

ENGINEERED ALPHA KLOTHO POLYPEPTIDES AND USES THEREOF

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. provisional application no. 63/597,872, filed November 10, 2023 and U.S. provisional application no. 63/657,247, filed June 7, 2024, the contents of each of which are incorporated herein in their entireties by reference thereto.

2. SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically and is hereby incorporated by reference in its entirety. Said copy, created on November 5, 2024, is named RGN-019WO_SL.xml and is 231,499 bytes in size.

3. BACKGROUND

[0003] Alpha-Klotho, often referred to as Klotho (KL), is a single pass transmembrane protein located at the plasma membrane and expressed primarily in the kidney, parathyroid gland, and choroid plexus. The intracellular region of human KL (hKL) is relatively short and lacks any functional domains, whereas the extra cellular region comprises two primary domains, KL1 and KL2, which have amino-acid sequence homology to family 1 glycosidases.

[0004] KL can be proteolytically cleaved to produce a 130 kDa soluble form of KL comprising the KL extracellular region and released into the circulation. Despite its sequence homology to glycosidases, KL extracellular region does not exhibit glycosidase enzymatic activity. Instead, the extracellular portion of KL functions as an FGF23 coreceptor through FGF receptors and is involved in the regulation of P_i and vitamin D metabolism, ion homeostasis, and oxidative stress.

[0005] KL has been associated with maintenance of kidney health. For instance, KL deficiency is implicated in human chronic kidney disease (Barker *et al.*, 2014, Nephrol Dial Transplant 30(2):223-233), which affects more than 700 million individuals worldwide and is associated with more than one million deaths globally. KL also displays protective effects against aging-related conditions, and circulating levels of soluble KL decrease with age (Kim *et al.*, 2015, J Lifestyle Med. 5(1):1-6).

[0006] Accordingly, there exists a need to develop KL polypeptides that exhibit effective bioactivity and can be produced efficiently at scale.

4. SUMMARY

[0007] The present disclosure relates to engineered alpha klotho polypeptides.

[0008] Typically, the engineered alpha klotho polypeptides of the disclosure are soluble, e.g., lack a transmembrane domain.

[0009] Engineered alpha klotho polypeptides of the present disclosure comprise an alpha klotho moiety. Exemplary alpha klotho moieties are described in Section 6.3.

[0010] In some embodiments, the engineered klotho polypeptides of the disclosure comprise a KL2 domain having an amino acid substitution at position 521 and a C-terminal tail region which does not comprise a cysteine at position 970, if present (numbering relative to human KL; SEQ ID NO:1). As described herein, these modifications produced a protein having surprisingly enhanced activity, production yield, and stability relative to human KL.

[0011] An engineered alpha klotho polypeptide may further comprise a stabilization moiety, optionally linked to the alpha klotho moiety via a linker. Exemplary stabilization moieties are described in Section 6.4. Exemplary linkers are described in Section 6.5.

[0012] Exemplary engineered alpha klotho polypeptides of the disclosure are described in Section 6.2 and numbered embodiments 1 to 181.

[0013] The disclosure further provides nucleic acids encoding the engineered alpha klotho polypeptides of the disclosure. The disclosure further provides host cells and cell lines engineered to express the nucleic acids and engineered alpha klotho polypeptides of the disclosure. The disclosure further provides methods of producing an engineered alpha klotho polypeptide of the disclosure. Exemplary nucleic acids, host cells, and cell lines, and methods of producing an engineered alpha klotho polypeptide are described in Section 6.6 and numbered embodiments 182 to 188.

[0014] The disclosure further provides pharmaceutical compositions comprising the engineered alpha klotho polypeptides of the disclosure. Exemplary pharmaceutical compositions are described in Section 6.7 and numbered embodiment 189.

[0015] Further provided herein are methods of using the engineered alpha klotho polypeptides and the pharmaceutical compositions of the disclosure, *e.g.*, for activating FGFR signaling, for treating age-related conditions, for treating kidney disease, or for supplementing endogenous alpha klotho protein. Exemplary methods are described in Section 6.8 and numbered embodiments 190 to 205.

5. BRIEF DESCRIPTION OF THE FIGURES

[0016] FIG. 1 is a schematic illustration of an alpha klotho extracellular region, which comprises, from N-terminus to C-terminus: an alpha klotho-1 (KL1) domain (AAs 34-506), an interdomain region (AAs 507-514), an alpha klotho-2 (KL2) domain (AAs 515-950), and a C-terminal tail region (950-981). Amino acid numbering shown is relative to full length human alpha klotho (Genbank BAA23382.1; SEQ ID NO:1 and UniProtKB Q9UEF7-1; SEQ ID NO:2).

[0017] FIGS. 2A-2J are schematic illustrations of exemplary engineered alpha klotho polypeptides.

[0018] FIG. 2A shows an engineered alpha klotho polypeptide chain comprising, in N- to C-terminal orientation, a KL1 domain, an inter-domain region, a KL2 domain having a C521S mutation, and a C-terminal tail region. In some embodiments, an engineered alpha klotho polypeptide of the disclosure, *e.g.*, as illustrated in FIG. 2A, is monomeric, consisting of only a single polypeptide chain, and may further comprise a signal sequence.

[0019] FIG. 2B shows an engineered alpha klotho polypeptide comprising, in N- to C-terminal orientation, a KL1 domain, an inter-domain region, a KL2 domain having a C521S mutation, a C-terminal tail region, an optional linker (L), and a stabilization moiety. In some embodiments, an engineered alpha klotho polypeptide of the disclosure, *e.g.*, as illustrated in FIG. 2B, is monomeric, consisting of only a single polypeptide chain, and may further comprise a signal sequence.

[0020] FIG. 2C shows an engineered alpha klotho polypeptide comprising, in N- to C-terminal orientation, a KL1 domain having F353V and C370S mutations, an inter-domain region, a KL2 domain having a C521S mutation, and a C-terminal tail region. In some embodiments, an engineered alpha klotho polypeptide of the disclosure, *e.g.*, as illustrated

in FIG. 2C, is monomeric, consisting of only a single polypeptide chain, and may further comprise a signal sequence.

[0021] FIG. 2D shows an engineered alpha klotho polypeptide comprising, in N- to C-terminal orientation, a KL1 domain having F353V and C370S mutations, an inter-domain region, a KL2 domain having a C521S mutation, a C-terminal tail region, an optional linker (L), and a stabilization moiety. In some embodiments, an engineered alpha klotho polypeptide of the disclosure, e.g., as illustrated in FIG. 2D, is monomeric, consisting of only a single polypeptide chain, and may further comprise a signal sequence.

[0022] FIG. 2E shows an engineered alpha klotho polypeptide comprising, in N- to C-terminal orientation, a KL1 domain having an F353V mutation, an inter-domain region, a KL2 domain having a C521S mutation, and a C-terminal tail region. In some embodiments, an engineered alpha klotho polypeptide of the disclosure, e.g., as illustrated in FIG. 2E, is monomeric, consisting of only a single polypeptide chain, and may further comprise a signal sequence.

[0023] FIG. 2F shows an engineered alpha klotho polypeptide comprising, in N- to C-terminal orientation, a KL1 domain having an F353V mutation, an inter-domain region, a KL2 domain having a C521S mutation, a C-terminal tail region, an optional linker (L), and a stabilization moiety. In some embodiments, an engineered alpha klotho polypeptide of the disclosure, e.g., as illustrated in FIG. 2F, is monomeric, consisting of only a single polypeptide chain, and may further comprise a signal sequence.

[0024] FIG. 2G shows an engineered alpha klotho polypeptide comprising, in N- to C-terminal orientation, a KL1 domain having a C370S mutation, an inter-domain region, a KL2 domain having a C521S mutation, and a C-terminal tail region. In some embodiments, an engineered alpha klotho polypeptide of the disclosure, e.g., as illustrated in FIG. 2G, is monomeric, consisting of only a single polypeptide chain, and may further comprise a signal sequence.

[0025] FIG. 2H shows an engineered alpha klotho polypeptide comprising, in N- to C-terminal orientation, a KL1 domain having a C370S mutation, an inter-domain region, a KL2 domain having a C521S mutation, a C-terminal tail region, an optional linker (L), and a stabilization moiety. In some embodiments, an engineered alpha klotho polypeptide of the

disclosure, e.g., as illustrated in FIG. 2H, is monomeric, consisting of only a single polypeptide chain, and may further comprise a signal sequence.

[0026] FIG. 2I shows an engineered alpha klotho polypeptide comprising, in N- to C-terminal orientation, a KL1 domain having an L111S mutation, an inter-domain region, a KL2 domain having a C521S mutation, and a C-terminal tail region. In some embodiments, an engineered alpha klotho polypeptide of the disclosure, e.g., as illustrated in FIG. 2I, is monomeric, consisting of only a single polypeptide chain, and may further comprise a signal sequence.

[0027] FIG. 2J shows an engineered alpha klotho polypeptide comprising, in N- to C-terminal orientation, a KL1 domain having an L111S mutation, an inter-domain region, a KL2 domain having a C521S mutation, a C-terminal tail region, an optional linker (L), and a stabilization moiety. In some embodiments, an engineered alpha klotho polypeptide of the disclosure, e.g., as illustrated in FIG. 2J, is monomeric, consisting of only a single polypeptide chain, and may further comprise a signal sequence.

[0028] For each of the constructs depicted in FIGS. 2A-2J, the C-terminal tail region may be a full-length C-terminal tail (optionally with one or more amino acid substitutions) or may be a truncated C-terminal tail region relative to human KL. In some embodiments, the C-terminal tail region of the constructs depicted in FIGS. 2A-2J comprises a C-terminal deletion of between 12 and 31 amino acids relative to human KL. All amino acid numbering is relative to full length human alpha klotho (Genbank BAA23382.1; SEQ ID NO:1 and UniProtKB Q9UEF7-1; SEQ ID NO:2).

[0029] FIG. 3 shows the amino acid sequence of full-length human klotho (UniProt KB Q9UEF7-1; SEQ ID NO:2). The first 33 amino acids underlined with the dotted line correspond to the native hKL signal sequence. Italicized amino acid residues correspond to the KL1 domain, bolded amino acid residues correspond to the KL2 domain, and the amino acid residues underlined with the solid line represent inter-domain region (the region located between the KL1 and KL2 domains). The amino acid residues presented inside the solid box show the C-terminal ADAM10/ADAM17 cleavage target sequence, while the dotted vertical line between the two bolded and underlined amino acid residues indicates the site of cleavage. The amino acid residues presented inside the dotted box correspond to the transmembrane region and the amino acid residues underlined with the double-lines

correspond to the intracellular region of hKL. The amino acids between the KL2 domain and the transmembrane region correspond to the C-terminal tail region.

[0030] FIGS. 4A-4C show the effects of the C-terminal truncation and surface Cys residue engineering on the production and activity of engineered KL polypeptide constructs. FIG. 4A shows a graph displaying the post-purification yield and aggregation of His-tagged constructs REGN14416 (KL981 His), REGN14225 (KL958 His), REGN 14226 (KL958 C521S His), and REGN14227 (KL958 C521S C910S His), where the bars represent the production yield, and the line plot illustrates the post-size exclusion chromatography (postSEC) percentage of high molecular weight species (HMW %), which is used as a measure of aggregation. FIG. 4B is a bar graph depicting the fold change in phosphorylated extracellular signal-regulated kinase (pERK) levels in NIH3T3 cells as a result of engineered KL polypeptide and FGF23 co-treatment, relative to engineered KL polypeptide-alone treatment. FIG. 4C is a bar graph depicting the fold change in pERK levels in NHDF cells as a result of engineered KL polypeptide and FGF23 co-treatment, relative to engineered KL polypeptide-alone treatment.

[0031] FIGS. 5A-5C illustrate the effect of pH on the production yield and activity of the His and/or HSA-tagged KL958 constructs. FIG. 5A shows non-reduced (NR) and reduced (R) polyacrylamide gel electrophoresis (PAGE) images depicting the yield and aggregation differences when His- or HSA-tagged constructs were eluted either with a His column at pH 7 or with an HSA column at pH 3. The presence of a smear above 169 kDa in the NR condition indicates aggregate formation. FIG. 5B is a bar graph showing the results of a pERK homogeneous time-resolved fluorescence-based (HTRF) assay, which depicts pERK-associated fluorescence in untreated cells as well as in cells that were treated with FGF23, a positive control human alpha klotho protein (h aKL), or FGF23 together with h aKL. FIG. 5C is a bar graph showing pERK HTRF in cells treated with the construct, KL958 SS HSA His, eluted either with a His column at pH 7 or with an HSA column at pH 3, in the presence or absence of FGF23. Background level corresponds to the pERK HTRF level of the untreated cells in FIG. 5B.

[0032] FIGS. 6A-6D are cartoon depictions of engineered KL polypeptide comprising different stabilization moieties. FIG. 6A is a cartoon illustration of an engineered KL polypeptide with a C-terminal HSA stabilization moiety. FIG. 6B is a cartoon illustration of an

engineered KL polypeptide dimer with a C-terminal Fc stabilization moiety. FIG. 6C is a cartoon illustration of an engineered KL polypeptide with a C-terminal Fc1.5 stabilization moiety. FIG. 6D is a cartoon illustration of an engineered KL polypeptide with a C-terminal monomeric Fc (moFc) stabilization moiety, where the moFc is a non-dimerizing Fc comprising one or more mutations on the hydrophobic surface of its CH3 region. Small ovals at the C-termini of the constructs shown in FIGS. 6A-6D represent optional additional tags (OATs) that can be used to facilitate purification of the constructs. Non-limiting examples of such additional tags include His tag (HHHHHH; SEQ ID NO:73) and Twin-Strep-tag® (WSHPQFEKGGGSGGGSGGSAWSHPQFEK; SEQ ID NO:74).

[0033] FIGS. 7A-7E illustrate the production yield and purity of four distinct constructs, each comprising a different C-terminal tag linked to REGN 14226 (KL958 C521S). FIG. 7A is a polyacrylamide gel electrophoresis image showing the yields of reduced and non-reduced constructs comprising Fc, moFc, Fc1.5, and HSA stabilization moieties, respectively. All constructs also comprised a Twin-Strep-tag. The scissor icon above a lane indicates that the sample in that lane was treated with 3C protease to remove the Twin-Strep-tag prior to loading. FIG. 7B shows the size exclusion chromatography (SEC) purification profile of the Fc-tagged KL958 C521S polypeptide construct, KL958(C521S)-Fc. FIG. 7C shows the size exclusion chromatography (SEC) purification profile of the moFc-tagged KL958 C521S polypeptide construct, KL958(C521S)-MoFc. FIG. 7D shows the size exclusion chromatography (SEC) purification profile of the Fc1.5-tagged KL958 C521S polypeptide construct, KL958(C521S)-Fc1.5. FIG. 7E shows the size exclusion chromatography (SEC) purification profile of the HSA-tagged KL958 C521S polypeptide construct, KL958(C521S)-HSA. Striped peaks represent the high molecular weight (HMW) proteins and aggregates. Checker-patterned peaks correspond to monomeric KL958 C521S polypeptide constructs.

[0034] FIGS. 8A-8B show the results of modified high salt purification of the KL polypeptide construct KL958(C521S)-HSA at neutral pH. FIG. 8A is a polyacrylamide gel electrophoresis image showing the yields of reduced and non-reduced KL958(C521S)-HSA. Lane 1 was loaded with a molecular weight marker. Lanes 2 and 5 were loaded with non-reduced and reduced samples comprising aggregate and HMW proteins, respectively. Lanes 3 and 6 were loaded with non-reduced and reduced SEC elutes comprising the monomeric KL958(C521S)-HSA constructs. FIG. 8B shows the SEC purification profile of

KL958(C521S)-HSA. Peak 1 corresponds to aggregated and HMW proteins, peak 2 corresponds to monomeric KL958 C521S polypeptide constructs, and peak 3 corresponds to low molecular weight peptides.

[0035] FIGS. 9A-9F show stepwise improvement of KL958(C521S)-HSA purification with a three-step modified ion exchange chromatography (IEX) purification method. FIGS. 9A-9B show a polyacrylamide gel electrophoresis image of different fractions of proteins (FIG. 9A) and an SEC profile of the elute (FIG. 9B) obtained with the Q-Sepharose ion exchange step. FIGS. 9C-9D show a polyacrylamide gel electrophoresis image of different fractions of proteins (FIG. 9C) and an SEC profile of the elute (FIG. 9D) obtained with the HSA-affinity column purification step. FIGS. 9E-9F show a polyacrylamide gel electrophoresis image of the final elute (FIG. 9E) and an SEC profile of the final elute (FIG. 9F) obtained with the SD-200 purification step.

[0036] FIGS. 10A-10B show the effect of His and HSA tags on pharmacokinetic (PK) profiles of engineered KL polypeptide constructs. FIG. 10A is a graph displaying the average serum concentrations of KL polypeptide constructs over time. FIG. 10B is a graph displaying the dose-normalized PK profiles of KL polypeptide constructs.

[0037] FIGS. 11A-11C show the effect of His and HSA tags on engineered KL polypeptide-mediated proliferation of cultured 3T3 cells. FIG. 11A is a graph showing dose-dependent proliferation of cells treated with KL958(C521S)-His in the presence or absence of FGF23. FIG. 11B is a graph showing dose-dependent proliferation of cells treated with KL958(C521S)-HSA in the presence or absence of FGF23. FIG. 11C is a graph showing the differences in growth of cells treated with KL958(C521S)-His and FGF23, KL958(C521S)-HSA and FGF23, bFGF, or FGF23 alone, and untreated cells.

[0038] FIG. 12 is a graph showing the effect of different C-terminal truncations on the activity of certain engineered KL polypeptide constructs.

[0039] FIGS. 13A-13F illustrate exemplary engineered alpha klotho polypeptide constructs with the N-terminus to C-terminus configuration: [mROR1]-[KL1 domain]-[KL2 domain]-[G4S linker]-[HSA domain]. The first 33 amino acids of human KL, which correspond to the native KL signal sequence, are replaced with mROR1 signal sequence, and KL2 domains are truncated at residue 958. FIG. 13A shows a polypeptide construct comprising a C521S mutation in its KL2 domain. FIG. 13B shows a polypeptide construct comprising F352V and

C370S mutations in its KL1 domain and a C521S mutation in its KL2 domain. FIG. 13C shows a polypeptide construct comprising an F353V mutation in its KL1 domain and a C521S mutation in its KL2 domain. FIG. 13D shows a polypeptide construct comprising an C370S mutation in its KL1 domain and a C521S mutation in its KL2 domain. FIG. 13E shows a polypeptide construct comprising an L111S mutation in its KL1 domain and a C521S mutation in its KL2 domain. FIG. 13F shows a polypeptide construct comprising a C521S mutation in its KL2 domain and a C34S mutation in its HSA domain.

6. DETAILED DESCRIPTION

6.1. Definitions

[0040] About, Approximately: The terms “about”, “approximately” and the like are used throughout the specification in front of a number to show that the number is not necessarily exact (e.g., to account for fractions, variations in measurement accuracy and/or precision, timing, etc.). It should be understood that a disclosure of “about X” or “approximately X” where X is a number is also a disclosure of “X.” Thus, for example, a disclosure of an embodiment in which one sequence has “about X% sequence identity” to another sequence is also a disclosure of an embodiment in which the sequence has “X% sequence identity” to the other sequence.

[0041] And, Or: Unless indicated otherwise, an “or” conjunction is intended to be used in its correct sense as a Boolean logical operator, encompassing both the selection of features in the alternative (A or B, where the selection of A is mutually exclusive from B) and the selection of features in conjunction (A or B, where both A and B are selected). In some places in the text, the term “and/or” is used for the same purpose, which shall not be construed to imply that “or” is used with reference to mutually exclusive alternatives.

[0042] EC50: The term “EC50” refers to the half maximal effective concentration of a molecule, such as a polypeptide of the disclosure, which induces a response halfway between the baseline and maximum after a specified exposure time. The EC50 essentially represents the concentration of a polypeptide where 50% of its maximal effect is observed. In certain embodiments, the EC50 value equals the concentration of a polypeptide that gives half-maximal activation in a luciferase reporter assay as described in Section 9.1.5.

[0043] Fc Domain and Fc Region: The term “Fc domain” refers to a portion of a heavy chain that pairs with the corresponding portion of another heavy chain on a separate polypeptide chain. In some embodiments an Fc domain comprises a CH2 domain followed by a CH3 domain, with or without a hinge region N-terminal to the CH2 domain. In some embodiments an Fc domain is non-dimerizing. Optionally, a non-dimerizing Fc domain further comprises, in addition to a CH2 domain followed by a CH3 domain, an additional CH3 domain connected to the CH3 domain via a linker (e.g., an “Fc1.5 domain”). In some embodiments, the Fc domain comprises a CH3 domain incapable of dimerizing with another Fc domain (e.g., a “monomeric Fc”). The term “Fc region” refers to the region formed by association of two heavy chain Fc domains on separate polypeptide chains. The two Fc domains within the Fc region may be the same or different from one another.

[0044] Fibroblast Growth Factor Receptor or FGFR: The terms “fibroblast growth factor receptor” and “FGFR” as used herein refer to any one of FGFRs 1-4 from any vertebrate source, including mammals such as primates (e.g., humans, cynomolgus monkey (cyno)), dogs, and rodents (e.g., mice and rats), unless otherwise indicated, and includes naturally occurring variants of FGFR, e.g., splice variants or allelic variants (e.g., FGFR1c).

[0045] Host Cell: The term “host cell” as used herein refers to cells into which a nucleic acid of the disclosure has been introduced. The terms “host cell” and “recombinant host cell” are used interchangeably herein. It is understood that such terms refer to the particular subject cell and to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. Typical host cells are eukaryotic host cells, such as mammalian host cells. Exemplary eukaryotic host cells include yeast and mammalian cells, for example vertebrate cells such as a mouse, rat, monkey or human cell line, for example HKB11 cells, PER.C6 cells, HEK cells or CHO cells.

[0046] KL1 Domain or Alpha Klotho KL1 Domain: The terms “KL1 domain” and “alpha klotho KL1 domain,” as used herein, refer to an amino acid sequence corresponding to the KL1 domain of an alpha klotho protein (e.g., human alpha klotho or murine alpha klotho), as well as derivatives and variants thereof. Accordingly, a KL1 domain may be the amino acid sequence of a KL1 domain of an alpha klotho protein (e.g., human alpha klotho or murine

alpha klotho) or a sequence having one, two, three, four, five, or more amino acid substitutions relative to the wild-type sequence. For example, in some embodiments, a KL1 domain of an alpha klotho moiety has L111S, F352V, and/or C370S substitutions, numbering relative to human alpha klotho (GenBank accession number BAA23382.1 - SEQ ID NO:1). In some embodiments, a KL1 domain is an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5% or 100% sequence identity to SEQ ID NO:9 or SEQ ID NO:10. In some embodiments, a KL1 domain is an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% to SEQ ID NO:9 or SEQ ID NO:10 and one, two or all three substitutions L111S, F352V, and/or C370S (*e.g.*, L111S only, F352V only, C370S only, or both F352V and C370S).

[0047] KL2 Domain or Alpha Klotho KL2 Domain: The terms “KL2 domain” and “alpha klotho KL2 domain,” as used herein, refer to an amino acid sequence corresponding to the KL2 domain of an alpha klotho protein (*e.g.*, human alpha klotho or murine alpha klotho), as well as derivatives and variants thereof. Accordingly, a KL2 domain may be the amino acid sequence of a KL2 domain of an alpha klotho protein (*e.g.*, human alpha klotho or murine alpha klotho) or a sequence having one, two, three, four, five, or more amino acid substitutions relative to the wild-type sequence. For example, in some embodiments, a KL2 domain of an alpha klotho moiety has a C521S mutation, numbering relative to human alpha klotho (GenBank accession number BAA23382.1; SEQ ID NO:1). In some embodiments, a KL2 domain is an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity to SEQ ID NO:3 or SEQ ID NO:4. In particular embodiments, a KL2 domain is an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% sequence identity to SEQ ID NO:3 and comprising a C521S substitution, numbering relative to human alpha klotho (GenBank accession number BAA23382.1). In some embodiments, a KL2 domain is the amino acid sequence of SEQ ID NO:4.

[0048] Klotho Moiety, Alpha Klotho Moiety, or KL Moiety: The terms “klotho moiety”, “alpha klotho moiety” and “KL moiety,” as used herein, refer to an amino acid sequence comprising at least one KL1 domain and/or at least one KL2 domain. In particular embodiments, an alpha klotho moiety comprises, in N- to C-terminal orientation, a KL1 domain and a KL2 domain. In some embodiments, the alpha klotho moiety further comprises an interdomain region between the KL1 domain and KL2 domain. In some embodiments, the interdomain region comprises the amino acid sequence of SEQ ID NO:21 or a variant thereof having 1, 2, 3, 4, or 5 amino acid substitutions relative to SEQ ID NO:21. In some embodiments, the alpha klotho moiety further comprises, at its C-terminus, the C-terminal tail of the KL extracellular domain or a portion thereof. In some embodiments, the C-terminal tail comprises amino acids 1-31, 1-30, 1-29, 1-28, 1-27, 1-26, 1-25, 1-24, 1-23, 1-22, 1-21, 1-20, 1-19, 1-18, 1-17, 1-16, 1-15, 1-14, 1-13, 1-12, 1-11, 1-10, 1-9, or 1-8 of SEQ ID NO:7, or a variant thereof having 1, 2, 3, 4, or 5 amino acid substitutions relative to amino acids 1-31, 1-30, 1-29, 1-28, 1-27, 1-26, 1-25, 1-24, 1-23, 1-22, 1-21, 1-20, 1-19, 1-18, 1-17, 1-16, 1-15, 1-14, 1-13, 1-12, 1-11, 1-10, 1-9, or 1-8 of SEQ ID NO:7. In particular embodiments, the alpha klotho moiety comprises, in N- to C-terminal orientation: (i) a KL1 domain; (ii) an interdomain region; (iii) a KL2 domain; and (iv) a C-terminal tail region.

[0049] In some embodiments, the alpha klotho moiety comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.8% sequence identity to SEQ ID NO:13. In some embodiments, the alpha klotho moiety comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% sequence identity to SEQ ID NO:14. In some embodiments, the alpha klotho moiety comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.8% sequence identity to SEQ ID NO:15. In some embodiments, the alpha klotho moiety comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% sequence identity to SEQ ID NO:16.

[0050] Linker: The term “linker” as used herein refers to a connecting peptide between two moieties. For example, a linker can connect an alpha klotho moiety to a stabilization domain.

[0051] Operably Linked: The term “operably linked” as used herein refers to a functional relationship between two or more regions of a polypeptide chain in which the two or more regions are linked so as to produce a functional polypeptide, or two or more nucleic acid sequences, *e.g.*, to produce an in-frame fusion of two polypeptide components or to link a regulatory sequence to a coding sequence.

[0052] Polypeptide, Peptide, and Protein: The terms “polypeptide”, “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues.

[0053] Subject: The term “subject” includes human and non-human animals. Non-human animals include all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dogs, cows, chickens, amphibians, and reptiles. Except when noted, the terms “patient” or “subject” are used herein interchangeably.

[0054] Treat, Treatment, Treating: As used herein, the terms “treat”, “treatment” and “treating” refer to the reduction or amelioration of the progression, severity and/or duration of a disorder as described herein, the amelioration of one or more symptoms (preferably, one or more discernible symptoms) of a condition or disorder as described herein, or prevention of a condition or disorder as described herein, *e.g.*, kidney disease or an age-related condition or disorder, resulting from the administration of a molecule or composition (*e.g.*, one or more alpha klotho polypeptides of the disclosure). In specific embodiments, the terms “treat”, “treatment” and “treating” refer to the amelioration of at least one measurable physical parameter of a disorder, *e.g.*, kidney disease or an age-related disorder, not necessarily discernible by the patient. In other embodiments the terms “treat”, “treatment” and “treating” refer to the inhibition of the progression or onset of a disorder, either physically by, *e.g.*, stabilization of a discernible symptom, physiologically by, *e.g.*, stabilization of a physical parameter, or both.

6.2. Soluble Alpha Klotho Polypeptides

[0055] The present disclosure relates to engineered alpha klotho polypeptides (also “engineered KL polypeptides”) comprising an alpha klotho moiety. Typically, the alpha klotho moiety has a KL2 domain having an amino acid substitution at the position corresponding to

amino acid C521 of full-length human alpha klotho (SEQ ID NO:1) and also lacks a cysteine at the position corresponding to amino acid C970 of full-length human alpha klotho (SEQ ID NO:1), if present.

[0056] Amino acid residues C521 and C970 of full-length human alpha klotho (SEQ ID NO:1), without being bound by theory, are understood to be free cysteines which are not paired via a disulfide bond to any other cysteine in the natural protein. Although there are many other free cysteine residues in the alpha klotho protein, it was surprisingly discovered that modification of both of these cysteine residues particularly (e.g., by substitution of C521 and by deletion of a portion the alpha klotho protein comprising C970) results in an alpha klotho protein having significantly enhanced activity, production yield, and stability relative to wild-type. Accordingly, polypeptides of the present disclosure generally include an alpha klotho moiety comprising 1) a KL2 domain having an amino acid substitution at position 521, and 2) a C-terminal tail region which does not comprise a cysteine at position 970 (if present) or which comprises a C-terminal deletion of at least 12 amino acids such that position 970 is absent. Exemplary alpha klotho moieties are described in Section 6.3.

[0057] Soluble alpha klotho polypeptides of the present disclosure may, in some embodiments, further comprise a stabilization moiety, optionally linked to the alpha klotho moiety (e.g., N-terminally or C-terminally) via a linker. Exemplary stabilization moieties are described in Section 6.4, and include Fc domains (as described in Section 6.4.1), albumin moieties (as described in Section 6.4.2) and other stabilization moieties (e.g., as described in Section 6.4.3). Exemplary linkers are described in Section 6.5.

[0058] Exemplary engineered alpha klotho polypeptides are illustrated in FIGS. 2A-2J. Amino acid sequences of example engineered alpha klotho polypeptides are provided as SEQ ID NOs:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 63. Sequences of example nucleic acids encoding engineered alpha klotho polypeptides are provided as SEQ ID NOs:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, and 61.

6.3. Alpha Klotho Moieties

[0059] Naturally occurring alpha klotho (KL) is encoded by the *α -klotho* gene located on human chromosome 13. The product of the *α -klotho* gene is a single-pass transmembrane protein comprising, from N- to C-terminal direction, an extracellular region, a transmembrane region, and a cytoplasmic region. The extracellular region of KL has two domains

(sometimes "subdomains"), termed KL1 and KL2. In addition, an interdomain amino acid sequence is located between the KL1 and KL2 domains and a C-terminal tail region is located C-terminal to the KL2 domain.

[0060] An amino acid sequence of human KL (GenBank accession number BAA23382.1) is reproduced below with the signal sequence italicized, the KL1 domain underlined with a straight line, the KL2 domain in bold, and the transmembrane region underlined with a dotted line.

MPASAPRRRPRPPPSLSLLLVLLGLGGRRLRAEPGDGAQTWARVSRPPAPEAAGLF
QGTFPDGLFWAVGSAAYQTEGGWQQHGKGASIWDTFTHHPLAPPGDSRNASLPLGAP
SPLQPATGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLR
YYRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHF
GQVKYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLYNTS
FRPTQGGQVSIALSSHWINPRMTDHSIKECQKSLDFVLGWFAKPVFIDGDYPESMK
NNLSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLESPNLRQLLS
WIDLEFNHPQIFIVENGWVSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYT
AWSLMDGFEWHRGYSIRRGLEYVDFLSQDKMLLPKSSALFYQKLEKNGFPPLPENQ
PLEGTFPCDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKRLIKVDGVVTKKRKS
YCVDFAAIQPQIALQEMHVTHFRFSLDWALILPLGNQSQVNHTILQYYRCMASELV
RVNITPVVALWQPMAPNQGLPRLARQGAWENPYTALAFAEYARLCFQELGHHVKLW
ITMNEPYTRNMTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPF
SQKDKEVAERVLEFDIGWLAEPFIGSGDYPWVMRDWLNQRNNFLLPYFTEDEKKLIQ
GTFDLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNPSQVAVVPWGLRKV
LNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVYMQNYINEALKAHILDGINLCGY
FAYSFNDRTPRFGLYRYAADQFEPKASKHYRKIIDSNGFPGPETLERFCPEEFTV
CTECSFFHTRKSLLAFLAFLFFASIIISLSLIFYYSKKGRRSYK (SEQ ID NO:
 1)

[0061] An alternative amino acid sequence of human KL (UniProtKB accession number Q9UEF7-1), having a single amino acid substitution relative to SEQ ID NO:1, is reproduced below with the signal sequence italicized, the KL1 domain underlined with a straight line, the KL2 domain in bold, and the transmembrane region underlined with a dotted line.

