, are inserted at position 38-42 of SEQ ID NO:2 (positions 16-20 of SEQ ID NO:4 and 17-21 of SEQ ID NO:6). In this particular chimeric polypeptide, it is noted that five residues of a FGF19 polypeptide scaffold are being replaced by three residues from a FGF21 polypeptide, highlighting the option of replacing a particular residue at a particular position in a polypeptide scaffold with no amino acid (*i.e.*, making a deletion). Such chimeric polypeptides can exhibit the properties of reduced FGF4 activation and/or hepatocyte mitogenicity.

In another embodiment, a chimeric polypeptide comprises the amino acid sequence of a wild type FGF23 (SEQ ID NOs:14, 16 or 18) polypeptide scaffold, wherein one or more of the residues of WGG at positions 36-38 of SEQ ID NO:14 (positions 12-14 in SEQ ID NO:16 and 13-15 in SEQ ID NO:18) has been replaced with at (a) no amino acid (a deletion); or (b) an amino acid other than the amino acid located at the position in the wild type sequence. In another example, the residues of FGF23 at positions 36-38 of SEQ ID NO:14 (positions 12-14 of SEQ ID NO:16 and 13-15 in SEQ ID NO:18), *e.g.*, the residues WGG, are replaced by the FGF19 residues at position 38-42 of SEQ ID NO:2 (positions 16-20 of SEQ ID NO:4 and 17-21 of SEQ ID NO:6), *e.g.*, WGDPI (SEQ ID NO:49). In this particular chimeric polypeptide, it is noted that three residues of a FGF23 polypeptide scaffold are being replaced by five residues from a FGF19 polypeptide, highlighting the option of replacing a particular residue at a particular position in a region of a polypeptide scaffold with more than one amino acid, *i.e.*, replacing three amino acids in a region with five amino acids.

As with all of the chimeric polypeptides of the present invention, the particular amino acids to be replaced in a FGF19, FGF21 or FGF23 polypeptide scaffold can be substituted with either no amino acid or with any amino acid other than the residue that appears at that position in the wild type sequence. For example, FGF19 residues 38-42 of SEQ ID NO:2 (positions 16-20 of SEQ ID NO:4 and 17-21 of SEQ ID NO:6) comprise the five residue sequence WGDPI, and can be substituted with any sequence other than WGDPI and can also comprise less than five residues or more than five residues. A more specific example is the replacement of the five residues of FGF19, namely WGDPI, with

the three residues normally found in FGF21 at positions 42-44 of SEQ ID NO:8 (positions 14-16 of SEQ ID NO:10 and 15-17 of SEQ ID NO:12), namely GQV.

II.C. Chimeric Polypeptides Lacking or Incorporating the FGF19 β1-β2 Loop

The β1-β2 loop of FGF19 is thought to contribute to heparin binding activity, and analogous regions are found in FGF21 and FGF23. A chimeric polypeptide comprising this loop region is expected contribute to the activation of FGFR4, which is not normally activated by FGF21 or FGF23, for example, and can contribute to hepatocyte mitogenicity, again, a property not normally observed in FGF21 or FGF23. Similarly, a chimeric polypeptide lacking this loop region, in conjunction with other modifications, is expected to lack the ability to signal through FGFR4.

In one aspect, the present disclosure relates to chimeric polypeptides that are based on an FGF21 scaffold. In one aspect, a chimeric polypeptide comprises the amino acid sequence of a wild type FGF21 (SEQ ID NOs: 8, 10 or 12) polypeptide scaffold wherein one or more of the residues DDAQQTE (SEQ ID NO:54) at positions 52-58 of SEQ ID NO:8 (positions 24-30 in SEQ ID NO:10 and 25-31 in SEQ ID NO:12) has been replaced with (a) no amino acid (a deletion); or (b) an amino acid other than the amino acid located at the position in the wild type sequence.

The present disclosure relates, in one aspect, to chimeric polypeptides that are based on an FGF23 scaffold. In one aspect, a chimeric polypeptide comprises the amino acid sequence of a wild type FGF23 (SEQ ID NOs:14, 16 or 18) polypeptide scaffold wherein one or more of the residues ATARNS (SEQ ID NO:56) at positions 45-50 of SEQ ID NO:14 (positions 21-26 of SEQ ID NO:16 and 22-27 of SEQ ID NO:18) has been replaced with at least one of (a) no amino acid (a deletion); or (b) an amino acid other than the amino acid located at the position in the wild type sequence.

The present disclosure also relates to chimeric polypeptides that are based on a FGF19 scaffold. In one embodiment such a chimeric polypeptide comprises the amino acid sequence of a wild type FGF19 (SEQ ID NOs:2, 4 or 6) polypeptide scaffold wherein one or more of the residues SGPHGLSS (SEQ ID NO:52) at positions 50-57 of SEQ ID NO:2 (positions 28-35 of SEQ ID NO:4 and positions 29-36 of SEQ ID NO:6) has been replaced with at least one of (a) no amino acid (a deletion); or (b) an amino acid

other than the amino acid located at the position in the wild type sequence. In one example, the FGF21 residues at positions 52-58 of SEQ ID NO:8 (positions 24-30 of SEQ ID NO:10 and 25-31 of SEQ ID NO:12), *e.g.*, the residues DDAQQTE, (SEQ ID NO:54) are inserted at position 50-57 of a wild type FGF19 sequence. In this particular chimeric polypeptide, it is noted that eight residues of a FGF19 polypeptide are being replaced by seven residues from a FGF21 polypeptide, highlighting the option of replacing a particular residue at a particular position in a polypeptide scaffold with no amino acid. Such chimeric polypeptides can exhibit the properties of reduced FGF4 activation and/or hepatocyte mitogenicity in and of itself, or such a substitution can form one element in a combination chimeric polypeptide, as described herein.

As with all of the chimeric polypeptides of the present invention, the particular amino acids to be replaced can be substituted with either no amino acid or with any amino acid other than the residue that appears at that position in the wild type sequence. For example, FGF19 residues 50-57 of SEQ ID NO:2 (positions 28-35 of SEQ ID NO:4 and positions 29-36 of SEQ ID NO:6) is the eight residue sequence SGPHGLSS, and could be substituted with any sequence other than SGPHGLSS and could also comprise less than eight residues. A more specific example is the replacement of these residues with the seven FGF21 residues found at positions 52-58 of SEQ ID NO:8 (positions 24-30 of SEQ ID NO:10 and 25-31 of SEQ ID NO:12), namely DDAQQTE (SEQ ID NO:54).

II.D. Chimeric Polypeptides Lacking or Incorporating the FGF19 β10-β12 Segment

The β10-β12 segment of FGF19 is thought to contribute to heparin binding activity and is a region found analogously in FGF19, FGF21 and FGF23. Such a chimeric polypeptide can contribute to the activation of FGFR4, which is not normally activated by FGF21, as well as contribute to hepatocyte mitogenicity, again, a property not normally observed in FGF21. In various embodiments, the present disclosure relates to chimeric polypeptides that are based on FGF19, FGF21 and FGF23 scaffolds.

In one aspect, a chimeric polypeptide comprising the amino acid sequence of a wild type FGF21 polypeptide scaffold (*e.g.*, SEQ ID NOs:10, 12 or 14) wherein one or

more of the residues PGNKSPHRDPAPRGP (SEQ ID NO:60) at positions 147-161 of SEQ ID NO:8 (positions 119-133 in SEQ ID NO:10 and 120-134 in SEQ ID NO:12) has been replaced with at least one of (a) no amino acid (a deletion); or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence.

In one aspect, a chimeric polypeptide comprising the amino acid sequence of a wild type FGF23 polypeptide scaffold (*e.g.*, SEQ ID NOs:14, 16 or 18) wherein one or more of the residues GRAKRAFLPGMNPPPY (SEQ ID NO:62) at positions 139-154 of SEQ ID NO:14 (positions 115-130 in SEQ ID NO:16 and 116-131 in SEQ ID NO:18) has been replaced with (a) no amino acid (a deletion); or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence.

The present disclosure also relates to chimeric polypeptides that are based on a FGF19 scaffold. In one embodiment of such a chimeric polypeptide comprises the amino acid sequence of a wild type FGF19 polypeptide scaffold *(e.g.*, SEQ ID NOs:2, 4 or 6) wherein one or more of the residues SSAKQRQLYKNRGFLPL (SEQ ID NO:58) at positions 146-163 of SEQ ID NO:2 (positions 124-140 in SEQ ID NO:4 and 125-141 in SEQ ID NO:6) has been replaced with (a) no amino acid (a deletion); or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence. In one example, the FGF21 residues at positions 147-161 of SEQ ID NO:8 (corresponding to positions 119-133 of SEQ ID NO:10 and 120-134 of SEQ ID NO:12), *e.g.*, PGNKSPHRDPAPRGP (SEQ ID NO:60), are inserted at position 146-162 of FGF19 sequence SEQ ID NO:2 (corresponding to positions 124-140 of SEQ ID NO:4 and 125-141 of SEQ ID NO:6). In this particular chimeric polypeptide, it is noted that 17 residues of a FGF19 polypeptide are being replaced by 15 residues from a FGF21 polypeptide, highlighting the option of replacing a particular residue at a particular position in a polypeptide scaffold with no amino acid. Such chimeric polypeptides are expected to exhibit the properties of reduced FGFR4 activation and/or hepatocyte mitogenicity in and of itself, or such a substitution can form one element in a combination chimeric polypeptide, as described herein.

As with all of the chimeric polypeptides of the present invention, the particular amino acids to be replaced can be substituted with either no amino acid or with any amino acid other than the residue that appears at that position in the wild type sequence.

For example, FGF19 residues 146-162 of SEQ ID NO:2 (corresponding to positions 124-140 of SEQ ID NO:4 and 125-141 of SEQ ID NO:6) is the 17 residue sequence SSAKQRQLYKNRGFLPL (SEQ ID NO:58), and could be substituted with any sequence other than SSAKQRQLYKNRGFLPL and could also comprise less than 17 residues. A more specific example is the replacement of these residues with the 15 FGF21 residues found at positions 147-161 of SEQ ID NO:8 (corresponding to positions 119-133 of SEQ ID NO:10 and 120-134 of SEQ ID NO:12), namely PGNKSPHRDPAPRGP (SEQ ID NO:60).

II.E. Chimeric Combination Polypeptides

The present disclosure also relates to chimeric combination polypeptides. A chimeric combination polypeptide is a chimeric polypeptide in which two or more regions of a polypeptide scaffold have been replaced at each position of each region with either no amino acid or an amino acid not normally found at the position in the wild type polypeptide scaffold. Chimeric combination polypeptides can therefore be engineered to demonstrate enhanced or reduced properties normally associated with the polypeptide scaffold, or properties not normally associated with the polypeptide scaffold. In various embodiments, a chimeric combination polypeptide can have the property of exhibiting decreased FGFR4-mediated signaling.

By way of example, a chimeric combination polypeptide can be built on a FGF19 scaffold. The amino acids in two or more particular regions of a FGF19 scaffold, such as the region comprising positions 38-42 and/or the region comprising positions 50-57 and/or the region comprising positions 146-162 of SEQ ID NO:2 (corresponding to positions 16-20, 28-35 and 124-140 of SEQ ID NO:4 and 17-21, 29-36 and 125-141 of SEQ ID NO:6), can each be replaced by either residues not found at each of positions 38-42 and/or 50-57 and/or 146-162 in SEQ ID NO:2, (positions 16-20, 28-35 and 124-140 of SEQ ID NO:4, or positions 17-21, 29-36 and 125-141 of SEQ ID NO:6), or by no amino acid. In one particular embodiment, a chimeric combination polypeptide can have amino acids from regions of FGF21 substituted for the wild type regions of the FGF19 polypeptide scaffold. One possible sequence of this chimeric polypeptide comprises an FGF19 scaffold in which (a) one or more of the residues WGDPI at positions 38-42 of

SEQ ID NO:2 has been substituted with (i) no amino acid (a deletion); or (ii) an amino acid other than the amino acid located at the position in the wild type amino acid sequence; (b) one or more of the residues SGPHGLSS at positions 50-57 of SEQ ID NO:2 (corresponding to positions 28-35 of SEQ ID NO:4 and positions 29-36 of SEQ ID NO:6) has been substituted with (i) no amino acid (a deletion); or (ii) an amino acid other than the amino acid located at the position in the wild type amino acid sequence; and (c) one or more of the residues SSAKQRQLYKNRGFLPL at positions 146-163 of SEQ ID NO:2 (corresponding to positions 124-140 of SEQ ID NO:4 and 125-141 of SEQ ID NO:6) has been substituted with (i) no amino acid; or (ii) an amino acid other than the amino acid located at the position in the wild type amino acid.

As disclosed herein, by replacing at least one amino acid of the 38-42 region of a FGF19 polypeptide scaffold of SEQ ID NO:2 (corresponding to positions 14-20 of SEQ ID NO:4 and 15-21 of SEQ ID NO:6) with either no amino acid or a non wild type residue at least one of these positions, the ability of FGF19 to activate FGFR4 is diminished and hepatocyte mitogenicity is also diminished. By replacing at least one of the 50-57 region of a FGF19 polypeptide scaffold of SEQ ID NO:2 (corresponding to positions 28-35 of SEQ ID NO:4 and 29-36 of SEQ ID NO:6), which comprises the heparin binding β1-β2 loop and/or at least one amino acid of the 146-162 region of a FGF19 polypeptide scaffold of SEQ ID NO:2 (corresponding to positions 124-140 of SEQ ID NO:4 and 125-141 of SEQ ID NO:6), which comprises the β10-β12 segment, with either no amino acid or a non-wild type residue at least one of these positions, FGFR4 activation and hepatocyte mitogenicity of FGF19 can be still further diminished or eliminated. It is significant to note, however, that a chimeric combination polypeptide comprising substitutions at one or more regions can still maintain other biological activities, such as the ability to lower blood glucose levels or decrease body weight. Thus, a chimeric combination polypeptide can be engineered to achieve various goals and to exhibit a desired activity profile by substituting the amino acids of two or more regions of a polypeptide scaffold with amino acids not found at each of the positions of the regions in the wild type polypeptide scaffold or with no amino acid (a deletion).

In another example, a chimeric combination polypeptide can be built on a FGF21 scaffold. The amino acids in two or more particular regions of a FGF21 scaffold (*e.g.*,

SEQ ID NOs:8, 10 or 12), such as the region comprising positions 42-44 and/or the region comprising positions 52-58 and/or the region comprising positions 147-161 of SEQ ID NO:8 (corresponding to positions 14-16, 24-30 and 119-133 of SEQ ID NO:10 and 15-17, 25-31 and 120-134 of SEQ ID NO:12), can each be replaced with residues not found at each of positions 42-44 and/or 52-58 and/or 147-161 of SEQ ID NO:8 (corresponding to positions 14-16, 24-30 and 119-133 of SEQ ID NO:10 and 15-17, 25-31 and 120-134 of SEQ ID NO:12), or with no amino acid. In one particular embodiment of such a chimeric combination polypeptide could have amino acids from regions of FGF19 substituted for the wild type regions of the FGF21 polypeptide scaffold. One possible sequence of such a chimeric polypeptide comprises a polypeptide scaffold in which (a) one or more of the residues GQV at positions 42-44 of SEQ ID NO:8 (corresponding to positions 14-16 of SEQ ID NO:10 and 15-17 of SEQ ID NO:12) has been substituted with (i) no amino acid (a deletion); or (ii) an amino acid other than the amino acid located at the position in the wild type amino acid sequence; (b) one or more of the residues DDAQQTE at positions 52-58 of SEQ ID NO:8 (corresponding to positions 24-30 in SEQ ID NO:10 and 25-31 of SEQ ID NO:12) has been substituted with (ii) no amino acid (a deletion); or (ii) an amino acid other than the amino acid located at the position in the wild type amino acid sequence; and (c) one or more of the residues PGNKSPHRDPAPRGP at positions 147-161 of SEQ ID NO:8 (corresponding to positions 119-133 in SEQ ID NO:10 and 120-134 in SEQ ID NO:12) has been substituted with (i) no amino acid (a deletion); or (ii) an amino acid other than the amino acid located at the position in the wild type amino acid.

As described herein, by replacing at least one amino acid of the 42-44 region of a FGF21 polypeptide scaffold of SEQ ID NO:8 (and corresponding residues in SEQ ID NOs:10 and 12) with either no amino acid or a non wild type residue at one or more of these positions, the ability of FGF21 to activate FGFR4 is imparted and hepatocyte mitogenicity is also imparted. By replacing at least one of the 52-58 region of a FGF21 polypeptide scaffold of SEQ ID NO:8 (and corresponding residues of SEQ ID NOs:10 and 12), which comprises the heparin binding β1-β2 loop, and/or one or more amino acids of the 147-161 region of a FGF19 polypeptide scaffold of SEQ ID NO:8 (and corresponding residues of SEQ ID NOs:10 and 12), which comprises the β10-β12

segment, with no amino acid or a non-wild type residue at one or more of these positions, FGFR4 activation and hepatocyte mitogenicity of FGF19 can be further augmented.

In yet another example, a chimeric combination polypeptide can be built on a FGF23 scaffold. The amino acids in two or more particular regions of a FGF23 scaffold (*e.g.*, SEQ ID NOs:14, 16 or 18), such as the region comprising positions 36-38 and/or the region comprising positions 45-50 and/or the region comprising positions 139-154 of SEQ ID NO:14 (corresponding to positions 12-14, 21-26 and 115-130 of SEQ ID NO:16 and 13-15, 22-27 and 116-131 of SEQ ID NO:18), can each be replaced by residues not found at each of positions 36-38 and/or 45-50 and/or 139-154 in SEQ ID NO:14 (corresponding to positions 12-14, 21-26 and 115-130 of SEQ ID NO:16 and 13-15, 22-27 and 116-131 of SEQ ID NO:18), or by no amino acid. In one particular embodiment of such a chimeric combination polypeptide can have amino acids from regions of FGF19 substituted for the wild type regions of the FGF23 polypeptide scaffold. One possible sequence of such a chimeric polypeptide comprises a polypeptide scaffold in which (a) one or more of the residues WGG at positions 36-38 of SEQ ID NO:14 (corresponding to positions 12-14 in SEQ ID NO:16 and 13-15 in SEQ ID NO:18) has been substituted with (i) no amino acid; or (ii) an amino acid other than the amino acid located at the position in the wild type amino acid sequence; (b) one or more of the residues ATARNS at positions 45-50 of SEQ ID NO:14 (corresponding to positions 21-26 in SEQ ID NO:16 and 22-27 in SEQ ID NO:18) has been substituted with at least one of (i) no amino acid; or (ii) an amino acid other than the amino acid located at the position in the wild type amino acid sequence; and (c) one or more of the residues GRAKRAFLPGMNPPPY at positions 139-154 of SEQ ID NO:14 (corresponding to positions 115-130 of SEQ ID NO:16 and 116-131 of SEQ ID NO:18) has been substituted with at least one of (i) no amino acid; or (ii) an amino acid other than the amino acid located at the position in the wild type amino acid.

As described herein, by replacing at least one amino acid of the 36-38 region of a FGF23 polypeptide scaffold of SEQ ID NO:14 (corresponding to positions 12-14 in SEQ ID NO:16 and 13-15 in SEQ ID NO:18) with either no amino acid or a non wild type residue at least one of these positions, the ability of FGF23 to activate FGFR4 is imparted and hepatocyte mitogenicity is also imparted. By replacing one or more of the 45-50

region of a FGF23 polypeptide scaffold of SEQ ID NO:14 (corresponding to positions 21-26 in SEQ ID NO:16 and 22-27 in SEQ ID NO:18), which comprises the heparin binding β1-β2 loop and/or at least one amino acid of the 139-154 region of a FGF23 polypeptide sequence of SEQ ID NO:14 (corresponding to positions 115-130 of SEQ ID NO:16 and 116-131 of SEQ ID NO:18), which comprises the β10-β12 segment, with either no amino acid or a non-wild type residue at one or more of these positions, FGFR4 activation and hepatocyte mitogenicity of FGF23 can be further augmented.

III. Truncated Chimeric Polypeptides

N and C-terminally truncated forms of the chimeric polypeptides described herein form another aspect of the present disclosure. As used herein, the term "truncated chimeric polypeptide" refers to a chimeric polypeptide in which one or more amino acid residues have been removed from the amino-terminal (or N-terminal) end of the chimeric polypeptide, amino acid residues have been removed from the carboxyl-terminal (or C-terminal) end of the chimeric polypeptide, or one or more amino acid residues have been removed from both the amino-terminal and carboxyl-terminal ends of the chimeric polypeptide. A truncated chimeric polypeptide can be truncated, for example, by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20 or more residues from the C-terminal end, N-terminal end or both C- and N-terminal ends of the polypeptide.

The activity of N-terminally truncated chimeric polypeptides and C-terminally truncated chimeric polypeptides can be assayed as described herein, for example by employing an *in vitro* ERK assay as described in Examples 2.1 and 2.2. Specific details of the *in vitro* assays that can be used to examine the activity of truncated chimeric polypeptides can be found in Example 2 and herein.

The activity of the truncated chimeric polypeptides disclosed herein can also be assessed in an *in vivo* assay as can be done for any of the chimeric polypeptides disclosed herein, for example in a BrdU incorporation assay as shown in Example 2.4, or by examining the effects of the truncated chimeric polypeptide on one or more metabolic parameters in a diseased model (*e.g.*, ob/ob or DIO mice), or by examining the effects of a truncated chimeric polypeptide on tissues functions, such as ERK phosphorylation levels. Generally, to assess the *in vivo* activity of a truncated chimeric polypeptide, the

truncated chimeric polypeptide can be administered to a test animal intraperitoneally. After a desired incubation period (*e.g.*, 5, 10, 15, 20, 30, 40, 50 or 60 or more minutes), a blood sample can be drawn, and blood glucose levels can be measured and/or tissues can be harvested for subsequent analysis. Specific details of the *in vivo* assays that can be used to examine the activity of truncated chimeric polypeptides can be found in Example 10. In another assay, a test animal can be administered a BrdU label, subsequently sacrificed and the liver examined for BrdU incorporation and/or for observable morphological changes.

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

The truncated chimeric polypeptides disclosed herein, and indeed all of the chimeric polypeptides disclosed herein, can be prepared as described in Example 1. Those of ordinary skill in the art, familiar with standard molecular biology techniques, can employ that knowledge, coupled with the instant disclosure, to make and use the truncated chimeric polypeptides described herein. Standard techniques can be used for recombinant DNA, oligonucleotide synthesis, tissue culture, and transformation (*e.g.*, electroporation, lipofection). See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, *supra*, which is incorporated herein by reference for any purpose. Enzymatic reactions and purification techniques can be performed according to manufacturer's specifications, as commonly accomplished in the art, or as described herein. Unless specific definitions are provided, the nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

The truncated chimeric polypeptides of the present invention can also be fused to another entity, which can impart additional properties to the truncated chimeric polypeptide. In one embodiment of the present invention, a truncated chimeric polypeptide can be fused to an Fc sequence as described herein. Such a fusion can be

generated using known molecular biological methods and/or the guidance provided herein. The benefits of such fusion polypeptides, as well as methods for making such fusion polypeptides, are discussed in more detail herein.

