ANTIGEN-BINDING PROTEINS THAT ACTIVATE THE LEPTIN RECEPTOR

TECHNICAL FIELD

[0001] The present invention is related to antibodies and antigen-binding fragments of antibodies that bind human leptin receptor (LEPR), and therapeutic and diagnostic methods of using those antibodies.

SEQUENCE LISTING

[0002] An official copy of the sequence listing is submitted concurrently with the specification electronically via EFS-Web as an ASCII formatted sequence listing with a file name of 2016_10_11_10178WO01_Sequence_Listing_as_Filed_ST25.TXT, a creation date of October 11, 2016, and a size of about 99.6 kilobytes. The sequence listing contained in this ASCII formatted document is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND

[0003] Leptin is a polypeptide hormone predominantly expressed by adipose tissue and is involved in the regulation of metabolism, energy balance and food intake. Leptin activity is mediated by interaction with, and signaling through, the leptin receptor. Leptin receptor, (also known as "LEPR," "WSX," "OB receptor," "OB-R," and "CD295") is a single-pass transmembrane receptor of the class I cytokine receptor family with a large (818 amino acid) extracellular domain. Leptin deficiency, leptin resistance, and certain LEPR signaling-defective/signaling impaired mutations, are associated with obesity, type 2 diabetes, dyslipidemia, lipodystrophies, hepatic steatosis, non-alcoholic and alcoholic fatty liver diseases, severe insulin resistance, Leprechaunism/Donohue syndrome, Rabson-Mendenhall syndrome, and related complications. Therapeutic approaches to address leptin resistance, leptin deficiency, and hypoleptinemia (*e.g.*, lipodystrophy) have mostly focused on the delivery of supplemental leptin or leptin analogues to affected individuals. Such approaches, however, have generally shown limited efficacy, particularly in leptin-resistant individuals, and are frequently associated with adverse side effects. Thus, a need exists in the art for alternative approaches to treating leptin resistance and other conditions associated with leptin deficiency or hypoleptinemia.

BRIEF SUMMARY OF THE INVENTION

[0004] The present invention provides antibodies and antigen-binding fragments thereof that bind human leptin receptor (LEPR). The antibodies of the present invention are agonist antibodies; *i.e.*, binding of the anti-LEPR antibodies of the invention to LEPR causes, *inter alia*, activation of leptin receptor signaling in cells. In certain embodiments, the antibodies of the

present invention do not compete with leptin for binding to LEPR. The antibodies of the present invention are useful, *e.g.*, for mimicking, substituting for, or supplementing the normal biological activity of leptin in a subject. The antibodies and antigen-binding fragments of the present invention are therefore useful in the therapeutic treatment of diseases and disorders associated with leptin resistance and leptin deficiency.

[0005] The antibodies of the invention can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to affect functionality, e.g., to eliminate residual effector functions (Reddy et al., 2000, J. Immunol. 164:1925-1933).

[0006] Exemplary anti-LEPR antibodies of the present invention are listed in Tables 1 and 2 herein. Table 1 sets forth the amino acid sequence identifiers of the heavy chain variable regions (HCVRs), light chain variable regions (LCVRs), heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3), and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) of the exemplary anti-LEPR antibodies. Table 2 sets forth the nucleic acid sequence identifiers of the HCVRs, LCVRs, HCDR1, HCDR2 HCDR3, LCDR1, LCDR2 and LCDR3 of the exemplary anti-LEPR antibodies.

[0007] The present invention provides antibodies or antigen-binding fragments thereof that specifically bind LEPR, comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0008] The present invention also provides antibodies or antigen-binding fragments thereof that specifically bind LEPR, comprising an LCVR comprising an amino acid sequence selected from any of the LCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0009] The present invention also provides antibodies or antigen-binding fragments thereof that specifically bind LEPR, comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 1 paired with any of the LCVR amino acid sequences listed in Table 1. According to certain embodiments, the present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCVR/LCVR amino acid sequence pair contained within any of the exemplary anti-LEPR antibodies listed in Table 1. In certain embodiments, the HCVR/LCVR amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 2/10, 18/10, 26/10, 34/10, 42/10, 50/10, 58/66, 74/66 and 82/66.

[0010] The present invention also provides antibodies or antigen-binding fragments thereof that specifically bind LEPR, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 1 or a

substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0011] The present invention also provides antibodies or antigen-binding fragments thereof that specifically bind LEPR, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0012] The present invention also provides antibodies or antigen-binding fragments thereof that specifically bind LEPR, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0013] The present invention also provides antibodies or antigen-binding fragments thereof that specifically bind LEPR, comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0014] The present invention also provides antibodies or antigen-binding fragments thereof that specifically bind LEPR, comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0015] The present invention also provides antibodies or antigen-binding fragments thereof that specifically bind LEPR, comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0016] The present invention also provides antibodies or antigen-binding fragments thereof that specifically bind LEPR, comprising an HCDR3 and an LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising any of the HCDR3 amino acid sequences listed in Table 1 paired with any of the LCDR3 amino acid sequences listed in Table 1. According to certain embodiments, the present invention provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3/LCDR3 amino acid sequence pair contained within any of the exemplary anti-LEPR antibodies listed in Table 1. In certain embodiments, the HCDR3/LCDR3 amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 8/16, 24/16, 32/16, 40/16, 48/16, 56/16, 64/72, 80/72 and 88/72.

[0017] The present invention also provides antibodies or antigen-binding fragments thereof that specifically bind LEPR, comprising a set of six CDRs (*i.e.*, HCDR1, HCDR2, HCDR3, LCDR1,

LCDR2, and LCDR3) contained within any of the exemplary anti-LEPR antibodies listed in Table 1. In certain embodiments, the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 amino acid sequences set is selected from the group consisting of SEQ ID NOs: 4, 6, 8, 12, 14, 16; 20, 22, 24, 12, 14, 16; 28, 30, 32, 12, 14, 16; 36, 38, 40, 12, 14, 16; 44, 46, 48, 12, 14, 16; 52, 54, 56, 12, 14, 16; 60, 62, 64, 68, 70, 72; 76, 78, 80, 68, 70, 72; and 84, 86, 88, 68, 70, 72.

[0018] In a related embodiment, the present invention provides antibodies, or antigen-binding fragments thereof that specifically bind LEPR, comprising a set of six CDRs (*i.e.*, HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary anti-LEPR antibodies listed in Table 1. For example, the present invention includes antibodies or antigen-binding fragments thereof that specifically bind LEPR, comprising the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/10, 26/10, 34/10, 42/10, 50/10, 58/66, 74/66 and 82/66. Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See*, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[0019] The present invention also provides nucleic acid molecules encoding anti-LEPR antibodies or portions thereof. For example, the present invention provides nucleic acid molecules encoding any of the HCVR amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0020] The present invention also provides nucleic acid molecules encoding any of the LCVR amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0021] The present invention also provides nucleic acid molecules encoding any of the HCDR1 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule

comprises a polynucleotide sequence selected from any of the HCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0022] The present invention also provides nucleic acid molecules encoding any of the HCDR2 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0023] The present invention also provides nucleic acid molecules encoding any of the HCDR3 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0024] The present invention also provides nucleic acid molecules encoding any of the LCDR1 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0025] The present invention also provides nucleic acid molecules encoding any of the LCDR2 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0026] The present invention also provides nucleic acid molecules encoding any of the LCDR3 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0027] The present invention also provides nucleic acid molecules encoding an HCVR, wherein the HCVR comprises a set of three CDRs (*i.e.*, HCDR1, HCDR2, HCDR3), wherein the HCDR1, HCDR2, HCDR3 amino acid sequence set is as defined by any of the exemplary anti-LEPR antibodies listed in Table 1.

[0028] The present invention also provides nucleic acid molecules encoding an LCVR, wherein the LCVR comprises a set of three CDRs (*i.e.*, LCDR1, LCDR2, LCDR3), wherein the LCDR1, LCDR2, LCDR3 amino acid sequence set is as defined by any of the exemplary anti-LEPR antibodies listed in Table 1.

[0029] The present invention also provides nucleic acid molecules encoding both an HCVR and an LCVR, wherein the HCVR comprises an amino acid sequence of any of the HCVR amino

acid sequences listed in Table 1, and wherein the LCVR comprises an amino acid sequence of any of the LCVR amino acid sequences listed in Table 1. In certain embodiments, the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto, and a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto. In certain embodiments according to this aspect of the invention, the nucleic acid molecule encodes an HCVR and LCVR, wherein the HCVR and LCVR are both derived from the same anti-LEPR antibody listed in Table 1.

[0030] The present invention also provides recombinant expression vectors capable of expressing a polypeptide comprising a heavy or light chain variable region of an anti-LEPR antibody. For example, the present invention includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 1. Also included within the scope of the present invention are host cells into which such vectors have been introduced, as well as methods of producing the antibodies or portions thereof by culturing the host cells under conditions permitting production of the antibodies or antibody fragments, and recovering the antibodies and antibody fragments so produced.

[0031] In another aspect, the invention provides a pharmaceutical composition comprising a recombinant human antibody or fragment thereof which specifically binds LEPR and a pharmaceutically acceptable carrier. In a related aspect, the invention features a composition which is a combination of an anti-LEPR antibody and a second therapeutic agent. In one embodiment, the second therapeutic agent is any agent that is advantageously combined with an anti-LEPR antibody.

[0032] In yet another aspect, the invention provides therapeutic methods for enhancing or stimulating LEPR signaling using an anti-LEPR antibody or antigen-binding portion of an antibody of the invention. The therapeutic methods according to this aspect of the invention comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an antibody or antigen-binding fragment of an antibody of the invention to a subject in need thereof. The disorder treated is any disease or condition which is improved, ameliorated, inhibited or prevented by stimulating or activating LEPR signaling, or otherwise mimicking the natural activity of leptin *in vitro* or *in vivo*.

[0033] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0034] Figure 1 depicts the binding of dimeric human LEPR to human Leptin in the presence of increasing concentrations of test anti-LEPR antibodies or control molecules, as measured by ELISA (absorbance at 450nm).

[0035] Figures 2A-2C illustrates the extent of LEPR signaling in HEK293 cells expressing either wild-type LEPR (circles), a signaling-defective LEPR mutant (A409E, squares), or a signaling-impaired LEPR mutant (P316T, triangles). LEPR signaling is expressed as ratio of pSTAT3-Y705 / STAT3, measured by densitometry from Western blots prepared from cells treated with increasing concentrations of leptin (Figure **2A**), H4H16650 (Figure **2B**), or H4H16679 (Figure **2C**).

[0036] Figure 3 shows the average daily food intake of leptin-deficient mice dosed with either an isotype control antibody at 3 mg/kg, or a LEPR antibody selected from H4H16650P2, H4H16679P2, H4H17319P2 or H4H17321P2 at 3 mg/kg.

[0037] Figure 4 shows the average percent change in body weight of mice dosed with either an isotype control antibody at 3 mg/kg, or a LEPR antibody selected from H4H16650P2, H4H16679P2, H4H17319P2 or H4H17321P2 at 3 mg/kg.

[0038] Figure 5 shows the average fat mass for animals in each antibody treatment group quantified by μCT 1 day prior to (bars not shaded) and 6 days following antibody treatment (shaded bars) expressed as mean ± SEM.

[0039] Figure 6 shows the percent change in body weight of mice fed 30 mg/kg of an antibody selected from H4H18482P2, H4H18487P2, H4H18492P2 or an isotype control.

[0040] Figures 7A-7B. Figure **7A** shows the fat mass of mice before dosing with anti-LEPR antibodies H4H18482P2, H4H18487P2 or H4H18492P2. Figure **7B** shows the fat mass of mice treated with 30 mg/kg of H4H18482P2, H4H18487P2 or H4H18492P2.

[0041] Figure 8. Figure **8** shows that anti-LEPR antibodies tested activated monkey (Mf) LEPR in an IMR-32/STAT3-luc/Mf LEPR cell line.

DETAILED DESCRIPTION OF THE INVENTION

[0042] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0043] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For

example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0044] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

Definitions

[0045] The expression “leptin receptor,” "LEPR," and the like, as used herein, refers to the human leptin receptor, comprising the amino acid sequence as set forth in SEQ ID NO:113 (see also UniProtKB/Swiss-Prot Accession No. P48357). Alternative names for LEPR used in the scientific literature include "OB receptor," "OB-R," and "CD295." LEPR is also referred to as "WSX" (*see*, *e.g.*, US Patent No. 7,524,937). The expression "LEPR" includes both monomeric and multimeric (*e.g.*, dimeric) LEPR molecules. As used herein, the expression "monomeric human LEPR" means a LEPR protein or portion thereof that does not contain or possess any multimerizing domains and that exists under normal conditions as a single LEPR molecule without a direct physical connection to another LEPR molecule. An exemplary monomeric LEPR molecule is the molecule referred to herein as "hLEPR.mmh" comprising the amino acid sequence of SEQ ID NO:114 (*see*, *e.g.*, Example 3, herein). As used herein, the expression "dimeric human LEPR" means a construct comprising two LEPR molecules connected to one another through a linker, covalent bond, non-covalent bond, or through a multimerizing domain such as an antibody Fc domain. An exemplary dimeric LEPR molecule is the molecule referred to herein as "hLEPR.mFc" comprising the amino acid sequence of SEQ ID NO:115 (*see*, *e.g.*, Example 3, herein), or the molecule referred to herein as "hLEPR.hFc" comprising the amino acid sequence of SEQ ID NO:116. As used herein, expressions such "anti-LEPR antibody," "antibody that specifically binds LEPR," "LEPR-specific binding protein," and the like, unless specifically indicated otherwise, refer to molecules that bind full length human LEPR, monomeric human LEPR, dimeric human LEPR, or other constructs that comprise or consist of the LEPR extracellular domain.

[0046] All references to proteins, polypeptides and protein fragments herein are intended to refer to the human version of the respective protein, polypeptide or protein fragment unless explicitly specified as being from a non-human species. Thus, the expression "LEPR" means human LEPR unless specified as being from a non-human species, *e.g.*, "mouse LEPR," "monkey LEPR," etc.

[0047] As used herein, the expression "cell surface-expressed LEPR" means one or more LEPR protein(s), or the extracellular domain thereof, that is/are expressed on the surface of a cell *in vitro* or *in vivo*, such that at least a portion of a LEPR protein is exposed to the extracellular side of the cell membrane and is accessible to an antigen-binding portion of an

antibody. A "cell surface-expressed LEPR" can comprise or consist of a LEPR protein expressed on the surface of a cell which normally (*e.g.*, in the native or wild-type state) expresses LEPR protein. Alternatively, "cell surface-expressed LEPR" can comprise or consist of LEPR protein expressed on the surface of a cell that normally does not express human LEPR on its surface but has been artificially engineered to express LEPR on its surface.

[0048] As used herein, the expressions such as "anti-LEPR antibody," or "antibody that binds human leptin receptor," include both monovalent antibodies with a single specificity, as well as bispecific antibodies comprising a first arm that binds LEPR and a second arm that binds a second (target) antigen, wherein the anti-LEPR arm comprises any of the HCVR/LCVR or CDR sequences as set forth in Table 1 herein.