MPASAPRRRPRPPPSLSLLLVLLGLGGRRLRAEPGDGAQTWARFSRPPAPEAAGLF
QGTFPDGLFWAVGSAAYQTEGGWQQHGKGASIWDTFTHHPLAPPGDSRNASLPLGAP
SPLQPATGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLR
YYRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHF
GQVKYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLYNTS
FRPTQGGQVSIALSSHWINPRMTDHSIKECQKSLDFVLGWFAKPVFIDGDYPESMK
NNLSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLESPNLRQLLS
WIDLEFNHPQIFIVENGWVSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYT
AWSLMDGFEWHRGYSIRRGLEYVDFLSQDKMLLPKSSALFYQKLEKNGFPPLPENQ
PLEGTFPCDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKRLIKVDGVVTKKRKS
YCVDFAAIQPQIALQEMHVTHFRFSLDWALILPLGNQSQVNHTILQYYRCMASELV

RVNITPVVALWQPMAPNQGLPRLLARQGAWENPYTALAFAEYARLCFQELGHHVKLW
 ITMNEPYTRNMTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPF
 SQKDKEVAERVLEFDIGWLAEPFIGSGDYPWVRDNLNQRNNFLLPYFTEDEKKLIQ
 GTFDFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNSPSQVAVVPWGLRKV
 LNWLKFKYGDLPYIIISNGIDDGLHAEDDQLRVYYMQNYINEALKAHILDGINLCGY
 FAYSFNDRTPRFGLYRYAADQFEPKASMKHYRKIIDSNGFPGPETLERFCPEEFTV
 CTECSFFHTRKSLLAFLFFASIIISLSLIFYYSKKGRRSYK (SEQ ID NO:
 2)

[0062] The extracellular region of human KL (FIG. 1) consists of amino acids 34 through 981 and, as discussed above, includes a KL1 domain (amino acids 34-506; SEQ ID NO:9), an interdomain region (amino acids 507-514; SEQ ID NO:21), a KL2 domain (amino acids 515-950; SEQ ID NO:3), and a C-terminal tail region (amino acids 951-981; SEQ ID NO:7). An alpha klotho moiety, as discussed herein in the context of an engineered alpha klotho polypeptide, comprises at least a portion of the extracellular region of human KL or a variant or derivative thereof.

[0063] In certain aspects, the alpha klotho moiety comprises an alpha klotho KL2 domain. In some embodiments, the alpha klotho KL2 domain has at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or at least about 99.8% sequence identity to the KL2 domain of human KL (SEQ ID NO:3). The alpha klotho KL2 domain may comprise 1, 2, 3, 4, 5, or more amino acid mutations (*e.g.*, deletions, additions, or substitutions) relative to the KL2 domain of human KL. In some embodiments, the alpha klotho KL2 domain has an amino acid substitution at the position corresponding to C521 of full-length human KL (SEQ ID NO:1). In some embodiments, this amino acid substitution is a cysteine to serine substitution. In other embodiments, this amino acid substitution is a substitution of cysteine with a different amino acid that is not a serine.

[0064] In some embodiments, the alpha klotho moiety lacks a cysteine at the position corresponding to C970 of full-length human KL (SEQ ID NO:1), if present. Accordingly, in some aspects, the alpha klotho moiety comprises a position corresponding to C970 of full-length human KL (SEQ ID NO:1), where the position is not a cysteine. For example, the alpha klotho moiety may comprise an amino acid substitution at the position corresponding

to C970, which substitution may be, for example, a cysteine to serine substitution. Alternatively, in some aspects, the alpha klotho moiety lacks a position corresponding to C970 of full-length human KL (SEQ ID NO:1) entirely. For example, the alpha klotho moiety may comprise only a portion of the C-terminal tail region of the extracellular region of human KL (SEQ ID NO:7), which portion lacks the position corresponding to C970 of full-length human KL. Thus, in some embodiments, the alpha klotho moiety lacks the C-terminal 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids of the C-terminal tail of full-length human KL (SEQ ID NO:7). In some embodiments, the alpha klotho moiety lacks the C-terminal 23 amino acids of the C-terminal tail of full-length human KL (SEQ ID NO:7). Without being bound by theory, it is believed that an alpha klotho moiety having both a C521 mutation (e.g., C521S) and lacking a cysteine at position 970 (or lacking position 970 entirely), in the context of an engineered alpha klotho polypeptide, provides for a significant increase in both production yield and bioactivity compared with an alpha klotho moiety comprising a wild type human KL2 domain and C-terminal tail region. Accordingly, in some embodiments, the alpha klotho moiety (1) comprises a KL2 domain having a C521S mutation (numbering relative to SEQ ID NO:1) and (2) lacks the C-terminal 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids of the C-terminal tail of full-length human KL (SEQ ID NO:7). In some embodiments, the alpha klotho moiety (1) comprises a KL2 domain having a C521S mutation (numbering relative to SEQ ID NO:1) and (2) lacks an amino acid sequence having 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:8.

[0065] In certain aspects, the alpha klotho moiety further comprises an amino acid sequence having at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% sequence identity to the KL1 domain of human KL (SEQ ID NO:9 or SEQ ID NO:10). The alpha klotho KL1 domain may comprise 1, 2, 3, 4, 5, or more amino acid mutations (e.g., deletions, additions, or substitutions) relative to the KL1 domain of human KL. In some embodiments, the alpha klotho KL1 domain has an amino acid substitution at the position corresponding to L111 of full-length human KL (SEQ ID NO:1). In some embodiments, this amino acid substitution is a

leucine to serine substitution. In some embodiments, the alpha klotho KL1 domain has an amino acid substitution at the position corresponding to F352 of full-length human KL (SEQ ID NO:1). In some embodiments, this amino acid substitution is a phenylalanine to valine substitution. In some embodiments, the alpha klotho KL1 domain has an amino acid substitution at the position corresponding to C370 of full-length human KL (SEQ ID NO:1). In some embodiments, this amino acid substitution is a cysteine to serine substitution.

[0066] Sequences of certain example alpha klotho moieties of the present disclosure are provided in Table 1, below. In some embodiments, the alpha klotho moiety has at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5% sequence identity, or 100% sequence identity to a sequence of Table 1.

Table 1 – Example Alpha Klotho Moieties		
Name	Sequence	SEQ ID NO:
hKL ECD (BAA23382.1)	EPGDGAQTWARVSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQTEGG WQQHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPATGDVAS DSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYY RRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDY AELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLV AHNLLLAHAKVWHLYNTSFRPTQGGQVSIALSSHWINPRRMTDHSIKE CQKSLDFVLGWFAKPVFIDGDYPESMKNNLSSILPDFTESEKKFIKGT DFFALCFGPTLSFQLLDPHMKFRQLESPNLRQLLSWIDLEFNHPQIFIV ENGWVFSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYTAWSLM DGFVWHRGYSIRRGFLFYVDFLSQDKMLLPKSSALFYQKLIKNGFPPL PENQPLEGTFPCDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKR LIKVDGVVTKKRKSYCVDFAAIQPQIALLQEMHVTHFRFSLDWALILPLG NQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPRLLAR QGAWENPYTALAFAEYARLCFQELGHHVKLWITMNEPYTRNMTYSAG HNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEV AERVLEFDIGWLAEPFGSGDYPWWMRDWLNQRNNFLLPYFTEDEKK LIQGTDFDLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNPSQV AVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVYYMQ NYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQFEPKASM KHRYKIIDSNGFPGPETLERFCPEEFTVCTECSFFHTRKS	69
hKL ECD (Q9UEF7-1)	EPGDGAQTWARFSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQTEGG WQQHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPATGDVAS DSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYY	13

Table 1 – Example Alpha Klotho Moieties		
Name	Sequence	SEQ ID NO:
	RLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDY AELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLV AHNLLLAHAKVWHLYNTSFRPTQGGQVSIALSSHWINPRRMTDHSIKE CQKSLDFVLGWFAKPVFIDGDYPESMKNNLSSILPDFTESEKKFIKGT DFFALCFGPTLSFQLLDPHMKFRQLESPNLRQLLSWIDLEFNHPQIFIV ENGWVSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYTAWSLM DGFWEHRGYSIRRGIFYVDLFSQDKMLLPKSSALFYQKLIKNGFPPL PENQPLEGTFPCDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKR LIKVDGVVTKKRKSYCVDFAAIQPIALLQEMHVTHFRFSLDWALILPLG NQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPRLLAR QGAWENPYTALAFAEYARLCFQELGHHVKLWTMNEPYTRNMTYSAG HNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEV AERVLEFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKK LIQGTDFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNPSQV AVVPWGLRKVLNWLKFKYGDLPYIISNGIDDLHAEDDQLRVVYMQ NYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQFEPKASM KHYRKIIDSNGFPGPETLERFCPEEFTVCTECSFFHTRKS	
hKL ECD – C521S (BAA23382.1)	EPGDGAQTWARVSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQTEGG WQQHKGKASIWDFTTHPLAPPGDSRNASPLGAPSPLQPATGDVAS DSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVNPREGRLYY RLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDY AELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLV AHNLLLAHAKVWHLYNTSFRPTQGGQVSIALSSHWINPRRMTDHSIKE CQKSLDFVLGWFAKPVFIDGDYPESMKNNLSSILPDFTESEKKFIKGT DFFALCFGPTLSFQLLDPHMKFRQLESPNLRQLLSWIDLEFNHPQIFIV ENGWVSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYTAWSLM DGFWEHRGYSIRRGIFYVDLFSQDKMLLPKSSALFYQKLIKNGFPPL PENQPLEGTFPSDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKR LIKVDGVVTKKRKSYCVDFAAIQPIALLQEMHVTHFRFSLDWALILPLG NQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPRLLAR QGAWENPYTALAFAEYARLCFQELGHHVKLWTMNEPYTRNMTYSAG HNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEV AERVLEFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKK LIQGTDFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNPSQV AVVPWGLRKVLNWLKFKYGDLPYIISNGIDDLHAEDDQLRVVYMQ NYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQFEPKASM KHYRKIIDSNGFPGPETLERFCPEEFTVCTECSFFHTRKS	70
hKL ECD – C521S (Q9UEF7-1)	EPGDGAQTWARFSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQTEGG WQQHKGKASIWDFTTHPLAPPGDSRNASPLGAPSPLQPATGDVAS DSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVNPREGRLYY RLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDY AELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLV AHNLLLAHAKVWHLYNTSFRPTQGGQVSIALSSHWINPRRMTDHSIKE CQKSLDFVLGWFAKPVFIDGDYPESMKNNLSSILPDFTESEKKFIKGT DFFALCFGPTLSFQLLDPHMKFRQLESPNLRQLLSWIDLEFNHPQIFIV ENGWVSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYTAWSLM	14

Table 1 – Example Alpha Klotho Moieties		
Name	Sequence	SEQ ID NO:
	DGFEWHRGYSIRRGIFYVDLFSQDKMLLPKSSALFYQKLIKNGFPPL PENQPLEGTFPSDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKR LIKVDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHFRFSLDWALILPLG NQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPRLLAR QGAWENPYTALAFAEYARLCFQELGHHVKLWITMNEPYTRNMTYSAG HNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEV AERVLEFDIGWLAEPFGSGDYPWWMRDWLNQRNNFLLPYFTEDEKK LIQGTDFLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNSPSQV AVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVYYMQ NYINEALKAHILDGINLCGYFAYSFNDRTPRFGLYRYAADQFEPKASM KHYRKIDSNGFPGPETLERFCPEEFTVCTECSFFHTRKS	
hKL ECD – 23aa truncation (wt) (BAA23382.1)	EPGDGAQTWARVSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQTEGG WQQHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPATGDVAS DSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYY RRLLERLRELGVQPVTLYHWDLPQRLQDAYGGWANRALADHFRDY AELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLV AHNLLLAHAKVWHLYNTSFRPTQGGQVSIALSSHWINPRRMTDHSIKE CQKSLDFVLGWFAKPVFIDGDYPESMKNNLSSILPDFTSEKFFIKGTA DFFALCFGPTLSFQLDPHMKFRQLESPNLRQLLSWIDLEFNHPQIFIV ENGWVFSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYTAWSLM DGFEWHRGYSIRRGIFYVDLFSQDKMLLPKSSALFYQKLIKNGFPPL PENQPLEGTFPCDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKR LIKVDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHFRFSLDWALILPLG NQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPRLLAR QGAWENPYTALAFAEYARLCFQELGHHVKLWITMNEPYTRNMTYSAG HNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEV AERVLEFDIGWLAEPFGSGDYPWWMRDWLNQRNNFLLPYFTEDEKK LIQGTDFLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNSPSQV AVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVYYMQ NYINEALKAHILDGINLCGYFAYSFNDRTPRFGLYRYAADQFEPKASM KHYRKIDSNGFPGPET	71
hKL ECD – 23aa truncation (wt) (Q9UEF7-1)	EPGDGAQTWARFSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQTEGG WQQHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPATGDVAS DSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYY RRLLERLRELGVQPVTLYHWDLPQRLQDAYGGWANRALADHFRDY AELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLV AHNLLLAHAKVWHLYNTSFRPTQGGQVSIALSSHWINPRRMTDHSIKE CQKSLDFVLGWFAKPVFIDGDYPESMKNNLSSILPDFTSEKFFIKGTA DFFALCFGPTLSFQLDPHMKFRQLESPNLRQLLSWIDLEFNHPQIFIV ENGWVFSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYTAWSLM DGFEWHRGYSIRRGIFYVDLFSQDKMLLPKSSALFYQKLIKNGFPPL PENQPLEGTFPCDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKR LIKVDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHFRFSLDWALILPLG NQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPRLLAR QGAWENPYTALAFAEYARLCFQELGHHVKLWITMNEPYTRNMTYSAG HNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEV	15

Table 1 – Example Alpha Klotho Moieties		
Name	Sequence	SEQ ID NO:
	AERVLEFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKK LIQGTDFLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNSPSQV AVVPWGLRKVLNWLKFKYGDLPYIISNGIDDLHAEDDQLRVVYMQ NYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQFEPKASM KHYRKIIDSNGFPGPET	
hKL ECD – 23aa truncation (C521S) (BAA23382.1)	EPGDGAQTWARVSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQTEGG WQQHKGKASIWDTFTHHPLAPPGDSRNASPLGAPSPLQPATGDVAS DSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYY RLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDY AELCFRHFGGQVKYWTIDNPYVVAWHGYATGRLAPGIRGSPRLGYLV AHNLLAHAKVWHLYNTSFRPTQGGQVSIALSSHWINPRRMTDHSIKE CQKSLDFVLGWFAKPVFIDGDYPESMKNNLSSILPDFTESEKKFIKGT DFFALCFGPTLSFQLLDPHMKFRQLESPNLRQLLSWIDLEFNHPQIFIV ENGWVSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYTAWSLM DGFVHWRGYSIRRGIFYVDLSDQDKMLLPKSSALFYQKLEKNGFPPL PENQPLEGTFPSDFAWGVVDNYIQVDTTSLSQFTDLNVYLWDVHHSKR LIKVDGVVTKKRKSYCVDFAAIQPIALLQEMHVTHFRFSLDWALILPLG NQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPRLAR QGAWENPYTALAFAEYARLCFQELGHHVKLWTMNEPYTRNMTYSAG HNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEV AERVLEFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKK LIQGTDFLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNSPSQV AVVPWGLRKVLNWLKFKYGDLPYIISNGIDDLHAEDDQLRVVYMQ NYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQFEPKASM KHYRKIIDSNGFPGPET	72
hKL ECD – 23aa truncation (C521S) (Q9UEF7-1)	EPGDGAQTWARVSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQTEGG WQQHKGKASIWDTFTHHPLAPPGDSRNASPLGAPSPLQPATGDVAS DSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYY RLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDY AELCFRHFGGQVKYWTIDNPYVVAWHGYATGRLAPGIRGSPRLGYLV AHNLLAHAKVWHLYNTSFRPTQGGQVSIALSSHWINPRRMTDHSIKE CQKSLDFVLGWFAKPVFIDGDYPESMKNNLSSILPDFTESEKKFIKGT DFFALCFGPTLSFQLLDPHMKFRQLESPNLRQLLSWIDLEFNHPQIFIV ENGWVSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYTAWSLM DGFVHWRGYSIRRGIFYVDLSDQDKMLLPKSSALFYQKLEKNGFPPL PENQPLEGTFPSDFAWGVVDNYIQVDTTSLSQFTDLNVYLWDVHHSKR LIKVDGVVTKKRKSYCVDFAAIQPIALLQEMHVTHFRFSLDWALILPLG NQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPRLAR QGAWENPYTALAFAEYARLCFQELGHHVKLWTMNEPYTRNMTYSAG HNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEV AERVLEFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKK LIQGTDFLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNSPSQV AVVPWGLRKVLNWLKFKYGDLPYIISNGIDDLHAEDDQLRVVYMQ NYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQFEPKASM KHYRKIIDSNGFPGPET	16

Table 1 – Example Alpha Klotho Moieties		
Name	Sequence	SEQ ID NO:
hKL ECD – 23aa truncation (C521S + C370S) (BAA23382.1)	EPGDGAQTTWARVSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQTEGGWQQHGKGASIWDFTTHHPLAPPGDSRNASLPLGAPSPLQPATGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYYRRLLERLRELGVQPVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFGGQVKYWTIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFIDGDYPESMKNNLSSILPDFTESEKKFIKGTADFFALSFGPTLSFQLLDPHMKFRQLESPNLRQLLSWIDLEFNHPQIFIVENGWVSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGIFYVDFLSQDKMLLPKSSALFYQKLIKENGFPPLPENQPLEGTFPSDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHFRFSLDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPRLLARQGAWENPYTALAFAEYARLCFQELGHHVKLWTMNEPYTRNMTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEYAERVLEFDIGWLAEPFGSGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQGTDFDLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNSPSQVAVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVYYMQNYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQFEPKASKHYRKIIDSNGFPGPET	91
hKL ECD – 23aa truncation (C521S + C370S) (Q9UEF7-1)	EPGDGAQTTWARVSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQTEGGWQQHGKGASIWDFTTHHPLAPPGDSRNASLPLGAPSPLQPATGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYYRRLLERLRELGVQPVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFGGQVKYWTIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFIDGDYPESMKNNLSSILPDFTESEKKFIKGTADFFALSFGPTLSFQLLDPHMKFRQLESPNLRQLLSWIDLEFNHPQIFIVENGWVSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGIFYVDFLSQDKMLLPKSSALFYQKLIKENGFPPLPENQPLEGTFPSDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHFRFSLDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPRLLARQGAWENPYTALAFAEYARLCFQELGHHVKLWTMNEPYTRNMTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEYAERVLEFDIGWLAEPFGSGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQGTDFDLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNSPSQVAVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVYYMQNYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQFEPKASKHYRKIIDSNGFPGPET	68

6.4. Stabilization Moieties

[0067] The engineered alpha klotho polypeptides of the disclosure can comprise a stabilization moiety that can extend the molecule's serum half-life *in vivo*. Serum half-life is often divided into an alpha phase and a beta phase. Either or both phases may be improved significantly by addition of an appropriate stabilization moiety. For example, the stabilization moiety can increase the serum half-life of the engineered alpha klotho polypeptide by more than 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, 200, 400, 600, 800, 1000% or more relative to a corresponding stabilization alpha klotho polypeptide not containing the stabilization moiety. For the purpose of this disclosure, serum half-life can refer to the half-life in humans or other mammals (e.g., mice or non-human primates).

[0068] Stabilization moieties include but are not limited to polyoxyalkylene moieties (e.g., polyethylene glycol), sugars (e.g., sialic acid), and well-tolerated protein moieties (e.g., Fc and fragments and variants thereof, transferrin, and serum albumin). In some embodiments, the stabilization moiety is human serum albumin (or a variant thereof having 1, 2, 3, 4, 5, or more amino acid substitutions relative to human serum albumin). In some embodiments, the stabilization moiety is an Fc domain.

[0069] Other stabilization moieties that can be used in the engineered alpha klotho polypeptides of the disclosure include those described in Kontermann *et al.*, 2011, Current Opinion in Biotechnology 22:868-76. Such stabilization moieties include, but are not limited to, human serum albumin, human serum albumin binders (e.g., Adnectin PKE, AlbuAb, ABD), XTEN®, PAS (recombinant PEG mimetics based on the three amino acids proline, alanine, and serine), carbohydrates (e.g., hydroxyethyl starch (HES)), glycosylation, polysialic acid, and fatty acids.

[0070] In some embodiments, the engineered alpha klotho polypeptide comprising a stabilization moiety will preferably retain at least about 25%, 50%, 60%, 70%, 80%, 85%, 90%, 95% or 100% of the biological activity associated with the unmodified engineered alpha klotho polypeptide. In some embodiments, biological activity refers to its ability to bind to FGFR, or both FGFR and FGF23, as assessed by K_D , k_{on} , or k_{off} .

[0071] The stabilization moiety can be connected to one or more other components of the engineered alpha klotho polypeptides of the disclosure (e.g., the alpha klotho moiety) via a linker, for example as described in Section 6.5. In some embodiments, the stabilization

moiety is C-terminal to the alpha klotho moiety. In some embodiments, the stabilization moiety is N-terminal to the alpha klotho moiety.

[0072] In some embodiments, the stabilization moiety is an Fc domain or a variant of fragment thereof, as described in Section 6.4.1. In some embodiments, the stabilization moiety is a non-dimerizing Fc domain, as described in Section 6.4.1.1. In some embodiments, the stabilization moiety is an Fc1.5 domain, as described in Section 6.4.1.1.1.

[0073] In other embodiments, the stabilization moiety is a serum albumin or a variant or fragment thereof, as described in Section 6.4.2.

[0074] Additional stabilization moieties are also contemplated herein, including a polyethylene glycol moiety or other polymer, as described in Section 6.4.3.

6.4.1. Fc Domains

[0075] In some embodiments, the engineered alpha klotho polypeptides of the disclosure comprise one or more Fc domains as a stabilization moiety.

[0076] The Fc domains can be derived from any suitable species. In one embodiment the Fc domain is derived from a human Fc domain. In some embodiments, the alpha klotho moiety is fused to an IgG Fc domain. The alpha klotho moiety may be fused to the N-terminus or the C-terminus of the IgG Fc domain.

[0077] The Fc domains can be derived from any suitable class of antibody, including IgA (including subclasses IgA1 and IgA2), IgD, IgE, IgG (including subclasses IgG1, IgG2, IgG3 and IgG4), and IgM. In one embodiment, the Fc domain is derived from IgG1, IgG2, IgG3 or IgG4. In one embodiment the Fc domain is derived from IgG1. In one embodiment the Fc domain is derived from IgG4.

[0078] Exemplary sequences of Fc domains from IgG1, IgG2, IgG3, and IgG4 are provided in Table Y-1, below.

TABLE Y-1 Fc Sequences		
Fc	Sequence	SEQ ID NO
hlgG1 Fc (amino acids 114-330 of UniprotKB P01857-1)	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK	23
hlgG2 Fc (amino acids 111-326 of UniprotKB P01859-1)	APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DISVEWESNGQPENNYKTTTPMLDSGDSFFLYSKLTVDKSRWQQGNV FSCSMHEALHNHYTQKSLSLSPGK	24
hlgG3 Fc (amino acids 161-377 of UniprotKB P01860-1)	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYV DGVEVHNAKTKPREEQYNSTFRVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESSGQPENNYNTTPMLDSGDSFFLYSKLTVDKSRWQQGNIF SCSMHEALHNRTQKSLSLSPGK	25
hlgG4 Fc (amino acids 111-327 of UniprotKB P01861-1)	APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGN VFSCSMHEALHNHYTQKSLSLSPGK	26

[0079] In some embodiments, the Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:23. In cases where an Fc domain comprises at least 90% sequence identity and less than 100% sequence identity to SEQ ID NO:23 (e.g., between 90% and 99% sequence identity to SEQ ID NO:23), an Fc domain may also comprise one or more amino acid substitutions described herein, for example one or more substitutions that prevent dimerization (e.g., as described in Section 6.4.1.1) and/or one or more substitutions that alter effector function (e.g., as described in Section 6.4.1.2).

[0080] In some embodiments, the Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:24. In cases where an Fc

domain comprises at least 90% sequence identity and less than 100% sequence identity to SEQ ID NO:24 (*e.g.*, between 90% and 99% sequence identity to SEQ ID NO:24), an Fc domain may also comprise one or more amino acid substitutions described herein, for example one or more substitutions that prevent dimerization (*e.g.*, as described in Section 6.4.1.1) and/or one or more substitutions that alter effector function (*e.g.*, as described in Section 6.4.1.2).

[0081] In some embodiments, the Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:25. In cases where an Fc domain comprises at least 90% sequence identity and less than 100% sequence identity to SEQ ID NO:25 (*e.g.*, between 90% and 99% sequence identity to SEQ ID NO:25), an Fc domain may also comprise one or more amino acid substitutions described herein, for example one or more substitutions that prevent dimerization (*e.g.*, as described in Section 6.4.1.1) and/or one or more substitutions that alter effector function (*e.g.*, as described in Section 6.4.1.2).

[0082] In some embodiments, the Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:26. In cases where an Fc domain comprises at least 90% sequence identity and less than 100% sequence identity to SEQ ID NO:26 (*e.g.*, between 90% and 99% sequence identity to SEQ ID NO:26), an Fc domain may also comprise one or more amino acid substitutions described herein, for example one or more substitutions that prevent dimerization (*e.g.*, as described in Section 6.4.1.1) and/or one or more substitutions that alter effector function (*e.g.*, as described in Section 6.4.1.2).

[0083] In native antibodies, the heavy chain Fc domain of IgA, IgD and IgG is composed of two heavy chain constant domains (CH2 and CH3) and that of IgE and IgM is composed of three heavy chain constant domains (CH2, CH3 and CH4). These dimerize to create an Fc region. The alpha klotho polypeptides of the present disclosure can comprise Fc domains

comprising heavy chain constant domains from one or more different classes of antibody, for example one, two or three different classes.

[0084] In some other embodiments, the stabilization moiety comprises two Fc domains forming a dimer. The two Fc domains can be the same or different from one another. In certain embodiments, the two Fc domains are identical. However, the Fc domains that allow for heterodimerization can be used to manufacture engineered alpha klotho polypeptides comprising different polypeptide components. An engineered alpha klotho polypeptide with different polypeptide components may comprise, for example, an alpha klotho moiety and another polypeptide, or a first alpha klotho moiety fused to another polypeptide and a second alpha klotho moiety that is not fused to another polypeptide.

6.4.1.1. Non-dimerizing Fc domains

[0085] In some embodiments, the Fc domain is a non-dimerizing (or “monomeric”) Fc domain, which refers to an Fc domain that has a reduced ability to self-associate relative to a wild type Fc domain, or which lacks the ability to self-associate entirely, *e.g.*, as described in Helm *et al.*, 1996, J. Biol. Chem. 271: 7494-7500 or Ying *et al.*, 2012, J Biol Chem. 287(23):19399–19408. An example non-dimerizing Fc domain comprises amino acid substitutions in the positions corresponding to T366 and/or Y407 in CH3 (numberings according to Kabat EU index), as described in U.S. Patent Publication No. 2019/0367611, incorporated herein by reference. Particular amino acid substitutions which may be included in a non-dimerizing Fc domain include, for example, L351S, T366R, L368H, P395K, L242C, K334C, L351S, P343C, A431C, L351Y, T366Y, L368A, P395R, F405R, Y407M, K409A, F405E, Y407K, L351K, T366S, P395V, Y407A, and K409Y (numberings according to Kabat EU index). A non-dimerizing Fc domain of the present disclosure may include any 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the abovementioned substitutions, or more.

[0086] Exemplary sequences of non-dimerizing Fc domains are provided in Table Y-2, below. Bolded residues show locations of amino acid substitutions relative to wild type IgG sequence.

TABLE Y-2 Non-dimerizing Fc Sequences		
Non-dimerizing Fc	Sequence	SEQ ID NO
IgG1 mFc (L351S, T366R, L368H, P395K; Kabat EU numbering)	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTSPPSRDELTKNQV SL <u>RCH</u> VKGFYPDSIAVEWESNGQPENNYKTT <u>K</u> PVLDSDGSFFLY SKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK	27
IgG1 smFc (L242C, K334C, L351S, T366R, L368H, P395K; Kabat EU numbering)	APELLGGPSVFCFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIE <u>C</u> TISKAKGQPREPQVYTSPPSRDELTKNQV SL <u>RCH</u> VKGFYPDSIAVEWESNGQPENNYKTT <u>K</u> PVLDSDGSFFLY SKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK	28
IgG1 ssmFc (L242C, K334C, P343C, L351S, T366R, L368H, P395K, A431C; Kabat EU numbering)	APELLGGPSVFCFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIE <u>C</u> TISKAKGQ <u>C</u> REPQVYTSPPSRDELTKNQV SL <u>RCH</u> VKGFYPDSIAVEWESNGQPENNYKTT <u>K</u> PVLDSDGSFFLY SKLTVDKSRWQQGNVSCSVMHE <u>C</u> LHNHYTQKSLSLSPGK	29
IgG1 mFc.1 (L351Y, T366Y, L368A, P395R, F405R, Y407M, K409A; Kabat EU numbering)	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTYPPSRDELTKNQV SL <u>YCA</u> VKGFYPDSIAVEWESNGQPENNYKTT <u>R</u> PVLDSDGSF <u>RLM</u> <u>SALT</u> VDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK	30
IgG1 mFc.23 (L351S, T366R, L368H, P395K, F405E, Y407K, K409A; Kabat EU numbering)	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTSPPSRDELTKNQV SL <u>RCH</u> VKGFYPDSIAVEWESNGQPENNYKTT <u>K</u> PVLDSDGSF <u>ELK</u> <u>SALT</u> VDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK	31
IgG1 mFc.67 (L351K, T366S, P395V, F405R, Y407A, K409Y; Kabat EU numbering)	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTKPPSRDELTKNQV SL <u>SCL</u> VKGFYPDSIAVEWESNGQPENNYKTT <u>V</u> PVLDSDGSF <u>RLA</u> <u>S</u> <u>YLT</u> VDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK	32

[0087] In some embodiments, the non-dimerizing Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:27. In some embodiments, the non-dimerizing Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:28. In some embodiments, the non-dimerizing Fc domain comprises an amino acid sequence having at least about 90%, at

least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:29. In some embodiments, the non-dimerizing Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:30. In some embodiments, the non-dimerizing Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:31. In some embodiments, the non-dimerizing Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:32.

6.4.1.1.1. Fc1.5 Domains

[0088] In some embodiments, the Fc domain further comprises, in addition to a CH2 and CH3 domain, an additional CH3 domain connected to the first CH3 domain via a linker (e.g., a linker as described in Section 6.5). An Fc domain comprising such a configuration (CH2 – CH3 – linker – CH3) is sometimes referred to herein as an “Fc1.5 domain” or simply “Fc1.5” for convenience. The linker between the first and second CH3 domains of an Fc1.5 domain is preferably of sufficient length and flexibility so as to permit dimerization of the first CH3 domain with the second CH3 domain. Thus, in some embodiments, the stabilization moiety is an Fc1.5 domain comprising a linker of at least 5, at least 10, at least 15, or at least 20 amino acids in length connecting the first and second CH3 domains.

[0089] Exemplary sequences of Fc1.5 domains are provided in Table Y-3, below.

TABLE Y-3 Fc1.5 Sequences		
Fc1.5	Sequence	SEQ ID NO
Fc1.5 (IgG1; (G ₄ S) ₃)	GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGG	33

TABLE Y-3 Fc1.5 Sequences		
Fc1.5	Sequence	SEQ ID NO
	GGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGQPREPQ VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGK	
Fc1.5 (IgG1; (G ₄ S) ₇)	GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTV DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSGG GGSGGGGSGGGGSGGGGSGGGGSGGGGSGQPREPQVYTLPP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQK SLSLSPGK	34
Fc1.5 (IgG1; (G ₄ S) ₆)	GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTV DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSGG GGSGGGGSGGGGSGGGGSGGGGSGGGGSGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSG SFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG K	35
Fc1.5 (IgG1; (G ₄ S) ₅)	GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTV DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSGG GGSGGGGSGGGGSGGGGSGGGGSGGGGSGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSK LTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK	36
Fc1.5 (IgG1; (G ₄ S) ₄)	GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTV DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSGG GGSGGGGSGGGGSGGGGSGGGGSGGGGSGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTVDK SRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK	37
Fc1.5 (IgG1; (G ₄ S) ₃)	GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTV DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGGGGSGG GGSGGGGSGGGGSGGGGSGGGGSGGGGSGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS	38

TABLE Y-3 Fc1.5 Sequences		
Fc1.5	Sequence	SEQ ID NO
	DIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG GNVFSCSVMHEALHNHYTQKSLSLSPGK	
Fc1.5 (IgG1; (G ₄ S) ₂)	GGPSVFLFPPKPKDTLMISRTPEVTCWVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGG GGSGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK	39
Fc1.5 (IgG1; (G ₄ S))	GGPSVFLFPPKPKDTLMISRTPEVTCWVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK	40

[0090] In some embodiments, the Fc1.5 domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:33. In some embodiments, the Fc1.5 domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:34. In some embodiments, the Fc1.5 domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:35. In some embodiments, the Fc1.5 domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%,

at least about 99%, or 100% sequence identity to SEQ ID NO:36. In some embodiments, the Fc1.5 domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:37. In some embodiments, the Fc1.5 domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:38. In some embodiments, the Fc1.5 domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:39. In some embodiments, the Fc1.5 domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:40.

6.4.1.2. Fc Domains with Altered Effector Function

[0091] In some embodiments, the Fc domain comprises one or more amino acid substitutions that reduce binding to an Fc receptor and/or effector function.

[0092] In a particular embodiment the Fc receptor is an Fcγ receptor. In one embodiment the Fc receptor is a human Fc receptor. In one embodiment the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an activating human Fcγ receptor, more specifically human FcγRIIIa, FcγRI or FcγRIIa, most specifically human FcγRIIIa. In one embodiment the effector function is one or more selected from the group of complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and cytokine secretion. In a particular embodiment, the effector function is ADCC.

