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(54) Benævnelse: **Opløselig fibroblastvækstfaktorreceptor 3 (SFGFR3)-polypeptider og anvendelser deraf**

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DESCRIPTION

FIELD OF THE INVENTION

[0001] The invention features soluble fibroblast growth factor receptor 3 (sFGFR3) polypeptides and compositions thereof. The invention also features methods to treat skeletal growth retardation disorders, such as achondroplasia.

BACKGROUND OF THE INVENTION

[0002] Fibroblast growth factor receptor 3 (FGFR3) is a member of the fibroblast growth factor (FGFR) family, in which there is high amino acid sequence conservation between family members. Members of the FGFR family are differentiated by both ligand binding affinities and tissue distribution. A full-length FGFR polypeptide contains an extracellular domain (ECD), a hydrophobic transmembrane domain, and a cytoplasmic tyrosine kinase domain. The ECD of FGFR polypeptides interacts with fibroblast growth factors (FGFs) to mediate downstream signaling, which ultimately influences cellular differentiation. In particular, activation of the FGFR3 protein plays a role in bone development by inhibiting chondrocyte proliferation at the growth plate and limiting bone elongation.

[0003] Gain-of-function point mutations in FGFR3 are known to cause several types of human skeletal growth retardation disorders, such as achondroplasia, thanatophoric dysplasia type I (TDI), thanatophoric dysplasia type II (TDII), severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN), hypochondroplasia, and craniosynostosis syndromes (e.g., Muenke syndrome, Crouzon syndrome, and Crouzonodermoskeletal syndrome). Loss-of-function point mutations in FGFR3 are also known to cause skeletal growth retardation disorders, such as camptodactyly, tall stature, and hearing loss syndrome (CATSHL). Achondroplasia is the most common form of short-limb dwarfism and is characterized by disproportionate shortness of limbs and relative macrocephaly. Approximately 97% of achondroplasia is caused by a single point mutation in the gene encoding FGFR3, in which a glycine residue is substituted with an arginine residue at position 380 of the FGFR3 amino acid sequence. Upon ligand binding, the mutation decreases the elimination of the receptor/ligand complex resulting in prolonged intracellular signaling. This prolonged FGFR3 signaling inhibits the proliferation and differentiation of the cartilage growth plate, consequently impairing endochondral bone growth.

[0004] There exists a need for improved therapeutics that target dysfunctional FGFR3 for treating skeletal growth retardation disorders, such achondroplasia.

SUMMARY OF THE INVENTION

[0005] The invention features soluble fibroblast growth factor receptor 3 (sFGFR3) polypeptides with an amino acid sequence that consists of an amino acid sequence with at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 4, and uses thereof for the treatment of skeletal growth retardation disorders (e.g., achondroplasia) in a patient (e.g., a human, particularly an infant, a child, or an adolescent).

[0006] A first aspect of the invention features a soluble fibroblast growth factor receptor 3 (sFGFR3)

polypeptide with an amino acid sequence that consists of an amino acid sequence with at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 4. In particular, the polypeptide includes an amino acid sequence according to SEQ ID NO: 33 (e.g., the polypeptide includes or consists of SEQ ID NO: 33).

[0007] The sFGFR3 polypeptide may include an amino acid substitution that removes a cysteine residue at position 253 of SEQ ID NO: 1. For example, the cysteine residue at position 253 is substituted with a serine residue or, e.g., another conservative amino acid substitution, such as alanine, glycine, proline, or threonine For instance, the sFGFR3 polypeptide can be an isolated sFGFR3 polypeptide.

[0008] The sFGFR3 polypeptide may consists of the amino acid sequence of SEQ ID NO: 4.

[0009] Also disclosed is a polynucleotide (e.g., an isolated polynucleotide) that encodes the sFGFR3 polypeptide of the first aspect of the invention (optionally with a signal peptide, e.g., the signal peptide can have the amino acid sequence of SEQ ID NO: 6 or 35 or an amino acid sequence having at least 92%, 95%, 97%, or 99% sequence identity to SEQ ID NO: 6 or 35). including a nucleic acid sequence having at least 85%, 90%, 92%, 95%, 97%, or 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 21 or 37 (e.g., the polynucleotide includes or consists of the nucleic acid of SEQ ID NO: 21 or 37). TAlso dislosed is a vector (e.g., an isolated vector) including the polynucleotide, such as a plasmid, an artificial chromosome, a viral vector, or a phage vector. Additionally disclosed is a host cell (e.g., an isolated host cell) including the polynucleotide, such as a HEK 293 cell or CHO cell.

[0010] The invention features a composition including the sFGFR3 polypeptide of the first aspect of the invention. Also disclosed is a composition including the polynucleotide that encodes the sFGFR3 polypeptide of the first aspect of the invention. In addition, the vector or host cell that includes the polynucleotide encoding the sFGFR3 polypeptide can be formulated in a composition. The composition can further include a pharmaceutically acceptable excipient, carrier, or diluent. The composition including the sFGFR3 polypeptide, polynucleotide, or vector can be formulated for administration at a dose of about 0.002 mg/kg to about 30 mg/kg, such as about 0.001 mg/kg to about 10 mg/kg. The composition including the host cell can be formulated for administration at a dose of about 1×10^3 cells/mL to about 1×10^{12} cells/mL. The composition can be formulated for daily, weekly, or monthly administration, such as seven times a week, six times a week, five times a week, four times a week, three times a week, twice a week, weekly, every two weeks, or once a month. For example, the composition including the sFGFR3 polypeptide, polynucleotide, or vector is formulated for administration at a dose of about 0.25 mg/kg to about 10 mg/kg once or twice a week. The composition can be formulated for parenteral administration (e.g., subcutaneous administration, intravenous administration, intramuscular administration, intra-arterial administration, intrathecal administration, or intraperitoneal administration), enteral administration, or topical administration. Preferably, the composition is formulated for subcutaneous administration. The invention also features a medicament that includes the composition including the sFGFR3 polypeptide of the first aspect of the invention. Further disclosed is a medicament that includes the composition including the polynucleotide, vector or host cell.

[0011] Further described is a method of delivering an sFGFR3 polypeptide to tissue (e.g., skeletal tissue) in a patient (e.g. a human) having a skeletal growth retardation disorder (e.g., achondroplasia) including administering to the patient an effective amount of the sFGFR3 polypeptide of the first aspect of the invention, a polynucleotide encoding the sFGFR3 polypeptide, a vector containing the polynucleotide, a host cell containing the polynucleotide or vector, or a composition containing the polypeptide, polynucleotide, vector, or host cell.

[0012] A second aspect of the invention features the polypeptide of the first aspect of the invention or a composition containing the polypeptide for use in a method of treating a skeletal growth retardation disorder (e.g., a FGFR3-related skeletal disease) in a patient (e.g., a human) that includes administering the polypeptide of the first aspect of the invention. Also described is a polynucleotide encoding the polypeptide, a vector containing the polynucleotide, a host cell containing the polynucleotide or vector, or a composition containing the polynucleotide, vector, or host cell for use in a method of treating a skeletal growth retardation disorder (e.g., a FGFR3-related skeletal disease) in a patient (e.g., a human) that includes administering the polynucleotide, vector, a host cell, or composition.The FGFR3-related skeletal disease is selected from the group consisting of achondroplasia, thanatophoric dysplasia type I (TDI), thanatophoric dysplasia type II (TDII), severe achondroplasia with developmental delay and acanthosis nigricans (SADDEN), hypochondroplasia, a craniosynostosis syndrome (e.g., Muenke syndrome, Crouzon syndrome, and Crouzonodermoskeletal syndrome), and camptodactyly, tall stature, and hearing loss syndrome (CATSHL). In particular, the skeletal growth retardation disorder is achondroplasia.

[0013] The FGFR3-related skeletal disease can be caused by expression in the patient of a constitutively active FGFR3, such as an amino acid substitution of a glycine residue with an arginine residue at position 380 of SEQ ID NO: 5 or 32. In particular, the patient may be diagnosed with the skeletal growth retardation disorder (e.g., prior to treatment). For instance, the patient exhibits one or more symptoms of the skeletal growth retardation disorder selected from the group consisting of short limbs, short trunk, bowlegs, a waddling gait, skull malformations, cloverleaf skull, craniosynostosis, wormian bones, anomalies of the hands, anomalies of the feet, hitchhiker thumb, and chest anomalies, such that the patient exhibits an improvement in the one or more symptoms of the skeletal growth retardation disorder after administration of the sFGFR3 polypeptide (or a polynucleotide encoding the polypeptide, a vector containing the polynucleotide, a host cell containing the polynucleotide or vector, or a composition containing the polypeptide, polynucleotide, vector, or host cell). Additionally, the patient may have not been previously administered the sFGFR3 polypeptide (or a polynucleotide encoding the polypeptide, a vector containing the polynucleotide, a host cell containing the polynucleotide or vector, or a composition containing the polypeptide, polynucleotide, vector, or host cell). For example, the patient may be an infant, a child, an adolescent, or an adult.

[0014] For example, the polypeptide (or polynucleotide or vector) is administered to the patient at a dose of about 0.002 mg/kg to about 30 mg/kg (e.g., a dose of about 0.001 mg/kg to about 10 mg/kg). The polypeptide may be administered to the patient one or more times daily, weekly (e.g., twice a week, three times a week, four times a week, five times a week, six times a week, or seven times a week), every two weeks, monthly, or yearly. For example, the polypeptide is administered to the patient at a dose of about 0.25 mg/kg to about 30 mg/kg at least about once or twice a week or more (e.g., the polypeptide is administered to the patient at a dose of about 2.5 mg/kg or about 10 mg/kg once or twice weekly). The polypeptide can be administered to the patient in a composition including a pharmaceutically acceptable excipient, carrier, or diluent. The polypeptide can be administered to the patient parenterally (e.g., subcutaneously, intravenously, intramuscularly, intra-arterially, intrathecally, or intraperitoneally), enterally, or topically. Preferably, the composition is administered to the patient by subcutaneous injection. Additionally, the polypeptide can bind to fibroblast growth factor 1 (FGF1), fibroblast growth factor 2 (FGF2), fibroblast growth factor 9 (FGF9), fibroblast growth factor 18 (FGF18), fibroblast growth factor 19 (FGF19), or fibroblast growth factor 21 (FGF21). In particular, the binding can be characterized by an equilibrium dissociation constant (K_d) of about 0.2 nM to about 20 nM, such as the binding is characterized by a K_d of about 1 nM to about 10 nM (e.g., about 1 nm, about 2 nm, about 3 nm, about 4 nm, about 5 nm, about 6 nm, about 7 nm, about 8 nm, about 9 nm, or about 10 nm). The polypeptide can exhibit greater binding affinity to FGF1, FGF2, FGF9, and FGF18 relative to the binding affinity of the polypeptide to FGF19 and FGF21.

[0015] The polypeptide can have an in vivo half-life of between about 2 hours to about 25 hours (e.g., 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, or 25 hours). Administration of the sFGFR3 polypeptide can increase survival of the patient and/or restore the shape of the foramen magnum of the patient. Preferably, administration of the polypeptide provides one or more, or all, of the following: an increase in survival of the patient, an improvement in locomotion of the patient, an improvement in abdominal breathing in the patient, an increase in body and/or bone length of the patient, an improvement in the cranial ratio of the patient, and/or restoration of the foramen magnum shape in the patient, e.g., relative to an untreated patient (e.g., an untreated achondroplasia patient).

[0016] Further disclosed is a method of producing the sFGFR3 polypeptide of the first aspect of the invention, which includes culturing the host cell described above (e.g., a CHO cell or HEK 293 cell) in a culture medium under conditions suitable to effect expression of the sFGFR3 polypeptide and recovering the sFGFR3 polypeptide from the culture medium. In particular, the recovering includes chromatography, such as affinity chromatography (e.g., ion exchange chromatography or anti-FLAG chromatography, such as immunoprecipitation) or size exclusion chromatography.

[0017] The sFGFR3 polypeptide of the invention can be encoded by a polynucleotide including a nucleic acid sequence having at least 85%, 90%, 92%, 95%, 97%, or 99% sequence identity to the nucleic acid sequence of SEQ ID NO: 21 or 37 (e.g., the polynucleotide includes or consists of the nucleic acid of SEQ ID NO: 21 or 37).

[0018] The composition including the host cell can be administered to the patient (e.g., a human) at a dose of about 1×10^3 cells/mL to about 1×10^{12} cells/mL. For example, the sFGFR3 polypeptide, polynucleotide, vector, or host cell is administered to the patient one or more times daily, weekly, monthly, or yearly (e.g., the sFGFR3 polypeptide is administered to the patient seven times a week, six times a week, five times a week, four times a week, three times a week, twice a week, weekly, every two weeks, or once a month).

[0019] The disclosure features a method of manufacturing the sFGFR3 polypeptide of the first aspect of the invention by deleting the signal peptide, the transmembrane domain, and a portion of the intracellular domain from a FGFR3 polypeptide (e.g., to manufacture a polypeptide having the amino acid sequence of SEQ ID NO: 33). In particular, the intracellular domain consists of amino acid residues 436 to 806 of SEQ ID NO: 32. The disclosure also features a method of manufacturing the sFGFR3 polypeptide by introducing an amino acid substitution that removes a cysteine residue at position 253 of SEQ ID NO: 1. For example, the cysteine residue at position 253 is substituted with a serine residue or, e.g., another conservative amino acid substitution, such as alanine, glycine, proline, or threonine.

[0020] The invention also features a kit including the sFGFR3 polypeptide of the first aspect of the invention (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 4 or 33). The disclosure further features a kit including the polynucleotide (e.g., a polynucleotide having the nucleic acid sequence of SEQ ID NO: 21 or 37), the vector (e.g., a plasmid, an artificial chromosome, a viral vector, or a phage vector), or the host cell (e.g., a HEK 293 cell or a CHO cell) described above.

[0021] The kit optionally includes instructions for using the kit.

Definitions

[0022] As used herein, "a" and "an" means "at least one" or "one or more" unless otherwise indicated. In addition, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[0023] As used herein, "about" refers to an amount that is ± 10% of the recited value and is preferably ±5% of the recited value, or more preferably ±2% of the recited value. For instance, the term "about" can be used to modify all dosages or ranges recited herein by ± 10% of the recited values or range endpoints.

[0024] The term "domain" refers to a conserved region of the amino acid sequence of a polypeptide (e.g. a FGFR3 polypeptide) having an identifiable structure and/or function within the polypeptide. A domain can vary in length from, e.g., about 20 amino acids to about 600 amino acids. Exemplary domains include the immunoglobulin domains of FGFR3 (e.g., Ig-like C2-type domain 1, Ig-like C2-type domain 2, and Ig-like C2-type domain 3).

[0025] The term "dosage" refers to a determined quantity of an active agent (e.g., an sFGFR3 polypeptide or variant thereof, such as a polypeptide having the amino acid sequence of SEQ ID NO: 4 or 33) calculated to produce a desired therapeutic effect (e.g., treatment of a skeletal growth retardation disorder, such as achondroplasia) when the active agent is administered to a patient (e.g., a patient having a skeletal growth retardation disorder, such as achondroplasia). A dosage may be defined in terms of a defined amount of the active agent or a defined amount coupled with a particular frequency of administration. A dosage form can include an sFGFR3 polypeptide or fragment thereof in association with any suitable pharmaceutical excipient, carrier, or diluent.

[0026] The terms "effective amount," "amount effective to," and "therapeutically effective amount" refer to an amount of an sFGFR3 polypeptide, a vector encoding a sFGR3, and/or an sFGFR3 composition that is sufficient to produce a desired result, for example, the treatment of a skeletal growth retardation disorder (e.g., achondroplasia).

[0027] The terms "extracellular domain" and "ECD" refer to the portion of a FGFR3 polypeptide that extends beyond the transmembrane domain into the extracellular space. The ECD mediates binding of a FGFR3 to one or more fibroblast growth factors (FGFs). For instance, an ECD includes the Ig-like C2-type domains 1-3 of a FGFR3 polypeptide. In particular, the ECD includes the Ig-like C2-type domain 1 of a wildtype (wt) FGFR3 polypeptide (e.g., amino acids 36-88 of a wt FGFR3 polypeptide having the amino acid sequence of SEQ ID NO: 5 (a mature FGFR3 protein without a signal sequence) or amino acids 57-110 of a wt FGFR3 polypeptide having the amino acid sequence of SEQ ID NO: 32 (a precursor FGFR3 protein with the signal sequence)), the Ig-like C2-type domain 2 of a wildtype (wt) FGFR3 polypeptide (e.g., amino acids 139-234 of a wt FGFR3 polypeptide having the amino acid sequence of SEQ ID NO: 5 or amino acids 161-245 of a wt FGFR3 polypeptide having the amino acid sequence of SEQ ID NO: 32), and the Ig-like C2-type domain 3 of a wt FGFR3 polypeptide (e.g., amino acids 247-335 of a wt FGFR3 polypeptide having the amino acid sequence of SEQ ID NO: 5 or amino acids 268-310 of a wt FGFR3 polypeptide having the amino acid sequence of SEQ ID NO: 32). An ECD of a FGFR3 can also include a fragment of the wildtype FGFR3 Ig-like C2-type domain 3, for instance, aa 247-288 of SEQ ID NO: 1, which can further include, e.g., an amino acid substitution of a cysteine residue with a serine residue or another conservative amino acid substitution (e.g., alanine, glycine, proline, or threonine) at position 253 of SEQ ID NO: 1 (e.g., aa 247-288 of SEQ ID NO: 2). Additionally, an ECD can include an Ig-like C2-type domain 3 of, e.g., aa 247-335 of SEQ ID NO: 4. Thus, exemplary ECDs of FGFR3 polypeptides include, e.g., those polypeptides having the amino acid sequence of aa 1-288 of SEQ ID NOs: 1 and 2 or aa 1-335

of SEQ ID NOs: 4 and 33. In particular, the ECD of a FGFR3 polypeptide includes aa 1-335 of SEQ ID NO: 33.

[0028] The term "FGFR3-related skeletal disease," as used herein, refers to a skeletal disease that is caused by an abnormal increase in the activation of FGFR3, such as by expression of a gain-of-function mutant of the FGFR3. The phrase "gain-of-function mutant of the FGFR3" refers to a mutant of the FGFR3 exhibiting a biological activity, such as triggering downstream signaling, which is higher than the biological activity of the corresponding wild-type FGFR3 (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 5) in the presence of a FGF ligand. FGFR3-related skeletal diseases can include an inherited or a sporadic disease. Exemplary FGFR3-related skeletal diseases include, but are not limited to, achondroplasia, thanatophoric dysplasia type I (TDI), thanatophoric dysplasia type II (TDII), severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN), hypochondroplasia, a craniosynostosis syndrome (e.g., Muenke syndrome, Crouzon syndrome, and Crouzonodermoskeletal syndrome), and camptodactyly, tall stature, and hearing loss syndrome (CATSHL).

[0029] The terms "fibroblast growth factor" and "FGF" refer to a member of the FGF family, which includes structurally related signaling molecules involved in various metabolic processes, including endocrine signaling pathways, development, wound healing, and angiogenesis. FGFs play key roles in the proliferation and differentiation of a wide range of cell and tissue types. The term preferably refers to FGF1, FGF2, FGF9, FGF18, FGF19, and FGF21, which have been shown to bind FGFR3. For instance, FGFs can include human FGF1 (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 13), human FGF2 (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 14), human FGF9 (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 15), human FGF18 (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 16), human FGF19 (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 38), and human FGF21 (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 39).

[0030] The terms "fibroblast growth factor receptor 3," "FGFR3," or "FGFR3 receptor," as used herein, refer to a polypeptide that specifically binds one or more FGFs (e.g., FGF1, FGF2, FGF9, FGF18, FGF19, and/or FGF21). The human *FGFR3* gene, which is located on the distal short arm of chromosome 4, encodes an 806 amino acid protein precursor (fibroblast growth factor receptor 3 isoform 1 precursor), which contains 19 exons, and includes a signal peptide (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 6 or 35). Mutations in the FGFR3 amino acid sequence that lead to skeletal growth disorders, (e.g., achondroplasia), include, e.g., the substitution of a glycine residue at position 380 with an arginine residue (i.e., G380R). The naturally occurring human FGFR3 gene has a nucleotide sequence as shown in Genbank Accession number NM_000142.4 and the naturally occurring human FGFR3 protein has an amino acid sequence as shown in Genbank Accession number NP_000133, herein represented by SEQ ID NO: 5. The wildtype FGFR3 (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 5) consists of an extracellular immunoglobulin-like membrane domain including Ig-like C2-type domains 1-3 (amino acid residues 1 to 335), a transmembrane domain (amino acid residues 345 to 377), and an intracellular domain (amino acid residues 378 to 784). FGFR3s can include fragments and/or variants (e.g., splice variants, such as splice variants utilizing alternate exon 8 rather than exon 9) of the full-length, wild-type FGFR3 (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 5).

[0031] The terms "fragment" and "portion" refer to a part of a whole, such as a polypeptide or nucleic acid molecule that contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more of the entire length of the reference nucleic acid molecule or

polypeptide. A fragment or portion may contain, e.g., 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 500, 600, 700, or more amino acid residues, up to the entire length of the reference polypeptide (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 5 or 32). For example, a FGFR3 fragment can include any polypeptide having at least 200, 205, 210, 215, 220, 225, 235, 230, 240, 245, 250, 255, 260, 265, 275, 280, 285, 290, or 300 consecutive amino acids of SEQ ID NO: 1 or 2. Additionally, a FGFR3 fragment can include any polypeptide having at least 200, 205, 210, 215, 220, 225, 235, 230, 240, 245, 250, 255, 260, 265, 275, 280, 285, 290, 300, 305, 310, 315, 320, 325, 330, 335, 345, or 345 consecutive amino acids of SEQ ID NO: 4 or 33.

[0032] As used herein, the term "host cell" refers to a vehicle that includes the necessary cellular components, e.g., organelles, needed to express an sFGFR3 polypeptide from a corresponding polynucleotide. The nucleic acid sequence of the polynucleotide is typically included in a nucleic acid vector (e.g., a plasmid, an artificial chromosome, a viral vector, or a phage vector) that can be introduced into the host cell by conventional techniques known in the art (e.g., transformation, transfection, electroporation, calcium phosphate precipitation, and direct microinjection). A host cell may be a prokaryotic cell, e.g., a bacterial or an archaeal cell, or a eukaryotic cell, e.g., a mammalian cell (e.g., a Chinese Hamster Ovary (CHO) cell or a Human Embryonic Kidney 293 (HEK 293)). Preferably, the host cell is a mammalian cell, such as a CHO cell.

[0033] By "isolated" is meant separated, recovered, or purified from its natural environment. For example, an isolated sFGFR3 polypeptide (e.g., an sFGFR3 polypeptide or variant thereof, such as a polypeptide having the amino acid sequence of SEQ ID NO:r 4) can be characterized by a certain degree of purity after isolating the sFGFR3 polypeptide from, e.g., cell culture media. An isolated sFGFR3 polypeptide can be at least 75% pure, such that the sFGFR3 polynucleotide constitutes at least 75% by weight of the total material (e.g., polypeptides, polynucleotides, cellular debris, and environmental contaminants) present in the preparation (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 99%, or at least 99.5% by weight of the total material present in the preparation). Likewise, an isolated polynucleotide encoding an sFGFR3 polypeptide (e.g., a polynucleotide having the nucleic acid sequence of SEQ ID NO: 21or 37), or an isolated host cell (e.g., CHO cell, a HEK 293 cell, L cell, C127 cell, 3T3 cell, BHK cell, or COS-7 cell) can be at least 75% pure, such that the polynucleotide or host cell constitutes at least 75% by weight of the total material (e.g., polypeptides, polynucleotides, cellular debris, and environmental contaminants) present in the preparation (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 99%, or at least 99.5% by weight of the total material present in the preparation).

[0034] "Polynucleotide" and "nucleic acid molecule," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or analogs thereof, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide can include modified nucleotides, such as methylated nucleotides and analogs thereof. If present, modification to the nucleotide structure can be imparted before or after assembly of the polymer. The sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after synthesis, such as by conjugation with a label.

[0035] The terms "patient" and "subject" refer to a mammal, including, but not limited to, a human (e.g., a human having a skeletal growth retardation disorder, such as achondroplasia) or a non-human mammal (e.g., a non-human mammal having a skeletal growth retardation disorder, such as achondroplasia), such

as a bovine, equine, canine, ovine, or feline. Preferably, the patient is a human having a skeletal growth retardation disorder (e.g., achondroplasia), particularly an infant, a child, or an adolescent having a skeletal growth retardation disorder (e.g., achondroplasia).

[0036] The terms "parenteral administration," "administered parenterally," and other grammatically equivalent phrases, as used herein, refer to a mode of administration of compositions including an sFGFR3 polypeptide (e.g., an sFGFR3 polypeptide or variant thereof, such as a polypeptide having the amino acid sequence of SEQ ID NO: 4, or 33) other than enteral and topical administration, usually by injection, and include, without limitation, subcutaneous, intradermal, intravenous, intranasal, intraocular, pulmonary, intramuscular, intra-arterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intrapulmonary, intraperitoneal, transtracheal, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, intracerebral, intracranial, intracarotid, and intrasternal injection and infusion.

[0037] By "pharmaceutically acceptable diluent, excipient, carrier, or adjuvant" is meant a diluent, excipient, carrier, or adjuvant, respectively that is physiologically acceptable to the subject (e.g., a human) while retaining the therapeutic properties of the pharmaceutical composition (e.g., an sFGFR3 polypeptide or variant thereof, such as a polypeptide having the amino acid sequence of SEQ ID NO: 4 or 33) with which it is administered. One exemplary pharmaceutically acceptable carrier is physiological saline. Other physiologically acceptable diluents, excipients, carriers, or adjuvants and their formulations are known to one skilled in the art.

[0038] By "pharmaceutical composition" is meant a composition containing an active agent, such as an sFGFR3 (e.g., an sFGFR3 polypeptide or variant thereof, such as a polypeptide having the amino acid sequence of SEQ ID NO: 4 or 33), formulated with at least one pharmaceutically acceptable excipient, carrier, or diluent. The pharmaceutical composition may be manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment of a disease or event (e.g., a skeletal growth retardation disorder, such achondroplasia) in a patient (e.g., a patient having a skeletal growth retardation disorder, such as a patient having achondroplasia). Pharmaceutical compositions can be formulated, e.g., for parenteral administration, such as for subcutaneous administration (e.g. by subcutaneous injection) or intravenous administration (e.g., as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use), or for oral administration (e.g., as a tablet, capsule, caplet, gelcap, or syrup).

[0039] As used herein, the term "sequence identity" refers to the percentage of amino acid (or nucleic acid) residues of a candidate sequence, e.g., an FGFR3 polypeptide, that are identical to the amino acid (or nucleic acid) residues of a reference sequence, e.g., an sFGFR3 polypeptide or variant thereof, such as a polypeptide having the amino acid sequence of SEQ ID NO: 4, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent identity (e.g., gaps can be introduced in one or both of the candidate and reference sequences for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). Alignment for purposes of determining percent identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software, such as BLAST, BLAST-2, BLAST-P, BLAST-N, BLAST-X, WU-BLAST-2, ALIGN, ALIGN-2, CLUSTAL, or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For instance, the percent amino acid (or nucleic acid) sequence identity of a given candidate sequence to, with, or against a given reference sequence (which can alternatively be phrased as a given candidate sequence that has or includes a certain percent amino acid (or nucleic acid) sequence identity to, with, or against a given reference sequence) is calculated as follows:

100 x (fraction of A/B)

100 x (fraction of A/B)

where A is the number of amino acid (or nucleic acid) residues scored as identical in the alignment of the candidate sequence and the reference sequence, and where B is the total number of amino acid (or nucleic acid) residues in the reference sequence. In particular, a reference sequence aligned for comparison with a candidate sequence can show that the candidate sequence exhibits from, e.g., 50% to 100% identity across the full length of the candidate sequence or a selected portion of contiguous amino acid (or nucleic acid) residues of the candidate sequence. The length of the candidate sequence aligned for comparison purpose is at least 30%, e.g., at least 40%, e.g., at least 50%, 60%, 70%, 80%, 90%, or 100% of the length of the reference sequence. When a position in the candidate sequence is occupied by the same amino acid (or nucleic acid) residue as the corresponding position in the reference sequence, then the molecules are identical at that position.

[0040] By "signal peptide" is meant a short peptide (e.g., 5-30 amino acids in length, such as 22 amino acids in length) at the N-terminus of a polypeptide that directs a polypeptide towards the secretory pathway (e.g., the extracellular space). The signal peptide is typically cleaved during secretion of the polypeptide. The signal sequence may direct the polypeptide to an intracellular compartment or organelle, e.g., the Golgi apparatus. A signal sequence may be identified by homology, or biological activity, to a peptide with the known function of targeting a polypeptide to a particular region of the cell. One of ordinary skill in the art can identify a signal peptide by using readily available software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, or PILEUP/PRETTYBOX programs). A signal peptide can be one that is, for example, substantially identical to the amino acid sequence of SEQ ID NO: 6 or 35.

[0041] The term "skeletal growth retardation disorder," as used herein, refers to a skeletal disease characterized by deformities and/or malformations of the bones. These disorders include, but are not limiting to, skeletal growth retardation disorders caused by growth plate (physeal) fractures, idiopathic skeletal growth retardation disorders, or FGFR3-related skeletal diseases. In particular, a patient having a skeletal growth retardation disorder (e.g., achondroplasia) may have bones that are shorter than the bones of a healthy patient. For example, the skeletal growth retardation disorder may include a skeletal dysplasia, e.g., achondroplasia, homozygous achondroplasia, heterozygous achondroplasia, achondrogenesis, acrodysostosis, acromesomelic dysplasia, atelosteogenesis, camptomelic dysplasia, chondrodysplasia punctata, rhizomelic type of chondrodysplasia punctata, cleidocranial dysostosis, congenital short femur, craniosynostosis (e.g., Muenke syndrome, Crouzon syndrome, Apert syndrome, Jackson-Weiss syndrome, Pfeiffer syndrome, or Crouzonodermoskeletal syndrome), dactyly, brachydactyly, camptodactyly, polydactyly, syndactyly, diastrophic dysplasia, dwarfism, dyssegmental dysplasia, enchondromatosis, fibrochondrogenesis, fibrous dysplasia, hereditary multiple exostoses, hypochondroplasia, hypophosphatasia, hypophosphatemic rickets, Jaffe-Lichtenstein syndrome, Kniest dysplasia, Kniest syndrome, Langer-type mesomelic dysplasia, Marfan syndrome, McCune-Albright syndrome, micromelia, metaphyseal dysplasia, Jansen-type metaphyseal dysplasia, metatrophic dysplasia, Morquio syndrome, Nievergelt-type mesomelic dysplasia, neurofibromatosis, osteoarthritis, osteochondrodysplasia, osteogenesis imperfecta, perinatal lethal type of osteogenesis imperfecta, osteopetrosis, osteopoikilosis, peripheral dysostosis, Reinhardt syndrome, Roberts syndrome, Robinow syndrome, short-rib polydactyly syndromes, short stature, spondyloepiphyseal dysplasia congenita, and spondyloepimetaphyseal dysplasia.

[0042] The terms "soluble fibroblast growth factor receptor 3," "soluble FGFR3," and "sFGFR3" refer to a FGFR3 that is characterized by the absence or functional disruption of all or a substantial part of the transmembrane domain and any polypeptide portion that would anchor the FGFR3 polypeptide to a cell membrane (e.g., a tyrosine kinase domain). An sFGFR3 polypeptide is a non-membrane bound form of

an FGFR3 polypeptide. In particular, the transmembrane domain of FGFR3 extends from amino acid residues 345 to 377 of the wild-type FGFR3 sequence (e.g, a polypeptide having the amino acid sequence of SEQ ID NO: 5) or amino acid residues 367 to 399 of the wild-type FGFR3 sequence including a signal peptide (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 32).

[0043] Any of the above sFGFR3 polypeptides or variants thereof can optionally include a signal peptide at the N-terminal position, such as amino acids 1 to 22 of SEQ ID NO: 6 (MGAPACALALCVAVAIVAGASS) or amino acids 1 to 19 of SEQ ID NO: 35 (e.g., MMSFVSLLLVGILFHATQA).

[0044] By "treating" and "treatment" is meant a reduction (e.g., by at least about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%, or even 100%) in the progression or severity of a skeletal growth retardation disorder (e.g., achondroplasia), or in the progression, severity, or frequency of one or more symptoms of a skeletal growth retardation disorder (e.g., achondroplasia) in a patient (e.g., a human, such as an infant, a child, or an adolescent). Treatment can occur for a treatment period, in which an sFGFR3 polypeptide is administered for a period of time (e.g., days, months, years, or longer) to treat a patient (e.g., a human, such as an infant, a child, or an adolescent) having a skeletal growth retardation disorder, such as achondroplasia. Exemplary symptoms of achondroplasia that can be treated with an sFGFR3 (e.g., an sFGFR3 polypeptide or variant thereof, such as a polypeptide having the amino acid sequence of SEQ ID NO: 4 or 33) include, but are not limited to, short stature, a long trunk, shortened limbs, an adult height of between about 42 to about 56 inches, a relatively large head, a forehead that is prominent, underdeveloped portions of the face, genu valgum (e.g., "knock-knee"), a waddling gait, short and stubby fingers, short and stubby toes, limited ability to straighten the arm at the elbow, an excessive curve of the lower back, dental problems (e.g. from overcrowding of teeth), weight control problems, neurological problems, respiratory problems, and/or pain and numbness in the lower back and/or spine.

[0045] The term "variant," with respect to a polypeptide, refers to a polypeptide (e.g., an sFGFR3 polypeptide or variant thereof, such as a polypeptide having the amino acid sequence of SEQ ID NO: 4) that differs by one or more changes in the amino acid sequence from the polypeptide from which the variant is derived (e.g., the parent polypeptide). The term "variant," with respect to a polynucleotide, refers to a polynucleotide (e.g., a polynucleotide encoding a sFGFR3 polypeptide, such as a polynucleotide having the nucleic acid sequence of SEQ ID NO: 21 or 37) that differs by one or more changes in the nucleic acid sequence from the polynucleotide from which the variant is derived (e.g., the parent polynucleotide). The changes in the amino acid or nucleic acid sequence of the variant can be, e.g., amino acid or nucleic acid substitutions, insertions, deletions, N-terminal truncations, or C-terminal truncations, or any combination thereof. In particular, the amino acid substitutions may be conservative and/or non-conservative substitutions. A variant can include any polynucleotide having at least 50% (e.g., 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to a polynucleotide having the nucleic acid sequence of SEQ ID NO: 21 or 37.

[0046] By "vector" is meant a DNA construct that includes one or more polynucleotides, or fragments thereof, encoding an sFGFR3 polypeptide (e.g., an sFGFR3 polypeptide or variant thereof, such as a polypeptide having the amino acid sequence of SEQ ID NO: 4 or 33, or a sFGFR3 polypeptide including a signal peptide, such as a polypeptide having the amino acid sequence of SEQ ID NO: 18 or 34). The vector can be used to infect a cell (e.g., a host cell or a cell of a patient having a human skeletal growth retardation disorder, such as achondroplasia), which results in the translation of the polynucleotides of the vector into a sFGFR3 polypeptide. One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Certain vectors are capable of

autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids.

[0047] The term "unit dosage form(s)" refers to physically discrete unit(s) suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with any suitable pharmaceutical excipient, carrier, or diluent.

[0048] The recitation herein of numerical ranges by endpoints is intended to include all numbers subsumed within that range (e.g., a recitation of 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, and 5).

[0049] Other features and advantages of the invention will be apparent from the following Detailed Description and from the claims.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0050]

FIGS. 1A-1D are graphs showing sensorgrams of the sFGFR3 polypeptides. Sensorgrams are shown for sFGFR3_Del1 (SEQ ID NO: 7), sFGFR3_Del4 (SEQ ID NO: 1), and sFGFR3_Del4-LK1-LK2 (SEQ ID NO: 10; Fig. 1A); sFGFR3_Del1 (SEQ ID NO: 7) and sFGFR3_Del1-D3 (SEQ ID NO: 9; Fig. 1B); sFGFR3_Del4-LK1-LK2 (SEQ ID NO: 10), sFGFR3_Del4-LK1-LK2-C253S (SEQ ID NO: 11), and sFGFR3_Del4-LK1-LK2-D3 (SEQ ID NO: 12; Fig. 1C); and sFGFR3_Del4 (SEQ ID NO: 1), sFGFR3_Del4-C253S (SEQ ID NO: 2), and sFGFR3_Del4-D3 (SEQ ID NO: 33; Fig. 1D).

FIGS. 2A-2C are images of Western blots of the sFGFR3 polypeptides. Western blots under reducing (R) and non-reducing (NR) conditions are shown for sFGFR3_Del1, sFGFR3_Del1-C253S (SEQ ID NO: 8), and sFGFR3_Del1-D3 (Fig. 2A); sFGFR3_Del4-LK1-LK2, sFGFR3_Del4-LK1-LK2-C253S, and sFGFR3_Del4-LK1-LK2-D3 (Fig. 2B); and sFGFR3_Del4, sFGFR3_Del4-C253S, and sFGFR3_Del4-D3 (Fig. 2C).

FIGS. 3A-3B are graphs showing a sensorgram (Fig. 3A) and proliferation assays of sFGFR3_Del4, sFGFR3_Del4-C253S, and sFGFR3_Del4-D3 (Fig. 3B) using *Fgfr3*$^{ach/+}$ chondrocyte cells in the presence of FGF2.

FIG. 4 is a graph showing luciferase signaling in Serum Response Element-Luciferase (SRE-Luc) HEK cells expressing FGFR3^{G380R} incubated with sFGFR3_Del4-D3 at 0 nM, 70 nM, and 280nM with or without 1 ng/mL of hFGF2 (* indicates p value < 0.05; *** indicates a p value < 0.001 compared to sFGFR3_Del4-D3 at 0 nM).

FIG. 5 is a graph showing the percentage of living animals (wild type (wt) and *Fgfr3*$^{ach/+}$ mice) after 3 days of treatment with a low dose (0.25 mg/kg) of sFGFR3_Del4-D3 relative to age (days). The percentage of living wt mice receiving vehicle (PBS) is also shown.

FIG. 6 is an image showing the amino acid residues corresponding to the Ig-like C2-type domains 1 (IgI),

2 (IgII), and 3 (IgIII) of wildtype FGFR3 polypeptide (SEQ ID NO: 5 or 32), sFGFR3_Del4-C253S (SEQ ID NO: 2), and a variant of sFGFR3_Del4-D3 (SEQ ID NO:33). sFGFR3_Del4-C253S includes an amino acid substitution of a cysteine residue with a serine residue at position 253 of SEQ ID NO: 1.

FIGS. 7A-7B are images of Western blots of the sFGFR3 polypeptides. Western blots under reducing (R) and non-reducing (NR) conditions are shown for 2.3 mg/ml and 23 mg/ml sFGFR3_Del1-D3 (Fig. 7A) and 1.5 mg/ml and 15 mg/ml sFGFR3_Del1-C253S (Fig. 7B).

FIGS. 8A-8B are graphs showing the melting temperature (T_m) of sFGFR3_Del4-C253S in 20 mM phosphate, 40mM NaCl, pH 7.5 buffer and 40 mM citrate, 40mM NaCl, pH 6.5 buffer (Fig. 8A) and the T_m of sFGFR3_Del4-D3 in 20 mM phosphate, 40mM NaCl, pH 7.5 buffer and 20 mM citrate, 40mM NaCl, pH 6.5 buffer (Fig. 8B).

FIGS. 9A-9C are graphs showing the fast protein liquid chromatography (FPLC) elution profiles of sFGFR3_Del4-D3. FPLC elution profiles are shown for Fig. 9A: sFGFR3_Del4-D3 at 0 minutes, 2 hours, and 24 hours in cpm/fraction (Fig. 9A); Fig. 9B: sFGFR3_Del4-D3 administered by intravenous bolus at 1 minute, 15 minutes, 30 minute, 2 hours, and 24 hours in cpm/fraction and as normalized to the highest peak (shown in Fig. 9B cont.); Fig 9C: sFGFR3_Del4-D3 administered by subcutaneous injection at 30 minutes, 2 hours, 4 hours, and 24 hours in cpm/fraction and as normalized to the highest peak (shown in Fig. 9C cont.).

FIGS. 10A-10B are graphs showing the percentage (%) of proliferation of *Fgfr3*$^{ach/+}$ chondrocyte cells in the presence of the sFGFR3 polypeptides. *Fgfr3*$^{ach/+}$ chondrocyte proliferation is shown for 1 ug/ml, 10 ug/ml, and 50 ug/ml of sFGFR3_Del4-D3 (Fig. 10A) and for 1 ug/ml, 10 ug/ml, and 50 ug/ml of sFGFR3_Del4-C253S (Fig. 10B).

FIG. 11 is a graph showing the PK profiles of 2.5 mg/kg sFGFR3_Del4-D3 administered subcutaneously and 2.5 mg/kg sFGFR3_Del4-D3 administered intravenously.

FIG. 12 is a graph showing the concentration of ^{125}I- sFGFR3_Del4-D3 in kidney, liver, spleen, lung, and heart tissue at 30 minutes, 120 minutes, and 1440 minutes after intravenous administration. The concentration is expressed as the percent of injected dose per gram (%ID/g).

FIG. 13 is a graph showing the concentration of ^{125}I- sFGFR3_Del4-D3 in kidney, liver, spleen, lung, and heart tissue at 30 minutes, 120 minutes, 240 minutes, 480 minutes, and 1440 minutes after subcutaneous administration. The concentration is expressed as %ID/g.

FIG. 14A-14B are graphs showing the concentration (c) and volume of distribution (V_d) of ^{125}I-sFGFR3_Del4-D3 in brain tissue. Shown is the c of ^{125}I-sFGFR3_Del4-D3 before and after correction for vascular content and degradation at 30 minutes, 2 hours, and 24 hours after intravenous bolus (Fig. 14A) and the V_d of ^{125}I-sFGFR3_Del4-D3 and RSA at 30 minutes, 2 hours, and 24 hours after intravenous bolus (Fig. 14B).

FIG. 15 is a graph showing the percentage of surviving *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del4-D3. Shown are the surviving wild type mice, *Fgfr3*$^{ach/+}$ mice administered PBS as vehicle, *Fgfr3*$^{ach/+}$ mice administered 2.5 mg/kg sFGFR3_Del4-D3 once weekly, *Fgfr3*$^{ach/+}$ mice administered 2.5 mg/kg sFGFR3_Del4-D3 twice weekly, and *Fgfr3*$^{ach/+}$ mice administered 10 mg/kg sFGFR3_Del4-D3 twice weekly over 22 days.

FIG. 16 is a graph showing the percentage (%) of locomotor and abdominal breathing complications in *Fgfr3*$^{ach/+}$ mice administered PBS as vehicle, 2.5 mg/kg sFGFR3_Del4-D3 once weekly, 2.5 mg/kg sFGFR3_Del4-D3 twice weekly, and 10 mg/kg sFGFR3_Del4-D3 twice weekly.

FIGS. 17A-17D are graphs and an x-ray radiograph showing the length of *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del4-D3. Shown are the axial length (Fig. 17A), tail length (Fig. 17B), and tibia length (Fig. 17C) of wild type mice administered PBS as vehicle, and *Fgfr3*$^{ach/+}$ mice administered PBS as vehicle, 2.5 mg/kg sFGFR3_Del4-D3 once weekly, 2.5 mg/kg sFGFR3_Del4-D3 twice weekly, and 10 mg/kg sFGFR3_Del4-D3 twice weekly. Also shown is the x-ray radiograph (Fig. 17D) of wild type mice administered PBS as vehicle and *Fgfr3*$^{ach/+}$ mice administered PBS as vehicle, 2.5 mg/kg sFGFR3_Del4-D3 twice weekly, and 10 mg/kg sFGFR3_Del4-D3 twice weekly. All measurements are in millimeters (mm).

FIGS. 18A-18B are a graph showing the cranium ratio and an x-ray radiograph showing the skulls of *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del4-D3, respectively. Shown in the graph (Fig. 18A) is the cranium ratio (L/W) of wild type mice administered PBS as vehicle and *Fgfr3*$^{ach/+}$ mice administered PBS as vehicle, 2.5 mg/kg sFGFR3_Del4-D3 once weekly, 2.5 mg/kg sFGFR3_Del4-D3 twice weekly, and 10 mg/kg sFGFR3_Del4-D3 twice weekly. Shown in the x-ray radiograph (Fig. 18B) is the skulls of wild type mice administered PBS as vehicle, *Fgfr3*$^{ach/+}$ mice administered PBS as vehicle, wild type mice administered 10 mg/kg sFGFR3_Del4-D3 twice weekly, and *Fgfr3*$^{ach/+}$ mice administered 10 mg/kg sFGFR3_Del4-D3 twice weekly.

FIGS. 19A-19E are graphs showing the kinetic profile for the binding of different concentrations of hFGF1, FGF2, hFGF9, hFGF18, hFGF19, and hFGF21 to immobilized SFGFR3_DEL4-D3 in real time. Shown are the kinetic profiles for binding of hFGF1 at concentrations of 0.5 nM to 12 nM to immobilized SFGFR3_DEL4-D3 (FIG. 19A); hFGF2 at concentrations of 2 nM to 10 nM to immobilized SFGFR3_DEL4-D3 (FIG. 19B); hFGF9 at concentrations of 1 nM to 5 nM to immobilized SFGFR3_DEL4-D3 (FIG. 19C); hFGF18 at concentrations of 1 nM to 10 nM to immobilized SFGFR3_DEL4-D3 (FIG. 19D); hFGF19 at concentrations of 2 nM to 20 nM to immobilized SFGFR3_DEL4-D3 (FIG. 19E); and hFGF21 at concentrations of 100 nM to 10000 nM to immobilized SFGFR3_DEL4-D3 (FIG. 19F). The darker, overlapping lines represent the 2:1 binding model used to generate the K_d values.

FIG. 20 is an image of a Western blot of non-induced wild type ATDC5 and retrovirally infected ATDC5 cells expressing FGFR3^{G380R}.

FIG 21 is a graph showing the induction of proliferation of ATDC5 FGFR3^{G380R} cells in the presence of SFGFR3_DEL4-D3 for three experiments. Untreated ATDC5 FGFR3^{G380R} cells were used as a control.

DETAILED DESCRIPTION OF THE INVENTION

[0051] We have discovered that soluble fibroblast growth factor receptor 3 (sFGFR3) polypeptides and variants thereof can be used to treat skeletal growth retardation disorders, such as achondroplasia, in a patient (e.g., a human, particularly an infant, a child, or an adolescent). In particular, sFGFR3 polypeptides of the invention feature a deletion of, e.g., amino acids 289 to 400 of SEQ ID NO: 5 or amino acids 311 to 422 of SEQ ID NO: 32, to provide the following exemplary sFGFR3 polypeptides:

sFGFR3_Del4 including an extended Ig-like C2-type domain 3 (sFGFR3_Del4-D3; SEQ ID NO: 33) and the sFGFR3 polypeptide having the amino acid sequence of SEQ ID NO: 4. See U.S. Provisional Application No. 62/276,222 and International Application No. PCT/US16/12553 for a description of sFGFR3_Del4 (SEQ ID NO: 1),.

Soluble Fibroblast Growth Factor Receptor 3 (sFGFR3) Polypeptides

[0052] The invention features sFGFR3 polypeptides and variants thereof formulated for the treatment of skeletal growth retardation disorders (e.g., achondroplasia). The sFGFR3 polypeptides have at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 4

[0053] The sFGFR3 polypeptides and variants thereof can also include fragments of the amino acid sequence of SEQ ID NO: 33 having at least 99% sequence identity to SEQ ID NO: 33 provided they have also the sequence identity to SEQ ID NO: 4 defined above. The cysteine residue at position 253 of SEQ ID NO: 4 or 33, if present, can be substituted with a serine residue or a conservative amino acid substitution, such as alanine, glycine, proline, or threonine.

[0054] An sFGFR3 polypeptide described herein (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 33), and SEQ ID NO: 4) can include a signal peptide at the N-terminal position. An exemplary signal peptide can include, but is not limited to, amino acids 1 to 22 of SEQ ID NO: 6 (e.g., MGAPACALALCVAVAIVAGASS) or amino acids 1 to 19 of SEQ ID NO: 35 (e.g., MMSFVSLLLVGILFHATQA). Accordingly, there are both secreted forms, which lack the N-terminal signal peptide, and non-secreted forms, which include the N-terminal signal peptide. For instance, a secreted sFGFR3 polypeptide can include the amino acid sequence of SEQ ID NOs: 4 or 33. One skilled in the art will appreciate that the position of the N-terminal signal peptide will vary in different sFGFR3 polypeptides and can include, for example, the first 5, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, or more amino acid residues on the N-terminus of the polypeptide. One of skill in the art can predict the position of a signal sequence cleavage site, e.g., by an appropriate computer algorithm such as that described in Bendtsen et al. (J. Mol. Biol. 340(4):783-795, 2004) and available on the Web at cbs.dtu.dk/services/SignalP/.