IV. Chimeric Polypeptide Variants

In another aspect, chimeric polypeptide variants are provided. A chimeric polypeptide variant comprises an amino acid sequence that exhibits enhanced or decreased FGFR4-mediated signaling and is at least about 85 percent identical to the amino acid sequence of FGF19 (e.g., SEQ ID NO: 4), but wherein the specific residues comprising the modification(s) that enhance or decrease FGF19's FGFR4-mediated signaling activity have not been further modified. In other words, with the exception of residues in the chimeric polypeptide that have been modified in order to confer enhanced or decreased FGFR4-mediated signaling, about 15 percent of all other amino acid residues in the chimeric polypeptide sequence can be modified. In the particular example of a chimeric polypeptide exhibiting decreased FGFR4-mediated activity in which one or more of the residues WGDPI at positions 16-20 of the FGF19 polypeptide scaffold has been substituted with (a) no amino acid; or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence (SEQ ID NO:4), up to 15 percent of all amino acid residues other than the residues changed at position 16-20 of the FGF19 polypeptide scaffold could be modified.

In still other embodiments, a chimeric polypeptide comprises an amino acid sequence that exhibits enhanced or decreased FGFR4-mediated signaling and that is at least about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the amino acid sequence of the polypeptide scaffold (e.g., SEQ ID NO: 4), but wherein the specific residues conferring the chimeric polypeptide's enhanced or decreased FGFR4-mediated signaling properties have not been further modified.

Also provided are nucleic acids encoding such chimeric polypeptide variants. Thus, a nucleic acid molecule encoding an amino acid sequence that exhibits enhanced or decreased FGFR4-mediated signaling and is at least about 85 percent identical to the amino acid sequence of FGF19 (e.g., SEQ ID NO: 4), but wherein the specific residues comprising the modification(s) that enhance or decrease FGF19's FGFR4-mediated

signaling activity have not been further modified, is provided. In other words, with the exception of nucleotides that encode residues in the chimeric polypeptide that have been modified in order to confer decreased FGFR4-mediated signaling or other properties, nucleotides encoding about 15 percent of all other amino acids in the chimeric polypeptide can be modified. Again using the case of a chimeric polypeptide showing decreased FGFR4-mediated signaling in which one or more of the residues WGDPI at positions 16-20 of the FGF19 polypeptide scaffold has been substituted with (a) no amino acid; or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence as an example, nucleotides encoding up to 15 percent of all amino acids other than the nucleotides encoding residues at positions 16-20 of the FGF19 polypeptide scaffold could be modified.

Also provided is a nucleic acid molecule encoding a chimeric polypeptide showing decreased FGFR4-mediated signaling and comprising an amino acid sequence that is at least about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the amino acid sequence of SEQ ID NO: 4, but wherein the specific residues comprising the modification(s) that decrease FGF19's FGFR4-mediated signaling activity have not been further modified.

V. Chimeric Fusion Polypeptides

Chimeric fusion polypeptides form another aspect of the present disclosure. As used herein, the term "chimeric fusion polypeptide" or "chimeric fusion protein" refers to a fusion of an amino acid sequence comprising one or more amino acid residues (including longer sequences such as a heterologous protein or peptide) at the N-terminus or C-terminus of any of the chimeric polypeptides disclosed herein.

Heterologous peptides and polypeptides include, but are not limited to, an epitope to allow for the detection and/or isolation of a chimeric polypeptide; a transmembrane receptor protein or a portion thereof, such as an extracellular domain or a transmembrane and intracellular domain; a ligand or a portion thereof which binds to a transmembrane receptor protein; an enzyme or portion thereof which is catalytically active; a polypeptide or peptide which promotes oligomerization, such as a leucine zipper domain; a polypeptide or peptide which increases stability, such as an immunoglobulin constant

region (an "Fc" domain); a functional or non-functional antibody, or a heavy or light chain thereof; and a polypeptide which has an activity, such as a therapeutic activity, different from the chimeric polypeptides of the present invention. Also encompassed by the present invention are chimeric polypeptides fused to human serum albumin (HSA).

Chimeric fusion polypeptides can be made by fusing heterologous sequences at either the N-terminus or at the C-terminus of a chimeric polypeptide. As described herein, a heterologous sequence can be an amino acid sequence or a non-amino acid-containing polymer. Heterologous sequences can be fused either directly to the chimeric polypeptide or via a linker or adapter molecule. A linker or adapter molecule can be one or more amino acid residues (or -mers), *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, or 9 residues (or -mers), preferably from 10 to 50 amino acid residues (or -mers), *e.g.*, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50 residues (or -mers), and more preferably from 15 to 35 amino acid residues (or -mers). Examples of linkers include the peptides of SEQ ID NOs:63-70. A linker or adapter molecule can also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of the fused moieties.

V.A. Fc Fusions

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

The resulting chimeric fusion polypeptide can be purified, for example, by the use of a Protein A affinity column. Peptides and proteins fused to an Fc region have been found to exhibit a substantially greater half-life *in vivo* than the unfused counterpart. Also, a fusion to an Fc region allows for dimerization/multimerization of the fusion

polypeptide. The Fc region can be a naturally occurring Fc region, or can be altered to improve certain qualities, such as therapeutic qualities, circulation time, or reduced aggregation.

Useful modifications of protein therapeutic agents by fusion with the "Fc" domain of an antibody are discussed in detail in International Publication No. WO 00/024782, which is hereby incorporated by reference in its entirety. This document discusses linkage to a "vehicle" such as polyethylene glycol (PEG), dextran, or an Fc region.

V.B. Fusion Protein Linkers

When forming a chimeric fusion polypeptide of the present disclosure, a linker can, but need not, be employed. When present, the linker's chemical structure may not always be critical, since it serves primarily as a spacer. The linker can be made up of amino acids linked together by peptide bonds. In some embodiments of the present invention, the linker is made up of from 1 to 20 amino acids linked by peptide bonds, wherein the amino acids are selected from the 20 naturally occurring amino acids. In various embodiments, the 1 to 20 amino acids are selected from the amino acids glycine, serine, alanine, proline, asparagine, glutamine, and lysine. In some embodiments, a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. In some embodiments, linkers are polyglycines (such as $(Gly)_4$ (SEQ ID NO:63) and $(Gly)_5$ (SEQ ID NO:64)), polyalanines, combinations of glycine and alanine (such as poly(Gly-Ala)), or combinations of glycine and serine (such as poly(Gly-Ser)). Other suitable linkers include: $(Gly)_5$-Ser-$(Gly)_3$-Ser-$(Gly)_4$-Ser (SEQ ID NO:65), $(Gly)_4$-Ser-$(Gly)_4$-Ser-$(Gly)_4$-Ser (SEQ ID NO:66), $(Gly)_3$-Lys-$(Gly)_4$ (SEQ ID NO:67), $(Gly)_3$-Asn-Gly-Ser-$(Gly)_2$ (SEQ ID NO:68), $(Gly)_3$-Cys-$(Gly)_4$ (SEQ ID NO:69), and Gly-Pro-Asn-Gly-Gly (SEQ ID NO:70). Linkers of any length or composition can be employed in the formation of a chimeric fusion polypeptide.

The linkers described herein are exemplary, and linkers that are much longer and which include other residues are contemplated by the present invention. Non-peptide linkers are also contemplated by the present invention. For example, alkyl linkers such as $–NH–(CH_2)_S–C(O)–$, wherein s = 2 to 20, could be used. These alkyl linkers can further be substituted by any non-sterically hindering group, including, but not limited to, a

lower alkyl (*e.g.*, C1–C6), lower acyl, halogen (*e.g.*, Cl, Br), CN, NH_2, or phenyl. An exemplary non-peptide linker is a polyethylene glycol linker, wherein the linker has a molecular weight of 100 to 5000 kD, for example, 100 to 500 kD.

VI. Chemically Modified Chimeric Polypeptides

Chemically modified forms of the chimeric polypeptides described herein, including their truncated forms, can be prepared by one skilled in the art, using the present disclosure coupled with techniques known in the art. Such chemically modified chimeric polypeptides are altered such that the chemically modified chimeric polypeptide is different from the unmodified chimeric polypeptide, either in the type or location of the molecules naturally attached to the chimeric polypeptide. Chemically modified chimeric polypeptides can include molecules formed by the deletion of one or more naturally-attached chemical groups.

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

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

Suitable water-soluble polymers or mixtures thereof include, but are not limited to, N-linked or O-linked carbohydrates, sugars, phosphates, polyethylene glycol (PEG) (including the forms of PEG that have been used to derivatize proteins, including mono-

(C_1-C_{10}), alkoxy-, or aryloxy-polyethylene glycol), monomethoxy-polyethylene glycol, dextran (such as low molecular weight dextran of, for example, about 6 kD), cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), and polyvinyl alcohol. Also encompassed by the present invention are bifunctional crosslinking molecules that can be used to prepare covalently attached chimeric polypeptides multimers. Also encompassed by the present invention are chimeric polypeptides covalently attached to polysialic acid.

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

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

In embodiments of the present invention wherein the polymer is PEG, the PEG group can be of any convenient molecular weight, and can be linear or branched. The average molecular weight of the PEG group will preferably range from about 2 kD to about 100 kDa, and more preferably from about 5 kDa to about 50 kDa, *e.g.*, 10, 20, 30, 40, or 50 kDa. The PEG groups will generally be attached to the chimeric polypeptide

via acylation or reductive alkylation through a reactive group on the PEG moiety (*e.g.*, an aldehyde, amino, thiol, or ester group) to a reactive group on the chimeric polypeptide (*e.g.*, an aldehyde, amino, or ester group).

The PEGylation of a polypeptide, including the chimeric polypeptides of the present invention, can be specifically carried out using any of the PEGylation reactions known in the art. Such reactions are described, for example, in the following references: Francis *et al.*, (1992), *Focus on Growth Factors* 3: 4-10; European Patent Nos. 0 154 316 and 0 401 384; and U.S. Patent No. 4,179,337. For example, PEGylation can be carried out via an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule (or an analogous reactive water-soluble polymer) as described herein. For the acylation reactions, a selected polymer should have a single reactive ester group. For reductive alkylation, a selected polymer should have a single reactive aldehyde group. A reactive aldehyde is, for example, polyethylene glycol propionaldehyde, which is water stable, or mono C_1-C_{10} alkoxy or aryloxy derivatives thereof (*see, e.g.,* U.S. Patent No. 5,252,714).

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

Polysaccharide polymers are another type of water-soluble polymer that can be used for protein modification. Therefore, the chimeric polypeptides of the present invention fused to a polysaccharide polymer form embodiments of the present invention. Dextrans are polysaccharide polymers comprised of individual subunits of glucose predominantly linked by alpha 1-6 linkages. The dextran itself is available in many

molecular weight ranges, and is readily available in molecular weights from about 1 kD to about 70 kD. Dextran is a suitable water-soluble polymer for use as a vehicle by itself or in combination with another vehicle (*e.g.*, a Fc). *See*, *e.g.*, International Publication No. WO 96/11953. The use of dextran conjugated to therapeutic or diagnostic immunoglobulins has been reported. *See*, *e.g.*, European Patent Publication No. 0 315 456, which is hereby incorporated by reference. The present invention also encompasses the use of dextran of about 1 kD to about 20 kD.

In general, chemical modification can be performed under any suitable condition used to react a protein with an activated polymer molecule. Methods for preparing chemically modified polypeptides will generally comprise the steps of: (a) reacting the polypeptide with the activated polymer molecule (such as a reactive ester or aldehyde derivative of the polymer molecule) under conditions whereby a chimeric polypeptide becomes attached to one or more polymer molecules, and (b) obtaining the reaction products. The optimal reaction conditions will be determined based on known parameters and the desired result. For example, the larger the ratio of polymer molecules to protein, the greater the percentage of attached polymer molecule. In one embodiment of the present invention, chemically modified chimeric polypeptides can have a single polymer molecule moiety at the amino-terminus (*see*, *e.g.*, U.S. Patent No. 5,234,784)

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

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

VII. Pharmaceutical Compositions

Therapeutic compositions comprising the disclosed chimeric polypeptides are within the scope of the present disclosure, and are specifically contemplated in light of the identification of several chimeric polypeptides exhibiting desirable properties. Such chimeric polypeptide pharmaceutical compositions can comprise a therapeutically effective amount of a chimeric polypeptide in admixture with a pharmaceutically or physiologically acceptable formulation agent (*e.g.*, a carrier, formulation material, etc) selected for suitability with the mode of administration.

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

The pharmaceutical composition can contain formulation materials for modifying, maintaining, or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine, or lysine), antimicrobials, antioxidants (such as ascorbic acid, sodium sulfite, or sodium hydrogen-sulfite), buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, or other organic acids), bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)), complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin, or hydroxypropyl-beta-cyclodextrin), fillers, monosaccharides, disaccharides, and other carbohydrates (such as glucose, mannose, or dextrins), proteins (such as serum albumin, gelatin, or immunoglobulins), coloring, flavoring and diluting agents, emulsifying agents, hydrophilic polymers (such as polyvinylpyrrolidone), low molecular weight polypeptides, salt-forming counterions (such as sodium), preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, or hydrogen peroxide), solvents (such as glycerin, propylene glycol, or polyethylene glycol), sugar alcohols (such as mannitol or sorbitol), suspending agents, surfactants or wetting agents (such as pluronics; PEG; sorbitan esters; polysorbates such as polysorbate 20 or polysorbate 80; triton; tromethamine; lecithin; cholesterol or tyloxapal), stability enhancing agents (such as sucrose or sorbitol), tonicity enhancing agents (such as alkali

metal halides – preferably sodium or potassium chloride – or mannitol sorbitol), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants (*see, e.g., Remington's Pharmaceutical Sciences* (18th Ed., A.R. Gennaro, ed., Mack Publishing Company 1990), and subsequent editions of the same, incorporated herein by reference for any purpose).

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

The primary vehicle or carrier in a pharmaceutical composition can be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier for injection can be water, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which can further include sorbitol or a suitable substitute. In one embodiment of the present disclosure, chimeric polypeptide compositions can be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (*Remington's Pharmaceutical Sciences*, *supra*) in the form of a lyophilized cake or an aqueous solution. Further, the chimeric polypeptide product can be formulated as a lyophilizate using appropriate excipients such as sucrose.

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

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

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

In one embodiment, a pharmaceutical composition can be formulated for inhalation. For example, a chimeric polypeptide can be formulated as a dry powder for inhalation. Chimeric polypeptide inhalation solutions can also be formulated with a propellant for aerosol delivery. In yet another embodiment, solutions can be nebulized. Pulmonary administration is further described in International Publication No. WO 94/20069, which describes the pulmonary delivery of chemically modified proteins.

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

Another pharmaceutical composition can involve an effective quantity of chimeric polypeptides in a mixture with non-toxic excipients that are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or another appropriate vehicle, solutions can be prepared in unit-dose form. Suitable excipients include, but are

not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

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

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

The chimeric polypeptide pharmaceutical composition to be used for *in vivo* administration typically must be sterile. This can be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method can be conducted either prior to, or following, lyophilization and reconstitution. The composition for parenteral administration can be stored in lyophilized form or in a solution. In addition, parenteral compositions generally are placed into a

container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

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

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

The effective amount of a chimeric polypeptide pharmaceutical composition to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which the chimeric polypeptide is being used, the route of administration, and the size (body weight, body surface, or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician can titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage can range from about 0.1 μg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage can range from 0.1 μg/kg up to about 100 mg/kg; or 1 μg/kg up to about 100 mg/kg; or 5 μg/kg, 10 μg/kg, 15 μg/kg, 20 μg/kg, 25 μg/kg, 30 μg/kg, 35 μg/kg, 40 μg/kg, 45 μg/kg, 50 μg/kg, 55 μg/kg, 60 μg/kg, 65 μg/kg, 70 μg/kg, 75 μg/kg, up to about 100 mg/kg. In yet other embodiments, the dosage can be 50 μg/kg, 100 μg/kg, 150 μg/kg, 200 μg/kg, 250 μg/kg, 300 μg/kg, 350 μg/kg, 400 μg/kg, 450 μg/kg, 500 μg/kg, 550 μg/kg, 600 μg/kg, 650 μg/kg, 700 μg/kg, 750 μg/kg, 800 μg/kg, 850 μg/kg, 900 μg/kg, 950 μg/kg, 100 μg/kg, 200 μg/kg, 300 μg/kg, 400 μg/kg, 500 μg/kg, 600 μg/kg, 700 μg/kg, 800 μg/kg, 900 μg/kg, 1000 μg/kg, 2000 μg/kg, 3000 μg/kg, 4000 μg/kg, 5000 μg/kg, 6000 μg/kg, 7000 μg/kg, 8000 μg/kg, 9000 μg/kg, 10 mg/kg or more.

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

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

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

VIII. Therapeutic and Other Uses of a Chimeric Polypeptide

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

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

IX. Antigen Binding Proteins

As used herein, an antigen binding protein is a protein comprising a portion that binds to an antigen and, optionally, a scaffold or framework portion that allows the antigen binding portion to adopt a conformation that promotes binding of the antigen binding protein to the antigen. Examples of antigen binding proteins include human antibody, a humanized antibody; a chimeric antibody; a recombinant antibody; a single chain antibody; a diabody; a triabody; a tetrabody; a Fab fragment; a F(ab')2 fragment; an IgD antibody; an IgE antibody; an IgM antibody; an IgG1 antibody; an IgG2 antibody; an IgG3 antibody; or an IgG4 antibody, and fragments thereof. The antigen binding protein can comprise, for example, an alternative protein scaffold or artificial scaffold with grafted CDRs or CDR derivatives. Such scaffolds include, but are not limited to, antibody-derived scaffolds comprising mutations introduced to, for example, stabilize the three-dimensional structure of the antigen binding protein as well as wholly synthetic scaffolds comprising, for example, a biocompatible polymer. *See*, *e.g.*, Korndorfer et al.,

2003, *Proteins: Structure, Function, and Bioinformatics*, 53(1):121-129 (2003); Roque et al., *Biotechnol. Prog.* 20:639-654 (2004). In addition, peptide antibody mimetics ("PAMs") can be used, as well as scaffolds based on antibody mimetics utilizing fibronectin components as a scaffold.

Antigen binding proteins that specifically bind to the chimeric polypeptides of the present invention but do not specifically bind to wild-type polypeptide scaffolds are contemplated and are within the scope of the present disclosure. An antigen binding protein (e.g., an antibody) is said to "specifically bind" its target antigen when the dissociation constant (K_D) is $\leq 10^{-8}$ M. The antibody specifically binds antigen with "high affinity" when the K_D is $\leq 5x\ 10^{-9}$ M, and with "very high affinity" when the K_D is $\leq 5x\ 10^{-10}$ M.

When an antigen binding protein is an antibody, the antibody can be polyclonal, including monospecific polyclonal; monoclonal (MAbs); recombinant; chimeric; humanized, such as complementarity-determining region (CDR)-grafted; human; single chain; and/or bispecific; as well as fragments; variants; or chemically modified molecules thereof. Antibody fragments include those portions of the antibody that specifically bind to an epitope on a chimeric polypeptide. Examples of such fragments include Fab and F(ab') fragments generated by enzymatic cleavage of full-length antibodies. Other binding fragments include those generated by recombinant DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions.

Polyclonal antibodies directed toward a chimeric polypeptide generally are produced in animals (*e.g.*, rabbits or mice) by means of multiple subcutaneous or intraperitoneal injections of the chimeric polypeptide and an adjuvant. It can be useful to conjugate a chimeric polypeptide to a carrier protein that is immunogenic in the species to be immunized, such as keyhole limpet hemocyanin, serum, albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents such as alum are used to enhance the immune response. After immunization, the animals are bled and the serum is assayed for anti-chimeric polypeptide antibody titer.

Monoclonal antibodies directed toward chimeric polypeptides can be produced using any method that provides for the production of antibody molecules by continuous

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

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

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

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

Sandwich assays typically involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected

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

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

The invention also relates to a kit comprising anti-chimeric polypeptide antibodies and other reagents useful for detecting chimeric polypeptide levels in biological samples. Such reagents can include a detectable label, blocking serum, positive and negative control samples, and detection reagents. Such a kit can further comprise a set of instructions indicating how the reagents and kit can be used.

EXAMPLES

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

Example 1

Expression and Purification of Recombinant Scaffold Polypeptides and Chimeric Proteins

Nucleotide sequences encoding wild type FGF19 without the secretory leader peptide (residues 23-216, SEQ ID NO:4) and chimeric polypeptides were cloned into the pET30 vector (Novagen). Briefly, nucleotides for wild type FGF19 and chimeric

polypeptides were generated through polymerase chain reaction (PCR), both PCR products and pET30 vector were digested with restriction enzyme Nde I and BamH I and ligated with ligase. DNA constructs were transformed into BL21(DE3) *E .coli* (Novagen). Protein expression was induced with IPTG at 37 °C. The purification process was the same as previously described (Wu *et al.,* (2008) *J. Biol Chem.* 283:33304-9). FGF21 without the secretory leader peptide, (residues 29-209, SEQ ID NO:10) was purified as previously described (Xu *et al.*, (2008) *Diabetes* 58:250-59).

A description of some of the polypeptides that were generated is shown in Table 2:

TABLE 2

Sequence Identifier	Composition of Sequence	SEQ ID NO: NT	SEQ ID NO:PP
FGF19	hFGF19	1	2
Mature FGF19	hFGF19 lacking signal sequence	3	4
Mature FGF19 + N-terminal Met	hFGF19 lacking signal sequence with N-terminal Met added	5	6
FGF21	hFGF21	7	8
Mature FGF21	hFGF21 lacking signal sequence	9	10
Mature FGF21 + N-terminal Met	hFGF21 lacking signal sequence with N-terminal Met added	11	12
FGF23	hFGF23	13	14
Mature FGF23	hFGF23 lacking signal sequence	15	16
Mature FGF23 + N-terminal Met	hFGF23 lacking signal sequence with N-terminal Met added	17	18
FGF19dCTD	M::hFGF19(23-177)	19	20
FGF19/21-1	M::hFGF19(23-80)::hFGF21(82-209)	21	22
FGF19/21-2	M::hFGF19(23-49)::hFGF21(52-209)	23	24
FGF19/21-3	M::hFGF19(23-42)::hFGF21(45-209)	25	26
FGF19/21-4	M::hFGF19(23-37)::hFGF21(42-209)	27	28

Sequence Identifier	Composition of Sequence	SEQ ID NO: NT	SEQ ID NO:PP
FGF19/21-5	M::hFGF19(23-32)::hFGF21(37-209)	29	30
FGF21/19$^{38-42}$	M::hFGF21(29-41)::hFGF19(38-42)::hFGF21(45-209)	31	32
FGF19/21$^{42-44}$	M::hFGF19(23-37)::hFGF21(42-44)::hFGF19(43-216)	33	34
FGF19-1	M::hFGF19(23-49)::hFGF21(52-58)::hFGF19(58-216)	35	36
FGF19-2	M::hFGF19(23-145)::hFGF21(147-161)::hFGF19(163-216)	37	38
FGF19-3	M::hFGF19(23-49)::hFGF21(52-58)::hFGF19(58-145)::hFGF21(147-161)::hFGF19(163-216)	39	40
FGF19-4	M::hFGF19(23-37)::hFGF21(42-44)::hFGF19(43-49)::hFGF21(52-58)::hFGF19(58-216)	41	42
FGF19-5	M::hFGF19(23-37)::hFGF21(42-44)::hFGF19(43-145)::hFGF21(148-162)::hFGF19(163-216)	43	44
FGF19-6	M::hFGF19(23-37)::hFGF21(42-44)::hFGF19(43-49)::hFGF21(52-58)::hFGF19(58-145)::hFGF21(148-162)::hFGF19(163-216)	45	46

In Table 2, each construct is presented in the N to C terminal direction; "M" indicates an N-terminal methionine, hFGF19 (X-Y) indicates a region of human FGF19 stretching between residue X and residue Y of a wild type FGF19 amino acid sequence, and hFGF21 (X-Y) indicates a region of human FGF21 stretching between residue X and residue Y of a wild type FGF21 amino acid sequence. For example, in the case of FGF19/21-1 in Table 2, this sequence comprises M::hFGF19(23-80)::hFGF21(82-209)

means sequence is composed of methionine, followed by human FGF19 sequences 23 to 80, then followed by human FGF21 sequences 82 to 209.