[0093] In one embodiment, the Fc domain comprises an amino acid substitution at a position selected from the group of E233, L234, L235, N297, P331 and P329 (numberings according to Kabat EU index). In a more specific embodiment, the Fc domain comprises an amino acid substitution at a position selected from the group of L234, L235 and P329

(numberings according to Kabat EU index). In some embodiments, the Fc domain comprises the amino acid substitutions L234A and L235A (numberings according to Kabat EU index). In one such embodiment, the Fc domain is an IgD Fc domain, particularly a human IgD Fc domain. In one embodiment, the Fc domain comprises an amino acid substitution at position P329. In a more specific embodiment, the amino acid substitution is P329A or P329G, particularly P329G (numberings according to Kabat EU index). In one embodiment, the Fc domain comprises an amino acid substitution at position P329 and a further amino acid substitution at a position selected from E233, L234, L235, N297 and P331 (numberings according to Kabat EU index). In a more specific embodiment, the further amino acid substitution is E233P, L234A, L235A, L235E, N297A, N297D or P331S. In particular embodiments, the Fc domain comprises amino acid substitutions at positions P329, L234 and L235 (numberings according to Kabat EU index). In more particular embodiments, the Fc domain comprises the amino acid mutations L234A, L235A and P329G (“P329G LALA”, “PGLALA” or “LALAPG”).

[0094] In one embodiment, the Fc domain is an IgG1 Fc domain, particularly a human IgG1 Fc domain. In some embodiments, the IgG1 Fc domain is a variant IgG1 comprising D265A, N297A mutations (EU numbering) to reduce effector function.

[0095] In another embodiment, the Fc domain is an IgG4 Fc domain with reduced binding to Fc receptors. Exemplary IgG4 Fc domains with reduced binding to Fc receptors may comprise an amino acid sequence selected from Table F below. In some embodiments, the Fc domain includes only the bolded portion of the sequences shown below:

TABLE F	
Fc Domain	Sequence
SEQ ID NO:1 of WO2014/121087	Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly

TABLE F	
Fc Domain	Sequence
	Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys (SEQ ID NO:83)
SEQ ID NO:2 of WO2014/121087	Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys (SEQ ID NO:84)
SEQ ID NO:30 of WO2014/121087	Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His

TABLE F	
Fc Domain	Sequence
	Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys (SEQ ID NO:85)
SEQ ID NO:31 of WO2014/121087	Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys (SEQ ID NO:86)
SEQ ID NO:37 of WO2014/121087	Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys

TABLE F	
Fc Domain	Sequence
	Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys (SEQ ID NO:87)
SEQ ID NO:38 of WO2014/121087	Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys (SEQ ID NO:88)

[0096] In a particular embodiment, the IgG4 with reduced effector function comprises the bolded portion of the amino acid sequence of SEQ ID NO:31 of WO2014/121087, sometimes referred to herein as IgG4s or hIgG4s.

6.4.2. Albumin Moieties

[0097] Human serum albumin (HSA) has a long average half-life of approximately 19 days. HSA is the most abundant protein in human plasma and serves a variety of functions including maintenance of plasma pH, transport of fatty acids and other metabolites, and

maintenance of blood pressure, among others. The amino acid sequence of mature HSA (lacking the signal sequence and propeptide) is shown below.

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DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQCPFEDHVKLVNEVTEFAKTCVADE
SAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPR
LVRPEVDVMCTAFHDNEETFLKKYLYEIIARRHPYFYAPELLFFAKRYKAAFTGCCQA
ADKAACLLPKLDELRLDEGKASSAKQRLKCSLQKFGERAFKAWAVARLSQRFPKAEF
AEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLE
KSHCIAEVENDEMPADLP SLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYS
VVLLLRRLAKTYETTL EKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCLEFQGLG
EYKFNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVV
LNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADI
CTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCKKADDKETCFAE
EGKKLVAAASQAALGL (SEQ ID NO:19)

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[0098] An albumin moiety of an engineered alpha klotho polypeptide of the disclosure may be HSA, a portion thereof, or a variant thereof. Thus, in some embodiments, the albumin moiety comprises the sequence of wild-type, mature HSA. In some embodiments, the albumin moiety comprises an amino acid sequence having 1, 2, 3, 4, 5, or more amino acid substitutions relative to wild-type, mature HSA. For example, in some embodiments, the albumin moiety comprises an amino acid sequence having a C34S mutation relative to wild-type, mature HSA. Without being bound by theory, a C34S in HSA is believed to improve stability by virtue of elimination of a free cysteine. Additional HSA variants are recognized in the art and contemplated herein including, for example, K573P mutant HSA. In some embodiments, the albumin moiety comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:19. In some embodiments, the albumin moiety comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:20.

6.4.3. Other Stabilization Moieties

[0099] In some embodiments, the engineered alpha klotho polypeptide comprises polyethylene glycol (PEG) or another hydrophilic polymer as a stabilization moiety, for example a copolymer of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane,

ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, a propylene glycol homopolymer, a polypropylene oxide/ethylene oxide co-polymer, a polyoxyethylated polyol (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. The polymer may be of any molecular weight, and may be branched or unbranched.

[0100] Serum albumin can be engaged in half-life extension through modules with the capacity to non-covalently interact with albumin. Accordingly, the engineered alpha klotho polypeptides of the disclosure can include as a stabilization moiety an albumin-binding protein. The albumin-binding protein can be either conjugated or genetically fused to one or more other components of the engineered alpha klotho polypeptides of the disclosure. Proteins with albumin-binding activity are known from certain bacteria. For example, streptococcal protein G contains several small albumin-binding domains composed of roughly 50 amino acid residues (6 kDa). Additional examples of serum albumin binding proteins such as those described in U.S. Publication Nos. 2007/0178082 and 2007/0269422. Fusion of an albumin binding domain to a protein results in a strongly extended half-life (see Kontermann *et al.*, 2011, Current Opinion in Biotechnology 22:868-76).

6.5. Linkers

[0101] In certain aspects, the present disclosure provides engineered alpha klotho polypeptides in which two or more components of are connected to one another by a peptide linker. By way of example and not limitation, linkers can be used to connect (a) an alpha klotho moiety and a stabilization moiety and/or (b) a stabilization moiety and another moiety, e.g., a purification tag moiety.

[0102] A peptide linker can range from 2 amino acids to 60 or more amino acids, and in certain aspects a peptide linker ranges from 3 amino acids to 50 amino acids, from 4 to 30 amino acids, from 5 to 25 amino acids, from 10 to 25 amino acids, 10 amino acids to 60 amino acids, from 12 amino acids to 20 amino acids, from 20 amino acids to 50 amino acids, or from 25 amino acids to 35 amino acids in length.

[0103] In particular aspects, a peptide linker is at least 5 amino acids, at least 6 amino acids or at least 7 amino acids in length and optionally is up to 30 amino acids, up to 40 amino acids, up to 50 amino acids or up to 60 amino acids in length.

[0104] In some embodiments of the foregoing, the linker ranges from 5 amino acids to 50 amino acids in length, *e.g.*, ranges from 5 to 50, from 5 to 45, from 5 to 40, from 5 to 35, from 5 to 30, from 5 to 25, or from 5 to 20 amino acids in length. In other embodiments of the foregoing, the linker ranges from 6 amino acids to 50 amino acids in length, *e.g.*, ranges from 6 to 50, from 6 to 45, from 6 to 40, from 6 to 35, from 6 to 30, from 6 to 25, or from 6 to 20 amino acids in length. In yet other embodiments of the foregoing, the linker ranges from 7 amino acids to 50 amino acids in length, *e.g.*, ranges from 7 to 50, from 7 to 45, from 7 to 40, from 7 to 35, from 7 to 30, from 7 to 25, or from 7 to 20 amino acids in length.

[0105] Charged (*e.g.*, charged hydrophilic linkers) and/or flexible linkers are particularly preferred.

[0106] Examples of flexible linkers that can be used in the engineered alpha klotho polypeptides of the disclosure include those disclosed by Chen *et al.*, 2013, Adv Drug Deliv Rev. 65(10): 1357-1369 and Klein *et al.*, 2014, Protein Engineering, Design & Selection 27(10): 325-330. Particularly useful flexible linkers are or comprise repeats of glycines and serines, *e.g.*, a monomer or multimer of G_nS or SG_n, where n is an integer from 1 to 10, *e.g.*, 1 2, 3, 4, 5, 6, 7, 8, 9 or 10. In one embodiment, the linker is or comprises a monomer or multimer of repeat of G₄S *e.g.*, (GGGGS)_n (SEQ ID NO:75).

[0107] Polyglycine linkers can suitably be used in the engineered alpha klotho polypeptides of the disclosure. In some embodiments, a peptide linker comprises two consecutive glycines (2Gly), three consecutive glycines (3Gly), four consecutive glycines (4Gly), five consecutive glycines (5Gly), six consecutive glycines (6Gly), seven consecutive glycines (7Gly), eight consecutive glycines (8Gly) or nine consecutive glycines (9Gly).

[0108] Exemplary linker sequences are set forth in Table L below. An engineered alpha klotho polypeptide of the disclosure may comprise one or more linkers of Table L. For example, in some embodiments, an engineered alpha klotho polypeptide of the disclosure comprises an alpha klotho moiety and a stabilization moiety connected by a linker of Table L.

TABLE L		
Linker Sequences		
Linker Sequence	Designation	SEQ ID NO:
(GGGGS) _n	L1	75

TABLE L Linker Sequences		
Linker Sequence	Designation	SEQ ID NO:
(GGGS) _n	L2	98
(GGS) _n	L3	N/A
(GS) _n	L4	N/A
(GSGGS) _n	L5	99
AAAGG	L6	100
ADAAP	L7	101
ADAAPTVSIFP	L8	102
ADAAPTVSIFPP	L9	103
AKTTAP	L10	104
AKTTAPSVYPLAP	L11	105
AKTTPKLEEGEFSEARV	L12	106
AKTTPKLGG	L13	107
AKTTPP	L14	108
AKTTPPSVTPLAP	L15	109
ASTKGP	L16	110
ASTKGPSVFPLAPASTKGPSVFPLAP	L17	111
EGKSSGSGSESKST	L18	112
GEGESGEGESGEGES	L19	113
GEGESGEGESGEGESGEGES	L20	114
GEGGSGEGGSGEGGS	L21	115
GENKVEYAPALMALS	L22	116
GGEGSGGEGSGGEGS	L23	117
GGGESGGEGSGGEGS	L24	118
GGGESGGGESGGGES	L25	119
GGGGSGGGGS	L26	82
GGGGSGGGGSGGGGS	L27	81
GGGGSGGGGSGGGGSGGGGS	L28	80
GGGKSGGGKSGGGKS	L29	120
GGGKSGGKGSGKGGS	L30	121
GGGS	L31	98
GGSG	L32	122
GGKGSGGKGSGGKGS	L33	123
GGG	L34	N/A
GGSG	L35	124
GGSGG	L36	125
GGSGG	L37	125
GGSGGGGSG	L38	126
GGSGGGGSGGGGS	L39	127
GHEAAVMQVQYPAS	L40	128
GKGGSGKGGSGKGGS	L41	129

TABLE L Linker Sequences		
Linker Sequence	Designation	SEQ ID NO:
GKGKSGKKGKSGKGS	L42	130
GKGKSGKKGKSGKKGKGS	L43	131
GKPGSGKPGSGKPGS	L44	132
GKPGSGKPGSGKPGSGKPSGS	L45	133
GPAKELTPLKEAKVS	L46	134
GSAGSAAGSGEF	L47	135
GSGGG	L48	136
GSGSG	L49	137
GSS	L50	N/A
GSSG	L51	138
GSSGGSGGSG	L52	139
GSSGGSGGSGG	L53	140
GSSGGSGGSGGS	L54	141
GSSGGSGGSGGSG	L55	142
GSSGGSGGSGGSGGGS	L56	143
GSSGGSGGSGS	L57	144
GSSGT	L58	145
GSSSG	L59	146
GSTSGSGKPGSGEGSTKG	L60	147
GTAAAGAGAAAGGAAAGAAG	L61	148
GTSGSSGSGSGGSGSGGGG	L62	149
IRPRAIGGSKPRVA	L63	150
KESGSVSSEQLAQFRSLD	L64	151
KTTTPKLEEGEFSEAR	L65	152
PRGASKSGSASQTGSAPGS	L66	153
QPKAAP	L67	154
QPKAAPSVTLFPP	L68	155
RADAAAA(G4S) ₄	L69	156
RADAAAAGGPGS	L70	157
RADAAP	L71	158
RADAAPTVS	L72	159
SAKTTTP	L73	160
SAKTTTPKLEEGEFSEARV	L74	161
SAKTTPKLGG	L75	162
STAGDTHLGGEDFD	L76	163
TVAAP	L77	164
TVAAPSVFIFPP	L78	165
TVAAPSVFIFPPTVAAPSVFIFPP	L79	166

[illegible]

[illegible]

linker L75. In some embodiments, the engineered KL polypeptide comprises linker L76. In some embodiments, the engineered KL polypeptide comprises linker L77. In some embodiments, the engineered KL polypeptide comprises linker L78. In some embodiments, the engineered KL polypeptide comprises linker L79.

6.6. Nucleic Acids and Host Cells

[0110] In another aspect, the disclosure provides nucleic acids encoding the engineered alpha klotho polypeptides of the disclosure. The nucleic acids of the disclosure can be DNA (e.g., plasmid) or RNA (e.g., mRNA).

[0111] In some embodiments, a nucleic acid of the present disclosure comprises a nucleic acid sequence having at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% sequence identity to any one of SEQ ID NOs:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, and 61.

[0112] In some aspects, the disclosure provides host cells and vectors containing the nucleic acids of the disclosure. The nucleic acids may be present in a single vector or separate vectors present in the same host cell or separate host cell, as described in more detail herein below.

6.6.1. Vectors

[0113] The disclosure provides vectors comprising nucleotide sequences encoding an alpha klotho polypeptide or a component thereof described herein, for example a polypeptide chains of an alpha klotho polypeptide. The vectors include, but are not limited to, a virus, plasmid, cosmid, lambda phage or a yeast artificial chromosome (YAC).

[0114] Numerous vector systems can be employed. For example, one class of vectors utilizes DNA elements which are derived from animal viruses such as, for example, bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (Rous Sarcoma Virus, MMTV or MOMLV) or SV40 virus. Another class of vectors utilizes RNA elements derived from RNA viruses such as Semliki Forest virus, Eastern Equine Encephalitis virus and Flaviviruses.

[0115] Additionally, cells which have stably integrated the DNA into their chromosomes can be selected by introducing one or more markers which allow for the selection of transfected host cells. The marker may provide, for example, prototrophy to an auxotrophic host, biocide resistance (e.g., antibiotics), or resistance to heavy metals such as copper, or the like. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, or introduced into the same cell by co-transformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcriptional promoters, enhancers, and termination signals.

[0116] Once the expression vector or DNA sequence containing the constructs has been prepared for expression, the expression vectors can be transfected or introduced into an appropriate host cell. Various techniques may be employed to achieve this, such as, for example, protoplast fusion, calcium phosphate precipitation, electroporation, retroviral transduction, viral transfection, gene gun, lipid-based transfection, or other conventional techniques. Methods and conditions for culturing the resulting transfected cells and for recovering the expressed polypeptides are known to those skilled in the art, and may be varied or optimized depending upon the specific expression vector and mammalian host cell employed, based upon the present description.

6.6.2. Host Cells

[0117] The disclosure also provides host cells comprising a nucleic acid of the disclosure.

[0118] In one embodiment, the host cells are genetically engineered to comprise one or more nucleic acids described herein.

[0119] In one embodiment, the host cells are genetically engineered by using an expression cassette. The phrase "expression cassette," refers to nucleotide sequences, which are capable of affecting expression of a gene in hosts compatible with such sequences. Such cassettes may include a promoter, an open reading frame with or without introns, and a termination signal. Additional factors necessary or helpful in effecting expression may also be used, such as, for example, an inducible promoter.

[0120] The disclosure also provides host cells comprising the vectors described herein. The cell can be, but is not limited to, a eukaryotic cell, a bacterial cell, an insect cell, or a human cell. Suitable eukaryotic cells include, but are not limited to, Vero cells, HeLa cells, COS

cells, CHO cells, HEK293 cells, BHK cells and MDCKII cells. Suitable insect cells include, but are not limited to, Sf9 cells.

6.7. Pharmaceutical Compositions

[0121] The engineered alpha klotho polypeptides of the disclosure may be in the form of compositions comprising the engineered alpha klotho polypeptide and one or more carriers, excipients and/or diluents. The compositions may be formulated for specific uses, such as for veterinary uses or pharmaceutical uses in humans. The form of the composition (*e.g.*, dry powder, liquid formulation, *etc.*) and the excipients, diluents and/or carriers used will depend upon the intended uses of the engineered alpha klotho polypeptide and, for therapeutic uses, the mode of administration.

[0122] For therapeutic uses, the compositions may be supplied as part of a sterile, pharmaceutical composition that includes a pharmaceutically acceptable carrier. This composition can be in any suitable form (depending upon the desired method of administering it to a patient). The pharmaceutical composition can be administered to a patient by a variety of routes such as orally, transdermally, subcutaneously, intranasally, intravenously, intramuscularly, intratumorally, intrathecally, topically, or locally. The most suitable route for administration in any given case will depend on the particular alpha klotho polypeptide, the subject, and the nature and severity of the disease and the physical condition of the subject. Typically, the pharmaceutical composition will be administered intravenously or subcutaneously.

[0123] Pharmaceutical compositions can be conveniently presented in unit dosage forms containing a predetermined amount of an engineered alpha klotho polypeptide of the disclosure per dose. The quantity of engineered alpha klotho polypeptide included in a unit dose will depend on the disease being treated, as well as other factors as are well known in the art. Such unit dosages may be in the form of a lyophilized dry powder containing an amount of engineered alpha klotho polypeptide suitable for a single administration, or in the form of a liquid. Dry powder unit dosage forms may be packaged in a kit with a syringe, a suitable quantity of diluent and/or other components useful for administration. Unit dosages in liquid form may be conveniently supplied in the form of a syringe pre-filled with a quantity of alpha klotho polypeptide suitable for a single administration.

[0124] The pharmaceutical compositions may also be supplied in bulk from containing quantities of alpha klotho polypeptide suitable for multiple administrations.

[0125] Pharmaceutical compositions may be prepared for storage as lyophilized formulations or aqueous solutions by mixing an alpha klotho polypeptide having the desired degree of purity with optional pharmaceutically-acceptable carriers, excipients or stabilizers typically employed in the art (all of which are referred to herein as "carriers"), i.e., buffering agents, stabilizing agents, preservatives, isotonicifiers, non-ionic detergents, antioxidants, and other miscellaneous additives. See, Remington's Pharmaceutical Sciences, 16th edition (Osol, ed. 1980). Such additives should be nontoxic to the recipients at the dosages and concentrations employed.

[0126] Buffering agents help to maintain the pH in the range which approximates physiological conditions. They may be present at a wide variety of concentrations, but will typically be present in concentrations ranging from about 2 mM to about 50 mM. Suitable buffering agents for use with the present disclosure include both organic and inorganic acids and salts thereof such as citrate buffers (e.g., monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, etc.), succinate buffers (e.g., succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, etc.), tartrate buffers (e.g., tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, etc.), fumarate buffers (e.g., fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, etc.), gluconate buffers (e.g., gluconic acid-sodium glyconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium glyconate mixture, etc.), oxalate buffer (e.g., oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, etc.), lactate buffers (e.g., lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, etc.) and acetate buffers (e.g., acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, etc.). Additionally, phosphate buffers, histidine buffers and trimethylamine salts such as Tris can be used.

[0127] Preservatives may be added to retard microbial growth, and can be added in amounts ranging from about 0.2%-1 % (w/v). Suitable preservatives for use with the present

disclosure include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (e.g., chloride, bromide, and iodide), hexamethonium chloride, and alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol. Isotonicifiers sometimes known as “stabilizers” can be added to ensure isotonicity of liquid compositions of the present disclosure and include polyhydric sugar alcohols, for example trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol. Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, *etc.*, organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinositol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (e.g., peptides of 10 residues or fewer); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophylic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trehalose; and trisaccharides such as raffinose; and polysaccharides such as dextran. Stabilizers may be present in amounts ranging from 0.5 to 10 wt % per wt of alpha klotho polypeptide.

[0128] Non-ionic surfactants or detergents (also known as “wetting agents”) may be added to help solubilize the glycoprotein as well as to protect the glycoprotein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, *etc.*), polyoxamers (184, 188, *etc.*), and pluronic polyols. Non-ionic surfactants may be present in a range of about 0.05 mg/mL to about 1.0 mg/mL, for example about 0.07 mg/mL to about 0.2 mg/mL.

[0129] Additional miscellaneous excipients include bulking agents (e.g., starch), chelating agents (e.g., EDTA), antioxidants (e.g., ascorbic acid, methionine, vitamin E), and cosolvents.

[0130] The engineered alpha klotho polypeptides of the disclosure can be formulated as pharmaceutical compositions comprising the engineered alpha klotho polypeptides, for example containing one or more pharmaceutically acceptable excipients or carriers. To prepare pharmaceutical or sterile compositions comprising the engineered alpha klotho polypeptides of the present disclosure, an engineered alpha klotho polypeptide preparation can be combined with one or more pharmaceutically acceptable excipient or carrier.

[0131] For example, formulations of engineered alpha klotho polypeptides can be prepared by mixing engineered alpha klotho polypeptides with physiologically acceptable carriers, excipients, or stabilizers in the form of, e.g., lyophilized powders, slurries, aqueous solutions, lotions, or suspensions (see, e.g., Hardman et al., 2001, Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y.; Gennaro, 2000, Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N.Y.; Avis, et al. (eds.), 1993, Pharmaceutical Dosage Forms: General Medications, Marcel Dekker, NY; Lieberman, et al. (eds.), 1990, Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY; Lieberman, et al. (eds.), 1990, Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY; Weiner and Kotkoskie, 2000, Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y.).

[0132] An effective amount for a particular subject may vary depending on factors such as the condition being treated, the overall health of the subject, the method route and dose of administration and the severity of side effects (see, e.g., Maynard, *et al.* (1996) A Handbook of SOPs for Good Clinical Practice, Interpharm Press, Boca Raton, Fla.; Dent (2001) Good Laboratory and Good Clinical Practice, Urch Publ., London, UK).

[0133] A composition of the present disclosure may also be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Selected routes of administration for engineered alpha klotho polypeptides include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other general routes of administration, for example by injection or

infusion. General administration may represent modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Alternatively, a composition of the disclosure can be administered via a non-general route, such as a topical, epidermal, or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually, or topically. In one embodiment, the engineered alpha klotho polypeptides are administered by infusion. In another embodiment, an engineered alpha klotho polypeptide of the disclosure is administered subcutaneously.

6.8. Therapeutic Indications and Methods of Use

[0134] The present disclosure provides methods for using and applications for the engineered alpha klotho polypeptides of the disclosure.

[0135] The engineered alpha klotho polypeptides of the disclosure can be used in therapeutic methods for treatment of a variety of disorders including age-related conditions, metabolic disorders, and kidney disease.

[0136] In certain aspects, the disclosure provides a method of treating an age-related condition comprising administering to a subject in need thereof an engineered alpha klotho polypeptide as described herein. Age-related conditions include, but are not limited to, hearing loss, cataracts and refractive errors, osteoarthritis, chronic obstructive pulmonary disease, diabetes, and dementia. An engineered alpha klotho polypeptide of the disclosure may be administered to a subject suffering from or diagnosed with an age-related condition. An engineered alpha klotho polypeptide may also be administered to a subject at risk for developing an age-related condition. For example, a subject having an increased risk for developing an age-related condition may be administered an engineered alpha klotho polypeptide prophylactically, thereby reducing the risk of developing the age-related condition.

[0137] In certain aspects, the disclosure provides a method of treating kidney disease comprising administering to a subject in need thereof an engineered alpha klotho polypeptide as described herein. Kidney disease includes both acute kidney injury and chronic kidney disease. Accordingly, an engineered alpha klotho polypeptide may be

administered to a subject following acute kidney injury or following diagnosis of chronic kidney disease. An engineered alpha klotho polypeptide may also be administered to a subject at risk for developing a kidney disease. For example, a subject having an increased risk for chronic kidney disease may be administered an engineered alpha klotho polypeptide prophylactically, thereby reducing the risk of developing chronic kidney disease.

[0138] Also disclosed are methods of supplementing loss of endogenous alpha klotho comprising administering to a subject in need thereof an engineered alpha klotho polypeptide as described herein. Endogenous levels of alpha klotho protein decrease with age, and engineered alpha klotho polypeptides may be used to supplement this loss. Accordingly, in some embodiments, an engineered alpha klotho polypeptide of the disclosure is administered to a subject having reduced alpha klotho protein levels relative to prior levels in the same subject (e.g., reduced by at least about 5%, 10%, 15%, 20%, 25%, or 30% relative to alpha klotho proteins levels in the same subject from at least 6 months prior).

[0139] Methods of activating FGFR signaling are also disclosed herein using engineered alpha klotho polypeptides. Exemplary engineered alpha klotho polypeptides of the present disclosure can interact with FGF23 and activate FGFR signaling in a cell. Accordingly, disclosed is a method of activating FGFR signaling in a cell comprising contacting the cell with an engineered alpha klotho polypeptide of the disclosure. The cell may be any cell expressing an FGFR on its surface. In some embodiments, the cell is a kidney cell.

7. SEQUENCES

[0140] Certain sequences of the disclosure are provided in Table S below.

Table S - Sequences		
Name	Sequence	SEQ ID NO:
Human alpha klotho (BAA23382.1)	MPASAPRRRPRPPPSLSLLLVLGLGGRRRLRAEPGDGAQTTWAR VSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQTEGGWQQHKGKA SIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPATGDVASDSYNNV FRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYYRLL ERLRELGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYA ELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGY LVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALSSHWINPRRMTD HSIKECQKSLDFVLGWFAKPVFIDGDYPESMKNNLSSILPDFTESE KKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLESPNLRQLLSWID	1

Table S - Sequences		
Name	Sequence	SEQ ID NO:
	LEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKFIMETLKAIKLDG VDVIGYTAWSLMDGFEWHRGYSIRRGIFYVDFLSQDKMLLPKSSA LFYQKLIKNGFPPLPENQPLEGTFPCDFAWGVVDNYIQVDTTLSQ FTDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALQ EMHVTHFRFSLDWALILPLGNQSQVNHTILQYYRCMASELVRVNIT PVVALWQPMAPNQGLPRLLARQGAWENPYTALAFAEYARLCFQE LGHHVKLWITMNEPYTRNMTYSAGHNLLKAHALAWHVYNEKFRH AQNGKISIALQADWIEPACPFSSQKDKEVAERVLFDIGWLAEPFG SGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQGTDFDLALSHYTT ILVDSEKEDPIKYNDYLEVQEMTDITWLNSPSQVAVVPWGLRKVLN WLKFKYGDLPYIISNGIDDLHAEDDQLRVYYMQNYINEALKAHI LDGINLCGYFAYSFNDRTPRFGLYRYAADQFEPKASMKHYRKIID SNGFPGPETLERFCPEEFTVCTECSFFHTRKSLLAFLFFASIIISL SLIFYYSKKGRRSYK	
Human alpha klotho (Q9UEF7-1)	MPASAPRRRPPPPPSLSLLVLLGLGGRRRLRAEPGDGAQTWAR FSRPPAPEAAGLFQGTFPDGLWAVGSAAYQTEGGWQQHGKGA SIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPATGDVASDSYNNV FRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYYRRL ERLRELGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYA ELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGY LVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALSSHWINPRRMTD HSIKECQKSLDFVLGWFAKPVFIDGDYPESMKNLSSILPDFTESE KKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLESPNLRQLLSWID LEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKFIMETLKAIKLDG VDVIGYTAWSLMDGFEWHRGYSIRRGIFYVDFLSQDKMLLPKSSA LFYQKLIKNGFPPLPENQPLEGTFPCDFAWGVVDNYIQVDTTLSQ FTDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALQ EMHVTHFRFSLDWALILPLGNQSQVNHTILQYYRCMASELVRVNIT PVVALWQPMAPNQGLPRLLARQGAWENPYTALAFAEYARLCFQE LGHHVKLWITMNEPYTRNMTYSAGHNLLKAHALAWHVYNEKFRH AQNGKISIALQADWIEPACPFSSQKDKEVAERVLFDIGWLAEPFG SGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQGTDFDLALSHYTT ILVDSEKEDPIKYNDYLEVQEMTDITWLNSPSQVAVVPWGLRKVLN WLKFKYGDLPYIISNGIDDLHAEDDQLRVYYMQNYINEALKAHI LDGINLCGYFAYSFNDRTPRFGLYRYAADQFEPKASMKHYRKIID SNGFPGPETLERFCPEEFTVCTECSFFHTRKSLLAFLFFASIIISL SLIFYYSKKGRRSYK	2
KL2 (wt)	LEGTFPCDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKRLIK VDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHFRFSLDWALILPL GNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPR LLARQGAWENPYTALAFAEYARLCFQELGHHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPAC PFSSQKDKEVAERVLFDIGWLAEPFGSGDYPWWMRDWLNQRNN FLLPYFTEDEKKLIQGTDFDLALSHYTTILVDSEKEDPIKYNDYLEVQ EMTDITWLNSPSQVAVVPWGLRKVLNWLKFKYGDLPYIISNGID	3

Table S - Sequences		
Name	Sequence	SEQ ID NO:
	DGLHAEDDQLRVYYMQNYINEALKAHILDGINLCGYFAYSFNDRTA PRFGLYRYAADQFEPKASMKHYRKIIDS	
KL2 (C521S)	LEGTFPSDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKRLIK VDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHFRFSLDWALILPL GNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPR LLARQGAWENPYTALAFAYARLCFQELGHHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPAC PFSQKDKEVAERVLEFDIGWLAEPFGSGDYPWWMRDWLNQRNN FLLPYFTEDEKKLIQGTDFDLALSHYTTILVDSEKEDPIKYNDYLEVQ EMTDITWLNSPSQVAVVPWGLRKVLNWLKFKYGDLPYIISNGID DGLHAEDDQLRVYYMQNYINEALKAHILDGINLCGYFAYSFNDRTA PRFGLYRYAADQFEPKASMKHYRKIIDS	4
KL2 + c-term loop (wt)	LEGTFPCDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKRLIK VDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHFRFSLDWALILPL GNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPR LLARQGAWENPYTALAFAYARLCFQELGHHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPAC PFSQKDKEVAERVLEFDIGWLAEPFGSGDYPWWMRDWLNQRNN FLLPYFTEDEKKLIQGTDFDLALSHYTTILVDSEKEDPIKYNDYLEVQ EMTDITWLNSPSQVAVVPWGLRKVLNWLKFKYGDLPYIISNGID DGLHAEDDQLRVYYMQNYINEALKAHILDGINLCGYFAYSFNDRTA PRFGLYRYAADQFEPKASMKHYRKIIDSNGFPGPETLERFCPEEFT VCTECSSFHTRKS	5
KL2 + c-term loop (C521S)	LEGTFPSDFAWGVVDNYIQVDTTLSQFTDLNVYLWDVHHSKRLIK VDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHFRFSLDWALILPL GNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPNQGLPR LLARQGAWENPYTALAFAYARLCFQELGHHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPAC PFSQKDKEVAERVLEFDIGWLAEPFGSGDYPWWMRDWLNQRNN FLLPYFTEDEKKLIQGTDFDLALSHYTTILVDSEKEDPIKYNDYLEVQ EMTDITWLNSPSQVAVVPWGLRKVLNWLKFKYGDLPYIISNGID DGLHAEDDQLRVYYMQNYINEALKAHILDGINLCGYFAYSFNDRTA PRFGLYRYAADQFEPKASMKHYRKIIDSNGFPGPETLERFCPEEFT VCTECSSFHTRKS	6
C-term tail region	NGFPGPETLERFCPEEFTVCTECSSFHTRKS	7
C-terminal 23AA of C-term tail region	LERFCPEEFTVCTECSSFHTRKS	8
KL1 (BAA23382.1)	EPGDGAQTWARVSRPPAPEAAGLFQGTFPDGLWAVGSAAYQT EGGWQQHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSIQCQKSLDFVLGWFAKPVFIDGDYPESMKNN	9

Table S - Sequences		
Name	Sequence	SEQ ID NO:
	LSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYYLKKF IMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLS QDKMLLPKSSALFYQKLIKNGF	
KL1 (Q9UEF7-1)	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQT EGGWQQHKGKASIWDTFTHHPLAPPGDSRNASPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFIDGDYPESMKNN LSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYYLKKF IMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLS QDKMLLPKSSALFYQKLIKNGF	10
KL1 through KL2 (wt - Q9UEF7-1)	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQT EGGWQQHKGKASIWDTFTHHPLAPPGDSRNASPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFIDGDYPESMKNN LSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYYLKKF IMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLS QDKMLLPKSSALFYQKLIKNGFPPLPENQPLEGTFPCDFAWGVV DNYIQVDTTSLSQFTDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCV DFAAIQPKIALLQEMHVTHFRFSLDWALILPLGNQSQVNHTILQYY RCMASELVRVNITPVVALWQPMAPNQGLPRLLARQGAWENPYTA LAFAYEARLCFQELGHHVKLWITMNEPYTRNMTYSAGHNLLKAHA LAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEVAERVL EFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQ GTFDFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNPSQ VAVVPWGLRKLVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVY YMQNYINEALKAHILDGINLCGYFAYSFNDRTAPRFLYRYAADQF EPKASMKHYRKIDS	11
KL1 through KL2 (C521S - Q9UEF7-1)	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQT EGGWQQHKGKASIWDTFTHHPLAPPGDSRNASPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFIDGDYPESMKNN LSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYYLKKF IMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLS	12