[0049] The term "antibody", as used herein, means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen (*e.g.*, LEPR). The term "antibody" includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-LEPR antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0050] The term "antibody", as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one

or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0051] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0052] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0053] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0054] As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.*, bispecific). A multispecific antigen-binding fragment of an antibody will

typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, including the exemplary bispecific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

[0055] In certain embodiments of the invention, the anti-LEPR antibodies of the invention are human antibodies. The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0056] The antibodies of the invention may, in some embodiments, be recombinant human antibodies. The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0057] The present invention encompasses antibodies having one or more mutations in the hinge, C_H2 or C_H3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0058] The antibodies of the invention may be isolated antibodies. An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an "isolated antibody" for

purposes of the present invention. An isolated antibody also includes an antibody *in situ* within a recombinant cell. Isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0059] The present invention includes variants of the anti-LEPR antibodies disclosed herein comprising one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the antibodies were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[0060] The present invention includes anti-LEPR antibodies and antigen-binding fragments thereof that comprise amino acid sequences that are substantially similar or substantially identical to one or more variable domain or CDR amino acid sequences as found in any of the exemplary anti-LEPR antibodies disclosed herein.

[0061] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443-1445. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0062] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. *See, e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of

the regions of the best overlap between the query and search sequences (Pearson (2000) supra). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. *See, e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215:403-410 and Altschul *et al.* (1997) Nucleic Acids Res. 25:3389-402.

Anti-LEPR Antibodies Comprising Fc Variants

[0063] According to certain embodiments of the present invention, anti-LEPR antibodies are provided comprising an Fc domain comprising one or more mutations which enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, the present invention includes anti-LEPR antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., H/L/R/S/P/Q or K) and/or 434 (*e.g.,* H/F or Y); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification (*e.g.,* 308F or 308P).
[0064] For example, the present invention includes anti-LEPR antibodies comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); and 433K and 434F (*e.g.*, H433K and N434F). All possible combinations of the foregoing Fc domain mutations, and other mutations within the antibody variable domains disclosed herein, are contemplated within the scope of the present invention.
[0065] The anti-LEPR antibodies of the present invention may comprise a modified Fc domain having reduced effector function. As used herein, a "modified Fc domain having reduced effector function" means any Fc portion of an immunoglobulin that has been modified, mutated, truncated, etc., relative to a wild-type, naturally occurring Fc domain such that a molecule comprising the modified Fc exhibits a reduction in the severity or extent of at least one effect selected from the group consisting of cell killing (*e.g.*, ADCC and/or CDC), complement activation, phagocytosis and opsonization, relative to a comparator molecule comprising the

wild-type, naturally occurring version of the Fc portion. In certain embodiments, a "modified Fc domain having reduced effector function" is an Fc domain with reduced or attenuated binding to an Fc receptor (*e.g.*, FcγR).

[0066] In certain embodiments of the present invention, the modified Fc domain is a variant IgG1 Fc or a variant IgG4 Fc comprising a substitution in the hinge region. For example, a modified Fc for use in the context of the present invention may comprise a variant IgG1 Fc wherein at least one amino acid of the IgG1 Fc hinge region is replaced with the corresponding amino acid from the IgG2 Fc hinge region. Alternatively, a modified Fc for use in the context of the present invention may comprise a variant IgG4 Fc wherein at least one amino acid of the IgG4 Fc hinge region is replaced with the corresponding amino acid from the IgG2 Fc hinge region. Non-limiting, exemplary modified Fc regions that can be used in the context of the present invention are set forth in US Patent Application Publication No. 2014/0243504.

[0067] Other modified Fc domains and Fc modifications that can be used in the context of the present invention include any of the modifications as set forth in US 2014/0171623; US 8,697,396; US 2014/0134162; WO 2014/043361. Methods of constructing antibodies or other antigen-binding fusion proteins comprising a modified Fc domain as described herein are known in the art.

Biological Characteristics of the Antibodies

[0068] The present invention includes antibodies and antigen-binding fragments thereof that bind human LEPR and activate LEPR signaling. Such antibodies may be referred to herein as "agonist antibodies." In the context of the present invention, "activation of LEPR signaling" means the stimulation of an intracellular effect that normally results from the interaction of leptin with LEPR in cells that express LEPR. In certain embodiments, "activation of LEPR signaling" means the transcriptional activation of STAT3, which can be detected using any method that can measure or identify, directly or indirectly, STAT3 activity, *e.g.*, using a labeled version of STAT3 expressed in a reporter cell line. For example, the present invention includes antibodies and antigen-binding fragments thereof that activate LEPR signaling in a cell-based reporter assay, *e.g.*, using a cell based assay format as defined in Example 7 herein, or a substantially similar assay. Cell-based reporter assays that detect LEPR activation, such as the assay set forth in Example 7 herein, can produce a detectable signal that may be expressed in terms of an EC_{50} value (*i.e.*, the antibody concentration required to produce half-maximal signaling) and/or a percentage of the maximal signaling observed in the presence of leptin. In certain exemplary embodiments of the present invention, anti-LEPR antibodies are provided that activate LEPR signaling with an EC_{50} value of less than about 12.0 nM in a cell-based reporter assay, *e.g.*, using an assay format as defined in Example 7 herein, or a substantially similar assay. In certain exemplary embodiments of the present invention, anti-LEPR antibodies are provided that

activate LEPR signaling with maximum percent activation relative to leptin signaling of greater than about 65% in a cell-based reporter assay, *e.g.*, using an assay format as defined in Example 7 herein, or a substantially similar assay.

[0069] The present invention includes antibodies and antigen-binding fragments thereof that bind monomeric human LEPR with high affinity. For example, the present invention includes anti-LEPR antibodies that bind monomeric human LEPR (*e.g.*, hLEPR.mmh, SEQ ID NO:114) with a K_D of less than about 150 nM as measured by surface plasmon resonance at 25ºC or 37ºC, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. According to certain embodiments, anti-LEPR antibodies are provided that bind monomeric human LEPR at 25ºC with a K_D of less than about 150 nM, less than about 140 nM, less than about 130 nM, less than about 120 nM, less than about 110 nM, less than about 100 nM, less than about 90 nM, less than about 80 nM, less than about 70 nM, less than about 60 nM, less than about 50 nM, less than about 40 nM, less than about 30 nM, less than about 20 nM, less than about 10 nM, less than about 9 nM, less than about 8 nM, less than about 7 nM, less than about 6 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 900 pM, less than about 800 pM, less than about 700 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, or less than about 300 pM, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay.

[0070] The present invention also includes antibodies and antigen-binding fragments thereof that bind monomeric human LEPR (*e.g.*, hLEPR.mmh, SEQ ID NO:114) with a dissociative half-life (t½) of greater than about 50 minutes as measured by surface plasmon resonance at 25ºC or 37ºC, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. According to certain embodiments, anti-LEPR antibodies are provided that bind monomeric human LEPR at 25ºC with a t½ of greater than about 50 minutes, greater than about 55 minutes, greater than about 60 minutes, greater than about 65 minutes, or longer, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay.

[0071] The present invention also includes antibodies and antigen-binding fragments thereof that bind dimeric human LEPR (*e.g.*, hLEPR.mFc, SEQ ID NO:115) with high affinity. For example, the present invention includes anti-LEPR antibodies that bind dimeric human LEPR with a K_D of less than about 1.5 nM as measured by surface plasmon resonance at 25ºC or 37ºC, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. According to certain embodiments, anti-LEPR antibodies are provided that bind dimeric human LEPR at 25ºC with a K_D of less than about 150 nM, less than about 130 nM, less than about 110 nM, less than about 80 nM, less than about 70 nM, less than about 60 nM, less than about 50 nM, less than about 40 nM, less than about 30 nM, less than about 20 nM, or less than about 10 nM, as measured by surface plasmon resonance, *e.g.*, using an assay format as

defined in Example 3 herein, or a substantially similar assay.

[0072] The present invention also includes antibodies and antigen-binding fragments thereof that bind dimeric human LEPR (*e.g.*, hLEPR.mFc, SEQ ID NO:115) with a dissociative half-life (t½) of greater than about 10 minutes as measured by surface plasmon resonance at 25ºC or 37ºC, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay. According to certain embodiments, anti-LEPR antibodies are provided that bind dimeric human LEPR at 25ºC with a t½ of greater than about 10, greater than about 15 minutes, greater than about 20 minutes, greater than about 25 minutes, greater than about 30 minutes, greater than about 40 minutes, greater than about 50 minutes, greater than about 60 minutes, greater than about 70 minutes, or longer, as measured by surface plasmon resonance, *e.g.*, using an assay format as defined in Example 3 herein, or a substantially similar assay.

[0073] The present invention also includes antibodies and antigen-binding fragments thereof that bind LEPR in complex with human leptin ("LEPR in complex with human leptin" may also be represented by the expression "leptin:LEPR"). For example the present invention includes antibodies and antigen-binding fragments thereof that are capable of binding to a pre-formed complex comprising hLEPR and human leptin. That is, according to certain embodiments, the interaction between anti-LEPR antibodies and LEPR is not inhibited by the presence of leptin in complex with LEPR; likewise, the interaction between leptin and LEPR, according to this aspect of the invention, is not inhibited by the presence of an anti-LEPR antibody. An exemplary assay format for determining whether an antibody or antigen-binding fragment thereof binds to LEPR in complex with human leptin is set forth in Example 4 herein.

[0074] Similarly, the present invention also includes antibodies and antigen-binding fragments thereof that bind LEPR and do not block the LEPR:leptin interaction. For example the present invention includes antibodies and antigen-binding fragments thereof that are capable of binding LEPR, thereby producing an antibody:LEPR complex, wherein the resulting antibody:LEPR complex is capable of interacting with leptin to produce a three-member complex comprising the antibody, LEPR and leptin. An exemplary assay format for determining whether an antibody or antigen-binding fragment thereof is capable of binding LEPR in a manner that does not block or interfere with the interaction between LEPR and leptin is set forth in Example 5 herein.

[0075] The present invention also includes antibodies and antigen-binding fragments thereof that bind cell surface-expressed LEPR in the presence and/or absence of human leptin. Cell surface-expressed LEPR means LEPR or a portion thereof (*e.g.*, an extracellular portion of LEPR) expressed on the surface of a cell, either naturally or in an engineered cell line, such that an antibody or antigen-binding fragment thereof is capable of binding to the LEPR molecule. In certain embodiments, cell surface-expressed LEPR includes recombinant complexes comprising an extracellular domain of LEPR connected to a cell via a tag or anchor (e.g., a GPI anchor as illustrated in Example 6 herein). According to this aspect of the invention, antibodies are provided which are capable of binding cell surface-expressed LEPR in the absence of leptin,

and are also capable of binding cell surface-expressed LEPR in the presence of leptin (i.e., under circumstances wherein leptin is capable of binding to cell surface-expressed leptin). That is, according to certain embodiments, the interaction between anti-LEPR antibodies and cell surface-expressed LEPR is not inhibited by the presence of leptin in complex with cell surface-expressed LEPR. Antibodies according to this aspect of the invention are capable of forming a three-member complex on the surface of a cell comprising the antibody, cell surface-expressed LEPR and leptin. An exemplary assay format for determining whether an antibody or antigen-binding fragment thereof is capable of binding cell surface-expressed LEPR in the presence and absence of human leptin is set forth in Example 6 herein.

[0076] The antibodies of the present invention may possess one or more of the aforementioned biological characteristics, or any combination thereof. The foregoing list of biological characteristics of the antibodies of the invention is not intended to be exhaustive. Other biological characteristics of the antibodies of the present invention will be evident to a person of ordinary skill in the art from a review of the present disclosure including the working Examples herein.

Epitope Mapping and Related Technologies

[0077] The present invention also includes anti-LEPR antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes anti-LEPR antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences set forth in Table 1 herein. In certain embodiments, the present invention provides anti-LEPR antibodies comprising variant HCVR, LCVR and/or CDR amino acid sequences relative to the sequences set forth in Table 1 herein (*e.g.*, comprising conservative amino acid substitutions), wherein such variant antibodies nonetheless exhibit one or more functions and/or properties of the exemplary anti-LEPR antibodies disclosed herein.

[0078] The extracellular domain of human LEPR contains an N-terminal cytokine receptor homology domain (CRH-1), an immunoglobulin-like (Ig) domain, and a second CRH domain (CRH-2) that is referred to as the leptin-binding domain (LBD). (Carpenter *et al.* (2012) Structure 20:487-97). Furthermore, LEPR shares the greatest homology and similar extracellular domain size and organization with granulocyte colony stimulating factor (GCSF) and glycoprotein 130 (gp13). (Haniu *et al.* (1998) J Biol Chem 273(44): 28691-699).

[0079] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either

conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

[0080] The present invention includes anti-LEPR antibodies that interact with one or more epitopes found within amino acids M1-D839 of human LEPR (SEQ ID NO: 113). As set forth in Example 11, 201 peptides from human LEPR had significantly reduced deuteration uptake when bound to antibody H4H16650P2. The peptides corresponding to amino acids 162-169 (amino acids LYVLPEVL of human LEPR, SEQ ID NO: 113) and 170-191 (amino acids EDSPLVPQKGSF of human LEPR, SEQ ID NO: 113) had slower deuteration rates when bound to H4H16650P2, indicating that this antibody binds at least two human LEPR epitopes having the sequences LYVLPEVL or EDSPLVPQKGSF (amino acids 162-169 or 170-191, respectively of SEQ ID NO: 113).

[0081] The epitope to which the antibodies of the present invention bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids of a LEPR protein. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) of LEPR. In some embodiments, the epitope is located on or near the leptin-binding domain of LEPR. In other embodiments, the epitope is located at a region distinct from the leptin-binding domain of LEPR, *e.g.*, at a location on the surface of LEPR at which an antibody, when bound to such an epitope, does not interfere with leptin binding to LEPR.

[0082] Various techniques known to persons of ordinary skill in the art can be used to identify the amino acids within an epitope recognized by a particular antibody. Exemplary techniques include, *e.g.*, alanine scanning mutational analysis, peptide blot analysis, and peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer, 2000, *Protein Science* 9:487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water to allow hydrogen-deuterium exchange to occur at all residues except for the residues protected by the antibody (which remain deuterium-labeled). After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring (1999) *Analytical Biochemistry* 267(2):252-259; Engen and Smith (2001) *Anal. Chem. 73*:256A-265A. X-ray crystallography analysis of an antibody in complex with its antigen may also be used to identify the amino acids within a polypeptide with which an antibody interacts.

[0083] The present invention further includes anti-LEPR antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 1 herein). Likewise, the present invention also includes anti-LEPR antibodies that compete for binding to LEPR with any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 1 herein).