Example 2

Experimental Methods

The following experimental methods were employed in Examples 3-10.

2.1 Western-blot Analysis of FGF Signaling

L6 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and penicillin/streptomycin. Cells were transfected with expression vectors using the Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer's protocol.

Analysis of FGF signaling in L6 cells were performed as described before. Cell cultures were collected 10 min after the treatment of FGF19 or chimeras and snap frozen in liquid nitrogen, homogenized in the lysis buffer and subjected to western blot analysis using anti-phospho-p44/42 MAP kinase (ERK1/2) antibody and anti-ERK antibody (Cell Signaling).

2.2 MSD Assay for FGF Signaling

L6 cells plated in 24 well plates (10^6 cells/well) were transfected with various FGF receptors, including FGFR1c and FGFR4 and αKlotho or βKlotho and serum starved in 0.2% bovine serum albumin overnight before FGF treatment. Media was aspirated after 10 min and plates were snap frozen in liquid nitrogen. Cells in each well were lysed in 60 µl of complete lysis buffer and total and phosphorylated ERK was measured using MSD whole cell lysate Phospho-ERK1/2 kit (Meso Scale Discovery, Gaithersburg, Maryland) according to the manufacturer's instructions.

2.3 Glucose Uptake Assay

3T3L1 preadipocytes (ATCC CL-173) were cultured and induced to differentiate. Glucose uptake was assayed as described in Kharitonenkov, *et al.,* (2005) *J Clin Invest* 115, 1627-1635.

2.4 *In Vivo* Hepatocyte BrdU Labeling Assay

For all BrDU studies described herein, on day 1 of the study an osmotic minipump (ALZET®, model 1007D) containing 5-bromo-2'-deoxyuridine (BrdU; Sigma Chemical Co., St. Louis, MO) (16 mg/mL) was implanted subcutaneously into each of 7-10 8 week-old female FVB mouse (Charles River Laboratories, Charles River, MA). Each mouse was given an IP injection of either phosphate-buffered saline (PBS; vehicle), or various proteins as indicated daily at 2 mg/kg/day beginning on day 2 of the study and continuing for 6 consecutive days. Samples of liver and duodenum were collected from each mouse on the day following the last IP injection and placed in 10% neutral-buffered formalin in preparation for paraffin-embedding, sectioning, and light microscopic evaluation. Sections of all collected tissues were stained by an immunohistochemical method described herein to visualize BrdU incorporation as a marker of mitotic activity. Tissue sections were examined at random by routine light microscopy without knowledge of treatment group. The number of hepatocyte nuclei stained for BrdU incorporation was assigned a score on a semiquantitative scale where 0 = no increase above expected levels in vehicle-treated (control) mice and ± = equivocal, 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked increase above control levels. The localization (centrilobular or diffusely scattered through hepatic lobules) of the hepatocytes stained for BrdU incorporation was also recorded.

Cellular incorporation of BrdU was detected by digesting deparaffinized tissue sections with 0.1% Protease (Sigma, St. Louis, MO) and treating the sections with 2N hydrochloric acid. Sections were blocked with CAS BLOCK (Zymed Laboratories, San Francisco, CA), incubated with rat antibody to BrdU (Accurate, Westbury, NY; catalog no. OBT0030, lot no. H9180), and bound rat antibody was detected with biotinylated rabbit antibody to rat IgG (Vector Laboratories, Burlingame, CA; catalog no. BA 4001, lot no. S0907). Tissue sections were quenched with Peroxidase Blocking Solution (DAKO Corp., Carpinteria, CA) and retained biotin was detected with Vectastain Elite ABC kit (Vector Laboratories). Reaction sites were visualized with DAB+Substrate-Chromagen System (DAKO Corp.). Sections were counterstained with hematoxylin.

Example 3

FGF21 Does not Increase Hepatocyte Proliferation *In Vivo*

Because FGF21 is in the same subfamily with FGF19, and both show significant similarities in receptor/co-receptor requirements and in regulation of glucose and lipid metabolism, the effects of each on hepatocyte proliferation was studied. Using *in vivo* BrdU labeling, enhanced hepatocyte proliferation around the central vein was observed in FGF19 transgenic animals as well as in nontransgenic animals 6 days post daily injection of recombinant FGF19 protein. *See*, Nicholes *et al.,* (2002) *Am J Pathol* 160, 2295-2307. Using a similar BrdU labeling method, we examined the effects of FGF21 treatment on hepatocyte proliferation and compared its activity to that of FGF19. As shown in Figure 1A, histopathological examination of the liver sections from FGF19 treated animals showed increased BrdU labeled hepatocytes concentrating in centrilobular regions of hepatic lobules, consistent with published observations (*see, e.g.*, Nicholes *et al.,* (2002) *Am J Pathol* 160, 2295-2307). In contrast, livers from FGF21 treated animals did not show increased numbers of BrdU-labeled hepatocytes in pericentral regions, nor was increased BrdU labeling noted in any other area of the liver, suggesting that FGF21 did not enhance hepatocyte proliferation under the conditions tested and, therefore, is distinct from FGF19. Figure 1B graphically depicts the incorporation level of the BrdU label at the conclusion of the experiment, and highlights the observation that FGF19 led to BrdU incorporation, while the PBS control and FGF21 did not.

Example 4

FGF19, but not FGF21, Activates FGFR4 Mediated ERK Phosphorylation, and Selective Activation of FGFR4 in Liver Induces Pericentral Hepatocyte Proliferation

To better understand the mechanism for FGF19 induced hepatocyte proliferation and its differences from FGF21, the receptor and co-receptor requirements between FGF19 and FGF21 were first compared. The rat myoblast cell line L6, which expresses very low levels of endogenous FGF receptors, was transfected with FGFR1c, 2c, 3c or 4 together with βKlotho. Receptor activation was determined by Western blot analysis of phospho-ERK levels in treated cells. As shown in Figures 2A-2D, while both FGF19 and

FGF21 were able to activate FGFR1c (Figure 2A), 2c (Figure 2B), and 3c (Figure 2C), only FGF19 and not FGF21 induced ERK phosphorylation via FGFR4 (Figure 2D) (*see, e.g.*, Kurosu *et al.,* (2007) *J. Biol Chem.* 282, 26687-26695, and Lin *et al.*, (2007) *J. Bio. Chem.* 282, 27277-27284). Given that FGFR4 is the predominant receptor expressed in hepatocytes, the effect of FGFR4 activation as measured by ERK phosphorylation on the enhanced hepatocyte proliferation observed in FGF19 treated animals was studied.

In this experiment a variant of FGF19, FGF19dCTD, identified as a selective FGFR4 agonist, was employed. As illustrated in Figure 3A, FGF19dCTD is a truncated form of FGF19 in which the C-terminal residues 178-216 have been removed. Because this region is critical for co-receptor βKlotho interaction, FGF19dCTD cannot activate FGFRs 1c, 2c, and 3c which depend on βKlotho for activation by both FGF19 and FGF21 (Figure 3B; see also Wu *et al.*, (2008) *J. Biol Chem.* 283(48):33304-9). FGF19dCTD can, however, still activate FGFR4 both *in vitro* (Figure 3B) and *in vivo*. Accordingly FGF19dCTD was employed to examine the effects of selective FGFR4 activation on hepatocyte proliferation. Analysis of BrdU immunostained liver sections from FGF19dCTD treated animals also showed enhanced BrdU labeling indicating increased mitotic activity almost as defined as was observed in animals treated with wild type FGF19. Thus, activation of FGFR4 alone can be sufficient to cause increased hepatocyte proliferation.

Example 5

Identification of a Region of FGF19 That is Critical for FGFR4 Activation

Upon consideration of the results shown in Example 4, the region(s) of FGF19 responsible for FGFR4 signaling were identified and studied. Because FGF19 and FGF21 share significant sequence homology but differ in the ability to activate FGFR4 signaling, chimeric proteins comprising regions of FGF19 and FGF21 were generated. The approach taken was to sequentially replace regions of an FGF21 wild type sequence with regions of FGF19, in order to identify the region responsible for FGFR4 activity.

Results from experiments using FGF19dCTD indicated that the C-terminal region of FGF19 is not essential for FGFR4 activation; therefore, a series of FGF19/FGF21 chimeric polypeptides was generated which sequentially replaced the N-terminal region

of FGF21 with the corresponding region of FGF19; these chimeric polypeptides are illustrated graphically in Figure 4A. The properties of these chimeric polypeptides were then assessed in *in vitro* receptor activity assays, adipocyte glucose uptake assays, and *in vivo* hepatocyte proliferation assays. As shown in the top panel of Figure 4B, all the chimeric polypeptides activated ERK phosphorylation in L6 cells transfected with FGFR1c and βKlotho. Consistently, because FGFR1c is the predominant receptor expressed in adipocytes, all the chimeric polypeptides induced glucose uptake in differentiated mouse 3T3-L1 adipocytes with similar potency and efficacy (see Figure 4C). These results suggest that all the chimeric polypeptides are functional, and that fusions between FGF19 and FGF21 yielded properly folded and active proteins.

These chimeric polypeptides, however, displayed differences in FGFR4 selective assays. For example, in L6 cells transfected with FGFR4 and βKlotho, ERK-phosphorylation was only observed with the chimeric polypeptides FGF19/21-1, FGF19/21-2 and FGF19/21-3, which share FGF19 residues 23-42 (Figure 4B). ERK-phosphorylation was not observed with FGF19/21-4 or FGF19/21-5, which comprise shorter N-terminal fragments derived from FGF19 (Figure 4B, bottom panel). These results indicate that critical FGFR4 activating residues are contained within FGF19 residues 38 to 42.

The effects of these chimeric polypeptides on hepatocyte proliferation were then tested in an *in vivo* BrdU incorporation assay. Examination of BrdU immunostained liver sections from treated animals showed that, like FGF19, the chimeric polypeptides FGF19/21-1, FGF19/21-2, FGF19/21-3, and FGF19/21-4 all exhibited increased BrdU labeling in the pericentral hepatocytes, however, such increases were not observed with animals treated with FGF19/21-5 and FGF19/21-6 (Figure 4D). Therefore, BrdU labeling correlated directly with each molecule's ability to activate FGFR4 mediated ERK phosphorylation (as shown in Figure 4A).

Example 6

Residues 38-42 of FGF19 Confer FGFR4 Activation and Increased Hepatocyte Proliferation

A comparison between FGF19/21-4 (containing the first 15 residues from a mature wild type FGF19 polypeptide) and FGF19/21-5 (containing the first 10 residues from a mature wild type FGF19 polypeptide) revealed a difference in each chimeric polypeptide's ability to activate FGFR4 and to induce hepatocyte proliferation. Because the two chimeric polypeptides only differ by 5 amino acids, a study was undertaken to determine whether these 5 residues, namely the residues at positions 38-42 a full length FGF19 (residues 16-20 in the mature form) were sufficient to confer FGFR4 activation. Another chimeric polypeptide designated "FGF21/19$^{38-42}$" (SEQ ID NO:32), comprising a FGF21 scaffold, in which residues 38-42 of a full length wild type FGF19 amino acid sequence (residues 16-20 in the mature form) replaced residues 42-44 of a full length FGF21 (residues 14-16 in the mature form), was constructed and is depicted graphically in Figure 5A. Similar to FGF19 and FGF21, FGF21/19$^{38-42}$ induced ERK-phosphorylation in L6 cells transfected with FGFR1c and βKlotho (Figure 5B) and was active in adipocyte glucose uptake assays. Similar to FGF19, however in contrast to FGF21, the FGF21/19$^{38-42}$ chimeric polypeptide induced ERK-phosphorylation in L6 cells transfected with FGFR4 and βKlotho (Figure 5B). Histopathological examination analysis of liver sections from FGF21/19$^{38-42}$ treated animals showed enhanced BrdU labeling in pericentral hepatocytes similar to FGF19 treatment, distinct from FGF21 (Figure 5C). These results indicate that introduction of these 5 residues from FGF19 conferred a gain-of-function phenotype on FGF21 with respect to FGFR4 activation *in vitro* and induction of hepatocyte proliferation *in vivo*.

Example 7

Replacing Residues 38-42 from FGF19 Does Not Completely Abolish FGFR4 Activation and Hepatocyte Proliferation

Using a FGF19 C-terminal truncation variant and novel FGF19/FGF21 chimeric molecules, it was determined that hepatocyte FGFR4 activation measured by ERK phosphorylation may lead to increased hepatocyte proliferation. In side-by-side direct

comparison studies, it was also determined that FGF21 is different from FGF19 and it lacks the ability to activate FGFR4 and does not induce hepatocyte proliferation as measured using *in vivo* BrdU labeling. In addition, these observations demonstrated the importance of the FGF19 N-terminal region to FGFR4 activation, and identified residues 38-42 of full length FGF19 to be sufficient to confer FGFR4 activation and to increase hepatocyte proliferation, as a construct in which the replacement of the corresponding region in FGF21 with these 5 amino acid residues (the construct designated FGF21/19$^{38-42}$ in Figure 6A) provided gain-of-function activity to FGF21 in the form of FGFR4 activation and induction of increased hepatocyte proliferation (Figure 6A). Additionally, the issue of whether the mutations in this region of FGF19 would abolish FGF19's ability to activate FGFR4 and eliminate its ability to induce hepatocyte proliferation was studied.

The alignment of FGF19, FGF21 and FGF23 around these 5 amino acid residues is shown in Figure 6B. These 5 residues are underlined in FGF19 sequence, the corresponding region in FGF23 contains only 3 amino acids, WGG, and similarly, the corresponding region of FGF21 contains only 3 amino acids, $G^{42}Q^{43}V^{44}$. A construct comprising the swap of this region between FGF19 and FGF21 was constructed as shown in Figure 6A. For FGF21/19$^{38-42}$, as described previously, the residues GQV at positions 42-44 in full length FGF21 (positions 14-16 in mature FGF21) were replaced with the corresponding FGF19 residues WGDPI (SEQ ID NO:49) at positions 38-42 of full length FGF19 (positions 16-20 of mature FGF19); and for FGF19/21$^{42-44}$ (SEQ ID NO:34), which is the reverse swap, the residues WGDPI at positions 38-42 in full length FGF19 (positions 16-20 in mature FGF19) were replaced with the corresponding residues GQV found at positions 42-44 of full length FGF21 (positions 14-16 of mature FGF21). If this region is the only region that contributes to FGFR4 activation, the substitution of FGF21 sequence into FGF19 would abolish that activity.

To test the activities of these chimeric FGF molecules in receptor activation assay, the previously described rat myoblast cell line L6, which expresses negligible levels of endogenous FGF receptors and βKlotho, was utilized. FGFRs were either transfected alone or together with βKlotho, and the signaling was monitored by the ERK phosphorylation levels (Wu *et al.*, (2008) *J. Biol. Chem.* 283(48):33304-9). In this assay

format, FGF19/21$^{42-44}$ still activated FGFR4 signaling in the presence of co-receptor βKlotho and its ability to activate FGFR1c/βKlotho complex was also unaffected. See Figures 6A and 6C.

The effects of FGF19/21$^{42-44}$ on hepatocyte proliferation was examined *in vivo* by measuring the incorporation of the label BrdU into hepatocytes post daily intraperitoneally (i.p.) injection of the recombinant protein for 7 days. Consistent with the previously observed link between liver FGFR4 activation and enhanced hepatocyte proliferation, histopathological examination of liver sections from FGF19/21$^{42-44}$ treated animals showed enhanced BrdU labeling in pericentral hepatocytes similar to FGF19 (Figure 6E). There results indicate that additional regions of FGF19 can independently contribute to FGFR4 activation.

One surprising finding is that FGF19/21$^{42-44}$ is no longer able to activate FGFR4 in the absence of βKlotho (Figure 6C lower panel FGFR4 alone transfection). This suggests that heparin induced FGF19/21$^{42-44}$/FGFR4 activation has been affected by this substitution. This is further confirmed by solid-phase binding assay where addition of heparin no longer stimulates FGF19/21$^{42-44}$ interaction with FGFR4 (Figure 6D) similar to effects observed with mutations in the heparin binding sites of β1- β2 and β10- β12 regions shown in Figure 7. Since FGF21/19$^{38-42}$ does not interact with or activate FGFR4 in the presence of heparin (Figure 6D), the effect of the N-terminal 5 residues of FGF19 (38-42) on heparin induced FGFR4 interaction may be an indirect effect.

Example 8

Replacing Heparin Binding Loops in FGF19 Abolished βKlotho-Independent FGFR4 Activation by FGF19

FGF19 subfamily members have reduced affinity to heparin/heparin sulfate, and the presence of co-receptors α or βKlotho facilitate the binding and activation of FGFRs by this subfamily members to compensate for the weak heparin binding affinity. The only exception is the FGF19/FGFR4 interaction. At relatively high concentrations of heparin, FGF19 can bind and activate FGFR4 in the absence of βKlotho in both *in vitro* and *in vivo*.

The published apo-FGF19 and FGF23 structures (PDB codes:2P23 and 2P39; Goetz *et al.*, (2007) *Mol. Cell. Biol.* 27:3417-28) provided some insights into the weakened affinity toward heparin for this subfamily. The β1-β2 loop (SEQ ID NOs:52, 54 and 56) and β10-β12 regions (SEQ ID NOs:58, 60 and 62), which are shown in Figure 7 and have been shown to be responsible for high affinity binding of heparin by other FGF family members, are much larger in this subfamily and could potentially form steric clashes with heparin in the ternary complex with FGFR, and therefore result in lower affinity toward heparin (Goetz *et al.*, (2007) *Mol. Cell. Biol.* 27:3417-28).

A FGF21 model built based on the published apo-FGF19 structure revealed that in addition to the potential steric clash, the surface charges for FGF21 in these regions are also less favorable for heparin binding and may explain the even lower affinity of FGF21 toward heparin compared with FGF19 (Goetz *et al.*, (2007) *Mol. Cell. Biol.* 27:3417-28). Since this is one of the major difference between FGF19 and FGF21, a modeled FGF19/FGFR structure based on the published FGF2/FGFR1 complex structure (PDB code: 1FQ9) revealed that these putative heparin binding domains are positioned opposite to the 5 amino acid residues at positions 38-42 in full length FGF19 and may also contact the receptor (Schlessinger *et al.*, (2000) *Mol. Cell* 6:743-50). In light of this observation, the issue of whether these regions contribute to FGFR4 activation by FGF19 was studied.

To examine the differences between FGF19 and FGF21 in the putative heparin binding domains, chimeric constructs in which the heparin interacting β1-β2 loop and β10-β12 segments in FGF19 were replaced with the corresponding sequences from FGF21 were designed and expressed. These chimeric constructs are shown graphically in Figure 8A. In Figure 8A, FGF19-1 corresponds to a chimera in which residues 52-58 of full length FGF21 (positions 24-30 in mature FGF21) replaced residues 50-57 of FGF19 (positions 28-35 in mature FGF19), FGF19-2 corresponds to a chimera in which residues 147-161 of full length FGF21 (positions 119-133 in mature FGF21) replaced residues 146-162 of FGF19 (positions 124-140 of mature FGF19) and FGF19-3 corresponds to a chimera in which residues 52-58 of full length FGF21 (positions 24-30 of mature FGF21) replaced residues 50-57 of full length FGF19 (positions 28-35 in mature FGF19) and residues 147-161 of full length FGF21 (positions 119-133 in mature FGF21) replaced residues 146-162 of full length FGF19 (positions 124-140 of mature

FGF21). These chimeric polypeptides were used to investigate the contribution of these domains to heparin binding and FGFR4 activation.

As demonstrated by the results of a solid-phase binding assay, replacing the β1-β2 loop and β10-β12 segment of FGF19 individually or in combination abolished heparin induced FGF19/FGFR4 interaction (Figure 8B) while preserving the ability to interact with FGFR4 in the presence of βKlotho (Figure 8C), is consistent with the roles of these two regions in interacting with heparin.

To further evaluate these findings in a functional assay, receptors were again transfected into L6 cells. FGFR4 was either transfected alone or together with βKlotho, and the signaling was monitored by the ERK phosphorylation levels. Consistent with solid-phase binding results, in contrast to FGF19, the chimeric substitutions in these putative heparin binding domains abolished heparin dependent FGFR4 activation (Figure 8D, lower panel), while βKlotho dependent FGFR1c and FGFR4 activation were preserved (Figure 8D upper panels). These results indicate that the mutations in the putative heparin binding domain indeed abolished heparin dependent receptor activities.

It has been shown that wild type FGF19 can activate FGFR4 either through heparin or βKlotho. One variant of FGF19, namely FGF19dCTD, can selectively activate FGFR4 only in a heparin dependent manner, and this activation still induced enhanced hepatocyte proliferation. In the case of FGF19-1 mutant described herein, the heparin dependent FGFR4 activation was abolished while preserving βKlotho dependent FGFR4 activation. With respect to FGF19dCTD and FGF19-1, each appeared to retain part of the wild type FGF19 function, and although both are able to activate FGFR4, signaling is mediated through different cofactors.

The issue of whether there is a qualitative difference in FGFR4 signaling mediated through βKlotho versus signaling mediated through heparin with respect to stimulation of hepatocyte proliferation was then studied. Histopathological examination of liver sections from FGF19-1 treated animals showed enhanced BrdU labeling in pericentral hepatocytes similar to FGF19 treatment (Figure 8E), suggesting that both heparin and βKlotho induced FGFR4 activation results in enhanced hepatocyte proliferation. As is the case with wild type FGF19, FGF19-1 is also still active in other metabolic assays, able to induce glucose uptake into adipocyte cells and reduced plasma

glucose levels in an ob/ob diabetic animal model, indicating that heparin domain mutations did not affect other FGF19 mediated functions.

Example 9

A Chimeric Protein in Which Residues 38-42 of Full Length FGF19 and both Heparin Binding Regions in FGF19 are Replaced Exhibits Decreased FGFR4 Activation and Hepatocyte Proliferation

The single changes in the 5 amino acid region of residues 38-42 of full length FGF19 (positions 14-20 in mature FGF19) and the heparin binding domains did not completely abolish FGFR4 activation, so a chimeric protein in which the replacement of all three of these regions was studied. Additional chimeric polypeptides combining residues 38-42 of full length FGF19 and one or both of the heparin interaction regions were constructed and expressed. These chimeric polypeptides were designated FGF19-4, FGF19-5 and FGF19-6, respectively, and are shown graphically in Figure 9A. The activities of these chimeric polypeptides were tested *in vitro* and *in vivo* assays.