Table S - Sequences		
Name	Sequence	SEQ ID NO:
	QDKMLLPKSSALFYQKLEKNGFPPLPENQPLEGTFPSDFAWGVV DNYIQVDTTLSQFTDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCV DFAAIQPQIALLQEMHVTHFRFSLDWALILPLGNQSQVNHTILQYY RCMASELVRVNITPVVALWQPMAPNQGLPRLLARQGAWENPYTA LAFAEYARLCFQELGHHVKLWITMNEPYTRNMTYSAGHNLLKAHA LAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEVAERVL EFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQ GTFDFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNPSQ VAVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVY YMQNYINEALKAHILDGINLCGYFAYSFNDRTPRFGLYRYAADQF EPKASMKHYRKIDS	
hKL ECD - wt	EPGDGAQQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQT EGGWQQHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFIDGDYPESMKNN LSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKF IMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLS QDKMLLPKSSALFYQKLEKNGFPPLPENQPLEGTFPCDFAWGVV DNYIQVDTTLSQFTDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCV DFAAIQPQIALLQEMHVTHFRFSLDWALILPLGNQSQVNHTILQYY RCMASELVRVNITPVVALWQPMAPNQGLPRLLARQGAWENPYTA LAFAEYARLCFQELGHHVKLWITMNEPYTRNMTYSAGHNLLKAHA LAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEVAERVL EFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQ GTFDFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNPSQ VAVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVY YMQNYINEALKAHILDGINLCGYFAYSFNDRTPRFGLYRYAADQF EPKASMKHYRKIDSNGFPGPETLERFCPEEFTVCTECSEFFHTRKS	13
hKL ECD - C521S	EPGDGAQQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQT EGGWQQHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFIDGDYPESMKNN LSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKF IMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLS QDKMLLPKSSALFYQKLEKNGFPPLPENQPLEGTFPSDFAWGVV DNYIQVDTTLSQFTDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCV DFAAIQPQIALLQEMHVTHFRFSLDWALILPLGNQSQVNHTILQYY RCMASELVRVNITPVVALWQPMAPNQGLPRLLARQGAWENPYTA	14

Table S - Sequences		
Name	Sequence	SEQ ID NO:
	LAFAEYARLCFQELGHHVKLWITMNEPYTRNMTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEVAERVL EFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQ GTFDLFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNPSQ VAVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVY YMQNYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQF EPKASMKHYRKIDSNGFPGPETLERFCPEEFTVCTECSSFHTRKS	
hKL ECD – 23aa truncation (wt)	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQT EGGWQQHKGKASIWDFTFHHPLAPPGDSRNASLPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSIECQKSLDFVLGWFAKPVFIDGDYPESMKNN LSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTRDDAKYMYLKKF IMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLS QDKMLLPKSSALFYQKLEKNGFPPLPENQPLEGTFPCDFAWGVV DNYIQVDTTLSQFTDLNVYLWDVHHSKRLIKVDGVTTKRKS YCV DFAAIQPQIALLQEMHVTFRFSLDWALILPLGNQSQVNHTILQYY RCMASELVRVNITPVVALWQPMAPNQGLPRLLARQGAWENPYTA LAFAEYARLCFQELGHHVKLWITMNEPYTRNMTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEVAERVL EFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQ GTFDLFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNPSQ VAVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVY YMQNYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQF EPKASMKHYRKIDSNGFPGPET	15
hKL ECD – 23aa truncation (C521S)	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQT EGGWQQHKGKASIWDFTFHHPLAPPGDSRNASLPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSIECQKSLDFVLGWFAKPVFIDGDYPESMKNN LSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTRDDAKYMYLKKF IMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLS QDKMLLPKSSALFYQKLEKNGFPPLPENQPLEGTFPSDFAWGVV DNYIQVDTTLSQFTDLNVYLWDVHHSKRLIKVDGVTTKRKS YCV DFAAIQPQIALLQEMHVTFRFSLDWALILPLGNQSQVNHTILQYY RCMASELVRVNITPVVALWQPMAPNQGLPRLLARQGAWENPYTA LAFAEYARLCFQELGHHVKLWITMNEPYTRNMTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEVAERVL EFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQ GTFDLFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNPSQ	16

Table S - Sequences		
Name	Sequence	SEQ ID NO:
	VAVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVY YMQNYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQF EPKASMKHYRKIIDSNGFPGPET	
hKL ECD – 23aa truncation (C521S + C370S)	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQT EGGWQQHKGASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFQGVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFIDGDYPESMKNN LSSILPDFTESEKKFIKGTADFFALSFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKF IMETLKAIKLDGVVIGYTAWSLMDGFEWHRGYSIRRGIFYVDFLS QDKMLLPKSSALFYQKLEKNGFPPLPENQPLEGTFPSDFAWGVV DNYIQVDTTLSQFTDLNVYLWDVHHSKRLIKVDGVVTKRKSYSYCV DFAAIQPIALLQEMHVTHFRFSLDWALILPLGNQSQVNHTILQYY RCMASELVRVNITPVVALWQPMAPNQGLPRLLARQGAWENPYTA LAFAYEARLCFQELGHHVKLWITMNEPYTRNMTYSAGHNLLKAHA LAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEVAERVL EFDIGWLAEPFYGSGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQ GTFDFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNPSQ VAVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVY YMQNYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQF EPKASMKHYRKIIDSNGFPGPET	68
hKL Transmembrane domain	LLAFIAFLFFASIIISLSLIFY	17
hKL Intracellular domain	YSKKGRRSYK	18
Human Serum Albumin (wt)	DAHKSEVAHRFKDLGEENFKALVLIAMFAQYLQQCPFEDHVKLVNE VTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADC CAKQEPERNECFQHKDDNPPLRLVRPEVDMCTAFHDNEETF LKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAAACLLP KLDELDRDEGKASSAKQRLKCSLQKFGERAFAKAWAVARLSQRFP KAFAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQD SISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDV CKNYAEAKDVFLGMFLYFYARRHPDYSVLLLRALAKTYETTLKCK CAAADPHECYAKVFDEFKPLVEEPQNLKQNCLEFQELGEYKFN ALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAE DYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDE TYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKE QLKAVMDDFAAFVEKCKADDKETCFEEGKKLVAASQAALGL	19
Human Serum Albumin (C34S)	DAHKSEVAHRFKDLGEENFKALVLIAMFAQYLQQSPFEDHVKLVNE VTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADC CAKQEPERNECFQHKDDNPPLRLVRPEVDMCTAFHDNEETF LKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAAACLLP	20

Table S - Sequences		
Name	Sequence	SEQ ID NO:
	KLDEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFP KA EFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQD SISSKLKECCEKPLLEKSHCIAEVENDEMPADLP SLAADFVESKDV CKNYAEAKDVFLGMFLYEYARRHPDYSVLLLR LAKTYETTLEKC CAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQN ALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAE DYLSVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDE TYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKE QLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGL	
Human KL interdomain region	PPLPENQP	21
Human KL C-terminal tail region (truncated by 23AA)	NGFPGPET	22
KL1 (BAA23382.1) (C370S)	EPGDGAQTWARVSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQT EGGWQQHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSI ECQKSLDFVLGWFAKPVFIDGDYPESMKNN LSSILPDFTESEKKFIKGTADFFALSFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFS GTTKRDDAKYMYYLKKF IMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLS QDKMLLPKSSALFYQK LIEKNGF	64
KL1 (Q9UEF7-1) (C370S)	EPGDGAQTWARVSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQT EGGWQQHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSI ECQKSLDFVLGWFAKPVFIDGDYPESMKNN LSSILPDFTESEKKFIKGTADFFALSFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFS GTTKRDDAKYMYYLKKF IMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLS QDKMLLPKSSALFYQK LIEKNGF	65
KL1 through KL2 (C370S+C521S - Q9UEF7-1)	EPGDGAQTWARVSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQT EGGWQQHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSI ECQKSLDFVLGWFAKPVFIDGDYPESMKNN	66

Table S - Sequences		
Name	Sequence	SEQ ID NO:
	LSSILPDFTESEKKFIKGTADFFALSFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKF IMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLS QDKMLLPKSSALFYQKLEKNGFPPLPENQPLEGTFPSDFAWGVV DNYIQVDTTLSQFTDLNVYLWDVHHSKRLIKVDGVTKKRKSVCV DFAAIQPQIALLQEMHVTHFRFSLDWALILPLGNQSQVNHTILQYY RCMASELVRVNITPVVALWQPMAPNQGLPRLLARQGAWENPYTA LAFAEYARLCFQELGHHVKLWITMNEPYTRNMTYSAGHNLLKAHA LAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEVAERVL EFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQ GTFDFLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNPSQ VAVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVY YMQNYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQF EPKASMKHYRKIDS	
hKL ECD – C370S+C521S	EPGDGAQTWARFSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQT EGGWQQHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSIECQKSLDFVLGWFAKPVFIDGDYPESMKNN LSSILPDFTESEKKFIKGTADFFALSFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKF IMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLS QDKMLLPKSSALFYQKLEKNGFPPLPENQPLEGTFPSDFAWGVV DNYIQVDTTLSQFTDLNVYLWDVHHSKRLIKVDGVTKKRKSVCV DFAAIQPQIALLQEMHVTHFRFSLDWALILPLGNQSQVNHTILQYY RCMASELVRVNITPVVALWQPMAPNQGLPRLLARQGAWENPYTA LAFAEYARLCFQELGHHVKLWITMNEPYTRNMTYSAGHNLLKAHA LAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKDKEVAERVL EFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQ GTFDFLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNPSQ VAVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVY YMQNYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQF EPKASMKHYRKIDSNGFPGPETLERFCPEEFTVCTECSFFHTRKS	67
hKL ECD – 23aa truncation (C370S+C521S)	EPGDGAQTWARFSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQT EGGWQQHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPA TGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVP NREGLRYYRLLERLRELGVQPVVTLYHWDLPQRLQDAYGGWAN RALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLA PGIRGSPRLGYLVAHNLLLAHAKVWHLYNTSFRPTQGGQVSIALS SHWINPRRMTDHSIECQKSLDFVLGWFAKPVFIDGDYPESMKNN LSSILPDFTESEKKFIKGTADFFALSFGPTLSFQLLDPHMKFRQLES PNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKF IMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLS QDKMLLPKSSALFYQKLEKNGFPPLPENQPLEGTFPSDFAWGVV	68

Table S - Sequences		
Name	Sequence	SEQ ID NO:
	DNYIQVDTTLSQFTDLNVYLWDVHHSKRLIKVDGWVTKKRKSYCV DFAAIQPQIALQEMHVTHFRFSLDWALILPLGNQSQVNHTILQYY RCMASELVRVNITPVVALWQPMAPNQGLPRLLARQGAWENPYTA LAFAEYARLCFQELGHHVKLWITMNEPYTRNMTYSAGHNLLKAHA LAWHVYNEKFRHAQNGKISIALQADWIEPACPFSQKDKEVAERVL EFDIGWLAEPFIGSGDYPWWMRDWLNQRNNFLLPYFTEDEKKLIQ GTFDFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNPSQ VAVVPWGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVY YMQNYINEALKAHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQF EPKASMKHYRKIIDSNGFPGPET	

8. SPECIFIC EMBODIMENTS

[0141] While various specific embodiments have been illustrated and described, it will be appreciated that various changes can be made without departing from the spirit and scope of the disclosure(s). The present disclosure is exemplified by the numbered embodiments set forth below.

[0142] In the numbered embodiments that follow, the alpha klotho moieties are preferably derived from a mammalian alpha klotho, the albumin moieties are preferably derived from a mammalian albumin, Fc domains are preferably derived from a mammalian antibody, and the subjects are preferably mammals. More preferably, the mammal is human.

1. A polypeptide which:

(a) comprises an alpha klotho KL2 domain having (i) at least about 80% sequence identity to SEQ ID NO:3 and (ii) an amino acid substitution at the position corresponding to amino acid C521 of SEQ ID NO:1; and

(b) lacks a cysteine at the amino acid corresponding to amino acid C970 of SEQ ID NO:1, if present.
2. The polypeptide of embodiment 1 which lacks an amino acid corresponding to amino acid C970 of SEQ ID NO:1.

3. The polypeptide of embodiment 1 which lacks a cysteine at the amino acid corresponding to C973 of SEQ ID NO:1, if present.
4. The polypeptide of embodiment 3 which lacks an amino acid corresponding to amino acid C973 of SEQ ID NO:1.
5. The polypeptide of embodiment 1 which lacks a cysteine at the amino acid corresponding to C963 of SEQ ID NO:1, if present.
6. The polypeptide of embodiment 5 which lacks an amino acid corresponding to amino acid C963 of SEQ ID NO:1.
7. The polypeptide of any one of embodiments 1 to 6, wherein the amino acid substitution at the position corresponding to amino acid C521 of SEQ ID NO:1 is a cysteine to serine mutation.
8. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 12 amino acids as compared to the amino acid sequence of SEQ ID NO:13.
9. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 13 amino acids as compared to the amino acid sequence of SEQ ID NO:13.
10. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 14 amino acids as compared to the amino acid sequence of SEQ ID NO:13.
11. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 15 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

12. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 16 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

13. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 17 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

14. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 18 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

15. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 19 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

16. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 20 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

17. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 21 amino acids as compared to the amino acid sequence of SEQ ID NO:13

18. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 22 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

19. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 23 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

20. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 24 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

21. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 25 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

22. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 26 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

23. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 27 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

24. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 28 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

25. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 29 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

26. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 30 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

27. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of at least 31 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

28. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 12 and 31 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

29. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 13 and 31 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

30. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 14 and 31 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

31. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 15 and 31 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

32. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 16 and 31 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

33. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 17 and 31 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

34. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 18 and 31 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

35. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 19 and 31 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

36. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 20 and 31 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

37. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 21 and 31 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

38. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 22 and 31 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

39. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 23 and 31 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

40. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 12 and 23 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

41. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 13 and 23 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

42. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 14 and 23 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

43. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 15 and 23 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

44. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 16 and 23 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

45. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 17 and 23 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

46. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 18 and 23 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

47. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 19 and 23 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

48. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 20 and 23 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

49. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 21 and 23 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

50. The polypeptide of any one of embodiments 1 to 7 which comprises a C-terminal deletion of between 22 and 23 amino acids as compared to the amino acid sequence of SEQ ID NO:13.

51. The polypeptide of any one of embodiments 1 to 50, which lacks an amino acid sequence having at least 95% sequence identity to SEQ ID NO:8.

52. The polypeptide of any one of embodiments 1 to 51, which lacks an amino acid sequence having at least 98% sequence identity to SEQ ID NO:8.

53. The polypeptide of any one of embodiments 1 to 52, which lacks the amino acid sequence of SEQ ID NO:8.

54. The polypeptide of any one of embodiments 1 to 53, wherein the alpha klotho KL2 domain has at least about 85% sequence identity to SEQ ID NO:3.

55. The polypeptide of any one of embodiments 1 to 54, wherein the alpha klotho KL2 domain has at least about 90% sequence identity to SEQ ID NO:3.

56. The polypeptide of any one of embodiments 1 to 55, wherein the alpha klotho KL2 domain has at least about 95% sequence identity to SEQ ID NO:3.

57. The polypeptide of any one of embodiments 1 to 56, wherein the alpha klotho KL2 domain has at least about 99% sequence identity to SEQ ID NO:3.

58. The polypeptide of any one of embodiments 1 to 57, wherein the alpha klotho KL2 domain has at least about 99.5% sequence identity to SEQ ID NO:3.

59. The polypeptide of any one of embodiments 1 to 53, wherein the alpha klotho KL2 domain has at least about 85% sequence identity to SEQ ID NO:4.

60. The polypeptide of any one of embodiments 1 to 54, wherein the alpha klotho KL2 domain has at least about 90% sequence identity to SEQ ID NO:4.

61. The polypeptide of any one of embodiments 1 to 55, wherein the alpha klotho KL2 domain has at least about 95% sequence identity to SEQ ID NO:4.

62. The polypeptide of any one of embodiments 1 to 56, wherein the alpha klotho KL2 domain has at least about 99% sequence identity to SEQ ID NO:4.

63. The polypeptide of any one of embodiments 1 to 57, wherein the alpha klotho KL2 domain has at least about 99.5% sequence identity to SEQ ID NO:4.

64. The polypeptide of any one of embodiments 1 to 63, wherein the alpha klotho KL2 domain comprises the amino acid sequence of SEQ ID NO:4.

65. The polypeptide of any one of embodiments 1 to 64, further comprising an alpha klotho KL1 domain having at least about 80% sequence identity to SEQ ID NO:9.

66. The polypeptide of embodiment 65, wherein the alpha klotho KL1 domain has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO:9.

67. The polypeptide of embodiment 65, wherein the alpha klotho KL1 domain has at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:9.

68. The polypeptide of embodiment 65, wherein the alpha klotho KL1 domain has at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:9.

69. The polypeptide of embodiment 65, wherein the alpha klotho KL1 domain comprises the amino acid sequence of SEQ ID NO:9.

70. The polypeptide of embodiment 65, wherein the alpha klotho KL1 domain has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO:10.

71. The polypeptide of embodiment 65, wherein the alpha klotho KL1 domain has at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:10.

72. The polypeptide of embodiment 65, wherein the alpha klotho KL1 domain has at least about 99% sequence identity to the amino acid sequence of SEQ ID NO:10.

73. The polypeptide of embodiment 65, wherein the alpha klotho KL1 domain comprises the amino acid sequence of SEQ ID NO:10.

74. The polypeptide of any one of embodiments 65 to 73, wherein the alpha klotho KL1 domain comprises an amino acid substitution at the position corresponding to position C370 of SEQ ID NO:1.

75. The polypeptide of embodiment 74, wherein the amino acid substitution at the position corresponding to position C370 of SEQ ID NO:1 is a cysteine to serine substitution.

76. The polypeptide of any one of embodiments 65 to 75, wherein the alpha klotho KL1 domain comprises the amino acid sequence of SEQ ID NO:9.

77. The polypeptide of any one of embodiments 1 to 76 which comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:11.

78. The polypeptide of any one of embodiments 1 to 76 which comprises an amino acid sequence having at least about 98% sequence identity to SEQ ID NO:11.

79. The polypeptide of any one of embodiments 1 to 76 which comprises an amino acid sequence having at least about 99% sequence identity to SEQ ID NO:11.

80. The polypeptide of any one of embodiments 1 to 77 which comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:12.

81. The polypeptide of any one of embodiments 1 to 77 which comprises an amino acid sequence having at least about 98% sequence identity to SEQ ID NO:12.

82. The polypeptide of any one of embodiments 1 to 77 which comprises an amino acid sequence having at least about 99% sequence identity to SEQ ID NO:12.

83. The polypeptide of any one of embodiments 1 to 77 which comprises an amino acid sequence having at least about 99.5% sequence identity to SEQ ID NO:12.

84. The polypeptide of any one of embodiments 80 to 83 which comprises an amino acid sequence having one amino acid substitution relative to SEQ ID NO:12.

85. The polypeptide of any one of embodiments 80 to 83 which comprises an amino acid sequence having two amino acid substitutions relative to SEQ ID NO:12.

86. The polypeptide of any one of embodiments 80 to 83 which comprises an amino acid sequence having three amino acid substitutions relative to SEQ ID NO:12.

87. The polypeptide of any one of embodiments 80 to 83 which comprises an amino acid sequence having four amino acid substitutions relative to SEQ ID NO:12.

88. The polypeptide of any one of embodiments 80 to 83 which comprises an amino acid sequence having five amino acid substitutions relative to SEQ ID NO:12.

89. The polypeptide of any one of embodiments 80 to 83 which comprises an amino acid sequence having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions relative to SEQ ID NO:12.

90. The polypeptide of any one of embodiments 80 to 83 which comprises an amino acid sequence having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid variations (substitutions, deletions, or insertions) relative to SEQ ID NO:12.

91. The polypeptide of any one of embodiments 1 to 77 which comprises the amino acid sequence of SEQ ID NO:12.

92. The polypeptide of any one of embodiments 1 to 91 which comprises an amino acid sequence having at least about 90% sequence identity to SEQ ID NO:15.

93. The polypeptide of any one of embodiments 1 to 91 which comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:15.

94. The polypeptide of any one of embodiments 1 to 91 which comprises an amino acid sequence having at least about 90% sequence identity to SEQ ID NO:16.

95. The polypeptide of any one of embodiments 1 to 91 which comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:16.
96. The polypeptide of any one of embodiments 92 to 95, wherein the amino acid sequence is between 850 and 950 amino acids in length.
97. The polypeptide of any one of embodiments 92 to 95, wherein the amino acid sequence is between 900 and 925 amino acids in length.
98. The polypeptide of any one of embodiments 92 to 95, wherein the amino acid sequence is between 910 and 920 amino acids in length.
99. The polypeptide of any one of embodiments 92 to 95, wherein the amino acid sequence is between 915 and 920 amino acids in length.
100. The polypeptide of any one of embodiments 1 to 99 which comprises the amino acid sequence of SEQ ID NO:16.
101. The polypeptide of any one of embodiments 1 to 100 which lacks an amino acid sequence having at least 95% sequence identity to SEQ ID NO:17.
102. The polypeptide of any one of embodiments 1 to 100 which lacks an amino acid sequence having at least 98% sequence identity to SEQ ID NO:17.
103. The polypeptide of any one of embodiments 1 to 100 which lacks the amino acid sequence of SEQ ID NO:17.
104. The polypeptide of any one of embodiments 1 to 103, further comprising a signal peptide.
105. The polypeptide of embodiment 104, wherein the signal peptide is an alpha klotho signal peptide.

106. The polypeptide of embodiment 104, wherein the signal peptide is not an alpha klotho signal peptide.

107. The polypeptide of embodiment 106, wherein the signal peptide is a serum albumin (SA) signal peptide.

108. The polypeptide of embodiment 106, wherein the signal peptide is an azurocidin (AZ) signal peptide.

109. The polypeptide of embodiment 106, wherein the signal peptide is an SP1 signal peptide.

110. The polypeptide of any one of embodiments 1 to 103 which lacks a signal peptide.

111. The polypeptide of any one of embodiments 1 to 110, further comprising a stabilization moiety.

112. The polypeptide of embodiment 111, wherein the stabilization moiety is C-terminal to the alpha klotho KL2 domain.

113. The polypeptide of embodiment 111, wherein the stabilization moiety is N-terminal to the alpha klotho KL2 domain.

114. The polypeptide of any one of embodiments 111 to 113, wherein the stabilization moiety comprises an Fc domain.

115. The polypeptide of embodiment 114, wherein the Fc domain comprises a CH2 domain and a CH3 domain.

116. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO:23.

117. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:23.

118. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:23.

119. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO:23.

120. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO:23.

121. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO:23.

122. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises the amino acid sequence of SEQ ID NO:23.

123. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO:26.

124. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:26.

125. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:26.

126. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO:26.

127. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO:26.

128. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO:26.

129. The polypeptide of embodiment 114 or 115, wherein the Fc domain comprises the amino acid sequence of SEQ ID NO:26.

130. The polypeptide of embodiment 114, wherein the Fc domain further comprises an additional CH3 domain.

131. The polypeptide of embodiment 130, wherein the additional CH3 domain is connected to the CH3 domain via a linker.

132. The polypeptide of embodiment 130 or 131, wherein the Fc domain comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOs:33-40.

133. The polypeptide of embodiment 130 or 131, wherein the Fc domain comprises an amino acid sequence having at least 85% sequence identity to any one of SEQ ID NOs:33-40.

134. The polypeptide of embodiment 130 or 131, wherein the Fc domain comprises an amino acid sequence having at least 90% sequence identity to any one of SEQ ID NOs:33-40.

135. The polypeptide of embodiment 130 or 131, wherein the Fc domain comprises an amino acid sequence having at least 95% sequence identity to any one of SEQ ID NOs:33-40.

136. The polypeptide of embodiment 130 or 131, wherein the Fc domain comprises an amino acid sequence having at least 98% sequence identity to any one of SEQ ID NOs:33-40.

137. The polypeptide of embodiment 130 or 131, wherein the Fc domain comprises an amino acid sequence having at least 99% sequence identity to any one of SEQ ID NOs:33-40.

138. The polypeptide of embodiment 130 or 131, wherein the Fc domain comprises the amino acid sequence of any one of SEQ ID NOs:33-40.

139. The polypeptide of embodiment 114, wherein the Fc domain is a non-dimerizing Fc domain.

140. The polypeptide of embodiment 139, wherein the non-dimerizing Fc domain comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOs:27-32.

141. The polypeptide of embodiment 139, wherein the non-dimerizing Fc domain comprises an amino acid sequence having at least 85% sequence identity to any one of SEQ ID NOs:27-32.

142. The polypeptide of embodiment 139, wherein the non-dimerizing Fc domain comprises an amino acid sequence having at least 90% sequence identity to any one of SEQ ID NOs:27-32.

143. The polypeptide of embodiment 139, wherein the non-dimerizing Fc domain comprises an amino acid sequence having at least 95% sequence identity to any one of SEQ ID NOs:27-32.

144. The polypeptide of embodiment 139, wherein the non-dimerizing Fc domain comprises an amino acid sequence having at least 98% sequence identity to any one of SEQ ID NOs:27-32.

145. The polypeptide of embodiment 139, wherein the non-dimerizing Fc domain comprises an amino acid sequence having at least 99% sequence identity to any one of SEQ ID NOs:27-32.

146. The polypeptide of embodiment 139, wherein the non-dimerizing Fc domain comprises the amino acid sequence of any one of SEQ ID NOs:27-32.

147. The polypeptide of any one of embodiments 111 to 113, wherein the stabilization moiety is an albumin moiety.

148. The polypeptide of embodiment 147, wherein the albumin moiety is human serum albumin.

149. The polypeptide of embodiment 147, wherein the albumin moiety is a human serum albumin variant.

150. The polypeptide of embodiment 149, wherein the human serum albumin variant has the amino acid substitution C34S.

151. The polypeptide of embodiment 149 or 150, wherein the albumin moiety comprises an amino acid sequence having at least about 90% sequence identity to SEQ ID NO:20.

152. The polypeptide of embodiment 149 or 150, wherein the albumin moiety comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:20.

153. The polypeptide of embodiment 149 or 150, wherein the albumin moiety comprises an amino acid sequence having at least about 98% sequence identity to SEQ ID NO:20.

154. The polypeptide of embodiment 149 or 150, wherein the albumin moiety comprises the amino acid sequence of SEQ ID NO:20.

155. The polypeptide of any one of embodiments 111 to 154, further comprising a linker.

156. The polypeptide of embodiment 155, wherein the polypeptide comprises, in N- to C-terminal order: an alpha klotho moiety comprising the v KL2 domain, the linker, and the stabilization moiety.

157. The polypeptide of embodiment 155, wherein the polypeptide comprises, in N- to C-terminal order: the stabilization moiety, the linker, and an alpha klotho moiety comprising the alpha klotho KL2 domain.

158. The polypeptide of any one of embodiments 155 to 157, wherein the linker comprises one or more amino acid sequences set forth in Table L.

159. The polypeptide of any one of embodiments 1 to 158, wherein the polypeptide is a monomer.

160. The polypeptide of any one of embodiments 1 to 158, wherein the polypeptide is a dimer.

161. A polypeptide, optionally a polypeptide of any one of embodiments 1 to 160, comprising an amino acid sequence having at least 80% sequence identity to SEQ ID NO:16, wherein the position corresponding to amino acid 488 of SEQ ID NO:16 is not a cysteine and which lacks an amino acid sequence having at least 80% sequence identity to SEQ ID NO:8.

162. The polypeptide of embodiment 161, wherein the position corresponding to amino acid 488 of SEQ ID NO:16 is a serine.

163. The polypeptide of embodiment 161 or 162, which comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 16.

164. The polypeptide of embodiment 161 or 162, which comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 16.

165. The polypeptide of embodiment 161 or 162, which comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 16.

166. The polypeptide of embodiment 161 or 162, which comprises an amino acid sequence having at least 96% sequence identity to SEQ ID NO: 16.

167. The polypeptide of embodiment 161 or 162, which comprises an amino acid sequence having at least 97% sequence identity to SEQ ID NO: 16.

168. The polypeptide of embodiment 161 or 162, which comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 16.

169. The polypeptide of embodiment 161 or 162, which comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 16.

170. The polypeptide of embodiment 161 or 162, which comprises the amino acid sequence of SEQ ID NO: 16.

171. The polypeptide of any one of embodiments 161 to 170, which lacks an amino acid sequence having at least 90% sequence identity to SEQ ID NO:8.

172. The polypeptide of any one of embodiments 161 to 170, which lacks an amino acid sequence having at least 95% sequence identity to SEQ ID NO:8.

173. The polypeptide of any one of embodiments 161 to 170, which lacks the amino acid sequence of SEQ ID NO:8.

174. The polypeptide of any one of embodiments 1 to 173, which comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:62.

175. The polypeptide of embodiment 174, which comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO:62.

176. The polypeptide of embodiment 174, which comprises an amino acid sequence having at least 96% sequence identity to SEQ ID NO:62.

177. The polypeptide of embodiment 174, which comprises an amino acid sequence having at least 97% sequence identity to SEQ ID NO:62.

178. The polypeptide of embodiment 174, which comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO:62.

179. The polypeptide of embodiment 174, which comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO:62.

180. The polypeptide of embodiment 174, which comprises an amino acid sequence having at least 99.5% sequence identity to SEQ ID NO:62.

181. The polypeptide of embodiment 174, which comprises the amino acid sequence of SEQ ID NO:62.

182. A nucleic acid encoding the polypeptide of any one of embodiments 1 to 181.

183. A host cell engineered to express the polypeptide of any one of embodiments 1 to 181 or the nucleic acid of embodiment 182.

184. A method of producing the polypeptide of any one of embodiments 1 to 181, comprising culturing the host cell of embodiment 183 and recovering the polypeptide expressed thereby.

185. The method of embodiment 184, further comprising purifying the polypeptide.

186. The method of embodiment 185, wherein the polypeptide is purified at a pH between 6.5 and 7.5.

187. The method of embodiment 185 or 186, wherein the polypeptide is purified at a pH between 6.9 and 7.1.

188. The method of any one of embodiments 185 or 187, wherein the polypeptide is purified at a pH of about 7.0.

189. A pharmaceutical composition comprising the polypeptide of any one of embodiments 1 to 181 and an excipient.

190. A method of activating FGFR signaling in a cell, the method comprising contacting the cell with the polypeptide of any one of embodiments 1 to 181.

191. The method of embodiment 190, wherein the cell is a kidney cell.

192. The method of embodiment 190 or 191, wherein the method is an in vitro method.

193. The method of embodiment 190 or 191, wherein the method is an in vivo method.

194. The method of embodiment 193, wherein the method comprises administering the polypeptide to a subject in need thereof.

195. The method of embodiment 194, wherein the subject is at risk for developing an age-related condition.

196. The method of embodiment 194, wherein the subject is a patient suffering from an age-related condition.

197. The method of embodiment 194, wherein the subject is at risk for developing kidney disease.

198. The method of embodiment 197, wherein the kidney disease is acute kidney injury.

199. The method of embodiment 197, wherein the kidney disease is chronic kidney disease.

200. A method of treating a subject suffering from an age-related condition comprising administering to the subject the polypeptide of any one of embodiments 1 to 181 or the pharmaceutical composition of embodiment 189.

201. A method of preventing an age-related condition comprising administering to a subject in need thereof the polypeptide of any one of embodiments 1 to 181 or the pharmaceutical composition of embodiment 189.

202. A method of treating a subject suffering from kidney disease comprising administering to the subject the polypeptide of any one of embodiments 1 to 181 or the pharmaceutical composition of embodiment 189.

203. A method of preventing kidney disease comprising administering to a subject in need thereof the polypeptide of any one of embodiments 1 to 181 or the pharmaceutical composition of embodiment 189.

204. The method of embodiment 202 or 203, wherein the kidney disease is acute kidney injury.

205. The method of embodiment 202 or 203, wherein the kidney disease is chronic kidney disease.

9. EXAMPLES

9.1. Materials and Methods

9.1.1. Design and Production of KL Polypeptide Constructs

[0143] KL polypeptide constructs were designed using the wildtype human alpha-Klotho (hKL) amino acid sequence (UniProtKB Accession Number Q9UEF7-1; FIG. 3). Where applicable, 29-amino acid signal sequence from murine inactive tyrosine-protein kinase transmembrane receptor ROR1 (mROR1) was used to replace the native KL signal sequence to increase production yield; the hKL amino acid sequence was truncated at the C-terminus to remove the C-terminal protease cleavage site; one or more Cys amino acids were mutated to Ser to remove free surface Cys residues; and one or more stabilization moieties were added via a linker to the N- or C-termini of constructs. Certain exemplary KL polypeptide constructs are shown in FIGS. 2A-2J.

