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(54) Title: FZD8 EXTRACELLULAR DOMAINS AND FZD8 EXTRACELLULAR DOMAIN FUSION MOLECULES AND TREATMENTS USING SAME

(57) Abstract: Methods of treatment using Fzd8 extracellular domains (ECDs), Fzd8 ECD fusion molecules, and/or antibodies that bind Fzd8 are provided. Such methods include, but are not limited to, methods of treating obesity and obesity -related conditions. Fzd8 ECDs and Fzd8 ECD fusion molecules are also provided. Polypeptide and polynucleotide sequences, vectors, host cells, and compositions comprising or encoding such molecules are provided. Methods of making and using Fzd8 ECDs, Fzd8 ECD fusion molecules, and antibodies that bind Fzd8 are also provided.

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FZD8 EXTRACELLULAR DOMAINS AND FZD8 EXTRACELLULAR DOMAIN FUSION MOLECULES AND TREATMENTS USING SAME

[001] This application claims the benefit of U.S. Provisional Application No. 61/359,214, filed June 28, 2010, which is incorporated by reference herein in its entirety for any purpose.

FIELD OF THE INVENTION

[002] This invention relates in general to treatment of human diseases and pathological conditions. In some embodiments, the present invention relates to methods and compositions in treatments for obesity and obesity-related conditions.

BACKGROUND OF THE INVENTION

[003] Obesity is one of the most serious public health problems today. In addition to being one of the leading preventable causes of death worldwide, obesity is associated with many serious medical conditions, including congestive heart failure, ischemic heart disease, deep vein thrombosis, stroke, diabetes, infertility, high blood pressure, high cholesterol, high triglyceride levels, fatty liver disease, breathing difficulties, osteoarthritis, and some forms of cancer.

[004] The first-line treatment for obesity remains dieting and physical exercise, although the success rate for such regimens is low. The most effective treatment is currently bariatric surgery, in which the size of the stomach is reduced by one of several different means. Bariatric surgery is an expensive treatment, however, and the potential complications, including incisional hernia, infections, and pneumonia, can be serious. In addition, the mortality rate with bariatric surgery is about 2 in 1000.

[005] There is a clear need for less costly and invasive alternative treatments for obesity.

SUMMARY OF THE INVENTION

[006] The invention provides methods and compositions for treating obesity and obesity-related conditions based at least in part on identification that administration of a frizzled-8 (Fzd8) extracellular domain (ECD)-Fc fusion molecule results in significant weight loss, and a reduction in fat mass. Fzd8 presents as an important and advantageous therapeutic target, and the invention provides Fzd8 ECDs, Fzd8 ECD fusion molecules, and antibodies that block ligand binding to Fzd8 as therapeutic agents for use in targeting pathological conditions associated with obesity. Accordingly, the invention provides methods and compositions related to Fzd8.

[007] In some embodiments, methods of treating obesity and/or an obesity-related condition are provided. In some embodiments, an obesity-related condition is selected from heart disease, diabetes, breathing difficulties, osteoarthritis, high blood pressure, high cholesterol, high triglycerides, and high free fatty acids. In some embodiments, methods of lowering a blood glucose level are provided. In some embodiments, methods of reducing NPY expression in the hypothalamus are provided.

[008] In some embodiments, the methods comprise administering to a subject a Fzd8 ECD. In some embodiments, the methods comprise administering to a subject a Fzd8 ECD fusion molecule comprising a Fzd8 ECD and at least one fusion partner. In some embodiments, Fzd8 ECDs are provided. In some embodiments, Fzd8 ECD fusion molecules are provided. In some embodiments, pharmaceutical compositions comprising a Fzd8 ECD and/or a Fzd8 ECD fusion molecule are provided. In some embodiments, polynucleotides comprising nucleic acid sequences that encode the polypeptide portion of a Fzd8 ECD or a Fzd8 ECD fusion molecule are provided. In some embodiments, polynucleotides comprising nucleic acid sequences that encode Fzd8 ECD fusion molecules are provided.

[009] In some embodiments, the Fzd8 ECD has a sequence selected from SEQ ID NOs: 3, 4, 12, and 13. In some embodiments, the Fzd8 ECD consists of a sequence selected from SEQ ID NOs: 3, 4, 12, and 13. In some embodiments, the Fzd8 ECD fusion molecule comprises an amino acid sequence selected from SEQ ID NO.: 5, 6, 14, 15, 17, and 18. In some embodiments, the Fzd8 ECD fusion molecule consists of an amino acid sequence selected from SEQ ID NO.: 5, 6, 14, 15, 17, and 18. In some embodiments, at least one fusion partner is selected from an Fc, albumin, and polyethylene glycol. In some embodiments, at least one fusion partner is Fc. In some embodiments, the Fc of the Fzd8 ECD fusion molecule is an IgGi, IgG_2, IgG_3 or IgG_4 Fc region. In some embodiments, the Fc of the Fzd8 fusion molecule is an IgGi Fc region. In some embodiments, the Fc of the Fzd8 fusion molecule is an IgGi C237S Fc region. In some embodiments, the Fc of the Fzd8 fusion molecule is an IgG_4 Fc region. In some embodiments, at least one fusion partner is an Fc and polyethylene glycol. In some embodiments, at least one fusion partners is polyethylene glycol. In some embodiments, the Fzd8 ECD or Fzd8 ECD fusion molecule comprises a signal peptide. In some embodiments, the fusion molecule comprises a linker between the Fzd8 ECD and one or more fusion partners. In some embodiments, the Fzd8 ECD fusion molecule is glycosylated and/or sialylated. In some

embodiments, the polypeptide portion of the fusion molecule is expressed in Chinese hamster ovary (CHO) cells.

[010] In some embodiments, a Fzd8 ECD or Fzd8 ECD fusion molecule lowers blood glucose levels when administered to a subject. In some embodiments, a Fzd8 ECD or Fzd8 ECD fusion molecule reduces NPY expression when administered to a subject. In some embodiments, a Fzd8 ECD or Fzd8 ECD fusion molecule reduces NPY expression when administered to a mouse.

[01 1] Any embodiment described herein or any combination thereof applies to any and all Fzd8 ECDs or Fzd8 ECD fusion molecules, methods and uses of the invention described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[012] **FIG.** 1 shows the body weight over time of mice fed a high-fat diet after receiving vehicle, Fzd2 ECD-Fc, Fzd8 ECD-Fc, or SFRP4 ECD-Fc by hydrodynamic tail vein transfection, as described in Example 1.

[013] **FIG. 2** shows lipid levels in mice fed a high-fat diet after receiving vehicle or Fzd8 ECD-Fc by hydrodynamic tail vein transfection, as described in Example 1. Figure 2A shows serum triglyceride levels. Figure 2B shows cholesterol levels. Figure 2C shows free fatty acid (FFA) levels.

[014] **FIG. 3** shows epididymal (A), inguinal (B), and renal (C) fat pad weights in mice fed a high-fat diet after receiving vehicle or Fzd8 ECD-Fc by hydrodynamic tail vein transfection, as described in Example 1.

[015] **FIG. 4** shows a duodenum from a mouse that received vehicle (A) and a mouse that received Fzd8 ECD-Fc (B) by hydrodynamic tail vein transfection, as described in Example 1.

[016] **FIG. 5** shows the body weight over time of mice fed a high-fat diet after receiving vehicle, Fzd8 ECD-Fc, or LRRP1 ECD-Fc by hydrodynamic tail vein transfection, as described in Example 2.

[017] **FIG. 6** shows the fat mass (A), lean mass (B), and lean mass to fat mass ratio (C) of mice fed a high-fat diet after receiving vehicle or Fzd8 ECD-Fc by hydrodynamic tail vein transfection, as described in Example 2.

[018] **FIG. 7** shows the body weight (A) and percent change in body weight (B) over time of mice fed a regular diet after receiving vehicle or Fzd8 ECD-Fc by hydrodynamic tail vein transfection (TVT), as described in Example 3.

[019] **FIG. 8** shows the food consumption over time of mice fed a regular diet after receiving vehicle or Fzd8 ECD-Fc by hydrodynamic tail vein transfection, as described in Example 3. Figure 8A shows the weight of food consumed. Figure 8B shows the percent food consumed by Fzd8-treated mice relative to vehicle-treated mice.

[020] **FIG. 9** shows the epididymal fat pad weight of mice fed a regular diet after receiving vehicle or Fzd8 ECD-Fc by hydrodynamic tail vein transfection, as described in Example 3.

[021] **FIG. 10** shows (A) mean cumulative food consumption and (B) mean body weight of rats fed a high fat diet after receiving vehicle or Fzd8 ECD-Fc by hydrodynamic tail vein transfection, as described in Example 4.

[022] **FIG. 11** shows mean body weight of mice fed a high fat diet after receiving vehicle or Fzd8 ECD-Fc by hydrodynamic tail vein transfection, as described in Example 5.

[023] **FIG. 12** shows (A) mean body weight and (B) glucose tolerance of mice fed a high fat diet after receiving vehicle or Fzd8 ECD-Fc by hydrodynamic tail vein transfection, as described in Example 6.

[024] **FIG. 13** shows (A) mean body weight, (b) mean fat mass, and (C) mean lean mass of mice fed a high fat diet after receiving Fzd8 ECD-Fc by hydrodynamic tail vein transfection, and of mice fed either a high fat diet or a high-fat restricted diet after receiving vehicle by hydrodynamic tail vein transfection, as described in Example 7.

[025] **FIG. 14** shows mean body weight of mice fed a high fat diet after administration of rhFzd8 ECD-Fc or vehicle, as described in Example 8.

[026] **FIG. 15** shows NPY expression in the hypothalamus of mice fed a high fat diet after receiving Fc or Fzd8 ECD-Fc by hydrodynamic tail vein transfection, as described in Example 9.

[027] **FIG. 16** shows mean body weight of mice fed a high fat diet after receiving Fc, Fzd8 ECD-Fc, or Fzd5 ECD-Fc by hydrodynamic tail vein transfection, as described in Example 10.

DETAILED DESCRIPTION

[028] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Definitions

[029] Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those

of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[030] Certain techniques used in connection with recombinant DNA, oligonucleotide synthesis, tissue culture and transformation (e.g., electroporation, lipofection), enzymatic reactions, and purification techniques are known in the art. Many such techniques and procedures are described, e.g., in Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), among other places. In addition, certain techniques for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients are also known in the art.

[03 1] In this application, the use of "or" means "and/or" unless stated otherwise. In the context of a multiple dependent claim, the use of "or" refers back to more than one preceding independent or dependent claim in the alternative only. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

[032] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[033] The terms **"nucleic acid molecule"** and **"polynucleotide"** may be used interchangeably, and refer to a polymer of nucleotides. Such polymers of nucleotides may contain natural and/or non-natural nucleotides, and include, but are not limited to, DNA, RNA, and PNA. **"Nucleic acid sequence"** refers to the linear sequence of nucleotides that comprise the nucleic acid molecule or polynucleotide.

[034] The terms **"polypeptide"** and **"protein"** are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Furthermore, for purposes of the present invention, a "polypeptide" refers to a protein which includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed

mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[035] The term **"Frizzled-8"** or **"Fzd8"** refer herein to the full-length Fzd8 protein, which includes the N-terminal ECD, the transmembrane domain, and the intracellular domain, with or without an N-terminal signal peptide. In some embodiments, Fzd8 is a human Fzd8. In some embodiments, human Fzd8 has an amino acid sequence corresponding to SEQ ID NO.: 1 or to SEQ ID NO.: 2.

[036] The term **"Fzd8 extracellular domain" ("Fzd8 ECD")** includes full-length Fzd8 ECDs, Fzd8 ECD fragments, and Fzd8 ECD variants. As used herein, the term **"Fzd8 ECD"** refers to a Fzd8 polypeptide that lacks the intracellular and transmembrane domains, with or without a signal peptide. In some embodiment, the Fzd8 ECD is a human full-length Fzd8 ECD having an amino acid sequence corresponding to SEQ ID NO.: 3 or to SEQ ID NO: 4. The term **"full-length Fzd8 ECD",** as used herein, refers to a Fzd8 ECD that extends to the last amino acid of the extracellular domain, and may or may not include an N-terminal signal peptide. As defined herein, the last amino acid of the full-length Fzd8 ECD is at position 275. Thus, a human full-length Fzd8 ECD may consist of the amino acid sequence corresponding to SEQ ID NO.: 4 (mature form) or to SEQ ID NO.: 3 (with the signal peptide). As used herein, the term **"Fzd8 ECD fragment"** refers to a Fzd8 ECD having one or more residues deleted from the N and/or C terminus of the full-length ECD and that retains the ability to bind to a Wnt ligand. In some embodiments, a Fzd8 fragment retains the ability to bind to at least one ligand selected from Wntl, Wnt2, Wnt3, Wnt3a, and Wnt7b. The Fzd8 ECD fragment may or may not include an N-terminal signal peptide. In some embodiments, the Fzd8 ECD fragment is a human Fzd8 ECD fragment having an amino acid sequence corresponding to SEQ ID NO.: 12 (mature form) or to SEQ ID NO.: 13 (with the signal peptide). As used herein, the term **"Fzd8 ECD variants"** refers to Fzd8 ECDs that contain amino acid additions, deletions, and substitutions and that remain capable of binding to a Wnt ligand. Such variants may be at least 90%, 92%, 95%, 97%, 98%, or 99% identical to the parent Fzd8 ECD. The % identity of two polypeptides can be measured by a similarity score determined by comparing the amino acid sequences of the two polypeptides using the Bestfit program with the default settings for determining similarity. Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981) to find the best segment of similarity between two sequences.

[037] A polypeptide having an amino acid sequence at least, for example, 95% identical to a reference amino acid sequence of a Fzd8 ECD polypeptide is one in which the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids, up to 5% of the total amino acid residues in the reference sequence, may be inserted into the reference sequence. These alterations of the reference sequence may occur at the N- or C- terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence, or in one or more contiguous groups within the reference sequence.

[038] As a practical matter, whether any particular polypeptide is at least 70%, 80%, 90%, or 95% identical to, for instance, an amino acid sequence or to a polypeptide sequence encoded by a nucleic acid sequence set forth in the Sequence Listing can be determined conventionally using known computer programs, such the Bestfit program. When using Bestfit or other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

[039] As used herein, the terms **"hFzd8-ECD.275"** and **"hFzd8.275"** may be used interchangeably to refer to the full-length human Fzd8 ECD corresponding to SEQ ID NO: 3 (with signal peptide) or to SEQ ID NO: 4 (without signal peptide; mature form).

[040] As used herein, the terms **"hFzd8-ECD.155"** and **"hFzd8.155"** may be used interchangeably to refer to the human Fzd8 ECD corresponding to SEQ ID NO: 13 (with signal peptide) or to SEQ ID NO: 12 (without signal peptide; mature form).

[041] The term **"Fzd8 ECD fusion molecule"** refers to a molecule comprising a Fzd8 ECD, and one or more **"fusion partners."** In some embodiment, the Fzd8 ECD and the fusion partner are covalently linked **("fused").** If the fusion partner is also a polypeptide **("the fusion partner polypeptide"),** the Fzd8 ECD and the fusion partner polypeptide may be part of a continuous amino acid sequence, and the fusion partner

polypeptide may be linked to either the N terminus or the C terminus of the Fzd8 ECD. In such cases, the Fzd8 ECD and the fusion partner polypeptide may be translated as a single polypeptide from a coding sequence that encodes both the Fzd8 ECD and the fusion partner polypeptide (the **"Fzd8 ECD fusion protein").** In some embodiments, the Fzd8 ECD and the fusion partner are covalently linked through other means, such as, for example, a chemical linkage other than a peptide bond. Many known methods of covalently linking polypeptides to other molecules (for example, fusion partners) may be used. In other embodiments, the Fzd8 ECD and the fusion partner may be fused through a **"linker,"** which is comprised of at least one amino acid or chemical moiety.

[042] In some embodiments, the Fzd8 polypeptide and the fusion partner are noncovalently linked. In some such embodiments, they may be linked, for example, using binding pairs. Exemplary binding pairs include, but are not limited to, biotin and avidin or streptavidin, an antibody and its antigen, etc.

[043] Exemplary fusion partners include, but are not limited to, an immunoglobulin Fc domain, albumin, and polyethylene glycol. The amino acid sequences of some exemplary Fc domains are shown in SEQ ID NOs: 8 to 10. In some embodiments, there is a two amino acid residue linker consisting of an N-terminal glycine residue followed by a serine residue (GS) located between the Fzd8 ECD and the Fc. The amino acid sequence of a some exemplary N-terminal GS linker followed by an Fc is shown in SEQ ID NO: 11.