These combination chimeric polypeptides were no longer able to activate FGFR4 signaling in L6 cells in the absence or presence of βKlotho but were still able to activate FGFR1c signaling (Figure 9B), therefore, selectively abolishing FGFR4 activity. Consistent with this observation, histopathological examination of liver sections from FGF19-4, -5, and -6, treated animals did not show increased numbers of BrdU-labeled hepatocytes in pericentral regions, nor was increased BrdU labeling noted in any other area of the liver (Figure 9C). Therefore, enhanced hepatocyte proliferation associated with wild type FGF19 was abolished by the combined mutagenesis of the 5 FGF19 amino acid residues WGDPI and heparin domains.

To rule out the possibility that the lack of positive BrdU labeling is due to differences in the degradation and clearance of the chimeric proteins in serum, the serum concentration of the chimeras was measured at various time points after injection into the mice and similar pharmacokinetic properties of the chimeric proteins to wild type FGF19 were observed.

Example 10

Chimeric Molecules Lacking the Ability to Activate FGFR4 Can Still Regulate Glucose Homeostasis

Since the chimeric FGF19 molecules FGF19-4, FGF19-5, and FGF19-6, which comprise the combined substitutions of the 5 amino acids from positions 38-42 of full length FGF19, namely residues WGDPI, and also one or both of the heparin binding domains, were still able to activate FGFR1c/βKlotho receptor signaling in L6 cells (Figure 9B), their ability to regulate glucose homeostasis was tested.

The effect of these chimeric proteins on glucose uptake into adipocytes was first tested. Similar to wild type FGF19 protein, the chimeric proteins were also able to stimulate glucose uptake independent of insulin into 3T3L1 adipocytes *in vitro* (Figure 10A).

To further investigate the ability of the chimeric proteins to regulate glucose homeostasis, ob/ob mice were injected intraperitoneally with FGF19 or FGF19-4 and blood glucose levels were measured at 0, 1, 3, and 5 hrs post injection; the values are reported as area under the curve (AUC) means ± S.E.M. over this time period (Figure 10B). Plasma glucose levels were significantly reduced in mice injected with both FGF19 and FGF19-4 with comparable potency and efficacy (Figure 10B). These results indicate that FGF19-4 only selectively lost its ability to induce FGFR4 mediated hepatocyte proliferation, but retained its ability to modulate glucose regulation.

Replacing the β1-β2 loop segment of FGF19 only (FGF19-1) abolished heparin induced FGF19/FGFR4 interaction while preserving the ability to interact with FGFR4 in the presence of βKlotho. Similar to wild type FGF19, FGF19-1 is also still active in other metabolic assays, able to induce glucose uptake into adipocyte cells (Figure 10C) and reduced plasma glucose levels in *ob/ob* diabetic animals model (Figure 10D), suggesting that heparin domain mutations did not affect other FGF19 mediated functions.

Example 11

Pharmacokinetic Analysis of the Chimeric Proteins

The pharmacokinetic profiles of the chimeric constructs FGF19/21-1, FGF19/21-2, FGF19/21-3, FGF19/21-4 and FGF19/21-5 were studied. The following protocol was

employed. Following i.p. injections of 2 mg/kg FGF19/21 chimeric proteins (n = 5), C57BL6 mouse serum samples were collected 15 min, 1 hour, 3 hours, and 6 hours after the injections. FGF19/21 chimeric protein concentrations were determined by an enzyme-linked immunosorbent assay (ELISA) developed at Amgen. The antibodies used as capture and detection reagents were generated in-house. A mouse monoclonal antibody raised against human FGF21 was used as the capture antibody and was specific for an epitope near the C-terminus on human FGF21. A biotin-conjugated rabbit polyclonal antibody raised against human FGF21 was used as the detection antibody and recognized multiple epitopes on human FGF21.

The ELISA was performed as follows. The capture antibody was bound onto a 96-well polystyrene microplate. Standards and quality control samples were prepared by spiking the FGF19/21 chimera into mouse plasma. Standards, quality controls, matrix blank, and unknown samples were loaded into the wells after pretreatment in assay buffer. After a two hour incubation followed by washing, the biotin-conjugated detection antibody was added to the wells. After a one hour incubation followed by washing, a streptavidin-horseradish peroxidase (HRP) conjugate (R&D Systems, Inc) was added to the wells. After a 30 minute incubation followed by washing, a tetramethylbenzidine (TMB) peroxidase substrate solution was added to the wells. In the presence of HRP, a colorimetric signal was produced that was proportional to the amount of FGF19/21 chimera bound by the capture antibody. The color development was stopped and the intensity of the color (optical density, OD) was measured at 450-650 nm with a plate reader. The conversion of OD units to concentration for the unknown samples was achieved through a software-mediated comparison to a standard curve assayed on the same plate. The data were regressed using SoftMax Pro 5 (Molecular Devices Corp.) data reduction package.

The results of the study are presented in Figure 11.

Example 12

Deletion or Mutation of FGF19 Residue W38

Abolishes FGFR4 and FGFR1c Function

16 mutants within the residues WGDPI (SEQ ID NO:49) region at positions 38-42 of the FGF19-1 polypeptide (in which residues 50-57 of FGF19 were replaced with residues 52-58 of FGF21; see Figure 8) were expressed and purified as described in Example 1. L6 cells transfected with either FGFR1c/βKlotho or FGFR4/βKlotho were treated with those purified proteins. Activities of mutants on FGFR1c or FGFR4 were determined by measuring ERK phosphorylation levels 15 min after treatments and are summarized in Figure 12.

The bar graphs of Figure 13 reflects the level of FGFR1c-mediated activity of FGF19 mutants administered at concentrations of 0, 2.5, 16 and 100 nM. The bar graphs of Figure 14 reflects the level of FGFR1c-mediated activity of FGF19 mutants administered at concentrations of 0, 2.5, 7.4, 44, 67 and 200 nM.

The bar graphs of Figure 15 reflect the level of FGFR4-mediated activity of FGF19 mutants administered at concentrations of 0, 2.5, 16 and 100 nM. The bar graphs of Figure 15 reflects the level of FGFR4-mediated activity of FGF19 mutants administered at concentrations of 0, 2.5, 7.4, 44, 67 and 200 nM.

Deletion of W38, P41, and I42 abolished activation of both FGFR1c and FGFR4 receptor by the mutant FGF19 proteins, while deletion of G39 reduced activity on both receptors and deletion of D40 selectively removed FGFR1c activity with much less effect on FGFR4 activity.

Each of the 5 amino acids was then individually mutated to alanine to study their involvement in receptor activation. While some of the mutations in the GDPI (SEQ ID NO:71) sequence to alanine affected either potency and/or efficacy, only W38A completely abolished activation of both FGFR1c and FGFR4.

The results of this study indicate that W38 is a critical residue for FGF19-induced FGFR activation. The results further indicate that deletion or mutagenesis of this residue to selectively decrease or remove FGFR4-mediated activity from FGF19 would require a change from wild type at position 38, particularly a deletion or mutation. This decrease in FGFR4-mediated activity may be achievable by mutating or deleting W38 alone or

may require additional deletions or mutations in the WGDPI or surrounding region. One such example is FGF19-4 (residues 38-42 of FGF19 were replaced with residues 42-44 of FGF21 and residues 50-57 of FGF19 were replaced with residues 52-58 of FGF21; see Figure 8) in which concurrent deletion of W38, P41 and mutation of I42V resulted in such selective FGFR1c-mediated activity.

By mitigating the mitogenicity of FGF19, a mutant form of FGF19 comprising a mutation or deletion at position 38 could make FGF19 a therapeutically relevant molecule and an attractive candidate for pharmaceutical development.

Claims

What is claimed is:

1. A chimeric polypeptide comprising a wild type mature FGF19 polypeptide scaffold comprising SEQ ID NO:4, further comprising a modification that decreases FGFR4-mediated signaling activity.

2. The chimeric polypeptide of claim 1, wherein the modification comprises substituting one or more of the residues WGDPI at positions 16-20 of the FGF19 polypeptide scaffold with (a) no amino acid; or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence.

3. The chimeric polypeptide of claim 2, wherein the tryptophan residue of the WGDPI sequence is deleted.

4. The chimeric polypeptide of claim 2, wherein the residues WGDPI are substituted with 1-5 contiguous residues present in a wild type FGF21 or a wild type FGF23 amino acid sequence.

5. The chimeric polypeptide of claim 4, wherein the 1-5 contiguous residues are present in a wild type FGF21 amino acid sequence.

6. The chimeric polypeptide of claim 5, wherein the 1-5 contiguous residues are GQV.

7. The chimeric polypeptide of claim 1, wherein the modification comprises substituting one or more of the residues SGPHGLSS at positions 28-35 of the FGF19 polypeptide scaffold with (a) no amino acid; or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence.

8. The chimeric polypeptide of claim 7, wherein the residues SGPHGLSS are substituted with 1-8 contiguous residues present in a wild type FGF21 or a wild type FGF23 amino acid sequence.

9. The chimeric polypeptide of claim 8, wherein the 1-8 contiguous residues are present in a wild type FGF21 amino acid sequence.

10. The chimeric polypeptide of claim 9, wherein the 1-8 contiguous residues are DDAQQTE.

11. The chimeric polypeptide of claim 1, wherein the modification comprises substituting one or more of the residues SSAKQRQLYKNRGFLPL at positions 124-140 of the FGF19 polypeptide scaffold with (a) no amino acid; or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence.

12. The chimeric polypeptide of claim 11, wherein the residues SSAKQRQLYKNRGFLPL are substituted with 1-17 contiguous residues present in either a wild type FGF21 or a wild type FGF23 amino acid sequence.

13. The chimeric polypeptide of claim 12, wherein the 1-17 contiguous residues are present in a wild type FGF21 amino acid sequence.

14. The chimeric polypeptide of claim 13, wherein the 1-17 contiguous residues are PGNKSPHRDPAPRGP.

15. A chimeric polypeptide that exhibits decreased FGFR4-mediated signaling activity comprising a wild type FGF19 polypeptide scaffold comprising SEQ ID NO:4, wherein one or more of the residues WGDPI at positions 16-20 of SEQ ID NO:4 has been substituted with (a) no amino acid; or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence; and one or both of:

(i) one or more of the residues SGPHGLSS at positions 28-35 of SEQ ID NO:4 has been substituted with (1) no amino acid; or (2) an amino acid other than the amino acid located at the position in the wild type amino acid sequence; and

(ii) one or more of the residues SSAKQRQLYKNRGFLPL at positions 124-140 of SEQ ID NO:4 has been substituted with (1) no amino acid; or (2) an amino acid other than the amino acid located at the position in the wild type amino acid.

16. The chimeric polypeptide of claim 15, wherein the tryptophan residue of the WGDPI sequence is deleted.

17. The chimeric polypeptide of claim 15, wherein the residues WGDPI are substituted with 1-5 contiguous residues present in a wild type FGF21 or a wild type FGF23 amino acid sequence.

18. The chimeric polypeptide of claim 17, wherein the 1-5 contiguous residues are present in wild type FGF21 amino acid sequence.

19. The chimeric polypeptide of claim 17, wherein the 1-5 contiguous residues are GQV.

20. The chimeric polypeptide of claim 15, wherein the residues SGPHGLSS are substituted with 1-8 contiguous residues present in a wild type FGF21 or a wild type FGF23 amino acid sequence.

21. The chimeric polypeptide of claim 20, wherein the 1-8 contiguous residues are present in a wild type FGF21 amino acid sequence.

22. The chimeric polypeptide of claim 21, wherein the 1-8 contiguous residues are DDAQQTE.

23. The chimeric polypeptide of claim 15, wherein the residues SSAKQRQLYKNRGFLPL are substituted with 1-17 contiguous residues present in a wild type FGF21 or wild type FGF23 amino acid sequence.

24. The chimeric polypeptide of claim 23, wherein the 1-17 contiguous residues are present in a wild type FGF21 amino acid sequence.

25. The chimeric polypeptide of claim 24, wherein the 1-17 contiguous residues are PGNKSPHRDPAPRGP.

26. The chimeric polypeptide of claim 15, wherein the residues WGDPI at positions 16-20 of SEQ ID NO:4 are substituted with GQV; and one or both of:

(a) the residues SGPHGLSS at positions 28-35 of SEQ ID NO:4 are substituted with DDAQQTE; and

(b) the residues SSAKQRQLYKNRGFLPL at positions 124-140 of SEQ ID NO:4 are substituted with PGNKSPHRDPAPRGP.

27. A nucleic acid molecule encoding the chimeric polypeptide of claim 1 or 15.

28. A vector comprising the nucleic acid molecule of claim 27.

29. A host cell comprising the nucleic acid molecule of claim 27.

30. A pharmaceutical composition comprising the chimeric polypeptide of claims 1 or 15 and a pharmaceutically acceptable carrier.

31. A method of treating a metabolic disease selected from the group consisting of diabetes and obesity comprising administering to a human patient in need thereof the pharmaceutical composition of claim 30.

32. An antigen binding protein that specifically binds to a chimeric polypeptide of claim 1 or 15.

33. A chimeric fusion polypeptide comprising the chimeric polypeptide of claim 1 or 15 fused to a heterogenous moiety.

34. The chimeric fusion polypeptide of claim 33, wherein the heterogenous moiety is selected from the group consisting of an Fc region of an IgG molecule and a PEG molecule.

35. The chimeric polypeptide of claim 1 or 15, wherein SEQ ID NO:4 is truncated on the N terminus by 1-15 amino acids, the C terminus by 1-15 amino acids, or on both the N terminus by 1-15 amino acids and the C terminus by 1-15 amino acids.

36. The chimeric polypeptide of claim 1 or 15, which, except for the modification that decreases FGFR4-mediated signaling activity, comprises a polypeptide scaffold that is 95% or more identical to SEQ ID NO:4.

FIG. 1A

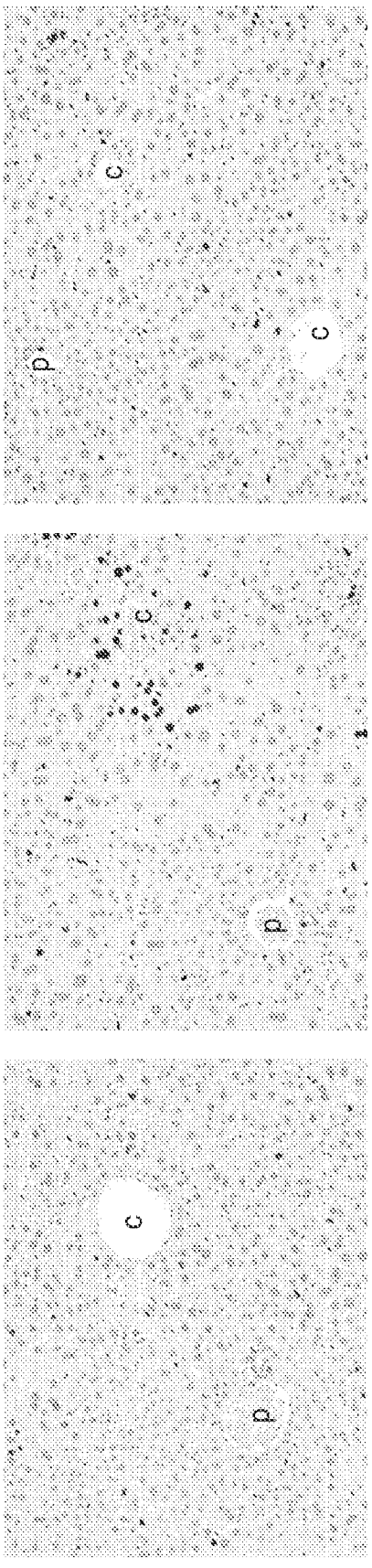

PBS
FGF19
FGF21
c
p

FIG. 1B

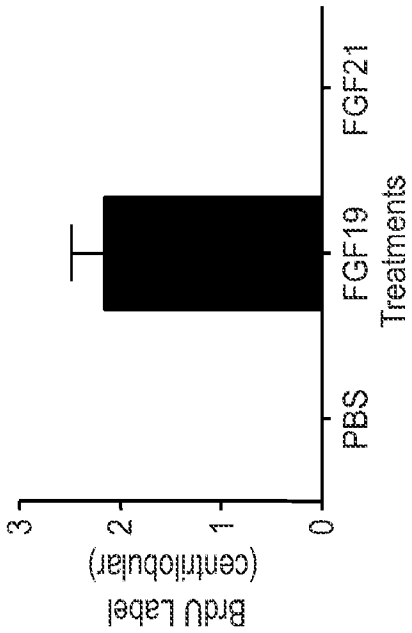

BrdU Label
(centrilobular)
0
1
2
3
PBS
FGF19
FGF21
Treatments

FIG. 2A

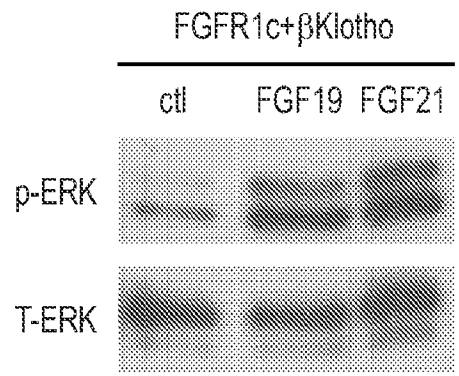


FIG. 2B

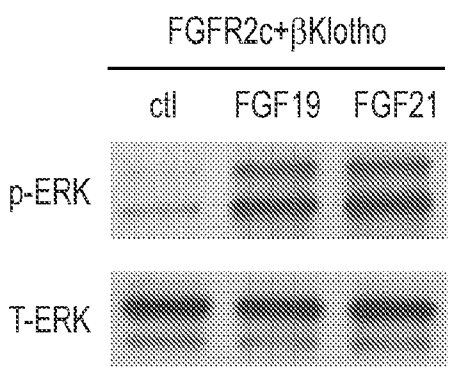


FIG. 2C

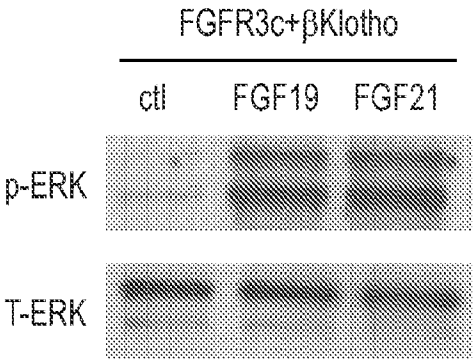


FIG. 2D

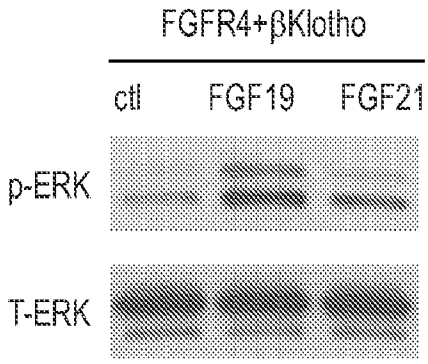


FIG. 3A

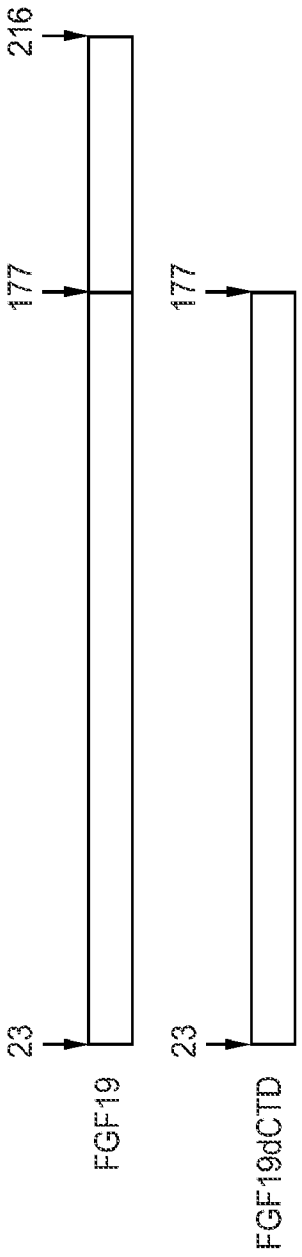


FIG. 3C

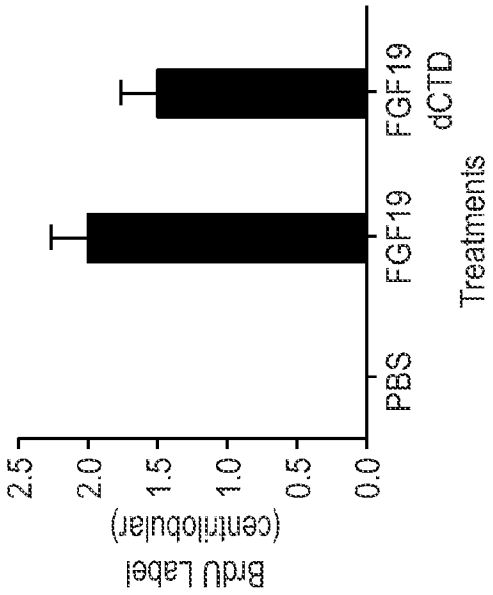


FIG. 3B

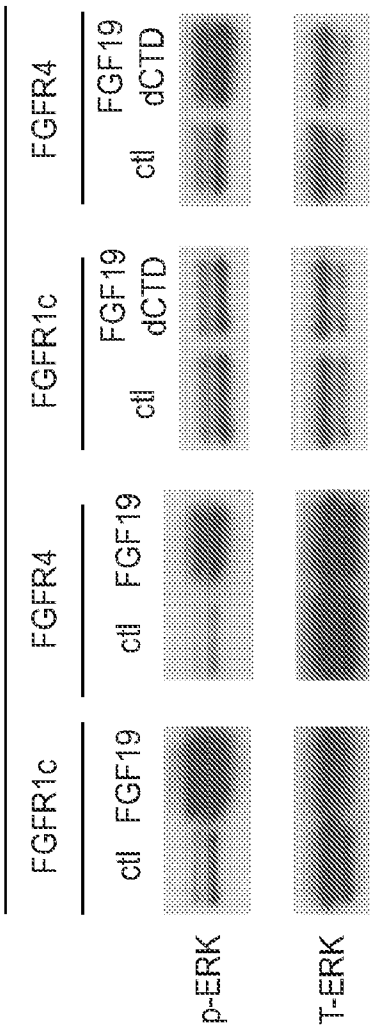


FIG. 4A

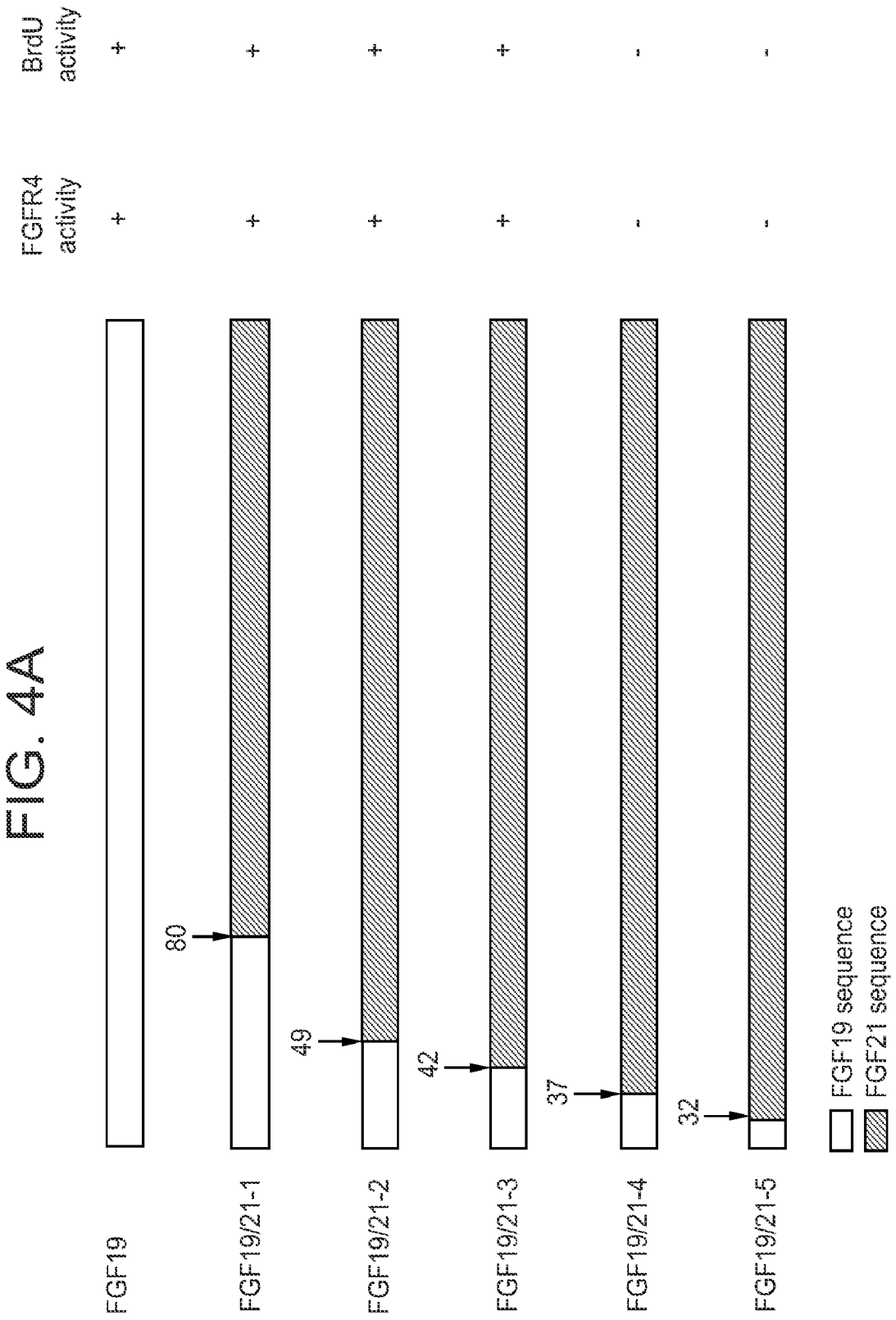

FGFR4 activity
BrdU activity
+
+
+
+
+
+
-
-
-
-
80
49
42
37
32
FGF19
FGF19/21-1
FGF19/21-2
FGF19/21-3
FGF19/21-4
FGF19/21-5
FGF19 sequence
FGF21 sequence

