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(54) **Title**: METHODS AND COMPOSITIONS USING FGF23 FUSION POLYPEPTIDES

(57) **Abstract**: The present disclosure is directed to methods, kits and compositions for preventing or treating age-related conditions or metabolic disorders. The fusion polypeptides of the disclosure include FGF23 or an active fragment thereof. In one embodiment, the fusion polypeptide comprises (a) a polypeptide comprising fibroblast growth factor 23 (FGF23), or a functionally active variant or derivative thereof, wherein FGF23 has a mutation at one or more of the positions Q 156, C206 and C244; and (b) either a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life, or a polypeptide comprising at least one extracellular subdomain of a Klotho protein, or a functionally active variant or derivative thereof; and, optionally (c) a linker. The Klotho fusion proteins are useful in the treatment and prevention of a variety of age-related conditions and metabolic disorders. In another embodiment, the fusion polypeptide comprises a FGF (such as FGF23), or a functionally active variant or derivative thereof; and a modified Fc fragment, or a functionally active variant or derivative thereof. In various embodiments of the fusion polypeptides, FGF23 has mutations which decrease aggregation and protease-mediated cleavage.

METHODS AND COMPOSITIONS USING FGF23 FUSION POLYPEPTIDES

This application claims priority to U.S. Application Serial No. 12/696693, filed January 29, 2010, the contents of which are incorporated herein by reference in their entirety.

1. BACKGROUND

Fibroblast growth factors (FGFs) constitute a family of homologous polypeptide growth factors expressed in many organisms (Ornitz and Itoh, Genome Biol. 2: reviews, 3005.1-3005.12 (2001)). Among vertebrate species, FGFs are highly conserved in both gene structure and amino-acid sequence, having between 13-71% amino acid identity with one another. In humans, there are 22 known members of the FGF family (FGF15 is the mouse ortholog of human FGF19, hence there is no human FGF15). During early development, FGFs regulate cell proliferation, migration, and differentiation, but in the adult organism, FGFs maintain homeostasis, function in tissue repair, and respond to injury.

FGFs function as growth factors by binding and thereby activating cell-surface FGF receptors. FGF receptors (FGFRs) are tyrosine kinase receptors that activate signal transduction through autophosphorylation of FGFR, phosphorylation of FRS2 (FGF receptor substrate 2) and ERK1/2 (extracellular signal-regulated protein kinase 1/2), and activating Egr-1 (early growth response-1). FGFs also have a high affinity for heparin sulfate proteoglycans. When bound to FGFs, heparin sulfate enhances the activation of FGFRs.

The alpha-Klotho gene encodes a 130 kDa single pass type I transmembrane protein with an extracellular domain and a short cytoplasmic domain. The extracellular domain of alpha-Klotho protein comprises two subdomains termed, KL-D1 and KL-D2. These two subdomains share sequence homology to β-glucosidase of bacteria and plants. The extracellular domain of the alpha-Klotho protein may be bound to the cell surface by the transmembrane domain or may be cleaved and released into the extracellular milieu. Cleavage of the extracellular domain appears to be facilitated by local low extracellular Ca^{2+} concentrations.

In addition to alpha-Klotho, a homolog of alpha-Klotho, beta-Klotho, has been identified (Ito et al., Mech. Dev. 98:115-9 (2000)). Beta-Klotho is also a single pass type I transmembrane protein with extracellular KL-D1 and KL-D2 subdomains.

Modulation of alpha-Klotho expression has been demonstrated to produce aging related characteristics in mammals. Mice homozygous for a loss of function mutation in the alpha-Klotho gene develop characteristics resembling human aging, including shortened lifespan, skin atrophy, muscle wasting, arteriosclerosis, pulmonary emphysema and osteoporosis (Kuro-o et al., Nature, 390:45-51 (1997)). In contrast, overexpression of the alpha-Klotho gene in mice extends lifespan and increases resistance to oxidative stress relative to wild-type mice (Kurosu et al., Science 309:1829–1833 (2005); Yamamoto et al., J. Biol. Chem. 280:38029–38034 (2005)).

Recent studies have demonstrated strikingly similar biological characteristics between FGF23-deficient mice and alpha-Klotho-deficient mice (Shimada et al., J. Clin. Invest. 113:561-568 (2004); Yoshida et al. Endocrinology 143:683-689 (2002)), indicating functional crosstalk between FGF23 and alpha-Klotho. These studies led to the identification of alpha-Klotho as an obligatory partner of FGF23, in terms of both binding and signaling through its cognate FGF receptors (Urakawa et al., Nature 22:1524-6 (2007)). The alpha-Klotho gene is mainly expressed in kidney, parathyroid gland and choroid plexus. It is hypothesized that the tissue-specific expression of alpha-Klotho restricts activation of FGF23 signaling to those tissues.

Similar to FGF23/alpha-Klotho, beta-Klotho is an obligatory partner of FGF19 and FGF21, both in binding and in signaling through their respective cognate FGF receptors (Ogawa et al., Proc. Natl. Acad. Sci. USA 104:7432-7 (2007); Lin et al., J. Biol Chem. 282:27227-84 (2007); and Wu et al., J. Biol. Chem. 282:29069-72 (2007)). Such studies have also demonstrated the involvement of beta-Klotho in regulating tissue-specific metabolic activity. Beta-Klotho was initially shown to act with FGF21 as a cofactor for regulating carbohydrate and lipid metabolism in adipose tissue. Beta-Klotho in conjunction with FGF19 regulates bile acid metabolism in liver, thus explaining elevated bile synthesis in beta-Klotho deficient mice (Ito et al., J Clin Invest. 2005 Aug;115(8):2202-8).

U.S. Patent No. 6,579,850 describes polypeptides and compositions comprising an alpha-Klotho polypeptide. Human and mouse alpha-Klotho polypeptides are disclosed. The patent also disclosed that compositions comprising the polypeptides are useful in treating a syndrome resembling premature aging, treating adult diseases, and suppressing aging.

U.S. Patent No. 7,223,563 describes isolated nucleic acids encoding the FGF23 polypeptide sequence or recombinant cells comprising such an isolated nucleic acid. The

patent further relates to methods of diagnosing and treating hypophosphatemic and hyperphosphatemic disorders, osteoporosis, dermatomyositis, and coronary artery disease.

U.S. Patent No. 7,259,248 describes isolated nucleic acids encoding the FGF21 polypeptide sequence. The patent further relates to methods of diagnosing and treating liver disease, conditions related to thymic function, and methods of treating conditions of the testis.

2. SUMMARY OF THE INVENTION

The present disclosure is directed to methods, uses, kits and compositions for preventing or treating age-related conditions or metabolic disorders with fusion polypeptides or soluble polypeptides. The fusion polypeptides of the present disclosure are formed of a FGF (e.g., FGF23); and either a Klotho protein or an active fragment thereof (e.g., sKlotho) and/or a Fc fragment (e.g., FcLALA); and, optionally, a linker. In some embodiments, the FGF23 is mutated. In some embodiments, the present disclosure provides a Klotho fusion polypeptide comprising a Klotho protein or an active fragment thereof and a fibroblast growth factor or an active fragment thereof. In some embodiments, the fusion polypeptide comprises a Klotho polypeptide, a FGF (such as FGF23) and a modified Fc fragment. The Fc fragment can, for example, have decreased binding to Fc-gamma-receptor and increased serum half-life. Fusion proteins comprising sKlotho, FGF23 and FcLALA (a modified Fc fragment having decreased affinity for Fc-gamma-receptorand/or increased serum half-life) are described in SEQ ID NOs. 46, 47, 48, and 49. In some embodiments, the fusion polypeptide or protein comprises a FGF (e.g., FGF23), or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof; and a modified Fc fragment, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof. Fusion proteins comprising FGF23 and FcLALA are described in SEQ ID NOs. 50, 51, 52 and 53. In some embodiments, the fusion polypeptide has one or more mutations in FGF23 which decrease aggregation and/or protease-mediated cleavage.

In a first aspect, the disclosure provides a fusion polypeptide having at least one extracellular subdomain of a Klotho protein and a fibroblast growth factor or an active fragment thereof. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity (e.g., decreased Ka or increased Kd) for Fc-gamma-receptorand/or increased serum half-life. The Klotho extracellular domain may be derived

from either the alpha or beta Klotho isoforms. Further, although the FGF component of the Klotho fusion polypeptide is described primarily with reference to fibroblast growth factor-19, fibroblast growth factor-21 and fibroblast growth factor-23, it is contemplated that any of the twenty-three known FGFs can be used in practicing the disclosure. The reader of the instant application may assume that each of every combination of alpha or beta extracellular domain with each human FGF protein or an active fragment thereof are individually and specifically contemplated.

According to the present disclosure, the extracellular domain of the Klotho protein can include one or both of the KL-D1 and KL-D2 domains of a Klotho protein, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof. In some embodiments, the Klotho fusion polypeptide of the disclosure has at least two extracellular subdomains of a Klotho protein, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof. For example, the at least two extracellular subdomains can be at least two KL-D1 domains in tandem repeats, at least two KL-D2 domains in tandem repeats, or at least one KL-D1 domain and at least one KL-D2 domain. In various embodiments, the fusion polypeptide of the disclosure comprises amino acids 28-292 of the full length alpha Klotho protein, or amino acids 28-982 (SEQ ID NO: 7). In another embodiment, the fusion polypeptide of the disclosure comprises amino acids 52-997 of the full length beta Klotho protein.

In one embodiment of the present disclosure, the components of a fusion polypeptide comprise: (a) a polypeptide comprising fibroblast growth factor 23 (FGF23), or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof, wherein FGF23 has a mutation at one or more of the positions Q156, C206 and C244; and (b) either a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life, or a polypeptide comprising at least one extracellular subdomain of a Klotho protein, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof; and, optionally (c) a linker. The components can be, for example, chemically linked or fused in frame by a peptide bond. They may also linked via a linker. Non-limiting examples of polypeptide linker are SEQ ID NOs: 11, 12, 13, 14, 15, 16, 17, and 18. Such linkers may comprise at least one and up to about 30 repeats of SEQ ID

NOs:11, 12, 13, 14, 15, 16, 17 and 18. In another non-limiting embodiment, the fusion comprises (2) a FGF or an active fragment thereof and (3) a modified Fc fragment. The various components of the fuion can be operatively linked in any order; the polypeptide (1) can be operatively linked to the N-terminus of the polypeptide for (2) or (3); the polypeptide for (2) can be operatively linked to the N-terminus of the polypeptide for (1) or (3); the polypeptide for (3) can be operatively linked to the N-terminus of the polypeptide for (1) or (2).

According to the present disclosure, the extracellular subdomain of a Klotho protein, the fibroblast growth factor and the (optional) modified Fc fragment having decreased affinity for Fc-gamma-receptorand/or increased serum half-life can be operatively linked to one another in a variety of orientations and manners. For example, the extracellular subdomain of the Klotho protein can be operatively linked to the N-terminus of the fibroblast growth factor or alternatively the fibroblast growth factor can be operatively linked to the N-terminus of an extracellular subdomain of the Klotho protein.

In one embodiment, the present disclosure provides a fusion polypeptide comprising a sKlotho of a Klotho protein and a linker. In another embodiment, the present disclosure provides a fusion polypeptide comprising a sKlotho of the alpha Klotho protein and a linker. In another embodiment, the present disclosure provides a fusion polypeptide comprising a sKlotho of the beta Klotho protein and a linker. In yet another embodiment, the present disclosure provides a human FGF protein or an active fragment thereof (e.g., without signal peptide) and a linker. In one embodiment the disclosure provides fusion proteins, nucleic acid molecules or pharmaceutical composition for use in therapy or as medicament for use in the treatment of a pathological disorder. Pharmaceutical compositions comprising the fusion proteins of the disclosure and their uses for treating or preventing age-related conditions or metabolic disorders are also encompassed by the present disclosure. In some embodiments, the fusion protein further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptorand/or increased serum half-life.

In one embodiment, the present disclosure provides a fusion polypeptide comprising a sKlotho of alpha Klotho protein with signal peptide fused (directly or indirectly via a linker) to FGF-23. In another embodiment, the present disclosure provides a fusion polypeptide comprising a sKlotho of alpha Klotho protein without signal peptide fused (directly or indirectly via a linker) to FGF-23. In another

embodiment, the present disclosure provides sKlotho of alpha Klotho protein with signal peptide fused (directly or indirectly via a linker) to FGF-23 without signal peptide. In another embodiment, the present disclosure provides a fusion polypeptide comprising sKlotho of alpha Klotho protein without signal peptide fused (directly or indirectly via a linker) to FGF-23 without signal peptide. In some embodiments, the fusion protein further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptorand/or increased serum half-life.

In one embodiment, the present disclosure provides a fusion polypeptide comprising a sKlotho of alpha Klotho protein with signal peptide fused (directly or indirectly via a linker) to FGF-23 (R179Q) variant. In another embodiment, the present disclosure provides a fusion polypeptide comprising a sKlotho of alpha Klotho protein without signal peptide fused (directly or indirectly via a linker) to FGF-23 (R179Q) variant. In another embodiment, the present disclosure provides sKlotho of alpha Klotho protein with signal peptide fused (directly or indirectly via a linker) to FGF-23 (R179Q) variant without signal peptide. In another embodiment, the present disclosure provides a fusion polypeptide comprising sKlotho of alpha Klotho protein without signal peptide fused (directly or indirectly via a linker) to FGF-23 (R179Q) variant without signal peptide. In some embodiments, the fusion protein further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptorand/or increased serum half-life.

In one embodiment, the present disclosure provides a fusion polypeptide comprising: (a) a polypeptide comprising fibroblast growth factor 23 (FGF23), or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof, wherein FGF23 has a mutation at one or more of the positions Q156, C206 and C244; and (b) either a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life, or a polypeptide comprising at least one extracellular subdomain of a Klotho protein, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof; and, optionally (c) a linker. Such fusion polypeptides are disclosed in SEQ ID NOs: 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, and 68.

In one embodiment, the present disclosure provides a fusion polypeptide comprising (1) sKlotho of alpha Klotho protein with signal peptide, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino

acid substitution and/or one amino acid deletion) thereof; (2) a linker; and (3) FGF-23 (R179Q) variant without signal peptide, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof. In another embodiment, the present disclosure provides a fusion polypeptide comprising (1) sKlotho of alpha Klotho protein without signal peptide, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof; (2) a linker; and (3) FGF-23 (R179Q) variant without signal peptide, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof. In some embodiments, the fusion polypeptides of the disclosure are glycosylated. In some embodiments, the fusion protein further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptorand/or increased serum half-life.

In one embodiment, the present disclosure provides a fusion polypeptide comprising (1) sKlotho of alpha Klotho protein with signal peptide (SEQ ID NO: 44 or SEQ ID NO: 45), or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof (2) a linker comprising SEQ ID NO: 11; and (3) FGF-23 (R179Q) variant without signal peptide (SEQ ID NO: 43), or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof. In another embodiment, the present disclosure provides a fusion polypeptide comprising (1) sKlotho of alpha Klotho protein without signal peptide (SEQ ID NO: 7), or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof (2) a linker comprising SEQ ID NO: 11; and (3) FGF-23 (R179Q) variant without signal peptide (SEQ ID NO: 43), or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof In one embodiment, the present disclosure provides a fusion polypeptide comprising the amino acid sequence of SEQ ID NO: 19, 20, 40, or 41. In some embodiments, the fusion polypeptides of the disclosure are glycosylated.

In one embodiment, the present disclosure provides a fusion polypeptide comprising sKlotho of alpha Klotho protein with signal peptide (SEQ ID NO: 44 or SEQ ID NO: 45), or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof;

and a linker comprising SEQ ID NO: 11. In another embodiment, the present disclosure provides a fusion polypeptide comprising sKlotho of alpha Klotho protein without signal peptide (SEQ ID NO: 7); and a linker comprising SEQ ID NO: 11. In some embodiments, the fusion polypeptides of the disclosure are glycosylated. In some embodiments, the fusion protein further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptorand/or increased serum half-life.

In one embodiment, the present disclosure provides a fusion polypeptide comprising a human FGF protein or an active fragment thereof (e.g., without the signal peptide); and a linker comprising SEQ ID NO: 11. In some embodiments, the fusion polypeptides of the disclosure are glycosylated. In some embodiments, the fusion protein further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptorand/or increased serum half-life.

In one embodiment, the present disclosure provides a fusion polypeptide comprising a human FGF protein (e.g., FGF23) or an active fragment thereof (e.g., without the signal peptide); a linker (e.g., a linker comprising SEQ ID NO: 11); and sKlotho (with or without a signal peptide), or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof) or a Fc-gramma-receptor (e.g., FcLALA); wherein the FGF (e.g., FGF23) has one or more mutations at these residues: R179, Q156, C206, and/or C244. In various embodiments, the mutations are R179Q, Q156A, C206S, and/or C244S. Even though these mutations are conserved in the human, rhesus, bovine, mouse and rat FGF23, mutating them does not prevent FGF23 activity. Rather, mutating these amino acids unexpectedly enhances the qualities of the proteins, by reducing aggregation, reducing undesired protease-induced cleavage, and increasing protein production from cells. In various embodiments, the fusion protein comprising one or more FGF23 mutation is glycosylated.

In one embodiment, the present disclosure provides a pharmaceutical composition (e.g., in an intra-muscular administering form) comprising (e.g., as a sole pharmaceutically active ingredient) a fusion polypeptide (e.g., glycosylated or non-glycosylated) that comprises (1) FGF-23 (R179Q) variant without signal peptide (SEQ ID NO: 43), or a variant comprising additional mutations which reduce aggregation and/or protease-mediated cleavage, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof (2) optionally, a linker comprising SEQ ID NO: 11; and (3) sKlotho of

alpha Klotho protein with signal peptide (SEQ ID NO: 44 or SEQ ID NO: 45), or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof or a modified Fc fragment having decreased affinity for Fc-gamma-receptorand/or increased serum half-life; and uses of the pharmaceutical composition in therapy or as medicament for the treatment of a pathological disorder, for example treating and/or preventing age-related conditions, such as muscular atrophy. In another embodiment, the present disclosure provides a pharmaceutical composition (e.g., in an intra-muscular administering form) comprising (e.g., as a sole pharmaceutically active ingredient) a fusion polypeptide (e.g., glycosylated or non-glycosylated) that comprises (1) FGF-23 (R179Q) variant without signal peptide (SEQ ID NO: 43), or a variant comprising additional mutations which reduce aggregation and/or protease-mediated cleavage, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof (2) a linker comprising SEQ ID NO: 11; and (3) sKlotho of alpha Klotho protein without signal peptide (SEQ ID NO: 7), or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof, or a modified Fc fragment having decreased affinity for Fc-gamma-receptorand/or increased serum half-life, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof; and uses of the pharmaceutical composition in therapy or as medicament for the treatment of a pathological disorder, for example treating and/or preventing age-related conditions, such as muscular atrophy. In one embodiment, the present disclosure provides a pharmaceutical composition (e.g., in an intra-muscular administering form) comprising (e.g., as a sole pharmaceutically active ingredient) a fusion polypeptide (e.g., glycosylated or non-glycosylated) comprising the amino acid sequence of SEQ ID NO: 19, 20, 40, or 41; and uses of the pharmaceutical composition in therapy or as medicament for the treatment of a pathological disorder, for example treating and/or preventing age-related conditions, such as muscular atrophy.

In one embodiment, the present disclosure provides a pharmaceutical composition (e.g., in an intra-muscular administering form) comprising (e.g., as a sole pharmaceutically active ingredient) a fusion polypeptide (e.g., glycosylated or non-glycosylated) that comprises sKlotho of alpha Klotho protein with signal peptide (SEQ ID NO: 44 or SEQ ID NO: 45); and a linker comprising SEQ ID NO: 11; and uses of the

pharmaceutical composition for treating and/or preventing age-related conditions, such as muscular atrophy. In another embodiment, the present disclosure provides a pharmaceutical composition (e.g., in an intra-muscular administering form) comprising (e.g., as a sole pharmaceutically active ingredient) a fusion polypeptide (e.g., glycosylated or non-glycosylated) comprising sKlotho of alpha Klotho protein without signal peptide (SEQ ID NO: 7); and a linker comprising SEQ ID NO: 11; and uses of the pharmaceutical composition in therapy or as medicament for the treatment of a pathological disorder,for example treating and/or preventing age-related conditions, such as muscular atrophy. In some embodiments, the fusion protein further comprises a modified Fc fragment.

In one embodiment, the present disclosure provides a pharmaceutical composition comprising (e.g., as a sole pharmaceutically active ingredient) a fusion polypeptide (e.g., glycosylated or non-glycosylated) that comprises a human FGF protein or an active fragment thereof (e.g., without the signal peptide); and a linker comprising SEQ ID NO: 11.

Pharmaceutical compositions comprising the fusion proteins of the disclosure and their uses in therapy or as medicament for the treatment of a pathological disorder therapy, for example treating or preventing age-related conditions (e.g., muscle atrophy) or metabolic disorders (e.g., diabete) are also encompassed by the present disclosure.

In one embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% identical to SEQ ID NO: 19. In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% identical to SEQ ID NO: 20.

In one embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% identical to SEQ ID NO: 40. In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% identical to SEQ ID NO: 41.

In one embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least

95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 46. In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 47.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 48. In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 49.

In one embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 50. In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 51.

In one embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 52. In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 53.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 54.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least

95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 55.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 56.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 57.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 58.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 59.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 60.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 61.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 62.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 63.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 64.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 65.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 66.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 67.

In another embodiment, the present disclosure provides a fusion polypeptide that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 68.

In one embodiment, the present disclosure provides a fusion polypeptide comprising a sKlotho of beta Klotho protein with signal peptide fused (directly or indirectly via a linker) to FGF-19 or an active fragment thereof. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life. In another embodiment, the present disclosure provides a fusion polypeptide comprising a sKlotho of beta Klotho protein without signal peptide fused (directly or indirectly via a linker) to FGF-19 or an active

fragment thereof. In another embodiment, the present disclosure provides a fusion polypeptide comprising a sKlotho of beta Klotho protein with signal peptide fused (directly or indirectly via a linker) to FGF-21 or an active fragment thereof. In another embodiment, the present disclosure provides a fusion polypeptide comprising a sKlotho of beta Klotho protein without signal peptide fused (directly or indirectly via a linker) to FGF-21 or an active fragment thereof.

The disclosure provides nucleic acid sequences encoding any of the Klotho fusion polypeptides described herein and host cells containing the nucleic acids. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life.

The disclosure also provides composition having any of the Klotho fusion polypeptides contemplated herein. The compositions of the disclosure can further include heparin. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life.

The disclosure also provides a method for treating or preventing an age-related condition in an individual. An individual (e.g., human) is administered a therapeutically effective dose of a pharmaceutical composition containing a Klotho fusion polypeptide, having at least one extracellular subdomain of a Klotho protein (e.g., alpha Klotho protein) and a fibroblast growth factor or an active fragment thereof so as to treat or prevent the age-related condition. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life. In particular, the disclosure provides a method of treating or preventing muscle wasting comprising administering to an individual (e.g., human) an therapeutically effective amount of a fusion polypeptide having at least one extracellular subdomain of an alpha Klotho protein and a fibroblast growth factor (or an active fragment thereof).

Additionally, the disclosure provides a method for treating or preventing a metabolic disorder in an individual. An individual is administered a therapeutically effective dose of a pharmaceutical composition containing a fusion polypeptide of the disclosure, having at least one extracellular subdomain of a Klotho protein and a fibroblast growth factor (or an active fragment thereof) so as to treat the metabolic disorder. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life. In particular, a fusion polypeptide of the disclosure having at least one extracellular

subdomain of a beta-Klotho protein and a fibroblast growth factor 21 is useful for treating a metabolic disorder.

Klotho-FGF23 fusion polypeptides of the disclosure can be used for treating or preventing hyperphosphatemia or calcinosis in an individual. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life. A pharmacologically effective dose of a pharmaceutical composition containing the Klotho fusion polypeptide of the disclosure, having at least one extracellular subdomain of a Klotho protein and a fibroblast growth factor, is administered to treat or prevent hyperphosphatemia or calcinosis. In particular, a Klotho fusion polypeptide of the disclosure having at least one extracellular subdomain of an alpha Klotho protein and a fibroblast growth factor 23 is useful for treating hyperphosphatemia or calcinosis.

Klotho-FGF23 fusion polypeptides of the disclosure can be used for treating or preventing chronic renal disease or chronic renal failure in an individual. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life. A therapeutically effective dose of a pharmaceutical composition containing the Klotho fusion polypeptide of the disclosure, having at least one extracellular subdomain of a Klotho protein (e.g., alpha Klotho protein) and a fibroblast growth factor, is administered to treat or prevent chronic renal disease or chronic renal failure.

Klotho-FGF23 fusion polypeptides of the disclosure can be used for treating or preventing cancer (e.g., breast cancer) in an individual. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life. A therapeutically effective dose of a pharmaceutical composition containing the Klotho fusion polypeptide of the disclosure, having at least one extracellular subdomain of a Klotho protein (e.g., alpha Klotho protein) and a fibroblast growth factor, is administered to treat or prevent cancer or breast cancer.

The present disclosure provides fusion polypeptides comprising at least one extracellular subdomain of Klotho protein and a FGF or an active fragment thereof for use in medicine. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life. In one embodiment, the present disclosure provides fusion polypeptides comprising at least one extracellular subdomain of Klotho protein and a FGF or an active fragment

thereof for use in treating or preventing muscle atrophy. The present disclosure also provides a method of treating or preventing an age related condition (e.g., muscle atrophy) comprising administering to an individual in need thereof a therapeutically effective dose of a pharmaceutical composition comprising a soluble Klotho protein.

The disclosure futhermore provides the above described peptides and fusion polypeptides or pharmaceutical compositions comprising said peptides for use in therapy, as a medicament or for use in the treatment of a pathological disorder, for example age-related condition, metabolic disorder, hyperphosphatemia or calcinosis, chronic renal disease or chronic renal failure or to prevent cancer or breast cancer, in an individual. Additonally, the disclosure further provides use of a polypeptide, nucleic acid or pharmaceutical composition of the invention in the manifacture of a medicament for the treatment of a pathological disorder, particularly for the treatment of the above mentioned disorders, preferably age related conditions like muscle atrophy.

The disclosure also includes kits for treating or preventing an age-related disorder or metabolic disorder in an individual. The kit includes instructions for use and a purified Klotho fusion polypeptide having at least one extracellular subdomain of a Klotho protein and a fibroblast growth factor. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life.

The disclosure also provides a kit for producing a Klotho fusion polypeptide of the disclosure. The kit of the disclosure includes instructions for use and a nucleic acid encoding a Klotho fusion polypeptide, having at least one extracellular subdomain of Klotho protein and a fibroblast growth factor. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life.

In one embodiment of the disclosure, the fusion polypeptide comprises: (a) a polypeptide comprising a fibroblast growth factor, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof; and (b) a modified Fc fragment, or a functionally active variant or derivative (e.g., a variant comprising at least one conservative amino acid substitution and/or one amino acid deletion) thereof, having decreased affinity for Fc-gamma-receptor and/or increased serum half-life

In one embodiment of the disclosure, the polypeptide of (a) and the polypeptide of (b) are connected by a polypeptide linker. The linker can be repeated 1 to 30 times, or more.

In one embodiment of the disclosure, the polypeptide linker comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18.

In one embodiment of the disclosure, the polypeptide of (a) is connected by a peptide bond to the N-terminus of said polypeptide linker, and the polypeptide of (b) is connected by a peptide bond to the C-terminus of said polypeptide linker.

In one embodiment of the disclosure, the fusion polypeptide further comprises a signal peptide.

In one embodiment of the disclosure, the signal peptide is the IgG signal peptide.

In one embodiment of the disclosure, the fibroblast growth factor is fibroblast growth factor-23 or a fibroblast growth factor-23 variant (R179Q).

In one embodiment of the disclosure, the fibroblast growth factor is fibroblast growth factor-19 or fibroblast growth factor-21.

In one embodiment of the disclosure, fusion polypeptide comprises an amino acid sequence which is 95% or more identical to the amino acid sequence of SEQ ID NO: 51, or SEQ ID NO: 53.

In one embodiment of the disclosure, fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 51, or SEQ ID NO: 53.

In one embodiment of the disclosure, fusion polypeptide comprises FcLALA.

3. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates several different embodiments of the Klotho fusion polypeptides of the disclosure. The represented fusion polypeptides include one or more Klotho extracellular subdomains operatively linked to a fibroblast growth factor. Polypeptides containing one or more Klotho extracellular subdomains include, for example, an extracellular domain of Klotho (e.g., aa 1 to 982 of human Klotho), or an active fragment of Klotho.

Figure 2 illustrates the amino acid and nucleic acid sequences of several Klotho fusion polypeptides of the disclosure and components thereof (e.g., Klotho extracellular

domain, FGF). Fusion proteins comprising sKlotho, FGF23 and FcLALA (a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life) are described in SEQ ID NOs. 46, 47, 48, and 49. Fusion proteins comprising FGF23 and FcLALA are described in SEQ ID NOs. 50, 51, 52 and 53.

Figures 3A-3C depict protein expression of an sKlotho-FGF23 fusion protein. Figure 3A shows that sKlotho-FGF23 fusion protein was detected in conditioned media by Western blotting with anti-FGF23 antibodies. Figure 3B shows that sKlotho-FGF23 fusion protein was detected in conditioned media by SDS-PAGE and Coomassie blue staining Figure 3C shows a highly purified sKlotho-FGF23-6xHis fusion protein, analyzed by SDS-PAGE and Coomassie blue staining.

Figure 4 illustrates the results of an Egr-1 luciferase assay comparing the activation level of Egr-1 in cells treated with conditioned media containing either a Klotho fusion polypeptide, a FGF 23 polypeptide only, a soluble Klotho (sKlotho) polypeptide only, and a soluble Klotho polypeptide in combination with a FGF 23 polypeptide in the absence or presence of heparin (20 μg/ml).

Figures 5A-5B depict the results of an Egr-1 luciferase assay comparing the activation level of Egr-1 in cells treated with purified Klotho fusion polypeptide, FGF 23 polypeptide, or soluble Klotho polypeptide in the absence or presence of heparin. Figure 5A shows an the results of an experiment comparing the activation level of Egr-1 in cells treated with FGF 23 alone, sKlotho-His (10 nM or 20 nM) and a combination of FGF 23 and sKlotho-His (10 nM or 20 nM) in the absence or presence of heparin (20 μg/ml). Figure 5B shows Egr-1 luciferase reporter activity in cells treated with sKlotho-FGF23-His fusion (0 nM, 0.6 nM, 1.21 nM, 2.41 nM, 4.83 nM, 9.65 nM, and 19.3 nM).

Figures 6A-6B illustrate the effect of treatment with a purified sKlotho fusion polypeptide on C2C12 muscle cells. Figure 6A shows measurements of myotube diameter in C2C12 muscle cells treated with either IGF-1 (10 nM), FGF2 (20ng/ml), or a purified Klotho fusion polypeptide (20 nM), in the absence or presence of dexamethasone (100 μM). Figure 6B shows the phosphorylation of signaling pathway proteins in C2C12 muscle cells by IGF-1 (10 nM), FGF2 (20ng/ml), or a purified Klotho fusion polypeptide (20 nM), in the absence or presence of rapamycin (40 nM).

Figure 7 shows activation of EGR-1-luc reporter gene by sKlotho-FGF23(R179Q)-FcLALA fusion proteins.

Figure 8 shows the activation of EGR-1-luc reporter gene by FGF23(R179Q)-FcLALA proteins.

Figure 9 shows the pharmacokinetic profile of FGF23(R179Q) vs FGF23(R179Q)-FcLALAv2.

Figures 10A and 10B show the in vivo efficacy of sKlotho-FGF23 fusion in enhancing muscle growth after dexamethasone-induced muscle atrophy.

Figure 11. This figure shows activation of EGR-1-luc reporter gene by FGF23(R179Q)-FcLALA and Q156A, C206S, C244S and C206S/C244S mutants.

Figure 12 shows protein qualities and dimerization of WT (wild-type), Q156A, C206S, C244S and C206S/C244S mutants of FGF23(R179Q)-FcLaLa.

4. DETAILED DESCRIPTION

The present disclosure is directed to methods, kits and compositions for preventing or treating age-related conditions and metabolic disorders; and to the use of said compostions in therapy, as a medicament or for use in the treatment of a pathological disorder. The fusion polypeptides of the disclosure include a Klotho protein or active fragment thereof. In some embodiments, the fusion polypeptides of the disclosure include a Klotho protein or an active fragment thereof operatively linked to a fibroblast growth factor polypeptide or active fragment thereof. In some embodiments, the fusion further comprises a modified Fc fragment with decreased ability to bind FcRn and/or increased stability in serum. In another embodiment, the fusion polypeptide comprises a FGF (e.g., FGF23) and a modified Fc fragment with decreased ability to bind FcRn and/or increased stability in serum.

The fusion proteins or sKlotho of the present disclosure are useful in the treatment and prevention of a variety of age-related conditions including sarcopenia, skin atrophy, muscle wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, memory loss, wrinkles, impaired kidney function, and age-related hearing loss; and metabolic disorders including Type II Diabetes, Metabolic Syndrome, hyperglycemia, and obesity.

The present disclosure is based at least in part on the finding that despite the physical constraints (e.g., large size of both the Klotho and FGF polypeptides) the Klotho-FGF fusion polypeptides are highly effective in activating an FGF receptor. This finding is unexpected given that fusion of these two proteins would likely interfere with the heterodimerization and thus the activities of the proteins; e.g., the binding domains of the proteins may be perturbed by the fusion or the proteins may be mis-oriented spatially if put together in a "cis" formation.

The fusion polypeptides described herein are advantageous because they allow the administration of a single therapeutic protein that has enhanced activity compared to Klotho or FGF administered alone or together as separate polypeptides. The use of Klotho and FGF as a single fusion polypeptide rather than as two separate polypeptides (i.e., a Klotho polypeptide and a separate FGF polypeptide) is more effective at activating the FGF receptor.

Definitions

"Klotho polypeptide", "Klotho protein", or "Klotho" as used herein, includes active fragments, derivatives, mimetics, variants and chemically modified compounds or hybrids thereof of wild-type "Klotho". A Klotho active fragment has the ability to bind to an FGF polypeptide. Generally, a Klotho active polypeptide contains at least a Klotho subdomain (e.g., KL-D1 and KL-D2). Wild-type Klotho has the amino acid sequence as is found in nature. Example Klotho polypeptides suitable for use with the present disclosure include alpha-Klotho (SEQ ID NO: 2) and beta-Klotho (SEQ ID NO: 4). Nucleotide and amino acid sequences of the alpha-Klotho and beta-Klotho are found in the GenBank database at Accession No. NM_004795; NP_004786 and NM_175737; NP__783864, respectively. Klotho polypeptides include those described in U.S. Patent No. 6,579,850, the content of which is herein incorporated by reference in its entirety. The Klotho polypeptides include those from other species besides humans, including alpha-Klotho from mouse (NP_038851), rat (NP_112626), rabbit (NP_001075692) and beta-Klotho from mouse (NP_112457). Species predicted to have alpha-Klotho include chimpanzee (XP_522655), macaque (XP_001101127), horse (XP_001495662), cow (XP_001252500), platypus (XP_001510981), and chicken (XP_417105). Species predicted to have beta-Klotho include chimpanzee (XP_526550), macaque (XP_001091413), horse (XP_001495248), dog (XP_536257), rat (XP_001078178), platypus (XP_001512722), and chicken (XP_423224). The Klotho polypeptides have an

amino acid sequence that is substantially identical to the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4; i.e., at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical at the amino acid sequences of SEQ ID NO: 2 or SEQ ID NO: 4, or active fragment thereof.

"Fusion polypeptide" or "fusion protein", as used herein, shall mean a polypeptide comprising two or more different polypeptides or active fragments thereof that are not naturally present in the same polypeptide. In some embodiments, the two or more different polypeptides are operatively linked together covalently, e.g., chemically linked or fused in frame by a peptide bond. As used herein a "Klotho fusion polypeptide" is a fusion polypeptide which includes an amino acid sequence from a Klotho polypeptide or active fragment thereof. A fusion polypeptide can comprise, as non-limiting examples, Klotho (e.g., sKlotho), FGF (e.g., FG23), and (optionally) a modified Fc fragment (e.g., a modified Fc fragment with decreased binding affinity to FC-gamma-receptor and/or increased serum half-life). Examples of this type of fusion polypeptide are presented in SEQ ID NOs. 46 to 49. In another embodiment, the fusion proteins comprise FGF (e.g., FGF23) and a modified Fc (e.g., FcLALA). Fusion proteins comprising FGF23 and FcLALA are described in SEQ ID NOs. 50, 51, 52 and 53. FcLALA is a Fc fragment with a LALA mutation (L234A, L235A), which triggers ADCC with lowered efficiency, and binds and activates human complement weakly. Hessell et al. 2007 Nature 449:101-104.

"Fibroblast growth factor" and "FGF" are used interchangeably herein and shall refer to polypeptides that regulate cell proliferation, migration, differentiation, homeostasis, tissue repair and response to injury in an animal, including a human subject. FGFs have the ability to bind to a fibroblast growth factor receptor and regulate its activity, including autophosphorylation of FGFR, phosphorylation of FRS2 (FGF receptor substrate 2) and ERK1/2 (extracellular signal-regulated protein kinase 1/2), and activating Egr-1 (early growth response-1). The term "FGF" includes active fragments, derivatives, mimetics, variants and chemically modified compounds or hybrids thereof of wild-type "FGF", e.g., as known in the art and as described in U.S. Patent No. 7,223,563 and U.S. Patent No. 7,259,248, the contents of which are incorporated by reference in their entirety. Wild-type FGF has an amino acid sequence as is found in nature. Example fibroblast growth factors suitable for use with the present disclosure include fibroblast growth factor-19 (FGF19; SEQ ID NO: 31), fibroblast growth factor-21 (FGF21; SEQ ID NO: 33), and fibroblast growth factor-23 (FGF23; SEQ ID NO: 35). The FGF

polypeptides include those from other species besides humans, including murine FGFs. Generally, FGF polypeptides have an amino acid sequence that is substantially identical to the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 33 or SEQ ID NO: 35; i.e., having an amino acid sequence is which is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more or 100% identical to the amino acid sequences of SEQ ID NO: 31 SEQ ID NO: 33 or SEQ ID NO: 35, or active fragments thereof. Additional non-limiting examples of FGF, particularly FGF23, are provided at aa 1002-1228 of SEQ ID NO: 47; aa 1002-1228 of SEQ ID NO: 49; aa 1-251 of SEQ ID NO: 51, and aa 1-251 of SEQ ID NO: 53; and sequences which are at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more or 100% identical to these sequences. Nucleotides encoding these sequences are provided in SEQ ID NOs: 46, 48, 50 and 52.

The term "FGF", includes active fragments of the full-length polypeptide. Active FGF fragments that are able to bind to their corresponding FGF receptors are known in the art and also contemplated for use in the present disclosure. One skilled in the art would appreciate, based on the sequences disclosed herein, that overlapping fragments of the FGFs can be generated using standard recombinant technology, for example, that described in Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York) and Ausubel et al. (1997, Current Protocols in Molecular Biology, Green & Wiley, New York). One skilled in the art would appreciate, based on the disclosure presented herein, that the biological activity of FGF fragments could be tested by methods well known in the art and described herein, including binding to the FGF receptor. Similarly, cell culture models which possess the necessary FGF signal transduction machinery (i.e. FGF receptor) may be transfected with FGF fragments and subsequently tested for alterations in FGF signaling, relative to wild type FGF.

FGFs are grouped into seven subfamilies based on the homology of the FGF core homology domain (approximately 120 amino acids long), which is flanked by N- and C-terminal sequences that are highly variable in both length and primary sequence, particularly among different FGF subfamilies (Goetz et al., Molecular and Cellular Biology, 2007, Vol. 27, 3417-3428). An FGF active polypeptide generally contains at least an FGF core homology domain. In some embodiments, an FGF active polypeptide may contain, in addition to an FGF core homology domain, flanking sequences which may confer additional specificity in binding FGF receptors. FGF19, FGF21, and FGF23 are grouped in the FGF19 subfamily because the core region of these ligands share high

sequence identity relative to other FGFs (FGF19 v. FGF21: 38% identity; FGF19 v. FGF23: 36% identity). FGF19 subfamily members act analogously to signaling molecules of the endocrine system and regulate diverse physiological processes uncommon to classical FGFs (e.g., FGF19: energy and bile acid homeostasis; FGF21: glucose and lipid metabolism; and FGF 23: phosphate and vitamin D homeostasis).

"Fibroblast growth factor receptor" and "FGFR" as used herein refer to any one of FGFRs 1-4 known in the art, or splice variants thereof (e.g., FGFR1c). Example fibroblast growth factor receptors suitable for use with the present disclosure include fibroblast growth factor receptor-19 (e.g., FGFR4-beta Klotho), fibroblast growth factor receptor-21 (e.g., FGFR1c-alpha Klotho), and fibroblast growth factor receptor-23 (e.g., FGFR1c-alpha Klotho, FGFR3-alpha Klotho, FGFR4-alpha Klotho).

"Extracellular domain", as used herein, refers to the fragment of a transmembrane protein existing outside of a cell (e.g., not including the intracellular or transmembrane region). The "extracellular domain of the Klotho protein", "soluble Klotho", or "sKlotho" (e.g., SEQ ID NO: 7; SEQ ID NO: 39), refers to an extracellular domain of the Klotho polypeptide that is capable of binding a fibroblast growth factor, and/or capable of enabling the binding of a fibroblast growth factor to a fibroblast growth factor receptor by binding to the fibroblast growth factor. The Klotho extracellular domain corresponds to amino acid residues 28-982 of the full length alpha Klotho sequence (SEQ ID NO: 2) and to amino acid residues 52-997 of the full length beta Klotho sequence (SEQ ID NO: 4).

"Extracellular subdomain of Klotho protein" and "extracellular subdomain of Klotho protein" are used interchangeably herein and shall refer to a region in the extracellular domain of the Klotho polypeptide that is capable of binding a fibroblast growth factor, and/or is capable of enabling the binding of a fibroblast growth factor to a fibroblast growth factor receptor by binding to the fibroblast growth factor. In various embodiments, the fusion comprises a polypeptide comprising at least one extracellular subdomain of a Klotho protein; a polypeptide comprising a fibroblast growth factor; and, optionally, a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life. The Klotho extracellular domain has two homologous subdomains that are repeated, i.e., KL-D1 (SEQ ID NO: 5) and KL-D2 (SEQ ID NO: 6). KL-D1 and KL-D2 correspond respectively to amino acid residues 58-506 and 517-953 of the full length alpha Klotho polypeptide (SEQ ID NO: 2) and respectively to amino acid residues 77-508 and 571-967 of the full length beta Klotho polypeptide (SEQ ID NO: 4) and are suitable for use with the present disclosure. Generally, a polypeptide that

contains at least one Klotho subdomain is a Klotho active polypeptide. The Klotho extracellular subdomain for use with the polypeptide of the disclosure may be an alpha Klotho or beta Klotho KL-D1 domain with an amino acid sequence that is substantially identical to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 37, respectively. Further, the Klotho KL-D1 domain may have an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 37. The Klotho extracellular subdomain may also be an alpha or beta Klotho polypeptide KL-D2 domain that is substantially identical to the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 38, respectively. In a further embodiment, the KL-D2 domain has an amino acid sequence that is at least at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 38. In some embodiments, the fusion comprises at least two extracellular subdomains of the Klotho protein (e.g., KL-D1 and KL-D2; KL-D1 and KL-D1 in tandem repeats; KL-D2 and KL-D2 in tandem repeats, etc.).

"Modified Fc fragment", as used herein, shall mean an Fc fragment of an antibody comprising a modified sequence. The Fc fragment is a portion of an antibody comprising the CH2, CH3 and part of the hinge region. The modified Fc fragment can be derived from, for example, IgG1, IgG2, IgG3, or IgG4. FcLALA is a modified Fc fragment with a LALA mutation (L234A, L235A), which triggers ADCC with lowered efficiency, and binds and activates human complement weakly. Hessell et al. 2007 Nature 449:101-104. Additional modifications to the Fc fragment are described in, for example, U.S. Patent No. 7,217,798. For example, in various modified Fc fragments: (a) amino acid residue 250 is glutamic acid and amino acid residue 428 is phenylalanine; or (b) amino acid residue 250 is glutamine and amino acid residue 428 is phenylalanine; or (c) amino acid residue 250 is glutamine and amino acid residue 428 is leucine. In some embodiments, amino acid residues 250 and 428 differ from the residues present in an unmodified Fc-fusion protein by amino acid residue 250 being glutamic acid or glutamine and amino acid residue 428 being leucine or phenylalanine, and wherein amino acid residues are numbered by the EU numbering system, as described in U.S. Patent No. 7,217,798. In some embodiments, the modified Fc-fusion protein has a higher affinity for FcRn at pH 6.0 than at pH 8.0. Preferably, the modified Fc fragment has decreased affinity to FcRn and/or increased serum half-life. Non-limiting examples of modified Fc fragments include that at aa (amino acids) 1234-1459 of SEQ ID NO: 47; aa 1234 to 1450 of SEQ

ID NO: 49; aa 257 to 482 of SEQ ID NO: 51; and aa 257 to 473 of SEQ ID NO: 53; and sequences which are at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more or 100% identical to these sequences. Nucleotides encoding these sequences are provided in SEQ ID NOs: 46, 48, 50 and 52.

"Signal peptide", as used herein, shall mean a peptide chain (3-60 amino acids long) that directs the post-translational transport of a protein to the endoplasmic reticulum and may be cleaved off. Example signal peptides suitable for use with the present disclosure include the Klotho signal peptide (SEQ ID NO: 19) and the IgG signal peptide (SEQ ID NO: 20). Note that upon secretion and cleavage by the producer cell line, the signal peptide (e.g., of the peptides corresponding to SEQ ID NO: 19 and SEQ ID NO: 20) is cleaved off. Thus, after secretion and cleavage of the signal peptide by the producer cell lines, the peptide of SEQ ID NO: 19 would generate the peptide of SEQ ID NO: 41.

"Linker", as used herein, shall mean a functional group (e.g., chemical or polypeptide) that covalently attaches two or more polypeptides or nucleic acids so that they are connected with one another. As used herein, a "peptide linker" refers to one or more amino acids used to couple two proteins together (e.g., to couple the extracellular domain of Klotho and fibroblast growth factor-23). Peptide linkers suitable for use with the present disclosure include, but are not limited to, polypeptides with amino acid sequences represented by SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18. A polypeptide linker can comprise at least 1 and up to about 30 repeats of any of these amino acid sequences.

"Operatively linked", as used herein, shall mean the linking of two or more biomolecules so that the biological functions, activities, and/or structure associated with the biomolecules are at least retained. In reference to polypeptides, the term means that the linking of two or more polypeptides results in a fusion polypeptide that retains at least some of the respective individual activities of each polypeptide component. The two or more polypeptides may be linked directly or via a linker. In reference to nucleic acids, the term means that a first polynucleotide is positioned adjacent to a second polynucleotide that directs transcription of the first polynucleotide when appropriate molecules (e.g., transcriptional activator proteins) are bound to the second polynucleotide.