[044] The term **"signal peptide"** refers to a sequence of amino acid residues located at the N terminus of a polypeptide that facilitates secretion of a polypeptide from a mammalian cell. A signal peptide may be cleaved upon export of the polypeptide from the mammalian cell, forming a mature protein. Signal peptides may be natural or synthetic, and they may be heterologous or homologous to the protein to which they are attached. Exemplary signal peptides include, but are not limited to, the signal peptides of Fzd8, such as, for example, the amino acid sequence of SEQ ID NOs: 7. Exemplary signal peptides also include signal peptides from heterologous proteins. A **"signal sequence"** refers to a polynucleotide sequence that encodes a signal peptide. In some embodiments, a Fzd8 ECD lacks a signal peptide. In some embodiments, a Fzd8 ECD includes at least one signal peptide, which may be a native Fzd8 signal peptide or a heterologous signal peptide.

[045] In some embodiments, a Fzd8 ECD amino acid sequence is derived from that of a non-human mammal. In such embodiments, the Fzd8 ECD amino acid sequence

may be derived from mammals including, but not limited to, rodents (including mice, rats, hamsters), rabbits, simians, felines, canines, equines, bovines, porcines, ovines, caprines, mammalian laboratory animals, mammalian farm animals, mammalian sport animals, and mammalian pets. Fzd8 ECD fusion molecules incorporating a non-human Fzd8 ECD are termed **"non-human Fzd8 ECD fusion molecules."** Similar to the human Fzd8 ECD fusion molecules, non-human fusion molecules may comprise a fusion partner, optional linker, and a Fzd8 ECD. Such non-human fusion molecules may also include a signal peptide. A **"non-human Fzd8 ECD fragment"** refers to a non-human Fzd8 ECD having one or more residues deleted from the N and/or C terminus of the full-length ECD and that retains the ability to bind to a Wnt ligand of the non-human animal from which the sequence was derived. A **"non-human Fzd8 ECD variant"** refers to Fzd8 ECDs that contain amino acid additions, deletions, and substitutions and that remain capable of binding to a Wnt ligand from the animal from which the sequence was derived.

[046] The term **"antibody"** as used herein refers to a molecule comprising at least complementarity-determining region (CDR) 1, CDR2, and CDR3 of a heavy chain and at least CDR1, CDR2, and CDR3 of a light chain, wherein the molecule is capable of binding to antigen. The term antibody includes, but is not limited to, fragments that are capable of binding antigen, such as Fv, single-chain Fv (scFv), Fab, Fab', and (Fab')2. The term antibody also includes, but is not limited to, chimeric antibodies, humanized antibodies, and antibodies of various species such as mouse, human, cynomolgus monkey, etc.

[047] The term **"vector"** is used to describe a polynucleotide that may be engineered to contain a cloned polynucleotide or polynucleotides that may be propagated in a host cell. A vector may include one or more of the following elements: an origin of replication, one or more regulatory sequences (such as, for example, promoters and/or enhancers) that regulate the expression of the polypeptide of interest, and/or one or more selectable marker genes (such as, for example, antibiotic resistance genes and genes that may be used in colorimetric assays, e.g., β-galactosidase). The term **"expression vector"** refers to a vector that is used to express a polypeptide of interest in a host cell.

[048] A **"host cell"** refers to a cell that may be or has been a recipient of a vector or isolated polynucleotide. Host cells may be prokaryotic cells or eukaryotic cells. Exemplary eukaryotic cells include mammalian cells, such as primate or non-primate animal cells; fungal cells; plant cells; and insect cells. Exemplary mammalian cells

include, but are not limited to, 293 and CHO cells, and their derivatives, such as 293 -6E and DG44 cells, respectively.

[049] The term **"isolated"** as used herein refers to a molecule that has been separated from at least some of the components with which it is typically found in nature. For example, a polypeptide is referred to as "isolated" when it is separated from at least some of the components of the cell in which it was produced. Where a polypeptide is secreted by a cell after expression, physically separating the supernatant containing the polypeptide from the cell that produced it is considered to be "isolating" the polypeptide. Similarly, a polynucleotide is referred to as "isolated" when it is not part of the larger polynucleotide (such as, for example, genomic DNA or mitochondrial DNA, in the case of a DNA polynucleotide) in which it is typically found in nature, or is separated from at least some of the components of the cell in which it was produced, e.g., in the case of an RNA polynucleotide. Thus, a DNA polynucleotide that is contained in a vector inside a host cell may be referred to as "isolated" so long as that polynucleotide is not found in that vector in nature.

[050] The terms **"subject"** and **"patient"** are used interchangeably herein to refer to a human. In some embodiments, methods of treating other mammals, including, but not limited to, rodents, simians, felines, canines, equines, bovines, porcines, ovines, caprines, mammalian laboratory animals, mammalian farm animals, mammalian sport animals, and mammalian pets, are also provided.

[05 1] The term **"obesity"** refers to a medical condition that includes an accumulation of excess body fat such that an obese individual has a body mass index (BMI) of greater than 25. In some embodiments, an obese individual has a BMI of greater than 26, greater than 27, greater than 28, greater than 29, or greater than 30. BMI may be calculated using standard methods, such as BMI = ((weight in pounds)x703)/(height in inches)^2.

[052] The term **"obesity-related condition"** refers to a condition that is associated with obesity. Such conditions include, but are not limited to, heart disease, diabetes (such as type 2 diabetes), breathing difficulties, osteoarthritis, high blood pressure, high cholesterol (including high LDL cholesterol), high triglycerides, and high free fatty acids.

[053] **"Treatment"** (and grammatical variations thereof such as "treat" or "treating"), as used herein, includes any administration or application of a therapeutic for condition in a mammal, including a human, and includes inhibiting the condition or

progression of the condition, inhibiting or slowing the condition or its progression, arresting its development, partially or fully relieving the condition, or curing the condition, for example, by causing regression, or restoring or repairing a lost, missing, or defective function; or stimulating an inefficient process.

[054] In the case of obesity, a treatment includes any administration or application of a therapeutic that results in at least a 10% reduction in body weight. In some embodiments, a treatment results in at least a 10% increase in the ratio of lean mass to fat mass in a subject.

[055] The terms **"inhibition"** or **"inhibit"** refer to a decrease or cessation of any phenotypic characteristic or to the decrease or cessation in the incidence, degree, or likelihood of that characteristic.

[056] An agent is said to **"lower blood glucose levels"** or **"lower a blood glucose level,"** if the agent lowers blood glucose levels in a subject by at least 15% after administration of the agent, relative to blood glucose levels in the subject before administration of the agent. In some embodiments, an agent lowers blood glucose levels by at least 20%, at least 25%, at least 30%, or at least 35% after administration. In some embodiments, "after administration" is 8 hours, 12 hours, 24 hours, 2 days, 3 days, 5 days, 1 week, 10 days, 2 weeks, or 1 month after first administration of the agent.

[057] An agent is said to **"lower fasting blood glucose levels"** or **"lower a fasting blood glucose level,"** if the agent lowers fasting blood glucose levels in a subject by at least 15% after administration of the agent, relative to fasting blood glucose levels in the subject before administration of the agent. In some embodiments, an agent lowers fasting blood glucose levels by at least 20%, at least 25%, at least 30%, or at least 35% after administration. In some embodiments, "after administration" is 8 hours, 12 hours, 24 hours, 2 days, 3 days, 5 days, 1 week, 10 days, 2 weeks, or 1 month after first administration of the agent. In some embodiments, the length of the fast prior to measuring fasting blood glucose levels is at least 4 hours, at least 6 hours, at least 8 hours, at least 12 hours, at least 18 hours, or at least 24 hours.

[058] An agent is said to **"reduce NPY expression"** if the agent reduces the levels of NPY mRNA and/or protein in the hypothalamus of a test animal after administration of the agent, relative to the levels of NPY mRNA and/or protein in the hypothalamus of an untreated test animal. In some embodiments, an agent reduces the levels of NPY mRNA and/or protein by at least 15%, at least 20%, at least 25%, at least 30%, or at least 35% after administration. In some embodiments, "after administration" is

8 hours, 12 hours, 24 hours, 2 days, 3 days, 5 days, 1 week, 10 days, 2 weeks, or 1 month after first administration of the agent. Nonlimiting exemplary test animals include rodents (such as mice and rats), simians, felines, canines, equines, bovines, porcines, ovines, and caprines.

[059] A **"pharmaceutically acceptable carrier"** refers to a non-toxic solid, semisolid, or liquid filler, diluent, encapsulating material, formulation auxiliary, or carrier conventional in the art for use with a therapeutic agent that together comprise a **"pharmaceutical composition"** for administration to a subject. A pharmaceutically acceptable carrier is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. The pharmaceutically acceptable carrier is appropriate for the formulation employed. For example, if the therapeutic agent is to be administered orally, the carrier may be a gel capsule. If the therapeutic agent is to be administered subcutaneously, the carrier ideally is not irritable to the skin and does not cause injection site reaction.

Therapeutic Compositions and Methods

Methods of Treating Conditions using Fzd8 ECDs and Fzd8 ECD Fusion Molecules

[060] Methods of treating obesity and obesity-related conditions comprising administering a Fzd8 ECD, Fzd8 ECD fusion molecule, and/or an antibody that binds Fzd8 are provided. Nonlimiting exemplary Fzd8 ECDs that are useful for treating obesity and obesity-related conditions include ECDs having a sequence selected from SEQ ID NOs: 3, 4, 12, and 16. Nonlimiting exemplary Fzd8 ECD fusion molecules that are useful for treating obesity and obesity-related conditions include fusion molecules having a sequence selected from SEQ ID NOs: 5, 6, 14, 15, 17, and 18.

[061] Exemplary obesity-related conditions that may be treated with a Fzd8 ECD, Fzd8 ECD fusion molecule, and/or an antibody that binds Fzd8 include, but are not limited to, heart disease, diabetes (such as type 2 diabetes), breathing difficulties, osteoarthritis, high blood pressure, high cholesterol (including high LDL cholesterol), high triglycerides, and high free fatty acids.

[062] Methods of lowering blood glucose levels in a subject comprising administering a Fzd8 ECD, Fzd8 ECD fusion molecule, and/or an antibody that binds Fzd8 are also provided. In some embodiments, methods of lowering fasting blood glucose levels in a subject comprising administering a Fzd8 ECD, Fzd8 ECD fusion molecule, and/or an antibody that binds Fzd8 are provided. Nonlimiting exemplary Fzd8 ECDs that

are useful for lowering blood glucose levels include ECDs having a sequence selected from SEQ ID NOs: 3, 4, 12, and 16. Nonlimiting exemplary Fzd8 ECD fusion molecules that are useful for treating obesity and obesity-related conditions include fusion molecules having a sequence selected from SEQ ID NOs: 5, 6, 14, 15, 17, and 18.

[063] Methods of reducing NPY expression in the hypothalamus of a subject comprising administering a Fzd8 ECD, Fzd8 ECD fusion molecule, and/or an antibody that binds Fzd8 are also provided. Nonlimiting exemplary Fzd8 ECDs that are useful for lowering blood glucose levels include ECDs having a sequence selected from SEQ ID NOs: 3, 4, 12, and 16. Nonlimiting exemplary Fzd8 ECD fusion molecules that are useful for treating obesity and obesity-related conditions include fusion molecules having a sequence selected from SEQ ID NOs: 5, 6, 14, 15, 17, and 18.

Routes of Administration and Carriers

[064] In some embodiments, a Fzd8 ECD, a Fzd8 ECD fusion molecule, and/or an antibody that binds Fzd8 is administered subcutaneously. In some embodiments, a Fzd8 ECD, a Fzd8 ECD fusion molecule, and/or an antibody that binds Fzd8 is administered intravenously. In some embodiments, a Fzd8 ECD, a Fzd8 ECD fusion molecule, and/or an antibody that binds Fzd8 may be administered *in vivo* by various routes, including, but not limited to, oral, intra-arterial, parenteral, intranasal, intramuscular, intracardiac, intraventricular, intratracheal, buccal, rectal, intraperitoneal, intradermal, topical, transdermal, and intrathecal, or otherwise by implantation or inhalation. The subject compositions may be formulated into preparations in solid, semi-solid, liquid, or gaseous forms; including, but not limited to, tablets, capsules, powders, granules, ointments, solutions, suppositories, enemas, injections, inhalants, and aerosols. One or more nucleic acid molecules that encode a Fzd8 ECD, a Fzd8 ECD fusion molecule, and/or an antibody that binds Fzd8 may be coated onto gold microparticles and delivered intradermally by a particle bombardment device, or "gene gun," as described in the literature (see, e.g., Tang et al, *Nature* 356:152-154 (1992)). The appropriate formulation and route of administration may be selected according to the intended application.

[065] In various embodiments, compositions comprising Fzd8 ECDs, Fzd8 ECD fusion molecules, and/or an antibodies that bind Fzd8 are provided in formulations with a wide variety of pharmaceutically acceptable carriers (see, e.g., Gennaro, *Remington: The Science and Practice of Pharmacy with Facts and Comparisons: Drugfacts Plus*, 20th ed. (2003); Ansel et al, *Pharmaceutical Dosage Forms and Drug Delivery Systems*, 7th ed.,

Lippencott Williams and Wilkins (2004); Kibbe et al, *Handbook of Pharmaceutical Excipients*, 3rd ed., Pharmaceutical Press (2000)). Various pharmaceutically acceptable carriers, which include vehicles, adjuvants, and diluents, are available. Moreover, various pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are also available. Nonlimiting exemplary carriers include saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

[066] In various embodiments, compositions comprising Fzd8 ECDs, Fzd8 ECD fusion molecules, and/or an antibodies that bind Fzd8 may be formulated for injection, including subcutaneous administration, by dissolving, suspending, or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids, or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives. In various embodiments, the compositions may be formulated for inhalation, for example, using pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen, and the like. The compositions may also be formulated, in various embodiments, into sustained release microcapsules, such as with biodegradable or non-biodegradable polymers. A non-limiting exemplary biodegradable formulation includes poly lactic acid-glycolic acid polymer. A non-limiting exemplary non-biodegradable formulation includes a polyglycerin fatty acid ester. Nonlimiting exemplary methods of making such formulations are described, for example, in EP 1 125 584 Al.

[067] Pharmaceutical packs and kits comprising one or more containers, each containing one or more doses of a Fzd8 ECD, a Fzd8 ECD fusion molecule, and/or an antibody to Fzd8 are also provided. In some embodiments, a unit dosage is provided wherein the unit dosage contains a predetermined amount of a composition comprising a Fzd8 ECD, a Fzd8 ECD fusion molecule, and/or an antibody to Fzd8, with or without one or more additional agents. In some embodiments, such a unit dosage is supplied in single-use prefilled syringe for injection. In various embodiments, the composition contained in the unit dosage may comprise saline, sucrose, or the like; a buffer, such as phosphate, or the like; and/or be formulated within a stable and effective pH range. Alternatively, in some embodiments, the composition may be provided as a lyophilized powder that may be reconstituted upon addition of an appropriate liquid, for example, sterile water. In some embodiments, the composition comprises one or more substances that inhibit protein

aggregation, including, but not limited to, sucrose and arginine. In some embodiments, a composition of the invention comprises heparin and/or a proteoglycan.

[068] Pharmaceutical compositions are administered in an amount effective for treatment or prophylaxis of the specific indication. The therapeutically effective amount is typically dependent on the weight of the subject being treated, his or her physical or health condition, the extensiveness of the condition to be treated, or the age of the subject being treated. In general, the Fzd8 ECDs, Fzd8 ECD fusion molecules, and antibodies to Fzd8 may be administered in an amount in the range of about 10 μg/kg body weight to about 100 mg/kg body weight per dose. In some embodiments, the Fzd8 ECDs, Fzd8 ECD fusion molecules, and antibodies to Fzd8 may be administered in an amount in the range of about 50 μg/kg body weight to about 5 mg/kg body weight per dose. In some embodiments, the Fzd8 ECDs, Fzd8 ECD fusion molecules, and antibodies to Fzd8 may be administered in an amount in the range of about 100 μg/kg body weight to about 10 mg/kg body weight per dose. In some embodiments, the Fzd8 ECDs, Fzd8 ECD fusion molecules, and antibodies to Fzd8 may be administered in an amount in the range of about 100 μg/kg body weight to about 20 mg/kg body weight per dose. In some embodiments, the Fzd8 ECDs, Fzd8 ECD fusion molecules, and antibodies to Fzd8 may be administered in an amount in the range of about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose.

[069] The Fzd8 ECD, Fzd8 ECD fusion molecule, or antibody to Fzd8 compositions may be administered as needed to subjects. Determination of the frequency of administration may be made by persons skilled in the art, such as an attending physician based on considerations of the condition being treated, age of the subject being treated, severity of the condition being treated, general state of health of the subject being treated and the like. In some embodiments, an effective dose of the Fzd8 ECD, Fzd8 ECD fusion molecule, and/or antibody to Fzd8 is administered to a subject one or more times. In various embodiments, an effective dose of the Fzd8 ECD, Fzd8 ECD fusion molecule, and/or antibody to Fzd8 is administered to the subject once a month, more than once a month, such as, for example, every two months or every three months. In some embodiments, an effective dose of the Fzd8 ECD, Fzd8 ECD fusion molecule, and/or antibody to Fzd8 is administered less than once a month, such as, for example, every three weeks, every two weeks or every week. An effective dose of the Fzd8 ECD, Fzd8 ECD fusion molecule, and/or antibody to Fzd8 is administered to the subject at least once. In some embodiments, the effective dose of the Fzd8 ECD, Fzd8 ECD fusion molecule,

and/or antibody to Fzd8 may be administered multiple times, including for periods of at least a month, at least six months, or at least a year.