FIG. 4B

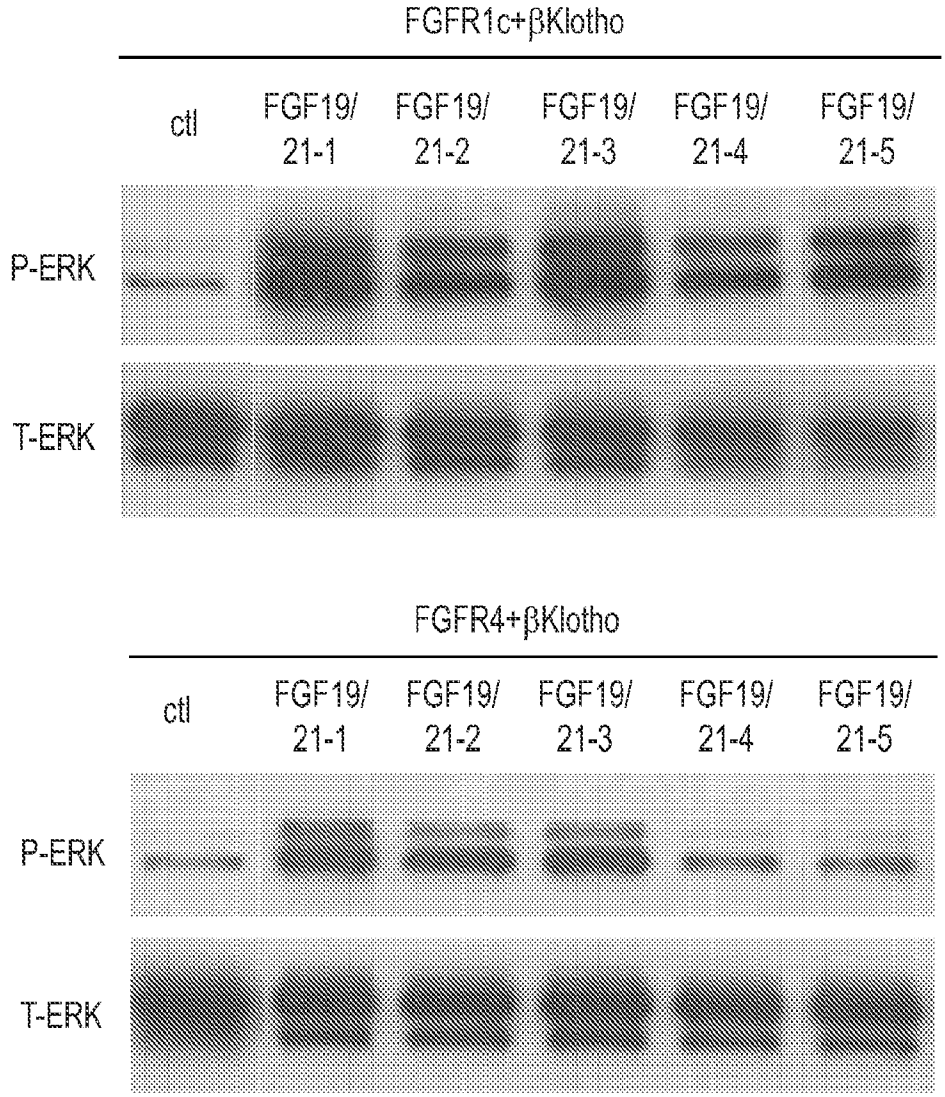

FGFR1c+βKlotho
ctl
FGF19/ 21-1
FGF19/ 21-2
FGF19/ 21-3
FGF19/ 21-4
FGF19/ 21-5
P-ERK
T-ERK
FGFR4+βKlotho
ctl
FGF19/ 21-1
FGF19/ 21-2
FGF19/ 21-3
FGF19/ 21-4
FGF19/ 21-5
P-ERK
T-ERK

FIG. 4C

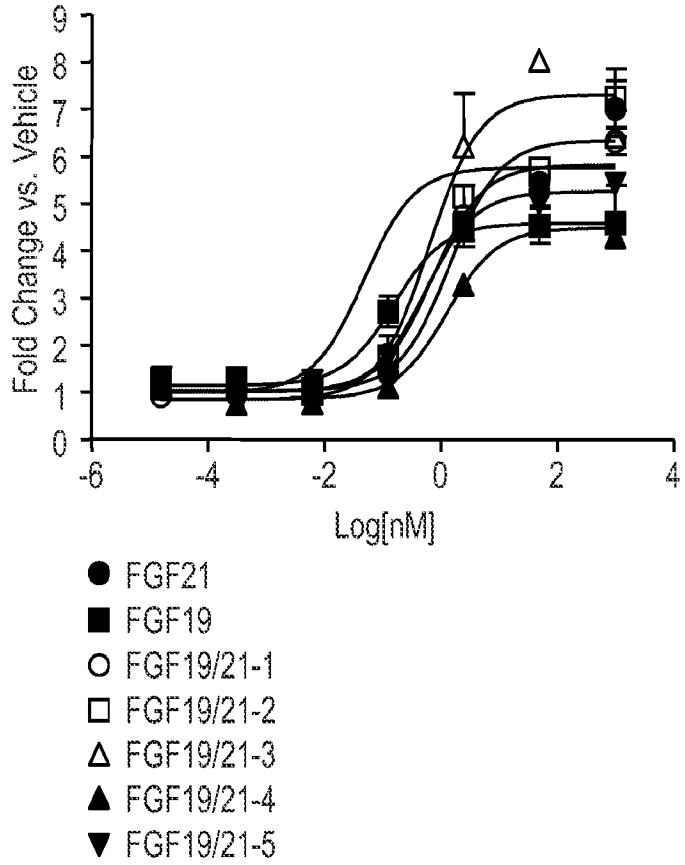

Fold Change vs. Vehicle
0
1
2
3
4
5
6
7
8
9
-6
-4
-2
0
2
4
Log[nM]
FGF21
FGF19
FGF19/21-1
FGF19/21-2
FGF19/21-3
FGF19/21-4
FGF19/21-5

FIG. 4D

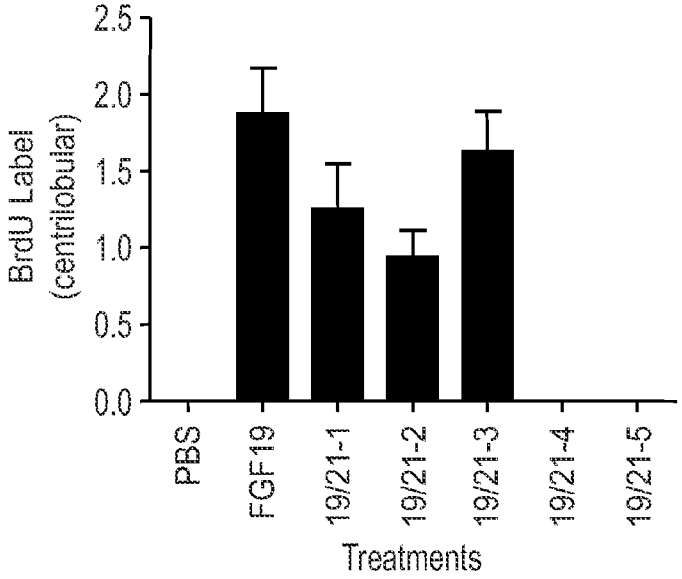

BrdU Label (centrilobular)
0.0
0.5
1.0
1.5
2.0
2.5
PBS
FGF19
19/21-1
19/21-2
19/21-3
19/21-4
19/21-5
Treatments

FIG. 5A

FIG. 5B

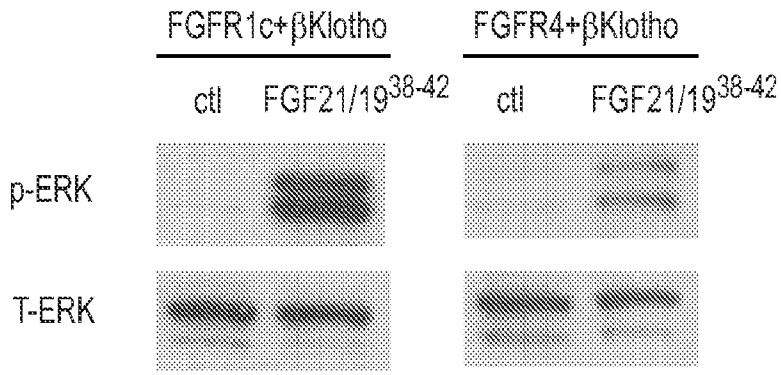
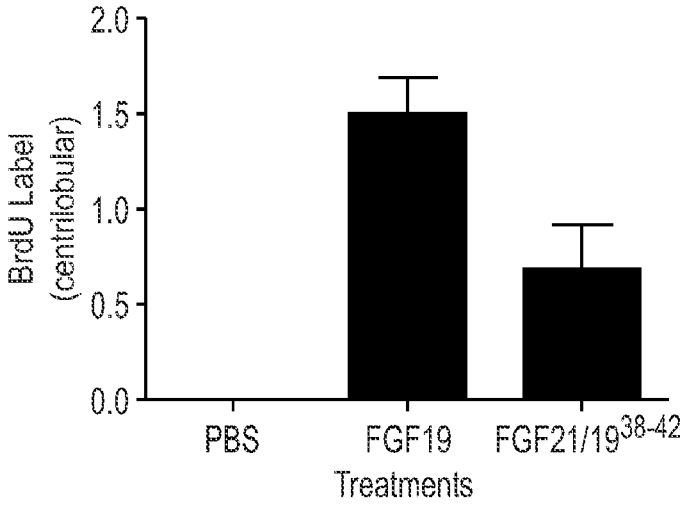


FIG. 5C



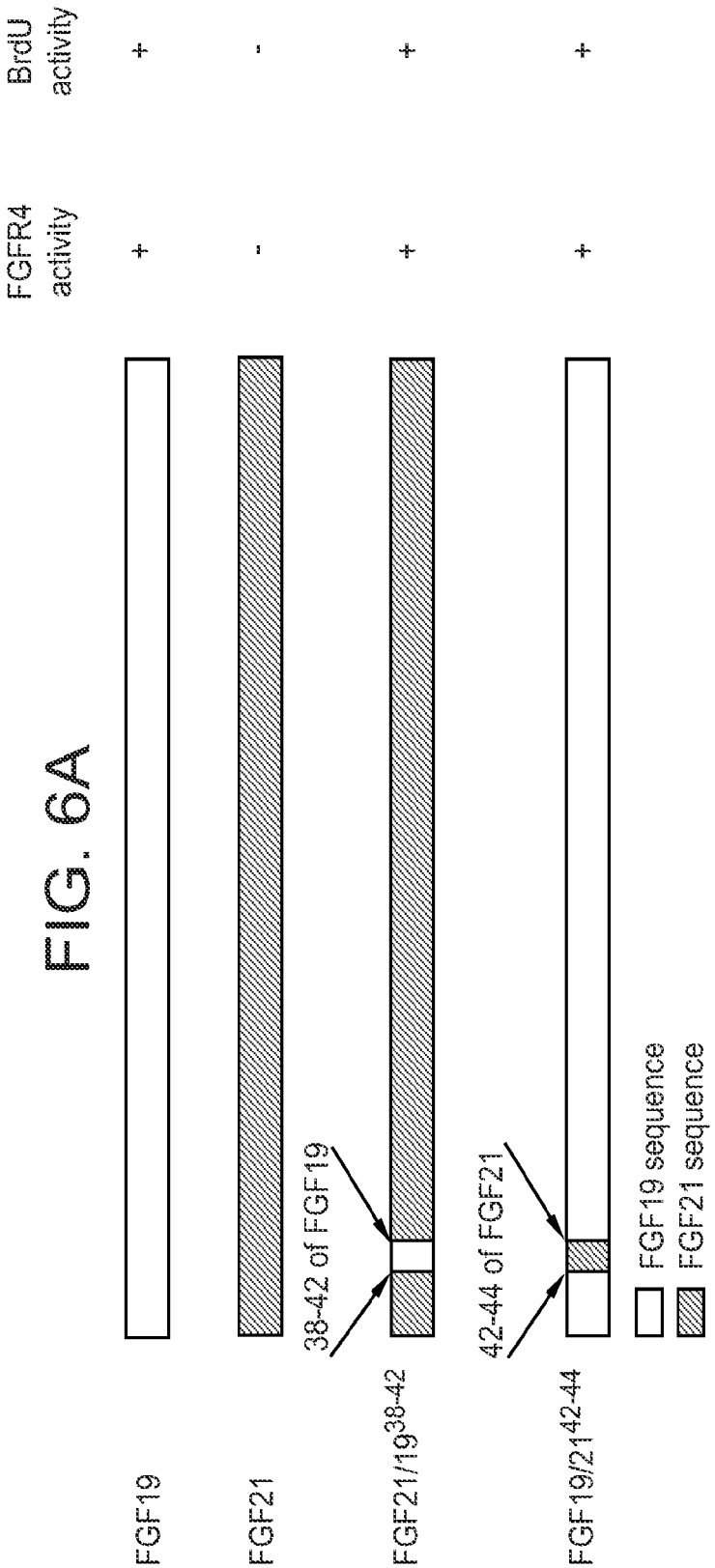


FIG. 6A

FIG. 6B

FGF19 29 DAGPHVHYGWGDPIRLRHLYT 49

FGF21 33 DSSPLLQFG--GQVRQRYLYT 51

FGF23 27 NASPLLGSSWGG---LIHLYT 44

FIG. 6C

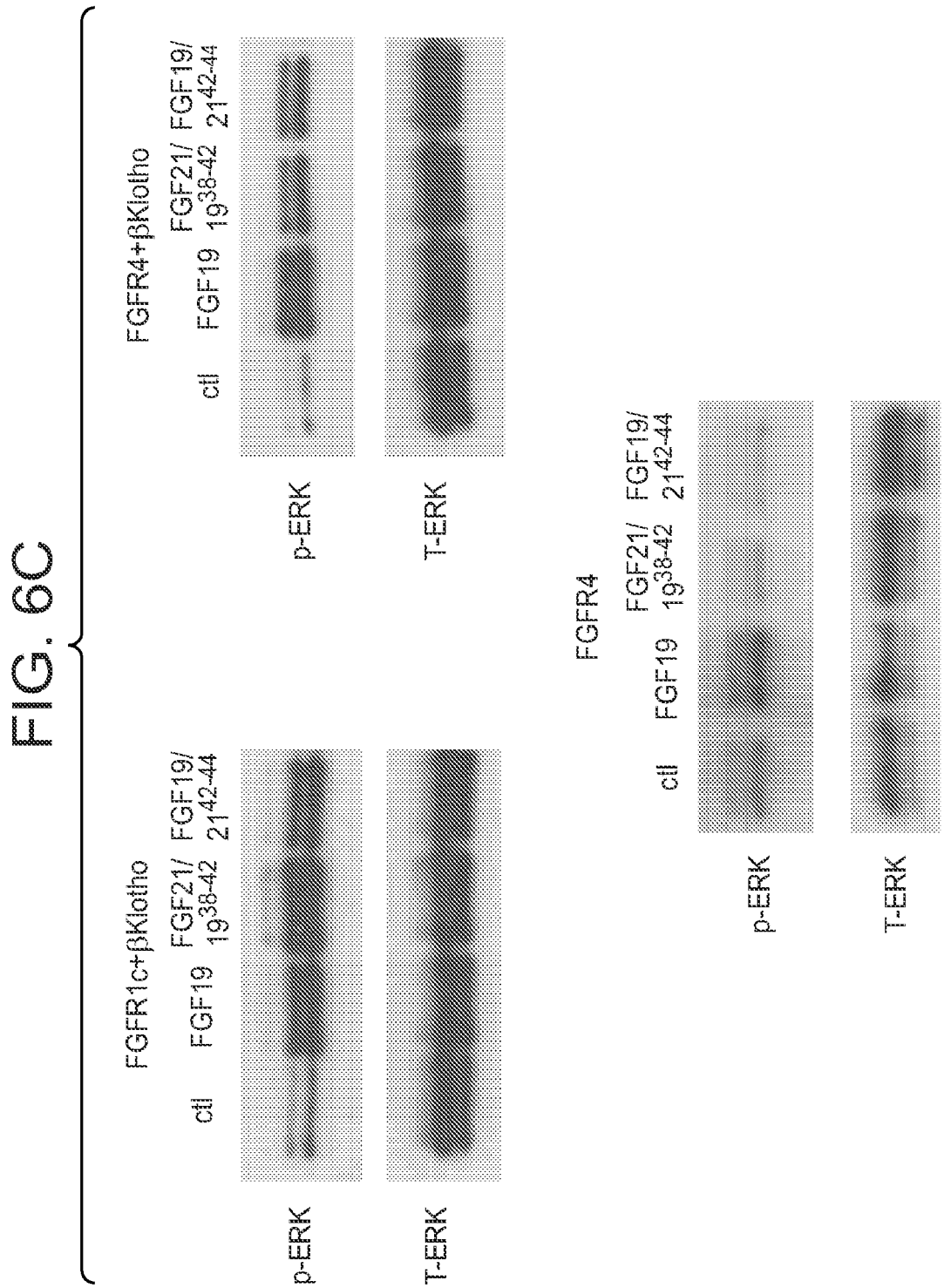

FGFR1c+βKlotho
ctl
FGF19
FGF21/ 19^38-42
FGF19/ 21^42-44
p-ERK
T-ERK
FGFR4+βKlotho
ctl
FGF19
FGF21/ 19^38-42
FGF19/ 21^42-44
p-ERK
T-ERK
FGFR4
ctl
FGF19
FGF21/ 19^38-42
FGF19/ 21^42-44
p-ERK
T-ERK

FIG. 6D-1

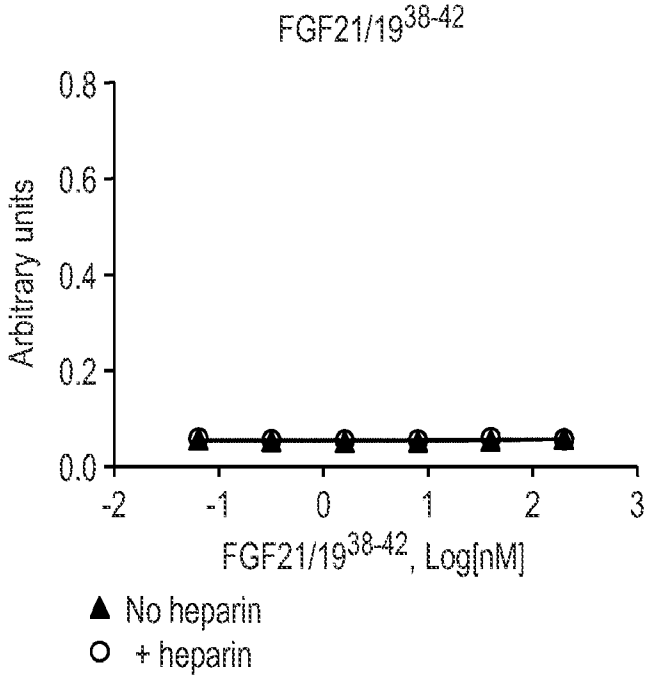


FIG. 6D-2

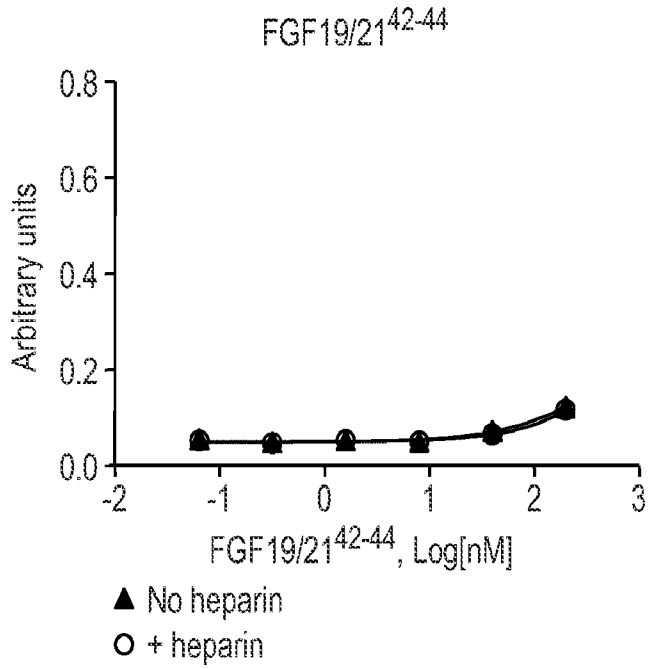


FIG. 6E

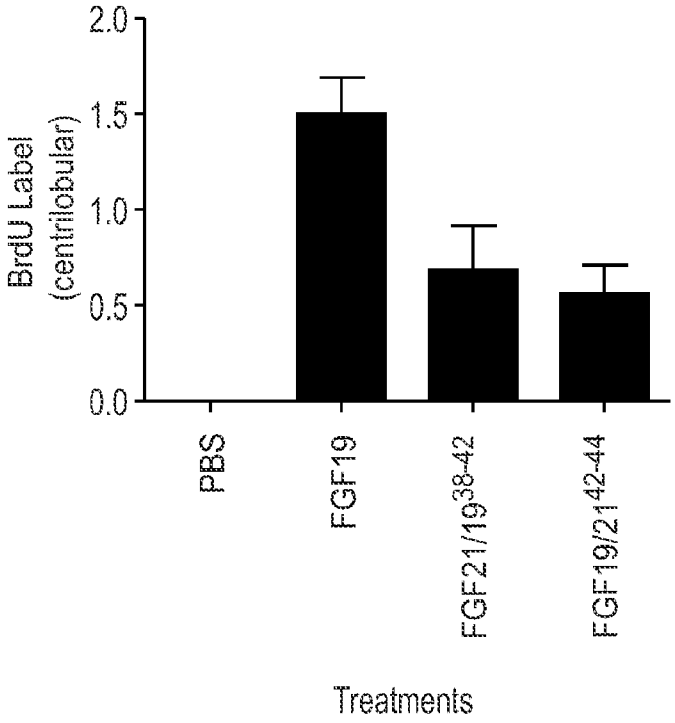

2.0
1.5
1.0
0.5
0.0
BrdU Label
(centrilobular)
PBS
FGF19
FGF21/19 38-42
FGF19/21 42-44
Treatments