[0055] Additionally, sFGFR3 polypeptides (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 33) or (SEQ ID NO: 4)) of the invention can be glycosylated. In particular, a sFGFR3 polypeptide can be altered to increase or decrease the extent to which the sFGFR3 polypeptide is glycosylated. Addition or deletion of glycosylation sites to an sFGFR3 polypeptide can be accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed. For example, N-linked glycosylation, in which an oligosaccharide is attached to the amide nitrogen of an asparagine residue, can occur at position Asn76, Asn148, Asn169, Asn 203, Asn240, Asn272, and/or Asn 294 of the amino acid sequence of sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33), and variants thereof. One or more of these Asn residues can also be substituted to remove the glycosylation site. For instance, O-linked glycosylation, in which an oligosaccharide is attached to an oxygen atom of an amino acid residue, can occur at position Ser109, Thr126, Ser199, Ser274, Thr281, Ser298, Ser299, and/or Thr301 of the amino acid sequence of sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33). Additionally, O-linked glycosylation can occur at position Ser310 and/or Ser321 of sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33). One or more of these Ser or Thr residues can also be substituted to remove the glycosylation site.

sFGFR3 Fusion Polypeptides

[0056] sFGFR3 polypeptides of the invention (e.g., sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) can be fused to a functional domain from a heterologous polypeptide (e.g., a fragment crystallizable region of an immunoglobulin (Fc region; such as a polypeptide having the amino acid sequence of SEQ ID NOs: 25 and 26) or human serum albumin (HSA; such as a polypeptide having the amino acid sequence of SEQ ID NO: 27)) to provide a sFGFR3 fusion polypeptide. Optionally, a flexible linker, can be included between the sFGFR3 polypeptide and the heterologous polypeptide (e.g., an Fc region or HSA), such as a serine or glycine-rich sequence (e.g., a poly-glycine or a poly-glycine/serine linker, such as SEQ ID NOs: 28 and 29).

[0057] For example, the sFGFR3 polypeptides (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) can be a fusion polypeptide including, e.g., an Fc region of an immunoglobulin at the N-terminal or C-terminal domain. In particular, useful Fc regions can include the Fc fragment of any immunoglobulin molecule, including IgG, IgM, IgA, IgD, or IgE and their various subclasses (e.g., IgG-1, IgG-2, IgG-3, IgG-4, IgA-1, IgA-2) from any mammal (e.g., a human). For instance, the Fc fragment human IgG-1 (SEQ ID NO: 25) or a variant of human IgG-1, such as a variant including a substitution of asparagine at position 297 of SEQ ID NO: 25 with alanine (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 26). The Fc fragments of the invention can include, for example, the CH2 and CH3 domains of the heavy chain and any portion of the hinge region. The sFGFR3 fusion polypeptides of the invention can also include, e.g., a monomeric Fc, such as a CH2 or CH3 domain. The Fc region may optionally be glycosylated at any appropriate one or more amino acid residues known to those skilled in the art. An Fc fragment as described herein may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, or more additions, deletions, or substitutions relative to any of the Fc fragments described herein.

[0058] Additionally, the sFGFR3 polypeptides (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) can be conjugated to other molecules at the N-terminal or C-terminal domain for the purpose of improving the solubility and stability of the protein in aqueous solution. Examples of such molecules include human serum albumin (HSA), PEG, PSA, and bovine serum albumin (BSA). For instance, the sFGFR3 polypeptides can be conjugated to human HSA (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 27) or a fragment thereof.

[0059] The sFGFR3 fusion polypeptides can include a peptide linker region between the sFGFR3 polypeptide (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) and the heterologous polypeptide (e.g., an Fc region or HSA). The linker region may be of any sequence and length that allows the sFGFR3 to remain biologically active, e.g., not sterically hindered. Exemplary linker lengths are between 1 and 200 amino acid residues, e.g., 1-5, 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65, 66-70, 71-75, 76-80, 81-85, 86-90, 91-95, 96-100, 101-110, 111-120, 121-130, 131-140, 141-150, 151-160, 161-170, 171-180, 181-190, or 191-200 amino acid residues. For instance, linkers include or consist of flexible portions, e.g., regions without significant fixed secondary or tertiary structure. Preferred ranges are 5 to 25 and 10 to 20 amino acids in length. Such flexibility is generally increased if the amino acids are small and do not have bulky side chains that impede rotation or bending of the amino acid chain. Thus, preferably the peptide linker of the present invention has an increased content of small amino acids, in particular of glycines, alanines, serines, threonines, leucines and isoleucines.

[0060] Exemplary flexible linkers are glycine-rich linkers, e.g., containing at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100% glycine residues. Linkers may also contain, e.g., serine-rich linkers, e.g., containing at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100% serine residues. In some cases, the amino acid sequence of a linker consists only of glycine and serine residues. For example, the linker can be the amino acid sequence of GGGGAGGGG (SEQ ID NO: 28) or GGGGSGGGGSGGGGS (SEQ ID NO: 29). A linker can optionally be glycosylated at any appropriate one

or more amino acid residues. The linker can also be absent, in which the FGFR3 polypeptide and the heterologous polypeptide (e.g., an Fc region or HSA) are fused together directly, with no intervening residues.

Polynucleotides encoding the sFGFR3 Polypeptides

[0061] The disclosure further described polynucleotides encoding the sFGFR3 polypeptides (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)) that can be used to treat skeletal growth retardation disorders (e.g., achondroplasia) in a patient (e.g., a human, such as an infant, a child, or an adolescent), such as SEQ ID NOs: 21 or 37. For example, the polynucleotide can be the nucleic acid sequence of SEQ ID NO: 21 or 37, which encodes sFGFR3_Del4-D3 (SEQ ID NO: 33), having at least 85% sequence identity (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to the nucleic acid sequence of SEQ ID NO: 21 or 37. The disclosure also describes polynucleotides encoding sFGFR3 fusion polypeptides (e.g., a sFGFR3 polypeptide fused to a heterologous polypeptide, such as a Fc region or HSA) and polynucleotides encoding sFGFR3 polypeptides without a signal peptide (e.g., polypeptides having the amino acid sequence of SEQ ID NOs: 4 or 33) or with a signal peptide (e.g., polypeptides having the amino acid sequence of SEQ ID NOs: 18, 19, and 34). Additionally, the invention includes polynucleotides include one or more mutations to alter any of the glycosylation sites described herein.

[0062] Optionally, the sFGFR3 polynucleotides (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)) can be codon optimized to alter the codons in the nucleic acid, in particular to reflect the typical codon usage of the host organism (e.g., a human) without altering the sFGFR3 polypeptide encoded by the nucleic acid sequence of the polynucleotide. Codon-optimized polynucleotides (e.g., a polynucleotide having the nucleic acid sequence of SEQ ID NO: 21 or 37) can, e.g., facilitate genetic manipulations by decreasing the GC content and/or for expression in a host cell (e.g., a HEK 293 cell or a CHO cell). Codon-optimization can be performed by the skilled person, e.g. by using online tools such as the JAVA Codon Adaption Tool (www.jcat.de) or Integrated DNA Technologies Tool (www.eu.idtdna.com/CodonOpt) by simply entering the nucleic acid sequence of the polynucleotide and the host organism for which the codons are to be optimized. The codon usage of different organisms is available in online databases, for example, www.kazusa.or.jp/codon.

Host cells for expression of the sFGFR3 polypeptides

[0063] Mammalian cells can be used as host cells for expression of the sFGFR3 polypeptides (e.g. sFGFR3_Del4-D3 (SEQ ID NO: or 33) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)). Exemplary mammalian cell types useful in the methods include, but are not limited to, human embryonic kidney (HEK; e.g., HEK 293) cells, Chinese Hamster Ovary (CHO) cells, L cells, C127 cells, 3T3 cells, BHK cells, COS-7 cells, HeLa cells, PC3 cells, Vero cells, MC3T3 cells, NS0 cells, Sp2/0 cells, VERY cells, BHK, MDCK cells, W138 cells, BT483 cells, Hs578T cells, HTB2 cells, BT20 cells, T47D cells, NS0 cells, CRL7O3O cells, and HsS78Bst cells, or any other suitable mammalian host cell known in the art. Alternatively, *E. coli* cells can be used as host cells for expression of the sFGFR3 polypeptides. Examples of *E. coli* strains include, but are not limited to, *E. coli* 294 (ATCC®31,446), *E. coli* λ 1776 (ATCC® 31,537, *E. coli* BL21 (DE3) (ATCC® BAA-1025), *E. coli* RV308 (ATCC® 31,608), or any other suitable *E. coli* strain known in the art.

Vectors including polynucleotides encoding the sFGFR3 polypeptides

[0064] The disclosure also describes recombinant vectors including any one or more of the polynucleotides described above. The vectors of the invention can be used to deliver a polynucleotide encoding a sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)), which can include mammalian, viral, and bacterial expression vectors. For example, the vectors can be plasmids, artificial chromosomes (e.g. BAG, PAC, and YAC), and virus or phage vectors, and may optionally include a promoter, enhancer, or regulator for the expression of the polynucleotide. The vectors can also contain one or more selectable marker genes, such as an ampicillin, neomycin, and/or kanamycin resistance gene in the case of a bacterial plasmid or a resistance gene for a fungal vector. Vectors can be used *in vitro* for the production of DNA or RNA or used to transfect or transform a host cell, such as a mammalian host cell for the production of a sFGFR3 polypeptide encoded by the vector. The vectors can also be adapted to be used *in vivo* in a method of gene therapy.

[0065] Exemplary viral vectors that can be used to deliver a polynucleotide encoding a sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)) include a retrovirus, adenovirus (e.g., Ad2, Ad5, Ad11, Ad12, Ad24, Ad26, Ad34, Ad35, Ad40, Ad48, Ad49, Ad50, and Pan9 (also known as AdC68)), parvovirus (e.g., adeno-associated viruses), coronavirus, negative strand RNA viruses such as orthomyxovirus (e.g., influenza virus), rhabdovirus (e.g., rabies and vesicular stomatitis virus), paramyxovirus (e.g. measles and Sendai), positive strand RNA viruses, such as picornavirus and alphavirus, and double stranded DNA viruses including adenovirus, herpesvirus (e.g., Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (e.g., vaccinia, modified vaccinia Ankara (MVA), fowlpox and canarypox). Other viruses useful for delivering polynucleotides encoding sFGFR3 polypeptides include Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, and hepatitis virus. Examples of retroviruses include avian leukosis-sarcoma, mammalian C-type, B-type viruses, D-type viruses, HTLV-BLV group, lentivirus, and spumavirus (Coffin, J. M., Retroviridae: The viruses and their replication, In Fundamental Virology, Third Edition, B. N. Fields, et al., Eds., Lippincott-Raven Publishers, Philadelphia, 1996).

Methods of Production

[0066] Polynucleotides encoding sFGFR3 polypeptides (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33), or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)) can be produced by any method known in the art. For instance, a polynucleotide is generated using molecular cloning methods and is placed within a vector, such as a plasmid, an artificial chromosome, a viral vector, or a phage vector. The vector is used to transform the polynucleotide into a host cell appropriate for the expression of the sFGFR3 polypeptide.

Nucleic acid vector construction and host cells

[0067] The sFGFR3 polypeptide (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)) can be produced from a host cell. The polynucleotides (e.g., polynucleotides having the nucleic acid sequence of SEQ ID NO: 21 or 37 and variants thereof) encoding sFGFR3 polypeptides can be included in vectors that can be introduced into the host cell by

conventional techniques known in the art (e.g., transformation, transfection, electroporation, calcium phosphate precipitation, direct microinjection, or infection). The choice of vector depends in part on the host cells to be used. Generally, host cells are of either prokaryotic (e.g., bacterial) or eukaryotic (e.g., mammalian) origin.

[0068] A polynucleotide encoding an sFGFR3 polypeptide (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)) can be prepared by a variety of methods known in the art. These methods include, but are not limited to, oligonucleotide-mediated (or site-directed) mutagenesis and PCR mutagenesis. A polynucleotide encoding an sFGFR3 polypeptide can be obtained using standard techniques, e.g., gene synthesis. Alternatively, a polynucleotide encoding a wild-type sFGFR3 polypeptide (e.g., a polypeptide having the amino acid sequence of SEQ ID NO: 5 or 32) can be mutated to contain specific amino acid substitutions (e.g., an amino acid substitution of a cysteine residue with a serine residue or a conservative amino acid substitution, such as alanine, glycine, proline, or threonine, at position 253 of SEQ ID NO: 33 and/or position 316 of SEQ ID NO: 4) using standard techniques in the art, e.g., QuikChange™ mutagenesis. Polynucleotides encoding an sFGFR3 polypeptide can be synthesized using, e.g., a nucleotide synthesizer or PCR techniques.

[0069] Polynucleotides encoding sFGFR3 polypeptide (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33), or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)) can be inserted into a vector capable of replicating and expressing the polynucleotide in prokaryotic or eukaryotic host cells. Exemplary vectors useful in the methods can include, but are not limited to, a plasmid, an artificial chromosome, a viral vector, and a phage vector. For example, a viral vector can include the viral vectors described above, such as a retroviral vector, adenoviral vector, or poxviral vector (e.g., vaccinia viral vector, such as Modified Vaccinia Ankara (MVA)), adeno-associated viral vector, and alphaviral vector)) containing the nucleic acid sequence of a polynucleotide encoding the sFGFR3 polypeptide. Each vector can contain various components that may be adjusted and optimized for compatibility with the particular host cell. For example, the vector components may include, but are not limited to, an origin of replication, a selection marker gene, a promoter, a ribosome binding site, a signal sequence, the nucleic acid sequence of the polynucleotide encoding the sFGFR3 polypeptide, and/or a transcription termination sequence.

[0070] The above-described vectors may be introduced into appropriate host cells (e.g., HEK 293 cells or CHO cells) using conventional techniques in the art, e.g., transformation, transfection, electroporation, calcium phosphate precipitation, and direct microinjection. Once the vectors are introduced into host cells for the production of an sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)), host cells are cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the polynucleotides (e.g. SEQ ID NOs: 21 and variants thereof) encoding the sFGFR3 polypeptide. Methods for expression of therapeutic proteins, such as sFGFR3 polypeptides, are known in the art, see, for example, Paulina Balbas, Argelia Lorence (eds.) Recombinant Gene Expression: Reviews and Protocols (Methods in Molecular Biology), Humana Press; 2nd ed. 2004 (July 20, 2004) and Vladimir Voynov and Justin A. Caravella (eds.) Therapeutic Proteins: Methods and Protocols (Methods in Molecular Biology) Humana Press; 2nd ed. 2012 (June 28, 2012).

sFGFR3 polypeptide production, recovery, and purification

[0071] Host cells (e.g., HEK 293 cells or CHO cells) used to produce the sFGFR3 polypeptide (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)) can be grown in media known in the art and suitable for culturing of the selected host cells.

Examples of suitable media for mammalian host cells include Minimal Essential Medium (MEM), Dulbecco's Modified Eagle's Medium (DMEM), Expi293™ Expression Medium, DMEM with supplemented fetal bovine serum (FBS), and RPMI-1640. Examples of suitable media for bacterial host cells include Luria broth (LB) plus necessary supplements, such as a selection agent, e.g., ampicillin. Host cells are cultured at suitable temperatures, such as from about 20 °C to about 39 °C, e.g., from 25 °C to about 37 °C, preferably 37 °C, and CO_2 levels, such as 5 to 10% (preferably 8%). The pH of the medium is generally from about 6.8 to 7.4, e.g., 7.0, depending mainly on the host organism. If an inducible promoter is used in the expression vector, sFGFR3 polypeptide expression is induced under conditions suitable for the activation of the promoter.

[0072] An sFGFR3 polypeptide (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)) can be recovered from the supernatant of the host cell. Alternatively, the sFGFR3 polypeptide can be recovered by disrupting the host cell (e.g., using osmotic shock, sonication, or lysis), followed by centrifugation or filtration to remove the sFGFR3 polypeptide. Upon recovery of the sFGFR3 polypeptide, the sFGFR3 polypeptide can then be further purified. An sFGFR3 polypeptide can be purified by any method known in the art of protein purification, such as protein A affinity, other chromatography (e.g., ion exchange, affinity, and size-exclusion column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins (see Process Scale Purification of Antibodies, Uwe Gottschalk (ed.) John Wiley & Sons, Inc., 2009).

[0073] The sFGFR3 polypeptide (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)) can be conjugated to a detectable label for purification. Examples of suitable labels for use in purification of the sFGFR3 polypeptides include, but are not limited to, a protein tag, a fluorophore, a chromophore, a radiolabel, a metal colloid, an enzyme, or a chemiluminescent, or bioluminescent molecule. In particular, protein tags that are useful for purification of the sFGFR3 polypeptides can include, but are not limited to, chromatography tags (e.g., peptide tags consisting of polyanionic amino acids, such as a FLAG-tag, or a hemagglutinin "HA" tag), affinity tags (e.g., a poly(His) tag, chitin binding protein (CBP), maltose binding protein (MBP), or glutathione-S-transferase (GST)), solubilization tags (e.g., thioredoxin (TRX) and poly(NANP)), epitope tags (e.g., V5-tag, Myc-tag, and HA-tag), or fluorescence tags (e.g., GFP, GFP variants, RFP, and RFP variants).

Methods of Treatment

[0074] Disclosed herein are methods for treating a skeletal growth retardation disorder in a patient, such as a patient having achondroplasia (e.g., a human having achondroplasia). In particular, the patient is one that exhibits or is likely to develop one or more symptoms of a skeletal growth retardation disorder (e.g., achondroplasia). The method involves administering an sFGFR3 polypeptide (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) to the patient having a skeletal growth retardation disorder, such as a patient having achondroplasia (e.g., a human having achondroplasia). In particular, the method involves administering sFGFR3_Del4-D3 (SEQ ID NO: 33) to the patient having a skeletal growth retardation disorder, such as a patient having achondroplasia (e.g., a human having achondroplasia). For example, the patient is an infant or child having a skeletal growth retardation disorder, such as an infant, a child, or an adolescent having achondroplasia (e.g., a human having achondroplasia).

[0075] The patient (e.g., a human) can be treated before symptoms of a skeletal growth retardation disorder (e.g., achondroplasia) appear or after symptoms of a skeletal growth retardation disorder (e.g.,

achondroplasia) develop. In particular, patients that can be treated are those exhibiting symptoms including, but not limited to, short limbs, short trunk, bowlegs, a waddling gait, skull malformations, cloverleaf skull, craniosynostosis, wormian bones, anomalies of the hands, anomalies of the feet, hitchhiker thumb, and/or chest anomalies. Furthermore, treatment with an sFGFR3 polypeptide can result in an improvement in one or more of the aforementioned symptoms of a skeletal growth retardation disorder (e.g., relative to an untreated patient), such as achondroplasia.

[0076] The patient (e.g., a human) can be diagnosed with a skeletal growth retardation disorder, such as achondroplasia, before administration of an sFGFR3 polypeptide. Additionally, the patient having a skeletal growth retardation disorder, such as achondroplasia, can be one that has not previously been treated with an sFGFR3 polypeptide.

Skeletal Growth Retardation Disorders

[0077] Skeletal growth retardation disorders can be treated by administering an sFGFR3 polypeptide as described herein to a patient (e.g., a human) in need thereof. The method involves administering to the patient (e.g., a human) having the skeletal growth retardation disorder an sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)). Skeletal growth retardation disorders that can be treated with the sFGFR3 polypeptides are characterized by deformities and/or malformations of the bones and can include, but are not limited to, FGFR3-related skeletal diseases. In particular, the patient is treated with sFGFR3_Del4-D3 (SEQ ID NO: 33).

[0078] Administration of an sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) can treat a skeletal growth retardation disorder including, but not limited to, achondroplasia, achondrogenesis, acrodysostosis, acromesomelic dysplasia, atelosteogenesis, camptomelic dysplasia, chondrodysplasia punctata, rhizomelic type of chondrodysplasia punctata, cleidocranial dysostosis, congenital short femur, Crouzon syndrome, Apert syndrome, Jackson-Weiss syndrome, Pfeiffer syndrome, Crouzonodermoskeletal syndrome, dactyly, brachydactyly, camptodactyly, polydactyly, syndactyly, diastrophic dysplasia, dwarfism, dyssegmental dysplasia, enchondromatosis, fibrochondrogenesis, fibrous dysplasia, hereditary multiple exostoses, hypophosphatasia, hypophosphatemic rickets, Jaffe-Lichtenstein syndrome, Kniest dysplasia, Kniest syndrome, Langer-type mesomelic dysplasia, Marfan syndrome, McCune-Albright syndrome, micromelia, metaphyseal dysplasia, Jansen-type metaphyseal dysplasia, metatrophic dysplasia, Morquio syndrome, Nievergelt-type mesomelic dysplasia, neurofibromatosis (such as type 1 (e.g., with bone manifestations or without bone manifestations), type 2, or schwannomatosis), osteoarthritis, osteochondrodysplasia, osteogenesis imperfecta, perinatal lethal type of osteogenesis imperfecta, osteopetrosis, osteopoikilosis, peripheral dysostosis, Reinhardt syndrome, Roberts syndrome, Robinow syndrome, short-rib polydactyly syndromes, short stature, spondyloepiphyseal dysplasia congenita, and spondyloepimetaphyseal dysplasia.

[0079] For instance, the sFGFR3 polypeptides of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) can be used to treat symptoms associated with a skeletal growth retardation disorder, including the disorders described above, such as achondroplasia. Non-limiting examples of symptoms of skeletal growth retardation disorders that can be treated with the sFGFR3 polypeptides, include short limbs and trunk, bowlegs, a waddling gait, skull malformations (e.g., a large head), cloverleaf skull, craniosynostosis (e.g., premature fusion of the bones in the skull), wormian bones (e.g., abnormal thread-like connections between the bones in the skull), anomalies of the hands and feet (e.g., polydactyly or extra fingers), "hitchhiker" thumbs and abnormal fingernails and toenails, and chest anomalies (e.g., pear-shaped chest

or narrow thorax). Additional symptoms that can treated by administering sFGFR3 polypeptides can also include non-skeletal abnormalities in patients having skeletal growth retardation disorders, such as anomalies of the eyes, mouth, and ears, such as congenital cataracts, myopia, cleft palate, or deafness; brain malformations, such as hydrocephaly, porencephaly, hydranencephaly, or agenesis of the corpus callosum; heart defects, such as atrial septal defect, patent ductus arteriosus, or transposition of the great vessels; developmental delays; or mental disabilities.

[0080] Treatment with the sFGFR3 polypeptides of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) can also increase survival of patients (e.g., humans) with skeletal growth retardation disorders (e.g., achondroplasia). For example, the survival rate of patients treated with the sFGFR3 polypeptides can increase by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more relative to, e.g., an untreated patient with a skeletal growth retardation disorder (e.g., achondroplasia), over a treatment period of days, months, years, or longer. In particular, administration of sFGFR3_Del4-D3 can increase survival of patients (e.g., humans) with skeletal growth retardation disorders (e.g., relative to an untreated patient), such as achondroplasia.

[0081] Any skeletal growth retardation disorder that is a FGFR3-related skeletal disease (e.g., caused by or associated with overactivation of FGFR3 as result of a gain-of-function FGFR3 mutation) can be treated by administering an sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) to a patient (e.g., a human). For example, FGFR3-related skeletal diseases can include, but are not limited to, achondroplasia, thanatophoric dysplasia type I (TDI), thanatophoric dysplasia type II (TDII), severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN), hypochondroplasia, and craniosynostosis (e.g., Muenke syndrome, Crouzon syndrome, and Crouzonodermoskeletal syndrome).

[0082] Patients (e.g., humans) with mutations in the *FGFR3* gene associated with different FGFR3-related skeletal disorders, such as achondroplasia, hypochondroplasia, SADDAN, TDI, and TDII, can be treated with sFGFR3 polypeptides of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)). For example, the sFGFR3 polypeptides can be administered to treat achondroplasia resulting from the G380R, G375C, G346E or S279C mutations of the *FGFR3* gene. Administration of the sFGFR3 polypeptides can be used to treat the following exemplary FGFR3-related skeletal disorders: hypochondroplasia resulting from the G375C, G346E or S279C mutations of the *FGFR3* gene; TDI resulting from the R248C, S248C, G370C, S371C, Y373C, X807R, X807C, X807G, X807S, X807W and K650M mutations of the *FGFR3* gene; TDII resulting from the K650E mutation of the *FGFR3* gene; and SADDAN resulting from the K650M mutation of the *FGFR3* gene.

[0083] Any of the aforementioned mutations in the *FGFR3* gene (e.g., the G380R mutation of the *FGFR3* gene) can be detected in a sample from the patient (e.g., a human with achondroplasia, hypochondroplasia, SADDAN, TDI, and TDII) prior to or after treatment with an sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)). Additionally, the parents of the patient and/or fetal samples (e.g., fetal nucleic acid obtained from maternal blood, placental, or fetal samples) can be tested by methods known in the art for the mutation in the *FGFR3* gene to determine their need for treatment.

Achondroplasia

[0084] Achondroplasia is the most common cause of dwarfism in humans and can be treated by administering sFGFR3 polypeptides as described herein. In particular, achondroplasia can be treated by

administering an sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)). Accordingly, administration of the sFGFR3 polypeptides can result in an improvement in symptoms including, but not limited to, growth retardation, skull deformities, orthodontic defects, cervical cord compression (with risk of death, e.g., from central apnea or seizures), spinal stenosis (e.g., leg and lower back pain), hydrocephalus (e.g., requiring cerebral shunt surgery), hearing loss due to chronic otitis, cardiovascular disease, neurological disease, respiratory problems, fatigue, pain, numbness in the lower back and/or spine, and/or obesity.

[0085] Patients treated using the sFGFR3 polypeptides of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) can include infants, children, and adults with achondroplasia. In particular, infants are often diagnosed with achondroplasia at birth, and thus, treatment with the sFGFR3 polypeptides can begin as early as possible in the patient's life, e.g., shortly after birth, or prior to birth (*in utero*).

[0086] Symptoms of achondroplasia in patients (e.g., humans) can also be monitored prior to or after a patient is treated with an sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 3)). For instance, symptoms of achondroplasia can be monitored prior to treatment to assess the severity of achondroplasia and condition of the patient prior to performing the methods.

[0087] The methods can include diagnosis of achondroplasia in a patient and monitoring the patient for changes in the symptoms of achondroplasia, such as changes in body weight and skull size (e.g., skull length and/or skull width) of the patient. Changes in body weight and skull size can be monitored over a period of time, e.g., 1, 2, 3, 4 or more times per month or per year or approximately every 1, 2, 3, 4, 5, 6, 7, 8, 12 or 16 weeks over the course of treatment with the sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)). Body weight and/or skull size of the patient having achondroplasia can also be determined at treatment specific events, such as before and/or after administration of the sFGFR3 polypeptide.

[0088] For example, body weight and/or skull size can be measured in response to administration of the sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)). Body weight can be measured by weighing the patient having achondroplasia on a scale, preferably in a standardized manner, such as with the same or no clothes or at a certain time of the day, preferably in a fasting state (e.g., in the morning before breakfast or after at least 1, 2, 3, 4, 5 or more hours of fasting). Skull size can be represented by length, height, width, and/or circumference of the skull. Measurements can be performed using any known or self-devised standardized method. For a human subject, the measurement of skull circumference is preferred, which can be measured using a flexible and non-stretchable material, such as a tape, wrapped around the widest possible circumference of the head (e.g. from the most prominent part of the forehead around to the widest part of the back of the head). The height of the skull of the subject (e.g., human) can also be determined from the underside of the chin to the uppermost point of the head. Preferably, any measurement is performed more than once, e.g. at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more times.

Administration of sFGFR3 Polypeptides

[0089] An sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) can be administered by any route known in the art, such as by parenteral administration, enteral administration, or topical administration. In particular, the sFGFR3 polypeptide can be administered to the patient having a skeletal growth retardation disorder (e.g., achondroplasia) subcutaneously (e.g., by subcutaneous injection), intravenously, intramuscularly, intra-arterially, intrathecally, or intraperitoneally.

[0090] An sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) can be administered to a patient (e.g., a human) at a predetermined dosage, such as in an effective amount to treat a skeletal growth retardation disorder (e.g., achondroplasia), without inducing significant toxicity. For example, sFGFR3 polypeptides can be administered to a patient having skeletal growth retardation disorders (e.g., achondroplasia) in individual doses ranging from about 0.002 mg/kg to about 50 mg/kg (e.g., from 2.5 mg/kg to 30 mgkg, from 0.002 mg/kg to 20 mg/kg, from 0.01 mg/kg to 2 mg/kg, from .2 mg/kg to 20 mg/kg, from 0.01 mg/kg to 10 mg/kg, from 10 mg/kg to 100 mg/kg, from 0.1 mg/kg to 50 mg/kg, 0.5 mg/kg to 20 mg/kg, 1.0 mg/kg to 10 mg/kg, 1.5 mg/kg to 5 mg/kg, or 0.2 mg/kg to 3 mg/kg). In particular, the sFGFR3 polypeptide can be administered in individual doses of, e.g., 0.001 mg/kg to 50 mg/kg, such as 2.5 mg/kg to about 10 mg/kg.

[0091] Exemplary doses of an sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) for administration to a patient (e.g., a human) having a skeletal growth retardation disorder (e.g., achondroplasia) include, e.g., 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, or 50 mg/kg. These doses can be administered one or more times (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 or more times) per day, week, month, or year. For example, an sFGFR3 polypeptide can be administered to patients in a weekly dosage ranging, e.g., from about 0.0014 mg/kg/week to about 140 mg/kg/week, e.g., about 0.14 mg/kg/week to about 105 mg/kg/week, or, e.g., about 1.4 mg/kg/week to about 70 mg/kg/week (e.g., 2.5 mg/kg/week, 5 mg/kg/week, 10 mg/kg/week, 20 mg/kg/week, 30 mg/kg/week, 40 mg/kg/week, or 50 mg/kg/week).

Gene Therapy

[0092] An sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO:4 or 33)) can also be delivered through gene therapy, where a polynucleotide encoding the sFGFR3 polypeptide is delivered to tissues of interest and expressed *in vivo.* Gene therapy methods are discussed, e.g., in Verme et al. (Nature 389: 239-242, 1997), Yamamoto et al. (Molecular Therapy 17: S67-S68, 2009), and Yamamoto et al., (J. Bone Miner. Res. 26: 135-142, 2011).

[0093] An sFGFR3 polypeptide of the invention (e.g. sFGFR3_Del4-D3 (SEQ ID NO: 4 or 33)) can be produced by the cells of a patient (e.g., a human) having a skeletal growth retardation disorder (e.g., achondroplasia) by administrating a vector (e.g., a plasmid, an artificial chromosome (e.g. BAG, PAC, and YAC), or a viral vector) containing the nucleic acid sequence of a polynucleotide encoding the sFGFR3 polypeptide. For example, a viral vector can be a retroviral vector, adenoviral vector, or poxviral vector (e.g., vaccinia viral vector, such as Modified Vaccinia Ankara (MVA)), adeno-associated viral vector, or alphaviral vector. The vector, once inside a cell of the patient (e.g., a human) having a skeletal growth retardation disorder (e.g., achondroplasia), by, e.g., transformation, transfection, electroporation, calcium phosphate precipitation, or direct microinjection, will promote expression of the sFGFR3 polypeptide, which is then secreted from the cell. The invention further includes cell-based therapies, in which the patient (e.g., a human) is administered a cell expressing the sFGFR3 polypeptide.

Pharmaceutical Compositions

[0094] Pharmaceutical compositions of the invention can include an sFGFR3 polypeptide (e.g. sFGFR3_Del4-C253S (SEQ ID NO: 2), sFGFR3_Del4-D3 (SEQ ID NO: 33), and variants thereof (SEQ ID

NO: 4) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)), polynucleotide, vector, and/or host cell. Compositions including an sFGFR3 polypeptide, polynucleotide, vector, and/or host cell can be formulated at a range of dosages, in a variety of formulations, and in combination with pharmaceutically acceptable excipients, carriers, or diluents.

[0095] A pharmaceutical composition including an sFGFR3 polypeptide (e.g. sFGFR3_Del4-C253S (SEQ ID NO: 2), sFGFR3_Del4-D3 (SEQ ID NO: 33), and variants thereof (SEQ ID NO: 4) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)), polynucleotide, vector, and/or host cell can be formulated at a specific dosage, such as a dosage that is effective for treating a patient (e.g., a human) skeletal growth retardation disorder (e.g., achondroplasia), without inducing significant toxicity. For example, the compositions can be formulated to include between about 1 mg/mL and about 500 mg/mL of the sFGFR3 polypeptide (e.g., between 10 mg/mL and 300 mg/mL, 20 mg/mL and 120 mg/mL, 40 mg/mL and 200 mg/mL, 30 mg/mL and 150 mg/mL, 40 mg/mL and 100 mg/mL, 50 mg/mL and 80 mg/mL, or 60 mg/mL and 70 mg/mL of the sFGFR3 polypeptide).

[0096] The pharmaceutical compositions including an sFGFR3 polypeptide (e.g. sFGFR3_Del4-C253S (SEQ ID NO: 2), sFGFR3_Del4-D3 (SEQ ID NO: 33), and variants thereof (SEQ ID NO: 4) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)), polynucleotide, vector, and/or host cell can be prepared in a variety of forms, such as a liquid solution, dispersion or suspension, powder, or other ordered structure suitable for stable storage. For example, compositions including an sFGFR3 polypeptide intended for systemic or local delivery can be in the form of injectable or infusible solutions, such as for parenteral administration (e.g., subcutaneous, intravenous, intramuscular, intra-arterial, intrathecal, or intraperitoneal administration). sFGFR3 compositions for injection (e.g., subcutaneous or intravenous injection) can be formulated using a sterile solution or any pharmaceutically acceptable liquid as a vehicle. Pharmaceutically acceptable vehicles include, but are not limited to, sterile water, physiological saline, and cell culture media (e.g., Dulbecco's Modified Eagle Medium (DMEM), α-Modified Eagles Medium (a-MEM), F-12 medium). Formulation methods are known in the art, see e.g., Banga (ed.) Therapeutic Peptides and Proteins: Formulation, Processing and Delivery Systems (2nd ed.) Taylor & Francis Group, CRC Press (2006).

[0097] Compositions including an sFGFR3 polypeptide (e.g. sFGFR3_Del4-C253S (SEQ ID NO: 2), sFGFR3_Del4-D3 (SEQ ID NO: 33), and variants thereof (SEQ ID NO: 4) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)), polynucleotide, vector, and/or host cell can be provided to patients (e.g., humans) having skeletal growth retardation disorders (e.g. achondroplasia) in combination with pharmaceutically acceptable excipients, carriers, or diluents. Acceptable excipients, carriers, or diluents can include buffers, antioxidants, preservatives, polymers, amino acids, and carbohydrates. Aqueous excipients, carriers, or diluents can include water, water-alcohol solutions, emulsions or suspensions including saline, buffered medical parenteral vehicles including sodium chloride solution, Ringer's dextrose solution, dextrose plus sodium chloride solution, Ringer's solution containing lactose, and fixed oils. Examples of non-aqueous excipients, carriers, or diluents are propylene glycol, polyethylene glycol, vegetable oil, fish oil, and injectable organic esters.

[0098] Pharmaceutically acceptable salts can also be included in the compositions including an sFGFR3 polypeptide (e.g. sFGFR3_Del4-C253S (SEQ ID NO: 2), sFGFR3_Del4-D3 (SEQ ID NO: 33), and variants thereof (SEQ ID NO: 4) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)), polynucleotide, vector, and/or host cell. Exemplary pharmaceutically acceptable salts can include mineral acid salts (e.g., hydrochlorides, hydrobromides, phosphates, and sulfates) and salts of organic acids (e.g., acetates, propionates, malonates, and benzoates). Additionally, auxiliary substances, such as wetting or emulsifying agents and pH buffering substances, can be present. A thorough discussion of

pharmaceutically acceptable excipients, carriers, and diluents is available in Remington: The Science and Practice of Pharmacy, 22nd Ed., Allen (2012).

[0099] Pharmaceutical compositions including an sFGFR3 polypeptide (e.g. sFGFR3_Del4-C253S (SEQ ID NO: 2), sFGFR3_Del4-D3 (SEQ ID NO: 33), and variants thereof (SEQ ID NO: 4) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)), polynucleotide, vector, and/or host cell can also be formulated with a carrier that will protect the sFGFR3 polypeptide against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. For example, the sFGFR3 composition can be entrapped in microcapsules prepared by coacervation techniques or by interfacial polymerization, such as hydroxymethylcellulose, gelatin, or poly-(methylmethacylate) microcapsules; colloidal drug delivery systems (e.g., liposomes, albumin microspheres, microemulsions, nano-particles, or nanocapsules); or macroemulsions. Additionally, an sFGFR3 composition can be formulated as a sustained-release composition. For example, sustained-release compositions can include semi-permeable matrices of solid hydrophobic polymers containing the sFGFR3 polypeptides, polynucleotides, vectors, or host cells, in which the matrices are in the form of shaped articles, such as films or microcapsules.

Kits

[0100] Kits of the invention can include one or more sFGFR3 polypeptides (e.g. sFGFR3_Del4-C253S (SEQ ID NO: 2), sFGFR3_Del4-D3 (SEQ ID NO: 33), and variants thereof (SEQ ID NO: 4) or a sFGFR3 polypeptide including a signal peptide (SEQ ID NO: 18 or 34)), polynucleotides, vectors, and/or cells as described herein. For example, the sFGFR3 polypeptide, polynucleotide, vector, and/or cell can be present in a container (e.g., a glass vial) in liquid form (e.g., in water or a buffered salt solution, such as, 2 mM to 20 mM of sodium phosphate, pH 6.5 or 7.0, and 25 mM to 250 mM sodium chloride). Alternatively, the sFGFR3 polypeptide, polynucleotide, and/or vector is present in a container (e.g., a glass vial) in lyophilized form, which can optionally include a diluent (e.g., water or a buffered salt solution) for reconstitution of the lyophilized sFGFR3 polypeptide, polynucleotide, vector, and/or cell into liquid form prior to administration. The sFGFR3 polypeptide, polynucleotide, vector, and/or cell can also be present in a kit in another formulation as described herein. The kit components can be provided in dosage form to facilitate administration, and optionally, can include materials required for administration and/or instructions for patient treatment consistent with the methods. For example, the kit can include instructions for use, which guides the user (e.g., the physician) with respect to the administration of the sFGFR3 polypeptide, polynucleotide, vector, and/or cell.

EXAMPLES

[0101] The following examples are intended to illustrate, rather than limit, the disclosure. These studies feature the administration of the sFGFR3 polypeptides of sFGFR3_Del4-C253S (SEQ ID NO: 2) and sFGFR3_Del4-D3 (SEQ ID NO: 33) to patients (e.g., humans) having achondroplasia, to treat achondroplasia and symptoms associated therewith.

Example 1: Production of sFGFR3 Polypeptides

[0102] sFGFR3_Del4-C253S (SEQ ID NO: 2) and sFGFR3_Del4-D3 (SEQ ID NO: 33) were produced by

transient transfection in three different suspension cell types: HEK 293 freestyle, CHO-S freestyle cells and Expi CHO-S cells. For production in HEK 293 freestyle and CHO-S freestyle cells, transfection was performed using polyethylenimine (PEIpro® - Polyplus-transfection), according to the manufacturer's directions. Proteins were harvested after three days. For sFGFR3 polypeptide production in Expi CHO-S cells, transfection was performed using Expifectamine as described by the manufacturer using the High Titer production protocol. A time course was performed and sFGFR3 polypeptides were optimally harvested after 12 days. Western blots were then performed using 50 ng of sFGFR3 polypeptide. Classical western blot protocols were used with B9 as a primary antibody (anti FGFR3, sc-13121, Santa Cruz) diluted 1:2000 in blocking buffer and anti-mouse IgG secondary antibody (Anti-mouse IgG, #7076, Cell signaling) diluted 1:5000 in blocking buffer.

Example 2: Purification of sFGFR3 Polypeptides

[0103] sFGFR3_Del4-C253S and sFGFR3_Del4-D3 were each purified using a two-step purification process including ion exchange chromatography and size exclusion chromatography.

[0104] For ion exchange chromatography, 300 mL of culture supernatant was purified by cross flow filtration (ÄKTA™ flux, GE Healthcare) using 5 µm and 0.2 µm capsules (KGF-A0504 TT and KMP-HEC 9204 TT, GE Healthcare, respectively). The purified sample including sFGFR3_Del4-C253S or sFGFR3_Del4-D3 was then loaded on an equilibrated column at 20 mL/min, after adjusting the sample's conductivity to 14 mS/cm (ÄKTA™ pure 25 (GE Healthcare)). Columns used were HiPrep Q FF 26/10 (GE Healthcare) with a bed volume of 53 mL. The binding buffer was 1X PBS and the elution buffer was PBS 1X + 1 M NaCl. The column was washed with four column volumes of 1X PBS. Elution of sFGFR3_Del4-C253S and sFGFR3_Del4-D3 was performed by two steps of 5% NaCl and 10% NaCl using four column volumes of each. Both 5% NaCl and 10% NaCl were pooled and concentrated by cross flow filtration (ÄKTA™ flux, GE Healthcare). The remaining volume was then concentrated on a 30 kDa filter by centrifugation at 4°C, 3,900 g for 10 min (MILLLIPORE® UFC903024 AMICON® Ultra-15 Centrifugal Filter Concentrator). For size exclusion chromatography, the remaining volume was loaded on a HiLoad 26/600 SUPERDEX™ 200 prep grade (28-9893-36, GE Healthcare) with a bed volume of 320 mL. Loading volume did not exceed 12.8 mL. Elution was performed in 1X PBS.

Example 3: Kinetic Assays and Dissociation Constant (K_d) Measurements of sFGFR3 Polypeptides

[0105] Calibration Free Concentration Analysis and kinetic assays of sFGFR3_Del4-C253S and sFGFR3_Del4-D3 were performed with a Sensor Chip CM5 (GE Healthcare). Human FGF2 (hFGF2) was covalently immobilized to the Sensor Chip CM5 at a level of about 5000 RU by amine coupling. To achieve 5000 RU, hFGF2 was immobilized for 420 seconds at a flow rate 10 µl/min and a concentration 25 µg/ml. Running buffer was HBS-EP+ Buffer (GE Healthcare). Regeneration buffer was 100mM sodium acetate with 2M sodium chloride pH 4.5. FGF binding, dissociation constant (K_d) measurements, and kinetic parameters were determined by Surface Plasmon Resonance using a BIACORE™ T200 (GE Healthcare). The model used for kinetic assays and K_d determination was a 1:1 binding algorithm.

Example 4: Proliferation Assays of sFGFR3 Polypeptides

[0106] Both ATDC5 and ATDC5 FGFR3^{G380R} cell lines were seeded at a density of 25,000 cells/cm^2 in NUNC™ MICROWELL™ 96-Well Optical-Bottom Plates with Polymer Base (ThermoFisher Scientific, Catalog No. 165305). After a 24 hour incubation period, cells were depleted for 48 hour in 0.5 % BSA and then stimulated for 72 hour with sFGFR3_Del4-C253S or sFGFR3_Del4-D3 with and without hFGF2 (Peprotech). Cell proliferation was then measured using the CyQUANT® Direct Cell Proliferation Assay (Molecular Probes, Catalog No. C35012). After stimulation, 10µL of CyQUANT® Direct Cell Proliferation (Invitrogen; 1mL 1X PBS, 250µL background suppressor, and 50µL nuclear stain) was added per well. ATDC5 and ATDC5 FGFR3^{G380R} cells were then incubated at room temperature in the dark for 2 hours. Fluorescence was read using the VARIOSKAN™ LUX multimode microplate reader (ThermoFisher Scientific).

Example 5: Luciferase Assays of sFGFR3 Polypeptides

[0107] Serum Response Element-Luciferase (SRE-Luc) HEK cells expressing FGFR3^{G380R} were seeded at a density of 100,000 cells/cm^2 in a standard culture 96 well plate. Cells were then depleted for 24 hours with 0.5% heat inactivated Fetal Bovine Serum (hiFBS), before being treated with sFGFR3_Del4-D3 at concentrations of 0 nm, 70 nm, and 280 nm with or without 1 ng/ml of hFGF2 for 24h. The culture plate was equilibrated to room temperature for 15 minutes prior to adding 100µL per well of Firefly Luc One-Step Glow Assay Working Solution (ThermoFisher Scientific, Catalog No. 16197), then shaken at 600 rpm for 3 minutes. The plate was incubated at room temperature for 10 minutes and each cell lysate was transferred to a white opaque 96 well plate to increase luminescence signal and decrease cross contamination. The luminescence signal was read using the VARIOSKAN™ LUX multimode microplate reader (ThermoFisher Scientific).

Example 6: *In vivo* Efficacy Study of sFGFR3 Polypeptides

[0108] Experiments were performed on transgenic *Fgfr3*$^{ach/+}$ animals in which expression of the mutant FGFR3 is driven by the Col2a1 promoter/enhancer. Mice were exposed to a 12 hour light/dark cycle and had free access to standard laboratory food and water. Genotypes were verified by PCR of genomic DNA using the primers 5'-AGGTGGCCTTTGACACCTACCAGG-3' (SEQ ID NO: 30) and 5'-TCTGTTGTGTTTCCTCCCTGTTGG-3' (SEQ ID NO: 31), which amplify 360 bp of the FGFR3 transgene.

[0109] sFGFR3_Del4-D3 produced using CHO cells was evaluated at a subcutaneous dose of 0.25 mg/kg twice weekly. At day 3, all newborn mice from a single litter received the same dose. Control litters received 10 µl of PBS (vehicle). Thereafter, subcutaneous injections of sFGFR3_Del4-D3 (0.25 mg/kg) were administered twice a week for three weeks, alternatively on the left and right sides of the back. Mice were observed daily with particular attention to locomotion and urination alterations. Breeding was performed to generate litters with half wild type and half heterozygous *Fgfr3*$^{ach/+}$ mice. To avoid bias due to phenotype penetrance variations, experiments were performed on at least two litters (one treated and one control) from the same breeders. Previous data indicated there was no statistical difference between males and females, and thus, males and females were considered one group for all analyses.

[0110] At day 22, all animals were sacrificed by lethal injection of pentobarbital, and gender was determined. All subsequent measurements and analyses were performed without knowledge of mice genotype to avoid investigator bias. Genotyping was performed at the end of the study to reveal the correspondence of data with a specific genotype. Since achondroplasia is a disease with phenotypic variability, all animals were included in the study. Animals dead before day 22 were used to investigate the impact of treatment on premature death. Surviving animals at day 22 were used for all analyses. All experiments and data measurements were performed by blinded experimenters at all time points.

[0111] Following sacrifice at day 22, body weights were measured. Cadavers were carefully skinned, eviscerated, and skeletal measurements were performed based on X-rays. Organs were harvested, weighed, and stored in 10% formalin for further histological analysis using standard paraffin-embedded techniques. Organs were then observed for macroscopic abnormalities, such as modification of color or texture and presence of nodules. The Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985; http://grants1.nih.gov/grants/olaw/references/phspol.htm) and the European commission guidelines for the protection of animals used for scientific purposes (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm) were followed during all animal experiments. All procedures were approved by the Institutional Ethic Committee for the use of Laboratory Animals (CIEPAL Azur) (approval # NCE-2012-52).

Example 7: The Cell Line used to produce sFGFR3 Polypeptides did not impact Activity

[0112] The FGF2 binding activity, Kd, and effect on cellular signaling of sFGFR3_Del1 (SEQ ID NO: 7), sFGFR3_Del4 (SEQ ID NO: 1), and sFGFR3_Del4-LK1-LK2 (SEQ ID NO: 10) produced in suspension HEK 293 cells or CHO cells were compared. HEK 293 cells or CHO cells differ in post-translation modification of proteins. Expression of the sFGFR3 polypeptides in different cell lines did not impact Kd, binding activity, or the effect of the sFGFR3 polypeptides on intracellular signaling inhibition (FIGS. 1A-1D).