Combination Therapy

[070] Fzd8 ECDs, Fzd8 ECD fusion molecules, and/or antibodies to Fzd8 may be administered alone or with other modes of treatment. They may be provided before, substantially contemporaneous with, or after other modes of treatment, for example, diet, physical exercise, surgery, or the administration of another therapeutic molecule. In some embodiments, a Fzd8 ECD, Fzd8 ECD fusion molecule, and/or antibody to Fzd8 is administered with an appetite suppressant. Nonlimiting exemplary therapeutic molecules that may be administered with a Fzd8 ECD, Fzd8 ECD fusion molecule, and/or antibody to Fzd8 include phentermine, Xenical® (orlistat), Meridia® (sibutramin HC1 monohydrate), Alii® (orlistat), lorcaserin, Qnexa® (phentermine/topiramate), Contrave® (naltrexone SR/bupropion SR), Victoza (liraglutide), cetilistat, Symlin with metreleptin (pramlintide/metreleptin), Histalean (betahistine), Empatic™ (zonisamide SR/bupropion SR), tesofensine, SLx-4090 (Surface Logix), AR9281 (Arete), velneperit, davalintide, TTP435 (TransTech), TM30339 (7TM Pharma), and obinepitide.

Fzd8 Extracellular Domains

[071] Nonlimiting exemplary Fzd8 ECDs include full-length Fzd8 ECDs, Fzd8 ECD fragments, and Fzd8 ECD variants. Fzd8 ECDs may include or lack a signal peptide. Exemplary Fzd8 ECDs include, but are not limited to, Fzd8 ECDs having amino acid sequences selected from SEQ ID NOs: 3, 4, 12, and 13. In some embodiments, a Fzd8 ECD is isolated. In some embodiments, an Fzd8 ECD binds at least one ligand selected from Wntl, Wnt2, Wnt3, Wnt3a, and Wnt7b.

[072] In some embodiments, administration of a human Fzd8 ECD fusion molecule to mice fed a normal diet results in weight loss and reduced fat pad weights. In addition, administration of a human Fzd8 ECD fusion molecule to mice fed a high fat diet results in very little weight gain (compared to a gain of about 30% body weight in vehicle-treated mice), reduced fat mass, and increased lean to fat ratio. Further, mice administered a Fzd8 ECD fusion molecule consumed less than vehicle-treated mice, but had normal digestive tracks.

Fzd8 ECD Fragments

[073] Non-limiting exemplary Fzd8 ECD fragments include human Fzd8 ECD ending at amino acid 155 (counting from the first amino acid of the signal peptide,

whether or not the signal peptide is present). In some embodiments, a Fzd8 ECD fragment ends at an amino acid between amino acid 155 and amino acid 275.

[074] Fzd8 ECD fragments may include or lack a signal peptide. Exemplary Fzd8 ECD fragments include, but are not limited to, Fzd8 ECD fragments having amino acid sequences selected from SEQ ID NOs: 12 and 13. In some embodiments, an Fzd8 ECD fragment binds at least one ligand selected from Wntl, Wnt2, Wnt3, Wnt3a, and Wnt7b.

Fusion Partners and Conjugates

[075] As discussed, a Fzd8 ECD of the present invention may be combined with a fusion partner polypeptide, resulting in a Fzd8 ECD fusion protein. These fusion partner polypeptides may facilitate purification, and the Fzd8 ECD fusion proteins may show an increased half-life *in vivo*. Fusion partner polypeptides that have a disulfide-linked dimeric structure due to the IgG portion may also be more efficient in binding and neutralizing other molecules than the monomeric Fzd8 ECD fusion protein or the Fzd8 ECD alone. Suitable fusion partners of a Fzd8 ECD include, for example, polymers, such as water soluble polymers, the constant domain of immunoglobulins; all or part of human serum albumin (HSA); fetuin A; fetuin B; a leucine zipper domain; a tetranectin trimerization domain; mannose binding protein (also known as mannose binding lectin), for example, mannose binding protein 1; and an Fc region, as described herein and further described in U.S. Patent No. 6,686,179.

[076] A Fzd8 ECD fusion molecule of the invention may be prepared by attaching polyaminoacids or branch point amino acids to the Fzd8 ECD. For example, the polyaminoacid may be a carrier protein that serves to increase the circulation half life of the Fzd8 ECD (in addition to the advantages achieved via a fusion molecule). For the therapeutic purpose of the present invention, such polyaminoacids should ideally be those that have or do not create neutralizing antigenic response, or other adverse responses. Such polyaminoacids may be chosen from serum album (such as HSA), an additional antibody or portion thereof, for example the Fc region, fetuin A, fetuin B, leucine zipper nuclear factor erythroid derivative-2 (NFE2), neuroretinal leucine zipper, tetranectin, or other polyaminoacids, for example, lysines. As described herein, the location of attachment of the polyaminoacid may be at the N terminus or C terminus, or other places in between, and also may be connected by a chemical linker moiety to the selected molecule.

Polymers

[077] Polymers, for example, water soluble polymers, are useful in the present invention as the Fzd8 ECD to which the polymer is attached will not precipitate in an aqueous environment, such as typically found in a physiological environment. Polymers employed in the invention will be pharmaceutically acceptable for the preparation of a therapeutic product or composition.

[078] Suitable, clinically acceptable, water soluble polymers include, but are not limited to, polyethylene glycol (PEG), polyethylene glycol propionaldehyde, copolymers of ethylene glycol/propylene glycol, monomethoxy-polyethylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol (PVA), polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-l,3,6-trioxane, ethylene/maleic anhydride copolymer, poly (β-amino acids) (either homopolymers or random copolymers), poly(n-vinyl pyrrolidone) polyethylene glycol, polypropylene glycol homopolymers (PPG) and other polyakylene oxides, polypropylene oxide/ethylene oxide copolymers, polyoxyethylated polyols (POG) (e.g., glycerol) and other polyoxyethylated polyols, polyoxyethylated sorbitol, or polyoxyethylated glucose, colonic acids or other carbohydrate polymers, Ficoll, or dextran and mixtures thereof.

[079] As used herein, polyethylene glycol (PEG) is meant to encompass any of the forms that have been used to derivatize other proteins, such as mono-(Cl-ClO) alkoxy- or aryloxy-polyethylene glycol. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water.

[080] Polymers used herein, for example water soluble polymers, may be of any molecular weight and may be branched or unbranched. In some embodiments, the polymers have an average molecular weight of between about 2 kDa to about 100 kDa (the term "about" indicating that in preparations of a polymer, some molecules will weigh more, some less, than the stated molecular weight). The average molecular weight of each polymer may be between about 5 kDa and about 50 kDa, or between about 12 kDa and about 25 kDa. Generally, the higher the molecular weight or the more branches, the higher the polymerprotein ratio. Other sizes may also be used, depending on the desired therapeutic profile; for example, the duration of sustained release; the effects, if any, on biological activity; the ease in handling; the degree or lack of antigenicity; and other known effects of a polymer on a Fzd8 ECD of the invention.

[081] Polymers employed in the present invention are typically attached to a Fzd8 ECD with consideration of effects on functional or antigenic domains of the polypeptide.

In general, chemical derivatization may be performed under any suitable condition used to react a protein with an activated polymer molecule. Activating groups which can be used to link the polymer to the active moieties include sulfone, maleimide, sulfhydryl, thiol, triflate, tresylate, azidirine, oxirane, and 5-pyridyl.

[082] Polymers of the invention are typically attached to a heterologous polypeptide at the alpha (a) or epsilon (ε) amino groups of amino acids or a reactive thiol group, but it is also contemplated that a polymer group could be attached to any reactive group of the protein that is sufficiently reactive to become attached to a polymer group under suitable reaction conditions. Thus, a polymer may be covalently bound to a Fzd8 ECD via a reactive group, such as a free amino or carboxyl group. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residue. Those having a free carboxyl group may include aspartic acid residues, glutamic acid residues, and the C-terminal amino acid residue. Those having a reactive thiol group include cysteine residues.

[083] Methods for preparing fusion molecules conjugated with polymers, such as water soluble polymers, will each generally involve (a) reacting a Fzd8 ECD with a polymer under conditions whereby the polypeptide becomes attached to one or more polymers and (b) obtaining the reaction product. Reaction conditions for each conjugation may be selected from any of those known in the art or those subsequently developed, but should be selected to avoid or limit exposure to reaction conditions such as temperatures, solvents, and pH levels that would inactivate the protein to be modified. In general, the optimal reaction conditions for the reactions will be determined case-by-case based on known parameters and the desired result. For example, the larger the ratio of polymenpolypeptide conjugate, the greater the percentage of conjugated product. The optimum ratio (in terms of efficiency of reaction in that there is no excess unreacted polypeptide or polymer) may be determined by factors such as the desired degree of derivatization (e.g., mono-, di-, tri-, etc.), the molecular weight of the polymer selected, whether the polymer is branched or unbranched and the reaction conditions used. The ratio of polymer (for example, PEG) to a polypeptide will generally range from 1:1 to 100: 1. One or more purified conjugates may be prepared from each mixture by standard purification techniques, including among others, dialysis, salting-out, ultrafiltration, ion-exchange chromatography, gel filtration chromatography, and electrophoresis.

[084] One may specifically desire an N-terminal chemically modified Fzd8 ECD. One may select a polymer by molecular weight, branching, etc., the proportion of

polymers to Fzd8 ECD molecules in the reaction mix, the type of reaction to be performed, and the method of obtaining the selected N-terminal chemically modified Fzd8 ECD. The method of obtaining the N-terminal chemically modified Fzd8 ECD preparation (separating this moiety from other monoderivatized moieties if necessary) may be by purification of the N-terminal chemically modified Fzd8 ECD material from a population of chemically modified protein molecules.

[085] Selective N-terminal chemical modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N terminus with a carbonyl group-containing polymer is achieved. For example, one may selectively attach a polymer to the N terminus of the protein by performing the reaction at a pH that allows one to take advantage of the pKa differences between the ε-amino group of the lysine residues and that of the a-amino group of the N-terminal residue of the protein. By such selective derivatization, attachment of a polymer to a protein is controlled: the conjugation with the polymer takes place predominantly at the N terminus of the protein and no significant modification of other reactive groups, such as the lysine side chain amino groups, occurs. Using reductive alkylation, the polymer may be of the type described above and should have a single reactive aldehyde for coupling to the protein. Polyethylene glycol propionaldehyde, containing a single reactive aldehyde, may also be used.

[086] In one embodiment, the present invention contemplates the chemically derivatized Fzd8 ECD to include mono- or poly- (e.g., 2-4) PEG moieties. Pegylation may be carried out by any of the pegylation reactions available. Methods for preparing a pegylated protein product will generally include (a) reacting a polypeptide with polyethylene glycol (such as a reactive ester or aldehyde derivative of PEG) under conditions whereby the protein becomes attached to one or more PEG groups; and (b) obtaining the reaction product(s). In general, the optimal reaction conditions will be determined case by case based on known parameters and the desired result.

[087] There are a number of PEG attachment methods available to those skilled in the art. *See*, for example, EP 0 401 384; Malik et al, *Exp. Hematol*, 20:1028-1035 (1992); Francis, *Focus on Growth Factors*, 3(2):4-10 (1992); EP 0 154 316; EP 0 401 384; WO 92/16221; WO 95/34326; and the other publications cited herein that relate to pegylation.

[088] Pegylation may be carried out, e.g., via an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule. Thus, protein products according to the present invention include pegylated proteins wherein the PEG group(s) is (are) attached via acyl or alkyl groups. Such products may be mono-pegylated or poly-pegylated (for example, those containing 2-6 or 2-5 PEG groups). The PEG groups are generally attached to the protein at the a- or ε-amino groups of amino acids, but it is also contemplated that the PEG groups could be attached to any amino group attached to the protein that is sufficiently reactive to become attached to a PEG group under suitable reaction conditions.

[089] Pegylation by acylation generally involves reacting an active ester derivative of polyethylene glycol (PEG) with a Fzd8 ECD of the invention. For acylation reactions, the polymer(s) selected typically have a single reactive ester group. Any known or subsequently discovered reactive PEG molecule may be used to carry out the pegylation reaction. An example of a suitable activated PEG ester is PEG esterified to N-hydroxysuccinimide (NHS). As used herein, acylation is contemplated to include, without limitation, the following types of linkages between the therapeutic protein and a polymer such as PEG: amide, carbamate, urethane, and the like, see for example, Chamow, *Bioconjugate Chem.,* 5:133-140 (1994). Reaction conditions may be selected from any of those currently known or those subsequently developed, but should avoid conditions such as temperature, solvent, and pH that would inactivate the polypeptide to be modified.

[090] Pegylation by acylation will generally result in a poly-pegylated protein. The connecting linkage may be an amide. The resulting product may be substantially only (e.g., > 95%) mono-, di-, or tri-pegylated. However, some species with higher degrees of pegylation may be formed in amounts depending on the specific reaction conditions used. If desired, more purified pegylated species may be separated from the mixture (particularly unreacted species) by standard purification techniques, including among others, dialysis, salting-out, ultrafiltration, ion-exchange chromatography, gel filtration chromatography, and electrophoresis.

[091] Pegylation by alkylation generally involves reacting a terminal aldehyde derivative of PEG with a polypeptide in the presence of a reducing agent. For the reductive alkylation reaction, the polymer(s) selected should have a single reactive aldehyde group. An exemplary reactive PEG aldehyde is polyethylene glycol propionaldehyde, which is water stable, or mono C1-C10 alkoxy or aryloxy derivatives thereof, see for example, U.S. Pat. No. 5,252,714.

Markers

[092] Moreover, Fzd8 ECDs of the present invention may be fused to marker sequences, such as a peptide that facilitates purification of the fused polypeptide. The marker amino acid sequence may be a hexa-histidine peptide such as the tag provided in a pQE vector (Qiagen), among others, many of which are commercially available. As described in Gentz et al, *Proc. Natl. Acad. Sci.* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the hemagglutinin (HA) tag, corresponds to an epitope derived from the influenza HA protein. (Wilson et al, *Cell* 37:767 (1984)). Any of these above fusions may be engineered using the Fzd8 ECDs of the present invention.

Oligomerization Domain Fusion Partners

[093] In various embodiments, oligomerization offers some functional advantages to a fusion protein, including, but not limited to, multivalency, increased binding strength, and the combined function of different domains. Accordingly, in some embodiments, a fusion partner comprises an oligomerization domain, for example, a dimerization domain. Exemplary oligomerization domains include, but are not limited to, coiled-coil domains, including alpha-helical coiled-coil domains; collagen domains; collagen-like domains; and certain immunoglobulin domains. Exemplary coiled-coil polypeptide fusion partners include, but are not limited to, the tetranectin coiled-coil domain; the coiled-coil domain of cartilage oligomeric matrix protein; angiopoietin coiled-coil domains; and leucine zipper domains. Exemplary collagen or collagen-like oligomerization domains include, but are not limited to, those found in collagens, mannose binding lectin, lung surfactant proteins A and D, adiponectin, ficolin, conglutinin, macrophage scavenger receptor, and emilin.

Antibody Fc Immunoglobulin Domain Fusion Partners

[094] Many Fc domains that may be used as fusion partners are known in the art. In some embodiments, a fusion partner is an Fc immunoglobulin domain. An Fc fusion partner may be a wild-type Fc found in a naturally occurring antibody, a variant thereof, or a fragment thereof. Non-limiting exemplary Fc fusion partners include Fes comprising a hinge and the CH2 and CH3 constant domains of a human IgG, for example, human IgGl, IgG2, IgG3, or IgG4. Additional exemplary Fc fusion partners include, but are not limited to, human IgA and IgM. In some embodiments, an Fc fusion partner comprises a C237S mutation, for example, in an IgGl. In some embodiments, an Fc fusion partner comprises a hinge, CH2, and CH3 domains of human IgG2 with a P331S mutation, as described in

U.S. Patent No. 6,900,292. Certain exemplary Fc domain fusion partners are shown in SEQ ID NOs: 8 to 11.

Albumin Fusion Partners and Albumin-binding Molecule Fusion Partners

[095] In some embodiments, a fusion partner is an albumin. Exemplary albumins include, but are not limited to, human serum album (HSA) and fragments of HSA that are capable of increasing the serum half-life or bioavailability of the polypeptide to which they are fused. In some embodiments, a fusion partner is an albumin-binding molecule, such as, for example, a peptide that binds albumin or a molecule that conjugates with a lipid or other molecule that binds albumin. In some embodiments, a fusion molecule comprising HSA is prepared as described, e.g., in U.S. Patent No. 6,686,179.