"Specifically binds", as used herein, shall refer to the ability of a first molecule to bind to a target molecule out of many, different types of molecules to which it may be

exposed because of the ability of the first molecule to adopt a particular structure conducive to forming non-covalent interactions between itself and the other target molecule. The first molecule binds to the target forming a stable complex while there is substantially less recognition, contact, or complex formation of the first molecule with any other non-specific molecules.

"Polypeptide variant" or "protein variant", as used herein, refers to polypeptides in which one or more amino acids have been substituted by different amino acids from a reference sequence. It is well understood in the art that some amino acids may be substituted by others with broadly similar properties without changing the nature of the activity of the polypeptide (conservative substitutions) as described hereinafter. These terms also encompass polypeptides in which one or more amino acids have been added or deleted, or replaced with different amino acids, e.g., protein isoforms. An example variant of fibroblast growth factor-23 suitable for use with the present disclosure is the fibroblast growth factor-23 variant (R179Q).

"Pharmaceutical composition", as used herein, shall mean a composition containing a compound (e.g., a fusion polypeptide of the disclosure) that may be administered to treat or prevent a disease or disorder in an individual.

"Individual" or "subject", as used herein, shall refer to a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.

"Treat", as used herein, shall mean decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease. In the context of the disclosure, the administration of the polypeptides of the disclosure may be used to treat age-related conditions, including sarcopenia, skin atrophy, muscle wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, memory loss, wrinkles, impaired kidney function, and age-related hearing loss; and metabolic disorders, including Type II Diabetes, Metabolic Syndrome, hyperglycemia, and obesity.

"Prevent", as used herein, shall refer to a decrease in the occurrence of a disorder or decrease in the risk of acquiring a disorder or its associated symptoms in a subject. In the context of the disclosure, the administration of the polypeptides of the disclosure may be used to prevent age-related conditions, including sarcopenia, skin atrophy, muscle

wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, memory loss, wrinkles, impaired kidney function, and age-related hearing loss; and metabolic disorders, including Type II Diabetes, Metabolic Syndrome, hyperglycemia, and obesity. The prevention may be complete, e.g., the total absence of an age-related condition or metabolic disorder. The prevention may also be partial, such that the likelihood of the occurrence of the age-related condition or metabolic disorder in a subject is less likely to occur than had the subject not received the present disclosure.

"Disease", as used herein, shall mean any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ.

"Age-related condition", as used herein, shall mean any disease or disorder whose incidence in a population or severity in an individual correlates with the progression of age. In one embodiment, the age-related condition is a disease or disorder whose incidence is at least 1.5 fold higher among human individuals greater than 60 years of age relative to human individuals between the ages of 30-40 and in a selected population of greater than 100,000 individuals. Age-related conditions relevant to the present disclosure include, but are not limited to, sarcopenia, skin atrophy, muscle wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, memory loss, wrinkles, impaired kidney function, and age-related hearing loss.

"Metabolic disorder", as used herein, shall mean any disease or disorder that damages or interferes with normal function in a cell, tissue, or organ by affecting the production of energy in cells or the accumulation of toxins in a cell, tissue, organ, or individual. Metabolic disorders relevant to the present disclosure include, but are not limited to, Type II Diabetes, Metabolic Syndrome, hyperglycemia, and obesity.

An "effective dose" or "effective amount" is an amount sufficient to effect a beneficial or desired clinical result. In the context of the disclosure, it is an amount of a Klotho fusion polypeptide or sKlotho effective to produce the intended pharmacological, therapeutic or preventive result. A therapeutically effective dose results in the prevention or amelioration of the disorder or one or more symptoms of the disorder, (e.g., an age-

related condition or metabolic disorder). Therapeutically effective doses will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like which can be readily be determined by one of ordinary skill in the art.

"Klotho nucleic acid molecule", as used herein is a gene encoding a Klotho protein. An example human Klotho gene is provided at GenBank Accession No. NM_004795 (SEQ ID NO: 1). Additional non-limiting examples of Klotho are provided at aa 1-982 of SEQ ID NO: 47 and aa 1-982 of SEQ ID NO: 49; and sequences which are at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more or 100% identical to these sequences.

"Fragment", as used herein, refers to a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or up to 3000 nucleotides or amino acids.

The term "substantially identical" refers to a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, 70%, 75%, 80% or 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical at the amino acid level or nucleic acid to the sequence used for comparison.

The present disclosure is directed to methods, kits and compositions for preventing or treating age-related conditions and metabolic disorders; and to the use of said compostions in therapy, as a medicament or for use in the treatment of a pathological disorder. In some embodiments, the disclosure provides a fusion polypeptide having at least one extracellular subdomain of a Klotho protein. In some embodiments, the fusion polypeptides further comprise a fibroblast growth factor or an active fragment thereof. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptorand/or increased serum half-life. In other embodiments, the fusion comprises an FGF (e.g., FGF19, FGF21, FGF23 or FGF23 variant R179Q) fused to a modified Fc (e.g., FcLALA). FcLALA is a Fc fragment with a LALA mutation (L234A, L235A), which triggers ADCC with lowered efficiency, and binds and activates human complement weakly. The Klotho extracellular domain may be

derived from either the alpha or beta Klotho isoforms. Further, although the FGF component of the Klotho fusion polypeptide is described primarily with reference to fibroblast growth factor-19, fibroblast growth factor-21 and fibroblast growth factor-23, it is contemplated that any of the twenty-three known FGFs or an active fragment thereof can be used in practicing the disclosure.

The extracellular domain of the Klotho protein can include one or both of the KL-D1 and KL-D2 domains of a Klotho protein. In some embodiments, the Klotho fusion polypeptide has at least two extracellular subdomains of a Klotho protein. For example, the at least two extracellular subdomains can be at least two KL-D1 domains in tandem repeats, at least two KL-D2 domains in tandem repeats, or at least one KL-D1 domain and at least one KL-D2 domain.

The extracellular subdomain of a Klotho protein and the fibroblast growth factor (or an active fragment thereof) can be operatively linked to one another in a variety of orientations and manners. For example, the extracellular subdomain of the Klotho protein can be operatively linked to the N-terminus of the fibroblast growth factor or alternatively the fibroblast growth factor can be operatively linked to the N-terminus of the at least one extracellular subdomain of the Klotho protein.

The fusion polypeptide of the disclosure may include one or both of the Klotho extracellular domains, i.e., KL-D1 (SEQ ID NO: 5) and KL-D2 (SEQ ID NO: 6). KL-D1 and KL-D2 correspond respectively to amino acid residues 58-506 and 517-953 of the full length alpha Klotho polypeptide (SEQ ID NO: 2) and to amino acid residues 77-508 and 571-967 of the full length beta Klotho polypeptide (SEQ ID NO: 4) and are suitable for use with the present disclosure. The Klotho fusion polypeptide may have a KL-D1 domain of an alpha Klotho polypeptide having an amino acid sequence that is substantially identical to the amino acid sequence of SEQ ID NO: 5 or of a beta Klotho polypeptide having an amino acid sequence that is substantially identical to the amino acid sequence of SEQ ID NO: 37. Specifically, the Klotho fusion polypeptide may have an amino acid sequence that is at least at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO: 5 or SEQ ID NO: 37. The Klotho fusion polypeptide may have a KL-D2 domain of an alpha Klotho polypeptide with an amino acid sequence that is substantially identical to the amino acid sequence of SEQ ID NO: 6 or of a beta Klotho polypeptide having an amino acid sequence that is substantially identical to the amino acid sequence of SEQ ID NO: 38. Specifically,, the Klotho fusion

polypeptide may have an amino acid sequence that is at least at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO: 6 or SEQ ID NO: 38, respectively.

In some embodiments, the Klotho fusion polypeptide of the disclosure is soluble and is capable of binding to an FGF receptor.

The Klotho fusion polypeptides of the disclosure can contain a polypeptide linker which connects the polypeptide having at least one extracellular subdomain of a Klotho protein and the fibroblast growth factor and the (optional) modified Fc fragment. Suitable linkers are well known in the art and generally contain several Gly and several Ser residues, e.g., $(Gly_4\ Ser)_3$ (SEQ ID NO: 11), Gly_4 Ser polypeptide (SEQ ID NO: 12), Gly (SEQ ID NO: 13), Gly Gly (SEQ ID NO: 14), Gly Ser (SEQ ID NO: 15), Gly_2 Ser (SEQ ID NO: 16), Ala (SEQ ID NO: 17), and Ala Ala (SEQ ID NO: 18). In some embodiments, the linker will have at least 2 and up to about 30 repeats of an amino acid sequence represented by any one of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

When a polypeptide linker is present in the Klotho fusion polypeptide of the disclosure, the polypeptide having at least one extracellular subdomain of a Klotho protein may be connected by a peptide bond to the N-terminus of the linker polypeptide with the FGF connected by a peptide bond to the C-terminus of the polypeptide linker. Alternatively, the FGF may be connected by a peptide bond to the N-terminus of the linker polypeptide with the polypeptide having at least one extracellular subdomain of Klotho connected by a peptide bond to the C-terminus of the polypeptide linker. A chemical linker can also be used to link the two polypeptides.

The Klotho fusion polypeptide of the disclosure may include a signal peptide. Example signal peptides for use with the Klotho fusion polypeptide include, but are not limited to the Klotho signal peptide (SEQ ID NO: 8) and the IgG signal peptide (SEQ ID NO: 9).

In some embodiments, the disclosure provides a fusion between a FGF (e.g., FGF19, FGF21, FGF23, or FGF23 variant R179Q) and a modified Fc (e.g., FcLALA). The fusion can also optionally comprise linkers between the FGF and Fc portions. The fusion can also optionally comprise a signal peptide. In various embodiments, the disclosure encompasses nucleic acids encoding these fusion polypeptides, vectors comprising these nucleic acids, and host cells containing these nucleic acids.

4.1. Klotho and Fibroblast growth factor polypeptides

The Klotho fusion polypeptides of the disclosure are expected to exhibit biological activities comparable to FGF in nature, such as binding to an FGF receptor and inducing the phosphorylation of an FGF receptor, FRS2 (FGF receptor substrate 2) and ERK1/2 (extracellular signal-regulated protein kinase 1/2) and activating Egr-1 (early growth response-1) gene. FGF is a secreted peptide growth factor that binds the FGF receptor. The amino acid and nucleic acid sequences of FGF are readily available to those of skill in the art. For example, example nucleotide sequences for FGF19, FGF21, and FGF23 can be found in the GenBank database at Accession numbers: NM_005117, NM_019113, and NM_020638, respectively, and herein as SEQ ID NOs: 30, 32, and 34, respectively. Example amino sequences for FGF19, FGF21, and FGF23 can be found in the GenBank database at Accession numbers: NP_005108, NP_061986, and NP_065689, respectively, and herein as SEQ ID NOs: 31, 35, and 35, respectively. Additionally, FGF may include one or more alterations which aid in the expression of the protein, e.g., the FGF23 (R179Q) variant (SEQ ID NO: 36).

The Klotho protein is a 130 kDa single pass type I transmembrane protein with an extracellular domain and a short cytoplasmic domain. The amino acid and nucleic acid sequences of Klotho are readily available to those of skill in the art. For example, example nucleotide sequences for alpha-Klotho and beta-Klotho can be found in the GenBank database at Accession numbers: NM_004795 and NM_175737, respectively, and herein as SEQ ID NOs: 7 and 8, respectively. Example amino acid sequences for alpha-Klotho and beta-Klotho can be found in the GenBank database at Accession numbers: NP_004786 and NP_783864, respectively, and herein as SEQ ID NOs: 2 and 4, respectively.

The Klotho fusion polypeptide of the disclosure can bind to a fibroblast growth factor receptor and has an alpha-Klotho or beta-Klotho extracellular domain operatively linked to either fibroblast growth factor-19 (SEQ ID NO: 31), fibroblast growth factor-21 (SEQ ID NO: 33), fibroblast growth factor-23 (SEQ ID NO: 35), or variants thereof (which include fibroblast growth factor-23 variant (R179Q) (SEQ ID NO: 36)).

Specifically, the Klotho fusion polypeptide of the disclosure may include an alpha-Klotho (SEQ ID NO: 2) which is operatively coupled to fibroblast growth factor-23 (SEQ ID NO: 35) or fibroblast growth factor-23 variant (R179Q) (SEQ ID NO: 36). Additionally, the Klotho fusion polypeptide of the disclosure may have beta-Klotho (SEQ ID NO: 4), which is operatively coupled to fibroblast growth factor-19 (SEQ ID NO: 31).

The Klotho fusion polypeptide of the disclosure may include a beta-Klotho (SEQ ID NO: 4), which is operatively coupled to fibroblast growth factor-21 (SEQ ID NO: 33).

The disclosure includes homologs of the various Klotho and FGF genes and proteins encoded by those genes. A "homolog," in reference to a gene refers to a nucleotide sequence that is substantially identical over at least part of the gene or to its complementary strand or a part thereof, provided that the nucleotide sequence encodes a protein that has substantially the same activity/function as the protein encoded by the gene which it is a homolog of. Homologs of the genes described herein can be identified by percent identity between amino acid or nucleotide sequences for putative homologs and the sequences for the genes or proteins encoded by them (e.g., nucleotide sequences for genes encoding Klotho and FGF or their complementary strands). Percent identity may be determined, for example, by visual inspection or by using various computer programs known in the art or as described herein. Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative amino acid substitutions typically include substitutions within the following groups:

glycine and alanine;

valine, isoleucine and leucine;

aspartic acid, glutamic acid, asparagine and glutamine;

serine and threonine;

lysine and arginine; and

phenylalanine and tyrosine.

Thus, mutating a glycine to alanine would be a conservative amino acid substititon, as would mutating an alanine to a glycine; mutating a valine to an isoleucine or leucine would be a conservative amino acid substation, as would replacing an isoleucine with valine or leucine, as would replacing leucine with valine or isoleucine, etc. The disclosure provides variants of all the amino acid sequences disclosed herein with at least one conservative amino acid substitution.

In an example approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

In one embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 19.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 20.

In one embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 40.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 41, or a variant thereof comprising at least one conservative amino acid substition.

In one embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 46.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 47, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 48.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 49, or a variant thereof comprising at least one conservative amino acid substition.

In one embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 50.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 51, or a variant thereof comprising at least one conservative amino acid substition.

In one embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 52.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 53, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 54, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 55, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 56, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 57, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 58, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 59, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 60, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 61, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 62, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 63, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 64, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 65, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 66, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 67, or a variant thereof comprising at least one conservative amino acid substition.

In another embodiment, the present disclosure provides a fusion polypeptide of SEQ ID NO: 68, or a variant thereof comprising at least one conservative amino acid substition.

As used herein, the terms "homology" and "homologous" are not limited to designate proteins having a theoretical common genetic ancestor, but includes proteins which may be genetically unrelated that have, nonetheless, evolved to perform similar functions and/or have similar structures. Functional homology to the various proteins described herein also encompasses proteins that have an activity of the corresponding protein of which it is a homolog. For proteins to have functional homology, it is not required that they have significant identity in their amino acid sequences, but, rather, proteins having functional homology are so defined by having similar or identical activities. For example, with respect to a Klotho molecule, the polypeptide should have the functional characteristics of binding to an FGF polypeptide and enable the binding of the FGF to an FGFR. With respect to an FGF molecule, the polypeptide should have the functional characteristics of binding to an FGFR and causing the activation of FGFR (e.g., phosphorylation). Assays for assessing FGF binding to the FGF receptor and/or activation of the FGF signaling pathway are known in the art and described herein (See Example 2). Assays for assessing Klotho activity are also known in the art and described

herein (e.g., binding to a FGF polypeptide). Proteins with structural homology are defined as having analogous tertiary (or quaternary) structure and do not necessarily require amino acid identity or nucleic acid identity for the genes encoding them. In certain circumstances, structural homologs may include proteins which maintain structural homology only at the active site or binding site of the protein.

In addition to structural and functional homology, the present disclosure further encompasses proteins having amino acid identity to the various Klotho and FGF amino acid sequences described herein. To determine the percent identity/homology of two amino acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the amino acid sequence of one protein for optimal alignment with the amino acid sequence of another protein). The amino acid residues at corresponding amino acid positions are then compared. When a position in one sequence is occupied by the same amino acid residue as the corresponding position in the other, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity= # of identical positions/total # of positions multiplied by 100).

The amino acid sequences of molecules of the disclosure described herein have an amino acid sequence which is at least about 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more identical or homologous to an amino acid sequence described herein.

The nucleic acid sequences of molecules of the disclosure described herein have a nucleotide sequence which hybridizes to or is at least about 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more identical or homologous to a nucleotide sequence described herein.

Nucleic acid molecules appropriate for use in the fusion polypeptides of the disclosure may have a Klotho or FGF nucleotide sequence which hybridizes under stringent conditions to the complement of a nucleic acid molecule encoding Klotho or FGF, respectively. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least about 70%, 80%, 85%, 90% or more homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in Ausubel et al. *Current Protocols in Molecular Biology*, Wiley Interscience, New York (2001), 6.3.1-6.3.6. A specific, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium

chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C.

4.2. Klotho-FGF fusion polypeptides of the disclosure

In some embodiments of the disclosure, a Klotho fusion polypeptide has a polypeptide chain having a first polypeptide sequence of a Klotho polypeptide or an active fragment thereof and a second polypeptide sequence encoding FGF or an active fragment thereof. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life.

The disclosure includes fusion polypeptides which are at least about 95% or more homologous to an amino acid sequence presented in SEQ ID NO: 19-28. The amino acid sequence of SEQ ID NO: 19 encodes a Klotho fusion polypeptide having a Klotho extracellular domain N-terminally linked to the FGF23 (R179Q) variant (SEQ ID NO: 36). The amino acid sequence of SEQ ID NO: 20 encodes a Klotho fusion polypeptide having an IgG signal peptide N-terminally linked to a Klotho extracellular domain lacking a signal peptide N-terminally linked to the FGF23 (R179Q) variant. The amino acid sequence of SEQ ID NO: 21 encodes a Klotho fusion polypeptide having a KL-D1 extracellular subdomain N-terminally linked to the FGF23 (R179Q) variant. The amino acid sequence of SEQ ID NO: 22 encodes a Klotho fusion polypeptide having a KL-D2 extracellular subdomain N-terminally linked to the FGF23 (R179Q) variant. The amino acid sequence of SEQ ID NO: 23 encodes a Klotho fusion polypeptide having two KL-D1 extracellular subdomains N-terminally linked to the FGF23 (R179Q) variant. The amino acid sequence of SEQ ID NO: 24 encodes a Klotho fusion polypeptide having two KL-D2 extracellular subdomains N-terminally linked to the FGF23 (R179Q) variant. The amino acid sequence of SEQ ID NO: 25 encodes a Klotho fusion polypeptide having the FGF23 (R179Q) variant N-terminally linked to a Klotho extracellular domain. The amino acid sequence of SEQ ID NO: 26 encodes a Klotho fusion polypeptide having the FGF23 (R179Q) variant N-terminally linked to a KL-D1 extracellular subdomain. The amino acid sequence of SEQ ID NO: 27 encodes a Klotho fusion polypeptide having the FGF23 (R179Q) variant N-terminally linked to a KL-D2 extracellular subdomain. The amino acid sequence of SEQ ID NO: 28 encodes a Klotho fusion polypeptide having the FGF23 (R179Q) variant N-terminally linked to two KL-D1 extracellular subdomains. The amino acid sequence of SEQ ID NO: 29 encodes a Klotho fusion polypeptide having the FGF23

(R179Q) variant N-terminally linked to two KL-D2 extracellular subdomains. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life.

The Klotho fusion polypeptide of the disclosure may include an amino acid sequence which is at least about 95% identical to the amino acid sequence set forth in SEQ ID NO: 7. The amino acid sequence of SEQ ID NO: 7 encodes a Klotho extracellular domain lacking a signal peptide. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life.

The subject fusion proteins are described herein and can be made using methods known in the art. For example, the fusion polypeptides of the disclosure may be constructed as described in U.S. No. Patent 6,194,177. The use of Klotho polypeptides is described in U.S. Patent No. 6,579,850. The use of FGF nucleic acid molecules is described in U.S. Patent No. 7,223,563.

In some embodiments, a nucleic acid molecule encoding the Klotho is cloned by PCR and ligated, in frame, with a nucleic acid molecule encoding FGF. In some embodiments, the fusion further comprises a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life. The nucleic acid encoding the fusion polypeptide is operatively linked to a promoter to allow for expression. The nucleic acid molecule encoding the fusion polypeptide is subsequently transfected into a host cell for expression. The sequence of the final construct can be confirmed by sequencing.

When preparing the fusion proteins of the present disclosure, a nucleic acid molecule encoding an extracellular subdomain of Klotho will be fused in frame to the nucleic acid molecule encoding FGF and the (optional) nucleic acid encoding the modified Fc fragment. Expression of the resulting nucleic acid molecule results in the extracellular subdomain of Klotho being fused N-terminal in relation to the FGF polypeptide. Fusions are also possible in which the extracellular subdomain of Klotho is fused C-terminal in relation to the FGF polypeptide. Methods for making fusion proteins are well known in the art.

The fusion polypeptides of the disclosure have at least two polypeptides that are covalently linked, in which one polypeptide comes from one protein sequence or domain, e.g., Klotho, and the other polypeptide comes from another protein sequence or domain, e.g., FGF. In some embodiments, the fusion further comprises a modified Fc fragment

having decreased affinity for Fc-gamma-receptor and/or increased serum half-life. In another embodiment, the disclosure comprises a FGF fused to a modified Fc fragment. Klotho and/or FGF and/or the (optional) modified Fc fragment, of the fusion polypeptides of the disclosure, can be joined by methods well known to those of skill in the art. These methods include both chemical and recombinant means.

Nucleic acids encoding the domains to be incorporated into the fusion polypeptides of the disclosure can be obtained using routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this disclosure include Sambrook and Russell, Molecular Cloning, A Laboratory Manual (3rd ed. 2001); Kriegler, Gene Transfer and Expression: A Laboratory Manual (1990); and Current Protocols in Molecular Biology (Ausubel et al., eds., 1994-1999). In nucleic acids encoding a Klotho fusion polypeptide of the disclosure, the nucleic acid sequence encoding alpha-Klotho or beta-Klotho, represented by SEQ ID NO: 1 and SEQ ID NO: 3, respectively, may be used. In nucleic acids encoding a Klotho fusion polypeptide, the nucleic acid sequence encoding FGF19, FGF21, or FGF23, represented by SEQ ID NO: 30, SEQ ID NO: 32 and SEQ ID NO: 34, respectively, may be used. Nucleic acid sequences of molecules of the disclosure described herein comprise a nucleotide sequence which hybridizes to or is at least about 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more identical or homologous to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 30, SEQ ID NO: 32, or SEQ ID NO: 34.

Nucleic acid sequences that encode the various components of the fusion [Klotho, and/or FGF peptide and/or the (optional) modified Fc fragment] can be obtained using any of a variety of methods. For example, the nucleic acid sequences encoding the polypeptides may be cloned from cDNA and genomic DNA libraries by hybridization with probes, or isolated using amplification techniques with oligonucleotide primers. More commonly, amplification techniques are used to amplify and isolate the Klotho and FGF sequences using a DNA or RNA template (see, e.g., Dieffenfach & Dveksler, PCR Primers: A Laboratory Manual (1995)). Alternatively, overlapping oligonucleotides can be produced synthetically and joined to produce one or more of the domains. Nucleic acids encoding Klotho or FGF can also be isolated from expression libraries using antibodies as probes.

According to the present disclosure, the various components of the fusion [Klotho, and/or, FGF and/or the (optional) modified Fc fragment] can be linked either directly or via a covalent linker, including amino acid linkers, such as a polyglycine linker, or

another type of chemical linker, including, carbohydrate linkers, lipid linkers, fatty acid linkers, polyether linkers, such as PEG, etc. (See for example, Hermanson, Bioconjugate techniques (1996)). The polypeptides forming the fusion/fusion polypeptide are typically linked C-terminus to N-terminus, although they can also be linked C-terminus to C-terminus, N-terminus to N-terminus, or N-terminus to C-terminus. One or more polypeptide domains may be inserted at an internal location within a fusion polypeptide of the disclosure. The polypeptides of the fusion protein can be in any order. The fusion polypeptides may be produced by covalently linking a chain of amino acids from one protein sequence, e.g., an extracellular subdomain of Klotho, to a chain of amino acids from another protein sequence, e.g., FGF, by preparing a recombinant polynucleotide contiguously encoding the fusion protein. The different chains of amino acids in a fusion protein may be directly spliced together or may be indirectly spliced together via a chemical linking group or an amino acid linking group. The amino acid linking group can be about 200 amino acids or more in length, or generally 1 to 100 amino acids. In some embodiments, proline residues are incorporated into the linker to prevent the formation of significant secondary structural elements by the linker. Linkers can often be flexible amino acid subsequences that are synthesized as part of a recombinant fusion protein. Such flexible linkers are known to persons of skill in the art.

According to the present disclosure, the amino acid sequences of the fusion [an extracellular subdomain of Klotho and/or the FGF and/or the (optional) modified Fc fragment] may be linked via a peptide linker. Example peptide linkers are well known in the art and described herein. For example, peptide linkers generally include several Gly and several Ser residues, such as: $(Gly_4\ Ser)_3$ (SEQ ID NO: 11), Gly_4 Ser polypeptide (SEQ ID NO: 12), Gly (SEQ ID NO: 13), Gly Gly (SEQ ID NO: 14), Gly Ser (SEQ ID NO: 15), Gly_2 Ser (SEQ ID NO: 16), Ala (SEQ ID NO: 17), and Ala Ala (SEQ ID NO: 18). Specifically, a peptide linker for use in a fusion protein of the disclosure may act as a flexible hinge.

The signal sequence of Klotho or FGF may be excluded prior to incorporation of Klotho into a fusion protein of the disclosure. The signal sequence for Klotho or FGF of the fusion protein may be included, e.g., the polypeptide represented by SEQ ID NO: 19. However, such sequences may also be omitted and replaced with the signal sequence of a different protein, e.g., the IgG signal sequence (SEQ ID NO: 9). Generally, the pharmaceutical compositions of the disclosure will contain the mature form of Klotho and FGF.

Generally, introns are excluded from either one or both the Klotho or the FGF moieties prior to incorporation into a fusion polypeptide.

The fusion polypeptides of the disclosure may include one or more polymers covalently attached to one or more reactive amino acid side chains. By way of example, not limitation, such polymers include polyethylene glycol (PEG), which can be attached to one or more free cysteine sulfhydryl residues, thereby blocking the formation of disulfide bonds and aggregation when the protein is exposed to oxidizing conditions. In addition, PEGylation of the fusion polypeptides of the disclosure is expected to provide such improved properties as increased half-life, solubility, and protease resistance. The fusion polypeptides of the disclosure may alternatively be modified by the covalent addition of polymers to free amino groups such as the lysine epsilon or the N-terminal amino group. Particular specific cysteines and lysines for covalent modification will be those not involved in receptor binding, heparin binding, or in proper protein folding. It will be apparent to one skilled in the art that the methods for assaying the biochemical and/or biological activity of the fusion polypeptides may be employed in order to determine if modification of a particular amino acid residue affects the activity of the protein as desired. Other similar suitable modifications are contemplated and known in the art.

The disclosure is also directed to the expression of a fusion polypeptide that is at least about 95% or more homologous to an amino acid sequence presented in SEQ ID NO: 19-28.

The present disclosure encompasses a fusion polypeptide comprising: (a) a polypeptide comprising at least one extracellular subdomain of a Klotho protein, or a functionally active variant or derivative thereof; (b) a polypeptide comprising a fibroblast growth factor, or a functionally active variant or derivative thereof; and (c) a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life. By "a functionally active variant or derivative thereof" is meant a variant or derivative comprising a longer, shorter or altered amino acid sequence than the corresponding wild-type polypeptide, while retaining the biological activity. Thus "a functionally active variant or derivative" of an extracellular subdomain of a Klotho protein or a fibroblast growth factor comprises fewer, more, or an altered amino acid sequence than a wild-type extracellular subdomain of a Klotho protein or a fibroblast growth factor, but still retains at least one biological activity of the wild-type polypeptide sequence. A functionally active variant or derivative of a polypeptide disclosed herein

can also comprise the same amino acid sequence of a polypeptide disclosed herein, but vary in post-translational modification (e.g., pegylation, methylation and/or glycosylation), or have additional moieties or elements added to it. In various embodiments, the variant or derivative of FGF23 comprises R179Q or does not.

In one embodiment, a functionally active variant or derivative polypeptide includes an amino acid sequence at least about 60% identical to a sequence disclosed herein (e.g., at least one extracellular domain of a Klotho protein or a fibroblast growth factor). Preferably, the polypeptide is at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more identical to a sequence disclosed herein.

As used herein, percent identity of two amino acid sequences (or of two nucleic acid sequences) is determined using the algorithm of Karlin and Altschul (PNAS USA 87:2264-2268, 1990), modified as in Karlin and Altschul, PNAS USA 90:5873-5877, 1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al. (J. Mol. Biol. 215:403-410, 1990). BLAST nucleotide searches are performed with the NBLAST program, score=100, wordlength=12. BLAST protein searches are performed with the XBLAST program, score=50, wordlength=3. To obtain gapped alignment for comparison purposes GappedBLAST is utilized as described in Altschul et al. (Nucleic Acids Res. 25:3389-3402, 1997). When utilizing BLAST and GappedBLAST programs the default parameters of the respective programs (e.g., XBLAST and NBLAST) are used to obtain nucleotide sequences homologous to a nucleic acid molecule of the invention.

Identity or identical means amino acid sequence (or nucleic acid sequence) similarity and has an art recognized meaning. Sequences with identity share identical or similar amino acids (or nucleic acids). Thus, a candidate sequence sharing 85% amino acid sequence identity with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 85% of the amino acids in the candidate sequence are identical to the corresponding amino acids in the reference sequence, and/or constitute conservative amino acid changes.

Functionally active variants of a polypeptide disclosed herein retain substantially the same functional activity of the original polypeptide or fragment. Naturally occurring functionally active variants such as allelic variants and species variants and non-naturally

occurring functionally active variants are included in the invention and can be produced by, for example, mutagenesis techniques or by direct synthesis.

A functionally active variant or derivative differs by about or at least, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more amino acid residues from a polypeptide disclosed herein. Where this comparison requires alignment the sequences are aligned for maximum homology. The site of variation can occur anywhere in the polypeptide, as long as activity substantially similar to a polypeptide disclosed herein.

Guidance concerning how to make variants and derivatives with phenotypically silent amino acid substitutions is provided in Bowie et al., Science, 247:1306-1310 (1990), which teaches that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, the amino acid positions which have been conserved between species can be identified. See e.g., FIG. 5. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions in which substitutions have been tolerated by natural selection indicate positions which are not critical for protein function. Thus, positions tolerating amino acid substitution can be modified while still maintaining specific binding activity of the polypeptide.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site-directed mutagenesis or alanine-scanning mutagenesis (the introduction of single alanine mutations at every residue in the molecule) can be used (Cunningham et al., Science, 244:1081-1085 (1989)).

Methods of introducing a mutation into amino acids of a protein is well known to those skilled in the art. See, e.g., Ausubel (ed.), Current Protocols in Molecular Biology, John Wiley and Sons, Inc. (1994); T. Maniatis, E. F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor laboratory, Cold Spring Harbor, N.Y. (1989)). Mutations can also be introduced using commercially available kits such as "QuikChange.TM. Site-Directed Mutagenesis Kit" (Stratagene). The generation of a

polypeptide functionally active variant or derivative to a polypeptide by replacing an amino acid that does not influence the function of a polypeptide can be accomplished by one skilled in the art.

A variant or derivative can have, for example, one or more conservative substitutions while still retaining at least one biological activity. A conservative substitution is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

Particular example variants and derivatives include, without limitation, functionally active variants and derivatives of a polypeptide comprising at least one extracellular subdomain of a Klotho protein, e.g., a polypeptide comprising at least about 100, 150, 200, 250, 300, 350, 375, 400, or 425 contiguous amino acids of an extracellular domain of Klotho (e.g., SEQ ID NO: 5 or 6), with no more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more amino acid residue differences from the wild-type sequence (as disclosed in SEQ ID NO: 5 or 6), while retaining at least one biological activity of the wild-type polypeptide. For example, a functionally active variant or derivative of a polypeptide comprising at least one extracellular subdomain of a Klotho protein comprises a polypeptide comprising at least about 400 contiguous amino acids of SEQ ID NO: 5 or 6, with no more than about 100 amino acid residue differences. For example, a functionally active variant or derivative of a polypeptide comprising at least one extracellular subdomain of a Klotho protein comprises a polypeptide comprising at least about 400 contiguous amino acids of SEQ ID NO: 5 or 6, with no more than about 50 amino acid residue differences. For example, a functionally active variant or derivative of a polypeptide comprising at least one extracellular subdomain of a Klotho protein comprises a polypeptide comprising at least about 425 contiguous amino acids of SEQ ID NO: 5 or 6, with no more than about 25 amino acid residue differences. For example, a functionally active variant or derivative of a polypeptide comprising at least one extracellular subdomain of a Klotho protein comprises a polypeptide comprising at least about 425 contiguous amino acids of SEQ ID NO: 5 or 6, with no more than about 10 amino acid residue differences. In

another example, a functionally active variant or derivative of a polypeptide comprising at least one extracellular subdomain of a Klotho protein comprises a polypeptide comprising at least about 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 925, 950 or 982 contiguous amino acids of SEQ ID NO: 7, with no more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 75, 80, 85, 90, 95, 100, 110, 120, 140, 150, 160, 170, 180, 190, or 200 amino acid residue differences from the wild-type sequence. For example, a functionally active variant or derivative of a polypeptide comprising at least one extracellular subdomain of a Klotho protein comprises a polypeptide comprising at least about 500 contiguous amino acids of SEQ ID NO: 7, with no more than about 100 amino acid residue differences. For example, a functionally active variant or derivative of a polypeptide comprising at least one extracellular subdomain of a Klotho protein comprises a polypeptide comprising at least about 600 contiguous amino acids of SEQ ID NO: 7, with no more than about 100 amino acid residue differences. For example, a functionally active variant or derivative of a polypeptide comprising at least one extracellular subdomain of a Klotho protein comprises a polypeptide comprising at least about 700 contiguous amino acids of SEQ ID NO: 7, with no more than about 100 amino acid residue differences. For example, a functionally active variant or derivative of a polypeptide comprising at least one extracellular subdomain of a Klotho protein comprises a polypeptide comprising at least about 800 contiguous amino acids of SEQ ID NO: 7, with no more than about 100 amino acid residue differences. For example, a functionally active variant or derivative of a polypeptide comprising at least one extracellular subdomain of a Klotho protein comprises a polypeptide comprising at least about 900 contiguous amino acids of SEQ ID NO: 7, with no more than about 100 amino acid residue differences. For example, a functionally active variant or derivative of a polypeptide comprising at least one extracellular subdomain of a Klotho protein comprises a polypeptide comprising at least about 900 contiguous amino acids of SEQ ID NO: 7, with no more than about 50 amino acid residue differences.

Particular example variants and derivatives include, without limitation, functionally active variants and derivatives of a polypeptide comprising a fibroblast growth factor, e.g., a polypeptide comprising at least about 100, 125, 150, 150, 175, 200, 225, or 250 contiguous amino acids of a fibroblast growth factor, e.g., FGF19 (SEQ ID NO: 31), FGF21 (SEQ ID NO: 33), or FGF23 (SEQ ID NO: 35), with no more than about

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more amino acid residue differences from the wild-type sequence (as disclosed in SEQ ID NOs: 31, 33 or 35), while retaining at least one biological activity of the wild-type polypeptide. In various embodiments, the variant or derivative can comprise the R179Q variation or not. For example, a functionally active variant or derivative of a polypeptide comprising a fibroblast growth factor comprises a polypeptide comprising at least about 150 contiguous amino acids of SEQ ID NOs: 31, 33 or 35, with no more than about 25 amino acid residue differences. For example, a functionally active variant or derivative of a polypeptide comprising a fibroblast growth factor comprises a polypeptide comprising at least about 175 contiguous amino acids of SEQ ID NOs: 31, 33 or 35, with no more than about 25 amino acid residue differences. For example, a functionally active variant or derivative of a polypeptide comprising a fibroblast growth factor comprises a polypeptide comprising at least about 200 contiguous amino acids of SEQ ID NOs: 31, 33 or 35, with no more than about 25 amino acid residue differences. For example, a functionally active variant or derivative of a polypeptide comprising a fibroblast growth factor comprises a polypeptide comprising at least about 225 contiguous amino acids of SEQ ID NO: 35, with no more than about 50 amino acid residue differences. For example, a functionally active variant or derivative of a polypeptide comprising a fibroblast growth factor comprises a polypeptide comprising at least about 225 contiguous amino acids of SEQ ID NO: 35, with no more than about 25 amino acid residue differences.

4.3. Expression of fusion polypeptides of the disclosure

In order to express the fusion protein of the disclosure, DNA molecules obtained by any of the methods described herein or those that are known in the art, can be inserted into appropriate expression vectors by techniques well known in the art. For example, a double stranded cDNA can be cloned into a suitable vector by homopolymeric tailing or by restriction enzyme linking involving the use of synthetic DNA linkers or by blunt-ended ligation. DNA ligases are usually used to ligate the DNA molecules and undesirable joining can be avoided by treatment with alkaline phosphatase.

Therefore, the disclosure includes vectors (e.g., recombinant plasmids and bacteriophages) that include nucleic acid molecules (e.g., genes or recombinant nucleic acid molecules encoding genes) as described herein. The term "recombinant vector"

includes a vector (e.g., plasmid, phage, phasmid, virus, cosmid, fosmid, or other purified nucleic acid vector) that has been altered, modified or engineered such that it contains greater, fewer or different nucleic acid sequences than those included in the native or natural nucleic acid molecule from which the recombinant vector was derived. For example, a recombinant vector may include a nucleotide sequence encoding a Klotho-FGF23 fusion operatively linked to regulatory sequences, e.g., promoter sequences, terminator sequences and/or artificial ribosome binding sites (RBSs), as defined herein. Recombinant vectors which allow for expression of the genes or nucleic acids included in them are referred to as "expression vectors."

For eukaryotic hosts, different transcriptional and translational regulatory sequences may be employed, depending on the nature of the host. They may be derived from viral sources, such as adenovirus, bovine papilloma virus, Simian virus or the like, where the regulatory signals are associated with a particular gene which has a high level of expression. Examples include, but are not limited to, the TK promoter of the Herpes virus, the SV40 early promoter, the yeast ga14 gene promoter, etc. Transcriptional initiation regulatory signals may be selected which allow for repression or activation, so that expression of the genes can be modulated.

In some of the molecules of the disclosure described herein, one or more DNA molecules having a nucleotide sequence encoding one or more polypeptide chains of a fusion polypeptide are operatively linked to one or more regulatory sequences, which are capable of integrating the desired DNA molecule into a host cell. Cells which have been stably transformed by the introduced DNA can be selected, for example, by introducing one or more markers which allow for selection of host cells which contain the expression vector. A selectable marker gene can either be linked directly to a nucleic acid sequence to be expressed, or be introduced into the same cell by co-transfection. Additional elements may also be needed for optimal synthesis of proteins described herein. It would be apparent to one of ordinary skill in the art which additional elements to use.

Factors of importance in selecting a particular plasmid or viral vector include, but are not limited to, the ease with which recipient cells that contain the vector are recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

Once the vector(s) is constructed to include a DNA sequence for expression, it may be introduced into an appropriate host cell by one or more of a variety of suitable

methods that are known in the art, including but not limited to, for example, transformation, transfection, conjugation, protoplast fusion, electroporation, calcium phosphate-precipitation, direct microinjection, etc.

Host cells may either be prokaryotic or eukaryotic. Examples of eukaryotic host cells include, for example, mammalian cells, such as human, monkey, mouse, and Chinese hamster ovary (CHO) cells. Such cells facilitate post-translational modifications of proteins, including, for example, correct folding or glycosylation. Additionally, yeast cells can also be used to express fusion polypeptides of the disclosure. Like most mammalian cells, yeast cells also enable post-translational modifications of proteins, including, for example, glycosylation. A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number plasmids that can be utilized for production of proteins in yeast. Yeast transcription and translation machinery can recognize leader sequences on cloned mammalian gene products, thereby enabling the secretion of peptides bearing leader sequences (i.e., pre-peptides). A particularmethod of high-yield production of the fusion polypeptides of the disclosure is through the use of dihydrofolate reductase (DHFR) amplification in DHFR-deficient CHO cells, by the use of successively increasing levels of methotrexate as described in U.S. Patent No. 4,889,803. The polypeptide obtained may be in a glycosylated form.

After the introduction of one or more vector(s), host cells are usually grown in a selective medium, which selects for the growth of vector-containing cells. Purification of the recombinant proteins can be carried out by any of the methods known in the art or described herein, for example, any conventional procedures involving extraction, precipitation, chromatography and electrophoresis. A further purification procedure that may be used for purifying proteins is affinity chromatography using monoclonal antibodies which bind a target protein. Generally, crude preparations containing a recombinant protein are passed through a column on which a suitable monoclonal antibody is immobilized. The protein usually binds to the column via the specific antibody while the impurities pass through. After washing the column, the protein is eluted from the gel by changing pH or ionic strength, for example.

4.4. Assays for assessing fusion polypeptide activity

Assays described herein (See Example 2) and those known in the art can be used for detecting Klotho or FGF activity of the fusion polypeptides of the disclosure. Suitable activity assays include receptor binding assays, cellular proliferation assays and cell

signaling assays. For example, a binding assay which may be used for determining whether a fusion polypeptide has Klotho or FGF activity includes, assaying the binding of a fusion polypeptide to an FGF receptor. FGF receptor binding assays include, but are not limited to, both competitive and non-competitive assay. For example, FGF receptor binding can be detected by contacting cells expressing an FGF receptor with a labeled FGF (for example, radio-active label) and increasing concentrations of an unlabeled Klotho-FGF fusion polypeptide. The two ligands that compete for binding to the same receptor are added to a reaction mixture containing the cell. The cells are subsequently washed and labeled FGF is measured. A decrease in the amount of the labeled FGF to its receptor in the presence of the unlabeled fusion polypeptide is indicative of binding of the Klotho-FGF fusion polypeptide to the receptor. Alternatively, the Klotho-FGF fusion polypeptide may be labeled and direct binding of the fusion polypeptide to the cell is detected.

Klotho or FGF activity can also be measured by determining whether the fusion polypeptide induces a cellular response. For example, in some embodiments, an assay for detecting the biological activity of a Klotho-FGF fusion polypeptide involves contacting cells which express an FGF receptor with a fusion polypeptide, assaying a cellular response such as, for example, cell proliferation or Egr-1 activation, myotube diameter in C2C12 cells, and comparing the cellular response in the presence and absence of the fusion polypeptide. An increase in the cellular response in the presence of the fusion polypeptide complex relative to the absence indicates that the fusion polypeptide has biological activity. Also, an increase in a downstream signaling event from the receptor can also be measured as indicia of biological activity (e.g., phosphorylation of FGFR, FRS2, ERK1/2, p70S6K etc.).

4.5 Pharmaceutical compositions and methods of treatment

The disclosure also pertains to pharmaceutical compositions containing one or more fusion polypeptides of the disclosure and a pharmaceutically acceptable diluent or carrier. The pharmaceutical compositions can further include a pharmaceutically effective dose of heparin. Such pharmaceutical compositions may be included in a kit or container. Such kit or container may be packaged with instructions pertaining to the extended *in vivo* half-life or the *in vitro* shelf life of the fusion polypeptides. Optionally associated with such kit or container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or

biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. Such compositions may be used in methods of treating, preventing, or ameliorating a disease or a disease symptom (e.g., age-related condition or metabolic disorder) in a patient, preferably a mammal and most preferably a human, by administering the pharmaceutical composition to the patient.

In general, a therapeutically effective amount of a pharmaceutical composition of the disclosure is from about 0.0001 mg/kg to 0.001 mg/kg; 0.001 mg/kg to about 10 mg/kg body weight or from about 0.02 mg/kg to about 5 mg/kg body weight. Commonly, a therapeutically effective amount of a fusion polypeptide is from about 0.001 mg to about 0.01 mg, about 0.01 mg to about 100 mg, or from about 100 mg to about 1000 mg, for example. Preferably, a therapeutically effective amount of a fusion polypeptide is from about 0.001 mg/kg to 2mg/kg.

The optimal pharmaceutical formulations for a fusion polypeptide can be determined by one or ordinary skilled in the art depending upon the route of administration and desired dosage. (See, for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990), Mack Publishing Co., Easton, Pa., the entire disclosure of which is hereby incorporated by reference).

The fusion polypeptides of the disclosure may be administered as a pharmaceutical composition that may be in the form of a solid, liquid or gas (aerosol). Typical routes of administration may include, without limitation, oral, topical, parenteral, sublingual, rectal, vaginal, intradermal and intranasal. Parenteral administration includes subcutaneous injections, intravenous, intramuscular, intraperitoneal, intrapleural, intrasternal injection or infusion techniques. Preferably, the compositions are administered parenterally. More preferably, the compositions are administered intravenously. Pharmaceutical compositions of the disclosure can be formulated so as to allow a polypeptide of the disclosure to be bioavailable upon administration of the composition to a subject. Compositions can take the form of one or more dosage units, where, for example, a tablet can be a single dosage unit, and a container of a polypeptide of the disclosure in aerosol form can hold a plurality of dosage units.

Materials used in preparing the pharmaceutical compositions can be non-toxic in the amounts used. It will be evident to those of ordinary skill in the art that the optimal dosage of the active ingredient(s) in the pharmaceutical composition will depend on a variety of factors. Relevant factors include, without limitation, the type of subject (e.g., human), the overall health of the subject, the type of age-related condition or metabolic

disorder the subject in need of treatment of, the use of the composition as part of a multi-drug regimen, the particular form of the polypeptide of the disclosure, the manner of administration, and the composition employed.

The pharmaceutically acceptable carrier or vehicle may be particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) can be liquid, with the compositions being, for example, an oral syrup or injectable liquid. In addition, the carrier(s) can be gaseous, so as to provide an aerosol composition useful in, e.g., inhalatory administration.

The term "carrier" refers to a diluent, adjuvant or excipient, with which a polypeptide of the disclosure is administered. Such pharmaceutical carriers can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The carriers can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents can be used. In one embodiment, when administered to a subject, the polypeptides of the disclosure and pharmaceutically acceptable carriers are sterile. Water is a particular carrier when the polypeptide of the disclosure is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The composition may be intended for oral administration, and if so, the composition is preferably in solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the composition can be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like form. Such a solid composition typically contains one or more inert diluents. In addition, one or more of the following can be present: binders such as ethyl cellulose, carboxymethylcellulose, microcrystalline cellulose, or gelatin; excipients such as starch, lactose or dextrins, disintegrating agents such as alginic acid, sodium alginate, Primogel,

corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin, a flavoring agent such as peppermint, methyl salicylate or orange flavoring, and a coloring agent.