Example 8: Improved Production of sFGFR3_Del4-C253S and sFGFR3_Del4-D3

[0113] The sFGFR3 polypeptides of sFGFR3_Del1 (SEQ ID NO: 7), sFGFR3_Del4 (SEQ ID NO: 1), and sFGFR3_Del4-LK1-LK2 (SEQ ID NO: 10) were each modified to include either an amino acid substitution of a cysteine residue with a serine residue at position 253 or an extended Ig-like C2-type domain 3 (SEQ ID NO: 33). These modifications of sFGFR3_Del1 and sFGFR3_Del4-LK1-LK2 had no or minimal effect on production of the sFGFR3 polypeptides, since aggregation was still visible (FIGS. 2A and 2B, respectively). Surprisingly, modification of sFGFR3_Del4 to include either an amino acid substitution of a cysteine residue with a serine residue at position 253 (sFGFR3_Del4-C253S) or an extended Ig-like C2-type domain 3 (SEQ ID NO: 33)) improved production of the sFGFR3 polypeptides. In particular, there was minimal aggregation of sFGFR3_Del4-C253S and sFGFR3_Del4-D3 under both reducing and non-reducing conditions (FIG. 2C). The inclusion of C253S or D3 also resulted in a relative increase in production compared to sFGFR3_Del4, a two-fold increase in sFGFR3_Del4-C253S production and a 3-fold increase in sFGFR3_Del4-D3 production.

[0114] Additionally, sFGFR3_Del4, sFGFR3_Del4-C253S, and sFGFR3_Del4-D3 exhibited similar Kd and were not affected by cell type specific changes in post translational modifications. In Expi CHO cells, the Kd of sFGFR3_Del4 was 0.8 nM, the Kd of sFGFR3_Del4-C253S was 0.6 nM, and the Kd of

sFGFR3_Del4-D3 was 0.7 nM (FIG. 3A and Table 1).

Table 1. Dissociation constant (Kd) of sFGFR3 polypeptides.

sFGFR3 Polypeptide	Kd (nM)
sFGFR3_Del4	0.8
sFGFR3_Del4-C253S	0.6
sFGFR3_Del4-D3	0.7

Example 9: sFGFR3_Del4-C253S and sFGFR3_Del4-D3 are Equally Active *In Vitro*

[0115] sFGFR3_Del4, sFGFR3_Del4-C253S, and sFGFR3_Del4-D3 restored proliferation of ATDC5 cells genetically modified to overexpress the $FGFR3^{ach}$ mutation (ATDC5 $FGFR3^{G380R}$ cell lines). At a dose of 36 nM, sFGFR3_Del4 produced using HEK 293 cells increased proliferation to 115.5%, sFGFR3_Del4 produced using CHO-S cells increased proliferation to 116%, sFGFR3_Del4-C253S produced using CHO-S cells increased proliferation to 114.4%, and sFGFR3_Del4-D3 using CHO-S cells increased proliferation to 120.1% (FIG. 3B).

[0116] sFGFR3_Del4-D3 was also tested in the $FGFR3^{G380R}$ expressing SRE(-Luc) HEK cell line at doses of 0 nM, 70 nM, and 280nM with or without 1 ng/ml of hFGF2 (FIG. 4; n=8). Data shown in FIG. 4 are the mean +/- standard error of the mean (SEM). These data followed a normal law and have equal variance based on the D'Agostino- Pearson omnibus normality test. Statistical comparisons with and without sFGFR3_Del4-D3 were performed using a student t-test. As shown in FIG. 4, sFGFR3_Del4-D3 decreases luciferase signalling in the SRE cell line.

Example 10: sFGFR3_Del4-D3 restores Bone Growth, prevents Mortality, and restores Foramen Magnum Shape in Mice with Achondroplasia

[0117] An in vivo efficacy study was performed as in Example 6 using a low dose (0.25 mg/kg) of sFGFR3_Del4-D3. A total of 60 mice were included in the vehicle group, with 32 wild type (wt) mice and 28 $Fgfr3^{ach/+}$ mice. The treated group included 40 mice, with 19 wt mice and 21 $Fgfr3^{ach/+}$ mice. Surprisingly, the low dose of sFGFR3_Del4-D3 almost completely prevented the premature death of mice with achondroplasia (FIG. 5). In the control group, 53.6% of the $Fgfr3^{ach/+}$ mice died before weaning, whereas only 4.8% of mice in the treated group died before day 22 and 20% of mice died following treatment with sFGFR3_Del1 at 0.25 mg/kg (Table 2; see also Garcia et al. Sci. Transl. Med. 5:203ra124, 2013).

[0118] sFGFR3_Del4-D3 also partially restored bone growth with correction of the initial discrepancy between wt and $Fgfr3^{ach/+}$ mice on the axial and appendicular skeleton (Table 2). In contrast to prior results of treatment with a low dose of sFGFR3_Del1, treatment with low dose of sFGFR3_Del4-D3 restored normal foramen magnum shape.

Table 2. In vivo results of administering a high dose of sFGFR3_Del1, a low dose of sFGFR3_Del1, and a low dose of sFGFR3_Del4-D3 to mice with achondroplasia

	2.5 mg/kg sFGFR3_Del1 (Garcia et al.)	0.25 mg/kg sFGFR3_Del1 (Garcia et al.)	0.25 mg/kg sFGFR3_Del4-D3
Mortality	12%	20%	4.8%
Axial correction	77%	24%	10%
Appendicular correction	150-215%	18-42%	11-42%
Foramen shape correction (ratio W/H	Not determined	Not determined	111%

Example 11: Treatment of Achondroplasia by Administration of sFGFR3_Del4-C253S

[0119] A human patient (e.g., an infant, child, adolescent, or adult) suffering from achondroplasia can be treated by administering sFGFR3_Del4-C253S (FIG. 6; SEQ ID NO: 2) by an appropriate route (e.g., by subcutaneous injection) at a particular dosage (e.g., between 0.0002 mg/kg/day to about 20 mg/kg/day, such as 0.001 mg/kg/day to 7 mg/kg/day) over a course of days, weeks, months, or years. The progression of achondroplasia that is treated with sFGFR3_Del4-C253S can be monitored by one or more of several established methods. A physician can monitor the patient by direct observation in order to evaluate how the symptoms of achondroplasia exhibited by the patient have changed in response to treatment. For instance, a physician may monitor changes in body weight, skull length, and/or skull width of the patient over a period of time, e.g., 1, 2, 3, 4 or more times per month or per year or approximately every 1, 2, 3, 4, 5, 6, 7, 8, 12, or 16 weeks over the course of treatment with sFGFR3_Del4-C253S. Body weight and/or skull size of the patient or changes thereof can also be determined at treatment specific events, e.g. before and/or after administration of sFGFR3_Del4-C253S. For example, body weight and/or skull size are measured in response to administration of sFGFR3_Del4-C253S.

Example 12: Treatment of Achondroplasia by Administration of sFGFR3_Del4-D3

[0120] Additionally, a human patient (e.g., an infant, child, adolescent, or adult) suffering from achondroplasia can be treated by administering the sFGFR3 polypeptide of sFGFR3_Del4-D3 (SEQ ID NO: 33) by an appropriate route (e.g., by subcutaneous injection) at a particular dosage (e.g., between 0.0002 mg/kg/day to about 20 mg/kg/day, such as 0.001 mg/kg/day to 7 mg/kg/day) over a course of days, weeks, months, or years. The progression of achondroplasia that is treated with sFGFR3_Del4-D3 can be monitored by one or more of several established methods. A physician can monitor the patient by direct observation in order to evaluate how the symptoms of achondroplasia exhibited by the patient have changed in response to treatment. For instance, a physician may monitor changes in body weight, skull length, and/or skull width of the patient over a period of time, e.g., 1, 2, 3, 4 or more times per month or per year or approximately every 1, 2, 3, 4, 5, 6, 7, 8, 12, or 16 weeks over the course of treatment with sFGFR3_Del4-D3. Body weight and/or skull size of the patient or changes thereof can also be determined at treatment specific events, e.g. before and/or after administration of sFGFR3_Del4-D3. For example, body weight and/or skull size are measured in response to administration of sFGFR3_Del4-D3.

Example 13: Production of sFGFR3_Del4-D3 and sFGFR3_Del4-C253S

[0121] The sFGFR3_Del4-D3 and sFGFR3_Del4-C253S polypeptides were purified as described in Example 2. Modification of sFGFR3_Del4 to include either an extended Ig-like C2-type domain 3 (FGFR3_Del4-D3) or an amino acid substitution of a cysteine residue with a serine residue at position 253 (sFGFR3_Del4-C253S) improved production of the sFGFR3 polypeptides. In particular, there was less than about 2% aggregation of sFGFR3_Del4-D3 and sFGFR3_Del4-C253S (as observed upon loading using a concentration of 2.3 mg/ml or 23 mg/ml for FGFR3_Del4-D3 and 1.5 mg/ml and 15 mg/ml of sFGFR3_Del4-C253S) under both reducing and non-reducing conditions using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; FIGS. 7A and 7B, respectively). Following production of sFGFR3_Del4-D3 and sFGFR3_Del4-C253S in fed-batch cultures, the top five clones were separated using capillary electrophoresis to yield 0.93 to 1.0 g/L and 0.98 to 1.1 g/L of sFGFR3_Del4-D3 and sFGFR3_Del4-C253S, respectively. Viral filtration using ion-exchange chromatography resulted in a yield of greater than 60% for both sFGFR3_Del4-D3 and sFGFR3_Del4-C253S.

Example 14: Pharmacokinetics and Tissue Distribution of sFGFR3_Del4-D3 *in vivo*

[0122] *In vivo* studies were performed to investigate the pharmacokinetic parameters of sFGFR3_Del4-D3, the uptake of sFGFR3_Del4-D3 across the blood brain barrier, and the tissue distribution of sFGFR3_Del4-D3 in kidney, liver, spleen, lung, and heart. The studies described herein included four arms with five groups of C57BL/6J mice per arm and a total of four mice (n=4) per group (Table 3). Mice were male and weighed 25 to 30 grams.

Table 3. Overview of mice used in studies of sFGFR3_Del4-D3.

Arm	sFGFR3_Del4-D3 (mg/kg)	Route	PK	BBB	Tissue distribution
1	0.25	SC	yes	no	no
2	2.5	SC	yes	no	yes
3	2.5	IV	yes	Yes	yes
4	10	SC	yes	no	no

[0123] Group 1 was sampled at 1 minute, 15 minutes, and 30 minutes; group 2 was sampled at 4 hours; group 3 was sampled at 24 hours; group 4 was sampled at 36 hours; and group 5 was sampled at 48 hours. For Group 1, an indwelling intra-arterial catheter (PE-10) was inserted into one common carotid artery under isoflurane anesthesia and used for repeated blood sampling at the 30 minute final sampling time point. For intravenous injection, ^{125}I-sFGFR3_Del4-D3 was injected intravenously into the jugular vein, which was exposed by skin incision under isoflurane anesthesia. Group 1 mice remained anesthetized throughout the experiments. Repeated blood samples (2 × ~50µL) were drawn from the arterial catheter at 1 minute and 15 minutes after intravenous injection. For groups 2 to 5, after injection of ^{125}I-sFGFR3_Del4-D3, the skin was closed with a surgical clip, and the mice were allowed to wake up and returned to the cage. At 5 minutes before termination time for group 3, mice were re-anesthetized and received an intravenous bolus of ^{3}H-albumin into the jugular vein. The ^{3}H tracer dose was targeted to yield a ratio of ^{125}I to ^{3}H in blood, which is suitable for double isotope labeling with a lower dose at later sampling times. At the terminal sampling time (2 hours, 3 hours, 24 hours, 36 hours, and 48 hours), a blood sample was collected, and the animal was euthanized. The brain was sampled for homogenization and determination of tissue concentration of tracers. Endpoints of the studies included pharmacokinetic parameters for sFGFR3_Del4-D3 (terminal half life), uptake of sFGFR3_Del4-D3 across the blood brain barrier, and the tissue distribution of sFGFR3_Del4-D3 in kidney, liver, spleen, lung, and heart.

Example 15: Thermal and Plasma Stability of sFGFR3_Del4-D3 and sFGFR3_Del4-C253S

[0124] The thermal stability of sFGFR3_Del4-D3 and sFGFR3_Del4-C253S in mouse plasma was investigated using differential scanning colorimetry. For sFGFR3_Del4-D3, two buffers (20 mM phosphate, 40mM NaCl, pH 7.5, and 20 mM citrate, 40mM NaCl, pH 6.5) were added to polypeptide samples. For sFGFR3_Del4-C253S, two buffers (20 mM phosphate, 40mM NaCl, pH 7.5, and 40 mM citrate, 40mM NaCl, pH 6.5) were added to polypeptide samples. The melting temperature (T_m) for sFGFR3_Del4-C253S in the 20 mM phosphate, 40mM NaCl, pH 7.5 buffer was 52°C and 56°C, and the T_m for sFGFR3_Del4-C253S in the 40 mM citrate, 40mM NaCl, pH 6.5 buffer was 55°C and 60°C (FIG. 8A). For sFGFR3_Del4-D3, two buffers (20 mM phosphate, 40mM NaCl, pH 7.5, and 20 mM citrate, 40mM NaCl, pH 6.5) were added to polypeptide samples. The T_m for sFGFR3_Del4-D3 in the 20 mM phosphate, 40mM NaCl, pH 7.5 buffer was 50°C and 54°C, and the T_m for sFGFR3_Del4-D3 in the 20 mM citrate, 40mM NaCl, pH 6.5 buffer was 53°C and 58°C (FIG. 8B). These results indicate that both sFGFR3_Del4-D3 and sFGFR3_Del4-C253S show two domains of polypeptide stability and unfolding.

[0125] The *ex vivo* plasma stability of sFGFR3_Del4-D3 with a Histidine tag was determined by labeling purified sFGFR3_Del4-D3 with ^{125}I-tracer using the Bolton-Hunter method, followed by purification on PD-10 (Sephadex® G-25) columns. The trichloroacetic acid (TCA) precipitability of peak fractions was also determined to confirm stability of the ^{125}I-tracer. Mouse plasma (n = 4) pre-warmed to 37°C was spiked with the ^{125}I-sFGFR3_Del4-D3 to a concentration of ~10 cpm/mL and then vortexed. The plasma samples were incubated with the ^{125}I-sFGFR3_Del4-D3 in an Eppendorf ThermoMixer® under gentle rotation (300 rpm). Aliquots were then collected for TCA precipitation (10 µl sample and 100 µl 2% BSA) and for injection onto an Fast Performance Liquid Chromatography (FPLC) column (20 µl sample and 150 µl 10 mM PBS, pH 7.4) at intervals of 0, 30, 60, 120, 180, and 360 minutes. Aliquots were stored on ice until TCA precipitation or FPLC injection was performed.

[0126] For TCA precipitation, 1 mL ice cold 10% TCA was added to plasma samples, incubated for 10 minutes on ice, centrifuged at 4,000g for 5 minutes, and then the supernatant and pellet were separated and both were counted in a gamma counter. For evaluation of the *ex vivo* plasma stability, 100 µl of the sample was injected on an FPLC column (Superdex® 200 10/300 GL) and eluted at a rate of 0.75 ml/min for 1.5 column volumes. Fractions of 1 ml were collected from the column and then measured in a gamma counter. The plasma stability of sFGFR3_Del4-D3 at 37°C was determined to be 95% at 0 minutes, 95% at 2 hours, and ~92% at 24 hours with only minor aggregation (FIG. 9A).

[0127] The *in vivo* stability of sFGFR3_Del4-D3 in plasma after administration by intravenous and subcutaneous injection was also determined. sFGFR3_Del4-D3 was labeled with ^{125}I-tracer using the Bolton-Hunter method, followed by purification on PD-10 (Sephadex® G-25) columns. The ^{125}I-labeled sFGFR3_Del4-D3 (10 µCi in ~50 µL PBS) was administered by intravenous or subcutaneous injection into anesthetized C57Bl/6 mice. The ^{125}I-tracer protein dose (approximately 0.1 mg/kg) was complemented with unlabeled protein to a total dose of 2.5 mg/kg. Rat serum albumin used as a vascular marker was labeled with [^{3}H]-NSP (N-succininidyl[2,3-^{3}H]Propionate; Perkin Elmer) and purified on PD-10 (Sephadex® G25) columns.

[0128] For the stability of sFGFR3_Del4-D3 in plasma after intravenous bolus injection, FPLC elution

profiles showed no degradation products in plasma up to 15 minutes (FIG. 9B). At 30 minutes after administration of sFGFR3_Del4-D3, a small amount of low molecular weight degradation products appeared, which increased by 2 hours, but largely disappeared by 24 hours. For the stability of sFGFR3_Del4-D3 in plasma after subcutaneous injection, FPLC elution profiles showed some degradation products in plasma at 30 minutes, with increased degradation by 2 hours and 4 hours (FIG. 9C). The low amount of tracer left in plasma after 24 hours appears largely as the intact sFGFR3_Del4-D3 polypeptide. The lower panel chromatograms for FIGS. 9B and 9C are presented as normalized to the highest peak in each individual run for easier comparison of the elution patterns.

Example 16: Ligand Binding Activity of sFGFR3_Del4-D3 and sFGFR3_Del4-C253S

[0129] Experiments were performed to characterize the binding affinity of sFGFR3_Del4-D3 and sFGFR3_Del4-C253S for human FGF2. The dissociation constant (Kd) of sFGFR3_Del4-D3 and Kd of sFGFR3_Del4-C253S for FGF2 were determined as described in Example 3 with a regeneration buffer of 20mM phosphate, 40mM NaCl, pH 7.5. Concentrations of 13 nM, 6.5 nM, 3.25 nM, and 1.75 nM were tested for both sFGFR3_Del4-D3 and sFGFR3_Del4-C253S. The Kd of sFGFR3_Del4-D3 was determined to be ~3.6 nm, and the Kd of sFGFR3_Del4-C253S was determined to be ~6.9 nm. These results indicate that sFGFR3_Del4-D3 and sFGFR3_Del4-C253S have binding activity for FGF2 in the low nM range.

Example 17: sFGFR3_Del4-D3 and sFGFR3_Del4-C253S Exhibit Functional Activity *in vitro*

[0130] Functional activity of sFGFR3_Del4-D3 and sFGFR3_Del4-C253S was tested using a proliferation assay. Proliferation assays using ATDC5 cells genetically modified to overexpress the $FGFR3^{ach}$ mutation (ATDC5 $FGFR3^{G380R}$ cell lines) were performed as described in Example 4 with concentrations of 1 ug/ml, 10 ug/ml, and 50 ug/ml for sFGFR3_Del4-D3 and sFGFR3_Del4-C253S. At each of these concentrations, sFGFR3_Del4-C253S and sFGFR3_Del4-D3 restored proliferation of the $FGFR3^{G380R}$ cells (FIG. 10A and 10B). The EC50 was determined to be about 10 nM for both sFGFR3_Del4-D3 and sFGFR3_Del4-C253S based on a concentration of 1 ug/ml. These results indicate that sFGFR3_Del4-D3 and sFGFR3_Del4-C253S are biologically active in the low nM range.

Example 18: Pharmacokinetic Profile of sFGFR3_Del4-D3 and sFGFR3_Del4-C253S

[0131] The pharmacokinetic (PK) profile of sFGFR3_Del4-D3 administered subcutaneously or intravenously at a dose of 2.5 mg/kg was used to determine the terminal elimination half-life of sFGFR3_Del4-D3 (FIG. 11). Samples were collected at 30 minutes, 2 hours, 4 hours, 8 hours, 24 hours, 36 hours, and 48 hours for mice administered sFGFR3_Del4-D3 subcutaneously. Samples were collected at 1 minute, 15 minutes, 30 minutes, 2 hours, 24 hours, and 36 hours for mice administered sFGFR3_Del4-D3 intravenously. The subcutaneous terminal elimination half-life of 2.5 mg/kg sFGFR3_Del4-D3 was -20 hours, while the intravenous terminal elimination half-life of 2.5 mg/kg sFGFR3_Del4-D3 was ~7 hours. From the PK profile, the T_{max} was ~8 hours, the C_{max} was ~ 4.5 nM, and the estimated bioavailability was ~30% for 2.5 mg/kg sFGFR3_Del4-D3 administered subcutaneously. There was rapid clearance of sFGFR3_Del4-D3 administered intravenously during the α phase followed by a slower β phase clearance, with a similar intravenous PK profile for sFGFR3_Del4-C253S.

Example 19: The Kidney and Liver are the Main Clearance Routes of sFGFR3_Del4-D3

[0132] Clearance of sFGFR3_Del4-D3 was evaluated in kidney, liver, spleen, lung, and heart tissue after 30 minutes, 120 minutes, and 1440 minutes following intravenous administration of 2.5 mg/kg sFGFR3_Del4-D3 and after 30 minutes, 120 minutes, 240 minutes, 480 minutes, and 1440 minutes following subcutaneous administration of 2.5 mg/kg sFGFR3_Del4-D3. The liver and kidney were the major route of sFGFR3_Del4-D3 clearance for intravenous administration (FIG. 12). The kidney was the major route of sFGFR3_Del4-D3 clearance for subcutaneous administration (FIG. 13).

Example 20: sFGFR3_Del4-D3 does not Cross the Blood Brain Barrier

[0133] Pharmacokinetic studies were also performed to determine the uptake of sFGFR3_Del4-D3 across the blood brain barrier in wild-type mice. After intravenous bolus injection, brain tissue uptake of sFGFR3_Del4-D3 was measured at three time points (30 minutes, 2 hours, and 24 hours). sFGFR3_Del4-D3 was injected as radiolabeled tracer (^{125}I- sFGFR3_Del4-D3) with 2.5 mg/kg unlabeled sFGFR3_Del4-D3. The injected dose of ^{125}I- sFGFR3_Del4-D3 was about 10 µCi per animal, which corresponds to less than 0.1 mg/kg. After euthanizing the mice at 30 minutes, 2 hours, and 24 hours, the concentration of ^{125}I- sFGFR3_Del4-D3 in organs and plasma was measured by liquid scintillation counting.

[0134] The ^{125}I- sFGFR3_Del4-D3 concentration was corrected for metabolism in plasma and in brain samples by measuring the fraction of trichloroacetic acid (TCA) precipitable material (e.g., intact tracer). The validity of the TCA correction was also confirmed by injecting samples on a size exclusion fast protein liquid chromatography (FPLC) column. The organ concentration of ^{125}I- sFGFR3_Del4-D3 was corrected for intravascular content (V_0) by injecting radiolabeled albumin (^{3}H-RSA) shortly before sacrificing the animal. The apparent organ volume of distribution of RSA represents V_0. The dose of albumin was negligible (on the order of 1% of the physiological concentration). For all organs other than the brain, the concentrations were calculated by subtracting the vascular content and taking into account the TCA precipitable fraction in plasma. However, no correction was made for the uptake of degraded material into these organs other than the brain because no TCA precipitation was performed.

[0135] The brain concentrations were calculated by the following formula: $C_{brain(corr.)} = [V_d(sFGFR3\_Del4\text{-}D3) - V_0] \times C_{plasma\ (terminal)}$, in which V_d(sFGFR3_Del4-D3) is the volume of distribution of sFGFR3_Del4-D3 in brain (calculated as C_{brain} / C_{plasma}), V_0 is the volume of albumin distributed in the brain, and $C_{plasma(terminal)}$ is the plasma concentration of sFGFR3_Del4-D3 at the terminal sampling time. All concentrations were expressed as the percent of injected dose per gram or ml (%ID/g or %ID/mL), respectively, and the dose of the intravenous bolus equals 100%. These values can be converted to [mg/g] or [mg/mL] by multiplication with the injected dose: (body weight in g /1000 g) × 2.5 mg. All body weights were in the range of 25 g - 30 g.

[0136] There was no detectable brain uptake of ^{125}I- sFGFR3_Del4-D3, as indicated by corrected brain concentrations (after correction for vascular content and degradation (TCA precipitability)) at at any of the measured time points (FIG. 14A). Additionally, the V_d of RSA (=V0) and ^{125}I- sFGFR3_Del4-D3 was not

significantly different at any of the measured time points (30 minutes, 2 hours, and 24 hours) as determined by a paired t-test (FIG. 14B). In conclusion, there is no measurable uptake of sFGFR3_Del4-D3 into brain tissue of mice at 30 minutes, 2 hours, and 24 hours at a dose of 2.5 mg/kg injected as an intravenous bolus.

Example 21: *In Vivo* Efficacy of sFGFR3_Del4-D3 for the Treatment of Achondroplasia

[0137] sFGFR3_Del4-D3 and sFGFR3_Del4-C253S were each evaluated at a subcutaneous dose of 2.5 mg/kg once or twice weekly or 10 mg/kg twice weekly. Breeding was performed to generate 30 litters with half wild type and half heterozygous *Fgfr3*$^{ach/+}$ mice (Table 4).

Table 4. Subcutaneous administration of sFGFR3_Del4-D3 and sFGFR3_Del4-C253S to wild type (WT) and *Fgfr3*$^{ach/+}$ mice.

		PBS (pooled)	2.5mg 1× week	2.5mg 2× week	10mg 2× week
sFGFR3_Del4-D3					
	WT	65	26	22	23
	Fgfr3$^{ach/+}$	43	26	25	30
					total N= 260
sFGFR3_Del4-C253S					
	WT	65	26	22	23
	Fgfr3$^{ach/+}$	27	22	18	28
					total N= 231
	% survival	62.8	84.6	72.0	93.3
	% mortality	37.2	15.4	28.0	6.7

[0138] At day 3, all newborn mice from a single litter received the same dose. Control litters received 10 µl of PBS (vehicle). Thereafter, subcutaneous injections of sFGFR3_Del4-D3 and sFGFR3_Del4-C253S were administered at doses of 2.5 mg/kg once or twice weekly or 10 mg/kg twice a week for three weeks, alternatively on the left and right sides of the back. Mice were observed daily with particular attention to locomotion and urination alterations and weighed on days of injection. Mice with complications were observed twice a day for surveillance. Previous data indicated there was no statistical difference between males and females, and thus, males and females were considered one group for all analyses.

[0139] At day 22, all animals were sacrificed by lethal injection of pentobarbital, and gender was determined. All subsequent measurements and analyses were performed without knowledge of mice genotype to avoid investigator bias. Genotyping was performed at the end of the study to reveal the correspondence of data with a specific genotype. Since achondroplasia is a disease with phenotypic variability, all animals were included in the study. Animals dead before day 22 were used to investigate the impact of treatment on premature death. Surviving animals at day 22 were used for all analyses. All experiments and data measurements were performed by blinded experimenters at all time points.

[0140] Subcutaneous administration of sFGFR3_Del4-D3 at 2.5 mg/kg once or twice weekly or 10 mg/kg

twice weekly increased survival of *Fgfr3*$^{ach/+}$ mice relative to *Fgfr3*$^{ach/+}$ mice receiving PBS (FIG. 15 and Table 4). In particular, administration of 10 mg/kg sFGFR3_Del4-D3 twice weekly resulted in 93% survival of *Fgfr3*$^{ach/+}$ mice, administration of 2.5 mg/kg sFGFR3_Del4-D3 once weekly resulted in 84% survival in *Fgfr3*$^{ach/+}$ mice, and administration of 2.5 mg/kg sFGFR3_Del4-D3 twice weekly resulted in 72% survival in *Fgfr3*$^{ach/+}$ mice, while the survival of *Fgfr3*$^{ach/+}$ mice receiving PBS was 62.8%. The mortality of *Fgfr3*$^{ach/+}$ mice administered 10 mg/kg sFGFR3_Del4-D3 twice weekly was 6.7%, the mortality of *Fgfr3*$^{ach/+}$ mice administered 2.5 mg/kg sFGFR3_Del4-D3 once weekly was 15.4%, the mortality of *Fgfr3*$^{ach/+}$ mice administered 2.5 mg/kg sFGFR3_Del4-D3 twice weekly was 28.0%, and the mortality of *Fgfr3*$^{ach/+}$ mice administered PBS was 37.2%. Statistical analysis of *Fgfr3*$^{ach/+}$ mice survival following treatment with sFGFR3_Del4-D3 was performed using the Agostino and Pearson omnibus normality test following by a t-test. All investigated groups passed the normality tests. The P-values from these analyses are shown below, in which * represent a P-value of <0.05 and *** represents a P-value of <0.001 (Table 5).

Table 5. P-values for subcutaneous administration of sFGFR3_Del4-D3 to wild type (WT) and *Fgfr3*$^{ach/+}$ mice.

Group Comparison	P Value
Wt vs ach	***
Fgfr3$^{ach/+}$ PBS vs *Fgfr3*$^{ach/+}$ 2.5 mg/kg, 1x	***
Fgfr3$^{ach/+}$ PBS vs *Fgfr3*$^{ach/+}$ 2.5 mg/kg, 2x	*
Fgfr3$^{ach/+}$ PBS vs *Fgfr3*$^{ach/+}$ 10 mg/kg, 2x	***
Wt PBS vs *Fgfr3*$^{ach/+}$ 10 mg/kg, 2x	ns

[0141] Subcutaneous administration of sFGFR3_Del4-D3 at 2.5 mg/kg once or twice weekly or 10 mg/kg twice weekly also decreased the severity and frequency of locomotor problems and complications in abdominal breathing in *Fgfr3*$^{ach/+}$ mice relative to *Fgfr3*$^{ach/+}$ mice receiving PBS (FIG. 16). In particular, locomotor problems decreased the most in *Fgfr3*$^{ach/+}$ mice administered subcutaneously 10 mg/kg sFGFR3_Del4-D3 twice weekly followed by mice administered sFGFR3_Del4-D3 2.5 mg/kg twice weekly and mice administered sFGFR3_Del4-D3 2.5 mg/kg once weekly. Complications in abdominal breathing decreased the most in *Fgfr3*$^{ach/+}$ mice administered subcutaneously 10 mg/kg sFGFR3_Del4-D3 twice weekly followed by mice administered sFGFR3_Del4-D3 2.5 mg/kg once weekly and then mice administered sFGFR3_Del4-D3 2.5 mg/kg twice weekly. These results show that sFGFR3_Del4-D3 reduces symptoms of achondroplasia in *Fgfr3*$^{ach/+}$ mice.

[0142] Subcutaneous administration of sFGFR3_Del4-D3 also significantly increased total body length, including axial length and tail length, and long bones ($p = 0.07$) in *Fgfr3*$^{ach/+}$ mice receiving 2.5 mg/kg sFGFR3_Del4-D3 once or twice weekly or 10 mg/kg sFGFR3_Del4-D3 twice weekly relative to *Fgfr3*$^{ach/+}$ mice receiving PBS (FIGS. 17A-17C). Tail and body length (axial length) were measured using the same digital caliper on whole skeletons. Tibia length was measured on digital X-rays. Administration of 10 mg/kg sFGFR3_Del4-D3 twice weekly resulted in 51% axial correction (body and tail length) of *Fgfr3*$^{ach/+}$ mice, followed by 43% axial correction in *Fgfr3*$^{ach/+}$ receiving 2.5 mg/kg sFGFR3_Del4-D3 twice weekly, and 39% axial correction in *Fgfr3*$^{ach/+}$ mice receiving 2.5 mg/kg sFGFR3_Del4-D3 once weekly. Increases in

bone and body length were also evident from x-ray radiographs of *Fgfr3*$^{ach/+}$ mice administered 2.5 mg/kg or 10 mg/kg sFGFR3_Del4-D3 twice weekly relative to *Fgfr3*$^{ach/+}$ mice receiving PBS (FIG. 17D). Administration of 10 mg/kg sFGFR3_Del4-D3 twice weekly resulted in 86% appendicular correction (tibia and femur length) of *Fgfr3*$^{ach/+}$ mice, followed by 68% appendicular correction in *Fgfr3*$^{ach/+}$ receiving 2.5 mg/kg sFGFR3_Del4-D3 twice weekly and 54% appendicular correction in *Fgfr3*$^{ach/+}$ mice receiving 2.5 mg/kg sFGFR3_Del4-D3 once weekly.

[0143] Subcutaneous administration of sFGFR3_Del4-D3 also resulted in a dose-dependent improvement in cranial ratio (length/width (L/W)) in *Fgfr3*$^{ach/+}$ mice relative to *Fgfr3*$^{ach/+}$ mice receiving PBS (FIG. 18A). *Fgfr3*$^{ach/+}$ mice subcutaneously administered 10 mg/kg sFGFR3_Del4-D3 twice weekly exhibited the greatest improvement in the cranium ratio (L/W), followed by *Fgfr3*$^{ach/+}$ mice administered 2 mg/kg sFGFR3_Del4-D3 twice weekly and *Fgfr3*$^{ach/+}$ mice administered 2 mg/kg sFGFR3_Del4-D3 once weekly. In particular, administration of 10 mg/kg sFGFR3_Del4-D3 twice weekly resulted in 37% skull shape correction (L/W ratio) of *Fgfr3*$^{ach/+}$ mice, followed by 29% skull shape correction in *Fgfr3*$^{ach/+}$ receiving 2.5 mg/kg sFGFR3_Del4-D3 twice weekly and 19% skull shape correction in *Fgfr3*$^{ach/+}$ mice receiving 2.5 mg/kg sFGFR3_Del4-D3 once weekly. Improvements in the cranial ratio were also evident from x-ray radiographs of *Fgfr3*$^{ach/+}$ mice administered 10 mg/kg sFGFR3_Del4-D3 relative to *Fgfr3*$^{ach/+}$ mice receiving PBS (FIG. 18B). Bone measurements (presented in mm and mean ± SEM) for body length, tail, femur, tibia, and cranial ratio are shown below (Table 6). These results indicate the dose-dependent *in vivo* efficacy of sFGFR3_Del4-D3 as demonstrated by increased survival, reduced number of complications, increased bone growth, and improvements in skeletal proportions of *Fgfr3*$^{ach/+}$ mice.

Table 6. Bone measurements (presented in mm and mean ± SEM) for body length, tail, femur, tibia, and cranial ratio of WT and *Fgfr3*$^{ach/+}$ mice administered subcutaneously sFGFR3_Del4-D3.

	Efficacy of sFGFR3_Del4-D3				
	WT	PBS in ***Fgfr3*$^{ach/+}$ mice**	2.5 mg/kg once weekly	2.5 mg/kg twice weekly	10 mg/kg twice weekly
Body length	144.8 ± 0.53	129.2 ± 1.98	135 ± 1.48	135.5 ± 1.75	135.2 ± 1.58
Tail	77.65 ± 0.39	70.25 ± 1.1	73.37 ± 1.66	73.69 ± 1.5	74.95 ± 0.91
Femur	10.94 ± 0.05	10.14 ± 0.13	10.47 ± 0.08	10.58 ± 0.09	10.63 ± 0.10
Tibia	14.19 ± 0.05	13.67 ± 0.14	14.02 ± 0.10	14.09 ± 0.12	14.25 ± 0.12
Cranial ratio	1.99 ± 0.01	1.79 ± 0.01	1.83 ± 0.02	1.85 ± 0.01	1.86 ± 0.02

[0144] Additionally, comparison of the bone measurements for *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del1 at a dosage of 2.5 mg/kg twice weekly show that administration sFGFR3_Del4-D3 at a dosage of 2.5 mg/kg twice weekly was comparable to or more effective in increasing the bone, tail, femur, and tibia length and improving the cranial ratio of *Fgfr3*$^{ach/+}$ mice (Table 7). In particular, the body length of *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del4-D3 improved to 135.5 ± 1.75 mm relative to 134.4 ± 1.17

mm for *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del1; the tail length of *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del4-D3 improved to 73.69 ± 1.5 mm relative to 71.58 ± 0.86 mm for *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del1; the femur length of *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del4-D3 improved to 10.58 ± 0.09 mm relative to 10.01 ± 0.06 mm for *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del1; the tibia length of *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del4-D3 improved to 14.09 ± 0.12 mm relative to 13.27 ± 0.31 mm for *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del1; and the cranial ratio of *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del4-D3 improved to 1.85 ± 0.01 mm relative to 1.81 ± 0.02 mm for *Fgfr3*$^{ach/+}$ mice administered sFGFR3_Del1.

Table 7. Bone measurements (presented in mm and mean ± SEM) for body length, tail, femur, tibia, and cranial ratio of WT and *Fgfr3*$^{ach/+}$ mice administered subcutaneously sFGFR3_Del1 (data described in Garcia et al. *Sci. Transl. Med.* 5:203ra124, 2013).

Efficacy of sFGFR3_Del1				
	WT	PBS in ***Fgfr3***$^{ach/+}$ **mice**	0.25 mg/kg twice weekly	2.5 mg/kg twice weekly
body length	133.9 ± 0.8	118.5 ± 1.76	132.4 ± 1.26	134.4 ± 1.17
tail	71.9 ± 0.49	64.48 ± 1.1	71.05 ± 0.99	71.58 ± 0.86
femur	10.05 ± 0.17	9.67 ± 0.16	9.85 ± 0.10	10.01 ± 0.06
tibia	13.43 ± 0.19	12.62 ± 0.18	12.87 ± 0.14	13.27 ± 0.31
cranial ratio	1.94 ± 0.01	1.75 ± 0.01	1.77 ± 0.02	1.81 ± 0.02

Example 22: No Organ Toxicity Associated with Administration of sFGFR3_Del4-D3

[0145] Histopathological studies were performed to characterize organ toxicity associated with sFGFR3_Del4-D3 administration. Wild type mice (6 males and 6 females per dose) were administered PBS, 2.5 mg/kg sFGFR3_Del4-D3 once weekly, 2.5 mg/kg sFGFR3_Del4-D3 twice weekly, or 10 mg/kg sFGFR3_Del4-D3 twice weekly. Organs investigated included the kidney, skin, salivary glands, mandibular lymph nodes, gall bladder, spleen, pancreas, lungs, heart, aorta, jejunum, colon, and liver. There were no histopathological results indicating organ toxicity in wild-type mice administered any of the doses of sFGFR3_Del4-D3. These results indicate that there was no toxicity associated with administration of sFGFR3_Del4-D3 up 10 mg/kg twice weekly.

Example 23: Determination of Binding Affinity of sFGFR3_Del4-D3 to Fibroblast Growth Factors

[0146] We determined that sFGFR3_Del4-D3 binds to Fibroblast Growth Factors (FGF) ligands and acts as a decoy to prevent the binding of FGFs to the membrane bound FGFR3. Surface Plasmon Resonance was performed using a BIACORE™ T200 (GE Healthcare) to determine the K_d values for different human FGFs (hFGFs) binding to immobilized sFGFR3_Del4-D3. In particular, K_d values for the paracrine hFGFs of hFGF1 (FIG. 19A), hFGF2 (FIG. 19B), hFGF9 (FIG. 19C), and hFGF18 (FIG. 19D) and the endocrine hFGFs of hFGF19 (FIG. 19E) and hFGF21 (FIG. 19F) were determined. All four paracrine FGF ligands bound sFGFR3_Del4-D3 with nanomolar (nM) affinity (Table 8).

Table 8. Summary of K_d determination and values for human, paracrine FGFs (hFGF1, hFGF2,

hFGF9, and hFGF18) and human, endocrine FGFs (hFGF19 and hFGF21).

Paracrine FGFs	Binding	k_{a1} (1/Ms)	k_{a2} (1/Ms)	k_{d1} (1/s)	k_{d2} (1/s)	K_D (M) Kinetic	Chi^2 (RU^2) average	K_D (M) Steady state	Chi^2 (RU^2) average
FGF1	2:1 binding & stead y state	2.0* 10^{+11}	1.2* 10^{-3}	1610	6.4 * 10^{-4}	**2.6* 10^{-9}** (+/-1.9*10^{-9}, n = 3)	0.138	**5.7* 10^{-9}** (+/-2.1*10^{-9}, n=3)	0.247
FGF2	1:1 binding	9.0* 10^{+5}		4.75* 10^{-4}		**6.1* 10^{-10}** (+/-1.7*10^{-10}, n = 3)	13.6		
FGF9	2:1 binding & stead y state	2.3* 10^{+6}	3.0* 10^{-2}	2.6* 10^{-2}	3.6* 10^{-3}	**1.8* 10^{-9}** (+/-0.25*10^{-9}, n = 3)	0.14	**3.6* 10^{-9}** (n = 1)	0.25
FGF18	1:1 binding & stead y state	2.0* 10^{+5}		9.1* 10^{-3}		**4.5* 10^{-9}** (+/-2.5*10^{-9}, n = 3)	9.7	**6.4*10^{-9}** (+/-0.89*10^{-9}, n=4)	11.8
Endocrine FGFs									
FGF19	2:1 binding	5.4* 10^{+4}	7.3* 10^{-3}	1.5* 10^{-1}	3.6* 10^{-3}	**4.8* 10^{-7}** (+/-3.2*10^{-7}, n = 3)	0.05		
FGF21	2:1 binding	258	1.8* 10^{-2}	5.5* 10^{-3}	1.4* 10^{-3}	**2.8* 10^{-5}** (n = 2)	0.56		

[0147] For FGF2 and FGF18, a good fit was achieved with a 1:1 binding model, which is the most direct model of binding affinity. This model describes a 1:1 binding interaction at the surface of the chip with immobilized SFGFR3_DEL4-D3 binding different FGFs: A + B = AB with single on- and off rate. The 2:1 model also describes a 1:1 interaction of FGF binding to SFGFR3_DEL4-D3, but also assumes a conformational change that stabilizes the complex: A + B = AB = AB* and represents two on- and off-rates. This model assumes that the conformationally changed complex (SFGFR3_DEL4-D3 bound to FGF) can only dissociate by reversing the conformational change. The experimental data for hFGF1, hFGF9, hFGF19, and hFGF21 were determined to fit the 2:1 model very well, and thus, K_d for hFGF1, hFGF9, hFGF19, and hFGF21 were derived from the 2:1 model.

[0148] Despite hFGF1, hFGF9, hFGF19, and hFGF21 all having a K_d in the low nM range, the kinetic profiles of these hFGFs differed significantly. For example, FGF1 binds sFGFR3_Del4-D3 with a very fast on-rate and off-rate, while FGF2 does not bind sFGFR3_Del4-D3 with as fast of an on-rate or off-rate as FGF1, resulting in an overall smaller K_d for FGF2 compared to FGF1 (Table 8). A significantly lower affinity was measured between sFGFR3_Del4-D3 and hFGF19 or hFGF21, which are members of the endocrine FGF15/FGF19 subfamily, relative to the paracrine hFGFs (Table 8 and FIGS. 19D and 19E). The FGF15/FGF19 subfamily uses Klotho instead of proteoglycans as a co-factor and has evolved into

endocrine-acting growth factors, which are important for the systemic regulation of metabolic parameters, such as phosphate, bile acid, carbohydrate, and lipid metabolism.

[0149] These results demonstrate that there was a high affinity interaction of sFGFR3_Del4-D3 with hFGF1, hFGF2, hFGF9, and hFGF18, while there was a low affinity interaction of sFGFR3_Del4-D3 with FGF19 and FGF21. The low affinity of sFGFR3_Del4-D3 for FGF19 and FGF21 is advantageous as sFGFR3_Del4-D3 will have a low probability of interfering with the function of these FGFs *in vivo.*

Example 24: *In Vitro* Proliferation Assay of sFGFR3_Del4-D3

[0150] Following binding of FGFs, FGFR3 dimerizes to initiate signaling cascades. Several downstream signaling pathways are associated with FGF signaling. In chondrocytes, dimerized FGFR3 results in an anti-proliferative signal/early differentiation signal into the chondrocyte, which eventually leads to inhibition of bone growth. For example, the RAS/MAPK pathway propagates signals to negatively affect proliferation, terminal differentiation, and post-mitotic matrix synthesis, and the STAT1 pathway mediates the inhibition of chondrocyte proliferation in concert with the cell cycle regulators p107 and 130 and cell cycle inhibitor p21Waf/Cip1. Gene expression studies suggest a number of other pathways are also involved in down-regulation of growth-promoting molecules or induction of anti-proliferative functions.

[0151] To study FGFR3-decoy induced inhibition of $FGFR3^{G380R}$ in a chondrocytic cell model, studies were performed to determine the effect of sFGFR3_Del4-D3 on the proliferation of ATDC5 cells genetically modified to overexpress the $FGFR3^{ach}$ mutation (ATDC5 $FGFR3^{G380R}$ cells). The chondrocytic cell line ATDC5 cell, which was first isolated from the differentiating teratocarcinoma stem cell line AT805, is commonly used as a model for *in vitro* chondrocyte research. ATDC5 cells were first infected with a retroviral expression vector and a stable cell line expressing $FGFR3^{G380R}$ was generated. The expression of $FGFR3^{G380R}$ in the ATDC5 cell line was determined via Western blot (FIG. 20). Extracts of ATDC5 cells expressing $FGFR3^{G380R}$ at passage one (G380R #1) and two (G380R #2) after resistant cell selection and extracts of control ATDC5 cells were blotted and detected with antibodies for total phosphorylation of FGFR3 (pFGFR3), the specific phosphotyrosine 724 in FGFR3 (pFGFR3 Y724), and total FGFR3 expression (FGFR3). Total extracellular signal-related kinase expression was used as loading control (ERK). Addition of SFGFR3_DEL4-D3 to the ATDC5 $FGFR3^{G380R}$ cells dose-dependently increased the proliferation index of the ATDC5 $FGFR3^{G380R}$ cells by two-fold with an EC_{50} of 1.25 +/- 0.27 nM (FIG. 21). These results demonstrate that addition of SFGFR3_DEL4-D3 to ATDC5 $FGFR3^{G380R}$ cells overcomes the negative growth signal mediated by $FGFR3^{G380R}$ in a cellular model of achondroplasia and are in line with the anti-proliferative signal mediated by FGFR3 in chondrocytes, which is more pronounced when the chondrocytes express a FGFR3 including the G380R mutation.