[0084] One can determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-LEPR antibody by using routine methods known in the art and exemplified herein. For example, to determine if a test antibody binds to the same epitope as a reference anti-LEPR antibody of the invention, the reference antibody is allowed to bind to a LEPR protein. Next, the ability of a test antibody to bind to the LEPR molecule is assessed. If the test antibody is able to bind to LEPR following saturation binding with the reference anti-LEPR antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-LEPR antibody. On the other hand, if the test antibody is not able to bind to the LEPR molecule following saturation binding with the reference anti-LEPR antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-LEPR antibody of the invention. Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, Biacore, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art. In accordance with certain embodiments of the present invention, two antibodies bind to the same (or overlapping) epitope if, *e.g.*, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, e.g., Junghans et al., *Cancer Res.* 1990:50:1495-1502). Alternatively, two antibodies are deemed to bind to the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies are deemed to have "overlapping epitopes" if only a subset of the amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0085] To determine if an antibody competes for binding (or cross-competes for binding) with a reference anti-LEPR antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a LEPR protein under saturating conditions followed by assessment of binding of the test antibody to the LEPR molecule. In a second orientation, the test antibody is allowed to bind to a LEPR molecule under saturating conditions followed by assessment of binding of the reference antibody to the LEPR molecule. If, in both orientations, only the first (saturating) antibody is capable of binding

to the LEPR molecule, then it is concluded that the test antibody and the reference antibody compete for binding to LEPR. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the same epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Preparation of Human Antibodies

[0086] The anti-LEPR antibodies of the present invention can be fully human antibodies. Methods for generating monoclonal antibodies, including fully human monoclonal antibodies are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to human LEPR.

[0087] Using VELOCIMMUNE™ technology, for example, or any other similar known method for generating fully human monoclonal antibodies, high affinity chimeric antibodies to LEPR are initially isolated having a human variable region and a mouse constant region. As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, ligand blocking activity, selectivity, epitope, etc. If necessary, mouse constant regions are replaced with a desired human constant region, for example wild-type or modified IgG1 or IgG4, to generate a fully human anti-LEPR antibody. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region. In certain instances, fully human anti-LEPR antibodies are isolated directly from antigen-positive B cells.

Bioequivalents

[0088] The anti-LEPR antibodies and antibody fragments of the present invention encompass proteins having amino acid sequences that vary from those of the described antibodies but that retain the ability to bind human LEPR. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the anti-LEPR antibody-encoding DNA sequences of the present invention encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an anti-LEPR antibody or antibody fragment that is essentially bioequivalent to an anti-LEPR antibody or antibody fragment of the invention. Examples of such variant amino acid and DNA sequences are discussed above.

[0089] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose

under similar experimental conditions, either single does or multiple dose. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

[0090] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0091] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[0092] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[0093] Bioequivalence may be demonstrated by in vivo and in vitro methods. Bioequivalence measures include, e.g., (a) an in vivo test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an in vitro test that has been correlated with and is reasonably predictive of human in vivo bioavailability data; (c) an in vivo test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

[0094] Bioequivalent variants of anti-LEPR antibodies of the invention may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antibodies may include anti-LEPR antibody variants comprising amino acid changes which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations which eliminate or remove glycosylation.

Species Selectivity and Species Cross-Reactivity

[0095] The present invention, according to certain embodiments, provides anti-LEPR antibodies that bind to human LEPR but not to LEPR from other species. The present invention also includes anti-LEPR antibodies that bind to human LEPR and to LEPR from one or more

non-human species. For example, the anti-LEPR antibodies of the invention may bind to human LEPR and may bind or not bind, as the case may be, to one or more of mouse, rat, guinea pig, hamster, gerbil, pig, cat, dog, rabbit, goat, sheep, cow, horse, camel, cynomologous, marmoset, rhesus or chimpanzee LEPR. According to certain exemplary embodiments of the present invention, anti-LEPR antibodies are provided which specifically bind human LEPR and cynomolgus monkey (*e.g.*, *Macaca fascicularis*) LEPR. Other anti-LEPR antibodies of the invention bind human LEPR but do not bind, or bind only weakly, to cynomolgus monkey LEPR.

Multispecific Antibodies

[0096] The antibodies of the present invention may be monospecific or multispecific (*e.g.*, bispecific). Multispecific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, e.g., Tutt et al., 1991, *J. Immunol.* 147:60-69; Kufer *et al.*, 2004, *Trends Biotechnol.* 22:238-244. The anti-LEPR antibodies of the present invention can be linked to or co-expressed with another functional molecule, e.g., another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bi-specific or a multispecific antibody with a second binding specificity.

[0097] The present invention includes bispecific antibodies wherein one arm of an immunoglobulin binds human LEPR, and the other arm of the immunoglobulin is specific for a second antigen. The LEPR-binding arm can comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 1 herein.

[0098] An exemplary bispecific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bispecific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bispecific antibody

format described above are contemplated within the scope of the present invention.

[0099] Other exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab^2 bispecific formats (*see*, *e.g.*, Klein *et al.* 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats). Bispecific antibodies can also be constructed using peptide/nucleic acid conjugation, *e.g.*, wherein unnatural amino acids with orthogonal chemical reactivity are used to generate site-specific antibody-oligonucleotide conjugates which then self-assemble into multimeric complexes with defined composition, valency and geometry. (*See*, *e.g.*, Kazane *et al.*, *J. Am. Chem. Soc.* [*Epub*: *Dec. 4, 2012*]).

Therapeutic Formulation and Administration

[0100] The invention provides pharmaceutical compositions comprising the anti-LEPR antibodies or antigen-binding fragments thereof of the present invention. The pharmaceutical compositions of the invention are formulated with suitable carriers, excipients, and other agents that provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™, Life Technologies, Carlsbad, CA), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) *J Pharm Sci Technol* 52:238-311.

[0101] The dose of antibody administered to a patient may vary depending upon the age and the size of the patient, target disease, conditions, route of administration, and the like. The preferred dose is typically calculated according to body weight or body surface area. In an adult patient, it may be advantageous to intravenously administer the antibody of the present invention normally at a single dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. Effective dosages and schedules for administering anti-LEPR antibodies may be determined empirically; for example, patient progress can be monitored by periodic assessment, and the dose adjusted accordingly. Moreover, interspecies scaling of dosages can be performed using well-known methods in the art (*e.g.*, Mordenti *et al.*, 1991,

Pharmaceut. Res. 8:1351).

[0102] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, e.g., Wu et al., 1987, *J. Biol. Chem.* 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0103] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0104] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (Sanofi-Aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (Sanofi-Aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park IL), to name only a few.

[0105] In certain situations, the pharmaceutical composition can be delivered in a controlled

release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:201). In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, *CRC Pres.*, Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, *Science* 249:1527-1533.

[0106] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (*e.g.*, propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0107] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Therapeutic Uses of the Antibodies

[0108] The present invention includes methods comprising administering to a subject in need thereof a therapeutic composition comprising an anti-LEPR antibody (*e.g.*, an anti-LEPR antibody comprising any of the HCVR/LCVR or CDR sequences as set forth in Table 1 herein). The therapeutic composition can comprise any of the anti-LEPR antibodies disclosed herein, or antigen-binding fragments thereof, and a pharmaceutically acceptable carrier or diluent.

[0109] The antibodies of the invention are useful, *inter alia*, for the treatment, prevention and/or amelioration of any disease or disorder associated with or mediated by leptin deficiency, leptin

resistance, hypoleptinemia, or otherwise treatable by stimulating or activating LEPR signaling or mimicking the natural activity of leptin *in vitro* or *in vivo*. For example, the antibodies and antigen-binding fragments thereof of the present invention are useful for treating lipodystrophy conditions. Exemplary lipodystrophy conditions that are treatable by the antibodies and antigen-binding fragments of the present invention include, *e.g.*, congenital generalized lipodystrophy, acquired generalized lipodystrophy, familial partial lipodystrophy, acquired partial lipodystrophy, centrifugal abdominal lipodystrophy, lipoatrophia annularis, localized lipodystrophy, and HIV-associated lipodystrophy.

[0110] The present invention also includes anti-LEPR antibodies and antigen-binding fragments thereof that are useful for restoring leptin signaling to cells, tissues and organs expressing one or more LEPR mutations associated with obesity. For example, certain LEPR mutants have been identified that exhibit no, or reduced signaling in the presence of leptin and are associated with obesity and related disorders. As used herein, a LEPR mutant that exhibits no signaling in the presence of leptin is referred to as a "signaling-defective LEPR mutant." An exemplary signaling-defective LEPR mutation is LEPR-A409E (Farooqi *et al.*, 2007, *N Engl J Med* 356(3): 237-247). As used herein, a LEPR mutant that exhibits reduced signaling in the presence of leptin (as compared to wild-type LEPR) is referred to as a "signaling-impaired LEPR mutant." An exemplary signaling-impaired LEPR mutation is LEPR-P316T (Mazen *et al.*, 2011, *Mol Genet Metab* 102:461-464). Thus, the present invention includes anti-LEPR antibodies and antigen-binding fragments thereof that are useful for the treatment, prevention and/or amelioration of diseases and disorders caused by or associated with one or more signaling-defective (*e.g.*, A409E) and/or signaling-impaired (*e.g.*, P316T) LEPR mutants.

[0111] The anti-LEPR antibodies and antigen-binding fragments thereof of the present invention are also useful for the treatment or prevention of one or more diseases or disorders selected from the group consisting of obesity, metabolic syndrome, diet-induced food craving, functional hypothalamic amenorrhea, type 1 diabetes, type 2 diabetes, insulin resistance, severe insulin resistance including severe insulin resistance due to mutation in insulin receptor, severe insulin resistance not caused by mutation in the insulin receptor, severe insulin resistance caused by a mutation in downstream signaling pathways or induced by other causes, non-alcoholic and alcoholic fatty liver diseases, Alzheimer's disease, leptin deficiency, leptin resistance, lipodystrophies, Leprechaunism/Donohue syndrome, Rabson-Mendenhall syndrome.

[0112] In the context of the methods of treatment described herein, the anti-LEPR antibody may be administered as a monotherapy (*i.e.*, as the only therapeutic agent) or in combination with one or more additional therapeutic agents (examples of which are described elsewhere herein).

Combination Therapies and Formulations

[0113] The present invention includes compositions and therapeutic formulations comprising

any of the anti-LEPR antibodies described herein in combination with one or more additional therapeutically active components, and methods of treatment comprising administering such combinations to subjects in need thereof.

[0114] The anti-LEPR antibodies of the present invention may be co-formulated with and/or administered in combination with one or more additional therapeutically active component(s), such as. *e.g.*, pharmaceutical products prescribed for the treatment of obesity, hypercholesterolemia, hyperlipidemia, type 2 diabetes, type 1 diabetes, appetite control, infertility, etc. Examples of such additional therapeutically active components include, *e.g.*, recombinant human leptin (*e.g.*, metreleptin [MYALEPT]), PCSK9 inhibitors (*e.g.*, anti-PCSK9 antibodies [alirocumab, evolocumab, bococizumab, lodelcizumab, ralpancizumab, etc.]), statins (atorvastatin, rosuvastatin, cerivastatin, pitavastatin, fluvastatin, simvastatin, lovastatin, pravastatin, etc.), ezetimibe, insulin, insulin variants, insulin secretagogues, metformin, sulfonylureas, sodium glucose cotransporter 2 (SGLT2) Inhibitors (*e.g.*, dapaglifozin, canaglifozin, empagliflozin, etc.), GLP-1 agonists/analogues (*e.g.*, extendin-4, exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, etc.), glucagon (GCG) inhibitors (*e.g.*, anti-GCG antibodies), glucagon receptor (GCGR) inhibitors (*e.g.*, anti-GCGR antibodies, small molecule GCGR antagonists, GCGR-specific antisense oligonucleotides, anti-GCGR aptamers [e.g., Spiegelmers], etc.), angiopoietin-like protein (ANGPTL) inhibitors (*e.g.*, anti-ANGPTL3 antibodies, anti-ANGPTL4 antibodies, anti-ANGPTL8 antibodies, etc.), Phentermine, Orlistat, Topiramate, Bupropion, Topiramate/Phentermine, Bupropion/Naltrexone, Bupropion/Zonisamide, Pramlintide/Metrelepin, Lorcaserin, Cetilistat, Tesofensine, Velneperit, etc.

[0115] The additional therapeutically active component(s), *e.g.*, any of the agents listed above or derivatives thereof, may be administered just prior to, concurrent with, or shortly after the administration of an anti-LEPR antibody of the present invention; (for purposes of the present disclosure, such administration regimens are considered the administration of an anti-LEPR antibody "in combination with" an additional therapeutically active component). The present invention includes pharmaceutical compositions in which an anti-LEPR antibody of the present invention is co-formulated with one or more of the additional therapeutically active component(s) as described elsewhere herein.

Administration Regimens

[0116] According to certain embodiments of the present invention, multiple doses of an anti-LEPR antibody (or a pharmaceutical composition comprising a combination of an anti-LEPR antibody and any of the additional therapeutically active agents mentioned herein) may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of an anti-LEPR

antibody of the invention. As used herein, "sequentially administering" means that each dose of anti-LEPR antibody is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of an anti-LEPR antibody, followed by one or more secondary doses of the anti-LEPR antibody, and optionally followed by one or more tertiary doses of the anti-LEPR antibody.

[0117] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the anti-LEPR antibody of the invention. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose," "loading dose," "starting dose," and the like); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of anti-LEPR antibody, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of anti-LEPR antibody contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (*e.g.*, 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

Diagnostic and Analytic Uses of the Antibodies

[0118] The anti-LEPR antibodies of the present invention may also be used to detect and/or measure LEPR, or LEPR-expressing cells in a sample, *e.g.*, for diagnostic purposes. For example, an anti-LEPR antibody, or fragment thereof, may be used to diagnose a condition or disease characterized by aberrant expression (*e.g.*, over-expression, under-expression, lack of expression, etc.) of LEPR. Exemplary diagnostic assays for LEPR may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-LEPR antibody of the invention, wherein the anti-LEPR antibody is labeled with a detectable label or reporter molecule. Alternatively, an unlabeled anti-LEPR antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, beta-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure LEPR in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), fluorescence-activated cell sorting (FACS), and positron emission tomography (PET) scanning.

[0119] Samples that can be used in LEPR diagnostic assays according to the present invention

include any tissue or fluid sample obtainable from a patient which contains detectable quantities of LEPR protein, or fragments thereof, under normal or pathological conditions. Generally, levels of LEPR in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with a disease or condition associated with abnormal LEPR levels or activity) will be measured to initially establish a baseline, or standard, level of LEPR. This baseline level of LEPR can then be compared against the levels of LEPR measured in samples obtained from individuals suspected of having a LEPR related disease or condition.

EXAMPLES

[0120] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Generation of Antigen-Binding Proteins that Specifically bind the Leptin Receptor (LEPR)

[0121] Anti-LEPR antibodies were obtained by immunizing a VELOCIMMUNE® mouse (*i.e.*, an engineered mouse comprising DNA encoding human immunoglobulin heavy and kappa light chain variable regions) with an immunogen comprising the extracellular domain of LEPR. The antibody immune response was monitored by a LEPR-specific immunoassay. Using previously described techniques, fully human anti-LEPR antibodies were isolated and purified.

[0122] Certain biological properties of the exemplary anti-LEPR antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2. Heavy and Light Chain Variable Region Amino Acid and Nucleic Acid Sequences

[0123] Table 1 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected anti-LEPR antibodies of the invention. The corresponding nucleic acid sequence identifiers are set forth in Table 2.