When the pharmaceutical composition is in the form of a capsule, e.g., a gelatin capsule, it can contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol, cyclodextrin or a fatty oil.

The pharmaceutical composition can be in the form of a liquid, e.g., an elixir, syrup, solution, emulsion or suspension. The liquid can be useful for oral administration or for delivery by injection. When intended for oral administration, a composition can contain one or more of a sweetening agent, preservatives, dye/colorant and flavour enhancer. In a composition for administration by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent can also be included.

The liquid compositions of the disclosure, whether they are solutions, suspensions or other like form, can also include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or digylcerides which can serve as the solvent or suspending medium, polyethylene glycols, glycerin, cyclodextrin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral composition can be enclosed in an ampoule, a disposable syringe or a multiple-dose vial made of glass, plastic or other material. Physiological saline is a particular specific adjuvant. An injectable composition is preferably sterile.

The pharmaceutical compositions contain an effective amount of a compound of the disclosure (e.g., fusion polypeptide) such that a suitable dosage will be obtained. The pharmaceutical compositions may contain the known effective amount of the compounds as currently prescribed for their respective disorders.

The route of administration of the polypeptide of the disclosure used in the prophylactic and/or therapeutic regimens which will be effective in the prevention, treatment, and/or management of a age-related condition or metabolic disorder can be based on the currently prescribed routes of administration for other therapeutics known in the art. The polypeptides of the disclosure can be administered by any convenient route,

for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.). Administration can be systemic or local. Various delivery systems are known, e.g., microparticles, microcapsules, capsules, etc., and may be useful for administering a polypeptide of the disclosure. More than one polypeptides of the disclosure may be administered to a subject. Methods of administration may include, but are not limited to, oral administration and parenteral administration; parenteral administration including, but not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, sublingual, intranasal, intracerebral, intraventricular, intrathecal, intravaginal, transdermal, rectally, by inhalation, or topically to the ears, nose, eyes, or skin.

The polypeptides of the disclosure may be administered parenterally. Specifically, the polypeptides of the disclosure may be administered intravenously.

Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent, or via perfusion in a fluorocarbon or synthetic pulmonary surfactant. The polypeptides of the disclosure can also be formulated as a suppository, with traditional binders and carriers such as triglycerides.

The polypeptides of the disclosure can be delivered in a controlled release system. For example, a pump can be used (see Sefton, *CRC Crit. Ref. Biomed. Eng.* 1987, *14*, 201; Buchwald *et al.*, Surgery 1980, *88*: 507; Saudek *et al.*, *N. Engl. J. Med.* 1989, 321: 574). Polymeric materials can also be used for controlled release of the polypeptides of the disclosure (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, FL, 1974; *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York, 1984; Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* 1983, *23*, 61; *see also* Levy *et al.*, *Science* 1985, *228*, 190; During *et al.*, *Ann. Neurol.*, 1989, *25*, 351; Howard *et al.*, *J. Neurosurg.*, 1989, *71*, 105). Specifically, a controlled-release system can be placed in proximity of the target of the polypeptides of the disclosure, e.g., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, 1984, pp. 115-138). Other controlled-release systems discussed in the review by Langer (*Science* 1990, *249*, 1527-1533) can be used.

Polymeric materials used to achieve controlled or sustained release of the polypeptides of the disclosure are disclosed, e.g., in U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent

No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. Preferably, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable.

In general, a therapeutically effective amount of a pharmaceutical composition of the disclosure is from about 0.0001 mg/kg to 0.001 mg/kg; 0.001 mg/kg to about 10 mg/kg body weight or from about 0.02 mg/kg to about 5 mg/kg body weight.

In other embodiments, the prophylactic and/or therapeutic regimen involves administering to a patient one or more doses of an effective amount of a polypeptide of the disclosure, wherein the dose of an effective amount achieves a plasma level of at least 0.01 μg/mL to at least 400 μg/mL of the polypeptide of the disclosure.

A prophylactic and/or therapeutic regimen may involve administering to a patient a plurality of doses of an effective amount of a polypeptide of the disclosure, wherein the plurality of doses maintains a plasma level of at least 0.01 μg/mL, to 400 μg/mL of the polypeptide of the disclosure. The prophylactic and/or therapeutic regimen may be administered for at least 1 day, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months or 9 months.

The prophylactic and/or therapeutic regimen may involve administration of a polypeptide of the disclosure in combination with one or more additional therapeutics. The recommended dosages of the one or more therapeutics currently used for the prevention, treatment, and/or management of an age-related condition or metabolic disorder can be obtained from any reference in the art including, but not limited to, Hardman *et al.*, eds., *Goodman & Gilman's The Pharmacological Basis Of Basis Of Therapeutics, 10th ed.*, McGraw-Hill, New York, 2001; *Physician's Desk Reference* (60th ed., 2006), which is incorporated herein by reference in its entirety.

The disclosure includes methods of treating disorders wherein agonistic activity of Klotho protein and FGF are desirable. The disclosure furthermore includes the use of the disclosed proteins, fusion proteins, nucleic acid molecules or pharmaceutical composition in therapy or as medicament for the treatment of a pathological disorder wherein agonistic

activity of Klotho protein and FGF are desirable. Examples of such methods or uses of the disclosure include, but are not limited to age-related condition or metabolic disorders.

The disclosure includes methods for treating or preventing an age-related condition in an individual; and the use of the disclosed proteins, fusion proteins, nucleic acid molecules or pharmaceutical composition in therapy or as medicament for treating or preventing an age-related condition in an individual. An individual in need of treatment is administered a pharmacologically effective dose of a pharmaceutical composition containing a Klotho fusion polypeptide, having at least one extracellular subdomain of a Klotho protein and a fibroblast growth factor and an (optional) modified Fc fragment, so as to treat or prevent the age-related condition. In some embodiments, the Klotho fusion polypeptide is co-administered with a pharmacologically effective dose of heparin. Age-related conditions include sarcopenia, skin atrophy, muscle wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, memory loss, wrinkles, impaired kidney function, and age-related hearing loss. In some embodiments, the Klotho fusion polypeptide contains at least one extracellular domain of an alpha Klotho protein. In a particular embodiment, a Klotho fusion protein containing at least one extracellular domain of alpha Klotho protein and fibroblast growth factor 23 is administered to an individual in need of treatment for muscle wasting.

The disclosure is also directed to a method for treating or preventing a metabolic disorder in an individual; and to the use of the disclosed proteins, fusion proteins, nucleic acid molecules or pharmaceutical composition in therapy or as medicament for treating or preventing metabolic disorder in an individual. An individual in need of treatment is administered a pharmacologically effective dose of a pharmaceutical composition containing a Klotho fusion polypeptide, having at least one extracellular subdomain of a Klotho protein and a fibroblast growth factor so as to treat the metabolic disorder, and an (optional) modified Fc fragment having decreased binding to FcRn and/or increased serum half-life and/or stability. In some embodiments, the Klotho fusion polypeptide is co-administered with a pharmacologically effective dose of heparin. The method may be used in the treatment or prevention of Type II Diabetes, Metabolic Syndrome, hyperglycemia, and obesity. In a particular embodiment, a Klotho fusion protein containing at least one extracellular domain of a beta-Klotho protein and fibroblast

growth factor 21 is administered to an individual in need of treatment for a metabolic disorder.

The disclosure also provides methods for treating or preventing hyperphosphatemia or calcinosis in an individual; and the use of the disclosed proteins, fusion proteins, nucleic acid molecules or pharmaceutical composition in therapy or as medicament for treating or preventing hyperphosphatemia or calcinosis in an individual. An individual in need of treatment is administered a pharmacologically effective dose of a pharmaceutical composition containing a Klotho fusion polypeptide, having at least one extracellular subdomain of a Klotho protein, a fibroblast growth factor and an (optional) modified Fc fragment so as to treat hyperphosphatemia or calcinosis. In some embodiments, the Klotho fusion polypeptide is co-administered with a pharmacologically effective dose of heparin. In a particular embodiment, a Klotho fusion protein containing at least one extracellular domain of an alpha Klotho protein and fibroblast growth factor 23 and an (optional) modified Fc fragment is administered to an individual in need of treatment for a hyperphosphatemia or calcinosis.

The disclosure is also directed to a method for treating or preventing chronic renal disease or chronic renal failure in an individual; and to the use of the disclosed proteins, fusion proteins, nucleic acid molecules or pharmaceutical composition in therapy or as medicament for treating or preventing chronic renal disease or chronic renal failure in an individual. An individual in need of treatment is administered a pharmacologically effective dose of a pharmaceutical composition containing a Klotho fusion polypeptide, having at least one extracellular subdomain of a Klotho protein, a fibroblast growth factor and an (optional) modified Fc fragment so as to treat chronic renal disease or chronic renal failure. In some embodiments, the Klotho fusion polypeptide is co-administered with a pharmacologically effective dose of heparin. In some embodiments, a Klotho fusion protein containing at least one extracellular domain of an alpha Klotho protein is administered to an individual in need of treatment for chronic renal disease or chronic renal failure.

The disclosure also includes methods for treating or preventing cancer in an individual; and the use of the disclosed proteins, fusion proteins, nucleic acid molecules or pharmaceutical composition in therapy or as medicament for treating or preventing cancer in an individual. An individual in need of treatment is administered a pharmacologically effective dose of a pharmaceutical composition containing a Klotho fusion polypeptide, having at least one extracellular subdomain of a Klotho protein, a

fibroblast growth factor and an (optional) modified Fc fragment so as to treat cancer. The method may be used in the treatment or prevention of breast cancer. In some embodiments, the Klotho fusion polypeptide is co-administered with a pharmacologically effective dose of heparin. In some embodiments, a Klotho fusion protein containing at least one extracellular domain of an alpha Klotho protein is administered to an individual in need of treatment for cancer.

In methods of treating disorders by administering a pharmaceutical composition containing a Klotho fusion polypeptide; or when using pharmaceutical composition containing a Klotho fusion polypeptide in therapy, the Klotho fusion polypeptide and an (optional) modified Fc fragment has at least one extracellular subdomain of a Klotho protein and a fibroblast growth factor. In a particular embodiment, the Klotho fusion protein contains at least one extracellular domain of a beta Klotho protein and fibroblast growth factor 21.

In another embodiment, the fusion comprises a FGF (e.g., FGF19, FGF21, FGF23 or FGF23 variant) and a modified Fc fragment with decreased binding to FcRn and/or increased serum stability. This type of fusion can be used in various diseases, as described above, or used to treat or prevent any FGF-related disease known in the art. The fusion can be administered to an individual in need thereof.

The fusion polypeptide compositions can be administered according to any method of administration known to those of skill in the art and described herein. Particular specific methods of administration include subcutaneous or intravenous. Other effective modes of administration are described herein.

4.6. Methods of Treatment and Assays for Assessing Efficacy

Methods or uses of the disclosure which provide administering the fusion polypeptides described herein to an individual can be used to treat a variety of disorders including an age-related disorder or a metabolic disorder. Without being limited by any particular theory, fusion polypeptides may be used to treat disorders in which there is dysregulation of Klotho or FGF. Example disorders include metabolic disorders and age-related disorders. For example, both FGF23 or Klotho knock-out mice display a variety of similar phenotypes including, low physical activity, growth retardation, muscle wasting, skin atrophy, atherosclerosis, short life spans, etc. (See Razzaque and Lanske, *J. of Endrocrinology*, 194:1-10 (2007), which is herein incorporated by reference).

In particular, fusion polypeptides of the disclosure are particularly useful in the treatment of aging-related disorders, including muscle wasting. Without being bound to theory, the ability of Klotho and FGF23 to control mineral (e.g., phosphate and calcium) and vitamin D homeostasis may be the means by which these proteins modulate aging and muscle atrophy.

On the other hand, fusion polypeptides of the disclosure may be used for treating a metabolic disorder. For example, beta-Klotho and FGF19 have been shown to control bile acid homeostasis by regulating cholesterol 7-α-hydroxylase (CYP7A1). A non-limiting example of bile homeostasis disorder is cholestasis. The beta-Klotho and FGF21 have been shown to induce lipolysis in adipocytes and, therefore, reduced fat storage and increased glucose uptake. Non-limiting examples of lipolysis/fat storage disorders are obesity and associated metabolic and cardiovascular diseases.

Based at least in part on the finding that FGF23 is able to stimulate excretion of phosphate in the urine and thereby reduce phosphate levels in the serum, Klotho-FGF23 fusion polypeptides of the disclosure can be used for treating or preventing hyperphosphatemia or calcinosis in an individual. For example, it has been shown that a homozygous missense mutation in Klotho resulting in a deficiency in Klotho in a patient can cause severe tumoral calcinosis and artery calcification (Ichikawa et al., *J. Clin. Invest.* 117:2684-2691 (2007), which is herein incorporated by reference). An individual is administered a pharmacologically effective dose of a pharmaceutical composition containing the Klotho fusion polypeptide, having at least one extracellular subdomain of a Klotho protein, a fibroblast growth factor and an (optional) modified Fc fragment so as to treat or prevent hyperphosphatemia or calcinosis. In particular, a Klotho fusion polypeptide containing at least one extracellular domain of an alpha Klotho protein, a fibroblast growth factor and an (optional) modified Fc fragment is useful for treating hyperphosphatemia or calcinosis.

Klotho fusion polypeptides of the disclosure can also be used for treating or preventing chronic renal disease or chronic renal failure in an individual. For example, it has been shown that Klotho expression is reduced in kidney of patients with chronic renal failure, compared to that in unaffected kidneys (Koh et al., *Biochem. Biophys. Res. Comm.* 280:1015-1020 (2001), which is herein incorporated by reference). An individual is administered a pharmacologically effective dose of a pharmaceutical composition containing the Klotho fusion polypeptide, having at least one extracellular subdomain of a Klotho protein, a fibroblast growth factor and an (optional) modified Fc fragment so as to

treat or prevent chronic renal disease or chronic renal failure. In particular, a Klotho fusion polypeptide containing at least one extracellular domain of an alpha Klotho protein is useful for treating chronic renal disease or chronic renal failure.

Klotho fusion polypeptides of the disclosure can also be used for treating or preventing cancer in an individual. For example, it has been shown that Klotho expression is reduced in breast cancer tissue, compared to normal breast cancer tissue (Wolf et al., *Oncogene* (2008) advance online publication, which is herein incorporated by reference). An individual is administered a pharmacologically effective dose of a pharmaceutical composition containing the Klotho fusion polypeptide, having at least one extracellular subdomain of a Klotho protein, a fibroblast growth factor and an (optional) modified Fc fragment so as to treat or prevent cancer or breast cancer. In particular, a Klotho fusion protein containing at least one extracellular domain of an alpha Klotho protein is useful for treating cancer or breast cancer.

Methods for evaluating the efficacy and/or determining the effective dose of a Klotho fusion polypeptide of the disclosure on an age-related disorder or metabolic disorder include organismal based assays, e.g., using a mammal (e.g., a mouse, rat, primate, or some other non-human), or other animal (e.g., Xenopus, zebrafish, or an invertebrate such as a fly or nematode). The Klotho fusion polypeptide can be administered to the organism once or as a regimen (regular or irregular). A parameter of the organism is then evaluated, e.g., an age-associated parameter. Klotho fusion polypeptides that are of interest result in a change in the parameter relative to a reference, e.g., a parameter of a control organism. Other parameters (e.g., related to toxicity, clearance, and pharmacokinetics) can also be evaluated.

The Klotho fusion polypeptide of the disclosure may be evaluated using an animal that has a particular disorder, e.g., a disorder described herein, e.g., an age-related disorder, a metabolic disorder. These disorders can also provide a sensitized system in which the test polypeptide's effects on physiology can be observed. Example disorders include: denervation, disuse atrophy; metabolic disorders (e.g., disorder of obese and/or diabetic animals such as db/db mouse and ob/ob mouse); cerebral, liver ischemia; cisplatin/taxol/vincristine models; various tissue (xenograph) transplants; transgenic bone models; pain syndromes (include inflammatory and neuropathic disorders); Paraquat, genotoxic, and oxidative stress models; and tumor I models.

For measuring an age-related disorder, the animal model can be an animal that has an altered phenotype when calorically restricted. For example, F344 rats provide a useful

assay system for evaluating a Klotho fusion polypeptide. When calorically restricted, F344 rats have a 0 to 10% incidence of nephropathy. However, when fed ad libitum, they have a 60 to 100% incidence of nephropathy.

To evaluate a Klotho fusion polypeptide of the disclosure, it is administered to the animal (e.g., an F344 rat or other suitable animal) and a parameter of the animal is evaluated, e.g., after a period of time. The animal can be fed ad libitum or normally (e.g., not under caloric restriction, although some parameters can be evaluated under such conditions). Typically, a cohort of such animals is used for the assay. Generally, a test polypeptide can be indicated as favorably altering lifespan regulation in the animal if the test polypeptide affects the parameter in the direction of the phenotype of a similar animal subject to caloric restriction. Such test polypeptides may cause at least some of the lifespan regulatory effects of caloric restriction, e.g., a subset of such effects, without having to deprive the organism of caloric intake.

The parameter to be tested may be an age-associated or disease associated parameter, e.g., a symptom of the disorder associated with the animal model. For example, the test polypeptide can be administered to a SH Rat, and blood pressure is monitored. A test polypeptide that is favorably indicated can cause an amelioration of the symptom relative to a similar reference animal not treated with the polypeptide. Other parameters relevant to a disorder or to aging can include: antioxidant levels (e.g.. antioxidant enzyme levels or activity), stress resistance (e.g., paraquat resistance), core body temperature, glucose levels, insulin levels, thyroid-stimulating hormone levels, prolactin levels, and leutinizing hormone levels.

To measure the effectiveness of the polypeptides of the disclosure for treating an age-related disorder, an animal having decreased Klotho expression may be used, e.g., mouse with a mutant Klotho; See Kuroo, et al. Nature, 390; 45 (1997) and U.S. Pub. No. 2003/0119910, both of which are herein incorporated by reference in their entirety. For example, the test polypeptide is administered to the mutant mouse and age-related parameters are monitored. A test polypeptide that is favorably indicated can cause an amelioration of the symptom relative to a similar reference animal not treated with the polypeptide. A parameter relevant to a metabolic disorder or to aging can be assessed by measurement of body weight, examination on the acquisition of reproductive ability, measurement of blood sugar level, observation of life span, observation of skin, observation of motor functions such as walking, and the like. The assessment can also be made by measurement of thymus weight, observation of the size of calcified nodules

formed on the inner surface of thoracic cavity, and the like. Further, quantitative determination of mRNA for the Klotho gene or Klotho protein is also useful for the assessment.

Still other in vivo models and organismal assays include evaluating an animal for a metabolic parameter, e.g., a parameter relevant to an insulin disorder, type II diabetes. Example metabolic parameters include: glucose concentration, insulin concentration, and insulin sensitivity.

Another example system features tumors, e.g., in an animal model. The tumors can be spontaneous or induced. For example, the tumors can be developed from cells that have a variety of genetic constitutions, e.g., they can be p53+ or p53-. It is also possible to use organisms that an autoimmune disorder, e.g., an NZB mouse, which is predisposed to SLE. To evaluate features of bone disease, it is possible, for example, to use an animal that has an ovariectomy as a model,. e.g., for osteoporosis. Similarly, for joint disease, the model can be based on adjuvant arthritis (e.g., mice can be immunized with cartilage proteoglycans, high mobility group proteins, streptococcal cell wall material, or collagens); for kidney disease, kd/kd mice can be used. Animal models of cognition, particularly learning and memory are also available. Animal models of diabetes and its complications are also available, e.g., the streptozotocin model. Canine models can be used, for example, for evaluating stroke and ischemia.

In assessing whether a test polypeptide is capable of altering life span regulation, a number of age-associated parameters or biomarkers can be monitored or evaluated. Example age associated parameters include: (i) lifespan of the cell or the organism; (ii) presence or abundance of a gene transcript or gene product in the cell or organism that has a biological age dependent expression pattern; (iii) resistance of the cell or organism to stress; (iv) one so or more metabolic parameters of the cell or organism (example parameters include circulating insulin levels, blood glucose levels; fat content; core body temperature and so forth); (v) proliferative capacity of the cell or a set of cells present in the organism; and (vi) physical appearance or behavior of the cell or organism.

The term "average lifespan" refers to the average of the age of death of a cohort of organisms. In some cases, the "average lifespan" is assessed using a cohort of genetically identical organisms under controlled environmental conditions. Deaths due to mishap are discarded. Where average lifespan cannot be determined (e.g., for humans) under controlled environmental conditions, reliable statistical information (e.g., from actuarial tables) for a sufficiently large population can be used as the average lifespan.

Characterization of molecular differences between two such organisms, e.g., one reference organism and one organism treated with a Klotho fusion polypeptide can reveal a difference in the physiological state of the organisms. The reference organism and the treated organism are typically the same chronological age. The term "chronological age" as used herein refers to time elapsed since a preselected event, such as conception, a defined embryological or fetal stage, or, more preferably, birth. A variety of criteria can be used to determine whether organisms are of the "same" chronological age for the comparative analysis. Typically, the degree of accuracy required is a function of the average lifespan of a wildtype organism. For example, for the nematode *C. elegans*, for which the laboratory wildtype strain N2 lives an to average of about 16 days under some controlled conditions, organisms of the same age may have lived for the same number of days. For mice, organism of the same age may have lived for the same number of weeks or months; for primates or humans, the same number of years (or within 2, 3, or 5 years); and so forth. Generally, organisms of the same chronological age may have lived for an amount of time within 15, 10, 5, 3, 2 or 1% of the average lifespan of a wildtype organism of that species. Preferably, the organisms are adult organisms, e.g., the organisms have lived for at least an amount of time in which the average wildtype organism has matured to an age at which it is competent to reproduce.

The organismal screening assay can be performed before the organisms exhibit overt physical features of aging. For example, the organisms may be adults that have lived only 10, 30, 40, 50, 60, or 70% of the average lifespan of a wildtype organism of the same species. Age-associated changes in metabolism, immune competence, and chromosomal structure have been reported. Any of these changes can be evaluated, either in a test subject (e.g., for an organism based assay), or for a patient (e.g., prior, during or after treatment with a therapeutic described herein.

A marker associated with caloric restriction can also be evaluated in a subject organism of a screening assay (or a treated subject). Although these markers may not be age-associated, they may be indicative of a physiological state that is altered when the Klotho pathway is modulated. The marker can be an mRNA or protein whose abundance changes in calorically restricted animals. WO01/12851 and U.S. Patent No. 6,406, 853 describe example markers. Cellular models derived from cells of an animal described herein or analogous to an animal model described herein can be used for a cell-based assay.

Models for evaluating the effect of a test polypeptide on muscle atrophy include: 1) rat medial gastrocnemius muscle mass loss resulting from denervation, e.g., by severing the right sciatic nerve at mid-thigh; 2) rat medial gastrocnemius muscle mass loss resulting from immobilization, e.g., by fixed the right ankle joint at 90 degrees of flexion; 3) rat medial gastrocnemius muscle mass loss resulting from hind limb suspension; (see, e.g., U.S. 2003-0129686); 4) skeletal muscle atrophy resulting from treatment with the cachectic cytokine, interleukin-1 (IL-1) (R. N. Cooney, S. R. Kimball, T. C. Vary, *Shock* 7, 1-16 (1997)); and 5) skeletal muscle atrophy resulting from treatment with the glucocorticoid, dexamethasone (A. L. Goldberg, *J. Biol. Chem.* 244, 3223-9 (1969).)

Example animal models for AMD include: laser-induced mouse model simulating exudative (wet) macular degeneration Bora *et al., Proc. Natl. Acad. Sci.* U S A., 100:2679-84 (2003); a transgenic mouse expressing a mutated form of cathepsin D resulting in features associated with the "geographic atrophy" form of AMD (Rakoczy *et al., Am. J. Pathol.*, 161:1515-24 (2002)); and a transgenic mouse over expressing VEGF in the retinal pigment epithelium resulting in CNV. Schwesinger *et al., Am. J. Pathol.* 158:1161-72 (2001).

Example animal models of Parkinson's disease include primates rendered Parkinsonian by treatment with the dopaminergic neurotoxin 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP) (see, e.g., U.S. Patent Publication No. 20030055231 and Wichmann *et al., Ann. N.Y. Acad. Sci.*, 991:199-213 (2003); 6-hydroxydopamine-lesioned rats (e.g., Lab. Anim. Sci.,49:363-71 (1999)) ; and transgenic invertebrate models (e.g., Lakso *et al., J. Neurochem.* 86:165-72 (2003) and Link, *Mech. Ageing Dev.*, 122:1639-49 (2001)).

Example molecular models of Type II diabetes include: a transgenic mouse having defective Nkx-2.2 or Nkx-6.1; (U.S. Patent No. 6,127,598); Zucker Diabetic Fatty fa/fa (ZDF) rat. (U.S. Patent No. 6,569,832); and Rhesus monkeys, which spontaneously develop obesity and subsequently frequently progress to overt type 2 diabetes (Hotta *et al., Diabetes*, 50:1126-33 (2001); and a transgenic mouse with a dominant-negative IGF-I receptor (KR-IGF-IR) having Type 2 diabetes-like insulin resistance.

Example animal and cellular models for neuropathy include: vincristine induced sensory-motor neuropathy in mice (U.S. Patent No. 5,420,112) or rabbits (Ogawa *et al., Neurotoxicology,* 21:501-11 (2000)); a streptozotocin (STZ)-diabetic rat for study of

autonomic neuropathy (Schmidt *et al., Am. J. Pathol.*, 163:21-8 (2003)); and a progressive motor neuropathy (pmn) mouse (Martin *et al., Genomics*, 75:9-16 (2001)).

Example animal models of hyperphosphatemia or tumoral calcinosis include Klotho knockout mice and FGF23 knockout mice (Yoshida et al., *Endocrinology* 143:683-689 (2002)).

Example animal models of chronic renal disease or chronic renal failure include COL4A3+/-mice (Beirowski et al., *J. Am. Soc. Nephrol.* 17:1986-1994 (2006)).

Example animal models of cancer include the transplantation or implantation of cancer cells or tissue into nude mice, as is known in the art (Giovanella et al., *Adv. Cancer Res.* 44:69-120 (1985)). For example, animal models of breast cancer include nude mice transplanted or implanted with breast cancer cells or tissue (e.g., Yue et al., *Cancer Res.* 54:5092-5095 (1994); Glinsky et al., *Cancer Res.* 56:5319-5324 (1996); Visonneau *Am. J. Path.* 152:1299-1311 (1998)).

The compositions can be administered to a subject, e.g., an adult subject, particularly a healthy adult subject or a subject having an age-related disease. In the latter case, the method can include evaluating a subject, e.g., to characterize a symptom of an age-related disease or other disease marker, and thereby identifying a subject as having a neurodegenerative disease, e.g., Alzheimer's or an age-related disease or being pre-disposed to such a disease.

Skeletal Muscle Atrophy

Methods or uses of the disclosure which provide administering the Klotho fusion polypeptide to an individual can be used to treat skeletal muscle atrophy. Muscle atrophy includes numerous neuromuscular, metabolic, immunological and neurological disorders and diseases as well as starvation, nutritional deficiency, metabolic stress, diabetes, aging, muscular dystrophy, or myopathy. Muscle atrophy occurs during the aging process. Muscle atrophy also results from reduced use or disuse of the muscle. Symptoms include a decline in skeletal muscle tissue mass. In human males, muscle mass declines by one-third between the ages of 50 and 80. Some molecular features of muscle atrophy include the upregulation of ubiquitin ligases, and the loss of myofibrillar proteins (Furuno et al., *J. Biol. Chem.*, 265:8550-8557, 1990). The breakdown of these proteins can be followed, e.g., by measuring 3-methyl-histidine production, which is a specific constituent of actin, and in certain muscles of myosin (Goodman, Biochem. J. 241:121-12, 1987 and Lowell, et al., Metabolism, 35:1121-112, 1986; Stein and Schluter, *Am. J. Physiol. Endocrinol.*

Metab. 272: E688-E696, 1997). Release of creatine kinase (a cell damage marker) (Jackson, et al., Neurology, 41: 101 104, 1991) can also be indicative.

Non-insulin-dependent Diabetes

Methods or uses of the disclosure which provide administering the Klotho fusion polypeptide to an individual can be used to treat Non-insulin-dependent Diabetes. Non-insulin-dependent Diabetes is also called "adult onset" diabetes and Type 2 diabetes. Type 2 diabetes also includes "non-obese type 2" and "obese type 2." Type II diabetes can be characterized by (1) reduced pancreatic-beta-islet-cell secretion of insulin such that less than necessary amounts of insulin are produced to keep blood glucose levels in balance and/or (2) "insulin resistance," wherein the body fails to respond normally to insulin. (U.S. Patent No. 5,266,561 and U.S. Patent No. 6,518,069) . For example, glucose-stimulated insulin levels typically fail to rise above 4.0 nmol/L. (U.S. Patent No. 5,266,561). Example symptoms of Type II diabetes include: hyperglycemia while fasting (U.S. Patent No. 5,266,561); fatigue; excessive thirst; frequent urination; blurred vision; and an increased rate of infections. Molecular indications of Type II diabetes include islet amyloid deposition in the pancreases.

Neuropathy

Neuropathy can include a central and/or peripheral nerve dysfunction caused by systemic disease, hereditary condition or toxic agent affecting motor, sensory, sensorimotor or autonomic nerves. (see, e.g., US Patent Application No. 20030013771). Symptoms can vary depending upon the cause of the nerve damage and the particular types of nerves affected. For example, symptoms of motor neuropathy include clumsiness in performing physical tasks or as muscular weakness, exhaustion after minor exertion, difficulty in standing or walking and attenuation or absence of a neuromuscular reflex. (U.S. Patent Application No. 20030013771) symptoms of autonomic neuropathy include constipation, cardiac irregularities and attenuation of the postural hypotensive reflex. (U.S. Patent Application No. 20030013771), symptoms of sensory neuropathy include pain and numbness; tingling in the hands, legs or feet; and extreme sensitivity to touch, and symptoms of retinopathy include blurred vision, sudden loss of vision, black spots, and flashing lights.

Alzheimer's Disease

Methods or uses of the disclosure which provide administering the Klotho fusion polypeptide to an individual can be used to treat Alzheimer's Disease (AD). Alzheimer's Disease is a complex neurodegenerative disease that results in the irreversible loss of neurons. It provides merely one example of a neurodegenerative disease that is also an age-related condition. Clinical hallmarks of Alzheimer's Disease include progressive impairment in memory, judgment, orientation to physical surroundings, and language. Neuropathological hallmarks of AD include region-specific neuronal loss, amyloid plaques, and neurofibrillary tangles. Amyloid plaques are extracellular plaques containing the amyloid peptide (also known as Ap, or Ap42), which is a cleavage product of the, 8-amyloid precursor protein (also known as APP). Neurofibrillary tangles are insoluble intracellular aggregates composed of filaments of the abnormally hyperphosphorylated microtubule-associated protein, taut Amyloid plaques and neurofibrillary tangles may contribute to secondary events that lead to neuronal loss by apoptosis (Clark and Karlawish, *Ann. Intern. Med.* 138(5):400-410 (2003). For example, p-amyloid induces caspase-2-dependent apoptosis in cultured neurons (Troy *et al. J Neurosci.* 20(4):1386-1392). The deposition of plaques in viva may trigger apoptosis of proximal neurons in a similar manner.

A variety of criteria, including genetic, biochemical, physiological, and cognitive criteria, can be used to evaluate AD in a subject. Symptoms and diagnosis of AD are known to medical practitioners. Some example symptoms and markers of AD are presented below. Information about these indications and other indications known to be associated with AD can be used as an "AD-related parameter." An AD related parameter can include qualitative or quantitative information. An example of quantitative information is a numerical value of one or more dimensions, e.g., a concentration of a protein or a tomographic map. Qualitative information can include an assessment, e.g., a physician's comments or a binary ("yes"/"no") and so forth. An AD-related parameter includes information that indicates that the subject is not diagnosed with AD or does not have a particular indication of AD, e.g., a cognitive test result that is not typical of AD or a genetic APOE polymorphism not associated with AD.

Progressive cognitive impairment is a hallmark of AD. This impairment can present as decline in memory, judgment, decision making, orientation to physical surroundings, and language (Nussbaum and Ellis, *New Eng J. Med.* 348(14):1356 35 1364 (2003)). Exclusion of other forms of dementia can assist in making a diagnosis of AD. Neuronal death leads to progressive cerebral atrophy in AD patients. Imaging

techniques (e.g., magnetic resonance imaging, or computer assisted tomography) can be used to detect AD-associated lesions in the brain and/or brain atrophy.

AD patients may exhibit biochemical abnormalities that result from the pathology of the disease. For example, levels of tan protein in the cerebrospinal fluid is elevated in AD patients (Andreasen, N. *et al. Arch Neurol.* 58:349-350 (2001)).

Levels of amyloid beta 42 (A,B42) peptide can be reduced in CSF of AD patients. Levels of Ap42 can be increased in the plasma of AD patients (Ertekein-Taner, N., *et al. Science* 290:2303 2304 (2000)). Techniques to detect biochemical abnormalities in a sample from a subject include cellular, immunological, and other biological methods known in the art. For general guidance, see, e.g., techniques described in Sambrook & Russell, Molecular Cloning: A Laboratory Manual, 3r Edition, Cold Spring Harbor Laboratory, N.Y. (2001), Ausubel et al., Current Protocols in Molecular Biology (Greene Publishing Associates and Wiley Interscience, N.Y. (1989), (Harrow, E. and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), and updated editions thereof.

For example, antibodies, other immunoglobulins, and other specific binding ligands can be used to detect a biomolecule, e.g., a protein or other antigen associated with AD. For example, one or more specific antibodies can be used to probe a sample. Various formats are possible, e.g., ELISAs, fluorescence-based assays, Western blots, and protein arrays. Methods of producing polypeptide arrays are described in the art, e.g., in De Wildt et al. (2000). *Nature Biotech.* 18, 989-994; Lucking et al. (1999). *Anal. Biochem.* 270, 103-111; Ge, H. (2000). *Nucleic Acids Res.* 28, e3, I-VII; MacBeath, G., and Schreiber, S.L. (2000). *Science* 289, 1760 to 1 763; and WO 99/5 1 773A1.

In one assay, a non-human animal model of AD (e.g., a mouse model) is used, e.g., to evaluate a polypeptide or a therapeutic regimen. For example, U.S. Patent No. 6,509,515 describes one such model animal which is naturally able to be used with learning and memory tests. The animal expresses an amyloid precursor protein (APP) sequence at a level in brain tissues such that the animal develops a progressive necrologic disorder within a short period of time from birth, generally within a year from birth, preferably within 2 to 6 months, from birth. The APP protein sequence is introduced into the animal, or an ancestor of the animal, at an embryonic stage, preferably the one cell, or fertilized oocyte, stage, and generally not later than about the 8-cell stage. The zygote or embryo is then developed to term in a pseudo-pregnant as foster female. The amyloid precursor protein genes are introduced into an animal embryo so as to be chromosomally

incorporated in a state which results in super endogenous expression of the amyloid precursor protein and the development of a progressive necrologic disease in the cortico-limbic areas of the brain, areas of the brain which are prominently affected in progressive necrologic disease states such as AD. The gliosis and clinical manifestations in affected transgenic animals model necrologic disease. The progressive aspects of the neurologic disease are characterized by diminished exploratory and/or locomotor behavior and diminished deoxyglucose uptake/utilization and hypertrophic gliosis in the cortico-limbic regions of the brain. Further, the changes that are seen are similar to those that are seen in some aging animals. Other animal models are also described in US 5,387,742; 5,877,399; 6,358,752; and 6, 187,992.

Parkinson's Disease

Methods or uses of the disclosure which provide administering the Klotho fusion polypeptide to an individual can be used to treat Parkinson's Disease. Parkinson's disease includes neurodegeneration of dopaminergic neurons in the substantia nigra resulting in the degeneration of the nigrostriatal dopamine system that regulates motor function. This pathology, in turn, leads to motor dysfunctions.(see, e.g., and Lotharius *et al., Nat. Rev. Neurosci.*, 3:932-42 (2002)). Example motor symptoms include: akinesia, stooped posture, gait difficulty, postural instability, catalepsy, muscle rigidity, and tremor. Example non-motor symptoms include: depression, lack of motivation, passivity, dementia and gastrointestinal dysfunction (see, e. g., Fahn, *Ann. N.Y. Acad. Sci.*, 991:1-14 (2003) and Pfeiffer, *Lancet Neurol.*, 2:107-16 (2003)) Parkinson's has been observed in 0.5 to 1 percent of persons 65 to 69 years of age and 1 to 3 percent among persons 80 years of age and older. (see, e.g., Nussbaum *et al., N. Engl. J. Med.*, 348:1356-64 (2003)). Molecular markers of Parkinson's disease include reduction in aromatic L amino acid decarboxylase (AADC) (see, e.g., US App.. No. 20020172664); and loss of dopamine content in the nigrostriatal neurons (see, e.g., Fahn, Ann. *N.Y. Acad. Sci.*, 991:1-14 (2003) and Lotharius et al., Nat. Rev. Neurosci., 3:932-42 (2002)). In some familial cases, PD is linked to mutations in single genes encoding alpha-synuclein and parkin (an E3 ubiquitin ligase) proteins. (e.g., Riess et al., J. Neurol. 250 Suppl 1:I3 10 (2003) and Nussbaum et al., N. Engl. J. Med., 348:1356-64 (2003)). A missense mutation in a neuron-specific C-terminal ubiquitin hydrolase gene is also associated with Parkinson's. (e.g., Nussbaum et al., N. Engl. J. Med., 348:1356-64 (2003))

Huntington's Disease

Methods or uses of the disclosure which provide administering the Klotho fusion polypeptide to an individual can be used to treat Huntington's Disease. Methods for evaluating the efficacy and/or determining the effective dose of a Klotho fusion polypeptide on Huntington's Disease include organismal based assays, e.g., using a mammal (e.g., a mouse, rat, primate, or some other non-human), or other animal (e.g., Xenopus, zebrafish, or an invertebrate such as a fly or nematode). A number of animal model system for Huntington's disease are available. See, e.g., Brouillet, Functional *Neurology* 15(4): 239-251 (2000); Ona *et al. Nature* 399: 263-267 (1999), Bates *et al. Hum Mol Genet.* 6(10):1633-7 (1997); Hansson *et al. J. of Neurochemistry* 78: 694-703; and Rubinsztein, D. C., *Trends in Genetics*, Vol. 1S, No. 4, pp. 202-209 (a review on various animal and non-human models of HD).

An example of such an animal model is the transgenic mouse strain is the R6/2 line (Mangiarini et al. Cell 87: 493-506 (1996)). The R6/2 mice are transgenic Huntington's disease mice, which over-express exon 1 of the human HD gene (under the control of the endogenous promoter). The exon 1 of the R6/2 human HD gene has an expanded CAG/polyglutamine repeat lengths (150 CAG repeats on average). These mice develop a progressive, ultimately fatal neurological disease with many features of human Huntington's disease. Abnormal aggregates, constituted in part by the N terminal part of Huntingtin (encoded by HD exon 1), are observed in R6/2 mice, both 45 in the cytoplasm and nuclei of cells (Davies *et al. Cell* 90: 537-548 (1997)). For example, the human Huntingtin protein in the transgenic animal is encoded by a gene that includes at least 55 CAG repeats and more preferably about 150 CAG repeats. These transgenic animals can develop a Huntington's disease-like phenotype.

These transgenic mice are characterized by reduced weight gain, reduced lifespan and motor impairment characterized by abnormal gait, resting tremor, hindlimb clasping and hyperactivity from 8 to 10 weeks after birth (for example the R6/2 strain; see Mangiarini et al. *Cell* 87: 493-506 (1996)). The phenotype worsens progressively toward hypokinesia. The brains of these transgenic mice also demonstrate neurochemical and histological abnormalities, such as changes in neurotransmitter receptors (glutamate, dopaminergic), decreased concentration of N-acetylaspartate (a marker of neuronal integrity) and reduced striatum and brain size. Accordingly, evaluating can include assessing parameters related to neurotransmitter levels, neurotransmitter receptor levels, brain size and striatum size. In addition, abnormal aggregates containing the transgenic

part of or full-length human Huntingtin protein are present in the brain tissue of these animals (e.g., the R6/2 transgenic mouse strain). See, e.g., Mangiarini *et al. Cell* 87: 493-506 (1996), Davies *et al. Cell* 90: 537-548 (1997), Brouillet, Functional Neurology 15(4): 239-251 (2000) and *Cha et al. Proc. Natl. Acad. Sci.* USA 95: 6480-6485 (1998).

To test the effect of the test polypeptide or known polypeptide described in the application in an animal model, different concentrations of test polypeptide are administered to the transgenic animal, for example by injecting the test polypeptide into circulation of the animal. A Huntington's disease-like symptom may be evaluated in the animal. The progression of the Huntington's disease-like symptoms, e.g., as described above for the mouse model, is then monitored to determine whether treatment with the test polypeptide results in reduction or delay of symptoms. In another assay, disaggregation of the Huntingtin protein aggregates in these animals is monitored. The animal can then be sacrificed and brain slices are obtained. The brain slices are then analyzed for the presence of aggregates containing the transgenic human Huntingtin protein, a portion thereof, or a fusion protein comprising human Huntingtin protein, or a portion thereof. This analysis can includes, for example, staining the slices of brain tissue with anti-Huntingtin antibody and adding a secondary antibody conjugated with FITC which recognizes the anti-Huntington's antibody (e.g., the anti-Huntingtin antibody is mouse anti-human antibody and the secondary antibody is specific for human antibody) and visualizing the protein aggregates by fluorescent microscopy.

A variety of methods are available to evaluate and/or monitor Huntington's disease. A variety of clinical symptoms and indicia for the disease are known. Huntington's disease causes a movement disorder, psychiatric difficulties and cognitive changes. The degree, age of onset, and manifestation of these symptoms can vary. The movement disorder can include quick, random, dance-like movements called chorea.

Example motor evaluations include: ocular pursuit, saccade initiation, saccade velocity, dysarthria, tongue protrusion, finger tap ability, pronate/supinate, a lo fist-hand-palm sequence, rigidity of arms, bradykinesia, maximal dystonia (trunk, upper and lower extremities), maximal chorea (e.g., trunk, face, upper and lower extremities), gait, tandem walking, and retropulsion. An example treatment can cause a change in the Total Motor Score 4 (TMS-4), a subscale of the UHDRS, e.g., over a one-year period.

Cancer

Methods or uses of the disclosure which provide administering the Klotho fusion polypeptide to an individual can be used to treat cacner. Cancer includes any disease that is caused by or results in inappropriately high levels of cell division, inappropriately low levels of apoptosis, or both. Examples of cancers include, without limitation, leukemias (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, nile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodenroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma). Lymphoproliferative disorders are also considered to be proliferative diseases.

All patents, patent applications, and published references cited herein are hereby incorporated by reference in their entirety. While this disclosure has been particularly shown and described with references to embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the disclosure encompassed by the appended claims.

5. EXAMPLES

Example 1. Expression and purification of Klotho fusion polypeptides

Expression of the Klotho fusion polypeptide

The polypeptides of the disclosure were made by transiently transfecting HEK293T cells with an expression vector encoding a Klotho fusion polypeptide having the extracellular domain of alpha Klotho and the FGF23 (R179Q) variant. Conditioned media containing expressed polypeptides were generated by transient transfection of the respective expression plasmids for Klotho, FGF23, and the Klotho-FGF23(R179Q) fusion protein. The transfections were performed in 6-well plates using Lipofectamine 2000 (Invitrogen, Cat # 11668-019). Five hours after transfection, the transfection mix was replaced with 3 ml DMEM plus 1% FBS. Conditioned media were collected 72 hours after the addition of 3 ml DMEM plus 1% FBS. Samples of conditioned medium from various transiently transfected HEK293T cells were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by Western blot (Figure 3A) or stained with Coomassie blue (Figure 3B).

SDS-polyacrylamide gel electrophoresis was performed on various samples (lane 1, Control; lane 2, FGF23; lane 3, sKlotho; lanes 4-6, sKlotho-FGF23). Coomassie blue staining revealed the expression of a high, >180 kDa band (Figure 3B, indicated by arrow on the right) that was not present in lanes 1-3, which contained samples that had not been transfected with the vector encoding the Klotho fusion polypeptide. The quality of the Klotho fusion polypeptide secreted into the media was evaluated by Western blot (Figure 3A). An anti-FGF23 rat monoclonal IgG2A (R&D Systems, Cat# MAB26291) was used as the primary antibody to detect the Klotho fusion polypeptides by Western blot. The Western blot confirmed that the additional bands observed in the Coomassie stained gels were Klotho fusion polypeptides. The Western blot confirmed that the Klotho fusion polypeptides had the expected molecular weight for the Klotho fusion polypeptide. This analysis shows the expression of the Klotho-FGF23(R179Q) fusion protein.

Purification of the Klotho fusion polypeptide

The polypeptides of the disclosure were purified from conditioned media from a culture of HEK293T cells transiently transfected with an expression vector encoding a Klotho fusion polypeptide having the extracellular domain of alpha Klotho and the FGF23 R179Q variant. To generate conditioned medium, an expression vector encoding sKlotho-FGF23-6xHis was transfected (500 μg DNA in 18 ml of OptiMEM 1 (GIBCO, Cat #11058) mixed with 18 ml of 2 μg/ml polyethlinimine (PEI) into HEK293 cells grown in suspension in expression medium (464 ml of HEK293T cells at 10^6 cells/ml in Freestype 293 expression medium (GIBCO, Cat #12338)). After transfection, the culture was allowed to grow (120 hours; 37°C in a 5% CO_2 incubator; shaking at 125 rpm). At

the end of incubation, conditioned medium was harvested by centrifugation (1000 rpm for five minutes). The conditioned medium was then applied to a nickel-agarose column. The sKlotho-FGF23-6xHis bound tightly to the column and was eluted with 50 mM imidazole. The resulting purified material was then dialyzed in PBS to remove imidazole. A sample of the purified sKlotho-FGF23-6xHis was separated by SDS-PAGE (lane 1, purified sKlotho-FGF23-6xHis; lane 2, molecular weight marker) and analyzed by staining with Coomassie blue (Figure 3C). The stained SDS-PAGE gel confirmed that the purified sKlotho-FGF23-6xHis had the expected molecular weight. The inability to detect bands corresponding to proteins other than full-length sKlotho-FGF23-6xHis in the lane loaded with the purified material also showed that the sKlotho-FGF23-6xHis was purified.

Example 2. *In vitro* assay assessing the activity of the Klotho fusion polypeptide.