SEQUENCE LISTING

[0152]

<110> THERACHON INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE UNIVERSITE NICE SOPHIA ANTIPOLIS

<120> SOLUBLE FIBROBLAST GROWTH FACTOR RECEPTOR 3 (SFGFR3) POLYPEPTIDES AND

USES THEREOF

<130> 901-10 PCT

<150> 62/467,478
<151> 2017-03-06

<150> 62/359,607
<151> 2016-07-07

<160> 39

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Asp Ala Val Glu Leu Ser Cys Pro Pro Pro Gly Gly Gly Pro Met Gly
        35                  40                  45

Pro Thr Val Trp Val Lys Asp Gly Thr Gly Leu Val Pro Ser Glu Arg
    50                  55                  60

Val Leu Val Gly Pro Gln Arg Leu Gln Val Leu Asn Ala Ser His Glu
65                  70                  75                  80

Asp Ser Gly Ala Tyr Ser Cys Arg Gln Arg Leu Thr Gln Arg Val Leu
                85                  90                  95

Cys His Phe Ser Val Arg Val Thr Asp Ala Pro Ser Ser Gly Asp Asp
            100                 105                 110

Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr Gly Val Asp Thr Gly Ala
        115                 120                 125

Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp Lys Lys Leu Leu Ala Val
    130                 135                 140

Pro Ala Ala Asn Thr Val Arg Phe Arg Cys Pro Ala Ala Gly Asn Pro
145                 150                 155                 160

Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly Arg Glu Phe Arg Gly Glu
                165                 170                 175

His Arg Ile Gly Gly Ile Lys Leu Arg His Gln Gln Trp Ser Leu Val
            180                 185                 190

Met Glu Ser Val Val Pro Ser Asp Arg Gly Asn Tyr Thr Cys Val Val
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        195                 200                 205

Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr Tyr Thr Leu Asp Val Leu
    210                 215                 220

Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
225                 230                 235                 240

Gln Thr Ala Val Leu Gly Ser Asp Val Glu Phe His Cys Lys Val Tyr
                245                 250                 255

Ser Asp Ala Gln Pro His Ile Gln Trp Leu Lys His Val Glu Val Asn
            260                 265                 270

Gly Ser Lys Val Gly Pro Asp Gly Thr Pro Tyr Val Thr Val Leu Lys
        275                 280                 285

Val Ser Leu Glu Ser Asn Ala Ser Met Ser Ser Asn Thr
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            20                  25                  30

Asp Ala Val Glu Leu Ser Cys Pro Pro Pro Gly Gly Gly Pro Met Gly

        35                  40                  45

Pro Thr Val Trp Val Lys Asp Gly Thr Gly Leu Val Pro Ser Glu Arg
    50                  55                  60

Val Leu Val Gly Pro Gln Arg Leu Gln Val Leu Asn Ala Ser His Glu
65                  70                  75                  80

Asp Ser Gly Ala Tyr Ser Cys Arg Gln Arg Leu Thr Gln Arg Val Leu
                85                  90                  95

Cys His Phe Ser Val Arg Val Thr Asp Ala Pro Ser Ser Gly Asp Asp
            100                 105                 110

Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr Gly Val Asp Thr Gly Ala
        115                 120                 125

Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp Lys Lys Leu Leu Ala Val
    130                 135                 140

Pro Ala Ala Asn Thr Val Arg Phe Arg Cys Pro Ala Ala Gly Asn Pro
145                 150                 155                 160

Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly Arg Glu Phe Arg Gly Glu
```

```
                165                 170                 175

His Arg Ile Gly Gly Ile Lys Leu Arg His Gln Gln Trp Ser Leu Val
            180                 185                 190

Met Glu Ser Val Val Pro Ser Asp Arg Gly Asn Tyr Thr Cys Val Val
        195                 200                 205

Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr Tyr Thr Leu Asp Val Leu
    210                 215                 220

Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
225                 230                 235                 240

Gln Thr Ala Val Leu Gly Ser Asp Val Glu Phe His Ser Lys Val Tyr
                245                 250                 255

Ser Asp Ala Gln Pro His Ile Gln Trp Leu Lys His Val Glu Val Asn
            260                 265                 270

Gly Ser Lys Val Gly Pro Asp Gly Thr Pro Tyr Val Thr Val Leu Lys
        275                 280                 285

Val Ser Leu Glu Ser Asn Ala Ser Met Ser Ser Asn Thr
    290                 295                 300
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            20                  25                  30

Ser Ile Gly Phe Ser His His Ser Ala Trp Leu Val Val Leu Pro
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            20                  25                  30

Asp Ala Val Glu Leu Ser Cys Pro Pro Pro Gly Gly Gly Pro Met Gly
        35                  40                  45
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Pro Thr Val Trp Val Lys Asp Gly Thr Gly Leu Val Pro Ser Glu Arg
    50                  55                  60

Val Leu Val Gly Pro Gln Arg Leu Gln Val Leu Asn Ala Ser His Glu
65                  70                  75                  80

Asp Ser Gly Ala Tyr Ser Cys Arg Gln Arg Leu Thr Gln Arg Val Leu
                85                  90                  95

Cys His Phe Ser Val Arg Val Thr Asp Ala Pro Ser Ser Gly Asp Asp
            100                 105                 110

Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr Gly Val Asp Thr Gly Ala
        115                 120                 125

Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp Lys Lys Leu Leu Ala Val
    130                 135                 140

Pro Ala Ala Asn Thr Val Arg Phe Arg Cys Pro Ala Ala Gly Asn Pro
145                 150                 155                 160

Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly Arg Glu Phe Arg Gly Glu
                165                 170                 175

His Arg Ile Gly Gly Ile Lys Leu Arg His Gln Gln Trp Ser Leu Val
            180                 185                 190

Met Glu Ser Val Val Pro Ser Asp Arg Gly Asn Tyr Thr Cys Val Val
        195                 200                 205

Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr Tyr Thr Leu Asp Val Leu
    210                 215                 220

Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
225                 230                 235                 240

Gln Thr Ala Val Leu Gly Ser Asp Val Glu Phe His Cys Lys Val Tyr
                245                 250                 255

Ser Asp Ala Gln Pro His Ile Gln Trp Leu Lys His Val Glu Val Asn
            260                 265                 270

Gly Ser Lys Val Gly Pro Asp Gly Thr Pro Tyr Val Thr Val Leu Lys
        275                 280                 285

Thr Ala Gly Ala Asn Thr Thr Asp Lys Glu Leu Glu Val Leu Ser Leu
    290                 295                 300

Asn Val Thr Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn
305                 310                 315                 320

Ser Ile Gly Phe Ser His His Ser Ala Trp Leu Val Val Leu Pro Val
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            20                  25                  30

Asp Ala Val Glu Leu Ser Cys Pro Pro Pro Gly Gly Gly Pro Met Gly
        35                  40                  45

Pro Thr Val Trp Val Lys Asp Gly Thr Gly Leu Val Pro Ser Glu Arg
    50                  55                  60

Val Leu Val Gly Pro Gln Arg Leu Gln Val Leu Asn Ala Ser His Glu
65                  70                  75                  80

Asp Ser Gly Ala Tyr Ser Cys Arg Gln Arg Leu Thr Gln Arg Val Leu
                85                  90                  95

Cys His Phe Ser Val Arg Val Thr Asp Ala Pro Ser Ser Gly Asp Asp
            100                 105                 110

Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr Gly Val Asp Thr Gly Ala
        115                 120                 125

Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp Lys Lys Leu Leu Ala Val
    130                 135                 140

Pro Ala Ala Asn Thr Val Arg Phe Arg Cys Pro Ala Ala Gly Asn Pro
145                 150                 155                 160

Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly Arg Glu Phe Arg Gly Glu
                165                 170                 175

His Arg Ile Gly Gly Ile Lys Leu Arg His Gln Gln Trp Ser Leu Val
            180                 185                 190

Met Glu Ser Val Val Pro Ser Asp Arg Gly Asn Tyr Thr Cys Val Val
        195                 200                 205

Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr Tyr Thr Leu Asp Val Leu
    210                 215                 220

Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
225                 230                 235                 240

Gln Thr Ala Val Leu Gly Ser Asp Val Glu Phe His Cys Lys Val Tyr
                245                 250                 255

Ser Asp Ala Gln Pro His Ile Gln Trp Leu Lys His Val Glu Val Asn
            260                 265                 270

Gly Ser Lys Val Gly Pro Asp Gly Thr Pro Tyr Val Thr Val Leu Lys
        275                 280                 285

Thr Ala Gly Ala Asn Thr Thr Asp Lys Glu Leu Glu Val Leu Ser Leu
    290                 295                 300

His Asn Val Thr Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly
305                 310                 315                 320
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            340                 345                 350

Gly Ile Leu Ser Tyr Gly Val Gly Phe Phe Leu Phe Ile Leu Val Val
        355                 360                 365

Ala Ala Val Thr Leu Cys Arg Leu Arg Ser Pro Pro Lys Lys Gly Leu
    370                 375                 380

Gly Ser Pro Thr Val His Lys Ile Ser Arg Phe Pro Leu Lys Arg Gln
385                 390                 395                 400

Val Ser Leu Glu Ser Asn Ala Ser Met Ser Ser Asn Thr Pro Leu Val
                405                 410                 415

Arg Ile Ala Arg Leu Ser Ser Gly Glu Gly Pro Thr Leu Ala Asn Val
            420                 425                 430

Ser Glu Leu Glu Leu Pro Ala Asp Pro Lys Trp Glu Leu Ser Arg Ala
        435                 440                 445

Arg Leu Thr Leu Gly Lys Pro Leu Gly Glu Gly Cys Phe Gly Gln Val
    450                 455                 460

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465                 470                 475                 480

Val Thr Val Ala Val Lys Met Leu Lys Asp Asp Ala Thr Asp Lys Asp

                485                 490                 495

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            500                 505                 510

His Lys Asn Ile Ile Asn Leu Leu Gly Ala Cys Thr Gln Gly Gly Pro
        515                 520                 525

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545                 550                 555                 560

Lys Pro Pro Glu Glu Gln Leu Thr Phe Lys Asp Leu Val Ser Cys Ala
                565                 570                 575

Tyr Gln Val Ala Arg Gly Met Glu Tyr Leu Ala Ser Gln Lys Cys Ile
            580                 585                 590

His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Thr Glu Asp Asn Val
        595                 600                 605

Met Lys Ile Ala Asp Phe Gly Leu Ala Arg Asp Val His Asn Leu Asp
    610                 615                 620

Tyr Tyr Lys Lys Thr Thr Asn Gly Arg Leu Pro Val Lys Trp Met Ala
625                 630                 635                 640

Pro Glu Ala Leu Phe Asp Arg Val Tyr Thr His Gln Ser Asp Val Trp
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            660                 665                 670

Tyr Pro Gly Ile Pro Val Glu Glu Leu Phe Lys Leu Leu Lys Glu Gly
        675                 680                 685

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    690                 695                 700

Met Arg Glu Cys Trp His Ala Ala Pro Ser Gln Arg Pro Thr Phe Lys
705                 710                 715                 720

Gln Leu Val Glu Asp Leu Asp Arg Val Leu Thr Val Thr Ser Thr Asp
                725                 730                 735

Glu Tyr Leu Asp Leu Ser Ala Pro Phe Glu Gln Tyr Ser Pro Gly Gly
            740                 745                 750

Gln Asp Thr Pro Ser Ser Ser Ser Ser Gly Asp Asp Ser Val Phe Ala
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            20                  25                  30

Asp Ala Val Glu Leu Ser Cys Pro Pro Pro Gly Gly Gly Pro Met Gly
        35                  40                  45

Pro Thr Val Trp Val Lys Asp Gly Thr Gly Leu Val Pro Ser Glu Arg
    50                  55                  60

Val Leu Val Gly Pro Gln Arg Leu Gln Val Leu Asn Ala Ser His Glu
65                  70                  75                  80
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                85                  90                  95

Cys His Phe Ser Val Arg Val Thr Asp Ala Pro Ser Ser Gly Asp Asp
            100                 105                 110

Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr Gly Val Asp Thr Gly Ala
        115                 120                 125

Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp Lys Lys Leu Leu Ala Val
    130                 135                 140

Pro Ala Ala Asn Thr Val Arg Phe Arg Cys Pro Ala Ala Gly Asn Pro
145                 150                 155                 160

Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly Arg Glu Phe Arg Gly Glu
                165                 170                 175

His Arg Ile Gly Gly Ile Lys Leu Arg His Gln Gln Trp Ser Leu Val
            180                 185                 190

Met Glu Ser Val Val Pro Ser Asp Arg Gly Asn Tyr Thr Cys Val Val
        195                 200                 205

Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr Tyr Thr Leu Asp Val Leu
    210                 215                 220

Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
225                 230                 235                 240

Gln Thr Ala Val Leu Gly Ser Asp Val Glu Phe His Cys Lys Val Tyr
                245                 250                 255

Ser Asp Ala Gln Pro His Ile Gln Trp Leu Lys His Val Glu Val Asn
            260                 265                 270

Gly Ser Lys Val Gly Pro Asp Gly Thr Pro Tyr Val Thr Val Leu Lys
        275                 280                 285

Val Ser Leu Glu Ser Asn Ala Ser Met Ser Ser Asn Thr Pro Leu Val
    290                 295                 300

Arg Ile Ala Arg Leu Ser Ser Gly Glu Gly Pro Thr Leu Ala Asn Val
305                 310                 315                 320

Ser Glu Leu Glu Leu Pro Ala Asp Pro Lys Trp Glu Leu Ser Arg Ala
                325                 330                 335

Arg Leu Thr Leu Gly Lys Pro Leu Gly Glu Gly Cys Phe Gly Gln Val
            340                 345                 350

Val Met Ala Glu Ala Ile Gly Ile Asp Lys Asp Arg Ala Ala Lys Pro

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Val Thr Val Ala Val Lys Met Leu Lys Asp Asp Ala Thr Asp Lys Asp
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Leu Ser Asp Leu Val Ser Glu Met Glu Met Met Lys Met Ile Gly Lys
385                 390                 395                 400
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Leu Arg Ala Arg Arg Pro Pro Gly Leu Asp Tyr Ser Phe Asp Thr Cys
        435                 440                 445

Lys Pro Pro Glu Glu Gln Leu Thr Phe Lys Asp Leu Val Ser Cys Ala
    450                 455                 460

Tyr Gln Val Ala Arg Gly Met Glu Tyr Leu Ala Ser Gln Lys Cys Ile
465                 470                 475                 480

His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Thr Glu Asp Asn Val
                485                 490                 495

Met Lys Ile Ala Asp Phe Gly Leu Ala Arg Asp Val His Asn Leu Asp
            500                 505                 510

Tyr Tyr Lys Lys Thr Thr Asn Gly Arg Leu Pro Val Lys Trp Met Ala
        515                 520                 525

Pro Glu Ala Leu Phe Asp Arg Val Tyr Thr His Gln Ser Asp Val Trp
    530                 535                 540

Ser Phe Gly Val Leu Leu Trp Glu Ile Phe Thr Leu Gly Gly Ser Pro
545                 550                 555                 560

Tyr Pro Gly Ile Pro Val Glu Glu Leu Phe Lys Leu Leu Lys Glu Gly
                565                 570                 575

His Arg Met Asp Lys Pro Ala Asn Cys Thr His Asp Leu Tyr Met Ile
            580                 585                 590

Met Arg Glu Cys Trp His Ala Ala Pro Ser Gln Arg Pro Thr Phe Lys
        595                 600                 605

Gln Leu Val Glu Asp Leu Asp Arg Val Leu Thr Val Thr Ser Thr Asp
    610                 615                 620

Glu Tyr Leu Asp Leu Ser Ala Pro Phe Glu Gln Tyr Ser Pro Gly Gly
625                 630                 635                 640

Gln Asp Thr Pro Ser Ser Ser Ser Ser Gly Asp Asp Ser Val Phe Ala
                645                 650                 655

His Asp Leu Leu Pro Pro Ala Pro Pro Ser Ser Gly Gly Ser Arg Thr
            660                 665                 670
```

<210> 8

<211> 672

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 8

```
Glu Ser Leu Gly Thr Glu Gln Arg Val Val Gly Arg Ala Ala Glu Val
1               5                   10                  15
```

```
Pro Gly Pro Glu Pro Gly Gln Gln Glu Gln Leu Val Phe Gly Ser Gly
            20                  25                  30

Asp Ala Val Glu Leu Ser Cys Pro Pro Pro Gly Gly Gly Pro Met Gly
        35                  40                  45

Pro Thr Val Trp Val Lys Asp Gly Thr Gly Leu Val Pro Ser Glu Arg
    50                  55                  60

Val Leu Val Gly Pro Gln Arg Leu Gln Val Leu Asn Ala Ser His Glu
65                  70                  75                  80

Asp Ser Gly Ala Tyr Ser Cys Arg Gln Arg Leu Thr Gln Arg Val Leu
                85                  90                  95

Cys His Phe Ser Val Arg Val Thr Asp Ala Pro Ser Ser Gly Asp Asp
            100                 105                 110

Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr Gly Val Asp Thr Gly Ala
        115                 120                 125

Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp Lys Lys Leu Leu Ala Val
    130                 135                 140

Pro Ala Ala Asn Thr Val Arg Phe Arg Cys Pro Ala Ala Gly Asn Pro

145                 150                 155                 160

Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly Arg Glu Phe Arg Gly Glu
                165                 170                 175

His Arg Ile Gly Gly Ile Lys Leu Arg His Gln Gln Trp Ser Leu Val
            180                 185                 190

Met Glu Ser Val Val Pro Ser Asp Arg Gly Asn Tyr Thr Cys Val Val
        195                 200                 205

Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr Tyr Thr Leu Asp Val Leu
    210                 215                 220

Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
225                 230                 235                 240

Gln Thr Ala Val Leu Gly Ser Asp Val Glu Phe His Ser Lys Val Tyr
                245                 250                 255

Ser Asp Ala Gln Pro His Ile Gln Trp Leu Lys His Val Glu Val Asn
            260                 265                 270

Gly Ser Lys Val Gly Pro Asp Gly Thr Pro Tyr Val Thr Val Leu Lys
        275                 280                 285

Val Ser Leu Glu Ser Asn Ala Ser Met Ser Ser Asn Thr Pro Leu Val
    290                 295                 300

Arg Ile Ala Arg Leu Ser Ser Gly Glu Gly Pro Thr Leu Ala Asn Val
305                 310                 315                 320

Ser Glu Leu Glu Leu Pro Ala Asp Pro Lys Trp Glu Leu Ser Arg Ala
                325                 330                 335
```

```
Arg Leu Thr Leu Gly Lys Pro Leu Gly Glu Gly Cys Phe Gly Gln Val
            340                 345                 350

Val Met Ala Glu Ala Ile Gly Ile Asp Lys Asp Arg Ala Ala Lys Pro
        355                 360                 365

Val Thr Val Ala Val Lys Met Leu Lys Asp Asp Ala Thr Asp Lys Asp
    370                 375                 380

Leu Ser Asp Leu Val Ser Glu Met Glu Met Met Lys Met Ile Gly Lys
385                 390                 395                 400

His Lys Asn Ile Ile Asn Leu Leu Gly Ala Cys Thr Gln Gly Gly Pro
                405                 410                 415

Leu Tyr Val Leu Val Glu Tyr Ala Ala Lys Gly Asn Leu Arg Glu Phe
            420                 425                 430

Leu Arg Ala Arg Arg Pro Pro Gly Leu Asp Tyr Ser Phe Asp Thr Cys
        435                 440                 445

Lys Pro Pro Glu Glu Gln Leu Thr Phe Lys Asp Leu Val Ser Cys Ala
    450                 455                 460

Tyr Gln Val Ala Arg Gly Met Glu Tyr Leu Ala Ser Gln Lys Cys Ile
465                 470                 475                 480

His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Thr Glu Asp Asn Val
                485                 490                 495

Met Lys Ile Ala Asp Phe Gly Leu Ala Arg Asp Val His Asn Leu Asp
            500                 505                 510

Tyr Tyr Lys Lys Thr Thr Asn Gly Arg Leu Pro Val Lys Trp Met Ala
        515                 520                 525

Pro Glu Ala Leu Phe Asp Arg Val Tyr Thr His Gln Ser Asp Val Trp
    530                 535                 540

Ser Phe Gly Val Leu Leu Trp Glu Ile Phe Thr Leu Gly Gly Ser Pro
545                 550                 555                 560

Tyr Pro Gly Ile Pro Val Glu Glu Leu Phe Lys Leu Leu Lys Glu Gly
                565                 570                 575

His Arg Met Asp Lys Pro Ala Asn Cys Thr His Asp Leu Tyr Met Ile
            580                 585                 590

Met Arg Glu Cys Trp His Ala Ala Pro Ser Gln Arg Pro Thr Phe Lys
        595                 600                 605

Gln Leu Val Glu Asp Leu Asp Arg Val Leu Thr Val Thr Ser Thr Asp
    610                 615                 620

Glu Tyr Leu Asp Leu Ser Ala Pro Phe Glu Gln Tyr Ser Pro Gly Gly
625                 630                 635                 640

Gln Asp Thr Pro Ser Ser Ser Ser Ser Gly Asp Asp Ser Val Phe Ala
                645                 650                 655

His Asp Leu Leu Pro Pro Ala Pro Pro Ser Ser Gly Gly Ser Arg Thr
            660                 665                 670
```

<210> 9

<211> 720

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 9

```
Glu Ser Leu Gly Thr Glu Gln Arg Val Val Gly Arg Ala Ala Glu Val
1               5                  10                  15

Pro Gly Pro Glu Pro Gly Gln Gln Glu Gln Leu Val Phe Gly Ser Gly
            20                  25                  30

Asp Ala Val Glu Leu Ser Cys Pro Pro Pro Gly Gly Gly Pro Met Gly
        35                  40                  45

Pro Thr Val Trp Val Lys Asp Gly Thr Gly Leu Val Pro Ser Glu Arg
    50                  55                  60

Val Leu Val Gly Pro Gln Arg Leu Gln Val Leu Asn Ala Ser His Glu
65                  70                  75                  80

Asp Ser Gly Ala Tyr Ser Cys Arg Gln Arg Leu Thr Gln Arg Val Leu
                85                  90                  95

Cys His Phe Ser Val Arg Val Thr Asp Ala Pro Ser Ser Gly Asp Asp
            100                 105                 110

Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr Gly Val Asp Thr Gly Ala
        115                 120                 125

Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp Lys Lys Leu Leu Ala Val
    130                 135                 140

Pro Ala Ala Asn Thr Val Arg Phe Arg Cys Pro Ala Ala Gly Asn Pro
145                 150                 155                 160

Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly Arg Glu Phe Arg Gly Glu
                165                 170                 175

His Arg Ile Gly Gly Ile Lys Leu Arg His Gln Gln Trp Ser Leu Val
            180                 185                 190

Met Glu Ser Val Val Pro Ser Asp Arg Gly Asn Tyr Thr Cys Val Val
        195                 200                 205

Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr Tyr Thr Leu Asp Val Leu
    210                 215                 220

Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
225                 230                 235                 240

Gln Thr Ala Val Leu Gly Ser Asp Val Glu Phe His Cys Lys Val Tyr
                245                 250                 255

Ser Asp Ala Gln Pro His Ile Gln Trp Leu Lys His Val Glu Val Asn
            260                 265                 270

Gly Ser Lys Val Gly Pro Asp Gly Thr Pro Tyr Val Thr Val Leu Lys
        275                 280                 285
```

```
Thr Ala Gly Ala Asn Thr Thr Asp Lys Glu Leu Glu Val Leu Ser Leu
    290                 295                 300

His Asn Val Thr Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly
305                 310                 315                 320

Asn Ser Ile Gly Phe Ser His His Ser Ala Trp Leu Val Val Leu Pro
                325                 330                 335

Val Ser Leu Glu Ser Asn Ala Ser Met Ser Ser Asn Thr Pro Leu Val
            340                 345                 350

Arg Ile Ala Arg Leu Ser Ser Gly Glu Gly Pro Thr Leu Ala Asn Val
        355                 360                 365

Ser Glu Leu Glu Leu Pro Ala Asp Pro Lys Trp Glu Leu Ser Arg Ala
    370                 375                 380

Arg Leu Thr Leu Gly Lys Pro Leu Gly Glu Gly Cys Phe Gly Gln Val
385                 390                 395                 400

Val Met Ala Glu Ala Ile Gly Ile Asp Lys Asp Arg Ala Ala Lys Pro
                405                 410                 415

Val Thr Val Ala Val Lys Met Leu Lys Asp Asp Ala Thr Asp Lys Asp
            420                 425                 430

Leu Ser Asp Leu Val Ser Glu Met Glu Met Met Lys Met Ile Gly Lys
        435                 440                 445

His Lys Asn Ile Ile Asn Leu Leu Gly Ala Cys Thr Gln Gly Gly Pro
    450                 455                 460

Leu Tyr Val Leu Val Glu Tyr Ala Ala Lys Gly Asn Leu Arg Glu Phe
465                 470                 475                 480

Leu Arg Ala Arg Arg Pro Pro Gly Leu Asp Tyr Ser Phe Asp Thr Cys
                485                 490                 495

Lys Pro Pro Glu Glu Gln Leu Thr Phe Lys Asp Leu Val Ser Cys Ala
            500                 505                 510

Tyr Gln Val Ala Arg Gly Met Glu Tyr Leu Ala Ser Gln Lys Cys Ile
        515                 520                 525

His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Thr Glu Asp Asn Val
    530                 535                 540

Met Lys Ile Ala Asp Phe Gly Leu Ala Arg Asp Val His Asn Leu Asp
545                 550                 555                 560

Tyr Tyr Lys Lys Thr Thr Asn Gly Arg Leu Pro Val Lys Trp Met Ala
                565                 570                 575

Pro Glu Ala Leu Phe Asp Arg Val Tyr Thr His Gln Ser Asp Val Trp
            580                 585                 590

Ser Phe Gly Val Leu Leu Trp Glu Ile Phe Thr Leu Gly Gly Ser Pro
        595                 600                 605

Tyr Pro Gly Ile Pro Val Glu Glu Leu Phe Lys Leu Leu Lys Glu Gly
    610                 615                 620
```

```
His Arg Met Asp Lys Pro Ala Asn Cys Thr His Asp Leu Tyr Met Ile
625                 630                 635                 640

Met Arg Glu Cys Trp His Ala Ala Pro Ser Gln Arg Pro Thr Phe Lys
                645                 650                 655

Gln Leu Val Glu Asp Leu Asp Arg Val Leu Thr Val Thr Ser Thr Asp
            660                 665                 670

Glu Tyr Leu Asp Leu Ser Ala Pro Phe Glu Gln Tyr Ser Pro Gly Gly
        675                 680                 685

Gln Asp Thr Pro Ser Ser Ser Ser Ser Gly Asp Asp Ser Val Phe Ala
    690                 695                 700

His Asp Leu Leu Pro Pro Ala Pro Pro Ser Ser Gly Gly Ser Arg Thr
705                 710                 715                 720
```

<210> 10

<211> 512

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 10

```
Glu Ser Leu Gly Thr Glu Gln Arg Val Val Gly Arg Ala Ala Glu Val
1               5                   10                  15

Pro Gly Pro Glu Pro Gly Gln Gln Glu Gln Leu Val Phe Gly Ser Gly
            20                  25                  30

Asp Ala Val Glu Leu Ser Cys Pro Pro Pro Gly Gly Gly Pro Met Gly
        35                  40                  45

Pro Thr Val Trp Val Lys Asp Gly Thr Gly Leu Val Pro Ser Glu Arg
    50                  55                  60

Val Leu Val Gly Pro Gln Arg Leu Gln Val Leu Asn Ala Ser His Glu
65                  70                  75                  80

Asp Ser Gly Ala Tyr Ser Cys Arg Gln Arg Leu Thr Gln Arg Val Leu
                85                  90                  95

Cys His Phe Ser Val Arg Val Thr Asp Ala Pro Ser Ser Gly Asp Asp
            100                 105                 110

Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr Gly Val Asp Thr Gly Ala
        115                 120                 125

Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp Lys Lys Leu Leu Ala Val
    130                 135                 140

Pro Ala Ala Asn Thr Val Arg Phe Arg Cys Pro Ala Ala Gly Asn Pro
145                 150                 155                 160

Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly Arg Glu Phe Arg Gly Glu
                165                 170                 175

His Arg Ile Gly Gly Ile Lys Leu Arg His Gln Gln Trp Ser Leu Val
            180                 185                 190
```

```
Met Glu Ser Val Val Pro Ser Asp Arg Gly Asn Tyr Thr Cys Val Val
        195                 200                 205

Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr Tyr Thr Leu Asp Val Leu
    210                 215                 220

Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
225                 230                 235                 240

Gln Thr Ala Val Leu Gly Ser Asp Val Glu Phe His Cys Lys Val Tyr
                245                 250                 255

Ser Asp Ala Gln Pro His Ile Gln Trp Leu Lys His Val Glu Val Asn
            260                 265                 270

Gly Ser Lys Val Gly Pro Asp Gly Thr Pro Tyr Val Thr Val Leu Lys
        275                 280                 285

Val Ser Leu Glu Ser Asn Ala Ser Met Ser Ser Asn Thr Ser Gly Ser
    290                 295                 300

Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Val Val Phe Pro
305                 310                 315                 320

Tyr Phe Pro Arg Leu Gly Arg Tyr Asn Leu Asn Phe His Glu Ala Gln
                325                 330                 335

Gln Ala Cys Leu Asp Gln Asp Ala Val Ile Ala Ser Phe Asp Gln Leu
            340                 345                 350

Tyr Asp Ala Trp Arg Gly Gly Leu Asp Trp Cys Asn Ala Gly Trp Leu
        355                 360                 365

Ser Asp Gly Ser Val Gln Tyr Pro Ile Thr Lys Pro Arg Glu Pro Cys
    370                 375                 380

Gly Gly Gln Asn Thr Val Pro Gly Val Arg Asn Tyr Gly Phe Trp Asp
385                 390                 395                 400

Lys Asp Lys Ser Arg Tyr Asp Val Phe Cys Phe Thr Ser Asn Phe Asn
                405                 410                 415

Gly Arg Phe Tyr Tyr Leu Ile His Pro Thr Lys Leu Thr Tyr Asp Glu
            420                 425                 430

Ala Val Gln Ala Cys Leu Asn Asp Gly Ala Gln Ile Ala Lys Val Gly
        435                 440                 445

Gln Ile Phe Ala Ala Trp Lys Ile Leu Gly Tyr Asp Arg Cys Asp Ala
    450                 455                 460

Gly Trp Leu Ala Asp Gly Ser Val Arg Tyr Pro Ile Ser Arg Pro Arg
465                 470                 475                 480

Arg Arg Cys Ser Pro Thr Glu Ala Ala Val Arg Phe Val Gly Phe Pro
                485                 490                 495

Asp Lys Lys His Lys Leu Tyr Gly Val Tyr Cys Phe Arg Ala Tyr Asn
            500                 505                 510
```

<210> 11

<211> 512

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 11

```
Glu Ser Leu Gly Thr Glu Gln Arg Val Val Gly Arg Ala Ala Glu Val
1               5                   10                  15

Pro Gly Pro Glu Pro Gly Gln Gln Glu Gln Leu Val Phe Gly Ser Gly
            20                  25                  30

Asp Ala Val Glu Leu Ser Cys Pro Pro Pro Gly Gly Gly Pro Met Gly
        35                  40                  45

Pro Thr Val Trp Val Lys Asp Gly Thr Gly Leu Val Pro Ser Glu Arg
    50                  55                  60

Val Leu Val Gly Pro Gln Arg Leu Gln Val Leu Asn Ala Ser His Glu
65                  70                  75                  80

Asp Ser Gly Ala Tyr Ser Cys Arg Gln Arg Leu Thr Gln Arg Val Leu
                85                  90                  95

Cys His Phe Ser Val Arg Val Thr Asp Ala Pro Ser Ser Gly Asp Asp
            100                 105                 110

Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr Gly Val Asp Thr Gly Ala
        115                 120                 125

Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp Lys Lys Leu Leu Ala Val
    130                 135                 140

Pro Ala Ala Asn Thr Val Arg Phe Arg Cys Pro Ala Ala Gly Asn Pro
145                 150                 155                 160

Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly Arg Glu Phe Arg Gly Glu
                165                 170                 175

His Arg Ile Gly Gly Ile Lys Leu Arg His Gln Gln Trp Ser Leu Val
            180                 185                 190

Met Glu Ser Val Val Pro Ser Asp Arg Gly Asn Tyr Thr Cys Val Val
        195                 200                 205

Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr Tyr Thr Leu Asp Val Leu
    210                 215                 220

Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
225                 230                 235                 240

Gln Thr Ala Val Leu Gly Ser Asp Val Glu Phe His Ser Lys Val Tyr
                245                 250                 255

Ser Asp Ala Gln Pro His Ile Gln Trp Leu Lys His Val Glu Val Asn
            260                 265                 270

Gly Ser Lys Val Gly Pro Asp Gly Thr Pro Tyr Val Thr Val Leu Lys
        275                 280                 285

Val Ser Leu Glu Ser Asn Ala Ser Met Ser Ser Asn Thr Ser Gly Ser
```

```
    290                 295                 300

Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Val Val Phe Pro
305                 310                 315                 320

Tyr Phe Pro Arg Leu Gly Arg Tyr Asn Leu Asn Phe His Glu Ala Gln
                325                 330                 335

Gln Ala Cys Leu Asp Gln Asp Ala Val Ile Ala Ser Phe Asp Gln Leu
            340                 345                 350

Tyr Asp Ala Trp Arg Gly Gly Leu Asp Trp Cys Asn Ala Gly Trp Leu
        355                 360                 365

Ser Asp Gly Ser Val Gln Tyr Pro Ile Thr Lys Pro Arg Glu Pro Cys
    370                 375                 380

Gly Gly Gln Asn Thr Val Pro Gly Val Arg Asn Tyr Gly Phe Trp Asp
385                 390                 395                 400

Lys Asp Lys Ser Arg Tyr Asp Val Phe Cys Phe Thr Ser Asn Phe Asn
                405                 410                 415

Gly Arg Phe Tyr Tyr Leu Ile His Pro Thr Lys Leu Thr Tyr Asp Glu
            420                 425                 430

Ala Val Gln Ala Cys Leu Asn Asp Gly Ala Gln Ile Ala Lys Val Gly
        435                 440                 445

Gln Ile Phe Ala Ala Trp Lys Ile Leu Gly Tyr Asp Arg Cys Asp Ala
    450                 455                 460

Gly Trp Leu Ala Asp Gly Ser Val Arg Tyr Pro Ile Ser Arg Pro Arg
465                 470                 475                 480

Arg Arg Cys Ser Pro Thr Glu Ala Ala Val Arg Phe Val Gly Phe Pro
                485                 490                 495

Asp Lys Lys His Lys Leu Tyr Gly Val Tyr Cys Phe Arg Ala Tyr Asn
            500                 505                 510
```

<210> 12

<211> 560

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 12

```
Glu Ser Leu Gly Thr Glu Gln Arg Val Val Gly Arg Ala Ala Glu Val
1               5                   10                  15

Pro Gly Pro Glu Pro Gly Gln Gln Glu Gln Leu Val Phe Gly Ser Gly
            20                  25                  30

Asp Ala Val Glu Leu Ser Cys Pro Pro Pro Gly Gly Gly Pro Met Gly
        35                  40                  45

Pro Thr Val Trp Val Lys Asp Gly Thr Gly Leu Val Pro Ser Glu Arg
    50                  55                  60
```

```
Val Leu Val Gly Pro Gln Arg Leu Gln Val Leu Asn Ala Ser His Glu
65                  70                  75                  80

Asp Ser Gly Ala Tyr Ser Cys Arg Gln Arg Leu Thr Gln Arg Val Leu
                85                  90                  95

Cys His Phe Ser Val Arg Val Thr Asp Ala Pro Ser Ser Gly Asp Asp
            100                 105                 110

Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr Gly Val Asp Thr Gly Ala
        115                 120                 125

Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp Lys Lys Leu Leu Ala Val
    130                 135                 140

Pro Ala Ala Asn Thr Val Arg Phe Arg Cys Pro Ala Ala Gly Asn Pro
145                 150                 155                 160

Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly Arg Glu Phe Arg Gly Glu
                165                 170                 175

His Arg Ile Gly Gly Ile Lys Leu Arg His Gln Gln Trp Ser Leu Val
            180                 185                 190

Met Glu Ser Val Val Pro Ser Asp Arg Gly Asn Tyr Thr Cys Val Val
        195                 200                 205

Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr Tyr Thr Leu Asp Val Leu
    210                 215                 220

Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
225                 230                 235                 240

Gln Thr Ala Val Leu Gly Ser Asp Val Glu Phe His Cys Lys Val Tyr
                245                 250                 255

Ser Asp Ala Gln Pro His Ile Gln Trp Leu Lys His Val Glu Val Asn
            260                 265                 270

Gly Ser Lys Val Gly Pro Asp Gly Thr Pro Tyr Val Thr Val Leu Lys
        275                 280                 285

Thr Ala Gly Ala Asn Thr Thr Asp Lys Glu Leu Glu Val Leu Ser Leu
    290                 295                 300

His Asn Val Thr Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly
305                 310                 315                 320

Asn Ser Ile Gly Phe Ser His His Ser Ala Trp Leu Val Val Leu Pro
                325                 330                 335

Val Ser Leu Glu Ser Asn Ala Ser Met Ser Ser Asn Thr Ser Gly Ser
            340                 345                 350

Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Val Val Phe Pro
        355                 360                 365

Tyr Phe Pro Arg Leu Gly Arg Tyr Asn Leu Asn Phe His Glu Ala Gln
    370                 375                 380

Gln Ala Cys Leu Asp Gln Asp Ala Val Ile Ala Ser Phe Asp Gln Leu
385                 390                 395                 400

Tyr Asp Ala Trp Arg Gly Gly Leu Asp Trp Cys Asn Ala Gly Trp Leu
```

```
                405                 410                 415

Ser Asp Gly Ser Val Gln Tyr Pro Ile Thr Lys Pro Arg Glu Pro Cys
            420                 425                 430

Gly Gly Gln Asn Thr Val Pro Gly Val Arg Asn Tyr Gly Phe Trp Asp
        435                 440                 445

Lys Asp Lys Ser Arg Tyr Asp Val Phe Cys Phe Thr Ser Asn Phe Asn
    450                 455                 460

Gly Arg Phe Tyr Tyr Leu Ile His Pro Thr Lys Leu Thr Tyr Asp Glu
465                 470                 475                 480

Ala Val Gln Ala Cys Leu Asn Asp Gly Ala Gln Ile Ala Lys Val Gly
                485                 490                 495

Gln Ile Phe Ala Ala Trp Lys Ile Leu Gly Tyr Asp Arg Cys Asp Ala
            500                 505                 510

Gly Trp Leu Ala Asp Gly Ser Val Arg Tyr Pro Ile Ser Arg Pro Arg
        515                 520                 525

Arg Arg Cys Ser Pro Thr Glu Ala Ala Val Arg Phe Val Gly Phe Pro
    530                 535                 540

Asp Lys Lys His Lys Leu Tyr Gly Val Tyr Cys Phe Arg Ala Tyr Asn
545                 550                 555                 560
```

<210> 13

<211> 155

<212> PRT

<213> Homo sapiens

<400> 13

```
Met Ala Glu Gly Glu Ile Thr Thr Phe Thr Ala Leu Thr Glu Lys Phe
1               5                   10                  15

Asn Leu Pro Pro Gly Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys Ser
            20                  25                  30

Asn Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp Gly
        35                  40                  45

Thr Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln Leu Ser Ala Glu
    50                  55                  60

Ser Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr Gly Gln Tyr Leu
65                  70                  75                  80

Ala Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr Pro Asn Glu
                85                  90                  95

Glu Cys Leu Phe Leu Glu Arg Leu Glu Glu Asn His Tyr Asn Thr Tyr
            100                 105                 110

Ile Ser Lys Lys His Ala Glu Lys Asn Trp Phe Val Gly Leu Lys Lys
        115                 120                 125

Asn Gly Ser Cys Lys Arg Gly Pro Arg Thr His Tyr Gly Gln Lys Ala
    130                 135                 140
```

```
Ile Leu Phe Leu Pro Leu Pro Val Ser Ser Asp
145                 150                 155
```

<210> 14

<211> 288

<212> PRT

<213> Homo sapiens

<400> 14

```
Met Val Gly Val Gly Gly Gly Asp Val Glu Asp Val Thr Pro Arg Pro
1               5                   10                  15

Gly Gly Cys Gln Ile Ser Gly Arg Gly Ala Arg Gly Cys Asn Gly Ile
            20                  25                  30

Pro Gly Ala Ala Ala Trp Glu Ala Ala Leu Pro Arg Arg Arg Pro Arg
        35                  40                  45

Arg His Pro Ser Val Asn Pro Arg Ser Arg Ala Ala Gly Ser Pro Arg
    50                  55                  60

Thr Arg Gly Arg Arg Thr Glu Glu Arg Pro Ser Gly Ser Arg Leu Gly
65                  70                  75                  80

Asp Arg Gly Arg Gly Arg Ala Leu Pro Gly Gly Arg Leu Gly Gly Arg
                85                  90                  95

Gly Arg Gly Arg Ala Pro Glu Arg Val Gly Gly Arg Gly Arg Gly Arg
            100                 105                 110

Gly Thr Ala Ala Pro Arg Ala Ala Pro Ala Ala Arg Gly Ser Arg Pro
        115                 120                 125

Gly Pro Ala Gly Thr Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala
    130                 135                 140

Leu Pro Glu Asp Gly Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys
145                 150                 155                 160

Asp Pro Lys Arg Leu Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile
                165                 170                 175

His Pro Asp Gly Arg Val Asp Gly Val Arg Glu Lys Ser Asp Pro His
            180                 185                 190

Ile Lys Leu Gln Leu Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys
        195                 200                 205

Gly Val Cys Ala Asn Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu
    210                 215                 220

Leu Ala Ser Lys Cys Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu
225                 230                 235                 240

Glu Ser Asn Asn Tyr Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp
                245                 250                 255

Tyr Val Ala Leu Lys Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr
            260                 265                 270
```

```
Gly Pro Gly Gln Lys Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser
        275                 280                 285
```

<210> 15

<211> 208

<212> PRT

<213> Homo sapiens

<400> 15

```
Met Ala Pro Leu Gly Glu Val Gly Asn Tyr Phe Gly Val Gln Asp Ala
1               5                   10                  15

Val Pro Phe Gly Asn Val Pro Val Leu Pro Val Asp Ser Pro Val Leu
            20                  25                  30

Leu Ser Asp His Leu Gly Gln Ser Glu Ala Gly Gly Leu Pro Arg Gly
        35                  40                  45

Pro Ala Val Thr Asp Leu Asp His Leu Lys Gly Ile Leu Arg Arg Arg
    50                  55                  60

Gln Leu Tyr Cys Arg Thr Gly Phe His Leu Glu Ile Phe Pro Asn Gly
65                  70                  75                  80

Thr Ile Gln Gly Thr Arg Lys Asp His Ser Arg Phe Gly Ile Leu Glu
                85                  90                  95

Phe Ile Ser Ile Ala Val Gly Leu Val Ser Ile Arg Gly Val Asp Ser
            100                 105                 110

Gly Leu Tyr Leu Gly Met Asn Glu Lys Gly Glu Leu Tyr Gly Ser Glu
        115                 120                 125

Lys Leu Thr Gln Glu Cys Val Phe Arg Glu Gln Phe Glu Glu Asn Trp
    130                 135                 140

Tyr Asn Thr Tyr Ser Ser Asn Leu Tyr Lys His Val Asp Thr Gly Arg
145                 150                 155                 160

Arg Tyr Tyr Val Ala Leu Asn Lys Asp Gly Thr Pro Arg Glu Gly Thr
                165                 170                 175

Arg Thr Lys Arg His Gln Lys Phe Thr His Phe Leu Pro Arg Pro Val
            180                 185                 190

Asp Pro Asp Lys Val Pro Glu Leu Tyr Lys Asp Ile Leu Ser Gln Ser
        195                 200                 205
```

<210> 16

<211> 207

<212> PRT

<213> Homo sapiens

<400> 16

```
Met Tyr Ser Ala Pro Ser Ala Cys Thr Cys Leu Cys Leu His Phe Leu
1               5                   10                  15

Leu Leu Cys Phe Gln Val Gln Val Leu Val Ala Glu Glu Asn Val Asp
            20                  25                  30

Phe Arg Ile His Val Glu Asn Gln Thr Arg Ala Arg Asp Asp Val Ser
        35                  40                  45
```

```
Arg Lys Gln Leu Arg Leu Tyr Gln Leu Tyr Ser Arg Thr Ser Gly Lys
    50                  55                  60

His Ile Gln Val Leu Gly Arg Arg Ile Ser Ala Arg Gly Glu Asp Gly
65                  70                  75                  80

Asp Lys Tyr Ala Gln Leu Leu Val Glu Thr Asp Thr Phe Gly Ser Gln
                85                  90                  95

Val Arg Ile Lys Gly Lys Glu Thr Glu Phe Tyr Leu Cys Met Asn Arg
            100                 105                 110

Lys Gly Lys Leu Val Gly Lys Pro Asp Gly Thr Ser Lys Glu Cys Val
        115                 120                 125

Phe Ile Glu Lys Val Leu Glu Asn Asn Tyr Thr Ala Leu Met Ser Ala
    130                 135                 140

Lys Tyr Ser Gly Trp Tyr Val Gly Phe Thr Lys Lys Gly Arg Pro Arg
145                 150                 155                 160

Lys Gly Pro Lys Thr Arg Glu Asn Gln Gln Asp Val His Phe Met Lys
                165                 170                 175

Arg Tyr Pro Lys Gly Gln Pro Glu Leu Gln Lys Pro Phe Lys Tyr Thr
            180                 185                 190

Thr Val Thr Lys Arg Ser Arg Arg Ile Arg Pro Thr His Pro Ala
        195                 200                 205
```

<210> 17

<211> 323

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 17

```
Met Gly Ala Pro Ala Cys Ala Leu Ala Leu Cys Val Ala Val Ala Ile
1               5                   10                  15

Val Ala Gly Ala Ser Ser Glu Ser Leu Gly Thr Glu Gln Arg Val Val
            20                  25                  30

Gly Arg Ala Ala Glu Val Pro Gly Pro Glu Pro Gly Gln Gln Glu Gln
        35                  40                  45

Leu Val Phe Gly Ser Gly Asp Ala Val Glu Leu Ser Cys Pro Pro Pro
    50                  55                  60

Gly Gly Gly Pro Met Gly Pro Thr Val Trp Val Lys Asp Gly Thr Gly
65                  70                  75                  80

Leu Val Pro Ser Glu Arg Val Leu Val Gly Pro Gln Arg Leu Gln Val
                85                  90                  95

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

Leu Thr Gln Arg Val Leu Cys His Phe Ser Val Arg Val Thr Asp Ala
```

```
Leu Thr Gln Arg Val Leu Cys His Phe Ser Val Arg Val Thr Asp Asp
        115                 120                 125

Pro Ser Ser Gly Asp Asp Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr
    130                 135                 140

Gly Val Asp Thr Gly Ala Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp
145                 150                 155                 160

Lys Lys Leu Leu Ala Val Pro Ala Ala Asn Thr Val Arg Phe Arg Cys
                165                 170                 175

Pro Ala Ala Gly Asn Pro Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly
            180                 185                 190

Arg Glu Phe Arg Gly Glu His Arg Ile Gly Gly Ile Lys Leu Arg His
        195                 200                 205

Gln Gln Trp Ser Leu Val Met Glu Ser Val Val Pro Ser Asp Arg Gly
    210                 215                 220

Asn Tyr Thr Cys Val Val Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr
225                 230                 235                 240

Tyr Thr Leu Asp Val Leu Glu Arg Ser Pro His Arg Pro Ile Leu Gln
                245                 250                 255

Ala Gly Leu Pro Ala Asn Gln Thr Ala Val Leu Gly Ser Asp Val Glu
            260                 265                 270

Phe His Cys Lys Val Tyr Ser Asp Ala Gln Pro His Ile Gln Trp Leu
        275                 280                 285

Lys His Val Glu Val Asn Gly Ser Lys Val Gly Pro Asp Gly Thr Pro
    290                 295                 300

Tyr Val Thr Val Leu Lys Val Ser Leu Glu Ser Asn Ala Ser Met Ser
305                 310                 315                 320

Ser Asn Thr
```

<210> 18

<211> 323

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 18

```
Met Gly Ala Pro Ala Cys Ala Leu Ala Leu Cys Val Ala Val Ala Ile
1               5                   10                  15

Val Ala Gly Ala Ser Ser Glu Ser Leu Gly Thr Glu Gln Arg Val Val
            20                  25                  30

Gly Arg Ala Ala Glu Val Pro Gly Pro Glu Pro Gly Gln Gln Glu Gln
        35                  40                  45

Leu Val Phe Gly Ser Gly Asp Ala Val Glu Leu Ser Cys Pro Pro Pro
    50                  55                  60

Glu Glu Glu Pro Met Glu Pro Thr Val Trp Val Lys Asp Gly Thr Gly
```

```
Gly Gly Gly Pro Met Gly Pro Thr Val Trp Val Lys Asp Gly Thr Gly
65                  70                  75                  80

Leu Val Pro Ser Glu Arg Val Leu Val Gly Pro Gln Arg Leu Gln Val
                85                  90                  95

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

Leu Thr Gln Arg Val Leu Cys His Phe Ser Val Arg Val Thr Asp Ala
        115                 120                 125

Pro Ser Ser Gly Asp Asp Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr
    130                 135                 140

Gly Val Asp Thr Gly Ala Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp
145                 150                 155                 160

Lys Lys Leu Leu Ala Val Pro Ala Ala Asn Thr Val Arg Phe Arg Cys
                165                 170                 175

Pro Ala Ala Gly Asn Pro Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly
            180                 185                 190

Arg Glu Phe Arg Gly Glu His Arg Ile Gly Gly Ile Lys Leu Arg His
        195                 200                 205

Gln Gln Trp Ser Leu Val Met Glu Ser Val Val Pro Ser Asp Arg Gly
    210                 215                 220

Asn Tyr Thr Cys Val Val Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr
225                 230                 235                 240

Tyr Thr Leu Asp Val Leu Glu Arg Ser Pro His Arg Pro Ile Leu Gln
                245                 250                 255

Ala Gly Leu Pro Ala Asn Gln Thr Ala Val Leu Gly Ser Asp Val Glu
            260                 265                 270

Phe His Cys Lys Val Tyr Ser Asp Ala Gln Pro His Ile Gln Trp Leu
        275                 280                 285

Lys His Val Glu Val Asn Gly Ser Lys Val Gly Pro Asp Gly Thr Pro
    290                 295                 300

Tyr Val Thr Val Leu Lys Val Ser Leu Glu Ser Asn Ala Ser Met Ser
305                 310                 315                 320

Ser Asn Thr
```

<210> 19

<211> 323

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 19

```
Met Gly Ala Pro Ala Cys Ala Leu Ala Leu Cys Val Ala Val Ala Ile
1               5                   10                  15
```

```
Val Ala Gly Ala Ser Ser Glu Ser Leu Gly Thr Glu Gln Arg Val Val
            20                  25                  30

Gly Arg Ala Ala Glu Val Pro Gly Pro Glu Pro Gly Gln Gln Glu Gln
        35                  40                  45

Leu Val Phe Gly Ser Gly Asp Ala Val Glu Leu Ser Cys Pro Pro Pro
    50                  55                  60

Gly Gly Gly Pro Met Gly Pro Thr Val Trp Val Lys Asp Gly Thr Gly
65                  70                  75                  80

Leu Val Pro Ser Glu Arg Val Leu Val Gly Pro Gln Arg Leu Gln Val
                85                  90                  95

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

Leu Thr Gln Arg Val Leu Cys His Phe Ser Val Arg Val Thr Asp Ala
        115                 120                 125

Pro Ser Ser Gly Asp Asp Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr
    130                 135                 140

Gly Val Asp Thr Gly Ala Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp
145                 150                 155                 160

Lys Lys Leu Leu Ala Val Pro Ala Ala Asn Thr Val Arg Phe Arg Cys
                165                 170                 175

Pro Ala Ala Gly Asn Pro Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly
            180                 185                 190

Arg Glu Phe Arg Gly Glu His Arg Ile Gly Gly Ile Lys Leu Arg His
        195                 200                 205

Gln Gln Trp Ser Leu Val Met Glu Ser Val Val Pro Ser Asp Arg Gly
    210                 215                 220

Asn Tyr Thr Cys Val Val Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr
225                 230                 235                 240

