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(54) **HUMAN G PROTEIN-COUPLED  
RECEPTORS AND MODULATORS THEREOF  
FOR THE TREATMENT OF  
METABOLIC-RELATED DISORDERS**

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**Related U.S. Application Data**

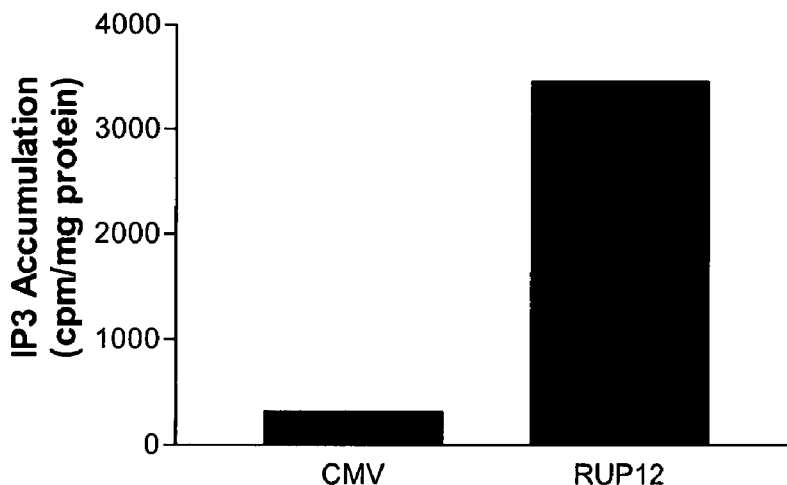
- (63) Continuation of application No. 10/314,048, filed on Dec. 6, 2002, which is a continuation-in-part of application No. 10/096,511, filed on Mar. 12, 2002, now abandoned, which is a continuation of application No. 09/995,543, filed on Nov. 27, 2001, now abandoned.
- (60) Provisional application No. 60/399,917, filed on Jul. 29, 2002. Provisional application No. 60/404,761, filed on Aug. 19, 2002. Provisional application No. 60/410,747, filed on Sep. 13, 2002.

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(52) **U.S. Cl.** ..... **514/341**; 435/7.1

(57) **ABSTRACT**

The present invention relates to methods of identifying whether a candidate compound is a modulator of a G protein-coupled receptor (GPCR). In preferred embodiments, the GPCR is human. In other preferred embodiments, the GPCR is coupled to Gi and lowers the level of intracellular cAMP. In other preferred embodiments, the GPCR is expressed endogenously by adipocytes. In further preferred embodiments, the GPCR inhibits intracellular lipolysis. In other further preferred embodiments, the GPCR is a nicotinic acid receptor. The present invention also relates to methods of using a modulator of said GPCR. Preferred modulator is agonist. Agonists of the invention are useful as therapeutic agents for the prevention or treatment of metabolic-related disorders, including dyslipidemia, atherosclerosis, coronary heart disease, stroke, insulin resistance, and type 2 diabetes.