Egr-1-luciferase

The biological activity of the expressed alpha Klotho fusion polypeptide was tested in Egr-1-luciferase reporter assays. Binding of the Klotho fusion polypeptide to the FGF23 receptor resulted in the downstream activation of Egr-1 and the expression of a luciferase reporter regulated by the Egr-1 promoter. The Egr-1-luciferase reporter gene was constructed based on that reported by Urakawa et al. (Nature, 2006, Vol 444, 770-774). HEK293T cells seeded in 48-well poly-D-lysine plate were transfected with the Egr-1-luciferase reporter gene together with a transfection normalization reporter gene (Renilla luciferase). Five hours after transfection of the Egr-1 luciferase reporter gene, the transfection mix was replaced with 3 ml DMEM plus 1% FBS. Conditioned media were collected 72 hours after the addition of 3 ml DMEM plus 1% FBS. Five hours later, the transfection mix was replaced with a sample to be tested for activity. In initial experiments, 50% conditioned medium (alone or containing Klotho, FGF23, Klotho and FGF23, and the Klotho-FGF23(R179Q) fusion protein) and 50% DMEM with 1% FBS in the presence or absence of 20 μg/ml heparin (Sigma, Cat#H8537; dissolved in DMEM as 2 mg/ml stock) were tested in the Egr-1-luciferase reporter assays (Figure 4). Further experiments used defined quantities of the purified polypeptides (Figures 5A and 5B). Cells were lysed 20 hours later in passive lysis buffer (Promega, Cat #E194A) and luciferase activities were determined using Dual-Glo Luciferase Assay System (Promega, Cat #E2940).

In initial experiments, Klotho fusion polypeptide activity was demonstrated in unfractionated conditioned medium. Using the Egr-1-luciferase reporter gene (Figure 4) these experiments quantified the fold changes in the expression of the luciferase reporter. Conditioned medium containing a combination of FGF23 and the extracellular domain of Klotho protein activated Egr-1-luciferase, but conditioned medium containing only FGF23 or conditioned medium containing only the extracellular domain of Klotho, did not activate Egr-1-luciferase. Conditioned medium containing the fusion protein sKlotho-FGF23(R179Q) activated the Egr-1-luciferase reporter gene in contrast to conditioned media containing either FGF23 or Klotho alone. In these experiments, conditioned medium containing the fusion protein sKlotho-FGF23(R179Q) activated the Egr-1-luciferase reporter gene significantly better than conditioned medium containing a combination of FGF23 and Klotho. In the presence of heparin, the inductions by conditioned medium containing the fusion protein sKlotho-FGF23(R179Q) and the conditioned medium containing a combination of FGF23 and Klotho were significantly enhanced. Table 1 lists the relative expression of various FGF-Klotho fusion polypeptides in conditioned medium and the relative activity of the unfractionated conditioned medium corresponding to the various FGF-Klotho fusion polypeptides in Egr-1-luciferase reporter assays.

Table 1. Expression and Activities of sKlotho-FGF23 fusion variants

	sKlotho-FGF23 fusion constructs	Expression	Activity in Egr-1-luc reporter gene
1	sKlotho-FGF23	good	yes
2	IgG sp-sKlotho-FGF23	good	yes
3	sKL-D1-FGF23	good	no
4	sKL-D2-FGF23	no	n.a.
5	s(KL-D1)2-FGF23	good	no
6	sKL-D1/D2-FGF23	no	n.a.
7	ssKlotho(ΔN-26)-FGF23	poor	no*
8	sKLD1-D2(Δ692-965)-FGF23	poor	no*
9	sKL-D1-D2(Δ507-798)-FGF23	poor	no*
10	FGF23-sKlotho	poor	no*

* lack of activity may be the result of low expression

Egr-1-luciferase reporter assays were also performed using defined quantities of proteins purified from the conditioned medium, using the purification procedure as described in Example 1. Consistent with previous results using unfractionated conditioned medium containing the expressed polypeptides, treatment with a combination of purified FGF23 and sKlotho resulted in luciferase reporter activity, but treatment with purified FGF23 alone did not (Figure 5A). The luciferase reporter activity from the combination of purified FGF23 and sKlotho was further dependent on the dose of purified sKlotho, and the effect could be enhanced by the presence of heparin (20 μg/ml). An effect of the sKlotho-FGF23-6xHis fusion polypeptide on luciferase activity could be detected at concentrations as low as about 1.21 nM (1.2 fold change) and at least up to about 19.3 nM (2.4 fold change) in Egr-1-luciferase reporter assays (Figure 5B). The activity of the sKlotho-FGF23-6xHis fusion polypeptide on luciferase activity was significantly enhanced in the presence of heparin (20 μg/ml). In the presence of heparin, the effect of the sKlotho-FGF23-6xHis fusion polypeptide on luciferase activity could be detected at a concentration as low as about 0.6 nM (2.0 fold change). The result showed that purified sKlotho-FGF23-6xHis dose-dependently induced the EGR-1-luc reporter gene, and that treatment with sKlotho-FGF23-6xHis.

Example 3. *In vitro* assay assessing the effect of the Klotho fusion polypeptide on muscle cells.

The biological effect of the expressed Klotho fusion polypeptide was tested on C2C12 myoblasts. Treatment of C2C12 myoblasts with IGF-1, FGF2, or sKlotho-FGF23 resulted in myotube growth and phosphorylation of signaling proteins. C2C12 myoblasts were seeded at a density of 40,000 cells/well in 6-well poly-D-lysine and fibronectin coated plates in growth medium (3 parts DMEM and 1 part F12), 10% FBS, 1% Glut; 1% P/S; 1% Linolic acid; 0.1% ITS: [insulin (10 mg/ml), transferrin (5.5 mg/ml), and selenium (5 ng/ml)]. After myoblasts reached confluence (3 days), medium was changed into differentiation medium (DMED with 2% horse serum; 1% Glut; 1% P/S).

For the myotube diameter experiments, three days after confluent media was changed into differentiation medium, cells were treated with IGF-1 (10 nM), FGF2 (20 ng/ml) or sKlotho-FGF23 (20 nM) in the absence or presence of dexamethasone (100 μM) for 24 hours in differentiation medium. At the end of treatment, cells were fixed

with glutaraldehyde (5% in PBS) and multiple fluorescent images were collected. Myotube diameter was measured using the Pipeline Pilot program to determine hypertrophy or atrophy.

For the signaling protein phosphorylation,experiments, three days after confluent media was changed into differentiation medium, cells were starved for four hours with DMEM without FBS and then treated with IGF-1 (10 nM), FGF2 (20 ng/ml) or sKlotho-FGF23 (20 nM) in the absence or presence of Rapamycin (40 nM) for 30 min. Cells were lysed in RIPA buffer in the presence of protease and phosphatase inhibitors. Western blot analysis was carried out and membranes were probed with different antibodies as indicated in the figure and developed on X-ray films, which were scanned.

The results of this study showed that sKlotho-FGF23 resulted in an increase in myotube diameter compared to the control and induced C2C12 myotube hypertrophy similar to results for IGF-1 and FGF2 (Figure 5A). In addition, treatment with sKlotho-FGF23, IGF-1, and FGF2 could partially reverse myotube atrophy induced by dexamethasone, based on measurements of myotube diameter. No difference was observed between sKlotho-FGF23 and FGF2 on myotube morphology (measured by thickness of the myotubes) in the absence or presence of dexamethasone. The trophic effects of sKlotho-FGF23, IGF-1, and FGF2 were statistically significant.

Consistent with the effects on C2C12 myotubes, sKlotho-FGF23 fusion protein signaling led to the phosphorylation of p70S6K and ERK, but not AKT or FoxO, in C2C12 myotubes (Figure 5B). The effect of sKlotho-FGF23 on signaling was similar to that of FGF2, but was distinct from that of IGF-1. The extent of ERK phosphorylation by sKlotho-FGF23 was observed to be less than that of IGF-1 or FGF2. The phosphorylation of p70S6K by sKlotho-FGF23 was rapamycin sensitive. In the experiments involving C2C12 cells, heparin was not required to activate signaling. These results show that a sKlotho-FGF23 fusion polypeptide activated signaling in C2C12 myotubes.

Example 4. Fusion polypeptides comprising sKlotho, FGF23 and FcLALA

Various fusion polypeptides are constructed using sKlotho, FGF23, and a modified Fc fragment of an antibody. These modified Fc molecules have altered (decreased) binding to FcRn and thus increased serum half-life. They also have modified bioavailability and alterered transport to mucosal surfaces and other targets in the body. In this example, the FGF23 and sKlotho are fused to FcLALA, which is described in U.S. Patent No. 7,217,798 and Hessell et al. 2007 Nature 449:101-104, Intervening between the various components of these fusion polypeptides are linkers, as described in Lode et

al. 1998 Proc. Natl. Acad. Sci. USA 95: 2475-2480. These fusions are inserted into constructs, e.g., pcDNA3.1
(Invitrogen, Carlsbad, CA), and expressed in HEK293 cells.

A. sKlotho-FGF23-FcLALA v1

A fusion is constructed which comprises: sKlotho, a linker, FGF23, another linker, and FcLALA. This embodiment, designated sKlotho-FGF23-FcLALA v1, is presented in SEQ ID NOs: 46 and 47, below.

The nucleotide sequence of sKlotho-FGF23-FcLALA v1 (wherein initiation **A**TG as 1) is presented as SEQ ID NO: 46.

The amino acid sequence of sKlotho-FGF23-FcLALA v1 is presented below as SEQ ID NO: 47.

In this sequence, the various components of the fusion are as follows:

sKlotho: 1-982; Linker1: 983-1001; FGF23: 1002-1228; Linker 2; 1229-1233; FcLALA: 1234-1459.

B. sKlotho-FGF23-FcLALA v2

A fusion is constructed which comprises: sKlotho, a linker, FGF23, another linker, and FcLALA. This embodiment is designated sKlotho-FGF23-FcLALA v2 and presented as SEQ ID NOs: 48 and 49, below.

The nucleotide sequence of sKlotho-FGF23-FcLALA v2 (wherein initiation **A**TG as 1) is presented as SEQ ID NO: 48.

The amino acid sequence of sKlotho-FGF23-FcLALA v2 is presented below as SEQ ID NO: 49.

In this sequence, the various components of the fusion are as follows:

sKlotho: (aa or amino acids) 1-982; Linker 1: 983-1001; FGF23: 1002-1228; Linker 2; 1229-1233; FcLALA: 1234-1450.

Other fusion polypeptides can be constructed by combining in various combinations the FGF, Klotho, modified Fc fragments, and (optionally) linker sequences, and variants and derivatives thereof, as described herein or known in the art.

Example 5. Fusion polypeptides comprising FGF23 and FcLALA.

Various fusion polypeptides are constructed using FGF23, and a modified Fc fragment of an antibody, as described in U.S. Patent No. 7,217,798. These modified Fc molecules have altered (decreased) binding to FcRn and thus increased serum half-life. They also have modified bioavailability and alterered transport to mucosal surfaces and

other targets in the body. In this example, FGF23 is fused to FcLALA, Intervening between the various components of these fusion polypeptides are linkers, as described in Lode et al. 1998 Proc. Natl. Acad. Sci. USA 95: 2475-2480. These fusions are inserted constructs, e.g., pcDNA3.1 (Invitrogen, Carlsbad, CA), and expressed in HEK293 cells.

C. FGF23-FcLALA v1

A fusion is constructed which comprises: FGF23, a linker, and FcLALA. This construct is designated FGF23-FcLALA v1 and presented below as SEQ ID NOs: 50 and 51.

The nucleotide sequence of FGF23-FcLALA v1 (wherein initiation **A**TG as 1) is presented below as SEQ ID NO: 50.

The amino acid sequence of FGF23(R179Q)-FcLALAv1 is presented below as SEQ ID NO: 51.

In this sequence, the various components of the fusion are as follows:

FGF23: (aa) 1-251; Linker: 252-256; FcLALA: 257-482.

D. FGF23-FcLALA v2

A fusion is constructed which comprises: FGF23-FcLALA v2, which comprises FGF23 and FcLALA.

The nucleotide sequence of FGF23-FcLALA v2 (wherein initiation **A**TG as 1) is presented below as SEQ ID NO: 52.

The amino acid sequence of FGF23(R179Q)-FcLALAv2 is presented below as SEQ ID NO: 53.

In this sequence, the various components of the fusion are as follows:

FGF23: 1-251; Linker: 252-256; FcLALA: 257-473.

Other fusion polypeptides can be constructed by combining in various combinations the FGF sequences, modified Fc fragments, and (optionally) linkers, and variants and derivatives thereof, as described herein or known in the art.

E. Activation of Egr-1-luc reporter gene by sKlotho-FGF23(R179Q)-FcLALA fusion proteins; activation of Egr-1-luc reporter gene by FGF23(R179Q)-FcLALA proteins; and pharmacokinetic profile of FGF23(R179Q) vs FGF23(R179Q)-FcLaLav2 are determined.

Figure 7 shows the activation of Egr-1-luc reporter gene by sKlotho-FGF23(R179Q)-FcLALA fusion proteins. HEK293T cells are transiently transfected with the Egr-1-luc reporter gene and incubated with the indicated conditioned media in the absence or

presence of 20 μg/ml heparin. Luciferase activities are then determined 18 hours later. The result shows that sklotho-FGF23-FcLALA fusion proteins induces the reporter gene activity. These inductions are significantly enhanced in the presence of heparin. sKF-Fcv1: sKlotho-FGF23-FcLALAv1; sKF-Fcv2: sKlotho-FGF23-FcLALAv2

Figure 8 shows the activation of Egr-1-luc reporter gene by FGF23(R179Q)-FcLALA proteins. HEK293T cells are transiently transfected with the Egr-1-luc reporter gene together with the full-length transmembrane form of Klotho and incubated with the indicated 30% conditioned media. Luciferase activities are then determined 18 hours later. The results show that FGF23-FcLALA fusion proteins induce the reporter gene activity in a similar manner as the FGF23.

Figure 9 shows the pharmacokinetic profile of FGF23(R179Q) vs FGF23(R179Q)-FcLALAv2. Four mice per group are injected subcutaneously with FGF23(R179Q)-6xHis or FGF23(R179Q)-FcLALAv2 at 2 mg/kg. At the indicated times, serum samples are collected and analyzed for FGF23 by ELISA. FGF23(R179Q)-FcLALA concentration in serum remains elevated at the 24 hr time point, while FGF23(R179Q)-6xHis is back to basal level. This results indicate that with the addition of FcLALA, the in vivo half-life of FGF23(R179Q) is significantly improved.

Example 6. In vivo efficacy of sKlotho-FGF23 fusion in enhancing muscle growth after dexamethasone-induced muscle atrophy

Experimental data shows that intramuscular injection of sKlotho-FGF23 significantly enhanced growth of muscle mass after dexamethasone-induced muscle atrophy. In this experiment, the peptide corresponding to that of SEQ ID NO: 41 is used.

Figure 10 shows absolute weights (A) and percent weight change (B) of the gastrocnemius-soleus-plantaris (GSP) muscles showing that intramuscular injection of sKlotho-FGF23 (KLOFGF) significantly enhanced regrowth of muscle mass after dexamethasone (DEX)-induced muscle atrophy compared with intramuscular injection of sKlotho (sKLO) or phosphate buffered saline (PBS).

Eighty male C57BL/6 mice, aged 15 weeks, are randomized by body weight into 8 groups each of 10 mice. Four groups receive water without DEX (W21d) while the other

four receive DEX in drinking water at 2.4 mg/kg/day for three weeks (D21d). After the three weeks, DEX treatment is stopped and one W21d and one D21d group is immediately sacrificed to establish the degree of muscle atrophy induced by the DEX treatment. The remaining three groups of W21d or D21d mice are allowed to recover for another 14 days (R14d) during which period they receive an intramuscular injection of 2x50 µl of PBS, sKlotho-FGF23 (KLOFGF; 1.6 mg/ml), or sKlotho (sKLO; 1.6 mg/ml), respectively, every other day into the right gastrocnemius-soleus-plantaris muscle complex. The mice are sacrificed 24h after the last intramuscular injection and the muscle weights determined and expressed as absolute weight (A) or percent change compared to the W21d+PBS group.

These data show the in vivo efficacy of sKlotho-FGF23 fusion in enhancing muscle growth after dexamethasone-induced muscle atrophy.

Example 7. Additional mutations in the FGF23 portion of fusion proteins which reduce aggregation, reduce undesired protease-induced cleavage, and increase production

Several mutations are investigated within the FGF23 portion of sKlotho-FGF23 and FGF23-FcLaLa fusion polypeptides. These include Q156, C206 and C244 (wherein the number is based on the FGF23 amino acid sequence). Example individual mutations include Q156A, C206S and C244S, and mutations at any of these sites can be combined with a mutation at R179 (e.g., R179Q). Example sequences are provided in SEQ ID NO: 54 to 68 of Figure 2.

C206 and C244 are suspected to be involved in dimerization; and Q156 is a site identified by the inventors as a protease sensitive site. Mutating these amino acids to any other amino acid enhances the qualities of the proteins, by reducing aggregation, reducing undesired protease-induced cleavage, and increasing protein production from cells, without interfering with FGF23 activity. This is an unexpected result, as these three positions are conserved in the FGF23 proteins found in human, rhesus, bovine, mouse and rat. This conservation is shown below in the comparison between SEQ ID NOs: 69, 70, 71, 72 and 73, with the Q156, C206 and C244 in bold, underlined font.

```
hFGF23          MLGARLRLWVCALCSVCSMSVLRAYPNASPLLGSSWGGLIHLYTATARNSYHLQIHKNGH
rhesus          MLGARLRLWVCALCSVCSMSVIRAYPNASPLLGSSWGGLIHLYTATARNSYHLQIHKNGH
bovine          MLGARLGLWVCTLSCV-----VQAYPNSSPLLGSSWGGLTHLYTATARNSYHLQIHGDGH
mouse           MLGTCLRLLVGVLCTVCSLGTARAYPDTSPLLGSNWGSLTHLYTATARTSYHLQIHRDGH
rat             MLGACLRLLVGALCTVCSLGTARAYSDTSPLLGSNWGSLTHLYTATARNSYHLQIHRDGH

hFGF23          VDGAPHQTIYSALMIRSEDAGFVVITGVMSRRYLCMDFRGNIFGSHYFDPENCRFQHQTL
rhesus          VDGAPHQTIYSALMIRSEDAGFVVITGVMSRRYLCMDFRGNIFGSHYFNPENCRFRHWTL
bovine          VDGSPQQTVYSALMIRSEDAGFVVITGVMSRRYLCMDFTGNIFGSHHFSPESCRFRQRTL
mouse           VDGTPHQTIYSALMITSEDAGSVVITGAMTRRFLCMDLHGNIFGSLHFSPENCKFRQWTL
rat             VDGTPHQTIYSALMITSEDAGSVVIIGAMTRRFLCMDLRGNIFGSYHFSPENCRFRQWTL

hFGF23          ENGYDVYHSPQYHFLVSLGRAKRAFLPGMNPPPYSQFLSRRNEIPLIHFNTPI-PRRHTR
rhesus          ENGYDVYHSPQHHFLVSLGRAKRAFLPGMNPPPYSQFLSRRNEIPLIHFNTPR-PRRHTR
bovine          ENGYDVYHSPQHRFLVSLGRAKRAFLPGTNPPPYAQFLSRRNEIPLPHFAATARPRRHTR
mouse           ENGYDVYLSQKHHYLVSLGRAKRIFQPGTNPPPFSQFLARRNEVPLLHFYTVR-PRRHTR
rat             ENGYDVYLSPKHHYLVSLGRSKRIFQPGTNPPPFSQFLARRNEVPLLHFYTAR-PRRHTR

hFGF23          SAEDDSERDPLNVLKPRARMTPAPASCSQELPSAEDNSPMASDPLGVVRGGRVNTHAGGT
rhesus          SAEDDSERDPLNVLKPRARMTPAPASCSQELPSAEDNSPVASDPLGVVRGGRVNTHAGGT
bovine          SAHDSG--DPLSVLKPRARATPVPAACSQELPSAEDSGPAASDPLGVLRGHRLDVRAGSA
mouse           SAEDPPERDPLNVLKPRPRATPVPVSCSRELPSAEEGGPAASDPLGVLRRGRGDARGGAG
rat             SAEDPPERDPLNVLKPRPRATPIPVSCSRELPSAEEGGPAASDPLGVLRRGRGDARRGAG

hFGF23          GPEGCRPFAKFI (SEQ ID NO: 69)
rhesus          GPEACRPFPKFI (SEQ ID NO: 70)
bovine          GAERCRPFPGFA (SEQ ID NO: 71)
mouse           GADRCRPFPRFV (SEQ ID NO: 72)
rat             GTDRCRPFPRFV (SEQ ID NO: 73)
```

The fact that these three mutations do not prevent FGF23 activity is shown in Figure 11. This figure shows activation of Egr-1-luc reporter gene by FGF23(R179Q)-FcLALA and Q156A, C206S, C244S and C206S/C244S mutants.

HEK293T cells are transiently transfected with the EGR-1-luc reporter gene together with the full-length transmembrane form of Klotho and indicated FGF23-FcLaLa mutants. Luciferase activities are then determined 18 hours later. The results show that C206S, C244S, C206S/C244S (three independent clones) and Q156A (three independent clones) mutants are equally effective as FGF23-FcLALA fusion proteins in activating EGR-1-Luc reporter gene activity.

Data showing that mutating C244 and C206 alter dimerization and aggregation of FGF23 is shown in Figure 12. This figure shows protein qualities of WT, Q156A, C206S, C244S and C206S/C244S mutants of FGF23(R179Q)-FcLaLa. Conditioned medium from HEK293T cells transient transfected with the indicated FGF23-FcLaLa expression vectors are analyzed by Western blot using an FGF23 antibody. The result shows that C206S/C244S mutation prevents protein dimerization and Q156A mutation has reduced proteolytic fragments.

Thus, surprisingly, even though these Q156, C206 and C244 residues are conserved across species, they can mutated without reducing FGF23 activity and can enhance the qualities of the protein by reducing aggregation and cleavage and by improving production.

Unless defined otherwise, the technical and scientific terms used herein have the same meaning as that usually understood by a specialist familiar with the field to which the disclosure belongs.

Unless indicated otherwise, all methods, steps, techniques and manipulations that are not specifically described in detail can be performed and have been performed in a manner known per se, as will be clear to the skilled person. Reference is for example again made to the standard handbooks and the general background art mentioned herein and to the further references cited therein.

Claims to the invention are non-limiting and are provided below.

Although particular embodiments and claims have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims, or the scope of subject matter of claims of any corresponding future application. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be made to the disclosure without departing from the spirit and scope of the disclosure as defined by the claims. The choice of nucleic acid starting material or clone of interest is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. Other aspects, advantages, and modifications considered to be within the scope of the following claims. Redrafting of claim scope in later filed corresponding applications may be due to limitations by the patent laws of various countries and should not be interpreted as giving up subject matter of the claims.

SEQUENCE LISTING (Figure 2)

Human Klotho nucleic acid sequence (NM_004795) (SEQ ID NO: 1)
Protein coding region: 9-3047

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Klotho amino acid sequence (NP_004786) (SEQ ID NO: 2)

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Protein coding region: 98-3232

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3061 cttattcctt gtgcagaaga aaccactgat attcctgggt tgttgcttct tctccaccct
3121 ggttctactc ttatcaattg ccatttttca aaggcagaag agaagaaagt tttggaaagc
3181 aaaaaactta caacacatac cattaaagaa aggcaagaga gttgttagct aaactgatct
3241 gtctgcatga tagacagttt aaaaattcat cccagttcc
```

beta-Klotho amino acid sequence (NP_783864) (SEQ ID NO: 4)

```
   1 mkpgcaagsp gnewiffstd eittryrntm sngglqrsvi lsalillrav tgfsgdgrai
  61 wsknpnftpv nesqlflydt fpknffwgig tgalqvegsw kkdgkgpsiw dhfihthlkn
 121 vsstngssds yiflekdlsa ldfigvsfyq fsiswprlfp dgivtvanak glqyystlld
 181 alvlrniepi vtlyhwdlpl alqekyggwk ndtiidifnd yatycfqmfg drvkywitih
 241 npylvawhgy gtgmhapgek gnlaavytvg hnlikahskv whnynthfrp hqkgwlsitl
 301 gshwiepnrs entmdifkcq qsmvsvlgwf anpihgdgdy pegmrkklfs vlpifseaek
 361 hemrgtadff afsfgpnnfk plntmakmgq nvslnlreal nwikleynnp riliaengwf
 421 tdsrvktedt taiymmknfl sqvlqairld eirvfgytaw slldgfewqd aytirrglfy
 481 vdfnskqker kpkssahyyk qiirengfsl kestpdvqgq fpcdfswgvt esvlkpesva
 541 sspqfsdphl yvwnatgnrl lhrvegvrlk trpaqctdfv nikkqlemla rmkvthyrfa
 601 ldwasvlptg nlsavnrqal ryyrcvvseg lklgisamvt lyypthahlg lpepllhadg
 661 wlnpstaeaf qayaglcfqe lgdlvklwit inepnrlsdi ynrsgndtyg aahnllvaha
 721 lawrlydrqf rpsqrgavsl slhadwaepa npyadshwra aerflqfeia wfaeplfktg
 781 dypaamreyi askhrrglss salprlteae rrllkgtvdf calnhfttrf vmheqlagsr
 841 ydsdrdiqfl qditrlsspt rlavipwgvr kllrwvrrny gdmdiyitas giddqaledd
 901 rlrkyylgky lqevlkayli dkvrikgyya fklaeekskp rfgfftsdfk akssiqfynk
 961 vissrgfpfe nsssrcsqtq entectvclf lvqkkplifl gccffstlvl llsiaifqrq
1021 krrkfwkakn lqhiplkkgk rvvs
```

Human Klotho domain 1 (KL-D1) amino acid sequence (SEQ ID NO: 5)

```
  58                                                             qgt
  61 fpdgflwavg saayqteggw qqhgkgasiw dtfthhplap pgdsrnaslp lgapsplqpa
 121 tgdvasdsyn nvfrdtealr elgvthyrfs iswarvlpng sagvpnregl ryyrrllerl
 181 relgvqpvvt lyhwdlpqrl qdayggwanr aladhfrdya elcfrhfggq vkywitidnp
 241 yvvawhgyat grlapgirgs prlgylvahn lllahakvwh lyntsfrptq ggqvsialss
 301 hwinprrmtd hsikecqksl dfvlgwfakp vfidgdypes mknnlssilp dftesekkfi
 361 kgtadffalc fgptlsfqll dphmkfrqle spnlrqllsw idlefnhpqi fivengwfvs
 421 gttkrddaky myylkkfime tlkaikldgv dvigytawsl mdgfewhrgy sirrglfyvd
 481 flsqdkmllp kssalfyqkl iekngf
```

Human Klotho domain 2 (KL-D2) amino acid sequence (SEQ ID NO: 6)

```
 517                                        gtfp cdfawgvvdn yiqvdttlsq
 541 ftdlnvylwd vhhskrlikv dgvvtkkrks ycvdfaaiqp qiallqemhv thfrfsldwa
 601 lilplgnqsq vnhtilqyyr cmaselvrvn itpvvalwqp mapnqglprl larqgawenp
 661 ytalafaeya rlcfqelghh vklwitmnep ytrnmtysag hnllkahala whvynekfrh
 721 aqngkisial qadwiepacp fsqkdkevae rvlefdigwl aepifgsgdy pwvmrdwlnq
 781 rnnfllpyft edekkliqgt fdflalshyt tilvdseked pikyndylev qemtditwln
 841 spsqvavvpw glrkvlnwlk fkygdlpmyi isngiddglh aeddqlrvyy mqnyinealk
 901 ahildginlc gyfaysfndr taprfglyry aadqfepkas mkhyrkiids ngf
```

Klotho extracellular domain (without signal peptide) amino acid sequence (SEQ ID NO: 7)

```
  28                                     epgdgaq twarfsrppa peaaglfqgt
  61 fpdgflwavg saayqteggw qqhgkgasiw dtfthhplap pgdsrnaslp lgapsplqpa
 121 tgdvasdsyn nvfrdtealr elgvthyrfs iswarvlpng sagvpnregl ryyrrllerl
```

```
181 relgvqpvvt lyhwdlpqrl qdayggwanr aladhfrdya elcfrhfggq vkywitidnp
241 yvvawhgyat grlapgirgs prlgylvahn lllahakvwh lyntsfrptq ggqvsialss
301 hwinprrmtd hsikecqksl dfvlgwfakp vfidgdypes mknnlssilp dftesekkfi
361 kgtadffalc fgptlsfqll dphmkfrqle spnlrqllsw idlefnhpqi fivengwfvs
421 gttkrddaky myylkkfime tlkaikldgv dvigytawsl mdgfewhrgy sirrglfyvd
481 flsqdkmllp kssalfyqkl ieknqfpplp enqplegtfp cdfawgvvdn yiqvdttlsq
541 ftdlnvylwd vhhskrlikv dgvvtkkrks ycvdfaaiqp qiallqemhv thfrfsldwa
601 lilplgnqsq vnhtilqyyr cmaselvrvn itpvvalwqp mapnqglprl larqgawenp
661 ytalafaeya rlcfqelghh vklwitmnep ytrnmtysag hnllkahala whvynekfrh
721 aqngkisial qadwiepacp fsqkdkevae rvlefdigwl aepifgsgdy pwvmrdwlnq
781 rnnfllpyft edekkliqgt fdflalshyt tilvdseked pikyndylev qemtditwln
841 spsqvavvpw glrkvlnwlk fkygdlpmyi isngiddglh aeddqlrvyy mqnyinealk
901 ahildginlc gyfaysfndr taprfglyry aadqfepkas mkhyrkiids ngfpgpetle
961 rfcpeeftvc tecsffhtrk sl
```

Klotho signal peptide amino acid sequence (SEQ ID NO: 8)

```
1 mpasapprrp rppppslsll lvllglggrr lra
```

IgG signal peptide amino acid sequence (SEQ ID NO: 9)

```
1 msvltqvlal lllwltgtrc rrlra
```

$(Gly_4\ Ser)_3$ polypeptide linker nucleic acid sequence (SEQ ID NO: 10)

```
1 ggaggtggag gttcaggagg tggaggttca ggaggtggag gttca
```

$(Gly_4\ Ser)_3$ polypeptide linker amino acid sequence (SEQ ID NO: 11)

```
1 GGGGSGGGGS GGGGS
```

$(Gly_4\ Ser)$ polypeptide linker amino acid sequence (SEQ ID NO: 12)

```
1 GGGGS
```

(Gly) polypeptide linker amino acid sequence (SEQ ID NO: 13)

```
1 G
```

(Gly Gly) polypeptide linker amino acid sequence (SEQ ID NO: 14)

```
1 GG
```

(Gly Ser) polypeptide linker amino acid sequence (SEQ ID NO: 15)

```
1 GS
```

$(Gly_2\ Ser)$ polypeptide linker amino acid sequence (SEQ ID NO: 16)

```
1 GGS
```

(Ala) polypeptide linker amino acid sequence (SEQ ID NO: 17)

1 A

(Ala Ala) polypeptide linker amino acid sequence (SEQ ID NO: 18)

1 AA

Klotho signal peptide-Klotho extracellular domain-FGF23 (R179Q) amino acid sequence (SEQ ID NO: 19)

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
 551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
 601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
 651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
 701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
 751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
 801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
 851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
 901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
 951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SLGSGGGGSG GGGSGGGGSL
1001 KYPNASPLLG SSWGGLIHLY TATARNSYHL QIHKNGHVDG APHQTIYSAL
1051 MIRSEDAGFV VITGVMSRRY LCMDFRGNIF GSHYFDPENC RFQHQTLENG
1101 YDVYHSPQYH FLVSLGRAKR AFLPGMNPPP YSQFLSRRNE IPLIHFNTPI
1151 PRRHTQSAED DSERDPLNVL KPRARMTPAP ASCSQELPSA EDNSPMASDP
1201 LGVVRGGRVN THAGGTGPEG CRPFAKFI*
```

IgG signal peptide-Klotho extracellular domain-FGF23 (R179Q) amino acid sequence (SEQ ID NO: 20)

```
   1 MSVLTQVLAL LLLWLTGLGG RRLRAEPGDG AQTWARFSRP PAPEAAGLFQ
  51 GTFPDGFLWA VGSAAYQTEG GWQQHGKGAS IWDTFTHHPL APPGDSRNAS
 101 LPLGAPSPLQ PATGDVASDS YNNVFRDTEA LRELGVTHYR FSISWARVLP
 151 NGSAGVPNRE GLRYYRRLLE RLRELGVQPV VTLYHWDLPQ RLQDAYGGWA
 201 NRALADHFRD YAELCFRHFG GQVKYWITID NPYVVAWHGY ATGRLAPGIR
 251 GSPRLGYLVA HNLLLAHAKV WHLYNTSFRP TQGGQVSIAL SSHWINPRRM
 301 TDHSIKECQK SLDFVLGWFA KPVFIDGDYP ESMKNNLSSI LPDFTESEKK
 351 FIKGTADFFA LCFGPTLSFQ LLDPHMKFRQ LESPNLRQLL SWIDLEFNHP
 401 QIFIVENGWF VSGTTKRDDA KYMYYLKKFI METLKAIKLD GVDVIGYTAW
 451 SLMDGFEWHR GYSIRRGLFY VDFLSQDKML LPKSSALFYQ KLIEKNGFPP
 501 LPENQPLEGT FPCDFAWGVV DNYIQVDTTL SQFTDLNVYL WDVHHSKRLI
 551 KVDGVVTKKR KSYCVDFAAI QPQIALLQEM HVTHFRFSLD WALILPLGNQ
 601 SQVNHTILQY YRCMASELVR VNITPVVALW QPMAPNQGLP RLLARQGAWE
 651 NPYTALAFAE YARLCFQELG HHVKLWITMN EPYTRNMTYS AGHNLLKAHA
 701 LAWHVYNEKF RHAQNGKISI ALQADWIEPA CPFSQKDKEV AERVLEFDIG
 751 WLAEPIFGSG DYPWVMRDWL NQRNNFLLPY FTEDEKKLIQ GTFDFLALSH
 801 YTTILVDSEK EDPIKYNDYL EVQEMTDITW LNSPSQVAVV PWGLRKVLNW
 851 LKFKYGDLPM YIISNGIDDG LHAEDDQLRV YYMQNYINEA LKAHILDGIN
 901 LCGYFAYSFN DRTAPRFGLY RYAADQFEPK ASMKHYRKII DSNGFPGPET
 951 LERFCPEEFT VCTECSFFHT RKSLGSGGGG SGGGGSGGGG SLKYPNASPL
```

```
1001 LGSSWGGLIH LYTATARNSY HLQIHKNGHV DGAPHQTIYS ALMIRSEDAG
1051 FVVITGVMSR RYLCMDFRGN IFGSHYFDPE NCRFQHQTLE NGYDVYHSPQ
1101 YHFLVSLGRA KRAFLPGMNP PPYSQFLSRR NEIPLIHFNT PIPRRHTQSA
1151 EDDSERDPLN VLKPRARMTP APASCSQELP SAEDNSPMAS DPLGVVRGGR
1201 VNTHAGGTGP EGCRPFAKFI *
```

KL-D1-FGF23 (R179Q) amino acid sequence (SEQ ID NO: 21)

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGSGG GGSGGGGSGG GGSLKYPNAS PLLGSSWGGL
 551 IHLYTATARN SYHLQIHKNG HVDGAPHQTI YSALMIRSED AGFVVITGVM
 601 SRRYLCMDFR GNIFGSHYFD PENCRFQHQT LENGYDVYHS PQYHFLVSLG
 651 RAKRAFLPGM NPPPYSQFLS RRNEIPLIHF NTPIPRRHTQ SAEDDSERDP
 701 LNVLKPRARM TPAPASCSQE LPSAEDNSPM ASDPLGVVRG GRVNTHAGGT
 751 GPEGCRPFAK FI*
```

KL-D2-FGF23 (R179Q) amino acid sequence (SEQ ID NO: 22)

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LPLPENQPLE GTFPCDFAWG
  51 VVDNYIQVDT TLSQFTDLNV YLWDVHHSKR LIKVDGVVTK KRKSYCVDFA
 101 AIQPQIALLQ EMHVTHFRFS LDWALILPLG NQSQVNHTIL QYYRCMASEL
 151 VRVNITPVVA LWQPMAPNQG LPRLLARQGA WENPYTALAF AEYARLCFQE
 201 LGHHVKLWIT MNEPYTRNMT YSAGHNLLKA HALAWHVYNE KFRHAQNGKI
 251 SIALQADWIE PACPFSQKDK EVAERVLEFD IGWLAEPIFG SGDYPWVMRD
 301 WLNQRNNFLL PYFTEDEKKL IQGTFDFLAL SHYTTILVDS EKEDPIKYND
 351 YLEVQEMTDI TWLNSPSQVA VVPWGLRKVL NWLKFKYGDL PMYIISNGID
 401 DGLHAEDDQL RVYYMQNYIN EALKAHILDG INLCGYFAYS FNDRTAPRFG
 451 LYRYAADQFE PKASMKHYRK IIDSNGFPGP ETLERFCPEE FTVCTECSFF
 501 HTRKSLGSGG GGSGGGGSGG GGSLKYPNAS PLLGSSWGGL IHLYTATARN
 551 SYHLQIHKNG HVDGAPHQTI YSALMIRSED AGFVVITGVM SRRYLCMDFR
 601 GNIFGSHYFD PENCRFQHQT LENGYDVYHS PQYHFLVSLG RAKRAFLPGM
 651 NPPPYSQFLS RRNEIPLIHF NTPIPRRHTQ SAEDDSERDP LNVLKPRARM
 701 TPAPASCSQE LPSAEDNSPM ASDPLGVVRG GRVNTHAGGT GPEGCRPFAK
 751 FI*
```

$(KL\text{-}D1)_2$-FGF23 (R179Q) amino acid sequence (SEQ ID NO: 23)

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGSGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW
 551 DTFTHHPLAP PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR
 601 ELGVTHYRFS ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT
 651 LYHWDLPQRL QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP
```

```
 701 YVVAWHGYAT GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ
 751 GGQVSIALSS HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES
 801 MKNNLSSILP DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE
 851 SPNLRQLLSW IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME
 901 TLKAIKLDGV DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP
 951 KSSALFYQKL IEKNGFPEFG SGGGGSGGGG SGGGGSLKYP NASPLLGSSW
1001 GGLIHLYTAT ARNSYHLQIH KNGHVDGAPH QTIYSALMIR SEDAGFVVIT
1051 GVMSRRYLCM DFRGNIFGSH YFDPENCRFQ HQTLENGYDV YHSPQYHFLV
1101 SLGRAKRAFL PGMNPPPYSQ FLSRRNEIPL IHFNTPIPRR HTQSAEDDSE
1151 RDPLNVLKPR ARMTPAPASC SQELPSAEDN SPMASDPLGV VRGGRVNTHA
1201 GGTGPEGCRP FAKFI*
```

$(KL\text{-}D2)_2$-FGF23 (R179Q) amino acid sequence (SEQ ID NO: 24)

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LPLPENQPLE GTFPCDFAWG
  51 VVDNYIQVDT TLSQFTDLNV YLWDVHHSKR LIKVDGVVTK KRKSYCVDFA
 101 AIQPQIALLQ EMHVTHFRFS LDWALILPLG NQSQVNHTIL QYYRCMASEL
 151 VRVNITPVVA LWQPMAPNQG LPRLLARQGA WENPYTALAF AEYARLCFQE
 201 LGHHVKLWIT MNEPYTRNMT YSAGHNLLKA HALAWHVYNE KFRHAQNGKI
 251 SIALQADWIE PACPFSQKDK EVAERVLEFD IGWLAEPIFG SGDYPWVMRD
 301 WLNQRNNFLL PYFTEDEKKL IQGTFDFLAL SHYTTILVDS EKEDPIKYND
 351 YLEVQEMTDI TWLNSPSQVA VVPWGLRKVL NWLKFKYGDL PMYIISNGID
 401 DGLHAEDDQL RVYYMQNYIN EALKAHILDG INLCGYFAYS FNDRTAPRFG
 451 LYRYAADQFE PKASMKHYRK IIDSNGFPGP ETLERFCPEE FTVCTECSFF
 501 HTRKSLGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD VHHSKRLIKV
 551 DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA LILPLGNQSQ
 601 VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL LARQGAWENP
 651 YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG HNLLKAHALA
 701 WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE RVLEFDIGWL
 751 AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT FDFLALSHYT
 801 TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW GLRKVLNWLK
 851 FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK AHILDGINLC
 901 GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS NGFGSGGGGS
 951 GGGGSGGGGS LKYPNASPLL GSSWGGLIHL YTATARNSYH LQIHKNGHVD
1001 GAPHQTIYSA LMIRSEDAGF VVITGVMSRR YLCMDFRGNI FGSHYFDPEN
1051 CRFQHQTLEN GYDVYHSPQY HFLVSLGRAK RAFLPGMNPP PYSQFLSRRN
1101 EIPLIHFNTP IPRRHTQSAE DDSERDPLNV LKPRARMTPA PASCSQELPS
1151 AEDNSPMASD PLGVVRGGRV NTHAGGTGPE GCRPFAKFI*
```

FGF23 (R179Q) -Klotho extracellular domain amino acid sequence (SEQ ID NO: 25)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
  51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
 101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
 151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
 201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
 251 IGSGGGGSGG GGSGGGGSLK EPGDGAQTWA RFSRPPAPEA AGLFQGTFPD
 301 GFLWAVGSAA YQTEGGWQQH GKGASIWDTF THHPLAPPGD SRNASLPLGA
 351 PSPLQPATGD VASDSYNNVF RDTEALRELG VTHYRFSISW ARVLPNGSAG
 401 VPNREGLRYY RRLLERLREL GVQPVVTLYH WDLPQRLQDA YGGWANRALA
 451 DHFRDYAELC FRHFGGQVKY WITIDNPYVV AWHGYATGRL APGIRGSPRL
 501 GYLVAHNLLL AHAKVWHLYN TSFRPTQGGQ VSIALSSHWI NPRRMTDHSI
 551 KECQKSLDFV LGWFAKPVFI DGDYPESMKN NLSSILPDFT ESEKKFIKGT
 601 ADFFALCFGP TLSFQLLDPH MKFRQLESPN LRQLLSWIDL EFNHPQIFIV
 651 ENGWFVSGTT KRDDAKYMYY LKKFIMETLK AIKLDGVDVI GYTAWSLMDG
 701 FEWHRGYSIR RGLFYVDFLS QDKMLLPKSS ALFYQKLIEK NGFPPLPENQ
 751 PLEGTFPCDF AWGVVDNYIQ VDTTLSQFTD LNVYLWDVHH SKRLIKVDGV
 801 VTKKRKSYCV DFAAIQPQIA LLQEMHVTHF RFSLDWALIL PLGNQSQVNH
 851 TILQYYRCMA SELVRVNITP VVALWQPMAP NQGLPRLLAR QGAWENPYTA
```

```
 901 LAFAEYARLC FQELGHHVKL WITMNEPYTR NMTYSAGHNL LKAHALAWHV
 951 YNEKFRHAQN GKISIALQAD WIEPACPFSQ KDKEVAERVL EFDIGWLAEP
1001 IFGSGDYPWV MRDWLNQRNN FLLPYFTEDE KKLIQGTFDF LALSHYTTIL
1051 VDSEKEDPIK YNDYLEVQEM TDITWLNSPS QVAVVPWGLR KVLNWLKFKY
1101 GDLPMYIISN GIDDGLHAED DQLRVYYMQN YINEALKAHI LDGINLCGYF
1151 AYSFNDRTAP RFGLYRYAAD QFEPKASMKH YRKIIDSNGF PGPETLERFC
1201 PEEFTVCTEC SFFHTRKSL*
```

FGF23 (R179Q) -KL-D1 amino acid sequence (SEQ ID NO: 26)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
  51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
 101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
 151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
 201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
 251 IQGTFPDGFL WAVGSAAYQT EGGWQQHGKG ASIWDTFTHH PLAPPGDSRN
 301 ASLPLGAPSP LQPATGDVAS DSYNNVFRDT EALRELGVTH YRFSISWARV
 351 LPNGSAGVPN REGLRYYRRL LERLRELGVQ PVVTLYHWDL PQRLQDAYGG
 401 WANRALADHF RDYAELCFRH FGGQVKYWIT IDNPYVVAWH GYATGRLAPG
 451 IRGSPRLGYL VAHNLLLAHA KVWHLYNTSF RPTQGGQVSI ALSSHWINPR
 501 RMTDHSIKEC QKSLDFVLGW FAKPVFIDGD YPESMKNNLS SILPDFTESE
 551 KKFIKGTADF FALCFGPTLS FQLLDPHMKF RQLESPNLRQ LLSWIDLEFN
 601 HPQIFIVENG WFVSGTTKRD DAKYMYYLKK FIMETLKAIK LDGVDVIGYT
 651 AWSLMDGFEW HRGYSIRRGL FYVDFLSQDK MLLPKSSALF YQKLIEKNGF
 652 *
```

FGF23 (R179Q) -KL-D2 amino acid sequence (SEQ ID NO: 27)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
  51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
 101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
 151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
 201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
 251 IGTFPCDFAW GVVDNYIQVD TTLSQFTDLN VYLWDVHHSK RLIKVDGVVT
 301 KKRKSYCVDF AAIQPQIALL QEMHVTHFRF SLDWALILPL GNQSQVNHTI
 351 LQYYRCMASE LVRVNITPVV ALWQPMAPNQ GLPRLLARQG AWENPYTALA
 401 FAEYARLCFQ ELGHHVKLWI TMNEPYTRNM TYSAGHNLLK AHALAWHVYN
 451 EKFRHAQNGK ISIALQADWI EPACPFSQKD KEVAERVLEF DIGWLAEPIF
 501 GSGDYPWVMR DWLNQRNNFL LPYFTEDEKK LIQGTFDFLA LSHYTTILVD
 551 SEKEDPIKYN DYLEVQEMTD ITWLNSPSQV AVVPWGLRKV LNWLKFKYGD
 601 LPMYIISNGI DDGLHAEDDQ LRVYYMQNYI NEALKAHILD GINLCGYFAY
 651 SFNDRTAPRF GLYRYAADQF EPKASMKHYR KIIDSNGF*
```

FGF23 (R179Q) -$(KL\text{-}D1)_2$ amino acid sequence (SEQ ID NO: 28)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
  51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
 101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
 151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
 201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
 251 IQGTFPDGFL WAVGSAAYQT EGGWQQHGKG ASIWDTFTHH PLAPPGDSRN
 301 ASLPLGAPSP LQPATGDVAS DSYNNVFRDT EALRELGVTH YRFSISWARV
 351 LPNGSAGVPN REGLRYYRRL LERLRELGVQ PVVTLYHWDL PQRLQDAYGG
 401 WANRALADHF RDYAELCFRH FGGQVKYWIT IDNPYVVAWH GYATGRLAPG
 451 IRGSPRLGYL VAHNLLLAHA KVWHLYNTSF RPTQGGQVSI ALSSHWINPR
 501 RMTDHSIKEC QKSLDFVLGW FAKPVFIDGD YPESMKNNLS SILPDFTESE
 551 KKFIKGTADF FALCFGPTLS FQLLDPHMKF RQLESPNLRQ LLSWIDLEFN
 601 HPQIFIVENG WFVSGTTKRD DAKYMYYLKK FIMETLKAIK LDGVDVIGYT
 651 AWSLMDGFEW HRGYSIRRGL FYVDFLSQDK MLLPKSSALF YQKLIEKNGF
 701 QGTFPDGFLW AVGSAAYQTE GGWQQHGKGA SIWDTFTHHP LAPPGDSRNA
```

```
 751 SLPLGAPSPL QPATGDVASD SYNNVFRDTE ALRELGVTHY RFSISWARVL
 801 PNGSAGVPNR EGLRYYRRLL ERLRELGVQP VVTLYHWDLP QRLQDAYGGW
 851 ANRALADHFR DYAELCFRHF GGQVKYWITI DNPYVVAWHG YATGRLAPGI
 901 RGSPRLGYLV AHNLLLAHAK VWHLYNTSFR PTQGGQVSIA LSSHWINPRR
 951 MTDHSIKECQ KSLDFVLGWF AKPVFIDGDY PESMKNNLSS ILPDFTESEK
1001 KFIKGTADFF ALCFGPTLSF QLLDPHMKFR QLESPNLRQL LSWIDLEFNH
1051 PQIFIVENGW FVSGTTKRDD AKYMYYLKKF IMETLKAIKL DGVDVIGYTA
1101 WSLMDGFEWH RGYSIRRGLF YVDFLSQDKM LLPKSSALFY QKLIEKNGF*
```