[0144] Constructs encoding KL polypeptides were generated in standard mammalian protein expression DNA vectors (pcDNA3.4 or similar) suitable for high yield protein production and containing standard elements such as promoter sequence, polyA sequence, regulatory elements, and resistance genes. Where applicable, sequences were codon optimized. The KL polypeptide constructs were expressed in suitable cells (e.g., Expi293 or CHO cells) by transient transfection. Proteins in cellular supernatants were purified using one of the methods described in Section 9.1.2, neutralized, dialyzed into a final buffer of phosphate buffered saline (PBS) with 5% glycerol, aliquoted, and stored at -80 °C. Samples were further analyzed by size exclusion chromatography (SEC) to determine the presence

of high or low molecular weight species relative to the species of interest as described in Section 9.1.2. Exemplary sequences are set forth in Table E1.

Table E1		
Name	Sequence	SEQ ID NO:
REGN14416 (KL981-His) nucleotide sequence	GAGCCGGGCGACGGCGCGCAGACCTGGGCCCGTTTCTCGCGGCCTCC TGCCCCCGAGGCCGCGGGCCTCTTCCAGGGCACCTTCCCCGACGGCTT CCTCTGGGCCGTGGGCAGCGCCGCTACCAGACCGAGGGCGGCTGGC AGCAGCACGGCAAGGGTGCGTCCATCTGGGATACGTTACCCACCACC CCCTGGCACCCCCGGGAGACTCCCGGAACGCCAGTCTGCCGTTGGGC GCCCCGTGCGCCGTGCAGCCCGCCACCGGGGACGTAGCCAGCGACAG CTACAACAACGTCTTCCGCGACACGGAGGCGCTGCGCGAGCTCGGGGT CACTCACTACCGCTTCTCCATCTCGTGGGCGCGAGTGCTCCCCAATGGC AGCGCGGGCGTCCCCAACCGCGAGGGGCTGCGCTACTACCGGCGCCT GCTGGAGCGGCTGCGGGAGCTGGGCGTGCAGCCCGTGGTCACCCTGT ACCACTGGGACCTGCCCCAGCGCCTGCAGGACGCCTACGGCGGCTGG GCCAACCGCGCCCTGGCCGACCACTTCAGGGATTACGCGGAGCTCTGC TTCCGCCACTTCGGCGGTCAAGTCAAGTACTGGATCACCATCGACAACC CCTACGTGGTGGCTTGGCACGGCTACGCCACCGGGCGCCTGGCCCCC GGCATCCGGGGCAGCCCGCGGCTCGGGTACCTGGTGGCGCACAACT CCTCCTGGCTCATGCCAAAGTCTGGCATCTCTACAATACTTCTTTCCGTC CCACTCAGGGAGGTCAAGGTGTCCATTGCCCTAAGCTCTCACTGGATCAA TCCTCGAAGAATGACCGACCACAGCATCAAAGAATGTCAAAAATCTCTG GACTTTGTACTAGGTTGGTTTGCCAAACCGTATTTATTGATGGTACTA TCCCCGAGAGCATGAAGAATAACCTTTCATCTATTCTGCCGTATTTTACTG AATCTGAGAAAAAGTTCATCAAAGGAAGTCTGACTTTTTTGTCTTTTGC TTTGGACCCACCTTGAGTTTTCAACTTTTGGACCTCACATGAAGTTCCG CCAATTGGAATCTCCCAACCTGAGGCAACTGCTTTCCTGGATTGACCTT GAATTTAACCATCCTCAAATATTTATTGTGGAATGGCTGGTTTGTCTCA GGGACCACCAAGAGAGATGATGCCAAATATATGTATTACCTCAAAAAGTT CATCATGGAACCTTAAAAGCCATCAAGCTGGATGGGGTGGATGTCATC GGGTATACCGCATGGTCCCTCATGGATGGTTTTCGAGTGGCACAGAGGT ACAGCATCAGGCGTGGACTCTTCTATGTTGACTTTCTAAGCCAGGACAA GATGTTGTTGCCAAAGTCTTCAGCCTTGTCTACCAAAAGCTGATAGAGA AAAATGGCTTCCCTCCTTTACCTGAAAATCAGCCCCTAGAAGGGACATTT CCCTGTGACTTTGCTTGGGGAGTTGTTGACAACCTACATTCAAGTAGATAC CACTCTGTCTCAGTTTACCGACCTGAATGTTTACCTGTGGGATGTCCACC ACAGTAAAAGGCTTATTAAGTGGATGGGGTTGTGACCAAGAAGAGGAA ATCCTACTGTGTTGACTTTGCTGCCATCCAGCCCCAGATCGCTTTACTCC AGGAAATGCACGTTACACATTTTCGCTTCTCCCTGGACTGGGCCCTGAT TCTCCCTCTGGGTAACCAAGTCCCAGGTGAACCACACCATCCTGCAGTAC TATCGCTGCATGGCCAGCGAGCTTGTCCGTGTCAACATCACCCCAAGTGG TGGCCCTGTGGCAGCCTATGGCCCCGAACCAAGGACTGCCGCGCCTCC TGGCCAGGCAGGGCGCCTGGGAGAACCCCTACACTGCCCTGGCCTTTG CAGAGTATGCCCCGACTGTGCTTTCAAGAGCTCGGCCATCAGTCAAGCT TTGGATAACGATGAATGAGCCGTATACAAGGAATATGACATACAGTGCT GGCCACAACCTTCTGAAGGCCCATGCCCTGGCTTGGCATGTGTACAATG AAAAGTTTAGGCATGCTCAGAATGGGAAAATATCCATAGCCTTGCAGGC TGATTGGATAGAACCTGCCTGCCCTTCTCCCAAAAGGACAAAGAGGTG	41

Table E1		
Name	Sequence	SEQ ID NO:
	GCTGAGAGAGTTTTGGAATTTGACATTGGCTGGCTGGCTGAGCCCATTT TCGGCTCTGGAGATTATCCATGGGTGATGAGGGACTGGCTGAACCAAAG AAACAATTTTCTTCTTCTTATTTCACTGAAGATGAAAAAAGCTAATCCA GGGTACCTTTGACTTTTTGGCTTTAAGCCATTATACCACCATCCTTGTAG ACTCAGAAAAAGAAGATCCAATAAAATACAATGATTACCTAGAAGTGCAA GAAATGACCGACATCACGTGGCTCAACTCCCCAGTCAGGTGGCGGTA GTGCCCTGGGGGTGCGCAAAGTGCTGAAGTGGCTGAAGTTCAAGTAC GGAGACCTCCCCATGTACATAATATCCAATGGAATCGATGACGGGCTGC ATGCTGAGGACGACCGAGCTGAGGGTGTATTATATGCAGAATTACATAAA CGAAGCTCTCAAAGCCACATACTGGATGGTATCAATCTTTGCGGACTACT TTGCTTATTCGTTTAACGACCGCACAGCTCCGAGGTTTGGCCTCTATCGT TATGCTGCAGATCAGTTTGAGCCCAAGGCATCCATGAAACATTACAGGA AAATTATTGACAGCAATGGTTTCCCGGGCCAGAACTCTGGAAAGATTT TGTCAGAGAATTCACCGTGTGTACTGAGTGCAGTTTTTTTACACCCCG AAAGTCTCACCATCACCATCACCATTGA	
REGN14416 (KL981-His) Amino acid sequence	EPGDGAQTWARFSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQTEGGWQ QHKGASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPATGDVASDSYNN VFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYRLLERLRE LGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFQGV KYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLY NTSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFID GDYPESMKNNLSSILPDTFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKF RQLESPNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKFI METLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLSQDKMLL PKSSALFYQKLIKNGFPPLPENQPLEGTFPCDFAWGVVDNYIQVDTTLSQF TDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHF RFSLDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPN QGLPRLARQGAWENPYTALAFAYEARLCFQELGHHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKD KEVAERVLEFDIGWLAEPFSGSDYPWVMRDWLNQRNNFLLPYFTEDEKKL IQGTFDFLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNNSPSQVAVVP WGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVYYMQNYINEALK AHILDGINLCGYFAYSFNDRTAPRFLYRYAADQFEPKASMKHYRKIIDSNG FPGPETLERFCPEEFTVCTECSFFHTRKSHHHHHH	42
REGN14225 (KL958-His) Nucleotide sequence	GAGCCGGGCGACGGCGCGCAGACCTGGGCCCGTTTCTCGCGGCCTCC TGCCCCCGAGGCCGCGGGCCTCTTCCAGGGCACCTTCCCCGACGGCTT CCTCTGGGCCGTGGGCAGCGCCGCTACCAGACCGAGGGCGGCTGGC AGCAGCACGGCAAGGGTGCGTCCATCTGGGATACGTTACCCACCACC CCCTGGCACCCCCGGGAGACTCCCGGAACGCCAGTCTGCCGTTGGGC GCCCCGTGCGCGCTGCAGCCCGCCACCGGGGACGTAGCCAGCGACAG CTACAACAACGTCTTCCGCGACACGGAGCGCTGCGCGAGCTCGGGGT CACTCACTACCGCTTCTCCATCTCGTGGGCGCGAGTGCTCCCCAATGGC AGCGCGGGCGTCCCCAACCGCGAGGGGCTGCGCTACTACCGGCGCCT GCTGGAGCGGCTGCGGGAGCTGGGCGTGACGCCCGTGGTCACCTGT ACCACTGGGACCTGCCCCAGCGCCTGCAGGACGCCTACGGCGGCTGG GCCAACCGCGCCCTGGCCGACCACTTCAGGGATTACGCGGAGCTCTGC TTCCGCCACTTCGGCGGTCAGGTCAAGTACTGGATCACCATCGACAACC CCTACGTGGTGGCTGGCACGGCTACGCCACCGGGCGCCTGGCCCCC	43

Table E1		
Name	Sequence	SEQ ID NO:
	GGCATCCGGGGCAGCCCGCGGCTCGGGTACCTGGTGGCGCACAACTCCTCCTGGCTCATGCCAAAGTCTGGCATCTCTACAATACTTCTTTCCGTC CCACTCAGGGAGGTCAGGTGTCCATTGCCCTAAGCTCTCACTGGATCAA TCCTCGAAGAATGACCGACCACAGCATCAAAGAATGTCAAAAATCTCTG GACTTTGTACTAGGTTGGTTTGCCAAACCCGTATTTATTGATGGTGACTA TCCCAGAGCATGAAGAATAACCTTTCATCTATTCTGCCTGATTTTACTG AATCTGAGAAAAAGTTCATCAAAGGAACTGCTGACTTTTTTGTCTTTGC TTTGGACCCACCTTGAGTTTTCAACTTTTGGACCCCTACATGAAGTTCCG CCAATTGGAATCTCCCAACCTGAGGCAACTGCTTTCCTGGATTGACCTT GAATTTAACCATCCTCAAAATTTTATTGTGGAAAATGGCTGGTTTGTCTCA GGGACCACCAAGAGAGATGATGCCAAATATATGTATTACCTCAAAAAGTT CATCATGGAAACCTTAAAAGCCATCAAGCTGGATGGGGTGGATGTCATC GGGTATACCGCATGGTCCCTCATGGATGGTTTCGAGTGGCACAGAGGTT ACAGCATCAGGCGTGGACTCTTCTATGTTGACTTTCCTAAGCCAGGACAA GATGTTGTTGCCAAAGTCTTCAGCCTTGTCTACCAAAAGCTGATAGAGA AAAATGGCTTCCCTCCTTTACCTGAAAATCAGCCCCTAGAAGGGACATTT CCCTGTGACTTTGCTTGGGGAGTTGTTGACAACTACATTCAAGTAGATAC CACTCTGTCTCAGTTTACCGACCTGAATGTTTACCTGTGGGATGTCCACC ACAGTAAAAGGCTTATTTAAAGTGGATGGGGTTGTGACCAAGAAGAGGAA ATCCTACTGTGTTGACTTTGCTGCCATCCAGCCCCAGATCGCTTTACTCC AGGAAATGCACGTTACACATTTTCGCTTCTCCCTGGACTGGGCCCTGAT TCTCCCTCTGGGTAACCAAGTCCCAGGTGAACCACACCATCCTGCAGTAC TATCGCTGCATGGCCAGCGAGCTTGTCCGTGTCAACATCACCCCAGTGG TGGCCCTGTGGCAGCCTATGGCCCCGAACCAAGGACTGCCGCGCCTCC TGGCCAGGCAGGGCGCCTGGGAGAACCCCTACACTGCCCTGGCCTTTG CAGAGTATGCCCCGACTGTGCTTTCAAGAGCTCGGCCATCACGTCAAGCT TTGGATAACGATGAATGAGCCGTATACAAGGAATATGACATACAGTGCT GGCCACAACCTTCTGAAGGCCCATGCCCTGGCTTGGCATGTGTACAATG AAAAGTTTAGGCATGCTCAGAATGGGAAAATATCCATAGCCTTGCAGGC TGATTGGATAGAACCTGCCTGCCCTTCTCCCAAAAGGACAAAGAGGTG GCTGAGAGAGTTTTGGAATTTGACATTGGCTGGCTGGCTGAGCCCATTT TCGGCTCTGGAGATTATCCATGGGTGATGAGGGACTGGCTGAACCAAAG AAACAATTTTCTTCTTCTTATTTCACTGAAGATGAAAAAAGCTAATCCA GGGTACCTTTGACTTTTTGGCTTTAAGCCATTATACCACCATCCTTGTA GACTCAGAAAAAGAAGATCCAATAAAATACAATGATTACCTAGAAGTGCAA GAAATGACCGACATCACGTGGCTCAACTCCCCAGTCAGGTGGCGGTA GTGCCCTGGGGTGGCGCAAAGTGTGAAGTGGCTGAAGTTCAAGTAC GGAGACCTCCCCATGTACATAATATCCAATGGAATCGATGACGGGCTGC ATGCTGAGGACGACCAAGCTGAGGGTGTATTATATGCAGAATTACATAAA CGAAGCTCTCAAAGCCACATACTGGATGGTATCAATCTTTCGGGATACT TTGCTTATTCGTTTAACGACCGCACAGCTCCGAGGTTTGGCCTCTATCGT TATGCTGCAGATCAGTTTGAGCCCAAGGCATCCATGAAACATTACAGGA AAATTATTGACAGCAATGGTTTCCCGGGCCCAAGAACTCACCATCACCAT CACCATTGA	

Table E1		
Name	Sequence	SEQ ID NO:
REGN14225 (KL958-His) Amino acid sequence	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQTEGGWQ QHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPATGDVASDSYNN VFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYRRLERLRE LGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFQGGQV KYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLY NTSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFID GDYPESMKNNLSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKF RQLESPNLRQLLSWIDLEFNHPQIFIVENGWFWVSGTTKRDDAKYMYLKKFI METLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLSQDKMLL PKSSALFYQKLEKNGFPPLPENQPLEGTFPCDFAWGVVDNYIQVDTTLSQF TDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALLLQEMHVTHF RFSLDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPN QGLPRLLARQGAWENPYTALAFAYARLCFQELGHHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKD KEVAERVLEFDIGWLAEPFGSGDYPWVMRDWLNQRNNFLLPYFTEDEKKL IQGTDFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNSPSQVAVVP WGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVYYMQNYINEALK AHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQFEPKASMKHYRKIIDSNG FPGPETHHHHHH	44
REGN14226 (C521S)-His) Nucleotide sequence	GAGCCGGGCGACGGCGCGCAGACCTGGGCCCGTTTCTCGCGGCCTCC TGCCCCCGAGGCCGCGGGCCTCTTCCAGGGCACCTTCCCCGACGGCTT CCTCTGGGCCGTGGGCAGCGCCGCTTACCAGACCGAGGGCGGCTGGC AGCAGCACGGCAAGGGTGCGTCCATCTGGGATACGTTACCCACCACC CCCTGGCACCCCCGGGAGACTCCCGGAACGCCAGTCTGCCGTTGGGC GCCCCGTGCGCCGTGCAGCCCGCCACCGGGGACGTAGCCAGCGACAG CTACAACAACGTCTTCCGCGACACGGAGGCGCTGCGCGAGCTCGGGGT CACTCACTACCGCTTCTCCATCTCGTGGGCGCGAGTGCTCCCCAATGGC AGCGCGGGCGTCCCCAACCGCGAGGGGCTGCGCTACTACCGGCGCCT GCTGGAGCGGCTGCGGGAGCTGGGCGTGCAGCCCGTGGTCAACCCTGT ACCACTGGGACCTGCCCCAGCGCCTGCAGGACGCCTACGGCGGCTGG GCCAACCGCGCCCTGGCCGACCACTTCAGGGATTACGCGGAGCTCTGC TTCCGCCACTTCGGCGGTCAAGTACTGGATCACCATCGACAACC CCTACGTGGTGGCCTGGCACGGCTACGCCACCGGGCGCCTGGCCCCC GGCATCCGGGGCAGCCCGCGGCTCGGGTACCTGGTGGCGCACAACT CCTCCTGGCTCATGCCAAAGTCTGGCATCTCTACAATACTTCTTCCGTC CCTCAGGGAGGTCAAGGTGTCCATTGCCCTAAGCTCTCACTGGATCAA TCCTCGAAGAATGACCGACCACAGCATCAAAGAATGTCAAAAATCTCTG GACTTTGTACTAGGTTGGTTTGCCAAACCGTATTTATTGATGGTGACTA TCCCGAGAGCATGAAGAATAACCTTTCATCTATTCTGCCTGATTTTACTG AATCTGAGAAAAAGTTCATCAAAGGAAGTCTGACTTTTTTGTCTTTTGC TTTGGACCCACCTTGAGTTTTCACTTTTGGACCCCTACATGAAGTTCCG CCAATTGGAATCTCCCAACCTGAGGCAACTGCTTTCCTGGATTGACCTT GAATTTAACCATCCTCAAAATTTATTGTGAAAAATGGCTGGTTTGTCTCA GGGACCACCAAGAGAGATGATGCCAAATATATGTATTACCTCAAAAAGTT CATCATGGAACCTTAAAAGCCATCAAGCTGGATGGGGTGGATGTCATC GGGTATACCGCATGGTCCCTCATGGATGGTTTCGAGTGGCACAGAGGTT ACAGCATCAGGCGTGGACTCTTCTATGTTGACTTTCTAAGCCAGGACAA GATGTTGTTGCCAAAGTCTTCAGCCTTGTCTACCAAAAGCTGATAGAGA	45

Table E1		
Name	Sequence	SEQ ID NO:
	AAAATGGCTTCCCTCCTTTACCTGAAAATCAGCCCCCTAGAAGGGACATTT CCCAGCGACTTTGCTTGGGGAGTTGTTGACAACCTACATTCAAGTAGATA CCACTCTGTCTCAGTTTACCGACCTGAATGTTTACCTGTGGGATGTCCAC CACAGTAAAAGGCTTATTAAGTGGATGGGGTTGTGACCAAGAAGAGGA AATCCTACTGTGTTGACTTTGCTGCCATCCAGCCCCAGATCGCTTTACTC CAGGAAATGCACGTTACACATTTTCGCTTCTCCCTGGACTGGGCCCTGA TTCTCCCTCTGGGTAACCAAGTCCCAGGTGAACCAACCATCCTGCAGTA CTATCGCTGCATGGCCAGCGAGCTTGTCCGTGTCAACATCACCCAGTG GTGGCCCTGTGGCAGCCTATGGCCCCGAACCAAGGACTGCCGCGCCTC CTGGCCAGGCAGGGCGCCTGGGAGAACCCCTACACTGCCCTGGCCTT GCAGAGTATGCCCCGACTGTGCTTTCAAGAGCTCGGCCATCACGTCAAGC TTTGATAACGATGAATGAGCCGTATACAAGGAATATGACATACAGTGCT GGCCACAACCTTCTGAAGGCCCATGCCCTGGCTTGGCATGTGTACAATG AAAAGTTTAGGCATGCTCAGAATGGGAAAATATCCATAGCCTTGCAGGC TGATTGGATAGAACCTGCCTGCCCTTCTCCCAAAGGACAAAGAGGTG GCTGAGAGAGTTTTGGAATTTGACATTGGCTGGCTGGCTGAGCCCATT TCGGCTCTGGAGATTATCCATGGGTGATGAGGGACTGGCTGAACCAAA AAACAATTTTCTTCTTCTTATTTCACTGAAGATGAAAAAAGCTAATCCA GGGTACCTTTGACTTTTTGGCTTTAAGCCATTATACCACCATCCTGTAG ACTCAGAAAAAGAAGATCCAATAAAATACAATGATTACCTAGAAGTGCAA GAAATGACCGACATCACGTGGCTCAACTCCCCAGTCAGGTGGCGTA GTGCCCTGGGGTTGCGCAAAGTGCTGAAGTGGCTGAAGTTCAAGTAC GGAGACCTCCCCATGTACATAATATCCAATGGAATCGATGACGGGCTGC ATGCTGAGGACGACCAAGCTGAGGGTGTATTATATGCAGAATTACATAAA CGAAGCTCTCAAAGCCACATACTGGATGGTATCAATCTTTCGGGATACT TTGCTTATTCGTTTAACGACCGCACAGCTCCGAGGTTTGGCCTCTATCGT TATGCTGCAGATCAGTTTGAGCCCAAGGCATCCATGAAACATTACAGGA AAATTATTGACAGCAATGGTTTCCCGGGCCAGAACTCACCATCACCAT CACCATTGA	
REGN14226 (KL958 (C521S)-His) Amino acid sequence	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQTEGGWQ QHKGKASIWDFTTHHPLAPPGDSRNASLPLGAPSPLQPATGDVASDSYNN VFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYRRLRLRE LGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFQGGV KYWITIDNPYVVAWHGYATGR LAPGIRGSPRLGYLVAHNLLLAHAKVWHLY NTSFRPTQGGQVSIALSSHWINPRMTDHSIKECQKSLDFVLGWFAKPVFID GDYPESMKNNLSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKF RQLESPNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKFI METLKAIKLDGVDVIGYTAWSLMDGF EWHRGYSIRRGIFYVDFLSQDKMLL PKSSALFYQKLIEKNGFPLPENQPLEGTFPSDFAWGVVDNYIQVDTTLSQF TDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHF RFSLDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPN QGLPRLARQGAWENPYTALAFAYARLCFQELGHVVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFQSKD KEVAERVLEFDIGWLAEPFGSGDYPWVMRDWLNQRNNFLLPYFTEDEKKL IQGTDFDLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNSPSQVAVVP WGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVVYMQNYINEALK AHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQFEPKASMKHYRKIIDSNG FPGPETHHHHHH	46

Table E1		
Name	Sequence	SEQ ID NO:
REGN14227 (KL958 (C521S C910S)-His) Nucleotide sequence	GAGCCGGGCGACGGCGCGCAGACCTGGGCCCCGTTTCTCGCGGCCTCC TGCCCCCGAGGCCGCGGGCCTCTTCCAGGGCACCTTCCCCGACGGCTT CCTCTGGGCCGTGGGCAGCGCCGCTACCAGACCGAGGGCGGCTGGC AGCAGCACGGCAAGGGTGCGTCCATCTGGGATACGTTACCCACCACC CCCTGGCACCCCCGGGAGACTCCCGGAACGCCAGTCTGCCGTTGGGC GCCCCGTGCGCGCTGCAGCCCGCCACCGGGGACGTAGCCAGCGACAG CTACAACAACGTCTTCCGCGACACGGAGGCGCTGCGCGAGCTCGGGGT CACTCACTACCGCTTCTCCATCTCGTGGGCGCGAGTGCTCCCCAATGGC AGCGCGGGCGTCCCCAACCGCGAGGGGCTGCGCTACTACCGGCGCCT GCTGGAGCGGCTGCGGGAGCTGGGCGTGCAGCCCGTGGTCACCCTGT ACCACTGGGACCTGCCCCAGCGCCTGCAGGACGCCACGGCGGCTGG GCCAACCGCGCCCTGGCCGACCACTTCAGGGATTACGCGGAGCTCTGC TTCCGCCACTTCGGCGGTCAGGTCAAGTACTGGATCACCATCGACAACC CCTACGTGGTGGCTTGGCACGGCTACGCCACCGGGCGCCTGGCCCCC GGCATCCGGGGCAGCCCGCGGCTCGGGTACCTGGTGGCGCACAACT CCTCCTGGCTCATGCCAAAGTCTGGCATCTCTACAATACTTCTTCCGTC CCACTCAGGGAGGTCAAGTGTCCATTGCCCTAAGCTCTCACTGGATCAA TCCTCGAAGAATGACCGACCACAGCATCAAAGAATGTCAAAAATCTCTG GACTTTGTACTAGGTTGGTTTGCCAAACCCGTATTTATTGATGGTGA TCCCGAGAGCATGAAGAATAACCTTTCATCTATTCTGCCTGATTTTACTG AATCTGAGAAAAAGTTCATCAAAGGAACTGCTGACTTTTTTGTCTTTTGC TTTGGACCCACCTTGAGTTTTCACTTTTGGACCCACATGAAGTTCCG CCAATTGGAATCTCCCAACCTGAGGCAACTGCTTTCCTGGATTGACCTT GAATTTAACCATCCTCAAAATTTTATTGTGGAATGGCTGGTTTGTCTCA GGGACCACCAAGAGAGATGATGCCAAATATATGTATTACCTCAAAAAGTT CATCATGGAACCTTAAAGCCATCAAGCTGGATGGGGTGGATGTCATC GGGTATACCGCATGGTCCCTCATGGATGGTTTCGAGTGCCACAGAGGT ACAGCATCAGGCGTGGACTCTTCTATGTTGACTTTCTAAGCCAGGACAA GATGTTGTTGCCAAAGTCTTCAGCCTTGTCTACCAAAAGCTGATAGAGA AAAATGGCTTCCCTCCTTTACCTGAAAATCAGCCCCCTAGAGGGACATTT CCCAGCGACTTTGCTTGGGGAGTTGTTGACAACCTACATTCAAGTAGATA CCACTCTGTCTCAGTTTACCGACCTGAATGTTTACCTGTGGGATGTCCAC CACAGTAAAAGGCTTATTAAAGTGGATGGGGTTGTGACCAAGAAGAGGA AATCCTACTGTGTTGACTTTGCTGCCATCCAGCCCCAGATCGCTTTACTC CAGGAAATGCACGTTACACATTTTCTGCTTCTCCCTGGACTGGGCCCTGA TTCTCCCTCTGGGTAACCAAGTCCCAGGTGAACCAACCATCCTGCAGTA CTATCGCTGCATGGCCAGCGAGCTTGTCCGTGTCAACATCACCCAGTG GTGGCCCTGTGGCAGCCTATGGCCCCGAACCAAGGACTGCCGCGCCTC CTGGCCAGGCAGGGCGCCTGGGAGAACCCCTACACTGCCCTGGCCTTT GCAGAGTATGCCCGACTGTGCTTTCAAGAGCTCGGCCATCACGTCAAGC TTTGGATAACGATGAATGAGCCGTATACAAGGAATATGACATACAGTGCT GGCCACAACCTTCTGAAGGCCCATGCCCTGGCTTGGCATGTGTACAATG AAAAGTTTAGGCATGCTCAGAATGGGAAAATATCCATAGCCTTGCAGGC TGATTGGATAGAACCTGCCTGCCCTTTCTCCAAAAGGACAAAGAGGTG GCTGAGAGAGTTTTGGAATTTGACATTGGCTGGCTGGCTGAGCCCATTT TCGGCTCTGGAGATTATCCATGGGTGATGAGGGACTGGCTGAACCAAAG AAACAATTTTCTTCTTCTTATTTCACTGAAGATGAAAAAAGCTAATCCA GGGTACCTTTGACTTTTTGGCTTTAAGCCATTATACCACCATCCTTGTAG	47

Table E1		
Name	Sequence	SEQ ID NO:
	ACTCAGAAAAAGAAGATCCAATAAAATACAATGATTACCTAGAAGTGCAA GAAATGACCGACATCACGTGGCTCAACTCCCCAGTCAGGTGGCGGTA GTGCCCTGGGGGTTGCGCAAAGTGCTGAAGTGGCTGAAGTTCAAGTAC GGAGACCTCCCCATGTACATAATATCCAATGGAATCGATGACGGGCTGC ATGCTGAGGACGACCAAGCTGAGGGTGTATTATATGCAGAATTACATAAA CGAAGCTCTCAAAGCCACATACTGGATGGTATCAATCTTAGCGGATAC TTTGCTTATTCGTTTAACGACCGCACAGCTCCGAGGTTTGGCCTCTATCG TTATGCTGCAGATCAGTTTGAGCCCAAGGCATCCATGAAACATTACAGG AAAATTATTGACAGCAATGGTTTCCCGGGCCAGAACTCACCATCACC ATCACCATTGA	
REGN14227 (KL958 (C521S C910S)-His) Amino acid sequence	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQTEGGWQ QHGKGASIWDTFTHHPLAPPGDSRNASLPLGAPSPQPATGDVASDSYNN VFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYRLLERLRE LGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFQQV KYWITIDNPYVVAWHGYATGR LAPGIRGSPRLGYLVAHNLLLAHAKVWHLY NTSFRPTQGGQVSIALSSHWINPRMTDHSIKECQKSLDFVLGWFAPVFD GDYPESMKNLSSILPDTFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKF RQLESPNLRLQLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKFI METLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGIFYVDFLSQDKMLL PKSSALFYQKLIKNGFPPLPENQPLEGTFPSDFAWGVVDNYIQVDTTSLQF TDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHF RFSLDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPN QGLPRLRLARQGAWENPYTALAFAYEARLCFQELGHVVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKD KEVAERVLEFDIGWLAEPFGSGDYPWVMRDWLNQRNNFLLPYFTEDEKKL IQGTFDFLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNSPSQVAVVP WGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVYVMQNYINEALK AHILDGINLSGYFAYSFNDRTPRFLYRYAADQFEPKASMKHYRKIDSNG FPGPETHHHHHH	48
REGN15823 (KL981-G4S- HSA) nucleotide sequence	GAGCCGGGCGACGGCGCGCAGACCTGGGCCCGTTTCTCGCGGCCTCC TGCCCCCGAGGCCGCGGGCCTCTTCCAGGGCACCTTCCCCGACGGCTT CCTCTGGGCCGTGGGCAGCGCCGCTACCAGACCGAGGGCGGCTGGC AGCAGCACGGCAAGGGTGCGTCCATCTGGGATACGTTACCCACCACC CCCTGGCACCCCCGGGAGACTCCCGGAACGCCAGTCTGCCGTTGGGC GCCCCGTGCGCGCTGCAGCCCCGCCACCGGGGACGTAGCCAGCGACAG CTACAACAACGTCTTCCGCGACACGGAGCGCTGCGCGAGCTCGGGGT CACTCACTACCGCTTCTCCATCTCGTGGGCGCGAGTGCTCCCCAATGGC AGCGCGGGCGTCCCCAACC CGAGGGGCTGCGCTACTACCGGCGCCT GCTGGAGCGGCTGCGGGAGCTGGGCGTGACAGCCCGTGGTACCCCTGT ACCACTGGGACCTGCCCCAGCGCCTGCAGGACGCCTACGGCGGCTGG GCCAACC GCGCCCTGGCCGACCACTTCAGGGATTACGCGGAGCTCTGC TTCCGCCACTTCGGCGGT CAGGTCAAGTACTGGATCACCATCGACAACC CCTACGTGGTGGCCTGGCACGGCTACGCCACCGGGCGCCTGGCCCCC GGCATCCGGGGCAGCCCGCGGCTCGGGTACCTGGTGGCGCACAACT CCTCCTGGCTCATGCCAAAGTCTGGCATCTCTACAATACTTCTTTCCGTC CCACTCAGGGAGGTCAGGTGTCCATTGCCCTAAGCTCTCACTGGATCAA TCCTCGAAGAATGACCGACCAACAGCATCAAAGAATGTCAAAAATCTCTG GACTTTGTACTAGGTTGGTTTGCCAAACCCGATTTATTGATGGTGACTA	49