Table 1: Amino Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H4H16650P2	2	4	6	8	10	12	14	16
H4H16679P2	18	20	22	24	10	12	14	16
H4H17319P2	26	28	30	32	10	12	14	16
H4H17321P2	34	36	38	40	10	12	14	16
H4H18417P2	42	44	46	48	10	12	14	16
H4H18438P2	50	52	54	56	10	12	14	16
H4H18445P2	58	60	62	64	10	12	14	16
H4H18446P2	66	68	70	72	10	12	14	16
H4H18449P2	74	76	78	80	10	12	14	16
H4H18482P2	82	84	86	88	90	92	94	96
H4H18487P2	98	100	102	104	90	92	94	96
H4H18492P2	106	108	110	112	90	92	94	96

Table 2: Nucleic Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H4H16650P2	1	3	5	7	9	11	13	15
H4H16679P2	17	19	21	23	9	11	13	15
H4H17319P2	25	27	29	31	9	11	13	15
H4H17321P2	33	35	37	39	9	11	13	15
H4H18417P2	41	43	45	47	9	11	13	15
H4H18438P2	49	51	53	55	9	11	13	15
H4H18445P2	57	59	61	63	9	11	13	15
H4H18446P2	65	67	69	71	9	11	13	15

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H4H18449P2	73	75	77	79	9	11	13	15
H4H18482P2	81	83	85	87	89	91	93	95
H4H18487P2	97	99	101	103	89	91	93	95
H4H18492P2	105	107	109	111	89	91	93	95

[0124] Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (*e.g.* "H4H," "H1M," "H2M," etc.), followed by a numerical identifier (*e.g.* "16650," "16679," etc.), followed by a "P" or "N" suffix. Thus, according to this nomenclature, an antibody may be referred to herein as, *e.g.*, "H4H16650P2," "H4H16679P2," etc. The Fc prefixes on the antibody designations used herein (H4H, H1M and H2M) indicate the particular Fc region isotype of the antibody. For example, an "H4H" antibody has a human IgG4 Fc, whereas an "H1M" antibody has a mouse IgG1 Fc, (all variable regions are fully human as denoted by the first 'H' in the antibody designation). As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (*e.g.*, an antibody with a mouse IgG1 Fc can be converted to an antibody with a human IgG4, etc.), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Tables 1 and 2 – will remain the same, and the binding properties are expected to be identical or substantially similar regardless of the nature of the Fc domain.
[0125] "Comparator mAb" as used in Examples herein refers to Fab9F8 described in Fazeli *et al.* (2006) J Immunol Methods 312:190-200 and Carpenter *et al.* (2012) Structure 20(3):487-97.

Example 3: Surface Plasmon Resonance Derived Binding Affinities and Kinetic Constants of Human Monoclonal Anti-LEPR Antibodies

[0126] Equilibrium dissociation constants (K_D values) for LEPR binding to purified anti-LEPR monoclonal antibodies were determined using a real-time surface plasmon resonance biosensor using a Biacore 4000 instrument. All binding studies were performed in 10mM HEPES, 150mM NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20, pH 7.4 (HBS-ET) running buffer at 25°C and 37°C. The Biacore sensor surface was first derivatized by amine coupling with a monoclonal mouse anti-human Fc antibody (GE, # BR-1008-39) to capture anti-LEPR monoclonal antibodies. Binding studies were performed on following LEPR reagents: human LEPR extracellular domain expressed with a C-terminal myc-myc-hexahistidine tag (hLEPR.mmh; SEQ ID NO: 114), macaca fascicularis LEPR extracellular domain expressed with a C-terminal myc-myc-hexahistidine tag (mfLEPR.mmh; SEQ ID NO: 117), human LEPR

extracellular domain expressed with a C-terminal mouse IgG2a Fc tag (hLEPR.mFc; SEQ ID NO: 115), mouse LEPR extracellular domain expressed with a C-terminal myc-myc-hexahistidine tag (mLEPR.mmh; SEQ ID NO: 118) and rat LEPR extracellular domain expressed with a C-terminal myc-myc-hexahistidine tag (rLEPR.mmh; SEQ ID NO: 119). Different concentrations of LEPR reagents were first prepared in HBS-ET running buffer (100nM – 3.7nM; 3-fold serial dilution) and were injected over anti-human Fc captured anti-LEPR monoclonal antibody surface for 4 minutes at a flow rate of 30µL/minute, while the dissociation of monoclonal antibody bound LEPR reagent was monitored for 10 minutes in HBS-ET running buffer. Kinetic association (k_a) and dissociation (k_d) rate constants were determined by fitting the real-time binding sensorgrams to a 1:1 binding model with mass transport limitation using Scrubber 2.0c curve-fitting software. Binding dissociation equilibrium constants (K_D) and dissociative half-lives (t½) were calculated from the kinetic rate constants as:

$$K_D\ (M) = \frac{kd}{ka}, \quad \text{and} \quad t½\ (min) = \frac{\ln(2)}{60 * kd}$$

[0127] Binding kinetics parameters for hLEPR.mmh, mfLEPR.MMH or hLEPR.mFc, binding to different anti-LEPR monoclonal antibodies of the invention at 25°C and 37°C are shown in Tables 3 through 8.

Table 3: Binding kinetics parameters of hLEPR-MMH binding to LEPR monoclonal antibodies at 25°C.

mAb Captured	mAb Capture Level (RU)	100nM hLEPR-MMH Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H16650P2	167 ± 0.3	51	2.81E+04	2.23E-04	7.93E-09	52
H4H16679P2	192 ± 0.7	39	2.34E+04	2.46E-04	1.05E-08	47
H4H18417P2	163 ± 0.4	28	6.14E+04	7.90E-03	1.29E-07	1.5
H4H18438P2	166 ± 0.4	22	3.00E+04	2.26E-03	7.54E-08	5.1
H4H18445P2	194 ± 1.1	45	4.42E+04	4.78E-03	1.08E-07	2.4
H4H18446P2	163 ± 2.4	16	1.81E+04	9.51E-04	5.25E-08	12
H4H18449P2	176 ± 1.3	54	2.91E+04	2.35E-04	8.08E-09	49
H4H18482P2	163 ± 0.4	47	6.31E+04	6.77E-03	1.07E-07	1.7

H4H18487P2	190 ± 1.2	42	4.73E+04	7.03E-03	1.48E-07	1.6
H4H18492P2	167 ± 3.1	87	8.10E+04	8.98E-04	1.11E-08	13
H4H17319P2	200 ± 0.4	36	2.61E+04	5.29E-04	2.03E-08	22
H4H17321P2	221 ± 0.5	32	2.36E+04	1.96E-04	8.31E-09	59
Isotype Control mAb	171 ± 0.4	4	NB*	NB*	NB*	NB*

*NB indicates that no binding was observed under the current experimental conditions.

Table 4: Binding kinetics parameters of hLEPR-MMH binding to LEPR monoclonal antibodies at 37°C.

mAb Captured	mAb Capture Level (RU)	100nM hLEPR-MMH Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H16650P2	210 ± 2.5	77	4.85E+04	9.58E-04	1.98E-08	12
H4H16679P2	239 ± 2	61	3.84E+04	8.42E-04	2.19E-08	14
H4H18417P2	206 ± 3.2	22	7.70E+04	1.80E-02	2.33E-07	0.6
H4H18438P2	206 ± 2.4	32	3.38E+04	5.76E-03	1.70E-07	2.0
H4H18445P2	234 ± 2	38	5.13E+04	1.68E-02	3.26E-07	0.7
H4H18446P2	188 ± 3.4	21	2.12E+04	2.56E-03	1.21E-07	4.5
H4H18449P2	206 ± 2.1	73	3.94E+04	8.15E-04	2.07E-08	14
H4H18482P2	188 ± 0.8	38	9.53E+04	1.93E-02	2.03E-07	0.6
H4H18487P2	219 ± 1.7	30	6.51E+04	1.86E-02	2.86E-07	0.6
H4H18492P2	192 ± 2.2	93	1.17E+05	4.18E-03	3.59E-08	2.8
H4H17319P2	264 ± 0.3	44	3.54E+04	3.41E-03	9.63E-08	3.4
H4H17321P2	290 ± 0.4	61	2.95E+04	4.38E-04	1.48E-08	26
Isotype Control mAb	193 ± 1.5	6	NB*	NB*	NB*	NB*

*NB indicates that no binding was observed under the current experimental conditions.

Table 5: Binding kinetics parameters of mfLEPR.MMH binding to LEPR monoclonal antibodies at 25°C.

mAb Captured	mAb Capture Level (RU)	100nM mfLEP. MMH Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H16650P2	166 ± 0.6	93	6.02E+04	1.37E-04	2.27E-09	84
H4H16679P2	191 ± 0.7	66	4.37E+04	1.41E-04	3.22E-09	82
H4H18417P2	162 ± 0.3	33	8.83E+04	1.23E-02	1.39E-07	0.9
H4H18438P2	166 ± 0.6	5	IC*	IC*	IC*	IC*
H4H18445P2	193 ± 0.6	58	5.90E+04	4.86E-03	8.24E-08	2.4
H4H18446P2	163 ± 2.8	23	1.93E+04	1.12E-03	5.83E-08	10
H4H18449P2	175 ± 0.5	6	IC*	IC*	IC*	IC*
H4H18482P2	163 ± 0.8	63	1.01E+05	6.74E-03	6.66E-08	1.7
H4H18487P2	189 ± 0.5	59	7.37E+04	6.79E-03	9.21E-08	1.7
H4H18492P2	165 ± 2.4	52	1.10E+05	1.20E-02	1.10E-07	1.0
H4H17319P2	213 ± 0.5	83	4.00E+04	4.63E-04	1.16E-08	25
H4H17321P2	236 ± 0.4	75	3.26E+04	1.33E-04	4.07E-09	87
Isotype Control mAb	171 ± 0.4	0	NB*	NB*	NB*	NB*

*NB indicates that no binding was observed under the current experimental conditions.

*IC indicates that observed binding was inclusive and was unable to fit the real time binding data under the current experimental conditions.

Table 6: Binding kinetics parameters of mfLEPR.MMH binding to LEPR monoclonal antibodies at 37°C.

mAb Captured	mAb Capture Level (RU)	100nM mfLEPR .MMH Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H16650P2	204 ± 1.7	134	1.22E+05	7.00E-04	5.76E-09	16
H4H16679P2	232 ± 1.1	104	6.49E+04	6.77E-04	1.04E-08	17

mAb Captured	mAb Capture Level (RU)	100nM mfLEPR .MMH Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H18417P2	202 ± 1.3	28	1.22E+05	2.63E-02	2.17E-07	0.4
H4H18438P2	203 ± 1.3	7	IC*	IC*	IC*	IC*
H4H18445P2	232 ± 0.9	48	7.17E+04	1.90E-02	2.64E-07	0.6
H4H18446P2	188 ± 2.9	30	2.53E+04	3.54E-03	1.40E-07	3.3
H4H18449P2	202 ± 1	6	IC*	IC*	IC*	IC*
H4H18482P2	187 ± 1.2	52	1.52E+05	2.04E-02	1.34E-07	0.6
H4H18487P2	216 ± 0.7	44	1.10E+05	1.95E-02	1.78E-07	0.6
H4H18492P2	191 ± 1.4	34	2.34E+05	3.94E-02	1.69E-07	0.3
H4H17319P2	274 ± 0.5	113	5.39E+04	3.24E-03	6.01E-08	3.6
H4H17321P2	304 ± 0.7	143	4.97E+04	2.57E-04	5.18E-09	45
Isotype Control mAb	190 ± 1	1	NB*	NB*	NB*	NB*

*NB indicates that no binding was observed under the current experimental conditions.

*IC indicates that observed binding was inclusive and was unable to fit the real time binding data under the current experimental conditions.

Table 7: Binding kinetics parameters of hLEPR.mFc binding to LEPR monoclonal antibodies at 25°C.

mAb Captured	mAb Capture Level (RU)	100nM hLEPR-mFc Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H16650P2	165 ± 0.2	102	1.06E+05	8.32E-05	7.85E-10	139
H4H16679P2	190 ± 1.2	78	5.84E+04	9.68E-05	1.66E-09	119
H4H18417P2	162 ± 0.6	90	1.40E+05	5.63E-04	4.04E-09	21
H4H18438P2	165 ± 1.2	51	5.19E+04	2.44E-04	4.70E-09	47
H4H18445P2	192 ± 0.4	76	1.22E+05	4.92E-04	4.03E-09	23
H4H18446P2	162 ± 2.8	20	3.20E+04	2.08E-04	6.48E-09	56

mAb Captured	mAb Capture Level (RU)	100nM hLEPR-mFc Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H18449P2	174 ± 0.6	116	7.05E+04	6.82E-05	9.64E-10	169
H4H18482P2	162 ± 0.5	88	1.44E+05	4.91E-04	3.42E-09	24
H4H18487P2	188 ± 0.6	85	1.06E+05	6.03E-04	5.70E-09	19
H4H18492P2	166 ± 3.2	129	2.27E+05	1.39E-04	6.13E-10	83
H4H17319P2	200 ± 0.5	69	4.77E+04	1.64E-04	3.45E-09	70
H4H17321P2	221 ± 0.4	65	4.10E+04	8.93E-05	2.18E-09	129
Isotype Control mAb	170 ± 0.7	-2	NB*	NB*	NB*	NB*

*NB indicates that no binding was observed under the current experimental conditions.

Table 8: Binding kinetics parameters of hLEPR.mFc binding to LEPR monoclonal antibodies at 37°C.

mAb Captured	mAb Capture Level (RU)	100nM hLEPR-mFc Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H16650P2	199 ± 1.9	145	1.57E+05	2.80E-04	1.79E-09	41
H4H16679P2	229 ± 2.3	116	1.21E+05	3.10E-04	2.56E-09	37
H4H18417P2	199 ± 1.1	111	1.85E+05	1.05E-03	5.64E-09	11
H4H18438P2	199 ± 0.6	82	7.02E+04	5.98E-04	8.53E-09	19
H4H18445P2	229 ± 2	104	1.56E+05	6.08E-04	3.89E-09	19
H4H18446P2	186 ± 2.5	34	4.27E+04	5.48E-04	1.28E-08	21
H4H18449P2	198 ± 1.6	148	1.33E+05	1.68E-04	1.26E-09	69
H4H18482P2	185 ± 1.3	109	1.89E+05	7.26E-04	3.84E-09	16
H4H18487P2	215 ± 1.5	99	1.23E+05	6.06E-04	4.93E-09	19
H4H18492P2	189 ± 1.8	160	4.33E+05	5.00E-04	1.16E-09	23
H4H17319P2	262 ± 0.5	100	8.51E+04	6.52E-04	7.66E-09	18

mAb Captured	mAb Capture Level (RU)	100nM hLEPR-mFc Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H17321P2	289 ± 0.4	110	5.53E+04	1.74E-04	3.15E-09	66
Isotype Control mAb	188 ± 0.8	1	NB*	NB*	NB*	NB*

*NB indicates that no binding was observed under the current experimental conditions.