FIG. 7

β1-β2 loop

FGF19	47	LYTSGPHGLSSCFL	60
FGF21	49	LYTDDAQ-QTEAHL	61
FGF23	42	LYTATAR--NSYHL	53

β10-β12 segment

FGF19	141	LPVSLSSAKQ RQLYKNRGFL PLSHFLPM	168
FGF21	142	LPLHLPGN-- KSPHRDPAPR GPARFLPL	167
FGF23	134	FLVSLGRAK- RAFLPGMNPP PYSQFLSR	160

FIG. 8A

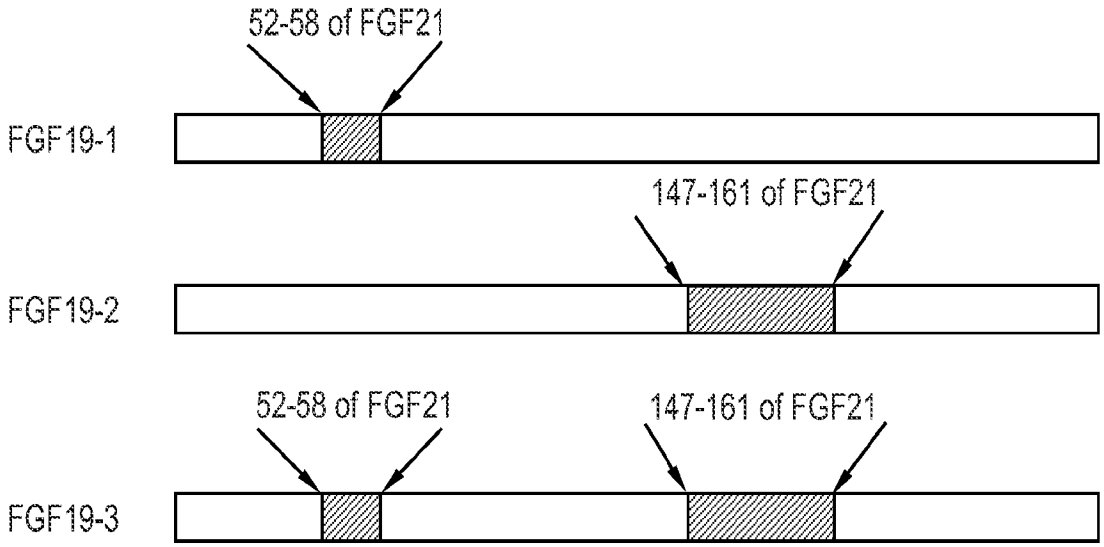

52-58 of FGF21
FGF19-1
147-161 of FGF21
FGF19-2
52-58 of FGF21
147-161 of FGF21
FGF19-3

FIG. 8B

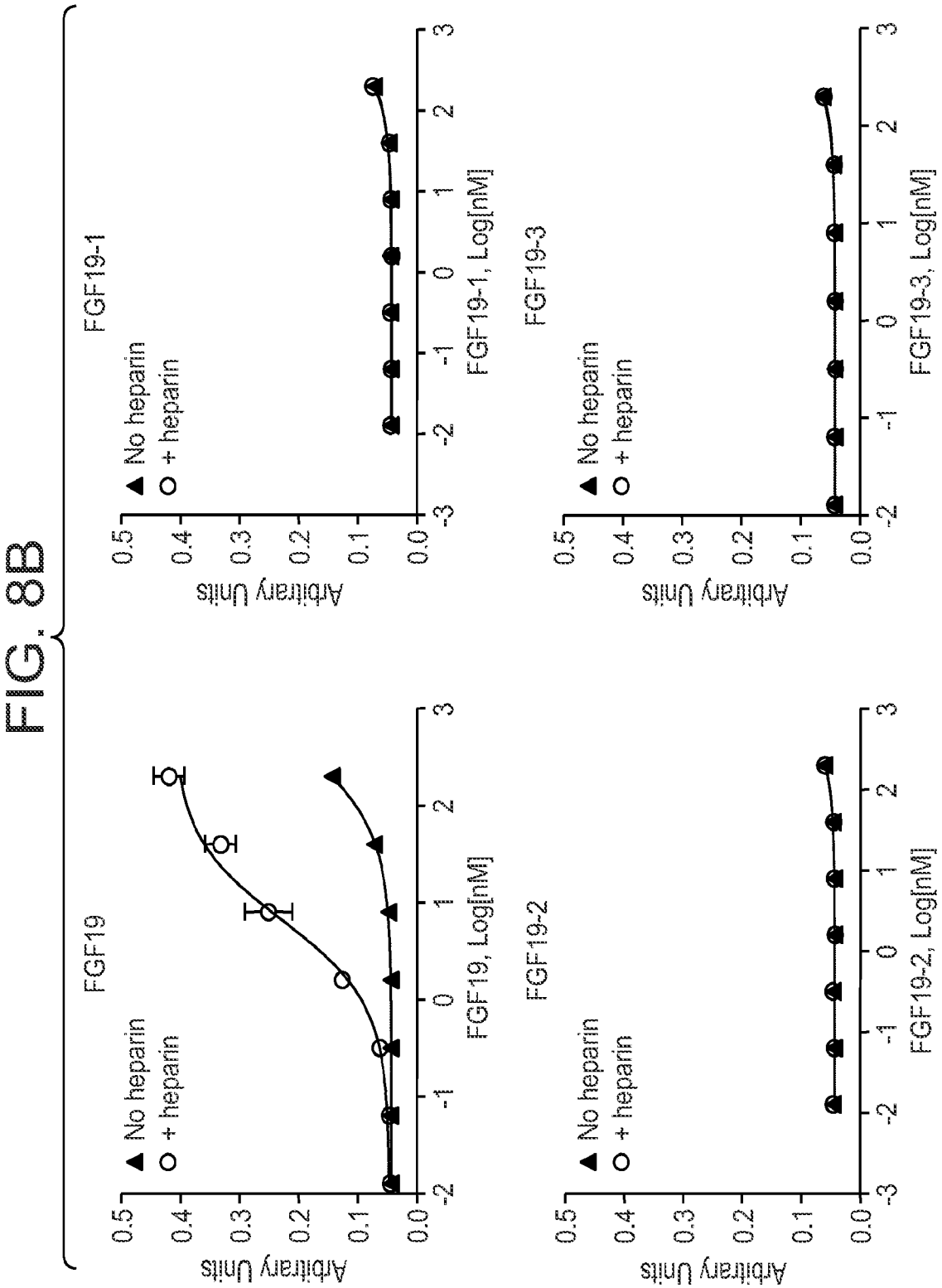

FGF19
No heparin
+ heparin
Arbitrary Units
FGF19, Log[nM]
FGF19-1
FGF19-1, Log[nM]
FGF19-2
FGF19-2, Log[nM]
FGF19-3
FGF19-3, Log[nM]

FIG. 8C

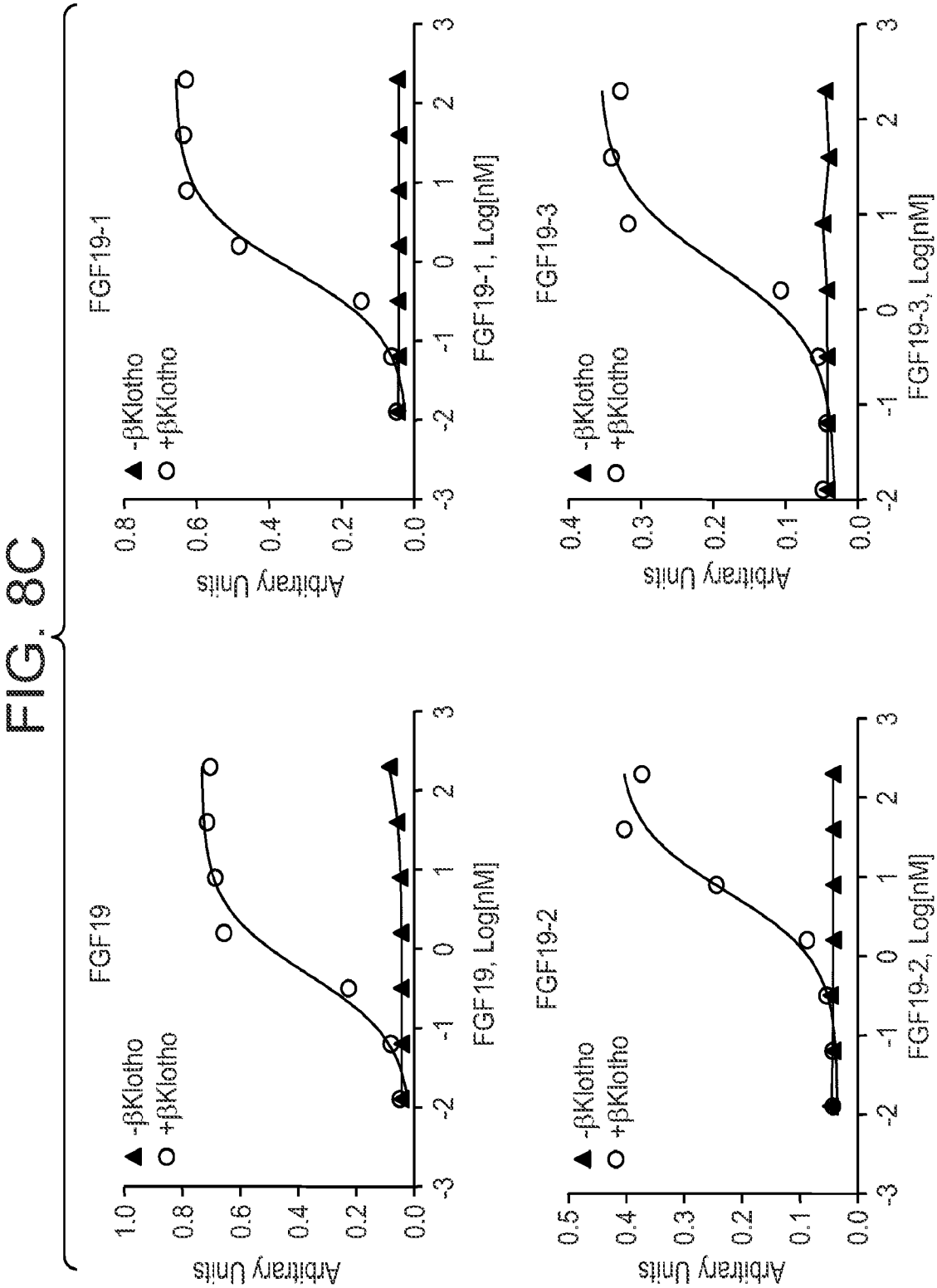

FGF19
-βKlotho
+βKlotho
Arbitrary Units
FGF19, Log[nM]
FGF19-1
FGF19-1, Log[nM]
FGF19-2
FGF19-2, Log[nM]
FGF19-3
FGF19-3, Log[nM]

FIG. 8D

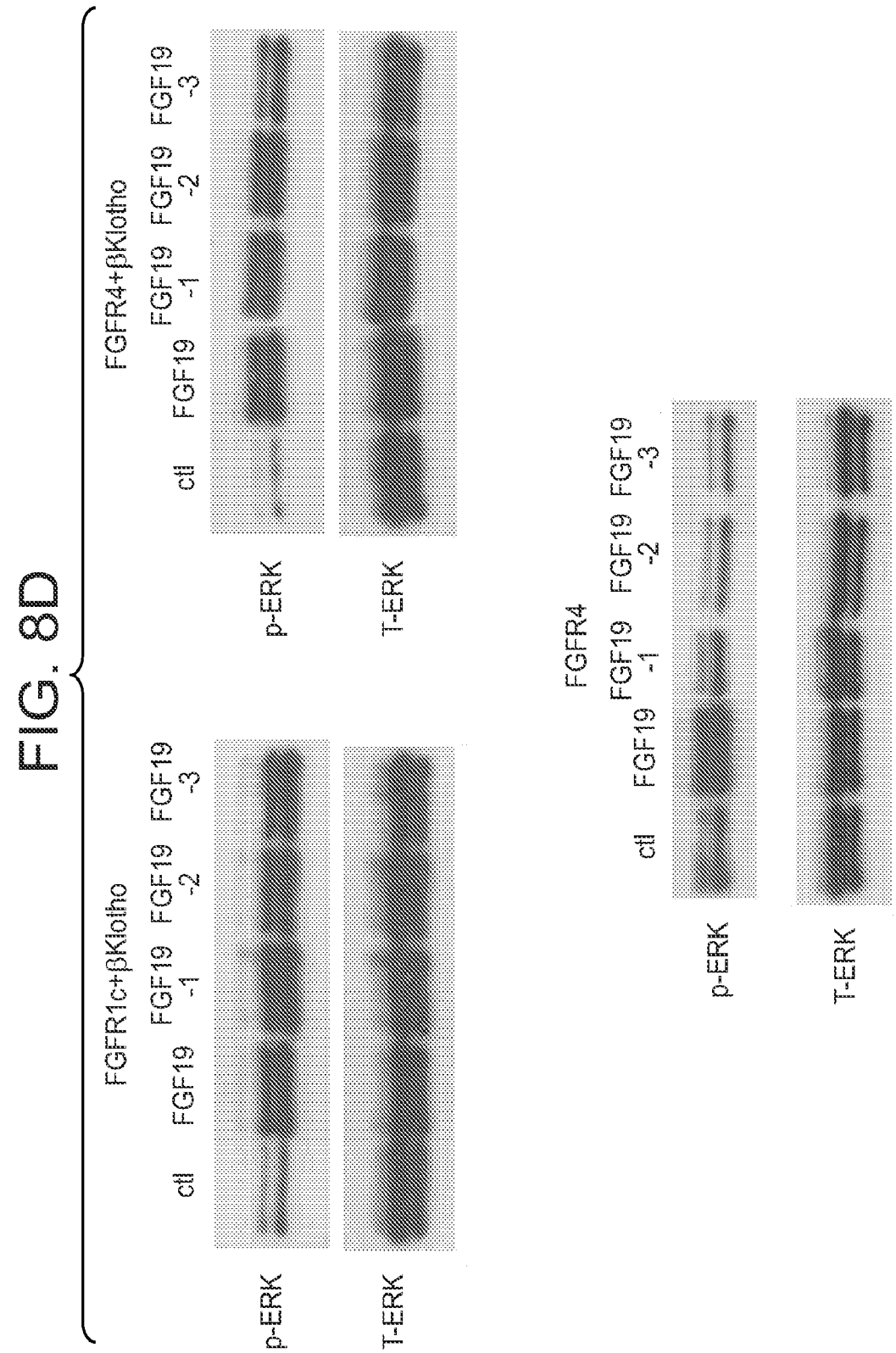

FGFR1c+βKlotho
ctl
FGF19
FGF19 -1
FGF19 -2
FGF19 -3
p-ERK
T-ERK
FGFR4+βKlotho
ctl
FGF19
FGF19 -1
FGF19 -2
FGF19 -3
p-ERK
T-ERK
FGFR4
ctl
FGF19
FGF19 -1
FGF19 -2
FGF19 -3
p-ERK
T-ERK

FIG. 8E

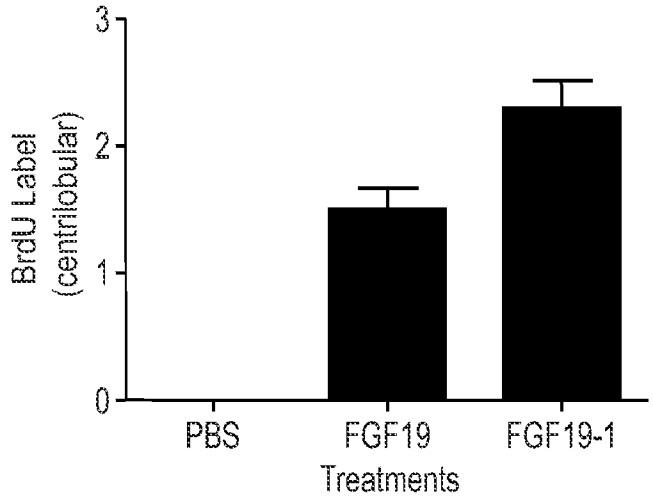

3
2
1
0
BrdU Label
(centrilobular)
PBS
FGF19
FGF19-1
Treatments

FIG. 9A

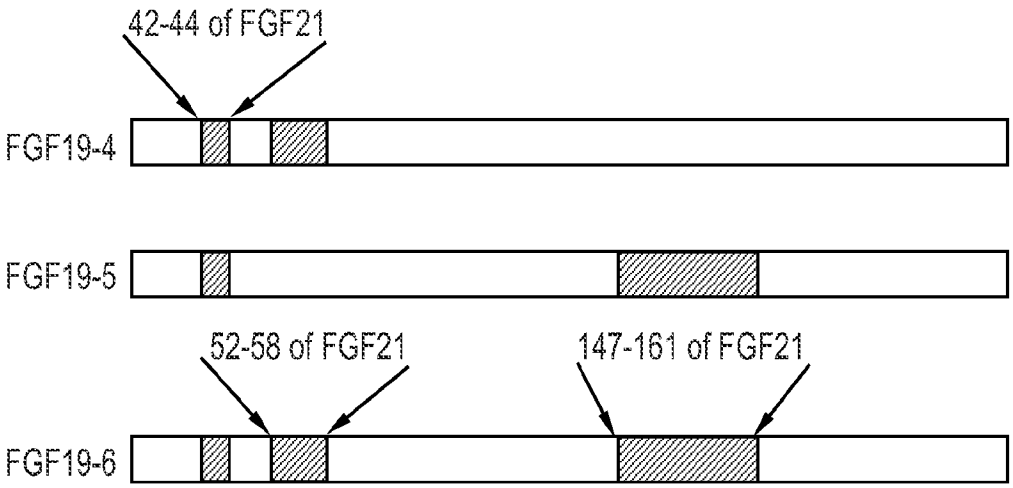

42-44 of FGF21
FGF19-4
FGF19-5
52-58 of FGF21
147-161 of FGF21
FGF19-6

FIG. 9B

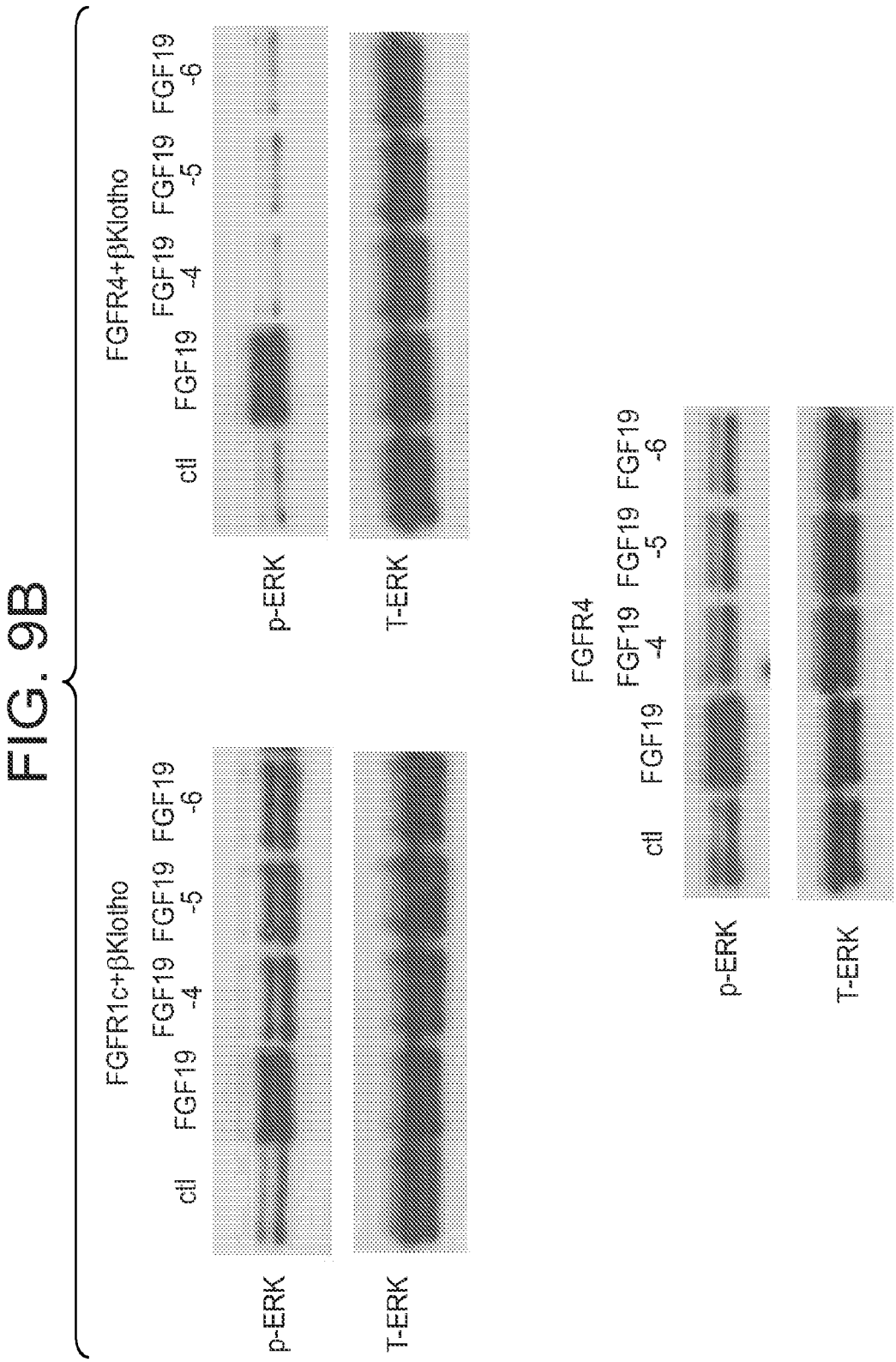

FGFR1c+βKlotho
ctl
FGF19
FGF19 -4
FGF19 -5
FGF19 -6
p-ERK
T-ERK
FGFR4+βKlotho
ctl
FGF19
FGF19 -4
FGF19 -5
FGF19 -6
p-ERK
T-ERK
FGFR4
ctl
FGF19
FGF19 -4
FGF19 -5
FGF19 -6
p-ERK
T-ERK

