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(54) **DB, THE RECEPTOR FOR LEPTIN,
NUCLEIC ACIDS ENCODING THE
RECEPTOR, AND USES THEREOF**

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(57) **ABSTRACT**

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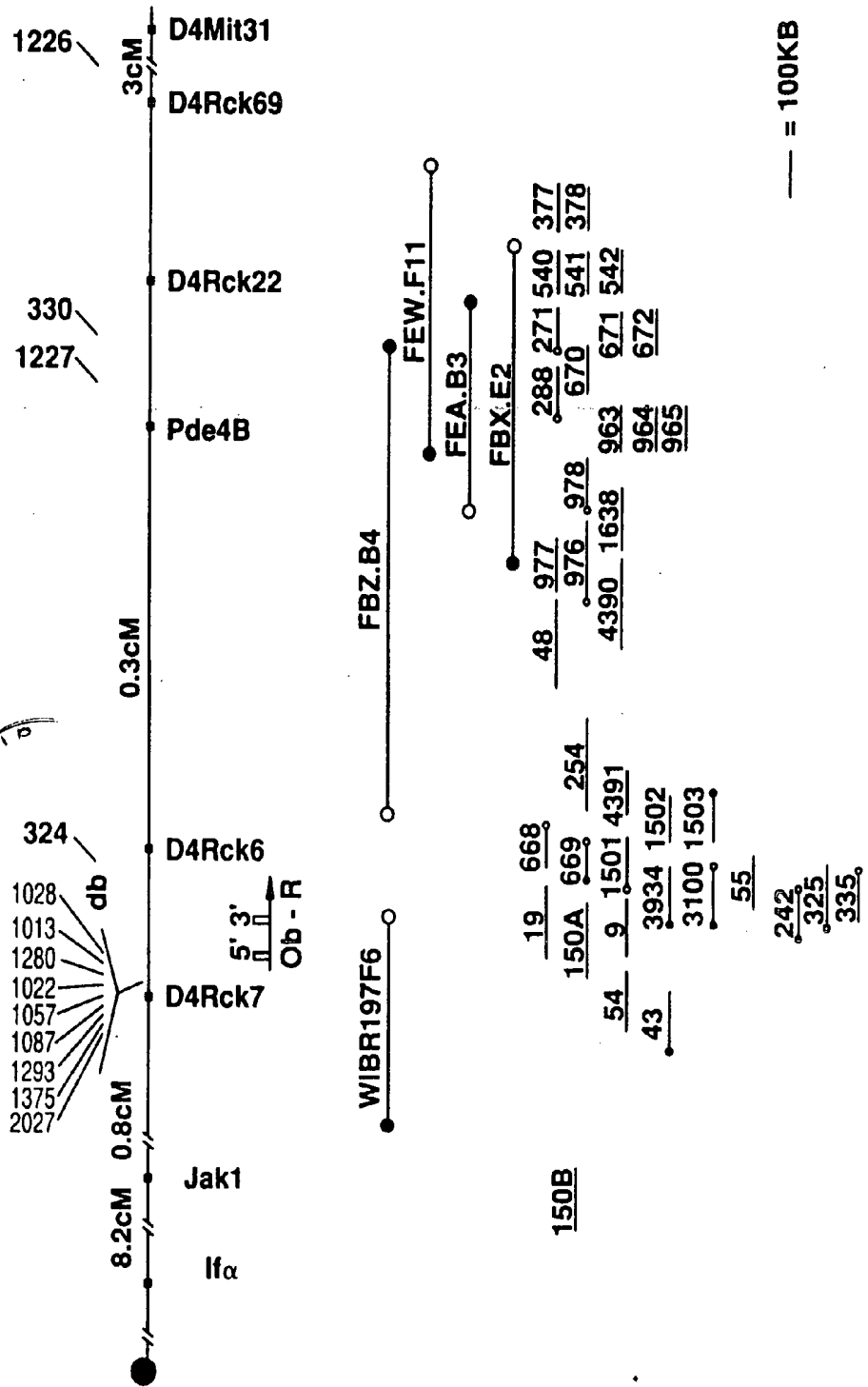
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Related U.S. Application Data

(62) Division of application No. 08/599,974, filed on Feb. 14, 1996, now Pat. No. 7,063,958.

The present invention relates to identification of a receptor for a satiety factor, which is involved in body weight homeostasis. Mutations in this receptor are associated with obese phenotypes. In particular, the present invention relates to identification and characterization of the receptor for leptin, including a naturally occurring soluble form of the receptor that is expected to modulate leptin activity, in particular to agonize leptin activity. The invention further relates to the nucleic acids encoding the receptor, and to methods for using the receptor, e.g., to identify leptin analogs, therapeutically, such as in gene therapy or in soluble form as an agonist or antagonist of leptin activity, or diagnostically.

FIG. 1A



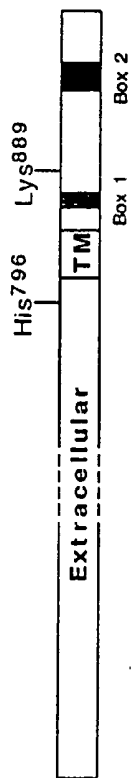


Fig. 2A

Ob-Ra

N886 F887 Q888 K889 RTDTL*

Ob-Rb

N886 F887 Q888 K889

PETFEQLFTKHAESVIFGPLLLEPEPISEIISVDTAWKN
 KDEMVPAAMVSLLLWTTDPPESSICISDQCNSANFSG
 SQSTQVTQEDECOQRPQSVKYATLVSNDKLVETDEEQG
 FIHSPVSNICISSNHSPLRQSFSSSSWETEAQTFFLLSD
 QQPTMISPQLSFGLDLELELGSEPEENHREKSVCYLG
 VTSVNRRESGVLITGEAGILCTFPAQCLFSDIRILQERC
 SHFVENILSLGTSGENFGPYMPOFQTCSTHSIKIMENK
 MCDFTV*

Ob-Rc

N886 F887 Q888 K889 VTV*

Ob-Rd

N886 F887 Q888 K889 DISFHEVFIFR*

Ob-Re

F793 Y794 I795 H796 GMCTVLFMD*

Fig. 2B

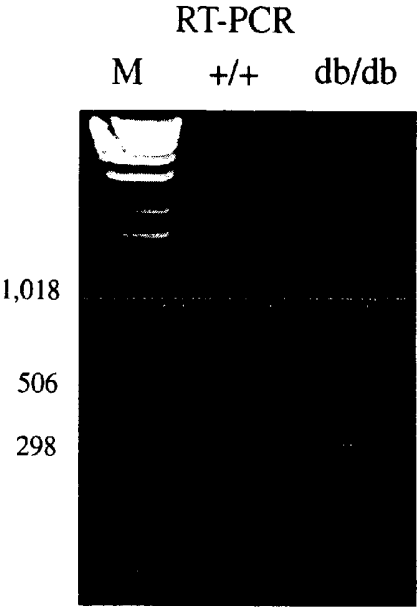


FIG.3A



FIG.3B

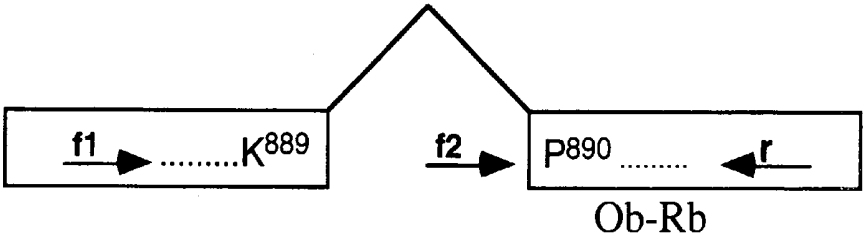


FIG.3C

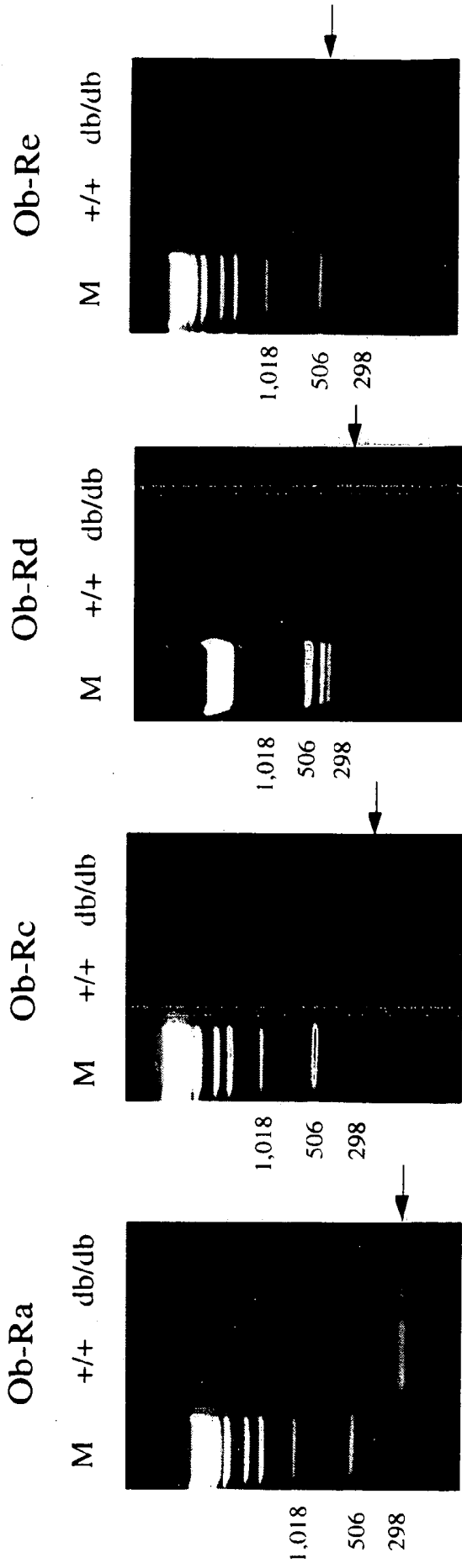
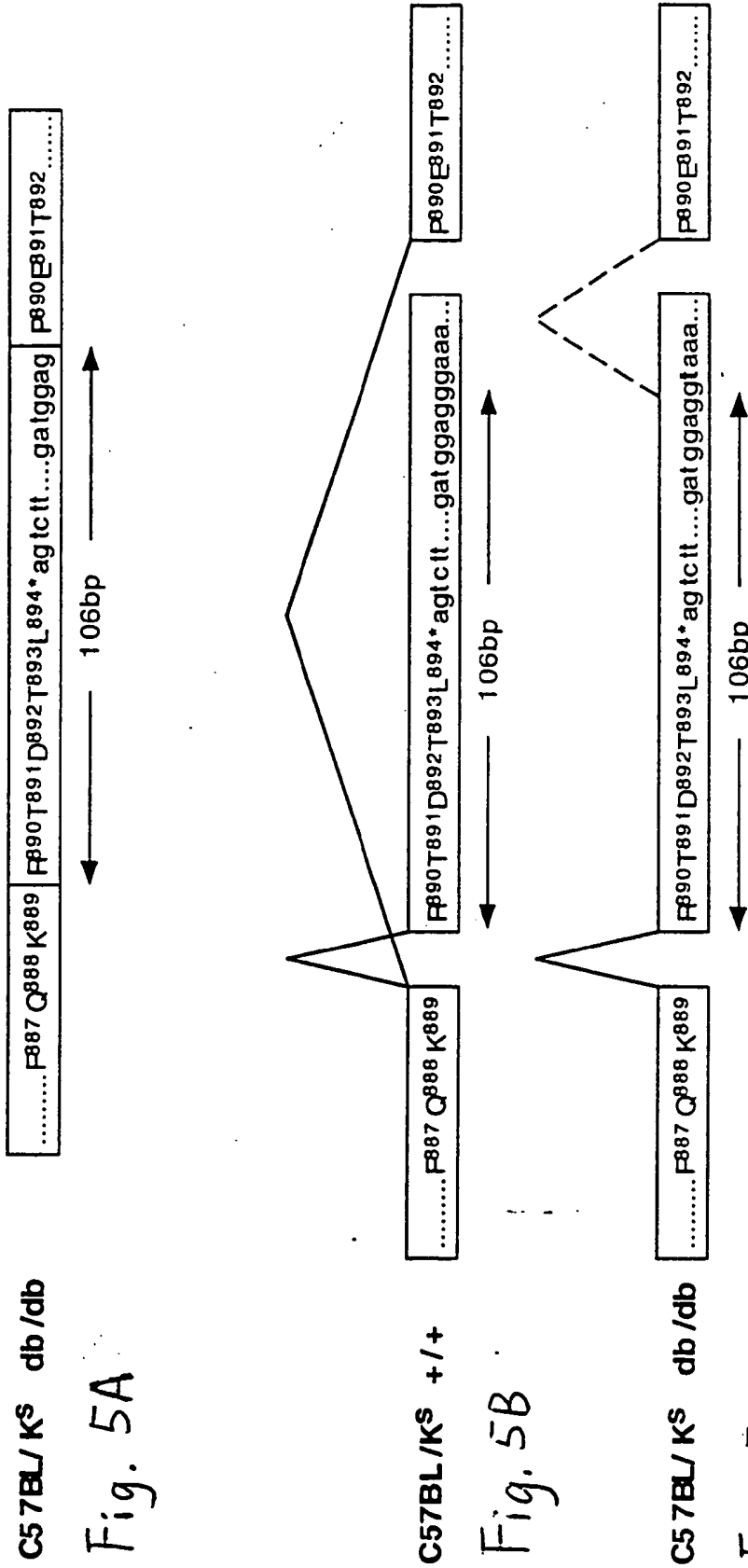


FIG. 4A

FIG. 4B

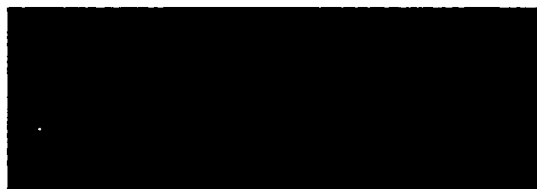
FIG. 4C

FIG. 4D

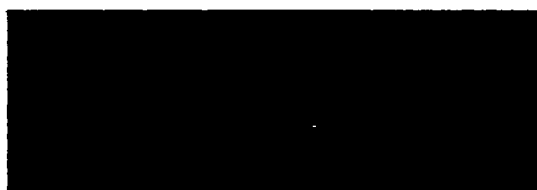


-B H_v L H K SI T F S

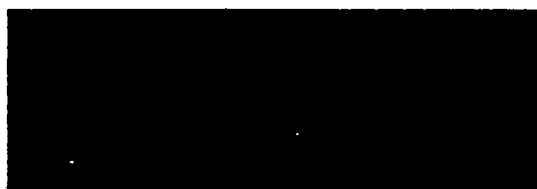
Fig. 6A Ob-Ra



" 6B Ob-Rb



" 6C Ob-Rc



" 6D Ob-Rd



" 6E Ob-Re



DB, THE RECEPTOR FOR LEPTIN, NUCLEIC ACIDS ENCODING THE RECEPTOR, AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to identification of a receptor for a satiety factor, which is involved in body weight homeostasis. Mutations in this receptor are associated with obese phenotypes. In particular, the present invention relates to identification and characterization of the receptor for leptin, including a naturally occurring soluble form of the receptor that is expected to modulate leptin activity, in particular to agonize leptin activity. The invention further relates to the nucleic acids encoding the receptor, and to methods for using the receptor, e.g., to identify leptin analogs, therapeutically, or diagnostically.

BACKGROUND OF THE INVENTION

[0002] Obesity, defined as an excess of body fat relative to lean body mass, is associated with important psychological and medical morbidities, the latter including hypertension, elevated blood lipids, and Type II or non-insulin-dependent diabetes mellitus (NIDDM). There are 6-10 million individuals with NIDDM in the U.S., including 18% of the population of 65 years of age [Harris et al., *Int. J. Obes.*, 11:275-283 (1987)]. Approximately 45% of males and 70% of females with NIDDM are obese, and their diabetes is substantially improved or eliminated by weight reduction [Harris, *Diabetes Care*, 14(3):639-648 (1991)]. As described below, both obesity and NIDDM are strongly heritable.

[0003] The assimilation, storage, and utilization of nutrient energy constitute a complex homeostatic system central to survival of metazoa. Among land-dwelling mammals, storage in adipose tissue of large quantities of metabolic fuel as triglycerides is crucial for surviving periods of food deprivation. The need to maintain a fixed level of energy stores without continual alterations in the size and shape of the organism requires the achievement of a balance between energy intake and expenditure.

[0004] An individual's level of adiposity is, to a large extent, genetically determined. Examination of the concordance rates of body weight and adiposity amongst mono- and dizygous twins or adoptees and their biological parents have suggested that the heritability of obesity (0.4-0.8) exceeds that of many other traits commonly thought to have a substantial genetic component, such as schizophrenia, alcoholism, and atherosclerosis [Stunkard et al., *N. Engl. J. Med.*, 322:1483-1487 (1990)]. Familial similarities in rates of energy expenditure have also been reported [Bogardus et al., *Diabetes*, 35:1-5 (1986)]. Genetic analysis in geographically delimited populations has suggested that a relatively small number of genes may account for the 30-50% of variance in body composition [Moll et al., *Am. J. Hum. Genet.*, 49:1243-1255 (1991)].

[0005] Rodent models of obesity include seven apparently single-gene mutations. The most intensively studied mouse obesity mutations are the ob (obese) and db (diabetes) genes. When present on the same genetic strain background, ob and db result in indistinguishable metabolic and behavioral phenotypes, suggesting that these genes may function in the same physiologic pathway [Coleman et al., *Diabetologia*, 14:141-148 (1978)]. Mice homozygous for either mutation

are hyperphagic and hypometabolic, leading to an obese phenotype that is notable at one month of age. The weight of these animals tends to stabilize at 60-70 g (compared with 30-35 g in control mice). ob and db animals manifest a myriad of other hormonal and metabolic changes that had made it difficult to identify the primary defect attributable to the mutation [Bray et al., *Am. J. Clin. Nutr.*, 50:891-902 (1989)]. As noted below, identification of the OB gene led to an understanding of one molecular element.

[0006] Each of the rodent obesity models is accompanied by alterations in carbohydrate metabolism resembling those in Type II diabetes in man. In some cases, the severity of the diabetes depends in part on the background mouse strain [Leiter, *Endocrinology*, 124:912-922 (1989)]. For both ob and db, congenic C57BL/Ks mice develop a severe diabetes with ultimate β cell necrosis and islet atrophy, resulting in a relative insulinopenia. Conversely, congenic C57BL/6J ob and db mice develop a transient insulin-resistant diabetes that is eventually compensated by β cell hypertrophy resembling human Type II diabetes.

[0007] The phenotype of ob and db mice resembles human obesity in ways other than the development of diabetes—the mutant mice eat more and expend less energy than do lean controls (as do obese humans). This phenotype is also quite similar to that seen in animals with lesions of the ventromedial hypothalamus, which suggests that both mutations may interfere with the ability to properly integrate or respond to nutritional information within the central nervous system. Support for this hypothesis comes from the results of parabiosis experiments [Coleman, *Diabetologia*, 9:294-298 (1973)] that suggest ob mice are deficient in a circulating satiety factor and that db mice are resistant to the effects of the ob factor (possibly due to an ob receptor defect). These experiments have led to the conclusion that obesity in these mutant mice may result from different defects in an afferent loop and/or integrative center of the postulated feedback mechanism that controls body composition.

[0008] Using molecular and classical genetic markers, the ob and db genes have been mapped to proximal chromosome 6 and midchromosome 4, respectively [Bahary et al., *Proc. Nat. Acad. Sci. USA*, 87:8642-8646 (1990); Friedman et al., *Genomics*, 11:1054-1062 (1991)]. In both cases, the mutations map to regions of the mouse genome that are syntenic with human, suggesting that, if there are human homologs of ob and db, they are likely to map, respectively, to human chromosomes 7q and 1 p. Defects in the db gene may result in obesity in other mammalian species: in genetic crosses between Zucker fa/fa rats and Brown Norway +/-rats, the fa mutation (rat chromosome 5) is flanked by the same loci that flank db in mouse [Truett et al., *Proc. Natl. Acad. Sci. USA*, 88:7806-7809 (1991)].

[0009] A major advance in understanding the molecular basis for obesity occurred with the cloning of the ob gene. The mouse obesity (ob) gene encodes an adipose tissue-derived signaling factor for body weight homeostasis [Zhang et al., *Nature*, 372:425 (1994); U.S. patent application Ser. No. 08/292,345 filed Aug. 17, 1994; U.S. patent application Ser. No. 08/483,211, filed Jun. 7, 1995, each of which is hereby incorporated by reference in its entirety]. Several recent studies have shown that recombinant OB protein (leptin) purified from *Escherichia coli* can correct the obesity related phenotypes in ob/ob mice when exogenously

administered [Campfield et al., *Science*, 269:546 (1995); Pellymouther et al., *Science*, 269:540, (1995); Halaas et al., *Science*, 269:543 (1995); Stephens et al., *Nature*, 377:530 (1995)]. Weight-reducing effects of recombinant leptin were also observed in normal mice and mice with diet-induced obesity. Although the target tissues that mediate the effects of leptin have not yet been defined, the instant inventors have predicted the brain as a target of leptin activity. Indeed, the work of Campfield et al. (supra) and Stephens et al. (supra) demonstrates that leptin introduced into the lateral or third brain ventricle is effective at low doses, arguing for a direct central affect of the leptin molecule.

[0010] Recent studies have suggested that obese humans and rodents (other than ob/ob mice) are not defective in their ability to produce leptin mRNA or protein and generally produce higher levels than lean individuals [Maffei et al., *Nature Med.*, 1:1155 (1995); Considine et al., *J. Clin. Invest.*, 95:2986 (1995); Lonnqvist et al., *Nature Med.*, 1:950 (1995); Hamilton et al., *Nature Med.*, 1:953 (1995)]. These data suggest that resistance to normal or elevated levels of leptin may be important factors in human obesity. However, a recent report of identification of a leptin receptor did not identify any mutations in the ob allele [Tartaglia et al., *Cell*, 83:1263-1271 (1995)].

[0011] Accordingly, there is a need in the art to identify a receptor for leptin.

[0012] There is a further need to characterize mutations in the leptin receptor, particularly as they may be associated with obesity.

[0013] There is a still further need to identify and characterize functions of the leptin receptor, or variants thereof.

[0014] These and other needs in the art are addressed by the present invention.

[0015] The citation of any reference herein should not be construed as an admission that such a reference is available as prior art to the application.

SUMMARY OF THE INVENTION

[0016] The present invention is directed to a leptin receptor (OB-R) polypeptide, nucleic acids encoding such polypeptide, non-coding nucleic acids flanking the coding sequences of the gene, oligonucleotides that hybridize to such nucleic acids, antibodies to the polypeptide, and diagnostic, therapeutic, and cosmetic compositions and methods utilizing the polypeptide, nucleic acids, or antibodies, or combinations thereof.

[0017] Thus, in a first aspect of the invention, the leptin receptor (also termed herein OB receptor or OB-R) is characterized by specific binding to leptin under physiological conditions; expression at high levels in cells of the hypothalamus, and expression at lower levels in adipose tissue, testes, heart, and brain; and having sequence similarity to gp130 cytokine receptors. In another embodiment, the leptin receptor is encoded by a nucleic acid which is identifiable with a polymerase chain reaction (PCR) probe selected from group consisting of a probe for clone 7 (forward primer SEQ ID NO:42 and reverse primer SEQ ID NO:43), a probe for clone 11 (forward primer SEQ ID NO:44 and reverse primer SEQ ID NO:45), and both clone 7 and clone 11. In a specific embodiments, leptin receptor is

encoded by a nucleic acid which is identifiable with a PCR probe selected from the group consisting of a probe for clone 42 (forward primer SEQ ID NO:26 and reverse primer SEQ ID NO:46); a probe for clone 46 (forward primer SEQ ID NO:47 and reverse primer SEQ ID NO:48); a probe for clone 58 (forward primer SEQ ID NO:49 and reverse primer SEQ ID NO:50); a probe for clone S14 (forward primer SEQ ID NO:51 and reverse primer SEQ ID NO:52); and a probe for clone S3 (forward primer SEQ ID NO:53 and reverse primer SEQ ID NO:54).

[0018] In specific Examples, infra, the leptin receptor is selected from the group consisting of OB-Ra (SEQ ID NO:2), OB-Rb (SEQ ID NO:4), OB-Rc (SEQ ID NO:6), OB-Rd (SEQ ID NO:8), and OB-Re (SEQ ID NO:10), or allelic variants thereof. Alternatively, the leptin receptor may have a sequence selected from the group consisting of:

[0019] N-terminal corresponding to OB-Ra through Lys⁴⁸⁹ and C-terminal corresponding to a C-terminal selected from the group consisting of OB-Rb, OB-Rc, and OB-Rd after Lys⁸⁸⁹;

[0020] N-terminal corresponding to OB-Rb or OB-Rc through Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra or OB-Rd after Lys⁸⁸⁹;

[0021] N-terminal corresponding to OB-Rd through Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra, OB-Rb, or OB-Rc;

[0022] N-terminal corresponding to OB-R from Pro⁶⁶⁴ to Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra, OB-Rb, OB-Rc, and OB-Rd; and

[0023] N-terminal corresponding to OB-R from Met⁷³³ to Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra, OB-Rb, OB-Rc, and OB-Rd; and

[0024] N-terminal selected from the group consisting of OB-Ra, OB-Rb, OB-Rd, and OB-R from Pro⁶⁶⁴ to His⁷⁹⁶, and OB-Re from His⁷⁹⁶;

[0025] N-terminal selected from the group consisting of OB-Ra, OB-Rb, OB-Rd, and OB-R from Me⁷³³ to His⁷⁹⁶, and OB-Re from His⁷⁹⁶, or allelic variants thereof.

[0026] In another embodiment, leptin receptor may have an N-terminal sequence is selected from the group consisting of

[0027] amino acid residues 1-889;

[0028] amino acid residues 23-889;

[0029] amino acid residues 28-889;

[0030] amino acid residues 133-889;

[0031] amino acid residues 733-889;

[0032] amino acid residues 1-796;

[0033] amino acid residues 23-796;

[0034] amino acid residues 28-796;

[0035] amino acid residues 133-796; and

[0036] amino acid residues 733-796; and

and a C-terminal sequence is selected from the group consisting of SEQ ID NO:11;

SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; and SEQ ID NO:15, wherein the numbering is based on the amino acid sequence of the full length transcribed murine leptin receptor, including the signal peptide, or allelic variants thereof.

[0037] In a specific embodiment, the leptin receptor is a soluble receptor. Such a soluble receptor may be selected from the group consisting of OB-Re; an N-terminal sequence which selected from the group consisting of OB-Ra, OB-Rb, OB-Rd, and OB-R from Pro⁶⁶⁴ to His⁷⁹⁶, and a C-terminal sequence which is OB-Re from His⁷⁹⁶; and OB-R from Met⁷³³ to His⁷⁹⁶, and a C-terminal sequence which is OB-Re from His⁷⁹⁶; an N-terminal sequence which is selected from the group consisting of

[0038] amino acid residues 1-796;

[0039] amino acid residues 23-796;

[0040] amino acid residues 28-796;

[0041] amino acid residues 133-796; and

[0042] amino acid residues 733-796; and

a C-terminal sequence which is SEQ ID NO:15; wherein the numbering is based on the amino acid sequence of the full length transcribed murine leptin receptor, including the signal peptide, or allelic variants thereof.

[0043] Alternatively, the leptin receptor comprises a transmembrane domain, and is an integral membrane protein. In this embodiment, the leptin receptor may further comprise a JAK binding motif selected from "Box 1," "Box 2," and "Box 1" and "Box 2", which motif is downstream of the transmembrane domain.

[0044] In one specific embodiment, the leptin receptor is a human leptin receptor. In another specific embodiment, exemplified infra, the leptin receptor is a murine leptin receptor. In a further specific embodiment, the leptin receptor is a human leptin receptor comprising a divergent amino acid substitution from the corresponding position of the murine leptin receptor. In another embodiment, the leptin receptor is a human leptin receptor comprising conservative amino acid substitutions. In a specific embodiment, conservative amino acid substitutions from murine leptin receptor are made in human leptin receptor. In yet another embodiment, conservative amino acid substitutions that enhance secondary structure, e.g., α -helical propensity, are made.

[0045] The present invention further provides an antigenic fragment of the leptin receptor. In a specific embodiment, the antigenic fragment is selected from the group consisting of SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:34.

[0046] The invention further relates to a derivative of the soluble form of the leptin receptor attached to a chemical moiety. Preferably, the chemical moiety is a water-soluble polymer. More preferably, the water soluble polymer is polyethylene glycol.

[0047] In another aspect, the invention provides an isolated nucleic acid encoding the leptin receptor, particularly as set forth above. In specific examples, infra, the invention provides cDNA encoding various splice forms of murine leptin receptor. In particular, the present invention provides nucleic acids having sequences corresponding or complementary to SEQ ID NO:1, 3, 5, 7, or 9.

[0048] More particularly, the invention provides an isolated DNA molecule encoding on expression a leptin receptor polypeptide selected from the group consisting of:

[0049] a polypeptide coding sequence of a DNA molecule of SEQ ID NO:1, 3, 5, 7, or 9;

[0050] a DNA molecule complementary to the DNA molecule defined in (a);

[0051] a DNA molecule which hybridizes to the DNA molecule of (a) or (b), or a hybridizable fragment thereof;

[0052] a DNA molecule which is identifiable with a polymerase chain reaction (PCR) probe selected from group consisting of a probe for clone 7 (forward primer SEQ ID NO:42 and reverse primer SEQ ID NO:43), a probe for clone 11 (forward primer SEQ ID NO:44 and reverse primer SEQ ID NO:45), and both clone 7 and clone 11; and

[0053] a DNA molecule that codes on expression for the polypeptide encoded by any of the foregoing DNA molecules.

[0054] Preferably the DNA molecule is human; in specific Examples, infra, the DNA molecule is murine. In specific embodiments, the DNA molecule codes on expression for a polypeptide selected from the group consisting of OB-Ra, OB-Rb, OB-Rc, OB-Rd, and OB-Re, or allelic variants thereof; a leptin receptor selected from the group consisting of:

[0055] N-terminal corresponding to OB-Ra through Lys⁴⁸⁹ and C-terminal corresponding to a C-terminal selected from the group consisting of OB-Rb, OB-Rc, and OB-Rd after Lys⁸⁸⁹;

[0056] N-terminal corresponding to OB-Rb or OB-Rc through Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra or OB-Rd after Lys⁸⁸⁹;

[0057] N-terminal corresponding to OB-Rd through Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra, OB-Rb, or OB-Rc;

[0058] N-terminal corresponding to OB-R from Pro⁶⁶⁴ to Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra, OB-Rb, OB-Rc, and OB-Rd; and

[0059] N-terminal corresponding to OB-R from Met⁷³³ to Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra, OB-Rb, OB-Rc, and OB-Rd; and

[0060] N-terminal selected from the group consisting of OB-Ra, OB-Rb, OB-Rd, and OB-R from Pro⁶⁶⁴ to His⁷⁹⁶, and OB-Re from His⁷⁹⁶;

[0061] N-terminal selected from the group consisting of OB-Ra, OB-Rb, OB-Rd, and OB-R from Met⁷³³ to His⁷⁹⁶, and OB-Re from His⁷⁹⁶, or allelic variants thereof;

a leptin receptor wherein the N-terminal sequence is selected from the group consisting of

[0062] amino acid residues 1-889;

[0063] amino acid residues 23-889;

[0064] amino acid residues 28-889;

[0065] amino acid residues 133-889;

[0066] amino acid residues 733-889;

[0067] amino acid residues 1-796;

[0068] amino acid residues 23-796;

[0069] amino acid residues 28-796;

[0070] amino acid residues 133-796; and

[0071] amino acid residues 733-796;

and the C-terminal sequence is selected from the group consisting of SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO: 14; and SEQ ID NO:15, wherein the numbering is based on the amino acid sequence of the full length transcribed murine leptin receptor, including the signal peptide, or allelic variants thereof.

[0072] The invention further contemplates, as a corollary to the coding nucleic acids described above, an oligonucleotide hybridizable under stringent conditions to the nucleic acid molecule encoding leptin receptor. In specific embodiments, exemplified infra, the oligonucleotide is selected from the group consisting of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, and SEQ ID NO:54. The oligonucleotide may be labeled.

[0073] In addition to the coding DNA, the present invention provides vectors comprising such DNA. A vector of the invention may be a cloning vector, or it may be an expression vector which comprises the DNA encoding leptin receptor operatively associated with an expression control sequence. Naturally, the invention extends to a unicellular host transformed or transfected with a DNA molecule, cloning vector, or expression vector of the invention. Such a unicellular host may be selected from the group consisting of bacteria, yeast, mammalian cells, plant cells, and insect cells, in tissue culture. In specific embodiments, the host may be selected from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Torulopsis*, CHO, R1.1, B-W, LM, COS 1, COS 7, BSC1, BSC40, BMT10, and Sf9 cells.

[0074] The invention further relates to a recombinant method for preparing a leptin receptor polypeptide comprising culturing a host cell comprising an expression vector of the invention under conditions that provide for expression of the leptin receptor polypeptide; and recovering the expressed polypeptide.

[0075] The invention further provides an antisense nucleic acid that hybridizes with an mRNA encoding leptin receptor, and a ribozyme which cleaves an mRNA encoding a leptin receptor.

[0076] In another embodiment, the invention provides a transgenic vector comprising a DNA molecule encoding leptin receptor, or an expression vector of the invention.

[0077] In another aspect, the invention provides an antibody specific for a leptin receptor. The antibody may be a monoclonal or polyclonal antibody. In a specific embodiment, the antibody may be labeled with a detectable label. Naturally, the invention extends to an immortal cell line that produces a monoclonal antibody.

[0078] In a specific embodiment, the invention provides a method for preparing an antibody specific for a leptin receptor, comprising: immunizing a host animal with the leptin receptor admixed with an adjuvant; and obtaining antibody from the immunized host animal. In another specific embodiment, exemplified infra, the method for preparing an antibody specific for a leptin receptor comprises conjugating a peptide having a sequence selected from the group consisting of SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:34 to a carrier protein; immunizing a host animal with the peptide-carrier protein conjugate of step (a) admixed with an adjuvant; and obtaining antibody from the immunized host animal.

[0079] In conjunction with the antibodies of the invention, the invention provides a method for measuring the presence of a leptin receptor in a sample, comprising contacting a sample suspected of containing a leptin receptor with an antibody that specifically binds to the leptin receptor under conditions which allow for the formation of reaction complexes comprising the antibody and the leptin receptor; and detecting the formation of reaction complexes comprising the antibody and leptin receptor in the sample, wherein detection of the formation of reaction complexes indicates the presence of leptin receptor in the sample. In a specific embodiment, the antibody is bound to a solid phase support. As a corollary to the method of measuring the presence of leptin receptor in a sample, the invention provides an in vitro method for evaluating the level of leptin receptor in a biological sample comprising detecting the formation of reaction complexes in a biological sample as described; and evaluating the amount of reaction complexes formed, which amount of reaction complexes corresponds to the level of leptin receptor in the biological sample. The invention further relates to an in vitro method for detecting or diagnosing the presence of a disease associated with elevated or decreased levels of leptin receptor in a subject comprising evaluating the level of leptin receptor in a biological sample from a subject as described; and comparing the level detected in step (a) to a level of leptin receptor present in normal subjects or in the subject at an earlier time, wherein an increase in the level of leptin receptor as compared to normal levels indicates a disease associated with elevated levels of leptin receptor, and decreased level of leptin receptor as compared to normal levels indicates a disease associated with decreased levels of leptin receptor.

[0080] The present invention also provides a pharmaceutical composition comprising a soluble leptin receptor, and a pharmaceutically acceptable carrier. Alternatively, a pharmaceutical composition of the invention may comprise a transgenic vector, e.g., a viral vector or naked DNA, for administration to a subject for gene therapy. Preferably, such a vector is targeted to the brain, more preferably the hypothalamus. The invention further provides a method for treating obesity in a subject comprising administering a therapeutically effective amount of the pharmaceutical composition of the invention. The method of treatment may

further comprise administering a treatment for diabetes, high blood pressure, and high cholesterol.

[0081] In another embodiment, the invention provides a body appearance improving cosmetic composition for reducing the body weight of an individual comprising a soluble leptin receptor, and an acceptable carrier. The invention further provides a method for improving the body appearance of an individual comprising administering the cosmetic composition of the invention.

[0082] Accordingly, it is a principal object of the present invention to provide modulators of body weight as defined herein in purified form, that exhibit certain characteristics and activities associated with control and variation of adiposity and fat content of mammals.

[0083] It is a further object of the present invention to provide methods for the detection and measurement of the modulators of weight control as set forth herein, as a means of the effective diagnosis and monitoring of pathological conditions wherein the variation in level of such modulators is or may be a characterizing feature.

[0084] It is a still further object of the present invention to provide a method and associated assay system for the screening of substances, such as drugs, agents and the like, that are potentially effective to either mimic or inhibit the activity of leptin binding to its receptor, e.g., agonists and antagonists of the modulators of the invention in mammals.

[0085] It is a still further object of the present invention to provide a method for the treatment of mammals to control body weight and fat content in mammals, and/or to treat certain of the pathological conditions of which abnormal depression or elevation of body weight is a characterizing feature.

[0086] It is a still further object of the present invention to prepare genetic constructs for use in genetic therapeutic protocols and/or pharmaceutical compositions for comparable therapeutic methods, which comprise or are based upon one or more of the modulators, binding partners, or agents that may control their production, or that may mimic or antagonize their activities.

[0087] Other objects and advantages will become apparent to those skilled in the art from a review of the ensuing description which proceeds with reference to the following illustrative drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0088] FIG. 1. Localization of the leptin receptor to the region of the db gene. The db mutation was segregated in two crosses totaling 750 meioses. A genetic map was compiled by genotyping the progeny of these crosses with the markers indicated in the map. Key recombinant animals are noted on the map as numbers above the line. A chromosome walk was initiated with the microdissection clone D4Rck22. The walk spanned 2.7 megabases and was composed of YACs (bold lines), BACs (italics) and P1 bacteriophage (numbers). Genotyping of the recombinant animals with two SSLP markers, D4Rck6 and D4Rck7 from the ends of these genomic clones, localized the db gene to the approximately 300 Kb interval between the recombination events in animals 324 and 1028. This interval was spanned by BACs 242 and 43. Southern blots and PCR revealed that the 5' ends of

the leptin receptor mapped to BAC 150A and the 3' end to BAC 19, indicating the gene is transcribed toward the telomeres.