**IP3 Assay in 293 Cells**

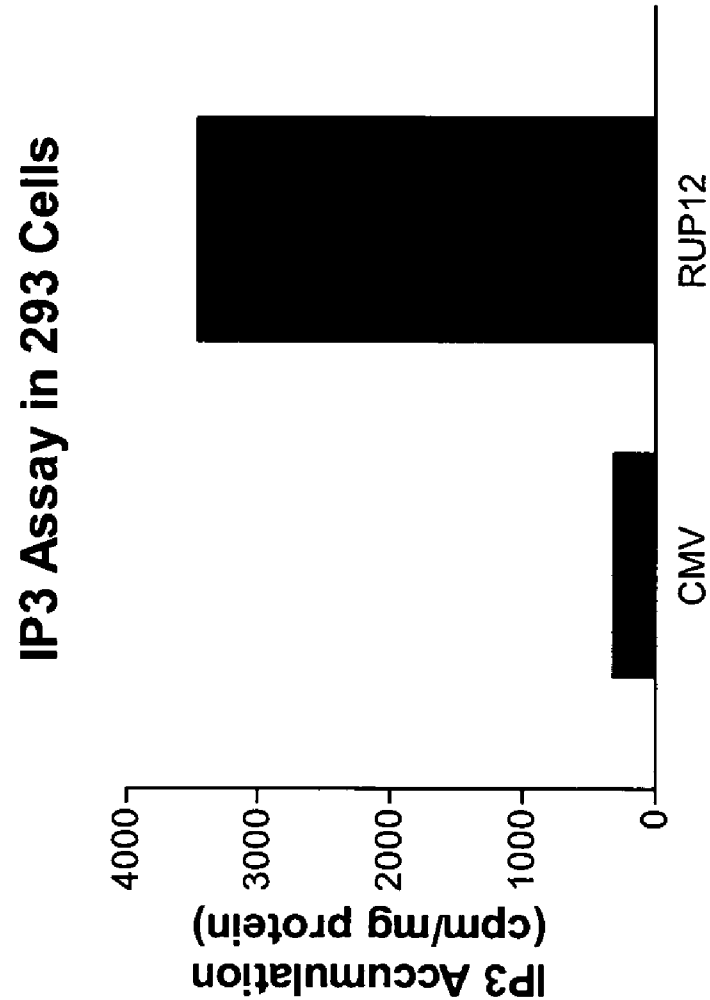


Figure 1

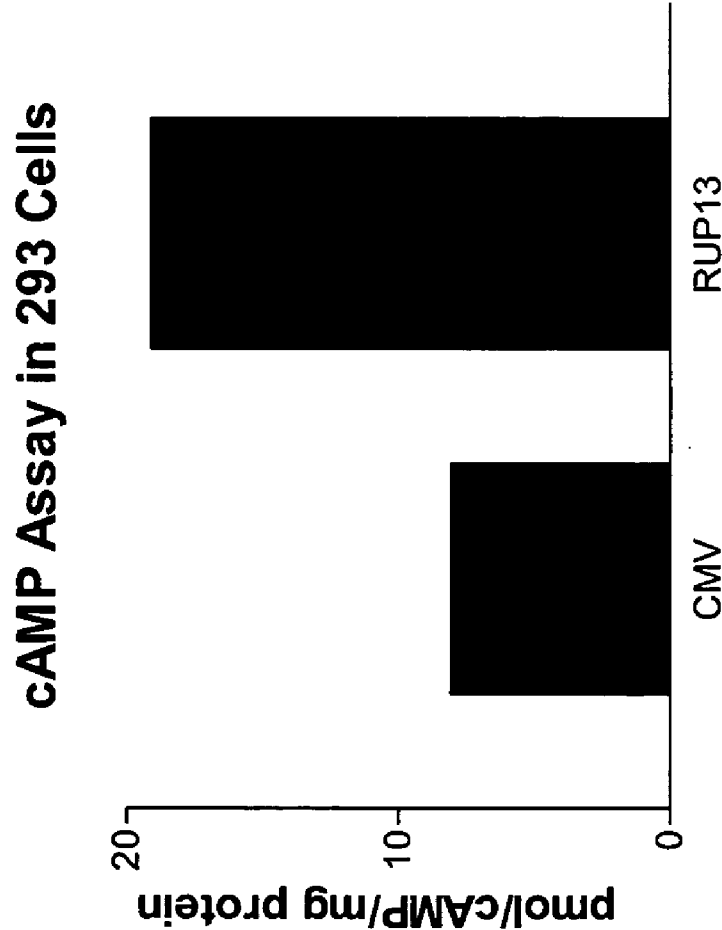