FIG. 9C

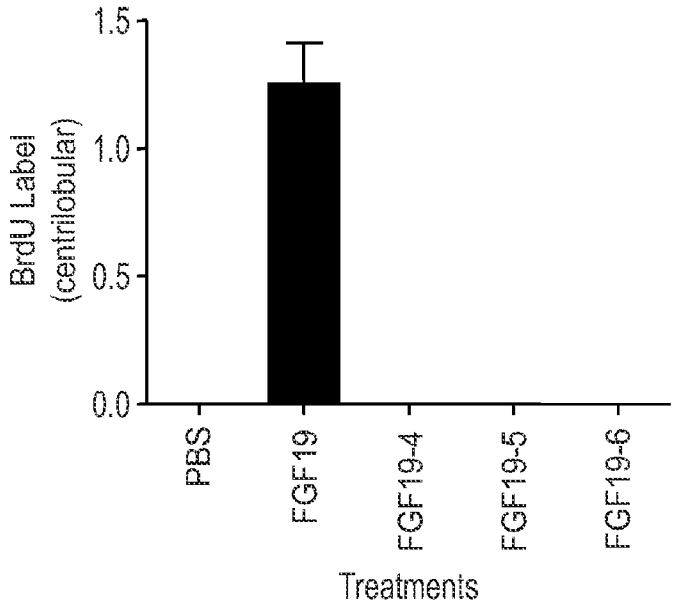

1.5
1.0
0.5
0.0
BrdU Label
(centrilobular)
PBS
FGF19
FGF19-4
FGF19-5
FGF19-6
Treatments

FIG. 10A

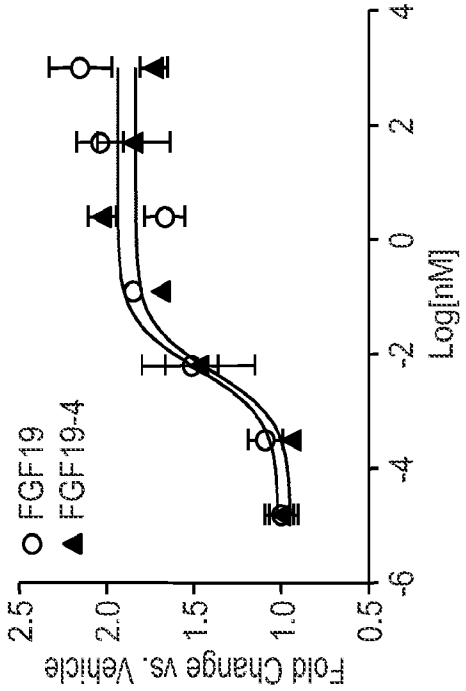


FIG. 10B-1

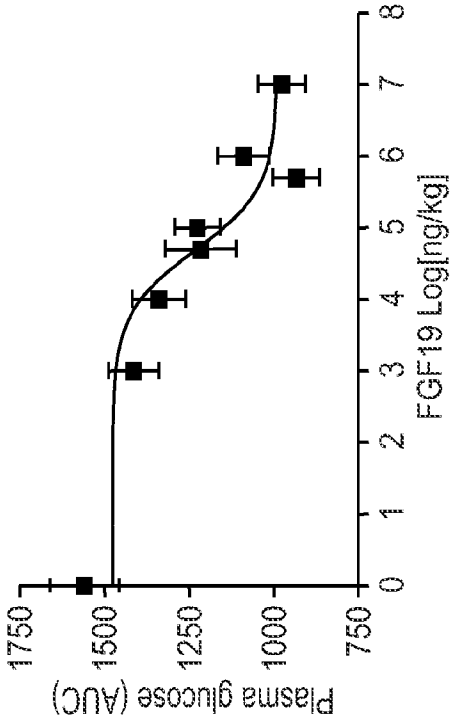


FIG. 10B-2

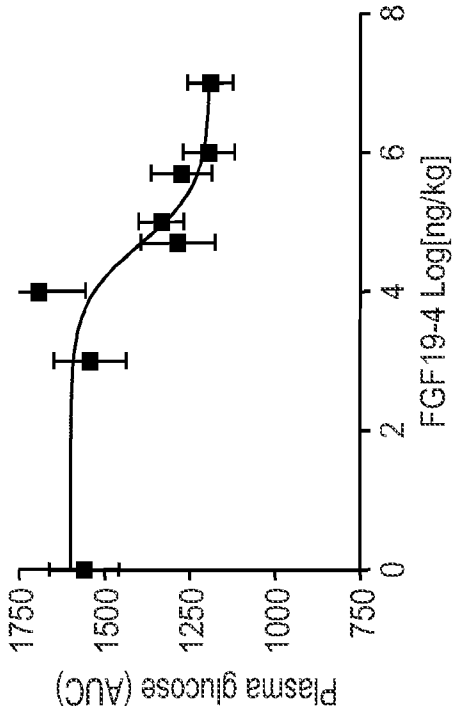


FIG. 10C

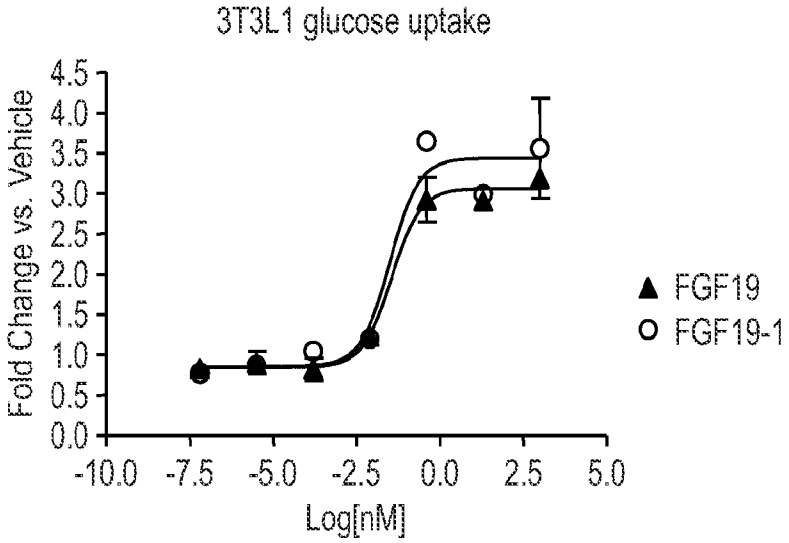


FIG. 10D

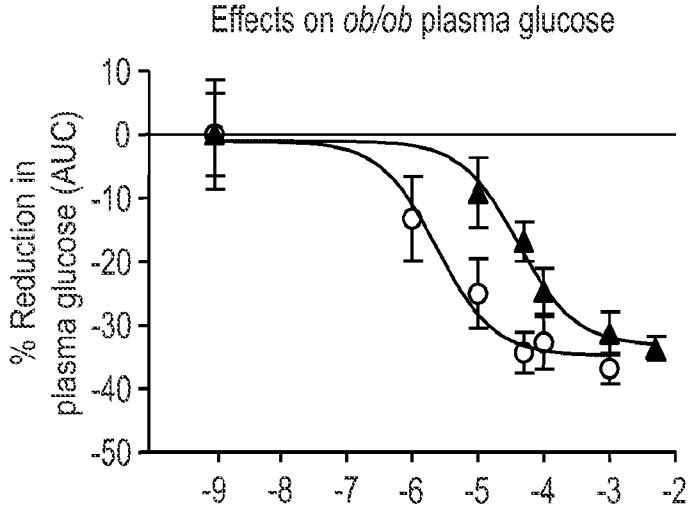


FIG. 11

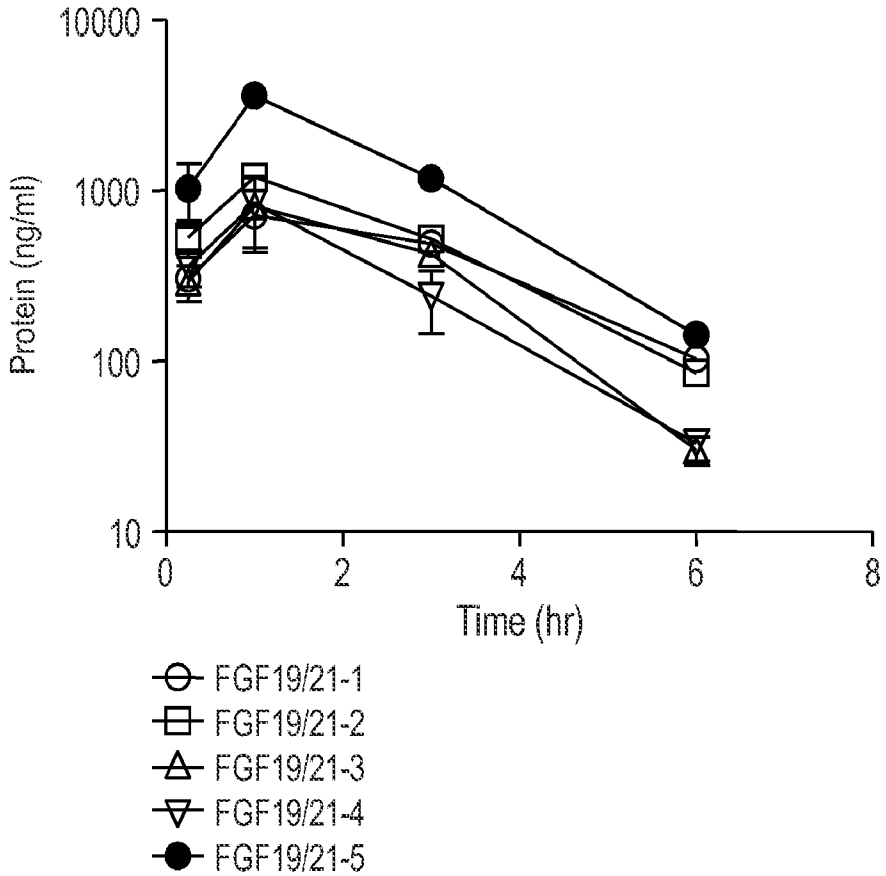

10000
1000
100
10
Protein (ng/ml)
0
2
4
6
8
Time (hr)
FGF19/21-1
FGF19/21-2
FGF19/21-3
FGF19/21-4
FGF19/21-5

FIG. 12

		FGFR1c	FGFR4
FGF19	WGDPI	+	+
FGF19-4	-GQ-V	+	-
FGF19-mut22	-GD-I	-	-
FGF19-mut23	WG-PI	-	+/-
FGF19-mut24	WGDPV	+	+
FGF19-mut25	WGD-I	-	-
FGF19-mut26	-GDPI	-	-
FGF19-mut27	-G-PI	-	-
FGF19-mut28	WGQPI	+	+
FGF19-mut29	WGAPI	+	+
FGF19-mut30	AGDPI	-	-
FGF19-mut31	WADPI	+	+
FGF19-mut32	WGDAI	+	+
FGF19-mut33	WGDPA	+	+
FGF19-mut34	W-DPI	+/-	+/-
FGF19-mut35	WGD-I	-	-
FGF19-mut36	WGDP-	-	-
FGF19-mut37	FGDPI	-	-