FGF23 (R179Q) -(KL-D2)$_2$ amino acid sequence (SEQ ID NO: 29)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
  51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
 101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
 151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
 201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
 251 IGTFPCDFAW GVVDNYIQVD TTLSQFTDLN VYLWDVHHSK RLIKVDGVVT
 301 KKRKSYCVDF AAIQPQIALL QEMHVTHFRF SLDWALILPL GNQSQVNHTI
 351 LQYYRCMASE LVRVNITPVV ALWQPMAPNQ GLPRLLARQG AWENPYTALA
 401 FAEYARLCFQ ELGHHVKLWI TMNEPYTRNM TYSAGHNLLK AHALAWHVYN
 451 EKFRHAQNGK ISIALQADWI EPACPFSQKD KEVAERVLEF DIGWLAEPIF
 501 GSGDYPWVMR DWLNQRNNFL LPYFTEDEKK LIQGTFDFLA LSHYTTILVD
 551 SEKEDPIKYN DYLEVQEMTD ITWLNSPSQV AVVPWGLRKV LNWLKFKYGD
 601 LPMYIISNGI DDGLHAEDDQ LRVYYMQNYI NEALKAHILD GINLCGYFAY
 651 SFNDRTAPRF GLYRYAADQF EPKASMKHYR KIIDSNGFGT FPCDFAWGVV
 701 DNYIQVDTTL SQFTDLNVYL WDVHHSKRLI KVDGVVTKKR KSYCVDFAAI
 751 QPQIALLQEM HVTHFRFSLD WALILPLGNQ SQVNHTILQY YRCMASELVR
 801 VNITPVVALW QPMAPNQGLP RLLARQGAWE NPYTALAFAE YARLCFQELG
 851 HHVKLWITMN EPYTRNMTYS AGHNLLKAHA LAWHVYNEKF RHAQNGKISI
 901 ALQADWIEPA CPFSQKDKEV AERVLEFDIG WLAEPIFGSG DYPWVMRDWL
 951 NQRNNFLLPY FTEDEKKLIQ GTFDFLALSH YTTILVDSEK EDPIKYNDYL
1001 EVQEMTDITW LNSPSQVAVV PWGLRKVLNW LKFKYGDLPM YIISNGIDDG
1051 LHAEDDQLRV YYMQNYINEA LKAHILDGIN LCGYFAYSFN DRTAPRFGLY
1101 RYAADQFEPK ASMKHYRKII DSNGF*
```

FGF19 nucleic acid sequence (NM_005117) (SEQ ID NO: 30)
Protein coding region (464-1114)

```
   1 gctcccagcc aagaacctcg gggccgctgc gcggtgggga ggagttcccc gaaacccggc
  61 cgctaagcga ggcctcctcc tcccgcagat ccgaacggcc tgggcggggt caccccggct
 121 gggacaagaa gccgccgcct gcctgcccgg gcccggggag ggggctgggg ctggggccgg
 181 aggcggggtg tgagtgggtg tgtgcggggg gcggaggctt gatgcaatcc cgataagaaa
 241 tgctcgggtg tcttgggcac ctacccgtgg ggcccgtaag gcgctactat ataaggctgc
 301 cggcccggag ccgccgcgcc gtcagagcag gagcgctgcg tccaggatct agggccacga
 361 ccatcccaac ccggcactca cagccccgca gcgcatcccg gtcgccgccc agcctcccgc
 421 acccccatcg ccggagctgc gccgagagcc ccagggaggt gccatgcgga gcgggtgtgt
 481 ggtggtccac gtatggatcc tggccggcct ctggctggcc gtggccgggc gccccctcgc
 541 cttctcggac gcggggcccc acgtgcacta cggctggggc gaccccatcc gcctgcggca
 601 cctgtacacc tccggccccc acgggctctc cagctgcttc ctgcgcatcc gtgccgacgg
 661 cgtcgtggac tgcgcgcggg gccagagcgc gcacagtttg ctggagatca aggcagtcgc
 721 tctgcggacc gtggccatca agggcgtgca cagcgtgcgg tacctctgca tgggcgccga
 781 cggcaagatg caggggctgc ttcagtactc ggaggaagac tgtgctttcg aggaggagat
 841 ccgcccagat ggctacaatg tgtaccgatc cgagaagcac cgcctcccgg tctccctgag
 901 cagtgccaaa cagcggcagc tgtacaagaa cagaggcttt cttccactct ctcatttcct
 961 gcccatgctg cccatggtcc cagaggagcc tgaggacctc aggggccact tggaatctga
1021 catgttctct tcgcccctgg agaccgacag catggaccca tttgggcttg tcaccggact
1081 ggaggccgtg aggagtccca gctttgagaa gtaactgaga ccatgcccgg gcctcttcac
1141 tgctgccagg ggctgtggta cctgcagcgt gggggacgtg cttctacaag aacagtcctg
1201 agtccacgtt ctgtttagct ttaggaagaa acatctagaa gttgtacata ttcagagttt
1261 tccattggca gtgccagttt ctagccaata gacttgtctg atcataacat tgtaagcctg
```

```
1321 tagcttgccc agctgctgcc tgggccccca ttctgctccc tcgaggttgc tggacaagct
1381 gctgcactgt ctcagttctg cttgaatacc tccatcgatg gggaactcac ttcctttgga
1441 aaaattctta tgtcaagctg aaattctcta atttttctc atcacttccc caggagcagc
1501 cagaagacag gcagtagttt taatttcagg aacaggtgat ccactctgta aaacagcagg
1561 taaatttcac tcaaccccat gtgggaattg atctatatct ctacttccag ggaccatttg
1621 cccttcccaa atccctccag gccagaactg actggagcag gcatggccca ccaggcttca
1681 ggagtagggg aagcctggag ccccactcca gccctgggac aacttgagaa ttccccctga
1741 ggccagttct gtcatggatg ctgtcctgag aataacttgc tgtcccggtg tcacctgctt
1801 ccatctccca gcccaccagc cctctgccca cctcacatgc ctccccatgg attggggcct
1861 cccaggcccc ccaccttatg tcaacctgca cttcttgttc aaaaatcagg aaaagaaaag
1921 atttgaagac cccaagtctt gtcaataact tgctgtgtgg aagcagcggg ggaagaccta
1981 gaacccttc cccagcactt ggttttccaa catgatattt atgagtaatt tattttgata
2041 tgtacatctc ttatttctt acattattta tgcccccaaa ttatatttat gtatgtaagt
2101 gaggtttgtt ttgtatatta aaatggagtt tgtttgtaaa aaaaaaaaaa aaaaaaa
```

FGF19 amino acid sequence (NP_005108) (SEQ ID NO: 31)

```
  1 MRSGCVVVHV WILAGLWLAV AGRPLAFSDA GPHVHYGWGD PIRLRHLYTS GPHGLSSCFL
 61 RIRADGVVDC ARGQSAHSLL EIKAVALRTV AIKGVHSVRY LCMGADGKMQ GLLQYSEEDC
121 AFEEEIRPDG YNVYRSEKHR LPVSLSSAKQ RQLYKNRGFL PLSHFLPMLP MVPEEPEDLR
181 GHLESDMFSS PLETDSMDPF GLVTGLEAVR SPSFEK
```

FGF21 nucleic acid sequence (NM_019113) (SEQ ID NO: 32)
Protein coding region 151-780

```
  1 CTGTCAGCTG AGGATCCAGC CGAAAGAGGA GCCAGGCACT CAGGCCACCT GAGTCTACTC
 61 ACCTGGACAA CTGGAATCTG GCACCAATTC TAAACCACTC AGCTTCTCCG AGCTCACACC
121 CCGGAGATCA CCTGAGGACC CGAGCCATTG ATGGACTCGG ACGAGACCGG GTTCGAGCAC
181 TCAGGACTGT GGGTTTCTGT GCTGGCTGGT CTTCTGCTGG GAGCCTGCCA GGCACACCCC
241 ATCCCTGACT CCAGTCCTCT CCTGCAATTC GGGGGCCAAG TCCGGCAGCG GTACCTCTAC
301 ACAGATGATG CCCAGCAGAC AGAAGCCCAC CTGGAGATCA GGGAGGATGG GACGGTGGGG
361 GGCGCTGCTG ACCAGAGCCC CGAAAGTCTC CTGCAGCTGA AAGCCTTGAA GCCGGGAGTT
421 ATTCAAATCT TGGGAGTCAA GACATCCAGG TTCCTGTGCC AGCGGCCAGA TGGGGCCCTG
481 TATGGATCGC TCCACTTTGA CCCTGAGGCC TGCAGCTTCC GGGAGCTGCT TCTTGAGGAC
541 GGATACAATG TTTACCAGTC CGAAGCCCAC GGCCTCCCGC TGCACCTGCC AGGGAACAAG
601 TCCCCACACC GGGACCCTGC ACCCCGAGGA CCAGCTCGCT TCCTGCCACT ACCAGGCCTG
661 CCCCCCGCAC TCCCGGAGCC ACCCGGAATC CTGGCCCCCC AGCCCCCCGA TGTGGGCTCC
721 TCGGACCCTC TGAGCATGGT GGGACCTTCC CAGGGCCGAA GCCCCAGCTA CGCTTCCTGA
781 AGCCAGAGGC TGTTTACTAT GACATCTCCT CTTTATTTAT TAGGTTATTT ATCTTATTTA
841 TTTTTTTATT TTTCTTACTT GAGATAATAA AGAGTTCCAG AGGAGAAAAA AAAAAAAAAA
901 AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
```

FGF21 amino acid sequence (NP_061986) (SEQ ID NO: 33)

```
  1 MDSDETGFEH SGLWVSVLAG LLLGACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQQTEAH
 61 LEIREDGTVG GAADQSPESL LQLKALKPGV IQILGVKTSR FLCQRPDGAL YGSLHFDPEA
121 CSFRELLLED GYNVYQSEAH GLPLHLPGNK SPHRDPAPRG PARFLPLPGL PPALPEPPGI
181 LAPQPPDVGS SDPLSMVGPS QGRSPSYAS
```

FGF23 nucleic acid sequence (NM_020638) (SEQ ID NO: 34)
Protein coding region 147-902

```
  1 cggcaaaaag gagggaatcc agtctaggat cctcacacca gctacttgca agggagaagg
 61 aaaaggccag taaggcctgg gccaggagag tcccgacagg agtgtcaggt ttcaatctca
121 gcaccagcca ctcagagcag ggcacgatgt tgggggcccg cctcaggctc tgggtctgtg
181 ccttgtgcag cgtctgcagc atgagcgtcc tcagagccta tcccaatgcc tccccactgc
241 tcggctccag ctggggtggc ctgatccacc tgtacacagc cacagccagg aacagctacc
301 acctgcagat ccacaagaat ggccatgtgg atggcgcacc ccatcagacc atctacagtg
361 ccctgatgat cagatcagag gatgctggct ttgtggtgat tacaggtgtg atgagcagaa
```

```
 421 gatacctctg catggatttc agaggcaaca tttttggatc acactatttc gacccggaga
 481 actgcaggtt ccaacaccag acgctggaaa acgggtacga cgtctaccac tctcctcagt
 541 atcacttcct ggtcagtctg ggccgggcga agagagcctt cctgccaggc atgaacccac
 601 ccccgtactc ccagttcctg tcccggagga acgagatccc cctaattcac ttcaacaccc
 661 ccataccacg gcggcacacc cggagcgccg aggacgactc ggagcgggac cccctgaacg
 721 tgctgaagcc ccgggcccgg atgaccccgg ccccggcctc ctgttcacag gagctcccga
 781 gcgccgagga caacagcccg atggccagtg acccattagg ggtggtcagg ggcggtcgag
 841 tgaacacgca cgctgggggа acgggcccgg aaggctgccg ccccttcgcc aagttcatct
 901 agggtcgctg gaagggcacc ctctttaacc catccctcag caaacgcagc tcttcccaag
 961 gaccaggtcc cttgacgttc cgaggatggg aaaggtgaca ggggcatgta tggaatttgc
1021 tgcttctctg gggtcccttc cacaggaggt cctgtgagaa ccaacctttg aggcccaagt
1081 catggggttt caccgccttc ctcactccat atagaacacc tttcccaata ggaaacccca
1141 acaggtaaac tagaaatttc cccttcatga aggtagagag aaggggtctc tcccaacata
1201 tttctcttcc ttgtgcctct cctctttatc acttttaagc ataaaaaaaa aaaaaaaaaa
1261 aaaaaaaaaa aaaagcagtg ggttcctgag ctcaagactt tgaaggtgta gggaagagga
1321 aatcggagat cccagaagct tctccactgc cctatgcatt tatgttagat gccccgatcc
1381 cactggcatt tgagtgtgca aaccttgaca ttaacagctg aatggggcaa gttgatgaaa
1441 acactacttt caagccttcg ttcttccttg agcatctctg gggaagagct gtcaaaagac
1501 tggtggtagg ctggtgaaaa cttgacagct agacttgatg cttgctgaaa tgaggcagga
1561 atcataatag aaaactcagc ctccctacag ggtgagcacc ttctgtctcg ctgtctccct
1621 ctgtgcagcc acagccagag ggcccagaat ggccccactc tgttcccaag cagttcatga
1681 tacagcctca ccttttggcc ccatctctgg tttttgaaaa tttggtctaa ggaataaata
1741 gcttttacac tggctcacga aaatctgccc tgctagaatt tgcttttcaa aatggaaata
1801 aattccaact ctcctaagag gcatttaatt aaggctctac ttccaggttg agtaggaatc
1861 cattctgaac aaactacaaa aatgtgactg ggaagggggc tttgagagac tgggactgct
1921 ctgggttagg ttttctgtgg actgaaaaat cgtgtccttt tctctaaatg aagtggcatc
1981 aaggactcag ggggaaagaa atcaggggac atgttataga agttatgaaa agacaaccac
2041 atggtcaggc tcttgtctgt ggtctctagg gctctgcagc agcagtggct cttcgattag
2101 ttaaaactct cctaggctga cacatctggg tctcaatccc cttggaaatt cttggtgcat
2161 taaatgaagc cttaccccat tactgcggtt cttcctgtaa gggggctcca ttttcctccc
2221 tctctttaaa tgaccaccta aaggacagta tattaacaag caaagtcgat tcaacaacag
2281 cttcttccca gtcacttttt tttttctcac tgccatcaca tactaacctt atactttgat
2341 ctattctttt tggttatgag agaaatgttg ggcaactgtt tttacctgat ggttttaagc
2401 tgaacttgaa ggactggttc ctattctgaa acagtaaaac tatgtataat agtatatagc
2461 catgcatggc aaatatttta atatttctgt tttcatttcc tgttggaaat attatcctgc
2521 ataatagcta ttggaggctc ctcagtgaaa gatcccaaaa ggattttggt ggaaaactag
2581 ttgtaatctc acaaactcaa cactaccatc aggggttttc tttatggcaa agccaaaata
2641 gctcctacaa tttcttatat ccctcgtcat gtggcagtat ttatttattt atttggaagt
2701 ttgcctatcc ttctatattt atagatattt ataaaaatgt aacccctttt tcctttcttc
2761 tgtttaaaat aaaaataaaa tttatctcag cttctgttag cttatcctct ttgtagtact
2821 acttaaaagc atgtcggaat ataagaataa aaaggattat gggaggggaa cattagggaa
2881 atccagagaa ggcaaaattg aaaaaaagat tttagaattt taaaattttc aaagatttct
2941 tccattcata aggagactca atgattttaa ttgatctaga cagaattatt taagttttat
3001 caatattgga tttctggt
```

FGF23 amino acid sequence (NP_065689) (SEQ ID NO: 35)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS YHLQIHKNGH
  61 VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG NIFGSHYFDP ENCRFQHQTL
 121 ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN PPPYSQFLSR RNEIPLIHFN TPIPRRHTRS
 181 AEDDSERDPL NVLKPRARMT PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG
 241 PEGCRPFAKF I
```

FGF23 (R179Q) amino acid sequence (SEQ ID NO: 36)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS YHLQIHKNGH
  61 VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG NIFGSHYFDP ENCRFQHQTL
 121 ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS
 181 AEDDSERDPL NVLKPRARMT PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG
 241 PEGCRPFAKF I
```

Human beta-Klotho domain 1 (b-KL-D1) amino acid sequence (SEQ ID NO: 37)

```
 77                   ydt fpknffwgig tgalqvegsw kkdgkgpsiw dhfihthlkn
121 vsstngssds yiflekdlsa ldfigvsfyq fsiswprlfp dgivtvanak glqyystlld
181 alvlrniepi vtlyhwdlpl alqekyggwk ndtiidifnd yatycfqmfg drvkywitih
241 npylvawhgy gtgmhapgek gnlaavytvg hnlikahskv whnynthfrp hqkgwlsitl
301 gshwiepnrs entmdifkcq qsmvsvlgwf anpihgdgdy pegmrkklfs vlpifseaek
361 hemrgtadff afsfgpnnfk plntmakmgq nvslnlreal nwikleynnp riliaengwf
421 tdsrvktedt taiymmknfl sqvlqairld eirvfgytaw slldgfewqd aytirrglfy
481 vdfnskqker kpkssahyyk qiirengf
```

Human beta-Klotho domain 2 (b-KL-D2) amino acid sequence (SEQ ID NO: 38)

```
      571                                  trpaqctdfv nikkqlemla
rmkvthyrfa
      601 ldwasvlptg nlsavnrqal ryyrcvvseg lklgisamvt lyypthahlg
lpepllhadg
      661 wlnpstaeaf qayaglcfqe lgdlvklwit inepnrlsdi ynrsgndtyg
aahnllvaha
      721 lawrlydrqf rpsqrgavsl slhadwaepa npyadshwra aerflqfeia
wfaeplfktg
      781 dypaamreyi askhrrglss salprlteae rrllkgtvdf calnhfttrf
vmheqlagsr
      841 ydsdrdiqfl qditrlsspt rlavipwgvr kllrwvrrny gdmdiyitas
giddqaledd
      901 rlrkyylgky lqevlkayli dkvrikgyya fklaeekskp rfgfftsdfk
akssiqfynk
      961 vissrgf
```

Beta-Klotho extracellular domain (without signal peptide) amino acid sequence (SEQ ID NO: 39)

```
 52                                                        gfsgdgrai
 61 wsknpnftpv nesqlflydt fpknffwgig tgalqvegsw kkdgkgpsiw dhfihthlkn
121 vsstngssds yiflekdlsa ldfigvsfyq fsiswprlfp dgivtvanak glqyystlld
181 alvlrniepi vtlyhwdlpl alqekyggwk ndtiidifnd yatycfqmfg drvkywitih
241 npylvawhgy gtgmhapgek gnlaavytvg hnlikahskv whnynthfrp hqkgwlsitl
301 gshwiepnrs entmdifkcq qsmvsvlgwf anpihgdgdy pegmrkklfs vlpifseaek
361 hemrgtadff afsfgpnnfk plntmakmgq nvslnlreal nwikleynnp riliaengwf
421 tdsrvktedt taiymmknfl sqvlqairld eirvfgytaw slldgfewqd aytirrglfy
481 vdfnskqker kpkssahyyk qiirengfsl kestpdvqgq fpcdfswgvt esvlkpesva
541 sspqfsdphl yvwnatgnrl lhrvegvrlk trpaqctdfv nikkqlemla rmkvthyrfa
601 ldwasvlptg nlsavnrqal ryyrcvvseg lklgisamvt lyypthahlg lpepllhadg
661 wlnpstaeaf qayaglcfqe lgdlvklwit inepnrlsdi ynrsgndtyg aahnllvaha
721 lawrlydrqf rpsqrgavsl slhadwaepa npyadshwra aerflqfeia wfaeplfktg
781 dypaamreyi askhrrglss salprlteae rrllkgtvdf calnhfttrf vmheqlagsr
841 ydsdrdiqfl qditrlsspt rlavipwgvr kllrwvrrny gdmdiyitas giddqaledd
901 rlrkyylgky lqevlkayli dkvrikgyya fklaeekskp rfgfftsdfk akssiqfynk
961 vissrgfpfe nsssrcsqtq entectvclf lvqkkpl
```

sKlotho without signal peptide – FGF23 amino acid sequence (without signal peptide) (SEQ ID NO: 40)

```
                                        EPGDGAQ TWARFSRPPA
 51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
```

```
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
 551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
 601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
 651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
 701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
 751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
 801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
 851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
 901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
 951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SLGSGGGGSG GGGSGGGGSL
1001 KYPNASPLLG SSWGGLIHLY TATARNSYHL QIHKNGHVDG APHQTIYSAL
1051 MIRSEDAGFV VITGVMSRRY LCMDFRGNIF GSHYFDPENC RFQHQTLENG
1101 YDVYHSPQYH FLVSLGRAKR AFLPGMNPPP YSQFLSRRNE IPLIHFNTPI
1151 PRRHTRSAED DSERDPLNVL KPRARMTPAP ASCSQELPSA EDNSPMASDP
1201 LGVVRGGRVN THAGGTGPEG CRPFAKFI*
```

sKlotho without signal peptide -FGF23 (R179Q) (without signal peptide) amino acid sequence (SEQ ID NO: 41)

```
                                          EPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
 551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
 601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
 651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
 701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
 751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
 801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
 851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
 901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
 951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SLGSGGGGSG GGGSGGGGSL
1001 KYPNASPLLG SSWGGLIHLY TATARNSYHL QIHKNGHVDG APHQTIYSAL
1051 MIRSEDAGFV VITGVMSRRY LCMDFRGNIF GSHYFDPENC RFQHQTLENG
1101 YDVYHSPQYH FLVSLGRAKR AFLPGMNPPP YSQFLSRRNE IPLIHFNTPI
1151 PRRHTQSAED DSERDPLNVL KPRARMTPAP ASCSQELPSA EDNSPMASDP
1201 LGVVRGGRVN THAGGTGPEG CRPFAKFI*
```

FGF23 without signal peptide (SEQ ID NO: 42)

```
                                YPNASP LLGSSWGGLI HLYTATARNS YHLQIHKNGH
  61 VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG NIFGSHYFDP ENCRFQHQTL
 121 ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN PPPYSQFLSR RNEIPLIHFN TPIPRRHTRS
 181 AEDDSERDPL NVLKPRARMT PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG
 241 PEGCRPFAKF I
```

FGF23(R179Q) without signal peptide (SEQ ID NO: 43)

```
                                  YPNASP LLGSSWGGLI HLYTATARNS YHLQIHKNGH
 61 VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG NIFGSHYFDP ENCRFQHQTL
121 ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS
181 AEDDSERDPL NVLKPRARMT PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG
241 PEGCRPFAKF I
```

sKlotho with Klotho signal peptide (SEQ ID NO: 44)

```
  1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
 51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SL
```

sKlotho with IgG Signal peptide (SEQ ID NO: 45)

```
  1 MSVLTQVLAL LLLWLTGLGG RRLRAEPGDG AQTWARFSRP PAPEAAGLFQ
 51 GTFPDGFLWA VGSAAYQTEG GWQQHGKGAS IWDTFTHHPL APPGDSRNAS
101 LPLGAPSPLQ PATGDVASDS YNNVFRDTEA LRELGVTHYR FSISWARVLP
151 NGSAGVPNRE GLRYYRRLLE RLRELGVQPV VTLYHWDLPQ RLQDAYGGWA
201 NRALADHFRD YAELCFRHFG GQVKYWITID NPYVVAWHGY ATGRLAPGIR
251 GSPRLGYLVA HNLLLAHAKV WHLYNTSFRP TQGGQVSIAL SSHWINPRRM
301 TDHSIKECQK SLDFVLGWFA KPVFIDGDYP ESMKNNLSSI LPDFTESEKK
351 FIKGTADFFA LCFGPTLSFQ LLDPHMKFRQ LESPNLRQLL SWIDLEFNHP
401 QIFIVENGWF VSGTTKRDDA KYMYYLKKFI METLKAIKLD GVDVIGYTAW
451 SLMDGFEWHR GYSIRRGLFY VDFLSQDKML LPKSSALFYQ KLIEKNGFPP
501 LPENQPLEGT FPCDFAWGVV DNYIQVDTTL SQFTDLNVYL WDVHHSKRLI
551 KVDGVVTKKR KSYCVDFAAI QPQIALLQEM HVTHFRFSLD WALILPLGNQ
601 SQVNHTILQY YRCMASELVR VNITPVVALW QPMAPNQGLP RLLARQGAWE
651 NPYTALAFAE YARLCFQELG HHVKLWITMN EPYTRNMTYS AGHNLLKAHA
701 LAWHVYNEKF RHAQNGKISI ALQADWIEPA CPFSQKDKEV AERVLEFDIG
751 WLAEPIFGSG DYPWVMRDWL NQRNNFLLPY FTEDEKKLIQ GTFDFLALSH
801 YTTILVDSEK EDPIKYNDYL EVQEMTDITW LNSPSQVAVV PWGLRKVLNW
851 LKFKYGDLPM YIISNGIDDG LHAEDDQLRV YYMQNYINEA LKAHILDGIN
901 LCGYFAYSFN DRTAPRFGLY RYAADQFEPK ASMKHYRKII DSNGFPGPET
951 LERFCPEEFT VCTECSFFHT RKSL*
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sKlotho-FGF23-FcLALA v1 (SEQ ID NO: 46)

```
   1 ATGCCCGCCA GCGCCCCGCC GCGCCGCCCG CGGCCGCCGC CGCCGTCGCT
     GTCGCTGCTG
  61 CTGGTGCTGC TGGGCCTGGG CGGCCGCCGC CTGCGTGCGG AGCCGGGCGA
     CGGCGCGCAG
 121 ACCTGGGCCC GTTTCTCGCG GCCTCCTGCC CCCGAGGCCG CGGGCCTCTT
     CCAGGGCACC
 181 TTCCCCGACG GCTTCCTCTG GGCCGTGGGC AGCGCCGCCT ACCAGACCGA
     GGGCGGCTGG
 241 CAGCAGCACG GCAAGGGTGC GTCCATCTGG GATACGTTCA CCCACCACCC
     CCTGGCACCC
 301 CCGGGAGACT CCCGGAACGC CAGTCTGCCG TTGGGCGCCC CGTCGCCGCT
     GCAGCCCGCC
 361 ACCGGGGACG TAGCCAGCGA CAGCTACAAC AACGTCTTCC GCGACACGGA
     GGCGCTGCGC
 421 GAGCTCGGGG TCACTCACTA CCGCTTCTCC ATCTCGTGGG CGCGAGTGCT
     CCCCAATGGC
 481 AGCGCGGGCG TCCCCAACCG CGAGGGGCTG CGCTACTACC GGCGCCTGCT
     GGAGCGGCTG
 541 CGGGAGCTGG GCGTGCAGCC CGTGGTCACC CTGTACCACT GGGACCTGCC
     CCAGCGCCTG
 601 CAGGACGCCT ACGGCGGCTG GGCCAACCGC GCCCTGGCCG ACCACTTCAG
     GGATTACGCG
 661 GAGCTCTGCT TCCGCCACTT CGGCGGTCAG GTCAAGTACT GGATCACCAT
     CGACAACCCC
 721 TACGTGGTGG CCTGGCACGG CTACGCCACC GGGCGCCTGG CCCCCGGCAT
     CCGGGGCAGC
 781 CCGCGGCTCG GGTACCTGGT GGCGCACAAC CTCCTCCTGG CTCATGCCAA
     AGTCTGGCAT
 841 CTCTACAATA CTTCTTTCCG TCCCACTCAG GGAGGTCAGG TGTCCATTGC
     CCTAAGCTCT
 901 CACTGGATCA ATCCTCGAAG AATGACCGAC CACAGCATCA AAGAATGTCA
     AAAATCTCTG
 961 GACTTTGTAC TAGGTTGGTT TGCCAAACCC GTATTTATTG ATGGTGACTA
     TCCCGAGAGC
1021 ATGAAGAATA ACCTTTCATC TATTCTGCCT GATTTTACTG AATCTGAGAA
     AAAGTTCATC
1081 AAAGGAACTG CTGACTTTTT TGCTCTTTGC TTTGGACCCA CCTTGAGTTT
     TCAACTTTTG
1141 GACCCTCACA TGAAGTTCCG CCAATTGGAA TCTCCCAACC TGAGGCAACT
     GCTTTCCTGG
1201 ATTGACCTTG AATTTAACCA TCCTCAAATA TTTATTGTGG AAAATGGCTG
     GTTTGTCTCA
1261 GGGACCACCA AGAGAGATGA TGCCAAATAT ATGTATTACC TCAAAAAGTT
     CATCATGGAA
1321 ACCTTAAAAG CCATCAAGCT GGATGGGGTG GATGTCATCG GGTATACCGC
     ATGGTCCCTC
1381 ATGGATGGTT TCGAGTGGCA CAGAGGTTAC AGCATCAGGC GTGGACTCTT
     CTATGTTGAC
1441 TTTCTAAGCC AGGACAAGAT GTTGTTGCCA AAGTCTTCAG CCTTGTTCTA
     CCAAAAGCTG
1501 ATAGAGAAAA ATGGCTTCCC TCCTTTACCT GAAAATCAGC CCCTAGAAGG
     GACATTTCCC
1561 TGTGACTTTG CTTGGGGAGT TGTTGACAAC TACATTCAAG TAGATACCAC
     TCTGTCTCAG
1621 TTTACCGACC TGAATGTTTA CCTGTGGGAT GTCCACCACA GTAAAAGGCT
     TATTAAAGTG
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1681 GATGGGGTTG TGACCAAGAA GAGGAAATCC TACTGTGTTG ACTTTGCTGC
CATCCAGCCC
1741 CAGATCGCTT TACTCCAGGA AATGCACGTT ACACATTTTC GCTTCTCCCT
GGACTGGGCC
1801 CTGATTCTCC CTCTGGGTAA CCAGTCCCAG GTGAACCACA CCATCCTGCA
GTACTATCGC
1861 TGCATGGCCA GCGAGCTTGT CCGTGTCAAC ATCACCCCAG TGGTGGCCCT
GTGGCAGCCT
1921 ATGGCCCCGA ACCAAGGACT GCCGCGCCTC CTGGCCAGGC AGGGCGCCTG
GGAGAACCCC
1981 TACACTGCCC TGGCCTTTGC AGAGTATGCC CGACTGTGCT TTCAAGAGCT
CGGCCATCAC
2041 GTCAAGCTTT GGATAACGAT GAATGAGCCG TATACAAGGA ATATGACATA
CAGTGCTGGC
2101 CACAACCTTC TGAAGGCCCA TGCCCTGGCT TGGCATGTGT ACAATGAAAA
GTTTAGGCAT
2161 GCTCAGAATG GGAAAATATC CATAGCCTTG CAGGCTGATT GGATAGAACC
TGCCTGCCCT
2221 TTCTCCCAAA AGGACAAAGA GGTGGCCGAG AGAGTTTTGG AATTTGACAT
TGGCTGGCTG
2281 GCTGAGCCCA TTTTCGGCTC TGGAGATTAT CCATGGGTGA TGAGGGACTG
GCTGAACCAA
2341 AGAAACAATT TTCTTCTTCC TTATTTCACT GAAGATGAAA AAAAGCTAAT
CCAGGGTACC
2401 TTTGACTTTT TGGCTTTAAG CCATTATACC ACCATCCTTG TAGACTCAGA
AAAAGAAGAT
2461 CCAATAAAAT ACAATGATTA CCTAGAAGTG CAAGAAATGA CCGACATCAC
GTGGCTCAAC
2521 TCCCCCAGTC AGGTGGCGGT AGTGCCCTGG GGGTTGCGCA AAGTGCTGAA
CTGGCTGAAG
2581 TTCAAGTACG GAGACCTCCC CATGTACATA ATATCCAACG GAATCGATGA
CGGGCTGCAT
2641 GCTGAGGACG ACCAGCTGAG GGTGTATTAT ATGCAGAATT ACATAAACGA
AGCTCTCAAA
2701 GCCCACATAC TGGATGGTAT CAATCTTTGC GGATACTTTG CTTATTCGTT
TAACGACCGC
2761 ACAGCTCCGA GGTTTGGCCT CTATCGTTAT GCTGCAGATC AGTTTGAGCC
CAAGGCATCC
2821 ATGAAACATT ACAGGAAAAT TATTGACAGC AATGGTTTCC CGGGCCCAGA
AACTCTGGAA
2881 AGATTTTGTC CAGAAGAATT CACCGTGTGT ACTGAGTGCA GTTTTTTTCA
CACCCGAAAG
2941 TCTTTAGGAT CCGGAGGTGG AGGTTCAGGA GGTGGAGGTT CAGGAGGTGG
AGGTTCACTT
3001 AAGTATCCCA ATGCCTCCCC ACTGCTCGGC TCCAGCTGGG GTGGCCTGAT
CCACCTGTAC
3061 ACAGCCACAG CCAGGAACAG CTACCACCTG CAGATCCACA AGAATGGCCA
TGTGGATGGC
3121 GCACCCCATC AGACCATCTA CAGTGCCCTG ATGATCAGAT CAGAGGATGC
TGGCTTTGTG
3181 GTGATTACAG GTGTGATGAG CAGAAGATAC CTCTGCATGG ATTTCAGAGG
CAACATTTTT
3241 GGATCACACT ATTTCGACCC GGAGAACTGC AGGTTCCAAC ACCAGACGCT
GGAAAACGGG
3301 TACGACGTCT ACCACTCTCC TCAGTATCAC TTCCTGGTCA GTCTGGGCCG
GGCGAAGAGA
3361 GCCTTCCTGC CAGGCATGAA CCCACCCCCG TACTCCCAGT TCCTGTCCCG
GAGGAACGAG
3421 ATCCCCCTAA TTCACTTCAA CACCCCCATA CCACGGCGGC ACACCCAGAG
CGCCGAGGAC
3481 GACTCGGAGC GGGACCCCCT GAACGTGCTG AAGCCCCGGG CCCGGATGAC
CCCGGCCCCG

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3541 GCCTCCTGTT CACAGGAGCT CCCGAGCGCC GAGGACAACA GCCCGATGGC
    CAGTGACCCA
3601 TTAGGGGTGG TCAGGGGCGG TCGAGTGAAC ACGCACGCTG GGGGAACGGG
    CCCGGAAGGC
3661 TGCCGCCCCT TCGCCAAGTT CATCGGAGGT GGAGGTTCAA AAACCCACAC
    GTGTCCTCCT
3721 TGTCCTGCCC CAGAAGCAGC AGGTGGTCCA TCAGTTTTTC TTTTCCCTCC
    CAAACCCAAG
3781 GATACGCTGA TGATCTCTCG CACGCCTGAG GTGACATGCG TCGTAGTAGA
    CGTGAGCCAC
3841 GAAGATCCCG AGGTGAAGTT CAATTGGTAT GTGGACGGAG TAGAAGTGCA
    TAACGCGAAA
3901 ACTAAGCCGC GCGAGGAACA ATATAACAGT ACTTACAGGG TGGTATCCGT
    GCTCACAGTC
3961 CTGCACCAGG ACTGGCTGAA CGGTAAGGAA TACAAGTGCA AAGTAAGCAA
    CAAGGCACTT
4021 CCCGCGCCTA TTGAGAAAAC AATCTCCAAG GCGAAGGGAC AACCAAGAGA
    ACCTCAGGTT
4081 TACACTCTCC CGCCTTCCAG GGAAGAGATG ACCAAAAAATC AAGTTTCCCT
    GACTTGCCTC
4141 GTCAAAGGAT TCTACCCTTC CGACATTGCT GTTGAATGGG AAAGCAATGG
    ACAACCAGAG
4201 AACAACTACA AGACAACACC CCCGGTGCTG GATAGTGACG GATCTTTCTT
    TCTCTACTCA
4261 AAGCTGACCG TGGATAAGTC CAGGTGGCAG CAGGGAAACG TGTTTTCCTG
    CTCTGTCATG
4321 CATGAAGCGC TGCATAATCA CTATACCCAG AAGTCTCTGA GCTTGAGCCC
    AGGCAAGTAA
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sKlotho-FGF23-FcLALA v1 (SEQ ID NO: 47)

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   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
 551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
 601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
 651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
 701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
 751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
 801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
 851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
 901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
 951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SLGSGGGGSG GGGSGGGGSL
1001 KYPNASPLLG SSWGGLIHLY TATARNSYHL QIHKNGHVDG APHQTIYSAL
1051 MIRSEDAGFV VITGVMSRRY LCMDFRGNIF GSHYFDPENC RFQHQTLENG
1101 YDVYHSPQYH FLVSLGRAKR AFLPGMNPPP YSQFLSRRNE IPLIHFNTPI
1151 PRRHTQSAED DSERDPLNVL KPRARMTPAP ASCSQELPSA EDNSPMASDP
1201 LGVVRGGRVN THAGGTGPEG CRPFAKFIGG GGSKTHTCPP CPAPEAAGGP
1251 SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK
1301 TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK
1351 AKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE
1401 NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ
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1451 KSLSLSPGK*

sKlotho-FGF23-FcLALA v2 (SEQ ID NO: 48)

```
   1 ATGCCCGCCA GCGCCCCGCC GCGCCGCCCG CGGCCGCCGC CGCCGTCGCT
     GTCGCTGCTG
  61 CTGGTGCTGC TGGGCCTGGG CGGCCGCCGC CTGCGTGCGG AGCCGGGCGA
     CGGCGCGCAG
 121 ACCTGGGCCC GTTTCTCGCG GCCTCCTGCC CCCGAGGCCG CGGGCCTCTT
     CCAGGGCACC
 181 TTCCCCGACG GCTTCCTCTG GGCCGTGGGC AGCGCCGCCT ACCAGACCGA
     GGGCGGCTGG
 241 CAGCAGCACG GCAAGGGTGC GTCCATCTGG GATACGTTCA CCCACCACCC
     CCTGGCACCC
 301 CCGGGAGACT CCCGGAACGC CAGTCTGCCG TTGGGCGCCC CGTCGCCGCT
     GCAGCCCGCC
 361 ACCGGGGACG TAGCCAGCGA CAGCTACAAC AACGTCTTCC GCGACACGGA
     GGCGCTGCGC
 421 GAGCTCGGGG TCACTCACTA CCGCTTCTCC ATCTCGTGGG CGCGAGTGCT
     CCCCAATGGC
 481 AGCGCGGGCG TCCCCAACCG CGAGGGGCTG CGCTACTACC GGCGCCTGCT
     GGAGCGGCTG
 541 CGGGAGCTGG GCGTGCAGCC CGTGGTCACC CTGTACCACT GGGACCTGCC
     CCAGCGCCTG
 601 CAGGACGCCT ACGGCGGCTG GGCCAACCGC GCCCTGGCCG ACCACTTCAG
     GGATTACGCG
 661 GAGCTCTGCT TCCGCCACTT CGGCGGTCAG GTCAAGTACT GGATCACCAT
     CGACAACCCC
 721 TACGTGGTGG CCTGGCACGG CTACGCCACC GGGCGCCTGG CCCCCGGCAT
     CCGGGGCAGC
 781 CCGCGGCTCG GGTACCTGGT GGCGCACAAC CTCCTCCTGG CTCATGCCAA
     AGTCTGGCAT
 841 CTCTACAATA CTTCTTTCCG TCCCACTCAG GGAGGTCAGG TGTCCATTGC
     CCTAAGCTCT
 901 CACTGGATCA ATCCTCGAAG AATGACCGAC CACAGCATCA AAGAATGTCA
     AAAATCTCTG
 961 GACTTTGTAC TAGGTTGGTT TGCCAAACCC GTATTTATTG ATGGTGACTA
     TCCCGAGAGC
1021 ATGAAGAATA ACCTTTCATC TATTCTGCCT GATTTTACTG AATCTGAGAA
     AAAGTTCATC
1081 AAAGGAACTG CTGACTTTTT TGCTCTTTGC TTTGGACCCA CCTTGAGTTT
     TCAACTTTTG
1141 GACCCTCACA TGAAGTTCCG CCAATTGGAA TCTCCCAACC TGAGGCAACT
     GCTTTCCTGG
1201 ATTGACCTTG AATTTAACCA TCCTCAAATA TTTATTGTGG AAAATGGCTG
     GTTTGTCTCA
1261 GGGACCACCA AGAGAGATGA TGCCAAATAT ATGTATTACC TCAAAAAGTT
     CATCATGGAA
1321 ACCTTAAAAG CCATCAAGCT GGATGGGGTG GATGTCATCG GGTATACCGC
     ATGGTCCCTC
1381 ATGGATGGTT TCGAGTGGCA CAGAGGTTAC AGCATCAGGC GTGGACTCTT
     CTATGTTGAC
1441 TTTCTAAGCC AGGACAAGAT GTTGTTGCCA AAGTCTTCAG CCTTGTTCTA
     CCAAAAGCTG
1501 ATAGAGAAAA ATGGCTTCCC TCCTTTACCT GAAAATCAGC CCCTAGAAGG
     GACATTTCCC
1561 TGTGACTTTG CTTGGGGAGT TGTTGACAAC TACATTCAAG TAGATACCAC
     TCTGTCTCAG
1621 TTTACCGACC TGAATGTTTA CCTGTGGGAT GTCCACCACA GTAAAAGGCT
     TATTAAAGTG
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1681 GATGGGGTTG TGACCAAGAA GAGGAAATCC TACTGTGTTG ACTTTGCTGC
CATCCAGCCC
1741 CAGATCGCTT TACTCCAGGA AATGCACGTT ACACATTTTC GCTTCTCCCT
GGACTGGGCC
1801 CTGATTCTCC CTCTGGGTAA CCAGTCCCAG GTGAACCACA CCATCCTGCA
GTACTATCGC
1861 TGCATGGCCA GCGAGCTTGT CCGTGTCAAC ATCACCCCAG TGGTGGCCCT
GTGGCAGCCT
1921 ATGGCCCCGA ACCAAGGACT GCCGCGCCTC CTGGCCAGGC AGGGCGCCTG
GGAGAACCCC
1981 TACACTGCCC TGGCCTTTGC AGAGTATGCC CGACTGTGCT TTCAAGAGCT
CGGCCATCAC
2041 GTCAAGCTTT GGATAACGAT GAATGAGCCG TATACAAGGA ATATGACATA
CAGTGCTGGC
2101 CACAACCTTC TGAAGGCCCA TGCCCTGGCT TGGCATGTGT ACAATGAAAA
GTTTAGGCAT
2161 GCTCAGAATG GGAAAATATC CATAGCCTTG CAGGCTGATT GGATAGAACC
TGCCTGCCCT
2221 TTCTCCCAAA AGGACAAAGA GGTGGCCGAG AGAGTTTTGG AATTTGACAT
TGGCTGGCTG
2281 GCTGAGCCCA TTTTCGGCTC TGGAGATTAT CCATGGGTGA TGAGGGACTG
GCTGAACCAA
2341 AGAAACAATT TTCTTCTTCC TTATTTCACT GAAGATGAAA AAAAGCTAAT
CCAGGGTACC
2401 TTTGACTTTT TGGCTTTAAG CCATTATACC ACCATCCTTG TAGACTCAGA
AAAAGAAGAT
2461 CCAATAAAAT ACAATGATTA CCTAGAAGTG CAAGAAATGA CCGACATCAC
GTGGCTCAAC
2521 TCCCCCAGTC AGGTGGCGGT AGTGCCCTGG GGGTTGCGCA AAGTGCTGAA
CTGGCTGAAG
2581 TTCAAGTACG GAGACCTCCC CATGTACATA ATATCCAACG GAATCGATGA
CGGGCTGCAT
2641 GCTGAGGACG ACCAGCTGAG GGTGTATTAT ATGCAGAATT ACATAAACGA
AGCTCTCAAA
2701 GCCCACATAC TGGATGGTAT CAATCTTTGC GGATACTTTG CTTATTCGTT
TAACGACCGC
2761 ACAGCTCCGA GGTTTGGCCT CTATCGTTAT GCTGCAGATC AGTTTGAGCC
CAAGGCATCC
2821 ATGAAACATT ACAGGAAAAT TATTGACAGC AATGGTTTCC CGGGCCCAGA
AACTCTGGAA
2881 AGATTTTGTC CAGAAGAATT CACCGTGTGT ACTGAGTGCA GTTTTTTTCA
CACCCGAAAG
2941 TCTTTAGGAT CCGGAGGTGG AGGTTCAGGA GGTGGAGGTT CAGGAGGTGG
AGGTTCACTT
3001 AAGTATCCCA ATGCCTCCCC ACTGCTCGGC TCCAGCTGGG GTGGCCTGAT
CCACCTGTAC
3061 ACAGCCACAG CCAGGAACAG CTACCACCTG CAGATCCACA AGAATGGCCA
TGTGGATGGC
3121 GCACCCCATC AGACCATCTA CAGTGCCCTG ATGATCAGAT CAGAGGATGC
TGGCTTTGTG
3181 GTGATTACAG GTGTGATGAG CAGAAGATAC CTCTGCATGG ATTTCAGAGG
CAACATTTTT
3241 GGATCACACT ATTTCGACCC GGAGAACTGC AGGTTCCAAC ACCAGACGCT
GGAAAACGGG
3301 TACGACGTCT ACCACTCTCC TCAGTATCAC TTCCTGGTCA GTCTGGGCCG
GGCGAAGAGA
3361 GCCTTCCTGC CAGGCATGAA CCCACCCCCG TACTCCCAGT TCCTGTCCCG
GAGGAACGAG
3421 ATCCCCCTAA TTCACTTCAA CACCCCCATA CCACGGCGGC ACACCCAGAG
CGCCGAGGAC
3481 GACTCGGAGC GGGACCCCCT GAACGTGCTG AAGCCCCGGG CCCGGATGAC
CCCGGCCCCG