Figure 2

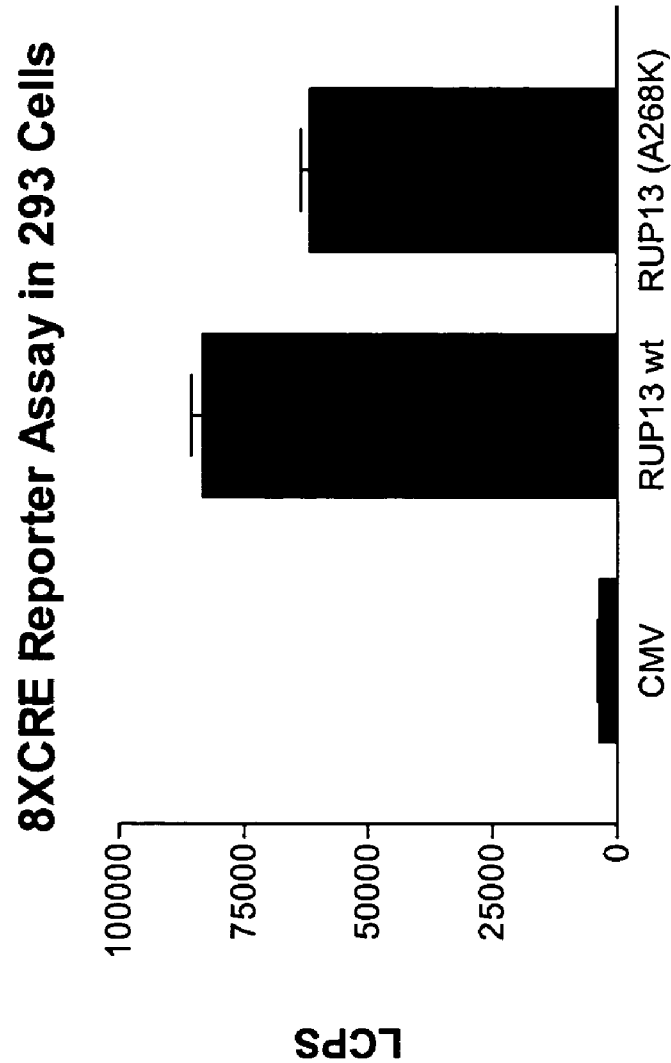


Figure 3

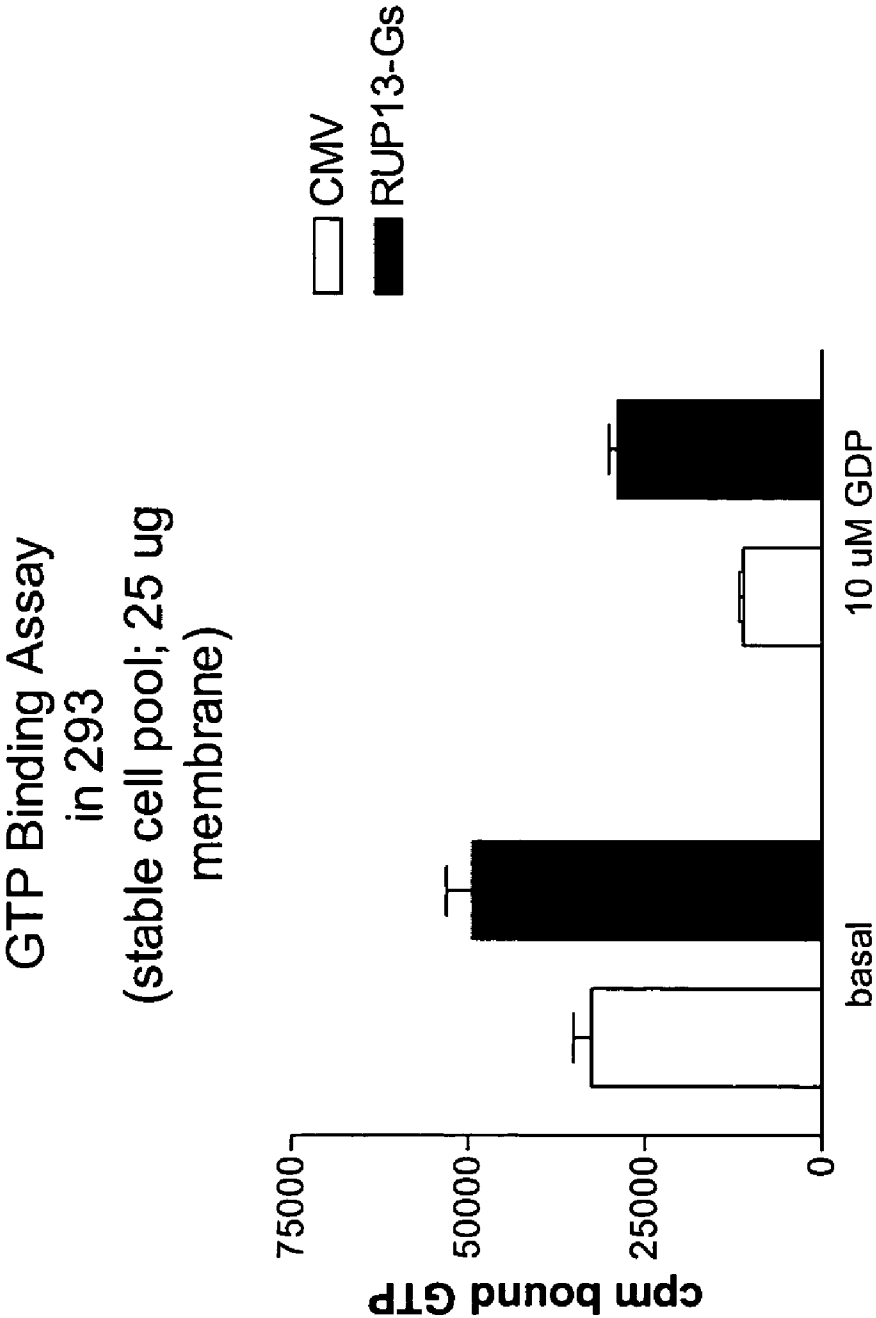


Figure 4