[0128] At 25°C, anti-LEPR monoclonal antibodies bound to hLEPR-MMH with K_D values ranging from 7.93nM to 148nM, as shown in Table 5. At 37°C, anti-LEPR monoclonal antibodies bound to hLEPR-MMH with K_D values ranging from 14.8nM to 326nM, as shown in Table 4.

[0129] Ten out of 12 anti-LEPR monoclonal antibodies of the invention bound to mfLEPR.MMH. At 25°C, anti-LEPR monoclonal antibodies bound to mfLEPR.MMH with K_D values ranging from 2.27nM to 139nM, as shown in Table 7. At 37°C, anti-LEPR monoclonal antibodies bound to mfLEPR.MMH with K_D values ranging from 5.18nM to 264nM, as shown in Table 8.

[0130] At 25°C, anti-LEPR monoclonal antibodies bound to hLEPR-mFc with K_D values ranging from 613pM to 5.7nM, as shown in Table 7. At 37°C, anti-LEPR monoclonal antibodies bound to hLEPR-mFc with K_D values ranging from 1.16nM to 12.8nM, as shown in Table 8.

[0131] None of the anti-LEPR monoclonal antibodies of the invention bound to mLEPR.MMH or rLEPR.MMH at 25°C or at 37°C (data not shown).

Example 4. Anti-LEPR Antibodies of the Invention Bind LEPR in the Presence of Leptin:LEPR Binding

[0132] Blocking of anti-LEPR antibodies from binding to LEPR by human Leptin was evaluated using a real-time surface plasmon resonance biosensor on a Biacore T200 instrument. The entire study was performed in 10mM HEPES pH 7.4, 150mM NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20 (HBS-ET running buffer) at 25°C. The Biacore CM5 sensor surface was first derivatized by amine coupling human Leptin (R&D Systems, # 398-LP) using standard EDC/NHS surface chemistry. A complex of human LEPR and human Leptin, was formed by injecting 20nM of human LEPR extracellular domain expressed with a C-terminal myc-myc-hexahistidine tag (hLEPR-MMH; SEQ ID NO: xx), over the human Leptin immobilized Biacore sensor surface at a flow rate of 10µL/minute or 25µL/minute for 4 minutes, to achieve a binding response of approximately 200RU. To evaluate whether antibody binding to hLEPR-MMH is blocked by human Leptin, 200nM of anti-LEPR monoclonal antibodies were injected over the preformed hLEPR-MMH:human Leptin complex, at a flow rate of 50µL/minute or 25µL/minute for 4 – 5 minutes. All the anti-LEPR antibodies of this invention bound to the complex of hLEPR-MMH and human Leptin ("Leptin:LEPR") with nearly similar signal strength and the observed

binding, expressed in RUs, are reported in Table 9. This result indicates that human Leptin does not block the binding of hLEPR-MMH to the anti-LEPR antibodies tested.

Table 9: Binding of anti-LEPR monoclonal antibodies to the pre-complex of hLEPR-MMH and human Leptin.

Antibody	hLEPR-MMH Bound (RU)	200nM mAb Bound (RU)
H4H16650P2	196	81
H4H16679P2	195	90
H4H17319P2	196	92

Example 5. Human Leptin receptor blocking ELISA

[0133] For the ELISA, human Leptin (hLeptin; R&D Systems, # 398-LP-01M) was coated at a concentration of 5 μg/mL in PBS on a 96-well microtiter plate overnight at 4°C. Nonspecific binding sites were subsequently blocked using a 0.5% (w/v) solution of BSA in PBS. A constant amount of 10nM of extracellular domain portion of LEPR protein that was expressed with a C-terminal human Fc tag (hLEPR.hFc; SEQ ID NO: 116) was titrated with anti-LEPR antibodies, hLeptin protein, or an isotype control antibody ranging from 8.5pM to 500nM in serial dilution. These antibody-protein or protein-protein complexes were then incubated for 1.5 hour at room temperature (RT). Complexes were subsequently transferred to microtiter plates coated with hLeptin and incubated for 2 hours at RT, the wells were washed, and plate-bound hLEPR.hFc was detected with an anti-human IgG polyclonal antibody conjugated with horseradish peroxidase (Jackson ImmunoResearch Inc, #109-035-098). Samples were developed with a TMB solution (BD Biosciences, #555214; substrate A and B mixed at 1:1 ratio as per manufacturer's instructions) to produce a colorimetric reaction and then neutralized with 1M sulfuric acid before measuring absorbance at 450nm on a Victor X5 plate reader.

[0134] Data analysis was performed using a sigmoidal dose-response model within Prism™ software (GraphPad). Percent blockade at maximum concentration of the antibody tested was calculated as an indicator of the ability of the antibodies to block the binding of 10nM of hLEPR.hFc to human Leptin on the plate. In the calculation, binding signal of 10nM of hLEPR.hFc without the presence of the antibody was referenced as 100% binding or 0% blocking; and the baseline signal of buffer alone without the presence of hLEPR.hFc was referenced as 0% binding or 100% blocking The blocking data at 500nM antibody concentration is summarized in Table 10.

[0135] As shown in Table 10, none of the anti-LEPR antibodies of the invention demonstrated >28% blocking of the binding of hLEPR.hFc to the hLeptin coated surface. However, the Comparator Antibody and the hLeptin, as the positive control, were able to block 99% of the hLEPR.hFc binding to the hLeptin coated surface. The isotype control antibody demonstrated no measurable blocking at concentrations up to 500nM.

Table 10: ELISA blocking of hLEPR.hFc binding to hLeptin by anti-LEPR antibodies

Antibody	500nM Ab Blocking of 10nM hLEPR.hFc Binding to hLeptin (% blockade)
H4H18487P2	5
H4H18417P2	16
H4H18482P2	25
H4H18492P2	-3
H4H18445P2	28
H4H18446P2	-5
H4H18449P2	8
H4H18438P2	15
H4H16650P2	-7
H4H16679P2	7
H4H173319P2	9
H4H173321P2	6
Controls	
Isotype control antibody	-3
Human Leptin	99
Comparator Antibody	99
Mouse IgG2a Isotype control	32

Example 6. Cell binding by FACS analysis with HEK293/Mycx2-hLepR(ecto)-GPI anchored cells

[0136] Leptin receptor, LEPR, is a single-pass transmembrane receptor of the class I cytokine receptor family (Tartaglia *et al.* (1997) *J Biol Chem* 7:272(10):6093-6). LEPR can bind to Leptin, a protein predominantly expressed by adipose tissue that is involved in regulation of food intake and metabolism (Friedman *et al.* (2014) *J Endocrinol* 223(1):T1-8).

[0137] In order to assess cell binding by anti-LEPR antibodies HEK293 stable cell lines were

generated. One cell line, known hereafter as HEK293/hLEPR-GPI, stably expressed the extracellular domain of human LEPR (amino acids 22-839 of accession # P48357 (SEQ ID NO:113), Isoform B) with an N-terminal myc-myc tag and C-terminal peptide sequence from human carboxypeptidase M that guides the addition of GPI (Glycosylphosphatidylinositol) (Deddish *et al.* (1990) *J. Biological Chemistry* 265:25:15083-89) such that the protein can be GPI-anchored to the membrane. Another HEK293 cell line was generated to stably express the full length human LEPR (amino acids 1-1165 of accession # P48357(SEQ ID NO:113), Isoform B) along with a luciferase reporter (Stat3-luciferase, Stat3-luc, SA Bioscience, #CLS-6028L), and is known hereafter as HEK293/Stat3-luc/hLEPR-FL. HEK293 cells with the Stat3-luciferase reporter only (HEK293/Stat3-luc) were also generated as a control cell line.

[0138] For the FACS analysis, HEK293 parental cells and HEK293/hLEPR-GPI cells were dissociated and plated onto 96-well v-bottom plates at 5 x 10^5 cells/well in PBS containing 2% FBS (FACS buffer). In order to test whether the ability of anti-hLEPR antibodies to bind to cells is affected by the presence of Leptin, FACS buffer with or without 1µM human Leptin (R&D Systems, # 398-LP) was incubated with the cells for 30 minutes at 4°C, followed by the addition of anti-LEPR antibodies or control antibodies at 10nM in FACS buffer. The cells were subsequently incubated for 30 minutes at 4°C, followed by washing and then incubation with 16µg/mL of Alexa Fluor®-647 conjugated secondary antibody (Jackson ImmunoResearch Laboratories Inc., # 109-547-003) for 30 minutes at 4°C. Cells were subsequently fixed using BD CytoFix™ (Becton Dickinson, # 554655), filtered, and analyzed on a HyperCyt Flow Cytometer (Beckman Coulter). Unstained and secondary antibody alone controls were also tested for all cell lines. The results were analyzed using ForeCyt (IntelliCyt) and FlowJo version 10 software to determine the geometric means of fluorescence for viable cells. The geometric mean of fluorescence for each sample was then normalized to the geometric mean of unstained cells to obtain relative binding per condition referred to as "binding ratios", and these binding ratios were recorded for each antibody tested.

[0139] As shown in Table 11, 9 anti-LEPR antibodies of the invention tested at 10nM demonstrated binding to HEK293/hLEPR-GPI cells with binding ratios ranging from 824 to 3374 fold without Leptin. The anti-LEPR antibodies also bound in the presence of 1µM Leptin with binding ratios of 398 and 4184 fold. As shown in Table 11, he Comparator Antibody tested at 10nM demonstrated binding to HEK293/hLEPR-GPI cells with a binding ratio of 2349-fold without Leptin but showed significantly less binding to cells in the presence of 1µM Leptin with binding ratio of 112. The anti-LEPR antibodies did not demonstrate any significant binding to the HEK293 parental cells with binding ratios with and without 1µM Leptin ranging from 1 to 9 fold. The isotype control antibodies and secondary antibodies alone samples also did not demonstrate significant binding to either cell line with or without Leptin, with binding ratios ranging from 1 to 6 fold.

[0140] As shown in Table 12, four antibodies of the invention tested at 70nM without Leptin, demonstrated binding to HEK293/hLEPR-GPI cells with binding ratios ranging from 707 to 1131 fold and to HEK293/Stat3-luc/hLEPR-FL cells with binding ratios ranging from 42 to 51. The anti-LEPR antibodies did not demonstrate any significant binding to the HEK293/Stat3-luc cells with binding ratios ranging from 1 to 8 fold. The isotype control antibodies and secondary antibodies alone samples also did not demonstrate significant binding to any of the cell lines tested, with binding ratios ranging from 1 to 2 fold.

Table 11: Binding of 10nM anti-LEPR antibodies to HEK293/hLEPR-GPI and HEK293 parental cells +/- 1μM Human Leptin

	Binding Ratio: Normalized to Unstained Sample of Each Cell Line				
	No added Leptin		1μM Leptin		Antibody Type
Antibody	HEK293 parental	HEK293/ hLEPR-GPI	HEK293 parental	HEK293/ hLEPR-GPI	
H4H16650P2	5	2420	4	3124	Agonist
H4H16679P2	5	2058	8	2223	Agonist
H4H18417P2	1	1835	2	2604	Potentiator
H4H18438P2	2	1486	3	2414	Potentiator
H4H18445P2	2	2016	3	2488	Potentiator
H4H18449P2	5	3374	9	3113	Potentiator
H4H18482P2	1	1966	3	2704	Potentiator
H4H18487P2	1	2422	3	2670	Potentiator
H4H18492P2	3	2603	7	4184	Potentiator
Comparitor	6	2349	3	112	N/A
Isotype control antibody	1	6	2	4	N/A
Secondary antibody alone	1	3	2	3	N/A
Unstained	1	1	1	1	N/A

*Classification of antibodies as “Agonist” or “Potentiator” is based in part on the results observed in Examples 7 and 8 herein.

Table 12: Binding of 70nM anti-LEPR antibodies to HEK293/hLEPR-GPI, HEK293/Stat3-hLEPR-FL, and HEK293/Stat3-luc parental cells

	Binding Ratio: Normalized to Unstained Sample of Each Cell Line			Antibody Type
Antibody	**HEK293/ Stat3-luc**	**HEK293/ hLEPR-GPI**	**HEK293/Stat3-luc hLEPR-FL**	
H4H16650P2	6	707	42	Agonist
H4H16679P2	8	1078	51	Agonist
H4H17319P2	7	1131	47	Agonist
H4H17321P2	7	1126	46	Agonist
Isotype control antibody	2	2	2	
Secondary antibody alone	1	1	1	
Unstained	1	1	1	

Example 7. Anti-LEPR Antibodies of the Invention Activate LEPR Signaling in the Presence or Absence of Leptin

[0141] A bioassay was developed to detect the transcriptional activation of STAT3 via LEPR activation using a reporter cell line that stably expresses full-length human LEPR (hLEPR; amino acids 1 through 1165 of accession number NP_002294.2) along with a luciferase reporter (STAT3-Luc; Qiagen, # CLS-6028L) in an IMR-32 cell line, a human neuroblastoma cell line. The resulting stable cell line, referred to as IMR-32/STAT3-Luc/hLEPR, was isolated and maintained in MEM-Earl medium supplemented with 10% FBS, NEAA, 1ug/mL Puromycin, 100ug/mL of Hygromycin B and Penicillin/Streptomycin/L-Glutamine (Complete Medium).

[0142] The resulting bioassay was used to measure the effect of anti-LEPR antibodies of the invention on LEPR signaling in the presence or absence of Leptin. For the bioassay, IMR-32/STAT3-Luc/hLEPR cells were plated at the density of 20,000 cells/100ul/well for 96well format in the complete medium, and the following day replaced with the appropriate volume of Opti-MEM medium supplemented with 1% BSA and 0.1% FBS (Assay Buffer) for 30 minutes. To measure the effect of the antibodies of the invention in the absence of Leptin, the anti-LEPR antibodies or an isotype control antibody and human Leptin (hLeptin; R&D Systems, #398-LP) were half-log serially diluted to final concentrations ranging from 100nM to 300fM in Assay Buffer, which were added to the cells and subsequently incubated overnight at 37°C in 5% CO2.

[0143] To measure the effect of the antibodies of the invention in the presence of Leptin, a fixed concentration of human Leptin at 200pM in Assay Buffer was added to the cells, immediately

followed by the addition of anti-LEPR antibodies or isotype control antibody that were half-log serially diluted to final concentrations ranging from 100nM to 300fM. The samples were then incubated overnight at 37°C in 5% CO2. OneGlo reagent (Promega, # E6051) was then added to the samples and luciferase activity was measured on an Envision Multilable Plate Reader (Perkin Elmer) in Luminescent mode. The relative light unit (RLU) values were obtained and the results were analyzed using nonlinear regression with GraphPad Prism software (GraphPad). The maximum RLU value obtained from the hLeptin dose response was defined as 100% activation in the IMR-32/STAT3-Luc/hLEPR assay.