[0089] FIG. 2. Several splice variants of the leptin receptor are present. (A) A schematic drawing of the leptin receptor, with putative motifs for JAK binding and signal transduction, "Box 1" and "Box 2" (shaded areas). "TM" indicates a putative transmembrane domain. A total of 8 cDNA clones were isolated from mouse brain. These cDNAs were found to correspond to five different splice variants of the leptin receptor. (B) Six of the clones had partly identical sequences upstream of lysine 889 of the leptin receptor (OB-Ra, OB-Rb, OB-Rc and OB-Rd), at which point the predicted proteins diverged. The predicted C-terminal amino acid sequences of these clones is shown (SEQ ID NOS: 11-14, respectively). OB-Ra, b, c, and d all predict a Box 1 motif. OB-Rb also predicts a peptide sequence potentially homologous to Box 2 (underlined). Two independent cDNA clones were identical to the leptin receptor upstream of histidine 796, at which point the sequences diverged (OB-Re)(SEQ ID NO:15). The nucleotide sequence predicts a soluble receptor.

[0090] FIG. 3. The db mutation results in abnormal RNA splicing and conversion of the splice variant OB-Rb to OB-Ra. (A) RT-PCR products from C57BL/Ks db/db and wild type mice were amplified using a primer pair specific for OB-Rb RNA (F1 and R₃). Electrophoresis revealed that the amplified fragment from these db mice was larger than from wild type animals. The PCR products of genomic DNA spanning the OB-Rb splice acceptor at Pro⁸⁹⁰ were of identical size in C57 BL/Ks db/db mice and littermate controls. (B) Primers F2 and R were used to amplify the genomic DNA. The F2 primer was selected after using vectorette PCR and BAC 242 to obtain the sequence of genomic DNA upstream of the splice acceptor at p⁸⁹⁰. (C) Localization of primers for RT-PCR and genomic PCR amplification.

[0091] FIG. 4. Hypothalamic RNA of wild type mice. The hypothalamic RT-PCR products for the C-terminal coding region of (A) OB-Ra, (B) OB-Re, (C) OB-Rd and (D) OB-Re were of normal size in db mice. The DNA sequence across the splice junction was normal in each of these RT-PCR products. This indicates that the splice donor at Lys⁸⁸⁹ is wild type.

[0092] FIG. 5. Identification of splice mutations in db mice. (A) DNA sequencing identified a 106 bp insertion in the mutant OB-Rb RNA at the splice junction between Lys⁸⁸⁹ and Pro⁸⁹⁰ (SEQ ID NO: 16). The sequence of the insertion was identical to the first 106 bp of the C-terminal exon of OB-RA. The insertion predicts a premature stop codon and changes the amino acid sequence of OB-Rb (SEQ ID NO:17) to OB-Ra. (B, C) The presumed genomic organization of the OB-Ra and OB-Rb 3' ends are shown. DNA sequencing of the OB-Ra exon from the C57 BL/K⁹ db/db mice (SEQ ID NO:18) and littermate controls (SEQ ID NO:19) revealed a G to T mutation 106 base pairs after the splice acceptor at R⁸⁹⁰. This mutation results in the appearance of a consensus splice donor site, AGGTAAA, which leads to the insertion of 106 bp of the C-terminal exon of OB-Ra into that of OB-Rb.

[0093] FIG. 6. Tissue distribution of the alternatively spliced leptin receptor. RT-PCR was performed from the

tissue sources indicated. In each case, one primer from a region of shared nucleotide sequence was used in combination with a primer specific for the alternatively spliced exon. (B) Brain, (H) Hypothalamus, (L) Liver, (H) Heart, (K) Kidney, (S) Spleen, (T) Testis, (F) Adipose Tissue, (S) Spleen.

DETAILED DESCRIPTION OF THE INVENTION

[0094] The present invention relates to the elucidation and discovery of a protein, termed herein ob receptor (OB-R) or leptin receptor, nucleic acids encoding the protein, including the OB-R gene (also termed herein DB—it should be noted that where all capitals are used it refers to the natural protein or gene; all lower case refers to a mutant protein or gene; italics indicates a gene or nucleic acid molecule; and normal type indicates a protein or polypeptide), including degenerate variations thereof, e.g., that incorporate optimal codons for expression in a particular expression system, which protein demonstrates the ability to participate in the control of mammalian body weight. In particular, the protein demonstrates the ability to bind leptin. In a specific embodiment, the protein mediates signal transduction upon binding to leptin.

[0095] The OB receptor of the invention may contain three important structural domains: an extracellular (or extracytoplasmic) domain, a transmembrane domain, and a cytoplasmic domain. The extracellular domain is postulated to bind leptin, leptin-protein complexes (such as leptin bound to a soluble leptin receptor), and may possibly bind other proteins or ligands. In a specific embodiment, a receptor of the invention comprises only an extracellular domain, i.e., it is a soluble receptor. The transmembrane domain comprises a stretch of highly non-polar amino acid residues that localize to the hydrophobic region of the cell membrane. In this respect, the term transmembrane domain has its ordinary meaning in molecular biology. Finally, the cytoplasmic domain of an OB receptor of the invention may contain none, one, or two JAK-binding consensus sequences, termed “Box 1” and “Box 2”. A receptor having “Box 1” and “Box 2” is believed competent for signal transduction via the JAK-Stat pathway upon bind ligand, e.g., leptin.

[0096] Furthermore, the protein has been identified as having numerous splice-forms. In one aspect, the splice variations lead to divergence of the C-terminal sequences. Thus, the protein can be found in a secreted form postulated to agonize leptin activity; it can be found as an integral membrane receptor that may facilitate leptin transfer across the blood-brain barrier, but that lacks domains involved in signal transduction; and it can be found as a integral membrane receptor containing domains involved in signal transduction. In another aspect, splice variations lead to divergence of the N-terminal polypeptide sequence.

[0097] The nucleic acids in object represent the coding sequences corresponding to the animal, specifically murine and human OB-R polypeptide, which, by mediating (or failing to mediate) signal transduction on binding leptin, is postulated to play a critical role in the regulation of body weight and adiposity. Data presented herein indicate that one splice variant of the polypeptide product of a nucleic acid of the invention may be secreted by the cells that express it, or it may be expressed as an integral membrane protein. In

either event, the polypeptide functions as a leptin receptor. Additional experimental data suggest that the naturally occurring splice- from of the OB-R polypeptide is very effective in treating obesity in mice carrying a mutation of the ob gene.

[0098] In addition, the Examples herein demonstrate that mRNA encoding the OB-R polypeptide, alternatively termed herein “leptin receptor,” is expressed in hypothalamus, testes, and adipocytes. Data also demonstrate expression of the protein in the choroid plexus.

[0099] In a further aspect, the OB-R polypeptide from one species is closely related (homologous) to the OB-R in another species. In particular, the human OB-R polypeptide is highly homologous to murine OB-R polypeptide. This observation is consistent with the data showing that human leptin is active in mice: for the hormone to be active inerspecies, one would expect a high degree of similarity or homology between the receptors from different species as well.

[0100] In its primary aspect, the present invention is directed to the identification of materials that function as modulators of mammalian body weight. In particular, the invention concerns the isolation, purification, and sequencing of certain nucleic acids that correspond to the OB-R gene (alternatively referred to herein and in the literature as DB) or its coding region in both mice and humans, as well as the corresponding polypeptides expressed by these nucleic acids. The invention thus comprises the discovery of nucleic acids having the nucleotide sequences set forth in SEQ ID NOS:1-5, and to degenerate variants, alleles and fragments thereof, all possessing the activity of modulating body weight and adiposity. The correspondence of the present nucleic acids to the OB-R gene portends their significant impact on conditions such as obesity as well as other maladies and dysfunctions where abnormalities in body weight are a contributory factor. The invention extends to the proteins expressed by the nucleic acids of the invention, and particularly to those proteins set forth in SEQ ID NOS:6-10, as well as to conserved variants and active fragments.

[0101] Of particular interest according to the invention are different splice variants of OB-R, e.g., as represented by OB-Ra, OB-Rb, OB-Rc, OB-Rd, and OB-Re. The present invention anticipates other OB-R splice variants as well.

[0102] Thus, in specific embodiments, the term OB-R refers to splice variants as follows (amino acid numbering correspond to the numbering applied to murine OB-R [Tartaglia et al., *Cell*, 83:1263 (1995)], which has been adopted herein):

[0103] 5 N-terminal corresponding to OB-Ra through Lys⁸⁸⁹ and C-terminal corresponding to a C-terminal selected from the group consisting of OB-Rb, OB-Rc, and OB-Rd after Lys⁸⁸⁹;

[0104] N-terminal corresponding to OB-Rb or OB-Rc through Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra or OB-Rd after Lys⁸⁸⁹;

[0105] N-terminal corresponding to OB-Rd through Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra, OB-Rb, or OB-Rc;

[0106] N-terminal corresponding to OB-R from Pro⁶⁶⁴ to Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra, OB-Rb, OB-Rc, and OB-Rd;

[0107] N-terminal corresponding to OB-R from Met⁷³³ to Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra, OB-Rb, OB-Rc, and OB-Rd;

[0108] N-terminal selected from the group consisting of OB-Ra, OB-Rb, OB-Rd, and OB-R from Pro⁶⁶⁴, to His⁷⁹⁶, and OB-Re from His⁷⁹⁶; and

[0109] N-terminal corresponding to OB-R from Met⁷³³ to His⁷⁹⁶, and OB-Re from His⁷⁹⁶.

[0110] Various forms of the OB-R, which may act as agonists (e.g., the naturally occurring secreted form of the OB-R) or antagonists (e.g., a truncated form of OB-R that only binds leptin), may be prepared in pharmaceutical compositions, with a suitable carrier and at a strength effective for administration by various means to a patient experiencing abnormal fluctuations in body weight or adiposity, either alone or as part of an adverse medical condition such as cancer or AIDS, for the treatment thereof. A variety of administrative techniques may be utilized, among them oral administration, nasal and other forms of transmucosal administration, parenteral techniques such as subcutaneous, intravenous and intraperitoneal injections, catheterizations and the like. Appropriate quantities of the soluble OB-R molecules may vary and in particular should be based upon the recommendations and prescription of a qualified physician or veterinarian.

[0111] In accordance with the above, an assay system for screening potential drugs effective to mimic or antagonize the activity of leptin may be prepared. The prospective drug may be contacted with a soluble form of the OB-R, or alternatively may be used with cells that express a receptor form of OB-R, to determine whether it binds to, or activates (or antagonizes) OB-R. For example, in an expression assay system, the culture may be examined to observe any changes in the activity of the cells, due either to the addition of the prospective drug alone, or due to the effect of added quantities of the known weight modulator.

[0112] As stated earlier, the molecular cloning of the OB-R gene described herein has led to the identification of a class of materials that function on the molecular level to modulate mammalian body weight. The discovery of the modulators of the invention has important implications for the diagnosis and treatment of nutritional disorders including, but not limited to, obesity, weight loss associated with cancer and the treatment of diseases associated with obesity such as hypertension, heart disease, and Type II diabetes. In addition, there are potential agricultural uses for the gene product in cases where one might wish to modulate the body weight of domestic animals. The discussion that follows with specific reference to the OB-R gene bears general applicability to the class of modulators that comprise a part of the present invention, and is therefore to be accorded such latitude and scope of interpretation.

[0113] In a particular embodiment, the functional activity of the OB-R polypeptide can be evaluated transgenically. The OB-R gene can be used in complementation studies employing transgenic mice. Transgenic vectors, including viral vectors, or cosmid clones (or phage clones) corresponding to the wild type locus of candidate gene, can be

constructed using the isolated OB-R gene. Cosmids may be introduced into transgenic mice using published procedures [Jaenisch, *Science*, 240:1468-1474 (1988)]. The constructs are introduced into fertilized eggs derived from an intercross between F1 progeny of a C57BL/6J db/db X DBA intercross. Genotype at the db loci in cosmid transgenic animals can be determined by typing animals with tightly linked RFLPs or microsatellites which flank the mutation and which are polymorphic between the progenitor strains. Complementation will be demonstrated when a particular construct renders a genetically obese F2 animal (as scored by RFLP analysis) lean and nondiabetic. Under these circumstances, final proof of complementation will require that the db/db animal carrying the transgene be mated to the db/db ovarian transplants. In this cross, all N2 animals which do not carry the transgene will be obese and insulin resistant/diabetic, while those that do carry the transgene will be lean and have normal glucose and insulin concentrations in plasma. In a genetic sense, the transgene acts as a suppressor mutation.

[0114] Alternatively, OB-R genes can be tested by examining their phenotypic effects when expressed in antisense orientation in wild-type animals. In this approach, expression of the wild-type allele is suppressed, which leads to a mutant phenotype. RNA-RNA duplex formation (antisense-sense) prevents normal handling of mRNA, resulting in partial or complete elimination of wild-type gene effect. This technique has been used to inhibit TK synthesis in tissue culture and to produce phenotypes of the Kruppel mutation in *Drosophila*, and the Shiverer mutation in mice Izant et al., *Cell*, 36:1007-1015 (1984); Green et al., *Annu. Rev. Biochem.*, 55:569-597 (1986); Katsuki et al., *Science*, 241:593-595 (1988). An important advantage of this approach is that only a small portion of the gene need be expressed for effective inhibition of expression of the entire cognate mRNA. The antisense transgene will be placed under control of its own promoter or another promoter expressed in the correct cell type, and placed upstream of the SV40 polyA site. This transgene can be used to make transgenic mice. Transgenic mice can also be mated ovarian transplants to test whether ob heterozygotes are more sensitive to the effects of the antisense construct.

[0115] In the long term, the OB-R gene product (the OB-R polypeptide or protein) is useful for identifying small molecule agonists and antagonists that affect its activity.

[0116] Various terms used throughout this specification shall have the definitions set out herein, for example, below.

[0117] The term "body weight modulator", "modulator", "modulators", and any variants not specifically listed, may be used herein interchangeably, and as used throughout the present application and claims refers in one instance to both nucleotides and to proteinaceous material, the latter including both single or multiple proteins. More specifically, the aforementioned terms extend to the nucleotides and to the DNA having the sequences described herein and presented in SEQ ID NOS: 1, 3, 5, 7, and 9. Likewise, the proteins having the amino acid sequence data described herein and presented in SEQ ID NOS: 2, 4, 6, 8, and 10 are likewise contemplated, as are the profile of activities set forth with respect to all materials both herein and in the claims.

[0118] Specific binding to leptin means that leptin is a ligand for OB-R, as that term is used to describe ligand-

receptor binding. Generally, such binding will have an affinity represented by an association constant of greater than $1 \times 10^7 M^{-1}$, preferably greater than $1 \times 10^8 M^{-1}$, and more preferably greater than $1 \times 10^9 M^{-1}$. However, the exact association constant may vary. Homology with gp130 refers to conservation of residues, particularly cysteine residues, motifs, and other important residues. The term "gp130" is used herein to refer generally to the class I cytokine receptor family, particularly interleukin-6 (IL-6) receptor, granulocyte colony-stimulating factor (G-CSF) receptor, ciliary neurotrophic factor (CNTF) receptor, and leukemia inhibitory factor (LIF) receptor.

[0119] Additionally, nucleotides displaying substantially equivalent or altered activity are likewise contemplated, including substantially homologous analogs and allelic variations. Likewise, proteins displaying substantially equivalent or altered activity, including proteins modified deliberately, as for example, by site-directed mutagenesis, or accidentally through mutations in hosts that produce the modulators are likewise contemplated.

[0120] The term "allelic variants" refers to the corresponding gene in different individuals that may have point mutations. For example, the various ob mutation represent allelic variants of OB-R.

[0121] The term "substantially homologous analogs" specifically includes the corresponding gene or protein from another species. In a specific embodiment, a substantially homologous analog of murine OB-R is human OB-R. The term can also include genes or proteins mutated or altered, e.g., by substitution of variant amino acid residues from one species in the polypeptide of another, so as to correspond to an analogous gene or protein as if from another species.

[0122] The term "gene" as used herein refers to a nucleic acid, such as DNA, which codes on expression for a protein. Unless stated otherwise, gene may include mRNA, cDNA, or genomic DNA.

[0123] A composition comprising "A" (where "A" is a single protein, DNA molecule, vector, recombinant host cell, etc.) is substantially free of "B" (where "B" comprises one or more contaminating proteins, DNA molecules, vectors, etc., but excluding racemic forms of A) when at least about 75% by weight of the proteins, DNA, vectors (depending on the category of species to which A and B belong) in the composition is "A". Preferably, "A" comprises at least about 90% by weight of the A+B species in the composition, most preferably at least about 99% by weight. It is also preferred that a composition, which is substantially free of contamination, contain only a single molecular weight species having the activity or characteristic of the species of interest.

[0124] A "BAC" is a bacterial artificial chromosome; "STS" refers to sequence tagged site; a "YAC" is a yeast artificial chromosome. Other terms have the standard meanings ordinarily intended in the art.

The OB-R Polypeptides

[0125] The terms "protein," which refers to the naturally occurring polypeptide, and "polypeptide" are used herein interchangeably with respect to the OB-R gene product and variants thereof. More particularly, OB-R refers to any of the splice forms of the OB-R (DB) gene product, such as but not limited to the product with two JAC binding boxes in the

cytoplasmic domain; the product with only one JAK binding box in the cytoplasmic domain; the product with no boxes; and the secreted (soluble) product. The term OB-R also refers to various splice-forms with divergent N-terminal amino acid sequences.

[0126] The term OB-R specifically encompasses different splice forms of the polypeptide, including but not limited to the follows:

Splice Form	Characteristics	Specific Embodiment
OB-Ra	Transmembrane protein with a "Box 1" but no "Box 2"; expected to bind leptin but does not directly mediate signal transduction via JAKs. Comprised of an extracellular domain, and a truncated cytoplasmic domain. N-terminus diverges from published OB-R sequence upstream of Cys ⁸⁸ .	SEQ ID NO: 2
OB-Rb	Transmembrane protein expected to mediate leptin signalling in hypothalamus and other cells. contains a larger cytoplasmic domain containing both a "Box 1" and "Box 2" sites. N-terminal portion appears to be truncated, diverging from the published OB-R sequence upstream of Pro ⁶⁶⁴ .	SEQ ID NO: 4
OB-Rc	Corresponds to OB-Rb with a tripeptide residue C-terminal to Lys ⁸⁸⁹ rather than the longer sequence; no "Box 2" site.	SEQ ID NO: 6
OB-Rd	Corresponds to published OB-R with a different eleven amino acid sequence C-terminal to Lys ⁸⁸⁹ .	SEQ ID NO: 8
OB-Re	Soluble/secreted receptor with a leptin-binding domain. Lacks a transmembrane or cytoplasmic domain, but comprises a large extracellular domain. Corresponds to published OB-R to His ⁷⁹⁶ , where it diverges.	SEQ ID NO: 10

[0127] The term OB-R specifically contemplates splice variants that incorporate different elements from the above-noted variants, e.g., as described above.

[0128] More particularly, the present invention is directed to OB-R with the N-terminal signal sequence cleaved. In one embodiment, amino acid residues 1-22 are cleaved. In another embodiment, amino acid residues 1-27 are cleaved.

[0129] As noted above, in specific embodiments polypeptides of the invention include those having the amino acid sequences set forth herein e.g., SEQ ID NOS:2, 4, 6, 8, and 10. The term further includes polypeptides modified with conservative amino acid substitutions, as well as biologically active fragments, analogs, and derivatives thereof. The term "biologically active," is used herein to refer to a specific effect of the polypeptide, including but not limited to specific binding, e.g., to leptin, an anti-OB-R antibody, or other recognition molecule; activation of signal transduction pathways on a molecular level; and/or induction (or inhibition by antagonists) of physiological effects mediated by the native leptin in vivo. OB-R polypeptides, including fragments, analogs, and derivatives, can be prepared syntheti-

cally, e.g., using the well known techniques of solid phase or solution phase peptide synthesis. Preferably, solid phase synthetic techniques are employed. Alternatively, OB-R polypeptides of the invention can be prepared using well known genetic engineering techniques, as described infra. In yet another embodiment, the soluble form of the OB-R polypeptide can be purified, e.g., by immunoaffinity purification, from a biological fluid, such as but not limited to plasma, serum, or urine, preferably human plasma, serum, or urine, and more preferably from a subject who overexpresses the polypeptide.

[0130] The structure of the OB-R polypeptide, preferably human OB-R polypeptide, can be analyzed by various methods known in the art. The protein sequence can be characterized by a hydrophilicity analysis [e.g., Hopp et al., *Proc. Natl. Acad. Sci. USA*, 78:3824 (1981)]. A hydrophilicity profile can be used to identify the hydrophobic and hydrophilic regions of the OB-R polypeptide, which may indicate regions buried in the interior of the folded polypeptide, the transmembrane domain, and regions accessible on the exterior of the polypeptide. In addition, secondary structural analysis [e.g., Chou et al., *Biochem.*, 13:222 (1974)] can also be done, to identify regions of OB-R polypeptide that assume specific secondary structures. Manipulation of the predicted or determined structure, including secondary structure prediction, can be accomplished using computer software programs available in the art.

[0131] By providing an abundant source of recombinant OB-R polypeptide, the present invention enables quantitative structural determination of the polypeptide. In particular, enough material is provided for nuclear magnetic resonance (NMR), infrared (IR), Raman, and ultraviolet (UV), especially circular dichroism (CD), spectroscopic analysis. In particular NMR provides very powerful structural analysis of molecules in solution, which more closely approximates their native environment [Marion et al., *Biochim. Biophys. Res. Comm.*, 113:967-974 (1983); Bar et al., *J. Magn. Reson.*, 65:355-360 (1985); Kimura et al., *Proc. Natl. Acad. Sci. USA*, 77:1681-1685 (1980)]. Other methods of structural analysis can also be employed. These include but are not limited to X-ray crystallography [Engstrom, *Biochem. Exp. Biol.*, 11:7-13 (1974)]. In a preferred aspect, either soluble form or a membrane-binding form of OB-R is co-crystallized with leptin to provide structural information about both molecules.

[0132] In yet a further embodiment, an analog of OB-R polypeptide can be tested to determine whether it cross-reacts with an antibody specific for native OB-R polypeptide, or specific fragments thereof. The degree of cross-reactivity provides information about structural homology or similarity of proteins, or about the accessibility of regions corresponding to portions of the polypeptide that were used to generate fragment-specific antibodies.

Fragments of the OB-R Polypeptide

[0133] In a particular embodiment, the present invention contemplates that naturally occurring fragments, or truncated forms, of the OB-R polypeptide may be important. As noted above, a large number of splice forms of OB-R have been found. Thus, the present invention encompasses a naturally occurring soluble form of the OB-R, as well as integral membrane forms that have 0, 1, or 2 JAK box

consensus sites. In addition to the naturally occurring splice isoforms of the polypeptide, the present invention further envisions recombinantly modified isoforms, e.g. by deletion of one or more of the cytoplasmic domain; the cytoplasmic consensus domain from the transmembrane domain to lysine-889; the box 1 or box two, or both regions; the cytoplasmic domain C-terminal of lysine-889; the transmembrane domain; the ligand binding domain; the extracytoplasmic domain; or portions thereof.

OB-R Polypeptide Chimeras

[0134] One or more of the splice-forms of the cytoplasmic domain can be used in a chimeric construct with another ligand-binding domain to artificially signal leptin binding [e.g., Capon et al., U.S. Pat. No. 5,359,046, issued Oct. 25, 1994; Sanchez et al., *J. Exp. Med.*, 178:1049 (1993); Burkhardt et al., *Mol. Cell. Biol.*, 14:1095]. In another embodiment, the extracytoplasmic (leptin-binding) domain can be joined to a different cytoplasmic signal transduction domain, or alternatively to a glycosyl-phosphatidylinositol linker domain to provide for activation of cells via gp130.

Analogs of the OB-R Polypeptide

[0135] The present invention specifically contemplates preparation of analogs of the OB-R polypeptide, which are characterized by being capable of a biological activity of OB-R polypeptide, e.g., of binding to leptin or to an anti-OB-R antibody. In one embodiment, the analog agonizes OB-R activity. Preferably, an OB-R agonist is more effective than the native protein. For example, an OB-R agonist analog may bind to leptin with higher affinity, thus amplifying the signal. Such an analog may be particularly desirable for gene therapy, where increased signal transduction efficiency can compensate for any deficiency in the level of expression. In another embodiment, the analog antagonizes OB-R activity. For example, an OB-R analog that binds to leptin, and inhibits leptin binding to signal-transduction competent OB-R, can competitively inhibit binding of native OB to the receptor, thus decreasing leptin activity in vivo. Such an OB-R antagonist analog is preferably a soluble form of the OB-R.

[0136] In one embodiment, an analog of OB-R polypeptide is the OB-R polypeptide modified by substitution of amino acids at positions on the polypeptide that are not essential for structure or function. For example, since it is expected that human OB-R polypeptide is biologically active in mouse, substitution of divergent amino acid residues in the human sequence as compared to the murine amino acid sequence will likely yield useful analogs of OB-R polypeptide. For example, the following residues in the human OB-R [numbering for human OB-R amino acids employs the numbering convention adopted in Tartaglia et al., *Cell*, 83:1263 (1995)] could be substituted with a divergent murine residue found at that position, or with a non-conservative amino acid substitution, such as one or more of: Phe for Ser³⁶; Asp for Tyr⁴⁴; Ser for Leu⁴⁹; Pro for Ser⁵⁴; Leu for Ser⁶⁰; Ala for His⁶³; Ala for Thr⁶⁶; Ala for Pro⁷⁰; Ile for Thr⁷⁷; Tyr for His⁷⁸; Pro for Ser⁸⁰; Gly for Arg⁹²; Gly for Asp⁹⁶; Thr for Ala¹⁰³ or Ile¹⁰⁶; Ser for Leu¹¹⁸; Gly for Asp¹²⁴; Thr for Lys¹³⁸; Pro for Ser¹⁴⁶; Asp for Val¹⁶⁴; Leu for Gln¹⁷⁷; Asp for Gly¹⁷⁹; Gly for Glu¹⁹²; deletion for Cys¹⁹³; His for Leu¹⁹⁷; Ser for Ile²²¹; Leu for Asn²³³; Leu for Ser²⁷³; deletion for Thr²⁷⁸; Ala for Asp²⁸⁵; Glu for

Lys²⁸⁶; Ser for Gly³¹⁰; Arg for Met³⁷⁰; Ile for Ser³⁷⁹; Ser for Phe³⁹⁴; Ala for Glu⁴¹⁷; Gly for Glu⁴⁵⁹; Ser for Ile⁴⁷⁶; Thr for Ile⁴⁸²; Thr for Ile⁵⁵¹; His for Tyr⁵⁸⁶; Lys for Ile⁶⁴⁸; Ala for Ser⁶⁸⁶; His for Cys⁶⁸⁷; Thr for Ile⁷⁵⁹; Ile for Asn⁷⁷⁶; Asp for Gly⁷⁸¹; Gly for Glu⁷⁸²; Gly for Ser⁸²⁷; Ala for Asp⁸³²; Arg for Pro⁸⁹²; Thr for Glu⁸⁹³; Asp for Thr⁸⁹⁴; or Leu for Glu⁸⁹⁶.

[0137] Also contemplated by the present invention are analogs comprising conservative amino acid substitutions. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity, which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. In some instances, one polar amino acid may be substituted with another to preserve local hydrophilicity; more likely, a substitution that conserves charge, or at least does not introduce the opposite charge, is required. Such alterations will not be expected to affect apparent molecular weight as determined by polyacrylamide gel electrophoresis, or isoelectric point.

[0138] In still another embodiment, amino acid residues can be substituted with residues to form analogs of OB-R polypeptide that demonstrate enhanced propensity for forming, or which form more stable, secondary structures. For example, α -helix structure would be preferred if Glu, Ala, Leu, His, Trp are introduced as substitutes for amino acid residues found in the native OB polypeptide. Preferably, conservative amino acid substitutions are employed, e.g., substituting aspartic acid with glutamic acid(s) (Glu); substituting isoleucine(s) with leucine; substituting glycine or valine, or any divergent amino acid (i.e., an amino acid that is not conserved between OB-R from different species), with alanine (e.g., serine at position 273 of the human OB-R polypeptide with alanine); substituting arginine or lysine with histidine; and substituting tyrosine and/or phenylalanine with tryptophan. Increasing the degree, or more importantly, the stability of α -helix structure may yield an OB-R analog with greater activity, increased binding affinity, or longer half-life. Also contemplated are truncated OB-R polypeptide analogs are generated that incorporate structure-forming, e.g., helix-forming, amino acid residues to compensate for the greater propensity of polypeptide fragments to lack stable structure.

[0139] In another embodiment, an analog of the OB-R polypeptide, preferably the human OB-R polypeptide, is a truncated form of the polypeptide. For example, it has already been demonstrated that the transmembrane domain is not essential, since a naturally occurring isoform of the polypeptide is encoded by cDNA that expresses a soluble protein. Similarly, it may be possible to delete some or all of the divergent amino acid residues (as compared to the murine OB-R). In addition, the invention contemplates providing an OB-R analog having the minimum amino acid sequence necessary for a biological activity. This can be

readily determined, e.g., by testing the activity of fragments of OB-R for the ability to bind to OB-R-specific antibodies, inhibit the activity of the native leptin (by competitive binding), or agonize the activity of native leptin.

[0140] The present invention specifically contemplates providing a soluble splice-form of the OB-R that is believed to agonize leptin activity. In particular, it is believed that OB-Rd (as referred to herein) binds leptin, and facilitates leptin binding to OB-Re (which is believed to be competent for signal transduction). Thus, in this embodiment, OB-R appears to behave analogously to other receptor systems [Kishimoto et al., *Cell*, 76:253 (1994); Davis et al., *Science*, 260:1805 (1993); Davis et al., *Science*, 259:1736 (1993)].

[0141] It will be appreciated by one of ordinary skill in the art that the foregoing fragment sizes are approximate, and that additional amino acids e.g. from one to about five, can be included or deleted from each or both ends, or from the interior of the polypeptide or fragments thereof, of the recited truncated analogs.

[0142] Analogs, such as fragments, may be produced, for example, by pepsin digestion of the OB-R. Other analogs, such as muteins, can be produced by standard site-directed mutagenesis of weight modulator peptide coding sequences.

Screening for Leptin Analogs

[0143] Various screening techniques are known in the art for screening for analogs of polypeptides. Various libraries of chemicals are available. Accordingly, the present invention contemplates screening such libraries, e.g., libraries of synthetic compounds generated over years of research, libraries of natural compounds, and combinatorial libraries, as described in greater detail, *infra*, for analogs of leptin. The invention contemplates screening such libraries for compounds that bind to OB-R, either in soluble or transmembrane forms. Preferably, such molecules agonize or antagonize signal transduction by OB-R. The present invention contemplates screens for small molecule ligands or ligand analogs and mimics, as well as screens for natural ligands that bind to and agonize or antagonize activate OB receptor *in vivo*.

[0144] Knowledge of the primary sequence of the receptor, and the similarity of that sequence with proteins of known function, can provide an initial clue as to the agonists or antagonists of the protein. Identification and screening of antagonists is further facilitated by determining structural features of the protein, e.g., using X-ray crystallography, neutron diffraction, nuclear magnetic resonance spectrometry, and other techniques for structure determination. These techniques provide for the rational design or identification of agonists and antagonists.

[0145] Another approach uses recombinant bacteriophage to produce large libraries. Using the "phage method" [Scott et al., *Science*, 249:386-390 (1990); Cwirla et al., *Proc. Natl. Acad. Sci. USA*, 87:6378-6382 (1990); Devlin et al., *Science*, 249:404-406 (1990)], very large libraries can be constructed (10^6 - 10^8 chemical entities). A second approach uses primarily chemical methods, of which the Geysen method [Geysen et al., *Molecular Immunology*, 23:709-715 (1986); Geysen et al., *J. Immunologic Method*, 102:259-274 (1987)] and the recent method of Fodor et al., *Science*, 251:767-773 (1991) are examples. Furka et al. 14th International Con-

gress of Biochemistry, Volume 5, Abstract FR:013 (1988); Furka, *Int. J. Peptide Protein Res.*, 37:487-493 (1991)]; Houghton (U.S. Pat. No. 4,631,211, issued Dec. 1986); and Rutter et al. (U.S. Pat. No. 5,010,175, issued Apr. 23, 1991) describe methods to produce a mixture of peptides that can be tested as agonists or antagonists.

[0146] In another aspect, synthetic libraries [Needels et al., *Proc. Natl. Acad. Sci. USA*, 90:10700-10704 (1993); Lam et al., International Patent Publication No. WO 92/00252, each of which is incorporated herein by reference in its entirety], and the like can be used to screen for OB receptor ligands according to the present invention.

[0147] In particular, assays for binding of soluble ligand to cells that express recombinant forms of the OB receptor ligand binding domain can be performed. The soluble ligands can be provided readily as recombinant or synthetic leptin polypeptide.

[0148] The screening can be performed with recombinant cells that express the OB receptor, or alternatively, using purified receptor protein, e.g., produced recombinantly, as described above. For example, the ability of labeled, soluble, or solubilized OB receptor, that includes the ligand-binding portion of the molecule, to bind ligand can be used to screen libraries, as described in the foregoing references.

Derivatives of OB Polypeptides

[0149] Generally, a soluble form of the present polypeptide may be derivatized by the attachment of one or more chemical moieties to the polypeptide moiety. The chemically modified derivatives may be further formulated for intraarterial, intraperitoneal, intramuscular, subcutaneous, intravenous, oral, nasal, rectal, buccal, sublingual, pulmonary, topical, transdermal, or other routes of administration. Chemical modification of biologically active proteins has been found to provide additional advantages under certain circumstances, such as increasing the stability and circulation time of the therapeutic protein and decreasing immunogenicity. See U.S. Pat. No. 4,179,337, Davis et al., issued Dec. 18, 1979. For a review, see Abuchowski et al., "Soluble Polymer-Enzyme Adducts", in *Enzymes as Drugs*, pp. 367-383, Holcenberg and Roberts, eds., Wiley-Interscience, New York, N.Y., (1981). A review article describing protein modification and fusion proteins is Francis, *Focus on Growth Factors*, 3:4-10 (1992).

Chemical Moieties For Derivatization

[0150] The chemical moieties suitable for derivatization may be selected from among water soluble polymers. The polymer selected should be water soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable. One skilled in the art will be able to select the desired polymer based on such considerations as whether the polymer/protein conjugate will be used therapeutically, and if so, the desired dosage, circulation time, resistance to proteolysis, and other considerations. For the present proteins and peptides, these may be ascertained using the assays provided herein.

Polymer Molecules

[0151] The water soluble polymer may be selected from the group consisting of, for example, polyethylene glycol,

copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols and polyvinyl alcohol. Polyethylene glycol propionaldehyde may provide advantages in manufacturing due to its stability in water.

[0152] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 2 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

Polymer/Protein Ratio

[0153] The number of polypeptide molecules attached to each polymer may vary, and one skilled in the art will be able to ascertain the effect on function. One may monoderivatize, or may provide for a di-, tri-, tetra- or some combination of derivatization, with the same or different chemical moieties (e.g., polymers, such as different weights of polyethylene glycols). The proportion of polymer molecules to protein (or peptide) molecules will vary, as will their concentrations in the reaction mixture. In general, the optimum ratio (in terms of efficiency of reaction in that there is no excess unreacted protein or polymer) will be determined by factors such as the desired degree of derivatization (e.g., mono, di-, tri-, etc.), the molecular weight of the polymer selected, whether the polymer is branched or unbranched, and the reaction conditions.

Attachment of the Chemical Moiety to the Protein

[0154] The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384 herein incorporated by reference (coupling PEG to G-CSF). See also Malik et al., *Exp. Hematol.*, 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues, glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecule(s). Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group. Attachment at residues important for receptor binding should be avoided if receptor binding is desired.

N-terminally Chemically Modified Proteins

[0155] One may specifically desire N-terminally chemically modified protein. Using polyethylene glycol as an illustration of the present compositions, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective N-terminal chemical modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminus) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved. For example, one may selectively N-terminally pegylate the protein by performing the reaction at a pH which allows one to take advantage of the pK_a differences between the ϵ -amino groups of the lysine residues and that of the α -amino group of the N-terminal residue of the protein. By such selective derivatization attachment of a water soluble polymer to a protein is controlled: the conjugation with the polymer takes place predominantly at the N-terminus of the protein and no significant modification of other reactive groups, such as the lysine side chain amino groups, occurs. Using reductive alkylation, the water soluble polymer may be of the type described above, and should have a single reactive aldehyde for coupling to the protein. Polyethylene glycol propionaldehyde, containing a single reactive aldehyde, may be used.

Nucleic Acids Associated with OB-R Polypeptide

[0156] As noted above, the present invention is directed to nucleic acids encoding OB-R polypeptides, as well as associated genomic non-coding sequences 5', 3', and intronic to the OB-R gene. Thus, in accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); Glover ed., *DNA Cloning: A Practical Approach*, Volumes I and II, IRL Press, Ltd., Oxford, U.K. (1985); Gait ed., *Oligonucleotide Synthesis*, Oxford University Press (1984); Hames et al., eds., *Nucleic Acid Hybridization*, Springer-Verlag (1985); Hames et al., eds. *Transcription And Translation*, Oxford University Press (1984); Freshney ed., *Animal Cell Culture*, Oxford University Press (1986); Perbal, *A Practical Guide To Molecular Cloning*, Wiley, New York (1984). Of particular relevance to the present invention are strategies for isolating, cloning, sequencing, analyzing, and characterizing a gene or nucleic acid based on the well known polymerase chain reaction (PCR) techniques.

[0157] A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication in vivo, i.e., capable of replication under its own control.

[0158] A "vector" is a replicon, such as a plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

[0159] A "cassette" refers to a segment of DNA that can be inserted into a vector at specific restriction sites. The segment of DNA encodes a polypeptide of interest, and the cassette and restriction sites are designed to ensure insertion of the cassette in the proper reading frame for transcription and translation.

[0160] "Heterologous" DNA refers to DNA not naturally located in the cell, or in a chromosomal site of the cell. Preferably, the heterologous DNA includes a gene foreign to the cell.

[0161] A cell has been "transfected" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. A cell has been "transformed" by exogenous or heterologous DNA when the transfected DNA effects a phenotypic change. Preferably, the transforming DNA should be integrated (covalently linked) into chromosomal DNA making up the genome of the cell.

[0162] A "clone" is a population of cells derived from a single cell or common ancestor by mitosis.