# 8XCRE Reporter Assay in 293 Cells

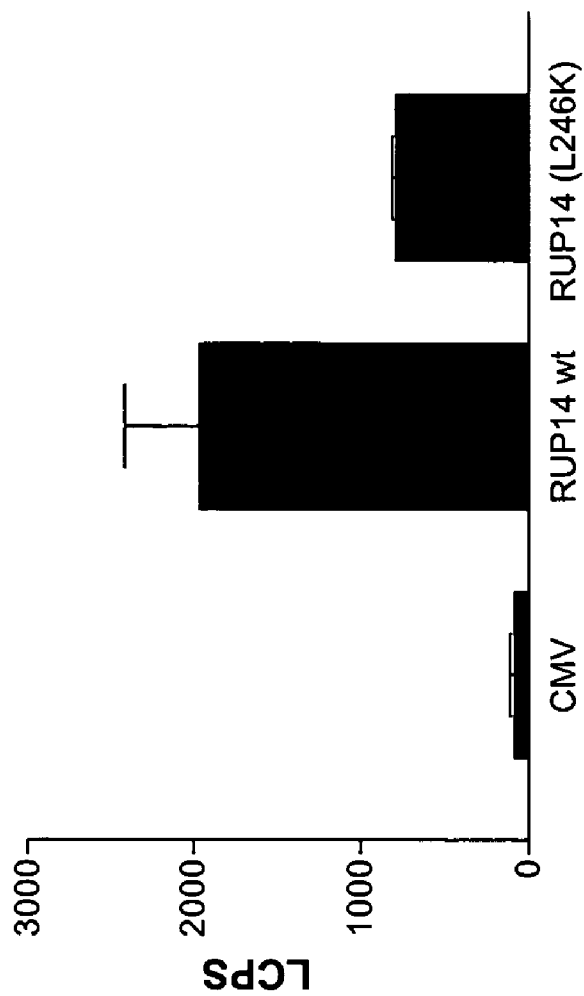


Figure 5

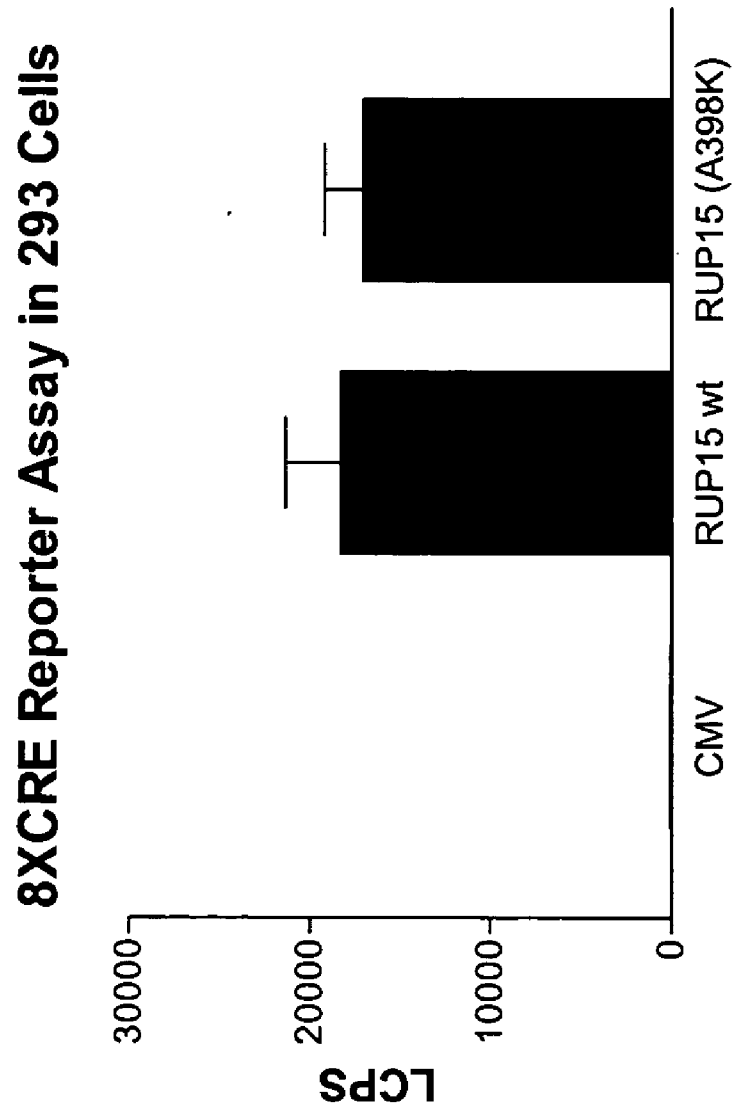


Figure 6