Exemplary Attachment of Fusion Partners

[096] The fusion partner may be attached, either covalently or non-covalently, to the N terminus or the C terminus of the Fzd8 ECD. The attachment may also occur at a location within the Fzd8 ECD other than the N terminus or the C terminus, for example, through an amino acid side chain (such as, for example, the side chain of cysteine, lysine, serine, or threonine).

[097] In either covalent or non-covalent attachment embodiments, a linker may be included between the fusion partner and the Fzd8 ECD. Such linkers may be comprised of at least one amino acid or chemical moiety. Exemplary methods of covalently attaching a fusion partner to a Fzd8 ECD include, but are not limited to, translation of the fusion partner and the Fzd8 ECD as a single amino acid sequence and chemical attachment of the fusion partner to the Fzd8 ECD. When the fusion partner and a Fzd8 ECD are translated as single amino acid sequence, additional amino acids may be included between the fusion partner and the Fzd8 ECD as a linker. In some embodiments, the linker is glycine-serine ("GS"; see, e.g., SEQ ID NO: 11). In some embodiments, the linker is selected based on the polynucleotide sequence that encodes it, to facilitate cloning the fusion partner and/or Fzd8 ECD into a single expression construct (for example, a polynucleotide containing a particular restriction site may be placed between the polynucleotide encoding the fusion partner and the polynucleotide encoding the Fzd8 ECD, wherein the polynucleotide containing the restriction site encodes a short amino acid linker sequence). When the fusion partner and the Fzd8 ECD are covalently coupled by chemical means, linkers of various sizes may typically be included during the coupling reaction.

[098] Exemplary methods of non-covalently attaching a fusion partner to a Fzd8 ECD include, but are not limited to, attachment through a binding pair. Exemplary binding pairs include, but are not limited to, biotin and avidin or streptavidin, an antibody and its antigen, etc.

Antibodies that Block Ligand Binding to Fzd8

[099] In some embodiments, antibodies that block binding of at least one ligand to Fzd8 are provided. In some embodiments, an antibody blocks binding of a ligand selected from Wntl, Wnt2, Wnt3, Wnt3a, and Wnt7b to Fzd8. See, e.g., PCT Publication No. WO 2007/053577. In some embodiments, an antibody binds to Fzd8 extracellular domain (ECD). In some embodiments, an antibody binds a polypeptide comprising an amino acid sequence selected from SEQ ID NOs: 3, 4, 12, and 13. In some embodiments, an antibody binds a polypeptide consisting of an amino acid sequence selected from SEQ ID NOs: 3, 4, 12, and 13. Nonlimiting exemplary antibodies that bind Fzd8 are described, e.g., in PCT Publication Nos. WO 2007/053577 and WO 2010/037041.

Signal Peptide

[0100] In order for some secreted proteins to express and secrete in large quantities, a signal peptide from a heterologous protein may be desirable. Employing heterologous signal peptides may be advantageous in that a resulting mature polypeptide may remain unaltered as the signal peptide is removed in the ER during the secretion process. The addition of a heterologous signal peptide may be required to express and secrete some proteins.

[0101] Nonlimiting exemplary signal peptide sequences are described, e.g., in the online Signal Peptide Database maintained by the Department of Biochemistry, National University of Singapore. *See* Choo et al, *BMC Bioinformatics,* 6: 249 (2005); and PCT Publication No. WO 2006/081430.

Co-Translational and Post-Translational Modifications

[0102] The invention encompasses Fzd8 ECDs and Fzd8 ECD fusion molecules that are differentially modified during or after translation, for example by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or linkage to an antibody molecule or other cellular ligand. Similarly, antibodies that bind Fzd8 ECD and block binding of at least one ligand to Fzd8 ECD that are differentially modified during or after translation are also provided. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin,

papain, V8 protease; $NABH_{4;}$ acetylation; formylation; oxidation; reduction; and/or metabolic synthesis in the presence of tunicamycin.

[0103] Additional post-translational modifications encompassed by the invention include, for example, for example, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression

Nucleic Acid Molecules Encoding Fzd8 ECDs, Fzd8 ECD Fusion Molecules, and/or Antibodies that Bind Fzd8

[0104] Nucleic acid molecules comprising polynucleotides that encode Fzd8 ECDs, Fzd8 ECD fusion molecules, or antibodies that bind Fzd8 ECD are provided. Nucleic acid molecules comprising polynucleotides that encode Fzd8 ECD fusion molecules in which the Fzd8 ECD and the fusion partner are translated as a single polypeptide are also provided. Such nucleic acid molecules may be constructed using recombinant DNA techniques conventional in the art.

[0105] In some embodiments, a polynucleotide encoding a Fzd8 ECD comprises a nucleotide sequence that encodes a signal peptide, which, when translated, will be fused to the N terminus of the Fzd8 ECD. As discussed above, the signal peptide may be the native Fzd8 signal peptide, or may be another heterologous signal peptide. In some embodiments, the nucleic acid molecule comprising the polynucleotide encoding the gene of interest is an expression vector that is suitable for expression in a selected host cell.

Fzd8 ECD, Fzd8 ECD Fusion Molecule, and Fzd8 Antibody Expression and Production

Vectors

[0106] Vectors comprising polynucleotides that encode Fzd8 ECDs are provided. Vectors comprising polynucleotides that encode Fzd8 ECD fusion molecules are also provided. Vectors comprising polynucleotides that encode one or both chains of an antibody that binds Fzd8 are also provided. Such vectors include, but are not limited to, DNA vectors, phage vectors, viral vectors, retroviral vectors, etc.

[0107] In some embodiments, a vector is selected that is optimized for expression of polypeptides in CHO or CHO-derived cells. Exemplary such vectors are described, e.g., in Running Deer et al, *Biotechnol. Prog.* 20:880-889 (2004).

[0108] In some embodiments, a vector is chosen for *in vivo* expression of Fzd8 ECDs and/or Fzd8 ECD fusion molecules and/or antibodies that bind Fzd8 in animals, including humans. In some such embodiments, expression of the polypeptide is under the control of a promoter that functions in a tissue-specific manner. For example, liver-specific promoters are described, e.g., in PCT Publication No. WO 2006/076288.

Host Cells

[0109] In various embodiments, Fzd8 ECDs, Fzd8 ECD fusion molecules, or antibodies that bind Fzd8 may be expressed in prokaryotic cells, such as bacterial cells; or in eukaryotic cells, such as fungal cells, plant cells, insect cells, and mammalian cells. Such expression may be carried out, for example, according to procedures known in the art. Exemplary eukaryotic cells that may be used to express polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S and DG44 cells; and NSO cells. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make certain desired post-translational modifications to the Fzd8 ECDs, Fzd8 ECD fusion molecules, or antibodies that bind Fzd8. For example, in some embodiments, CHO cells produce Fzd8 ECDs and/or Fzd8 ECD fusion molecules and/or antibodies that bind Fzd8 that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[01 10] Introduction of a nucleic acid into a desired host cell may be accomplished by any method known in the art, including but not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, etc. Nonlimiting exemplary methods are described, e.g., in Sambrook et al, *Molecular Cloning, A Laboratory Manual,* 3rd ed. Cold Spring Harbor Laboratory Press (2001). Nucleic acids may be transiently or stably transfected in the desired host cells, according to methods known in the art.

[01 11] In some embodiments, a polypeptide may be produced *in vivo* in an animal that has been engineered or transfected with a nucleic acid molecule encoding the polypeptide, according to methods known in the art.

Purification of Fzd8 ECD Polypeptides

[01 12] Fzd8 ECDs, Fzd8 ECD fusion molecules, and antibodies that bind Fzd8 may be purified by various methods known in the art. Such methods include, but are not limited to, the use of affinity matrices or hydrophobic interaction chromatography. Suitable affinity ligands include any ligands of the Fzd8 ECD or of the fusion partner, or antibodies thereto. Suitable affinity ligands in the case of an antibody that binds Fzd8

include, but are not limited to, Fzd8 itself and fragments thereof. Further, a Protein A, Protein G, Protein A/G, or an antibody affinity column may be used to bind to an Fc fusion partner to purify a Fzd8 ECD fusion molecule or to the Fc portion of an antibody to Fzd8. Antibodies to Fzd8 ECD may also be used to purify Fzd8 ECD or Fzd8 ECD fusion molecules. Hydrophobic interactive chromatography, for example, a butyl or phenyl column, may also suitable for purifying some polypeptides. Many methods of purifying polypeptides are known in the art.

EXAMPLES

[01 13] The examples discussed below are intended to be purely exemplary of the invention and should not be considered to limit the invention in any way. The examples are not intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (for example, amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Mice treated with Fzd8 ECD-Fc exhibited less weight gain on a high-fat diet than vehicle-treated mice

Study procedure

[01 14] Seven- to eight-week old male C57B1/6 mice (Jackson Labs, Bar Harbor, ME) were administered a DNA vector encoding Fzd8 ECD-Fc, Fzd2 ECD-Fc, secreted frizzled-related protein 4 (SFRP4) ECD-Fc, or vehicle (Ringer's Solution; Fisher Scientific, Pittsburgh, PA; AD-5038) by hydrodynamic tail vein transfection (TVT) substantially as described in Chen et al, *Human Gene Therapy* 16(1): 126-131 (2005). Eleven mice were included in each group, and each mouse (except vehicle-treated mice) received 20 μg of DNA in 2 ml Ringer's Solution by hydrodynamic tail vein transfection. Vehicle-treated mice received 2 ml Ringer's Solution by hydrodynamic tail vein transfection.

[01 15] Following hydrodynamic tail vein transfection, the mice were switched to a 60% high fat diet (Research Diets Inc., New Brunswick, NJ; Diet #D12492, 60% kcal fat). Fresh food weight was recorded for each mouse, and the mice were weighed and their food consumption recorded by measuring the difference between what was provided and what was left, every seven days. Mice were monitored for bloody stool and diarrhea on days when food was recorded.

[01 16] Mice were bled on day 5 to determine plasma protein expression and transfection efficiency. On day 21, body weight and food consumption were recorded. Blood was collected by cardiac puncture at the time of euthanasia with isoflurane for serum chemistry and lipid panels.

[01 17] Necroscopy was performed following euthanasia to record the weights of the testicular, inguinal, and renal fat pads, and to dissect the colon, caecum, and duodenum for histological analysis. The gastrointestinal tracts were fixed in 4% neutral buffered formalin for 24 hours. Fixed tissues were washed in PBS and stored in 70% ethanol. For histological analysis, the fixed tissues were embedded in paraffin and stained with H&E (hematoxylin and eosin).

Results

[01 18] Figure 1 shows body weight of the mice in the study. The mice that received Fzd8 ECD-Fc by TVT had significantly lower body weight by 14 days post-injection than the mice that received Fzd2 ECD-Fc, SFRP ECD-Fc, and vehicle. While the vehicle-, Fzd2 ECD-Fc-, and SFRP ECD-Fc-treated mice all gained about 7 grams (or about 30% of their initial body weight) over the course of the study, the Fzd8-treated mice gained only about 2 grams (approximately 12% of their initial body weight).

[01 19] Food consumption was monitored at various intervals during the course of the study (data not shown). Fzd8 ECD-Fc-treated mice consumed about 9% less food than vehicle-treated mice on day 5 post injection, about 13% less food on day 14 post injection, and about 11% less food on day 2 1 post injection. This suggests that Fzd8 treatment may lead to increased satiety signals, lower food consumption, and ultimately a decrease in body weight gain, relative to vehicle treated mice.

[0120] Figure 2 shows the results of the lipid panel for the vehicle- and Fzd8 ECD-Fc-treated mice. The Fzd8 ECD-Fc-treated mice showed no reduction in serum triglycerides or cholesterol (Figures 2A and 2B), but showed some reduction in serum free fatty acids, with a statistical p value of 0.07 (Figure 2C).

[0121] Figure 3 shows that the epididymal, inguinal, and renal fat pads of the Fzd8 ECD-Fc-treated mice weighed significantly less ($p<0.05$) than the fat pads of the vehicle-treated mice.

[0122] Finally, the Fzd8 ECD-Fc-treated mice did not display any histological abnormalities in the gastrointestinal tract. Figure 4 shows the duodenum from a vehicle-treated mouse (A) and a Fzd8 ECD-Fc-treated mouse (B).

Example 2: Mice treated with Fzd8 ECD-Fc and fed a high-fat diet had

higher lean to fat ratio than vehicle-treated mice

Study procedure

[0123] Seven- to eight-week old male C57B1/6 mice (Jackson Labs) were administered Fzd8 ECD-Fc, LRRPl ECD-Fc, or vehicle (Ringer's Solution; Fisher Scientific; AD-5038) by hydrodynamic tail vein transfection. Twelve mice were included in each group, and each mouse (except vehicle-treated mice) received 20 μg of DNA in 2 ml Ringer's Solution by hydrodynamic tail vein transfection. Vehicle-treated mice received 2 ml Ringer's Solution by hydrodynamic tail vein transfection.

[0124] Following hydrodynamic tail vein transfection, the mice were switched to a 60% high fat diet (Purina 60 kCal% Fat Blue Dye, Lot 090406024i). Body weight was recorded weekly. Mice were also monitored for bloody stool and diarrhea on days when weight was recorded. On day 28, the mice were euthanized using isoflurane and cardiac puncture, and blood was collected for serum chemistry and plasma fatty acid analysis.

[0125] Necroscopy was performed following euthanasia to collect the duodenum from each mouse for histological analysis. The duodenums were fixed in 4% neutral buffered formalin for 24 hours, washed in PBS, then stored in 70% ethanol. Fixed tissues were embedded in paraffin, cross-sectioned, and stained with H&E (hematoxylin and eosin). As before, no differences were observed between the duodenums of the vehicle-treated and Fzd8 ECD-Fc-treated mice (data not shown).

[0126] After tissue collection, the small incision was glued and the vehicle- and Fzd8-treated mice were placed in sealed bags and frozen for MRI analysis of body composition, including fat mass, lean mass, and lean to fat ratio. MRI analysis was done at Echo Medical Systems in Houston, TX, using an EchoMRI-100.

Results

[0127] Figure 5 shows body weight of the vehicle-, Fzd8 ECD-Fc-, and leucine-rich repeat protein 1 (LRRPl) ECD-Fc-treated mice over the course of the study. The vehicle- and LRRPl ECD-Fc-treated mice gained approximately 7 grams, or about 30% of their body weight, during the study. Fzd8 ECD-Fc-treated mice, in contrast, gained only about 1 gram during the study.

[0128] Figure 6 shows the fat mass, lean mass, and lean to fat ratio of the vehicle- and Fzd8-treated mice. The Fzd8 ECD-Fc-treated mice had significantly decreased fat mass compared to the vehicle-treated mice. See Figure 6A. The Fzd8 ECD-Fc-treated mice also had an increased lean to fat ratio compared to vehicle-treated mice. See Figure 6C. Finally, the Fzd8 ECD-Fc-treated mice also had decreased lean mass compared to

vehicle-treated mice. See Figure 6B. The decreased lean mass may be due to a reduced need for lean mass to support overall body mass in the lighter-weight Fzd8 ECD-Fc-treated mice. It should be noted that the study did not show a reduction in overall lean or fat mass, e.g., relative to mice fed a regular diet, but rather an attenuation of growth of lean and fat tissue in Fzd8 ECD-Fc-treated mice fed a high-fat diet relative to vehicle-treated mice also fed a high-fat diet.

Example 3: Mice treated with Fzd8 ECD-Fc that were fed a regular diet lost weight

Study procedure

[0129] Twenty six-week-old male C57B1/6 mice (Charles River Labs) were individually housed for 14 days. On day 15, food consumption measurements were started and conducted twice per week. One week later, ten mice were administered Fzd8 ECD-Fc and ten mice were administered vehicle (0.9% NaCl) by hydrodynamic tail vein transfection. Each Fzd8 ECD-Fc-treated mouse received 20 μg of DNA in 2 ml 0.9% NaCl by hydrodynamic tail vein transfection. Vehicle-treated mice received 2 ml 0.9% NaCl by hydrodynamic tail vein transfection.

[0130] Following hydrodynamic tail vein transfection, body weight and food consumption were recorded daily to every other day throughout the study. Mice were bled 5 days post transfection to assay plasma protein expression by ELISA.

[0131] On day 20 post transfection, body weight and food consumption were recorded before euthanasia using isoflurane and cardiac puncture. At necropsy, the epididymal fat pad was collected and weighed. Blood was collected in serum separator tubes, allowed to clot at room temperature, and spun for 10 minutes at 10,000 rpm (13,000 xg). Serum was separated and sent to Quality Clinical Labs for serum chemistry analysis of alanine aminotransferase (ALT), aspartate aminotransferase (ALT), potassium (K), calcium (Ca), total protein (PROT-T), albumin (ALB), and globulin (GLOB).