Table E1		
Name	Sequence	SEQ ID NO:
	TCCCGAGAGCATGAAGAATAACCTTTTCATCTATTCTGCCTGATTTTACTG AATCTGAGAAAAAGTTCATCAAAGGAACTGCTGACTTTTTTGTCTTTTGC TTTGGACCCACCTTGAGTTTTCAACTTTTGGACCCTCACATGAAGTTCCG CCAATTGGAATCTCCCAACCTGAGGCAACTGCTTTCCTGGATTGACCTT GAATTTAACCATCCTCAAATATTTATTGTGGAAAATGGCTGGTTTGTCTCA GGGACCACCAAGAGAGATGATGCCAAATATATGTATTACCTCAAAAAGTT CATCATGGAACCTTAAAAGCCATCAAGCTGGATGGGGTGGATGTCATC GGGTATACCGCATGGTCCCTCATGGATGGTTTCGAGTGGCACAGAGGTT ACAGCATCAGGCGTGGACTCTTCTATGTTGACTTTCTAAGCCAGGACAA GATGTTGTTGCCAAAGTCTTCAGCCTTGTCTACCAAAAGCTGATAGAGA AAAATGGCTTCCCTCCTTTACCTGAAAATCAGCCCTAGAAAGGACATTT CCCTGTGACTTTGCTTGGGGAGTTGTTGACAACTACATTCAAGTAGATAC CACTCTGTCTCAGTTTACCGACCTGAATGTTTACCTGTGGGATGTCCACC ACAGTAAAAGGCTTATTAAGTGGATGGGGTTGTGACCAAGAAGAGGAA ATCCTACTGTGTTGACTTTGCTGCCATCCAGCCCCAGATCGCTTTACTCC AGGAAATGCACGTTACACATTTTCGCTTCTCCCTGGACTGGGCCCTGAT TCTCCCTCTGGGTAACCAAGTCCCAGGTGAACCACACCATCCTGCAGTAC TATCGCTGCATGGCCAGCGAGCTTGTCCGTGTCAACATCACCCCAGTGG TGGCCCTGTGGCAGCCTATGGCCCCGAACCAAGGACTGCCGCGCCTCC TGGCCAGGCAGGGCGCCTGGGAGAACCCTTACACTGCCCTGGCCTTTG CAGAGTATGCCCCGACTGTGCTTTCAAGAGCTCGGCCATCAGTCAAGCT TTGGATAACGATGAATGAGCCGTATACAAGGAATATGACATACAGTGCT GGCCACAACCTTCTGAAGGCCCATGCCCTGGCTTGGCATGTGTACAATG AAAAGTTTAGGCATGCTCAGAATGGGAAAATATCCATAGCCTTGCAGGC TGATTGGATAGAACCTGCCTGCCCTTTCTCCCAAAAGGACAAAGAGGTG GCTGAGAGAGTTTTGGAATTTGACATTGGCTGGCTGGCTGAGCCCATTT TCGGCTCTGGAGATTATCCATGGGTGATGAGGGACTGGCTGAACCAAAG AAACAATTTTCTTCTTCTTATTTCACTGAAGATGAAAAAAGCTAATCCA GGGTACCTTTGACTTTTTTGGCTTTAAGCCATTATACCACCATCCTTGTAG ACTCAGAAAAAGAAGATCCAATAAAATACAATGATTACCTAGAAGTGCAA GAAATGACCGACATCACGTGGCTCAACTCCCCCAGTCAGGTGGCGGTA GTGCCCTGGGGTTGCGCAAAGTGCTGAACTGGCTGAAGTTCAAGTAC GGAGACCTCCCCATGTACATAATATCCAATGGAATCGATGACGGGCTGC ATGCTGAGGACGACCAAGCTGAGGGTGTATTATATGCAGAATTACATAAA CGAAGCTCTCAAAGCCACATACTGGATGGTATCAATCTTTGCGGATACT TTGCTTATTCGTTTAAACGACCGCACAGCTCCGAGGTTTGGCCTCTATCGT TATGCTGCAGATCAGTTTGAGCCCAAGGCATCCATGAAACATTACAGGA AAATTATTGACAGCAATGGTTTCCCGGGCCCAAGAACTCTGGAAGATTT TGTCAGAGAAGATTTACCGTGTGTACTGAGTGCAGTTTTTTTACACCCG AAAGTCTGGTGGAGGAGGTAGTGATGCACACAAGAGTGAGGTTGCTCAT CGGTTTAAAGATTTGGGAGAAGAAAATTTCAAAGCCTTGGTGTGATTGC CTTTGCTCAGTATCTTCAGCAGTGTCCATTTGAAGATCATGTAAATTAGT GAATGAAGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAGTCAGCTG AAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTATGCACA GTTGCAACTCTTCGTGAAACCTATGGTGAATGGCTGACTGCTGTGCAA AACAAGAACCTGAGAGAAATGAATGCTTCTTGCAACACAAAGATGACAAAC CCAAACCTCCCCGATTGGTGAGACCAGAGGTTGATGTGATGTGCACTG CTTTTCATGACAAATGAAGAGACATTTTGA AAAAATACTTATATGAAATTG	

Table E1		
Name	Sequence	SEQ ID NO:
	CCAGAAGACATCCTTACTTTTATGCCCCGGAACCTCTTTTCTTTGCTAAA AGGTATAAAGCTGCTTTTACAGAATGTTGCCAAGCTGCTGATAAAGCTGC CTGCCTGTTGCCAAAGCTCGATGAACCTTCGGGATGAAGGGAAGGCTTCG TCTGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAGAAA GAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGATTTCCCAA AGCTGAGTTTGCAAGTTTCCAAGTTAGTGACAGATCTTACCAAAGTCC ACACGGAATGCTGCCATGGAGATCTGCTTGAATGTGCTGATGACAGGGC GGACCTTGCCAAGTATATCTGTGAAAATCAAGATTGATCTCCAGTAAAC TGAAGGAATGCTGTGAAAAACCTCTGTTGGAAAAATCCCAGTGCATTGC CGAAGTGGAAAAATGATGAGATGCCTGCTGACTTGCCTTCATTAGCTGCT GATTTTGTGAAAGTAAGGATGTTTGCAAAAAATGCTGAGGGCAAGGA TGCTCTGCTGGGCATGTTTTGTATGAATATGCAAGAAGGCATCTGATT ACTCTGCTGCTGCTGCTGAGACTTGCCAAGACATATGAAACCACTCT AGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTATGCCAAAGTG TTCGATGAATTTAAACCTCTTGTGGAAGAGCCTCAGAAATTAATCAAACA AAATTGTGAGCTTTTTGAGCAGCTTGGAGAGTACAAATTCAGAATGCGC TATTAGTTCGTTACACCAAGAAAGTACCCCAAGTGCTCAACTCCAACCTT GTAGAGGTCTCAAGAAACCTAGGAAAAGTGGGCAGCAAAATGTTGTAAC ATCCTGAAGCAAAAAGAAATGCCCTGTGCAGAAGCATATCTATCCGTGGT CCTGAACCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGA GTCACCAAATGCTGCACAGAATCCTTGGTGAACAGGCGACCATGCTTTT CAGCTCTGGAAGTCGATGAAACATACGTTCCCAAAGAGTTTAATGCTGAA ACATTACCTTCCATGCAGATATATGCACACTTTCTGAGAAGGAGAGACA AATCAAGAAACAACTGCACCTGTTGAGCTCGTGAACACAAAGCCCAAG GCAACAAAAGAGCAACTGAAAGCTGTTATGGATGATTTTCGCAGCTTTTGT AGAGAAGTGCTGCAAGGCTGACGATAAGGAGACCTGCTTTGCCGAGGA GGGTAAAAAACTTGTTGCTGCAAGTCAAGCTGCCTTAGGCTTATGA	
REGN15823 (KL981-G4S- HSA) amino acid sequence	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGLFWAVGSAAYQTEGGWQ QHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSP LQPATGDVASDSYNN VFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYRRLERLRE LGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFGGQV KYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLY NTSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFID GDYPESMKNLNLSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKF RQLESPLNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKFI METLKAIKLDGVDVIGYTAWSLMDGFWEHGRGYSIRRLGFYVDFLSQDKMLL PKSSALFYQKLIKENGFPPLPENQPLEGTFPCDFAWGVVDNYIQVDDTLSQF TDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALLQEMHVTHF RFSLDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPN QGLPRLLARQGAWENPYTALAFAYARLCFQELGHHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNKGKISALQADWIEPACPFSSQKD KEVAERVLEFDIGWLAEPIFGSGDYPWVMRDWLNQRNNFLLPYFTEDEKKL IQGTFDFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNSPSQVAVVP WGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVYYMQNYINEALK AHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQFEPKASMKHYRKIIDSNG FPNGPETLERFCPEEFTVCTECSFFHTRKSGGGSGDAHKSEVAHRFKDLGE ENFKALVLIAFAQYLQCCPFEDHVKLVNEVTEFAKCTVADESAENCDSKSLHT LFGDKLCTVATILRETYGEMADCCAKQEPERNEFCFLQHKDDNP NLPRLVRP	50

Table E1		
Name	Sequence	SEQ ID NO:
	EVDVMCTAFHDNEETFLKKYLYEIAARRHPYFYAPELLFFAKRYKAAFTGCCQ AADKAACLLPKLDELDEGKASSAKQRLKASLQKFGERAFKAWAVARLSQ RFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISS KLKECCEKPLLEKSHCIAEVENDEMPADLP SLAADFVESKDVCKNYAEAKD VFLGMFLY EYARRHPDYSVLLLR LAKTYETTLEKCCAAADPHECYAKVFDE FKPLVEEPQNLIKQNCLEFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSR NLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCT ESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVE LVKHKPKATKEQLKAVMDDFAAFVEKCKADDKETCF AE EGKKLVAASQAA LGL	
REGN15548 (KL958 (C521S)- G4S-HSA) Nucleotide sequence	GAGCCGGGCGACGGCGCGCAGACCTGGGCCCGTTTCTCGCGGCCTCC TGCCCCCGAGGCCGCGGGCCTCTTCCAGGGCACCTTCCCCGACGGCTT CCTCTGGGCCGTGGGCAGCGCCGCTTACCAGACCGAGGGCGGCTGGC AGCAGCACGGCAAGGGTGCGTCCATCTGGGATACGTTCACCACCACC CCCTGGCACCCCCGGGAGACTCCCGGAACGCCAGTCTGCCGTTGGGC GCCCCGTGCGCGCTGCAGCCCCGCCACCGGGGACGTAGCCAGCGACAG CTACAACAACGTCTTCCGCGACACGGAGGCGCTGCGCGAGCTCGGGGT CACTCACTACCGCTTCTCCATCTCGTGGGCGCGAGTGCTCCCCAATGGC AGCGCGGGCGTCCCCAACCGCGAGGGGCTGCGCTACTACCGGCGCCT GCTGGAGCGGCTGCGGGAGCTGGGCGTGCAGCCCGTGGTCAACCTGT ACCACTGGGACCTGCCCCAGCGCCTGCAGGACGCCTACGGCGGCTGG GCCAACC GCGCCCTGGCCGACCACTTCAGGGATTACGCGGAGCTCTGC TTCCGCCACTTCGGCGGTCAAGTACTGGATCACCATCGACAACC CCTACGTGGTGGCCTGGCACGGCTACGCCACCGGGCGCCTGGCCCC GGCACTCCGGGGCAGCCCGCGGCTCGGGTACCTGGTGGCGCACAACT CCTCCTGGCTCATGCCAAAGTCTGGCATCTCTACAATACTTCTTTCCGTC CCACTCAGGGAGGTCAAGTGTCCATTGCCCTAAGCTCTCACTGGATCAA TCCTCGAAGAATGACCGACCAAGCATCAAAGAATGTCAAAAATCTCTG GACTTTGTACTAGGTTGGTTTGCCAAACCGTATTTATTGATGGTGACTA TCCCGAGAGCATGAAGAATAACCTTTCATCTATTCTGCCTGATTTTACTG AATCTGAGAAAAAGTTCATCAAAGGAAGTCTGACTTTTTTGTCTTTTGC TTTGGACCCACCTTGAGTTTTCAACTTTTGGACCCACATGAAGTTCCG CCAATTGGAATCTCCAACCTGAGGCAACTGCTTTCCTGGATTGACCTT GAATTTAACCATCCTCAAAATATTTATTGTGGAATGGCTGGTTTGTCTCA GGGACCACCAAGAGAGATGATGCCAAATATATGTATTACCTCAAAAAGTT CATCATGGAAACCTTAAAAGCCATCAAGCTGGATGGGGTGGATGTCATC GGGTATACCGCATGGTCCCTCATGGATGGTTTCGAGTGGCACAGAGGTT ACAGCATCAGGCGTGGACTCTTCTATGTTGACTTTCTAAGCCAGGACAA GATGTTGTTGCCAAAGTCTTCAGCCTTGTCTACCAAAAAGCTGATAGAGA AAAATGGCTTCCCTCCTTTACCTGAAAATCAGCCCCCTAGAAGGGACATTT CCCAGCGACTTTGCTTGGGGAGTTGTTGACAACTACATTCAAGTAGATA CCACTCTGTCTCAGTTTACCGACCTGAATGTTTACCTGTGGGATGTCCAC CACAGTAAAAGGCTTATTAAAGTGGATGGGGTTGTGACCAAGAAGAGGA AATCCTACTGTGTTGACTTTGCTGCCATCCAGCCCCAGATCGCTTTACTC CAGGAAATGCACGTTACACATTTTCGCTTCTCCCTGGACTGGGCCCTGA TTCTCCCTCTGGGTAACCAAGTCCAGGTGAACCAACCATCCTGCAGTA CTATCGCTGCATGGCCAGCGAGCTTGTCCGTGTCAACATCACCCCAAGT	51

Table E1		
Name	Sequence	SEQ ID NO:
	GTGGCCCTGTGGCAGCCTATGGCCCCGAACCAAGGACTGCCGCGCCTC CTGGCCAGGCAGGGCGCCTGGGAGAACCCCTACACTGCCCTGGCCTTT GCAGAGTATGCCCGACTGTGCTTTCAAGAGCTCGGCCATCACGTCAAGC TTTGGATAACGATGAATGAGCCGTATACAAGGAATATGACATACAGTGCT GGCCACAACCTTCTGAAGGCCCATGCCCTGGCTTGGCATGTGTACAATG AAAAGTTTAGGCATGCTCAGAATGGGAAAATATCCATAGCCTTGCAGGC TGATTGGATAGAACCTGCCTGCCCTTTCTCCAAAAGGACAAAGAGGTG GCTGAGAGAGTTTTTGAATTTGACATTGGCTGGCTGGCTGAGCCATTT TCGGCTCTGGAGATTATCCATGGGTGATGAGGGACTGGCTGAACCAAAG AAACAATTTTCTTCTTCTTATTTCACTGAAGATGAAAAAAGCTAATCCA GGGTACCTTTGACTTTTGGCTTTAAGCCATTATACCACCATCCTTGTA ACTCAGAAAAAGAAGATCCAATAAAATACAATGATTACCTAGAAGTGCAA GAAATGACCGACATCACGTGGCTCAACTCCCCAGTCAGGTGGCGGTA GTGCCCTGGGGGTTGCGCAAAGTGCTGAACTGGCTGAAGTTCAAGTAC GGAGACCTCCCCATGTACATAATATCCAATGGAATCGATGACGGGCTGC ATGCTGAGGACGACCAGCTGAGGGTGTATTATATGCAGAATTACATAAA CGAAGCTCTCAAAGCCACATACTGGATGGTATCAATCTTTCGGTACT TTGCTTATTCGTTTAACGACCGCACAGCTCCGAGGTTTGGCCTCTATCGT TATGCTGCAGATCAGTTTGAGCCCAAGGCATCCATGAAACATTACAGGA AAATTATTGACAGCAATGGTTTCCCGGGCCAGAACTGGTGGAGGAGG TAGTGATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGGA GAAGAAAATTTCAAAGCCTTGGTGTTGATTGCCCTTTCAGTATCTTCA GCAGTGTCATTTGAAGATCATGTAAATTAGTGAATGAAGTAACTGAAT TTGCAAAAACATGTGTTGCTGATGAGTCAGCTGAAAATTGTGACAAATCA CTTTCATACCCTTTTTGGAGACAAATTATGCACAGTTGCAACTCTTCGTGA AACCTATGGTGAAATGGCTGACTGCTGTGCAAAAACAAGAACCTGAGAGA AATGAATGCTTCTTGCAACACAAAGATGACAACCCAAACCTCCCCCGATT GGTGAGACCAGAGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAA GAGACATTTTTGAAAAATACTTATATGAAATTGCCAGAAGACATCCTTAC TTTTATGCCCCGGAACCTCTTTTCTTTGCTAAAAGGTATAAAGCTGCTTTT ACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTGTTGCCAAAGC TCGATGAACCTTCGGGATGAAGGGAAGGCTTCGTCTGCCAAACAGAGACT CAAGTGTCAGTCTCCAAAAATTTGGAGAAAGAGCTTTCAAAGCATGG GCAGTAGCTCGCCTGAGCCAGAGATTTCCCAAAGCTGAGTTTGCAGAAG TTTCCAAGTTAGTGACAGATCTTACCAAAGTCCACACGGAATGCTGCCAT GGAGATCTGCTTGAATGTGCTGATGACAGGGCGGACCTTGCCAAGTATA TCTGTGAAAATCAAGATTCGATCTCCAGTAACTGAAGGAATGCTGTGAA AAACCTCTGTTGGAAAAATCCCACTGCATTGCCGAAGTGGAATGATG AGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTGAAAGTAAG GATGTTTGCAAAAATATGCTGAGGCAAAGGATGTCTTCCTGGGCATGT TTTTGTATGAATATGCAAGAAGGCATCCTGATTACTCTGTCGTGCTGCTG CTGAGACTTGCCAAGACATATGAAACCACTCTAGAGAAGTGCTGTGCCG CTGCAGATCCTCATGAATGCTATGCCAAAGTGTTTCGATGAATTTAAACCT CTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAATTGTGAGCTTTTTGA GCAGCTTGGAGAGTACAAATTCAGAATGCGCTATTAGTTTCGTTACACCA AGAAAGTACCCCAAGTGCTCAACTCCAACCTTTGTAGAGGTTCAAGAAA CCTAGGAAAAGTGGGCAGCAATGTTGTAAACATCCTGAAGCAAAAAGA ATGCCCTGTGCAGAAGACTATCTATCCGTGGTCTGAACCAAGTTATGTG	

Table E1		
Name	Sequence	SEQ ID NO:
	TGTTGCATGAGAAAACGCCAGTAAGTGACAGAGTCACCAAATGCTGCAC AGAATCCTTGGTGAACAGGCGACCATGCTTTTCAGCTCTGGAAGTCGAT GAAACATACGTTCCCAAAGAGTTTAAATGCTGAAACATTCACCTTCCATGC AGATATATGCACACTTTCTGAGAAGGAGAGACAAATCAAGAAACAAACTG CACTTGTGAGCTCGTGAAACACAAGCCCAAGGCAACAAAAGAGCAACT GAAAGCTGTTATGGATGATTTGCGAGCTTTGTAGAGAAAGTCTGCAAG GCTGACGATAAGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTGTG CTGCAAGTCAAGCTGCCTTAGGCTTATGA	
REGN15548 (KL958 (C521S)- G4S-HSA) Amino acid sequence	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQTEGGWQ QHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPLQPATGDVASDSYNN VFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYRRLLERLRE LGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFGGQV KYWITIDNPYVVAWHGYATGR LAPGIRGSPRLGYLVAHNLLLAHAKVWHLY NTSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFID GDYPESMKNNLSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKF RQLESPNLRQLLSWIDLEFNHPQIFIVENGWVFSVGTTRDDAKYMYLKKFI METLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGLFYVDFLSQDKMLL PKSSALFYQKLIKNGFPPLPENQPLEGTFPSDFAWGVVDNYIQVDTTLSQF TDLNVYLWVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALQLQEMHVT RFLSDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPN QGLPRLARQGAWENPYTALAFAYEARLCFQELGHHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKD KEVAERVLEFDIGWLAEIFGSGDYPWVMRDWLNQRNNFLLPYFTEDEKKL IQGTDFLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNPSQVAVVP WGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVYMQNYINEALK AHILDGINLCGYFAYSFNDRTPRFLYRYAADQFEPKASMKHYRKIIDSNG FPGPETGGGSDAHKSEVAHRFKDLGEENFKALVLIQYLAQYLAQYLAQYLAQY KLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFQLQKDDNPPLPRLVRPEVDVMCTAFHDNEETFLKKYLYEI ARRHPYFYAPELLFFAKRYKAAFTCECCQAADKAACLLPKLDELREDEGKASS AKQRLKCASLQKFGERAFAKAWAVARLSQRFPAEFAEVSKLVDTLTKVHTE CCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLEKSHCIAEVEND EMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYFYARRHPDYSVLLLL RLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLQKNCLEFEQLG EYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAE DYL SVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKQTALVELVKHKPKATKEQLKAVMDDFAA FVEKCKKADDKETCFAEEGKKLVAASQAALGL	52
KL958 ((C521S C910S)- HSA-His) nucleotide sequence	GAGCCTGGGGACGGCGCTCAGACCTGGGCTCGTTTTCTCGTCCTCCT GCTCCTGAGGCTGCTGGCCTCTTCCAGGGCACGTTCCAGACGGCTTTT TGTGGGCGGTGGGTTCTGCGGCCTACCAGACGGAGGGGGGTTGGCAG CAGCATGGCAAGGGCGCCTCCATCTGGGATACTTTCACGCACCACCCC CTGGCTCCGCCTGGCGATTCCCGCAACGCCTCCCTGCCCTTAGGAGCC CCCTCTCCCCTGCAGCCGGCCACCGGCATGTAGCGTCCGATAGCTAC	53

Table E1		
Name	Sequence	SEQ ID NO:
	AATAACGTGTTCCGCGACACCGAGGCTTTGAGAGAGTTGGGCGTACTC ATTACCGCTTCTCTATCAGTTGGGCCCGCTACTGCCGAACGGTTCTGC CGGGGTGCCAAACAGGGAGGGGCTTCGCTACTACCGCCCGCTGCTGGA GCGTTTACGGGAGCTGGGAGTGCAGCCTGTCGTTACTCTGTACCACTG GGACCTCCCGCAGAGGCTGCAGGACGCCATGAGGCTGGGCCAACC GCGCCCTTGCGGACCATTTCGGGATTACGCGGAACTGTGCTTCCGCCA CTTCGGTGGCCAGGTGAAGTATTGGATCACCATTGACAATCCGTATGTG GTGGCTTGGCATGGATACGCGACCGGTAGGCTGGCCCCCGGCATCCGC GGGAGCCCTCGGTTGGGCTACCTGGTGGCGCACAACTGCTGTTGGCC CACGCTAAGGTGTGGCACCTGTACAACACCTCCTTTCGCCCCACCCAGG GCGGTCAGGTGTCCATCGCCCTCAGCTCCCACTGGATTAAACCCCGGC GCATGACCGACCACTCCATCAAAGAATGTCAGAAGTCCCTCGACTTTGT GCTGGGCTGGTTCGCCAAGCCCGTGTATTGACGGTGACTACCCGA GTCTATGAAGAACAACCTAAGCTCGATCCTGCCCGACTTCACAGAGTCT GAGAAAAAATTTATTAAGGGCACCGCGGACTTCTTCGCACTCTGCTTG GCCCTACCCTGTCTTTTACGCTTCTGGACCCCAACATGAAATTCAGACA GCTGGAGTCACCCAACCTGAGGCAGCTGCTGAGTTGGATCGATCTGGA ATTCAATCACCTCAGATCTTCATCGTGGAGAATGGATGGTTCGTCTCAG GCACCACCAAGCGCGACGATGCCAAGTACATGTACTATCTGAAGAAATT TATCATGGAGACACTCAAGGCGATCAAGCTGGACGGCGTCGATGTGATC GGTTACACCGCTTGGTCTTTGATGGATGGCTTCGAATGGCACCAGCGCT ACTCCATCCGTGCGGACTGTTCTACGTGGATTTCTGTCTCAGGACAA AATGCTCCTGCCAAAGTCCAGTGCAGTGTCTATCAGAAGCTAATCGAG AAAAATGGCTTCCACCTCTGCCTGAGAACCAGCCCTCGAAGGCACCT TCCCCTCGGACTTTCGCTGGGGCGTCTGTGGACAACCTATATACAAGTGGA CACCACACTTTCTCAGTTTACTGACCTGAACGTGTACCTGTGGGACGTG CACCACCTCTAAGCGACTTATCAAGGTGGATGGCGTGGTTACTAAGAAGC GCAAATCATATTGCGTGGATTTTGGGCCATCCAGCCTCAGATCGCCCT GCTGCAGGAAATGCACGTGACCCACTTTCGATTCTCTCTCGACTGGGCA CTGATTCTGCCACTTGGCAACCAGAGCCAGGTGAACCATACAATCCTGC AATATTACAGGTGTATGGCCAGCGAGCTGGTGGCGGTGAACATCACTCC GGTGGTCGCTCTCTGGCAACCCATGGCCCTAACCAAGGCCTGCCTCG CCTGCTGGCTCGCCAGGGTGGTGGGAAAACCCGTACACGGCCCTGGC CTTCGCCGAGTACGCACGTCTGTGCTTCCAGGAGCTCGGCCACCACGT CAAGTTGTGGATCACCATGAACGAGCCCTACACCCGTAATATGACCTAC AGCGCAGGCCACAACCTGTTGAAGGCCCATGCCCTGGCTTGGCACGTG TACAACGAGAAGTTCGCCACGCACAGAACGGTAAGATCTCCATCGCTC TTCAGGCGGACTGGATTGAGCCAGCCTGCCATTCTCGCAGAAGGACA AAGAGGTGGCCGAACGCGTCTCTGAATTCGACATCGGTTGGCTCGCTG AGCCCATCTTCGGCTCCGGCGACTACCCGTGGGTGATGCGTGACTGGC TGAACCAGCGCAACAACCTTCTGCTGCCCTATTTACCGAGGACGAGAA GAACTGATACAGGGAACCTTCGACTTCTAGCACTGTCGCACTACACG ACAATCCTGGTGGACAGCGAGAAGGAGGACCCGATCAAGTACAACGAC TACCTGGAGGTGCAGGAGATGACCGACATCACCTGGCTGAACTCGCCC AGCCAGGTCGCCGTGGTTCCGTGGGGCCTGCGCAAGGTACTGAACTGG CTGAAGTTCAAGTACGGCGACCTGCCCATGTATATCATCTCAACGGGA TTGACGATGGGCTACATGCTGAGGATGACCAGCTGCGGGTGTACTACAT GCAGAACTACATCAATGAGGCTCTTAAGGCTCACATTCTAGACGGGATT	

Table E1		
Name	Sequence	SEQ ID NO:
	AACTTATCGGGGTACTTCGCGTACTCCTTCAACGACCGCACGGCCCCGC GCTTTGGGCTCTACCGCTACGCTGCGGACCAGTTCGAGCCAAAAGCGT CCATGAAGCACTACCGGAAGATAATCGACAGCAATGGCTTCCCTGGACC CGAGACTGACGCACATAAGTCCGAGGTGCGCCACCGCTTCAAGGACCT GGGCGAGGAGAATTTCAAGGCCTTGGTCCTCATCGCCTTCGCCCAGTAT CTGCAGCAGTGCCCCATTTGAAGATCACGTGAAGCTGGTCAACGAGGTGA CGGAGTTCGCCAAGACCTGCGTGGCTGATGAGAGCGCCGAAAACCTGCG ATAAGAGCCTTCACACACTCTTCGGTGATAAGCTGTGCACCGTGGCCAC CCTGCGCGAGACGTACGGCGAGATGGCAGACTGTTGTGCCAAGCAGGA GCCCCGAGAGAAACGAGTGCTTTCTGCAGCACAAAGACGATAATCCAAAC TTGCCGCGCCTGGTGCGTCCCGAGGTAGATGTGATGTGCACCTGCGTTT CATGACAACGAGGAGACTTTCCTGAAGAAGTATCTCTATGAGATCGCCC GCCGCCACCCCTATTTCTACGCGCCGGAGCTACTTTTTTTCGCCAAGCG TTACAAGGCGGCCTTCACCGAATGCTGTGTCAGGCTGCCGACAAAGCTGC CTGCCTACTGCCTAAGCTGGACGAGCTCAGGGACGAGGGCAAAGCGAG TTCTGCTAAGCAGCGCCTGAAGTGCGCCTCCCTGCAGAAATTTGGGGA GCGCGCTTTCAAGGCCTGGGCGGTGGCGCGCCTGTGCGAGCGTTTTCC CAAAGCTGAGTTCGCGGAGGTGAGCAAATTGGTAACCGACCTGACCAA GGTGCACACTGAATGTTGTGTCATGGCGACCTGCTGGAGTGCGCCGATGA CCGCGCCGACTTAGCCAAGTACATCTGCGAAAATCAGGACTCTATCTCT TCTAAGCTCAAGGAATGTTGCGAGAAACCCCTGCTAGAGAAGTCCCCT GTATTGCTGAGGTGCGAAGCAGACGAGATGCCCGCCGACCTGCCCTCTC TGGCCGCCGACTTTGTGCGAGAGCAAGGACGTGTGCAAGAACTACGCGG AGGCTAAGGACGTGTTCTCGGCATGTTCTGTACGAGTACGCCCGAC GGCACCCCGACTACTCCGTAGTGCTTTTGTGCGTCTGGCTAAAACCTA CGAGACTACCCCTTGAGAAATGTTGTGCGAGCTGCAGACCCCGCATGAGTGT TACGCCAAAGTCTTCGACGAGTTCAAACCTCTGGTCAAGAGCCTCAGA ACCTGATCAAGCAGAACTGCGAGCTGTTGAGCAGCTAGGAGAGTACAA GTTCCAAAATGCTCTGCTGGTGCCTACACCAAGAAGGTCCCTCAGGTG TCCACCCCAACCCTGGTGGAGGTGTGCGCAACCTGGGTAAGGTGGGC TCCAAGTGTTGCAAGCACCCGGAGGCCAAGCGGATGCCTTGTGCTGAG GACTACCTGTCCGTGGTGTGTAACCAGCTGTGCGTGTGACAGAGAAGA CTCCGGTGTCCGATCGTGTGACGAAATGCTGCACTGAGAGTCTTGTGAA CCGCAGGCCTTGCTTTTCAGCCCTGGAGGTAGACGAGACTTACGTGCC CAAGGAGTTCAACGCTGAGACTTTACATTTACGCCGACATCTGCACT CTTAGCGAGAAGGAGCGGCAAATTAAGAAGCAGACGGCGCTGGTTGAA TTAGTTAAGCACAAAGCCGAAGGCTACCAAGGAACAGCTGAAGGCGGTG ATGGATGACTTCGCTGCATTTCGTCGAGAAATGTTGCAAAGCGGACGACA AGGAGACTTGCTTCGAGAGGAGGGCAAGAAGCTGGTGGCTGCTTCCC AGGCCGCCCTGGGGCTCCATCACCATCATCACCCTGA	
KL958 ((C521S C910S)- HSA-His) amino acid sequence	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQTEGGWQ QHGKGASIWDTFTHHPLAPPGDsrNASLPLGAPSPLQPATGDVASDSYNN VFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYRRLRLRLE LGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFQGGV KYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLY NTSFRPTQGGQVSIALSSHWINPRMTDHSIKECQKSLDFVLGWFAKPVFID GDYPESMKNNLSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKF RQLESPNLRQLLSWIDLEFNHPQIFIVENGWVFSVGTTRDDAKYMYLKKFI	54

Table E1		
Name	Sequence	SEQ ID NO:
	METLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGIFYVDFLSQDKMLL PKSSALFYQKLIKNGFPPLPENQPLEGTFPSDFAWGVVDNYIQVDTTLSQF TDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALLQEMHVTHF RFSLDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPN QGLPRLLARQGAWENPYTALAFAYARLCFQELGHHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKD KEVAERVLEFDIGWLAEPFGSGDYPWVMRDWLNQRNNFLLPYFTEDEKKL IQGTDFDLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNSPSQVAVVP WGLRKVLNWLKFKYGDLPYIISNGIDDLHAEDDQLRVYYMQNYINEALK AHILDGINLSGYFAYSFNDRTPRFGLYRYAADQFEPKASMKHYRKIIDSNG FPGPETDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNE VTEFAKTCVADESAENCDSLHTLFGDKLCTVATLRETYGEMADCCAKQEP ERNECFQHKDDNPPLRLVRPEVDVMCTAFHDNEETFLKKYLYEIAARRHP YFYAPELLFFAKRYKAAFTCECCQAADKAACLLPKLDELREDEGKASSAKQRLK CASLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDL LECADDRADLAKYICENQDSISSKLKECCEKPLEKSHCIAEVENDEMPADL PSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLRLAKTY ETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCLEFQLEGEYKFQ NALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSV VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAET FTFHADICTLSEKERQIKKQATLVELVKHKPKATKEQLKAVMDDFAAFVEKC CKADDKETCFAEEGKKLVAASQAALGLHHHHHH	
KL958 ((C521S)-Fc- Twin-Strep) nucleotide sequence	GAGCCTGGGGACGGCGCTCAGACCTGGGCTCGTTTTCTCGTCCTCCT GCTCCTGAGGCTGCTGGCCTCTTCCAGGGCACGTTCCAGACGGCTTTT TGTGGGCGGTGGGTTCTGCGGCCCTACCAGACGGAGGGGGGTTGGCAG CAGCATGGCAAGGGCGCCTCCATCTGGGATACTTTCACGCACCACCCC CTGGCTCCGCCTGGCGATTCCCGCAACGCCTCCCTGCCCTTAGGAGCC CCCTCTCCCTGCAGCCGGCCACCGGCGATGTAGCGTCCGATAGCTAC AATAACGTGTTCCGCGACACCGAGGCTTTGAGAGAGTTGGGCGTTACTC ATTACCGCTTCTCTATCAGTTGGGCCCCGCTACTGCCGAACGGTTCTGC CGGGGTGCCAAACAGGGAGGGGCTTCGCTACTACCGCCGCGCTGCTGGA GCGTTTACGGGAGCTGGGAGTGCAGCCTGTCGTTACTCTGTACCACTG GGACCTCCCGCAGAGGCTGCAGGACGCCTATGGAGGCTGGGCCAACCC GCGCCCTTGCGGACCATTTCGGGATTACGCGGAACTGTGCTTCGGCCA CTTCCGTGGCCAGGTGAAGTATTGGATCACCATTGACAATCCGTATGTG GTGGCTTGGCATGGATACGCGACCGGTAGGCTGGCCCCCGGCATCCGC GGGAGCCCTCGGTTGGGCTACCTGGTGGCGCACAACTGCTGTTGGCC CACGCTAAGGTGTGGCACCTGTACAACACCTCCTTTTCGCCCCACCCAGG GCGGTGAGGTGTCCATCGCCCTCAGCTCCCACTGGATTAACCCCCGGC GCATGACCGACCACTCCATCAAAGAATGTCAGAAGTCCCTCGACTTTGT GCTGGGCTGGTTGCGCAAGCCCGTGTTTATTGACGGTGACTACCCCGA GTCTATGAAGAACAACCTAAGCTCGATCCTGCCCGACTTCACAGAGTCT GAGAAAAAATTTATTAAGGGCACCGCGGACTTCTTCGCACTCTGCTTTG GCCCTACCTGTCTTTTCTAGCTTCTGGACCCCCACATGAAATTCAGACA GCTGGAGTCACCCAACCTGAGGCAGCTGCTGAGTTGGATCGATCTGGA ATTCAATCACCTCAGATCTTCATCGTGGAGAATGGATGGTTCTGCTCAG GCACCACCAAGCGCGACGATGCCAAGTACATGTACTATCTGAAGAAATT TATCATGGAGACACTCAAGGCGATCAAGCTGGACGGCGTCGATGTGATC	55