[0163] A "nucleic acid molecule" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules") in either single-stranded form, or a double-stranded helix. Double-stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary or quaternary forms. Thus, this term includes double-stranded DNA found, inter alia, in linear or circular DNA molecules (e.g., restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

[0164] A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single-stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (see Sambrook et al., 1989, supra). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a T_m of 55° C., can be used, e.g., 5×SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5×SSC, 0.5% SDS). Moderate stringency hybridization conditions correspond to a higher T_m , e.g., 40% formamide, with 5× or 6×SSC. High stringency

hybridization conditions correspond to the highest T_m , e.g., 50% formamide, 5× or 6×SSC. Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (see Sambrook et al., 1989, supra, 9.50-0.51). For hybridization with shorter nucleic acids, i.e., oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., 1989, supra, 11.7-11.8). Preferably a minimum length for a hybridizable nucleic acid is at least about 10 nucleotides; more preferably at least about 15 nucleotides; most preferably the length is at least about 20 nucleotides.

[0165] “Homologous recombination” refers to the insertion of a foreign DNA sequence of a vector in a chromosome. Preferably, the vector targets a specific chromosomal site for homologous recombination. For specific homologous recombination, the vector will contain sufficiently long regions of homology to sequences of the chromosome to allow complementary binding and incorporation of the vector into the chromosome. Longer regions of homology, and greater degrees of sequence similarity, may increase the efficiency of homologous recombination.

[0166] A DNA “coding sequence” is a double-stranded DNA sequence which is transcribed and translated into a polypeptide in a cell in vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Isolation of OB-R Coding and Flanking Sequences

[0167] The nucleic acids contemplated by the present invention include nucleic acids that code on expression for peptides such as those set forth in SEQ ID NOS:2, 4, 6, 8, and 10. Accordingly, while specific DNA has been isolated and sequenced in relation to the OB-R gene, any animal cell potentially can serve as the nucleic acid source for the molecular cloning of a gene encoding the polypeptides of the invention. The DNA may be obtained by standard procedures known in the art from cloned DNA (e.g., a DNA “library”), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell (See, for example, Sambrook et al., 1989, supra; Glover, 1985, supra). Clones derived from genomic DNA may contain regulatory and intronic DNA

regions in addition to coding regions; clones derived from cDNA will not contain intron sequences. Whatever the source, the gene should be molecularly cloned into a suitable vector for propagation of the gene.

[0168] In the molecular cloning of the gene from genomic DNA, the genomic DNA can be amplified using primers selected from the cDNA sequences. Alternatively, DNA fragments are generated, some of which will encode the desired gene. The DNA may be cleaved at specific sites using various restriction enzymes. One may also use DNase in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by sonication. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

[0169] Once the DNA fragments are generated, identification of the specific DNA fragment containing the desired OB-R-gene may be accomplished in a number of ways. For example, if an amount of a portion of a OB-R-gene or its specific RNA, or a fragment thereof, is available and can be purified and labeled, the generated DNA fragments may be screened by nucleic acid hybridization to a labeled probe [Benton et al., *Science*, 196:180 (1977); Grunstein et al., *Proc. Natl. Acad. Sci. USA*, 72:3961 (1975)]. The present invention provides such nucleic acid probes, which can be conveniently prepared from the specific sequences disclosed herein, e.g., a hybridizable probe having a nucleotide sequence corresponding to at least a 10, and preferably a 15, nucleotide fragment of the sequences depicted in SEQ ID NOS:1, 3, 5, 7, and 9. Preferably, a fragment is selected that is highly unique to the modulator peptides of the invention. Those DNA fragments with substantial homology to the probe will hybridize. As noted above, the greater the degree of homology, the more stringent the hybridization conditions that can be used. In one embodiment, low stringency hybridization conditions are used to identify a homologous modulator peptide. However, in a preferred aspect, and as demonstrated experimentally herein, a nucleic acid encoding a polypeptide of the invention will hybridize to a nucleic acid having a nucleotide sequence such as depicted in (SEQ ID NOS:1, 3, 5, 7, and 9), or a hybridizable fragment thereof, under moderately stringent conditions; more preferably, it will hybridize under high stringency conditions.

[0170] In another specific embodiment, the DNA of the invention can be identified using one of the PCR probes obtained by exon trapping and cDNA selection. For example, the primer pairs described in Example 3 can be used to will amplify a DNA of the invention.

[0171] Preferably, these primers will amplify DNA under moderately to high stringency conditions, e.g., using pre-hybridization at 65° using Rapid-hyb buffer (Amersham Life Sciences), followed by hybridization for 6 hours at 65°, followed by washing first with 2×SSC/0.1% SDS for 30 min at room temperature (RT), and a second wash at higher stringency with 0.3×SSC/0.1% SDS, RT, for 30 min. As will be appreciated by those of skill in the art, the stringency of the second wash is flexible and depends on the length of the probe and the degree of sequence similarity of each probe. For example, since human and mouse coding regions are about 78% homologous, the same hybridization conditions may be employed with a lower the stringency second wash

(e.g., twice with $2\times\text{SSC}/0.1\%$ SDS, RT). If this results in no signal with no-background, hybridization can be attempted at a lower temperature (lower stringency), e.g., 42°C . If there is too much background, the stringency of the second wash can be increased, (e.g., 0.5 or $0.3\times\text{SSC}$, 0.1% SDS, RT). According to the invention, the above-noted PCR probes will define a nucleic acid molecule, e.g., DNA, encoding OB-R from human as well as murine DNA libraries under similar hybridization conditions.

[0172] Alternatively, the presence of the gene may be detected by assays based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, can be selected which produce a protein that, e.g., has similar or identical electrophoretic migration, isoelectric focusing behavior, proteolytic digestion maps, tyrosine phosphatase activity, or antigenic properties as known for the present OB-R. For example, antibodies of the instant invention can conveniently be used to screen for homologs of OB-R from other sources.

[0173] A gene encoding a polypeptide of the invention can also be identified by mRNA selection, i.e., by nucleic acid hybridization followed by *in vitro* translation. In this procedure, fragments are used to isolate complementary mRNAs by hybridization. Such DNA fragments may represent available, purified modulator DNA. Immunoprecipitation analysis or functional assays (e.g., tyrosine phosphatase activity) of the *in vitro* translation products of the products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments, that contain the desired sequences. In addition, specific mRNAs may be selected by adsorption of polysomes isolated from cells to immobilized antibodies specifically directed against a modulator peptide.

[0174] A radiolabeled modulator peptide cDNA can be synthesized using the selected MRNA (from the adsorbed polysomes) as a template. The radiolabeled mRNA or cDNA may then be used as a probe to identify homologous modulator peptide DNA fragments from among other genomic DNA fragments.

[0175] As mentioned above, a DNA sequence encoding weight modulator peptides as disclosed herein can be prepared synthetically rather than cloned. The DNA sequence can be designed with the appropriate codons for the OB-R amino acid sequences. In general, one will select preferred codons for the intended host if the sequence will be used for expression. The complete sequence may be assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, *Nature*, 292:756 (1981); Nambair et al., *Science*, 223:1299 (1984); Jay et al., *J. Biol. Chem.*, 259:6311 (1984).

[0176] Synthetic DNA sequences allow convenient construction of genes which will express OB-R analogs, as described above. Alternatively, DNA encoding analogs can be made by site-directed mutagenesis of native OB-R genes or cDNAs, and analogs can be made directly using conventional polypeptide synthesis.

[0177] A general method for site-specific incorporation of unnatural amino acids into proteins is described in Noren et al, *Science*, 244:182-188 (1989). This method may be used to create analogs of the OB-R polypeptide with unnatural amino acids.

[0178] Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as a OB-R gene may be used in the practice of the present invention. These include but are not limited to allelic genes, homologous genes from other species, and nucleotide sequences comprising all or portions of OB-R genes which are altered by the substitution of different codons that encode the same amino acid residue within the sequence, thus producing a silent change. Likewise, the OB-R derivatives of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of a OB-R protein including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a conservative amino acid substitution, as described above in connection with OB-R analogs.

Non-coding Nucleic Acids

[0179] The present invention extends to the preparation of antisense nucleotides and ribozymes that may be used to interfere with the expression of the proteins at the translational level. This approach utilizes antisense nucleic acid and ribozymes to block translation of a specific mRNA, either by masking that mRNA with an antisense nucleic acid or cleaving it with a ribozyme.

[0180] Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule [See Weintraub, *Sci. Am.*, 262:40-46 (1990); Marcus-Sekura, *Anal. Biochem.*, 172:289-295 (1988)]. In the cell, they hybridize to that mRNA, forming a double-stranded molecule. The cell does not translate an mRNA complexed in this double-stranded form. Therefore, antisense nucleic acids interfere with the expression of mRNA into protein. Oligomers of about fifteen nucleotides and molecules that hybridize to the AUG initiation codon will be particularly efficient, since they are easy to synthesize and are likely to pose fewer problems than larger molecules when introducing them into weight modulator peptide-producing cells. Antisense methods have been used to inhibit the expression of many genes *in vitro* [(Marcus-Sekura, 1988 *supra*; Hambor et al., *J. Exp. Med.*, 168:1237-1245 (1988)].

[0181] Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA molecules in a manner somewhat analogous to DNA restriction endonucleases. Ribozymes were discovered from the observation that certain mRNAs have the ability to excise their own introns. By modifying the nucleotide sequence of these RNAs, researchers have been able to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it [Cech, *J. Am. Med. Assoc.*, 260:3030-3034 (1988)]. Because ribozymes are sequence-specific, only mRNAs with particular sequences are inactivated.

[0182] Investigators have identified two types of ribozymes, *Tetrahymena*-type and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize four-base sequences, while "hammerhead"-type recognize eleven- to eighteen-base sequences. The longer the recognition sequence, the more likely it is to occur exclusively in the target mRNA species. Therefore, hammerhead-type ribozymes are prefer-

able to *Tetrahymena*-type ribozymes for inactivating a specific mRNA species, and eighteen base recognition sequences are preferable to shorter recognition sequences.

[0183] The DNA sequences described herein may thus be used to prepare antisense molecules against and ribozymes that cleave mRNAs for weight modulator proteins and their ligands, thus inhibiting expression of the OB-R gene, and leading to increased weight gain and adiposity.

[0184] In another embodiment, short oligonucleotides complementary to the coding and complementary strands of the OB-R nucleic acid, or to non-coding regions of the OB-R gene 5', 3', or internal (intronic) to the coding region are provided by the present invention. Such nucleic acids are useful as probes, either as directly labeled oligonucleotide probes, or as primers for the polymerase chain reaction, for evaluating the presence of mutations in the ob-r gene, or the level of expression of OB-R mRNA. Preferably, the non-coding nucleic acids of the invention are from the human OB-R gene.

[0185] In a specific embodiment, the non-coding nucleic acids provide for homologous recombination for integration of an amplifiable gene and/or other regulatory sequences in proximity to the OB-R gene, e.g., to provide for higher levels of expression of the OB-R polypeptide, or to overcome a mutation in the ob-r gene regulatory sequences that prevent proper levels of expression of the OB-R polypeptide (See International Patent Publication WO 91/06666, published May 16, 1991 by Skoultchi; International Patent Publication No. WO 91/09955, published Jul. 11, 1991 by Chappel; see also International Patent Publication No. WO 90/14092, published Nov. 29, 1990, by Kucherlapati and Campbell).

Production of OB-R Polypeptide: Expression and Synthesis

[0186] Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

[0187] A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then trans-RNA spliced and translated into the protein encoded by the coding sequence.

[0188] A "signal sequence" is included at the beginning of the coding sequence of a protein to be expressed on the surface of a cell. This sequence encodes a signal peptide, N-terminal to the mature polypeptide, that directs the host cell to translocate the polypeptide. The term "translocation signal sequence" is also used herein to refer to this sort of signal sequence. Translocation signal sequences can be found associated with a variety of proteins native to eukaryotes and prokaryotes, and are often functional in both types of organisms. According to the present invention, amino acid residues 1-27 of the murine and human OB-R polypeptides (see SEQ ID NOS:8, 10) comprise the signal peptide. In another embodiment, amino acid residues 1-22 comprise the signal peptide [Tartaglia et al., *Cell*, 83:1263 (1995)].

[0189] A DNA sequence is "operatively linked" to an expression control sequence when the expression control

sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted upstream (5') of and in reading frame with the gene.

[0190] A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

[0191] Another feature of this invention is the expression of the DNA sequences disclosed herein. As is well known in the art, DNA sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and employing that expression vector to transform an appropriate unicellular host.

[0192] Such operative linking of a DNA sequence of this invention to an expression control sequence, of course, includes, if not already part of the DNA sequence, the provision of an initiation codon, ATG, in the correct reading frame upstream of the DNA sequence.

[0193] A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal, and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, e.g., *E. coli* plasmids col E1, pCR1, pBR322, pMB9, pUC or pUC plasmid derivatives, e.g., pGEX vectors, pET vectors, pmal-c, pFLAG, etc., and their derivatives, plasmids such as RP4; phage DNAs, e.g., the numerous derivatives of phage λ , such as NM989, and other phage DNA, e.g., M13 and filamentous single-stranded phage DNA; yeast plasmids such as the 2 μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like.

[0194] Any of a wide variety of expression control sequences—sequences that control the expression of a DNA sequence operatively linked to it—may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences include, for example, the early or late promoters of SV40, CMV, vaccinia, polyoma or adenovirus, the lac system, the trp system, the TAC system, the TRC system, the LTR system, the major operator and promoter regions of phage λ , the control

regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase (e.g., Pho5), the AOX 1 promoter of methylotrophic yeast, the promoters of the yeast α -mating factors, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

[0195] A wide variety of unicellular host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*; fungi such as yeasts (*Saccharomyces*, and methylotrophic yeast such as *Pichia*, *Candida*, *Hansenula*, and *Torulopsis*); and animal cells, such as CHO, R1.1, B-W and LM cells, African Green Monkey kidney cells (e.g., COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (e.g., Sf9), and human cells and plant cells in tissue culture.

[0196] It will be understood that not all vectors, expression control sequences and hosts will function equally well to express the DNA sequences of this invention. Neither will all hosts function equally well with the same expression system. However, one skilled in the art will be able to select the proper vectors, expression control sequences, and hosts without undue experimentation to accomplish the desired expression without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must function in it. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, will also be considered.

[0197] In selecting an expression control sequence, a variety of factors will normally be considered. These include, for example, the relative strength of the system, its controllability, and its compatibility with the particular DNA sequence or gene to be expressed, particularly as regards potential secondary structures. Suitable unicellular hosts will be selected by consideration of, e.g., their compatibility with the chosen vector, their secretion characteristics, their ability to fold proteins correctly, and their fermentation requirements, as well as the toxicity to the host of the product encoded by the DNA sequences to be expressed, and the ease of purification of the expression products.

[0198] Considering these and other factors, a person skilled in the art will be able to construct a variety of vector/expression control sequence/host combinations that will express the DNA sequences of this invention on fermentation or in large scale animal culture.

[0199] In a specific embodiment, an OB-R fusion protein can be expressed. An OB-R fusion protein comprises at least a functionally active portion of a non-OB-R protein joined via a peptide bond to at least a functionally active portion of an OB polypeptide. The non-OB-R sequences can be amino- or carboxy-terminal to the OB-R sequences. For example, in preparing "artificial" receptors, joining the OB-R encoding coding domain for the leptin binding (extracytoplasmic) portion at the 5' position will yield a protein that binds leptin and mediates some other action based on the non-OB-R protein's activity. Conversely, joining a different protein (such as a growth factor, cytokine, or hormone receptor binding coding domain) 5' to a OB-R cytoplasmic coding

domain (containing "Box 1" and "Box 2") will allow for activation via OB-R upon binding a different ligand than leptin. In another embodiment, a chimeric construct may simply facilitate expression of OB-R.

[0200] In another aspect, the pGEX vector [Smith et al., Gene 67:31-40 (1988)] can be used. This vector fuses the schistosoma japonicum glutathionine S-transferase cDNA to the sequence of interest. Bacterial proteins are harvested and recombinant proteins can be quickly purified on a reduced glutathione affinity column. The GST carrier can subsequently be cleaved from fusion proteins by digestion with site-specific proteases. After cleavage, the carrier and uncleaved fusion protein can be removed by absorption on glutathione agarose. Difficulty with the system occasionally arises when the encoded protein is insoluble in aqueous solutions.

[0201] Expression of recombinant proteins in bacterial systems may result in incorrect folding of the expressed protein, requiring refolding. The recombinant protein can be refolded prior to or after cleavage to form a functionally active OB polypeptide. The OB polypeptide may be refolded by the steps of (i) incubating the protein in a denaturing buffer that contains a reducing agent, and then (ii) incubating the protein in a buffer that contains an oxidizing agent, and preferably also contains a protein stabilizing agent or a chaotropic agent, or both. Suitable redox (reducing/oxidizing) agent pairs include, but are not limited to, reduced glutathione/glutathione disulfide, cystine/cysteine, cystamine/cysteamine, and 2-mercaptoethanol/2-hydroxyethylid-isulfide. In a particular aspect, the fusion protein can be solubilized in a denaturant, such as urea, prior to exchange into the reducing buffer. In preferred embodiment, the protein is also purified, e.g., by ion exchange or Ni-chelation chromatography, prior to exchange into the reducing buffer. Denaturing agents include but are not limited to urea and guanidine-HCl. The recombinant protein is then diluted about at least 10-fold, more preferably about 100-fold, into an oxidizing buffer that contains an oxidizing agent, such as but not limited to 0.1 M Tris-HCl, pH 8.0, 1 mM EDTA, 0.15 M NaCl, 0.3 M oxidized glutathione. The fusion protein is then incubated for about 1 to about 24 hours, preferably about 2 to about 16 hours, at room temperature in the oxidizing buffer. The oxidizing buffer may comprise a protein stabilizing agent, e.g., a sugar, an alcohol, or ammonium sulfate. The oxidizing buffer may further comprise a chaotropic agent at low concentration, to destabilize incorrect intermolecular interactions and thus promote proper folding. Suitable chaotropic agents include but are not limited to a detergent, a polyol, L-arginine, guanidine-HCl and polyethylene glycol (PEG). It is important to use a low enough concentration of the chaotropic agent to avoid denaturing the protein. The refolded protein can be concentrated by at least about 10-fold, more preferably by the amount it was diluted into the oxidizing buffer.

[0202] Alternatively, the invention contemplates periplasmic expression of a protein of the invention.

[0203] Bacterial fermentation processes can also result in a recombinant protein preparation that contains unacceptable levels of endotoxins. Therefore, the invention contemplates removal of such endotoxins, e.g., by using endotoxin-specific antibodies or other endotoxin binding molecules. The presence of endotoxins can be determined by standard

techniques, such as by employing E-TOXATE Reagents (Sigma, St. Louis, Mo.), or with bioassays.

[0204] In addition to the specific example, the present inventors contemplate use of baculovirus, mammalian, and yeast expression systems to express the ob protein. For example, in baculovirus expression systems, both non-fusion transfer vectors, such as but not limited to pVL941 (BamHI cloning site; Summers), pVL1393 (BamHI, SmaI, XbaI, EcoRI, NotI, XmaIII, BglII, and PstI cloning site; Invitrogen), pVL1392 (BglII, PstI, NotI, XmaIII, EcoRI, XbaI, SmaI, and BamHI cloning site; Summers and Invitrogen), and pBlueBacIII (BamHI, BglII, PstI, NcoI, and HindIII cloning site, with blue/white recombinant screening possible; Invitrogen), and fusion transfer vectors, such as but not limited to pAc700 (BamHI and KpnI cloning site, in which the BamHI recognition site begins with the initiation codon; Summers), pAc701 and pAc702 (same as pAc700, with different reading frames), pAc360 (BamHI cloning site 36 base pairs downstream of a polyhedrin initiation codon; Invitrogen(195)), and pBlueBacHisA, B, C (three different reading frames, with BamHI, BglII, PstI, NcoI, and HindIII cloning site, an N-terminal peptide for ProBond purification, and blue/white recombinant screening of plaques; Invitrogen (220)).

[0205] Mammalian expression vectors contemplated for use in the invention include vectors with inducible promoters, such as the dihydrofolate reductase (DHFR) promoter, e.g., any expression vector with a DHFR expression vector, or a DHFR/methotrexate co-amplification vector, such as pED [PstI, Sall, SbaI, SmaI, and EcoRI cloning site, with the vector expressing both the cloned gene and DHFR; see Kaufman, *Current Protocols in Molecular Biology*, 16.12 (1991)]. Alternatively, a glutamine synthetase/methionine sulfoximine co-amplification vector, such as pEE14 (HindIII, XbaI, SmaI, SbaI, EcoRI, and BclI cloning site, in which the vector expresses glutamine synthase and the cloned gene; Celltech). In another embodiment, a vector that directs episomal expression under control of Epstein Barr Virus (EBV) can be used, such as pREP4 (BamHI, SfiI, XhoI, NotI, NheI, HindIII, NheI, PvuII, and KpnI cloning site, constitutive RSV-LTR promoter, hygromycin selectable marker; Invitrogen), pCEP4 (BamHI, SfiI, XhoI, NotI, NheI, HindIII, NheI, PvuII, and KpnI cloning site, constitutive hCMV immediate early gene, hygromycin selectable marker; Invitrogen), pMEP4 (KpnI, PvuI, NheI, HindIII, NotI, XhoI, SfiI, BamHI cloning site, inducible methallothionein IIa gene promoter, hygromycin selectable marker; Invitrogen), pREP8 (BamHI, XhoI, NotI, HindIII, NheI, and KpnI cloning site, RSV-LTR promoter, histidinol selectable marker; Invitrogen), pREP9 (KpnI, NheI, HindIII, NotI, XhoI, SfiI, and BamHI cloning site, RSV-LTR promoter, G418 selectable marker; Invitrogen), and pEBVHis (RSV-LTR promoter, hygromycin selectable marker, N-terminal peptide purifiable via ProBond resin and cleaved by enterokinase; Invitrogen). Selectable mammalian expression vectors for use in the invention include pRc/CMV (HindIII, BstXI, NotI, SbaI, and ApaI cloning site, G418 selection; Invitrogen), pRc/RSV (HindIII, SpeI, BstXI, NotI, XbaI cloning site, G418 selection; Invitrogen), and others. Vaccinia virus mammalian expression vectors (see, Kaufman, 1991, supra) for use according to the invention include but are not limited to pSC11 (SmaI cloning site, TK- and β -gal selection), pMJ601 (Sall, SmaI, AflI, NarI, BspMII, BamHI, ApaI, NheI, SacII, KpnI, and HindIII cloning site; TK- and

β -gal selection), and pTKgptF1S (EcoRI, PstI, Sall, AccI, HindII, SbaI, BamHI, and Hpa cloning site, TK or XPRT selection).

[0206] Yeast expression systems can also be used according to the invention to express OB polypeptide. For example, the non-fusion pYES2 vector (XbaI, SphI, ShoI, NotI, GstXI, EcoRI, BstXI, BamHI, SacI, KpnI, and HindIII cloning site; Invitrogen) or the fusion pYESHisA, B, C (XbaI, SphI, ShoI, NotI, BstXI, EcoRI, BamHI, SacI, KpnI, and HindIII cloning site, N-terminal peptide purified with

[0207] ProBond resin and cleaved with enterokinase; Invitrogen), to mention just two, can be employed according to the invention.

[0208] It is further intended that body weight modulator polypeptides and analogs may be prepared from nucleotide sequences derived within the scope of the present invention.

[0209] In addition to recombinant expression of OB-R polypeptide, the present invention envisions and fully enables preparation of OB-R polypeptide, or fragments thereof, using the well known and highly developed techniques of solid phase peptide synthesis. The invention contemplates using both the popular Boc and Fmoc, as well as other protecting group strategies, for preparing ob polypeptide or fragments thereof. Various techniques for refolding and oxidizing the cysteine side chains to form a disulfide bond are also well-known in the art.

Antibodies to the OB-R Polypeptide

[0210] According to the invention, OB-R polypeptide produced recombinantly or by chemical synthesis, and fragments or other derivatives or analogs thereof, including fusion proteins, may be used as an immunogen to generate antibodies that recognize the OB-R polypeptide. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library.

[0211] A molecule is "antigenic" when it is capable of specifically interacting with an antigen recognition molecule of the immune system, such as an immunoglobulin (antibody) or T cell antigen receptor. An antigenic polypeptide contains at least about 5, and preferably at least about 10, amino acids. An antigenic portion of a molecule can be that portion that is immunodominant for antibody or T cell receptor recognition, or it can be a portion used to generate an antibody to the molecule by conjugating the antigenic portion to a carrier molecule for immunization. A molecule that is antigenic need not be itself immunogenic, i.e., capable of eliciting an immune response without a carrier.

[0212] An "antibody" is any immunoglobulin, including antibodies and fragments thereof, that binds a specific epitope. The term encompasses polyclonal, monoclonal, and chimeric antibodies, the last mentioned described in further detail in U.S. Pat. Nos. 4,816,397 and 4,816,567, as well as antigen binding portions of antibodies, including Fab, F(ab')₂, and F(v) (including single chain antibodies). Accordingly, the phrase "antibody molecule" in its various grammatical forms as used herein contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule containing the antibody combining site. An "antibody combining site" is that structural

portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically binds antigen.

[0213] Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contains the paratope, including those portions known in the art as Fab, Fab', F(ab')₂ and F(v), which portions are preferred for use in the therapeutic methods described herein.

[0214] Fab and F(ab')₂ portions of antibody molecules are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibody molecules by methods that are well-known. See for example, U.S. Pat. No. 4,342,566 to Theofilopolous et al. Fab' antibody molecule portions are also well-known and are produced from F(ab')₂ portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules is preferred herein.

[0215] The phrase "monoclonal antibody" in its various grammatical forms refers to an antibody having only one species of antibody combining site capable of immunoreacting with a particular antigen. A monoclonal antibody thus typically displays a single binding affinity for any antigen with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different antigen; e.g., a bispecific (chimeric) monoclonal antibody.

[0216] The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and also as a lymphoid system activator that non-specifically enhances the immune response [Hood et al., in *Immunology*, p. 384, Second Ed., Benjamin/Cummings, Menlo Park, Calif. (1984)]. Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or cellular immune response. Adjuvants include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*. Preferably, the adjuvant is pharmaceutically acceptable.

[0217] Various procedures known in the art may be used for the production of polyclonal antibodies to OB-R polypeptide, or fragment, derivative or analog thereof. For the production of antibody, various host animals can be immunized by injection with the OB-R polypeptide, or a derivative (e.g., fragment or fusion protein) thereof, including but not limited to rabbits, mice, rats, sheep, goats, etc. In one embodiment, the OB-R polypeptide or fragment thereof can be conjugated to an immunogenic carrier, e.g., bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). Specific OB-R antigenic fragments are disclosed in Example 2, *infra*. Various adjuvants may be used to increase the immunological response, depending on the host species,

including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*.

[0218] For preparation of monoclonal antibodies directed toward the OB-R polypeptide, or fragment, analog, or derivative thereof, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used. These include but are not limited to the hybridoma technique originally developed by Kohler et al., *Nature*, 256:495-497 (1975), as well as the trioma technique, the human B-cell hybridoma technique [Kozbor et al., *Immunology Today*, 4:72 (1983)], and the EBV-hybridoma technique to produce human monoclonal antibodies [Cole et al., in *Monoclonal Antibodies and Cancer Therapy*, pp. 77-96, Alan R. Liss, Inc., (1985)]. Immortal, antibody-producing cell lines can be created by techniques other than fusion, such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al., "Hybridoma Techniques" (1980); Hammerling et al., "Monoclonal Antibodies And T-cell Hybridomas" (1981); Kennett et al., "Monoclonal Antibodies" (1980); see also U.S. Pat. Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,451,570; 4,466,917; 4,472,500; 4,491,632; and 4,493,890.

[0219] In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human hybridomas [Cote et al., *Proc. Natl. Acad. Sci. USA*, 80:2026-2030 (1983)] or by transforming human B cells with EBV virus in vitro (Cole et al., 1985, *supra*). In fact, according to the invention, techniques developed for the production of "chimeric antibodies" [Morrison et al., *J. Bacteriol.*, 159:870 (1984); Neuberger et al., *Nature*, 312:604-608 (1984); Takeda et al., *Nature*, 314:452-454 (1985)] by splicing the genes from a mouse antibody molecule specific for an ob polypeptide together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention. Such human or humanized chimeric antibodies are preferred for use in therapy of human diseases or disorders (described *infra*), since the human or humanized antibodies are much less likely than xenogenic antibodies to induce an immune response, in particular an allergic response, themselves.

[0220] According to the invention, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce OB polypeptide-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries [Huse et al., *Science*, 246:1275-1281 (1989)] to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for an ob polypeptide, or its derivatives, or analogs.

[0221] Antibody fragments which contain the idiotype of the antibody molecule can be generated by known techniques. For example, such fragments include but are not

limited to: the F(ab')₂ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

[0222] In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g., radioimmunoassay, ELISA (enzyme-linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), Western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention. For example, to select antibodies which recognize a specific epitope of an OB polypeptide, one may assay generated hybridomas for a product which binds to an OB polypeptide fragment containing such epitope. For selection of an antibody specific to an OB polypeptide from a particular species of animal, one can select on the basis of positive binding with OB polypeptide expressed by or isolated from cells of that species of animal.

[0223] The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the OB-R polypeptide, e.g., for Western blotting, imaging OB-R polypeptide in situ, measuring levels thereof in appropriate physiological samples, etc.

[0224] In a specific embodiment, antibodies that agonize or antagonize the activity of OB-R polypeptide can be generated. Such antibodies can be tested using the assays described infra for identifying ligands.

[0225] In a particular aspect, antibodies are developed by immunizing rabbits with synthetic peptides predicted by the protein sequence or with recombinant proteins made using bacterial expression vectors. The choice of synthetic peptides is made after careful analysis of the predicted protein structure, as described above. In particular, peptide sequences between putative cleavage sites are chosen. Synthetic peptides are conjugated to a carrier such as KLH hemocyanin or BSA using carbodiimide and used in Freund's adjuvant to immunize rabbits. In order to prepare recombinant protein, the pGEX vector can be used to express the polypeptide (Smith et al., 1988, supra). Alternatively, one can use only hydrophilic domains to generate the fusion protein. The expressed protein will be prepared in quantity and used to immunize rabbits in Freund's adjuvant.

[0226] In a specific embodiment, infra, peptides corresponding to amino acid residues 145-158, 465-484, and 863-881 (from the murine OB-R polypeptide depicted in any one of SEQ ID NOS:8, 10) can be generated by solid phase peptide synthesis, conjugated to a carrier such as KLH, and used to immunize rabbits, rats, goats, chickens, etc.

[0227] In another specific embodiment, recombinant OB-R polypeptide is used to immunize chickens, and the chicken anti-OB-R antibodies are recovered from egg yolk, e.g., by affinity purification on an OB-R-column. Preferably, chickens used in immunization are kept under specific pathogen free (SPF) conditions.

[0228] In yet another embodiment, recombinant OB-R polypeptide is used to immunize rabbits, and the polyclonal antibodies are immunopurified prior to further use. The purified antibodies are particularly useful for semi-quantitative assays, particularly for detecting the presence of the circulating (soluble) splice form(s) of OB-R polypeptide in serum or plasma.

[0229] Panels of monoclonal antibodies produced against modulator peptides can be screened for various properties; i.e., isotype, epitope, affinity, etc. Of particular interest are monoclonal antibodies that neutralize the activity of the modulator peptides. Such monoclonals can be readily identified in activity assays for the weight modulators. High affinity antibodies are also useful when immunoaffinity purification of native or recombinant polypeptide is desired.

[0230] Preferably, the anti-modulator antibody used in the diagnostic and therapeutic methods of this invention is an affinity-purified polyclonal antibody. More preferably, the antibody is a monoclonal antibody (mAb). In addition, it is preferable for the anti-modulator antibody molecules used herein be in the form of Fab, Fab', F(ab')₂ or F(v) portions of whole antibody molecules.

Diagnosics

[0231] The present invention also relates to a variety of diagnostic applications, including methods for detecting the presence of conditions and/or stimuli that impact upon abnormalities in body weight or adiposity, by reference to their ability to elicit the activities which are mediated by the present OB-R polypeptides. As mentioned earlier, the peptides can be used to produce antibodies to themselves by a variety of known techniques, and such antibodies could then be isolated and utilized as in tests for the presence of particular transcriptional activity in suspect target cells. Alternatively, the nucleic acids of the invention can be employed in diagnosis.

Antibody-based Diagnosics

[0232] As suggested earlier, a diagnostic method useful in the present invention comprises examining a cellular sample or medium by means of an assay including an effective amount of an OB-R binding partner, such as an anti-modulator antibody or leptin, preferably an affinity-purified polyclonal antibody, and more preferably a mAb. In addition, it is preferable for the antibody molecules used herein be in the form of Fab, Fab', F(ab')₂ or F(v) portions or whole antibody molecules. As previously discussed, patients capable of benefiting from this method include those suffering from cancer, AIDS, obesity or other conditions where abnormal body weight is an element of the condition.

[0233] Also, antibodies including both polyclonal and monoclonal antibodies, may possess certain diagnostic applications and may for example, be utilized for the purpose of detecting and/or measuring conditions where abnormalities in body weight are or may be likely to develop.

[0234] The diagnostic methods can be used to detect OB-R in a biological sample from an individual. The biological sample can be a biological fluid, such as but not limited to, blood, serum, plasma, interstitial fluid, plural effusions, urine, cerebrospinal fluid, and the like. Preferably, soluble OB-R is detected in serum or urine, which are both readily obtained. Alternatively, OB-R can be detected from cellular sources, such as, but not limited to, brain tissue biopsies, adipocytes, testes, heart, and the like. For example, cells can be obtained from an individual by biopsy and lysed, e.g., by freeze-thaw cycling, or treatment with a mild cytolytic detergent such as, but not limited to, TRITON X-100®, digitonin, NONIDET P (NP)-40®, saponin, and the like, or combinations thereof (see, e.g., International Patent Publication WO 92/08981, published May 29, 1992). In yet another embodiment, samples containing both cells and body fluids can be used (see *ibid.*).

[0235] The presence of OB-R in cells or in a biological fluid can be ascertained by the usual immunological procedures applicable to such determinations. A number of useful procedures are known. Three such procedures which are especially useful utilize either the OB-R (particularly the secreted splice form) labeled with a detectable label, antibody Ab₁ labeled with a detectable label, or antibody Ab₂ labeled with a detectable label.

[0236] The procedures and their application are all familiar to those skilled in the art and accordingly may be utilized within the scope of the present invention. For example, a “competitive” procedure, is described in U.S. Pat. Nos. 3,654,090 and 3,850,752. A “sandwich” procedure, is described in U.S. Pat. Nos. RE 31,006 and 4,016,043. Still other procedures are known such as the “double antibody”, or “DASP” procedure.

[0237] The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others.

[0238] A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine and auramine. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate.

[0239] The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from ³H, ¹⁴C, ³²P, ³⁵S, ³⁶Cl, ⁵¹Cr, ⁵⁷Co, ⁵⁸Co, ⁵⁹Fe, ⁹⁰Y, ¹²⁵I, ¹³¹I, and ¹⁸⁶Re.

[0240] Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, β-glucuronidase, β-D-glucosidase, β-D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Pat. Nos. 3,654,090; 3,850,752; and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

[0241] In a further embodiment of this invention, test kits suitable for use by a medical specialist may be prepared to determine the presence or absence of OB-R in suspected

target cells or biological fluids. In accordance with the testing techniques discussed above, one class of such kits will contain at least the labeled OB-R polypeptide or its binding partner, for instance an antibody specific thereto, and directions, of course, depending upon the method selected, e.g., “competitive,” “sandwich,” “DASP” and the like. The kits may also contain peripheral reagents such as buffers, stabilizers, etc.

Nucleic Acid-based Diagnostics

[0242] As demonstrated in the examples, *infra*, nucleic acids of the invention can be used to detect defects associated with defects in the OB-R polypeptide associated with an obese phenotypes. For example, nucleic acid probes (e.g., in Northern analysis or RT-PCR analysis) can be used to determine whether an obese phenotype is associated with lack of expression of OB-R mRNA, or expression of non-functional OB-R mRNA, e.g., as in db/db mice (where the deficiency results from lack of an effective leptin receptor), or where a mutation yields a non-transcribed mRNA. Moreover, the nucleic acid-based diagnostic techniques of the invention can be used in conjunction with antibody-based techniques to further develop a molecular understanding of obese or anorexic phenotypes.

[0243] Human cDNA clones may be sequenced. This facilitates the determination of the complete sequence of the human gene. DNA sequences from the introns of the human OB-R gene may thus be obtained, and these can be used to prepare PCR primers to PCR amplify the coding sequence of the OB-R gene from human genomic DNA so as to identify mutations or allelic variants of the OB-R gene, all in accordance with protocols described in detail earlier herein.

[0244] The current hypothesis is that heterozygous mutations in the DB gene will be associated with mild/moderate obesity while homozygous mutations would be associated with severe obesity. This would allow the ascertainment of people at risk for the development of obesity and make possible the application of drug treatment and/or lifestyle changes before an increased body weight is fully developed.

[0245] Alternatively, the presence of microsatellites that segregate with mutant forms of human ob-r can be used for diagnosis. Various PCR primers, can be used in this respect.

[0246] The OB-R gene may also be useful diagnostically for measurements of its encoded RNA and protein in nutritional disorders. It will be of importance to know, in a particular nutritional disorder, whether OB-R RNA and/or its encoded protein is upregulated or downregulated. Thus, if an obese person has increased levels of OB-R, it would appear that the problem is downstream of OB-R, while if OB-R expression is reduced, it would appear that inappropriately low levels of OB may be cause of obesity (whether or not the defect is in the OB-R gene). Conversely, if a cancer or AIDS patient who lost weight had elevated levels of OB-R, it may be concluded that inappropriately high expression of OB-R is responsible for the weight loss.

[0247] The present invention is concerned with not only inappropriate levels of expression of OB-R, but also with expression of non-functional or dysfunctional splice forms. The nucleic acid diagnostics of the invention provide for determining whether the predominantly expressed form is

dysfunctional, e.g., for signal transduction. As demonstrated in the Examples, infra, db mutant mice (C57BL/Ks db/db) express a longer OB-R mRNA (as determined by RT-PCR).

Therapeutics

[0248] The polypeptides, nucleic acids, and antibodies of the invention have significant therapeutic potential. Preferably, a therapeutically effective amount of such an agent (e.g., soluble form of the protein, or DNA for gene therapy, or an antisense nucleic acid for antagonizing leptin activity) is administered in a pharmaceutically acceptable carrier, diluent, or excipient.

[0249] The phrase “pharmaceutically acceptable” refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similarly untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. In one embodiment, as used herein, the term “pharmaceutically acceptable” may mean approved by a regulatory agency of the federal or a state government or listed in the *U.S. Pharmacopeia* or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in Martin, *Remington's Pharmaceutical Sciences*, 18th Ed., Mack Publishing Co., Easton, Pa., (1990).