Results

[0132] Table 1 shows the level of expression of Fzd8 ECD-Fc in each mouse at 5 days post transfection. Expression levels for animals 1 through 10, which received vehicle, were 0 for each animal, and are not shown in Table 1. Mouse 19 died the day after hydrodynamic tail vein transfection, likely as a result of the procedure. All of the mice expressed Fzd8 ECD-Fc. The upper threshold of the assay was 2500. The expression levels of Fzd8 ECD-Fc in the mice were generally very high.

Table 1: Fzd8 ECD-Fc expression in transfected mice

Animal	Expression level
11	2500
12	2500
13	2500
14	2500
15	2500
16	790
18	690
19	died
20	1770

[0133] Blood was collected at the end of the study for a serum chemistry panel. Table 2 shows the results of that panel. There was no significant difference between vehicle- and Fzd8 ECD-Fc-treated mice for any of the factors tested in the panel.

Table 2: Serum chemistry

	AST (U/L)	ALT (U/L)	K (mEq/L)	CA (mg/dL)	PROT-T (g/dL)	ALB (g/dL)	GLOB (g/dL)
Vehicle	89	36.2	7.04	10.16	5.26	3.54	1.72
FZD8	88.57143	45.85714	6.914286	10.17143	5.128571	3.385714	1.742857
p Value	0.979593	0.120749	0.779125	0.946053	0.262711	0.063217	0.775257

[0134] In addition, the hematocrit for each mouse was determined, and no difference was found between the vehicle-treated mice and the Fzd8 ECD-Fc-treated mice (data not shown). Finally, no gross changes in the mice were observed, nor any differences between the vehicle- and Fzd8 ECD-Fc-treated mice.

[0135] Figure 7 shows body weight of the vehicle- and Fzd8 ECD-Fc-treated mice over the course of the study. The vehicle-treated mice gained 1 to 2 grams over the course of the study, while the Fzd8 ECD-Fc-treated mice lost 1 to 2 grams. See Figure 7A. This corresponds to a gain of about 5% of their body weight for the vehicle-treated mice, and a loss of about 5% of their body weight for the Fzd8 ECD-Fc-treated mice. See Figure 7B.

[0136] Figure 8 shows food consumption of the vehicle- and Fzd8 ECD-Fc-treated mice over the course of the study. After day 7, the Fzd8 ECD-Fc-treated animals ate significantly less than the vehicle-treated mice for most of the periods tested during the remainder of the study. See Figure 8A. The Fzd8 ECD-Fc-treated mice had about 90% of the food intake of the vehicle-treated mice from day 7 through the end of the study. See Figure 8B. These results suggest that Fzd8 ECD-Fc treatment may lead to increased

satiety signals, less food consumption, and ultimately a decrease in body weight gain, relative to vehicle treated mice.

[0137] Figure 9 shows epididymal fat pad weight in the mice following necropsy. The epididymal fat pads of the Fzd8 ECD-Fc-treated mice weighed significantly less than the fat pads from vehicle-treated mice.

Example 4: Fzd8 ECD-Fc administration prevents diet-induced obesity in rats

Study procedure

[0138] Ten five-week-old male Sprague-Dawley rats (Charles River Labs, Wilmington, MA) were administered Fzd8 ECD-Fc and ten five-week-old male Sprague-Dawley rats were administered vehicle (0.9% NaCl) by hydrodynamic tail vein transfection. Each Fzd8 ECD-Fc-treated rat received 500 μg of DNA in 16 ml 0.9% NaCl by hydrodynamic tail vein transfection. Vehicle-treated rats received 16 ml 0.9% NaCl by hydrodynamic tail vein transfection. The rats were switched to a high fat diet after hydrodynamic tail vein transfection.

[0139] Following hydrodynamic tail vein transfection, body weight and food consumption were recorded at various time points throughout the study. Food consumption was determined by measuring the remaining weight of food left in the feeder relative to the starting amount.

Results

[0140] Figure 10 shows the mean cumulative food consumption (A) and mean body weight (B) of the rats over the course of the 18 day study. After 18 days, the mean body weight of vehicle-treated rats was 325 grams, while the mean body weight of Fzd8 ECD-Fc-treated rats was 188 grams, a body weight difference of 43%. After 18 days, the cumulative food consumption was 278 grams for vehicle-treated rats, and 150 grams for Fzd8 ECD-Fc-treated rats, representing a cumulative food consumption difference of 46%.

Example 5: Fzd8 ECD-Fc administration causes weight loss in mice

Study procedure

[0141] Ten eight-week-old female C57BL/6 mice (Jackson Labs) were administered Fzd8 ECD-Fc and ten eight-week-old female C57BL/6 mice were administered vehicle (0.9% NaCl) by hydrodynamic tail vein transfection. Each Fzd8 ECD-Fc-treated mouse received 25 μg of DNA in 2 ml 0.9% NaCl by hydrodynamic tail vein transfection. Vehicle-treated mice received 2 ml 0.9% NaCl by hydrodynamic tail

vein transfection. After hydrodynamic tail vein transfection, the mice were switched to a high fat diet. Body weights were measured at various time points during the study.

Results

[0142] Figure 11 shows the mean body weight of the mice over the course of the two week study. At the start of the study, the mean body weight for all mice was 17.8 grams. After two weeks, the mean body weight of the Fzd8 ECD-Fc-treated mice was 13.6 grams, representing weight loss of 24%.

Example 6: Fzd8 ECD-Fc administration prevents diet-induced glucose intolerance and hyperglycemia in mice

Study procedure

[0143] Ten 13-week-old female C57BL/6 mice (Charles River Labs) were administered Fzd8 ECD-Fc and ten 13-week-old female C57BL/6 mice were administered vehicle (0.9% NaCl) by hydrodynamic tail vein transfection. Each Fzd8 ECD-Fc-treated mouse received 25 μg of DNA in 2 ml 0.9% NaCl by hydrodynamic tail vein transfection. Vehicle-treated mice received 2 ml 0.9% NaCl by hydrodynamic tail vein transfection. The mice were switched to a high fat diet after hydrodynamic tail vein transfection.

[0144] Body weight was measured at various time points during the study. Ten weeks after administration of Fzd8 ECD-Fc or vehicle, a glucose tolerance test (GTT) was conducted after a four hour fast. Blood glucose levels were measured with a glucometer from a blood drop at the tip of the tail after the four hour fast, and then at 30 minute intervals after a bolus intraperitoneal dose of 4.0 g/kg glucose.

Results

[0145] Figure 12 shows (A) mean body weight over the course of the ten week study and (B) mean glucose tolerance at 10 weeks after Fzd8 ECD-Fc administration. After ten weeks, the mean body weight of the vehicle-treated mice was 34 grams, while the mean body weight of the Fzd8 ECD-Fc-treated mice was 25 grams, a difference of 36%. Fasting blood glucose was 33% lower in Fzd8 ECD-Fc-treated mice compared to vehicle treated mice. Additionally, blood glucose levels at all time points after glucose administration were 32% - 35% lower in Fzd8 ECD-Fc-treated mice relative to vehicle treated mice.

Example 7: Fzd8 ECD-Fc administration leads to a body mass composition that is equivalent to the body mass composition in dieting mice

Study procedure

[0146] Ten eight-week-old male C57BL/6 mice (Charles River Labs) were administered Fzd8 ECD-Fc and ten eight-week-old male C57BL/6 mice were administered vehicle (0.9% NaCl) by hydrodynamic tail vein transfection. Each Fzd8 ECD-Fc-treated mouse received 25 μg of DNA in 2 ml 0.9% NaCl by hydrodynamic tail vein transfection. Vehicle-treated mice received 2 ml 0.9% NaCl by hydrodynamic tail vein transfection. After hydrodynamic tail vein transfection, the mice were maintained on a high fat diet, and the vehicle-treated mice were divided into two groups, one of which was diet-restricted, being fed 1.7 grams of food per day. The other vehicle-treated group had an average food consumption of 2.2 grams per day. Body weight and qNMR body mass composition analysis were conducted on day 22 using an EchoMRI-700™ with mouse attachment (Echo Medical Systems, Houston, TX).

Results

[0147] Figure 13 shows (A) mean body weight, (B) mean fat mass, and (C) mean lean mass of vehicle-treated, Fzd8 ECD-Fc-treated, and diet-restricted mice on day 22. The mean body weight of vehicle-treated, Fzd8 ECD-Fc-treated, and diet-restricted mice was 32 grams, 26 grams, and 26 grams, respectively. The mean fat mass of vehicle-treated, Fzd8 ECD-Fc-treated, and diet-restricted mice was 9.7 grams, 6.2 grams and 6.0 grams, respectively. Finally, the mean lean mass of vehicle-treated, Fzd8 ECD-Fc-treated, and diet-restricted mice was 21.7 grams, 19.5 grams, and 19.6 grams, respectively. The mean body weight, mean lean mass, and mean fat mass were indistinguishable between Fzd8 ECD-Fc-treated and diet-restricted mice in this experiment.

Example 8: Recombinant human Fzd8 ECD-Fc administration prevents obesity in mice

Production of recombinant human Fzd8 ECD-Fc

[0148] A pTT5 vector that expresses human Fzd8 ECD.155-Fc (SEQ ID NO: 5) was transiently transfected into CHO cells and the cells were grown in a shaker flask for 6 days in CD DG44 medium (Gibco/Invitrogen, Carlsbad, CA) + 8 mM glutamine + 0.18% Pluronic® F-68 polyol (Mediatech, Manassas, VA). Cells were pelleted by centrifugation at 5000 xg. The supernatant was loaded directly onto a 30 ml MabSelect SuRe protein A purification column (GE Healthcare, Waukesha, WI) equilibrated in lxPBS with 500 mM NaCl ("Buffer A"). The column was washed with Buffer A and the rhFzd8 ECD-Fc was eluted using a linear gradient to Buffer B (0.1 M glycine, pH 2.7, with 500 mM NaCl) over 20 column volumes. The eluent was collected into 1/10 fraction volume of 1M Tris, pH 8.

[0149] The pool of fractions from the protein A column containing rhFzd8 ECD-Fc was diluted by addition of 0.8 volumes of 1.6M ammonium sulfate, and loaded onto a 25 ml (1.6 x 12 cm) butyl sepharose HP column (GE Healthcare) equilibrated in lOmM potassium phosphate, pH 7, with 0.8 M ammonium sulfate ("Buffer C"). The column was then washed with Buffer C and the rhFzd8 ECD-Fc was eluted using a linear gradient to 10 mM potassium phosphate, pH 7 ("Buffer D") over 15 column volumes. The pool of fractions from the butyl HP column containing rhFzd8 ECD-Fc was dialyzed against lx PBS, and then concentrated using a spin filter with a 10 kD molecular weight cutoff membrane at 3500 xg in a swinging bucket rotor at 4°C.

Study procedure

[0150] Eight C57BL/6 mice (Charles River Labs) were administered 10 mg/kg rhFzd8 ECD-Fc in a volume of 0.33 ml 0.9% NaCl on days 0, 4, 8, and 12, and eight mice were administered 0.33 ml 0.9% NaCl on days 0, 4, 8, and 12. Twenty-four hours after the first dose, mice were switched to a high-fat diet. Body weight measurements were taken at various time points during the study.

Results

[0151] Figure 14 shows mean body weight of rhFzd8 ECD-Fc-treated and vehicle-treated mice. At the beginning of the study, the mean body weight of all of the mice was 21.7 grams. After 14 days, the mean body weight of rhFzd8 ECD-Fc-administered and vehicle-administered mice was 22.8 grams and 24.4 grams, respectively. The difference in mean body weight between the two groups was statistically significant, with $p<0.05$.

Example 9: Fzd8 ECD-Fc administration leads to a decrease in NPY expression in the hypothalamus of mice

Study procedure

[0152] Ten eight-week-old male C57BL/6 mice (Charles River Labs) were administered Fzd8 ECD-Fc and ten mice were administered human IgGl C237S Fc alone by hydrodynamic tail vein transfection. Each mouse received 20 μg of DNA in 2 ml of Ringer's solution by hydrodynamic tail vein transfection.

[0153] Mice were switched to a high fat diet after hydrodynamic tail vein transfection. Body weight was measured at certain time points throughout the study. Mice were bled on day 5 for measuring plasma protein expression in order to determine transfection efficiency. At 21 days after transfection, mice were euthanized by isoflurane and decapitation and the hypothalamus removed and flash-frozen in liquid nitrogen.

[0154] NPY is a neuropeptide that has been shown to correlate with appetite and food consumption. NPY mRNA expression in the hypothalamus increases during fasting and exogenous administration of NPY will lead to increased food consumption. NPY expression in the hypothalamus was measured by qRT-PCR using a QuantiTect Primer Assay (Qiagen, Valencia, CA), and normalized to β-glucuronidase (GUSB).

Results

[0155] Figure 15 shows mean NPY expression in the hypothalamus of Fc-treated mice and Fzd8 ECD-Fc-treated mice. NPY expression in Fzd8 ECD-Fc-treated mice was about 50% lower than NPY expression in Fc-treated mice.

Example 10: Fzd8 ECD-Fc, but not Fzd5 ECD-Fc, prevents diet-induced obesity in mice

Study procedure

[0156] Ten eight-week-old male C57BL/6 mice (Charles River Labs) were administered Fzd8 ECD-Fc, Ten mice were administered Fzd5 ECD-Fc, and ten mice were administered human IgGl C237S Fc alone by hydrodynamic tail vein transfection. Each mouse received 20 μg of DNA in 2 ml of Ringer's solution by hydrodynamic tail vein transfection.

[0157] Mice were switched to a high fat diet after hydrodynamic tail vein transfection. Body weight and food consumption were recorded twice per week throughout the 21-day study. Mice were bled on day 5 for measuring plasma protein expression in order to determine transfection efficiency. Fzd8 ECD-Fc was found to be present at average 876 μg/ml and Fzd5 ECD-Fc was found to be present at an average of 294 μg/ml.

Results

[0158] Figure 16 shows the mean body weight of Fzd8 ECD-Fc-treated mice, Fzd5 ECD-Fc-treated mice, and Fc-treated mice throughout the study. Both the Fzd5 ECD-Fc-treated and the Fc-treated mice gained significant weight during the study, while the Fzd8 ECD-Fc-treated mice gained little or no weight during the course of the study.

[0159] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literatures cited herein are expressly incorporated in their entirety by reference.

TABLE OF SEQUENCES

[0160] Table 3 lists certain sequences discussed herein. Fzd8 sequences are shown without the signal peptide, unless otherwise indicated.

Table 3: Sequences and Descriptions

SEQ ID NO	Description	Sequence
1	Human Fzd8 (with signal peptide)	MEWGYLLEVT SLLAALALLQ RSSGAAAASA KELACQEITV PLCKGIGYNY TYMPNQFNHD TQDEAGLEVH QFWPLVEIQC SPDLKFFLCS MYTPICLEDY KKPLPPCRSV CERAKAGCAP LMRQYGFAWP DRMRCDRLPE QGNPDTLCMD YNRTDLTTAA PSPPRRLPPP PPGEQPPSGS GHGRPPGARP PHRGGGRGGG GGDAAAPPAR GGGGGGKARP PGGGAAPCEP GCQCRAPMVS VSSERHPLYN RVKTGQIANC ALPCHNPFFS QDERAFTVFW IGLWSVLCFV STFATVSTFL IDMERFKYPE RPIIFLSACY LFVSVGYLVR LVAGHEKVAC SGGAPGAGGA GGAGGAAAGA GAAGAGAGGP GGRGEYEELG AVEQHVRYET TGPALCTVVF LLVYFFGMAS SIWWVILSLT WFLAAGMKWG NEAIAGYSQY FHLAAWLVPS VKSIAVLALS SVDGDPVAGI CYVGNQSLDN LRGFVLAPLV IYLFIGTMFL LAGFVSLFRI RSVIKQQDGP TKTHKLEKLM IRLGLFTVLY TVPAAVVVAC LFYEQHNRPR WEATHNCPCL RDLQPDQARR PDYAVFMLKY FMCLVVGITS GVWVWSGKTL ESWRSLCTRC CWASKGAAVG GGAGATAAGG GGGPGGGGGG GPGGGGGPGG GGGSLYSDVS TGLTWRSGTA SSVSYPKQMP LSQV
2	Human Fzd8 (without signal peptide)	ASA KELACQEITV PLCKGIGYNY TYMPNQFNHD TQDEAGLEVH QFWPLVEIQC SPDLKFFLCS MYTPICLEDY KKPLPPCRSV CERAKAGCAP LMRQYGFAWP DRMRCDRLPE QGNPDTLCMD YNRTDLTTAA PSPPRRLPPP PPGEQPPSGS GHGRPPGARP PHRGGGRGGG GGDAAAPPAR GGGGGGKARP PGGGAAPCEP GCQCRAPMVS VSSERHPLYN RVKTGQIANC ALPCHNPFFS QDERAFTVFW IGLWSVLCFV STFATVSTFL IDMERFKYPE RPIIFLSACY LFVSVGYLVR LVAGHEKVAC SGGAPGAGGA GGAGGAAAGA GAAGAGAGGP GGRGEYEELG AVEQHVRYET TGPALCTVVF LLVYFFGMAS SIWWVILSLT WFLAAGMKWG NEAIAGYSQY FHLAAWLVPS VKSIAVLALS SVDGDPVAGI CYVGNQSLDN LRGFVLAPLV IYLFIGTMFL LAGFVSLFRI RSVIKQQDGP TKTHKLEKLM IRLGLFTVLY TVPAAVVVAC LFYEQHNRPR WEATHNCPCL RDLQPDQARR PDYAVFMLKY FMCLVVGITS GVWVWSGKTL ESWRSLCTRC CWASKGAAVG GGAGATAAGG GGGPGGGGGG GPGGGGGPGG GGGSLYSDVS TGLTWRSGTA SSVSYPKQMP LSQV
3	Full-length human Fzd8 ECD (with signal peptide); SP-hFzd8-ECD.275	MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCK GIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVE IQCSPDLKFFL CSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDR MRC DRLPEQGNPDTLCMDYNRTDLTTAAPSPPRRLPPPPPGEQP PSGSGHGRPPGARPPHRGGGRGGGGGDAAAPPARGGGGGGKARP PGGGAAPCEPGCQCRAPMVSVSSERHPLYNRVKTGQIANCALPC HNPFFSQDERA
4	Full-length human Fzd8 ECD (without signal peptide); hFzd8-ECD.275	ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQ FWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAK AGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTA APSPPRRLPPPPPGEQPPSGSGHGRPPGARPPHRGGGRGGGGGD AAAPPARGGGGGGKARPPGGGAAPCEPGCQCRAPMVSVSSERHP LYNRVKTGQIANCALPCHNPFFSQDERA
12	hFzd8-ECD. 155	ASAKELACQE ITVPLCKGIG YNYTYMPNQF NHDTQDEAGL EVHQFWPLVE IQCSPDLKFF LCSMYTPICL EDYKKPLPPC RSVCERAKAG CAPLMRQYGF AWPDRMRCDR LPEQGNPDTL CMDYNRTD
13	SP-hFzd8-ECD. 155	MEWGYLLEVT SLLAALALLQ RSSGAAAASA KELACQEITV PLCKGIGYNY TYMPNQFNHD TQDEAGLEVH QFWPLVEIQC SPDLKFFLCS MYTPICLEDY KKPLPPCRSV CERAKAGCAP