Table E1		
Name	Sequence	SEQ ID NO:
	GGTTACACCGCTTGGTCTTTGATGGATGGCTTCGAATGGCACC GCGGCT ACTCCATCCGTCGCGGACTGTTCTACGTGGATTTCTGTCTCAGGACAA AATGCTCCTGCCAAAGTCCAGTGCACTGTTCTATCAGAAGCTAATCGAG AAAAATGGCTTCCACCTCTGCCTGAGAACCAGCCCCCTCGAAGGCACCT TCCCTCGGACTTCGCTGGGGCGTCGTGGACAACATATACAAGTGGA CACCACACTTTCTCAGTTTACTGACCTGAACGTGTACCTGTGGGACGTG CACCCTCTAAGCGACTTATCAAGGTGGATGGCGTGGTTACTAAGAAGC GCAAATCATATTGCGTGGATTTTGC GGCCATCCAGCCTCAGATCGCCCT GCTGCAGGAAATGCACGTGACCCACTTTCGATTCTCTCTCGACTGGGCA CTGATTCTGCCACTTGGCAACCAGAGCCAGGTGAACCATACAATCCTGC AATATTACAGGTGTATGGCCAGCGAGCTGGTGC GCGTGAACATCACTCC GGTGGTCGCTCTCTGGCAACCCATGGCCCCCTAACCAAGGCCTGCCTCG CCTGCTGGCTCGCCAGGGTGC GTGGGAAAACCCGTACACGGCCCTGGC CTTCGCCGAGTACGCACGTCTGTGCTTCCAGGAGCTCGGCCACCACGT CAAGTTGTGGATCACCATGAACGAGCCCTACACCCGTAATATGACCTAC AGCGCAGGCCACAACCTGTTGAAGGCCCATGCCCTGGCTTGGCACGTG TACAACGAGAAGTTCCGCCACGCACAGAACGGTAAGATCTCCATCGCTC TTCAGGCGGACTGGATTGAGCCAGCCTGCCCATTTCTCGCAGAAGGACA AAGAGGTGGCCGAACGCGTCTCTGAATTTGACATCGGTTGGCTCGCTG AGCCCATCTTCGGCTCCGGCGACTACCCGTGGGTGATGCGTGACTGGC TGAACCAGCGCAACAACCTTCTGCTGCCCTATTTACCGAGGACGAGAA GAAACTGATACAGGGAACCTTCGACTTTCTAGCACTGTCGCACTACACG ACAATCCTGGTGGACAGCGAGAAGGAGGACCCGATCAAGTACAACGAC TACCTGGAGGTGCAGGAGATGACCGACATCACCTGGCTGAACTCGCCC AGCCAGGTCCGCCGTGGTTCCGTGGGGCCTGCGCAAGGTACTGAACTGG CTGAAGTTCAAGTACGGCGACCTGCCCATGTATATCATCTCCAACGGGA TTGACGATGGGCTACATGCTGAGGATGACCAGCTGCGGGTGACTACAT GCAGAACTACATCAATGAGGCTCTTAAGGCTCACATTCTAGACGGGATT AACTTATGCGGGTACTTCGCGTACTCCTTCAACGACCGCACGGCCCCGC GCTTTGGGCTCTACCGCTACGCTGCGGACCAGTTCGAGCCAAAAGCGT CCATGAAGCACTACCGGAAGATAATCGACAGCAATGGCTTCCCTGGACC CGAGACTGGATCCGAGGCGGTTCCGGCCCCGAGGCCGCTGGCGGCC CTTCCGTGTTCTGTTCCCCCAAAGCCCAAGGACACCCTGATGATCTC TCGCACCCCTGAGGTGACCTGCGTGGTCTGTTGATGTGAGCCACGAAGA TCCAGAGGTTAAGTTCAACTGGTACGTGGACGGCGTCGAAGTCCACAAT GCCAAGACCAAGCCGAGGGAGGAGCAGTACAACCTCCACGTACCGCGTG GTGTCCGTTCTAACCGTGCTTACCAGGACTGGCTAAACGGCAAGGAGT ACAAGTGTAAGGTGTCAAACAAGGCCCTCCCTGCCCCCATTGAGAAGAC CATCTCGAAGGCGAAGGGACAGCCCCGCGAGCCTCAGGTCTACACGCT GCCCCATCCCGCGACGAGCTCACTAAGAACCAGGTGAGCCTTACTTGC CTGGTGAAGGGCTTTTACCCGTCCGACATCGCGGTAGAGTGGGAGAGC AACGGTCAGCCAGAGAATAACTACAAGACGACTCCCCCGGTGCTGGAC AGTGACGGCTCCTTCTTTCTGTATTCTAAGCTGACAGTCGACAAAAGCC GCTGGCAACAGGGTAACGTGTTCTCGTGTCTGTGTAATGCATGAGGCTCT GCACAACCATTACACCCAGAAGAGCCTGTCCCTGTCTCCCGGCAAAAGT TCCCTGGAGGTGCTGTTCCAGGGGCCAGGCAGCGCCTGGTCCCACCCC CAGTTTCGAGAAGGGCGGCGGTAGTGGAGGGGCGGATCTGGCGGCTC AGCTTGGAGCCACCCCCAGTTTCGAAAAGTGA	

Table E1		
Name	Sequence	SEQ ID NO:
KL958 ((C521S)-Fc-Twin-Strep) amino acid sequence	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQTEGGWQ QHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSP LQPATGDVASDSYNN VFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYRRLLERLRE LGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFGGQV KYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLY NTSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFID GDYPESMKNNLSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKF RQLESPNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKFI METLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLSQDKMLL PKSSALFYQK LIEKNGFPPLPENQPLEGTFPSDFAWGVVDNYIQVDTTLSQF TDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALLQEMHVTHF RFSLDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPN QGLPRLLARQGAWENPYTALAFAYARLCFQELGHHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKD KEVAERVLEFDIGWLAEPFGSDYPWVMRDWLNQRNNFLLPYFTEDEKKL IQGTDFLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNSPSQVAVVP WGLRKVLNWLKFKYGDLPYIISNGIDDLHAEDDQLRVYYMQNYINEALK AHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQFEPKASMKHYRKIIDSNG FPGPETGGGSAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMEALHNHYTQKSLSLSPGKGSLEVLFGPGSAWSHPQFEKGGGS GGGSGGSAWSHPQFEK	56
KL958 ((C521S)-monoFc-Twin-Strep) Nucleotide sequence	GAGCCTGGGGACGGCGCTCAGACCTGGGCTCGTTTTCTCGTCCTCCT GCTCCTGAGGCTGCTGGCCTCTTCCAGGGCACGTTCCCAGACGGCTTTT TGTGGGCGGTGGGTTCTGCGGCTACCAGACGGAGGGGTTGGCAG CAGCATGGCAAGGGCGCCTCCATCTGGGATACTTTCACGCACCACCCC CTGGCTCCGCCTGGCGATTCCCGCAACGCCTCCCTGCCCTTAGGAGCC CCCTCTCCCTGCGAGCCGGCCACCGGCATGTAGCGTCCGATAGCTAC AATAACGTGTTCCGCGACACCGAGGCTTTGAGAGAGTTGGGCGTTACTC ATTACCGCTTCTCTATCAGTTGGGCCCCGCTACTGCCGAACGGTTCTGC CGGGGTGCCAAACAGGGAGGGGCTTCGCTACTACCGCCGCTGCTGGA GCGTTTACGGGAGCTGGGAGTGCAGCCTGTCGTTACTCTGTACCACTG GGACCTCCCGCAGAGGCTGCAGGACGCCTATGGAGGCTGGGCCAACC GCGCCCCTTGCGGACCATTTTCGGGATTACGCGGAACGTGTGCTCCGCCA CTTCGGTGGCCAGGTGAAGTATTGGATCACCATTGACAATCCGTATGTG GTGGCTTGGCATGGATACGCGACCGGTAGGCTGGCCCCCGGCATCCGC GGGAGCCCTCGGTTGGGCTACCTGGTGGCGCACAACTGCTGTTGGCC CACGCTAAGGTGTGGCACCTGTACAACACCTCCTTTGCCCCACCCAGG GCGGTCAAGGTGTCCATCGCCCTCAGCTCCCACTGGATTAACCCCCGGC GCATGACCGACCACTCCATCAAAGAATGTCAGAAGTCCCTCGACTTTGT GCTGGGCTGGTTCGCCAAGCCCGTGTATTGACGGTGACTACCCCGA GTCTATGAAGAACAACCTAAGCTCGATCCTGCCCGACTTCACAGAGTCT GAGAAAAAATTTATTAAGGGCACCGCGGACTTCTTCGCACTCTGCTTG GCCCTACCCTGTCTTTTACGCTTCTGGACCCCAACATGAAATTCAGACA GCTGGAGTCACCCAACCTGAGGCAGCTGCTGAGTTGGATCGATCTGGA ATTCAATCACCTCAGATCTTCATCGTGGAGAATGGATGGTTCGTCTCAG	57

Table E1		
Name	Sequence	SEQ ID NO:
	GCACCACCAAGCGCGACGATGCCAAGTACATGTACTATCTGAAGAAATT TATCATGGAGACACTCAAGGCGATCAAGCTGGACGGCGTCGATGTGATC GGTTACACCGCTTGGTCTTTGATGGATGGCTTCGAATGGCACC GCGGCT ACTCCATCCGTCGCGGACTGTTCTACGTGGATTTCTGTCTCAGGACAA AATGCTCCTGCCAAAGTCCAGTGCAGTGTCTATCAGAAGCTAATCGAG AAAAATGGCTTCCACCTCTGCCTGAGAACCAGCCCCCTCGAAGGCACCT TCCCCTCGGACTTCGCCTGGGGCGTCGTGGACAACATATACAAGTGGA CACCACACTTTCTCAGTTTACTGACCTGAACGTGTACCTGTGGGACGTG CACCACCTAAGCGACTTATCAAGGTGGATGGCGTGGTTACTAAGAAGC GCAAATCATATTGCGTGGATTTTGGGCCATCCAGCCTCAGATCGCCCT GCTGCAGGAAATGCACGTGACCCACTTTTCGATTCTCTCTCGACTGGGCA CTGATTCTGCCACTTGGCAACCAGAGCCAGGTGAACCATAACAATCCTGC AATATTACAGGTGTATGGCCAGCGAGCTGGTGC GCGTGAACATCACTCC GGTGGTCGCTCTCTGGCAACCCATGGCCCCCTAACCAAGGCCTGCCTCG CCTGCTGGCTCGCCAGGGTGCCTGGGAAAACCCGTACACGGCCCTGGC CTTCGCCGAGTACGCACGTCTGTGCTTCCAGGAGCTCGGCCACCACGT CAAGTTGTGGATCACCATGAACGAGCCCTACACCCGTAATATGACCTAC AGCGCAGGCCACAACCTGTTGAAGGCCCATGCCCTGGCTTGGCACGTG TACAACGAGAAGTTCCGCCACGCACAGAACGGTAAGATCTCCATCGCTC TTCAGGCGGACTGGATTGAGCCAGCCTGCCCATTTCTCGCAGAAGGACA AAGAGGTGGCCGAACGCGTCCCTCGAATTTGACATCGGTTGGTCGCTG AGCCCATCTTCGGCTCCGGCGACTACCCGTGGGTGATGCGTGA CTGGC TGAACCAGCGCAACAACCTTCTGCTGCCCTATTTACCCGAGGACGAGAA GAACTGATACAGGGAACCTTCGACTTTCTAGCACTGTGCGACTACACG ACAATCCTGGTGGACAGCGAGAAGGAGGACCCGATCAAGTACAACGAC TACCTGGAGGTGCAGGAGATGACCGACATCACCTGGCTGAACTCGCCC AGCCAGGTCGCCGTGGTTCCGTGGGGCCTGCGCAAGGTACTGAACTGG CTGAAGTTCAAGTACGGCGACCTGCCCATGTATATCATCTCCAACGGGA TTGACGATGGGCTACATGCTGAGGATGACCAGCTGCGGGTGTACTACAT GCAGAACTACATCAATGAGGCTCTTAAGGCTCACATTCTAGACGGGATT AATTATGCGGGTACTTCGCGTACTCCTTCAACGACCGCACGGCCCCGC GCTTTGGGCTCTACCGCTACGCTGCGGACCAAGTTTCGAGCCAAAAGCGT CCATGAAGCACTACCGGAAGATAATCGACAGCAATGGCTTCCCTGGACC CGAGACTGGATCCGGAGGCGGTTCCGGCACCTGAGGCCGCCGGGGGTC CCTCCGTGTTCTGTTCCACCCAAGCCTAAGGACACCCTGATGATCTC CCGCACTCCTGAAGTCACTTGCGTGGTGGTTGATGTAAGTCATGAGGAC CCGGAGGTGAAATTTAATTGGTACGTGGACGGCGTGGAGGTGCACAAC GCCAAGACTAAGCCCCGCGAGGAGCAGTACAACCTCCACCTACCGGGTC GTGTCGGTTCTTACGGTGCTCCACCAGGACTGGCTCAACGGTAAGGAGT ACAAGTGCAAAGTATCTAACAAGGCGCTGCCCGCCCCCATTGAGAAAAC CATCTCTAAGGCTAAGGGACAGCCGCGCGAACCTCAGGTGTACACGAG CCCTCCCAGCCGCGACGAGCTGACCAAGAACCAGGTGAGCCTGAGGTG TCACGTGAAGGGCTTCTATCCATCCGACATCGCGGTGGAATGGGAGAG CAATGGCCAGCCAGAGAACAACTACAAGACCACAAAGCCGGTGCTAGAT TCTGATGGCTCCTTCTTCTGTATTCCAAGCTGACTGTGGACAAGTCCC GTTGGCAACAGGGCAACGTCTTTTCATGCTCTGTGATGCACGAGGCTTT GCACAACCATTACCCAGAAGAGCCTTTCGCTGTCCCCCGGCAAGGC AGCCTGGAAGTGCTGTTCCAGGGACCTGGCAGCGCCTGGAGCCACCT	

Table E1		
Name	Sequence	SEQ ID NO:
	CAGTTTGAGAAGGGCGGCGGAAGCGGCGGAGGAGGCTCTGGCGGCAG CGCCTGGTCCCACCCCCAGTTCGAGAAGTGA	
KL958 ((C521S)- monoFc- Twin-Strep) Amino acid sequence	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQTEGGWQ QHKGKASIWDTFTHHPLAPPGDSRNASLPLGAPSPATGDVASDSYNN VFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYRLLERLRE LGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFQQV KYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLY NTSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFID GDYPESMKNNLSSILPDTFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKF RQLESPNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKFI METLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLYVDFLSQDKMLL PKSSALFYQKLIKNGFPPLPENQPLEGTFPSDFAWGVVDNYIQVDTTLSQF TDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPIALLQEMHVTHF RFSLDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPN QGLPRLLARQGAWENPYTALAFAEYARLCFQELGHHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKD KEVAERVLEFDIGWLAEPFGSGDYPWVMRDWLNQRNNFLLPYFTEDEKKL IQGTDFDLALSHYTTILVDSEKEDPIKYNDYLEVQEMTDITWLNSSPSQVAVVP WGLRKVLNWLKFKYGDLPYIISNGIDDLHAEDDLRVYYMQNYINEALK AHILDGINLCGYFAYSFNDRTPRFGFLYRYAADQFEPKASMKHYRKIIDSNG FPGPETGGGSAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTSPPSRDELTKNQVSLRCHVKGF YPSDIAVEWESNGQPENNYKTTKPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMEALHNHYTQKSLSLSPGKGSLEVLFGPGSAWSHPQFEKGGGS GGGGSGGSAWSHPQFEK	58
KL958 ((C521S)- Fc1.5-Twin Strep) nucleotide sequence	GAGCCTGGGGACGGCGCTCAGACCTGGGCTCGTTTTCTCGTCCTCCT GCTCCTGAGGCTGCTGGCCTCTCCAGGGCACGTTCCCAGACGGCTTTT TGTGGGCGGTGGGTTCTGCGGCCTACCAGACGGAGGGGGGTTGGCAG CAGCATGGCAAGGGCGCCTCCATCTGGGATACTTTCACGCACCACCCC CTGGCTCCGCCTGGCGATTCCCGCAACGCCTCCCTGCCCTTAGGAGCC CCCTCTCCCCTGCAGCCGGCCACCGCGCATGTAGCGTCCGATAGCTAC AATAACGTGTTCCGCGACACCGAGGCTTTGAGAGAGTTGGGCGTTACTC ATTACCGCTTCTCTATCAGTTGGGCCCGCGTACTGCCGAACGGTTCTGC CGGGGTGCCAAACAGGGAGGGGCTTCGCTACTACCGCCGCTGCTGGA GCGTTTACGGGAGCTGGGAGTGCAGCCTGTCGTTACTCTGTACCACTG GGACCTCCCGCAGAGGCTGCAGGACGCCTATGGAGGCTGGGCCAACC GCGCCCTTGCGGACCATTTTCGGGATTACGCGGAACGTGTGCTTCGGCCA CTTCGGTGGCCAGGTGAAGTATTGGATCACCATTGACAATCCGTATGTG GTGGCTTGGCATGGATACGCGACCGGTAGGCTGGCCCCCGGCATCCGC GGGAGCCCTCGGTTGGGCTACCTGGTGGCGCACAACTGCTGTTGGCC CACGCTAAGGTGTGGCACCTGTACAACACCTCCTTTTCGCCCCACCCAGG GCGGTCAGGTGTCCATCGCCCTCAGCTCCCACTGGATTAACCCCCGGC GCATGACCGACCACTCCATCAAAGAATGTCAGAAGTCCCTCGACTTTGT GCTGGGCTGGTTCGCCAAGCCCGTGTATTGACGGTGACTACCCCGA GTCTATGAAGAACAACCTAAGCTCGATCCTGCCCGACTTCACAGAGTCT GAGAAAAAATTTATTAAGGGCACCGCGGACTTCTTCGCACTCTGCTTG	59

Table E1		
Name	Sequence	SEQ ID NO:
	GCCCTACCCTGTCTTTCAGCTTCTGGACCCCCACATGAAATTCAGACA GCTGGAGTCACCCAACCTGAGGCAGCTGCTGAGTTGGATCGATCTGGA ATTCAATCACCCCTCAGATCTTCATCGTGGAGAAATGGATGGTTCGTCTCAG GCACCACCAAGCGCGACGATGCCAAGTACATGTACTATCTGAAGAAATT TATCATGGAGACACTCAAGGCGATCAAGCTGGACGGCGTCGATGTGATC GGTTACACCGCTTGGTCTTTGATGGATGGCTTCAATGGCACC GCGGCT ACTCCATCCGTCGCGGACTGTTCTACGTGGATTTCTGTCTCAGGACAA AATGCTCCTGCCAAAGTCCAGTGCACTGTTCTATCAGAAGCTAATCGAG AAAAATGGCTTCCCACCTCTGCCTGAGAACCAGCCCCCTCGAAGGCACCT TCCCCCTCGGACTTCGCCTGGGGCGTCGTGGACAACATATACAAGTGGA CACCACACTTTTCTCAGTTTACTGACCTGAACGTGTACCTGTGGGACGTG CACCACCTCTAAGCGACTTATCAAGGTGGATGGCGTGGTTACTAAGAAGC GCAAATCATATTGCGTGGATTTTGC GGCCATCCAGCCTCAGATCGCCCT GCTGCAGGAAATGCACGTGACCCACTTTCGATTCTCTCTCGACTGGGCA CTGATTCTGCCACTTGGCAACCAGAGCCAGGTGAACCATACAATCCTGC AATATTACAGGTGTATGGCCAGCGAGCTGGTGC GCGTGAACATCACTCC GGTGGTCGCTCTCTGGCAACCCATGGCCCCCTAACCAAGGCCTGCCTCG CCTGCTGGCTCGCCAGGGTGC GTGGGAAAACCCGTACACGGCCCTGGC CTTCGCCGAGTACGCACGTCTGTGCTTCCAGGAGCTCGGCCACCACGT CAAGTTGTGGATCACCATGAACGAGCCCTACACCCGTAATATGACCTAC AGCGCAGGCCACAACCTGTTGAAGGCCCATGCCCTGGCTTGGCACGTG TACAACGAGAAGTTCCGCCACGCACAGAACGGTAAGATCTCCATCGCTC TTCAGGCGGACTGGATTGAGCCAGCCTGCCCCATTCTCGCAGAAGGACA AAGAGGTGGCCGAACGCGTCTCTGAATTTGACATCGGTTGGCTCGCTG AGCCCATCTTCGGCTCCGGCGACTACCCGTGGGTGATGCGTGACTGGC TGAACCAGCGCAACAACCTTCTGCTGCCCTATTTACCGAGGACGAGAA GAAACTGATACAGGGAACCTTCGACTTTCTAGCACTGTGCGCACTACACG ACAATCCTGGTGGACAGCGAGAAGGAGGACCCGATCAAGTACAACGAC TACCTGGAGGTGCAGGAGATGACCGACATCACCTGGCTGAACTCGCCC AGCCAGGTCCGCCGTGGTTCCGTGGGGCCTGCGCAAGGTACTGAACTGG CTGAAGTTCAAGTACGGCGACCTGCCCATGTATATCATCTCCAACGGGA TTGACGATGGGCTACATGCTGAGGATGACCAGCTGCGGGTGTACTACAT GCAGAACTACATCAATGAGGCTCTTAAGGCTCACATTCTAGACGGGATT AACTTATGCGGGTACTTTCGCTACTCCTTCAACGACCGCACGGCCCCGC GCTTTGGGCTCTACCGCTACGCTGCGGACCAGTTCGAGCCAAAAGCGT CCATGAAGCACTACCGGAAGATAATCGACAGCAATGGCTTCCCTGGACC CGAGACTGGATCCGGAGGCGGTTCCGGCCCCGAGGCGCTGGCGGGCC CTTCCGTGTTCTGTTCCCCC CAAAGCCCAAGGACACCCTGATGATCTC TCGCACCCCTGAGGTGACCTGCGTGGTCGTTGATGTGAGCCACGAAGA TCCAGAGGTTAAGTTCAACTGGTACGTGGACGGCGTCGAAGTCCACAAT GCCAAGACCAAGCCGAGGGAGGAGCAGTACAACCTCCACGTACCGCGTG GTGTCCGTTCTAACCGTGCTTACCAGGACTGGCTAAACGGCAAGGAGT ACAAGTGTAAGGTGTCAAACAAGGCCCTCCCTGCCCCCATTGAGAAGAC CATCTCGAAGGCGAAGGGACAGCCCCGCGAGCCTCAGGTCTACACGCT GCCCCCATCCCGCGACGAGCTCACTAAGAACCAGGTCAGCCTTACTTGC CTGGTGAAGGGCTTTTACCCGTCCGACATCGCGGTAGAGTGGGAGAGC AACGGTCAGCCAGAGAATAACTACAAGACGACTCCCCCGGTGCTGGAC AGTGACGGCTCCTTCTTTCTGTATTCTAAGCTGACAGTCGACAAAAGCC	

Table E1		
Name	Sequence	SEQ ID NO:
	GCTGGCAACAGGGTAACGTGTTCTCGTGTTCTGTAATGCATGAGGCTCTGCACAACCATTACACCCAGAAGAGCCTGTCCCTGTCTCCCGGCAAAGGAGGCGGCGGGTCCGGGGGCGGCGGTTCCGGGCGGTGGTGGCTCCGGGGGTGGAGGTTCTGGACAGCCGCGTGAACCTCAGGTGTACACCCTTCCCCCTCTCGGGACGAGCTACCAAAAACCAGGTGTCCCTGACCTGCCTGGTAAAGGGCTTCTATCCGTCGACATCGCCGTGGAGTGGGAGAGTAATGGGCAGCCGGAGAACAACTATAAAACCACACCTCCCGTGCTGGACAGCGATGGCTCCTTCTTCTGTACTCCAAGTTGACTGTGATAAATCGCGATGCGAGCAGGGCAACGTGTTTTCTTGCTCTGTGATGCACGAAGCACTGCACAACCACTACACCCAGAAGTCTTTGTCCCTGAGCCCGGGGAAAGGTTCCCTGGAGGTGCTGTTCCAGGGGCCAGGCAGCGCCTGGTCCCAACCCCAAGTTCGAGAAGGGCGGCGGTAGTGGAGGGGGCGGATCTGGCGGCTCAGCTTGGAGCCACCCCAAGTTCGAAAAGTGA	
KL958 ((C521S)- Fc1.5-Twin- Strep) amino acid sequence	EPGDGAQTWARFSRPPAPEAAGLFQGTFFPDGFLWAVGSAAYQTEGGWQ QHKGKASIWDTFTHHPLAPPGDSTRNASLPLGAPSPLQPATGDVASDSYNN VFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYRRLRLRE LGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFQGGV KYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLY NTSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFID GDYPESMKNLSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKF RQLESPNLRQLLSWIDLEFNHPQIFIVENGWVFSVGTTRDDAKYMYLKKFI METLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGFLFYVDFLSQDKMLL PKSSALFYQKLIKNGFPPLPENQPLEGTFPSDFAWGVVDNYIQVDTTLSQF TDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPIALLQEMHVTHF RFLSDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPN QGLPRLARQGAWENPYTALAFAYARLCFQELGHVHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKD KEVAERVLEFDIGWLAEPFSGSDYPWVMRDWLNQRNNFLLPYFTEDEKKL IQGTDFLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNPSQVAVVP WGLRKVLNWLKFKYGDLPYIISNGIDDLHAEDDQLRVYMQNYINEALK AHILDGINLCGYFAYSFNDRAPRFLYRYAADQFEPKASMKHYRKIIDSNG FPGPETGGGSAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVMHEALHNHYTQKSLSLS PGKGSLEVLFGQPGSAWSHPQFEKGGGSGGGGSGGSAWSHPQFEK	60
KL958 ((C521S)- HSA-Twin- Strep) nucleotide sequence	GAGCCGGGCGACGCGCGCAGACCTGGGCCCGTTTCTCGCGGCCTCC TGCCCCGAGGCCGCGGGCCTCTTCCAGGGCACCTTCCCCGACGGCTT CCTCTGGGCCGTGGGCAGCGCCGCTACCAGACCGAGGGCGGCTGGC AGCAGCACGGCAAGGGTGCCTCATCTGGGATACGTTACCCACCACC CCTGGCACCCCCGGGAGACTCCCGGAACGCCAGTCTGCCGTTGGGC GCCCCGTGCGCGCTGCAGCCCGCCACCGGGGACGTAGCCAGCGACAG CTACAACAACGTCTTCCGCGACACGGAGCGCTGCGCGAGCTCGGGGT CACTCACTACCGCTTCTCATCTCGTGGGCGCGAGTGCTCCCCAATGGC AGCGCGGGCGTCCCCAACCGCGAGGGGCTGCGCTACTACCGGCGCCT	61

Table E1		
Name	Sequence	SEQ ID NO:
	GCTGGAGCGGCTGCGGGAGCTGGGCGTGCAGCCCCGTGGTCACCCTGT ACCACTGGGACCTGCCCCAGCGCCTGCAGGACGCCTACGGCGGCTGG GCCAACCGCGCCCTGGCCGACCACTTCAGGGATTACGCGGAGCTCTGC TTCCGCCACTTCGGCGGTCAAGTACTGGATCACCATCGACAACC CCTACGTGGTGGCCTGGCACGGCTACGCCACCGGGCGCCTGGCCCC GGCATCCGGGGCAGCCCGCGGCTCGGGTACCTGGTGGCGCACAACT CCTCCTGGCTCATGCCAAAGTCTGGCATCTCTACAATACTTCTTTCCGTC CCTCAGGGAGGTCAAGGTGTCCATTGCCCTAAGCTCTCACTGGATCAA TCCTCGAAGAATGACCGACCACAGCATCAAAGAATGTCAAAAATCTCTG GACTTTGTACTAGTTGGTTTGCCAAACCCGTATTTATTGATGGTGACTA TCCCGAGAGCATGAAGAATAACCTTTTCATCTATTCTGCCTGATTTTACTG AATCTGAGAAAAAGTTCATCAAAGGAACTGCTGACTTTTTTGTCTTTTGC TTTGGACCCACCTTGAGTTTTCAACTTTTGGACCCCTACATGAAGTTCCG CCAATTGGAATCTCCCAACCTGAGGCAACTGCTTTCCTGGATTGACCTT GAATTTAACCATCCTCAAAATTTATTGTGAAAAATGGCTGGTTTGTCTCA GGGACCACCAAGAGAGATGATGCCAAATATATGTATTACCTCAAAAAGTT CATCATGGAACCTTAAAAGCCATCAAGCTGGATGGGGTGGATGTCATC GGGTATACCGCATGGTCCCTCATGGATGGTTTCGAGTGGCACAGAGGTT ACAGCATCAGGCGTGGACTCTTCTATGTTGACTTTCTAAGCCAGGACAA GATGTTGTTGCCAAAGTCTTCAGCCTTGTCTACCAAAAAGCTGATAGAGA AAAATGGCTTCCCTCCTTTACCTGAAAATCAGCCCCTAGAAGGGACATTT CCCAGCGACTTTGCTTGGGGAGTTGTTGACAACTACATTCAAGTAGATA CCTCTGTCTCAGTTTACCGACCTGAATGTTTACCTGTGGGATGTCCAC CACAGTAAAAGGCTTATTAAAGTGGATGGGGTTGTGACCAAGAAGAGGA AATCCTACTGTGTTGACTTTGCTGCCATCCAGCCCCAGATCGCTTTACTC CAGGAAATGCACGTTACACATTTTCGCTTCTCCCTGGACTGGGCCCTGA TTCTCCCTCTGGGTAACCAAGTCCAGGTGAACCACACCATCCTGCAGTA CTATCGCTGCATGGCCAGCGAGCTTGTCCGTGTCAACATCACCCAGTG GTGGCCCTGTGGCAGCCTATGGCCCCGAACCAAGGACTGCCGCGCCTC CTGGCCAGGCAGGGCGCCTGGGAGAACCCCTACACTGCCCTGGCCTTT GCAGAGTATGCCCCGACTGTGCTTTCAAGAGCTCGGCCATCACGTCAAGC TTTGGATAACGATGAATGAGCCGTATACAAGGAATATGACATACAGTGCT GGCCACAACCTTCTGAAGGCCCATGCCCTGGCTTGGCATGTGTACAATG AAAAGTTTAGGCATGCTCAGAATGGGAAAATATCCATAGCCTTGCAGGC TGATTGGATAGAACCTGCCTGCCCTTTCTCCAAAAGGACAAAGAGGTG GCTGAGAGAGTTTTGGAATTTGACATTGGCTGGCTGGCTGAGCCCATTT TCGGCTCTGGAGATTATCCATGGGTGATGAGGGACTGGCTGAACCAAAG AAACAATTTTCTTCTTCTTATTTCACTGAAGATGAAAAAAGCTAATCCA GGGTACCTTTGACTTTTTGGCTTTAAGCCATTATACCACCATCCTGTAG ACTCAGAAAAAGAAGATCCAATAAAATACAATGATTACCTAGAAGTGCAA GAAATGACCGACATCACGTGGCTCAACTCCCCAGTCAGGTGGCGGTA GTGCCCTGGGGGTGCGCAAAGTGTGAAGTGGCTGAAGTTCAAGTAC GGAGACCTCCCCATGTACATAATATCCAATGGAATCGATGACGGGCTGC ATGCTGAGGACGACCAGCTGAGGGTGTATTATATGCAGAATTACATAAA CGAAGCTCTCAAAGCCACATACTGGATGGTATCAATCTTTCGGGATACT TTGCTTATTGTTTAACGACCGCACAGCTCCGAGGTTTGGCCTCTATCGT TATGCTGCAGATCAGTTTGAGCCCAAGGCATCCATGAAACATTACAGGA AAATTATTGACAGCAATGGTTTCCCGGGCCAGAACTGGTGGAGGAGG	