FIG. 13A

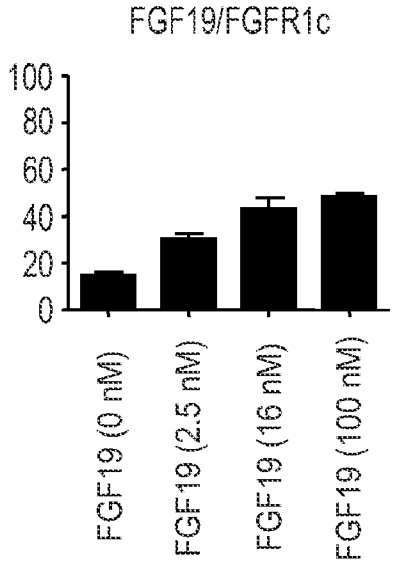


FIG. 13B

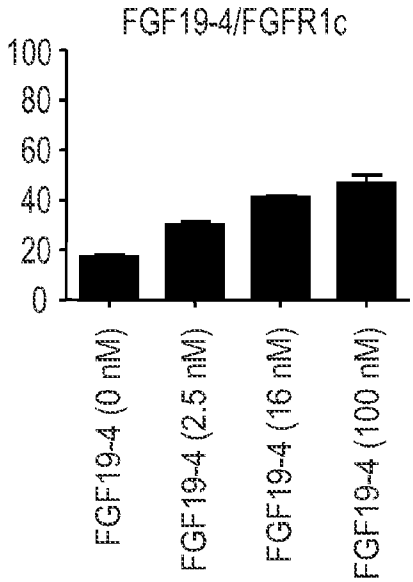


FIG. 13C

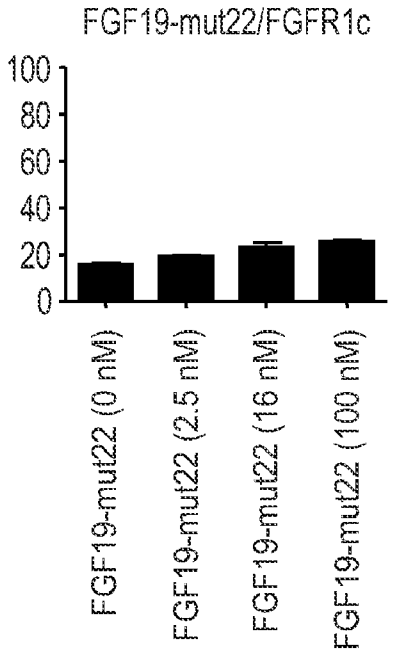


FIG. 13D

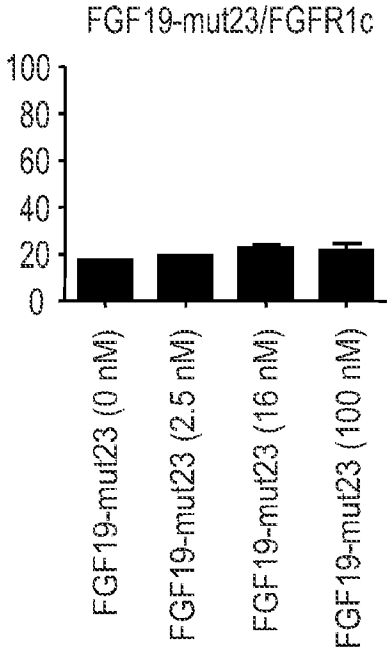


FIG. 13E

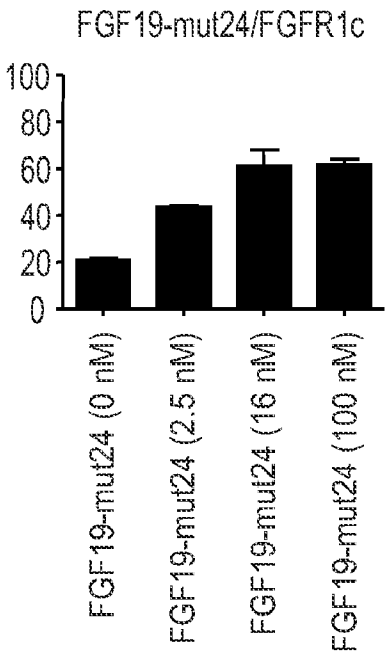


FIG. 13F

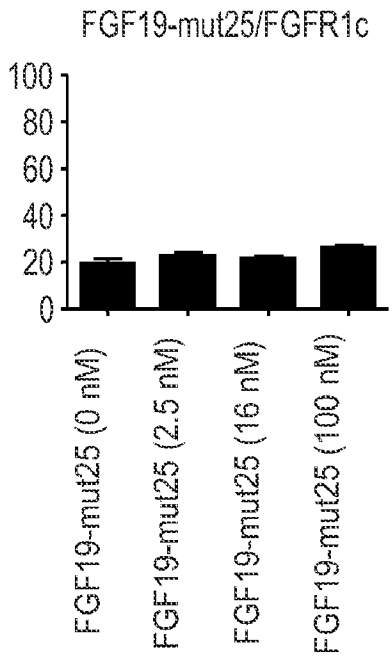


FIG. 13G

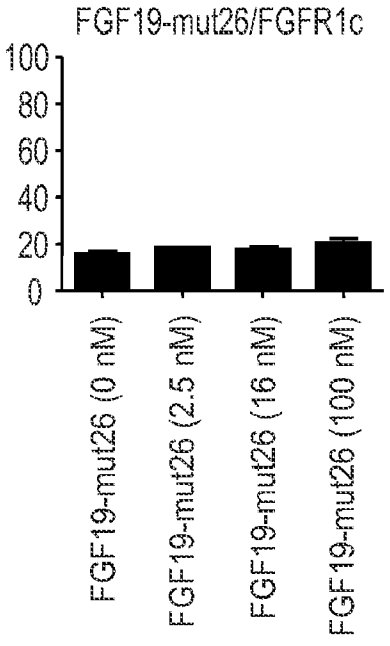


FIG. 13H

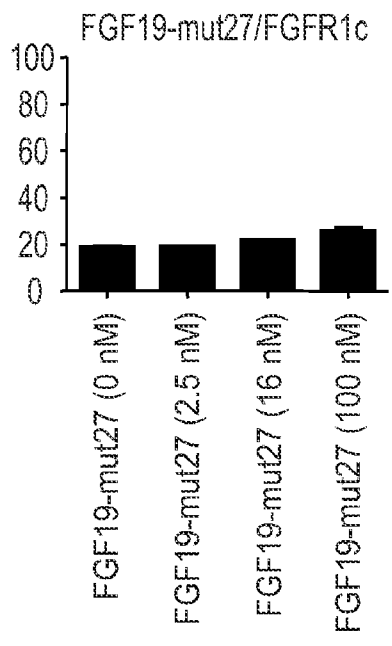


FIG. 13I

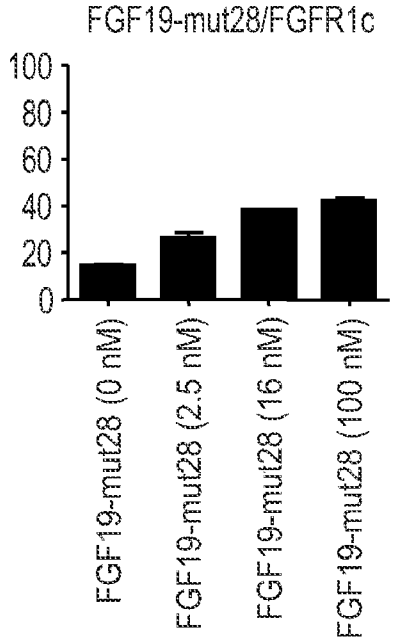


FIG. 13J

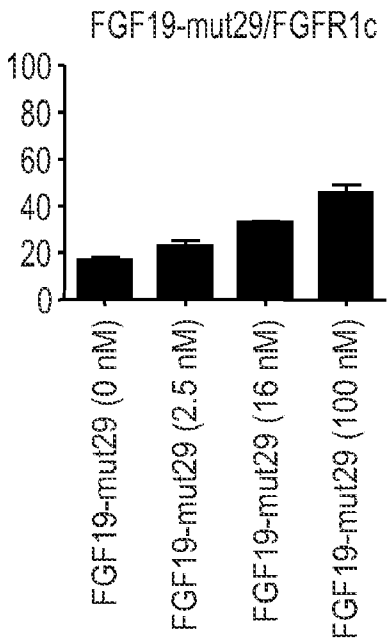


FIG. 13K

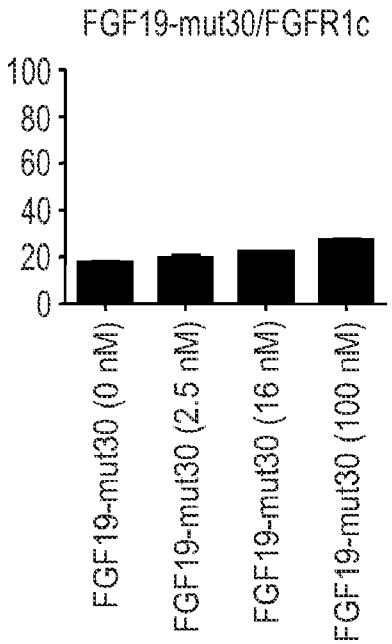


FIG. 14A

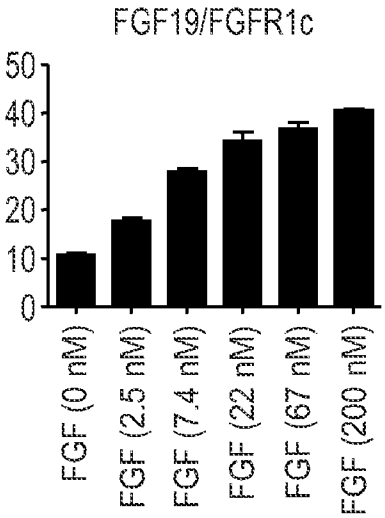


FIG. 14B

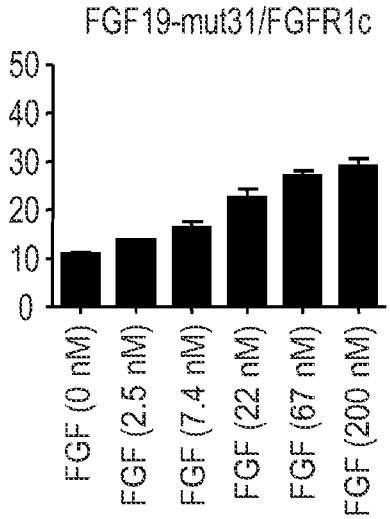


FIG. 14C

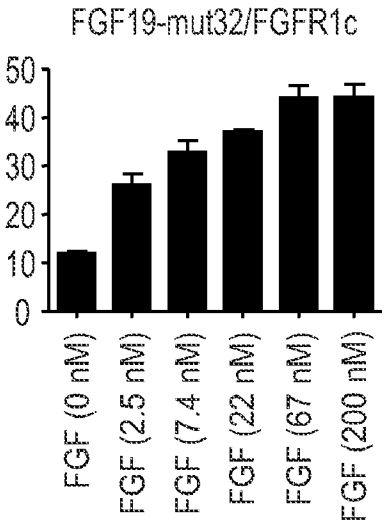


FIG. 14D

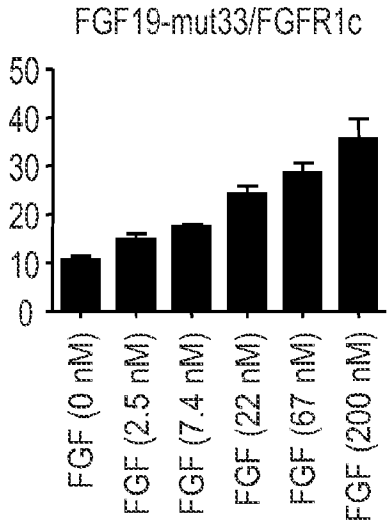


FIG. 14E

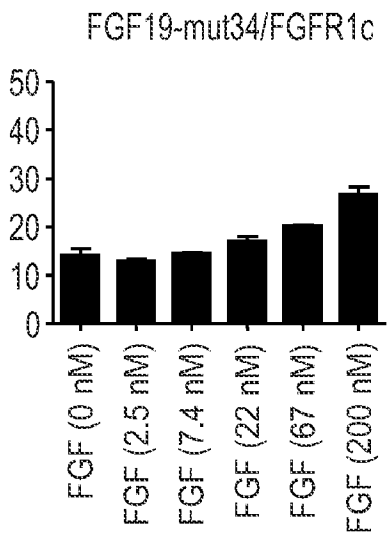


FIG. 14F

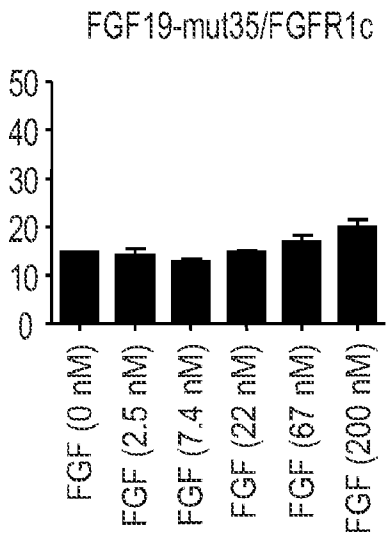


FIG. 14G

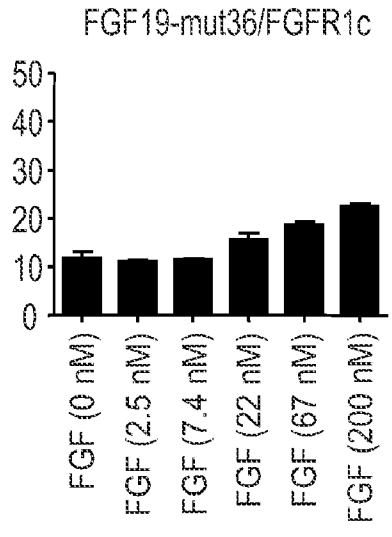


FIG. 14H

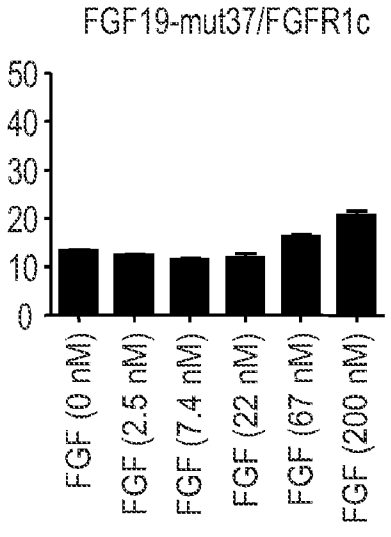


FIG. 15A

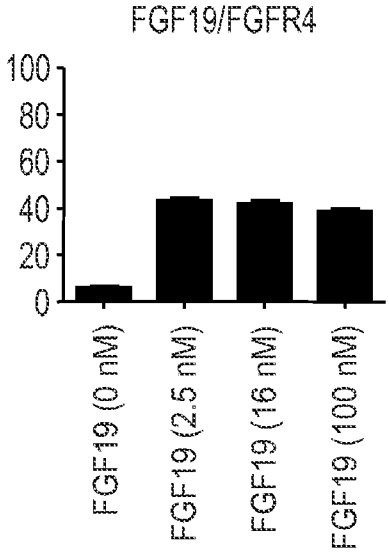


FIG. 15B

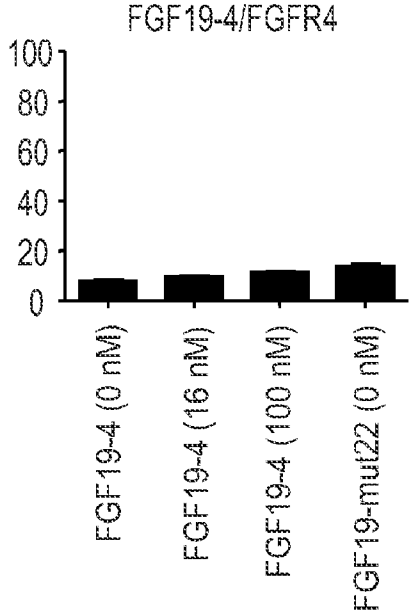


FIG. 15C

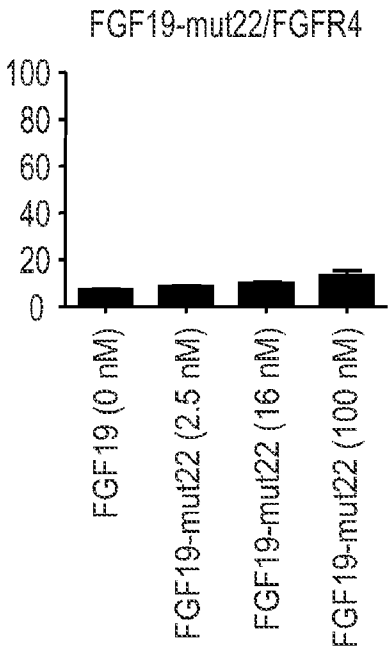


FIG. 15D

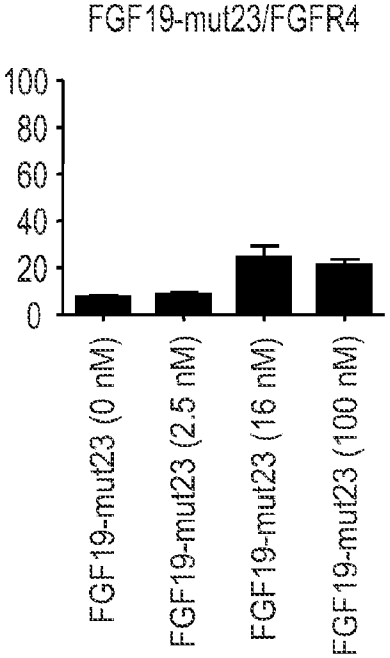


FIG. 15E

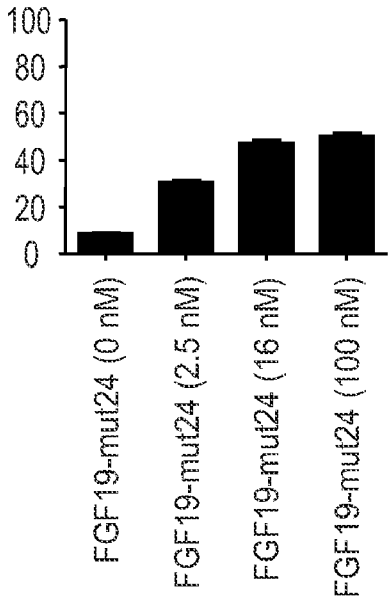


FIG. 15F

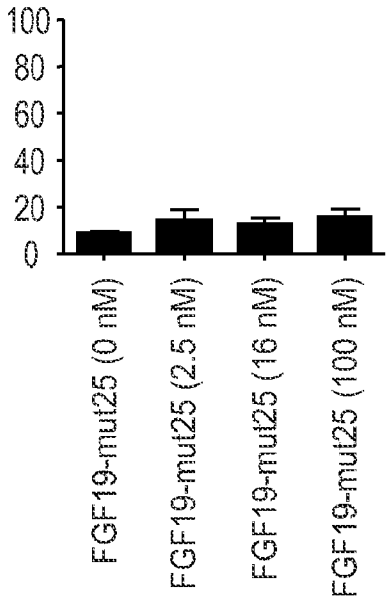


FIG. 15G

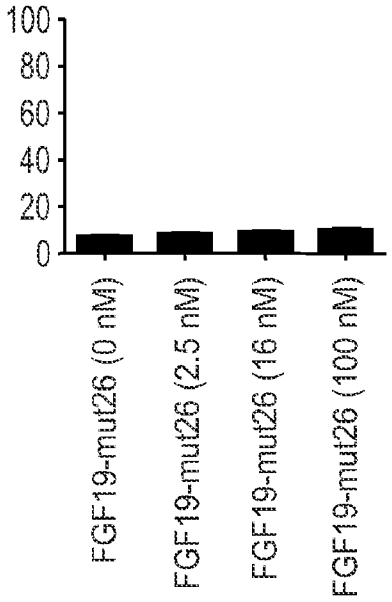


FIG. 15H

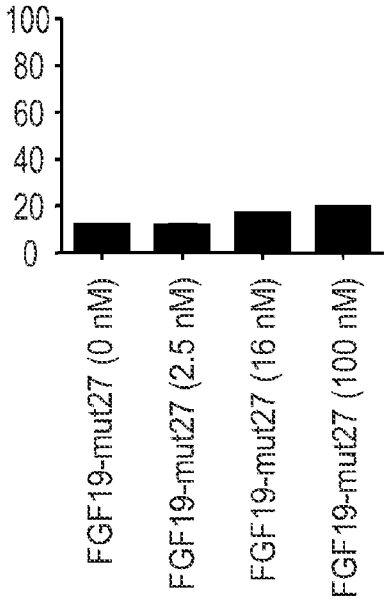


FIG. 15I

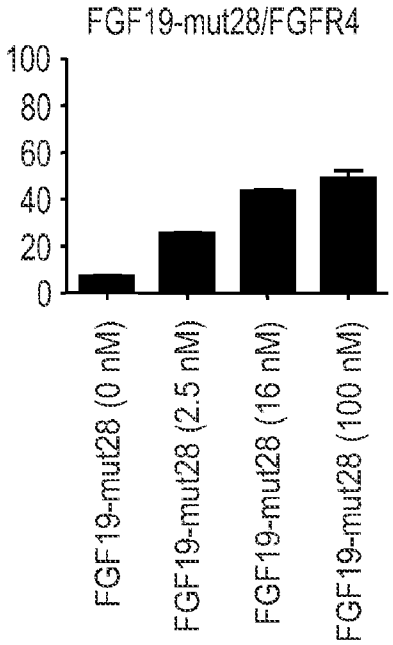


FIG. 15J

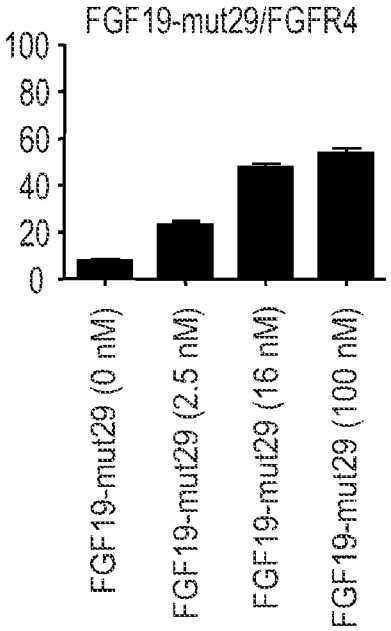


FIG. 15K

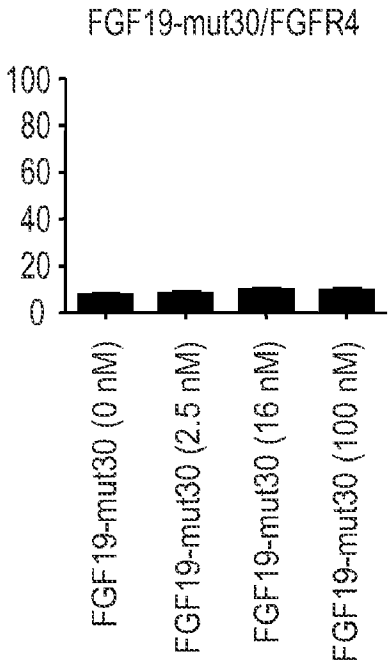


FIG. 16A

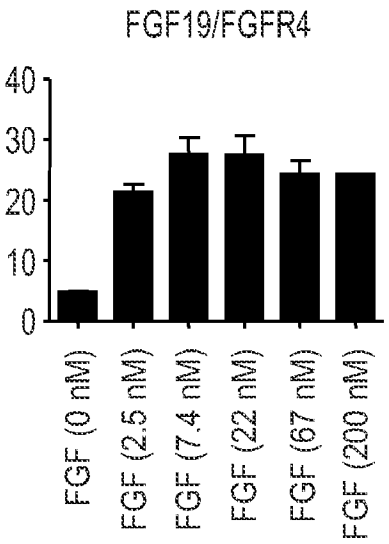


FIG. 16B

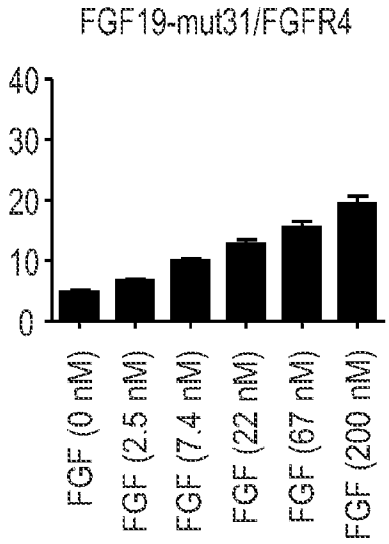


FIG. 16C

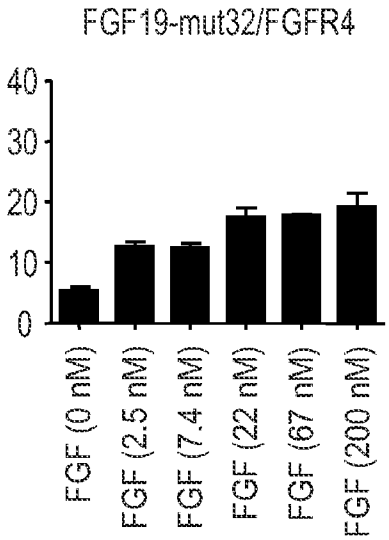


FIG. 16D

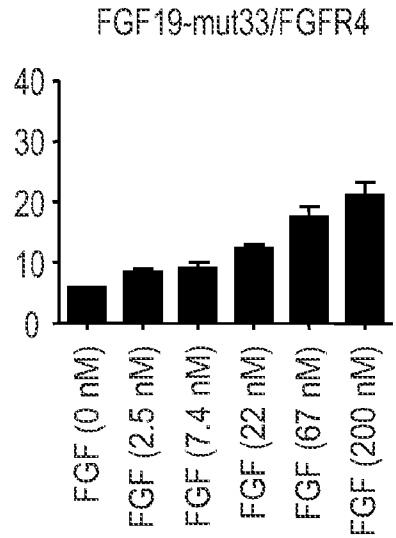


FIG. 16E

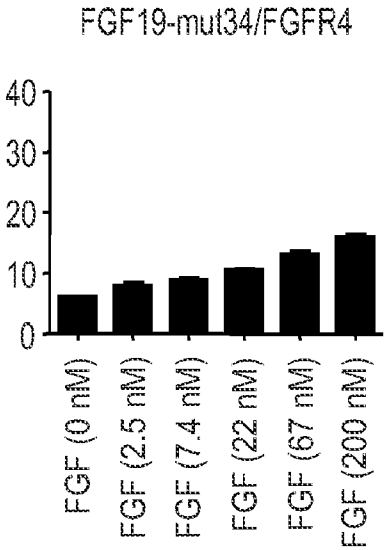


FIG. 16F

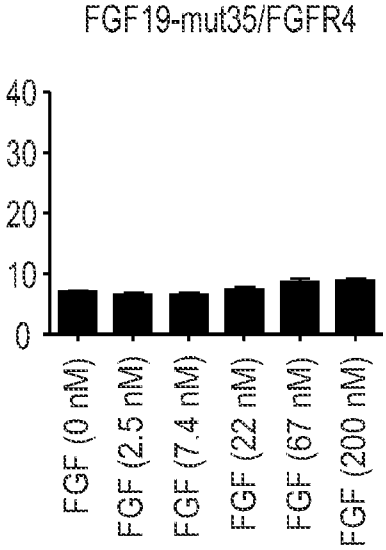


FIG. 16G

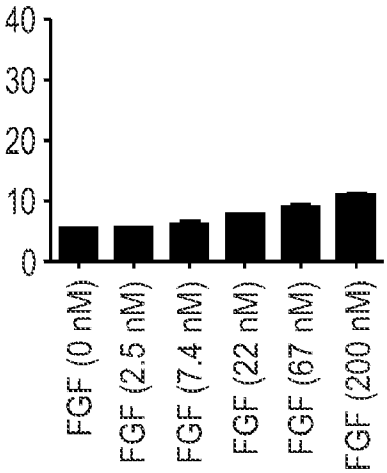
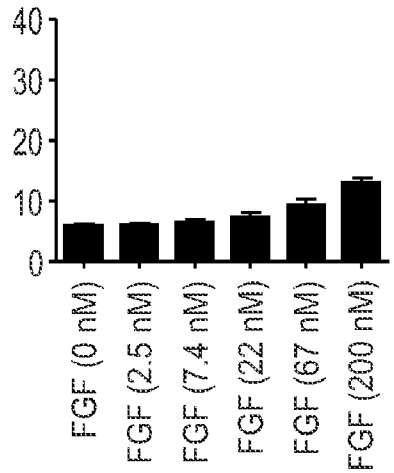


FIG. 16H



PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference A-1498-WO-PC	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2010/038894	International filing date *(day/month/year)* 16/06/2010	(Earliest) Priority Date *(day/month/year)* 17/06/2009
Applicant AMGEN INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 8 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box No. II)

3. [X] **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**
   [ ] the text is approved as submitted by the applicant
   [X] the text has been established by this Authority to read as follows:

   CHIMERIC FGF19 POLYPEPTIDES AND USES THEREOF

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      [ ] as suggested by the applicant
      [ ] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [X] none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2010/038894

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   [ ] on paper

   [X] in electronic form

   b. (time)

   [X] in the international application as filed

   [ ] together with the international application in electronic form

   [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/038894

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/50

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)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GOETZ REGINA ET AL: "Molecular insights into the klotho-dependent, endocrine mode of action of fibroblast growth factor 19 subfamily members." MOLECULAR AND CELLULAR BIOLOGY MAY 2007 LNKD- PUBMED:17339340, vol. 27, no. 9, May 2007 (2007-05), pages 3417-3428, XP002596984 ISSN: 0270-7306 cited in the application the whole document	1-6, 15-36
A	WO 02/36732 A2 (PROCHON BIOTECH LTD [IL]; BOGIN OREN [IL]; ADAR RIVKA [IL]; YAYON AVNE) 10 May 2002 (2002-05-10) claims 1,3,10	1-6, 15-36

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 August 2010	10/11/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pilat, Daniel

4

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WU X ET AL: "C-terminal tail of FGF19 determines its specificity toward Klotho co-receptors" JOURNAL OF BIOLOGICAL CHEMISTRY 20081128 US LNKD- DOI:10.1074/JBC.M803319200, vol. 283, no. 48, 28 November 2008 (2008-11-28), pages 33304-33309, XP002596985 the whole document	1-6, 15-36
X	WO 01/18209 A1 (CURAGEN CORP [US]; SHIMKETS RICHARD A [US]; VERNET CORINE [US]; BURGES) 15 March 2001 (2001-03-15) the whole document claims; table 2; sequence 2	1-5, 15-18, 20-24, 27-30, 32-36
X	HARMER NICHOLAS J ET AL: "The crystal structure of fibroblast growth factor (FGF) 19 reveals novel features of the FGF family and offers a structural basis for its unusual receptor affinity." BIOCHEMISTRY 27 JAN 2004 LNKD- PUBMED:14730967, vol. 43, no. 3, 27 January 2004 (2004-01-27), pages 629-640, XP002596986 ISSN: 0006-2960 the whole document page 631, column 1, paragraph 3	1-3, 27-30, 33-36
X	DATABASE UniProt [Online] 1 March 2001 (2001-03-01), "SubName: Full=Fibroblast growth factor 19;" XP002596987 retrieved from EBI accession no. UNIPROT:Q9DDN0 Database accession no. Q9DDN0 compound	1-5, 15-18, 20-24, 27-29, 35,36
X	DATABASE UniProt [Online] 5 July 2004 (2004-07-05), "SubName: Full=Fibroblast growth factor 19; Flags: Fragment;" XP002596988 retrieved from EBI accession no. UNIPROT:Q76B59 Database accession no. Q76B59 compound	1-5, 15-18, 20-24, 27-29, 35,36

-/--

4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/038894

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE UniProt [Online] 10 February 2009 (2009-02-10), "SubName: Full=FGF19;" XP002596989 retrieved from EBI accession no. UNIPROT:B7U4G3 Database accession no. B7U4G3 compound -----	1-5, 15-18, 20-24, 27-29, 35,36
X	DATABASE UniProt [Online] 22 July 2008 (2008-07-22), "SubName: Full=Fgf19 protein;" XP002596990 retrieved from EBI accession no. UNIPROT:B3DHS4 Database accession no. B3DHS4 compound -----	1-5, 15-18, 20-24, 27-29, 35,36
A	WO 99/27100 A1 (GENENTECH INC [US]; BOTSTEIN DAVID [US]; GODDARD AUDREY [US]; GURNEY A) 3 June 1999 (1999-06-03) claims 26-30 -----	32,34

4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2010/038894

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

2-6, 15-26(completely); 1, 27-36(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 2-6, 15-26(completely); 1, 27-36(partially)

a chimeric polypeptide comprising a wild type mature FGF19 polypeptide scaffold comprising SEQ ID NO:4, further comprising a modification that decreases FGFR4-mediated signaling activity, wherein the modification comprises substituting one or more of the residues WGDPI at positions 16-20 of the FGF19 polypeptide scaffold with (a) no amino acid; or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence and embodiments dependent thereon.

---

2. claims: 7-10(completely); 1, 27-36(partially)

a chimeric polypeptide comprising a wild type mature FGF19 polypeptide scaffold comprising SEQ ID NO:4, further comprising a modification that decreases FGFR4-mediated signaling activity, wherein the modification comprises substituting one or more of the residues SGPHGLSS at positions 28-35 of the FGF19 polypeptide scaffold with (a) no amino acid; or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence and embodiments dependent thereon.

---

3. claims: 11-14(completely); 27-36(partially)

a chimeric polypeptide comprising a wild type mature FGF19 polypeptide scaffold comprising SEQ ID NO:4, further comprising a modification that decreases FGFR4-mediated signaling activity, wherein the modification comprises substituting one or more of the residues SSAKQRQLYKNRGFLPL at positions 124-140 of the FGF19 polypeptide scaffold with (a) no amino acid; or (b) an amino acid other than the amino acid located at the position in the wild type amino acid sequence and embodiments dependent thereon.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2010/038894

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0236732 A2	10-05-2002	AU 1089302 A	15-05-2002
		CA 2427477 A1	10-05-2002
		EP 1583817 A2	12-10-2005
		US 2004014658 A1	22-01-2004
WO 0118209 A1	15-03-2001	AU 7368100 A	10-04-2001
WO 9927100 A1	03-06-1999	AT 397063 T	15-06-2008
		AU 733222 B2	10-05-2001
		CA 2309783 A1	03-06-1999
		DK 1032668 T3	29-09-2008
		EP 1032668 A1	06-09-2000
		JP 3653469 B2	25-05-2005
		JP 2002503446 T	05-02-2002
		NZ 504425 A	26-10-2001