[0250] The phrase “therapeutically effective amount” is used herein to mean an amount sufficient to reduce by at least about 15%, preferably by at least 50%, more preferably by at least 90%, and most preferably prevent, a clinically significant deficit in the activity, function and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in the host. Modulation of OB-R activity can be useful for reducing body weight (by increasing its activity) or increasing body weight (by decreasing its activity).

[0251] Administration of recombinant soluble OB-R polypeptide corresponding to OB-Re is expected to result in weight loss, in particular, a decrease in fat tissue. Soluble type OB-Re polypeptide can be prepared using standard bacterial and/or mammalian expression vectors, synthetically, or purified from plasma or serum, all as stated in detail earlier herein. Alternatively, increased expression of native soluble OB-R polypeptide may be induced by homologous recombination techniques, as described supra.

[0252] Reduction of leptin activity (by developing antagonists, inhibitors, use of neutralizing antibodies, or antisense molecules) should result in weight gain as might be desirable for the treatment of the weight loss associated with cancer, AIDS or anorexia nervosa. In one embodiment, a leptin-binding form of soluble OB-R that lacks portions necessary for signal transduction or enhancement can be employed.

Polypeptide-based Therapeutic Treatment

[0253] In the simplest analysis, the OB-R gene is intimately associated with determination of body weight in

animals, in particular, mice, rats, dogs, and man. The OB-R gene product, and, correspondingly, cognate molecules, appear to be part of a signaling pathway by which adipose tissue communicates with the brain and the other organs. It is believed that at least one splice form of the OB-R polypeptide (e.g., OB-Rb) is itself a signaling molecule, i.e., a receptor for the hormone leptin.

[0254] The soluble OB-R polypeptide, or functionally active fragment thereof, or an antagonist thereof, can be administered orally or parenterally, preferably parenterally. Because metabolic homeostasis is a continuous process, controlled release administration of soluble OB-R polypeptide (OB-Re) is preferred. For example, the polypeptide may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used [Langer et al., eds., *Medical Applications of Controlled Release*, CRC Pres., Boca Raton, Fla. (1974); Sefton, *CRC Crit. Ref. Biomed. Eng.*, 14:201 (1987); Buchwald et al., *Surgery*, 88:507 (1980); Saudek et al., *N. Engl. J. Med.*, 321:574 (1989)]. In another embodiment, polymeric materials can be used [Langer, 1974, supra; Sefton, 1987, supra; Smolen et al., eds., *Controlled Drug Bioavailability, Drug Product Design and Performance*, Wiley, New York (1984); Ranger et al., *J. Macromol. Sci. Rev. Macromol. Chem.*, 23:61 (1983); see also Levy et al., *Science*, 228:190 (1985); During et al., *Ann. Neurol.*, 25:351 (1989); Howard et al., *J. Neurosurg.*, 71:105 (1989)]. In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose [see, e.g., Goodson, in *Medical Applications of Controlled Release*, vol. 2, pp. 115-138 (1984)]. Other controlled release systems are discussed in the review by Langer, *Science*, 249:1527-1533 (1990). In another embodiment, the therapeutic compound can be delivered in a vesicle, in particular a liposome (see Langer, 1990 supra); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

[0255] In a further aspect, recombinant cells that have been transformed with the soluble splice form of the OB-R cDNA (e.g., OB-Re, which will be used herein to refer to a soluble OB-R agonist of leptin) and that express high levels of the polypeptide can be transplanted in a subject in need of enhancement of leptin activity. Preferably autologous cells transformed with OB-Re are transplanted to avoid rejection; alternatively, technology is available to shield non-autologous cells that produce soluble factors within a polymer matrix that prevents immune recognition and rejection.

[0256] The OB-Re polypeptide can be delivered by intravenous, intraarterial, intraperitoneal, intramuscular, or subcutaneous routes of administration. Alternatively, the OB-Re polypeptide, properly formulated, can be administered by nasal or oral administration. A constant supply of OB-Re can be ensured by providing a therapeutically effective dose (i.e., a dose effective to induce metabolic changes in a subject) at the necessary intervals, e.g., daily, every 12 hours, etc. These parameters will depend on the severity of the disease condition being treated, other actions, such as diet modification, that are implemented, the weight, age, and sex of the subject,

and other criteria, which can be readily determined according to standard good medical practice by those of skill in the art.

[0257] It can readily be appreciated by one of ordinary skill in the art that a soluble OB-R leptin antagonist can also be administered as described above for OB-Re.

Pharmaceutical Compositions

[0258] In yet another aspect of the present invention, provided are pharmaceutical compositions of the above. Such pharmaceutical compositions may be for administration for injection, or for oral, pulmonary, nasal or other forms of administration. In general, comprehended by the invention are pharmaceutical compositions comprising effective amounts of protein or derivative products of the invention together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Hylauronic acid may also be used. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present proteins and derivatives. See, e.g., *Martin, Remington's Pharmaceutical Sciences*, 18th Ed. (1990, Mack Publishing Co., Easton, Pa. 18042) pages 1435-1712 which are herein incorporated by reference. The compositions may be prepared in liquid form, or may be in dried powder, such as lyophilized form.

Oral Delivery

[0259] Contemplated for use herein are oral solid dosage forms, which are described generally in *Martin, Remington's Pharmaceutical Sciences*, 18th Ed. (1990 Mack Publishing Co. Easton Pa. 18042) at Chapter 89, which is herein incorporated by reference. Solid dosage forms include tablets, capsules, pills, troches or lozenges, cachets or pellets. Also, liposomal or proteinoid encapsulation may be used to formulate the present compositions (as, for example, proteinoid microspheres reported in U.S. Pat. No. 4,925,673). Liposomal encapsulation may be used and the liposomes may be derivatized with various polymers (E.g., U.S. Pat. No. 5,013,556). A description of possible solid dosage forms for the therapeutic is given by Marshall, in *Modern Pharmaceutics*, Chapter 10, Banker and Rhodes ed., (1979), herein incorporated by reference. In general, the formulation will include the protein (or chemically modified protein), and inert ingredients which allow for protection against the stomach environment, and release of the biologically active material in the intestine.

[0260] Also specifically contemplated are oral dosage forms of the above derivatized proteins. Protein may be chemically modified so that oral delivery of the derivative is efficacious. Generally, the chemical modification contemplated is the attachment of at least one moiety to the protein (or peptide) molecule itself, where said moiety permits (a) inhibition of proteolysis; and (b) uptake into the blood

stream from the stomach or intestine. Also desired is the increase in overall stability of the protein and increase in circulation time in the body. Examples of such moieties include: polyethylene glycol, copolymers of ethylene glycol and propylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone and polyproline. Abuchowski et al., 1981, supra; Newmark et al., *J. Appl. Biochem.*, 4:185-189 (1982). Other polymers that could be used are poly-1,3-dioxolane and poly-1,3,6-tioxocane. Preferred for pharmaceutical usage, as indicated above, are polyethylene glycol moieties.

[0261] For the protein (or derivative) the location of release may be the stomach, the small intestine (the duodenum, the jejunum, or the ileum), or the large intestine. One skilled in the art has available formulations which will not dissolve in the stomach, yet will release the material in the duodenum or elsewhere in the intestine. Preferably, the release will avoid the deleterious effects of the stomach environment, either by protection of the protein (or derivative) or by release of the biologically active material beyond the stomach environment, such as in the intestine.

[0262] To ensure full gastric resistance, a coating impermeable to at least pH 5.0 is essential. Examples of the more common inert ingredients that are used as enteric coatings are cellulose acetate trimellitate (CAT), hydroxypropylmethylcellulose phthalate (HPMCP), HPMCP 50, HPMCP 55, polyvinyl acetate phthalate (PVAP), Eudragit L30D, Aquateric, cellulose acetate phthalate (CAP), Eudragit L, Eudragit S, and Shellac. These coatings may be used as mixed films.

[0263] A coating or mixture of coatings can also be used on tablets, which are not intended for protection against the stomach. This can include sugar coatings, or coatings which make the tablet easier to swallow. Capsules may consist of a hard shell (such as gelatin) for delivery of dry therapeutic i.e. powder; for liquid forms, a soft gelatin shell may be used. The shell material of cachets could be thick starch or other edible paper. For pills, lozenges, molded tablets or tablet triturates, moist massing techniques can be used.

[0264] The therapeutic can be included in the formulation as fine multiparticulates in the form of granules or pellets of particle size about 1mm. The formulation of the material for capsule administration could also be as a powder, lightly compressed plugs or even as tablets. The therapeutic could be prepared by compression.

[0265] Colorants and flavoring agents may all be included. For example, the protein (or derivative) may be formulated (such as by liposome or microsphere encapsulation) and then further contained within an edible product, such as a refrigerated beverage containing colorants and flavoring agents.

[0266] One may dilute or increase the volume of the therapeutic with an inert material. These diluents could include carbohydrates, especially mannitol, α -lactose, anhydrous lactose, cellulose, sucrose, modified dextrans and starch. Certain inorganic salts may be also be used as fillers including calcium triphosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and Avicell.

[0267] Disintegrants may be included in the formulation of the therapeutic into a solid dosage form. Materials used

as disintegrants include but are not limited to starch including the commercial disintegrant based on starch, Explotab. Sodium starch glycolate, Amberlite, sodium carboxymethylcellulose, ultramylopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. Another form of the disintegrants are the insoluble cationic exchange resins. Powdered gums may be used as disintegrants and as binders and these can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also useful as disintegrants.

[0268] Binders may be used to hold the therapeutic agent together to form a hard tablet and include materials from natural products such as acacia, tragacanth, starch and gelatin. Others include methyl cellulose (MC), ethyl cellulose (EC) and carboxymethyl cellulose (CMC). Polyvinyl pyrrolidone (PVP) and hydroxypropylmethyl cellulose (HPMC) could both be used in alcoholic solutions to granulate the therapeutic.

[0269] An antifrictional agent may be included in the formulation of the therapeutic to prevent sticking during the formulation process. Lubricants may be used as a layer between the therapeutic and the die wall, and these can include but are not limited to: stearic acid including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes. Soluble lubricants may also be used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, and Carbowax 4000 and 6000.

[0270] Glidants that might improve the flow properties of the drug during formulation and to aid rearrangement during compression might be added. The glidants may include starch, talc, pyrogenic silica and hydrated silicoaluminate.

[0271] To aid dissolution of the therapeutic into the aqueous environment, a surfactant might be added as a wetting agent. Surfactants may include anionic detergents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents might be used and could include benzalkonium chloride or benzethonium chloride. The list of potential nonionic detergents that could be included in the formulation as surfactants are laurmacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. These surfactants could be present in the formulation of the protein or derivative either alone or as a mixture in different ratios.

[0272] Additives which potentially enhance uptake of the protein (or derivative) are for instance the fatty acids oleic acid, linoleic acid and linolenic acid.

[0273] Controlled release formulation may be desirable. The drug could be incorporated into an inert matrix which permits release by either diffusion or leaching mechanisms i.e., gums. Slowly degenerating matrices may also be incorporated into the formulation. Another form of a controlled release of this therapeutic is by a method based on the Oros therapeutic system (Alza Corp.), i.e. the drug is enclosed in a semipermeable membrane which allows water to enter and push drug out through a single small opening due to osmotic effects. Some enteric coatings also have a delayed release effect.

[0274] Other coatings may be used for the formulation. These include a variety of sugars which could be applied in a coating pan. The therapeutic agent could also be given in a film-coated tablet; the materials used in this instance are divided into 2 groups. The first are the nonenteric materials and include methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, methylhydroxy-ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl-methyl cellulose, sodium carboxymethyl cellulose, providone and the polyethylene glycols. The second group consists of the enteric materials that are commonly esters of phthalic acid.

[0275] A mix of materials might be used to provide the optimum film coating. Film coating may be carried out in a pan coater or in a fluidized bed or by compression coating.

Pulmonary Delivery

[0276] Also contemplated herein is pulmonary delivery of the present soluble protein (or derivatives thereof). The protein (or derivative) is delivered to the lungs of a mammal while inhaling and traverses across the lung epithelial lining to the blood-stream. Other reports of this include Adjei et al., *Pharmaceutical Research*, 7(6):565-569 (1990); Adjei et al., *International Journal of Pharmaceutics*, 63:135-144 (1990) (leuprolide acetate); Braquet et al., *Journal of Cardiovascular Pharmacology*, 13(suppl. 5): 143-146 (1989) (endotelin-1); Hubbard et al., *Annals of Internal Medicine*, 3(3):206-212 (1989) (α 1-antitrypsin); Smith et al., *J. Clin. Invest.*, 84:1145-1146 (1989) (α 1-proteinase); Oswein et al., "Aerosolization of Proteins", *Proceedings of Symposium on Respiratory Drug Delivery II*, Keystone, Colo., (March 1990) (recombinant human growth hormone); Debs et al., *J. Immunol.*, 140:3482-3488 (1988) and Platz et al., U.S. Pat. No. 5,284,656 (granulocyte colony stimulating factor). Contemplated for use in the practice of this invention are a wide range of mechanical devices designed for pulmonary delivery of therapeutic products, including but not limited to nebulizers, metered-dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art.

[0277] Some specific examples of commercially available devices suitable for the practice of this invention are the Ultravent nebulizer, manufactured by Mallinckrodt, Inc., St. Louis, Mo.; the Acorn II nebulizer, manufactured by Marquest Medical Products, Englewood, Colo.; the Ventolin metered dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, N.C.; and the Spinhaler powder inhaler, manufactured by Fisons Corp., Bedford, Mass.

[0278] All such devices require the use of formulations suitable for the dispensing of protein (or derivative). Typically, each formulation is specific to the type of device employed and may involve the use of an appropriate propellant material, in addition to the usual diluents, adjuvants and/or carriers useful in therapy. Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated. Chemically modified protein may also be prepared in different formulations depending on the type of chemical modification or the type of device employed.

[0279] Formulations suitable for use with a nebulizer, either jet or ultrasonic, will typically comprise protein (or derivative) dissolved in water at a concentration of about 0.1 to 25 mg of biologically active protein per ml of solution. The formulation may also include a buffer and a simple

sugar (e.g., for protein stabilization and regulation of osmotic pressure). The nebulizer formulation may also contain a surfactant, to reduce or prevent surface induced aggregation of the protein caused by atomization of the solution in forming the aerosol.

[0280] Formulations for use with a metered-dose inhaler device will generally comprise a finely divided powder containing the protein (or derivative) suspended in a propellant with the aid of a surfactant. The propellant may be any conventional material employed for this purpose, such as a chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, or combinations thereof. Suitable surfactants include sorbitan trioleate and soya lecithin. Oleic acid may also be useful as a surfactant.

[0281] Formulations for dispensing from a powder inhaler device will comprise a finely divided dry powder containing protein (or derivative) and may also include a bulking agent, such as lactose, sorbitol, sucrose, or mannitol in amounts which facilitate dispersal of the powder from the device, e.g., 50 to 90% by weight of the formulation. The protein (or derivative) should most advantageously be prepared in particulate form with an average particle size of less than 10 μm (or microns), most preferably 0.5 to 5 μm , for most effective delivery to the distal lung.

Nasal Delivery

[0282] Nasal delivery of the protein (or derivative) is also contemplated. Nasal delivery allows the passage of the protein to the blood stream directly after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with dextran or cyclodextran.

Methods of Treatment, Methods of Preparing a Medicament

[0283] In yet another aspect of the present invention, methods of treatment and manufacture of a medicament are provided. Conditions alleviated by or modulated by the administration of the present derivatives are those indicated above.

Dosages

[0284] For all of the above molecules, as further studies are conducted, information will emerge regarding appropriate dosage levels for treatment of various conditions in various patients, and the ordinary skilled worker, considering the therapeutic context, age and general health of the recipient, will be able to ascertain the proper dosage. Generally, for injection or infusion, dosage will be between 0.01 μg of biologically active protein/kg body weight, (calculating the mass of the protein alone, without chemical modification), and 10 mg/kg (based on the same). The dosing schedule may vary, depending on the circulation half-life of the protein or derivative used, whether the polypeptide is delivered by bolus dose or continuous infusion, and the formulation used.

Administration with Other Compounds

[0285] For therapy associated with obesity, one may administer the present soluble protein (or derivatives) in

conjunction with one or more pharmaceutical compositions used for treating other clinical complications of obesity, such as those used for treatment of diabetes (e.g., insulin), high blood pressure, high cholesterol, and other adverse conditions incident to obesity. Also, other appetite suppressants may be co-administered, e.g., amphetamines. Administration may be simultaneous (for example, administration of a mixture of the present protein and insulin) or may be in seriatim.

Nucleic Acid-based Therapeutic Treatment

[0286] An OB-R gene capable of mediating signal transduction, e.g., OB-Rb, could be introduced into human hypothalamus cells to develop gene therapy for obesity. Such therapy would be expected to decrease body weight. Conversely, introduction of antisense constructs into brain cells, particularly hypothalamus but also including choroid plexus, or other cells where OB-R is expressed, would reduce the levels of active OB-R polypeptide and would be predicted to increase body adiposity.

[0287] In one embodiment, a gene encoding an OB-R polypeptide is introduced in vivo in a viral vector. Such vectors include an attenuated or defective DNA virus, such as but not limited to herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), and the like. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. Defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Thus, brain tissue can be specifically targeted. Examples of particular vectors include, but are not limited to, a defective herpes virus 1 (HSV1) vector [Kaplitt et al., *Molec. Cell. Neurosci.*, 2:320-330 (1991)], an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al., *J. Clin. Invest.*, 90:626-630 (1992), and a defective adeno-associated virus vector [Samulski et al., *J. Virol.*, 61:3096-3101 (1987); Samulski et al., *J. Virol.*, 63:3822-3828 (1989)].

[0288] In another embodiment, the gene can be introduced in a retroviral vector, e.g., as described in Anderson et al., U.S. Pat. No. 5,399,346; Mann et al., *Cell*, 33:153 (1983); Temin et al., U.S. Pat. No. 4,650,764; Temin et al., U.S. Pat. No. 4,980,289; Markowitz et al., *J. Virol.*, 62:1120 (1988); Temin et al., U.S. Pat. No. 5,124,263; International Patent Publication No. WO 95/07358, published Mar. 16, 1995, by Dougherty et al.; and Kuo et al., *Blood*, 82:845 (1993).

[0289] Alternatively, the vector can be introduced in vivo by lipofection. For the past decade, there has been increasing use of liposomes for encapsulation and transfection of nucleic acids in vitro. Synthetic cationic lipids designed to limit the difficulties and dangers encountered with liposome mediated transfection can be used to prepare liposomes for in vivo transfection of a gene encoding a marker [Feigner et al., *Proc. Natl. Acad. Sci. USA*, 84:7413-7417 (1987); see Mackey et al., *Proc. Natl. Acad. Sci. USA*, 85:8027-8031 (1988)]. The use of cationic lipids may promote encapsulation of negatively charged nucleic acids, and also promote fusion with negatively charged cell membranes [Felgner et al., *Science*, 337:387-388 (1989)]. The use of lipofection to introduce exogenous genes into specific organs in vivo has certain practical advantages. Molecular targeting of lipo-

comes to specific cells represents one area of benefit. It is clear that directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting (see Mackey et al., 1988, supra). Targeted peptides, e.g., hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically.

[0290] It is also possible to introduce the vector in vivo as a naked DNA plasmid. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, micro-injection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun, or use of a DNA vector transporter (see, e.g., Wu et al., *J. Biol. Chem.*, 267:963-967 (1992); Wu et al., *J. Biol. Chem.*, 263:14621-14624 (1988); Hartmut et al., Canadian Patent Application No. 2,012,311, filed Mar. 15, 1990).

Agricultural Applications

[0291] The OB-R gene can also be isolated from domestic animals, and the corresponding OB-R polypeptide obtained thereby. It is expected that the probe derived from the murine OB-R gene hybridizes to corresponding homologous coding sequences from a large number of species of animals. As discussed for human therapies, recombinant proteins can also be prepared and administered to domestic animals. Administration of the soluble polypeptide can be implemented to produce leaner food animals, such as beef cattle, swine, poultry, sheep, etc. Preferably, an autologous OB polypeptide is administered, although the invention contemplates administration of non-autologous polypeptide as well. Since the soluble OB-R polypeptide consists of approximately 800 amino acid residues, it may be highly immunogenic. Thus, administration of autologous polypeptide is preferred.

[0292] Alternatively, the introduction of the cloned genes into transgenic domestic animals would allow one to potentially decrease body weight and adiposity by overexpressing an OB-R transgene. The simplest means of achieving this would be to target an OB-R transgene to brain using its own or another brain specific promoter.

[0293] Conversely, increases in body fat might be desirable in other circumstances such as for the development of Kobe beef or fatty liver to make foie gras. This could be accomplished by targeting an antisense OB-R transgene to brain, or by using gene knockout technology. Alternatively, where an increase in body weight at percentage of fat is desired, an inhibitor or antagonist of the OB-R polypeptide can be administered. Such inhibitors or antagonists include, but are not limited to, antibodies reactive with the polypeptide, and fragments of the polypeptide that bind but do not activate the OB receptor, i.e., antagonists of leptin.

Cosmetic Implications

[0294] The OB-R polypeptide has significant value for cosmetic use, in addition to the health benefits. In particular, since the OB-R polypeptides of the invention, including derivatives and agonist analogs thereof, are useful for modulation of the rate and quantity of fat cell deposition in an animal, they are useful for reducing unsightly fat tissue, e.g.,

fat deposits in the abdomen, hips, thighs, neck, and chin that do not necessarily amount to an obese condition, but which nevertheless detract from an individual's appearance. The fat reduction effect is thought to be accomplished, in part, by a reduction in appetite, i.e., a reduction in food intake, by an increase in basal metabolism, or both. Thus, the present soluble OB-Re polypeptide, or its derivatives or agonist analogs, is useful for administration to a subject to effect cosmetic changes in fat tissue deposits, whether by modulating fat deposition, reducing appetite, or both.

[0295] In addition, the present compositions and methods may be used in conjunction with various procedures, such as cosmetic surgeries designed to alter the overall appearance of a body (e.g., liposuction or laser surgeries designed to reduce body mass by aspirating or ablating fat tissue), exercise (especially running and weight training), low fat diet, hypnosis, biofeedback, as examples of the ways one may attempt to decrease the percentage of fat tissue and improve the appearance of the body.

[0296] Accordingly, the present invention relates to a method for effecting cosmetic fat tissue modulation in an individual comprising administering a fat modulating amount of a soluble OB-R polypeptide, or derivative or agonist analog thereof, to an individual who desires cosmetic fat tissue modulation to improve overall body appearance. In a particular aspect, the fat tissue modulation is a consequence of appetite suppression. Preferably, the fat tissue modulation is a reduction in fat tissue.

[0297] In a further embodiment, the invention relates to a method for effecting cosmetic fat tissue loss comprising combining a procedure for changing body appearance with administration of a fat modulating amount of a soluble OB-R polypeptide, or derivative or agonist analog thereof, to an individual who desires cosmetic fat tissue modulation to improve overall body appearance.

[0298] The invention may be better understood by reference to the following Examples, which are intended to be exemplary of the invention and not limiting thereof.

EXAMPLE 1

Isolation of DB cDNA Clones

[0299] Mutations in the mouse db gene result in severe obesity and diabetes in a syndrome that resembles morbid human obesity [Hummel et al., *Science*, 153:1127 (1966)]. Previous data suggested that the db gene encoded the receptor for the gene product of the ob locus, known as leptin [Coleman, *Diabetologia*, 14:141 (1978); Zhang et al., *Nature*, 372:425 (1994)]. Recently, a report that the leptin receptor was cloned from choroid plexus appeared; this clone was shown to map to the same region of chromosome 4 as db [Tartaglia et al., *Cell*, 83:1263 (1995)]. This receptor is a member of the family of receptors that associate with the JAK tyrosine kinases. However, mutations in this receptor were not identified in C57BL/6J db/db mice, suggesting that the mutation in these animals might be in a splice variant of this gene [Tartaglia et al., supra].

[0300] The present Example shows that the leptin receptor maps to the same 300 kB interval on mouse chromosome 4 as db. cDNA selection and exon trapping from this region identified several ESTs with sequences identical to the leptin receptor. Characterization of the corresponding cDNA

clones isolated from a mouse brain cDNA library revealed that there are at least five alternatively spliced forms of the leptin receptor, each with differences at their amino and/or carboxy terminus. One of the splice variants is expressed at a high level in the hypothalamus and at a lower level in other tissues. This transcript is mutant in C57BL/Ks db/db mice. This mutation is the result of abnormal splicing leading to a 106 bp insertion into the 3' end of the RNA, which results in a truncated cytoplasmic region that deletes "Box 2", a protein site known to interact with JAK proteins [Murakami et al., *Proc. Natl. Acad. Sci. USA*, 88:11349 (1991); Fukunaga, et al., *EMBO*, 10:2855 (1991)]; it is likely to be defective in signal transduction [Bahary et al., *Proc. Natl. Acad. Sci. USA*, 87:8642 (1990); Modl et al., *Dytogenetics Cell Genetics*, 67:232 (1995)]. These data suggest that the weight reducing effects of leptin are mediated via interactions with a receptor in the hypothalamus, and perhaps other tissues.

Materials and Methods

[0301] Isolation of genomic clones. YAC clones were isolated by PCR screening and sized on a CHEF MAPPER (Bio-Rad) [Green et al., *Proc. Natl. Acad. Sci. USA*, 87:1213 (1996); Kasumi et al., *Mammalian Genome*, 4:391 (1993)]. YAC ends were recovered using vectorette PCR and plasmid end rescue [Riley et al., *Nucl. Acids Res.*, 18:2887 (1990); Hermanson et al., *Nucl. Acids Res.*, 19:4943 (1991)]. P1 clones were isolated by sending specific pairs of PCR primers to Genome Systems Inc. (St. Louis, Mo.) who provided single picks of individual mouse P1 clones [Sternberg, *Trends Genet.*, 8:11 (1992)]. P1 ends were recovered using vectorette PCR [Hartl et al., *Bio Techniques*, 15:201 (1993)]. BACs were isolated as described [Shizuya, *Proc. Natl. Acad. Sci. USA*, 89:8794 (1992)]. Primer selection and PCR amplification were performed as described: initial denaturation at 94° C. for 3 min., 25 cycles of 94° for 1 min., 55° for 2 min. and 72° for 3 min [Zhang, 1994, supra.] The primers were:

D4Rck6f
5' ATCTTGGGTCTCTGAAGAA 3'; (SEQ ID NO: 20)

D4Rck6r
5' GAGATTGTCAGTCACAGCCTC 3'; (SEQ ID NO: 21)

D4Rck7f
5' ATCTGAATTGGAATCAATACAC 3'; (SEQ ID NO: 22)

D4Rck7r
5' AAATCTGTTATCCTTCTGAAAC 3'. (SEQ ID NO: 23)

[0302] Isolation of db clones. cDNA selection was performed as described using mouse brain hypothalamic RNA as the starting material [Morgan et al., *Nucl. Acids Res.*, 20:5173 (1992)]. Library screening, exon trapping, and DNA sequencing were performed as described [Zhang et al., 1994, supra, (see Example 3)]. The C-terminal sequences of OB-Ra, OB-Re, OB-Rd and OB-Re were found in different cDNA clones. The C-terminal sequence of OB-Rb was not full length. The C-terminus sequence of this variant was initially completed by sequencing genomic DNA. The template was prepared using vectorette PCR of BAC 242 with primers from the cDNA²⁹ [Riley et al., *Nucl. Acids Res.*, 18:2887 (1990)]. The sequence was confirmed by sequencing RT-PCR products.

[0303] Identification of mutations in db. RT-PCR and sequencing were performed as described. Genomic sequences at the splice acceptor of OB-Rb were obtained by vectorette PCR of BAC 242 with the OB-Rb reverse primer. For RT-PCR of OB-Ra, OB-Rb, OB-Re and OB-Rd the forward primer was the same

5' ACACTGTTAATTCACACCAGAG 3' (SEQ ID NO:24) (also labeled F1 in FIG. 3C). The reverse (r) primers were:

[0304] OB-Ra 5' AGTCATTCAAACCATTAGTTTAGG 3' (SEQ ID NO:25),

[0305] OB-Rb 5' TGGATAAACCCCTTGCTCTTCA 3' (SEQ ID NO:26),

[0306] OB-Rc 5' TGAACACAACAACATAAAGCCC 3' (SEQ ID NO:27),

[0307] OB-Rd 5' AGGCTCCCTCAGGGCCAC 3' (SEQ ID NO:28). The intron primer for OB-Rb (labeled F2 in FIG. 3C) was

[0308] 5' GTGACTGAATGAAGATGTAATATAC 3' (SEQ ID NO:29).

[0309] Tissue distribution of the alternatively spliced leptin receptor. RT-PCR was performed as described. The primer sequences for OB-Ra, OB-Rb, OB-Rc, and OB-Rd are shown above. The primers for OB-Re were:

f
5' TGTTATATCTGGTTATTGAATGG, (SEQ ID NO: 30)

r
5' CATTAAATGATTATTATCAGAATTGC 3'. (SEQ ID NO: 31)

Results and Discussion

[0310] A series of genetic crosses segregating db were established. These included 50 obese (db/db) progeny of a C57BL/Ks db/db x *Mus spretus* intercross and 350 obese (db/db) progeny of a C57BL/Ks db/db x *Mus castaneus* intercross. The assignment of genotype as the db locus was made as previously described [Bahary et al., *Proc. Natl. Acad. Sci. USA*, 37:8642 (1990)].

[0311] Several microsatellite markers flanking db were used to type DNA from each animal. These included a distal marker, D4Mit31 and a proximal marker, 1fnc. A genetic map in the region of db was compiled using these and other loci (FIG. 1). The mouse homologous of two previously cloned human genes were found in the region of db: JAK1 and PDE4B. Both of these genes map to human chromosome 1p31 suggesting that the human db gene maps to this chromosomal region [Modl et al., *Cytogenetics Cell Genetics*, 69:232 (1995); Milatovich et al., *Somatic Cell Mol. Gen.*, 20:75 (1994)].

[0312] A microdissection clone, D4Rck22, was found to be distal to db and recombinant in three animals [Bahary et al., *Mammalian Genome*, 4:511-515 (1993)]. D6Rck 22 was used as the starting point of a chromosome walk using yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs) and P1 bacteriophage clones [Zhang et al., 1994, supra; Steinberg, *Trends Genet.*, 8:11 (1994); Shizuya, *Proc. Natl. Acad. Sci. USA*, 89:8794 (1992)]. A 2.7 mB contig was assembled by chromosome walking from this

marker. Of note, an approximately 200 kB region was not identified in any available mouse YAC library (~12 genome equivalents screened). This gap in the contig was closed after chromosome walking with a series of BAC and P1 clones followed by the isolation of an additional YAC that extended an additional 500 kB proximal to this region. Recombinant animals were typed with genetic markers (both RFLPs and SSLPs) derived from the ends of the individual genomic clones. The db mutation was located between the distal recombination event in animals 324 and the proximal recombination events in animal 1028. Seven other proximal recombinations were noted with 50 kB, suggesting that this is a hot spot for recombination. The entire nonrecombinant interval corresponds to ~300 kB of DNA, and was spanned by two BACs, 43 and 242 (FIG. 1).

[0313] Candidate genes for db were isolated from BACs 43 and 242 using exon trapping and cDNA selection from mouse hypothalamus [Church et al, *Nature Genetics*, 6:98 (1994); Morgan et al, *Nucl. Acids. Res.*, 20:5173 (1992)]. A mouse brain cDNA library was screened with putative gene fragments. Analysis of eight brain cDNA clones indicated that six independent products of cDNA selection and two cDNAs identified using trapped exons were present on overlapping transcripts. The nucleotide sequence of each cDNA clone predicted N-terminal sequences at least partially identical to the mouse leptin receptor (OB-R). The position of sequences from the 5' and 3' end of the OB-R RNA was determined by the STS content of each BAC and are shown on the physical map (FIG. 1). These data indicate that the gene spans ~100 kB and is transcribed toward the telomere.

[0314] These cDNA clones diverge at the carboxy terminus. In four cases, the predicted sequences were at least partially identical up to lysine 889 of the leptin receptor, which includes the transmembrane domain. Beyond this point, the cDNAs predicts proteins with differences in the cytoplasmic domain designated OB-Ra (SEQ ID NO:11), OB-Rb (SEQ ID NO:12), OB-Rc (SEQ ID NO:13), and OB-Rd (SEQ ID NO:14) (FIG. 2B). OB-Re predicted a different amino acid sequence after His⁷⁹⁶ (SEQ ID NO:15), which appears to encode a soluble receptor (FIG. 2B). Clones for OB-Ra, OB-Rb, and OB-Re diverged at its N-terminus. In all cases, the divergent sequence also mapped to the BAC contig. OB-Ra corresponds generally to mouse OB-R [Tartaglia et al., *Cell*, 83:1263 (1995)].

[0315] The C-terminus of OB-Rb was 78 percent identical to the human OB receptor, suggesting that it is the mouse homologue [Tartaglia et al., supra]. Leptin receptor is a member of the gp-130 family of receptors that interact with JAK protein kinase. The cytoplasmic domains of gp-130 receptors are generally required for binding JAKs and signal transduction [Kishimoto et al., *Cell*, 76:253 (1994)]. The OB-Rb cDNA sequence predicts a potential "box 2" sequence (underlined in FIG. 2B), a protein motif required for binding with JAK protein kinases [Kishimoto, supra]. "Box 2" is conserved among many members of this receptor family and is required for signal transduction of the GCSF and IL6 receptors [Murakami et al., *Proc. Natl. Acad. Sci. USA*, 88:11349 (1991); Fukunaga et al., *EMBO J.*, 10:2855 (1991). None of the other transcripts predict a "Box 2" sequence. Of the eight cDNA clones characterized, OB-Ra was isolated three times and OB-Re two times. OB-Rb,

OB-Rc, and OB-Rd were each isolated once. Additional splice variants are likely to be identified.

[0316] C57BL/Ks db/db mice have a longer fragment length of OB-Rb specific RT-PCR products (it should be noted that this PCR amplified 3' nucleic acids, and thus is specific for all splice variants having a cytoplasmic domain characteristics of OB-OB-Rb, but provides no data on the extracellular domain) from hypothalamic RNA than wild type littermates (FIG. 3A). However, PCR amplified genomic DNA spanning the splice acceptor at Pro⁸⁹⁰ was of normal size in C57BL/Ks db/db compared to wild type (FIG. 3B). DNA sequencing of this fragment confirmed that the genomic sequences at the splice acceptor are wild type in db mice. In addition, both the size and nucleotide sequence of RT-PCR products corresponding to the other 3' ends were normal in the db mice, suggesting that the splice donor at Lys⁸⁸⁹ is also normal (FIG. 4). These data suggested that the longer OB-Rb-specific fragment from C57BL/Ks db/db mice was the result of abnormal splicing.

[0317] Sequencing of the RT-PCR products of OB-Rb from the mutant mice revealed a 106 bp insertion between the splice donor at Lys⁸⁸⁹ and splice acceptor at Pro⁸⁹⁰. The sequence of the inserted DNA was identical to the first 106 bp of the unique OB-Ra exon downstream of its splice acceptor at Arg⁸⁹⁰ (FIG. 5A). Sequencing of genomic DNA and RT-PCR products from the 3' untranslated region of OB-Ra of C57BL/Ks db/db mice identified a g→t mutation 106 bp after the splice acceptor (compare FIGS. 5B and 5C). This mutation results in the appearance of a consensus splice donor site, AGGTAAA (FIG. 5C) [Lodish et al., *Mol. Cell. Biol.*, Scientific American Books : New York, pp. 1-1344 (1986)]. This mutant splice donor results in the splicing of 106 bp of the OB-Ra terminal exon into the splice acceptor at Pro⁸⁹⁰ at OB-Rb RNA. The resulting mutant OB-Rb protein has a termination codon five amino acids after the splice and an identical amino acid sequence to OB-Ra. The mutant receptor is missing most of the cytoplasmic region including the potential "Box 2" motif. While RT-PCR demonstrated that the sizes of the other 3' ends were normal in C57BL/K⁹ db/db mice, it is possible that this alternative exon is inserted into other transcripts as well.

[0318] The OB-Rb leptin receptor is expressed at a high level in the hypothalamus relative to other tissues (FIG. 6). Lower level expression is seen in testes with an even lower level in adipose tissue. The other alternatively spliced mRNAs are expressed in several tissues including in some cases hypothalamus (FIG. 6). OB-Re, which encodes a putative soluble receptor, is highly expressed in adipose tissue and is expressed at a lower level in brain, heart, and testes (FIG. 6E).

[0319] The C57BL/Ks db/db mutation is coisogenic and results in the functional replacement of the cytoplasmic domain corresponding to OB-Rb by that of OB-Ra. These data, combined with the localization of the leptin receptor to precisely the same chromosomal region as db, strongly confirm that OB-Rb is allelic with db. The identification of mutations in the two other available alleles of db will provide additional information on the structure-function relationship of the protein. The fact that the C57BL/Ks db/db mutation is found in the unique C-terminus of OB-Rb explains why the sequence of OB-Ra was unchanged in C57BL/Ks db/db mice, and that binding of leptin to the

choroid plexus was normal in these animals. Leptin binding in C57BL/Ks db/db mice is likely to be normal in all locations. Rather, the obese phenotype appears to result from the inability of the OB-Ra C-terminus to initiate signal transduction when expressed in place of the C-terminus of OB-Rb. Elucidation of the signal transduction pathway and identification of possible sites of JAK binding to the cytoplasmic region of this receptor are contemplated.

[0320] These results suggest that the weight reducing effects of leptin are at least partially mediated via interactions with the OB-Rb receptor having a C-terminal (cytoplasmic) domain characteristic of OB-Rb in the hypothalamus, a brain region known to play an important role in regulating body weight. This is supported by the increased potency of leptin when administered directly into the CSF and the affects of leptin on the electrical activity of hypothalamic neurons. Leptin may modulate the activity of NPY, GLP-1 and other peptides known to affect feeding behavior in the hypothalamus and brain [Stephens et al., supra; Tarton et al., *Nature*, 379:69 (1996)]. It may also have effects other tissues expressing the leptin receptor including fat. The receptor expressed in choroid plexus, possibly OB-Ra or a splice variant sharing a similar C-terminus, may act to transport the protein to the CSF, a mechanism similar to that proposed for transport of insulin by the insulin receptor [Bahary et al., 1990, supra; Partridge et al., *Neurochem.*, 44:1771 (1985); Van Houten and Posner, *Nature*, 282:623 (1979); Wood and Park, *Am. J. Physiol.*, 233:E331-E334 (1979)].