		LMRQYGFAWP DRMRCDRLPE QGNPDTLCMD YNRTD
14	hFzd8-ECD.275-Fc	MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCK GIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVE IQCSPDLKFFL CSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDR MRC DRLPEQGNPDTLCMDYNRTDLTTAAPSPPRRLPPPPPGEQP PSGSGHGRPPGARPPHRGGGRGGGGGDAAAPPARGGGGGGKARP PGGGAAPCEPGCQCRAPMVSVSSERHPLYNRVKTGQIANCALPC HNPFFSQDERAGSEPKSSDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKE YKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
15	SP-hFzd8-ECD.275-Fc	ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQ FWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAK AGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDLTTA APSPPRRLPPPPPGEQPPSGSGHGRPPGARPPHRGGGRGGGGGD AAAPPARGGGGGGKARPPGGGAAPCEPGCQCRAPMVSVSSERHP LYNRVKTGQIANCALPCHNPFFSQDERAGSEPKSSDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
5	hFzd8-ECD. 155-Fc with GS linker	ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQ FWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAK AGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDGSEP KSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
6	SP-hFzd8-ECD. 155-Fc with GS linker	MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCK GIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVE IQCSPDLKFFL CSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDR MRC DRLPEQGNPDTLCMDYNRTDGSEPKSSDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
7	hFzd8 signal peptide	MEWGYLLEVTSLLAALALLQRSSGAAA
8	Fc C237S	EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
9	Exemplary Fc #1	ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK

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10	Exemplary Fc #2	ESKYGPPCPS CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK
11	Fc C237S with N-terminal GS linker	GSEPKSSDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
16	Fzd5 ECD-Fc	MARPDPSAPP SLLLLLAQL VGRAAAASKA PVCQEITVPM CRGIGYNLTH MPNQFNHDTQ DEAGLEVHQF WPLVEIQCSP DLRFFLCSMY TPICLPDYHK PLPPCRSVCE RAKAGCSPLM RQYGFAWPER MSCDRLPVLG RDAEVLCMDY NRSEGPAEPK SSDKTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK
17	hFzd8-ECD. 155-Fc without GS linker	ASAKELACQEITVPLCKGIGYNYTYMPNQFNHDTQDEAGLEVHQ FWPLVEIQCSPDLKFFLCSMYTPICLEDYKKPLPPCRSVCERAK AGCAPLMRQYGFAWPDRMRCDRLPEQGNPDTLCMDYNRTDEPKS SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKE YKCKVSNKAL PAP IEKT ISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK
18	SP-hFzd8-ECD. 155-Fc without GS linker	MEWGYLLEVTSLLAALALLQRSSGAAAASAKELACQEITVPLCK GIGYNYTYMPNQFNHDTQDEAGLEVHQFWPLVE IQCSPDLKFFL CSMYTPICLEDYKKPLPPCRSVCERAKAGCAPLMRQYGFAWPDR MRC DRLPEQGNPDTLCMDYNRTDEPKSSDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVE VHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKE YKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL VKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Attorney Docket No. 5641-00032

CLAIMS

1. A method of treating obesity or an obesity-related condition comprising administering to a subject a frizzled-8 (Fzd8) extracellular domain (ECD) or a Fzd8 ECD fusion molecule comprising a Fzd8 ECD and at least one fusion partner.

2. A method of lowering a blood glucose level comprising administering to a subject a frizzled-8 (Fzd8) extracellular domain (ECD) or a Fzd8 ECD fusion molecule comprising a Fzd8 ECD and at least one fusion partner.

3. The method of claim 1 or claim 2, wherein the Fzd8 ECD has a sequence selected from SEQ ID NOs: 3, 4, 12, and 13.

4. The method of any one of claims 1 to 3, wherein at least one fusion partner is selected from an Fc, albumin, and polyethylene glycol.

5. The method of claim 4, wherein at least one fusion partner is an Fc.

6. The method of any one of claims 1 to 5, wherein the fusion molecule comprises a linker between the Fzd8 ECD and one or more fusion partners.

7. The method of claim 1 or claim 2, wherein the Fzd8 ECD fusion molecule comprises an amino acid sequence selected from SEQ ID NO.: 5, 6, 14, 15, 17, and 18.

8. The method of claim 7, wherein the Fzd8 ECD fusion molecule consists of an amino acid sequence selected from SEQ ID NO.: 5, 6, 14, 15, 17, and 18.

9. The method of any one of claims 1 to 8, wherein the Fzd8 ECD or the polypeptide portion of the Fzd8 ECD fusion molecule is expressed in Chinese hamster ovary (CHO) cells.

10. The method of any one of claims 1 and 2 to 9, wherein the obesity-related condition is selected from heart disease, diabetes, breathing difficulties, osteoarthritis, high blood pressure, high cholesterol, high triglycerides, and high free fatty acids.

11. A frizzled-8 (Fzd8) extracellular domain (ECD) fusion molecule comprising a Fzd8 ECD and at least one fusion partner, wherein the Fzd8 ECD has a sequence selected from SEQ ID NO: 3, 4, 12, and 13, for use in a method of treating obesity or an obesity-related condition, or for use in a method of lowering a blood glucose level.

12. The Fzd8 ECD fusion molecule of claim 11, wherein at least one fusion partner is selected from an Fc, albumin, and polyethylene glycol.

13. The Fzd8 ECD fusion molecule of claim 12, wherein at least one fusion partner is an Fc.

14. The Fzd8 ECD fusion molecule of any one of claims 11 to 13, wherein the

Fzd8 ECD comprises a signal peptide.

15. The Fzd8 ECD fusion molecule of any one of claims 11 to 14, wherein the fusion molecule comprises a linker between the Fzd8 ECD and one or more fusion partners.

16. A Fzd8 ECD consisting of an amino acid sequence selected from SEQ ID NOs: 3, 4, 12, and 13 for use in a method of treating obesity or an obesity-related condition, or for use in a method of lowering a blood glucose level.

17. A Fzd8 ECD fusion molecule comprising an amino acid sequence selected from SEQ ID NO.: 3 to 6, 12 to 15, 17, and 18 for use in a method of treating obesity or an obesity-related condition, or for use in a method of lowering a blood glucose level.

18. A Fzd8 ECD fusion molecule consisting of an amino acid sequence selected from SEQ ID NO.: 5, 6, 14, 15, 17, and 18 for use in a method of treating obesity or an obesity-related condition, or for use in a method of lowering a blood glucose level.

19. The Fzd8 ECD or Fzd8 ECD fusion molecule of any one of claims 11 to 18, wherein the polypeptide portion of the Fzd8 ECD or Fzd8 ECD fusion molecule is expressed in Chinese hamster ovary (CHO) cells.

20. A pharmaceutical composition comprising a Fzd8 ECD or Fzd8 ECD fusion molecule of any one of claims 11 to 19, and a pharmaceutically acceptable carrier.

21. A polynucleotide comprising a nucleic acid sequence that encodes a Fzd8 ECD or the polypeptide portion of a Fzd8 ECD fusion molecule of any one of claims 11 to 19.

22. The polynucleotide of claim 21, wherein the nucleic acid sequence encodes a Fzd8 ECD fusion molecule.

23. The polynucleotide of claim 21, wherein the nucleic acid sequence encodes an amino acid sequence selected from SEQ ID NOs: 3 to 6, 12 to 15, 17, and 18.

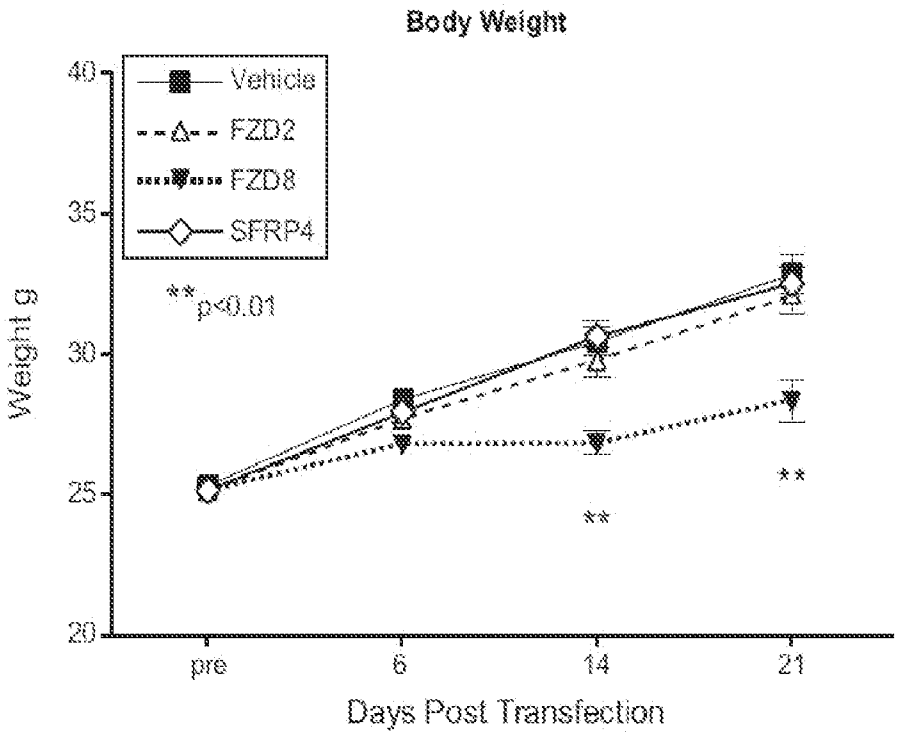

Body Weight
Vehicle
FZD2
FZD8
SFRP4
**p<0.01
Weight g
40
35
30
25
20
pre
6
14
21
**
**
**
Days Post Transfection

FIG. 1

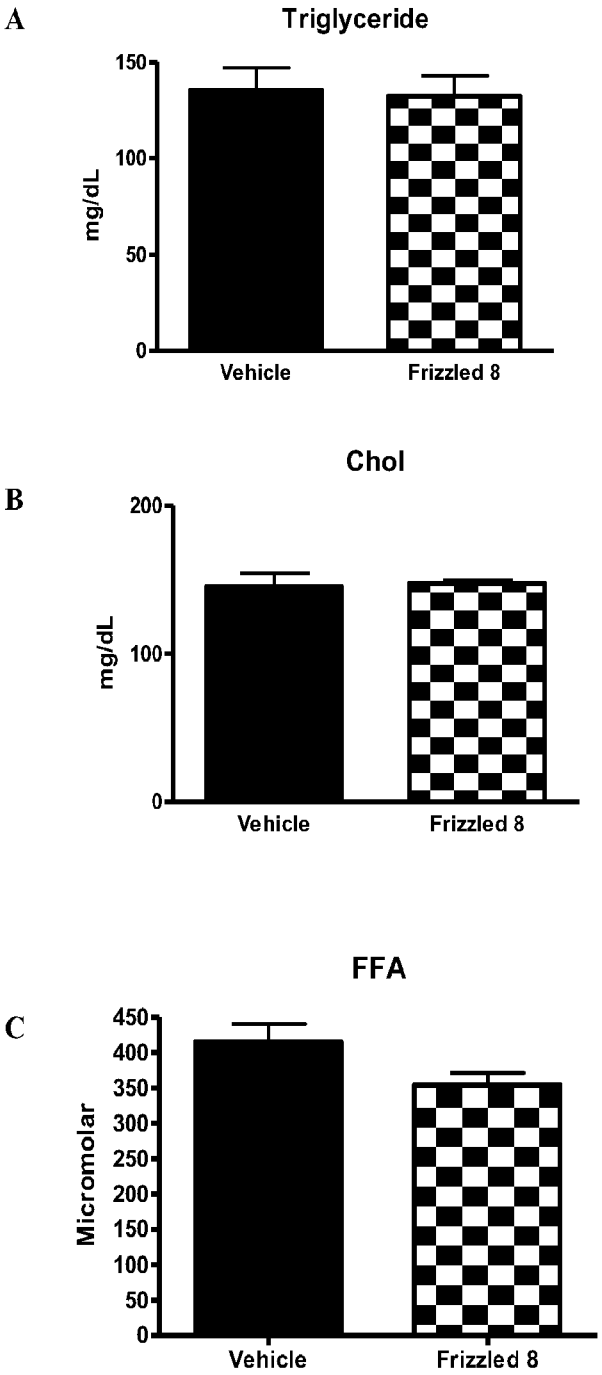

A
Triglyceride
150
100
50
0
mg/dL
Vehicle
Frizzled 8
B
Chol
200
100
0
mg/dL
Vehicle
Frizzled 8
C
FFA
450
400
350
300
250
200
150
100
50
0
Micromolar
Vehicle
Frizzled 8

FIG. 2

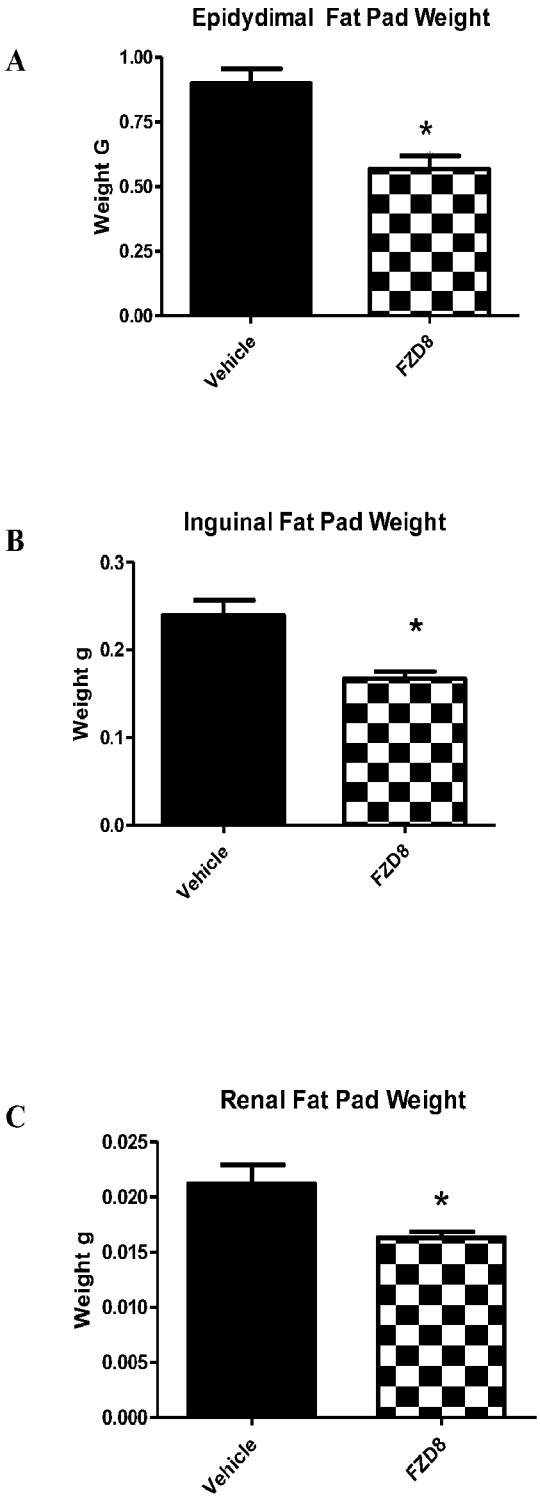

A
Epidydimal Fat Pad Weight
Weight G
1.00
0.75
0.50
0.25
0.00
*
Vehicle
FZD8
B
Inguinal Fat Pad Weight
Weight g
0.3
0.2
0.1
0.0
*
Vehicle
FZD8
C
Renal Fat Pad Weight
Weight g
0.025
0.020
0.015
0.010
0.005
0.000
*
Vehicle
FZD8