[0144] As shown in Table 13, in Study 1, in the absence of hLeptin, all of the anti-LEPR antibodies tested demonstrated weak stimulation of the IMR-32/STAT3-Luc/hLEPR cells with EC_{50} values ranging from 134pM to 11.9nM and maximal activation ranging from 5% to 13% respectively that of maximum activation obtained from the hLeptin dose response. In Study 2, in the absence of hLeptin, the 4 anti-LEPR antibodies tested demonstrated stimulation of the IMR-32/STAT3-Luc/hLEPR cells with EC_{50} values ranging from 61.9pM to 206.9pM and maximal activation ranging from 65% to 68% respective to the maximum activation obtained from the hLeptin dose response. In Study 1, in the presence of 200pM of hLeptin, all of the anti-LEPR antibodies tested demonstrated stimulation of the IMR-32/STAT3-Luc/hLEPR cells with EC_{50} values ranging from 20.2pM to 523pM and maximal activation ranging from 66% to 107% respectively that of maximum activation obtained from the hLeptin dose response. Because these antibodies enhanced leptin-induced LEPR signaling, these antibodies were classified as "potentiators", as defined herein. In Study 2, in the presence of 200pM of hLeptin, the 4 anti-LEPR antibodies tested demonstrated stimulation of the IMR-32/STAT3-Luc/hLEPR cells with EC_{50} values ranging from 51.9pM to 257.3pM with maximal activation ranging from 76% to 88% that of maximum activation obtained from the hLeptin dose response. LEPR signaling was not appreciably enhanced by these antibodies in the presence of leptin. The isotype control antibody did not demonstrate any measurable stimulation of the IMR-32/STAT3-Luc/hLEPR cells in any of the assays.

Table 13: Activation of hLEPR by anti-LEPR Antibodies

	IMR-32/LEPR without human Leptin		**IMR-32/LEPR with 200pM human Leptin**	
Antibody	**EC_{50} (M)**	**% activation**	**EC_{50} (M)**	**% activation**
Study 1				
H4H18445P2	1.19E-08	5	4.10E-10	97
H4H18446P2	3.73E-10	6	3.42E-11	68

H4H18449P2	2.12E-10	13	5.23E-11	66
H4H18438P2	1.49E-09	5	2.02E-11	76
H4H18482P2	2.69E-10	7	1.69E-10	94
H4H18487P2	8.01E-10	6	4.10E-10	107
H4H18492P2	1.34E-10	5	2.74E-11	94
H4H18417P2	1.53E-10	5	5.23E-10	87
Study 2				
H4H16650P2	6.19E-11	68	5.19E-11	88
H4H16679P2	8.62E-11	65	7.37E-11	88
H4H17319P2	1.867E-10	68	1.914E-10	76
H4H17321P2	2.069E-10	66	2.573E-10	76

Example 8. Anti-LEPR Antibodies of the Invention Activate Signaling in Cells Expressing Signaling-Defective or Signaling-Impaired LEPR Mutants

[0145] LEPR mutants have been identified that exhibit defective or impaired leptin-mediated signaling and are associated with early-onset obesity. For example, LEPR-A409E is a signaling-defective mutant LEPR protein that does not transduce leptin signals to STAT3; the A409E mutant was originally identified as a monogenic cause of early onset obesity. (Farooqi *et al.*, 2007, *N Engl J Med* 356(3): 237-247). LEPR-P316T is a signaling-impaired mutant LEPR protein that has also been shown to be associated with early-onset obesity. (Mazen *et al.*, 2011, *Mol Genet Metab* 102:461-464).

[0146] In this Example, the ability of anti-LEPR antibodies of the invention to stimulate LEPR signaling in cell lines expressing signaling-defective or signaling-impaired LEPR mutants was assessed. In particular, reporter cell lines (HEK293) were constructed expressing either wild-type LEPR, LEPR-A409E (signaling-defective) or LEPR-P316T (signaling-impaired). Cells were treated with either vehicle only, recombinant human leptin, control IgG, or agonist anti-LEPR antibodies of the present invention (H4H16650 or H4H16679), and the extent of LEPR signaling (as measured by Western blot detection of pSTAT3-Y705 expression relative to STAT3 expression) was determined.

[0147] The agonist anti-LEPR antibodies of the present invention (H4H16650 and H4H16679) were shown in these experiments to stimulate LEPR signaling in cells expressing the LEPR-A409E mutant or the LEPR-P316T mutant (as measured by STAT3 expression) in a dose-dependent manner (Figure 2, panels B and C). By contrast, leptin treatment induced only modest signaling in cells expressing the LEPR-P316T mutant, and no signaling in cells expressing the LEPR-A409E mutant. (Figure 2, panel A). Moreover, no LEPR signaling was detected in any of the cell lines treated with vehicle or IgG control antibody (data not shown).

Other signaling-defective or signaling-impaired LEPR mutants were tested in this assay but were not activated by anti-LEPR mutants (data not shown), suggesting that this rescue effect may be mutant-dependent.

[0148] The results of this Example indicate that the agonist anti-LEPR antibodies of the present invention may be useful in the treatment of diseases and disorders (*e.g.*, early-onset obesity) that are caused by or associated with certain signaling-defective or signaling-impaired LEPR mutants (*e.g.*, LEPR-P316T or LEPR-A409E).

Example 9: Octet cross-competition between different anti-LEPR monoclonal antibodies.

[0149] Binding competition between a panel of different anti-LEPR monoclonal antibodies was determined using a real time, label-free bio-layer interferometry assay on the Octet HTX biosensor platform (Pall ForteBio Corp.). The entire experiment was performed at 25°C in buffer containing 10mM HEPES, 150mM NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20, 1mg/mL BSA, pH7.4 (HBS-EBT) with the plate shaking at the speed of 1000rpm. To assess whether two antibodies were able to compete with one another for binding to their respective epitopes on recombinant human LEPR expressed with a C-terminal myc-myc-hexahistidine tag (hLEPR.mmh; SEQ ID NO: 114), around 0.25nm or 0.34nm of hLEPR-MMH was first captured onto anti-penta-His antibody coated Octet biosensor tips (Fortebio Inc, # 18-5122) by submerging the biosensor tips for 5 minutes in wells containing 20µg/mL of hLEPR-MMH. The antigen captured biosensor tips were then saturated with a first anti-LEPR monoclonal antibody (subsequently referred to as mAb-1) by dipping into wells containing 50µg/mL solution of mAb-1 for 210 seconds. The biosensor tips were then subsequently dipped into wells containing a 50µg/mL solution of a second anti-LEPR monoclonal antibody (subsequently referred to as mAb-2) for 150 seconds. The biosensor tips were washed in HBS-EBT buffer in between every step of the experiment. The real-time binding response was monitored during the entire course of the experiment and the binding response at the end of every step was recorded. The response of mAb-2 binding to hLEPR-MMH pre-complexed with mAb-1 was compared and competitive/non-competitive behavior of different anti-LEPR monoclonal antibodies was determined as shown in Table 14 and Table 15.

Table 14: Cross-competition between anti-LEPR monoclonal antibodies

First antibody (mAb-1) binding to captured hLEPR-MMH	Second antibody (mAb-2) shown to compete with mAb-1
H4H18492P2	H4H18417P2
	H4H18438P2
H4H18417P2	H4H18492P2

First antibody (mAb-1) binding to captured hLEPR-MMH	Second antibody (mAb-2) shown to compete with mAb-1
	H4H18438P2
H4H18438P2	H4H18492P2
	H4H18417P2
H4H16650P2	H4H16679P2
H4H16679P2	H4H16650P2
H4H18445P2	H4H18482P2
	H4H18487P2
	H4H18446P2
H4H18446P2	H4H18482P2
	H4H18487P2
	H4H18445P2
H4H18482P2	H4H18445P2
	H4H18487P2
H4H18487P2	H4H18445P2
	H4H18482P2
H4H18449P2	None
Comparator Antibody	None

Table 15: Cross-competition between anti-LEPR monoclonal antibodies

mAb-1	mAb-2 that competes with mAb-1
H4H17319P2	H4H17321P2
	H4H16650P2
	H4H16679P2
H4H17321P2	H4H17319P2

mAb-1	mAb-2 that competes with mAb-1
	H4H16650P2
	H4H16679P2
H4H16650P2	H4H17319P2
	H4H17321P2
	H4H16679P2
H4H16679P2	H4H17319P2
	H4H17321P2
	H4H16650P2

Example 10: In vivo efficacy of LEPR agonist antibodies H4H16650P2, H4H16679P2, H4H17319P2 and H4H17321P2 in an inducible mouse model of Leptin deficiency.

[0150] The effects of four specific agonist anti-LEPR antibodies of the invention, H4H16650P2, H4H16679P2, H4H17319P2, and H4H17321P2 on food intake, body weight and adiposity were determined in an inducible model of leptin deficiency in genetically engineered *LEPR*$^{Hu/Hu}$ mice, that express a leptin receptor which is composed of the human LEPR ectodomain sequence in place of the murine LEPR ectodomain sequence. The model of leptin deficiency was induced by hydrodynamic DNA delivery (HDD) of a plasmid encoding an hFc-tagged mouse LEPR ectodomain (referred to herein as mLEPR.hFc or "Leptin trap"; SEQ ID NO: 120). The Leptin trap when expressed is secreted and binds circulating Leptin. After HDD of 50 μg of the DNA construct encoding the Leptin trap, mice exhibited increased food consumption and increased adiposity and body weight.

[0151] Baseline daily food intake was measured between 7 and 4 days prior to administration of the Leptin trap (days -7 and -4). On day 0, thirty-five 13- to 17- week old male *LEPR*$^{Hu/Hu}$ mice were successfully subjected to HDD with the Leptin trap. On days 6 and 13 post HDD, retro-orbital bleeds were collected and body composition including adiposity was quantified by μCT. On day 7 post HDD, mice were randomized into five groups of 7 mice based on percent body weight change from day 0. Each group received via subcutaneous injection either a single dose of isotype control antibody at 3 mg/kg, H4H16650P2 at 3 mg/kg, H4H16679P2 at 3 mg/kg, H4H17319P2 at 3 mg/kg, or H4H17321 at 3 mg/kg. The isotype control antibody did not bind any known mouse protein. Food intake and body weight were measured for each animal for the duration of the study. Figure 3 summarizes the average daily food intake for each treatment

group. In Figure 3, the dotted line represents the average baseline food intake prior to HDD injection. The percent change in body weight from day 0 was calculated for each animal at each time point. Figure 4 summarizes the average percent change in body weight for animals in each antibody treatment group. Figure 5 summarizes the average fat mass for animals in each antibody treatment group quantified by μCT 1 day prior to and 6 days following antibody treatment. All results are expressed as mean ± SEM.

[0152] As shown in Figures 3 and 4, following HDD with the Leptin trap, similar increases in food intake and percent change in body weight were observed among the groups of mice before antibody treatment. As shown in Figure 3, mice treated with antibodies H4H16650P2 or H4H16679P2 at 3 mg/kg exhibited significant reductions in food intake starting at one day after antibody treatment (day 8 post HDD) and at subsequent time points measured as compared to mice injected with the isotype control antibody. Mice treated with antibodies H4H17319P2 or H4H17321P2 at 3 mg/kg exhibited a significant reduction in food intake at two days post antibody treatment (day 9 post HDD) and at the other subsequent time points measured as compared to mice injected with isotype control antibody. As shown in Figure 4, mice treated with antibody H4H16650P2 at 3 mg/kg exhibited a significant reduction in percent body weight change one day after antibody treatment (day 8 post HDD) and at other subsequent time points measured as compared to mice injected with isotype control antibody. One day after antibody treatment, on day 8, mice treated with the isotype control showed a 21.16 ± 1.27% increase in body weight from day 0, whereas mice treated with H4H16650P2 had a 15.57 ± 0.9% increase in body weight from day 0. Mice treated with antibodies H4H16679P2, H4H17319P2 or H4H17321P2 at 3 mg/kg exhibited a significant reduction in percent body weight change two days after antibody treatment (day 9 post HDD) and at other subsequent time points measured as compared to mice injected with isotype control antibody. On day 9, the % body weight changes from day 0 were 23.18 ± 1.22, 13.17 ± 1.05, 12.95 ± 1.26, 15.98 ± 1.78 and 15.83 ± 2.01 for mice treated with isotype control, H4H16650P2, H4H16679P2, H4H17319P2, or H4H17321P2, respectively. As shown in Figure 5, mice treated with isotype control antibody at 3 mg/kg demonstrated a significant increase in fat mass 6 days after antibody treatment (day 13 post HDD) as compared to 1 day prior to antibody treatment (day 6 post HDD). Mice treated with antibodies H4H16650P2, H4H16679P2, H4H17319P2, or H4H17321P2 at 3 mg/kg did not gain adipose mass after antibody treatment as compared to pre-antibody treatment. After 6 days of treatment (day 13 post HDD), mice treated with antibodies H4H16650P2, H4H16679P2 or H4H17319P2 at 3 mg/kg demonstrated significant decreases in fat mass as compared to mice treated with isotype control antibody at 3 mg/kg.

Example 11: Epitope mapping of H4H16650P2 binding to human leptin receptor (hLEPR.mmh) by hydrogen deuterium exchange.

[0153] Experiments were conducted to determine the amino acid residues of hLEPR.mmh

(amino acids M1-D839 of SEQ ID NO: 114) with which H4H16650P2 interacts. For this purpose H/D exchange epitope mapping with mass spectrometry was carried out. A general description of the H/D exchange method is set forth in, *e.g.*, Ehring (1999) Analytical Biochemistry 267(2):252-259; and Engen and Smith (2001) Anal. Chem. 73:256A-265A.

[0154] Experimental procedure. HDX-MS experiments were performed on an integrated Waters HDX/MS platform, consisting of a Leaptec HDX PAL system for the deuterium labeling, a Waters Acquity M-Class (Auxiliary solvent manager) for the sample digestion and loading, a Waters Acquity M-Class (μBinary solvent manager) for the analytical column gradient, and Synapt G2-Si mass spectrometer for peptic peptide mass measurement.

[0155] The labeling solution was prepared in 10 mM PBS buffer in D2O at pD 7.0 (equivalent to pH 6.6). For deterium labeling, 3.8 μL of hLEPR.mmh (8 pmol/μL) or hLEPR.mmh premixed with the antibody in 2:1 molar ratio was incubated with 56.2 μL D2O labeling solution for various time-points (e.g., undeuterated control = 0 sec, labeled for 1 min and 20 min). The deuteration was quenched by transferring 50 μL sample to 50 μL pre-chilled quench buffer (0.2 M TCEP, 6 M guanidine chloride in 100 mM phosphate buffer, pH 2.5) and the mixed sample was incubated at 1.0°C for two minutes. The quenched sample was then injected into a Waters HDX Manager for online pepsin/protease XIII digestion. The digested peptides were trapped onto an ACQUITY UPLC BEH C18 1.7-μm, 2.1 × 5 mm VanGuard pre-column at 0°C and eluted to an analytical column ACQUITY UPLC BEH C18 1.7-μm, 1.0 × 50 mm for a 9-minute gradient separation of 5%-40% B (mobile phase A: 0.1% formic acid in water, mobile phase B: 0.1% formic acid in acetonitrile). The mass spectrometer was set at cone voltage of 37 V, scan time of 0.5 s, and mass/charge range of 50-1700 Th.

[0156] For the identification of the peptides from human LEPR, LC-MSE data from undeuterated sample were processed and searched against the database including human LEPR, pepsin, and their randomized sequences via Waters ProteinLynx Global Server (PLGS) software. The identified peptides were imported to DynamX software and filtered by two criteria: 1) minimum products per amino acid: 0.2, and 2) replication file threshold: 3. DynamX software then automatically determined deuterium uptake of each peptide based on retention time and high mass accuracy (<10ppm) across multiple time points with 3 replicates at each time.