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3541 GCCTCCTGTT CACAGGAGCT CCCGAGCGCC GAGGACAACA GCCCGATGGC
    CAGTGACCCA
3601 TTAGGGGTGG TCAGGGGCGG TCGAGTGAAC ACGCACGCTG GGGGAACGGG
    CCCGGAAGGC
3661 TGCCGCCCCT TCGCCAAGTT CATCGGAGGT GGAGGTTCAG CCCCAGAAGC
    AGCAGGTGGT
3721 CCATCAGTTT TTCTTTTCCC TCCCAAACCC AAGGATACGC TGATGATCTC
    TCGCACGCCT
3781 GAGGTGACAT GCGTCGTAGT AGACGTGAGC CACGAAGATC CCGAGGTGAA
    GTTCAATTGG
3841 TATGTGGACG GAGTAGAAGT GCATAACGCG AAAACTAAGC CGCGCGAGGA
    ACAATATAAC
3901 AGTACTTACA GGGTGGTATC CGTGCTCACA GTCCTGCACC AGGACTGGCT
    GAACGGTAAG
3961 GAATACAAGT GCAAAGTAAG CAACAAGGCA CTTCCCGCGC CTATTGAGAA
    AACAATCTCC
4021 AAGGCGAAGG GACAACCAAG AGAACCTCAG GTTTACACTC TCCCGCCTTC
    CAGGGAAGAG
4081 ATGACCAAAA ATCAAGTTTC CCTGACTTGC CTCGTCAAAG GATTCTACCC
    TTCCGACATT
4141 GCTGTTGAAT GGGAAAGCAA TGGACAACCA GAGAACAACT ACAAGACAAC
    ACCCCCGGTG
4201 CTGGATAGTG ACGGATCTTT CTTTCTCTAC TCAAAGCTGA CCGTGGATAA
    GTCCAGGTGG
4261 CAGCAGGGAA ACGTGTTTTC CTGCTCTGTC ATGCATGAAG CGCTGCATAA
    TCACTATACC
4321 CAGAAGTCTC TGAGCTTGAG CCCAGGCAAG TAA
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sKlotho-FGF23-FcLALA v2 (SEQ ID NO: 49)

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   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
 551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
 601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
 651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
 701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
 751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
 801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
 851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
 901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
 951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SLGSGGGGSG GGGSGGGGSL
1001 KYPNASPLLG SSWGGLIHLY TATARNSYHL QIHKNGHVDG APHQTIYSAL
1051 MIRSEDAGFV VITGVMSRRY LCMDFRGNIF GSHYFDPENC RFQHQTLENG
1101 YDVYHSPQYH FLVSLGRAKR AFLPGMNPPP YSQFLSRRNE IPLIHFNTPI
1151 PRRHTQSAED DSERDPLNVL KPRARMTPAP ASCSQELPSA EDNSPMASDP
1201 LGVVRGGRVN THAGGTGPEG CRPFAKFIGG GGSAPEAAGG PSVFLFPPKP
1251 KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
1301 STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
1351 VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
1401 LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
1451 *
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FGF23-FcLALA v1 (SEQ ID NO: 50)

```
   1 ATGTTGGGGG CCCGCCTCAG GCTCTGGGTC TGTGCCTTGT GCAGCGTCTG
     CAGCATGAGC
  61 GTCCTCAGAG CCTATCCCAA TGCCTCCCCA CTGCTCGGCT CCAGCTGGGG
     TGGCCTGATC
 121 CACCTGTACA CAGCCACAGC CAGGAACAGC TACCACCTGC AGATCCACAA
     GAATGGCCAT
 181 GTGGATGGCG CACCCCATCA GACCATCTAC AGTGCCCTGA TGATCAGATC
     AGAGGATGCT
 241 GGCTTTGTGG TGATTACAGG TGTGATGAGC AGAAGATACC TCTGCATGGA
     TTTCAGAGGC
 301 AACATTTTTG GATCACACTA TTTCGACCCG GAGAACTGCA GGTTCCAACA
     CCAGACGCTG
 361 GAAAACGGGT ACGACGTCTA CCACTCTCCT CAGTATCACT TCCTGGTCAG
     TCTGGGCCGG
 421 GCGAAGAGAG CCTTCCTGCC AGGCATGAAC CCACCCCCGT ACTCCCAGTT
     CCTGTCCCGG
 481 AGGAACGAGA TCCCCCTAAT TCACTTCAAC ACCCCCATAC CACGGCGGCA
     CACCCAGAGC
 541 GCCGAGGACG ACTCGGAGCG GGACCCCCTG AACGTGCTGA AGCCCCGGGC
     CCGGATGACC
 601 CCGGCCCCGG CCTCCTGTTC ACAGGAGCTC CCGAGCGCCG AGGACAACAG
     CCCGATGGCC
 661 AGTGACCCAT TAGGGGTGGT CAGGGGCGGT CGAGTGAACA CGCACGCTGG
     GGGAACGGGC
 721 CCGGAAGGCT GCCGCCCCTT CGCCAAGTTC ATCGGAGGTG GAGGTTCAAA
     AACCCACACG
 781 TGTCCTCCTT GTCCTGCCCC AGAAGCAGCA GGTGGTCCAT CAGTTTTTCT
     TTTCCCTCCC
 841 AAACCCAAGG ATACGCTGAT GATCTCTCGC ACGCCTGAGG TGACATGCGT
     CGTAGTAGAC
 901 GTGAGCCACG AAGATCCCGA GGTGAAGTTC AATTGGTATG TGGACGGAGT
     AGAAGTGCAT
 961 AACGCGAAAA CTAAGCCGCG CGAGGAACAA TATAACAGTA CTTACAGGGT
     GGTATCCGTG
1021 CTCACAGTCC TGCACCAGGA CTGGCTGAAC GGTAAGGAAT ACAAGTGCAA
     AGTAAGCAAC
1081 AAGGCACTTC CCGCGCCTAT TGAGAAAACA ATCTCCAAGG CGAAGGGACA
     ACCAAGAGAA
1141 CCTCAGGTTT ACACTCTCCC GCCTTCCAGG GAAGAGATGA CCAAAAATCA
     AGTTTCCCTG
1201 ACTTGCCTCG TCAAAGGATT CTACCCTTCC GACATTGCTG TTGAATGGGA
     AAGCAATGGA
1261 CAACCAGAGA ACAACTACAA GACAACACCC CCGGTGCTGG ATAGTGACGG
     ATCTTTCTTT
1321 CTCTACTCAA AGCTGACCGT GGATAAGTCC AGGTGGCAGC AGGGAAACGT
     GTTTTCCTGC
1381 TCTGTCATGC ATGAAGCGCT GCATAATCAC TATACCCAGA AGTCTCTGAG
     CTTGAGCCCA
1441 GGCAAGTAA
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FGF23(R179Q)-FcLALAv1 (SEQ ID NO: 51)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
  51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
 101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
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151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
251 IGGGGSKTHT CPPCPAPEAA GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
301 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
351 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
401 TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS
451 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK*
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FGF23-FcLALA v2 (SEQ ID NO: 52)

```
  1 ATGTTGGGGG CCCGCCTCAG GCTCTGGGTC TGTGCCTTGT GCAGCGTCTG
    CAGCATGAGC
 61 GTCCTCAGAG CCTATCCCAA TGCCTCCCCA CTGCTCGGCT CCAGCTGGGG
    TGGCCTGATC
121 CACCTGTACA CAGCCACAGC CAGGAACAGC TACCACCTGC AGATCCACAA
    GAATGGCCAT
181 GTGGATGGCG CACCCCATCA GACCATCTAC AGTGCCCTGA TGATCAGATC
    AGAGGATGCT
241 GGCTTTGTGG TGATTACAGG TGTGATGAGC AGAAGATACC TCTGCATGGA
    TTTCAGAGGC
301 AACATTTTTG GATCACACTA TTTCGACCCG GAGAACTGCA GGTTCCAACA
    CCAGACGCTG
361 GAAAACGGGT ACGACGTCTA CCACTCTCCT CAGTATCACT TCCTGGTCAG
    TCTGGGCCGG
421 GCGAAGAGAG CCTTCCTGCC AGGCATGAAC CCACCCCCGT ACTCCCAGTT
    CCTGTCCCGG
481 AGGAACGAGA TCCCCCTAAT TCACTTCAAC ACCCCCATAC CACGGCGGCA
    CACCCAGAGC
541 GCCGAGGACG ACTCGGAGCG GGACCCCCTG AACGTGCTGA AGCCCCGGGC
    CCGGATGACC
601 CCGGCCCCGG CCTCCTGTTC ACAGGAGCTC CCGAGCGCCG AGGACAACAG
    CCCGATGGCC
661 AGTGACCCAT TAGGGGTGGT CAGGGGCGGT CGAGTGAACA CGCACGCTGG
    GGGAACGGGC
721 CCGGAAGGCT GCCGCCCCTT CGCCAAGTTC ATCGGAGGTG GAGGTTCAGC
    CCCAGAAGCA
781 GCAGGTGGTC CATCAGTTTT TCTTTTCCCT CCCAAACCCA AGGATACGCT
    GATGATCTCT
841 CGCACGCCTG AGGTGACATG CGTCGTAGTA GACGTGAGCC ACGAAGATCC
    CGAGGTGAAG
901 TTCAATTGGT ATGTGGACGG AGTAGAAGTG CATAACGCGA AAACTAAGCC
    GCGCGAGGAA
961 CAATATAACA GTACTTACAG GGTGGTATCC GTGCTCACAG TCCTGCACCA
    GGACTGGCTG
1021 AACGGTAAGG AATACAAGTG CAAAGTAAGC AACAAGGCAC TTCCCGCGCC
    TATTGAGAAA
1081 ACAATCTCCA AGGCGAAGGG ACAACCAAGA GAACCTCAGG TTTACACTCT
    CCCGCCTTCC
1141 AGGGAAGAGA TGACCAAAAA TCAAGTTTCC CTGACTTGCC TCGTCAAAGG
    ATTCTACCCT
1201 TCCGACATTG CTGTTGAATG GGAAAGCAAT GGACAACCAG AGAACAACTA
    CAAGACAACA
1261 CCCCCGGTGC TGGATAGTGA CGGATCTTTC TTTCTCTACT CAAAGCTGAC
    CGTGGATAAG
1321 TCCAGGTGGC AGCAGGGAAA CGTGTTTTCC TGCTCTGTCA TGCATGAAGC
    GCTGCATAAT
1381 CACTATACCC AGAAGTCTCT GAGCTTGAGC CCAGGCAAGT AA
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FGF23(R179Q)-FcLALAv2 (SEQ ID NO: 53)

```
  1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
 51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
251 IGGGGSAPEA AGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK
301 FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS
351 NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
401 SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS
451 CSVMHEALHN HYTQKSLSLS PGK*
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Amino acid sequence of sKlotho-FGF23 (R1156Q, C1183S) (SEQ ID NO: 54)

sKlotho: aa [amino acid] 1-982; Linker1: aa 983-1001; FGF23: aa 1002-1228

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
 551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
 601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
 651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
 701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
 751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
 801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
 851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
 901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
 951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SLGSGGGGSG GGGSGGGGSL
1001 KYPNASPLLG SSWGGLIHLY TATARNSYHL QIHKNGHVDG APHQTIYSAL
1051 MIRSEDAGFV VITGVMSRRY LCMDFRGNIF GSHYFDPENC RFQHQTLENG
1101 YDVYHSPQYH FLVSLGRAKR AFLPGMNPPP YSQFLSRRNE IPLIHFNTPI
1151 PRRHTQSAED DSERDPLNVL KPRARMTPAP ASSSQELPSA EDNSPMASDP
1201 LGVVRGGRVN THAGGTGPEG CRPFAKFI*
```

Amino acid sequence of sKlotho-FGF23 (R1156Q, C1221S) (SEQ ID NO: 55)

sKlotho: 1-982; Linker1: 983-1001; FGF23: 1002-1228;

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
 551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
 601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
 651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
 701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
 751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
 801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
 851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
 901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
 951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SLGSGGGGSG GGGSGGGGSL
1001 KYPNASPLLG SSWGGLIHLY TATARNSYHL QIHKNGHVDG APHQTIYSAL
1051 MIRSEDAGFV VITGVMSRRY LCMDFRGNIF GSHYFDPENC RFQHQTLENG
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54067A

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1101 YDVYHSPQYH FLVSLGRAKR AFLPGMNPPP YSQFLSRRNE IPLIHFNTPI
1151 PRRHTQSAED DSERDPLNVL KPRARMTPAP ASCSQELPSA EDNSPMASDP
1201 LGVVRGGRVN THAGGTGPEG SRPFAKFI*
```

Amino acid sequence of sKlotho-FGF23 (R1156Q, Q1133A) (SEQ ID NO: 56)

sKlotho: 1-982; Linker1: 983-1001; FGF23: 1002-1228

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
 551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
 601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
 651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
 701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
 751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
 801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
 851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
 901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
 951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SLGSGGGGSG GGGSGGGGSL
1001 KYPNASPLLG SSWGGLIHLY TATARNSYHL QIHKNGHVDG APHQTIYSAL
1051 MIRSEDAGFV VITGVMSRRY LCMDFRGNIF GSHYFDPENC RFQHQTLENG
1101 YDVYHSPQYH FLVSLGRAKR AFLPGMNPPP YSAFLSRRNE IPLIHFNTPI
1151 PRRHTQSAED DSERDPLNVL KPRARMTPAP ASCSQELPSA EDNSPMASDP
1201 LGVVRGGRVN THAGGTGPEG CRPFAKFI*
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Amino acid sequence of sKlotho-FGF23 (R1156Q, C1183S, C1221S) (SEQ ID NO: 57)

sKlotho: 1-982; Linker1: 983-1001; FGF23: 1002-1228

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
 551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
 601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
 651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
 701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
 751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
 801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
 851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
 901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
 951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SLGSGGGGSG GGGSGGGGSL
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54067A

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1001 KYPNASPLLG SSWGGLIHLY TATARNSYHL QIHKNGHVDG APHQTIYSAL
1051 MIRSEDAGFV VITGVMSRRY LCMDFRGNIF GSHYFDPENC RFQHQTLENG
1101 YDVYHSPQYH FLVSLGRAKR AFLPGMNPPP YSQFLSRRNE IPLIHFNTPI
1151 PRRHTQSAED DSERDPLNVL KPRARMTPAP ASSSQELPSA EDNSPMASDP
1201 LGVVRGGRVN THAGGTGPEG SRPFAKFI*
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Amino acid sequence of sKlotho-FGF23 (R1156Q, C1183S, C1221S, Q1133A) (SEQ ID NO: 58)

sKlotho: 1-982; Linker1: 983-1001; FGF23: 1002-1228

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
 551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
 601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
 651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
 701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
 751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
 801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
 851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
 901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
 951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SLGSGGGGSG GGGSGGGGSL
1001 KYPNASPLLG SSWGGLIHLY TATARNSYHL QIHKNGHVDG APHQTIYSAL
1051 MIRSEDAGFV VITGVMSRRY LCMDFRGNIF GSHYFDPENC RFQHQTLENG
1101 YDVYHSPQYH FLVSLGRAKR AFLPGMNPPP YSAFLSRRNE IPLIHFNTPI
1151 PRRHTQSAED DSERDPLNVL KPRARMTPAP ASSSQELPSA EDNSPMASDP
1201 LGVVRGGRVN THAGGTGPEG SRPFAKFI*
```

Amino acid sequence of FGF23(R179Q; C206S)-FcLALAv1 (SEQ ID NO: 59)

FGF23: 1-251; Linker: 252-256; FcLALA: 257-482

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
  51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
 101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
 151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
 201 PAPASSSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
 251 IGGGGSKTHT CPPCPAPEAA GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 301 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
 351 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
 401 TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS
 451 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK*
```

Amino acid sequence of FGF23(R179Q, C244S)-FcLALAv1 (SEQ ID NO: 60)

FGF23: 1-251; Linker: 252-256; FcLALA: 257-482

```
  1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
 51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGSRPFAKF
251 IGGGGSKTHT CPPCPAPEAA GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
301 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
351 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
401 TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS
451 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK*
```

Amino acid sequence of FGF23(R179Q, Q156A)-FcLALAv1 (SEQ ID NO: 61)

FGF23: 1-251; Linker: 252-256; FcLALA: 257-482

```
  1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
 51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
151 PPPYSAFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
251 IGGGGSKTHT CPPCPAPEAA GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
301 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
351 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
401 TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS
451 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK*
```

Amino acid sequence of FGF23(R179Q, C206S, C244S)-FcLALAv1 (SEQ ID NO: 62)

FGF23: 1-251; Linker: 252-256; FcLALA: 257-482

```
  1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
 51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
201 PAPASSSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGSRPFAKF
251 IGGGGSKTHT CPPCPAPEAA GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
301 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
351 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
401 TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS
451 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK*
```

Amino acid sequence of FGF23(R179Q, C206S, C244S, Q156A)-FcLALAv1 (SEQ ID NO: 63)

FGF23: 1-251; Linker: 252-256; FcLALA: 257-482

```
  1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
 51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
151 PPPYSAFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
201 PAPASSSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGSRPFAKF
251 IGGGGSKTHT CPPCPAPEAA GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
301 VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
351 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL
401 TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS
451 RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK*
```

Amino acid sequence of FGF23(R179Q, C206S)-FcLALAv2 (SEQ ID NO: 64)

FGF23: 1-251; Linker: 252-256; FcLALA: 257-473

```
  1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
 51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
201 PAPASSSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
251 IGGGGSAPEA AGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK
301 FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS
351 NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
401 SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS
451 CSVMHEALHN HYTQKSLSLS PGK*
```

Amino acid sequence of FGF23(R179Q,C244S)-FcLALAv2 (SEQ ID NO: 65)

FGF23: 1-251; Linker: 252-256; FcLALA: 257-473

```
  1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
 51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGSRPFAKF
251 IGGGGSAPEA AGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK
301 FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS
351 NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
401 SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS
```

451 CSVMHEALHN HYTQKSLSLS PGK*

Amino acid sequence of FGF23(R179Q,Q156A)-FcLALAv2 (SEQ ID NO: 66)

FGF23: 1-251; Linker: 252-256; FcLALA: 257-473

```
  1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
 51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
151 PPPYSAFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
251 IGGGGSAPEA AGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK
301 FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS
351 NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
401 SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS
451 CSVMHEALHN HYTQKSLSLS PGK*
```

Amino acid sequence of FGF23(R179Q, C206S, C244S)-FcLALAv2 (SEQ ID NO: 67)

FGF23: 1-251; Linker: 252-256; FcLALA: 257-473

```
  1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
 51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
201 PAPASSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGSRPFAKF
251 IGGGGSAPEA AGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK
301 FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS
351 NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
401 SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS
451 CSVMHEALHN HYTQKSLSLS PGK*
```

Amino acid sequence of FGF23(R179Q, C206S, C244S, Q156A)-FcLALAv2 (SEQ ID NO: 68)

FGF23: 1-251; Linker: 252-256; FcLALA: 257-473

```
  1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
 51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
151 PPPYSAFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
201 PAPASSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGSRPFAKF
251 IGGGGSAPEA AGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK
301 FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS
351 NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
401 SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS
```

54067A

```
451 CSVMHEALHN HYTQKSLSLS PGK*
```

What is claimed is:

1. A fusion polypeptide comprising: (a) a polypeptide comprising fibroblast growth factor 23 (FGF23), or a functionally active variant or derivative thereof, wherein FGF23 has a mutation at one or more of the positions Q156, C206 and C244; and (b) either a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life, or a polypeptide comprising at least one extracellular subdomain of a Klotho protein, or a functionally active variant or derivative thereof; and, optionally (c) a linker.

2. The fusion polypeptide of claim 1, wherein the polypeptide of (a) is operatively linked to the N-terminus of the polypeptide of (b).

3. The fusion polypeptide of claim 1, wherein the polypeptide of (b) is operatively linked to the N-terminus of the polypeptide of (a).

4. The fusion polypeptide of claim 1, wherein the polypeptide of (a) and the polypeptide of (b) are connected by a polypeptide linker.

5. The fusion polypeptide of claim 4, wherein the polypeptide linker comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18.

6. The fusion polypeptide of claim 4, wherein the polypeptide linker comprises at least 1 and up to about 30 repeats of an amino acid sequence selected from the group consisting of: SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18.

7. The fusion polypeptide of claim 4, wherein the polypeptide of (a) is connected by a peptide bond to the N-terminus of said polyppetide linker, and the polypeptide of (b) is connected by a peptide bond to the C-terminus of said polypeptide linker.

8. The fusion polypeptide of claim 4, wherein the polypeptide of (a) is connected by a peptide bond to the C-terminus of said polypeptide linker, and the polypeptide of (b) is connected by a peptide bond to the N-terminus of said polypeptide linker.

9. The fusion polypeptide of claim 1, wherein the extracellular subdomain of the Klotho protein is a KL-D1 domain or a KL-D2 domain.

10. The fusion polypeptide of claim 1, wherein the polypeptide of (a) comprises at least two extracellular subdomains of the Klotho protein.

11. The fusion polypeptide of claim 10, wherein the at least two extracellular subdomains of the Klotho protein are at least two KL-D1 domains in tandem repeats.

12. The fusion polypeptide of claim 10, wherein the at least two extracellular subdomains of the Klotho protein are at least two KL-D2 domains in tandem repeats.

13. The fusion polypeptide of claim 10, wherein the at least two extracellular subdomains of Klotho protein comprise a KL-D1 domain and a KL-D2 domain.

14. The fusion polypeptide of claim 1, wherein the polypeptide of (a) is the extracellular domain of the Klotho protein.

15. The fusion polypeptide of claim 1, further comprising a signal peptide.

16. The fusion polypeptide of claim 15, wherein the signal peptide is the Klotho signal peptide.

17. The fusion polypeptide of claim 15, wherein the signal peptide is the IgG signal peptide.

18. The fusion polypeptide of claim 1 that specifically binds to a fibroblast growth factor receptor.

19. The fusion polypeptide of claim 1, wherein the Klotho protein is alpha-Klotho.

20. The fusion polypeptide of claim 1, wherein the Klotho protein is beta-Klotho.

21. The fusion polypeptide of claim 19, wherein the fibroblast growth factor is fibroblast growth factor-23 (FGF23) or a fibroblast growth factor-23 variant (R179Q).

22. The fusion polypeptide of claim 20, wherein the fibroblast growth factor is fibroblast growth factor-19 or fibroblast growth factor-21.

23. The fusion polypeptide of claim 1 comprising an amino acid sequence which is 95% or more identical to the amino acid sequence of SEQ ID NO: 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, or 68.

24. The fusion polypeptide of claim 1 having the amino acid sequence of SEQ ID NO: 58, or SEQ ID NO: 68.

25. The fusion polypeptide of claim 1 comprising FcLALA.

26. A pharmaceutical composition comprising the fusion polypeptide of claim 1 and a pharmaceutically acceptable carrier.

27. A nucleic acid comprising a sequence that encodes the fusion polypeptide of claim 1.

28. A host cell containing the nucleic acid of claim 27.

29. A vector comprising the nucleic acid of claim 27.

30. A method for treating or preventing an age-related condition in an individual, comprising administering to an individual in need thereof a therapeutically effective dose of a pharmaceutical composition comprising a fusion polypeptide comprising: (a) a polypeptide comprising fibroblast growth factor 23 (FGF23), or a functionally active variant or derivative thereof, wherein FGF23 has a mutation at one or more of the positions Q156, C206 and C244; and (b) either a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life, or a polypeptide comprising at least one extracellular subdomain of a Klotho protein, or a functionally active variant or derivative thereof; and, optionally (c) a linker.

31. The method of claim 30, wherein the age-related condition is selected from the group consisting of sarcopenia, skin atrophy, muscle wasting, brain atrophy, atherosclerosis, arteriosclerosis, pulmonary emphysema, osteoporosis, osteoarthritis, immunologic incompetence, high blood pressure, dementia, Huntington's disease, Alzheimer's disease, cataracts, age-related macular degeneration, prostate cancer, stroke, diminished life expectancy, memory loss, wrinkles, impaired kidney function, and age-related hearing loss.

32. The method of claim 30, wherein the Klotho protein is alpha Klotho protein.

33. The method of claim 31, wherein the age-related condition is muscle wasting, the Klotho protein is alpha Klotho protein, and the fibroblast growth factor is fibroblast growth factor 23.

34. A method for treating or preventing a metabolic disorder in an individual, comprising administering to an individual in need thereof a therapeutically effective dose of a pharmaceutical composition comprising a fusion polypeptide, comprising: (a) a polypeptide comprising fibroblast growth factor 23 (FGF23), or a functionally active variant or derivative thereof, wherein FGF23 has a mutation at one or more of the positions Q156, C206 and C244; and (b) either a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life, or a polypeptide comprising at least one extracellular subdomain of

a Klotho protein, or a functionally active variant or derivative thereof; and, optionally (c) a linker.

35. The method of claim 34, wherein the metabolic disorder is selected from the group consisting of Type II Diabetes, Metabolic Syndrome, hyperglycemia, and obesity.

36. The method of claim 34, wherein the fusion polypeptide comprises: (a) a polypeptide that comprises at least one extracellular subdomain of a beta-Klotho protein; and (b) a polypeptide that comprises a fibroblast growth factor 21.

37. A method for treating or preventing hyperphosphatemia or calcinosis in an individual, comprising administering to an individual in need thereof a therapeutically effective dose of a pharmaceutical composition comprising a fusion polypeptide, comprising: (a) a polypeptide comprising fibroblast growth factor 23 (FGF23), or a functionally active variant or derivative thereof, wherein FGF23 has a mutation at one or more of the positions Q156, C206 and C244; and (b) either a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life, or a polypeptide comprising at least one extracellular subdomain of a Klotho protein, or a functionally active variant or derivative thereof; and, optionally (c) a linker.

38. The method of claim 37, wherein the fusion polypeptide comprises: (a) a polypeptide that comprises at least one extracellular subdomain of an alpha Klotho protein; and (b) a polypeptide that comprises a fibroblast growth factor 23.

39. A method for treating or preventing chronic renal disease or chronic renal failure in an individual, comprising administering to an individual in need thereof a therapeutically effective dose of a pharmaceutical composition comprising a fusion polypeptide, comprising: (a) a polypeptide comprising fibroblast growth factor 23 (FGF23), or a functionally active variant or derivative thereof, wherein FGF23 has a mutation at one or more of the positions Q156, C206 and C244; and (b) either a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life, or a polypeptide comprising at least one

extracellular subdomain of a Klotho protein, or a functionally active variant or derivative thereof; and, optionally (c) a linker.

40. The method of claim 39, wherein the Klotho protein is alpha Klotho protein.

41. A method for treating or preventing cancer in an individual, comprising administering to an individual in need thereof a therapeutically effective dose of a pharmaceutical composition comprising a fusion polypeptide, comprising: (a) a polypeptide comprising fibroblast growth factor 23 (FGF23), or a functionally active variant or derivative thereof, wherein FGF23 has a mutation at one or more of the positions Q156, C206 and C244; and (b) either a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life, or a polypeptide comprising at least one extracellular subdomain of a Klotho protein, or a functionally active variant or derivative thereof; and, optionally (c) a linker.

42. The method of claim 41, wherein the cancer is breast cancer.

43. The method of claim 41, wherein the Klotho protein is an alpha Klotho protein.

44. The fusion polypeptide of claim 1, wherein the Klotho protein is a human Klotho protein.

45. The fusion polypeptide of claim 1 for use in treating or preventing muscle atrophy.

46. A method of treating or preventing muscle atrophy comprising (consisting essentially of, or consisting of) administering to an individual in need thereof a therapeutically effective dose of a pharmaceutical composition comprising a soluble Klotho fusion protein of SEQ ID NO: 47, or SEQ ID NO: 49.

47. A method of treating or preventing muscle atrophy comprising (consisting essentially of, or consisting of) administering to an individual in need thereof a therapeutically effective

dose of a pharmaceutical composition comprising (a) a polypeptide comprising fibroblast growth factor 23 (FGF23), or a functionally active variant or derivative thereof, wherein FGF23 has a mutation at one or more of the positions Q156, C206 and C244; and (b) either a modified Fc fragment having decreased affinity for Fc-gamma-receptor and/or increased serum half-life, or a polypeptide comprising at least one extracellular subdomain of a Klotho protein, or a functionally active variant or derivative thereof; and, optionally (c) a linker.

REPLACEMENT SHEET

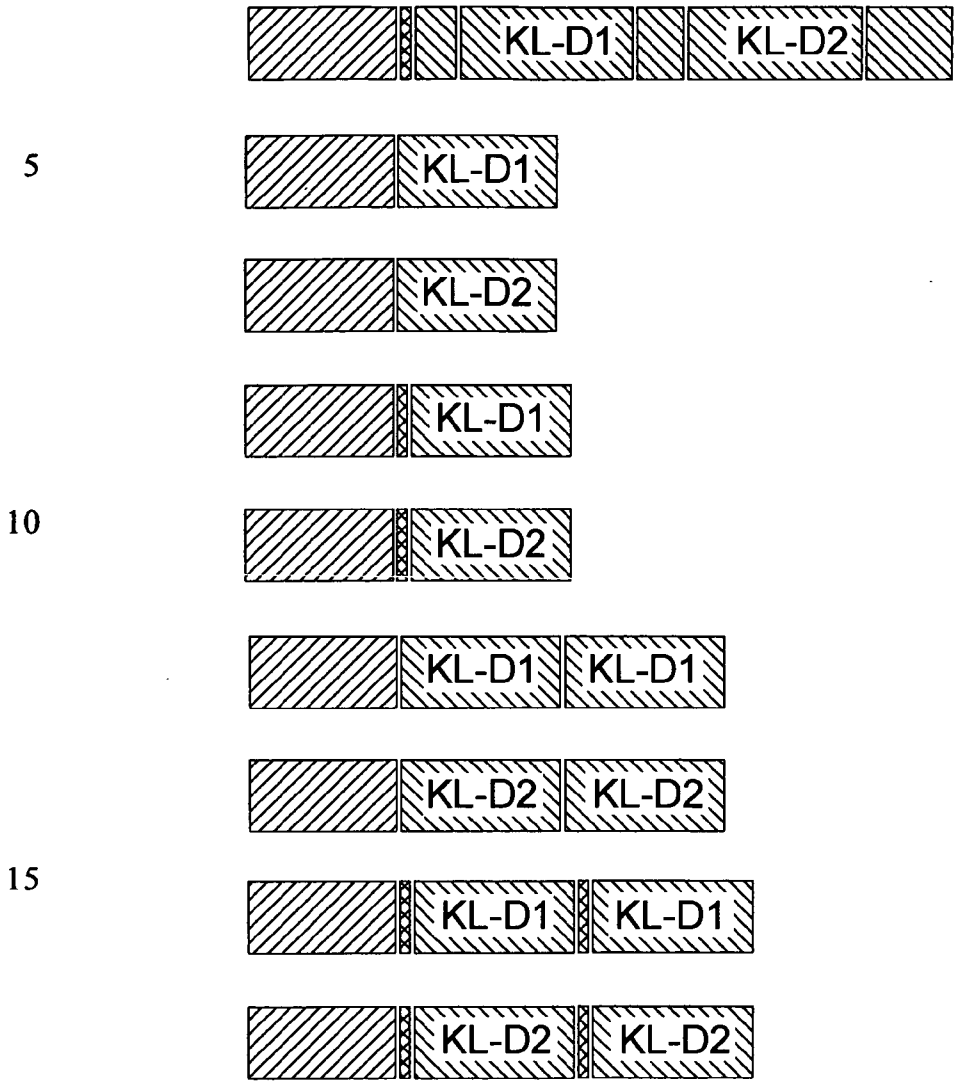
Figure 1

: Klotho (extracellular domain) or active fragment of Klotho;

: FGF23 (R179Q), FGF23, FGF 19, FGF21; : linker; : IgG signal peptide.

REPLACEMENT SHEET

Figure 1 (continued)



: Klotho (extracellular domain) or active fragment of Klotho;

: FGF23 (R179Q), FGF23, FGF 19, FGF21; : linker; : IgG signal peptide.

REPLACEMENT SHEET

Figure 2

Human Klotho nucleic acid sequence (NM_004795) (SEQ ID NO: 1)
Protein coding region: 9-3047

```
   1 cgcgcagcat gcccgccagc gccccgccgc gccgcccgcg gccgccgccg ccgtcgctgt
  61 cgctgctgct ggtgctgctg ggcctgggcg gccgccgcct gcgtgcggag ccgggcgacg
 121 gcgcgcagac ctgggcccgt ttctcgcggc ctcctgcccc cgaggccgcg ggcctcttcc
 181 agggcacctt ccccgacggc ttcctctggg ccgtgggcag cgccgcctac cagaccgagg
 241 gcggctggca gcagcacggc aagggtgcgt ccatctggga tacgttcacc caccaccccc
 301 tggcaccccc gggagactcc cggaacgcca gtctgccgtt gggcgccccg tcgccgctgc
 361 agcccgccac cggggacgta gccagcgaca gctacaacaa cgtcttccgc gacacggagg
 421 cgctgcgcga gctcggggtc actcactacc gcttctccat ctcgtgggcg cgagtgctcc
 481 ccaatggcag cgcgggcgtc cccaaccgcg aggggctgcg ctactaccgg cgcctgctgg
 541 agcggctgcg ggagctgggc gtgcagcccg tggtcaccct gtaccactgg gacctgcccc
 601 agcgcctgca ggacgcctac ggcggctggg ccaaccgcgc cctggccgac cacttcaggg
 661 attacgcgga gctctgcttc cgccacttcg gcggtcaggt caagtactgg atcaccatcg
 721 acaaccccta cgtggtggcc tggcacggct acgccaccgg gcgcctggcc cccggcatcc
 781 ggggcagccc gcggctcggg tacctggtgg cgcacaacct cctcctggct catgccaaag
 841 tctggcatct ctacaatact tctttccgtc ccactcaggg aggtcaggtg tccattgccc
 901 taagctctca ctggatcaat cctcgaagaa tgaccgacca cagcatcaaa gaatgtcaaa
 961 aatctctgga ctttgtacta ggttggtttg ccaaacccgt atttattgat ggtgactatc
1021 ccgagagcat gaagaataac ctttcatcta ttctgcctga ttttactgaa tctgagaaaa
1081 agttcatcaa aggaactgct gacttttttg ctctttgctt tggacccacc ttgagttttc
1141 aacttttgga ccctcacatg aagttccgcc aattggaatc tcccaacctg aggcaactgc
1201 tttcctggat tgaccttgaa tttaaccatc ctcaaatatt tattgtggaa aatggctggt
1261 ttgtctcagg gaccaccaag agagatgatg ccaaatatat gtattacctc aaaaagttca
1321 tcatggaaac cttaaaagcc atcaagctgg atggggtgga tgtcatcggg tataccgcat
1381 ggtccctcat ggatggtttc gagtggcaca gaggttacag catcaggcgt ggactcttct
1441 atgttgactt tctaagccag gacaagatgt tgttgccaaa gtcttcagcc ttgttctacc
1501 aaaagctgat agagaaaaat ggcttccctc ctttacctga aaatcagccc ctagaaggga
1561 catttccctg tgactttgct tggggagttg ttgacaacta cattcaagta gataccactc
1621 tgtctcagtt taccgacctg aatgtttacc tgtgggatgt ccaccacagt aaaaggctta
1681 ttaaagtgga tggggttgtg accaagaaga ggaaatccta ctgtgttgac tttgctgcca
1741 tccagcccca gatcgcttta ctccaggaaa tgcacgttac acattttcgc ttctccctgg
1801 actgggccct gattctccct ctgggtaacc agtcccaggt gaaccacacc atcctgcagt
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1921 ggcagcctat ggccccgaac caaggactgc cgcgcctcct ggccaggcag ggcgcctggg
1981 agaaccccta cactgccctg gcctttgcag agtatgcccg actgtgcttt caagagctcg
2041 gccatcacgt caagctttgg ataacgatga atgagccgta tacaaggaat atgacataca
2101 gtgctggcca caaccttctg aaggcccatg ccctggcttg gcatgtgtac aatgaaaagt
2161 ttaggcatgc tcagaatggg aaaatatcca tagccttgca ggctgattgg atagaacctg
2221 cctgcccttt ctcccaaaag gacaaagagg tggccgagag agttttggaa tttgacattg
2281 gctggctggc tgagcccatt ttcggctctg gagattatcc atgggtgatg agggactggc
2341 tgaaccaaag aaacaatttt cttcttcctt atttcactga agatgaaaaa aagctaatcc
2401 agggtacctt tgacttttg gctttaagcc attataccac catccttgta gactcagaaa
2461 aagaagatcc aataaaatac aatgattacc tagaagtgca agaaatgacc gacatcacgt
2521 ggctcaactc ccccagtcag gtggcggtag tgccctgggg gttgcgcaaa gtgctgaact
2581 ggctgaagtt caagtacgga gacctcccca tgtacataat atccaacgga atcgatgacg
2641 ggctgcatgc tgaggacgac cagctgaggg tgtattatat gcagaattac ataaacgaag
2701 ctctcaaagc ccacatactg gatggtatca atctttgcgg atactttgct tattcgttta
2761 acgaccgcac agctccgagg tttggcctct atcgttatgc tgcagatcag tttgagccca
2821 aggcatccat gaaacattac aggaaaatta ttgacagcaa tggtttcccg ggcccagaaa
2881 ctctggaaag attttgtcca gaagaattca ccgtgtgtac tgagtgcagt ttttttcaca
2941 cccgaaagtc tttactggct ttcatagctt ttctattttt tgcttctatt atttctctct
3001 cccttatatt ttactactcg aagaaaggca gaagaagtta caaatagttc tgaacatttt
```

REPLACEMENT SHEET

```
3061 tctattcatt cattttgaaa taattatgca gacacatcag ctgttaacca tttgcacctc
3121 taagtgttgt gaaactgtaa atttcataca tttgacttct agaaaacatt tttgtggctt
3181 atgacagagg ttttgaaatg ggcataggtg atcgtaaaat attgaataat gcgaatagtg
3241 cctgaatttg ttctcttttt gggtgattaa aaaactgaca ggcactataa tttctgtaac
3301 acactaacaa aagcatgaaa aataggaacc acaccaatgc aacatttgtg cagaaatttg
3361 aatgacaaga ttaggaatat tttcttctgc acccacttct aaatttaatg tttttctgga
3421 agtagtaatt gcaagagttc gaatagaaag ttatgtacca agtaaccatt tctcagctgc
3481 cataataatg cctagtggct tcccctctgt caaatctagt ttcctatgga aaagaagatg
3541 gcagatacag gagagacgac agagggtcct aggctggaat gttcctttcg aaagcaatgc
3601 ttctatcaaa tactagtatt aatttatgta tctggttaat gacatacttg gagagcaaat
3661 tatggaaatg tgtattttat atgatttttg aggtcctgtc taaaccctgt gtccctgagg
3721 gatctgtctc actggcatct tgttgagggc cttgcacata ggaaactttt gataagtatc
3781 tgcggaaaaa caaacatgaa tcctgtgata ttgggctctt caggaagcat aaagcaattg
3841 tgaaatacag tataccgcag tggctctagg tggaggaaag gaggaaaaag tgcttattat
3901 gtgcaacatt atgattaatc tgattataca ccatttttga gcagatcttg gaatgaatga
3961 catgaccttt ccctagagaa taaggatgaa ataatcactc attctatgaa cagtgacact
4021 actttctatt ctttagctgt actgtaattt ctttgagttg atagttttac aaattcttaa
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4141 tattttatgt ttttgctgct acttctgtgg aagtagcttt gaactagttt tactttgaac
4201 tttcacgctg aaacatgcta gtgatatcta gaaagggcta attaggtctc atcctttaat
4261 gccccttaaa taagtcttgc tgattttcag acagggaagt ctctctatta cactggagct
4321 gttttataga taagtcaata ttgtatcagg caagataaac caatgtcata acaggcattg
4381 ccaacctcac tgacacaggg tcatagtgta taataatata ctgtactata taatatatca
4441 tctttagagg tatgattttt tcatgaaaga taagcttttg gtaatattca ttttaaagtg
4501 gacttattaa aattggatgc tagagaatca agtttatttt atgtatatat ttttctgatt
4561 ataagagtaa tatatgttca ttgtaaaaat ttttaaaaca cagaaactat atgcaaagaa
4621 aaaataaaaa ttatctataa tctcagaacc cagaaatagc cactattaac atttcctacg
4681 tattttattt tacatagatc atattgtata tagttagtat ctttattaat ttttattatg
4741 aaactttcct ttgtcattat tagtcttcaa aagcatgatt tttaatagtt gttgagtatt
4801 ccaccacagg aatgtatcac aacttaaccg ttcccgtttg ttagactagt ttcttattaa
4861 tgttgatgaa tgttgtttaa aaataatttt gttgctacat ttactttaat ttccttgact
4921 gtaaagagaa gtaattttgc tccttgataa agtattatat taataataaa tctgcctgca
4981 acttttgcc ttctttcata atc
```

Klotho amino acid sequence (NP_004786) (SEQ ID NO: 2)

```
  1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA PEAAGLFQGT
 61 FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP PGDSRNASLP LGAPSPLQPA
121 TGDVASDSYN NVFRDTEALR ELGVTHYRFS ISWARVLPNG SAGVPNREGL RYYRRLLERL
181 RELGVQPVVT LYHWDLPQRL QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP
241 YVVAWHGYAT GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP DFTESEKKFI
361 KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW IDLEFNHPQI FIVENGWFVS
421 GTTKRDDAKY MYYLKKFIME TLKAIKLDGV DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD
481 FLSQDKMLLP KSSALFYQKL IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ
541 FTDLNVYLWD VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL LARQGAWENP
661 YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG HNLLKAHALA WHVYNEKFRH
721 AQNGKISIAL QADWIEPACP FSQKDKEVAE RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ
781 RNNFLLPYFT EDEKKLIQGT FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN
841 SPSQVAVVPW GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS NGFPGPETLE
961 RFCPEEFTVC TECSFFHTRK SLLAFIAFLF FASIISLSLI FYYSKKGRRS YK
```

REPLACEMENT SHEET

beta-Klotho nucleic acid sequence (NM_175737) (SEQ ID NO: 3)
Protein coding region: 98-3232

```
   1 atcctcagtc tcccagttca agctaatcat tgacagagct ttacaatcac aagcttttac
  61 tgaagctttg ataagacagt ccagcagttg gtggcaaatg aagccaggct gtgcggcagg
 121 atctccaggg aatgaatgga ttttcttcag cactgatgaa ataaccacac gctataggaa
 181 tacaatgtcc aacgggggat tgcaaagatc tgtcatcctg tcagcactta ttctgctacg
 241 agctgttact ggattctctg gagatggaag agctatatgg tctaaaaatc ctaatttac
 301 tccggtaaat gaaagtcagc tgtttctcta tgacactttc cctaaaaact ttttctgggg
 361 tattgggact ggagcattgc aagtggaagg gagttggaag aaggatggaa aaggaccttc
 421 tatatgggat catttcatcc acacacacct taaaaatgtc agcagcacga atggttccag
 481 tgacagttat atttttctgg aaaaagactt atcagccctg gattttatag gagtttcttt
 541 ttatcaattt tcaatttcct ggccaaggct tttccccgat ggaatagtaa cagttgccaa
 601 cgcaaaaggt ctgcagtact acagtactct tctggacgct ctagtgctta gaaacattga
 661 acctatagtt actttatacc actgggattt gcctttggca ctacaagaaa aatatggggg
 721 gtggaaaaat gataccataa tagatatctt caatgactat gccacatact gtttccagat
 781 gtttggggac cgtgtcaaat attggattac aattcacaac ccatatctag tggcttggca
 841 tgggtatggg acaggtatgc atgcccctgg agagaaggga aatttagcag ctgtctacac
 901 tgtgggacac aacttgatca aggctcactc gaaagtttgg cataactaca acacacattt
 961 ccgcccacat cagaagggtt ggttatcgat cacgttggga tctcattgga tcgagccaaa
1021 ccggtcggaa aacacgatgg atatattcaa atgtcaacaa tccatggttt ctgtgcttgg
1081 atggtttgcc aaccctatcc atggggatgg cgactatcca gaggggatga gaaagaagtt
1141 gttctccgtt ctacccattt tctctgaagc agagaagcat gagatgagag gcacagctga
1201 tttctttgcc ttttcttttg gacccaacaa cttcaagccc ctaaacacca tggctaaaat
1261 gggacaaaat gtttcactta atttaagaga agcgctgaac tggattaaac tggaatacaa
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1381 agacaccacg gccatctaca tgatgaagaa tttcctcagc caggtgcttc aagcaataag
1441 gttagatgaa atacgagtgt ttggttatac tgcctggtct ctcctggatg gctttgaatg
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1681 tgtcactgaa tctgttctta agcccgagtc tgtggcttcg tccccacagt tcagcgatcc
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1921 cactggcaac ctgtccgcgg tgaaccgaca ggccctgagg tactacaggt gcgtggtcag
1981 tgaggggctg aagcttggca tctccgcgat ggtcaccctg tattatccga cccacgccca
2041 cctaggcctc cccgagcctc tgttgcatgc cgacgggtgg ctgaacccat cgacggccga
2101 ggccttccag gcctacgctg ggctgtgctt ccaggagctg ggggacctgg tgaagctctg
2161 gatcaccatc aacgagccta accggctaag tgacatctac aaccgctctg gcaacgacac
2221 ctacggggcg gcgcacaacc tgctggtggc ccacgccctg gcctggcgcc tctacgaccg
2281 gcagttcagg ccctcacagc gcggggccgt gtcgctgtcg ctgcacgcgg actgggcgga
2341 acccgccaac ccctatgctg actcgcactg gagggcggcc gagcgcttcc tgcagttcga
2401 gatcgcctgg ttcgccgagc cgctcttcaa gaccggggac taccccgcgg ccatgaggga
2461 atacattgcc tccaagcacc gacgggggct ttccagctcg gccctgccgc gcctcaccga
2521 ggccgaaagg aggctgctca agggcacggt cgacttctgc gcgctcaacc acttcaccac
2581 taggttcgtg atgcacgagc agctggccgg cagccgctac gactcggaca gggacatcca
2641 gtttctgcag gacatcaccc gcctgagctc ccccacgcgc ctggctgtga ttccctgggg
2701 ggtgcgcaag ctgctgcggt gggtccggag gaactacggc gacatggaca tttacatcac
2761 cgccagtggc atcgacgacc aggctctgga ggatgaccgg ctccggaagt actacctagg
2821 gaagtacctt caggaggtgc tgaaagcata cctgattgat aaagtcagaa tcaaaggcta
2881 ttatgcattc aaactggctg aagagaaatc taaacccaga tttggattct tcacatctga
2941 ttttaaagct aaatcctcaa tacaatttta caacaaagtg atcagcagca ggggcttccc
3001 ttttgagaac agtagttcta gatgcagtca gacccaagaa aatacagagt gcactgtctg
3061 cttattcctt gtgcagaaga aaccactgat attcctgggt tgttgcttct tctccaccct
3121 ggttctactc ttatcaattg ccatttttca aaggcagaag agaagaaagt tttggaaagc
```

REPLACEMENT SHEET

```
3181 aaaaaactta caacacatac cattaaagaa aggcaagaga gttgttagct aaactgatct
3241 gtctgcatga tagacagttt aaaaattcat cccagttcc
```

beta-Klotho amino acid sequence (NP_783864) (SEQ ID NO: 4)