[0321] OB-Re, the putative soluble receptor is believed to bind to leptin in the circulation. It could function as a transport protein to agonize leptin activity [see, e.g., Davis et al., *Science*, 259:1736 (1993); Kishimoto et al., supra; Davis et al., *Science*, 260:1805 (1993)].

EXAMPLE 2

Preparation of Antibodies to the OB Polypeptide

[0322] In addition to use of the recombinant protein to generate polyclonal antibodies, a set of three peptide sequences from the deduced full length murine OB-R sequence (i.e., SEQ ID NOS:6-10) were identified. The four internal peptide fragments are:

Peptide A (amino acid numbers (SEQ ID NO: 32) 145-158):

Glu-Pro-Leu-Pro-Lys-Asn-Pro-Phe-Lys-Asn-Tyr-Asp-Ser-Lys

Peptide B (amino acid numbers (SEQ ID NO: 33) 465-484):

His-Arg-Arg-Ser-Leu-Tyr-Cys-Pro-Asp-Ser-Pro-Ser-Ile-His-Pro-Thr-Ser-Glu-Pro-Lys

Peptide C (amino acid numbers (SEQ ID NO: 34) 863-881):

Gln-Arg-Met-Lys-Lys-Leu-Phe-Trp-Asp-Asp-Val-Pro-Asn-Pro-Lys-Asn-Cys-Ser-Trp

[0323] These peptides are prepared using standard solid phase peptide synthesis. The purified synthetic peptides are conjugated to KLH, and the peptide-KLH conjugates are used to immunize rabbits using standard techniques. Polyclonal antisera specific for each peptide is recovered from the rabbits.

EXAMPLE 3

Preparation of PCR Probes from cDNA Selection and Exon Trapping Clones

[0324] This Example describes the cDNA selection clones that were identified to correspond to OB-R. PCR primers from these clones were used as probes for OB-R cDNA and genomic clones, and are useful for identifying OB-R DNA, as well as characterizing different OB-R splice variants.

[0325] Five cDNA selection clones were found to be useful as probes: clones 7 (SEQ ID NO:35), 11 (SEQ ID NO:36), 42 (SEQ ID NO:37), 46 (SEQ ID NO:38), and 58 (SEQ ID NO:39). Two cDNA selection clones identified by hybridization with exon trapping clones were also found to be useful probes: clones S3 (SEQ ID NO:40) and S14 (SEQ ID NO:41).

[0326] PCR primers were prepared from each of the above-noted clones for use as probes in identifying OB-R DNA. Table 1 reports the forward and reverse primers for each of the clones, and notes which splice variants of OB-R, as well as the predicted coding region, each probe labels.

TABLE 1

PCR Primer Probes for OB-R DNA				
Source Clone (direction)	Sequence	SEQ ID NO:	Splice variant	Recognition region
7 (forward)	CCGAGGGAATTGACAGCC	42	all	extracellular
7 (reverse)	CTCACTGTGTAGTGTGAGGAGG	43		
11 (f)	TCCTGTGGACAGAACCAGC	44	all	extracellular
11 (r)	TGACACAGCTGCTGCTCAG	45		
42 (f)	TGGATAAACCCCTTGCTTTCA	26	b	far 3' region
42 (r)	GGTCTCAGAGCACCCAGGTA	46		
46 (f)	AGAGAGATCCCTGACCCTAGTT	47	d	3' non-coding
46 (r)	AACTTCTGCCTTCTCATGTCA	48		
58 (f)	TTTCTCATCTAACAAGCAAGCA	49	b	far 5'
58 (r)	ATCTGTTTCTTGCGCAGGAT	50		
S14 (f)	CATTGTTGGGGCTCCAG	51	d, e	extracellular
S14 (r)	AATCGTTCTGCAAATCCAGG	52		
S3 (f)	TGAAGTCATAGATGATTCGCC	53	a, d, e	extracellular
S3 (r)	GTTCTGACCCGACGTCCTG	54		

[0327] As indicated in the table, probes from clones 7 and 11 have been useful in identifying all splice forms of OB-R identified to date. Probe 42 is useful to identify a splice variant with a cytoplasmic domain corresponding to OB-Rb, i.e., that is putatively signal transduction competent. Probes 46 and S14 are useful to identify splice variants having an N-terminal amino acid sequence corresponding to OB-Rd and OB-Re (which is identical to the N-terminal sequence of the published murine OB-R up to the C-terminal splice sites identified for these proteins; see FIG. 2B). Probe 58 is useful to identify an OB-R containing a unique 5' region found in the OB-Rb splice variant cDNA, which may be a non-coding region. S3 identifies nucleic acids encoding extracellular domains found in variants a, d, and e (corresponding to the published murine OB-R extracellular domain).

[0328] The hybridization conditions for screening mouse brain cDNA library were as follows: probes with a length of about 150-300bp long were labeled with ³²P-dCTP using hot-PCR. The filters were first pre-hybridized for at least one hour at 65° C. using RAPID-HYB buffer (Amersham LIFE SCIENCES). The labeled probe was added to a final concentration of 106 cpm/ml of RAPID-HYB solution and the

hybridization was done for at least 6 hours at 65° C. The filters were washed with 2×SSC/0.1% SDS, RT, for 30 min, followed by a more stringent wash with 0.3×SSC/0.1% SDS, RT, for ½ hour.

[0329] Thus, the probes described in this example are useful for identifying OB-R, as well as identifying unique splice variants. It is believed, for example, that a splice variant with an extracellular domain corresponding to OB-Ra, or OB-Rc/d/e may be joined with a cytoplasmic domain corresponding to OB-Rb.

[0330] The present invention is not to be limited in scope by the specific embodiments describe 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.

[0331] Where nucleotide or amino acid sequence lengths are provided, or molecular weight values given, they are approximate.

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agatgaatgg agcctgtgtc ttccactact ctcccaggag gttccagaat agcnaaagtg      240
tcagccagaa ttcttgaagt catagactgg agttagagat gaacataagc tcattgtaag      300
cctgggttac ttcttatcat ccttaatttt gaaagctaag agggcctaac catcaagaac      360
gtcctggagg aaagaatgth ttaacgccca ttattcagtc aaagaaatta agacttgaga      420

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gaaatgctca tttcttctct catgatggct ccttacacct tacttctacc gtacgatcca	480
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agagactcct gttctagtct gtgntataaa attcagcttg tggaaagcttt ctgaggggtt	600
ggcagcattc aattttacct gcaataggta aaggtaatct tttgggaagt gaagagtgtt	660
attagacatt tcagaaagaa caaacaggat tggggctgct atgtgttcta cacaggaatc	720
ttccataaca cagaataatt tatgtagata gagacaagat ggaaatgccc agggcccca	780
aatagccgct gttatttggt aacctcaag gttttctggt tgtttatctg tttcttgcgc	840
aggatcatct tccaagcaca tcctggggga acagtggcag agtcaactga gttcatgaaa	900
ctatggtgac atctgagctt ccttggttct tcacagaaca taagcagttc ctttcttgc	960
ttgttagatg agaaaacttc cttgtcagtc tgtctctacg actagaatgg aaagccttac	1020
tacttctat gtattcttaa tatttcaaat gcctaatta tgtttggctt ctctgtcttt	1080
aagggattta gtctctggat ttgaagaaat aaataaataa ataaaggaaa actaattttc	1140
tcgtgccgga tgactgctag ctgagctcag gcctactgca ttctacattt cgactctctc	1200
cctcttcccc agtgctttag cactggactg ggcagtcct ggctgtgtct aactcctggt	1260
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ggaaggagtt gaaaaccaa agataattaa tggtttcacc aaagatgcta tcgacaagca	1860
gcagaatgac gcagggctgt atgtcattgt acccataatt atttctctt gtgtcctact	1920
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aaacccaag aattgttctt gggcacaagg actgaatttc caaaagcctg aaacattnga	2040
gcactctttt accaagcatg cagaatcagt gatatttggg cctcttcttc tggagcctga	2100
accatttca gaagaaatca gtgtcgatac agcttgaaa aataaagatg agatggctcc	2160
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gttgatgag cttttggaac tggagggaa ttttctgaa gaaaatcaca gggagnagtc	2580
tgtctgttat ctaggagtca cctccgtccn cagaagagag agtgggtgtc ttttgactgg	2640
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ccaggagaga tgctcacact ttgtagaaaa taatttgagt ttagggacct ctggtgagaa 2760
ctttggtcct aacatgcccc aattccaaac ctggtccacg cacagtcaca agataatgga 2820
gaataagatg tgtgacttaa ctgtgtaa 2848

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<210> SEQ ID NO 4
<211> LENGTH: 582
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (79)..(79)
<223> OTHER INFORMATION: X can be any amino acid

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<400> SEQUENCE: 4

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Leu Arg Asp Leu Val Ser Gly Phe Glu Glu Ile Asn Lys Ile Lys Glu
1          5          10          15
Asn Phe Ser Arg Ala Gly Leu Leu Ala Glu Leu Arg Pro Thr Ala Phe
20          25          30
Tyr Ile Ser Thr Leu Ser Leu Phe Pro Ser Ala Leu Ala Leu Asp Trp
35          40          45
Ala Val Pro Gly Leu Val Leu Leu Phe Pro Gly Gly Asn Val Glu Leu
50          55          60
His Glu Phe Trp Tyr Lys His Cys Gly Leu Cys Ala Asn Ile Xaa Cys
65          70          75          80
Phe Leu Gln Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg
85          90          95
Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp
100         105         110
Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His
115         120         125
Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn
130         135         140
Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu
145         150         155         160
Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp
165         170         175
Thr Leu Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp
180         185         190
Lys Ile Leu Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser
195         200         205
Asn Val Lys Lys Phe Tyr Ile His Asp Asn Phe Ile Pro Ile Glu Lys
210         215         220
Tyr Gln Phe Ser Leu Tyr Pro Val Phe Met Glu Gly Val Gly Lys Pro
225         230         235         240
Lys Ile Ile Asn Gly Phe Thr Lys Asp Ala Ile Asp Lys Gln Gln Asn
245         250         255
Asp Ala Gly Leu Tyr Val Ile Val Pro Ile Ile Ile Ser Ser Cys Val
260         265         270
Leu Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu
275         280         285
Phe Trp Asp Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln Gly
290         295         300
Leu Asn Phe Gln Lys Pro Glu Thr Phe Glu Gln Leu Phe Thr Lys His

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305	310	315	320
Ala Glu Ser Val	Ile Phe Gly Pro Leu Leu Leu Glu Pro Glu Pro Ile		
	325	330	335
Ser Glu Glu Ile	Ser Val Asp Thr Ala Trp Lys Asn Lys Asp Glu Met		
	340	345	350
Val Pro Ala Ala	Met Val Ser Leu Leu Leu Thr Thr Pro Asp Pro Glu		
	355	360	365
Ser Ser Ser Ile	Cys Ile Ser Asp Gln Cys Asn Ser Ala Asn Phe Ser		
	370	375	380
Gly Ser Gln Ser	Thr Gln Val Thr Cys Glu Asp Glu Cys Gln Arg Gln		
	385	390	400
Pro Ser Val Lys	Tyr Ala Thr Leu Val Ser Asn Asp Lys Leu Val Glu		
	405	410	415
Thr Asp Glu Glu	Gln Gly Phe Ile His Ser Pro Val Ser Asn Cys Ile		
	420	425	430
Ser Ser Asn His	Ser Pro Leu Arg Gln Ser Phe Ser Ser Ser Ser Trp		
	435	440	445
Glu Thr Glu Ala	Gln Thr Phe Phe Leu Leu Ser Asp Gln Gln Pro Thr		
	450	455	460
Met Ile Ser Pro	Gln Leu Ser Phe Ser Gly Leu Asp Glu Leu Leu Glu		
	465	470	480
Leu Glu Gly Ser	Phe Pro Glu Glu Asn His Arg Glu Lys Ser Val Cys		
	485	490	495
Tyr Leu Gly Val	Thr Ser Val Asn Arg Arg Glu Ser Gly Val Leu Leu		
	500	505	510
Thr Gly Glu Ala	Gly Ile Leu Cys Thr Phe Pro Ala Gln Cys Leu Phe		
	515	520	525
Ser Asp Ile Arg	Ile Leu Gln Glu Arg Cys Ser His Phe Val Glu Asn		
	530	535	540
Asn Leu Ser Leu	Gly Thr Ser Gly Glu Asn Phe Val Pro Tyr Met Pro		
	545	550	555
Gln Phe Gln Thr	Cys Ser Thr His Ser His Lys Ile Met Glu Asn Lys		
	565	570	575
Met Cys Asp Leu	Thr Val		
	580		

<210> SEQ ID NO 5
 <211> LENGTH: 961
 <212> TYPE: DNA
 <213> ORGANISM: Mus musculus
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (160)..(160)
 <223> OTHER INFORMATION: N can be A, C, T or G
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (258)..(258)
 <223> OTHER INFORMATION: N can be A, C, T or G
 <400> SEQUENCE: 5

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ctccctcttc cccagtgtt tagcactgga ctgggcagtn cctggcctgg tctaactcct	180
gtttcctggt gggaatgtat aataagaact ccatgagttc tggataaac actgtggtct	240

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gtgtgctaataaatactngt gtttcttaca gccctgacg aaaaatgact cactgtgtag 300
tgtgaggagg tacgtggtga agcatcgtac tgcccacaat gggacgtggt cagaagatgt 360
gggaaatcgg accaatctca ctttctgtg gacagaacca ggcacactg ttacagttct 420
ggctgtcaat tcctctggcg cttccctgt gaattttaac cttaccttct catggcccat 480
gagtaaagt agtgctgtgg agtcactcag tgcttatccc ctgagcagca gctgtgtcat 540
ccttctctgg acactgtcac ctgatgatta tagtctgtta tatctggta ttgaatggaa 600
gatccttaataaagatgatg gaatgaagt gcttagaatt ccctcgaatg ttaaaaagtt 660
ttatatccac gataatttta ttcccatoga gaaatcacg tttagtcttt acccagtatt 720
tatggaagga gttgaaaaa caaagataat taatggtttc accaaagatg ctatcgacaa 780
gcagcagaat gacgcagggc tgtatgtcat tgtaccata attatttct cttgtgtcct 840
actgctcgga acactgttaa ttccacacca gagaatgaaa aagttgtttt gggacgatgt 900
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a 961

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<210> SEQ ID NO 6
<211> LENGTH: 319
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (58)..(58)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (67)..(67)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (68)..(68)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (84)..(84)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (86)..(86)
<223> OTHER INFORMATION: X can be any amino acid

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<400> SEQUENCE: 6

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Leu Arg Asp Leu Val Ser Gly Phe Glu Glu Ile Asn Lys Xaa Ile Lys
1           5           10          15
Glu Asn Xaa Phe Ser Arg Ala Gly Xaa Leu Leu Ala Glu Leu Arg Pro
20          25          30
Thr Ala Phe Tyr Ile Ser Thr Leu Ser Leu Phe Pro Ser Ala Leu Ala
35          40          45

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Leu Asp Trp Ala Val Pro Gly Leu Val Xaa Leu Leu Phe Pro Gly Gly
 50 55 60

Asn Val Xaa Xaa Glu Leu His Glu Phe Trp Tyr Lys His Cys Gly Leu
 65 70 75 80

Cys Ala Asn Xaa Ile Xaa Cys Phe Leu Gln Pro Leu Thr Lys Asn Asp
 85 90 95

Ser Leu Cys Ser Val Arg Arg Tyr Val Val Lys His Arg Thr Ala His
 100 105 110

Asn Gly Thr Trp Ser Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe
 115 120 125

Leu Trp Thr Glu Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser
 130 135 140

Leu Gly Ala Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met
 145 150 155 160

Ser Lys Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser
 165 170 175

Ser Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu
 180 185 190

Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly Met
 195 200 205

Lys Trp Leu Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His Asp
 210 215 220

Asn Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro Val Phe
 225 230 235 240

Met Glu Gly Val Gly Lys Pro Lys Ile Ile Asn Gly Phe Thr Lys Asp
 245 250 255

Ala Ile Asp Lys Gln Gln Asn Asp Ala Gly Leu Tyr Val Ile Val Pro
 260 265 270

Ile Ile Ile Ser Ser Cys Val Leu Leu Leu Gly Thr Leu Leu Ile Ser
 275 280 285

His Gln Arg Met Lys Lys Leu Phe Trp Asp Asp Val Pro Asn Pro Lys
 290 295 300

Asn Cys Ser Trp Ala Gln Gly Leu Asn Phe Gln Lys Val Thr Val
 305 310 315

<210> SEQ ID NO 7
 <211> LENGTH: 2703
 <212> TYPE: DNA
 <213> ORGANISM: Mus musculus
 <400> SEQUENCE: 7

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atgatgtgtc agaaattcta tgtggttttg ttacactggg aatttcttta tgtgatagct    60
gcacttaacc tggcatatcc aatctctccc tggaaattta agttgttttg tggaccaccg    120
aacacaaccg atgactcctt tctctcacct gctggagccc caaacaatgc ctcggtttg    180
aagggggcct ctgaagcaat tgttgaagct aaatttaatt caagtggat ctacgttcct    240
gagttatcca aaacagtctt ccaactgttc tttgggaatg agcaaggtca aaactgctct    300
gcactcacag acaactga agggaagaca ctggcttcag tagtgaaggc ttcagttttt    360
cgccagctag gtgtaaactg ggacatagag tgctggatga aaggggactt gacattattc    420
atctgtcata tggagccatt acctaagaac cccttcaaga attatgactc taaggtccat    480
cttttatatg atctgcctga agtcatagat gattcgcctc tgccccact gaaagacagc    540
    
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tttcagactg tccaatgcaa ctgcagtcctt cggggatgtg aatgtcatgt gccggtaccc	600
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tttcagtcac ctctgatgtc actgcagccc atgcttgttg tgaaaccoga tccaccctta	720
ggtttgcata tggaaatcac agatgatggg aatttaaaga tttcttggga cagccaaaca	780
atggcaccat ttccgcttca atatcagggt aatatattag agaattctac aattgtaaga	840
gaggctgctg aaattgtctc agctacatct ctgctggtag acagtggtct tcctggatct	900
tcatatgagg tccagggtgag gagcaagaga ctggatggtt caggagtctg gactgactgg	960
agttcacctc aagtctttac cacacaagat gttgtgtatt ttccacccaa aattctgact	1020
agtgttgat cgaatgcttc ttttcattgc atctacaaaa acgaaaacca gattatctcc	1080
tcaaacaga tagtttggtg gaggaatcta gctgagaaaa tccctgagat acagtacagc	1140
attgtgagtg accgagttag caaagttacc ttctccaacc tgaaagccac cagacctcga	1200
gggaagtta cctatgacgc agtgtactgc tgcaatgagc aggcgtgcca tcaccgctat	1260
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actaaatga cttgcagatg gtcaccagc acaatccaat cactagtggg aagcactgtg	1380
cagctgaggt atcacaggcg cagcctgtat tgcctgata gtccatctat tcatcctacg	1440
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atctttctat tatctggtta tacaatgtgg atcaggatca accattcttt aggttcactt	1560
gactcgccac caacgtgtgt ccttctctgac tccgtagtaa aaccactacc tccatctaac	1620
gtaaaagcag agattactgt aaacactgga ttattgaaag tatcttggga aaagccagtc	1680
tttccggaga ataacttca attccagatt cgatatggct taagtggaaa agaaatacaa	1740
tggaagacac atgaggtatt cgatgcaaaag tcaaagtctg ccagcctgct ggtgtcagac	1800
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ttcctgtgga cagaaccagc gcacactggt acagttctgg ctgtcaattc cctcggcgt	2160
tccctgtgga attttaacct taccttctca tggccatga gtaaagtgag tgcgtggag	2220
tcactcagtg cttatcccct gacgacgagc tgtgtcatcc tttcctggac actgtcacct	2280
gatgattata gtctgttata tctggttatt gaatggaaga tccttaatga agatgatgga	2340
atgaagtggc ttagaattcc ctgcaatggt aaaaagtttt atatccacga taattttatt	2400
cccacgaga aatatcagtt tagtctttac ccagtattha tggaggaggt tggaaaacca	2460
aagataatta atggtttcac caaagatgct atcgacaagc agcagaatga cgcagggctg	2520
tatgtcattg taccataat tatttctctt tgtgtcctac tgctcggaac actgttaatt	2580
tcacaccaga gaatgaaaaa gttgttttgg gacgatgttc caaacccaa gaattgttcc	2640
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tag	2703

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<210> SEQ ID NO 8
<211> LENGTH: 900
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 8

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1          5          10          15

Tyr Val Ile Ala Ala Leu Asn Leu Ala Tyr Pro Ile Ser Pro Trp Lys
          20          25          30

Phe Lys Leu Phe Cys Gly Pro Pro Asn Thr Thr Asp Asp Ser Phe Leu
          35          40          45

Ser Pro Ala Gly Ala Pro Asn Asn Ala Ser Ala Leu Lys Gly Ala Ser
          50          55          60

Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser Gly Ile Tyr Val Pro
65          70          75          80

Glu Leu Ser Lys Thr Val Phe His Cys Cys Phe Gly Asn Glu Gln Gly
          85          90          95

Gln Asn Cys Ser Ala Leu Thr Asp Asn Thr Glu Gly Lys Thr Leu Ala
          100          105          110

Ser Val Val Lys Ala Ser Val Phe Arg Gln Leu Gly Val Asn Trp Asp
          115          120          125

Ile Glu Cys Trp Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met
130          135          140

Glu Pro Leu Pro Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His
145          150          155          160

Leu Leu Tyr Asp Leu Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro
          165          170          175

Leu Lys Asp Ser Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly
          180          185          190

Cys Glu Cys His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu
          195          200          205

Leu Met Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro
210          215          220

Leu Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu
225          230          235          240

Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp
          245          250          255

Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr
          260          265          270

Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala
          275          280          285

Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly Ser Ser Tyr Glu Val
290          295          300

Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp
305          310          315          320

Ser Ser Pro Gln Val Phe Thr Thr Gln Asp Val Val Tyr Phe Pro Pro
          325          330          335

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

Lys Asn Glu Asn Gln Ile Ile Ser Ser Lys Gln Ile Val Trp Trp Arg
          355          360          365

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Asn Leu Ala Glu Lys Ile Pro Glu Ile Gln Tyr Ser Ile Val Ser Asp
 370 375 380

Arg Val Ser Lys Val Thr Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg
 385 390 395 400

Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys
 405 410 415

His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile
 420 425 430

Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser
 435 440 445

Pro Ser Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr
 450 455 460

His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr
 465 470 475 480

Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu Cys
 485 490 495

Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp Ile Arg
 500 505 510

Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys Val Leu
 515 520 525

Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn Val Lys Ala Glu
 530 535 540

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

Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu Ser Gly
 565 570 575

Lys Glu Ile Gln Trp Lys Thr His Glu Val Phe Asp Ala Lys Ser Lys
 580 585 590

Ser Ala Ser Leu Leu Val Ser Asp Leu Cys Ala Val Tyr Val Val Gln
 595 600 605

Val Arg Cys Arg Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser
 610 615 620

Ser Pro Ala Tyr Thr Leu Val Met Asp Val Lys Val Pro Met Arg Gly
 625 630 635 640

Pro Glu Phe Trp Arg Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg
 645 650 655

Asn Val Thr Leu Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys
 660 665 670

Ser Val Arg Arg Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr
 675 680 685

Trp Ser Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr
 690 695 700

Glu Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala
 705 710 715 720

Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys Val
 725 730 735

Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val
 740 745 750

Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu
 755 760 765

Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly Met Lys Trp Leu

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cgcagtgtac tgctgcaatg agcaggcgtg ccatcaccgc tatgtgtaac tatacgtgat 1320
cgatgtcaat atcaatataat catgtgaaac tgacgggtac ttaactaaaa tgacttgacag 1380
atggtcacc cgcacaatcc aatcactagt gggaagcact gtgcagctga ggtatcacag 1440
gcgcagcctg tattgtcctg atagtccatc tattcatcct acgtctgagc ccaaaaactg 1500
cgtcttacag agagacggct tttatgaatg tgttttccag ccaatctttc tattatctgg 1560
ctatacaatg tggatcagga tcaaccattc tttaggttca cttgactcgc caccaacgtg 1620
tgtccttct gactccgtag taaaccact acctccatct aacgtaaaag cagagattac 1680
tgtaaacact ggattattga aagtatcttg gaaaagcca gtctttccgg agaataacct 1740
tcaattccag attcgatatg gcttaagtgg aaaagaata caatggaaga cacatgaggt 1800
attcgatgca aagtcaaagt ctgccagcct gctgggtgca gacctctgtg cagtctatgt 1860
gggccaggtt cgtgccggc gggtggatgg actaggatat tggagtaatt ggagcagtc 1920
agcctatacg cttgtcatgg atgtaaaagt tcctatgaga gggcctgaat tttggagaaa 1980
aatggatggg gacgttacta aaaaggagag aaatgtcacc ttgctttgga agcccctgac 2040
gaaaaatgac tcactgtgta gtgtgaggag gtacgtggtg aagcatcgta ctgccacaa 2100
tgggacgtgg tcagaagatg tgggaaatcg gaccaatctc actttcctgt ggacagaacc 2160
agcgcacact gttacagttc tggctgtcaa ttccctcggc gcttccttg tgaattttaa 2220
ccttaccttc tcattggcca tgagtaaagt gagtgctgtg gagtoactca gtgcttatcc 2280
cctgagcagc agctgtgta tcctttcctg gacactgtca cctgatgatt atagtctgtt 2340
atatctgggtt attgaatgga agatccttaa tgaagatgat ggaatgaagt ggcttagaat 2400
tcctcgaat gttaaaaagt tttatatoca cggtatgtgt actgtacttt tcattgatta 2460
g 2461

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<210> SEQ ID NO 10
<211> LENGTH: 805
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 10

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Met Met Cys Gln Lys Phe Tyr Val Val Leu Leu His Trp Glu Phe Leu
1           5           10           15
Tyr Val Ile Ala Ala Leu Asn Leu Ala Tyr Pro Ile Ser Pro Trp Lys
          20           25           30
Phe Lys Leu Phe Cys Gly Pro Pro Asn Thr Thr Asp Asp Ser Phe Leu
          35           40           45
Ser Pro Ala Gly Ala Pro Asn Asn Ala Ser Ala Leu Lys Gly Ala Ser
          50           55           60
Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser Gly Ile Tyr Val Pro
          65           70           75           80
Glu Leu Ser Lys Thr Val Phe His Cys Cys Phe Gly Asn Glu Gln Gly
          85           90           95
Gln Asn Cys Ser Ala Leu Thr Asp Asn Thr Glu Gly Lys Thr Leu Ala
          100          105          110
Ser Val Val Lys Ala Ser Val Phe Arg Gln Leu Gly Val Asn Trp Asp
          115          120          125
Ile Glu Cys Trp Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met
          130          135          140

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Glu Pro Leu Pro Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His
 145 150 155 160
 Leu Leu Tyr Asp Leu Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro
 165 170 175
 Leu Lys Asp Ser Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly
 180 185 190
 Cys Glu Cys His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu
 195 200 205
 Leu Met Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro
 210 215 220
 Leu Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu
 225 230 235 240
 Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp
 245 250 255
 Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr
 260 265 270
 Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala
 275 280 285
 Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly Ser Ser Tyr Glu Val
 290 295 300
 Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp
 305 310 315 320
 Ser Ser Pro Gln Val Phe Thr Thr Gln Asp Val Val Tyr Phe Pro Pro
 325 330 335
 Lys Ile Leu Thr Ser Val Gly Ser Asn Ala Ser Phe His Cys Ile Tyr
 340 345 350
 Lys Asn Glu Asn Gln Ile Ile Ser Ser Lys Gln Ile Val Trp Trp Arg
 355 360 365
 Asn Leu Ala Glu Lys Ile Pro Glu Ile Gln Tyr Ser Ile Val Ser Asp
 370 375 380
 Arg Val Ser Lys Val Thr Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg
 385 390 395 400
 Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys
 405 410 415
 His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile
 420 425 430
 Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser
 435 440 445
 Pro Ser Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr
 450 455 460
 His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr
 465 470 475 480
 Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu Cys
 485 490 495
 Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp Ile Arg
 500 505 510
 Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys Val Leu
 515 520 525
 Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn Val Lys Ala Glu
 530 535 540

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

Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu Ser Gly
                565                570                575

Lys Glu Ile Gln Trp Lys Thr His Glu Val Phe Asp Ala Lys Ser Lys
                580                585                590

Ser Ala Ser Leu Leu Val Ser Asp Leu Cys Ala Val Tyr Val Val Gln
                595                600                605

Val Arg Cys Arg Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser
        610                615                620

Ser Pro Ala Tyr Thr Leu Val Met Asp Val Lys Val Pro Met Arg Gly
625                630                635                640

Pro Glu Phe Trp Arg Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg
                645                650                655

Asn Val Thr Leu Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys
        660                665                670

Ser Val Arg Arg Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr
        675                680                685

Trp Ser Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr
        690                695                700

Glu Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala
705                710                715                720

Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys Val
                725                730                735

Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val
                740                745                750

Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu
        755                760                765

Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly Met Lys Trp Leu
        770                775                780

Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His Gly Met Cys Thr
785                790                795                800

Val Leu Phe Met Asp
                805

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<210> SEQ ID NO 11
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 11