Tyr Thr Leu Asp Val Leu Glu Arg Ser Pro His Arg Pro Ile Leu Gln
                245                 250                 255

Ala Gly Leu Pro Ala Asn Gln Thr Ala Val Leu Gly Ser Asp Val Glu
            260                 265                 270

Phe His Ser Lys Val Tyr Ser Asp Ala Gln Pro His Ile Gln Trp Leu
        275                 280                 285

Lys His Val Glu Val Asn Gly Ser Lys Val Gly Pro Asp Gly Thr Pro
    290                 295                 300

Tyr Val Thr Val Leu Lys Val Ser Leu Glu Ser Asn Ala Ser Met Ser
305                 310                 315                 320

Ser Asn Thr
```

<210> 20

<211> 987

<212> DNA

<213> Artificial Sequence

<220>
<223> Synthetic construct

<400> 20
atgggagtga aggtgctgtt cgccctgatc tgtatcgccg tggccgaggc cgagtctctg 60
ggcacagaac agagagtcgt gggcagagcc gccgaagtgc ctggacctga acctggccag 120
caggaacagc tggtgtttgg cagcggcgac gccgtggaac tgagctgtcc tccacctggc 180
ggaggcccta tgggacctac cgtgtgggtc aaggatggca ccggactggt gcctagcgag 240
agggtgctcg tgggacctca gagactgcag gtgctgaacg ccagccacga ggatagcggc 300
gcctacagct gcagacagag actgacacag cgggtgctgt gccacttctc cgtcagagtg 360
accgacgccc ctagctccgg cgacgatgag gatggcgaag atgaggccga ggacaccggc 420
gtggacacag gcgctccata ctggaccaga cccgagcgga tggacaagaa actgctggcc 480
gtgcctgccg ccaacaccgt gcggtttaga tgtcctgccg ccggaaaccc cacccccagc 540
atcagctggc tgaagaacgg cagagagttc cggggcgagc acagaatcgg cggcatcaag 600
ctgagacacc agcagtggtc cctcgtgatg gaaagcgtgg tgcccagcga ccggggcaac 660
tacacctgtg tggtggaaaa caagttcggc agcatccggc agacctacac cctggacgtg 720
ctggaaagaa gcccccacag acccatcctg caggccggac tgcctgccaa tcagacagcc 780
gtgctgggca gcgacgtgga atttcacagc aaggtgtaca gcgacgccca gccccacatc 840
cagtggctga aacacgtgga agtgaacggc agcaaagtgg gccccgacgg cacccttat 900
gtgaccgtgc tgaaggtgtc cctggaaagc aacgccagca tgagcagcaa caccgactac 960
aaggacgacg acgacaagtg aaagctt 987

<210> 21
<211> 1131
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic construct

<400> 21
atgggagtga aggtgctgtt cgccctgatc tgtatcgccg tggccgaggc cgagtctctg 60
ggcacagaac agagagtcgt gggcagagcc gccgaagtgc ctggacctga acctggccag 120
caggaacagc tggtgtttgg cagcggcgac gccgtggaac tgagctgtcc tccacctggc 180
ggaggcccta tgggacctac cgtgtgggtc aaggatggca ccggactggt gcctagcgag 240
agggtgctcg tgggacctca gagactgcag gtgctgaacg ccagccacga ggatagcggc 300
gcctacagct gcagacagag actgacacag cgggtgctgt gccacttctc cgtcagagtg 360
accgacgccc ctagctccgg cgacgatgag gatggcgaag atgaggccga ggacaccggc 420
gtggacacag gcgctccata ctggaccaga cccgagcgga tggacaagaa actgctggcc 480
gtgcctgccg ccaacaccgt gcggtttaga tgtcctgccg ccggaaaccc cacccccagc 540
atcagctggc tgaagaacgg cagagagttc cggggcgagc acagaatcgg cggcatcaag 600
ctgagacacc agcagtggtc cctcgtgatg gaaagcgtgg tgcccagcga ccggggcaac 660
tacacctgtg tggtggaaaa caagttcggc agcatccggc agacctacac cctggacgtg 720
ctggaaagaa gcccccacag acccatcctg caggccggac tgcctgccaa tcagacagcc 780

gtgctgggca gcgacgtgga atttcactgc aaggtgtaca gcgacgccca gccccacatc 840

cagtggctga aacacgtgga agtgaacggc agcaaagtgg gccccgacgg caccccttat 900

gtgaccgtgc tgaaaaccgc tggcgccaat accaccgaca aagaactgga agtgctgagc 960

ctgcacaacg tgaccttcga ggatgccggc gagtacacct gtctggccgg caacagcatc 1020

ggcttcagcc accattctgc ctggctggtg gtgctgcccg tgtccctgga aagcaacgcc 1080

agcatgagca gcaacaccga ctacaaggac gacgacgaca agtgaaagct t 1131

<210> 22
<211> 2019
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic construct

<400> 22
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gagcctggcc agcaggaaca gctggtcttt ggctctggcg acgccgtgga actgagctgt 120

cctccacctg gcggaggccc tatgggacct accgtgtggg tcaaggatgg caccggactg 180

gtgcctagcg agagggtgct cgtgggacct cagagactgc aggtcctgaa cgccagccac 240

gaggatagcg gcgcctacag ctgcagacag agactgaccc agcgggtgct gtgccacttc 300

agcgtcagag tgaccgatgc ccccagcagc ggagatgacg aggatggcga ggatgaggcc 360

gaggatacag gcgtggacac aggcgcccct tactggacca gacccgagcg gatggacaag 420

aaactgctgg ccgtgcctgc cgccaacacc gtgcggttta gatgccctgc cgccggaaac 480

cccaccccca gcatctcttg gctgaagaac ggcagagagt tccggggcga gcaccggatc 540

ggcggcatta agctgagaca ccagcagtgg tccctggtca tggaaagcgt ggtgcccagc 600

gaccggggca actacacctg tgtggtggaa aacaagttcg gcagcatccg gcagacctac 660

accctggacg tgctggaaag aagcccccac agacctatcc tgcaggccgg actgcctgcc 720

aatcagacag ccgtgctggg cagcgacgtg gaatttcaca gcaaggtgta cagcgacgcc 780

cagccccaca tccagtggct gaagcacgtg gaagtgaacg gcagcaaagt gggccccgac 840

ggcacccctt acgtgaccgt gctgaaagtg tccctggaaa gcaacgccag catgagcagc 900

aacacccccc tcgtgcggat cgccagactg tctagcggag agggccctac cctggccaac 960

gtgtccgaac tggaactgcc cgccgacccc aagtgggagc tgagcagagc tagactgacc 1020

ctgggcaagc ctctgggcga gggctgtttt ggacaggtgg tcatggccga ggccatcggc 1080

atcgacaagg acagagccgc caagcctgtg accgtggccg tgaagatgct gaaggacgac 1140

gccaccgaca aggacctgag cgacctggtg tccgagatgg aaatgatgaa gatgatcggc 1200

aagcacaaga acatcatcaa cctgctgggc gcctgcaccc agggcggacc tctgtacgtg 1260

ctggtggaat acgccgccaa gggcaacctg agagagttcc tgagagccag aaggccccct 1320

ggcctggact acagcttcga tacctgcaag ccccccgaag aacagctgac cttcaaggat 1380

ctggtgtcct gcgcctatca ggtggccaga ggcatggaat acctggccag ccagaagtgc 1440

atccaccggg atctggccgc cagaaacgtg ctggtcaccg aggacaacgt gatgaagatc 1500

gccgacttcg gcctggcccg ggacgtgcac aacctggact actacaagaa aaccaccaac 1560

ggccggctgc ccgtgaagtg gatggcccct gaggccctgt tcgacagagt gtacacccac 1620

gagaggcagg tgtggtggtt ggggtggtg gtgtgggaga tgtttaggct gggggggagc 1680

cagagcgacg tgtggtcctt cggcgtgctg ctgtgggaga tctttaccct gtgcggcagc 1680

ccttaccccg gcatccctgt ggaagaactg ttcaagctgc tgaaagaggg ccacagaatg 1740

gacaagcccg ccaactgcac ccacgacctg tacatgatca tgagagagtg ctggcacgcc 1800

gctcccagcc agaggcctac ctttaagcag ctggtggaag atctggaccg ggtgctgacc 1860

gtgaccagca ccgacgagta cctggatctg agcgcccccc tcgagcagta ctctcctggc 1920

ggccaggata cccctagcag cagctctagc ggcgacgaca gcgtgttcgc ccacgatctg 1980

ctgcctccag cccctcctag ctctggcggc tctagaacc 2019

<210> 23
<211> 1491
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic construct

<400> 23
atggagagcc tgggcacaga acagagagtc gtgggcagag ccgccgaagt gcctggacct 60

gaacctggcc agcaggaaca gctggtcttt ggctctggcg acgccgtgga actgagctgt 120

cctccacctg gcggaggccc tatgggacct accgtgtggg tcaaggatgg caccggactg 180

gtgcctagcg agagggtgct cgtgggacct cagagactgc aggtcctgaa cgccagccac 240

gaggatagcg gcgcctacag ctgcagacag agactgaccc agcgggtgct gtgccacttc 300

agcgtcagag tgaccgatgc ccccagcagc ggagatgacg aggatggcga ggatgaggcc 360

gaggatacag gcgtggacac aggcgccccc tactggacca gacccgagcg gatggacaag 420

aaactgctgg ccgtgcctgc cgccaacacc gtgcggttta gatgccctgc cgccggaaac 480

cccaccccca gcatctcttg gctgaagaac ggcagagagt tccggggcga gcaccggatc 540

ggcggcatta agctgagaca ccagcagtgg tccctggtca tggaaagcgt ggtgcccagc 600

gaccggggca actacacctg tgtggtggaa aacaagttcg gcagcatccg gcagacctac 660

accctggacg tgctggaaag aagcccccac agacctatcc tgcaggccgg actgcctgcc 720

aatcagacag ccgtgctggg cagcgacgtg gaatttcact gcaaggtgta cagcgacgcc 780

cagccccaca tccagtggct gaagcacgtg gaagtgaacg gcagcaaagt gggccccgac 840

ggcaccccct acgtgaccgt gctgaaagtg tccctggaaa gcaacgccag catgagcagc 900

aacaccagcg gcagcggctc tggcagcgga tctggttctg gctccggcag cgtggtgttc 960

ccctacttcc cccggctggg ccggtacaac ctgaactttc atgaggccca gcaggcctgc 1020

ctggaccagg atgccgtgat cgccagcttc gaccagctgt acgatgcttg gagaggcggc 1080

ctggactggt gcaatgccgg ctggctgtct gacggcagcg tgcagtaccc catcaccaag 1140

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gacaaggaca agagcagata cgacgtgttc tgcttcacca gcaacttcaa cggccggttc 1260

tactacctga tccaccccac caagctgacc tacgacgagg ccgtgcaggc ctgtctgaac 1320

gatggcgccc agatcgccaa agtgggacag atcttcgccg cctggaagat cctgggctac 1380

gacagatgtg acgccggatg gctggccgac ggctccgtgc ggtatcccat cagccggcct 1440

agaagaagat gcagccctac cgaggccgcc gtcagattcg tgggcttccc c 1491

<210> 24
<211> 1710

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic construct

<400> 24
atgggagcac cagcttgtgc cctggctctg tgtgtggccg tggctattgt ggctggcgcc 60
tctagcgaga gcctgggcac agaacagaga gtcgtgggca gagccgccga agtgcctgga 120
cctgaacctg gccagcagga acagctggtc tttggctctg gcgacgccgt ggaactgagc 180
tgtcctccac ctggcggagg ccctatggga cctaccgtgt gggtcaagga tggcaccgga 240
ctggtgccta gcgagagggt gctcgtggga cctcagagac tgcaggtcct gaacgccagc 300
cacgaggata gcggcgccta cagctgcaga cagagactga cccagcgggt gctgtgccac 360
ttcagcgtca gagtgaccga tgcccccagc agcggagatg acgaggatgg cgaggatgag 420
gccgaggata caggcgtgga cacaggcgcc ccttactgga ccagacccga gcggatggac 480
aagaaactgc tggccgtgcc tgccgccaac accgtgcggt ttagatgccc tgccgccgga 540
aaccccaccc ccagcatctc ttggctgaag aacggcagag agttccgggg cgagcaccgg 600
atcggcggca ttaagctgag acaccagcag tggtccctgg tcatggaaag cgtggtgccc 660
agcgaccggg gcaactacac ctgtgtggtg gaaaacaagt tcggcagcat ccggcagacc 720
tacaccctgg acgtgctgga aagaagcccc cacagaccta tcctgcaggc cggactgcct 780
gccaatcaga cagccgtgct gggcagcgac gtggaatttc actgcaaggt gtacagcgac 840
gcccagcccc acatccagtg gctgaagcac gtggaagtga acggcagcaa agtgggcccc 900
gacggcaccc cttacgtgac cgtgctgaaa accgctggcg ccaacaccac cgacaaagaa 960
ctggaagtgc tgagcctgca caacgtgacc ttcgaggacg ccggcgagta cacctgtctg 1020
gccggcaata gcatcggctt cagccaccac tctgcctggc tggtggtgct gccaggcgga 1080
ggctctgtgt ccctggaaag caacgccagc atgagcagca acaccagcgg cagcggctct 1140
ggcagcggat ctggttctgg ctccggcagc gtggtgttcc cctacttccc ccggctgggc 1200
cggtacaacc tgaactttca tgaggcccag caggcctgcc tggaccagga tgccgtgatc 1260
gccagcttcg accagctgta cgatgcttgg agaggcggcc tggactggtg caatgccggc 1320
tggctgtctg acggcagcgt gcagtacccc atcaccaagc ccagagagcc ctgcggcgga 1380
cagaataccg tgcccggcgt gcggaactac ggcttctggg acaaggacaa gagcagatac 1440
gacgtgttct gcttcaccag caacttcaac ggccggttct actacctgat ccaccccacc 1500
aagctgacct acgacgaggc cgtgcaggcc tgtctgaacg atggcgccca gatcgccaaa 1560
gtgggacaga tcttcgccgc ctggaagatc ctgggctacg acagatgtga cgccggatgg 1620
ctggccgacg gctccgtgcg gtatcccatc agccggccta gaagaagatg cagccctacc 1680
gaggccgccg tcagattcgt gggcttcccc 1710

<210> 25
<211> 236
<212> PRT
<213> Homo sapiens

<400> 25
Gly Gly Gly Gly Ala Gly Gly Gly Gly Asp Lys Thr His Thr Cys Pro
1 5 10 15

```
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
            20                  25                  30

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
        35                  40                  45

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
    50                  55                  60

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
65                  70                  75                  80

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
                85                  90                  95

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
            100                 105                 110

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
        115                 120                 125

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
    130                 135                 140

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
145                 150                 155                 160

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
                165                 170                 175

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
            180                 185                 190

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
        195                 200                 205

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
    210                 215                 220

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
225                 230                 235
```

<210> 26

<211> 236

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 26

```
Gly Gly Gly Gly Ala Gly Gly Gly Gly Asp Lys Thr His Thr Cys Pro
1               5                   10                  15

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
            20                  25                  30

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
        35                  40                  45

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
```

```
    50                  55                  60

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
65                  70                  75                  80

Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg Val Val Ser Val Leu Thr
                85                  90                  95

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
            100                 105                 110

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
        115                 120                 125

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
    130                 135                 140

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
145                 150                 155                 160

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
                165                 170                 175

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
            180                 185                 190

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
        195                 200                 205

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His

    210                 215                 220

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
225                 230                 235
```

<210> 27

<211> 585

<212> PRT

<213> Homo sapiens

<400> 27

```
Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu
1               5                   10                  15

Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
            20                  25                  30

Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
        35                  40                  45

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
    50                  55                  60

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65                  70                  75                  80

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
                85                  90                  95

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
```

```
            100                 105                 110

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
        115                 120                 125

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
    130                 135                 140

Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145                 150                 155                 160

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
                165                 170                 175

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
            180                 185                 190

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu

        195                 200                 205

Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
    210                 215                 220

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225                 230                 235                 240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
                245                 250                 255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
            260                 265                 270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
        275                 280                 285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
    290                 295                 300

Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305                 310                 315                 320

Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
                325                 330                 335

Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
            340                 345                 350

Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
        355                 360                 365

Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
    370                 375                 380

Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
385                 390                 395                 400

Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
                405                 410                 415

Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
            420                 425                 430
```

```
Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
        435                 440                 445

Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
    450                 455                 460

Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
465                 470                 475                 480

Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
                485                 490                 495

Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
            500                 505                 510

Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
        515                 520                 525

Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
    530                 535                 540

Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
545                 550                 555                 560

Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
                565                 570                 575

Ala Ala Ser Gln Ala Ala Leu Gly Leu
            580                 585
```

<210> 28

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 28

```
Gly Gly Gly Gly Ala Gly Gly Gly Gly
1               5
```

<210> 29

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 29

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

<210> 30

<211> 24

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 30

```
Ala Gly Gly Thr Gly Gly Cys Cys Thr Thr Thr Gly Ala Cys Ala Cys
1               5                   10                  15

Cys Thr Ala Cys Cys Ala Gly Gly
            20
```

<210> 31

<211> 24

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 31

```
Thr Cys Thr Gly Thr Thr Gly Thr Gly Thr Thr Thr Cys Cys Thr Cys
1               5                   10                  15

Cys Cys Thr Gly Thr Thr Gly Gly
            20
```

<210> 32

<211> 806

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 32

```
Met Gly Ala Pro Ala Cys Ala Leu Ala Leu Cys Val Ala Val Ala Ile
1               5                   10                  15

Val Ala Gly Ala Ser Ser Glu Ser Leu Gly Thr Glu Gln Arg Val Val
            20                  25                  30

Gly Arg Ala Ala Glu Val Pro Gly Pro Glu Pro Gly Gln Gln Glu Gln
        35                  40                  45

Leu Val Phe Gly Ser Gly Asp Ala Val Glu Leu Ser Cys Pro Pro Pro
    50                  55                  60

Gly Gly Gly Pro Met Gly Pro Thr Val Trp Val Lys Asp Gly Thr Gly
65                  70                  75                  80

Leu Val Pro Ser Glu Arg Val Leu Val Gly Pro Gln Arg Leu Gln Val
                85                  90                  95

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

Leu Thr Gln Arg Val Leu Cys His Phe Ser Val Arg Val Thr Asp Ala
        115                 120                 125

Pro Ser Ser Gly Asp Asp Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr
    130                 135                 140
```

```
Gly Val Asp Thr Gly Ala Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp
145                 150                 155                 160

Lys Lys Leu Leu Ala Val Pro Ala Ala Asn Thr Val Arg Phe Arg Cys
                165                 170                 175

Pro Ala Ala Gly Asn Pro Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly
            180                 185                 190

Arg Glu Phe Arg Gly Glu His Arg Ile Gly Gly Ile Lys Leu Arg His
        195                 200                 205

Gln Gln Trp Ser Leu Val Met Glu Ser Val Val Pro Ser Asp Arg Gly
    210                 215                 220

Asn Tyr Thr Cys Val Val Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr
225                 230                 235                 240

Tyr Thr Leu Asp Val Leu Glu Arg Ser Pro His Arg Pro Ile Leu Gln
                245                 250                 255

Ala Gly Leu Pro Ala Asn Gln Thr Ala Val Leu Gly Ser Asp Val Glu
            260                 265                 270

Phe His Cys Lys Val Tyr Ser Asp Ala Gln Pro His Ile Gln Trp Leu
        275                 280                 285

Lys His Val Glu Val Asn Gly Ser Lys Val Gly Pro Asp Gly Thr Pro
    290                 295                 300

Tyr Val Thr Val Leu Lys Thr Ala Gly Ala Asn Thr Thr Asp Lys Glu
305                 310                 315                 320

Leu Glu Val Leu Ser Leu His Asn Val Thr Phe Glu Asp Ala Gly Glu
                325                 330                 335

Tyr Thr Cys Leu Ala Gly Asn Ser Ile Gly Phe Ser His His Ser Ala
            340                 345                 350

Trp Leu Val Val Leu Pro Ala Glu Glu Glu Leu Val Glu Ala Asp Glu
        355                 360                 365

Ala Gly Ser Val Tyr Ala Gly Ile Leu Ser Tyr Gly Val Gly Phe Phe
    370                 375                 380

Leu Phe Ile Leu Val Val Ala Ala Val Thr Leu Cys Arg Leu Arg Ser
385                 390                 395                 400

Pro Pro Lys Lys Gly Leu Gly Ser Pro Thr Val His Lys Ile Ser Arg
                405                 410                 415

Phe Pro Leu Lys Arg Gln Val Ser Leu Glu Ser Asn Ala Ser Met Ser
            420                 425                 430

Ser Asn Thr Pro Leu Val Arg Ile Ala Arg Leu Ser Ser Gly Glu Gly
        435                 440                 445

Pro Thr Leu Ala Asn Val Ser Glu Leu Glu Leu Pro Ala Asp Pro Lys
    450                 455                 460

Trp Glu Leu Ser Arg Ala Arg Leu Thr Leu Gly Lys Pro Leu Gly Glu
465                 470                 475                 480
```

```
Gly Cys Phe Gly Gln Val Val Met Ala Glu Ala Ile Gly Ile Asp Lys
                485                 490                 495

Asp Arg Ala Ala Lys Pro Val Thr Val Ala Val Lys Met Leu Lys Asp
            500                 505                 510

Asp Ala Thr Asp Lys Asp Leu Ser Asp Leu Val Ser Glu Met Glu Met
        515                 520                 525

Met Lys Met Ile Gly Lys His Lys Asn Ile Ile Asn Leu Leu Gly Ala
    530                 535                 540

Cys Thr Gln Gly Gly Pro Leu Tyr Val Leu Val Glu Tyr Ala Ala Lys
545                 550                 555                 560

Gly Asn Leu Arg Glu Phe Leu Arg Ala Arg Arg Pro Pro Gly Leu Asp
                565                 570                 575

Tyr Ser Phe Asp Thr Cys Lys Pro Pro Glu Glu Gln Leu Thr Phe Lys
            580                 585                 590

Asp Leu Val Ser Cys Ala Tyr Gln Val Ala Arg Gly Met Glu Tyr Leu
        595                 600                 605

Ala Ser Gln Lys Cys Ile His Arg Asp Leu Ala Ala Arg Asn Val Leu
    610                 615                 620

Val Thr Glu Asp Asn Val Met Lys Ile Ala Asp Phe Gly Leu Ala Arg
625                 630                 635                 640

Asp Val His Asn Leu Asp Tyr Tyr Lys Lys Thr Thr Asn Gly Arg Leu
                645                 650                 655

Pro Val Lys Trp Met Ala Pro Glu Ala Leu Phe Asp Arg Val Tyr Thr
            660                 665                 670

His Gln Ser Asp Val Trp Ser Phe Gly Val Leu Leu Trp Glu Ile Phe
        675                 680                 685

Thr Leu Gly Gly Ser Pro Tyr Pro Gly Ile Pro Val Glu Glu Leu Phe
    690                 695                 700

Lys Leu Leu Lys Glu Gly His Arg Met Asp Lys Pro Ala Asn Cys Thr
705                 710                 715                 720

His Asp Leu Tyr Met Ile Met Arg Glu Cys Trp His Ala Ala Pro Ser
                725                 730                 735

Gln Arg Pro Thr Phe Lys Gln Leu Val Glu Asp Leu Asp Arg Val Leu
            740                 745                 750

Thr Val Thr Ser Thr Asp Glu Tyr Leu Asp Leu Ser Ala Pro Phe Glu
        755                 760                 765

Gln Tyr Ser Pro Gly Gly Gln Asp Thr Pro Ser Ser Ser Ser Ser Gly
    770                 775                 780

Asp Asp Ser Val Phe Ala His Asp Leu Leu Pro Pro Ala Pro Pro Ser
785                 790                 795                 800

Ser Gly Gly Ser Arg Thr
                805
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<210> 33

<211> 349

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 33

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Glu Ser Leu Gly Thr Glu Gln Arg Val Val Gly Arg Ala Ala Glu Val
1               5                  10                  15

Pro Gly Pro Glu Pro Gly Gln Gln Glu Gln Leu Val Phe Gly Ser Gly
            20                  25                  30

Asp Ala Val Glu Leu Ser Cys Pro Pro Pro Gly Gly Gly Pro Met Gly
        35                  40                  45

Pro Thr Val Trp Val Lys Asp Gly Thr Gly Leu Val Pro Ser Glu Arg
    50                  55                  60

Val Leu Val Gly Pro Gln Arg Leu Gln Val Leu Asn Ala Ser His Glu
65                  70                  75                  80

Asp Ser Gly Ala Tyr Ser Cys Arg Gln Arg Leu Thr Gln Arg Val Leu
                85                  90                  95

Cys His Phe Ser Val Arg Val Thr Asp Ala Pro Ser Ser Gly Asp Asp
            100                 105                 110

Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr Gly Val Asp Thr Gly Ala
        115                 120                 125

Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp Lys Lys Leu Leu Ala Val
    130                 135                 140

Pro Ala Ala Asn Thr Val Arg Phe Arg Cys Pro Ala Ala Gly Asn Pro
145                 150                 155                 160

Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly Arg Glu Phe Arg Gly Glu
                165                 170                 175

His Arg Ile Gly Gly Ile Lys Leu Arg His Gln Gln Trp Ser Leu Val
            180                 185                 190

Met Glu Ser Val Val Pro Ser Asp Arg Gly Asn Tyr Thr Cys Val Val
        195                 200                 205

Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr Tyr Thr Leu Asp Val Leu
    210                 215                 220

Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
225                 230                 235                 240

Gln Thr Ala Val Leu Gly Ser Asp Val Glu Phe His Cys Lys Val Tyr
                245                 250                 255

Ser Asp Ala Gln Pro His Ile Gln Trp Leu Lys His Val Glu Val Asn
            260                 265                 270

Gly Ser Lys Val Gly Pro Asp Gly Thr Pro Tyr Val Thr Val Leu Lys
```

```
        275                 280                 285

Thr Ala Gly Ala Asn Thr Thr Asp Lys Glu Leu Glu Val Leu Ser Leu
    290                 295                 300

His Asn Val Thr Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly
305                 310                 315                 320

Asn Ser Ile Gly Phe Ser His His Ser Ala Trp Leu Val Val Leu Pro
                325                 330                 335

Val Ser Leu Glu Ser Asn Ala Ser Met Ser Ser Asn Thr
            340                 345
```

<210> 34

<211> 368

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 34

```
Met Met Ser Phe Val Ser Leu Leu Leu Val Gly Ile Leu Phe His Ala
1               5                   10                  15

Thr Gln Ala Glu Ser Leu Gly Thr Glu Gln Arg Val Val Gly Arg Ala
            20                  25                  30

Ala Glu Val Pro Gly Pro Glu Pro Gly Gln Gln Glu Gln Leu Val Phe
        35                  40                  45

Gly Ser Gly Asp Ala Val Glu Leu Ser Cys Pro Pro Pro Gly Gly Gly
    50                  55                  60

Pro Met Gly Pro Thr Val Trp Val Lys Asp Gly Thr Gly Leu Val Pro
65                  70                  75                  80

Ser Glu Arg Val Leu Val Gly Pro Gln Arg Leu Gln Val Leu Asn Ala
                85                  90                  95

Ser His Glu Asp Ser Gly Ala Tyr Ser Cys Arg Gln Arg Leu Thr Gln
            100                 105                 110

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

Gly Asp Asp Glu Asp Gly Glu Asp Glu Ala Glu Asp Thr Gly Val Asp
    130                 135                 140

Thr Gly Ala Pro Tyr Trp Thr Arg Pro Glu Arg Met Asp Lys Lys Leu
145                 150                 155                 160

Leu Ala Val Pro Ala Ala Asn Thr Val Arg Phe Arg Cys Pro Ala Ala
                165                 170                 175

Gly Asn Pro Thr Pro Ser Ile Ser Trp Leu Lys Asn Gly Arg Glu Phe
            180                 185                 190

Arg Gly Glu His Arg Ile Gly Gly Ile Lys Leu Arg His Gln Gln Trp
        195                 200                 205
```

```
Ser Leu Val Met Glu Ser Val Val Pro Ser Asp Arg Gly Asn Tyr Thr
    210                 215                 220

Cys Val Val Glu Asn Lys Phe Gly Ser Ile Arg Gln Thr Tyr Thr Leu
225                 230                 235                 240

Asp Val Leu Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu
                245                 250                 255

Pro Ala Asn Gln Thr Ala Val Leu Gly Ser Asp Val Glu Phe His Cys
            260                 265                 270

Lys Val Tyr Ser Asp Ala Gln Pro His Ile Gln Trp Leu Lys His Val
        275                 280                 285

Glu Val Asn Gly Ser Lys Val Gly Pro Asp Gly Thr Pro Tyr Val Thr
    290                 295                 300

Val Leu Lys Thr Ala Gly Ala Asn Thr Thr Asp Lys Glu Leu Glu Val
305                 310                 315                 320

Leu Ser Leu His Asn Val Thr Phe Glu Asp Ala Gly Glu Tyr Thr Cys
                325                 330                 335

Leu Ala Gly Asn Ser Ile Gly Phe Ser His His Ser Ala Trp Leu Val
            340                 345                 350

Val Leu Pro Val Ser Leu Glu Ser Asn Ala Ser Met Ser Ser Asn Thr
        355                 360                 365
```

<210> 35

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 35

```
Met Met Ser Phe Val Ser Leu Leu Leu Val Gly Ile Leu Phe His Ala
1               5                   10                  15

Thr Gln Ala
```

<210> 36

<211> 963

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 36

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atgatgtcct ttgtctctct gctcctggtt ggcatcctat tccatgccac ccaggccgag       60

tccttgggga cggagcagcg cgtcgtgggg cgagcggcag aagtcccggg cccagagccc      120

ggccagcagg agcagttggt cttcggcagc ggggatgctg tggagctgag ctgtcccccg      180

cccgggggtg gtcccatggg gcccactgtc tgggtcaagg atggcacagg gctggtgccc      240

tcggagcgtg tcctggtggg gccccagcgg ctgcaggtgc tgaatgcctc ccacgaggac      300
```

tccggggcct acagctgccg gcagcggctc acgcagcgcg tactgtgcca cttcagtgtg 360

cgggtgacag acgctccatc ctcgggagat gacgaagacg gggaggacga ggctgaggac 420

acaggtgtgg acacaggggc cccttactgg acacggcccg agcggatgga caagaagctg 480

ctggccgtgc cggccgccaa caccgtccgc ttccgctgcc cagccgctgg caaccccact 540

ccctccatct cctggctgaa gaacggcagg gagttccgcg gcgagcaccg cattggaggc 600

atcaagctgc ggcatcagca gtggagcctg gtcatggaaa gcgtggtgcc ctcggaccgc 660

ggcaactaca cctgcgtcgt ggagaacaag tttggcagca tccggcagac gtacacgctg 720

gacgtgctgg agcgctcccc gcaccggccc atcctgcagg cggggctgcc ggccaaccag 780

acggcggtgc tgggcagcga cgtggagttc cactccaagg tgtacagtga cgcacagccc 840

cacatccagt ggctcaagca cgtggaggtg aatggcagca aggtgggccc ggacggcaca 900

ccctacgtta ccgtgctcaa ggtgtccctg gagtccaacg cgtccatgag ctccaacaca 960

tga 963

<210> 37
<211> 1107
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic construct

<400> 37
atgatgtcct ttgtctctct gctcctggtt ggcatcctat tccatgccac ccaggccgag 60

tccttgggga cggagcagcg cgtcgtgggg cgagcggcag aagtcccggg cccagagccc 120

ggccagcagg agcagttggt cttcggcagc ggggatgctg tggagctgag ctgtcccccg 180

cccgggggtg gtcccatggg gcccactgtc tgggtcaagg atggcacagg gctggtgccc 240

tcggagcgtg tcctggtggg gccccagcgg ctgcaggtgc tgaatgcctc ccacgaggac 300

tccggggcct acagctgccg gcagcggctc acgcagcgcg tactgtgcca cttcagtgtg 360

cgggtgacag acgctccatc ctcgggagat gacgaagacg gggaggacga ggctgaggac 420

acaggtgtgg acacaggggc cccttactgg acacggcccg agcggatgga caagaagctg 480

ctggccgtgc cggccgccaa caccgtccgc ttccgctgcc cagccgctgg caaccccact 540

ccctccatct cctggctgaa gaacggcagg gagttccgcg gcgagcaccg cattggaggc 600

atcaagctgc ggcatcagca gtggagcctg gtcatggaaa gcgtggtgcc ctcggaccgc 660

ggcaactaca cctgcgtcgt ggagaacaag tttggcagca tccggcagac gtacacgctg 720

gacgtgctgg agcgctcccc gcaccggccc atcctgcagg ctgggctgcc tgctaaccag 780

acagcggtgc tgggcagcga cgtggagttc cactgcaagg tgtacagtga cgcacagccc 840

cacatccagt ggctcaagca cgtggaggtg aatggcagca aggtgggccc ggacggcaca 900

ccctacgtta ccgtgctcaa gacggcgggc gctaacacca ccgacaagga gctagaggtt 960

ctctccttgc acaacgtcac ctttgaggac gccggggagt acacctgcct ggcgggcaat 1020

tctattgggt tttctcatca ctctgcgtgg ctggtggtgc tgccagtgtc cctggagtcc 1080

aacgcgtcca tgagctccaa cacatga 1107

<210> 38
<211> 216
<212> PRT

<213> Homo sapiens

<400> 38

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Met Arg Ser Gly Cys Val Val Val His Val Trp Ile Leu Ala Gly Leu
1               5                  10                  15

Trp Leu Ala Val Ala Gly Arg Pro Leu Ala Phe Ser Asp Ala Gly Pro
            20                  25                  30

His Val His Tyr Gly Trp Gly Asp Pro Ile Arg Leu Arg His Leu Tyr
        35                  40                  45

Thr Ser Gly Pro His Gly Leu Ser Ser Cys Phe Leu Arg Ile Arg Ala
    50                  55                  60

Asp Gly Val Val Asp Cys Ala Arg Gly Gln Ser Ala His Ser Leu Leu
65                  70                  75                  80

Glu Ile Lys Ala Val Ala Leu Arg Thr Val Ala Ile Lys Gly Val His
                85                  90                  95

Ser Val Arg Tyr Leu Cys Met Gly Ala Asp Gly Lys Met Gln Gly Leu
            100                 105                 110

Leu Gln Tyr Ser Glu Glu Asp Cys Ala Phe Glu Glu Glu Ile Arg Pro
        115                 120                 125

Asp Gly Tyr Asn Val Tyr Arg Ser Glu Lys His Arg Leu Pro Val Ser
    130                 135                 140

Leu Ser Ser Ala Lys Gln Arg Gln Leu Tyr Lys Asn Arg Gly Phe Leu
145                 150                 155                 160

Pro Leu Ser His Phe Leu Pro Met Leu Pro Met Val Pro Glu Glu Pro
                165                 170                 175

Glu Asp Leu Arg Gly His Leu Glu Ser Asp Met Phe Ser Ser Pro Leu
            180                 185                 190

Glu Thr Asp Ser Met Asp Pro Phe Gly Leu Val Thr Gly Leu Glu Ala
        195                 200                 205

Val Arg Ser Pro Ser Phe Glu Lys
    210                 215
```

<210> 39

<211> 209

<212> PRT

<213> Homo sapiens

<400> 39

```
Met Asp Ser Asp Glu Thr Gly Phe Glu His Ser Gly Leu Trp Val Ser
1               5                  10                  15

Val Leu Ala Gly Leu Leu Leu Gly Ala Cys Gln Ala His Pro Ile Pro
            20                  25                  30

Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr
        35                  40                  45

Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg
    50                  55                  60
```

```
Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu
65                  70                  75                  80

Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val
                85                  90                  95