[0157] Results. Using the online pepsin/protease XIII column coupled with MS^E data acquisition, total 201 peptides from human LEPR were reproducibly identified in the absence or presence of the antibody, representing 70% sequence coverage. Five peptides had significantly reduced deuteration uptake (centroid delta values > 0.4 daltons with p-values < 0.05) when bound to H4H16650P2 as shown in the Table 16. The recorded peptide mass corresponds to the average value of the centroid MH+ mass from three replicates. These peptides, corresponding to amino acids 162-169 (amino acids LYVLPEVL of human LEPR; SEQ ID NO: 113), and to amino acids 170-181 (amino acids EDSPLVPQKGSF of human LEPR; SEQ ID NO: 113), had a slower deuteration rate when bound to H4H16650P2. These identified residues

also correspond to residues acids 162-169 and 170-181 of human LEPR as defined by Uniprot entry P48357 (SEQ ID NO. 113; Human leptin receptor)

Table 16: Human Leptin receptor peptides with significant protection upon binding to antibody H4H16650P2

Residues	1 min Deuteration			20 min Deuteration		
	hLEPR.mmh	hLEPR.mmh + H4H16650P2	Δ	hLEPR.mmh	hLEPR.mmh + H4H16650P2	Δ
162-169	949.03±0.03	947.99±0.02	-1.04	949.23±0.02	948.16±0.02	-1.03
163-169	835.82±0.03	834.79±0.02	-1.03	836.03±0.02	834.94±0.02	-1.08
170-181	1310.02±0.05	1309.12±0.03	-0.89	1309.77±0.02	1309.38±0.02	-0.39

Example 12: *In vivo* efficacy testing of LEPR potentiator antibodies in humanized LEPR mice.

[0158] The effects of three specific potentiator anti-LEPR antibodies of the invention, H4H18482P2, H4H18487P2 and H4H18492P2, on body weight and adiposity were determined in singly-housed genetically engineered *LEPR*$^{Hu/Hu}$ mice, that express a leptin receptor which is composed of the human LEPR ectodomain sequence in place of the murine LEPR ectodomain sequence (mLEPR.hFc, SEQ ID NO: 120).

[0159] On days -19 body composition including adiposity was quantified by μCT. On days 0, forty-eight 14 to 16-week old female *LEPR*$^{Hu/Hu}$ mice were randomized to four groups of 12 mice based on body weight. On days 0 and 11, mice from each group received via subcutaneous injection a single dose of isotype control antibody at 30 mg/kg, H4H18482P2 at 30 mg/kg, H4H18487P2 at 30 mg/kg or H4H18492P2 at 30 mg/kg. The isotype control antibody does not bind any known mouse protein. Body weight was measured for the duration of the study for each animal. The percent change in body weight from day 0 was calculated for each animal at each time point. Figure 6 summarizes the average percent change in body weight for animals in each treatment group. Figure 6 summarizes the average fat mass for animals in each antibody treatment group quantified by μCT 19 days prior to and 11 days following antibody treatment. All results are expressed as mean ± SEM.

[0160] As shown in Figure 6, decreases in percent change in body weight were observed following dosing with the LEPR potentiator antibodies, but not the isotype control antibody. As shown in Figure 6, mice treated with H4H18482P2 at 30 mg/kg exhibited significant decreases in percent body weight change starting two days after treatment (day 2), and at the other time points compared to mice injected with an isotype control antibody. Mice treated with H4H18487P2 at 30 mg/kg exhibited significant decreases in percent body weight change starting at day 2 and at the other time points compared to mice injected with isotype control

antibody. Mice treated with H4H18492P2 at 30 mg/kg exhibited a significant reduction in percent body weight change on days 4, 5 and 17 but not at other time points compared to mice injected with isotype control antibody. Mice treated with H4H18482P2 at 30 mg/kg exhibited a significant decrease in percent body weight change starting at day 6 and on subsequent days but not days 7, 14 and 17, compared to mice injected with H4H18492P2. Mice treated with H4H18487P2 at 30 mg/kg exhibited a significant decrease in percent body weight change starting at day 3 and at the other time points, but not days 4 and 5, compared to mice injected with H4H18492P2.

[0161] As shown in Figure 7A, there were no differences in fat mass between the groups prior to treatment (day -19). As shown in Figure 7B, mice treated with antibodies H4H18482 and H4H18487, but not H4H18492, at 30 mg/kg showed a statistically significant decrease in fat mass 17 days after treatment (day 12) as compared to the isotype control antibody.

[0162] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Example 13: Effect of anti-LEPR antibodies of the invention on monkey LEPR signaling

[0163] In order to assess transcriptional activation of monkey Leptin receptor, a stable cell line was developed. IMR-32 cells (human Neuroblastoma ATCC) were generated to stably express the extracellular domain of Macaca fascicularis LEPR (MfLEPR; amino acids 22 through 837 of accession number XP_005543194.1 with threonine at 827 changed to alanine) fused with the transmembrane and cytosolic domains of human LEPR (hLEPR; amino acids 840 through 1165 of accession number NP_002294.2) along with a luciferase reporter (STAT3-Luc; SABiosciences, # CLS-6028L). The resulting cell line, referred to hereafter as IMR-32/STAT3-Luc/MfLEPR was isolated and maintained in MEM-Earl medium supplemented with 10% FBS, NEAA, 1ug/mL Puromycin, 100ug/mL of Hygromycin B and Penicillin/Streptomycin/L-Glutamine.

[0164] The bioassay was performed to measure the effect of anti-LEPR antibodies of the invention on monkey LEPR signaling in the absence of Leptin. For the bioassay, IMR-32/STAT3-Luc/MfLEPR cells were plated at 10,000 cells/well in a 96-well plate in 0.1% FBS in Optimem with penicillin/streptomycin (assay buffer) and incubated overnight at 37°C in 5% CO_2. The following day human leptin (hLeptin), anti-LEPR antibodies or an isotype control antibody were serially diluted from 50nM to 0.8pM in the assay buffer (plus a sample containing buffer alone without test molecule) and added to the cells. After 5.5 hours at 37°C in 5% CO_2, luciferase activity was measured with OneGlo™ reagent (Promega, # E6031) and Victor™ X multilabel plate reader (Perkin Elmer). The results were analyzed using nonlinear regression (4-parameter logistics) with Prism™6 software (GraphPad) to obtain EC_{50} values. Percentage of

activation of antibodies was calculated as the maximum range of RLU achieved by the antibody relative to that of maximum range of RLU achieved by hLeptin.

[0165] As shown in Table 17, in the absence of hLeptin, all of the anti-LEPR antibodies tested showed activation of monkey LEPR signaling in IMR-32/STAT3-Luc/mfLEPR cells with EC_{50} values ranging from 266pM to 368pM and maximal activation ranging from 76% to 82% where 100% activation was obtained with hLeptin. hLeptin activated with an EC_{50} value of 333pM. The isotype control antibody did not demonstrate any measurable stimulation of the IMR-32/STAT3-Luc/mfLEPR cells.

Table 17: Activation of Macaca fascicularis LEPR by anti-LEPR antibodies

Leptin or Antibody	EC_{50} (M)	% Activation
Human Leptin	3.33E-11	100
H4H16650P2	2.66E-10	82
H4H16679P2	2.49E-10	80
H4H17319P2	3.65E-10	76
H4H17321P2	3.68E-10	78
Isotype control antibody	No Activation	No Activation

Example 14: Epitope binding to the full-length extracellular domain of human LEPR using Luminex MFI signal

[0166] To determine the epitope of human LEPR on which anti-LEPR antibodies of the invention bind, a Luminex FLEXMAP (FM3DD, LuminexCorp) flow cytometry based analysis was utilized to characterize the interaction of anti-LEPR antibodies with recombinant human LEPR protein domains. For the assay, approximately 3 million carboxylated Microplex^{R} microspheres (Luminex, Cat# LC1000A), were washed, vortexed and sonicated in 0.1 M $NaPO_4$, pH 6.2 (activation buffer) and then centrifuged to remove the supernatant. The microspheres were resuspended in 120 µL of activation buffer and the carboxylate groups (-COOH) were activated by addition of 15 µL of 50 mg/mL of N-hydroxysuccinimide (NHS, Thermo Scientific, Cat# 24500) followed by addition of 15 µL of 50 mg/mL of 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC, ThermoScientific, Cat# 22980) at 25°C. After 10 minutes, the pH of the reaction was reduced to 5.0 with the addition of 600 µL of 50 mM MES, pH 5 (coupling buffer), and the microspheres were vortexed, and centrifuged to remove supernatant. The activated beads were immediately mixed with 500 µL of 20 µg/mL monoclonal

anti-myc antibodies with either a mouse IgG or a human IgG, in coupling buffer and incubated for two hours at 25°C. The coupling reaction was quenched by addition of 50 µL of 1M Tris-HCl, pH 8.0 and the microspheres were rapidly vortexed, centrifuged, and washed four times with 1 mL of DPBS, to remove uncoupled proteins and other reaction components.

[0167] The transiently expressed LEPR proteins, including human LEPR extracellular domain expressed with a C-terminal myc-myc hexahistidine tag (human LEPR-MMH, SEQ ID NO: 113), human LEPR CRH1 (D1) expressed with a C-terminal myc-myc hexahistidine tag (human LEPR CRH1 (D1)-MMH, amino acids 1-208 of SEQ ID NO: 113 with a myc-myc hexahistidine tag, amino acids 209-236), human LEPR CRH1 (D1,D2) domain expressed with a C-terminal myc-myc hexahistidine tag (human LEPR CRH1 (D1,D2)-MMH, amino acids 1-318 of SEQ ID NO: 113 with a myc-myc hexahistidine tag, amino acids 319-346), human LEPR CRH1-Ig (D1,D2,D3) domain expressed with a C-terminal myc-myc hexahistidine tag (human LEPR CRH1 (D1,D2,D3)-MMH, amino acids 1-278 of SEQ ID NO: 113 with a myc-myc hexahistidine tag, amino acids 279-306), human LEPR CRH1-Ig (D2,D3) domain expressed with a C-terminal myc-myc hexahistidine tag (human LEPR CRH1-Ig (D2,D3)-MMH, amino acids 1-198 of SEQ ID NO: 113 with a myc-myc hexahistidine tag, amino acids 199-226), human LEPR Ig (D3) domain expressed with a C-terminal myc-myc hexahistidine tag (human LEPR Ig (D3)-MMH, amino acids 1-88 of SEQ iD NO: 113 with a myc-myc hexahistidine tag, amino acids 89-116), human LEPR CRH2 domain expressed with a C-terminal myc-myc hexahistidine tag (human LEPR CRH2-MMH, amino acids 1-207 of SEQ ID NO: 113 with a myc-myc-hexahistidine tag, amino acids 208-235), human LEPR FNIII domain expressed with a C-terminal myc-myc hexahistidine tag (human LEPR FNIII-MMH, amino acids 1-204 of SEQ ID NO: 113 with a myc-myc hexahistidine tage, amino acids 205-232), and human LEPR Ig-CRH2-FNIII domain expressed with a C-terminal myc-myc hexahistidine tag (human LEPR Ig-CRH2-FNIII-MMH, amino acids 1-510 of SEQ ID NO: 113 with a myc-myc-hexahistidine tag, amino acids 511-538), were suspended in serum free CHO-S-SFM II Medium (Thermo Fisher, Cat # 31033020) and were then clarified by centrifugation. Aliquots of microspheres with immobilized anti-myc monoclonal antibodies, prepared as described above, were added individually to 1 mL of the each of these protein supernatants. The microspheres were gently mixed, incubated for two hours at 25°C, washed twice with 1 mL of DBPS, centrifuged to remove the supernatant and finally resuspended in 1 mL of DPBS buffer. Forty eight µL of anti-myc IgG coupled microspheres from individual reactions with full length human LEPR and with each of the human LEPR domain proteins were withdrawn and mixed together in 3.6 mL of PBS + 20mg/mL BSA+0.05% sodium azide (blocking buffer).

[0168] From this mixed pool, 75 µL of microspheres were plated per well on a 96 well filter plate (Millipore, Cat. No: MSBVN1250) and mixed with 25 µL of individual anti- human LEPR monoclonal antibodies (0.5 or 5 µg/mL), incubated for two hours at 25°C and then washed twice

with 200 µL of DPBS with 0.05% Tween 20 (washing buffer). To detect and quantify the amounts of bound anti-LEPR antibody levels to individual microspheres, either 100 µL of 2.5 µg/mL R-Phycoerythrin conjugated goat F(ab')2 anti-human kappa (Southern Biotech, Cat# 2063-09) in blocking buffer or 100 µL of 1.25 µg/mL R-Phycoerythrin AffiniPure F(ab')2 Fragment Goat Anti-Mouse IgG, F(ab')2 Fragment Specific (Jackson Immunoresearch, Cat. No: 115-116-072) in blocking buffer, was added and incubated for 30 minutes at 25°C. After 30 minutes, the samples were washed twice with 200 µL of washing buffer and resuspended in 150 µL of wash buffer. The Median Fluorescence intensity (MFI) of the microspheres was measured in a Luminex Analyzer.

Table 18: Luminex MFI signal of anti-LEPR antibodies binding to myc tag captured full-length extracellular domain of human LEPR and isonalted human LEPR domains

Antibody	CRH1 (D1)	CRH1 (D1,D2)	CRH1-Ig (D1,D2,D3)	CRH1-Ig (D2,D3)	Ig (D3)	CRH 2	FNIII	Ig-CRH2-FNIII	Full Length extracellular domain	Probable Binding site
H4H18445 P2	12	30	22	40	19	17	230	14544	6573	FNIII
H4H18446 P2	17	682	205	645	25	65	32	16852	10536	Ig-CRH2-FNIII
H4H18482 P2	13	40	21	52	27	23	167	15316	7311	Ig-CRH2-FNIII
H4H18487 P2	12	51	29	62	22	27	174	16320	7329	Ig-CRH2-FNIII
H4H18417 P2	10	16048	3334	5502	17	39	14	37	4887	CRH1 D2
H4H18438 P2	13	18931	6572	8884	30	165	25	468	6251	CRH1 D2
H4H18492 P2	11	19371	6354	8685	19	18	16	186	6382	CRH1 D2
H4H18449 P2	20	2934	2056	42	24	15	13	43	7976	CRH1(D1-2)
H4H16650 P2	8	4722	2562	74	10	16	6	110	7603	CRH1(D1-2)
H4H16679 P2	12	4388	2797	34	14	33	10	42	7507	CRH1(D1-2)
H4H17319 P2	8	1246	938	14	8	91	20	8	3305	CRH1(D1-2)
H4H17321 P2	9	2649	1752	15	7	116	40	14	4696	CRH1(D1-2)
Comparator mAb	-14	19	-57	27	10	9404	73	7112	3908	CRH2

[0169] The results of the Luminex based analysis are tabulated in Table 18. Luminex MFI signal intensities indicate that the twelve anti-LEPR antibodies of the invention bound to the complete human LEPR extracellular domain. Anti-LEPR antibodies H4H18417P2, H4H18438P2, and H4H18492P2, bound to epitopes within the CRH1 D2 domain of human LEPR. Anti-LEPR antibodies H4H18449P2, H4H16650P and H4H16679P, bound to epitopes within the CRH1(D1-2) domain of human LEPR. Anti-LEPR antibody Comparator mAB, bound to epitopes within the CRH2 domain of human LEPR. Anti-LEPR antibody H4H18445P2 bound to epitopes within the FNIII domain of human LEPR. Anti-LEPR antibodies H4H18446P2, H4H18482P2 and H4H18487P2, bound to epitopes within the Ig-CRH2-FNIII domain of human LEPR.