**cAMP Assay in 293 Cells**

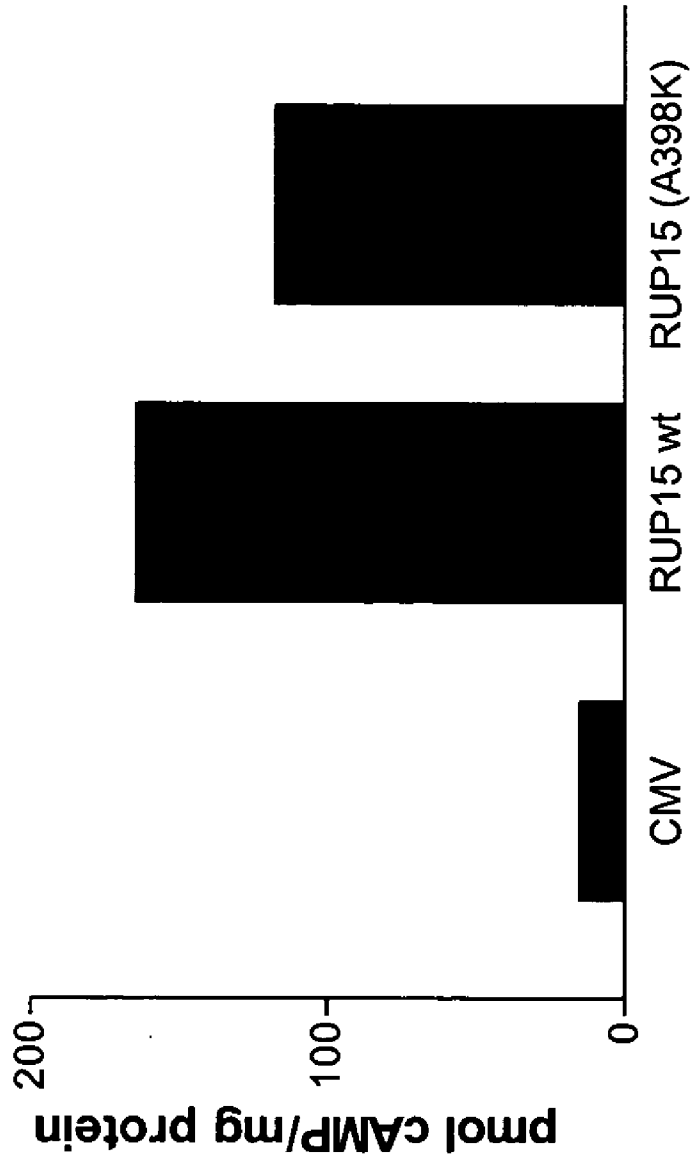


**Figure 7**

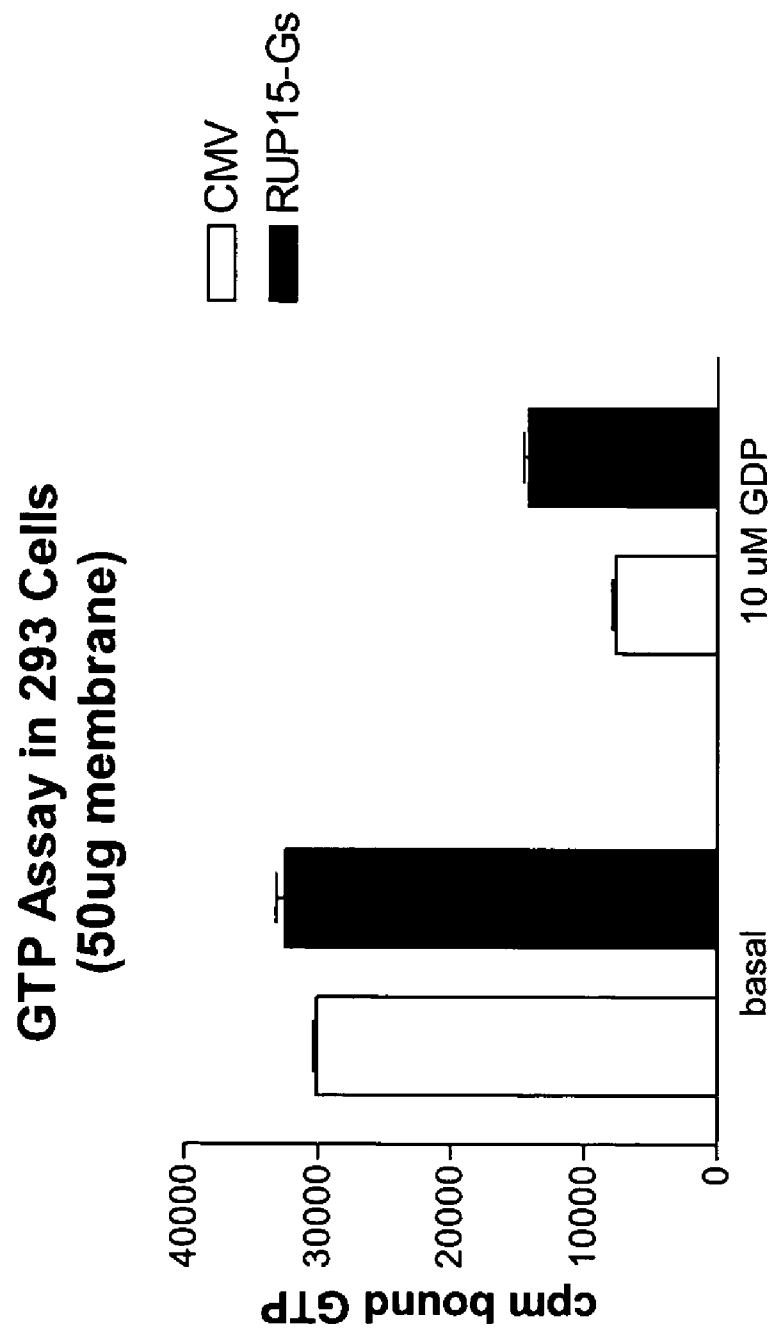