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

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

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

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

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

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

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

Ser
```

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Patentkrav

1. Opløselig fibroblastvækstfaktorreceptor 3 (sFGFR3)-polypeptid, hvis aminosyresekvens består af en aminosyresekvens med mindst 99 % sekvensidentitet med aminosyresekvensen af SEQ ID NO: 4.

2. sFGFR3-polypeptid ifølge krav 1, hvor:
(i) sFGFR3-polypeptidet er et isoleret sFGFR3-polypeptid; og/eller
(ii) sFGFR3-polypeptidet binder til fibroblastvækstfaktor 1 (FGF1), fibroblastvækstfaktor 2 (FGF2), fibroblastvækstfaktor 9 (FGF9) eller fibroblastvækstfaktor 18 (FGF18).

3. Farmaceutisk sammensætning omfattende sFGFR3-polypeptidet ifølge krav 1 eller 2 og et farmaceutisk acceptabelt hjælpestof, bærestof eller fortyndingsmiddel, fortrinsvis hvor den farmaceutiske sammensætning omfatter mellem ca. 1 mg/ml og ca. 500 mg/ml af sFGFR3-polypeptidet.

4. sFGFR3-polypeptid ifølge et hvilket som helst af kravene 1-2 til anvendelse inden for behandlingen af en skeletvæksthæmningsforstyrrelse i et individ, hvor eventuelt:
(i) sFGFR3-polypeptidet er indgivet i en dosis på ca. 0,002 mg/kg til ca. 20 mg/kg som for eksempel en dosis på ca. 0,01 mg/kg til ca. 10 mg/kg eller en dosis på ca. 0,2 mg/kg til ca. 20 mg/kg; og/eller
(ii) sFGFR3-polypeptidet er formuleret til daglig, ugentlig eller månedlig indgivelse.

5. sFGFR3-polypeptid til anvendelse ifølge krav 4, hvor:
(i) sFGFR3-polypeptidet er formuleret til indgivelse syv gange om ugen, seks gange om ugen, fem gange om ugen, fire gange om ugen, tre gange om ugen eller to gange om ugen; og/eller
(ii) sFGFR3-polypeptidet er formuleret til parenteral indgivelse, enteral indgivelse eller topisk indgivelse, hvor eventuelt sFGFR3-polypeptidet er formuleret til subkutan indgivelse, intravenøs indgivelse, intramuskulær indgivelse, intraarteriel indgivelse, intrathekal indgivelse eller intraperitoneal indgivelse.

6. sFGFR3-polypeptid til anvendelse ifølge krav 4 eller 5, hvor skeletvæksthæmningsforstyrrelsen er en FGFR3-relateret skeletsygdom, hvor eventuelt den FGFR3-relaterede skeletsygdom er udvalgt fra gruppen bestående af akondroplasi, thanatofor dysplasi type I (TDI), thanatofor dysplasi type II (TDII), alvorlig akondroplasi med udviklingsforsinkelse og acanthosis nigricans (SADDEN), hypochondroplasi, et craniosynostosis syndrom og camptodactyly, høj statur og høretabsyndrom (CATSHL).

7. sFGFR3-polypeptid til anvendelse ifølge krav 6, hvor:
(i) skeletvæksthæmningsforstyrrelsen er akondroplasi;
(ii) craniosynostosis syndromet er udvalgt fra gruppen bestående af Muenke-syndrom, Crouzon-syndrom og Crouzonodermoskeletsyndrom; eller
(iii) den FGFR3-relaterede skeletsygdom skyldes ekspression i individet af en konstitutivt aktiv FGFR3, hvor eventuelt den konstitutivt aktive FGFR3 omfatter en aminosyresubstitution af en glycinrest med en argininrest ved position 380 af SEQ ID NO: 5.

8. sFGFR3-polypeptid til anvendelse ifølge et hvilket som helst af kravene 4-7, hvor:
(i) individet er blevet diagnosticeret med skeletvæksthæmningsforstyrrelsen;
(ii) individet udviser et eller flere symptomer på skeletvæksthæmningsforstyrrelsen udvalgt fra gruppen bestående af korte lemmer, kort krop, hjulbenethed, en vraltende gang, kraniemisdannelser, kløverbladskranium, craniosynostosis, ormeknogler, anomalier i hænderne, anomalier i fødderne, blaffertommelfinger og brystanomalier;
(iii) individet udviser en forbedring i det ene eller de flere symptomer på skeletvæksthæmningsforstyrrelsen efter indgivelse af sFGFR3-polypeptidet;
(iv) individet har ikke tidligere fået indgivet sFGFR3-polypeptidet;
(v) individet er udvalgt fra gruppen bestående af et spædbarn, et barn og en voksen; og/eller
(vi) individet er et menneske.

9. sFGFR3-polypeptid til anvendelse ifølge et hvilket som helst af kravene 4-8, hvor:
(i) sFGFR3-polypeptidet øger individets overlevelse; og/eller
(ii) sFGFR3-polypeptidet genopretter foramen magnum-formen i individet.