Table E1		
Name	Sequence	SEQ ID NO:
	TAGTGATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGGA GAAGAAAATTTCAAAGCCTTGTTGATTGCCTTTGCTCAGTATCTTCA GCAGTGTCATTTGAAGATCATGTAAAATTAGTGAATGAAGTAACTGAAT TTGCAAAAACATGTGTTGCTGATGAGTCAGCTGAAAATTGTGACAAATCA CTTCATACCCTTTTTGAGACAAATTATGCACAGTTGCAACTCTTCGTGA AACCTATGGTGAAATGGCTGACTGCTGTGCAAAACAAGAACCTGAGAGA AATGAATGCTTCTTGCAACACAAAGATGACAACCCAAACCTCCCCGATT GGTGAGACCAGAGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAA GAGACATTTTTGAAAAATACTTATATGAAATTGCCAGAAGACATCCTTAC TTTTATGCCCGGAACCTCTTTCTTTGCTAAAAGGTATAAAGCTGCTTTT ACAGAAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTGTTGCCAAGC TCGATGAACTTCGGGATGAAGGGAAGGCTTCGTCTGCCAAACAGAGACT CAAGTGTGCCAGTCTCCAAAAATTTGGAGAAAGAGCTTTCAAAGCATGG GCAGTAGCTCGCCTGAGCCAGAGATTTCCCAAAGCTGAGTTTGCAGAAG TTTCCAAGTTAGTGACAGATCTTACCAAAGTCCACACGGAATGCTGCCAT GGAGATCTGCTTGAATGTGCTGATGACAGGGCGGACCTTGCCAAGTATA TCTGTGAAATCAAGATTCTGATCTCCAGTAAACTGAAGGAATGCTGTGAA AAACCTCTGTTGGAAAAATCCCACTGCATTGCCGAAGTGGAAAAATGATG AGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTGAAAGTAAG GATGTTTGCAAAACTATGCTGAGGCAAAGGATGTCTTCCTGGGCATGT TTTTGTATGAATATGCAAGAAGGCATCCTGATTACTCTGTCTGTCTGCTG CTGAGACTTGCCAAGACATATGAAACCACTCTAGAGAAGTGCTGTGCCG CTGCAGATCCTCATGAATGCTATGCCAAAGTGTTGATGAATTTAAACCT CTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAATTGTGAGCTTTTTGA GCAGCTTGAGAGTACAAATTCAGAATGCGCTATG GAGCCGGGCGACGGCGCGCAGACCTGGGCCCCGTTTCTCGCGGCCCTCC TGCCCCCGAGGCGCGGGCCTCTTCCAGGGCACCTTCCCCGACGGCTT CCTCTGGGCCGTGGGCAGCGCGCCTACCAGACCGAGGGCGGCTGGC AGCAGCACGGCAAGGGTGCGTCCATCTGGGATACGTTACCCACCACC CCCTGGCACCCCCGGGAGACTCCCGGAACGCCAGTCTGCCGTTGGGC GCCCCGTGCGCGCTGCAGCCCCGCCACCGGGGACGTAGCCAGCGACAG CTACAACAACGTCTTCCGCGACACGGAGGCGCTGCGCGAGCTCGGGGT CACTCACTACCGCTTCTCCATCTCGTGGGCGCGAGTGCTCCCCAATGGC AGCGCGGGCGTCCCCAACCGCGAGGGGCTGCGCTACTACCGGCGCCT GCTGGAGCGGCTGCGGGAGCTGGGCGTGCAGCCCGTGGTCACCCTGT ACCACTGGGACCTGCCCCAGCGCCTGCAGGACGCCTACGGCGGCTGG GCCAACCGCGCCCTGGCCGACCACTTCAGGGATTACGCGGAGCTCTGC TTCCGCCACTTCGGCGGTCAGGTCAAGTACTGGATCACCATCGACAACC CCTACGTGGTGGCCTGGCACGGCTACGCCACCGGGCGCCTGGCCCCC GGCATCCGGGGCAGCCCCGCGGCTCGGGTACCTGGTGGCGCACAACT CCTCCTGGCTCATGCCAAAGTCTGGCATCTCTACAATACTTCTTTCCGTC CCTCAGGGAGGTCAAGTGTCCATTGCCCTAAGCTCTCACTGGATCAA TCCTCGAAGAATGACCGACCACAGCATCAAAGAATGTCAAAAATCTCTG GACTTTGTACTAGGTTGGTTTGCCAAACCCGTATTTATTGATGGTGACTA TCCCGAGAGCATGAAGAATAACCTTTTCTATTCTGCCTGATTTTACTG AATCTGAGAAAAAGTTCATCAAAGGAACTGCTGACTTTTTTGTCTTTTGC TTTGGACCCACCTTGAGTTTTCACTTTTGGACCCCTCACATGAAGTTCCG CCAATTGGAATCTCCCAACCTGAGGCAACTGCTTTCCTGGATTGACCTT	

Table E1		
Name	Sequence	SEQ ID NO:
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Table E1		
Name	Sequence	SEQ ID NO:
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KL958 ((C521S)- G4S-HSA- Twin-Strep) amino acid sequence	EPGDGAQTWARFSRPPAPEAAGLFGQTFPDGFLWAVGSAAYQTEGGWQ QHGKGASIWDTFTHHPLAPPGDSRNASLPLGAPSLQPATGDVSDSYNN VFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYRRLRLRE LGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFQGV KYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLY NTSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFID GDYPESMKNNLSSILPDFTESEKKFIKTADFFALCFGPTLSFQLLDPHMKF RQLESPNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKFI METLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGIFYVDFLSQDKMLL PKSSALFYQKLIKNGFPPLPENQPLEGTFPSDFAWGVVDNYIQVDTTSLQF TDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHF RFSLDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPN QGLPRLLARQGAWENPYTALAFAEYARLCFQELGHHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKD KEVAERVLEFDIGWLAEPFGSGDYPWVMRDWLNQRNNFLLPYFTEDEKKL IQGTFDFLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNSPSQVAVVP WGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVYYMQNYINEALK AHILDGINLCGYFAYSFNDRTAPRFGLYRYAADQFEPKASMKHYRKIDSNG FPGPETGGGSDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQCCPFEDHV KLVNEVTEFAKTCVADESAENCDSKSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFQHKDDNPPLRLVRPEVDMCTAFHDNEETFLKKYLYEI ARRHYPFYAPELLFFAKRYKAAFTCCQAADKAACLLPKLDELDEGKASS AKQRLKASLQKQGERAFKAWAVARLSQRFPAEFAEVSKLVTDLTQKVHTE	62

Table E1		
Name	Sequence	SEQ ID NO:
	CCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVEND EMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYFYARRHPDYSVLLLL RLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCSELFQQLG EYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAE DYL SVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAA FVEKCKKADDKETCFAEEGKKLVAASQAALGLLEVLFGPGGSAWSHPQFE KGGGSGGGGSGGSAWSHPQFEK	
KL958 ((C370S)(C5 21S)-G4S- HSA amino acid sequence	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQTEGGWQ QHKGASIWDTFTHHPLAPPGDSRNASLPLGAPSPQPATGDVASDSYNN VFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLRYRRLERLRE LGVQPVVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHFQGGV KYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLY NTSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFID GDYPESMKNLSSILPDFTESEKKFIKGTADFFALSFGPTLSFQLLDPHMKF RQLESPNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKFI METLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGIFYVDFLSQDKMLL PKSSALFYQKLEKNGFPPLPENQPLEGTFPSDFAWGVVDNYIQVDTTLSQF TDLNVYLWDVHHSKRLIKVDGVVTKKRKSYCVDFAAIQPQIALQEMHVTHF RFLSDWALILPLGNQSQVNHTILQYYRCMASELVRVNITPVVALWQPMAPN QGLPRLLARQGAWENPYTALAFAEYARLCFQELGHHVKLWITMNEPYTRN MTYSAGHNLLKAHALAWHVYNEKFRHAQNGKISIALQADWIEPACPFSSQKD KEVAERVLEFDIGWLAEIFGSGDYPWVMRDWLNQRNFFLLPYFTEDEKKL IQGTDFLALSHYTTILVDSEKEDIKYNDYLEVQEMTDITWLNSPSQVAVVP WGLRKVLNWLKFKYGDLPYIISNGIDDGLHAEDDQLRVYMQNYINEALK AHILDGINLCGYFAYSFNDRRTAPRFGLYRYAADQFEPKASMKHYRKIIDSNG FPGPETGGGSDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHV KLVNEVTEFAKTCVADESAENCDSLHTLFGDKLCTVATLRETYGEMADCC AKQEPERNECFQLQHKKDDNPPLPRLVRPEVDVMCTAFHDNEETFLKKYLYEI ARRHPYFYAPELFFAKRYKAAFTGCCAADKAACLLPKLDELREDEGKASS AKQRLKCASLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLTQVHTE CCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVEND EMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYFYARRHPDYSVLLLL RLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCSELFQQLG EYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAE DYL SVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKE FNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAA FVEKCKKADDKETCFAEEGKKLVAASQAALGL	63

9.1.2. Purification of KL Polypeptide Constructs

9.1.2.1. Affinity Column Purification

[0145] For some evaluations, His- and HSA-tagged KL polypeptides were purified with Ni-NTA Agarose (Qiagen) or CaptureSelect™ Human Albumin Affinity Matrix (ThermoFisher). First the Ni-NTA and Albumin Affinity Matrix columns were equilibrated with 5 column volumes (CV) of equilibration buffer. Next, sterile filtered supernatant containing KL polypeptides was loaded over the pre-equilibrated column at a flow rate of 2.0 mL/min. Any non-specifically bound substances were washed out the columns using a wash buffer for 5CV at a flow rate of 2.0 mL/min. Finally, the affinity bound KL proteins were eluted from the column with 1CV elution buffer and further purified with SEC. The buffer compositions for His and HSA affinity columns are set forth in Table E2 below.

Table E2 Affinity Column Buffer Compositions			
Column Type	Buffer Name	Buffer Composition	Buffer pH
HSA	Equilibration	50mM Tris, 150 mM NaCl	8
	Wash	50mM Tris, 500 mM NaCl	8
	Elution	100mM Glycine	3
His	Equilibration	50mM Tris, 150 mM NaCl	8
	Wash	50mM Tris, 500 mM NaCl, 20 mM Imidazole	8
	Elution	50mM Tris, 500 mM NaCl, 250 mM Imidazole	8

9.1.2.2. Twin-Strep Purification

[0146] Twin-Strep-tagged KL polypeptides were purified with Strep-Tactin®XT resin (IBA). First, the column was equilibrated with 5 column volumes (CV) of PBS buffer (pH7.4). Next, sterile filtered supernatant containing KL polypeptides was loaded over the pre-equilibrated column at a flow rate of 2.0 mL/min. Any non-specifically bound substances were washed out the columns using PBS for 5CV at a flow rate of 2.0 mL/min. 1CV PBS and HRV-3C protease (SigmaAldrich, 1:100) were added to the column, incubated at 4 °C for 24 hrs, and the flow-through was harvested. Next, the column was washed with 3CV PBS and the resulting flow-through was harvested. Both flow-throughs were combined and further purified with SEC.

9.1.2.3. Modified High Salt Purification

[0147] A CM volume of 500 mL was collected from CHO cell lines expressing KL polypeptide constructs. CaptureSelect™ HSA Affinity Resin (ThermoFisher) in PBS was utilized for binding overnight at 4 °C. KL polypeptides were eluted from the resin with a high salt elution buffer (2.0 M MgCl₂ in Tris-HCl, pH 7.4). Aggregated and HMW proteins and other contaminations were removed with SEC.

9.1.2.4. Modified Ion Exchange Purification

[0148] A three-step modified ion exchange (IEX) purification was utilized to capture the KL polypeptides from large volume samples. In step one, sterile filtered supernatant containing KL polypeptides were loaded onto Q-Sepharose column. The column was washed with 20mM PBS pH6.5. Proteins were eluted with NaCl gradient (50mM to 2M). SDS-PAGE gels were used for guidance with the fraction collection. In the next step, CaptureSelect™ Human Albumin Affinity Matrix was used to specifically capture the HSA-tagged KL polypeptides and remove the contaminants. Proteins were eluted with Pierce™ Gentle Ag/Ab Elution Buffer, pH 6.6 (ThermoFisher). KL polypeptides were further purified with SEC.

9.1.3. Pharmacokinetic Analysis of KL Polypeptides

[0149] To assess the pharmacokinetic properties of KL polypeptides in plasma, adult C57BL/6J mice were intraperitoneally injected with a single dose of a KL polypeptide or a control antibody diluted in saline. Blood samples were collected at 0, 2, 8, 24, 48, 72, and 96 hours as well as at day 6 and 14 after dosing. Plasma was isolated by centrifuging the samples.

9.1.4. pERK HTRF Assay

[0150] Phospho-ERK (pERK) homogeneous time-resolved fluorescence-based (HTRF) assay utilizes ERK signaling as a readout of FGF receptor activity, triggered by co-binding of FGF23 and alpha-Klotho.

[0151] NIH3T3 cells (ATCC) in culture were washed twice with 10 mL Ca²⁺ and Mg²⁺-free PBS. 4 mL of TrypLE™ (ThermoFisher) was added to washed cells, which were then incubated at 37 °C, 5% CO₂ for 5 minutes. 10 mL of PBS was added to the cells, and cell clumps were broken up by repeated pipetting. The dissociated cell mixture was transferred to a 50 mL conical tube and centrifuged at 1,000 rpm for 5 minutes. The supernatant was

removed, and the cells were resuspended in 1 mL assay medium (0.5 % BSA in OptiMEM) by repeated pipetting. Next, 10 mL of assay medium was added to the resuspended cells, and cells were counted by Auto T4. Cells were diluted to 2.0×10^5 cells/mL in assay medium, seeded onto 96-well plates at 20,000 cells/well, and incubated overnight at 37 °C, 5% CO₂.

[0152] The next day, the media were replaced with 50 µL of assay medium/well and plates were incubated at 37 °C, 5% CO₂ for 2 hours. KL polypeptides were diluted to 80 nM in assay medium and further diluted 1:2. FGF23 and anti-pERK antibodies were diluted in assay medium to a concentration of 40 nM and 400 nM, respectively. Wells were pretreated with antibodies for 10 minutes. KL polypeptide dilutions and FGF23 were added to corresponding wells and plates were incubated at 37 °C, 5% CO₂ for 15 minutes. The supernatant in each well was replaced with 30 µL NP40 lysis buffer containing a protease and phosphatase inhibitor cocktail. HTRF detection steps were carried out by following manufacturer's advanced phospho-ERK (Thr202/Tyr204) cellular HTRF kit protocol (CiSBio).

9.1.5. SRE/ERK Luciferase Assay

[0153] HEK293.FGFR1KO.hFGFR1c.Sre-Luc reporter line was treated with a KL polypeptide of interest for 5 hours.

9.1.6. 3T3 Cell Proliferation Assay

[0154] 3T3 cells were maintained in culture according to suppliers' recommendations. For the assay, the cells were seeded in 96-well plates in culture medium and cultured for 24-48 hours at 37 °C, 5% CO₂ until use. Next, the cells were treated with a KL polypeptide or a control substance in culture medium. Cell proliferation was monitored every three hours for 66 hours and was expressed as percent phase confluence. Untreated cells were used as controls.

9.2. Example 1: Sequence Engineering of KL Polypeptides

[0155] In order to engineer the KL amino acid sequence for efficient production of bioactive KL polypeptides, one or more modifications were introduced to the full-length hKL sequence shown in FIG. 3, as described in Section 9.1.1. Resulting polypeptides were purified using His affinity columns followed by SEC as described in Section 9.1.2.1 and the bioactivity of the purified KL polypeptides was assessed using pERK HTRF assay as described in Section 9.1.4.

[0156] Four distinct KL peptide sequences, KL981, KL958, KL958 C521S, and KL958 C521S C910S were engineered as follows: KL981 was generated by replacing its native signal sequence corresponding to the first 33 amino acids with an mROR1 signal sequence (SS). KL958 was generated by truncating KL981 at the C-terminal to remove the C-terminal loop. KL958 C521S was generated by replacing the Cys residue at 521 of KL958 with a Ser. KL958 C521S C910S was generated by replacing the Cys residue at 910 of KL958 C521S with a Ser. A C-terminal His-tag was added to all four constructs.

[0157] First, the yield and purity of each construct were evaluated. KL958 C521S was associated with ~5-7 times higher yield than the other three constructs (FIG. 4A). The constructs truncated at the C-termini had a lower levels of high molecular weight species (HMW) than KL981 (FIG. 4A).

[0158] To determine whether sequence engineering could affect KL bioactivity, changes in phospho-ERK (pERK) levels were measured in NIH3T3 and NHDF cells upon KL and FGF23 co-treatment and quantified as fold changes in pERK relative to KL alone treatment. A positive control KL protein (hKL) was used for comparison. All four sequence-engineered constructs were associated with increased pERK fold change in NIH3T3 and NHDF cells (FIGS. 4B and 4C, respectively). Among the four engineered constructs, KL958 C521S displayed the highest pERK fold change in both cell lines and surpassed the pERK fold change observed with the positive control hKL protein.

[0159] In sum, C-terminal truncation combined with the C521S mutation not only resulted in relatively high yield of KL polypeptide with low levels of HMW, but it was also associated with enhanced bioactivity.

9.3. Example 2: Effect of Purification pH on KL Polypeptide Yield and Activity

[0160] In order to assess the effect of different affinity columns on the yield and activity of KL polypeptides, a double-tagged new construct, KL958 (C521S C910S)-HSA-His, was designed and produced as described in Section 9.1.1. Resulting polypeptides were purified using His or HSA affinity columns followed by SEC as described in Section 9.1.2.1 and the bioactivity of the purified KL polypeptides was assessed using pERK HTRF assay as described in Section 9.1.4.

[0161] The yield and purity of the double-tagged KL polypeptide KL958 (C521S C910S)-HSA-His was evaluated with SDS-PAGE under non-reducing (NR) and reducing (R) conditions by loading 10 µL of purified polypeptide sample per well. Although HSA affinity column purification achieved a visibly higher yield (*i.e.*, a much thicker band at ~160 kDa under reducing conditions), it was associated with high molecular weight aggregates (*i.e.*, a smear above ~160 kDa under non-reducing conditions) (FIG. 5A) and displayed no activity, as the pERK HTRF signal of KL + FGF23 co-treatment was lower than the cells-only background signal (FIGS. 5B and 5C). Purification of the same construct with a His-affinity column resulted in a higher pERK HTRF signal in cells co-treated with FGF23 (FIG. 5C). Given that the elution buffer pH values differ, these results suggested that the acidic pH of the HSA elution buffer inactivated the purified KL polypeptide.

9.4. Example 3: Effect of Different C-Terminal Tags on Yield and Purity of KL Polypeptides

[0162] KL polypeptide constructs were designed by linking different C-terminal tags to REGN 14226 (KL958 C521S), and produced as described in Section 9.1.1. Resulting polypeptides were purified using Twin-Strep purification method as described in Section 9.1.2.2.

[0163] A set of four KL polypeptide constructs were utilized in this example. KL958(C521S)-HSA comprised a C-terminal HSA and Twin-Strep-tag (FIG. 6A), KL958(C521S)-Fc comprised a C-terminal Fc and Twin-Strep-tag (FIG. 6B); KL958(C521S)-Fc1.5 comprised a C-terminal Fc1.5 and Twin-Strep-tag, wherein the Fc1.5 comprised one CH2 and two CH3 regions (FIG. 6C); and KL958(C521S)-moFc comprised a C-terminal moFc and Twin-Strep-tag (FIG. 6D).

[0164] Following Twin-Strep purification, samples were treated with 3C protease to remove the Twin-Strep-tag. The yield and purity of the 3C-treated and untreated constructs were evaluated via SDS-PAGE under reducing and non-reducing conditions. 3C-protease treated and untreated KL polypeptide constructs resulted in slightly lower molecular weight bands with comparable thickness for each group, suggesting that the protease treatment successfully removed Twin-Strep-tag from each polypeptide construct (FIG. 7A). The highest yield was achieved with KL958(C521S)-HSA and KL958(C521S)-Fc1.5, whereas KL958(C521S)-Fc was associated with high molecular weight aggregation (FIG. 7A).

[0165] The yield and purity of the KL polypeptide constructs comprising either an Fc, an Fc1.5, a moFc, or an HSA tag were also evaluated with SEC. Consistent with the SDS-PAGE results, the Fc-tagged KL polypeptide had the highest HMW peak, whereas the peak corresponding to the KL958 C521 polypeptide was relatively smaller (FIG. 7B). MoFc- and Fc1.5-tagged KL polypeptides had better yields than Fc-tagged polypeptide construct; however, the highest yield and lowest HMW levels were achieved with the HSA-tagged KL polypeptide construct (FIGS. 7C-7E).

9.5. Example 4: Enhancement of HSA-Tagged KL Polypeptide Purification

[0166] Experiments in Example 3 suggested that HSA-tagged KL polypeptide results in higher yields than KL polypeptides with other tags. Yet, the results of the experiments in Example 2 suggested that HSA affinity purification with a low pH elution buffer renders HSA-tagged KL polypeptides biologically inactive. The goal of the experiments in this present Example was to enhance the purification of biologically active KL958 (C521)-HSA polypeptides.

[0167] First, a modified high salt purification method was used as described in Section 9.1.2.3 by binding HSA-tagged KL polypeptides in a 500 mL of sample with an HSA Affinity resin, and eluting the proteins from the resin with a high salt buffer at pH 7.4. Next, HMW were removed with SEC, which accounted for approximately 75% of the total yield. SDS-PAGE was used to analyze the fractions that correspond to reduced and non-reduced HMW (peak 1 in FIG. 8B) and the eluted KL polypeptide (peak 2 in FIG. 8B). In non-reduced conditions, a thin band corresponding to the monomeric KL polypeptide was observed in the HMW lane, whereas in reduced conditions, the amount of monomeric KL polypeptide in the HMW lane was comparable to that of the eluted KL polypeptide lane (FIG. 8A), suggesting most the polypeptides in the HMW fraction were aggregates of KL polypeptides. The final yield of monomeric KL polypeptides purified with this method was 8 mg (16 mg/L).

[0168] In order to further enhance the large scale purification of KL polypeptides, a modified ion exchange (IEX) purification method was used as described in Section 9.1.2.4, which involved three steps: a Q-Sepharose step to concentrate the KL polypeptides (FIGS. 9A and 9B), followed by capturing of KL polypeptides with an HSA affinity resin (FIGS. 9C and 9D), which was then followed by SD-200 step to further remove any remaining contaminants

(FIGS. 9E and 9F). Purification of KL958 (C521)-HSA from a 25L sample with this approach resulted in approximately 5 mg/L monomeric KL polypeptides.

9.6. Example 5: Pharmacokinetic Profiles of His- and HSA-Tagged KL Polypeptides

[0169] Pharmacokinetic properties of His-tagged and HSA-tagged KL polypeptides were assessed in mice as described in Section 9.1.3. Briefly, mice received a single injection of one of the three doses of KL958 (C521)-HSA (3, 10, or 30 mg/kg) or 20 mg/kg KL958 (C521)-His. A positive control group of mice was dosed with a single injection of 3 mg/kg control mAb.

[0170] By 8 hours after dosing, KL958 (C521)-His was undetectable, suggesting that this polypeptide was cleared relatively rapidly. KL958 (C521)-HSA exhibited a slower clearance than the His-tagged KL polypeptide at every dose evaluated (FIG. 10A). Dose-normalization of the data revealed that although HSA-tagged KL polypeptide exhibited similar clearance with all three doses, clearance rates were slightly faster at 3 and 10 mg/kg after 24 hours (FIG. 10B).

9.7. Example 6: Effects of His- and HSA-Tagged KL Polypeptides on 3T3 Cell Proliferation

[0171] The proliferative effect of His-tagged and HSA-tagged KL polypeptides KL958 (C521)-His and KL958 (C521)-HSA was evaluated as described in Section 9.1.6.

[0172] Both KL polypeptides promoted 3T3 cell proliferation when co-incubated with 10 nM FGF23. Yet, the potency of KL958 (C521)-His was greater than that of KL958 (C521)-HSA (FIGS. 11A and 11B). Next, the same concentration (20 nM) of KL958 (C521)-His and KL958 (C521)-HSA was added to cells with or without 10 nM FGF23. In the presence of FGF23, both KL polypeptides resulted in 3T3 cell proliferation, but the percent phase confluence was more pronounced with the KL958 (C521)-His construct (FIG. 11C).

9.8. Example 7: Differences in C-Terminal Truncation Affect the Activity of KL Polypeptides

[0173] The experiments in the previous Examples assessed the activity of KL958 polypeptides that were derived from the full-length human KL981 by truncating the 23 amino acids at the C-terminus. To determine whether a construct with a slightly different length would result in a construct with similar activity, a KL polypeptide with a 20 amino acid

truncation at the C-terminus, KL961 (C521S)-HSA, was designed and produced as described in Section 9.1.1. The activity of KL958 (C521S)-HSA and KL961 (C521S)-HSA was measured as described in Section 9.1.5.

[0174] Both KL958 (C521S)-HSA and KL961 (C521S)-HSA displayed activity; however, the signal magnitude was higher with the KL958 (C521S)-HSA (FIG. 12). Similarly, the potency of KL958 (C521S)-HSA was higher with an EC50 value of 2.8E-08 M, which was approximately an order of magnitude higher than the EC50 value obtained with KL961 (C521S)-HSA.

9.9. Example 8: Targeted Sequence Engineering to Enhance KL Polypeptide Yield and Activity

[0175] A new set of KL-polypeptides were designed by introducing additional mutations to HSA-tagged KL958 C521S (FIGS. 13A-13F) and produced as described in Section 9.1.1 and purified as described in Section 9.1.2.2. The activity of the construct KL958 (C521S C370S)-G4S-HSA (FIG. 13D), was evaluated as described in Section 9.1.5. and compared to the activity of KL958 (C521)-HSA (FIG. 13A).

[0176] KL958 (C521S C370S)-G4S-HSA had a lower EC50 value and better yield relative to KL958 (C521)-G4S-HSA (Table E3).

Table E3 Targeted Sequence Engineering Associated Changes in Potency and Expression				
Construct	Yield (mg/L)	Expression level (rel. to KL958 (C521)-HSA)	EC50 (trial 1)	EC50 (trial 2)
KL958 (C521S)-G4S-HSA	4	1	11.9E-06	11.1E-06
KL958 (C521S C370S)-G4S-HSA	8.1	~2	9.7E-6	9.8E-06

WHAT IS CLAIMED IS:

1. A polypeptide which:
 - (a) comprises an alpha klotho KL2 domain having (i) at least about 80% sequence identity to SEQ ID NO:3 and (ii) an amino acid substitution at the position corresponding to amino acid C521 of SEQ ID NO:1; and
 - (b) lacks a cysteine at the amino acid corresponding to amino acid C970 of SEQ ID NO:1, if present.
2. The polypeptide of claim 1 which lacks an amino acid corresponding to amino acid C970 of SEQ ID NO:1.
3. The polypeptide of claim 1 which lacks a cysteine at the amino acid corresponding to C973 of SEQ ID NO:1, if present, optionally which lacks an amino acid corresponding to amino acid C973 of SEQ ID NO:1.
4. The polypeptide of claim 1 which lacks a cysteine at the amino acid corresponding to C963 of SEQ ID NO:1, if present, optionally which lacks an amino acid corresponding to amino acid C963 of SEQ ID NO:1.
5. The polypeptide of any one of claims 1 to 4, wherein the amino acid substitution at the position corresponding to amino acid C521 of SEQ ID NO:1 is a cysteine to serine mutation.
6. The polypeptide of any one of claims 1 to 5 which comprises a C-terminal deletion of at least 21 amino acids or at least 23 amino acids as compared to the amino acid sequence of SEQ ID NO:13
7. The polypeptide of any one of claims 1 to 6, which lacks an amino acid sequence having at least 95%, at least 98%, or 100% sequence identity to SEQ ID NO:8.

8. The polypeptide of any one of claims 1 to 7, wherein the alpha klotho KL2 domain has at least about 95% sequence identity to SEQ ID NO:3.
9. The polypeptide of any one of claims 1 to 8, wherein the alpha klotho KL2 domain has at least about 99% or 100% sequence identity to SEQ ID NO:4.
10. The polypeptide of any one of claims 1 to 9, further comprising an alpha klotho KL1 domain having at least about 80%, at least about 95%, at least about 99%, or 100% sequence identity to SEQ ID NO:9.
11. The polypeptide of claim 10, wherein the alpha klotho KL1 domain has at , at least about 80%, least about 95%, at least about 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:10.
12. The polypeptide of claim 10 or 11, wherein the alpha klotho KL1 domain comprises an amino acid substitution at the position corresponding to position C370 of SEQ ID NO:1, which is optionally a cysteine to serine substitution.
13. The polypeptide of any one of claims 1 to 12 which comprises an amino acid sequence having at least about 99% or 100% sequence identity to SEQ ID NO:12.
14. The polypeptide of any one of claims 1 to 13 which comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:15.
15. The polypeptide of any one of claims 1 to 14 which comprises an amino acid sequence having at least about 95%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:16.
16. The polypeptide of any one of claims 1 to 15, wherein the amino acid sequence is between 900 and 925 amino acids in length.
17. The polypeptide of any one of claims 1 to 16 which lacks the amino acid sequence of SEQ ID NO:17.

18. The polypeptide of any one of claims 1 to 17, further comprising a signal peptide.
19. The polypeptide of any one of claims 1 to 18, further comprising a stabilization moiety C-terminal to the alpha klotho KL2 domain.
20. The polypeptide of claim 18, further comprising a stabilization moiety N-terminal to the alpha klotho KL2 domain.
21. The polypeptide of claim 19 or 20, wherein the stabilization moiety comprises an Fc domain comprising a CH2 domain and a CH3 domain, the Fc domain optionally comprising an amino acid sequence having at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO:23 or SEQ ID NO:26.
22. The polypeptide of claim 21, wherein the Fc domain further comprises an additional CH3 domain connected to the CH3 domain via a linker.
23. The polypeptide of claim 21, wherein the Fc domain is a non-dimerizing Fc domain, optionally comprising an amino acid sequence having at least 98%, at least 99%, or 100% sequence identity to any one of SEQ ID NOs:27-32.
24. The polypeptide of claim 19 or 20, wherein the stabilization moiety is an albumin moiety.
25. The polypeptide of claim 24, wherein the albumin moiety is human serum albumin.
26. The polypeptide of claim 24, wherein the albumin moiety is a human serum albumin variant, optionally having the amino acid substitution C34S.
27. The polypeptide of claim 26, wherein the albumin moiety comprises the amino acid sequence of SEQ ID NO:20.

28. The polypeptide of any one of claims 19 to 27, further comprising a linker, optionally a linker comprising a sequence set forth in Table L.

29. The polypeptide of claim 28, wherein the polypeptide comprises, in N- to C-terminal order: an alpha klotho moiety comprising the KL2 domain, the linker, and the stabilization moiety.

30. The polypeptide of claim 28, wherein the polypeptide comprises, in N- to C-terminal order: the stabilization moiety, the linker, and an alpha klotho moiety comprising the alpha klotho KL2 domain.

31. The polypeptide of any one of claims 1 to 30, wherein the polypeptide is a monomer.

32. The polypeptide of any one of claims 1 to 30, wherein the polypeptide is a dimer.

33. A polypeptide, optionally a polypeptide of any one of claims 1 to 32, comprising an amino acid sequence having at least 80% sequence identity to SEQ ID NO:16, wherein the position corresponding to amino acid 488 of SEQ ID NO:16 is not a cysteine and which lacks an amino acid sequence having at least 80% sequence identity to SEQ ID NO:8.

34. The polypeptide of claim 33, wherein the position corresponding to amino acid 488 of SEQ ID NO:16 is a serine.

35. The polypeptide of claim 33 or 34, which comprises the amino acid sequence of SEQ ID NO: 16.

36. The polypeptide of any one of claims 33 to 35, which lacks the amino acid sequence of SEQ ID NO:8.

37. The polypeptide of any one of claims 1 to 36, which comprises the amino acid sequence of SEQ ID NO:62.

38. A nucleic acid encoding the polypeptide of any one of claims 1 to 37.

39. A host cell engineered to express the polypeptide of any one of claims 1 to 37 or the nucleic acid of claim 38.

40. A method of producing the polypeptide of any one of claims 1 to 37, comprising culturing the host cell of claim 39 and recovering the polypeptide expressed thereby.

41. The method of claim 40, further comprising purifying the polypeptide.

42. The method of claim 41, wherein the polypeptide is purified at a pH between 6.9 and 7.1.

43. A pharmaceutical composition comprising the polypeptide of any one of claims 1 to 37 and an excipient.

44. A method of activating FGFR signaling in a cell, the method comprising contacting the cell with the polypeptide of any one of claims 1 to 37.

45. The method of claim 44, wherein the cell is a kidney cell.

46. The method of claim 44 or 45, wherein the method is an in vitro method.

47. The method of claim 44 or 45, wherein the method is an in vivo method.

48. A method of preventing an age-related condition in a subject or of treating a subject suffering from an age-related condition, the method comprising administering to the subject the polypeptide of any one of claims 1 to 37 or the pharmaceutical composition of claim 43.

49. A method of preventing an age-related condition comprising administering to a subject in need thereof the polypeptide of any one of claims 1 to 37 or the pharmaceutical composition of claim 43.

50. A method of preventing kidney disease in a subject or of treating a subject suffering from kidney disease, the method comprising administering to the subject the polypeptide of any one of claims 1 to 37 or the pharmaceutical composition of claim 43.