Figure 8

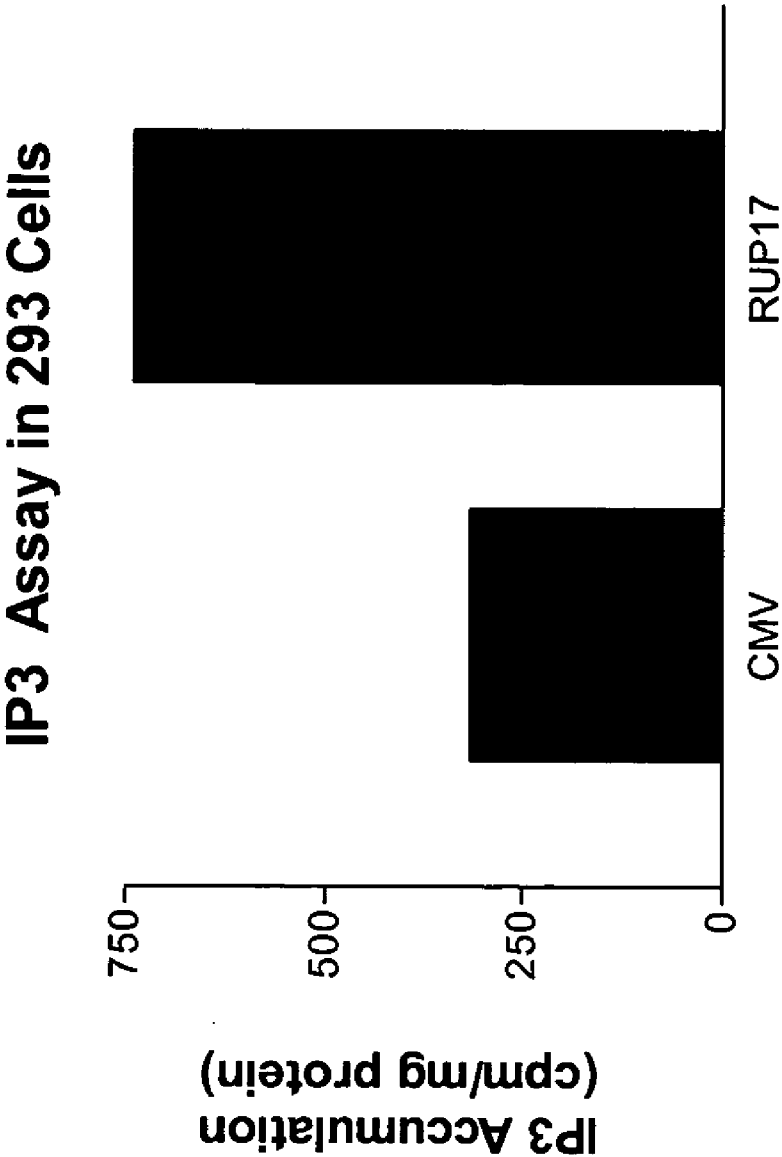


Figure 9

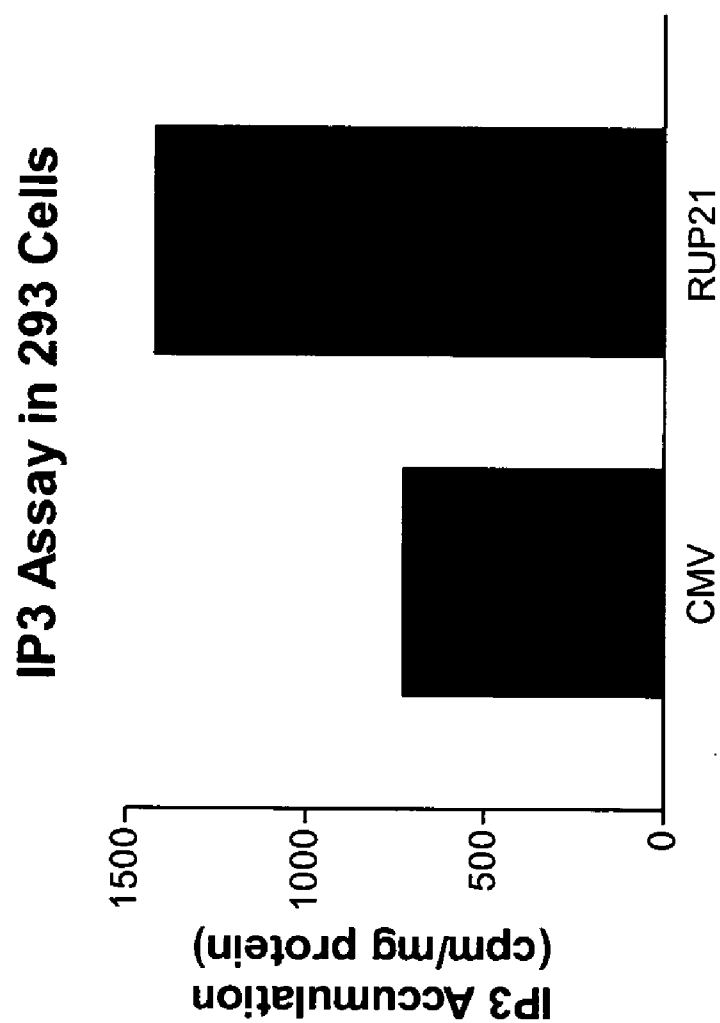


Figure 10