10. Kit omfattende sFGFR3-polypeptidet ifølge krav 1, hvor kittet eventuelt omfatter instruktioner til anvendelse af kittet.

DRAWINGS

FIG. 1A

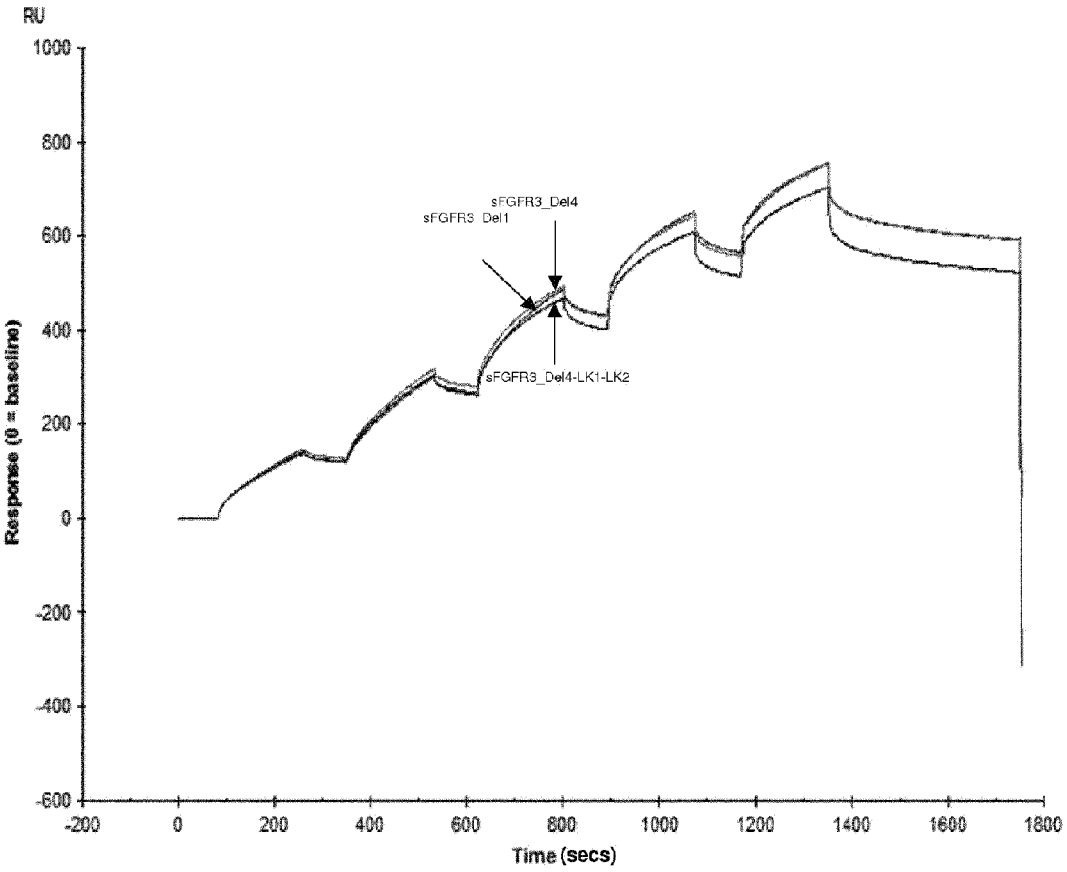


FIG. 1B

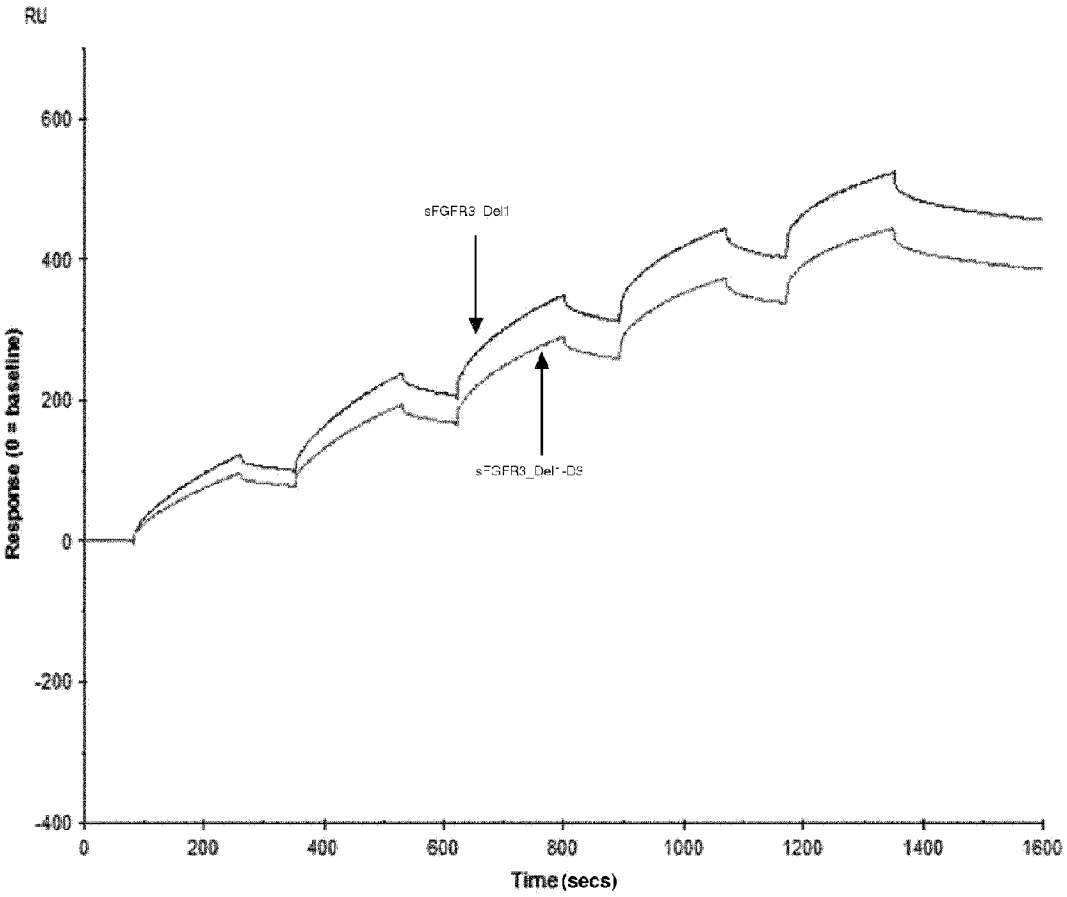

RU
600
400
200
0
-200
-400
Response (0 = baseline)
sFGFR3_Del1
0
200
400
600
800
1000
1200
1400
1600
Time (secs)

FIG. 1C

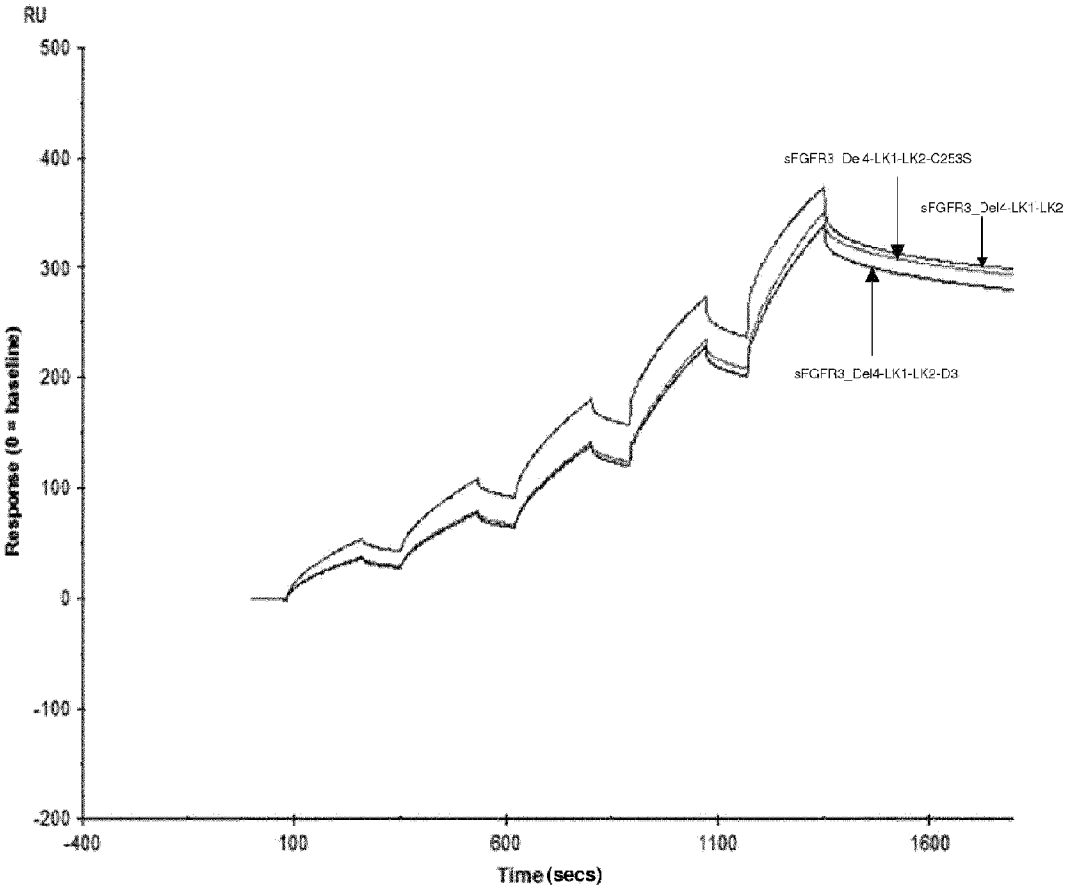

RU
500
400
300
200
100
0
-100
-200
Response (0 = baseline)
-400
100
600
1100
1600
Time (secs)
sFGFR3_Del4-LK1-LK2-C253S
sFGFR3_Del4-LK1-LK2
sFGFR3_Del4-LK1-LK2-D3

FIG. 1D

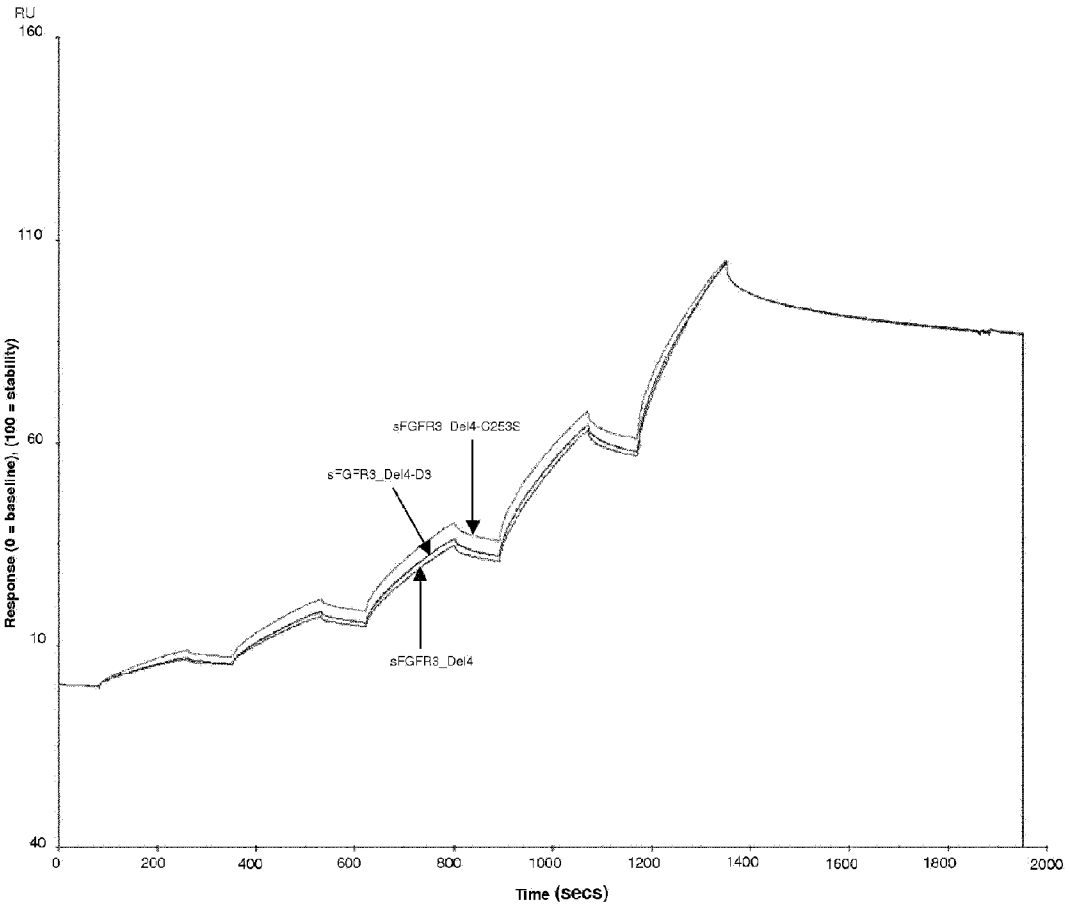

RU
160
110
60
10
40
Response (0 = baseline), (100 = stability)
sFGFR3_Del4-G253S
sFGFR3_Del4-D3
sFGFR3_Del4
0
200
400
600
800
1000
1200
1400
1600
1800
2000
Time (secs)

FIG. 2A

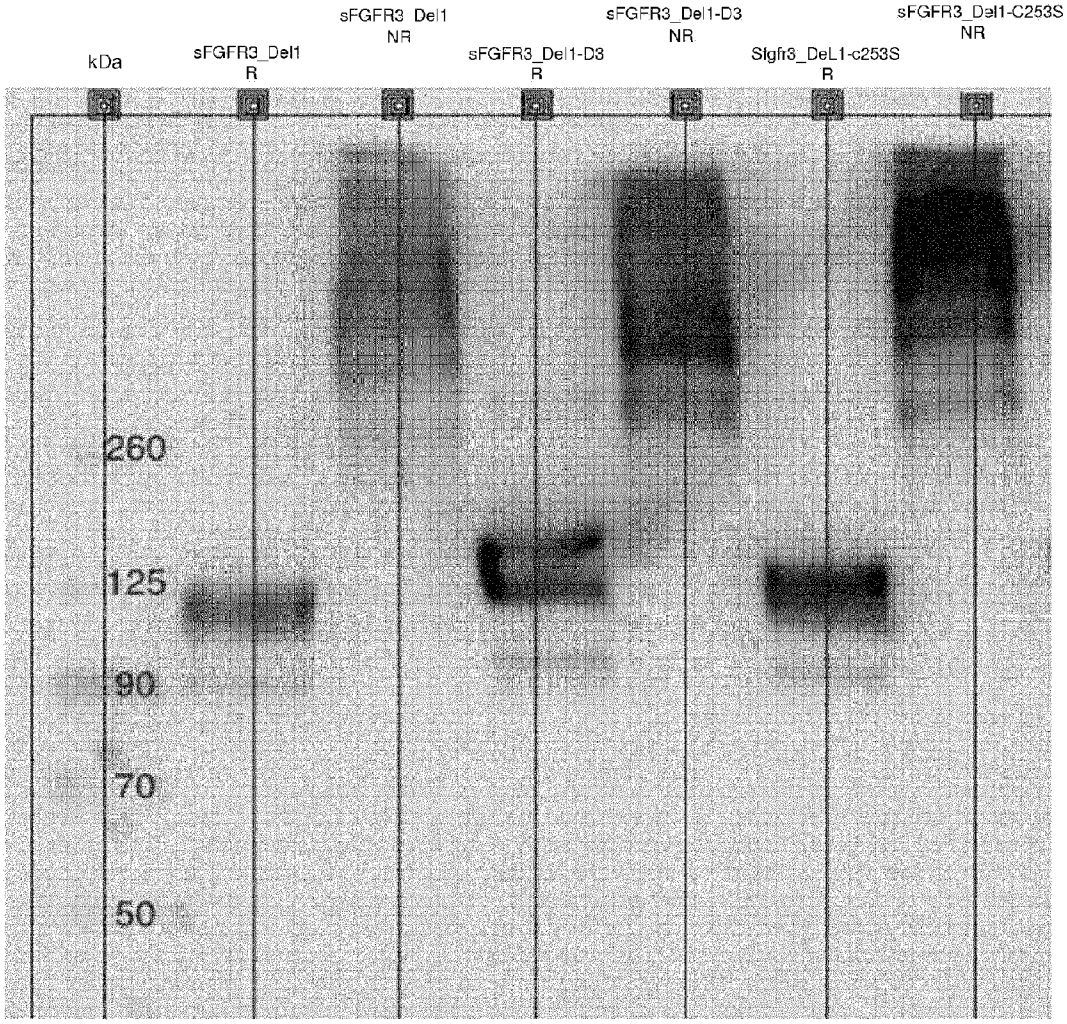

kDa
sFGFR3_Del1 R
sFGFR3_Del1 NR
sFGFR3_Del1-D3 R
sFGFR3_Del1-D3 NR
Sfgfr3_DeL1-c253S R
sFGFR3_Del1-C253S NR
260
125
90
70
50

FIG. 2B

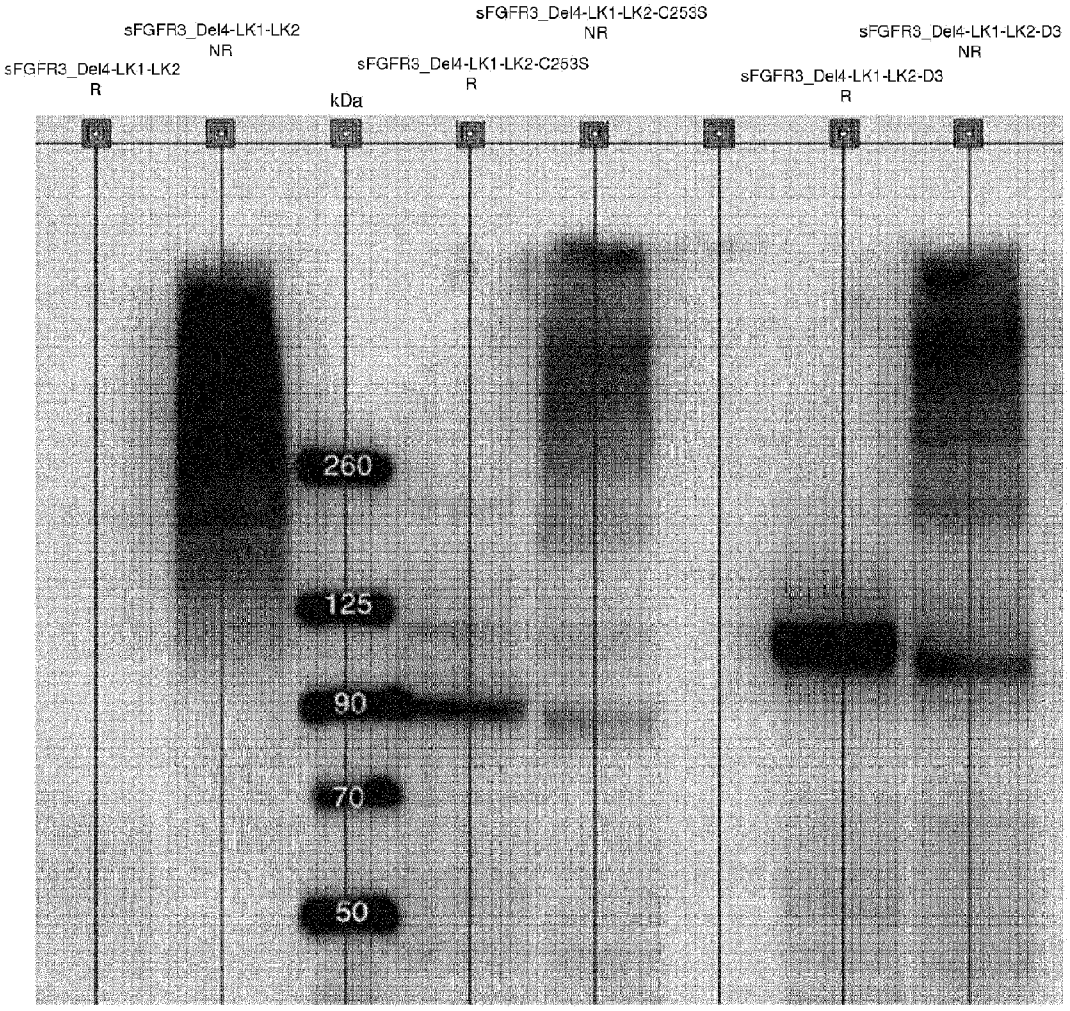

sFGFR3_Del4-LK1-LK2
R
sFGFR3_Del4-LK1-LK2
NR
kDa
sFGFR3_Del4-LK1-LK2-C253S
R
sFGFR3_Del4-LK1-LK2-C253S
NR
sFGFR3_Del4-LK1-LK2-D3
R
sFGFR3_Del4-LK1-LK2-D3
NR
260
125
90
70
50

FIG. 2C

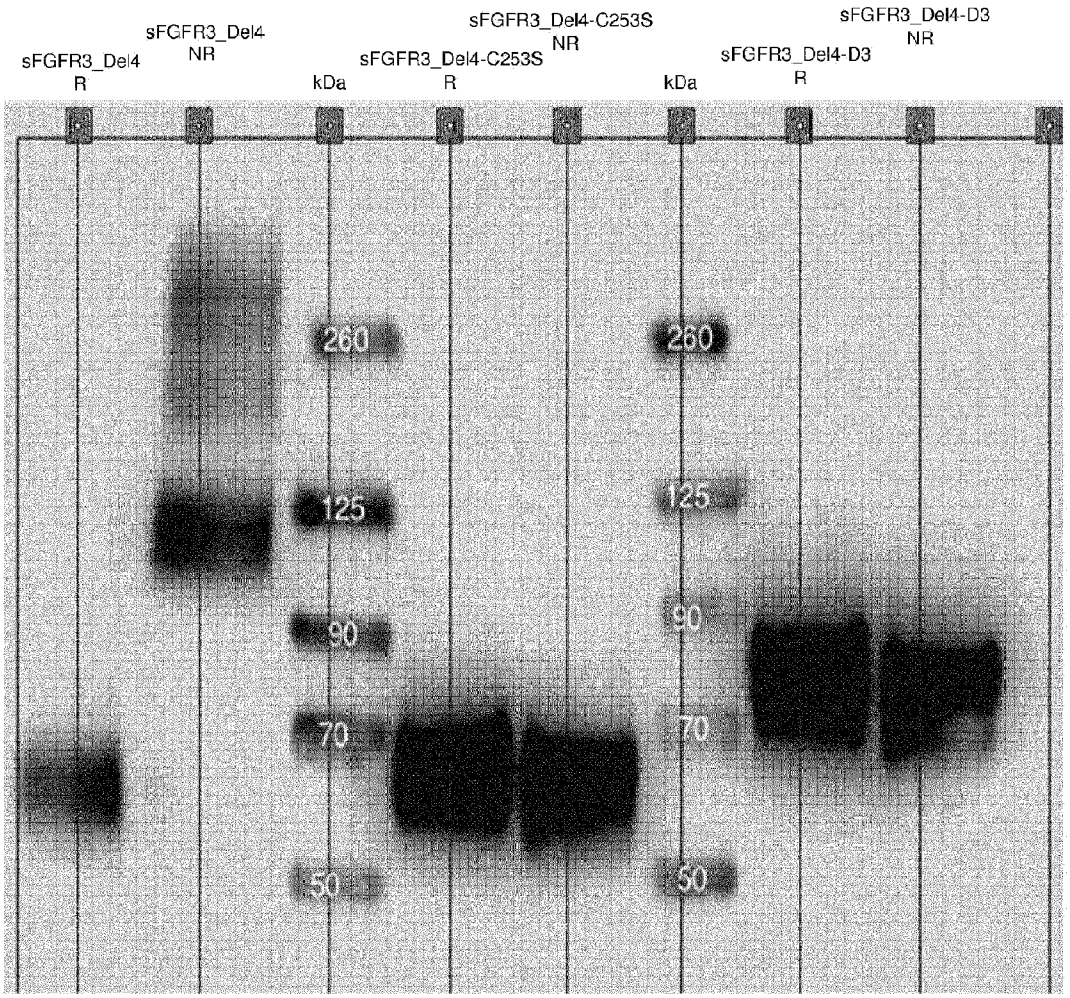
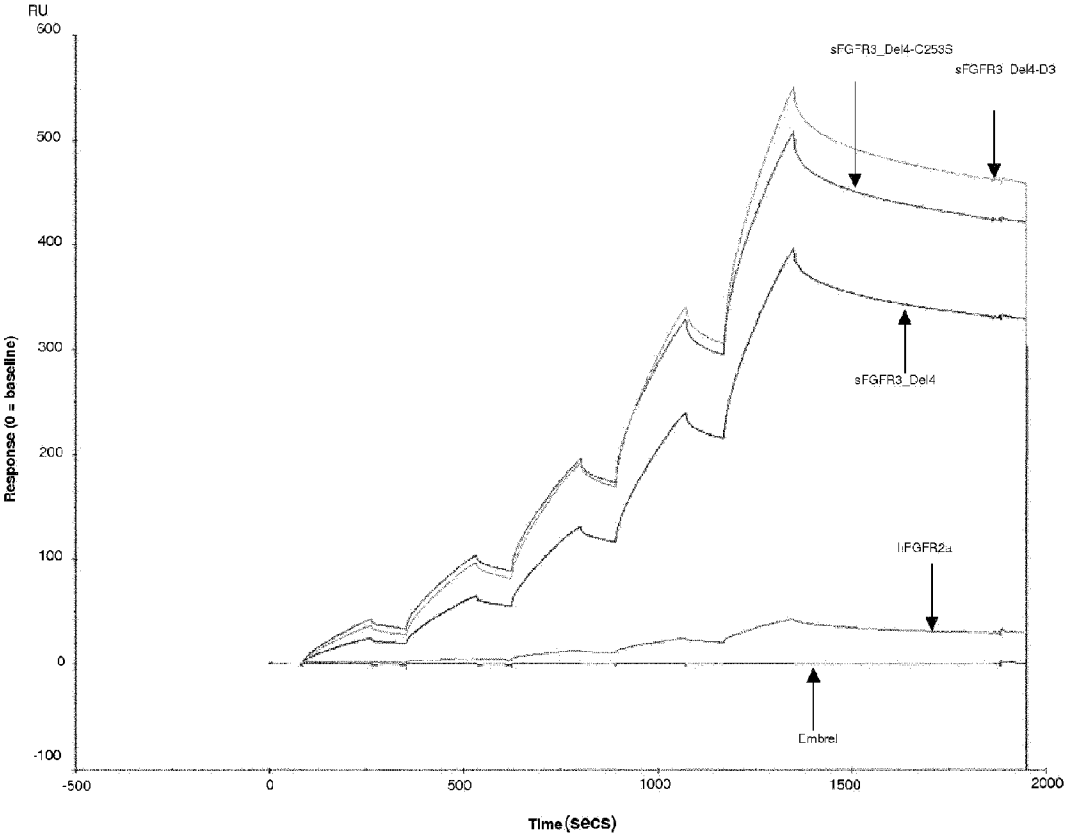

sFGFR3_Del4
R
sFGFR3_Del4
NR
kDa
sFGFR3_Del4-C253S
R
sFGFR3_Del4-C253S
NR
kDa
sFGFR3_Del4-D3
R
sFGFR3_Del4-D3
NR
260
125
90
70
50
260
125
90
70
50

FIG. 3A


RU
600
500
400
300
200
100
0
-100
Response (0 = baseline)
-500
0
500
1000
1500
2000
Time (secs)
sFGFR3_Del4-C253S
sFGFR3_Del4-D3
sFGFR3_Del4
hFGFR2-IIIc
Embrel

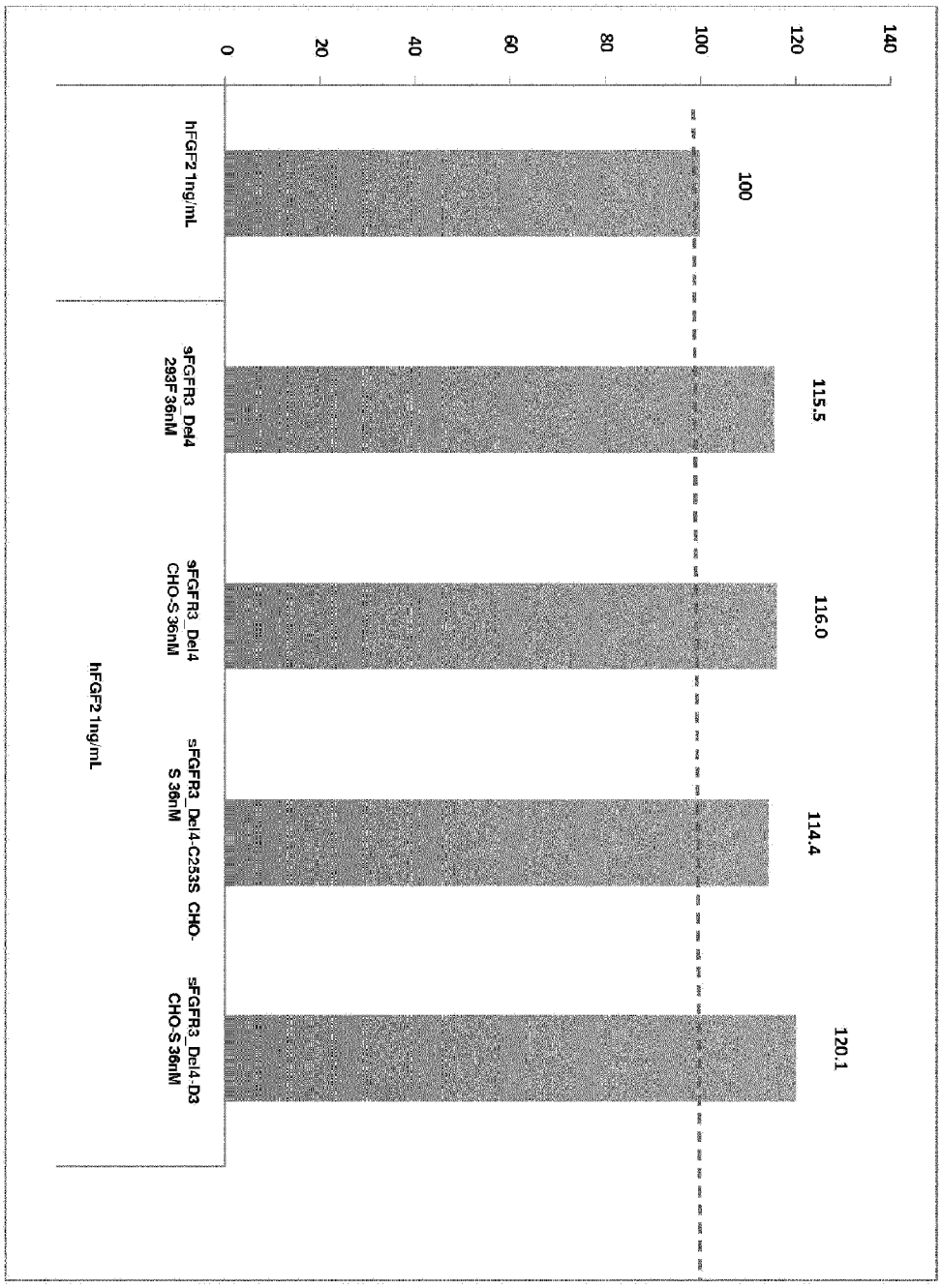

140
120
100
80
60
40
20
0
100
115.5
116.0
114.4
120.1
hFGF21ng/mL
sFGFR3_Del4 293F36nM
sFGFR3_Del4 CHO-S36nM
sFGFR3_Del4-C233S CHO-S 36nM
sFGFR3_Del4-D3 CHO-S36nM
hFGF21ng/mL

FIG. 3B

FIG. 4

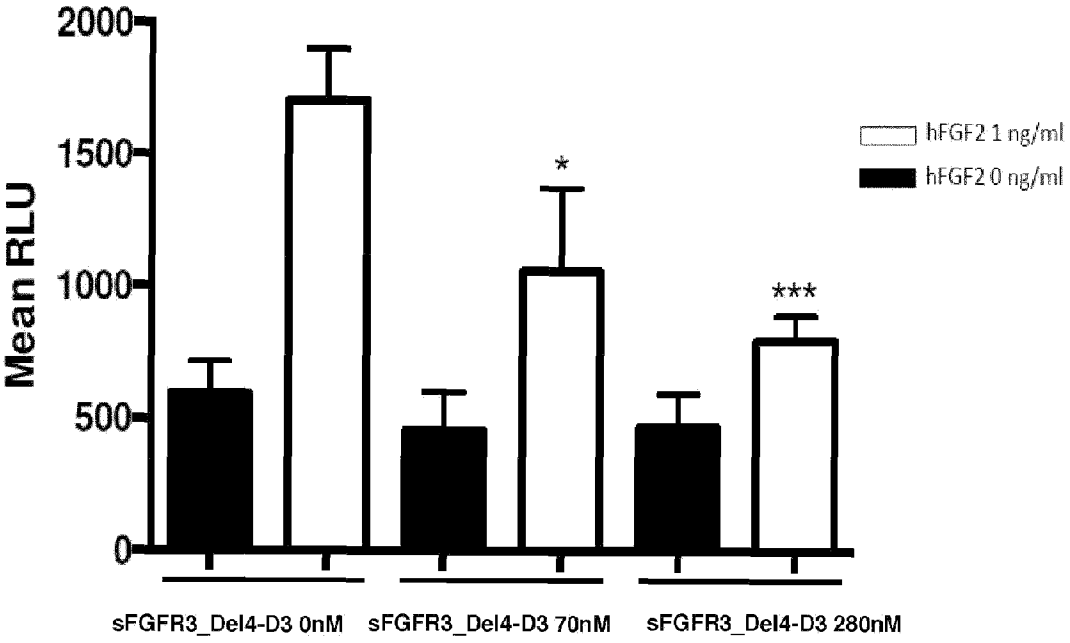
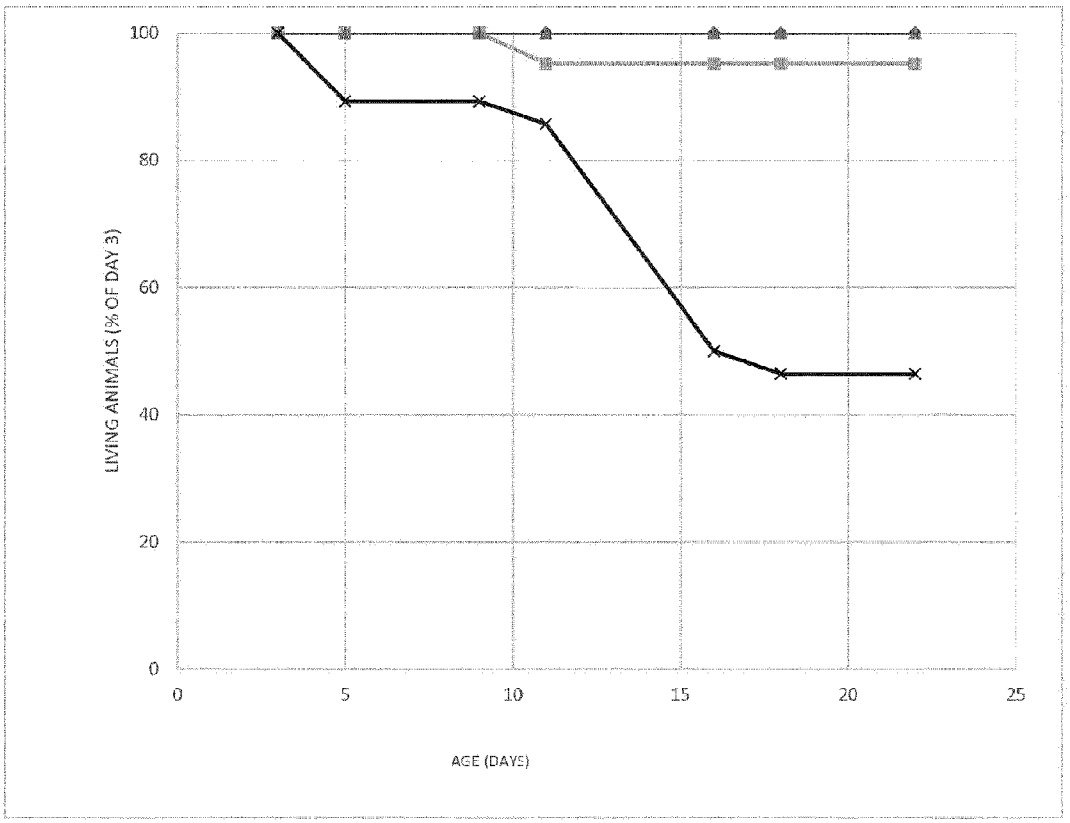

2000
1500
1000
500
0
Mean RLU
*
***
hFGF2 1 ng/ml
hFGF2 0 ng/ml
sFGFR3_Del4-D3 0nM
sFGFR3_Del4-D3 70nM
sFGFR3_Del4-D3 280nM

FIG. 5



▲ Fgfr3ach/+ sFGFR3_Del4-D3 0.25 mg/kg

▩ WT PBS

✖ Fgfr3ach/+ PBS

FIG. 6

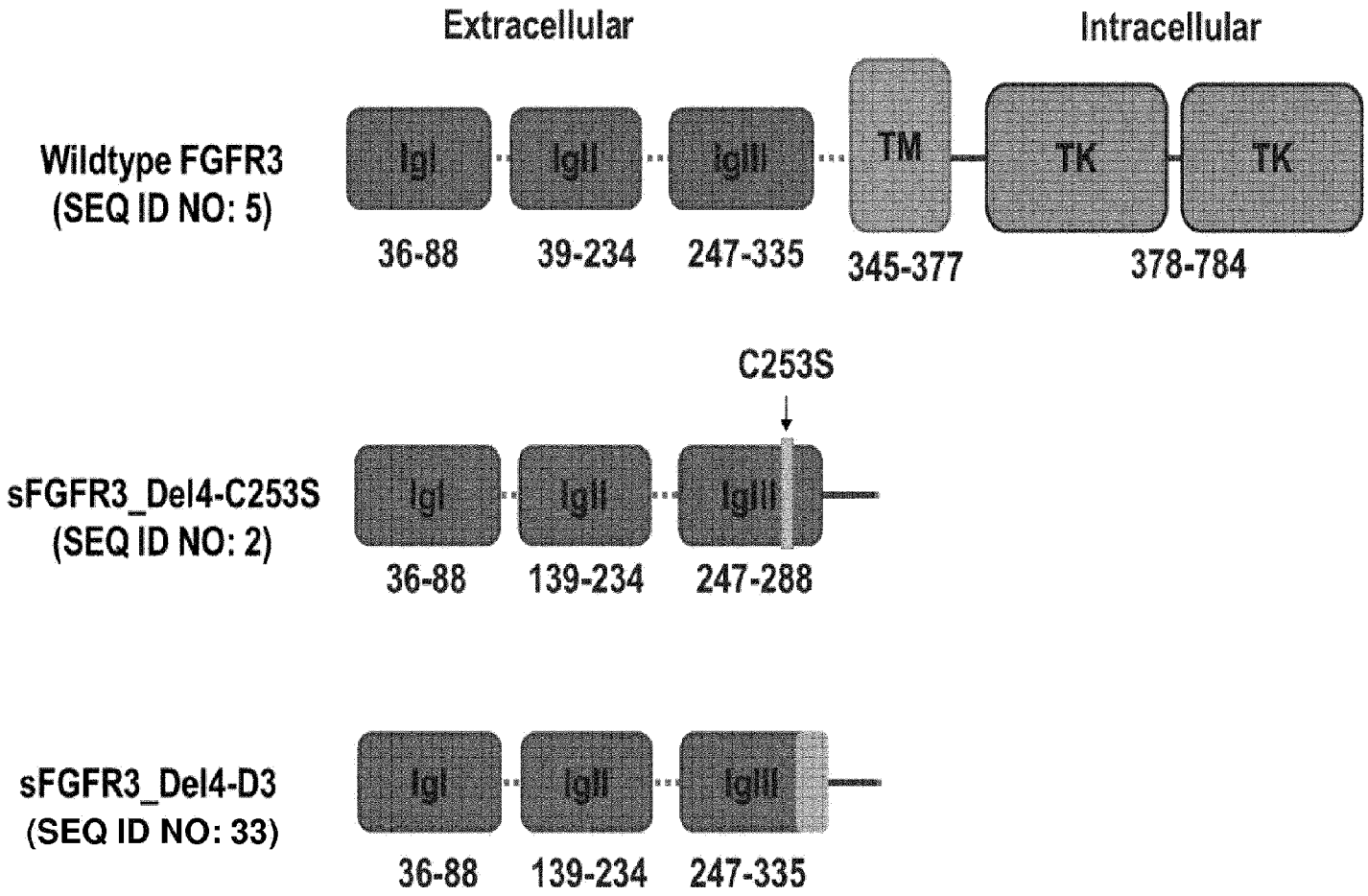

Extracellular
Intracellular
Wildtype FGFR3
(SEQ ID NO: 5)
IgI
IgII
IgIII
TM
TK
TK
36-88
39-234
247-335
345-377
378-784
C253S
sFGFR3_Del4-C253S
(SEQ ID NO: 2)
IgI
IgII
IgIII
36-88
139-234
247-288
sFGFR3_Del4-D3
(SEQ ID NO: 33)
IgI
IgII
IgIII
36-88
139-234
247-335

FIG. 7A

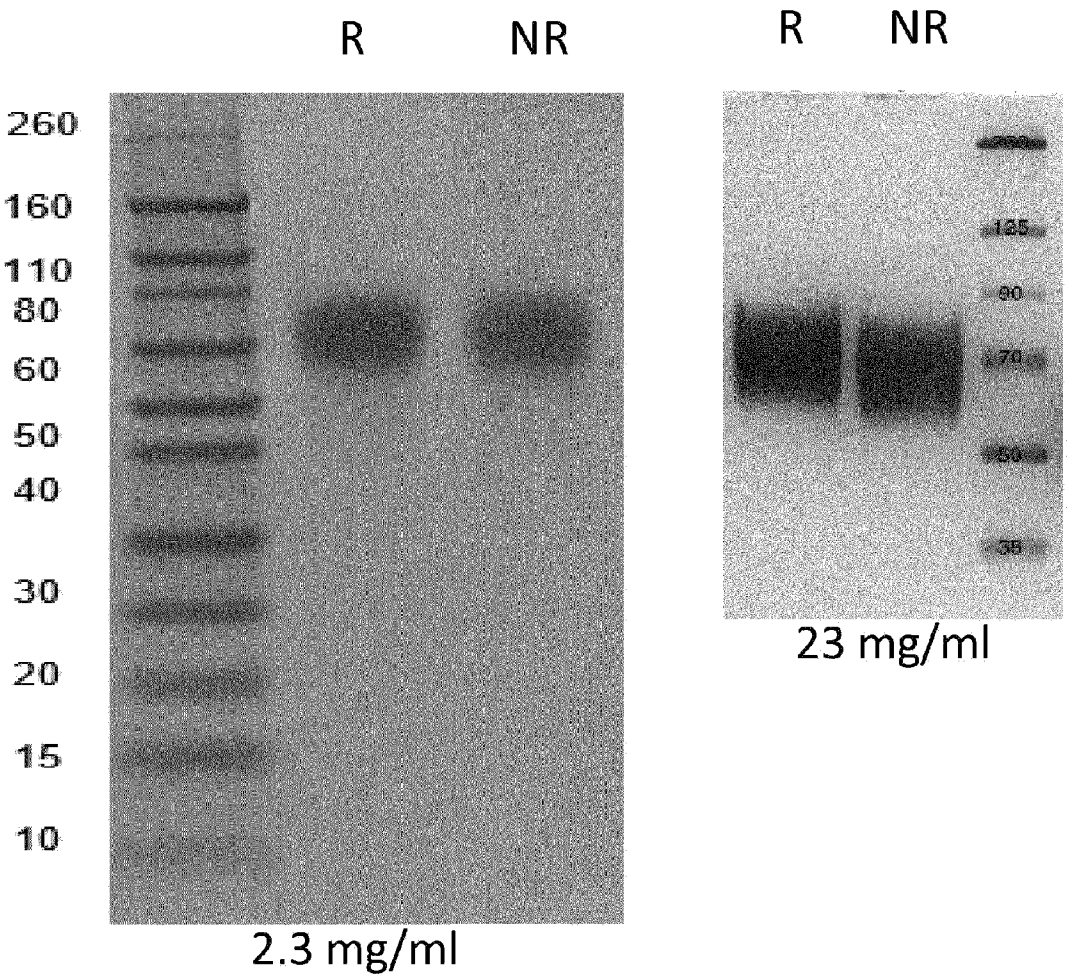

R
NR
260
160
110
80
60
50
40
30
20
15
10
2.3 mg/ml
R
NR
135
90
70
50
35
23 mg/ml

FIG. 7B

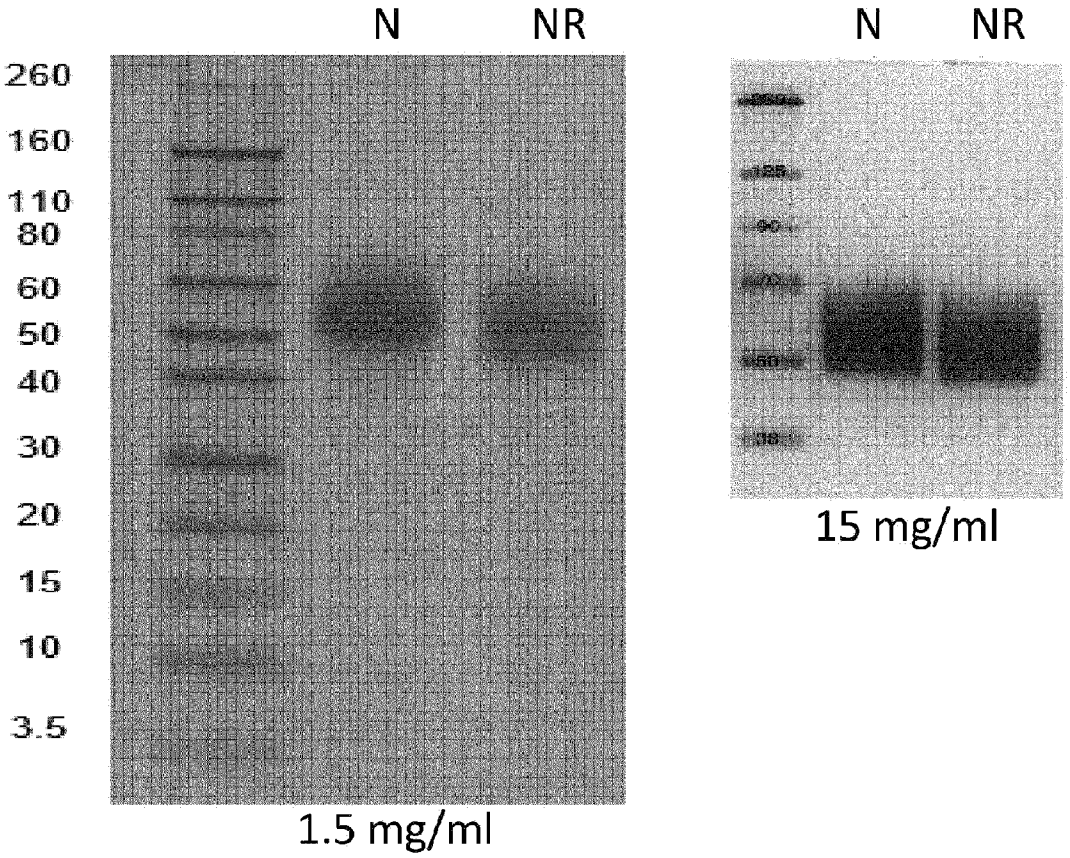


FIG. 8A

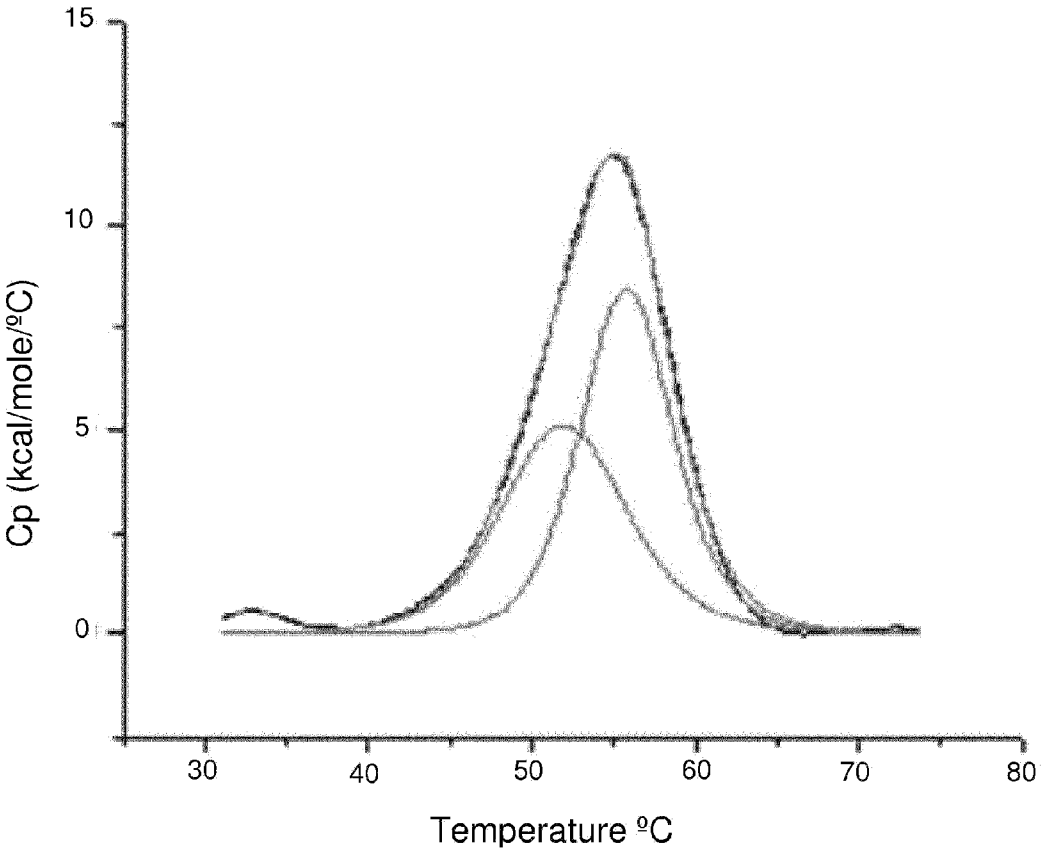


FIG. 8B

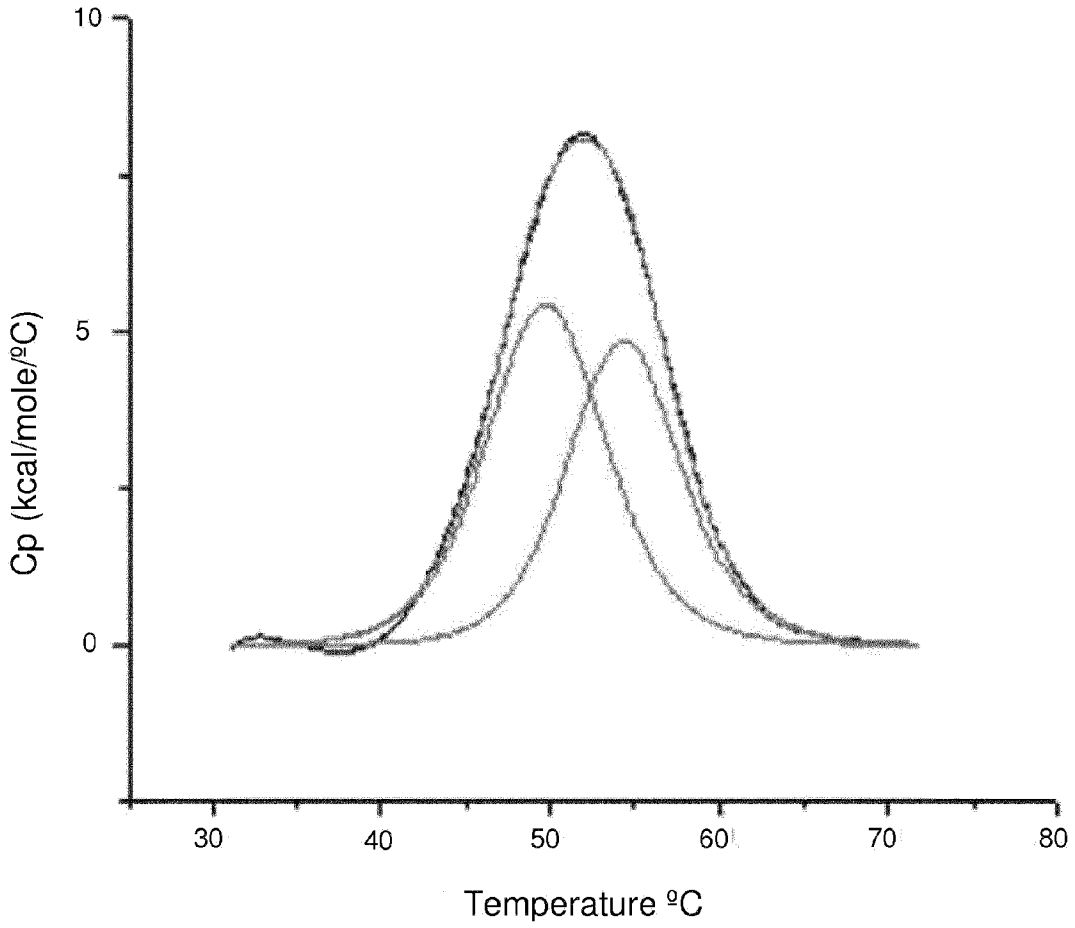


FIG. 9A

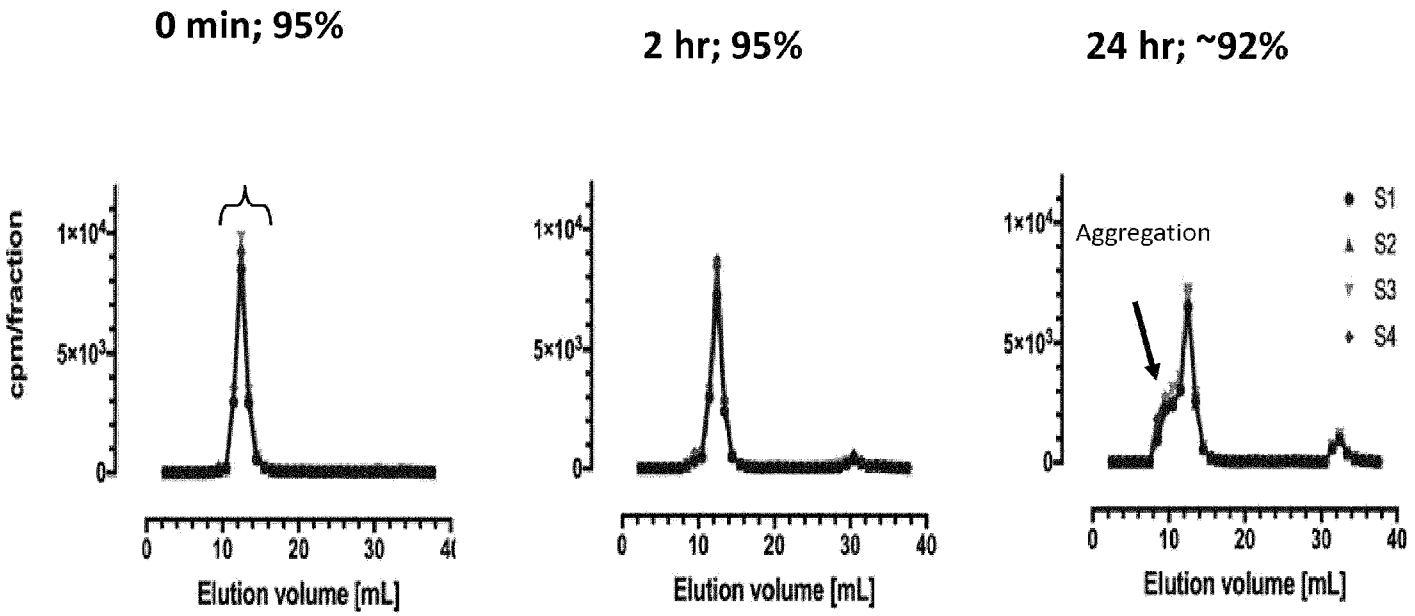

0 min; 95%
2 hr; 95%
24 hr; ~92%
cpm/fraction
1×10⁴
5×10³
0
0
10
20
30
40
Elution volume [mL]
Aggregation
S1
S2
S3
S4

FIG. 9B

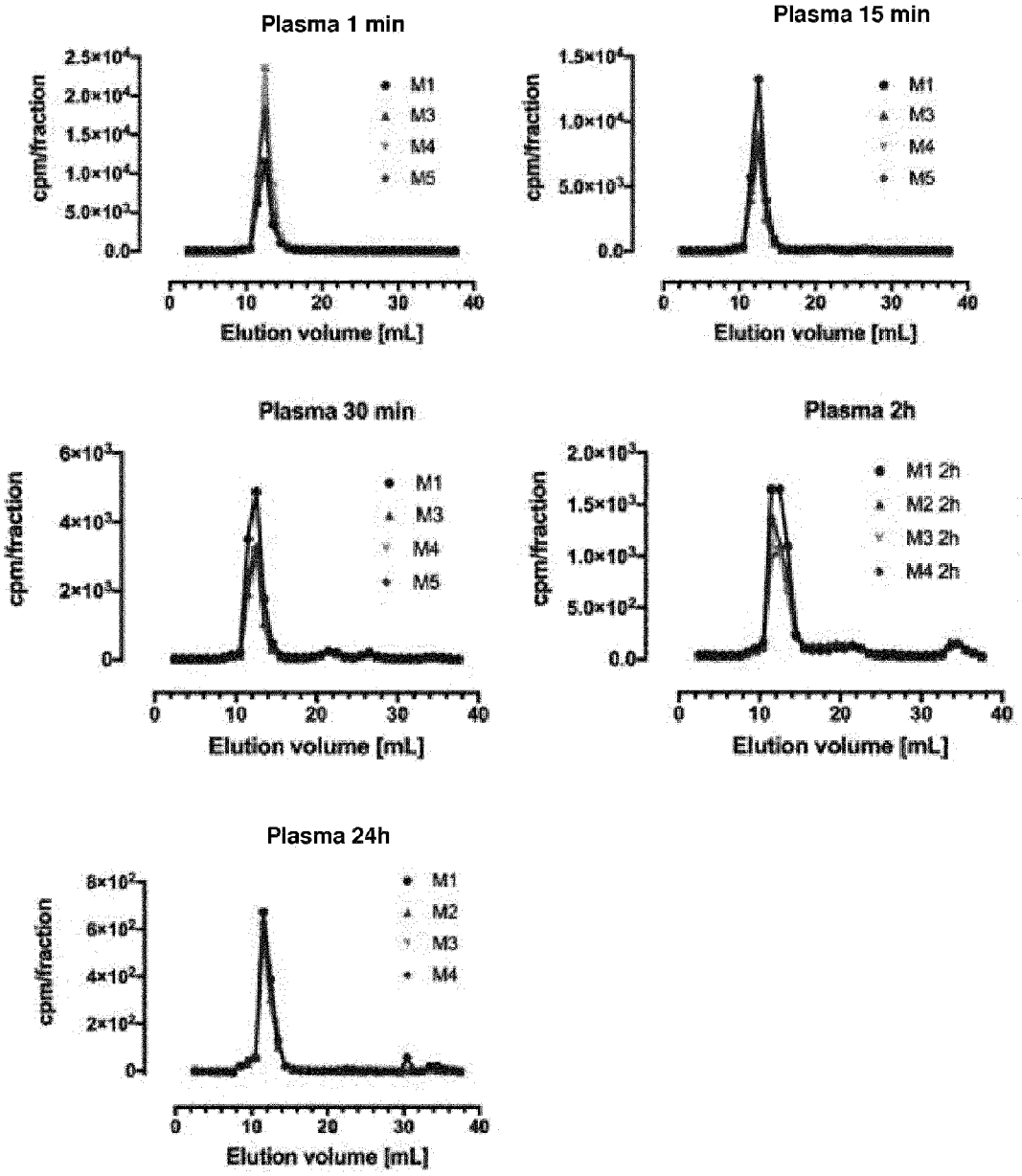
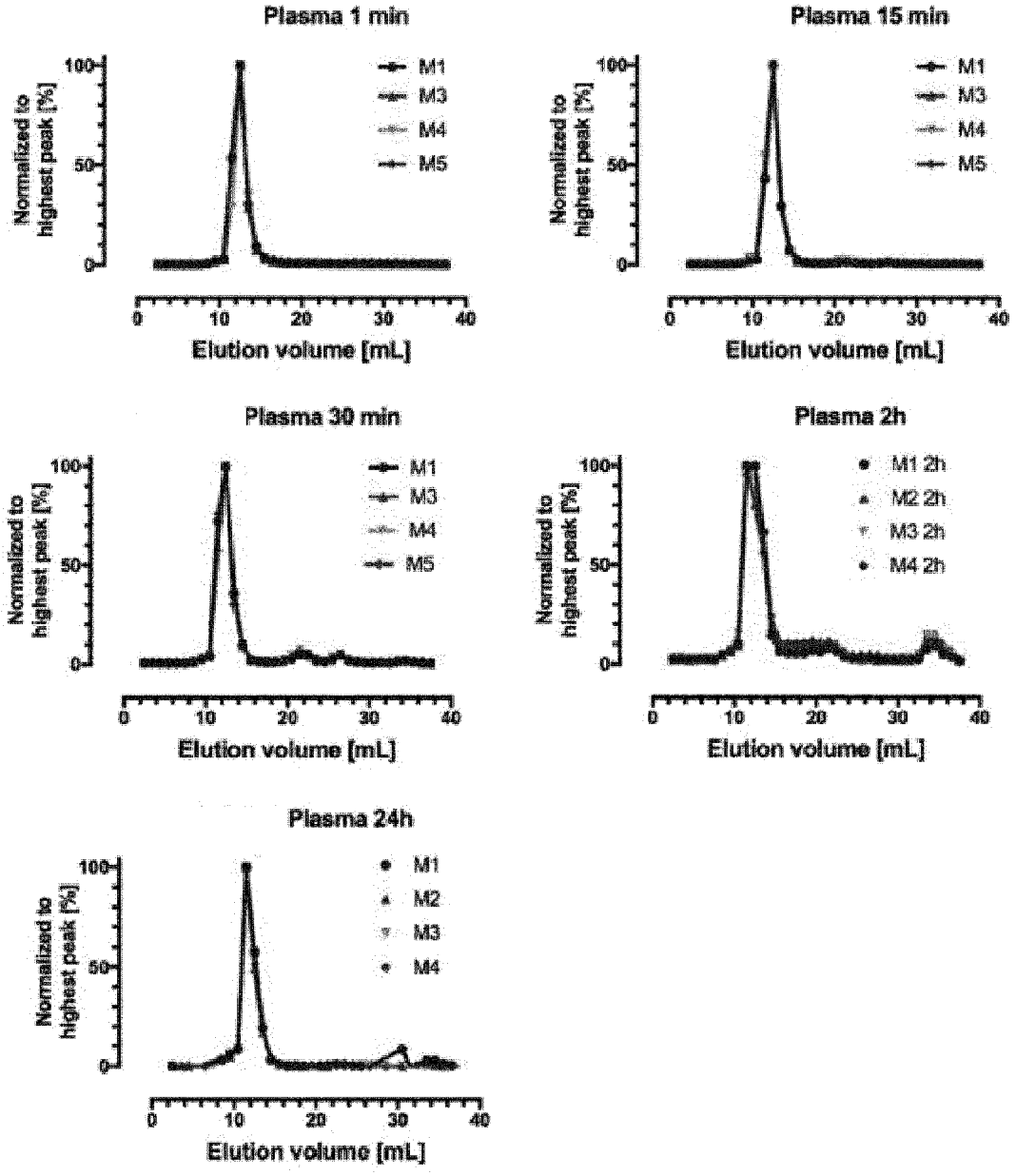

Plasma 1 min
cpm/fraction
2.5×10⁴
2.0×10⁴
1.5×10⁴
1.0×10⁴
5.0×10³
0.0
M1
M3
M4
M5
0
10
20
30
40
Elution volume [mL]
Plasma 15 min
cpm/fraction
1.5×10⁴
1.0×10⁴
5.0×10³
0.0
M1
M3
M4
M5
0
10
20
30
40
Elution volume [mL]
Plasma 30 min
cpm/fraction
6×10³
4×10³
2×10³
0
M1
M3
M4
M5
0
10
20
30
40
Elution volume [mL]
Plasma 2h
cpm/fraction
2.0×10³
1.5×10³
1.0×10³
5.0×10²
0.0
M1 2h
M2 2h
M3 2h
M4 2h
0
10
20
30
40
Elution volume [mL]
Plasma 24h
cpm/fraction
8×10²
6×10²
4×10²
2×10²
0
M1
M2
M3
M4
0
10
20
30
40
Elution volume [mL]

FIG. 9B cont.


Plasma 1 min
Normalized to highest peak [%]
100
50
0
M1
M3
M4
M5
0
10
20
30
40
Elution volume [mL]
Plasma 15 min
Normalized to highest peak [%]
100
50
0
M1
M3
M4
M5
0
10
20
30
40
Elution volume [mL]
Plasma 30 min
Normalized to highest peak [%]
100
50
0
M1
M3
M4
M5
0
10
20
30
40
Elution volume [mL]
Plasma 2h
Normalized to highest peak [%]
100
50
0
M1 2h
M2 2h
M3 2h
M4 2h
0
10
20
30
40
Elution volume [mL]
Plasma 24h
Normalized to highest peak [%]
100
50
0
M1
M2
M3
M4
0
10
20
30
40
Elution volume [mL]

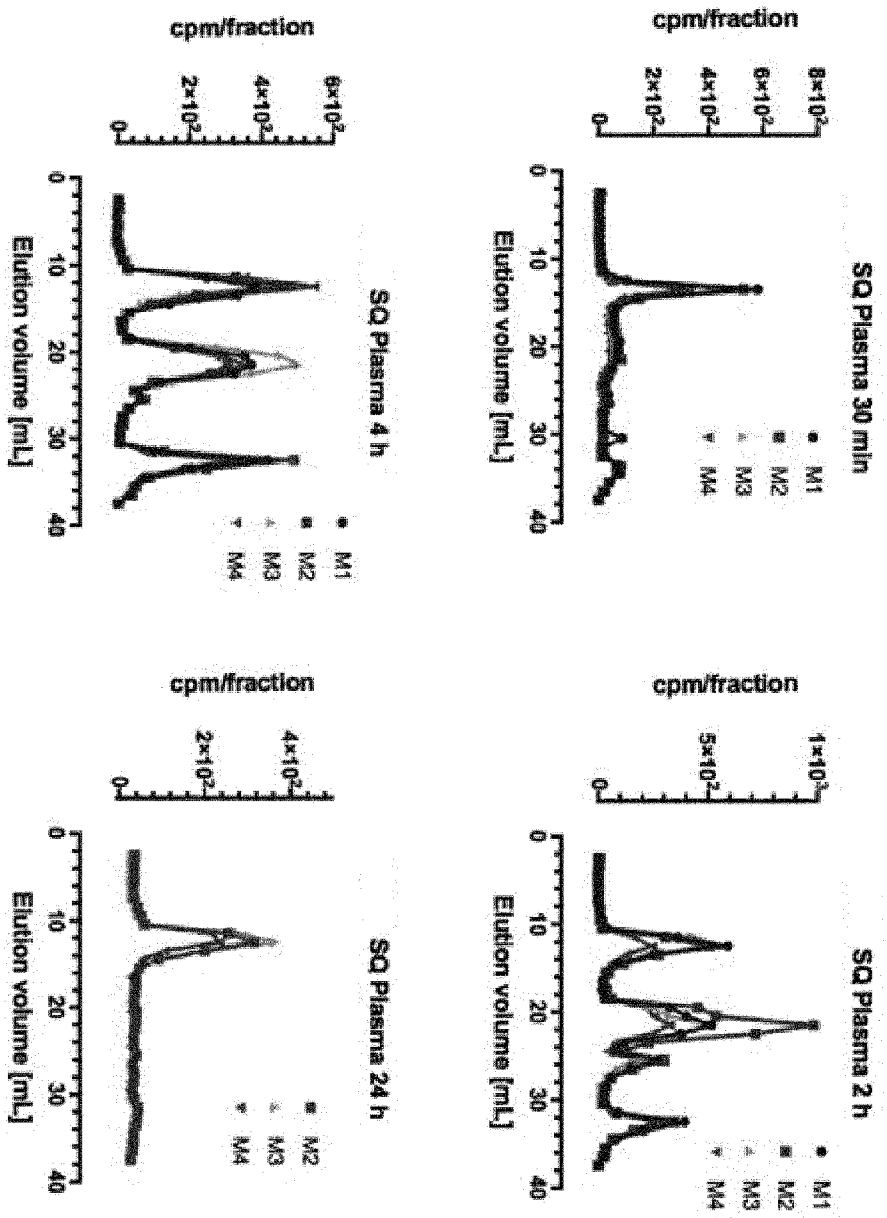

cpm/fraction
SQ Plasma 30 min
SQ Plasma 2 h
SQ Plasma 4 h
SQ Plasma 24 h
Elution volume [mL]
M1
M2
M3
M4

FIG. 9C

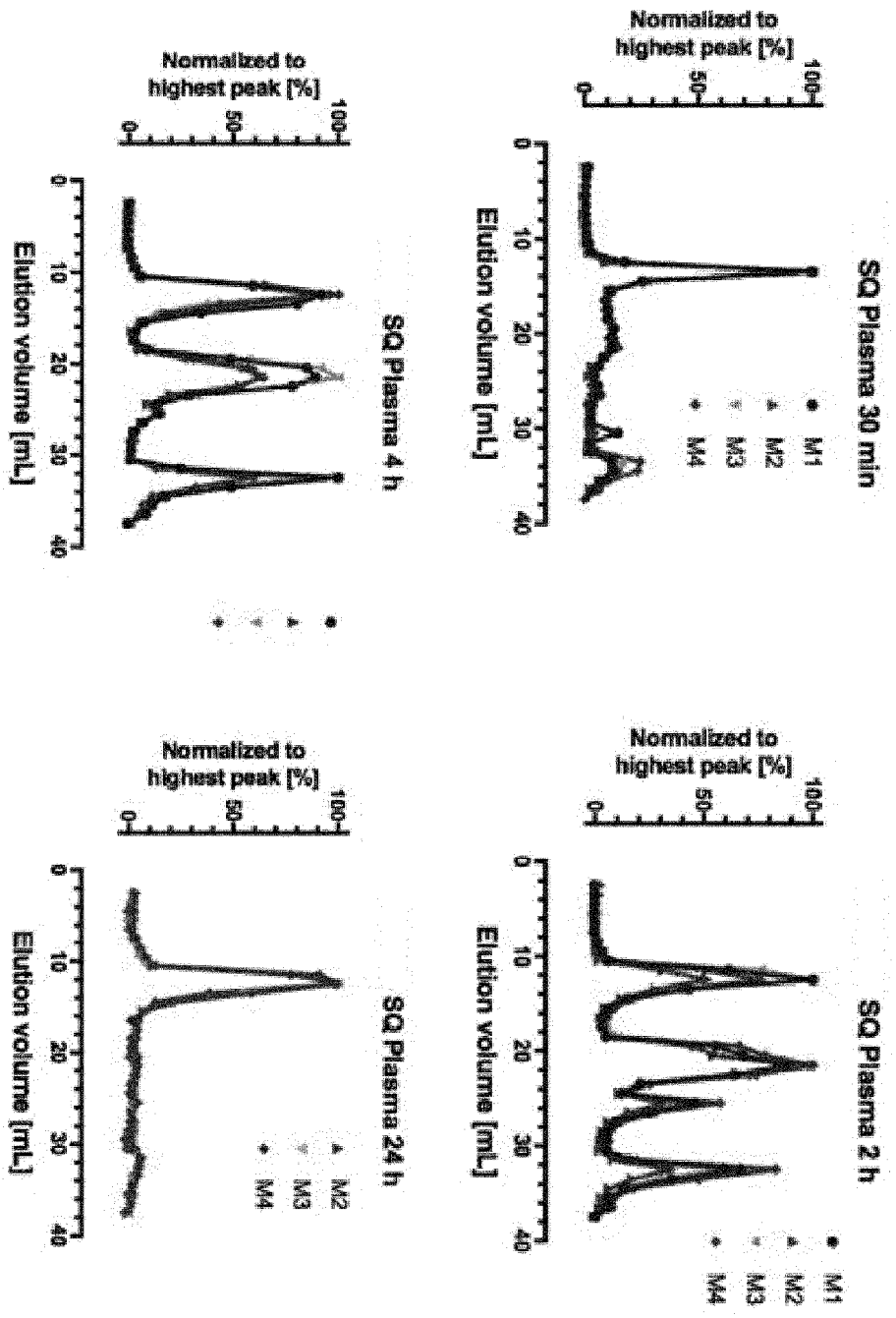

SQ Plasma 30 min
SQ Plasma 2 h
SQ Plasma 4 h
SQ Plasma 24 h
Normalized to highest peak [%]