```
   1 mkpgcaagsp gnewiffstd eittryrntm sngglqrsvi lsalillrav tgfsgdgrai
  61 wsknpnftpv nesqlflydt fpknffwgig tgalqvegsw kkdgkgpsiw dhfihthlkn
 121 vsstngssds yiflekdlsa ldfigvsfyq fsiswprlfp dgivtvanak glqyystlld
 181 alvlrniepi vtlyhwdlpl alqekyggwk ndtiidifnd yatycfqmfg drvkywitih
 241 npylvawhgy gtgmhapgek gnlaavytvg hnlikahskv whnynthfrp hqkgwlsitl
 301 gshwiepnrs entmdifkcq qsmvsvlgwf anpihgdgdy pegmrkklfs vlpifseaek
 361 hemrgtadff afsfgpnnfk plntmakmgq nvslnlreal nwikleynnp riliaengwf
 421 tdsrvktedt taiymmknfl sqvlqairld eirvfgytaw slldgfewqd aytirrglfy
 481 vdfnskqker kpkssahyyk qiirengfsl kestpdvqgq fpcdfswgvt esvlkpesva
 541 sspqfsdphl yvwnatgnrl lhrvegvrlk trpaqctdfv nikkqlemla rmkvthyrfa
 601 ldwasvlptg nlsavnrqal ryyrcvvseg lklgisamvt lyypthahlg lpepllhadg
 661 wlnpstaeaf qayaglcfqe lgdlvklwit inepnrlsdi ynrsgndtyg aahnllvaha
 721 lawrlydrqf rpsqrgavsl slhadwaepa npyadshwra aerflqfeia wfaeplfktg
 781 dypaamreyi askhrrglss salprlteae rrllkgtvdf calnhfttrf vmheqlagsr
 841 ydsdrdiqfl qditrlsspt rlavipwgvr kllrwvrrny gdmdiyitas giddqaledd
 901 rlrkyylgky lqevlkayli dkvrikgyya fklaeekskp rfgfftsdfk akssiqfynk
 961 vissrgfpfe nsssrcsqtq entectvclf lvqkkplifl gccffstlvl llsiaifqrq
1021 krrkfwkakn lqhiplkkgk rvvs
```

Human Klotho domain 1 (KL-D1) amino acid sequence (SEQ ID NO: 5)

```
  58                                                              qgt
  61 fpdgflwavg saayqteggw qqhgkgasiw dtfthhplap pgdsrnaslp lgapsplqpa
 121 tgdvasdsyn nvfrdtealr elgvthyrfs iswarvlpng sagvpnregl ryyrrllerl
 181 relgvqpvvt lyhwdlpqrl qdayggwanr aladhfrdya elcfrhfggq vkywitidnp
 241 yvvawhgyat grlapgirgs prlgylvahn lllahakvwh lyntsfrptq ggqvsialss
 301 hwinprrmtd hsikecqksl dfvlgwfakp vfidgdypes mknnlssilp dftesekkfi
 361 kgtadffalc fgptlsfqll dphmkfrqle spnlrqllsw idlefnhpqi fivengwfvs
 421 gttkrddaky myylkkfime tlkaikldgv dvigytawsl mdgfewhrgy sirrglfyvd
 481 flsqdkmllp kssalfyqkl iekngf
```

Human Klotho domain 2 (KL-D2) amino acid sequence (SEQ ID NO: 6)

```
 517                                          gtfp cdfawgvvdn yiqvdttlsq
 541 ftdlnvylwd vhhskrlikv dgvvtkkrks ycvdfaaiqp qiallqemhv thfrfsldwa
 601 lilplgnqsq vnhtilqyyr cmaselvrvn itpvvalwqp mapnqglprl larqgawenp
 661 ytalafaeya rlcfqelghh vklwitmnep ytrnmtysag hnllkahala whvynekfrh
 721 aqngkisial qadwiepacp fsqkdkevae rvlefdigwl aepifgsgdy pwvmrdwlnq
 781 rnnfllpyft edekkliqgt fdflalshyt tilvdseked pikyndylev qemtditwln
 841 spsqvavvpw glrkvlnwlk fkygdlpmyi isngiddglh aeddqlrvyy mqnyinealk
 901 ahildginlc gyfaysfndr taprfglyry aadqfepkas mkhyrkiids ngf
```

Klotho extracellular domain (without signal peptide) amino acid sequence (SEQ ID NO: 7)

```
  28                                       epgdgaq twarfsrppa peaaglfqgt
  61 fpdgflwavg saayqteggw qqhgkgasiw dtfthhplap pgdsrnaslp lgapsplqpa
 121 tgdvasdsyn nvfrdtealr elgvthyrfs iswarvlpng sagvpnregl ryyrrllerl
 181 relgvqpvvt lyhwdlpqrl qdayggwanr aladhfrdya elcfrhfggq vkywitidnp
```

REPLACEMENT SHEET

```
241 yvvawhgyat grlapgirgs prlgylvahn lllahakvwh lyntsfrptq ggqvsialss
301 hwinprrmtd hsikecqksl dfvlgwfakp vfidgdypes mknnlssilp dftesekkfi
361 kgtadffalc fgptlsfqll dphmkfrqle spnlrqllsw idlefnhpqi fivengwfvs
421 gttkrddaky myylkkfime tlkaikldgv dvigytawsl mdgfewhrgy sirrglfyvd
481 flsqdkmllp kssalfyqkl iekngfpplp enqplegtfp cdfawgvvdn yiqvdttlsq
541 ftdlnvylwd vhhskrlikv dgvvtkkrks ycvdfaaiqp qiallqemhv thfrfsldwa
601 lilplgnqsq vnhtilqyyr cmaselvrvn itpvvalwqp mapnqglprl larqgawenp
661 ytalafaeya rlcfqelghh vklwitmnep ytrnmtysag hnllkahala whvynekfrh
721 aqngkisial qadwiepacp fsqkdkevae rvlefdigwl aepifgsgdy pwvmrdwlnq
781 rnnfllpyft edekkliqgt fdflalshyt tilvdseked pikyndylev qemtditwln
841 spsqvavvpw glrkvlnwlk fkygdlpmyi isngiddglh aeddqlrvyy mqnyinealk
901 ahildginlc gyfaysfndr taprfglyry aadqfepkas mkhyrkiids ngfpgpetle
961 rfcpeeftvc tecsffhtrk sl
```

Klotho signal peptide amino acid sequence (SEQ ID NO: 8)

```
1 mpasapprrp rppppslsll lvllglggrr lra
```

IgG signal peptide amino acid sequence (SEQ ID NO: 9)

```
1 msvltqvlal lllwltgtrc rrlra
```

$(Gly_4\ Ser)_3$ polypeptide linker nucleic acid sequence (SEQ ID NO: 10)

```
1 ggaggtggag gttcaggagg tggaggttca ggaggtggag gttca
```

$(Gly_4\ Ser)_3$ polypeptide linker amino acid sequence (SEQ ID NO: 11)

```
1 GGGGSGGGGS GGGGS
```

$(Gly_4\ Ser)$ polypeptide linker amino acid sequence (SEQ ID NO: 12)

```
1 GGGGS
```

(Gly) polypeptide linker amino acid sequence (SEQ ID NO: 13)

```
1 G
```

(Gly Gly) polypeptide linker amino acid sequence (SEQ ID NO: 14)

```
1 GG
```

(Gly Ser) polypeptide linker amino acid sequence (SEQ ID NO: 15)

```
1 GS
```

$(Gly_2\ Ser)$ polypeptide linker amino acid sequence (SEQ ID NO: 16)

```
1 GGS
```

REPLACEMENT SHEET

(Ala) polypeptide linker amino acid sequence (SEQ ID NO: 17)

1 A

(Ala Ala) polypeptide linker amino acid sequence (SEQ ID NO: 18)

1 AA

Klotho signal peptide-Klotho extracellular domain-FGF23 (R179Q) amino acid sequence (SEQ ID NO: 19)

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
 551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
 601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
 651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
 701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
 751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
 801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
 851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
 901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
 951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SLGSGGGGSG GGGSGGGGSL
1001 KYPNASPLLG SSWGGLIHLY TATARNSYHL QIHKNGHVDG APHQTIYSAL
1051 MIRSEDAGFV VITGVMSRRY LCMDFRGNIF GSHYFDPENC RFQHQTLENG
1101 YDVYHSPQYH FLVSLGRAKR AFLPGMNPPP YSQFLSRRNE IPLIHFNTPI
1151 PRRHTQSAED DSERDPLNVL KPRARMTPAP ASCSQELPSA EDNSPMASDP
1201 LGVVRGGRVN THAGGTGPEG CRPFAKFI*
```

IgG signal peptide-Klotho extracellular domain-FGF23 (R179Q) amino acid sequence (SEQ ID NO: 20)

```
   1 MSVLTQVLAL LLLWLTGLGG RRLRAEPGDG AQTWARFSRP PAPEAAGLFQ
  51 GTFPDGFLWA VGSAAYQTEG GWQQHGKGAS IWDTFTHHPL APPGDSRNAS
 101 LPLGAPSPLQ PATGDVASDS YNNVFRDTEA LRELGVTHYR FSISWARVLP
 151 NGSAGVPNRE GLRYYRRLLE RLRELGVQPV VTLYHWDLPQ RLQDAYGGWA
 201 NRALADHFRD YAELCFRHFG GQVKYWITID NPYVVAWHGY ATGRLAPGIR
 251 GSPRLGYLVA HNLLLAHAKV WHLYNTSFRP TQGGQVSIAL SSHWINPRRM
 301 TDHSIKECQK SLDFVLGWFA KPVFIDGDYP ESMKNNLSSI LPDFTESEKK
 351 FIKGTADFFA LCFGPTLSFQ LLDPHMKFRQ LESPNLRQLL SWIDLEFNHP
 401 QIFIVENGWF VSGTTKRDDA KYMYYLKKFI METLKAIKLD GVDVIGYTAW
 451 SLMDGFEWHR GYSIRRGLFY VDFLSQDKML LPKSSALFYQ KLIEKNGFPP
 501 LPENQPLEGT FPCDFAWGVV DNYIQVDTTL SQFTDLNVYL WDVHHSKRLI
 551 KVDGVVTKKR KSYCVDFAAI QPQIALLQEM HVTHFRFSLD WALILPLGNQ
```

REPLACEMENT SHEET

```
 601 SQVNHTILQY YRCMASELVR VNITPVVALW QPMAPNQGLP RLLARQGAWE
 651 NPYTALAFAE YARLCFQELG HHVKLWITMN EPYTRNMTYS AGHNLLKAHA
 701 LAWHVYNEKF RHAQNGKISI ALQADWIEPA CPFSQKDKEV AERVLEFDIG
 751 WLAEPIFGSG DYPWVMRDWL NQRNNFLLPY FTEDEKKLIQ GTFDFLALSH
 801 YTTILVDSEK EDPIKYNDYL EVQEMTDITW LNSPSQVAVV PWGLRKVLNW
 851 LKFKYGDLPM YIISNGIDDG LHAEDDQLRV YYMQNYINEA LKAHILDGIN
 901 LCGYFAYSFN DRTAPRFGLY RYAADQFEPK ASMKHYRKII DSNGFPGPET
 951 LERFCPEEFT VCTECSFFHT RKSLGSGGGG SGGGGSGGGG SLKYPNASPL
1001 LGSSWGGLIH LYTATARNSY HLQIHKNGHV DGAPHQTIYS ALMIRSEDAG
1051 FVVITGVMSR RYLCMDFRGN IFGSHYFDPE NCRFQHQTLE NGYDVYHSPQ
1101 YHFLVSLGRA KRAFLPGMNP PPYSQFLSRR NEIPLIHFNT PIPRRHTQSA
1151 EDDSERDPLN VLKPRARMTP APASCSQELP SAEDNSPMAS DPLGVVRGGR
1201 VNTHAGGTGP EGCRPFAKFI *
```

KL-D1-FGF23 (R179Q) amino acid sequence (SEQ ID NO: 21)

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGSGG GGSGGGGSGG GGSLKYPNAS PLLGSSWGGL
 551 IHLYTATARN SYHLQIHKNG HVDGAPHQTI YSALMIRSED AGFVVITGVM
 601 SRRYLCMDFR GNIFGSHYFD PENCRFQHQT LENGYDVYHS PQYHFLVSLG
 651 RAKRAFLPGM NPPPYSQFLS RRNEIPLIHF NTPIPRRHTQ SAEDDSERDP
 701 LNVLKPRARM TPAPASCSQE LPSAEDNSPM ASDPLGVVRG GRVNTHAGGT
 751 GPEGCRPFAK FI*
```

KL-D2-FGF23 (R179Q) amino acid sequence (SEQ ID NO: 22)

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LPLPENQPLE GTFPCDFAWG
  51 VVDNYIQVDT TLSQFTDLNV YLWDVHHSKR LIKVDGVVTK KRKSYCVDFA
 101 AIQPQIALLQ EMHVTHFRFS LDWALILPLG NQSQVNHTIL QYYRCMASEL
 151 VRVNITPVVA LWQPMAPNQG LPRLLARQGA WENPYTALAF AEYARLCFQE
 201 LGHHVKLWIT MNEPYTRNMT YSAGHNLLKA HALAWHVYNE KFRHAQNGKI
 251 SIALQADWIE PACPFSQKDK EVAERVLEFD IGWLAEPIFG SGDYPWVMRD
 301 WLNQRNNFLL PYFTEDEKKL IQGTFDFLAL SHYTTILVDS EKEDPIKYND
 351 YLEVQEMTDI TWLNSPSQVA VVPWGLRKVL NWLKFKYGDL PMYIISNGID
 401 DGLHAEDDQL RVYYMQNYIN EALKAHILDG INLCGYFAYS FNDRTAPRFG
 451 LYRYAADQFE PKASMKHYRK IIDSNGFPGP ETLERFCPEE FTVCTECSFF
 501 HTRKSLGSGG GGSGGGGSGG GGSLKYPNAS PLLGSSWGGL IHLYTATARN
 551 SYHLQIHKNG HVDGAPHQTI YSALMIRSED AGFVVITGVM SRRYLCMDFR
 601 GNIFGSHYFD PENCRFQHQT LENGYDVYHS PQYHFLVSLG RAKRAFLPGM
 651 NPPPYSQFLS RRNEIPLIHF NTPIPRRHTQ SAEDDSERDP LNVLKPRARM
 701 TPAPASCSQE LPSAEDNSPM ASDPLGVVRG GRVNTHAGGT GPEGCRPFAK
 751 FI*
```

$(KL\text{-}D1)_2$-FGF23 (R179Q) amino acid sequence (SEQ ID NO: 23)

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
```

REPLACEMENT SHEET

```
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGSGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW
 551 DTFTHHPLAP PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR
 601 ELGVTHYRFS ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT
 651 LYHWDLPQRL QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP
 701 YVVAWHGYAT GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ
 751 GGQVSIALSS HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES
 801 MKNNLSSILP DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE
 851 SPNLRQLLSW IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME
 901 TLKAIKLDGV DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP
 951 KSSALFYQKL IEKNGFPEFG SGGGGSGGGG SGGGGSLKYP NASPLLGSSW
1001 GGLIHLYTAT ARNSYHLQIH KNGHVDGAPH QTIYSALMIR SEDAGFVVIT
1051 GVMSRRYLCM DFRGNIFGSH YFDPENCRFQ HQTLENGYDV YHSPQYHFLV
1101 SLGRAKRAFL PGMNPPPYSQ FLSRRNEIPL IHFNTPIPRR HTQSAEDDSE
1151 RDPLNVLKPR ARMTPAPASC SQELPSAEDN SPMASDPLGV VRGGRVNTHA
1201 GGTGPEGCRP FAKFI*
```

$(KL\text{-}D2)_2$-FGF23 (R179Q) amino acid sequence (SEQ ID NO: 24)

```
   1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LPLPENQPLE GTFPCDFAWG
  51 VVDNYIQVDT TLSQFTDLNV YLWDVHHSKR LIKVDGVVTK KRKSYCVDFA
 101 AIQPQIALLQ EMHVTHFRFS LDWALILPLG NQSQVNHTIL QYYRCMASEL
 151 VRVNITPVVA LWQPMAPNQG LPRLLARQGA WENPYTALAF AEYARLCFQE
 201 LGHHVKLWIT MNEPYTRNMT YSAGHNLLKA HALAWHVYNE KFRHAQNGKI
 251 SIALQADWIE PACPFSQKDK EVAERVLEFD IGWLAEPIFG SGDYPWVMRD
 301 WLNQRNNFLL PYFTEDEKKL IQGTFDFLAL SHYTTILVDS EKEDPIKYND
 351 YLEVQEMTDI TWLNSPSQVA VVPWGLRKVL NWLKFKYGDL PMYIISNGID
 401 DGLHAEDDQL RVYYMQNYIN EALKAHILDG INLCGYFAYS FNDRTAPRFG
 451 LYRYAADQFE PKASMKHYRK IIDSNGFPGP ETLERFCPEE FTVCTECSFF
 501 HTRKSLGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD VHHSKRLIKV
 551 DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA LILPLGNQSQ
 601 VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL LARQGAWENP
 651 YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG HNLLKAHALA
 701 WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE RVLEFDIGWL
 751 AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT FDFLALSHYT
 801 TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW GLRKVLNWLK
 851 FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK AHILDGINLC
 901 GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS NGFGSGGGGS
 951 GGGGSGGGGS LKYPNASPLL GSSWGGLIHL YTATARNSYH LQIHKNGHVD
1001 GAPHQTIYSA LMIRSEDAGF VVITGVMSRR YLCMDFRGNI FGSHYFDPEN
1051 CRFQHQTLEN GYDVYHSPQY HFLVSLGRAK RAFLPGMNPP PYSQFLSRRN
1101 EIPLIHFNTP IPRRHTQSAE DDSERDPLNV LKPRARMTPA PASCSQELPS
1151 AEDNSPMASD PLGVVRGGRV NTHAGGTGPE GCRPFAKFI*
```

FGF23 (R179Q) -Klotho extracellular domain amino acid sequence (SEQ ID NO: 25)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
```

REPLACEMENT SHEET

```
  51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
 101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
 151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
 201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
 251 IGSGGGGSGG GGSGGGGSLK EPGDGAQTWA RFSRPPAPEA AGLFQGTFPD
 301 GFLWAVGSAA YQTEGGWQQH GKGASIWDTF THHPLAPPGD SRNASLPLGA
 351 PSPLQPATGD VASDSYNNVF RDTEALRELG VTHYRFSISW ARVLPNGSAG
 401 VPNREGLRYY RRLLERLREL GVQPVVTLYH WDLPQRLQDA YGGWANRALA
 451 DHFRDYAELC FRHFGGQVKY WITIDNPYVV AWHGYATGRL APGIRGSPRL
 501 GYLVAHNLLL AHAKVWHLYN TSFRPTQGGQ VSIALSSHWI NPRRMTDHSI
 551 KECQKSLDFV LGWFAKPVFI DGDYPESMKN NLSSILPDFT ESEKKFIKGT
 601 ADFFALCFGP TLSFQLLDPH MKFRQLESPN LRQLLSWIDL EFNHPQIFIV
 651 ENGWFVSGTT KRDDAKYMYY LKKFIMETLK AIKLDGVDVI GYTAWSLMDG
 701 FEWHRGYSIR RGLFYVDFLS QDKMLLPKSS ALFYQKLIEK NGFPPLPENQ
 751 PLEGTFPCDF AWGVVDNYIQ VDTTLSQFTD LNVYLWDVHH SKRLIKVDGV
 801 VTKKRKSYCV DFAAIQPQIA LLQEMHVTHF RFSLDWALIL PLGNQSQVNH
 851 TILQYYRCMA SELVRVNITP VVALWQPMAP NQGLPRLLAR QGAWENPYTA
 901 LAFAEYARLC FQELGHHVKL WITMNEPYTR NMTYSAGHNL LKAHALAWHV
 951 YNEKFRHAQN GKISIALQAD WIEPACPFSQ KDKEVAERVL EFDIGWLAEP
1001 IFGSGDYPWV MRDWLNQRNN FLLPYFTEDE KKLIQGTFDF LALSHYTTIL
1051 VDSEKEDPIK YNDYLEVQEM TDITWLNSPS QVAVVPWGLR KVLNWLKFKY
1101 GDLPMYIISN GIDDGLHAED DQLRVYYMQN YINEALKAHI LDGINLCGYF
1151 AYSFNDRTAP RFGLYRYAAD QFEPKASMKH YRKIIDSNGF PGPETLERFC
1201 PEEFTVCTEC SFFHTRKSL*
```

FGF23 (R179Q) -KL-D1 amino acid sequence (SEQ ID NO: 26)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
  51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
 101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
 151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
 201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
 251 IQGTFPDGFL WAVGSAAYQT EGGWQQHGKG ASIWDTFTHH PLAPPGDSRN
 301 ASLPLGAPSP LQPATGDVAS DSYNNVFRDT EALRELGVTH YRFSISWARV
 351 LPNGSAGVPN REGLRYYRRL LERLRELGVQ PVVTLYHWDL PQRLQDAYGG
 401 WANRALADHF RDYAELCFRH FGGQVKYWIT IDNPYVVAWH GYATGRLAPG
 451 IRGSPRLGYL VAHNLLLAHA KVWHLYNTSF RPTQGGQVSI ALSSHWINPR
 501 RMTDHSIKEC QKSLDFVLGW FAKPVFIDGD YPESMKNNLS SILPDFTESE
 551 KKFIKGTADF FALCFGPTLS FQLLDPHMKF RQLESPNLRQ LLSWIDLEFN
 601 HPQIFIVENG WFVSGTTKRD DAKYMYYLKK FIMETLKAIK LDGVDVIGYT
 651 AWSLMDGFEW HRGYSIRRGL FYVDFLSQDK MLLPKSSALF YQKLIEKNGF
 652 *
```

FGF23 (R179Q) -KL-D2 amino acid sequence (SEQ ID NO: 27)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
  51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
 101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
 151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
 201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
 251 IGTFPCDFAW GVVDNYIQVD TTLSQFTDLN VYLWDVHHSK RLIKVDGVVT
 301 KKRKSYCVDF AAIQPQIALL QEMHVTHFRF SLDWALILPL GNQSQVNHTI
 351 LQYYRCMASE LVRVNITPVV ALWQPMAPNQ GLPRLLARQG AWENPYTALA
 401 FAEYARLCFQ ELGHHVKLWI TMNEPYTRNM TYSAGHNLLK AHALAWHVYN
 451 EKFRHAQNGK ISIALQADWI EPACPFSQKD KEVAERVLEF DIGWLAEPIF
```

REPLACEMENT SHEET

```
 501 GSGDYPWVMR DWLNQRNNFL LPYFTEDEKK LIQGTFDFLA LSHYTTILVD
 551 SEKEDPIKYN DYLEVQEMTD ITWLNSPSQV AVVPWGLRKV LNWLKFKYGD
 601 LPMYIISNGI DDGLHAEDDQ LRVYYMQNYI NEALKAHILD GINLCGYFAY
 651 SFNDRTAPRF GLYRYAADQF EPKASMKHYR KIIDSNGF*
```

FGF23 (R179Q) -(KL-D1)$_2$ amino acid sequence (SEQ ID NO: 28)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
  51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
 101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
 151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
 201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
 251 IQGTFPDGFL WAVGSAAYQT EGGWQQHGKG ASIWDTFTHH PLAPPGDSRN
 301 ASLPLGAPSP LQPATGDVAS DSYNNVFRDT EALRELGVTH YRFSISWARV
 351 LPNGSAGVPN REGLRYYRRL LERLRELGVQ PVVTLYHWDL PQRLQDAYGG
 401 WANRALADHF RDYAELCFRH FGGQVKYWIT IDNPYVVAWH GYATGRLAPG
 451 IRGSPRLGYL VAHNLLLAHA KVWHLYNTSF RPTQGGQVSI ALSSHWINPR
 501 RMTDHSIKEC QKSLDFVLGW FAKPVFIDGD YPESMKNNLS SILPDFTESE
 551 KKFIKGTADF FALCFGPTLS FQLLDPHMKF RQLESPNLRQ LLSWIDLEFN
 601 HPQIFIVENG WFVSGTTKRD DAKYMYYLKK FIMETLKAIK LDGVDVIGYT
 651 AWSLMDGFEW HRGYSIRRGL FYVDFLSQDK MLLPKSSALF YQKLIEKNGF
 701 QGTFPDGFLW AVGSAAYQTE GGWQQHGKGA SIWDTFTHHP LAPPGDSRNA
 751 SLPLGAPSPL QPATGDVASD SYNNVFRDTE ALRELGVTHY RFSISWARVL
 801 PNGSAGVPNR EGLRYYRRLL ERLRELGVQP VVTLYHWDLP QRLQDAYGGW
 851 ANRALADHFR DYAELCFRHF GGQVKYWITI DNPYVVAWHG YATGRLAPGI
 901 RGSPRLGYLV AHNLLLAHAK VWHLYNTSFR PTQGGQVSIA LSSHWINPRR
 951 MTDHSIKECQ KSLDFVLGWF AKPVFIDGDY PESMKNNLSS ILPDFTESEK
1001 KFIKGTADFF ALCFGPTLSF QLLDPHMKFR QLESPNLRQL LSWIDLEFNH
1051 PQIFIVENGW FVSGTTKRDD AKYMYYLKKF IMETLKAIKL DGVDVIGYTA
1101 WSLMDGFEWH RGYSIRRGLF YVDFLSQDKM LLPKSSALFY QKLIEKNGF*
```

FGF23 (R179Q) -(KL-D2)$_2$ amino acid sequence (SEQ ID NO: 29)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS
  51 YHLQIHKNGH VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG
 101 NIFGSHYFDP ENCRFQHQTL ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN
 151 PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS AEDDSERDPL NVLKPRARMT
 201 PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG PEGCRPFAKF
 251 IGTFPCDFAW GVVDNYIQVD TTLSQFTDLN VYLWDVHHSK RLIKVDGVVT
 301 KKRKSYCVDF AAIQPQIALL QEMHVTHFRF SLDWALILPL GNQSQVNHTI
 351 LQYYRCMASE LVRVNITPVV ALWQPMAPNQ GLPRLLARQG AWENPYTALA
 401 FAEYARLCFQ ELGHHVKLWI TMNEPYTRNM TYSAGHNLLK AHALAWHVYN
 451 EKFRHAQNGK ISIALQADWI EPACPFSQKD KEVAERVLEF DIGWLAEPIF
 501 GSGDYPWVMR DWLNQRNNFL LPYFTEDEKK LIQGTFDFLA LSHYTTILVD
 551 SEKEDPIKYN DYLEVQEMTD ITWLNSPSQV AVVPWGLRKV LNWLKFKYGD
 601 LPMYIISNGI DDGLHAEDDQ LRVYYMQNYI NEALKAHILD GINLCGYFAY
 651 SFNDRTAPRF GLYRYAADQF EPKASMKHYR KIIDSNGFGT FPCDFAWGVV
 701 DNYIQVDTTL SQFTDLNVYL WDVHHSKRLI KVDGVVTKKR KSYCVDFAAI
 751 QPQIALLQEM HVTHFRFSLD WALILPLGNQ SQVNHTILQY YRCMASELVR
 801 VNITPVVALW QPMAPNQGLP RLLARQGAWE NPYTALAFAE YARLCFQELG
 851 HHVKLWITMN EPYTRNMTYS AGHNLLKAHA LAWHVYNEKF RHAQNGKISI
 901 ALQADWIEPA CPFSQKDKEV AERVLEFDIG WLAEPIFGSG DYPWVMRDWL
 951 NQRNNFLLPY FTEDEKKLIQ GTFDFLALSH YTTILVDSEK EDPIKYNDYL
1001 EVQEMTDITW LNSPSQVAVV PWGLRKVLNW LKFKYGDLPM YIISNGIDDG
1051 LHAEDDQLRV YYMQNYINEA LKAHILDGIN LCGYFAYSFN DRTAPRFGLY
1101 RYAADQFEPK ASMKHYRKII DSNGF*
```

REPLACEMENT SHEET

FGF19 nucleic acid sequence (NM_005117) (SEQ ID NO: 30)
Protein coding region (464-1114)

```
   1 gctcccagcc aagaacctcg gggccgctgc gcggtgggga ggagttcccc gaaacccggc
  61 cgctaagcga ggcctcctcc tcccgcagat ccgaacggcc tgggcggggt caccccggct
 121 gggacaagaa gccgccgcct gcctgcccgg gcccggggag ggggctgggg ctggggccgg
 181 aggcggggtg tgagtgggtg tgtgcggggg gcggaggctt gatgcaatcc cgataagaaa
 241 tgctcgggtg tcttgggcac ctacccgtgg ggcccgtaag gcgctactat ataaggctgc
 301 cggcccggag ccgccgcgcc gtcagagcag gagcgctgcg tccaggatct agggccacga
 361 ccatcccaac ccggcactca cagccccgca gcgcatcccg gtcgccgccc agcctcccgc
 421 acccccatcg ccggagctgc gccgagagcc ccagggaggt gccatgcgga gcgggtgtgt
 481 ggtggtccac gtatggatcc tggccggcct ctggctggcc gtggccgggc gccccctcgc
 541 cttctcggac gcggggcccc acgtgcacta cggctggggc gaccccatcc gcctgcggca
 601 cctgtacacc tccggccccc acgggctctc cagctgcttc ctgcgcatcc gtgccgacgg
 661 cgtcgtggac tgcgcgcggg gccagagcgc gcacagtttg ctggagatca aggcagtcgc
 721 tctgcggacc gtggccatca agggcgtgca cagcgtgcgg tacctctgca tgggcgccga
 781 cggcaagatg caggggctgc ttcagtactc ggaggaagac tgtgctttcg aggaggagat
 841 ccgcccagat ggctacaatg tgtaccgatc cgagaagcac cgcctcccgg tctccctgag
 901 cagtgccaaa cagcggcagc tgtacaagaa cagaggcttt cttccactct ctcatttcct
 961 gcccatgctg cccatggtcc cagaggagcc tgaggacctc aggggccact tggaatctga
1021 catgttctct tcgccctgg agaccgacag catggaccca tttgggcttg tcaccggact
1081 ggaggccgtg aggagtccca gctttgagaa gtaactgaga ccatgcccgg gcctcttcac
1141 tgctgccagg ggctgtggta cctgcagcgt gggggacgtg cttctacaag aacagtcctg
1201 agtccacgtt ctgtttagct ttaggaagaa acatctagaa gttgtacata ttcagagttt
1261 tccattggca gtgccagttt ctagccaata gacttgtctg atcataacat tgtaagcctg
1321 tagcttgccc agctgctgcc tgggccccca ttctgctccc tcgaggttgc tggacaagct
1381 gctgcactgt ctcagttctg cttgaatacc tccatcgatg gggaactcac ttcctttgga
1441 aaaattctta tgtcaagctg aaattctcta atttttctc atcacttccc caggagcagc
1501 cagaagacag gcagtagttt taatttcagg aacaggtgat ccactctgta aaacagcagg
1561 taaatttcac tcaaccccat gtgggaattg atctatatct ctacttccag ggaccatttg
1621 cccttcccaa atcccttccag gccagaactg actggagcag gcatggccca ccaggcttca
1681 ggagtagggg aagcctggag ccccactcca gccctgggac aacttgagaa ttccccctga
1741 ggccagttct gtcatggatg ctgtcctgag aataacttgc tgtcccggtg tcacctgctt
1801 ccatctccca gcccaccagc cctctgccca cctcacatgc ctccccatgg attggggcct
1861 cccaggcccc ccaccttatg tcaacctgca cttcttgttc aaaaatcagg aaaagaaaag
1921 atttgaagac cccaagtctt gtcaataact tgctgtgtgg aagcagcggg ggaagaccta
1981 gaaccctttc cccagcactt ggttttccaa catgatattt atgagtaatt tattttgata
2041 tgtacatctc ttatttttctt acattattta tgcccccaaa ttatatttat gtatgtaagt
2101 gaggtttgtt ttgtatatta aaatggagtt tgtttgtaaa aaaaaaaaaa aaaaaaa
```

FGF19 amino acid sequence (NP_005108) (SEQ ID NO: 31)

```
  1 MRSGCVVVHV WILAGLWLAV AGRPLAFSDA GPHVHYGWGD PIRLRHLYTS GPHGLSSCFL
 61 RIRADGVVDC ARGQSAHSLL EIKAVALRTV AIKGVHSVRY LCMGADGKMQ GLLQYSEEDC
121 AFEEEIRPDG YNVYRSEKHR LPVSLSSAKQ RQLYKNRGFL PLSHFLPMLP MVPEEPEDLR
181 GHLESDMFSS PLETDSMDPF GLVTGLEAVR SPSFEK
```

FGF21 nucleic acid sequence (NM_019113) (SEQ ID NO: 32)
Protein coding region 151-780

```
  1 CTGTCAGCTG AGGATCCAGC CGAAAGAGGA GCCAGGCACT CAGGCCACCT GAGTCTACTC
 61 ACCTGGACAA CTGGAATCTG GCACCAATTC TAAACCACTC AGCTTCTCCG AGCTCACACC
121 CCGGAGATCA CCTGAGGACC CGAGCCATTG ATGGACTCGG ACGAGACCGG GTTCGAGCAC
```

REPLACEMENT SHEET

```
181 TCAGGACTGT GGGTTTCTGT GCTGGCTGGT CTTCTGCTGG GAGCCTGCCA GGCACACCCC
241 ATCCCTGACT CCAGTCCTCT CCTGCAATTC GGGGGCCAAG TCCGGCAGCG GTACCTCTAC
301 ACAGATGATG CCCAGCAGAC AGAAGCCCAC CTGGAGATCA GGGAGGATGG GACGGTGGGG
361 GGCGCTGCTG ACCAGAGCCC CGAAAGTCTC CTGCAGCTGA AAGCCTTGAA GCCGGGAGTT
421 ATTCAAATCT TGGGAGTCAA GACATCCAGG TTCCTGTGCC AGCGGCCAGA TGGGGCCCTG
481 TATGGATCGC TCCACTTTGA CCCTGAGGCC TGCAGCTTCC GGGAGCTGCT TCTTGAGGAC
541 GGATACAATG TTTACCAGTC CGAAGCCCAC GGCCTCCCGC TGCACCTGCC AGGGAACAAG
601 TCCCCACACC GGGACCCTGC ACCCCGAGGA CCAGCTCGCT TCCTGCCACT ACCAGGCCTG
661 CCCCCCGCAC TCCCGGAGCC ACCCGGAATC CTGGCCCCCC AGCCCCCCGA TGTGGGCTCC
721 TCGGACCCTC TGAGCATGGT GGGACCTTCC CAGGGCCGAA GCCCCAGCTA CGCTTCCTGA
781 AGCCAGAGGC TGTTTACTAT GACATCTCCT CTTTATTTAT TAGGTTATTT ATCTTATTTA
841 TTTTTTTATT TTTCTTACTT GAGATAATAA AGAGTTCCAG AGGAGAAAAA AAAAAAAAAA
901 AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
```

FGF21 amino acid sequence (NP_061986) (SEQ ID NO: 33)

```
  1 MDSDETGFEH SGLWVSVLAG LLLGACQAHP IPDSSPLLQF GGQVRQRYLY TDDAQQTEAH
 61 LEIREDGTVG GAADQSPESL LQLKALKPGV IQILGVKTSR FLCQRPDGAL YGSLHFDPEA
121 CSFRELLLED GYNVYQSEAH GLPLHLPGNK SPHRDPAPRG PARFLPLPGL PPALPEPPGI
181 LAPQPPDVGS SDPLSMVGPS QGRSPSYAS
```

FGF23 nucleic acid sequence (NM_020638) (SEQ ID NO: 34)
Protein coding region 147-902

```
   1 cggcaaaaag gagggaatcc agtctaggat cctcacacca gctacttgca agggagaagg
  61 aaaaggccag taaggcctgg gccaggagag tcccgacagg agtgtcaggt ttcaatctca
 121 gcaccagcca ctcagagcag ggcacgatgt tgggggcccg cctcaggctc tgggtctgtg
 181 ccttgtgcag cgtctgcagc atgagcgtcc tcagagccta tcccaatgcc tccccactgc
 241 tcggctccag ctggggtggc ctgatccacc tgtacacagc cacagccagg aacagctacc
 301 acctgcagat ccacaagaat ggccatgtgg atggcgcacc ccatcagacc atctacagtg
 361 ccctgatgat cagatcagag gatgctggct ttgtggtgat tacaggtgtg atgagcagaa
 421 gatacctctg catggatttc agaggcaaca tttttggatc acactatttc gacccggaga
 481 actgcaggtt ccaacaccag acgctggaaa acgggtacga cgtctaccac tctcctcagt
 541 atcacttcct ggtcagtctg ggccgggcga agagagcctt cctgccaggc atgaacccac
 601 ccccgtactc ccagttcctg tcccggagga acgagatccc cctaattcac ttcaacaccc
 661 ccataccacg gcggcacacc cggagcgccg aggacgactc ggagcgggac cccctgaacg
 721 tgctgaagcc ccgggccccgg atgaccccgg ccccggcctc ctgttcacag gagctcccga
 781 gcgccgagga caacagcccg atggccagtg acccattagg ggtggtcagg ggcggtcgag
 841 tgaacacgca cgctgggggа acgggcccgg aaggctgccg ccccttcgcc aagttcatct
 901 agggtcgctg gaagggcacc ctctttaacc catccctcag caaacgcagc tcttcccaag
 961 gaccaggtcc cttgacgttc cgaggatggg aaaggtgaca ggggcatgta tggaatttgc
1021 tgcttctctg gggtcccttc cacaggaggt cctgtgagaa ccaacctttg aggcccaagt
1081 catggggttt caccgccttc ctcactccat atagaacacc tttcccaata ggaaacccca
1141 acaggtaaac tagaaatttc cccttcatga aggtagagag aaggggtctc tcccaacata
1201 tttctcttcc ttgtgcctct cctctttatc acttttaagc ataaaaaaaa aaaaaaaaaa
1261 aaaaaaaaaa aaaagcagtg ggttcctgag ctcaagactt tgaaggtgta gggaagagga
1321 aatcggagat cccagaagct tctccactgc cctatgcatt tatgttagat gccccgatcc
1381 cactggcatt tgagtgtgca aaccttgaca ttaacagctg aatggggcaa gttgatgaaa
1441 acactacttt caagccttcg ttcttccttg agcatctctg gggaagagct gtcaaaagac
1501 tggtggtagg ctggtgaaaa cttgacagct agacttgatg cttgctgaaa tgaggcagga
1561 atcataatag aaaactcagc ctccctacag ggtgagcacc ttctgtctcg ctgtctccct
1621 ctgtgcagcc acagccagag ggcccagaat ggccccactc tgttcccaag cagttcatga
1681 tacagcctca ccttttggcc ccatctctgg tttttgaaaa tttggtctaa ggaataaata
1741 gcttttacac tggctcacga aaatctgccc tgctagaatt tgcttttcaa aatggaaata
1801 aattccaact ctcctaagag gcatttaatt aaggctctac ttccaggttg agtaggaatc
```

REPLACEMENT SHEET

```
1861 cattctgaac aaactacaaa aatgtgactg ggaagggggc tttgagagac tgggactgct
1921 ctgggttagg ttttctgtgg actgaaaaat cgtgtccttt tctctaaatg aagtggcatc
1981 aaggactcag ggggaaagaa atcaggggac atgttataga agttatgaaa agacaaccac
2041 atggtcaggc tcttgtctgt ggtctctagg gctctgcagc agcagtggct cttcgattag
2101 ttaaaactct cctaggctga cacatctggg tctcaatccc cttggaaatt cttggtgcat
2161 taaatgaagc cttaccccat tactgcggtt cttcctgtaa gggggctcca ttttcctccc
2221 tctctttaaa tgaccaccta aaggacagta tattaacaag caaagtcgat tcaacaacag
2281 cttcttccca gtcacttttt tttttctcac tgccatcaca tactaacctt atactttgat
2341 ctattctttt tggttatgag agaaatgttg ggcaactgtt tttacctgat ggttttaagc
2401 tgaacttgaa ggactggttc ctattctgaa acagtaaaac tatgtataat agtatatagc
2461 catgcatggc aaatatttta atatttctgt tttcatttcc tgttggaaat attatcctgc
2521 ataatagcta ttggaggctc ctcagtgaaa gatcccaaaa ggattttggt ggaaaactag
2581 ttgtaatctc acaaactcaa cactaccatc aggggttttc tttatggcaa agccaaaata
2641 gctcctacaa tttcttatat ccctcgtcat gtggcagtat ttatttattt atttggaagt
2701 ttgcctatcc ttctatattt atagatattt ataaaaatgt aacccctttt tcctttcttc
2761 tgtttaaaat aaaaataaaa tttatctcag cttctgttag cttatcctct ttgtagtact
2821 acttaaaagc atgtcggaat ataagaataa aaaggattat gggaggggaa cattagggaa
2881 atccagagaa ggcaaaattg aaaaaaagat tttagaattt taaaattttc aaagatttct
2941 tccattcata aggagactca atgattttaa ttgatctaga cagaattatt taagttttat
3001 caatattgga tttctggt
```

FGF23 amino acid sequence (NP_065689) (SEQ ID NO: 35)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS YHLQIHKNGH
  61 VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG NIFGSHYFDP ENCRFQHQTL
 121 ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN PPPYSQFLSR RNEIPLIHFN TPIPRRHTRS
 181 AEDDSERDPL NVLKPRARMT PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG
 241 PEGCRPFAKF I
```

FGF23 (R179Q) amino acid sequence (SEQ ID NO: 36)

```
   1 MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS YHLQIHKNGH
  61 VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG NIFGSHYFDP ENCRFQHQTL
 121 ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS
 181 AEDDSERDPL NVLKPRARMT PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG
 241 PEGCRPFAKF I
```

Human beta-Klotho domain 1 (b-KL-D1) amino acid sequence (SEQ ID NO: 37)

```
  77                    ydt fpknffwgig tgalqvegsw kkdgkgpsiw dhfihthlkn
 121 vsstngssds yiflekdlsa ldfigvsfyq fsiswprlfp dgivtvanak glqyystlld
 181 alvlrniepi vtlyhwdlpl alqekyggwk ndtiidifnd yatycfqmfg drvkywitih
 241 npylvawhgy gtgmhapgek gnlaavytvg hnlikahskv whnynthfrp hqkgwlsitl
 301 gshwiepnrs entmdifkcq qsmvsvlgwf anpihgdgdy pegmrkklfs vlpifseaek
 361 hemrgtadff afsfgpnnfk plntmakmgq nvslnlreal nwikleynnp riliaengwf
 421 tdsrvktedt taiymmknfl sqvlqairld eirvfgytaw slldgfewqd aytirrglfy
 481 vdfnskqker kpkssahyyk qiirengf
```

Human beta-Klotho domain 2 (b-KL-D2) amino acid sequence (SEQ ID NO: 38)

```
 571                                trpaqctdfv nikkqlemla rmkvthyrfa
 601 ldwasvlptg nlsavnrqal ryyrcvvseg lklgisamvt lyypthahlg lpepllhadg
 661 wlnpstaeaf qayaglcfqe lgdlvklwit inepnrlsdi ynrsgndtyg aahnllvaha
 721 lawrlydrqf rpsqrgavsl slhadwaepa npyadshwra aerflqfeia wfaeplfktg
```

REPLACEMENT SHEET

```
781 dypaamreyi askhrrglss salprlteae rrllkgtvdf calnhfttrf vmheqlagsr
841 ydsdrdiqfl qditrlsspt rlavipwgvr kllrwvrrny gdmdiyitas giddqaledd
901 rlrkyylgky lqevlkayli dkvrikgyya fklaeekskp rfgfftsdfk akssiqfynk
961 vissrgf
```

Beta-Klotho extracellular domain (without signal peptide) amino acid sequence (SEQ ID NO: 39)

```
 52                                                            gfsgdgrai
 61 wsknpnftpv nesqlflydt fpknffwgig tgalqvegsw kkdgkgpsiw dhfihthlkn
121 vsstngssds yiflekdlsa ldfigvsfyq fsiswprlfp dgivtvanak glqyystlld
181 alvlrniepi vtlyhwdlpl alqekyggwk ndtiidifnd yatycfqmfg drvkywitih
241 npylvawhgy gtgmhapgek gnlaavytvg hnlikahskv whnynthfrp hqkgwlsitl
301 gshwiepnrs entmdifkcq qsmvsvlgwf anpihgdgdy pegmrkklfs vlpifseaek
361 hemrgtadff afsfgpnnfk plntmakmgq nvslnlreal nwikleynnp riliaengwf
421 tdsrvktedt taiymmknfl sqvlqairld eirvfgytaw slldgfewqd aytirrglfy
481 vdfnskqker kpkssahyyk qiirengfsl kestpdvqgq fpcdfswgvt esvlkpesva
541 sspqfsdphl yvwnatgnrl lhrvegvrlk trpaqctdfv nikkqlemla rmkvthyrfa
601 ldwasvlptg nlsavnrqal ryyrcvvseg lklgisamvt lyypthahlg lpepllhadg
661 wlnpstaeaf qayaglcfqe lgdlvklwit inepnrlsdi ynrsgndtyg aahnllvaha
721 lawrlydrqf rpsqrgavsl slhadwaepa npyadshwra aerflqfeia wfaeplfktg
781 dypaamreyi askhrrglss salprlteae rrllkgtvdf calnhfttrf vmheqlagsr
841 ydsdrdiqfl qditrlsspt rlavipwgvr kllrwvrrny gdmdiyitas giddqaledd
901 rlrkyylgky lqevlkayli dkvrikgyya fklaeekskp rfgfftsdfk akssiqfynk
961 vissrgfpfe nsssrcsqtq entectvclf lvqkkpl
```

sKlotho without signal peptide – FGF23 amino acid sequence (without signal peptide) (SEQ ID NO: 40)

```
                                                    EPGDGAQ TWARFSRPPA
 51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SLGSGGGGSG GGGSGGGGSL
1001 KYPNASPLLG SSWGGLIHLY TATARNSYHL QIHKNGHVDG APHQTIYSAL
1051 MIRSEDAGFV VITGVMSRRY LCMDFRGNIF GSHYFDPENC RFQHQTLENG
1101 YDVYHSPQYH FLVSLGRAKR AFLPGMNPPP YSQFLSRRNE IPLIHFNTPI
1151 PRRHTRSAED DSERDPLNVL KPRARMTPAP ASCSQELPSA EDNSPMASDP
1201 LGVVRGGRVN THAGGTGPEG CRPFAKFI*
```

REPLACEMENT SHEET

sKlotho without signal peptide -FGF23 (R179Q) (without signal peptide) amino acid sequence (SEQ ID NO: 41)

```
                                                EPGDGAQ TWARFSRPPA
  51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
 101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
 151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
 201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
 251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
 301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
 351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
 401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
 451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
 501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
 551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
 601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
 651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
 701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
 751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
 801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
 851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
 901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
 951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SLGSGGGGSG GGGSGGGGSL
1001 KYPNASPLLG SSWGGLIHLY TATARNSYHL QIHKNGHVDG APHQTIYSAL
1051 MIRSEDAGFV VITGVMSRRY LCMDFRGNIF GSHYFDPENC RFQHQTLENG
1101 YDVYHSPQYH FLVSLGRAKR AFLPGMNPPP YSQFLSRRNE IPLIHFNTPI
1151 PRRHTQSAED DSERDPLNVL KPRARMTPAP ASCSQELPSA EDNSPMASDP
1201 LGVVRGGRVN THAGGTGPEG CRPFAKFI*
```

FGF23 without signal peptide (SEQ ID NO:42)