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Asn Phe Gln Lys Arg Thr Asp Thr Leu
1                5

```

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<210> SEQ ID NO 12
<211> LENGTH: 277
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 12

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Asn Phe Gln Lys Pro Glu Thr Phe Glu His Leu Phe Thr Lys His Ala
1                5                10                15

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

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<400> SEQUENCE: 15

Phe Tyr Ile His Gly Met Cys Thr Val Leu Phe Met Asp
 1 5 10

<210> SEQ ID NO 16

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 16

Pro Gln Lys Arg Thr Asp Thr Leu
 1 5

<210> SEQ ID NO 17

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 17

Pro Gln Lys Pro Glu Thr
 1 5

<210> SEQ ID NO 18

<211> LENGTH: 12

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 18

gatggaggga aa

12

<210> SEQ ID NO 19

<211> LENGTH: 12

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 19

gatggaggta aa

12

<210> SEQ ID NO 20

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 20

atcttgggtt ctctgaagaa

20

<210> SEQ ID NO 21

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 21

gagattgtca gtcacagcct c

21

<210> SEQ ID NO 22

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 22

atctgaattg gaatcaaata cac

23

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<210> SEQ ID NO 23
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 23

aaatctgtta tccttctgaa ac 22

<210> SEQ ID NO 24
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 24

acactgttaa tttcacacca gag 23

<210> SEQ ID NO 25
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 25

agtcattcaa accattagtt tagg 24

<210> SEQ ID NO 26
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 26

tggataaacc cttgctcttc a 21

<210> SEQ ID NO 27
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 27

tgaacacaac aacataaagc cc 22

<210> SEQ ID NO 28
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 28

aggctccctc agggccac 18

<210> SEQ ID NO 29
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 29

gtgactgaat gaagatgtaa tatac 25

<210> SEQ ID NO 30
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

-continued

<400> SEQUENCE: 30

tggttatct ggttattgaa tgg 23

<210> SEQ ID NO 31

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 31

cattaaatga ttattatca gaattgc 27

<210> SEQ ID NO 32

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 32

Glu Pro Leu Pro Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys
1 5 10

<210> SEQ ID NO 33

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 33

His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr
1 5 10 15Ser Glu Pro Lys
20

<210> SEQ ID NO 34

<211> LENGTH: 19

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 34

Gln Arg Met Lys Lys Leu Phe Trp Asp Asp Val Pro Asn Pro Lys Asn
1 5 10 15

Cys Ser Trp

<210> SEQ ID NO 35

<211> LENGTH: 166

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (5)..(5)

<223> OTHER INFORMATION: N can be A, C, T or G

<400> SEQUENCE: 35

agggnaagcg ccgaggggaat tgacagccag aactgtaaca gtgtgcgctg gttctgtcca 60

caggaaagtg agattgtcc gatttcccac atcttctgac caggtcccat tgtgggcagt 120

acgatgcttc accacgtacc tcctcact acacagtgag tcattt 166

<210> SEQ ID NO 36

<211> LENGTH: 320

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

-continued

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<400> SEQUENCE: 36
ggtgaagcat cgtactgccc acaatgggac gtggtcagaa gatgtgggaa atcggaccaa    60
tctcactttc ctgtggacag aaccagcgca cactgttaca gttctggctg tcaattccct    120
cggcgcttcc ctgtgaatt ttaaccttac cttctcatgg cccatgagta aagtgagtgc    180
tgtggagtca ctcaagtgtt atccccctgag cagcagctgt gtcacccctt cctggacact    240
gtcacctgat gattatagtc tgttatatct ggttattgaa tggaagatcc ttaatgaaga    300
tgatggaatg aagtggctta                                           320

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<210> SEQ ID NO 37
<211> LENGTH: 158
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 37
gattactgga gatgcagttg ctgacaggac tatggataaa cccttgcctc tcatcagttt    60
ccactagttt atcgttgctg accagagttg catatttaac tgagggttgt ctctgacact    120
catcctcaca ggttacctgg gtgctctgag acccagag                                           158

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<210> SEQ ID NO 38
<211> LENGTH: 192
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 38
agagagatcc ctgaccctag ttagatctgt tttcaggctc tgtgttcatt tgatgttcag    60
aagtcagcaa ggttctcata tgtcctgagt tagtaagatg tctcagggtt ccccatcag    120
ctaacaacca ctttgacatg agaaggcaga aagttaaaga aactactctg gtgttttact    180
taaagatacg ag                                           192

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<210> SEQ ID NO 39
<211> LENGTH: 168
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (55)..(55)
<223> OTHER INFORMATION: N can be A, C, T or G
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (62)..(62)
<223> OTHER INFORMATION: N can be A, C, T or G
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (72)..(72)
<223> OTHER INFORMATION: N can be A, C, T or G
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (143)..(143)
<223> OTHER INFORMATION: N can be A, C, T or G

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<400> SEQUENCE: 39
agactgacaa ggaagttttc tcatctaaca agcaagcaaa ggaactgctt atgtntctgtg    60
angaaccaag gnagctcaga tgtcaccata gtcacatga actcgagtga ctctgccact    120
gttccccag gatgtgcttg gangataatc ctgcgcaaga aacagata                                           168

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<210> SEQ ID NO 40
<211> LENGTH: 259
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (83)..(83)
<223> OTHER INFORMATION: N can be A, C, T or G
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (101)..(101)
<223> OTHER INFORMATION: N can be A, C, T or G
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (181)..(181)
<223> OTHER INFORMATION: N can be A, C, T or G

<400> SEQUENCE: 40

agaattatga ctctaaggtc catcttttat atgatctgcc tgaagtcata gatgattcgc      60
ctctgcccc  actgaaagac agntttcaga ctgtccaatg naactgcagt cttcggggat      120
gtgaatgtca tgtgccagta cccagagcca aactcaacta cgctctctctg atgtatttgg      180
naatcacatc tgccgggtgtg agttttcagt cacctctgat gtcactgcag cccatgcttg      240
ttgtgaaacc cgatccacc                                          259

<210> SEQ ID NO 41
<211> LENGTH: 250
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (193)..(193)
<223> OTHER INFORMATION: N can be A, C, T or G

<400> SEQUENCE: 41

cttcaacaat tggttcagaa gcccccttca aagccgaggc attgtttggg gctccagcag      60
gtgagagaaa ggagtcacatg gttgtgttcg gtggtccaca aaacaactta aatttccagg      120
gagagattgg atatgccagg ttaagtgcag ctatcacata aagaaattcc cagtgttaaca      180
aaaccacata gantttctaa cacatcatct ttcttcagag gtgtacacct ggatttgcag      240
aacgattcct                                          250

<210> SEQ ID NO 42
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 42

ccgaggggaat tgacagcc                                          18

<210> SEQ ID NO 43
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 43

ctcactgtgt agtgtgagga gg                                          22

<210> SEQ ID NO 44
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 44
tcctgtggac agaaccagc 19

<210> SEQ ID NO 45
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 45
tgacacagct gctgctcag 19

<210> SEQ ID NO 46
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 46
ggtctcagag caccaggta 20

<210> SEQ ID NO 47
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 47
agagagatcc ctgacctag tt 22

<210> SEQ ID NO 48
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 48
aaactttctgc cttccttctc atgtca 26

<210> SEQ ID NO 49
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 49
tttctcatct aacaagcaag ca 22

<210> SEQ ID NO 50
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 50
atctgtttct tgcgaggat 20

<210> SEQ ID NO 51
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 51
cattgtttgg ggctccag 18

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<210> SEQ ID NO 52
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 52
aatcgttctg caaatccagg                20

<210> SEQ ID NO 53
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 53
tgaagtcata gatgattcgc c            21

<210> SEQ ID NO 54
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 54
gttcgtaccc gacgtcactg                20

<210> SEQ ID NO 55
<211> LENGTH: 894
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 55
Met Met Cys Gln Lys Phe Tyr Val Val Leu Leu His Trp Glu Phe Leu
 1          5          10          15
Tyr Val Ile Ala Ala Leu Asn Leu Ala Tyr Pro Ile Ser Pro Trp Lys
 20          25          30
Phe Lys Leu Phe Cys Gly Pro Pro Asn Thr Thr Asp Asp Ser Phe Leu
 35          40          45
Ser Pro Ala Gly Ala Pro Asn Asn Ala Ser Ala Leu Lys Gly Ala Ser
 50          55          60
Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser Gly Ile Tyr Val Pro
 65          70          75          80
Glu Leu Ser Lys Thr Val Phe His Cys Cys Phe Gly Asn Glu Gln Gly
 85          90          95
Gln Asn Cys Ser Ala Leu Thr Asp Asn Thr Glu Gly Lys Thr Leu Ala
 100         105         110
Ser Val Val Lys Ala Ser Val Phe Arg Gln Leu Gly Val Asn Trp Asp
 115         120         125
Ile Glu Cys Trp Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met
 130         135         140
Glu Pro Leu Pro Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His
 145         150         155         160
Leu Leu Tyr Asp Leu Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro
 165         170         175
Leu Lys Asp Ser Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly
 180         185         190
Cys Glu Cys His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu
 195         200         205

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Leu Met Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro
 210 215 220
 Leu Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu
 225 230 235 240
 Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp
 245 250 255
 Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr
 260 265 270
 Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala
 275 280 285
 Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly Ser Ser Tyr Glu Val
 290 295 300
 Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp
 305 310 315 320
 Ser Ser Pro Gln Val Phe Thr Thr Gln Asp Val Val Tyr Phe Pro Pro
 325 330 335
 Lys Ile Leu Thr Ser Val Gly Ser Asn Ala Ser Phe His Cys Ile Tyr
 340 345 350
 Lys Asn Glu Asn Gln Ile Ile Ser Ser Lys Gln Ile Val Trp Trp Arg
 355 360 365
 Asn Leu Ala Glu Lys Ile Pro Glu Ile Gln Tyr Ser Ile Val Ser Asp
 370 375 380
 Arg Val Ser Lys Val Thr Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg
 385 390 395 400
 Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys
 405 410 415
 His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile
 420 425 430
 Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser
 435 440 445
 Pro Ser Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr
 450 455 460
 His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr
 465 470 475 480
 Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu Cys
 485 490 495
 Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp Ile Arg
 500 505 510
 Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys Val Leu
 515 520 525
 Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn Val Lys Ala Glu
 530 535 540
 Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser Trp Glu Lys Pro Val
 545 550 555 560
 Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu Ser Gly
 565 570 575
 Lys Glu Ile Gln Trp Lys Thr His Glu Val Phe Asp Ala Lys Ser Lys
 580 585 590
 Ser Ala Ser Leu Leu Val Ser Asp Leu Cys Ala Val Tyr Val Val Gln
 595 600 605
 Val Arg Cys Arg Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser

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610	615	620
Ser Pro Ala Tyr Thr Leu Val Met Asp Val Lys Val Pro Met Arg Gly 625 630 635 640		
Pro Glu Phe Trp Arg Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg 645 650 655		
Asn Val Thr Leu Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys 660 665 670		
Ser Val Arg Arg Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr 675 680 685		
Trp Ser Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr 690 695 700		
Glu Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala 705 710 715 720		
Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys Val 725 730 735		
Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val 740 745 750		
Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu 755 760 765		
Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly Met Lys Trp Leu 770 775 780		
Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His Asp Asn Phe Ile 785 790 795 800		
Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro Val Phe Met Glu Gly 805 810 815		
Val Gly Lys Pro Lys Ile Ile Asn Gly Phe Thr Lys Asp Ala Ile Asp 820 825 830		
Lys Gln Gln Asn Asp Ala Gly Leu Tyr Val Ile Val Pro Ile Ile Ile 835 840 845		
Ser Ser Cys Val Leu Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg 850 855 860		
Met Lys Lys Leu Phe Trp Asp Asp Val Pro Asn Pro Lys Asn Cys Ser 865 870 875 880		
Trp Ala Gln Gly Leu Asn Phe Gln Lys Arg Thr Asp Thr Leu 885 890		

<210> SEQ ID NO 56
 <211> LENGTH: 1165
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 56

Met Ile Cys Gln Lys Phe Cys Val Val Leu Leu His Trp Glu Phe Ile 1 5 10 15
Tyr Val Ile Thr Ala Phe Asn Leu Ser Tyr Pro Ile Thr Pro Trp Arg 20 25 30
Phe Lys Leu Ser Cys Met Pro Pro Asn Ser Thr Tyr Asp Tyr Phe Leu 35 40 45
Leu Pro Ala Gly Leu Ser Lys Asn Thr Ser Asn Ser Asn Gly His Tyr 50 55 60
Glu Thr Ala Val Glu Pro Lys Phe Asn Ser Ser Gly Thr His Phe Ser 65 70 75 80

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485					490					495					
Glu	Cys	Ile	Phe	Gln	Pro	Ile	Phe	Leu	Leu	Ser	Gly	Tyr	Thr	Met	Trp
			500					505					510		
Ile	Arg	Ile	Asn	His	Ser	Leu	Gly	Ser	Leu	Asp	Ser	Pro	Pro	Thr	Cys
		515					520					525			
Val	Leu	Pro	Asp	Ser	Val	Val	Lys	Pro	Leu	Pro	Pro	Ser	Ser	Val	Lys
	530					535					540				
Ala	Glu	Ile	Thr	Ile	Asn	Ile	Gly	Leu	Leu	Lys	Ile	Ser	Trp	Glu	Lys
545					550					555					560
Pro	Val	Phe	Pro	Glu	Asn	Asn	Leu	Gln	Phe	Gln	Ile	Arg	Tyr	Gly	Leu
				565					570					575	
Ser	Gly	Lys	Glu	Val	Gln	Trp	Lys	Met	Tyr	Glu	Val	Tyr	Asp	Ala	Lys
			580					585					590		
Ser	Lys	Ser	Val	Ser	Leu	Pro	Val	Pro	Asp	Leu	Cys	Ala	Val	Tyr	Ala
		595					600					605			
Val	Gln	Val	Arg	Cys	Lys	Arg	Leu	Asp	Gly	Leu	Gly	Tyr	Trp	Ser	Asn
	610					615					620				
Trp	Ser	Asn	Pro	Ala	Tyr	Thr	Val	Val	Met	Asp	Ile	Lys	Val	Pro	Met
625						630				635					640
Arg	Gly	Pro	Glu	Phe	Trp	Arg	Ile	Ile	Asn	Gly	Asp	Thr	Met	Lys	Lys
				645					650					655	
Glu	Lys	Asn	Val	Thr	Leu	Leu	Trp	Lys	Pro	Leu	Met	Lys	Asn	Asp	Ser
			660					665					670		
Leu	Cys	Ser	Val	Gln	Arg	Tyr	Val	Ile	Asn	His	His	Thr	Ser	Cys	Asn
		675					680					685			
Gly	Thr	Trp	Ser	Glu	Asp	Val	Gly	Asn	His	Thr	Lys	Phe	Thr	Phe	Leu
	690					695					700				
Trp	Thr	Glu	Gln	Ala	His	Thr	Val	Thr	Val	Leu	Ala	Ile	Asn	Ser	Ile
705					710					715					720
Gly	Ala	Ser	Val	Ala	Asn	Phe	Asn	Leu	Thr	Phe	Ser	Trp	Pro	Met	Ser
				725				730						735	
Lys	Val	Asn	Ile	Val	Gln	Ser	Leu	Ser	Ala	Tyr	Pro	Leu	Asn	Ser	Ser
			740				745						750		
Cys	Val	Ile	Val	Ser	Trp	Ile	Leu	Ser	Pro	Ser	Asp	Tyr	Lys	Leu	Met
		755					760					765			
Tyr	Phe	Ile	Ile	Glu	Trp	Lys	Asn	Leu	Asn	Glu	Asp	Gly	Glu	Ile	Lys
	770					775						780			
Trp	Leu	Arg	Ile	Ser	Ser	Ser	Val	Lys	Lys	Tyr	Tyr	Ile	His	Asp	His
785					790					795					800
Phe	Ile	Pro	Ile	Glu	Lys	Tyr	Gln	Phe	Ser	Leu	Tyr	Pro	Ile	Phe	Met
				805					810					815	
Glu	Gly	Val	Gly	Lys	Pro	Lys	Ile	Ile	Asn	Ser	Phe	Thr	Gln	Asp	Asp
			820						825				830		
Ile	Glu	Lys	His	Gln	Ser	Asp	Ala	Gly	Leu	Tyr	Val	Ile	Val	Pro	Val
	835						840					845			
Ile	Ile	Ser	Ser	Ser	Ile	Leu	Leu	Leu	Gly	Thr	Leu	Leu	Ile	Ser	His
	850					855					860				
Gln	Arg	Met	Lys	Lys	Leu	Phe	Trp	Glu	Asp	Val	Pro	Asn	Pro	Lys	Asn
865					870					875					880
Cys	Ser	Trp	Ala	Gln	Gly	Leu	Asn	Phe	Gln	Lys	Pro	Glu	Thr	Phe	Glu
				885					890						895

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His Leu Phe Ile Lys His Thr Ala Ser Val Thr Cys Gly Pro Leu Leu
 900 905 910
 Leu Glu Pro Glu Thr Ile Ser Glu Asp Ile Ser Val Asp Thr Ser Trp
 915 920 925
 Lys Asn Lys Asp Glu Met Met Pro Thr Thr Val Val Ser Leu Leu Ser
 930 935 940
 Thr Thr Asp Leu Glu Lys Gly Ser Val Cys Ile Ser Asp Gln Phe Asn
 945 950 955 960
 Ser Val Asn Phe Ser Glu Ala Glu Gly Thr Glu Val Thr Tyr Glu Ala
 965 970 975
 Glu Ser Gln Arg Gln Pro Phe Val Lys Tyr Ala Thr Leu Ile Ser Asn
 980 985 990
 Ser Lys Pro Ser Glu Thr Gly Glu Glu Gln Gly Leu Ile Asn Ser Ser
 995 1000 1005
 Val Thr Lys Cys Phe Ser Ser Lys Asn Ser Pro Leu Lys Asp Ser
 1010 1015 1020
 Phe Ser Asn Ser Ser Trp Glu Ile Glu Ala Gln Ala Phe Phe Ile
 1025 1030 1035
 Leu Ser Asp Gln His Pro Asn Ile Ile Ser Pro His Leu Thr Phe
 1040 1045 1050
 Ser Glu Gly Leu Asp Glu Leu Leu Lys Leu Glu Gly Asn Phe Pro
 1055 1060 1065
 Glu Glu Asn Asn Asp Lys Lys Ser Ile Tyr Tyr Leu Gly Val Thr
 1070 1075 1080
 Ser Ile Lys Lys Arg Glu Ser Gly Val Leu Leu Thr Asp Lys Ser
 1085 1090 1095
 Arg Val Ser Cys Pro Phe Pro Ala Pro Cys Leu Phe Thr Asp Ile
 1100 1105 1110
 Arg Val Leu Gln Asp Ser Cys Ser His Phe Val Glu Asn Asn Ile
 1115 1120 1125
 Asn Leu Gly Thr Ser Ser Lys Lys Thr Phe Ala Ser Tyr Met Pro
 1130 1135 1140
 Gln Phe Gln Thr Cys Ser Thr Gln Thr His Lys Ile Met Glu Asn
 1145 1150 1155
 Lys Met Cys Asp Leu Thr Val
 1160 1165

<210> SEQ ID NO 57
 <211> LENGTH: 1110
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (29)..(29)
 <223> OTHER INFORMATION: X can be any amino acid

<400> SEQUENCE: 57

Gly Leu Arg Ser Ala Ser Tyr Gln Pro Leu Lys Arg Phe Ser Arg Phe
 1 5 10 15
 Gln Ala Leu Ser Pro Cys Arg Ile Ser Thr Ser Leu Xaa Leu Val Pro
 20 25 30
 Asn Ser Ala Arg Gly Cys Phe Gly Asn Glu Gln Gly Gln Asn Cys Ser
 35 40 45

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Ala Leu Thr Asp Asn Thr Glu Gly Lys Thr Leu Ala Ser Val Val Lys
50 55 60

Ala Ser Val Phe Arg Gln Leu Gly Val Asn Trp Asp Ile Glu Cys Trp
65 70 75 80

Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro
85 90 95

Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp
100 105 110

Leu Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser
115 120 125

Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys His
130 135 140

Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu Leu Met Tyr Leu
145 150 155 160

Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro Leu Met Ser Leu
165 170 175

Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu Gly Leu His Met
180 185 190

Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp Asp Ser Gln Thr
195 200 205

Met Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr Leu Glu Asn Ser
210 215 220

Thr Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala Thr Ser Leu Leu
225 230 235 240

Val Asp Ser Val Leu Pro Gly Ser Ser Tyr Glu Val Gln Val Arg Ser
245 250 255

Lys Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp Ser Ser Pro Gln
260 265 270

Val Phe Thr Thr Gln Asp Val Val Tyr Phe Pro Pro Lys Ile Leu Thr
275 280 285

Ser Val Gly Ser Asn Ala Ser Phe His Cys Ile Tyr Lys Asn Glu Asn
290 295 300

Gln Ile Ile Ser Ser Lys Gln Ile Val Trp Trp Arg Asn Leu Ala Glu
305 310 315 320

Lys Ile Pro Glu Ile Gln Tyr Ser Ile Val Ser Asp Arg Val Ser Lys
325 330 335

Val Thr Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg Gly Lys Phe Thr
340 345 350

Tyr Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys His His Arg Tyr
355 360 365

Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser Cys Glu Thr
370 375 380

Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser Pro Ser Thr Ile
385 390 395 400

Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr His Arg Arg Ser
405 410 415

Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr Ser Glu Pro Lys
420 425 430

Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu Cys Val Phe Gln Pro
435 440 445

Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp Ile Arg Ile Asn His Ser

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450					455					460					
Leu	Gly	Ser	Leu	Asp	Ser	Pro	Pro	Thr	Cys	Val	Leu	Pro	Asp	Ser	Val
465					470					475					480
Val	Lys	Pro	Leu	Pro	Pro	Ser	Asn	Val	Lys	Ala	Glu	Ile	Thr	Val	Asn
				485					490					495	
Thr	Gly	Leu	Leu	Lys	Val	Ser	Trp	Glu	Lys	Pro	Val	Phe	Pro	Glu	Asn
			500					505					510		
Asn	Leu	Gln	Phe	Gln	Ile	Arg	Tyr	Gly	Leu	Ser	Gly	Lys	Glu	Ile	Gln
		515					520					525			
Trp	Lys	Thr	His	Glu	Val	Phe	Asp	Ala	Lys	Ser	Lys	Ser	Ala	Ser	Leu
	530					535					540				
Leu	Val	Ser	Asp	Leu	Cys	Ala	Val	Tyr	Val	Val	Gln	Val	Arg	Cys	Arg
545				550					555					560	
Arg	Leu	Asp	Gly	Leu	Gly	Tyr	Trp	Ser	Asn	Trp	Ser	Ser	Pro	Ala	Tyr
			565						570					575	
Thr	Leu	Val	Met	Asp	Val	Lys	Val	Pro	Met	Arg	Gly	Pro	Glu	Phe	Trp
			580					585					590		
Arg	Lys	Met	Asp	Gly	Asp	Val	Thr	Lys	Lys	Glu	Arg	Asn	Val	Thr	Leu
		595					600					605			
Leu	Trp	Lys	Pro	Leu	Thr	Lys	Asn	Asp	Ser	Leu	Cys	Ser	Val	Arg	Arg
	610					615					620				
Tyr	Val	Val	Lys	His	Arg	Thr	Ala	His	Asn	Gly	Thr	Trp	Ser	Glu	Asp
625				630						635				640	
Val	Gly	Asn	Arg	Thr	Asn	Leu	Thr	Phe	Leu	Trp	Thr	Glu	Pro	Ala	His
				645					650					655	
Thr	Val	Thr	Val	Leu	Ala	Val	Asn	Ser	Leu	Gly	Ala	Ser	Leu	Val	Asn
			660				665						670		
Phe	Asn	Leu	Thr	Phe	Ser	Trp	Pro	Met	Ser	Lys	Val	Ser	Ala	Val	Glu
		675					680					685			
Ser	Leu	Ser	Ala	Tyr	Pro	Leu	Ser	Ser	Ser	Cys	Val	Ile	Leu	Ser	Trp
	690					695					700				
Thr	Leu	Ser	Pro	Asp	Asp	Tyr	Ser	Leu	Leu	Tyr	Leu	Val	Ile	Glu	Trp
705				710						715				720	
Lys	Ile	Leu	Asn	Glu	Asp	Asp	Gly	Met	Lys	Trp	Leu	Arg	Ile	Pro	Ser
			725						730					735	
Asn	Val	Lys	Lys	Phe	Tyr	Ile	His	Asp	Asn	Phe	Ile	Pro	Ile	Glu	Lys
			740					745					750		
Tyr	Gln	Phe	Ser	Leu	Tyr	Pro	Val	Phe	Met	Glu	Gly	Val	Gly	Lys	Pro
		755					760					765			
Lys	Ile	Ile	Asn	Gly	Phe	Thr	Lys	Asp	Ala	Ile	Asp	Lys	Gln	Gln	Asn
	770					775					780				
Asp	Ala	Gly	Leu	Tyr	Val	Ile	Val	Pro	Ile	Ile	Ile	Ser	Ser	Cys	Val
785				790						795				800	
Leu	Leu	Leu	Gly	Thr	Leu	Leu	Ile	Ser	His	Gln	Arg	Met	Lys	Lys	Leu
				805					810					815	
Phe	Trp	Asp	Asp	Val	Pro	Asn	Pro	Lys	Asn	Cys	Ser	Trp	Ala	Gln	Gly
			820					825					830		
Leu	Asn	Phe	Gln	Lys	Pro	Glu	Thr	Phe	Glu	Gln	Leu	Phe	Thr	Lys	His
		835					840					845			
Ala	Glu	Ser	Val	Ile	Phe	Gly	Pro	Leu	Leu	Leu	Glu	Pro	Glu	Pro	Ile
	850					855					860				

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Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro
 85 90 95
 Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp
 100 105 110
 Leu Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser
 115 120 125
 Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys His
 130 135 140
 Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu Leu Met Tyr Leu
 145 150 155 160
 Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro Leu Met Ser Leu
 165 170 175
 Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu Gly Leu His Met
 180 185 190
 Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp Asp Ser Gln Thr
 195 200 205
 Met Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr Leu Glu Asn Ser
 210 215 220
 Thr Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala Thr Ser Leu Leu
 225 230 235 240
 Val Asp Ser Val Leu Pro Gly Ser Ser Tyr Glu Val Gln Val Arg Ser
 245 250 255
 Lys Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp Ser Ser Pro Gln
 260 265 270
 Val Phe Thr Thr Gln Asp Val Val Tyr Phe Pro Pro Lys Ile Leu Thr
 275 280 285
 Ser Val Gly Ser Asn Ala Ser Phe His Cys Ile Tyr Lys Asn Glu Asn
 290 295 300
 Gln Ile Ile Ser Ser Lys Gln Ile Val Trp Trp Arg Asn Leu Ala Glu
 305 310 315 320
 Lys Ile Pro Glu Ile Gln Tyr Ser Ile Val Ser Asp Arg Val Ser Lys
 325 330 335
 Val Thr Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg Gly Lys Phe Thr
 340 345 350
 Tyr Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys His His Arg Tyr
 355 360 365
 Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser Cys Glu Thr
 370 375 380
 Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser Pro Ser Thr Ile
 385 390 395 400
 Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr His Arg Arg Ser
 405 410 415
 Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr Ser Glu Pro Lys
 420 425 430
 Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu Cys Val Phe Gln Pro
 435 440 445
 Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp Ile Arg Ile Asn His Ser
 450 455 460
 Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys Val Leu Pro Asp Ser Val
 465 470 475 480
 Val Lys Pro Leu Pro Pro Ser Asn Val Lys Ala Glu Ile Thr Val Asn

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485				490				495							
Thr	Gly	Leu	Leu	Lys	Val	Ser	Trp	Glu	Lys	Pro	Val	Phe	Pro	Glu	Asn
			500						505				510		
Asn	Leu	Gln	Phe	Gln	Ile	Arg	Tyr	Gly	Leu	Ser	Gly	Lys	Glu	Ile	Gln
		515					520					525			
Trp	Lys	Thr	His	Glu	Val	Phe	Asp	Ala	Lys	Ser	Lys	Ser	Ala	Ser	Leu
	530					535					540				
Leu	Val	Ser	Asp	Leu	Cys	Ala	Val	Tyr	Val	Val	Gln	Val	Arg	Cys	Arg
545					550					555					560
Arg	Leu	Asp	Gly	Leu	Gly	Tyr	Trp	Ser	Asn	Trp	Ser	Ser	Pro	Ala	Tyr
			565						570					575	
Thr	Leu	Val	Met	Asp	Val	Lys	Val	Pro	Met	Arg	Gly	Pro	Glu	Phe	Trp
			580					585					590		
Arg	Lys	Met	Asp	Gly	Asp	Val	Thr	Lys	Lys	Glu	Arg	Asn	Val	Thr	Leu
	595						600					605			
Leu	Trp	Lys	Pro	Leu	Thr	Lys	Asn	Asp	Ser	Leu	Cys	Ser	Val	Arg	Arg
	610					615					620				
Tyr	Val	Val	Lys	His	Arg	Thr	Ala	His	Asn	Gly	Thr	Trp	Ser	Glu	Asp
625					630					635					640
Val	Gly	Asn	Arg	Thr	Asn	Leu	Thr	Phe	Leu	Trp	Thr	Glu	Pro	Ala	His
			645						650					655	
Thr	Val	Thr	Val	Leu	Ala	Val	Asn	Ser	Leu	Gly	Ala	Ser	Leu	Val	Asn
			660						665					670	
Phe	Asn	Leu	Thr	Phe	Ser	Trp	Pro	Met	Ser	Lys	Val	Ser	Ala	Val	Glu
	675						680					685			
Ser	Leu	Ser	Ala	Tyr	Pro	Leu	Ser	Ser	Ser	Cys	Val	Ile	Leu	Ser	Trp
	690				695						700				
Thr	Leu	Ser	Pro	Asp	Asp	Tyr	Ser	Leu	Leu	Tyr	Leu	Val	Ile	Glu	Trp
705					710					715					720
Lys	Ile	Leu	Asn	Glu	Asp	Asp	Gly	Met	Lys	Trp	Leu	Arg	Ile	Pro	Ser
			725						730					735	
Asn	Val	Lys	Lys	Phe	Tyr	Ile	His	Asp	Asn	Phe	Ile	Pro	Ile	Glu	Lys
			740						745					750	
Tyr	Gln	Phe	Ser	Leu	Tyr	Pro	Val	Phe	Met	Glu	Gly	Val	Gly	Lys	Pro
		755					760						765		
Lys	Ile	Ile	Asn	Gly	Phe	Thr	Lys	Asp	Ala	Ile	Asp	Lys	Gln	Gln	Asn
	770					775					780				
Asp	Ala	Gly	Leu	Tyr	Val	Ile	Val	Pro	Ile	Ile	Ile	Ser	Ser	Cys	Val
785					790					795					800
Leu	Leu	Leu	Gly	Thr	Leu	Leu	Ile	Ser	His	Gln	Arg	Met	Lys	Lys	Leu
			805						810					815	
Phe	Trp	Asp	Asp	Val	Pro	Asn	Pro	Lys	Asn	Cys	Ser	Trp	Ala	Gln	Gly
			820						825					830	
Leu	Asn	Phe	Gln	Lys	Val	Thr	Val								
	835					840									

<210> SEQ ID NO 59

<211> LENGTH: 848

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<220> FEATURE:

<221> NAME/KEY: MISC_FEATURE

<222> LOCATION: (29)..(29)

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<223> OTHER INFORMATION: X can be any amino acid

<400> SEQUENCE: 59

Gly Leu Arg Ser Ala Ser Tyr Gln Pro Leu Lys Arg Phe Ser Arg Phe
 1 5 10 15
 Gln Ala Leu Ser Pro Cys Arg Ile Ser Thr Ser Leu Xaa Leu Val Pro
 20 25 30
 Asn Ser Ala Arg Gly Cys Phe Gly Asn Glu Gln Gly Gln Asn Cys Ser
 35 40 45
 Ala Leu Thr Asp Asn Thr Glu Gly Lys Thr Leu Ala Ser Val Val Lys
 50 55 60
 Ala Ser Val Phe Arg Gln Leu Gly Val Asn Trp Asp Ile Glu Cys Trp
 65 70 75 80
 Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro
 85 90 95
 Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp
 100 105 110
 Leu Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser
 115 120 125
 Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys His
 130 135 140
 Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu Leu Met Tyr Leu
 145 150 155 160
 Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro Leu Met Ser Leu
 165 170 175
 Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu Gly Leu His Met
 180 185 190
 Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp Asp Ser Gln Thr
 195 200 205
 Met Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr Leu Glu Asn Ser
 210 215 220
 Thr Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala Thr Ser Leu Leu
 225 230 235 240
 Val Asp Ser Val Leu Pro Gly Ser Ser Tyr Glu Val Gln Val Arg Ser
 245 250 255
 Lys Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp Ser Ser Pro Gln
 260 265 270
 Val Phe Thr Thr Gln Asp Val Val Tyr Phe Pro Pro Lys Ile Leu Thr
 275 280 285
 Ser Val Gly Ser Asn Ala Ser Phe His Cys Ile Tyr Lys Asn Glu Asn
 290 295 300
 Gln Ile Ile Ser Ser Lys Gln Ile Val Trp Trp Arg Asn Leu Ala Glu
 305 310 315 320
 Lys Ile Pro Glu Ile Gln Tyr Ser Ile Val Ser Asp Arg Val Ser Lys
 325 330 335
 Val Thr Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg Gly Lys Phe Thr
 340 345 350
 Tyr Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys His His Arg Tyr
 355 360 365
 Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser Cys Glu Thr
 370 375 380

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Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser Pro Ser Thr Ile
 385 390 395 400
 Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr His Arg Arg Ser
 405 410 415
 Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr Ser Glu Pro Lys
 420 425 430
 Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu Cys Val Phe Gln Pro
 435 440 445
 Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp Ile Arg Ile Asn His Ser
 450 455 460
 Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys Val Leu Pro Asp Ser Val
 465 470 475 480
 Val Lys Pro Leu Pro Pro Ser Asn Val Lys Ala Glu Ile Thr Val Asn
 485 490 495
 Thr Gly Leu Leu Lys Val Ser Trp Glu Lys Pro Val Phe Pro Glu Asn
 500 505 510
 Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu Ser Gly Lys Glu Ile Gln
 515 520 525
 Trp Lys Thr His Glu Val Phe Asp Ala Lys Ser Lys Ser Ala Ser Leu
 530 535 540
 Leu Val Ser Asp Leu Cys Ala Val Tyr Val Val Gln Val Arg Cys Arg
 545 550 555 560
 Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser Ser Pro Ala Tyr
 565 570 575
 Thr Leu Val Met Asp Val Lys Val Pro Met Arg Gly Pro Glu Phe Trp
 580 585 590
 Arg Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg Asn Val Thr Leu
 595 600 605
 Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg
 610 615 620
 Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp
 625 630 635 640
 Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His
 645 650 655
 Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn
 660 665 670
 Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu
 675 680 685
 Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp
 690 695 700
 Thr Leu Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp
 705 710 715 720
 Lys Ile Leu Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser
 725 730 735
 Asn Val Lys Lys Phe Tyr Ile His Asp Asn Phe Ile Pro Ile Glu Lys
 740 745 750
 Tyr Gln Phe Ser Leu Tyr Pro Val Phe Met Glu Gly Val Gly Lys Pro
 755 760 765
 Lys Ile Ile Asn Gly Phe Thr Lys Asp Ala Ile Asp Lys Gln Gln Asn
 770 775 780
 Asp Ala Gly Leu Tyr Val Ile Val Pro Ile Ile Ile Ser Ser Cys Val

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785                790                795                800
Leu Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu
      805                810                815
Phe Trp Asp Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln Gly
      820                825                830
Leu Asn Phe Gln Lys Asp Ile Ser Leu His Glu Val Phe Ile Phe Arg
      835                840                845

<210> SEQ ID NO 60
<211> LENGTH: 314
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (79)..(79)
<223> OTHER INFORMATION: X can be any amino acid

<400> SEQUENCE: 60
Leu Arg Asp Leu Val Ser Gly Phe Glu Glu Ile Asn Lys Ile Lys Glu
1          5          10
Asn Phe Ser Arg Ala Gly Leu Leu Ala Glu Leu Arg Pro Thr Ala Phe
      20          25          30
Tyr Ile Ser Thr Leu Ser Leu Phe Pro Ser Ala Leu Ala Leu Asp Trp
      35          40          45
Ala Val Pro Gly Leu Val Leu Leu Phe Pro Gly Gly Asn Val Glu Leu
      50          55          60
His Glu Phe Trp Tyr Lys His Cys Gly Leu Cys Ala Asn Ile Xaa Cys
65          70          75          80
Phe Leu Gln Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg
      85          90          95
Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp
      100         105         110
Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His
      115         120         125
Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn
      130         135         140
Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu
145         150         155         160
Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp
      165         170         175
Thr Leu Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp
      180         185         190
Lys Ile Leu Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser
      195         200         205
Asn Val Lys Lys Phe Tyr Ile His Asp Asn Phe Ile Pro Ile Glu Lys
      210         215         220
Tyr Gln Phe Ser Leu Tyr Pro Val Phe Met Glu Gly Val Gly Lys Pro
      225         230         235         240
Lys Ile Ile Asn Gly Phe Thr Lys Asp Ala Ile Asp Lys Gln Gln Asn
      245         250         255
Asp Ala Gly Leu Tyr Val Ile Val Pro Ile Ile Ile Ser Ser Cys Val
      260         265         270
Leu Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu
      275         280         285

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Phe Trp Asp Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln Gly
290 295 300

Leu Asn Phe Gln Lys Arg Thr Asp Thr Leu
305 310

<210> SEQ ID NO 61
<211> LENGTH: 321
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (58)..(58)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (67)..(67)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (68)..(68)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (84)..(84)
<223> OTHER INFORMATION: X can be any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (86)..(86)
<223> OTHER INFORMATION: X can be any amino acid

<400> SEQUENCE: 61

Leu Arg Asp Leu Val Ser Gly Phe Glu Glu Ile Asn Lys Xaa Ile Lys
1 5 10 15
Glu Asn Xaa Phe Ser Arg Ala Gly Xaa Leu Leu Ala Glu Leu Arg Pro
20 25 30
Thr Ala Phe Tyr Ile Ser Thr Leu Ser Leu Phe Pro Ser Ala Leu Ala
35 40 45
Leu Asp Trp Ala Val Pro Gly Leu Val Xaa Leu Leu Phe Pro Gly Gly
50 55 60
Asn Val Xaa Xaa Glu Leu His Glu Phe Trp Tyr Lys His Cys Gly Leu
65 70 75 80
Cys Ala Asn Xaa Ile Xaa Cys Phe Leu Gln Pro Leu Thr Lys Asn Asp
85 90 95
Ser Leu Cys Ser Val Arg Arg Tyr Val Val Lys His Arg Thr Ala His
100 105 110
Asn Gly Thr Trp Ser Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe
115 120 125
Leu Trp Thr Glu Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser
130 135 140
Leu Gly Ala Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met
145 150 155 160

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Ser Lys Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser
165 170 175

Ser Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu
180 185 190

Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly Met
195 200 205

Lys Trp Leu Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His Asp
210 215 220

Asn Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro Val Phe
225 230 235 240

Met Glu Gly Val Gly Lys Pro Lys Ile Ile Asn Gly Phe Thr Lys Asp
245 250 255

Ala Ile Asp Lys Gln Gln Asn Asp Ala Gly Leu Tyr Val Ile Val Pro
260 265 270

Ile Ile Ile Ser Ser Cys Val Leu Leu Leu Gly Thr Leu Leu Ile Ser
275 280 285

His Gln Arg Met Lys Lys Leu Phe Trp Asp Asp Val Pro Asn Pro Lys
290 295 300

Asn Cys Ser Trp Ala Gln Gly Leu Asn Phe Gln Lys Arg Thr Asp Thr
305 310 315 320

Leu

<210> SEQ ID NO 62
<211> LENGTH: 320
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (79)..(79)
<223> OTHER INFORMATION: X can be any amino acid

<400> SEQUENCE: 62

Leu Arg Asp Leu Val Ser Gly Phe Glu Glu Ile Asn Lys Ile Lys Glu
1 5 10 15

Asn Phe Ser Arg Ala Gly Leu Leu Ala Glu Leu Arg Pro Thr Ala Phe
20 25 30

Tyr Ile Ser Thr Leu Ser Leu Phe Pro Ser Ala Leu Ala Leu Asp Trp
35 40 45

Ala Val Pro Gly Leu Val Leu Leu Phe Pro Gly Gly Asn Val Glu Leu
50 55 60

His Glu Phe Trp Tyr Lys His Cys Gly Leu Cys Ala Asn Ile Xaa Cys
65 70 75 80

Phe Leu Gln Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg
85 90 95

Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp
100 105 110

Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His
115 120 125

Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn
130 135 140

Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu
145 150 155 160

Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp

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Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser Gly Ile Tyr Val Pro
 65 70 75 80
 Glu Leu Ser Lys Thr Val Phe His Cys Cys Phe Gly Asn Glu Gln Gly
 85 90 95
 Gln Asn Cys Ser Ala Leu Thr Asp Asn Thr Glu Gly Lys Thr Leu Ala
 100 105 110
 Ser Val Val Lys Ala Ser Val Phe Arg Gln Leu Gly Val Asn Trp Asp
 115 120 125
 Ile Glu Cys Trp Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met
 130 135 140
 Glu Pro Leu Pro Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His
 145 150 155 160
 Leu Leu Tyr Asp Leu Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro
 165 170 175
 Leu Lys Asp Ser Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly
 180 185 190
 Cys Glu Cys His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu
 195 200 205
 Leu Met Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro
 210 215 220
 Leu Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu
 225 230 235 240
 Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp
 245 250 255
 Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr
 260 265 270
 Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala
 275 280 285
 Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly Ser Ser Tyr Glu Val
 290 295 300
 Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp
 305 310 315 320
 Ser Ser Pro Gln Val Phe Thr Thr Gln Asp Val Val Tyr Phe Pro Pro
 325 330 335
 Lys Ile Leu Thr Ser Val Gly Ser Asn Ala Ser Phe His Cys Ile Tyr
 340 345 350
 Lys Asn Glu Asn Gln Ile Ile Ser Ser Lys Gln Ile Val Trp Trp Arg
 355 360 365
 Asn Leu Ala Glu Lys Ile Pro Glu Ile Gln Tyr Ser Ile Val Ser Asp
 370 375 380
 Arg Val Ser Lys Val Thr Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg
 385 390 395 400
 Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys
 405 410 415
 His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile
 420 425 430
 Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser
 435 440 445
 Pro Ser Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr
 450 455 460
 His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr

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340				345				350							
Lys	Asn	Glu	Asn	Gln	Ile	Ile	Ser	Ser	Lys	Gln	Ile	Val	Trp	Trp	Arg
	355						360					365			
Asn	Leu	Ala	Glu	Lys	Ile	Pro	Glu	Ile	Gln	Tyr	Ser	Ile	Val	Ser	Asp
	370					375					380				
Arg	Val	Ser	Lys	Val	Thr	Phe	Ser	Asn	Leu	Lys	Ala	Thr	Arg	Pro	Arg
	385				390					395				400	
Gly	Lys	Phe	Thr	Tyr	Asp	Ala	Val	Tyr	Cys	Cys	Asn	Glu	Gln	Ala	Cys
				405					410					415	
His	His	Arg	Tyr	Ala	Glu	Leu	Tyr	Val	Ile	Asp	Val	Asn	Ile	Asn	Ile
			420					425				430			
Ser	Cys	Glu	Thr	Asp	Gly	Tyr	Leu	Thr	Lys	Met	Thr	Cys	Arg	Trp	Ser
		435					440					445			
Pro	Ser	Thr	Ile	Gln	Ser	Leu	Val	Gly	Ser	Thr	Val	Gln	Leu	Arg	Tyr
		450				455					460				
His	Arg	Arg	Ser	Leu	Tyr	Cys	Pro	Asp	Ser	Pro	Ser	Ile	His	Pro	Thr
					470					475				480	
Ser	Glu	Pro	Lys	Asn	Cys	Val	Leu	Gln	Arg	Asp	Gly	Phe	Tyr	Glu	Cys
				485					490					495	
Val	Phe	Gln	Pro	Ile	Phe	Leu	Leu	Ser	Gly	Tyr	Thr	Met	Trp	Ile	Arg
			500					505					510		
Ile	Asn	His	Ser	Leu	Gly	Ser	Leu	Asp	Ser	Pro	Pro	Thr	Cys	Val	Leu
		515					520					525			
Pro	Asp	Ser	Val	Val	Lys	Pro	Leu	Pro	Pro	Ser	Asn	Val	Lys	Ala	Glu
		530				535					540				
Ile	Thr	Val	Asn	Thr	Gly	Leu	Leu	Lys	Val	Ser	Trp	Glu	Lys	Pro	Val
					550					555				560	
Phe	Pro	Glu	Asn	Asn	Leu	Gln	Phe	Gln	Ile	Arg	Tyr	Gly	Leu	Ser	Gly
			565						570					575	
Lys	Glu	Ile	Gln	Trp	Lys	Thr	His	Glu	Val	Phe	Asp	Ala	Lys	Ser	Lys
			580					585				590			
Ser	Ala	Ser	Leu	Leu	Val	Ser	Asp	Leu	Cys	Ala	Val	Tyr	Val	Val	Gln
		595					600					605			
Val	Arg	Cys	Arg	Arg	Leu	Asp	Gly	Leu	Gly	Tyr	Trp	Ser	Asn	Trp	Ser
					610		615				620				
Ser	Pro	Ala	Tyr	Thr	Leu	Val	Met	Asp	Val	Lys	Val	Pro	Met	Arg	Gly
					630					635				640	