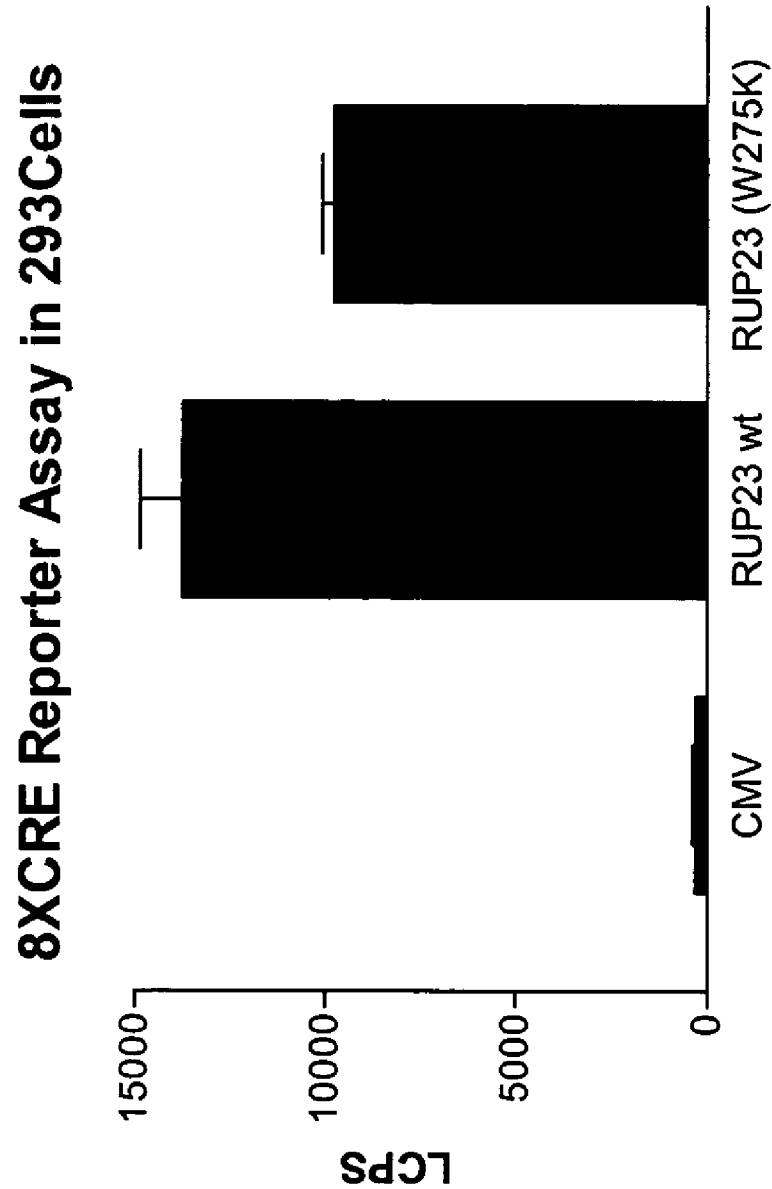


Figure 11

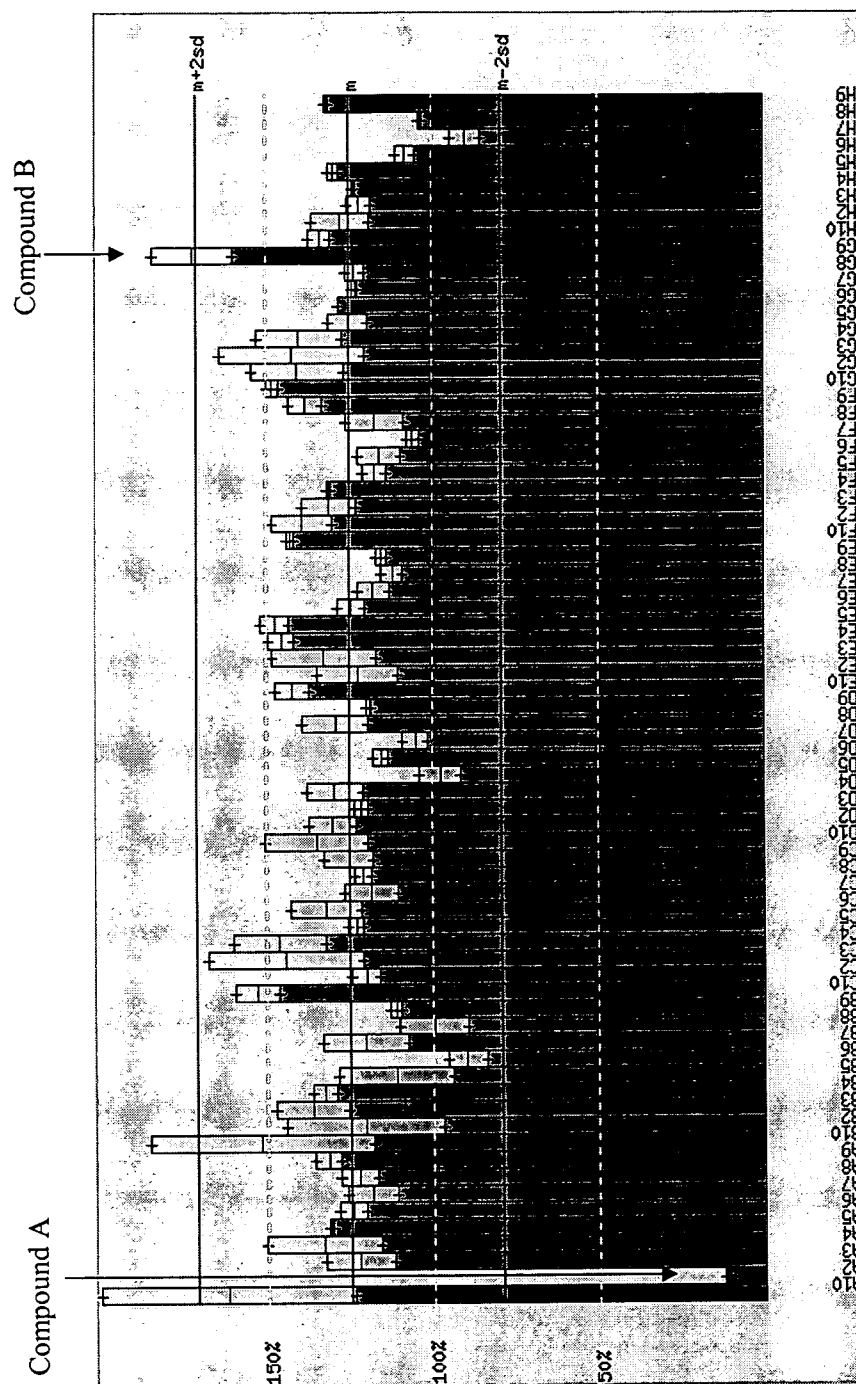
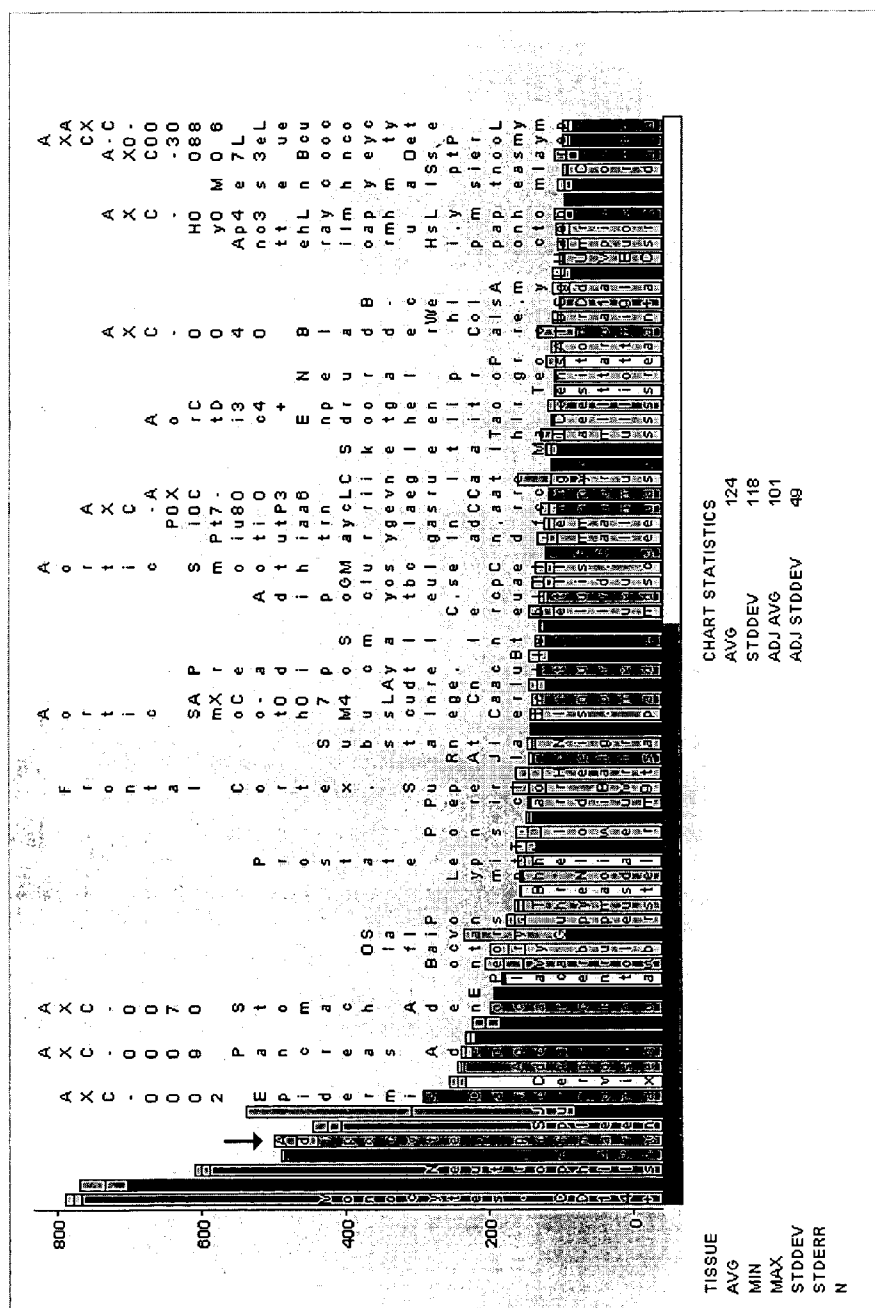