```
                                  YPNASP LLGSSWGGLI HLYTATARNS YHLQIHKNGH
  61 VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG NIFGSHYFDP ENCRFQHQTL
 121 ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN PPPYSQFLSR RNEIPLIHFN TPIPRRHTRS
 181 AEDDSERDPL NVLKPRARMT PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG
 241 PEGCRPFAKF I
```

FGF23(R179Q) without signal peptide (SEQ ID NO:43)

```
                                  YPNASP LLGSSWGGLI HLYTATARNS YHLQIHKNGH
  61 VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG NIFGSHYFDP ENCRFQHQTL
 121 ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN PPPYSQFLSR RNEIPLIHFN TPIPRRHTQS
 181 AEDDSERDPL NVLKPRARMT PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG
 241 PEGCRPFAKF I
```

REPLACEMENT SHEET

sKlotho with Klotho signal peptide (SEQ ID NO:44)

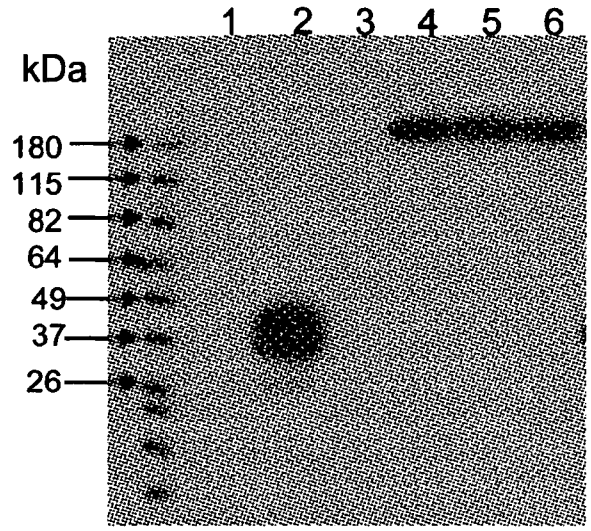
```
  1 MPASAPPRRP RPPPPSLSLL LVLLGLGGRR LRAEPGDGAQ TWARFSRPPA
 51 PEAAGLFQGT FPDGFLWAVG SAAYQTEGGW QQHGKGASIW DTFTHHPLAP
101 PGDSRNASLP LGAPSPLQPA TGDVASDSYN NVFRDTEALR ELGVTHYRFS
151 ISWARVLPNG SAGVPNREGL RYYRRLLERL RELGVQPVVT LYHWDLPQRL
201 QDAYGGWANR ALADHFRDYA ELCFRHFGGQ VKYWITIDNP YVVAWHGYAT
251 GRLAPGIRGS PRLGYLVAHN LLLAHAKVWH LYNTSFRPTQ GGQVSIALSS
301 HWINPRRMTD HSIKECQKSL DFVLGWFAKP VFIDGDYPES MKNNLSSILP
351 DFTESEKKFI KGTADFFALC FGPTLSFQLL DPHMKFRQLE SPNLRQLLSW
401 IDLEFNHPQI FIVENGWFVS GTTKRDDAKY MYYLKKFIME TLKAIKLDGV
451 DVIGYTAWSL MDGFEWHRGY SIRRGLFYVD FLSQDKMLLP KSSALFYQKL
501 IEKNGFPPLP ENQPLEGTFP CDFAWGVVDN YIQVDTTLSQ FTDLNVYLWD
551 VHHSKRLIKV DGVVTKKRKS YCVDFAAIQP QIALLQEMHV THFRFSLDWA
601 LILPLGNQSQ VNHTILQYYR CMASELVRVN ITPVVALWQP MAPNQGLPRL
651 LARQGAWENP YTALAFAEYA RLCFQELGHH VKLWITMNEP YTRNMTYSAG
701 HNLLKAHALA WHVYNEKFRH AQNGKISIAL QADWIEPACP FSQKDKEVAE
751 RVLEFDIGWL AEPIFGSGDY PWVMRDWLNQ RNNFLLPYFT EDEKKLIQGT
801 FDFLALSHYT TILVDSEKED PIKYNDYLEV QEMTDITWLN SPSQVAVVPW
851 GLRKVLNWLK FKYGDLPMYI ISNGIDDGLH AEDDQLRVYY MQNYINEALK
901 AHILDGINLC GYFAYSFNDR TAPRFGLYRY AADQFEPKAS MKHYRKIIDS
951 NGFPGPETLE RFCPEEFTVC TECSFFHTRK SL
```

sKlotho with IgG Signal peptide (SEQ ID NO:45)

```
  1 MSVLTQVLAL LLLWLTGLGG RRLRAEPGDG AQTWARFSRP PAPEAAGLFQ
 51 GTFPDGFLWA VGSAAYQTEG GWQQHGKGAS IWDTFTHHPL APPGDSRNAS
101 LPLGAPSPLQ PATGDVASDS YNNVFRDTEA LRELGVTHYR FSISWARVLP
151 NGSAGVPNRE GLRYYRRLLE RLRELGVQPV VTLYHWDLPQ RLQDAYGGWA
201 NRALADHFRD YAELCFRHFG GQVKYWITID NPYVVAWHGY ATGRLAPGIR
251 GSPRLGYLVA HNLLLAHAKV WHLYNTSFRP TQGGQVSIAL SSHWINPRRM
301 TDHSIKECQK SLDFVLGWFA KPVFIDGDYP ESMKNNLSSI LPDFTESEKK
351 FIKGTADFFA LCFGPTLSFQ LLDPHMKFRQ LESPNLRQLL SWIDLEFNHP
401 QIFIVENGWF VSGTTKRDDA KYMYYLKKFI METLKAIKLD GVDVIGYTAW
451 SLMDGFEWHR GYSIRRGLFY VDFLSQDKML LPKSSALFYQ KLIEKNGFPP
501 LPENQPLEGT FPCDFAWGVV DNYIQVDTTL SQFTDLNVYL WDVHHSKRLI
551 KVDGVVTKKR KSYCVDFAAI QPQIALLQEM HVTHFRFSLD WALILPLGNQ
601 SQVNHTILQY YRCMASELVR VNITPVVALW QPMAPNQGLP RLLARQGAWE
651 NPYTALAFAE YARLCFQELG HHVKLWITMN EPYTRNMTYS AGHNLLKAHA
701 LAWHVYNEKF RHAQNGKISI ALQADWIEPA CPFSQKDKEV AERVLEFDIG
751 WLAEPIFGSG DYPWVMRDWL NQRNNFLLPY FTEDEKKLIQ GTFDFLALSH
801 YTTILVDSEK EDPIKYNDYL EVQEMTDITW LNSPSQVAVV PWGLRKVLNW
851 LKFKYGDLPM YIISNGIDDG LHAEDDQLRV YYMQNYINEA LKAHILDGIN
901 LCGYFAYSFN DRTAPRFGLY RYAADQFEPK ASMKHYRKII DSNGFPGPET
951 LERFCPEEFT VCTECSFFHT RKSL*
```

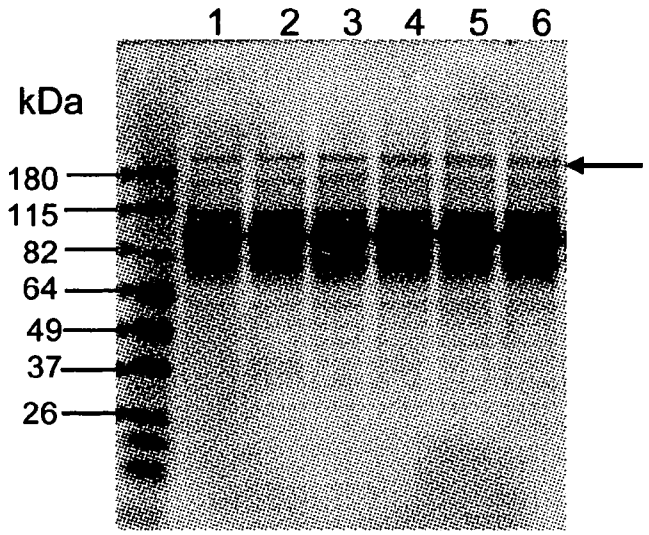
REPLACEMENT SHEET

Figure 3A



lane 1, Ctrl; lane 2, FGF23; lane 3, sKlotho; lanes 4-6, sKlotho-FGF23

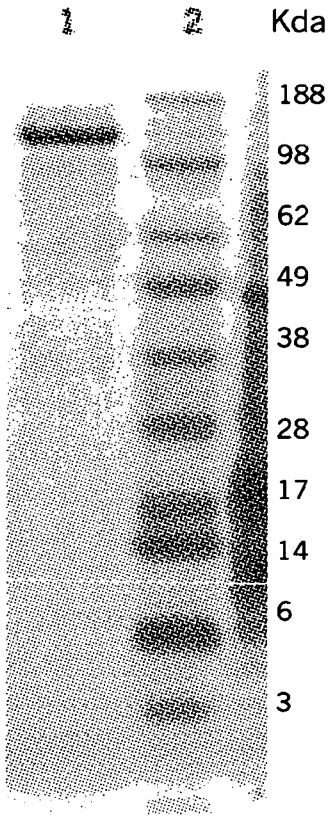
Figure 3B



lane 1, Ctrl; lane 2, FGF23; lane 3, sKlotho; lanes 4-6, sKlotho-FGF23

REPLACEMENT SHEET

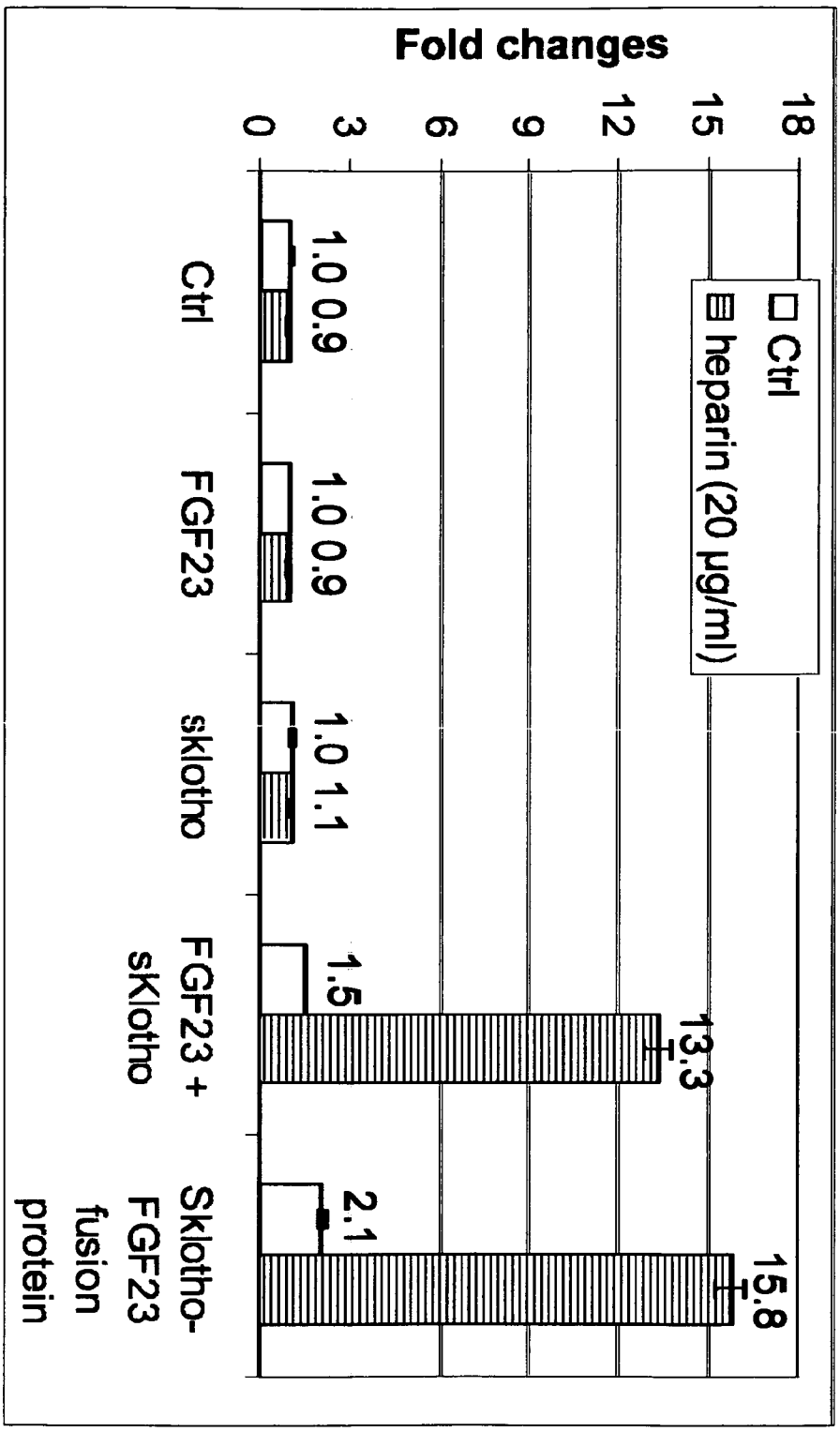
Figure 3C



lane 1, purified sKlotho-FGF23-6xHis;
lane 2, molecular weight marker

REPLACEMENT SHEET

Figure 4



REPLACEMENT SHEET

Figure 5A

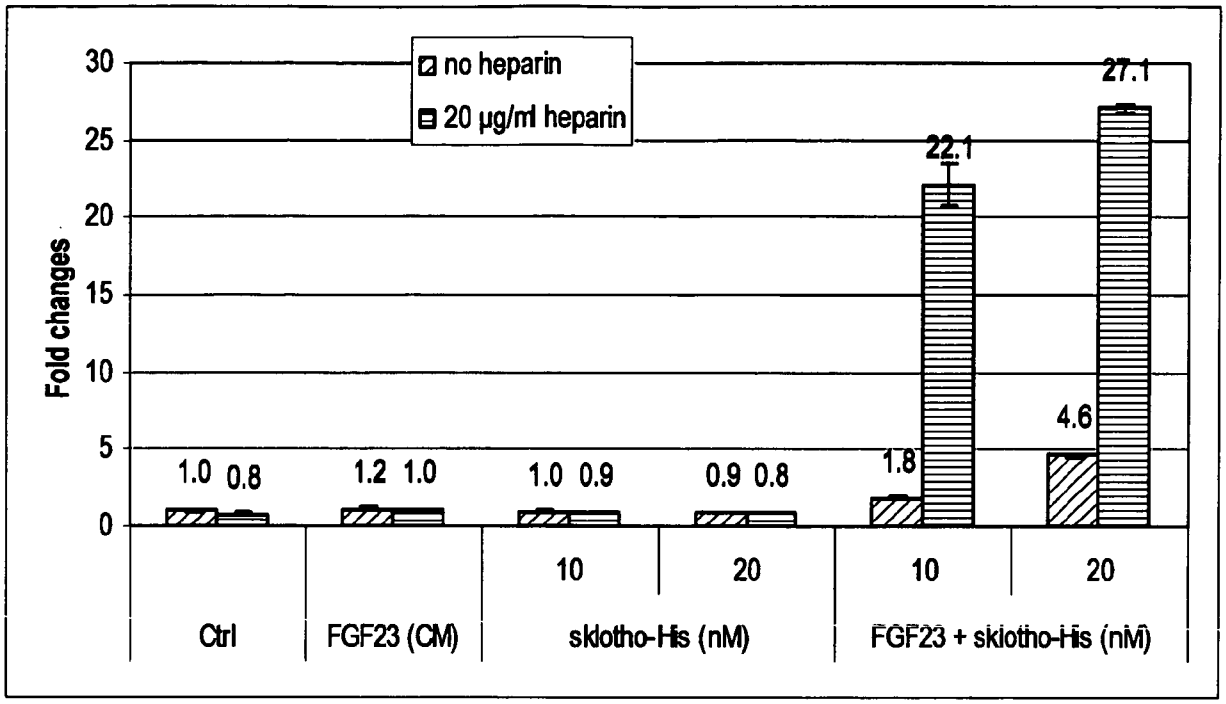
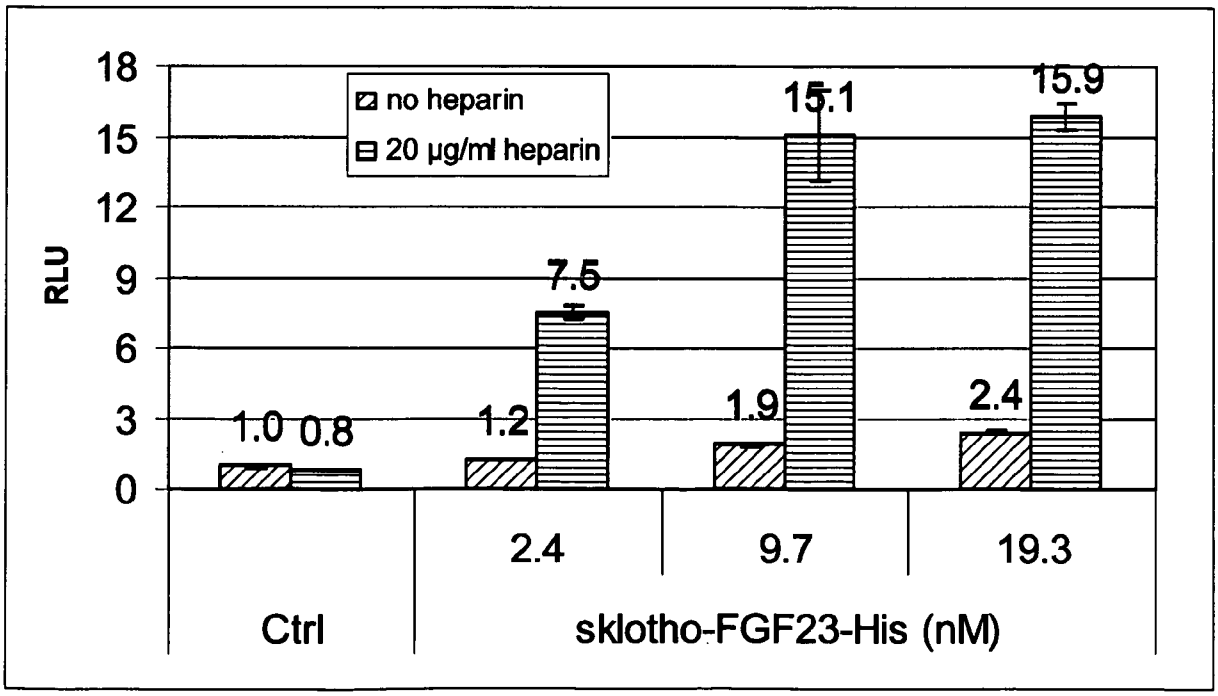
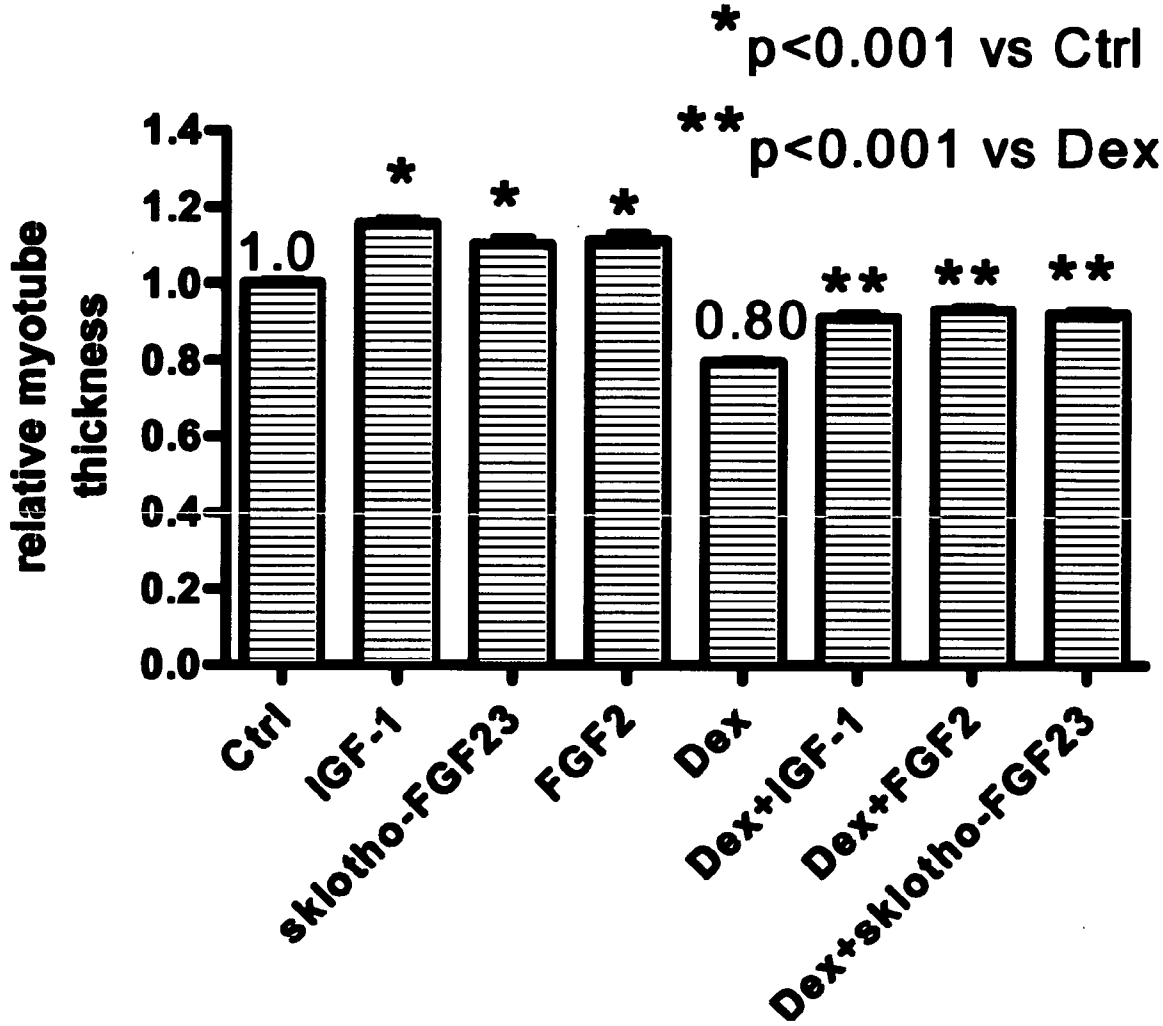


Figure 5B



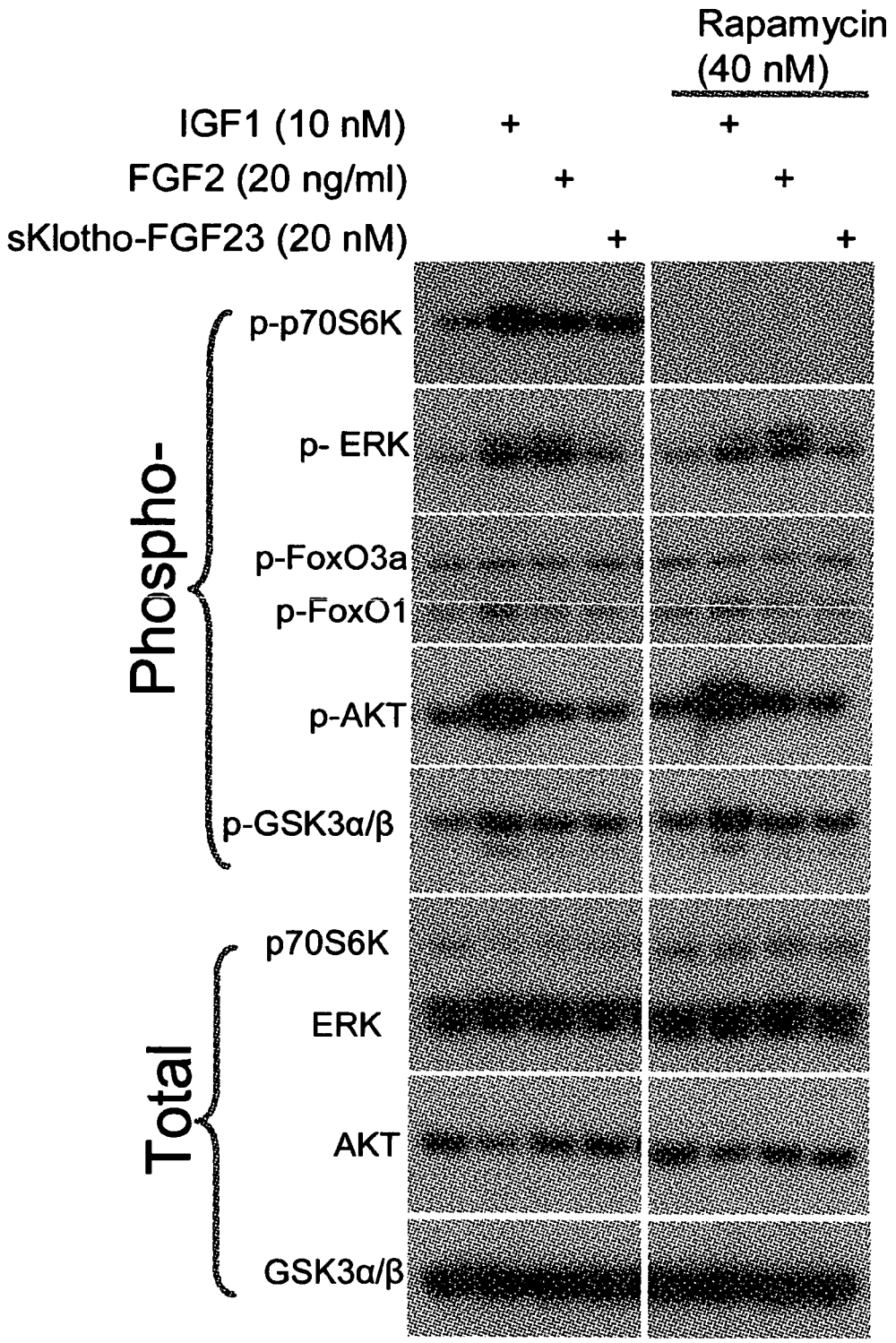
REPLACEMENT SHEET

Figure 6A



REPLACEMENT SHEET

Figure 6B



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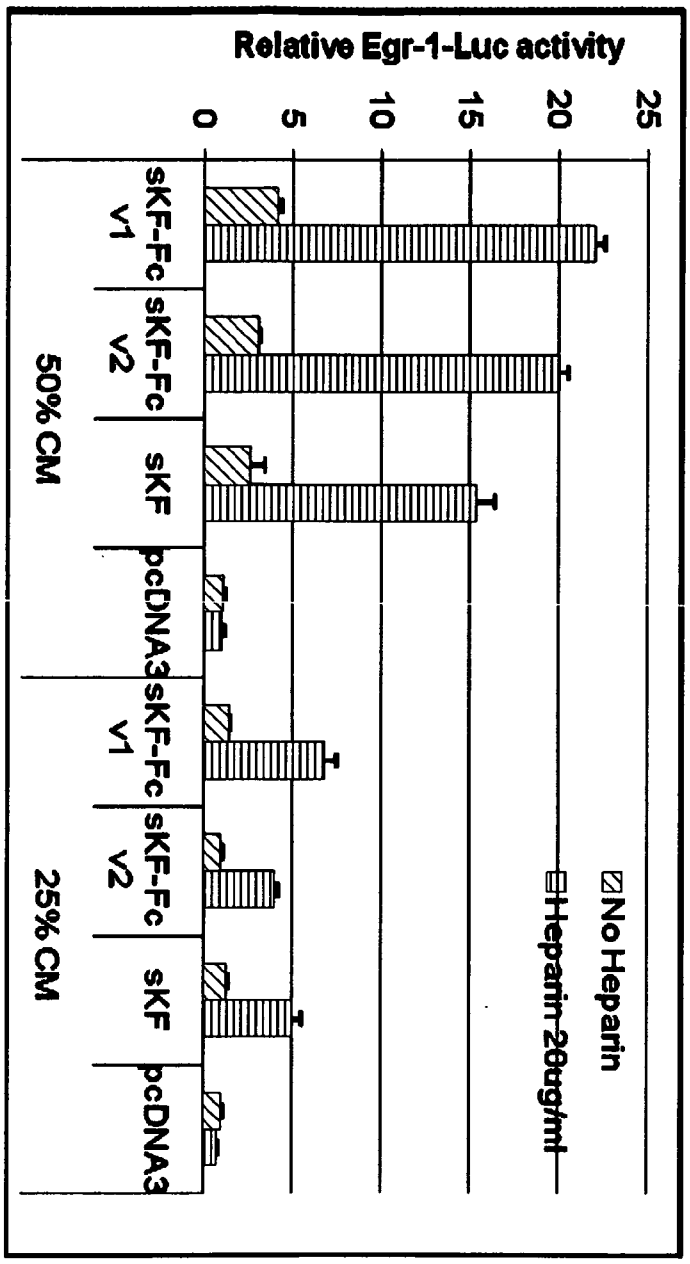


Figure 7

REPLACEMENT SHEET

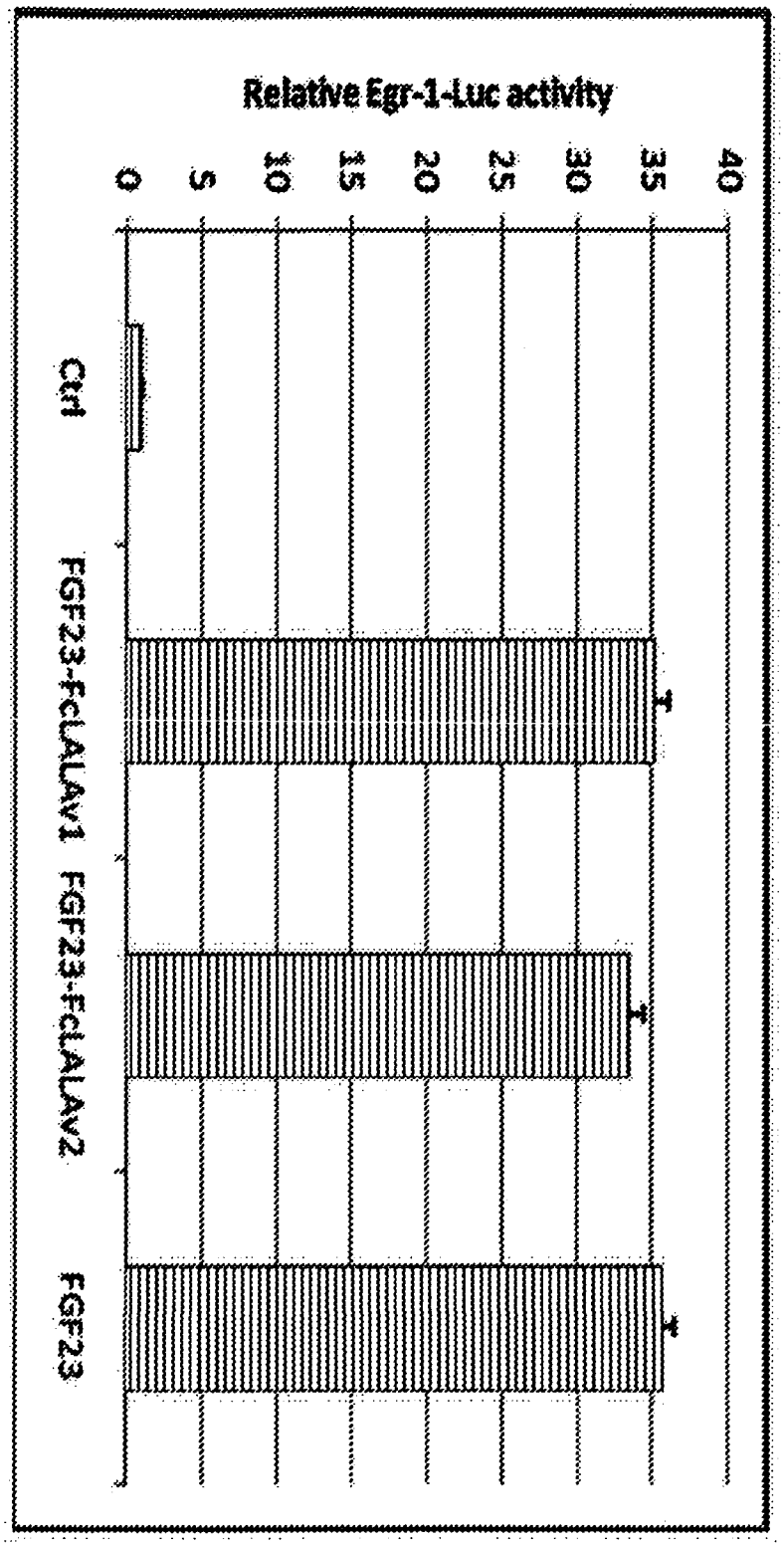
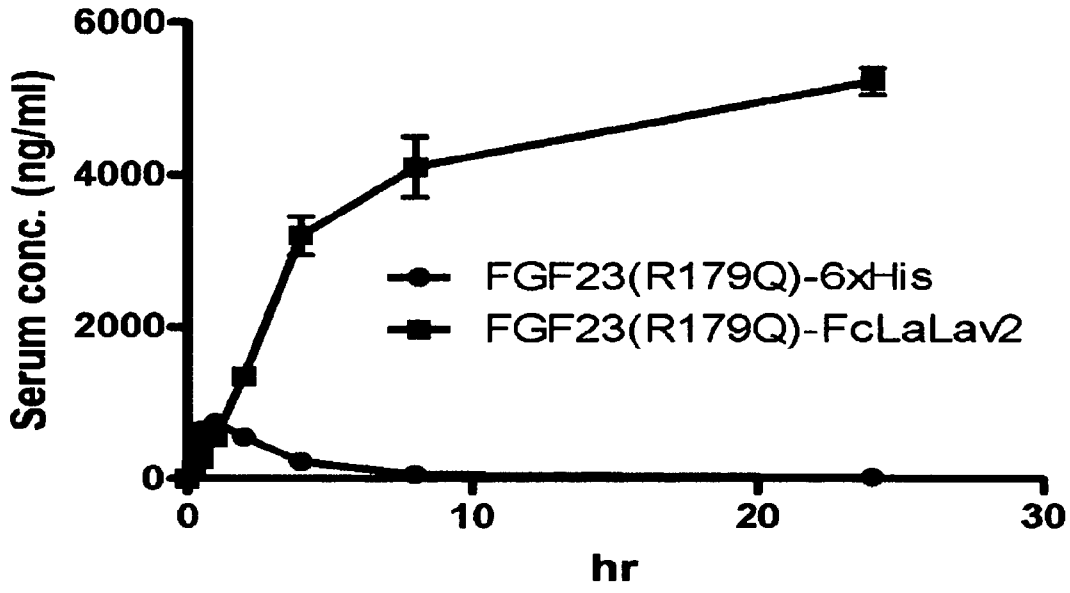


Figure 8

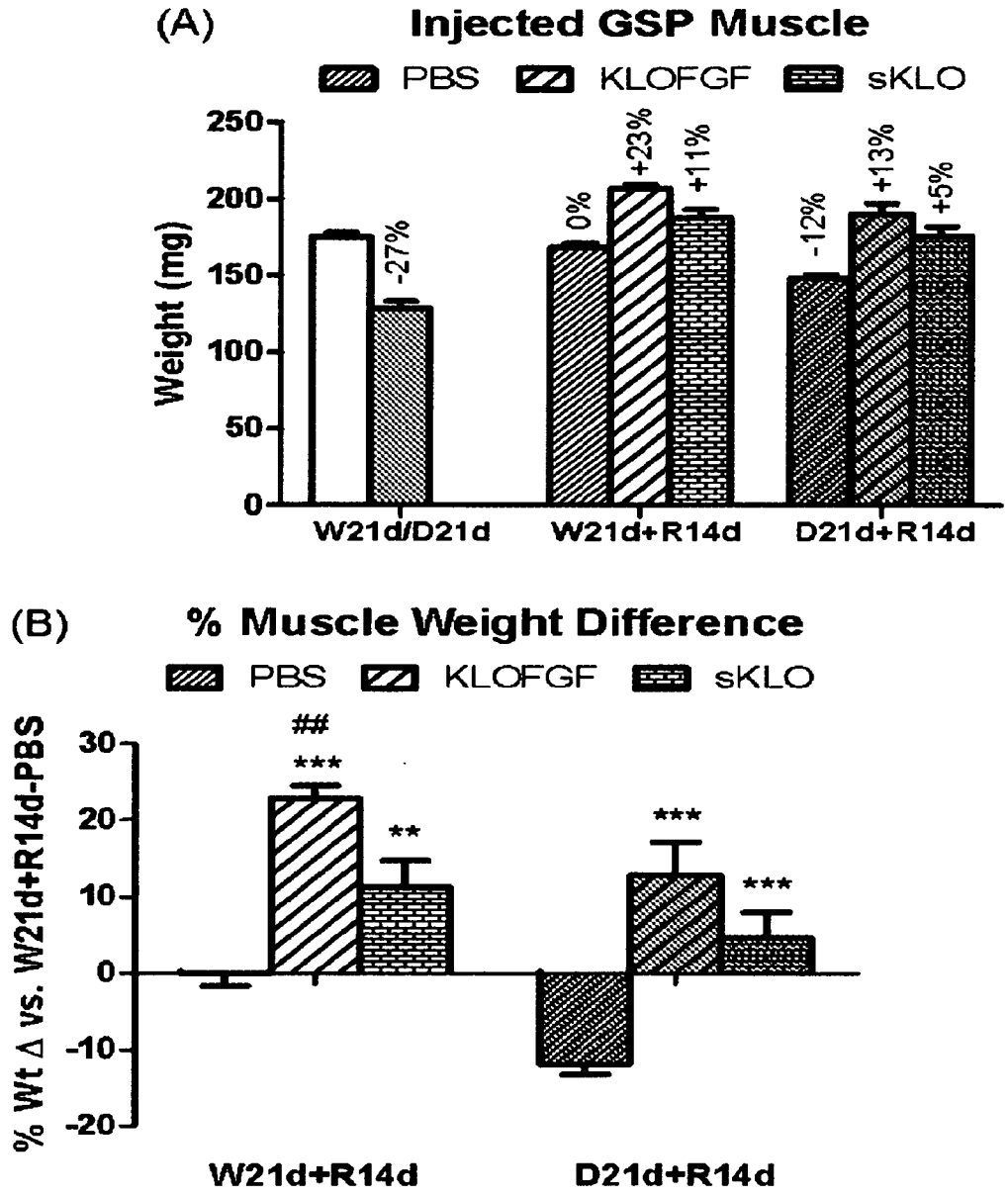
REPLACEMENT SHEET

Figure 9



REPLACEMENT SHEET

Figure 10



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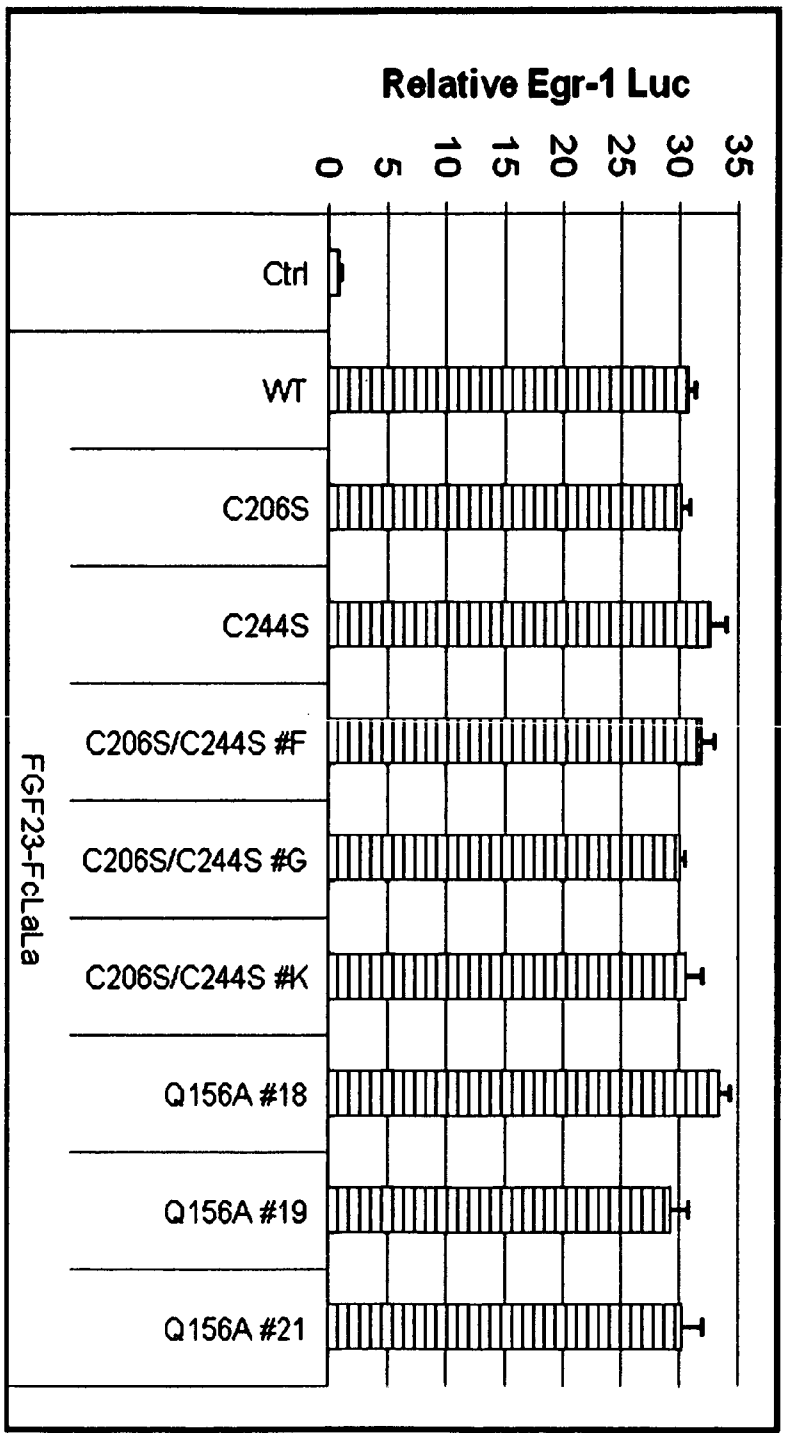
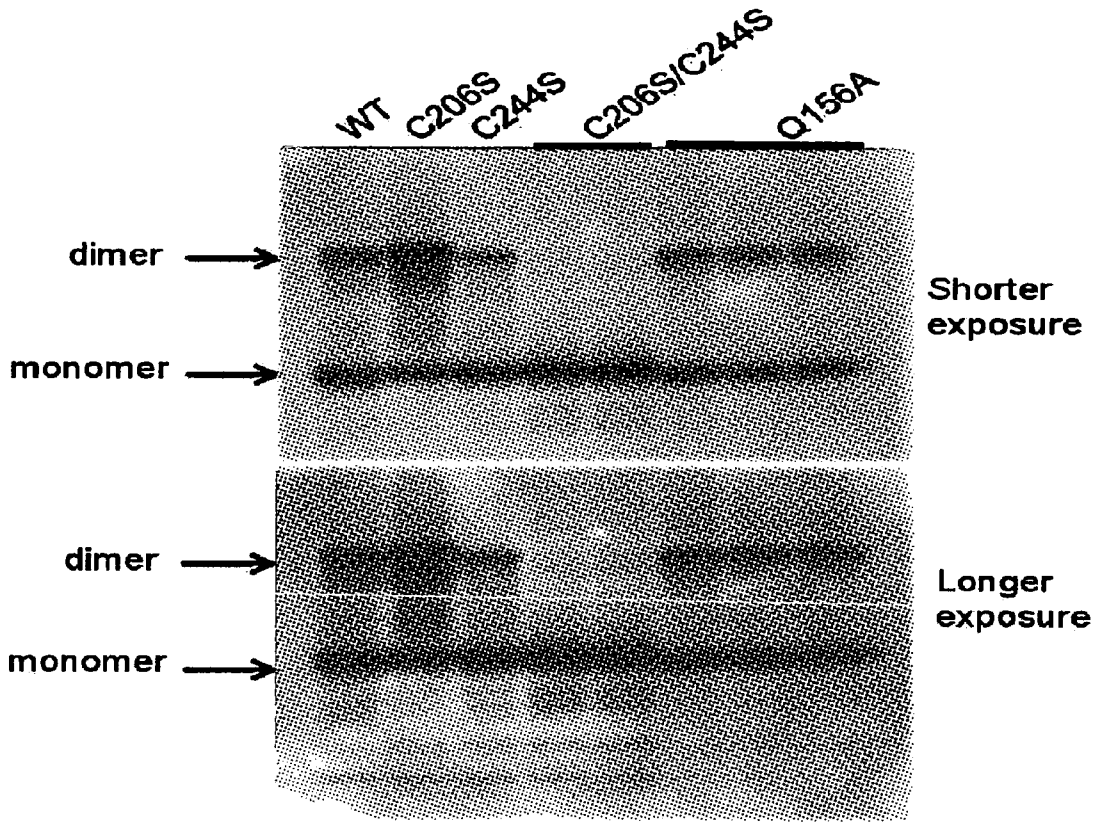


Figure 11

REPLACEMENT SHEET

Figure 12



INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2011/051112

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)

   [X] on paper

   [X] in electronic form

   b. (time)

   [X] in the international application as filed

   [X] 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/EP2011/051112

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/62 G01N33/574 G01N33/68
ADD.

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)
C12N G01N

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, EMBASE, INSPEC, WPI Data, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2009/095372 A1 (NOVARTIS AG [CH]; GLASS DAVID [US]; HU SHOU-IH [US]) 6 August 2009 (2009-08-06) the whole document In particular: Examples 1-3; Claims 1-62.	1-47
A	US 2006/160181 A1 (LUETHY ROLAND [US] ET AL) 20 July 2006 (2006-07-20) the whole document See in particular: paragraphs 125-143.	1-47
A	WO 2009/117622 A2 (AMBRX INC [US]; PINKSTAFF JASON [US]; HAYS PUTNAM ANNA-MARIA A [US]; E) 24 September 2009 (2009-09-24) the whole document See in particular: paragraphs 59 and 64.	1-47

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

[X] See patent family annex.

* Special categories of cited documents :

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"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
14 April 2011	28/04/2011

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	C.F. Angioni

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/051112

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 AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY INC. US, vol. 283, no. 48, 28 November 2008 (2008-11-28), pages 33304-33309, XP002632895, DOI: DOI:10.1074/JBC.M803319200 the whole document See in particular: Abstract; Experimental procedures section.	1-47

T	RAZZAQUE M S: "Therapeutic potential of klothoFGF23 fusion polypeptides: WO2009095372", EXPERT OPINION ON THERAPEUTIC PATENTS, INFORMA HEALTHCARE, GB, vol. 20, no. 7, 1 July 2010 (2010-07-01), pages 981-985, XP008135447, ISSN: 1354-3776 the whole document	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/051112

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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