Pro	Glu	Phe	Trp	Arg	Lys	Met	Asp	Gly	Asp	Val	Thr	Lys	Lys	Glu	Arg
				645					650					655	
Asn	Val	Thr	Leu	Leu	Trp	Lys	Pro	Leu	Thr	Lys	Asn	Asp	Ser	Leu	Cys
			660					665					670		
Ser	Val	Arg	Arg	Tyr	Val	Val	Lys	His	Arg	Thr	Ala	His	Asn	Gly	Thr
			675				680					685			
Trp	Ser	Glu	Asp	Val	Gly	Asn	Arg	Thr	Asn	Leu	Thr	Phe	Leu	Trp	Thr
						695					700				
Glu	Pro	Ala	His	Thr	Val	Thr	Val	Leu	Ala	Val	Asn	Ser	Leu	Gly	Ala
					710					715				720	
Ser	Leu	Val	Asn	Phe	Asn	Leu	Thr	Phe	Ser	Trp	Pro	Met	Ser	Lys	Val
			725						730					735	
Ser	Ala	Val	Glu	Ser	Leu	Ser	Ala	Tyr	Pro	Leu	Ser	Ser	Ser	Cys	Val
			740				745							750	

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Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu
 755 760 765

Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly Met Lys Trp Leu
 770 775 780

Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His Asp Asn Phe Ile
 785 790 795 800

Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro Val Phe Met Glu Gly
 805 810 815

Val Gly Lys Pro Lys Ile Ile Asn Gly Phe Thr Lys Asp Ala Ile Asp
 820 825 830

Lys Gln Gln Asn Asp Ala Gly Leu Tyr Val Ile Val Pro Ile Ile Ile
 835 840 845

Ser Ser Cys Val Leu Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg
 850 855 860

Met Lys Lys Leu Phe Trp Asp Asp Val Pro Asn Pro Lys Asn Cys Ser
 865 870 875 880

Trp Ala Gln Gly Leu Asn Phe Gln Lys Pro Glu Thr Phe Glu Gln Leu
 885 890 895

Phe Thr Lys His Ala Glu Ser Val Ile Phe Gly Pro Leu Leu Leu Glu
 900 905 910

Pro Glu Pro Ile Ser Glu Glu Ile Ser Val Asp Thr Ala Trp Lys Asn
 915 920 925

Lys Asp Glu Met Val Pro Ala Ala Met Val Ser Leu Leu Leu Thr Thr
 930 935 940

Pro Asp Pro Glu Ser Ser Ser Ile Cys Ile Ser Asp Gln Cys Asn Ser
 945 950 955 960

Ala Asn Phe Ser Gly Ser Gln Ser Thr Gln Val Thr Cys Glu Asp Glu
 965 970 975

Cys Gln Arg Gln Pro Ser Val Lys Tyr Ala Thr Leu Val Ser Asn Asp
 980 985 990

Lys Leu Val Glu Thr Asp Glu Glu Gln Gly Phe Ile His Ser Pro Val
 995 1000 1005

Ser Asn Cys Ile Ser Ser Asn His Ser Pro Leu Arg Gln Ser Phe
 1010 1015 1020

Ser Ser Ser Ser Trp Glu Thr Glu Ala Gln Thr Phe Phe Leu Leu
 1025 1030 1035

Ser Asp Gln Gln Pro Thr Met Ile Ser Pro Gln Leu Ser Phe Ser
 1040 1045 1050

Gly Leu Asp Glu Leu Leu Glu Leu Glu Gly Ser Phe Pro Glu Glu
 1055 1060 1065

Asn His Arg Glu Lys Ser Val Cys Tyr Leu Gly Val Thr Ser Val
 1070 1075 1080

Asn Arg Arg Glu Ser Gly Val Leu Leu Thr Gly Glu Ala Gly Ile
 1085 1090 1095

Leu Cys Thr Phe Pro Ala Gln Cys Leu Phe Ser Asp Ile Arg Ile
 1100 1105 1110

Leu Gln Glu Arg Cys Ser His Phe Val Glu Asn Asn Leu Ser Leu
 1115 1120 1125

Gly Thr Ser Gly Glu Asn Phe Val Pro Tyr Met Pro Gln Phe Gln
 1130 1135 1140

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 Thr Cys Ser Thr His Ser His Lys Ile Met Glu Asn Lys Met Cys
 1145 1150 1155

 Asp Leu Thr Val
 1160

 <210> SEQ ID NO 66
 <211> LENGTH: 892
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 66

 Met Met Cys Gln Lys Phe Tyr Val Val Leu Leu His Trp Glu Phe Leu
 1 5 10 15

 Tyr Val Ile Ala Ala Leu Asn Leu Ala Tyr Pro Ile Ser Pro Trp Lys
 20 25 30

 Phe Lys Leu Phe Cys Gly Pro Pro Asn Thr Thr Asp Asp Ser Phe Leu
 35 40 45

 Ser Pro Ala Gly Ala Pro Asn Asn Ala Ser Ala Leu Lys Gly Ala Ser
 50 55 60

 Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser Gly Ile Tyr Val Pro
 65 70 75 80

 Glu Leu Ser Lys Thr Val Phe His Cys Cys Phe Gly Asn Glu Gln Gly
 85 90 95

 Gln Asn Cys Ser Ala Leu Thr Asp Asn Thr Glu Gly Lys Thr Leu Ala
 100 105 110

 Ser Val Val Lys Ala Ser Val Phe Arg Gln Leu Gly Val Asn Trp Asp
 115 120 125

 Ile Glu Cys Trp Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met
 130 135 140

 Glu Pro Leu Pro Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His
 145 150 155 160

 Leu Leu Tyr Asp Leu Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro
 165 170 175

 Leu Lys Asp Ser Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly
 180 185 190

 Cys Glu Cys His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu
 195 200 205

 Leu Met Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro
 210 215 220

 Leu Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu
 225 230 235 240

 Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp
 245 250 255

 Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr
 260 265 270

 Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala
 275 280 285

 Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly Ser Ser Tyr Glu Val
 290 295 300

 Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp
 305 310 315 320

 Ser Ser Pro Gln Val Phe Thr Thr Gln Asp Val Val Tyr Phe Pro Pro
 325 330 335

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Lys Ile Leu Thr Ser Val Gly Ser Asn Ala Ser Phe His Cys Ile Tyr
 340 345 350
 Lys Asn Glu Asn Gln Ile Ile Ser Ser Lys Gln Ile Val Trp Trp Arg
 355 360 365
 Asn Leu Ala Glu Lys Ile Pro Glu Ile Gln Tyr Ser Ile Val Ser Asp
 370 375 380
 Arg Val Ser Lys Val Thr Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg
 385 390 395 400
 Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys
 405 410 415
 His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile
 420 425 430
 Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser
 435 440 445
 Pro Ser Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr
 450 455 460
 His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr
 465 470 475 480
 Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu Cys
 485 490 495
 Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp Ile Arg
 500 505 510
 Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys Val Leu
 515 520 525
 Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn Val Lys Ala Glu
 530 535 540
 Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser Trp Glu Lys Pro Val
 545 550 555 560
 Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu Ser Gly
 565 570 575
 Lys Glu Ile Gln Trp Lys Thr His Glu Val Phe Asp Ala Lys Ser Lys
 580 585 590
 Ser Ala Ser Leu Leu Val Ser Asp Leu Cys Ala Val Tyr Val Val Gln
 595 600 605
 Val Arg Cys Arg Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser
 610 615 620
 Ser Pro Ala Tyr Thr Leu Val Met Asp Val Lys Val Pro Met Arg Gly
 625 630 635 640
 Pro Glu Phe Trp Arg Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg
 645 650 655
 Asn Val Thr Leu Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys
 660 665 670
 Ser Val Arg Arg Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr
 675 680 685
 Trp Ser Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr
 690 695 700
 Glu Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala
 705 710 715 720
 Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys Val
 725 730 735

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Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val
740 745 750

Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu
755 760 765

Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly Met Lys Trp Leu
770 775 780

Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His Asp Asn Phe Ile
785 790 795 800

Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro Val Phe Met Glu Gly
805 810 815

Val Gly Lys Pro Lys Ile Ile Asn Gly Phe Thr Lys Asp Ala Ile Asp
820 825 830

Lys Gln Gln Asn Asp Ala Gly Leu Tyr Val Ile Val Pro Ile Ile Ile
835 840 845

Ser Ser Cys Val Leu Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg
850 855 860

Met Lys Lys Leu Phe Trp Asp Asp Val Pro Asn Pro Lys Asn Cys Ser
865 870 875 880

Trp Ala Gln Gly Leu Asn Phe Gln Lys Val Thr Val
885 890

<210> SEQ ID NO 67

<211> LENGTH: 231

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 67

Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg Tyr Val Val
1 5 10 15

Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp Val Gly Asn
20 25 30

Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His Thr Val Thr
35 40 45

Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn Phe Asn Leu
50 55 60

Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser
65 70 75 80

Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser
85 90 95

Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu
100 105 110

Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys
115 120 125

Lys Phe Tyr Ile His Asp Asn Phe Ile Pro Ile Glu Lys Tyr Gln Phe
130 135 140

Ser Leu Tyr Pro Val Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile
145 150 155 160

Asn Gly Phe Thr Lys Asp Ala Ile Asp Lys Gln Gln Asn Asp Ala Gly
165 170 175

Leu Tyr Val Ile Val Pro Ile Ile Ile Ser Ser Cys Val Leu Leu Leu
180 185 190

Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu Phe Trp Asp
195 200 205

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Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln Gly Leu Asn Phe
210 215 220

Gln Lys Arg Thr Asp Thr Leu
225 230

<210> SEQ ID NO 68

<211> LENGTH: 499

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 68

Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg Tyr Val Val
1 5 10 15

Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp Val Gly Asn
20 25 30

Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His Thr Val Thr
35 40 45

Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn Phe Asn Leu
50 55 60

Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser
65 70 75 80

Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser
85 90 95

Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu
100 105 110

Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys
115 120 125

Lys Phe Tyr Ile His Asp Asn Phe Ile Pro Ile Glu Lys Tyr Gln Phe
130 135 140

Ser Leu Tyr Pro Val Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile
145 150 155 160

Asn Gly Phe Thr Lys Asp Ala Ile Asp Lys Gln Gln Asn Asp Ala Gly
165 170 175

Leu Tyr Val Ile Val Pro Ile Ile Ile Ser Ser Cys Val Leu Leu Leu
180 185 190

Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu Phe Trp Asp
195 200 205

Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln Gly Leu Asn Phe
210 215 220

Gln Lys Pro Glu Thr Phe Glu Gln Leu Phe Thr Lys His Ala Glu Ser
225 230 235 240

Val Ile Phe Gly Pro Leu Leu Leu Glu Pro Glu Pro Ile Ser Glu Glu
245 250 255

Ile Ser Val Asp Thr Ala Trp Lys Asn Lys Asp Glu Met Val Pro Ala
260 265 270

Ala Met Val Ser Leu Leu Leu Thr Thr Pro Asp Pro Glu Ser Ser Ser
275 280 285

Ile Cys Ile Ser Asp Gln Cys Asn Ser Ala Asn Phe Ser Gly Ser Gln
290 295 300

Ser Thr Gln Val Thr Cys Glu Asp Glu Cys Gln Arg Gln Pro Ser Val
305 310 315 320

Lys Tyr Ala Thr Leu Val Ser Asn Asp Lys Leu Val Glu Thr Asp Glu

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	325		330		335
Glu Gln Gly Phe Ile His Ser Pro Val Ser Asn Cys Ile Ser Ser Asn	340		345		350
His Ser Pro Leu Arg Gln Ser Phe Ser Ser Ser Ser Trp Glu Thr Glu	355		360		365
Ala Gln Thr Phe Phe Leu Leu Ser Asp Gln Gln Pro Thr Met Ile Ser	370		375		380
Pro Gln Leu Ser Phe Ser Gly Leu Asp Glu Leu Leu Glu Leu Glu Gly	385		390		395
Ser Phe Pro Glu Glu Asn His Arg Glu Lys Ser Val Cys Tyr Leu Gly	405		410		415
Val Thr Ser Val Asn Arg Arg Glu Ser Gly Val Leu Leu Thr Gly Glu	420		425		430
Ala Gly Ile Leu Cys Thr Phe Pro Ala Gln Cys Leu Phe Ser Asp Ile	435		440		445
Arg Ile Leu Gln Glu Arg Cys Ser His Phe Val Glu Asn Asn Leu Ser	450		455		460
Leu Gly Thr Ser Gly Glu Asn Phe Val Pro Tyr Met Pro Gln Phe Gln	465		470		475
Thr Cys Ser Thr His Ser His Lys Ile Met Glu Asn Lys Met Cys Asp	485		490		495

Leu Thr Val

<210> SEQ ID NO 69
 <211> LENGTH: 229
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 69

Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg Tyr Val Val	1	5	10	15
Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp Val Gly Asn	20	25	30	
Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His Thr Val Thr	35	40	45	
Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn Phe Asn Leu	50	55	60	
Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser	65	70	75	80
Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser	85	90	95	
Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu	100	105	110	
Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys	115	120	125	
Lys Phe Tyr Ile His Asp Asn Phe Ile Pro Ile Glu Lys Tyr Gln Phe	130	135	140	
Ser Leu Tyr Pro Val Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile	145	150	155	160
Asn Gly Phe Thr Lys Asp Ala Ile Asp Lys Gln Gln Asn Asp Ala Gly	165	170	175	

Leu Tyr Val Ile Val Pro Ile Ile Ile Ser Ser Cys Val Leu Leu Leu

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180 185 190
 Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu Phe Trp Asp
 195 200 205
 Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln Gly Leu Asn Phe
 210 215 220
 Gln Lys Val Thr Val
 225

<210> SEQ ID NO 70
 <211> LENGTH: 237
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 70

Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg Tyr Val Val
 1 5 10 15
 Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp Val Gly Asn
 20 25 30
 Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His Thr Val Thr
 35 40 45
 Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn Phe Asn Leu
 50 55 60
 Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser
 65 70 75 80
 Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser
 85 90 95
 Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu
 100 105 110
 Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys
 115 120 125
 Lys Phe Tyr Ile His Asp Asn Phe Ile Pro Ile Glu Lys Tyr Gln Phe
 130 135 140
 Ser Leu Tyr Pro Val Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile
 145 150 155 160
 Asn Gly Phe Thr Lys Asp Ala Ile Asp Lys Gln Gln Asn Asp Ala Gly
 165 170 175
 Leu Tyr Val Ile Val Pro Ile Ile Ile Ser Ser Cys Val Leu Leu Leu
 180 185 190
 Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu Phe Trp Asp
 195 200 205
 Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln Gly Leu Asn Phe
 210 215 220
 Gln Lys Asp Ile Ser Leu His Glu Val Phe Ile Phe Arg
 225 230 235

<210> SEQ ID NO 71
 <211> LENGTH: 162
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 71

Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser
 1 5 10 15
 Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser

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210					215					220					
Gln	Cys	Asn	Ser	Ala	Asn	Phe	Ser	Gly	Ser	Gln	Ser	Thr	Gln	Val	Thr
225					230					235					240
Cys	Glu	Asp	Glu	Cys	Gln	Arg	Gln	Pro	Ser	Val	Lys	Tyr	Ala	Thr	Leu
				245					250					255	
Val	Ser	Asn	Asp	Lys	Leu	Val	Glu	Thr	Asp	Glu	Glu	Gln	Gly	Phe	Ile
				260					265					270	
His	Ser	Pro	Val	Ser	Asn	Cys	Ile	Ser	Ser	Asn	His	Ser	Pro	Leu	Arg
				275					280					285	
Gln	Ser	Phe	Ser	Ser	Ser	Ser	Trp	Glu	Thr	Glu	Ala	Gln	Thr	Phe	Phe
				290					295					300	
Leu	Leu	Ser	Asp	Gln	Gln	Pro	Thr	Met	Ile	Ser	Pro	Gln	Leu	Ser	Phe
305				310										315	320
Ser	Gly	Leu	Asp	Glu	Leu	Leu	Glu	Leu	Glu	Gly	Ser	Phe	Pro	Glu	Glu
				325					330					335	
Asn	His	Arg	Glu	Lys	Ser	Val	Cys	Tyr	Leu	Gly	Val	Thr	Ser	Val	Asn
				340					345					350	
Arg	Arg	Glu	Ser	Gly	Val	Leu	Leu	Thr	Gly	Glu	Ala	Gly	Ile	Leu	Cys
				355					360					365	
Thr	Phe	Pro	Ala	Gln	Cys	Leu	Phe	Ser	Asp	Ile	Arg	Ile	Leu	Gln	Glu
				370					375					380	
Arg	Cys	Ser	His	Phe	Val	Glu	Asn	Asn	Leu	Ser	Leu	Gly	Thr	Ser	Gly
385				390										395	400
Glu	Asn	Phe	Val	Pro	Tyr	Met	Pro	Gln	Phe	Gln	Thr	Cys	Ser	Thr	His
				405					410					415	
Ser	His	Lys	Ile	Met	Glu	Asn	Lys	Met	Cys	Asp	Leu	Thr	Val		
				420					425				430		

<210> SEQ ID NO 73

<211> LENGTH: 160

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 73

Met	Ser	Lys	Val	Ser	Ala	Val	Glu	Ser	Leu	Ser	Ala	Tyr	Pro	Leu	Ser
1				5					10					15	
Ser	Ser	Cys	Val	Ile	Leu	Ser	Trp	Thr	Leu	Ser	Pro	Asp	Asp	Tyr	Ser
			20					25					30		
Leu	Leu	Tyr	Leu	Val	Ile	Glu	Trp	Lys	Ile	Leu	Asn	Glu	Asp	Asp	Gly
		35					40					45			
Met	Lys	Trp	Leu	Arg	Ile	Pro	Ser	Asn	Val	Lys	Lys	Phe	Tyr	Ile	His
	50				55						60				
Asp	Asn	Phe	Ile	Pro	Ile	Glu	Lys	Tyr	Gln	Phe	Ser	Leu	Tyr	Pro	Val
65				70					75					80	
Phe	Met	Glu	Gly	Val	Gly	Lys	Pro	Lys	Ile	Ile	Asn	Gly	Phe	Thr	Lys
				85					90					95	
Asp	Ala	Ile	Asp	Lys	Gln	Gln	Asn	Asp	Ala	Gly	Leu	Tyr	Val	Ile	Val
			100					105					110		
Pro	Ile	Ile	Ile	Ser	Ser	Cys	Val	Leu	Leu	Leu	Gly	Thr	Leu	Leu	Ile
		115				120						125			
Ser	His	Gln	Arg	Met	Lys	Lys	Leu	Phe	Trp	Asp	Asp	Val	Pro	Asn	Pro
		130				135						140			

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Lys Asn Cys Ser Trp Ala Gln Gly Leu Asn Phe Gln Lys Val Thr Val
145 150 155 160

<210> SEQ ID NO 74
<211> LENGTH: 168
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 74

Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser
1 5 10 15

Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser
20 25 30

Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly
35 40 45

Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His
50 55 60

Asp Asn Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro Val
65 70 75 80

Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile Asn Gly Phe Thr Lys
85 90 95

Asp Ala Ile Asp Lys Gln Gln Asn Asp Ala Gly Leu Tyr Val Ile Val
100 105 110

Pro Ile Ile Ile Ser Ser Cys Val Leu Leu Leu Gly Thr Leu Leu Ile
115 120 125

Ser His Gln Arg Met Lys Lys Leu Phe Trp Asp Asp Val Pro Asn Pro
130 135 140

Lys Asn Cys Ser Trp Ala Gln Gly Leu Asn Phe Gln Lys Asp Ile Ser
145 150 155 160

Leu His Glu Val Phe Ile Phe Arg
165

<210> SEQ ID NO 75
<211> LENGTH: 142
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 75

Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg Tyr Val Val
1 5 10 15

Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp Val Gly Asn
20 25 30

Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His Thr Val Thr
35 40 45

Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn Phe Asn Leu
50 55 60

Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser
65 70 75 80

Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser
85 90 95

Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu
100 105 110

Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys
115 120 125

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Lys Phe Tyr Ile His Gly Met Cys Thr Val Leu Phe Met Asp
 130 135 140

<210> SEQ ID NO 76
 <211> LENGTH: 142
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 76

Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg Tyr Val Val
 1 5 10 15

Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp Val Gly Asn
 20 25 30

Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His Thr Val Thr
 35 40 45

Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn Phe Asn Leu
 50 55 60

Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser
 65 70 75 80

Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser
 85 90 95

Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu
 100 105 110

Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys
 115 120 125

Lys Phe Tyr Ile His Gly Met Cys Thr Val Leu Phe Met Asp
 130 135 140

<210> SEQ ID NO 77
 <211> LENGTH: 142
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 77

Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg Tyr Val Val
 1 5 10 15

Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp Val Gly Asn
 20 25 30

Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His Thr Val Thr
 35 40 45

Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn Phe Asn Leu
 50 55 60

Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser
 65 70 75 80

Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser
 85 90 95

Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu
 100 105 110

Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys
 115 120 125

Lys Phe Tyr Ile His Gly Met Cys Thr Val Leu Phe Met Asp
 130 135 140

<210> SEQ ID NO 78
 <211> LENGTH: 142

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<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 78

Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg Tyr Val Val
1           5           10           15
Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp Val Gly Asn
20           25           30
Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His Thr Val Thr
35           40           45
Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn Phe Asn Leu
50           55           60
Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser
65           70           75           80
Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser
85           90           95
Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu
100          105          110
Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys
115          120          125
Lys Phe Tyr Ile His Gly Met Cys Thr Val Leu Phe Met Asp
130          135          140

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<210> SEQ ID NO 79
<211> LENGTH: 73
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 79

Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser
1           5           10           15
Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser
20           25           30
Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly
35           40           45
Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His
50           55           60
Gly Met Cys Thr Val Leu Phe Met Asp
65           70

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<210> SEQ ID NO 80
<211> LENGTH: 889
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 80

Met Met Cys Gln Lys Phe Tyr Val Val Leu Leu His Trp Glu Phe Leu
1           5           10           15
Tyr Val Ile Ala Ala Leu Asn Leu Ala Tyr Pro Ile Ser Pro Trp Lys
20           25           30
Phe Lys Leu Phe Cys Gly Pro Pro Asn Thr Thr Asp Asp Ser Phe Leu
35           40           45
Ser Pro Ala Gly Ala Pro Asn Asn Ala Ser Ala Leu Lys Gly Ala Ser
50           55           60
Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser Gly Ile Tyr Val Pro

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65	70	75	80
Glu Leu Ser Lys Thr Val Phe His Cys Cys Phe Gly Asn Glu Gln Gly	85	90	95
Gln Asn Cys Ser Ala Leu Thr Asp Asn Thr Glu Gly Lys Thr Leu Ala	100	105	110
Ser Val Val Lys Ala Ser Val Phe Arg Gln Leu Gly Val Asn Trp Asp	115	120	125
Ile Glu Cys Trp Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met	130	135	140
Glu Pro Leu Pro Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His	145	150	155
Leu Leu Tyr Asp Leu Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro	165	170	175
Leu Lys Asp Ser Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly	180	185	190
Cys Glu Cys His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu	195	200	205
Leu Met Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro	210	215	220
Leu Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu	225	230	235
Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp	245	250	255
Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr	260	265	270
Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala	275	280	285
Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly Ser Ser Tyr Glu Val	290	295	300
Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp	305	310	315
Ser Ser Pro Gln Val Phe Thr Thr Gln Asp Val Val Tyr Phe Pro Pro	325	330	335
Lys Ile Leu Thr Ser Val Gly Ser Asn Ala Ser Phe His Cys Ile Tyr	340	345	350
Lys Asn Glu Asn Gln Ile Ile Ser Ser Lys Gln Ile Val Trp Trp Arg	355	360	365
Asn Leu Ala Glu Lys Ile Pro Glu Ile Gln Tyr Ser Ile Val Ser Asp	370	375	380
Arg Val Ser Lys Val Thr Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg	385	390	395
Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys	405	410	415
His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile	420	425	430
Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser	435	440	445
Pro Ser Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr	450	455	460
His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr	465	470	475
			480

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Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu Cys
 485 490 495

Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp Ile Arg
 500 505 510

Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys Val Leu
 515 520 525

Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn Val Lys Ala Glu
 530 535 540

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

Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu Ser Gly
 565 570 575

Lys Glu Ile Gln Trp Lys Thr His Glu Val Phe Asp Ala Lys Ser Lys
 580 585 590

Ser Ala Ser Leu Leu Val Ser Asp Leu Cys Ala Val Tyr Val Val Gln
 595 600 605

Val Arg Cys Arg Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser
 610 615 620

Ser Pro Ala Tyr Thr Leu Val Met Asp Val Lys Val Pro Met Arg Gly
 625 630 635 640

Pro Glu Phe Trp Arg Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg
 645 650 655

Asn Val Thr Leu Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys
 660 665 670

Ser Val Arg Arg Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr
 675 680 685

Trp Ser Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr
 690 695 700

Glu Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala
 705 710 715 720

Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys Val
 725 730 735

Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val
 740 745 750

Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu
 755 760 765

Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly Met Lys Trp Leu
 770 775 780

Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His Asp Asn Phe Ile
 785 790 795 800

Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro Val Phe Met Glu Gly
 805 810 815

Val Gly Lys Pro Lys Ile Ile Asn Gly Phe Thr Lys Asp Ala Ile Asp
 820 825 830

Lys Gln Gln Asn Asp Ala Gly Leu Tyr Val Ile Val Pro Ile Ile Ile
 835 840 845

Ser Ser Cys Val Leu Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg
 850 855 860

Met Lys Lys Leu Phe Trp Asp Asp Val Pro Asn Pro Lys Asn Cys Ser
 865 870 875 880

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 Trp Ala Gln Gly Leu Asn Phe Gln Lys
 885

<210> SEQ ID NO 81

<211> LENGTH: 867

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 81

Asn Leu Ala Tyr Pro Ile Ser Pro Trp Lys Phe Lys Leu Phe Cys Gly
 1 5 10 15
 Pro Pro Asn Thr Thr Asp Asp Ser Phe Leu Ser Pro Ala Gly Ala Pro
 20 25 30
 Asn Asn Ala Ser Ala Leu Lys Gly Ala Ser Glu Ala Ile Val Glu Ala
 35 40 45
 Lys Phe Asn Ser Ser Gly Ile Tyr Val Pro Glu Leu Ser Lys Thr Val
 50 55 60
 Phe His Cys Cys Phe Gly Asn Glu Gln Gly Gln Asn Cys Ser Ala Leu
 65 70 75 80
 Thr Asp Asn Thr Glu Gly Lys Thr Leu Ala Ser Val Val Lys Ala Ser
 85 90 95
 Val Phe Arg Gln Leu Gly Val Asn Trp Asp Ile Glu Cys Trp Met Lys
 100 105 110
 Gly Asp Leu Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro Lys Asn
 115 120 125
 Pro Phe Lys Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp Leu Pro
 130 135 140
 Glu Val Ile Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser Phe Gln
 145 150 155 160
 Thr Val Gln Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys His Val Pro
 165 170 175
 Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu Leu Met Tyr Leu Glu Ile
 180 185 190
 Thr Ser Ala Gly Val Ser Phe Gln Ser Pro Leu Met Ser Leu Gln Pro
 195 200 205
 Met Leu Val Val Lys Pro Asp Pro Pro Leu Gly Leu His Met Glu Val
 210 215 220
 Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp Asp Ser Gln Thr Met Ala
 225 230 235 240
 Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr Leu Glu Asn Ser Thr Ile
 245 250 255
 Val Arg Glu Ala Ala Glu Ile Val Ser Ala Thr Ser Leu Leu Val Asp
 260 265 270
 Ser Val Leu Pro Gly Ser Ser Tyr Glu Val Gln Val Arg Ser Lys Arg
 275 280 285
 Leu Asp Gly Ser Gly Val Trp Ser Asp Trp Ser Ser Pro Gln Val Phe
 290 295 300
 Thr Thr Gln Asp Val Val Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val
 305 310 315 320
 Gly Ser Asn Ala Ser Phe His Cys Ile Tyr Lys Asn Glu Asn Gln Ile
 325 330 335
 Ile Ser Ser Lys Gln Ile Val Trp Trp Arg Asn Leu Ala Glu Lys Ile
 340 345 350

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Pro Glu Ile Gln Tyr Ser Ile Val Ser Asp Arg Val Ser Lys Val Thr
 355 360 365

Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg Gly Lys Phe Thr Tyr Asp
 370 375 380

Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys His His Arg Tyr Ala Glu
 385 390 395 400

Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser Cys Glu Thr Asp Gly
 405 410 415

Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser Pro Ser Thr Ile Gln Ser
 420 425 430

Leu Val Gly Ser Thr Val Gln Leu Arg Tyr His Arg Arg Ser Leu Tyr
 435 440 445

Cys Pro Asp Ser Pro Ser Ile His Pro Thr Ser Glu Pro Lys Asn Cys
 450 455 460

Val Leu Gln Arg Asp Gly Phe Tyr Glu Cys Val Phe Gln Pro Ile Phe
 465 470 475 480

Leu Leu Ser Gly Tyr Thr Met Trp Ile Arg Ile Asn His Ser Leu Gly
 485 490 495

Ser Leu Asp Ser Pro Pro Thr Cys Val Leu Pro Asp Ser Val Val Lys
 500 505 510

Pro Leu Pro Pro Ser Asn Val Lys Ala Glu Ile Thr Val Asn Thr Gly
 515 520 525

Leu Leu Lys Val Ser Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu
 530 535 540

Gln Phe Gln Ile Arg Tyr Gly Leu Ser Gly Lys Glu Ile Gln Trp Lys
 545 550 555 560

Thr His Glu Val Phe Asp Ala Lys Ser Lys Ser Ala Ser Leu Leu Val
 565 570 575

Ser Asp Leu Cys Ala Val Tyr Val Val Gln Val Arg Cys Arg Arg Leu
 580 585 590

Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser Ser Pro Ala Tyr Thr Leu
 595 600 605

Val Met Asp Val Lys Val Pro Met Arg Gly Pro Glu Phe Trp Arg Lys
 610 615 620

Met Asp Gly Asp Val Thr Lys Lys Glu Arg Asn Val Thr Leu Leu Trp
 625 630 635 640

Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg Tyr Val
 645 650 655

Val Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp Val Gly
 660 665 670

Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His Thr Val
 675 680 685

Thr Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn Phe Asn
 690 695 700

Leu Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu Ser Leu
 705 710 715 720

Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp Thr Leu
 725 730 735

Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile
 740 745 750

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Leu Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser Asn Val
   755                               760           765

Lys Lys Phe Tyr Ile His Asp Asn Phe Ile Pro Ile Glu Lys Tyr Gln
   770                               775           780

Phe Ser Leu Tyr Pro Val Phe Met Glu Gly Val Gly Lys Pro Lys Ile
  785                               790           795           800

Ile Asn Gly Phe Thr Lys Asp Ala Ile Asp Lys Gln Gln Asn Asp Ala
                               805           810           815

Gly Leu Tyr Val Ile Val Pro Ile Ile Ile Ser Ser Cys Val Leu Leu
                               820           825           830

Leu Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu Phe Trp
   835                               840           845

Asp Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln Gly Leu Asn
   850                               855           860

Phe Gln Lys
865

<210> SEQ ID NO 82
<211> LENGTH: 862
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 82

Ile Ser Pro Trp Lys Phe Lys Leu Phe Cys Gly Pro Pro Asn Thr Thr
 1      5      10
Asp Asp Ser Phe Leu Ser Pro Ala Gly Ala Pro Asn Asn Ala Ser Ala
 20      25      30
Leu Lys Gly Ala Ser Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser
 35      40      45
Gly Ile Tyr Val Pro Glu Leu Ser Lys Thr Val Phe His Cys Cys Phe
 50      55      60
Gly Asn Glu Gln Gly Gln Asn Cys Ser Ala Leu Thr Asp Asn Thr Glu
 65      70      75      80
Gly Lys Thr Leu Ala Ser Val Val Lys Ala Ser Val Phe Arg Gln Leu
 85      90      95
Gly Val Asn Trp Asp Ile Glu Cys Trp Met Lys Gly Asp Leu Thr Leu
 100     105     110
Phe Ile Cys His Met Glu Pro Leu Pro Lys Asn Pro Phe Lys Asn Tyr
 115     120     125
Asp Ser Lys Val His Leu Leu Tyr Asp Leu Pro Glu Val Ile Asp Asp
 130     135     140
Ser Pro Leu Pro Pro Leu Lys Asp Ser Phe Gln Thr Val Gln Cys Asn
 145     150     155     160
Cys Ser Leu Arg Gly Cys Glu Cys His Val Pro Val Pro Arg Ala Lys
 165     170     175
Leu Asn Tyr Ala Leu Leu Met Tyr Leu Glu Ile Thr Ser Ala Gly Val
 180     185     190
Ser Phe Gln Ser Pro Leu Met Ser Leu Gln Pro Met Leu Val Val Lys
 195     200     205
Pro Asp Pro Pro Leu Gly Leu His Met Glu Val Thr Asp Asp Gly Asn
 210     215     220
Leu Lys Ile Ser Trp Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln
 225     230     235     240

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Tyr Gln Val Lys Tyr Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala
 245 250 255
 Glu Ile Val Ser Ala Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly
 260 265 270
 Ser Ser Tyr Glu Val Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly
 275 280 285
 Val Trp Ser Asp Trp Ser Ser Pro Gln Val Phe Thr Thr Gln Asp Val
 290 295 300
 Val Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly Ser Asn Ala Ser
 305 310 315 320
 Phe His Cys Ile Tyr Lys Asn Glu Asn Gln Ile Ile Ser Ser Lys Gln
 325 330 335
 Ile Val Trp Trp Arg Asn Leu Ala Glu Lys Ile Pro Glu Ile Gln Tyr
 340 345 350
 Ser Ile Val Ser Asp Arg Val Ser Lys Val Thr Phe Ser Asn Leu Lys
 355 360 365
 Ala Thr Arg Pro Arg Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys
 370 375 380
 Asn Glu Gln Ala Cys His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp
 385 390 395 400
 Val Asn Ile Asn Ile Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met
 405 410 415
 Thr Cys Arg Trp Ser Pro Ser Thr Ile Gln Ser Leu Val Gly Ser Thr
 420 425 430
 Val Gln Leu Arg Tyr His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro
 435 440 445
 Ser Ile His Pro Thr Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp
 450 455 460
 Gly Phe Tyr Glu Cys Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr
 465 470 475 480
 Thr Met Trp Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro
 485 490 495
 Pro Thr Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser
 500 505 510
 Asn Val Lys Ala Glu Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser
 515 520 525
 Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg
 530 535 540
 Tyr Gly Leu Ser Gly Lys Glu Ile Gln Trp Lys Thr His Glu Val Phe
 545 550 555 560
 Asp Ala Lys Ser Lys Ser Ala Ser Leu Leu Val Ser Asp Leu Cys Ala
 565 570 575
 Val Tyr Val Val Gln Val Arg Cys Arg Arg Leu Asp Gly Leu Gly Tyr
 580 585 590
 Trp Ser Asn Trp Ser Ser Pro Ala Tyr Thr Leu Val Met Asp Val Lys
 595 600 605
 Val Pro Met Arg Gly Pro Glu Phe Trp Arg Lys Met Asp Gly Asp Val
 610 615 620
 Thr Lys Lys Glu Arg Asn Val Thr Leu Leu Trp Lys Pro Leu Thr Lys
 625 630 635 640

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

Ala His Asn Gly Thr Trp Ser Glu Asp Val Gly Asn Arg Thr Asn Leu
    660                                665                    670

Thr Phe Leu Trp Thr Glu Pro Ala His Thr Val Thr Val Leu Ala Val
    675                                680                    685

Asn Ser Leu Gly Ala Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp
    690                                695                    700

Pro Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu
    705                                710                    715                    720

Ser Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr
    725                                730                    735

Ser Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp
    740                                745                    750

Gly Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile
    755                                760                    765

His Asp Asn Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro
    770                                775                    780

Val Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile Asn Gly Phe Thr
    785                                790                    795                    800

Lys Asp Ala Ile Asp Lys Gln Gln Asn Asp Ala Gly Leu Tyr Val Ile
    805                                810                    815

Val Pro Ile Ile Ile Ser Ser Cys Val Leu Leu Leu Gly Thr Leu Leu
    820                                825                    830

Ile Ser His Gln Arg Met Lys Lys Leu Phe Trp Asp Asp Val Pro Asn
    835                                840                    845

Pro Lys Asn Cys Ser Trp Ala Gln Gly Leu Asn Phe Gln Lys
    850                                855                    860

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<210> SEQ ID NO 83

<211> LENGTH: 757

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 83

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Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro
 1          5          10          15

Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp
 20          25          30

Leu Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser
 35          40          45

Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys His
 50          55          60

Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu Leu Met Tyr Leu
 65          70          75          80

Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro Leu Met Ser Leu
 85          90          95

Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu Gly Leu His Met
100         105         110

Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp Asp Ser Gln Thr
115         120         125

Met Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr Leu Glu Asn Ser
130         135         140

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Thr Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala Thr Ser Leu Leu
 145 150 155 160

Val Asp Ser Val Leu Pro Gly Ser Ser Tyr Glu Val Gln Val Arg Ser
 165 170 175

Lys Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp Ser Ser Pro Gln
 180 185 190

Val Phe Thr Thr Gln Asp Val Val Tyr Phe Pro Pro Lys Ile Leu Thr
 195 200 205

Ser Val Gly Ser Asn Ala Ser Phe His Cys Ile Tyr Lys Asn Glu Asn
 210 215 220

Gln Ile Ile Ser Ser Lys Gln Ile Val Trp Trp Arg Asn Leu Ala Glu
 225 230 235 240

Lys Ile Pro Glu Ile Gln Tyr Ser Ile Val Ser Asp Arg Val Ser Lys
 245 250 255

Val Thr Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg Gly Lys Phe Thr
 260 265 270

Tyr Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys His His Arg Tyr
 275 280 285

Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser Cys Glu Thr
 290 295 300

Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser Pro Ser Thr Ile
 305 310 315 320

Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr His Arg Arg Ser
 325 330 335

Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr Ser Glu Pro Lys
 340 345 350

Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu Cys Val Phe Gln Pro
 355 360 365

Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp Ile Arg Ile Asn His Ser
 370 375 380

Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys Val Leu Pro Asp Ser Val
 385 390 395 400

Val Lys Pro Leu Pro Pro Ser Asn Val Lys Ala Glu Ile Thr Val Asn
 405 410 415

Thr Gly Leu Leu Lys Val Ser Trp Glu Lys Pro Val Phe Pro Glu Asn
 420 425 430

Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu Ser Gly Lys Glu Ile Gln
 435 440 445

Trp Lys Thr His Glu Val Phe Asp Ala Lys Ser Lys Ser Ala Ser Leu
 450 455 460

Leu Val Ser Asp Leu Cys Ala Val Tyr Val Val Gln Val Arg Cys Arg
 465 470 475 480

Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser Ser Pro Ala Tyr
 485 490 495

Thr Leu Val Met Asp Val Lys Val Pro Met Arg Gly Pro Glu Phe Trp
 500 505 510

Arg Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg Asn Val Thr Leu
 515 520 525

Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg Arg
 530 535 540

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Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp
545                550                555                560

Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His
                565                570                575

Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn
                580                585                590

Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu
                595                600                605

Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp
610                615                620

Thr Leu Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp
625                630                635                640

Lys Ile Leu Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser
645                650                655

Asn Val Lys Lys Phe Tyr Ile His Asp Asn Phe Ile Pro Ile Glu Lys
660                665                670

Tyr Gln Phe Ser Leu Tyr Pro Val Phe Met Glu Gly Val Gly Lys Pro
675                680                685