FIG. 3

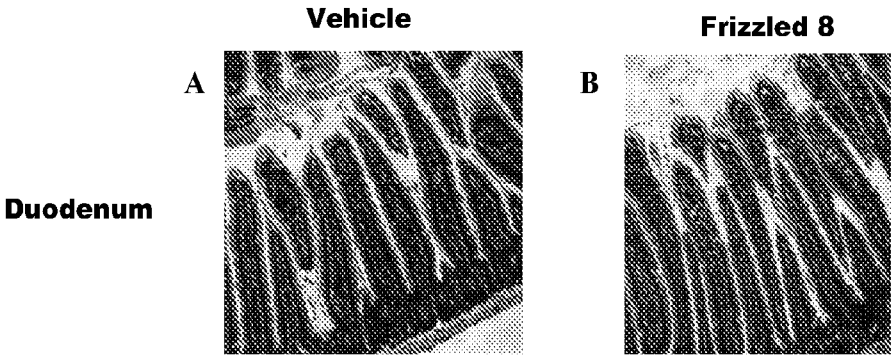

Vehicle
Frizzled 8
A
B
Duodenum

FIG. 4

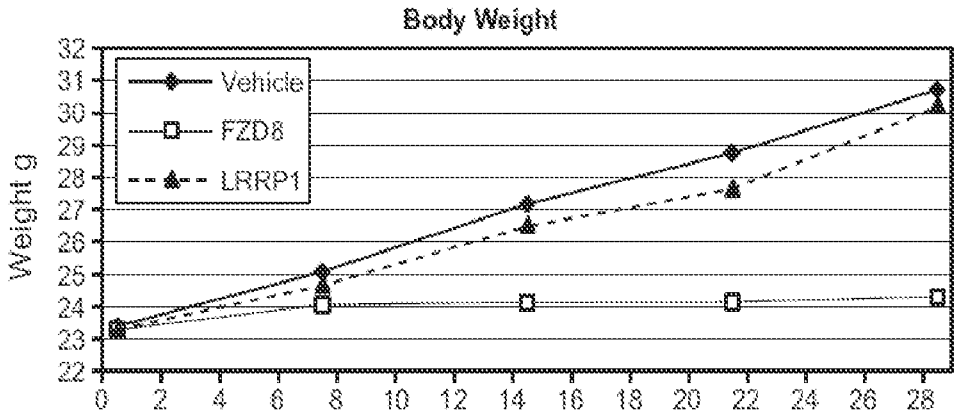

Body Weight
Weight g
Vehicle
FZD8
LRRP1
32
31
30
29
28
27
26
25
24
23
22
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28

FIG. 5

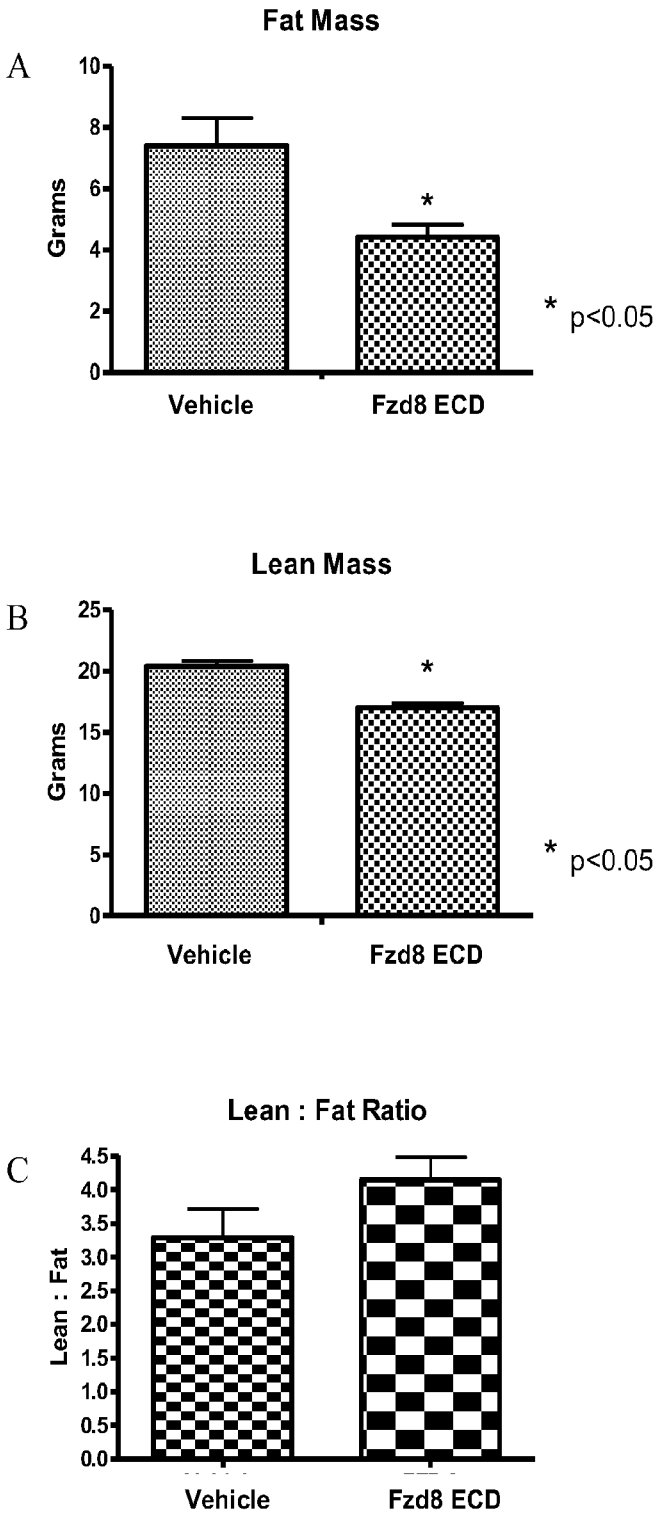

A
Fat Mass
Grams
10
8
6
4
2
0
*
* p<0.05
Vehicle
Fzd8 ECD
B
Lean Mass
Grams
25
20
15
10
5
0
*
* p<0.05
Vehicle
Fzd8 ECD
C
Lean : Fat Ratio
Lean : Fat
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
Fzd8 ECD

FIG. 6

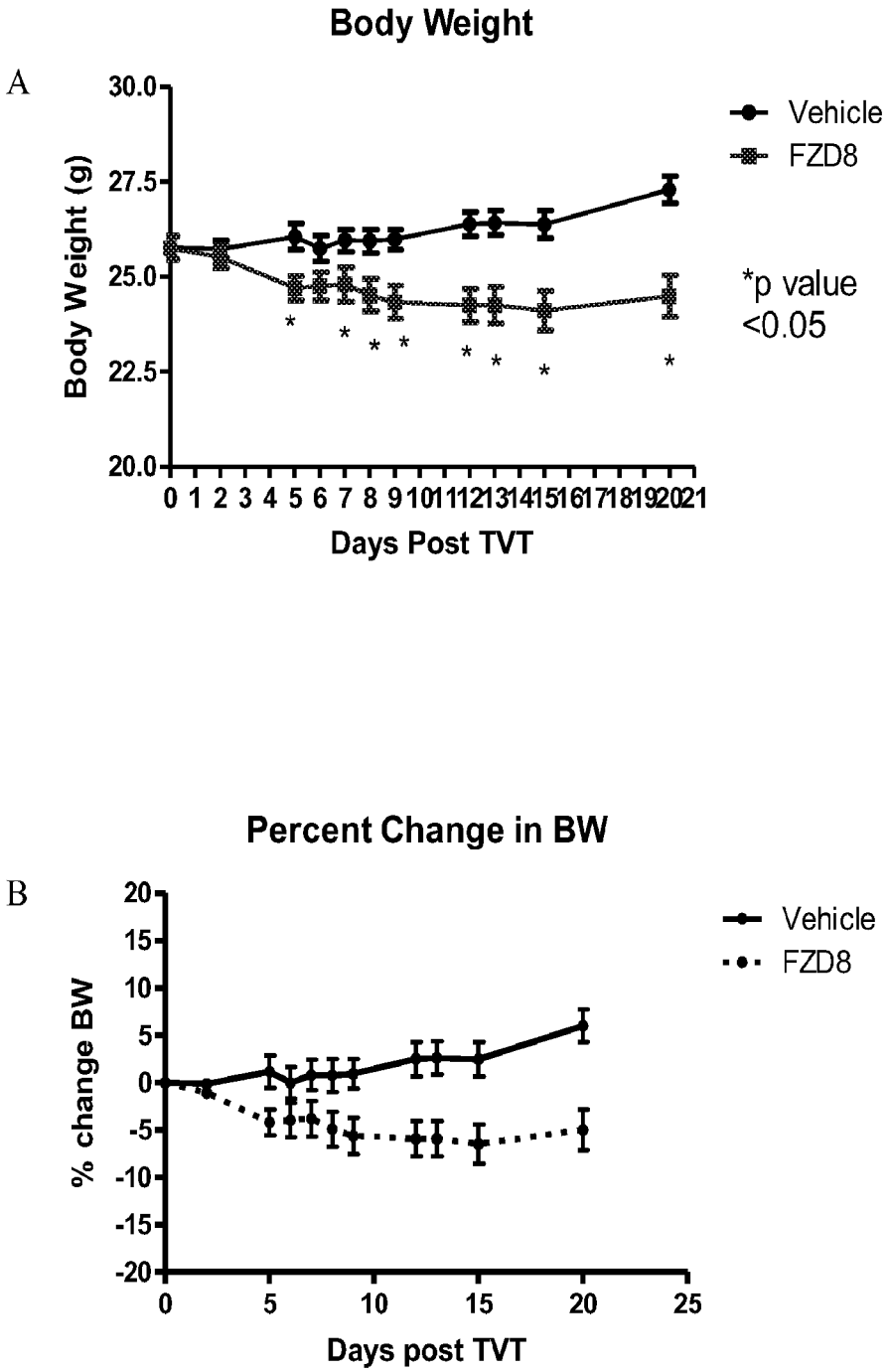
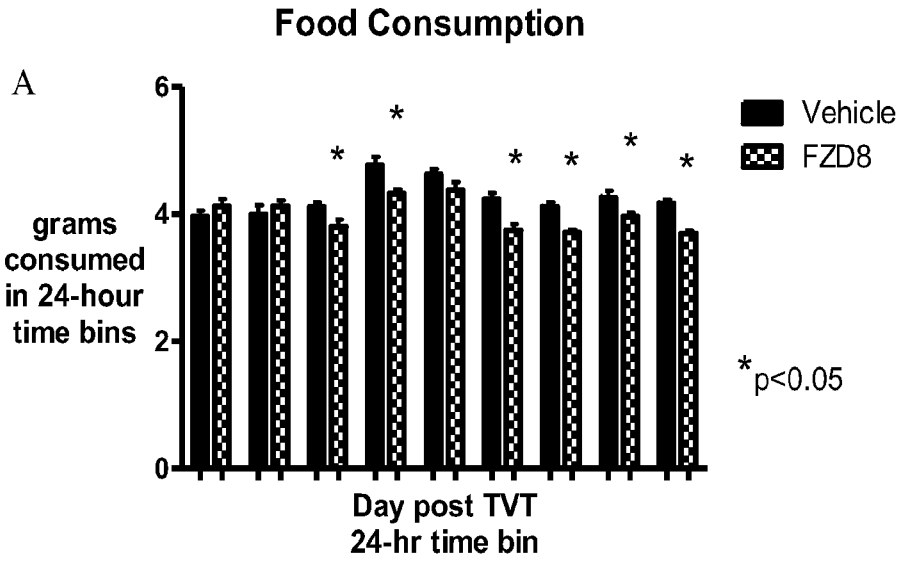

A
Body Weight
Body Weight (g)
30.0
27.5
25.0
22.5
20.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days Post TVT
Vehicle
FZD8
*p value
<0.05
B
Percent Change in BW
% change BW
20
15
10
5
0
-5
-10
-15
-20
0
5
10
15
20
25
Days post TVT
Vehicle
FZD8

FIG. 7


Food Consumption
A
grams consumed in 24-hour time bins
6
4
2
0
Vehicle
FZD8
*p<0.05
Day post TVT
24-hr time bin

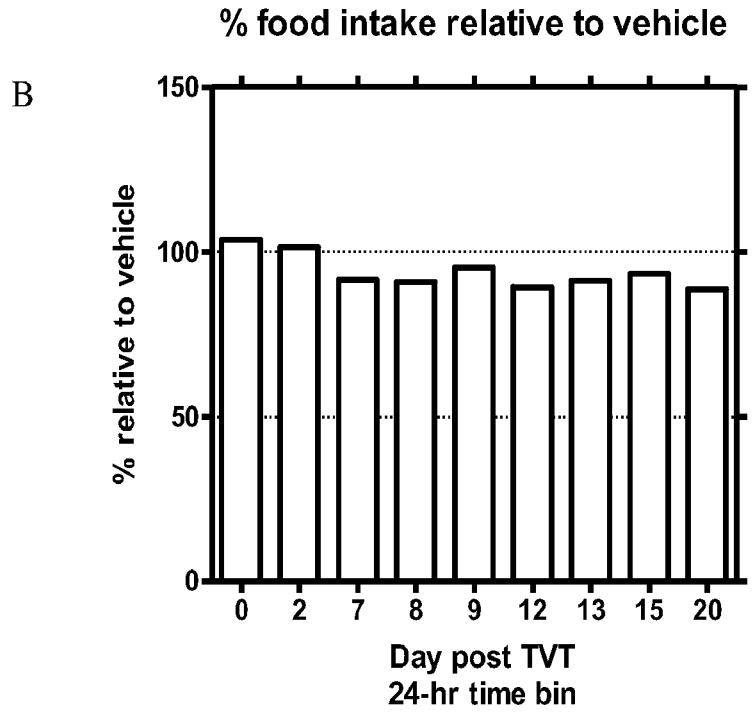

% food intake relative to vehicle
B
% relative to vehicle
150
100
50
0
0 2 7 8 9 12 13 15 20
Day post TVT
24-hr time bin

FIG. 8

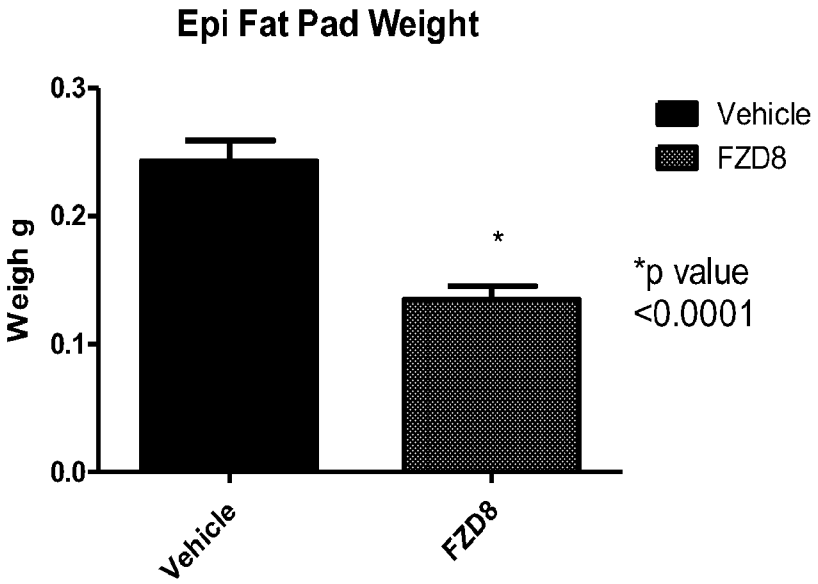
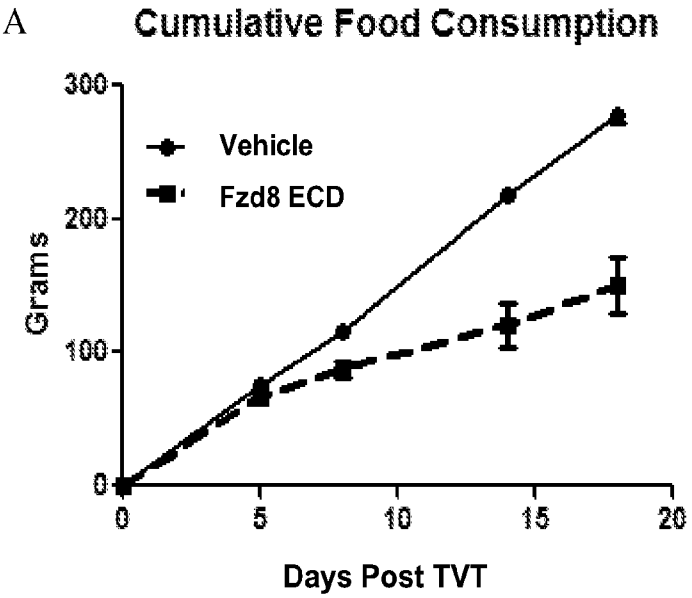