Elution volume [mL]
M1
M2
M3
M4

FIG. 9C cont.

FIG. 10A

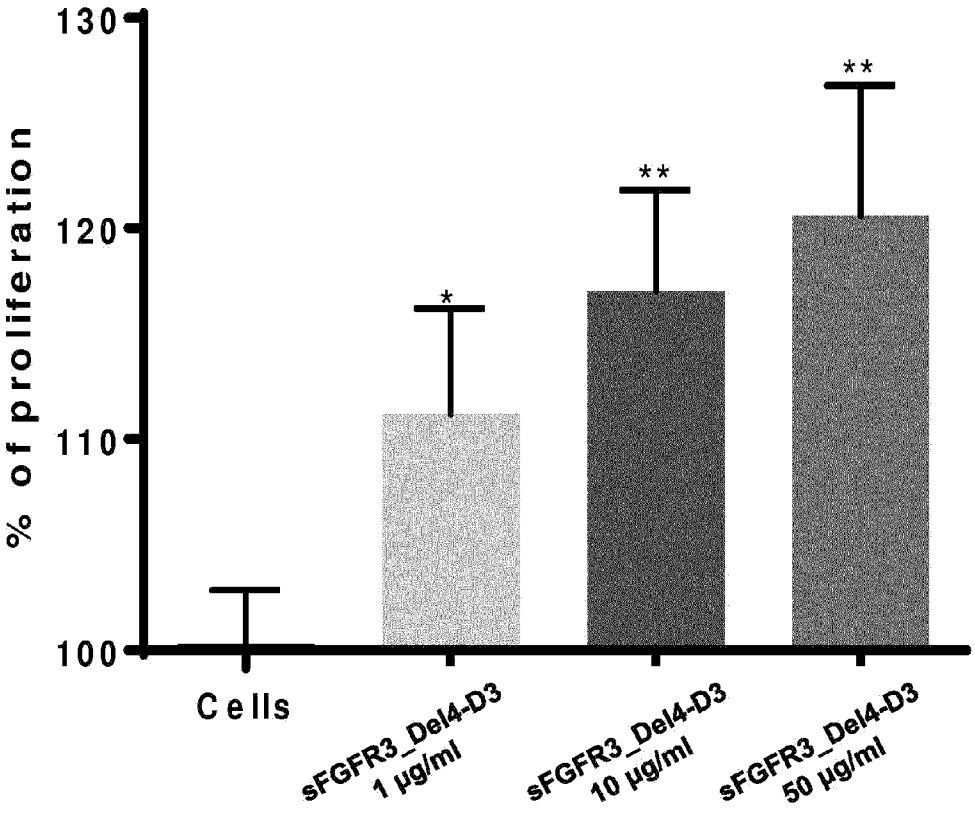
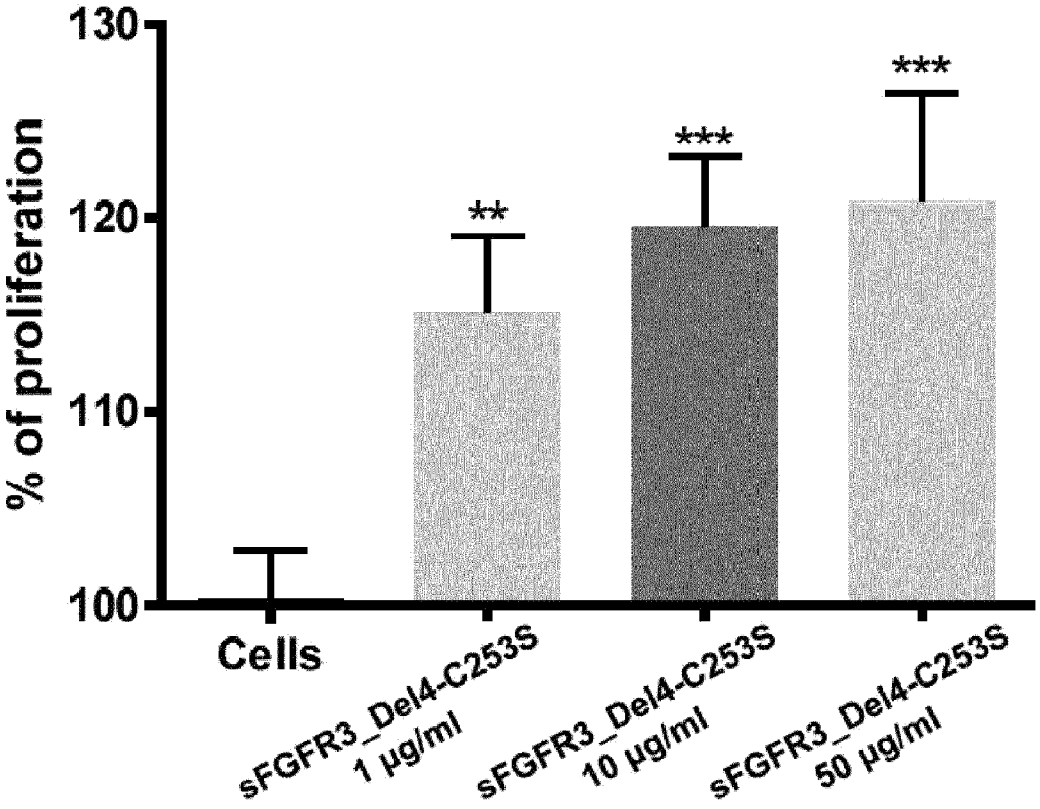

130
120
110
100
% of proliferation
*
**
**
Cells
sFGFR3_Del4-D3 1 µg/ml
sFGFR3_Del4-D3 10 µg/ml
sFGFR3_Del4-D3 50 µg/ml

FIG. 10B


130
120
110
100
% of proliferation
**
***
***
Cells
sFGFR3_Del4-C253S 1 µg/ml
sFGFR3_Del4-C253S 10 µg/ml
sFGFR3_Del4-C253S 50 µg/ml

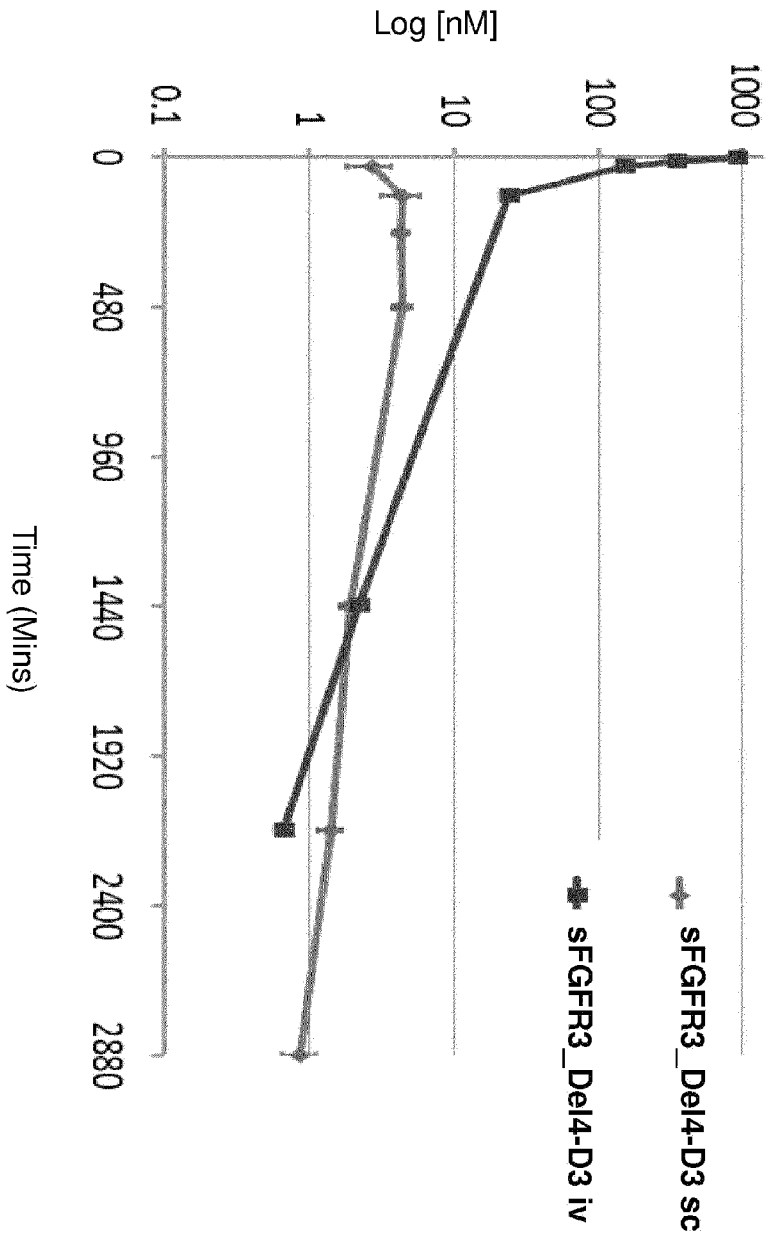

Log [nM]
1000
100
10
1
0.1
0
480
960
1440
1920
2400
2880
Time (Mins)
sFGFR3_Del4-D3 sc
sFGFR3_Del4-D3 iv

FIG. 11

FIG. 12

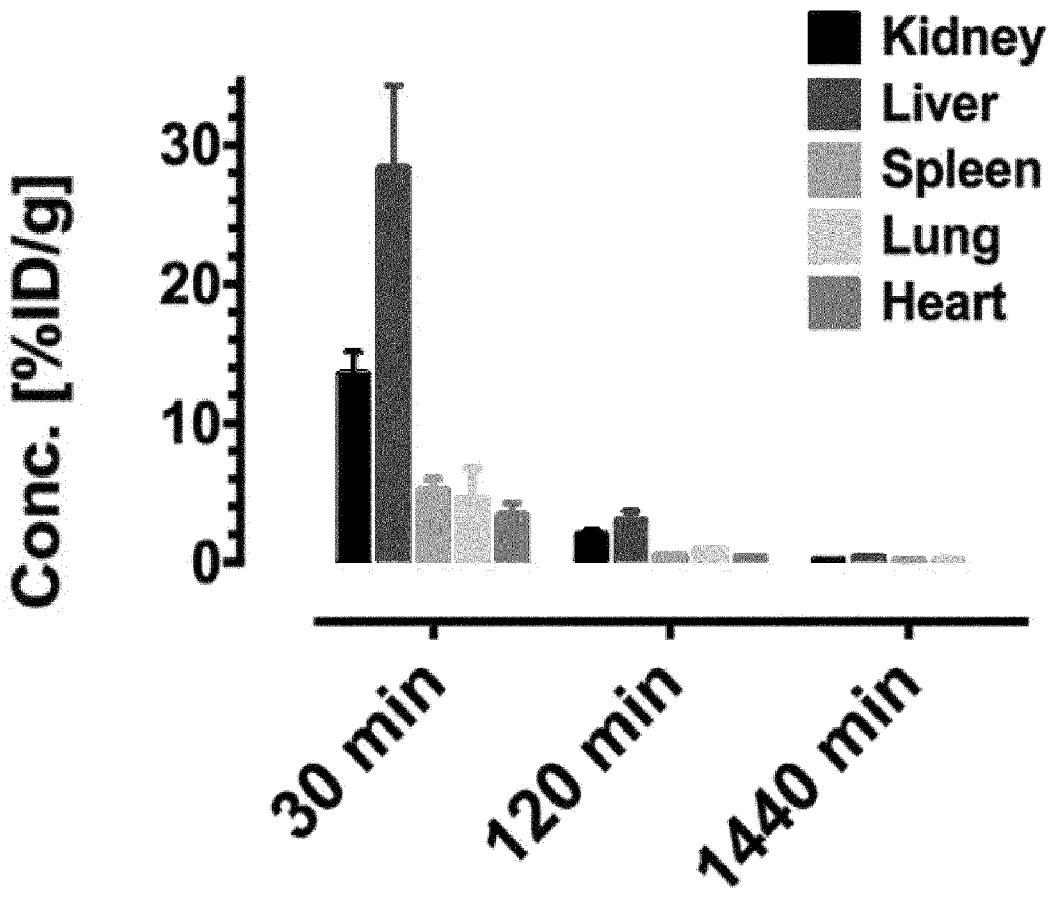
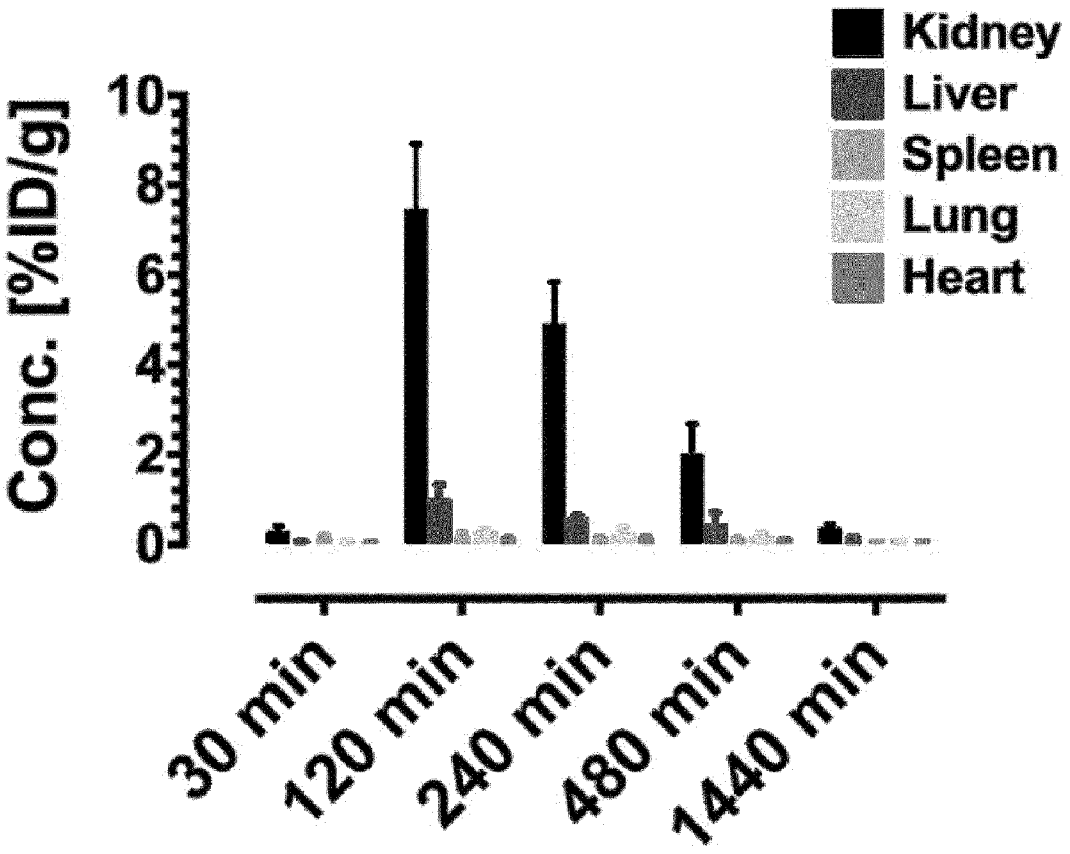

Kidney
Liver
Spleen
Lung
Heart
Conc. [%ID/g]
30
20
10
0
30 min
120 min
1440 min

FIG. 13


Kidney
Liver
Spleen
Lung
Heart
Conc. [%ID/g]
10
8
6
4
2
0
30 min
120 min
240 min
480 min
1440 min

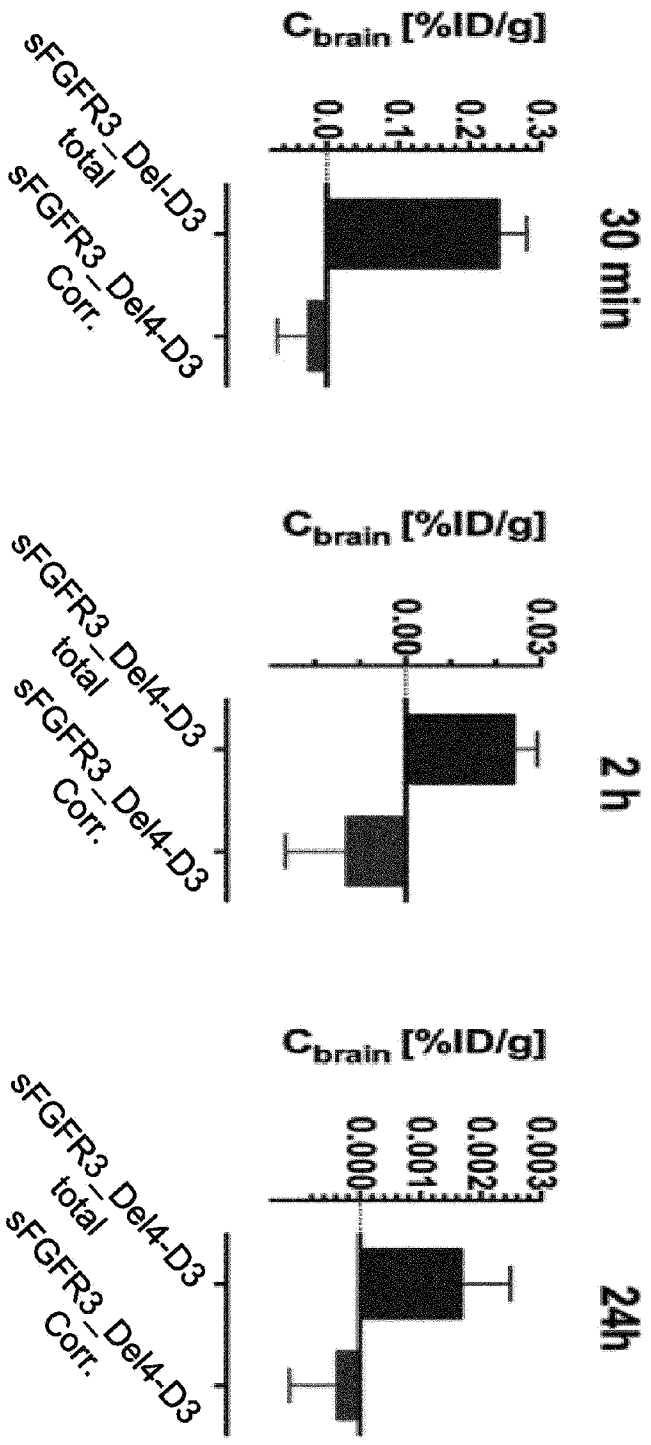

C brain [%ID/g]
0.0
0.1
0.2
0.3
sFGFR3_Del-D3 total
sFGFR3_Del4-D3 Corr.
30 min
C brain [%ID/g]
0.00
0.03
sFGFR3_Del4-D3 total
sFGFR3_Del4-D3 Corr.
2 h
C brain [%ID/g]
0.000
0.001
0.002
0.003
sFGFR3_Del4-D3 total
sFGFR3_Del4-D3 Corr.
24h

FIG. 14A

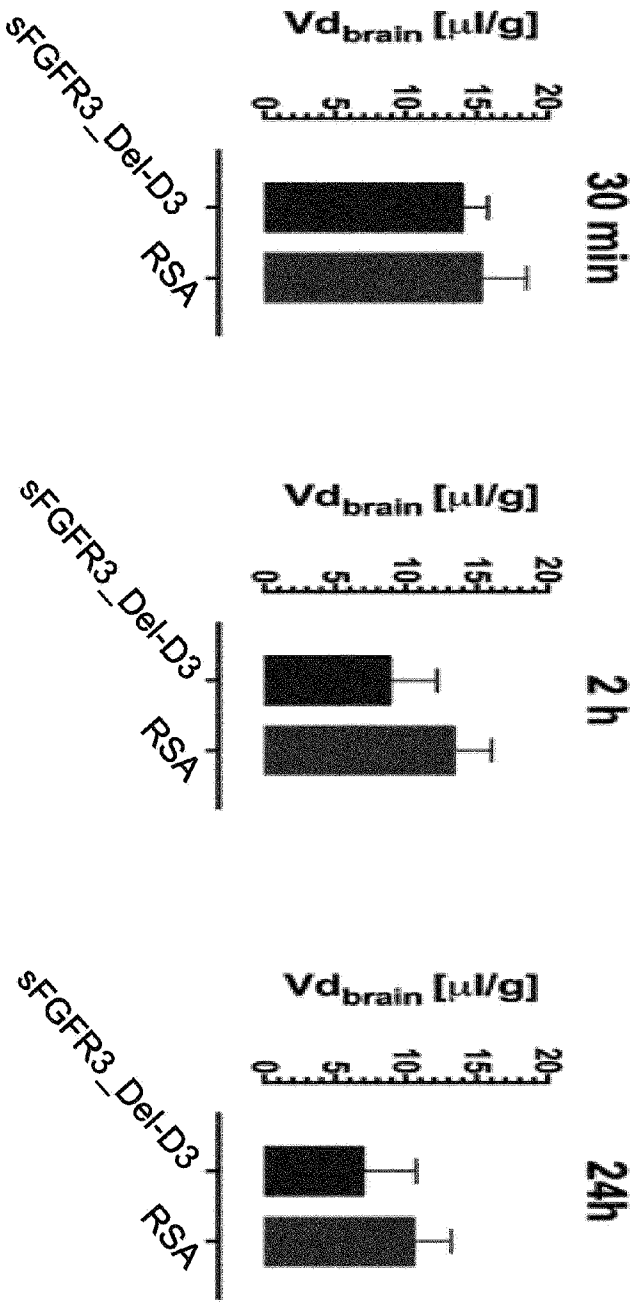

Vd brain [µl/g]
0
5
10
15
20
sFGFR3_Del-D3
RSA
30 min
Vd brain [µl/g]
0
5
10
15
20
sFGFR3_Del-D3
RSA
2 h
Vd brain [µl/g]
0
5
10
15
20
sFGFR3_Del-D3
RSA
24h

FIG. 14B

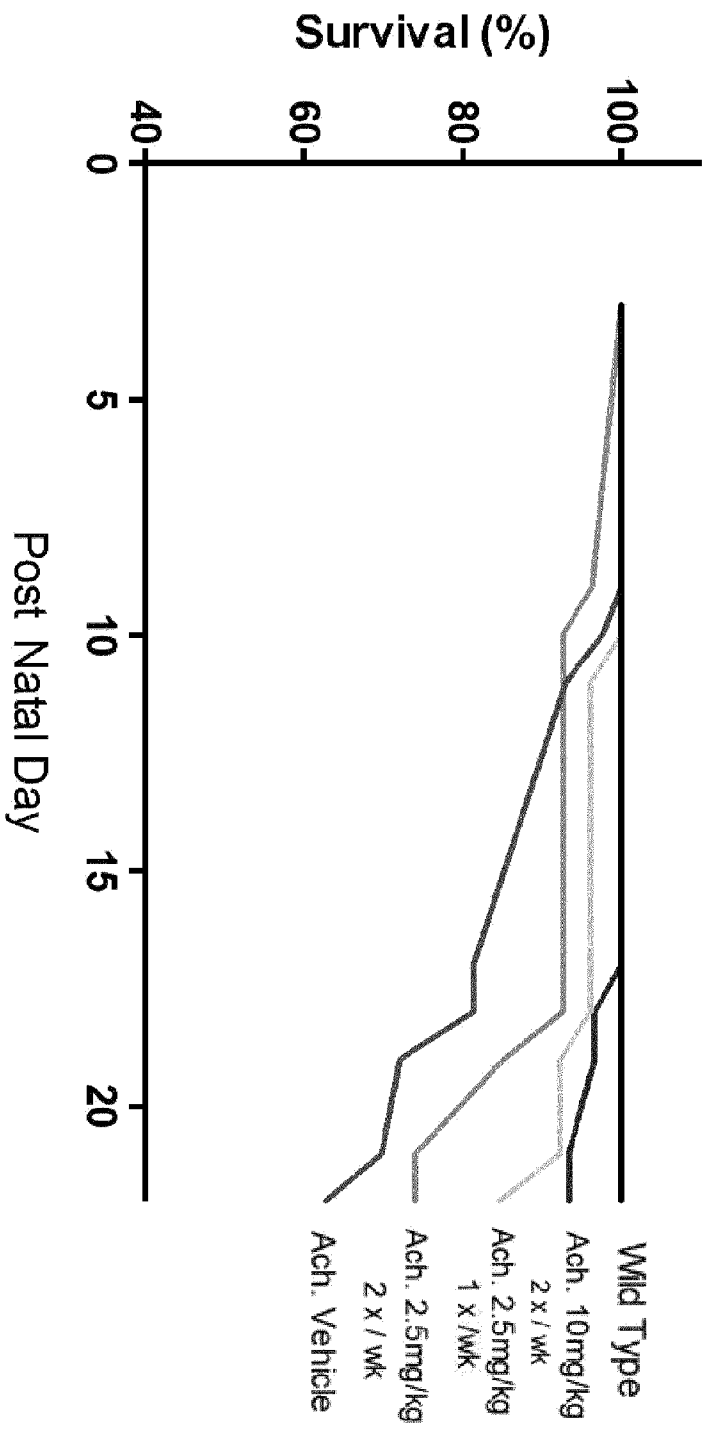
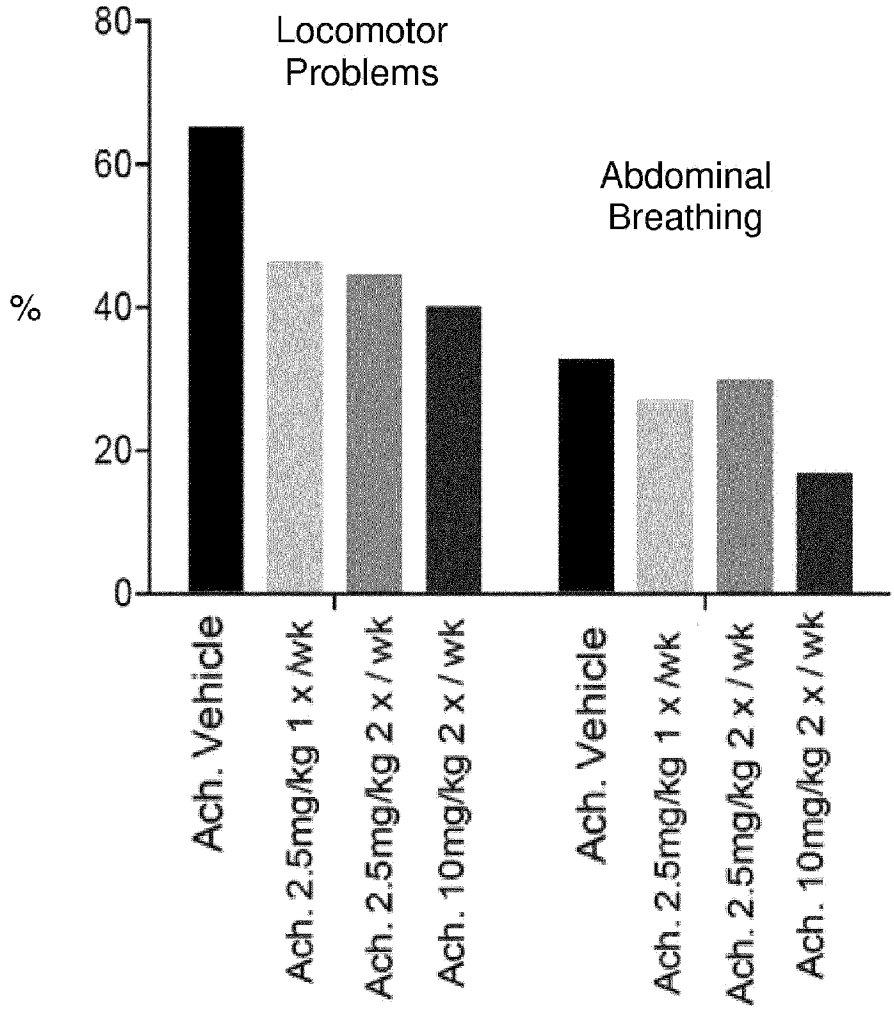

Survival Analysis
Survival (%)
100
80
60
40
0
5
10
15
20
Post Natal Day
Wild Type
Ach. 10mg/kg
2 x / wk
Ach. 2.5mg/kg
1 x /wk
Ach. 2.5mg/kg
2 x / wk
Ach. Vehicle

FIG. 15

FIG. 16


80
60
40
20
0
%
Locomotor Problems
Abdominal Breathing
Ach. Vehicle
Ach. 2.5mg/kg 1 x /wk
Ach. 2.5mg/kg 2 x / wk
Ach. 10mg/kg 2 x / wk
Ach. Vehicle
Ach. 2.5mg/kg 1 x /wk
Ach. 2.5mg/kg 2 x / wk
Ach. 10mg/kg 2 x / wk

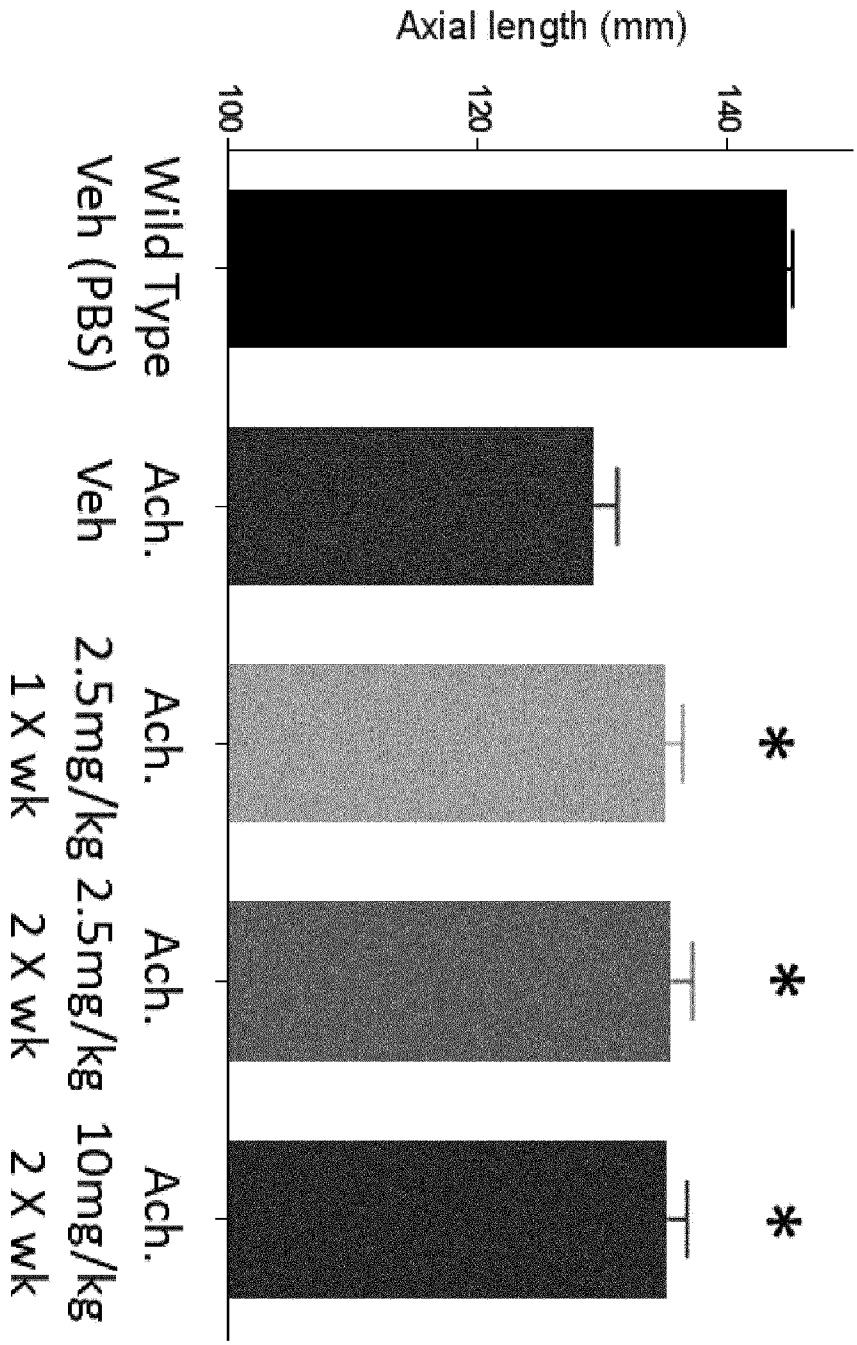

Axial length (mm)
100
120
140
Wild Type
Veh (PBS)
Ach.
Veh
Ach.
2.5mg/kg
1 X wk
Ach.
2.5mg/kg
2 X wk
Ach.
10mg/kg
2 X wk
*
*
*

FIG. 17A

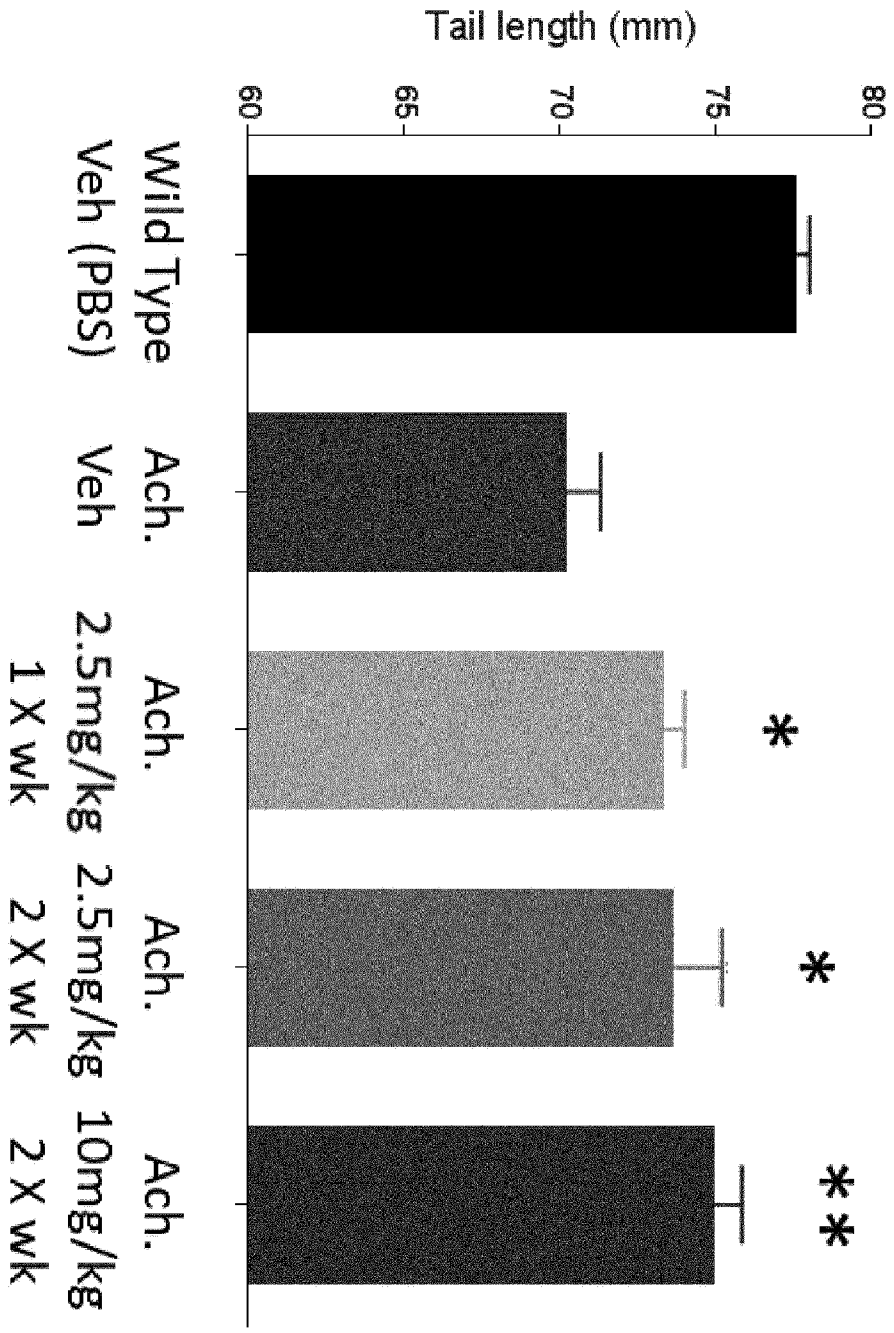

Tail length (mm)
60
65
70
75
80
Wild Type
Veh (PBS)
Ach.
Veh
Ach.
2.5mg/kg
1 X wk
Ach.
2.5mg/kg
2 X wk
Ach.
10mg/kg
2 X wk
*
*
**

FIG. 17B

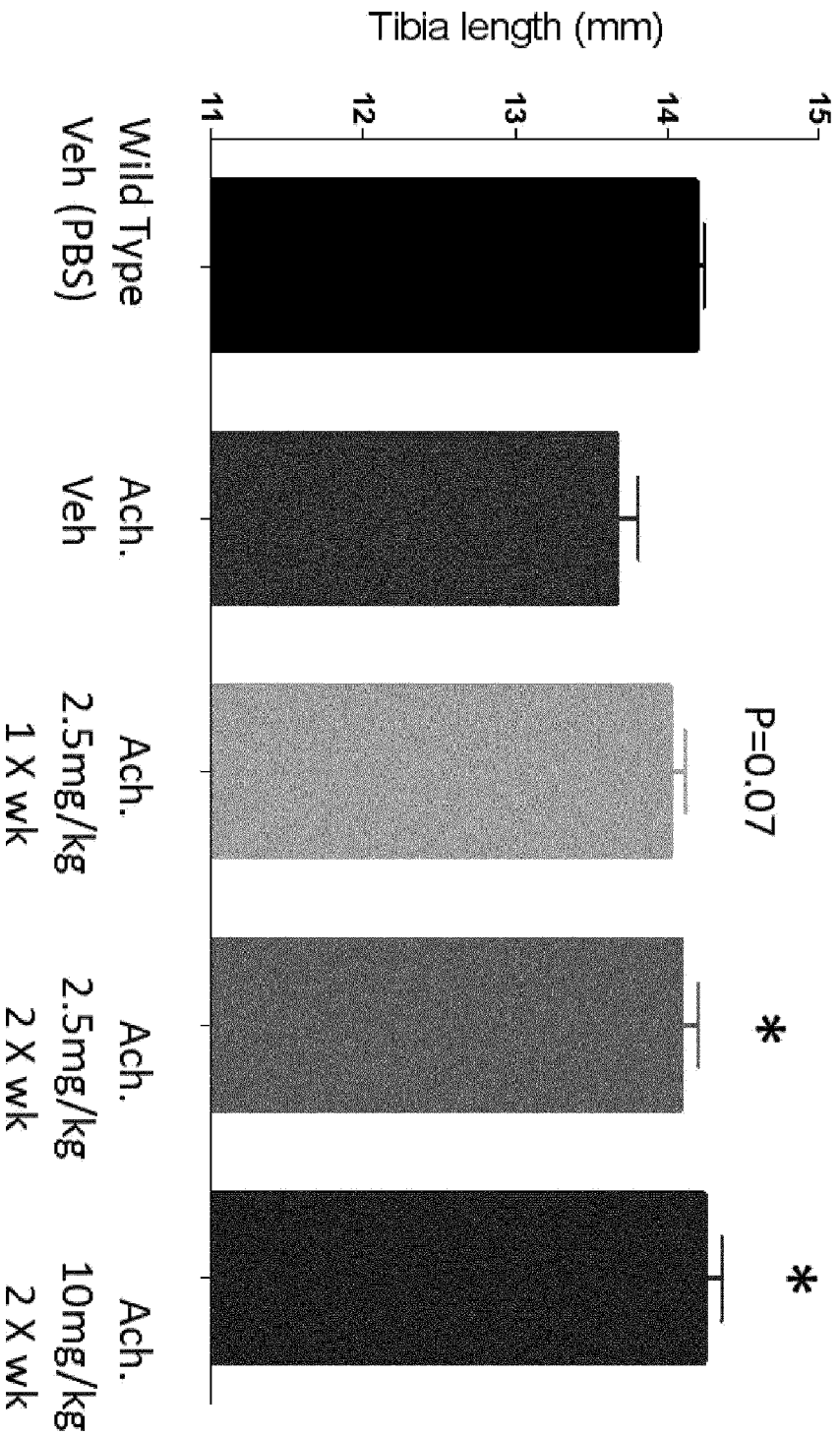
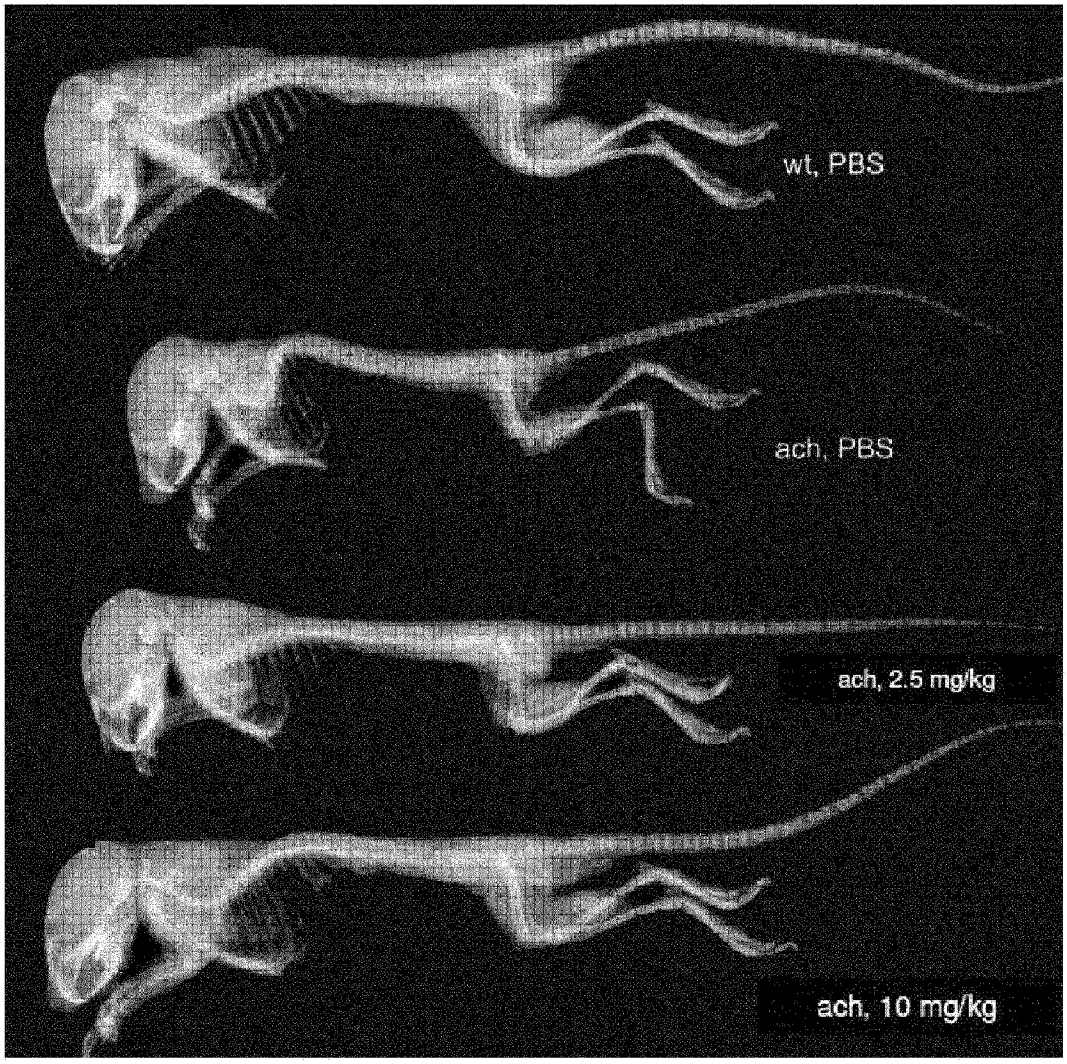

Tibia length (mm)
11
12
13
14
15
Wild Type
Veh (PBS)
Ach.
Veh
Ach.
2.5mg/kg
1 X wk
Ach.
2.5mg/kg
2 X wk
Ach.
10mg/kg
2 X wk
P=0.07
*
*

FIG. 17C

FIG. 17D



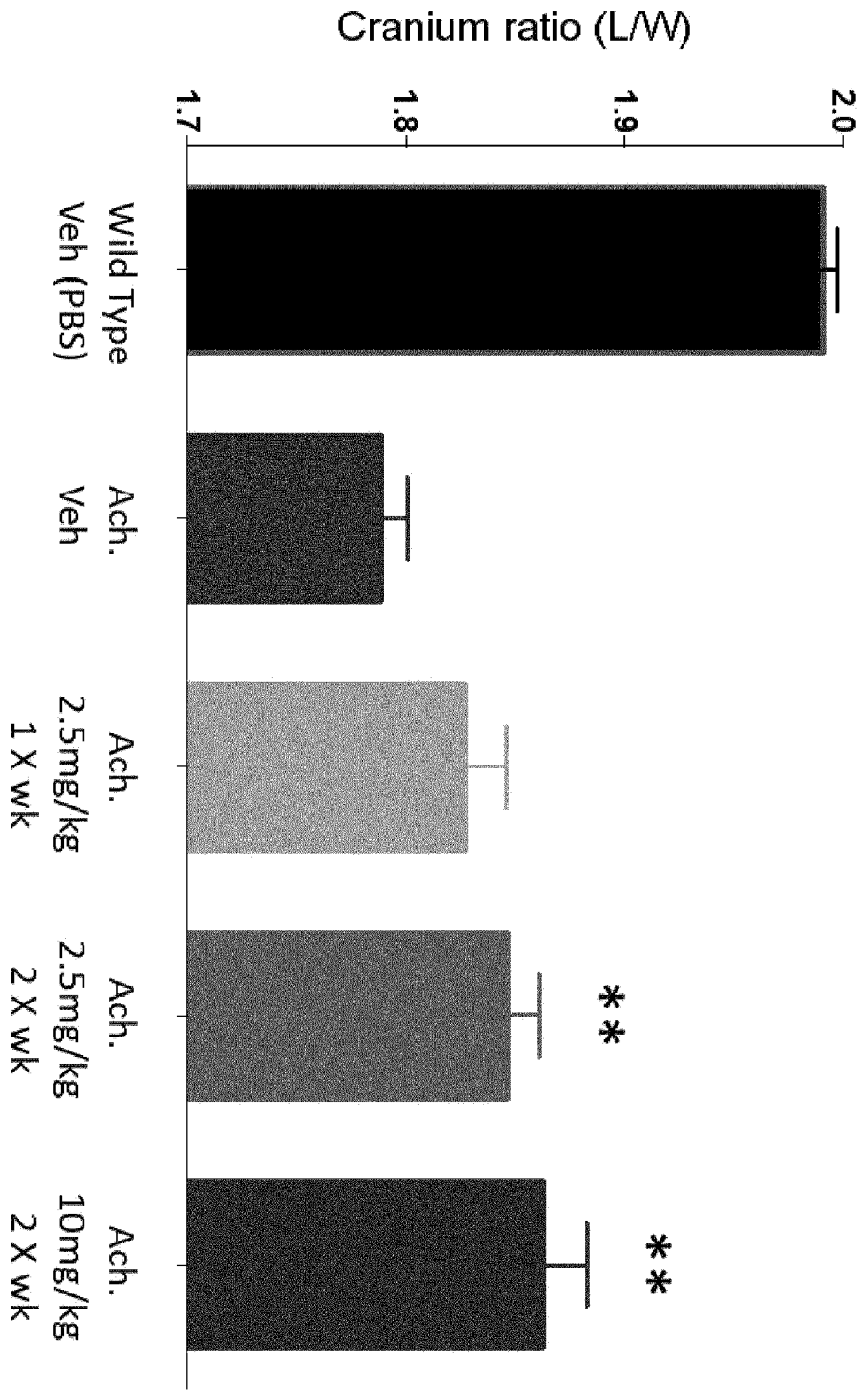
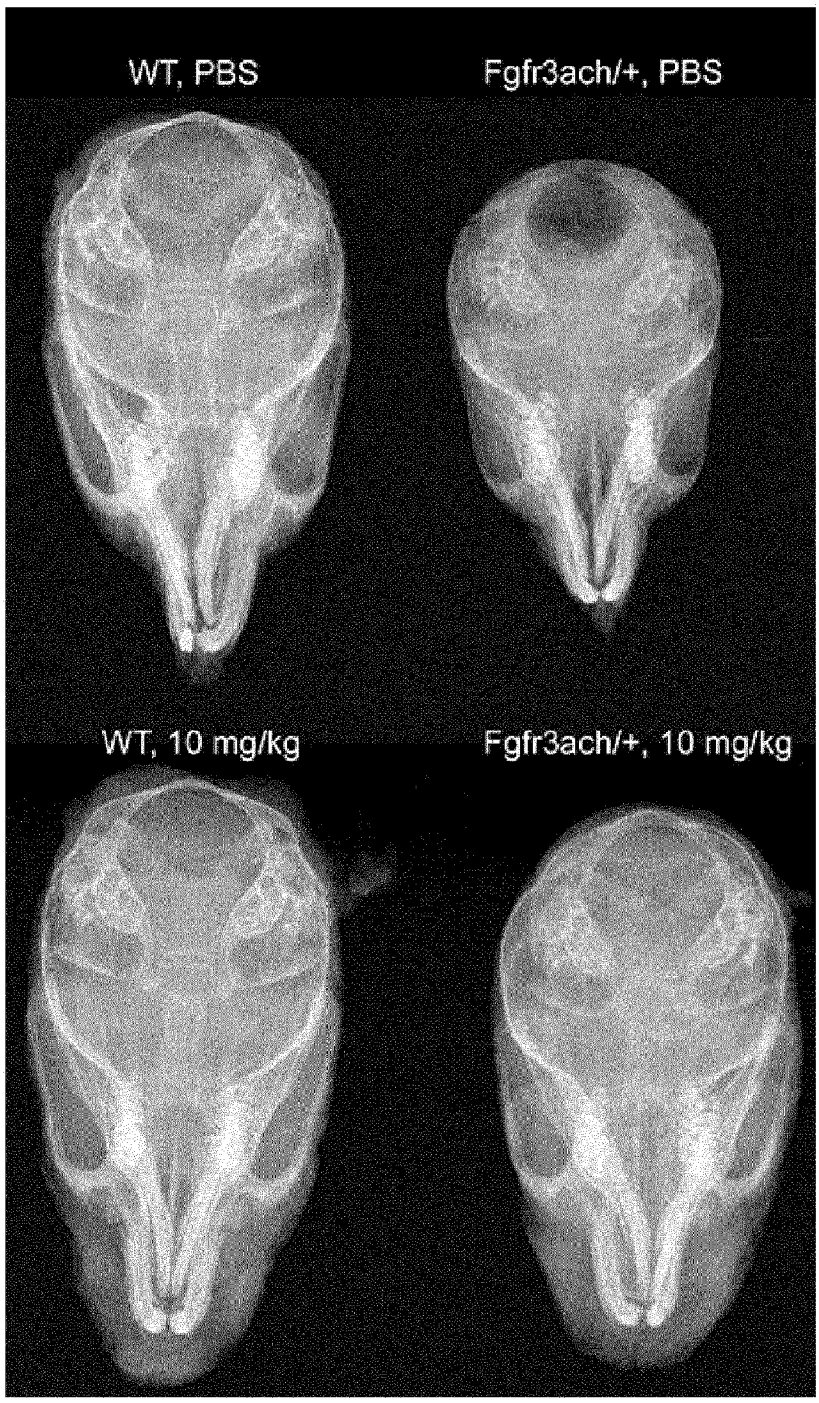

Cranium ratio (L/W)
1.7
1.8
1.9
2.0
Wild Type Veh (PBS)
Ach. Veh
Ach. 2.5mg/kg 1 X wk
Ach. 2.5mg/kg 2 X wk
Ach. 10mg/kg 2 X wk
**
**

FIG. 18A

FIG. 18B



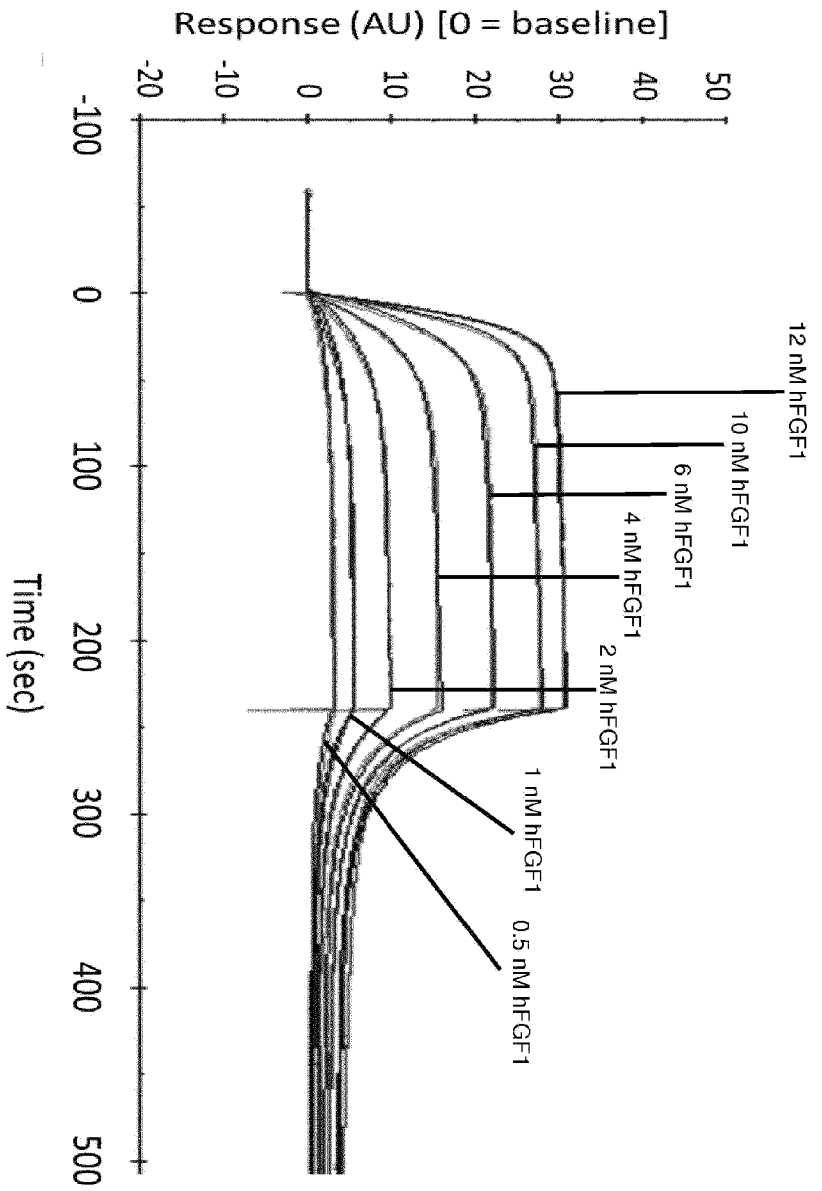

Response (AU) [0 = baseline]
-20
-10
0
10
20
30
40
50
-100
0
100
200
300
400
500
Time (sec)
12 nM hFGF1
10 nM hFGF1
6 nM hFGF1
4 nM hFGF1
2 nM hFGF1
1 nM hFGF1
0.5 nM hFGF1

FIG. 19A

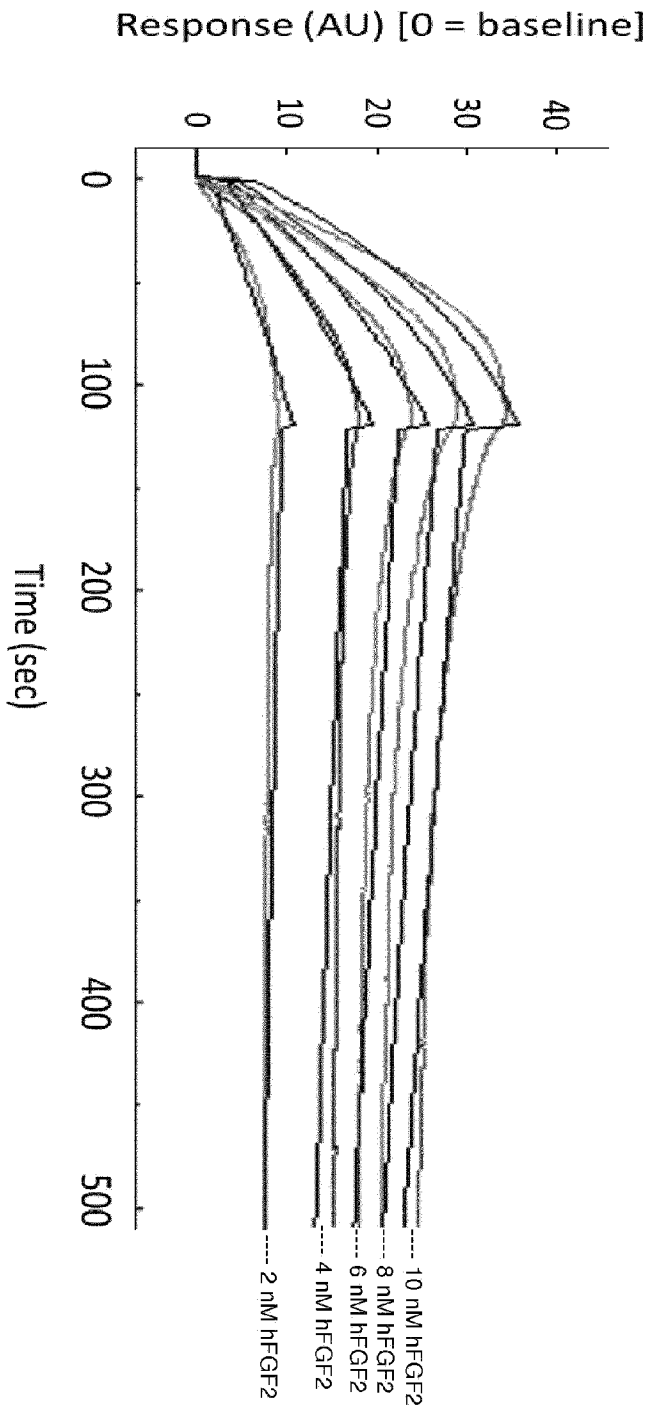

Response (AU) [0 = baseline]
0
10
20
30
40
Time (sec)
0
100
200
300
400
500
10 nM hFGF2
8 nM hFGF2
6 nM hFGF2
4 nM hFGF2
2 nM hFGF2

FIG. 19B

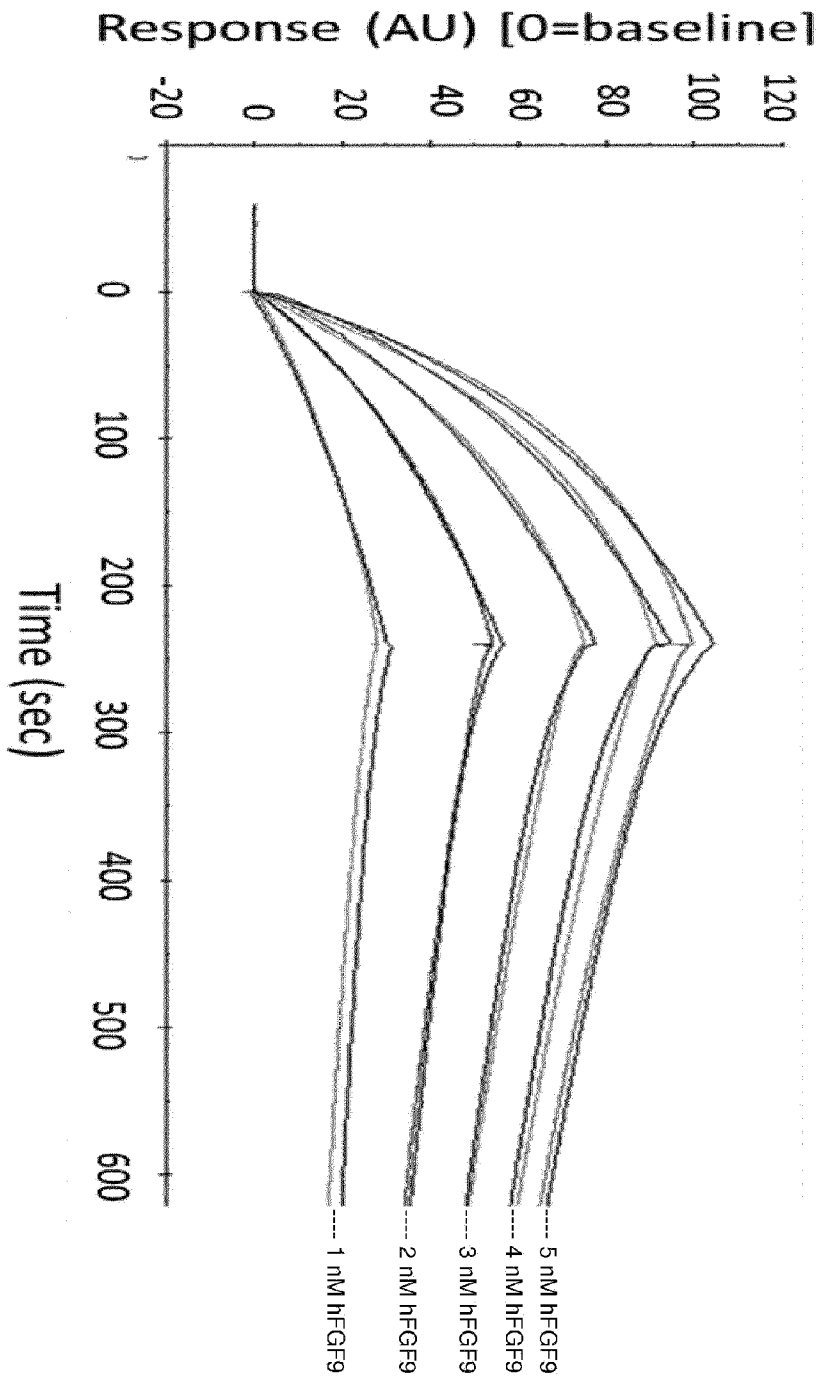

Response (AU) [0=baseline]
-20
0
20
40
60
80
100
120
Time (sec)
0
100
200
300
400
500
600
5 nM hFGF9
4 nM hFGF9
3 nM hFGF9
2 nM hFGF9
1 nM hFGF9

FIG. 19C

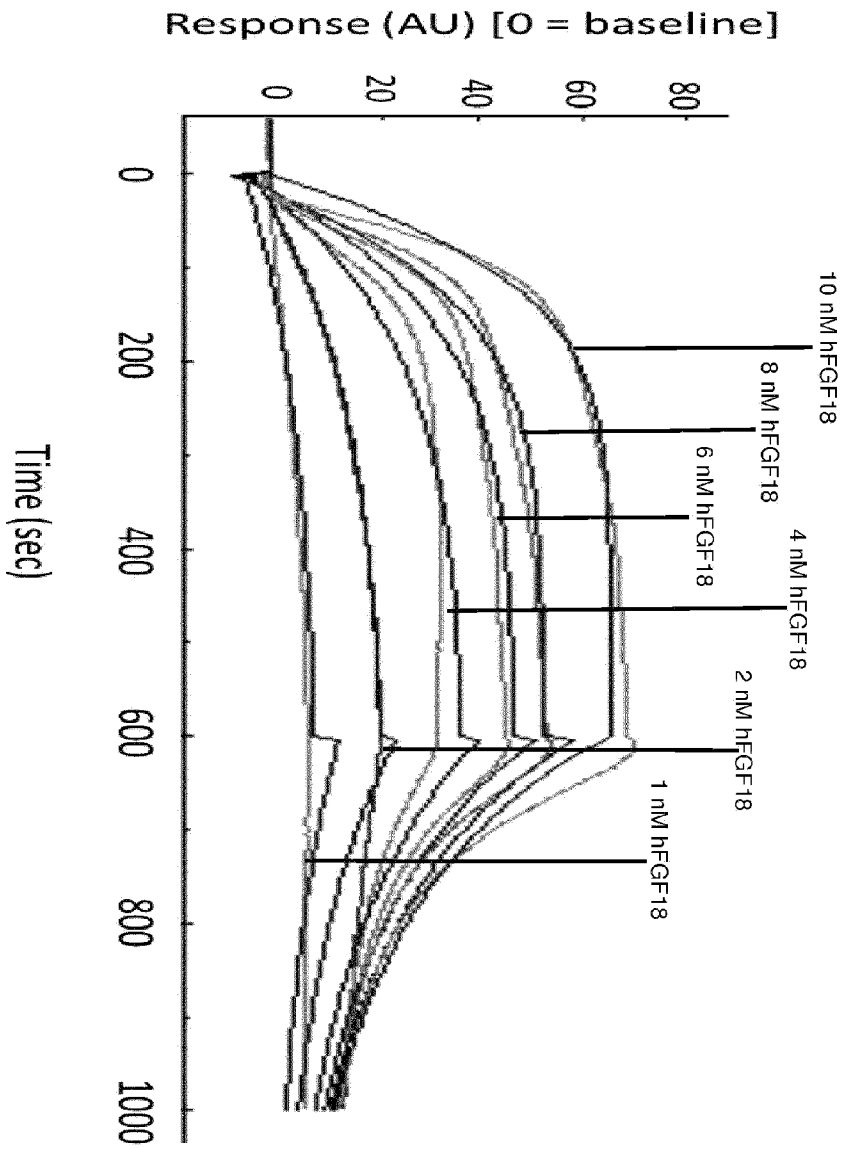

Response (AU) [0 = baseline]
0
20
40
60
80
Time (sec)
0
200
400
600
800
1000
10 nM hFGF18
8 nM hFGF18
6 nM hFGF18
4 nM hFGF18
2 nM hFGF18
1 nM hFGF18

FIG. 19D

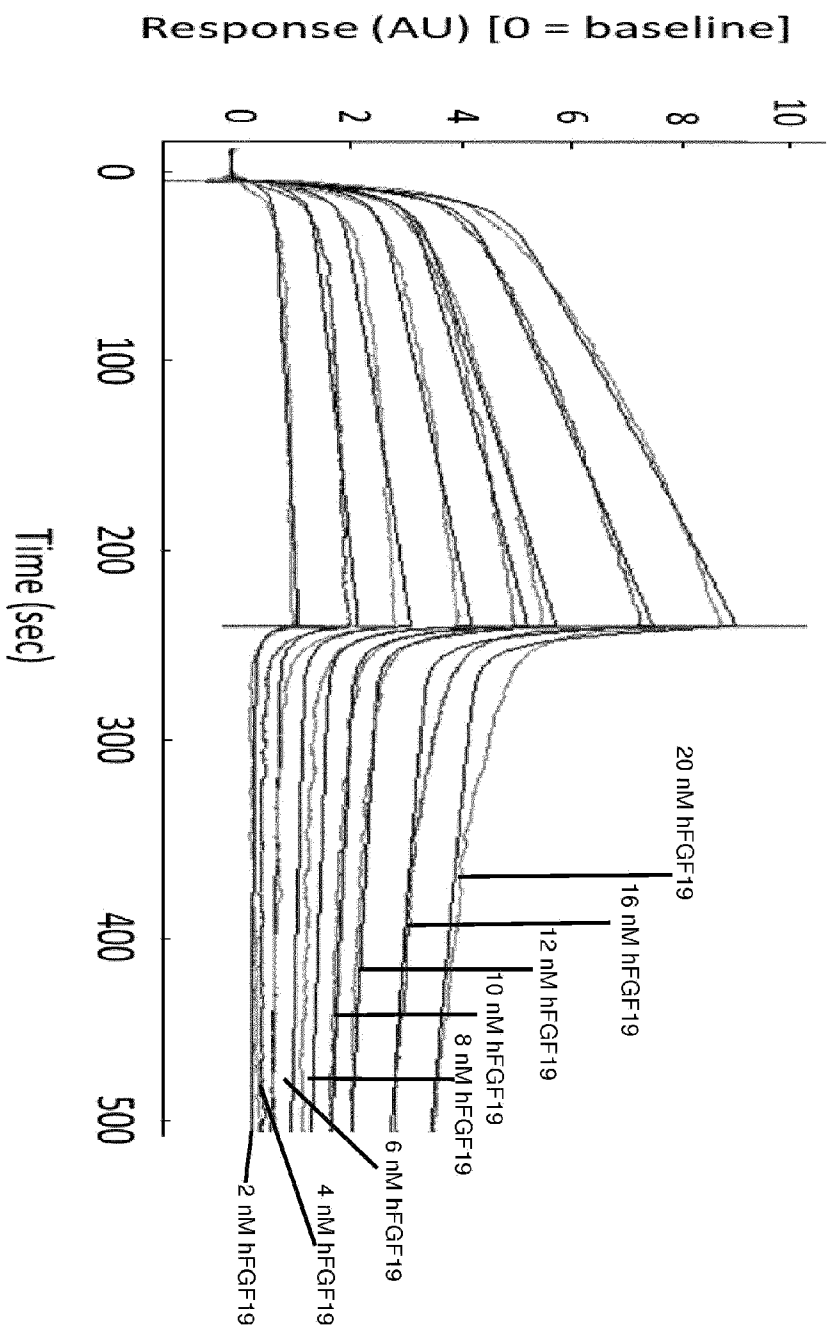

Response (AU) [0 = baseline]
0
2
4
6
8
10
Time (sec)
0
100
200
300
400
500
20 nM hFGF19
16 nM hFGF19
12 nM hFGF19
10 nM hFGF19
8 nM hFGF19
6 nM hFGF19
4 nM hFGF19
2 nM hFGF19

FIG. 19E

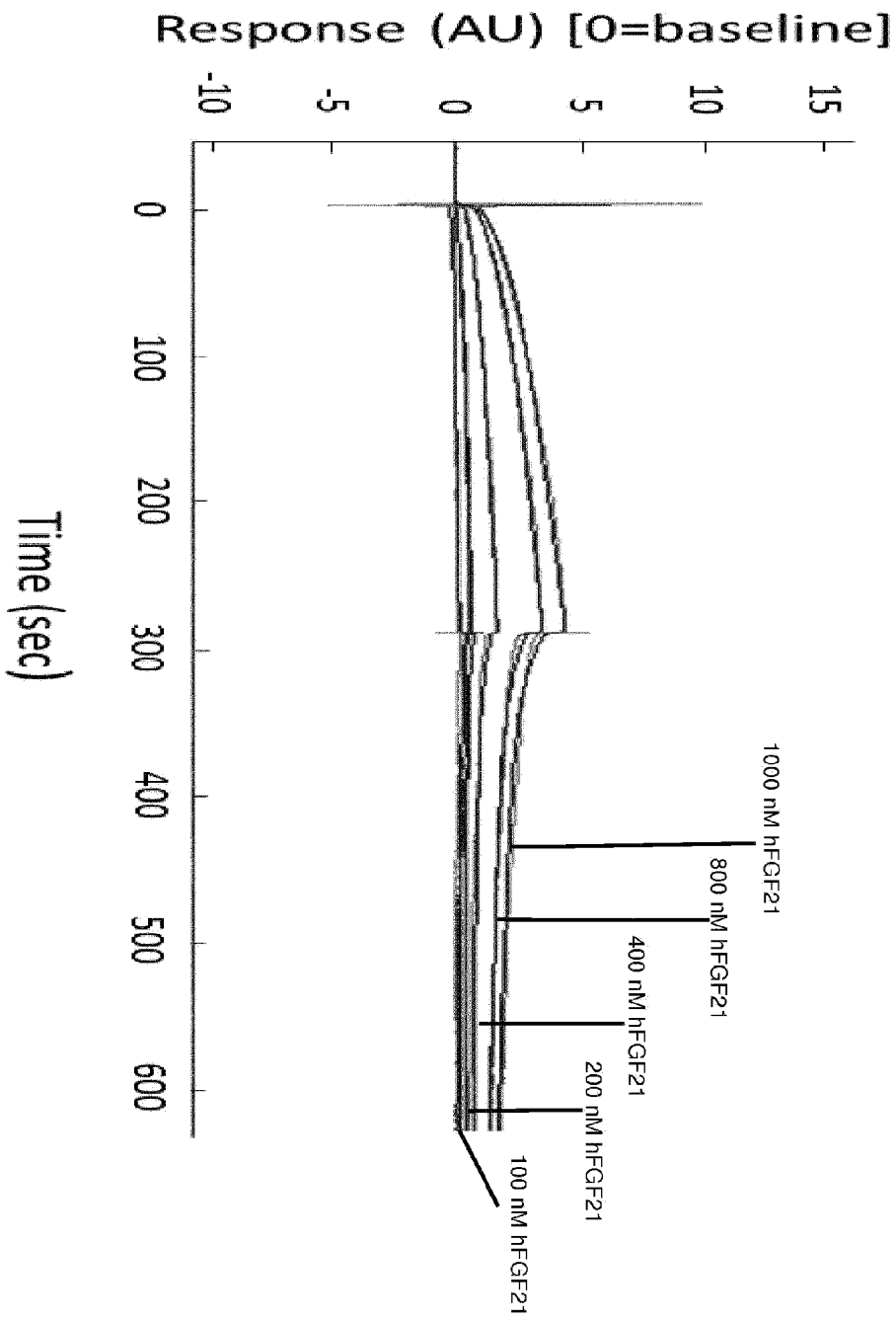

Response (AU) [0=baseline]
-10
-5
0
5
10
15
Time (sec)
0
100
200
300
400
500
600
1000 nM hFGF21
800 nM hFGF21
400 nM hFGF21
200 nM hFGF21
100 nM hFGF21

FIG. 19F

FIG. 20

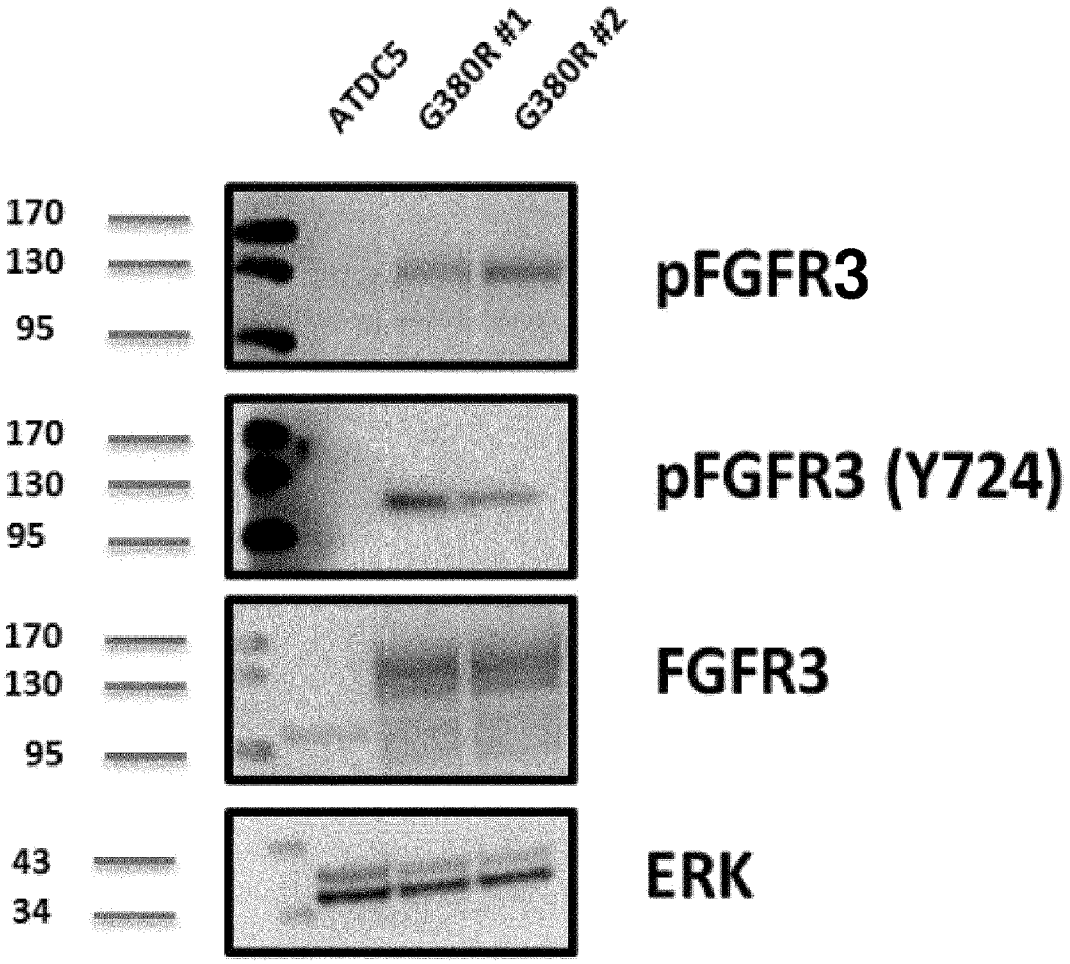
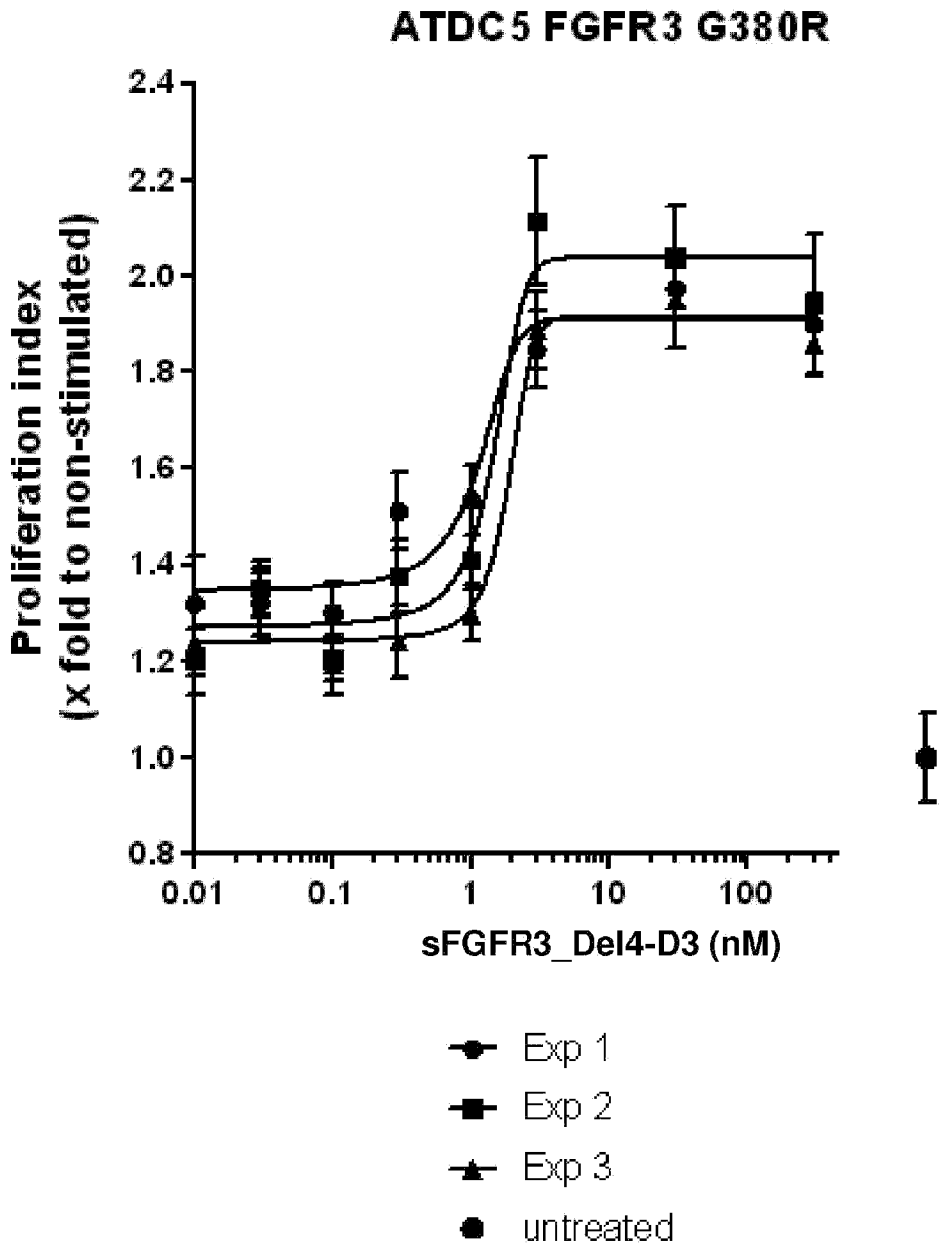

ATDC5
G380R #1
G380R #2
170
130
95
pFGFR3
170
130
95
pFGFR3 (Y724)
170
130
95
FGFR3
43
34
ERK

FIG. 21


ATDC5 FGFR3 G380R
Proliferation index
(x fold to non-stimulated)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.01
0.1
1
10
100
sFGFR3_Del4-D3 (nM)
Exp 1
Exp 2
Exp 3
untreated