Lys Ile Ile Asn Gly Phe Thr Lys Asp Ala Ile Asp Lys Gln Gln Asn
690                695                700

Asp Ala Gly Leu Tyr Val Ile Val Pro Ile Ile Ile Ser Ser Cys Val
705                710                715                720

Leu Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu
725                730                735

Phe Trp Asp Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln Gly
740                745                750

Leu Asn Phe Gln Lys
755

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<210> SEQ ID NO 84
<211> LENGTH: 157
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 84

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Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser
1                5                10                15

Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser
20                25                30

Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly
35                40                45

Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His
50                55                60

Asp Asn Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro Val
65                70                75                80

Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile Asn Gly Phe Thr Lys
85                90                95

Asp Ala Ile Asp Lys Gln Gln Asn Asp Ala Gly Leu Tyr Val Ile Val
100               105               110

Pro Ile Ile Ile Ser Ser Cys Val Leu Leu Leu Gly Thr Leu Leu Ile
115               120               125

Ser His Gln Arg Met Lys Lys Leu Phe Trp Asp Asp Val Pro Asn Pro
130               135               140

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340				345				350							
Lys	Asn	Glu	Asn	Gln	Ile	Ile	Ser	Ser	Lys	Gln	Ile	Val	Trp	Trp	Arg
	355						360					365			
Asn	Leu	Ala	Glu	Lys	Ile	Pro	Glu	Ile	Gln	Tyr	Ser	Ile	Val	Ser	Asp
	370					375					380				
Arg	Val	Ser	Lys	Val	Thr	Phe	Ser	Asn	Leu	Lys	Ala	Thr	Arg	Pro	Arg
	385				390					395				400	
Gly	Lys	Phe	Thr	Tyr	Asp	Ala	Val	Tyr	Cys	Cys	Asn	Glu	Gln	Ala	Cys
				405					410					415	
His	His	Arg	Tyr	Ala	Glu	Leu	Tyr	Val	Ile	Asp	Val	Asn	Ile	Asn	Ile
			420						425				430		
Ser	Cys	Glu	Thr	Asp	Gly	Tyr	Leu	Thr	Lys	Met	Thr	Cys	Arg	Trp	Ser
		435					440						445		
Pro	Ser	Thr	Ile	Gln	Ser	Leu	Val	Gly	Ser	Thr	Val	Gln	Leu	Arg	Tyr
		450				455					460				
His	Arg	Arg	Ser	Leu	Tyr	Cys	Pro	Asp	Ser	Pro	Ser	Ile	His	Pro	Thr
					470					475				480	
Ser	Glu	Pro	Lys	Asn	Cys	Val	Leu	Gln	Arg	Asp	Gly	Phe	Tyr	Glu	Cys
				485					490					495	
Val	Phe	Gln	Pro	Ile	Phe	Leu	Leu	Ser	Gly	Tyr	Thr	Met	Trp	Ile	Arg
			500						505				510		
Ile	Asn	His	Ser	Leu	Gly	Ser	Leu	Asp	Ser	Pro	Pro	Thr	Cys	Val	Leu
		515					520						525		
Pro	Asp	Ser	Val	Val	Lys	Pro	Leu	Pro	Pro	Ser	Asn	Val	Lys	Ala	Glu
		530				535					540				
Ile	Thr	Val	Asn	Thr	Gly	Leu	Leu	Lys	Val	Ser	Trp	Glu	Lys	Pro	Val
					550					555				560	
Phe	Pro	Glu	Asn	Asn	Leu	Gln	Phe	Gln	Ile	Arg	Tyr	Gly	Leu	Ser	Gly
			565						570					575	
Lys	Glu	Ile	Gln	Trp	Lys	Thr	His	Glu	Val	Phe	Asp	Ala	Lys	Ser	Lys
			580						585				590		
Ser	Ala	Ser	Leu	Leu	Val	Ser	Asp	Leu	Cys	Ala	Val	Tyr	Val	Val	Gln
		595					600						605		
Val	Arg	Cys	Arg	Arg	Leu	Asp	Gly	Leu	Gly	Tyr	Trp	Ser	Asn	Trp	Ser
						615					620				
Ser	Pro	Ala	Tyr	Thr	Leu	Val	Met	Asp	Val	Lys	Val	Pro	Met	Arg	Gly
					630					635				640	
Pro	Glu	Phe	Trp	Arg	Lys	Met	Asp	Gly	Asp	Val	Thr	Lys	Lys	Glu	Arg
				645					650					655	
Asn	Val	Thr	Leu	Leu	Trp	Lys	Pro	Leu	Thr	Lys	Asn	Asp	Ser	Leu	Cys
			660						665				670		
Ser	Val	Arg	Arg	Tyr	Val	Val	Lys	His	Arg	Thr	Ala	His	Asn	Gly	Thr
			675				680						685		
Trp	Ser	Glu	Asp	Val	Gly	Asn	Arg	Thr	Asn	Leu	Thr	Phe	Leu	Trp	Thr
						695					700				
Glu	Pro	Ala	His	Thr	Val	Thr	Val	Leu	Ala	Val	Asn	Ser	Leu	Gly	Ala
					710					715				720	
Ser	Leu	Val	Asn	Phe	Asn	Leu	Thr	Phe	Ser	Trp	Pro	Met	Ser	Lys	Val
			725						730					735	
Ser	Ala	Val	Glu	Ser	Leu	Ser	Ala	Tyr	Pro	Leu	Ser	Ser	Ser	Cys	Val
			740				745						750		

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Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu
   755                               760                               765
Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly Met Lys Trp Leu
   770                               775                               780
Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His
   785                               790                               795

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<210> SEQ ID NO 86
<211> LENGTH: 774
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 86

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Asn Leu Ala Tyr Pro Ile Ser Pro Trp Lys Phe Lys Leu Phe Cys Gly
 1                               5                               10                               15
Pro Pro Asn Thr Thr Asp Asp Ser Phe Leu Ser Pro Ala Gly Ala Pro
 20                               25                               30
Asn Asn Ala Ser Ala Leu Lys Gly Ala Ser Glu Ala Ile Val Glu Ala
 35                               40                               45
Lys Phe Asn Ser Ser Gly Ile Tyr Val Pro Glu Leu Ser Lys Thr Val
 50                               55                               60
Phe His Cys Cys Phe Gly Asn Glu Gln Gly Gln Asn Cys Ser Ala Leu
 65                               70                               75                               80
Thr Asp Asn Thr Glu Gly Lys Thr Leu Ala Ser Val Val Lys Ala Ser
 85                               90                               95
Val Phe Arg Gln Leu Gly Val Asn Trp Asp Ile Glu Cys Trp Met Lys
 100                              105                              110
Gly Asp Leu Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro Lys Asn
 115                              120                              125
Pro Phe Lys Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp Leu Pro
 130                              135                              140
Glu Val Ile Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser Phe Gln
 145                              150                              155                              160
Thr Val Gln Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys His Val Pro
 165                              170                              175
Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu Leu Met Tyr Leu Glu Ile
 180                              185                              190
Thr Ser Ala Gly Val Ser Phe Gln Ser Pro Leu Met Ser Leu Gln Pro
 195                              200                              205
Met Leu Val Val Lys Pro Asp Pro Pro Leu Gly Leu His Met Glu Val
 210                              215                              220
Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp Asp Ser Gln Thr Met Ala
 225                              230                              235                              240
Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr Leu Glu Asn Ser Thr Ile
 245                              250                              255
Val Arg Glu Ala Ala Glu Ile Val Ser Ala Thr Ser Leu Leu Val Asp
 260                              265                              270
Ser Val Leu Pro Gly Ser Ser Tyr Glu Val Gln Val Arg Ser Lys Arg
 275                              280                              285
Leu Asp Gly Ser Gly Val Trp Ser Asp Trp Ser Ser Pro Gln Val Phe
 290                              295                              300
Thr Thr Gln Asp Val Val Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val

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Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp Thr Leu
725 730 735

Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile
740 745 750

Leu Asn Glu Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser Asn Val
755 760 765

Lys Lys Phe Tyr Ile His
770

<210> SEQ ID NO 87
<211> LENGTH: 769
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 87

Ile Ser Pro Trp Lys Phe Lys Leu Phe Cys Gly Pro Pro Asn Thr Thr
1 5 10 15

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

Leu Lys Gly Ala Ser Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser
35 40 45

Gly Ile Tyr Val Pro Glu Leu Ser Lys Thr Val Phe His Cys Cys Phe
50 55 60

Gly Asn Glu Gln Gly Gln Asn Cys Ser Ala Leu Thr Asp Asn Thr Glu
65 70 75 80

Gly Lys Thr Leu Ala Ser Val Val Lys Ala Ser Val Phe Arg Gln Leu
85 90 95

Gly Val Asn Trp Asp Ile Glu Cys Trp Met Lys Gly Asp Leu Thr Leu
100 105 110

Phe Ile Cys His Met Glu Pro Leu Pro Lys Asn Pro Phe Lys Asn Tyr
115 120 125

Asp Ser Lys Val His Leu Leu Tyr Asp Leu Pro Glu Val Ile Asp Asp
130 135 140

Ser Pro Leu Pro Pro Leu Lys Asp Ser Phe Gln Thr Val Gln Cys Asn
145 150 155 160

Cys Ser Leu Arg Gly Cys Glu Cys His Val Pro Val Pro Arg Ala Lys
165 170 175

Leu Asn Tyr Ala Leu Leu Met Tyr Leu Glu Ile Thr Ser Ala Gly Val
180 185 190

Ser Phe Gln Ser Pro Leu Met Ser Leu Gln Pro Met Leu Val Val Lys
195 200 205

Pro Asp Pro Pro Leu Gly Leu His Met Glu Val Thr Asp Asp Gly Asn
210 215 220

Leu Lys Ile Ser Trp Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln
225 230 235 240

Tyr Gln Val Lys Tyr Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala
245 250 255

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

Ser Ser Tyr Glu Val Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly
275 280 285

Val Trp Ser Asp Trp Ser Ser Pro Gln Val Phe Thr Thr Gln Asp Val

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290	295	300
Val Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly Ser Asn Ala Ser 305 310 315 320		
Phe His Cys Ile Tyr Lys Asn Glu Asn Gln Ile Ile Ser Ser Lys Gln 325 330 335		
Ile Val Trp Trp Arg Asn Leu Ala Glu Lys Ile Pro Glu Ile Gln Tyr 340 345 350		
Ser Ile Val Ser Asp Arg Val Ser Lys Val Thr Phe Ser Asn Leu Lys 355 360 365		
Ala Thr Arg Pro Arg Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys 370 375 380		
Asn Glu Gln Ala Cys His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp 385 390 395 400		
Val Asn Ile Asn Ile Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met 405 410 415		
Thr Cys Arg Trp Ser Pro Ser Thr Ile Gln Ser Leu Val Gly Ser Thr 420 425 430		
Val Gln Leu Arg Tyr His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro 435 440 445		
Ser Ile His Pro Thr Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp 450 455 460		
Gly Phe Tyr Glu Cys Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr 465 470 475 480		
Thr Met Trp Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro 485 490 495		
Pro Thr Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser 500 505 510		
Asn Val Lys Ala Glu Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser 515 520 525		
Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg 530 535 540		
Tyr Gly Leu Ser Gly Lys Glu Ile Gln Trp Lys Thr His Glu Val Phe 545 550 555 560		
Asp Ala Lys Ser Lys Ser Ala Ser Leu Leu Val Ser Asp Leu Cys Ala 565 570 575		
Val Tyr Val Val Gln Val Arg Cys Arg Arg Leu Asp Gly Leu Gly Tyr 580 585 590		
Trp Ser Asn Trp Ser Ser Pro Ala Tyr Thr Leu Val Met Asp Val Lys 595 600 605		
Val Pro Met Arg Gly Pro Glu Phe Trp Arg Lys Met Asp Gly Asp Val 610 615 620		
Thr Lys Lys Glu Arg Asn Val Thr Leu Leu Trp Lys Pro Leu Thr Lys 625 630 635 640		
Asn Asp Ser Leu Cys Ser Val Arg Arg Tyr Val Val Lys His Arg Thr 645 650 655		
Ala His Asn Gly Thr Trp Ser Glu Asp Val Gly Asn Arg Thr Asn Leu 660 665 670		
Thr Phe Leu Trp Thr Glu Pro Ala His Thr Val Thr Val Leu Ala Val 675 680 685		
Asn Ser Leu Gly Ala Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp 690 695 700		

-continued

Pro Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu
705 710 715 720

Ser Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr
725 730 735

Ser Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp
740 745 750

Gly Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile
755 760 765

His

<210> SEQ ID NO 88

<211> LENGTH: 771

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 88

Asp Pro Ile Ser Pro Trp Lys Phe Lys Leu Phe Cys Gly Pro Pro Asn
1 5 10 15

Thr Thr Asp Asp Ser Phe Leu Ser Pro Ala Gly Ala Pro Asn Asn Ala
20 25 30

Ser Ala Leu Lys Gly Ala Ser Glu Ala Ile Val Glu Ala Lys Phe Asn
35 40 45

Ser Ser Gly Ile Tyr Val Pro Glu Leu Ser Lys Thr Val Phe His Cys
50 55 60

Cys Phe Gly Asn Glu Gln Gly Gln Asn Cys Ser Ala Leu Thr Asp Asn
65 70 75 80

Thr Glu Gly Lys Thr Leu Ala Ser Val Val Lys Ala Ser Val Phe Arg
85 90 95

Gln Leu Gly Val Asn Trp Asp Ile Glu Cys Trp Met Lys Gly Asp Leu
100 105 110

Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro Lys Asn Pro Phe Lys
115 120 125

Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp Leu Pro Glu Val Ile
130 135 140

Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser Phe Gln Thr Val Gln
145 150 155 160

Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys His Val Pro Val Pro Arg
165 170 175

Ala Lys Leu Asn Tyr Ala Leu Leu Met Tyr Leu Glu Ile Thr Ser Ala
180 185 190

Gly Val Ser Phe Gln Ser Pro Leu Met Ser Leu Gln Pro Met Leu Val
195 200 205

Val Lys Pro Asp Pro Pro Leu Gly Leu His Met Glu Val Thr Asp Asp
210 215 220

Gly Asn Leu Lys Ile Ser Trp Asp Ser Gln Thr Met Ala Pro Phe Pro
225 230 235 240

Leu Gln Tyr Gln Val Lys Tyr Leu Glu Asn Ser Thr Ile Val Arg Glu
245 250 255

Ala Ala Glu Ile Val Ser Ala Thr Ser Leu Leu Val Asp Ser Val Leu
260 265 270

Pro Gly Ser Ser Tyr Glu Val Gln Val Arg Ser Lys Arg Leu Asp Gly
275 280 285

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Ser Gly Val Trp Trp Ser Asp Trp Ser Ser Pro Gln Val Phe Thr Thr Gln
 290 295 300

Asp Val Val Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly Ser Asn
 305 310 315 320

Ala Ser Phe His Cys Ile Tyr Lys Asn Glu Asn Gln Ile Ile Ser Ser
 325 330 335

Lys Gln Ile Val Trp Trp Arg Asn Leu Ala Glu Lys Ile Pro Glu Ile
 340 345 350

Gln Tyr Ser Ile Val Ser Asp Arg Val Ser Lys Val Thr Phe Ser Asn
 355 360 365

Leu Lys Ala Thr Arg Pro Arg Gly Lys Phe Thr Tyr Asp Ala Val Tyr
 370 375 380

Cys Cys Asn Glu Gln Ala Cys His His Arg Tyr Ala Glu Leu Tyr Val
 385 390 395 400

Ile Asp Val Asn Ile Asn Ile Ser Cys Glu Thr Asp Gly Tyr Leu Thr
 405 410 415

Lys Met Thr Cys Arg Trp Ser Pro Ser Thr Ile Gln Ser Leu Val Gly
 420 425 430

Ser Thr Val Gln Leu Arg Tyr His Arg Arg Ser Leu Tyr Cys Pro Asp
 435 440 445

Ser Pro Ser Ile His Pro Thr Ser Glu Pro Lys Asn Cys Val Leu Gln
 450 455 460

Arg Asp Gly Phe Tyr Glu Cys Val Phe Gln Pro Ile Phe Leu Leu Ser
 465 470 475 480

Gly Tyr Thr Met Trp Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp
 485 490 495

Ser Pro Pro Thr Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro
 500 505 510

Pro Ser Asn Val Lys Ala Glu Ile Thr Val Asn Thr Gly Leu Leu Lys
 515 520 525

Val Ser Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe Gln
 530 535 540

Ile Arg Tyr Gly Leu Ser Gly Lys Glu Ile Gln Trp Lys Thr His Glu
 545 550 555 560

Val Phe Asp Ala Lys Ser Lys Ser Ala Ser Leu Leu Val Ser Asp Leu
 565 570 575

Cys Ala Val Tyr Val Val Gln Val Arg Cys Arg Arg Leu Asp Gly Leu
 580 585 590

Gly Tyr Trp Ser Asn Trp Ser Ser Pro Ala Tyr Thr Leu Val Met Asp
 595 600 605

Val Lys Val Pro Met Arg Gly Pro Glu Phe Trp Arg Lys Met Asp Gly
 610 615 620

Asp Val Thr Lys Lys Glu Arg Asn Val Thr Leu Leu Trp Lys Pro Leu
 625 630 635 640

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

Arg Thr Ala His Asn Gly Thr Trp Ser Glu Asp Val Gly Asn Arg Thr
 660 665 670

Asn Leu Thr Phe Leu Trp Thr Glu Pro Ala His Thr Val Thr Val Leu
 675 680 685

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Ala Val Asn Ser Leu Gly Ala Ser Leu Val Asn Phe Asn Leu Thr Phe
690 695 700

Ser Trp Pro Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser Ala Tyr
705 710 715 720

Pro Leu Ser Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp
725 730 735

Asp Tyr Ser Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu
740 745 750

Asp Asp Gly Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys Lys Phe
755 760 765

Tyr Ile His
770

<210> SEQ ID NO 89
 <211> LENGTH: 684
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 89

Ser Val Val Lys Ala Ser Val Phe Arg Gln Leu Gly Val Asn Trp Asp
1 5 10 15

Ile Glu Cys Trp Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met
20 25 30

Glu Pro Leu Pro Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His
35 40 45

Leu Leu Tyr Asp Leu Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro
50 55 60

Leu Lys Asp Ser Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly
65 70 75 80

Cys Glu Cys His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu
85 90 95

Leu Met Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro
100 105 110

Leu Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu
115 120 125

Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp
130 135 140

Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr
145 150 155 160

Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala
165 170 175

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

Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp
195 200 205

Ser Ser Pro Gln Val Phe Thr Thr Gln Asp Val Val Tyr Phe Pro Pro
210 215 220

Lys Ile Leu Thr Ser Val Gly Ser Asn Ala Ser Phe His Cys Ile Tyr
225 230 235 240

Lys Asn Glu Asn Gln Ile Ile Ser Ser Lys Gln Ile Val Trp Trp Arg
245 250 255

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

-continued

Arg Val Ser Lys Val Thr Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg
 275 280 285
 Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys
 290 295 300
 His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile
 305 310 315 320
 Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser
 325 330 335
 Pro Ser Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr
 340 345 350
 His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr
 355 360 365
 Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu Cys
 370 375 380
 Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp Ile Arg
 385 390 395 400
 Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys Val Leu
 405 410 415
 Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn Val Lys Ala Glu
 420 425 430
 Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser Trp Glu Lys Pro Val
 435 440 445
 Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu Ser Gly
 450 455 460
 Lys Glu Ile Gln Trp Lys Thr His Glu Val Phe Asp Ala Lys Ser Lys
 465 470 475 480
 Ser Ala Ser Leu Leu Val Ser Asp Leu Cys Ala Val Tyr Val Val Gln
 485 490 495
 Val Arg Cys Arg Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser
 500 505 510
 Ser Pro Ala Tyr Thr Leu Val Met Asp Val Lys Val Pro Met Arg Gly
 515 520 525
 Pro Glu Phe Trp Arg Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg
 530 535 540
 Asn Val Thr Leu Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys
 545 550 555 560
 Ser Val Arg Arg Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr
 565 570 575
 Trp Ser Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr
 580 585 590
 Glu Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala
 595 600 605
 Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys Val
 610 615 620
 Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val
 625 630 635 640
 Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu
 645 650 655
 Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly Met Lys Trp Leu
 660 665 670

-continued

Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His
 675 680

<210> SEQ ID NO 90
 <211> LENGTH: 64
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 90

Met Ser Lys Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser
 1 5 10 15

Ser Ser Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser
 20 25 30

Leu Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly
 35 40 45

Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His
 50 55 60

<210> SEQ ID NO 91
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 91

Gly Met Cys Thr Val Leu Phe Met Asp
 1 5

<210> SEQ ID NO 92
 <211> LENGTH: 227
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 92

Asp Arg Trp Gly Ser Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile
 1 5 10 15

Asn Ile Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg
 20 25 30

Trp Ser Pro Ser Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu
 35 40 45

Arg Tyr His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His
 50 55 60

Pro Thr Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr
 65 70 75 80

Glu Cys Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp
 85 90 95

Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys
 100 105 110

Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn Val Lys
 115 120 125

Ala Glu Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser Trp Glu Lys
 130 135 140

Pro Val Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu
 145 150 155 160

Ser Gly Lys Glu Ile Gln Trp Lys Thr His Glu Val Phe Asp Ala Lys
 165 170 175

Ser Lys Ser Ala Ser Leu Leu Val Ser Asp Leu Cys Ala Val Tyr Val

-continued

180	185	190
Val Gln Val Arg Cys Arg Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn		
195	200	205
Trp Ser Ser Pro Ala Tyr Thr Leu Val Met Asp Val Lys Val Pro Met		
210	215	220
Arg Gly Pro		
225		

<210> SEQ ID NO 93
 <211> LENGTH: 227
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 93

Asp Arg Trp Gly Ser Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile		
1	5	10
Asn Ile Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg		
20	25	30
Trp Ser Pro Ser Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu		
35	40	45
Arg Tyr His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His		
50	55	60
Pro Thr Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr		
65	70	75
Glu Cys Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp		
85	90	95
Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys		
100	105	110
Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn Val Lys		
115	120	125
Ala Glu Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser Trp Glu Lys		
130	135	140
Pro Val Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu		
145	150	155
Ser Gly Lys Glu Ile Gln Trp Lys Thr His Glu Val Phe Asp Ala Lys		
165	170	175
Ser Lys Ser Ala Ser Leu Leu Val Ser Asp Leu Cys Ala Val Tyr Val		
180	185	190
Val Gln Val Arg Cys Arg Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn		
195	200	205
Trp Ser Ser Pro Ala Tyr Thr Leu Val Met Asp Val Lys Val Pro Met		
210	215	220
Arg Gly Pro		
225		

<210> SEQ ID NO 94
 <211> LENGTH: 529
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 94

Asp Arg Trp Gly Ser Ser Val Phe Arg Gln Leu Gly Val Asn Trp Asp		
1	5	10
Ile Glu Cys Trp Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met		

-continued

20					25					30					
Glu	Pro	Leu	Pro	Lys	Asn	Pro	Phe	Lys	Asn	Tyr	Asp	Ser	Lys	Val	His
	35						40					45			
Leu	Leu	Tyr	Asp	Leu	Pro	Glu	Val	Ile	Asp	Asp	Ser	Pro	Leu	Pro	Pro
	50					55					60				
Leu	Lys	Asp	Ser	Phe	Gln	Thr	Val	Gln	Cys	Asn	Cys	Ser	Leu	Arg	Gly
	65					70					75				80
Cys	Glu	Cys	His	Val	Pro	Val	Pro	Arg	Ala	Lys	Leu	Asn	Tyr	Ala	Leu
				85					90					95	
Leu	Met	Tyr	Leu	Glu	Ile	Thr	Ser	Ala	Gly	Val	Ser	Phe	Gln	Ser	Pro
			100					105					110		
Leu	Met	Ser	Leu	Gln	Pro	Met	Leu	Val	Val	Lys	Pro	Asp	Pro	Pro	Leu
		115					120					125			
Gly	Leu	His	Met	Glu	Val	Thr	Asp	Asp	Gly	Asn	Leu	Lys	Ile	Ser	Trp
	130					135						140			
Asp	Ser	Gln	Thr	Met	Ala	Pro	Phe	Pro	Leu	Gln	Tyr	Gln	Val	Lys	Tyr
	145					150					155				160
Leu	Glu	Asn	Ser	Thr	Ile	Val	Arg	Glu	Ala	Ala	Glu	Ile	Val	Ser	Ala
				165					170					175	
Thr	Ser	Leu	Leu	Val	Asp	Ser	Val	Leu	Pro	Gly	Ser	Ser	Tyr	Glu	Val
			180					185					190		
Gln	Val	Arg	Ser	Lys	Arg	Leu	Asp	Gly	Ser	Gly	Val	Trp	Ser	Asp	Trp
		195					200					205			
Ser	Ser	Pro	Gln	Val	Phe	Thr	Thr	Gln	Asp	Val	Val	Tyr	Phe	Pro	Pro
		210				215					220				
Lys	Ile	Leu	Thr	Ser	Val	Gly	Ser	Asn	Ala	Ser	Phe	His	Cys	Ile	Tyr
		225				230			235					240	
Lys	Asn	Glu	Asn	Gln	Ile	Ile	Ser	Ser	Lys	Gln	Ile	Val	Trp	Trp	Arg
			245						250					255	
Asn	Leu	Ala	Glu	Lys	Ile	Pro	Glu	Ile	Gln	Tyr	Ser	Ile	Val	Ser	Asp
		260					265						270		
Arg	Val	Ser	Lys	Val	Thr	Phe	Ser	Asn	Leu	Lys	Ala	Thr	Arg	Pro	Arg
		275					280					285			
Gly	Lys	Phe	Thr	Tyr	Asp	Ala	Val	Tyr	Cys	Cys	Asn	Glu	Gln	Ala	Cys
	290					295					300				
His	His	Arg	Tyr	Ala	Glu	Leu	Tyr	Val	Ile	Asp	Val	Asn	Ile	Asn	Ile
	305					310					315			320	
Ser	Cys	Glu	Thr	Asp	Gly	Tyr	Leu	Thr	Lys	Met	Thr	Cys	Arg	Trp	Ser
			325						330					335	
Pro	Ser	Thr	Ile	Gln	Ser	Leu	Val	Gly	Ser	Thr	Val	Gln	Leu	Arg	Tyr
			340					345					350		
His	Arg	Arg	Ser	Leu	Tyr	Cys	Pro	Asp	Ser	Pro	Ser	Ile	His	Pro	Thr
		355					360					365			
Ser	Glu	Pro	Lys	Asn	Cys	Val	Leu	Gln	Arg	Asp	Gly	Phe	Tyr	Glu	Cys
		370				375					380				
Val	Phe	Gln	Pro	Ile	Phe	Leu	Leu	Ser	Gly	Tyr	Thr	Met	Trp	Ile	Arg
	385					390					395			400	
Ile	Asn	His	Ser	Leu	Gly	Ser	Leu	Asp	Ser	Pro	Pro	Thr	Cys	Val	Leu
			405					410						415	
Pro	Asp	Ser	Val	Val	Lys	Pro	Leu	Pro	Pro	Ser	Asn	Val	Lys	Ala	Glu
			420				425						430		

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Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser Trp Glu Lys Pro Val
    435                                440                445

Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu Ser Gly
    450                                455                460

Lys Glu Ile Gln Trp Lys Thr His Glu Val Phe Asp Ala Lys Ser Lys
    465                                470                475                480

Ser Ala Ser Leu Leu Val Ser Asp Leu Cys Ala Val Tyr Val Val Gln
    485                                490                495

Val Arg Cys Arg Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser
    500                                505                510

Ser Pro Ala Tyr Thr Leu Val Met Asp Val Lys Val Pro Met Arg Gly
    515                                520                525

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Pro

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<210> SEQ ID NO 95
<211> LENGTH: 529
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 95

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Asp Arg Trp Gly Ser Ser Val Phe Arg Gln Leu Gly Val Asn Trp Asp
1      5      10      15

Ile Glu Cys Trp Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met
20     25     30

Glu Pro Leu Pro Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His
35     40     45

Leu Leu Tyr Asp Leu Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro
50     55     60

Leu Lys Asp Ser Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly
65     70     75     80

Cys Glu Cys His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu
85     90     95

Leu Met Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro
100    105    110

Leu Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu
115    120    125

Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp
130    135    140

Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr
145    150    155    160

Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala
165    170    175

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

Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp
195    200    205

Ser Ser Pro Gln Val Phe Thr Thr Gln Asp Val Val Tyr Phe Pro Pro
210    215    220

Lys Ile Leu Thr Ser Val Gly Ser Asn Ala Ser Phe His Cys Ile Tyr
225    230    235    240

Lys Asn Glu Asn Gln Ile Ile Ser Ser Lys Gln Ile Val Trp Trp Arg
245    250    255

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-continued

Asn Leu Ala Glu Lys Ile Pro Glu Ile Gln Tyr Ser Ile Val Ser Asp
 260 265 270
 Arg Val Ser Lys Val Thr Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg
 275 280 285
 Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys
 290 295 300
 His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile
 305 310 315 320
 Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser
 325 330 335
 Pro Ser Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr
 340 345 350
 His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr
 355 360 365
 Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu Cys
 370 375 380
 Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp Ile Arg
 385 390 395 400
 Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys Val Leu
 405 410 415
 Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn Val Lys Ala Glu
 420 425 430
 Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser Trp Glu Lys Pro Val
 435 440 445
 Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu Ser Gly
 450 455 460
 Lys Glu Ile Gln Trp Lys Thr His Glu Val Phe Asp Ala Lys Ser Lys
 465 470 475 480
 Ser Ala Ser Leu Leu Val Ser Asp Leu Cys Ala Val Tyr Val Val Gln
 485 490 495
 Val Arg Cys Arg Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser
 500 505 510
 Ser Pro Ala Tyr Thr Leu Val Met Asp Val Lys Val Pro Met Arg Gly
 515 520 525

Pro

<210> SEQ ID NO 96
 <211> LENGTH: 214
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 96

Asp Arg Trp Gly Ser Leu Gly Val Asn Trp Asp Ile Glu Cys Trp Met
 1 5 10 15
 Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro Lys
 20 25 30
 Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp Leu
 35 40 45
 Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser Phe
 50 55 60
 Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys His Val
 65 70 75 80

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Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu Leu Met Tyr Leu Glu
      85                      90                      95
Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro Leu Met Ser Leu Gln
      100                      105                      110
Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu Gly Leu His Met Glu
      115                      120                      125
Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp Asp Ser Gln Thr Met
      130                      135                      140
Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr Leu Glu Asn Ser Thr
      145                      150                      155                      160
Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala Thr Ser Leu Leu Val
      165                      170                      175
Asp Ser Val Leu Pro Gly Ser Ser Tyr Glu Val Gln Val Arg Ser Lys
      180                      185                      190
Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp Ser Ser Pro Gln Val
      195                      200                      205
Phe Thr Thr Gln Asp Val
      210

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<210> SEQ ID NO 97
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 97

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Asp Arg Trp Gly Ser Leu Gly Val Asn Trp Asp Ile Glu Cys Trp Met
1      5      10      15
Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro Lys
20     25     30
Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp Leu
35     40     45
Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser Phe
50     55     60
Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys His Val
65     70     75     80
Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu Leu Met Tyr Leu Glu
85     90     95
Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro Leu Met Ser Leu Gln
100    105    110
Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu Gly Leu His Met Glu
115    120    125
Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp Asp Ser Gln Thr Met
130    135    140
Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr Leu Glu Asn Ser Thr
145    150    155    160
Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala Thr Ser Leu Leu Val
165    170    175
Asp Ser Val Leu Pro Gly Ser Ser Tyr Glu Val Gln Val Arg Ser Lys
180    185    190
Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp Ser Ser Pro Gln Val
195    200    205

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-continued

Phe Thr Thr Gln Asp Val
210

1-14. (canceled)

15. An antigenic fragment of the leptin receptor (OB-R) polypeptide.

16. The antigenic fragment of claim 15 which is selected from the group consisting of SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:34.

17. A derivative of the leptin receptor (OB-R) polypeptide which is a soluble receptor attached to a chemical moiety.

18. The derivative of claim 15 wherein the chemical moiety is a water-soluble polymer.

19. The derivative of claim 16 wherein the water soluble polymer is polyethylene glycol.

20-28. (canceled)

29. An oligonucleotide hybridizable under stringent conditions to a nucleic acid molecule encoding on expression a leptin receptor polypeptide selected from the group consisting of:

a. a polypeptide coding sequence of a DNA molecule of SEQ ID NO:1, 3, 5, 7, or 9;

b. a DNA molecule complementary to the DNA molecule defined in (a);

c. a DNA molecule which hybridizes to the DNA molecule of (a) or (b), or a hybridizable fragment thereof;

d. a DNA molecule which is identifiable with a polymerase chain reaction (PCR) probe selected from group consisting of a probe for clone 7 (forward primer SEQ ID NO:42 and reverse primer SEQ ID NO:43), a probe for clone 11 (forward primer SEQ ID NO:44 and reverse primer SEQ ID NO:45), and both clone 7 and clone 11; and

a DNA molecule that codes on expression for the polypeptide encoded by any of the foregoing DNA molecules.

30. An oligonucleotide hybridizable under stringent conditions to a nucleic acid molecule which codes on expression for a polypeptide selected from the group consisting of:

a. a leptin receptor selected from the group consisting of OB-Ra, OB-Rb, OB-Rc, OB-Rd, and OB-Re, or allelic variants thereof;

b. a leptin receptor selected from the group consisting of:

i. N-terminal corresponding to OB-Ra through Lys⁸⁸⁹ and C-terminal corresponding to a C-terminal selected from the group consisting of OB-Rb, OB-Rc, and OB-Rd after Lys⁸⁸⁹;

ii. N-terminal corresponding to OB-Rb or OB-Rc through Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra or OB-Rd after Lys⁸⁸⁹;

iii. N-terminal corresponding to OB-Rd through Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra, OB-Rb, or OB-Rc;

iv. N-terminal corresponding to OB-R from Pro⁶⁶⁴ to Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra, OB-Rb, OB-Rc, and OB-Rd;

v. N-terminal corresponding to OB-R from Met⁷³³ to Lys⁸⁸⁹, and C-terminal corresponding to OB-Ra, OB-Rb, OB-Rc, and OB-Rd;

vi. N-terminal selected from the group consisting of OB-Ra, OB-Rb, OB-Rd, and OB-R from Pro⁶⁶⁴, through His⁷⁹⁶, and OB-Re from His⁷⁹⁶, and

vii. N-terminal corresponding to OB-R from Met⁷³³ to His⁷⁹⁶, and OB-Re from His⁷⁹⁶,

or allelic variants thereof;

c. a leptin receptor wherein

i. the N-terminal sequence is selected from the group consisting of

(1) amino acid residues 1-889;

(2) amino acid residues 23-889;

(3) amino acid residues 28-889;

(4) amino acid residues 133-889;

(5) amino acid residues 733-889;

(6) amino acid residues 1-796;

(7) amino acid residues 23-796;

(8) amino acid residues 28-796;

(9) amino acid residues 133-796; and

(10) amino acid residues 733-796; and

ii. the C-terminal sequence is selected from the group consisting of

(1) SEQ ID NO:11;

(2) SEQ ID NO:12;

(3) SEQ ID NO:13;

(4) SEQ ID NO:14; and

(5) SEQ ID NO:15;

wherein the numbering is based on the amino acid sequence of the full length transcribed murine leptin receptor, including the signal peptide, or allelic variants thereof.

31. An oligonucleotide hybridizable under stringent conditions to a nucleic acid molecule having a nucleotide sequence corresponding or complementary to the DNA sequence set forth in SEQ ID NO:1, 3, 5, 7 or 9.

32. The oligonucleotide of claim 29, 30, or 31 selected from the group consisting of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID

NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, and SEQ ID NO:54.

33. The oligonucleotide of claim 32 which is labeled.

34-48. (canceled)

49. The oligonucleotide of claim 29, 30, or 31 which is an antisense nucleic acid that hybridizes with an mRNA encoding leptin receptor.

50. A ribozyme which cleaves an mRNA encoding a leptin receptor.

51. A transgenic vector comprising a DNA molecule encoding a leptin receptor (OB-R) polypeptide.

52. A transgenic vector comprising a DNA molecule encoding on expression a leptin receptor polypeptide selected from the group consisting of:

- a. a polypeptide coding sequence of a DNA molecule of SEQ ID NO:1, 3, 5, 7, or 9;
- b. a DNA molecule complementary to the DNA molecule defined in (a);
- c. a DNA molecule which hybridizes to the DNA molecule of (a) or (b), or a hybridizable fragment thereof;
- d. a DNA molecule which is identifiable with a polymerase chain reaction (PCR) probe selected from group consisting of a probe for clone 7 (forward primer SEQ ID NO:42 and reverse primer SEQ ID NO:43), a probe for clone 11 (forward primer SEQ ID NO:44 and reverse primer SEQ ID NO:45), and both clone 7 and clone 11; and

a DNA molecule that codes on expression for the polypeptide encoded by any of the foregoing DNA molecules.

53. An antibody specific for a leptin receptor (OB-R) polypeptide.

54. An antibody according to claim 53 which is a monoclonal or polyclonal antibody.

55. An antibody according to claim 53 labeled with a detectable label.

56. An immortal cell line that produces a monoclonal antibody according to claim 54.

57. A method for preparing an antibody specific for a leptin receptor, comprising:

- a. immunizing a host animal with a the leptin receptor (OB-R) polypeptide admixed with an adjuvant; and
- b. obtaining antibody from the immunized host animal.

58. A method for preparing an antibody specific for a leptin receptor, comprising:

- a. conjugating a peptide having a sequence selected from the group consisting of SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:34 to a carrier protein;

b. immunizing a host animal with the peptide-carrier protein conjugate of step (a) admixed with an adjuvant; and

c. obtaining antibody from the immunized host animal.

59. A method for measuring the presence of a leptin receptor in a sample, comprising:

a. contacting a sample suspected of containing a leptin receptor with an antibody that specifically binds to the leptin receptor under conditions which allow for the formation of reaction complexes comprising the antibody and the leptin receptor; and

b. detecting the formation of reaction complexes comprising the antibody and leptin receptor in the sample, wherein detection of the formation of reaction complexes indicates the presence of leptin receptor in the sample.

60. The method according to claim 59 wherein the antibody is bound to a solid phase support.

61. An in vitro method for evaluating the level of leptin receptor in a biological sample comprising:

a. detecting the formation of reaction complexes in a biological sample according to the method of claim 59 or 60; and

b. evaluating the amount of reaction complexes formed, which amount of reaction complexes corresponds to the level of leptin receptor in the biological sample.

62. An in vitro method for detecting or diagnosing the presence of a disease associated with elevated or decreased levels of leptin receptor in a subject comprising:

a. evaluating the level of leptin receptor in a biological sample from a subject according to claim 61; and

b. comparing the level detected in step (a) to a level of leptin receptor present in normal subjects or in the subject at an earlier time,

wherein an increase in the level of leptin receptor as compared to normal levels indicates a disease associated with elevated levels of leptin receptor, and decreased level of leptin receptor as compared to normal levels indicates a disease associated with decreased levels of leptin receptor.

63. (canceled)

64. A method for treating obesity in a subject comprising administering a therapeutically effective amount of a pharmaceutical composition comprising a soluble leptin receptor (OB-R) polypeptide and a pharmaceutically acceptable carrier.

65. The method according to claim 64, further comprising administering a treatment for diabetes, high blood pressure, and high cholesterol.

66. (canceled)

* * * * *

专利名称(译)	Db, 瘦蛋白受体, 编码受体的核酸及其用途		
公开(公告)号	US20070020686A1	公开(公告)日	2007-01-25
申请号	US11/410186	申请日	2006-04-24
[标]申请(专利权)人(译)	洛克菲勒大学		
申请(专利权)人(译)	洛克菲勒UNIVERSITY		
当前申请(专利权)人(译)	美国国立卫生研究院 (NIH), 美国DEPT.卫生与人类服务部 (DHHS), 美国政府的		
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摘要(译)

本发明涉及饱和因子受体的鉴定, 其涉及体重稳态。该受体的突变与肥胖表型相关。特别地, 本发明涉及瘦蛋白受体的鉴定和表征, 包括天然存在的可溶形式的受体, 其预期调节瘦蛋白活性, 特别是激动瘦蛋白活性。本发明进一步涉及编码受体的核酸, 以及使用该受体的方法, 例如, 在治疗上, 例如在基因治疗中或以可溶形式鉴定瘦蛋白类似物作为瘦蛋白活性的激动剂或拮抗剂, 或在诊断上。