Epi Fat Pad Weight
Weigh g
0.3
0.2
0.1
0.0
Vehicle
FZD8
*
*p value
<0.0001
Vehicle
FZD8

FIG. 9

A


Cumulative Food Consumption
300
200
100
0
Grams
Vehicle
Fzd8 ECD
0
5
10
15
20
Days Post TVT

B

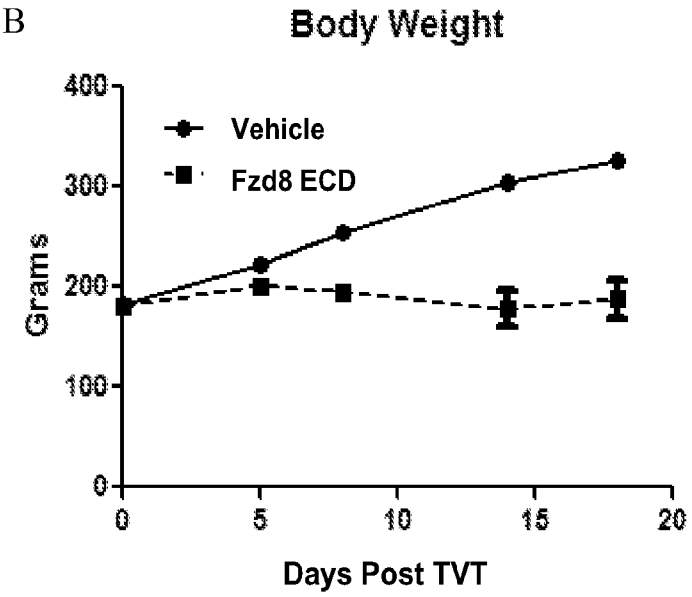

Body Weight
400
300
200
100
0
Grams
Vehicle
Fzd8 ECD
0
5
10
15
20
Days Post TVT

FIG. 10

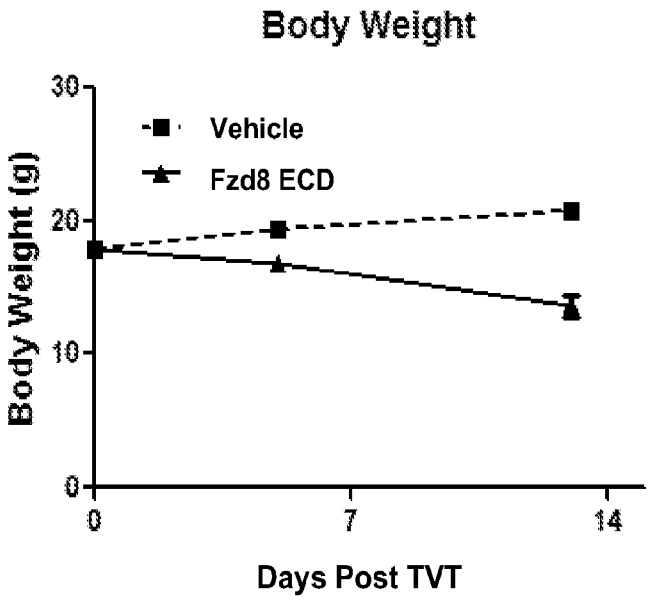
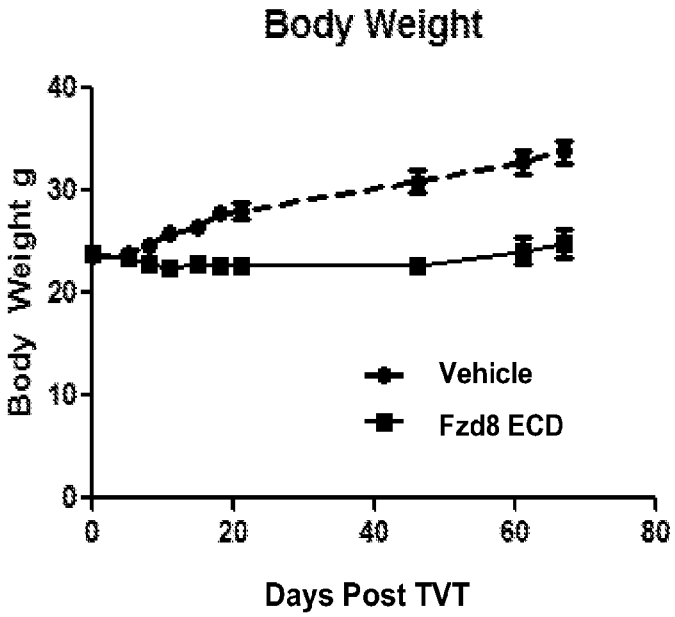

Body Weight
Vehicle
Fzd8 ECD
Body Weight (g)
30
20
10
0
0
7
14
Days Post TVT

FIG. 11

A


Body Weight
Body Weight g
40
30
20
10
0
0
20
40
60
80
Days Post TVT
Vehicle
Fzd8 ECD

B

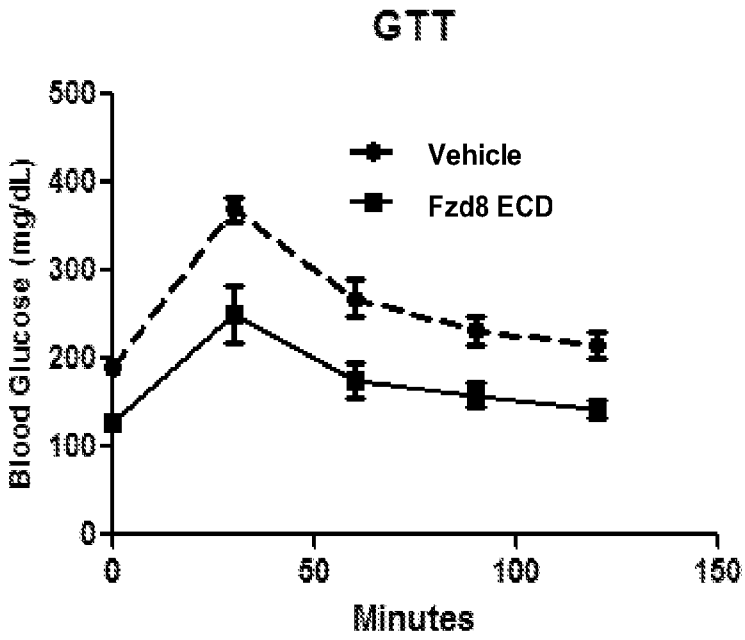

GTT
Blood Glucose (mg/dL)
500
400
300
200
100
0
0
50
100
150
Minutes
Vehicle
Fzd8 ECD

FIG. 12

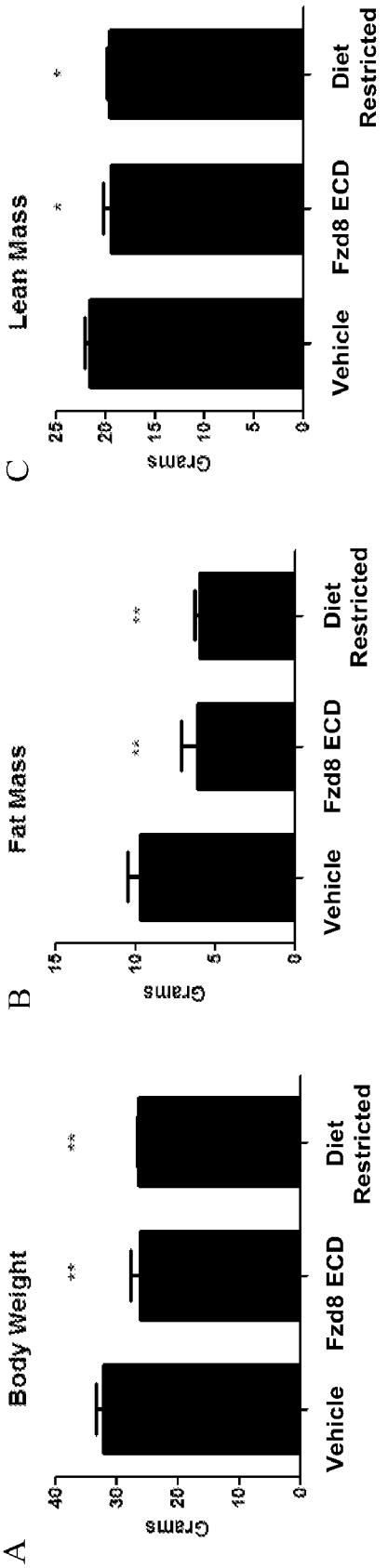

A
Body Weight
Grams
40
30
20
10
0
**
**
Vehicle
Fzd8 ECD
Diet Restricted
B
Fat Mass
Grams
15
10
5
0
**
**
Vehicle
Fzd8 ECD
Diet Restricted
C
Lean Mass
Grams
25
20
15
10
5
0
*
*
Vehicle
Fzd8 ECD
Diet Restricted

FIG. 13

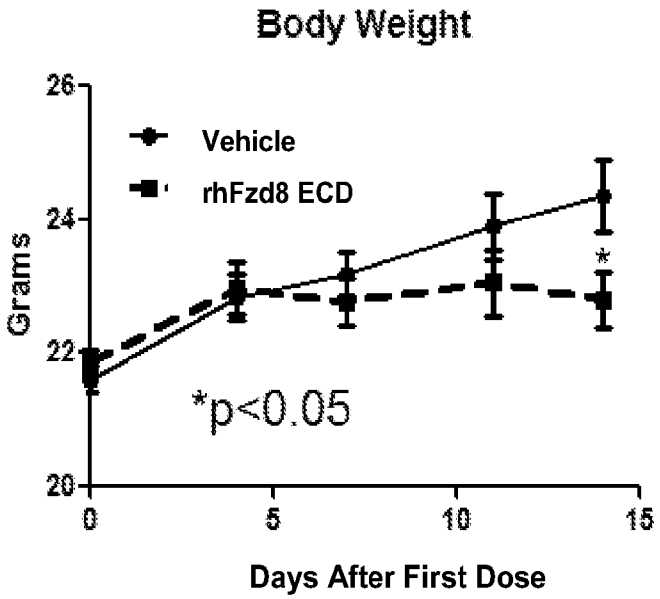

Body Weight
Vehicle
rhFzd8 ECD
Grams
26
24
22
20
*p<0.05
*
0
5
10
15
Days After First Dose

FIG. 14

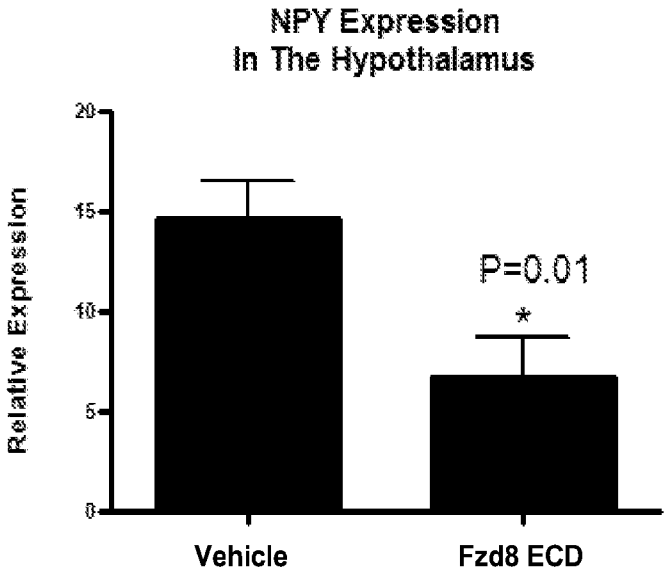

NPY Expression
In The Hypothalamus
Relative Expression
20
15
10
5
0
P=0.01
*
Vehicle
Fzd8 ECD

FIG. 15

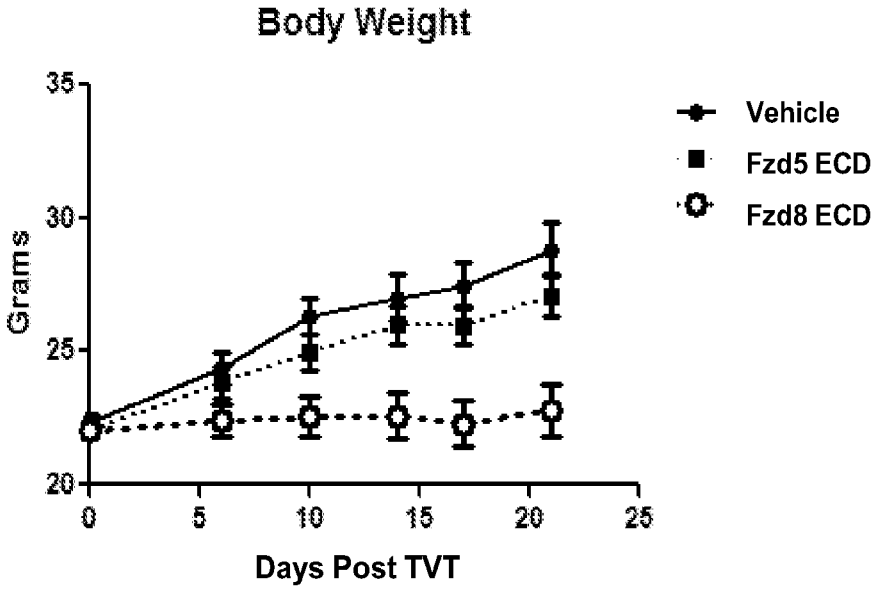

Body Weight
35
30
25
20
Grams
0
5
10
15
20
25
Days Post TVT
Vehicle
Fzd5 ECD
Fzd8 ECD

FIG. 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2011/042033

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

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

   a. (means)

   [x] on paper

   [x] in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   [x] subsequently to this Authority for the purpose of search

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

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/042033

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 C12N15/62 A61P3/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P C12N

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/130076 A1 (ASTRAZENECA AB [SE] ; BROCKBANK SARAH [GB] ; NEWHAM PETER [GB]) 7 December 2006 (2006-12-07)	1,3,10, 20
Y	abstract page 1, lines 8,9 page 3, lines 6,19,20 page 17, lines 4-10 page 19, line 21 page 22, lines 20-22	1-23

-/- .

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

[X] See patent family annex.

* Special categories of cited documents :

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

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Date of the actual completion of the international search	Date of mailing of the international search report
20 October 2011	31/10/2011

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

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/031009 A2 (GENENTECH INC [US] ; ERNST JAMES A [US] ; P0LAKIS PAUL [US] ; RUBINFELD B) 13 March 2008 (2008-03-13)	20-23
Y	abstract page 3, lines 11-13 page 5, lines 9,14,26-28 page 20, lines 8-18 page 29, lines 19,20 page 38; sequences 25,42 page 39, lines 11,12,20,21 page 41, lines 30,31 page 42, line 3 page 66, lines 14-18 page 89, lines 5-14; example 2	1-23
Y	KIM JAEY00N ET AL: "Comparative analysis of the secretory proteome of human adipose stromal vascular fraction cells during adipogenesis.", PR0TE0MICS FEB 2010 LNKD- PUBMED: 19953544, vol. 10, no. 3, February 2010 (2010-02) , pages 394-405 , XP002660552 , ISSN: 1615-9861 abstract page 403, column 1; figure 6	1-23
Y	HU ERDING ET AL: "Tissue restricted expression of two human Frzbs in preadipocytes and pancreas" , BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 247, no. 2, 18 June 1998 (1998-06-18) , pages 287-293 , XP002198883 , ISSN: 0006-291X, D0I: 10. 1006/BBRC. 1998.8784 abstract page 287, column 3 page 292, column 5	1-23
A	DANN C E ET AL: "Insights into Wnt binding and signalling from the structures of two Frizzled cysteine-rich domains" , NATURE, NATURE PUBLISHING GROUP, LONDON , GB, vol. 412, 5 July 2001 (2001-07-05) , pages 86-90, XP003000381 , ISSN: 0028-0836, D0I: 10. 1038/35083601 abstract page 86, column 1 figure 3	1-23

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2011/042033

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