Claims

What is claimed is:

1. An isolated antibody or antigen-binding fragment thereof that binds human leptin receptor (LEPR) and activates LEPR signaling.

2. The isolated antibody or antigen-binding fragment thereof of claim 1, wherein the antibody or antigen-binding fragment thereof exhibits one or more properties selected from the group consisting of:

(i) binds monomeric human LEPR at 25ºC with a K_D of less than about 150 nM as measured by surface plasmon resonance;

(ii) binds monomeric human LEPR at 25ºC with a $t_{½}$ of greater than about 1 minute as measured by surface plasmon resonance;

(iii) binds dimeric human LEPR at 25ºC with a K_D of less than about 5 nM as measured by surface plasmon resonance;

(iv) binds dimeric human LEPR at 25ºC with a $t_{½}$ of greater than about 15 minutes as measured by surface plasmon resonance;

(v) binds human LEPR in complex with human leptin;

(vi) does not block the LEPR:leptin interaction;

(vii) binds cell surface-expressed LEPR in the presence and absence of human leptin;

(viii) activates LEPR signaling with an EC_{50} of less than about 90 pM in a cell-based reporter assay; and

(ix) potentiates LEPR intracellular signaling when LEPR is bound to leptin.

3. The isolated antibody or antigen-binding fragment thereof of claim 1 or 2, wherein the antibody or antigen-binding fragment thereof activates LEPR signaling in a cell-based reporter assay at least 50% as effectively as leptin.

4. The isolated antibody or antigen-binding fragment thereof of claim 3, wherein the antibody or antigen-binding fragment thereof activates LEPR signaling in a cell-based reporter assay at least 70% as effectively as leptin.

5. An isolated antibody or antigen-binding fragment thereof that binds human leptin receptor (LEPR), wherein the antibody or antigen-binding fragment thereof comprises: (a) a

heavy chain variable region (HCVR) comprising the complementarity determining regions (CDRs) of a HCVR comprising the amino acid sequence of SEQ ID NO: 26, SEQ ID NO: 34, SEQ ID NO:2, SEQ ID NO:18, SEQ ID NO: 42, SEQ ID NO: 50, SEQ ID NO: 58, SEQ ID NO: 66, SEQ ID NO: 74, SEQ ID NO: 82, SEQ ID NO: 98 or SEQ ID NO: 106 and (b) a light chain variable region (LCVR) comprising the CDRs of a LCVR comprising the amino acid sequence of SEQ ID NO:10 or SEQ ID NO: 90.

6. The isolated antibody or antigen-binding fragment thereof of claim 5, wherein the antibody or antigen-binding fragment comprises the heavy and light chain CDRs of an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 26/10, 34/10, 2/10,18/10, 42/10, 50/10, 58/10, 66/10, 74/10, 82/90, 98/90 and 106/90.

7. The isolated antibody or antigen-binding fragment thereof of claim 5 or 6, wherein the antibody or antigen-binding fragment thereof comprises an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 26/10, 34/10, 2/10,18/10, 42/10, 50/10, 58/10, 66/10, 74/10, 82/90, 98/90 and 106/90.

8. An antibody or antigen-binding fragment thereof, which competes for binding to LEPR with a reference antibody comprising an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 26/10, 34/10, 2/10,18/10, 42/10, 50/10, 58/10, 66/10, 74/10, 82/90, 98/90 and 106/90.

9. An antibody or antigen-binding fragment thereof which binds to the same epitope on LEPR as a reference antibody comprising an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 26/10, 34/10, 2/10,18/10, 42/10, 50/10, 58/10, 66/10, 74/10, 82/90, 98/90 and 106/90.

10. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of any one of claims 1 to 9, and a pharmaceutically acceptable carrier or diluent.

11. The pharmaceutical composition of claim 10 for use in treating a disease or condition associated with or caused by leptin deficiency or leptin resistance in subject in need thereof.

12. The pharmaceutical composition of claim 10 for use in treating a disease or condition associated with or caused by leptin deficiency or leptin resistance, wherein the disease or condition associated with or caused by leptin deficiency or leptin resistance is selected from the group consisting of alcoholic fatty liver disease, non-alcoholic fatty liver disease, severe insulin resistance, severe insulin resistance caused by a mutation in downstream signaling pathways, severe insulin resistance not caused by mutation in the insulin receptor, lipodystrophy, obesity, metabolic syndrome, diet-induced food craving, functional hypothalamic

amenorrhea, type 1 diabetes, type 2 diabetes, insulin resistance, severe insulin resistance due to mutation in insulin receptor, Alzheimer's disease, leptin deficiency, leptin resistance, Leprechaunism/Donohue syndrome, and Rabson-Mendenhall syndrome.

13. The pharmaceutical composition of claim 10 for use in treating a lipodystrophy condition in a patient in need thereof, wherein the lipodystrophy condition is selected from the group consisting of congenital generalized lipodystrophy, acquired generalized lipodystrophy, familial partial lipodystrophy, acquired partial lipodystrophy, centrifugal abdominal lipodystrophy, lipoatrophia annularis, localized lipodystrophy, and HIV-associated lipodystrophy.

14. The pharmaceutical composition of claim 10 for use in treating a disease or condition associated with or caused by a signaling-defective or signaling-impaired LEPR mutation in a subject in need thereof.

15. The pharmaceutical composition of claim 14, wherein the signaling-defective or signaling-impaired LEPR mutation is LEPR-A409E or LEPR-P316T.

16. The pharmaceutical composition of claim 14 or 15, wherein the disease or condition associated with or caused by a signaling-defective or signaling-impaired LEPR mutation is early-onset obesity.

17. The pharmaceutical composition of any one of claims 11 through 16, wherein the composition is administratively combined with a second therapeutic agent, wherein the second therapeutic agent is selected from the group consisting of albiglutide, alirocumab, an anti-ANGPTL3 antibody, an anti-ANGPTL4 antibody, an anti-GCG antibody, an anti-GCGR antibody, an insulin secretagogue, an insulin variant, anti-ANGPTL8 antibody, atorvastatin, bococizumab, canaglifozin, cerivastatin, dapaglifozin, dulaglutide, empagliflozin, evolocumab, exenatide, extendin-4, fluvastatin, lixisenatide, lodelcizumab, lovastatin, pitavastatin, pramlintide and metrelepin, pravastatin, ralpancizumab, rosuvastatin, simvastatin, a recombinant human leptin, a PCSK9 inhibitor, a statin, ezetimibe, insulin, an insulin variant, an insulin secretagogue, metformin, a sulfonylurea, a sodium glucose cotransporter 2 (SGLT2) Inhibitor, a GLP-1 agonist/analogue, a glucagon (GCG) inhibitor, a glucagon receptor (GCGR) inhibitor, an angiopoietin-like protein (ANGPTL) inhibitor, Phentermine, Orlistat, Topiramate, Bupropion, Topiramate/Phentermine, Bupropion/Naltrexone, Bupropion/Zonisamide, Pramlintide/Metrelepin, Lorcaserin, Cetilistat, Tesofensine, and Velneperit.

18. The pharmaceutical composition of claim 12 wherein the disease or condition is lipodystrophy.

19. The pharmaceutical composition of claim 12 wherein the disease or condition is obesity.

20. The pharmaceutical composition of claim 12 wherein the disease or condition is leptin deficiency.

21. An isolated antibody or antigen-binding fragment thereof that specifically binds human LEPR , wherein the antibody or antigen binding fragment interacts with amino acids 162-169 of SEQ ID NO: 113 and amino acids 170-181 of SEQ ID NO: 113, as determined by hydrogen/deuterium exchange.

22. The isolated antibody or antigen-binding fragment thereof of claim 5 comprising:

(a) a light chain variable region comprising the light chain complementarity determining regions (CDRs) of a light chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 10; and

(b) a heavy chain variable region comprising the heavy chain CDRs of a heavy chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 2, SEQ ID NO: 26 or SEQ ID NO: 34.

23. The isolated antibody or antigen-binding fragment thereof of claim 22 comprising:

(a) a light chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 10; and

(b) a heavy chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 26, SEQ ID NO: 34 or SEQ ID NO: 2.

24. The isolated antibody or antigen-binding fragment thereof of claim 23 comprising:

a light chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 10; and

a heavy chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 2.

25. The antibody or antigen-binding fragment of claim 24 which is a full antibody having two of said heavy chain variable regions linked to human heavy chain constant domains and two of said light chain variable regions linked to human light chain constant domains, which chains are interconnected by disulfide bonds.

26. The antibody of claim 25 wherein the heavy chain variable regions are linked to human IgG1 heavy chain constant domains and the light chain variable regions are linked to human kappa light chain constant domains.

27. The antibody of claim 25 wherein the heavy chain variable regions are linked to human IgG4 heavy chain constant domains and the light chain variable regions are linked to human kappa light chain constant domains.

28. The isolated antibody or antigen-binding fragment thereof of claim 23 comprising:

a light chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 10; and

a heavy chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 26.

29. The antibody or antigen-binding fragment of claim 28 which is a full antibody having two of said heavy chain variable regions linked to human heavy chain constant domains and two of said light chain variable regions linked to human light chain constant domains, which chains are interconnected by disulfide bonds.

30. The antibody of claim 29 wherein the heavy chain variable regions are linked to human IgG1 heavy chain constant domains and the light chain variable regions are linked to human kappa light chain constant domains.

31. The antibody of claim 29 wherein the heavy chain variable regions are linked to human IgG4 heavy chain constant domains and the light chain variable regions are linked to human kappa light chain constant domains.

32. The isolated antibody or antigen-binding fragment thereof of claim 23 comprising:

a light chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 10; and

a heavy chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 34.

33. The antibody or antigen-binding fragment of claim 32 which is a full antibody having two of said heavy chain variable regions linked to human heavy chain constant domains and two of said light chain variable regions linked to human light chain constant domains, which chains are interconnected by disulfide bonds.

34. The antibody of claim 33 which wherein the heavy chain variable regions are linked to human IgG1 heavy chain constant domains and the light chain variable regions are linked to human kappa light chain constant domains.

35. The antibody of claim 33 which wherein the heavy chain variable regions are linked

to human IgG4 heavy chain constant domains and the light chain variable regions are linked to human kappa light chain constant domains.

36. A pharmaceutical composition comprising the antibody of claim 25 and a pharmaceutically acceptable carrier or diluent.

37. A pharmaceutical composition comprising the antibody of claim 29 and a pharmaceutically acceptable carrier or diluent.

38. A pharmaceutical composition comprising the antibody of claim 33 and a pharmaceutically acceptable carrier or diluent.

39. The antibody of claim 25 in combination with one or more additional therapeutically active components.

40. The antibody of claim 29 in combination with one or more additional therapeutically active components.

41. The antibody of claim 33 in combination with one or more additional therapeutically active components.

42. A pen delivery device comprising the pharmaceutical composition of claim 36.

43. A pen delivery device comprising the pharmaceutical composition of claim 37.

44. A pen delivery device comprising the pharmaceutical composition of claim 38.

45. The antibody of claim 25 in combination with a GLP-1 agonist/analogue, a glucagon (GCG) inhibitor, a glucagon receptor (GCGR) inhibitor, a PCSK9 inhibitor, a sodium glucose cotransporter 2 (SGLT2) Inhibitor, a statin, a sulfonylurea, an angiopoietin-like protein (ANGPTL) inhibitor, an insulin secretagogue, an insulin variant, recombinant human leptin, alirocumab, evolocumab, bococizumab, lodelcizumab, ralpancizumab, atorvastatin, rosuvastatin, cerivastatin, pitavastatin, fluvastatin, simvastatin, lovastatin, pravastatin, ezetimibe, insulin, metformin, dapaglifozin, canaglifozin, empagliflozin, extendin-4, exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, an anti-GCG antibody, an anti-GCGR antibody, an anti-ANGPTL3 antibody, an anti-ANGPTL4 antibody, anti-ANGPTL8 antibody, phentermine, orlistat, topiramate, bupropion, topiramate and phentermine, bupropion and naltrexone, bupropion and zonisamide, pramlintide and metrelepin, lorcaserin, cetilistat, tesofensine or velneperit.

46. The antibody of claim 29 in combination with a GLP-1 agonist/analogue, a glucagon (GCG) inhibitor, a glucagon receptor (GCGR) inhibitor, a PCSK9 inhibitor, a sodium glucose cotransporter 2 (SGLT2) Inhibitor, a statin, a sulfonylurea, an angiopoietin-like protein

(ANGPTL) inhibitor, an insulin secretagogue, an insulin variant, recombinant human leptin, alirocumab, evolocumab, bococizumab, lodelcizumab, ralpancizumab, atorvastatin, rosuvastatin, cerivastatin, pitavastatin, fluvastatin, simvastatin, lovastatin, pravastatin, ezetimibe, insulin, metformin, dapaglifozin, canaglifozin, empagliflozin, extendin-4, exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, an anti-GCG antibody, an anti-GCGR antibody, an anti-ANGPTL3 antibody, an anti-ANGPTL4 antibody, anti-ANGPTL8 antibody, phentermine, orlistat, topiramate, bupropion, topiramate and phentermine, bupropion and naltrexone, bupropion and zonisamide, pramlintide and metrelepin, lorcaserin, cetilistat, tesofensine or velneperit.

47. The antibody of claim 33 in combination with a GLP-1 agonist/analogue, a glucagon (GCG) inhibitor, a glucagon receptor (GCGR) inhibitor, a PCSK9 inhibitor, a sodium glucose cotransporter 2 (SGLT2) Inhibitor, a statin, a sulfonylurea, an angiopoietin-like protein (ANGPTL) inhibitor, an insulin secretagogue, an insulin variant, recombinant human leptin, alirocumab, evolocumab, bococizumab, lodelcizumab, ralpancizumab, atorvastatin, rosuvastatin, cerivastatin, pitavastatin, fluvastatin, simvastatin, lovastatin, pravastatin, ezetimibe, insulin, metformin, dapaglifozin, canaglifozin, empagliflozin, extendin-4, exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, an anti-GCG antibody, an anti-GCGR antibody, an anti-ANGPTL3 antibody, an anti-ANGPTL4 antibody, anti-ANGPTL8 antibody, phentermine, orlistat, topiramate, bupropion, topiramate and phentermine, bupropion and naltrexone, bupropion and zonisamide, pramlintide and metrelepin, lorcaserin, cetilistat, tesofensine or velneperit.

48. The antibody or antigen-binding fragment of claim 5 which is bispecific.