Figure 12



Figure 13B



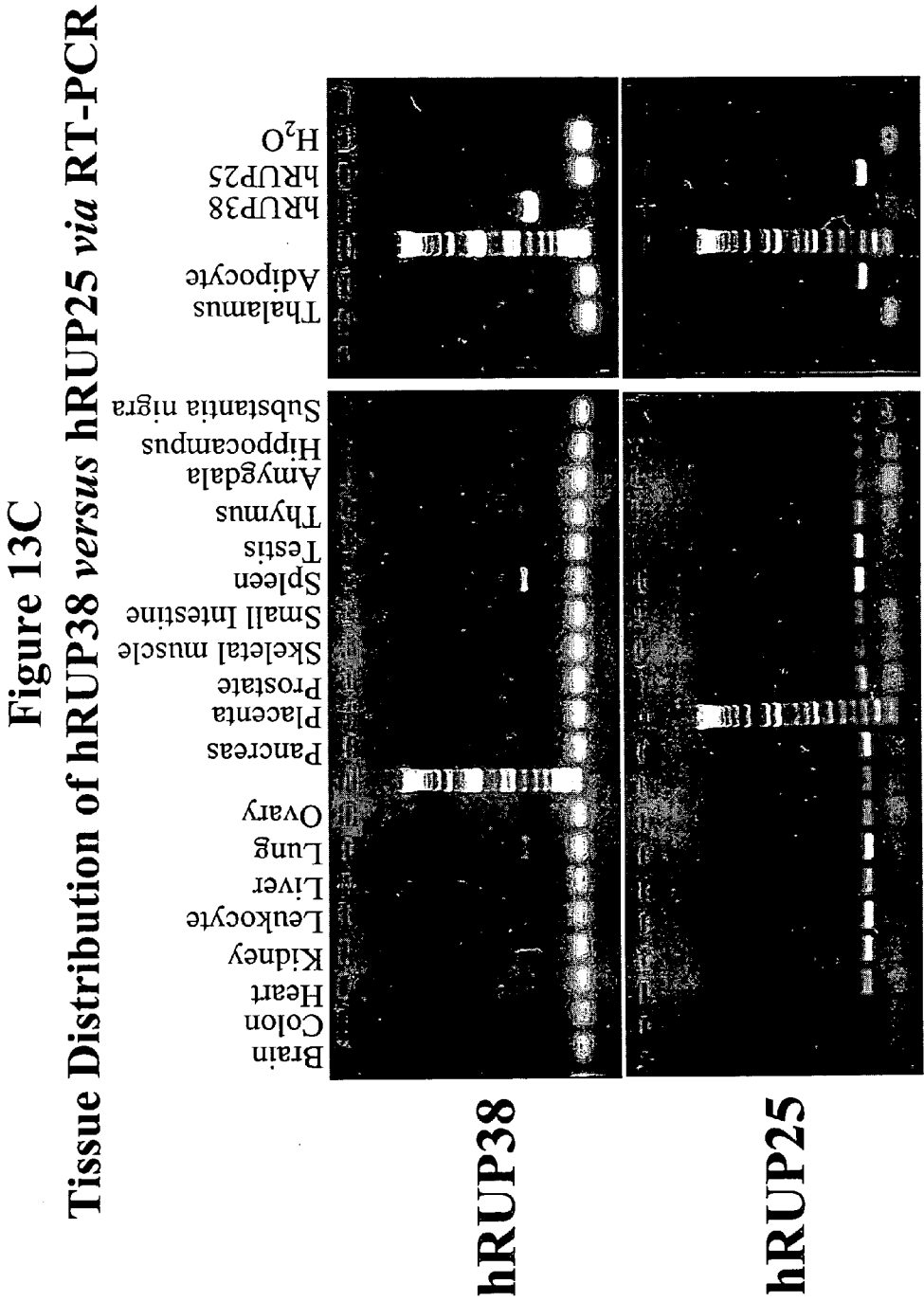


Figure 14A

hRUP25 G<sub>i</sub> - Coupled Constitutive Activity in  
Melanophore

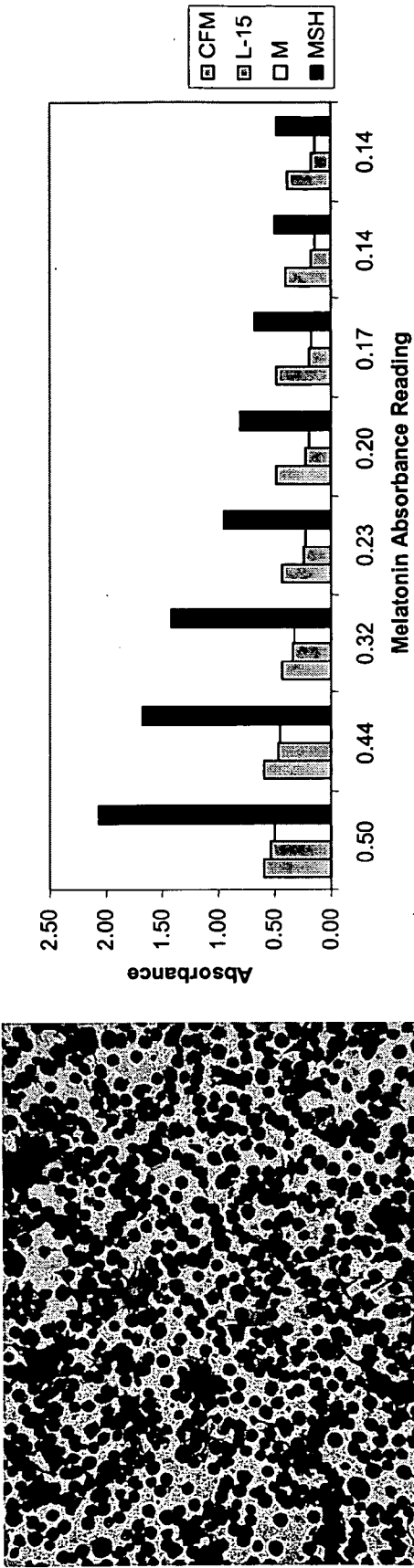


Figure 14B

hRUP38 G<sub>i</sub> - Coupled Constitutive Activity in  
Melanophore

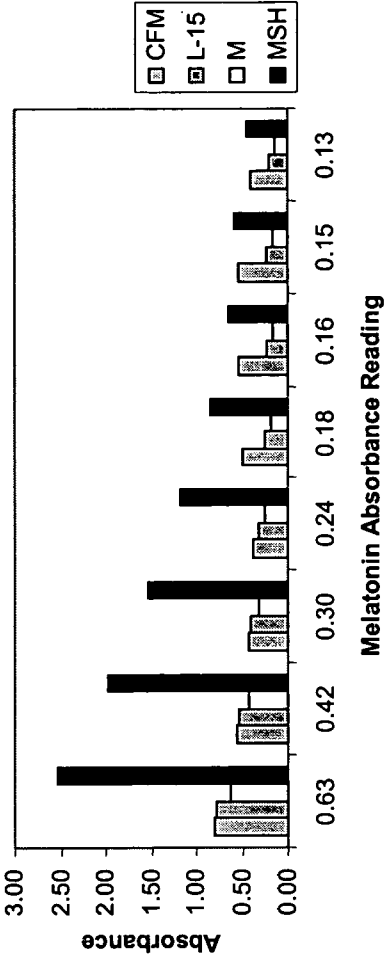
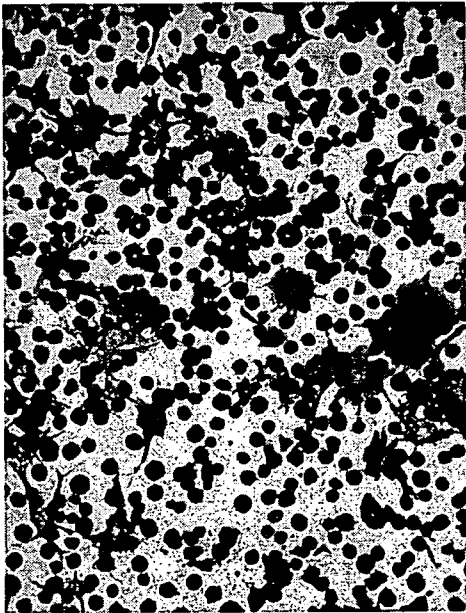


Figure 14C

hRUP19 G<sub>i</sub> - Coupled Constitutive Activity in  
Melanophore

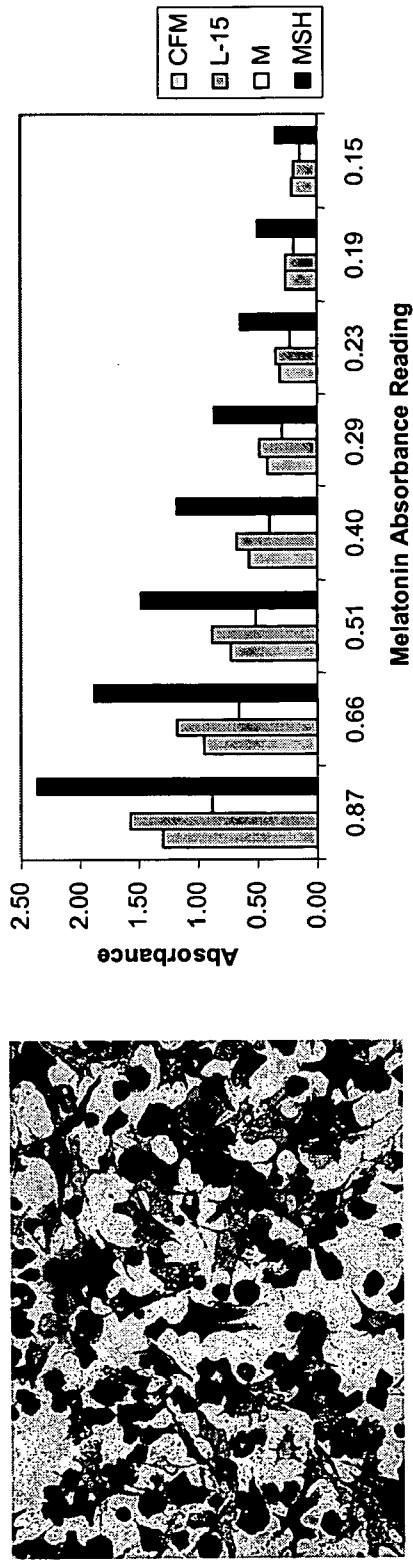


Figure 15A

**Action of Nicotinic Acid at RUP25  
Expressing Melanophores**

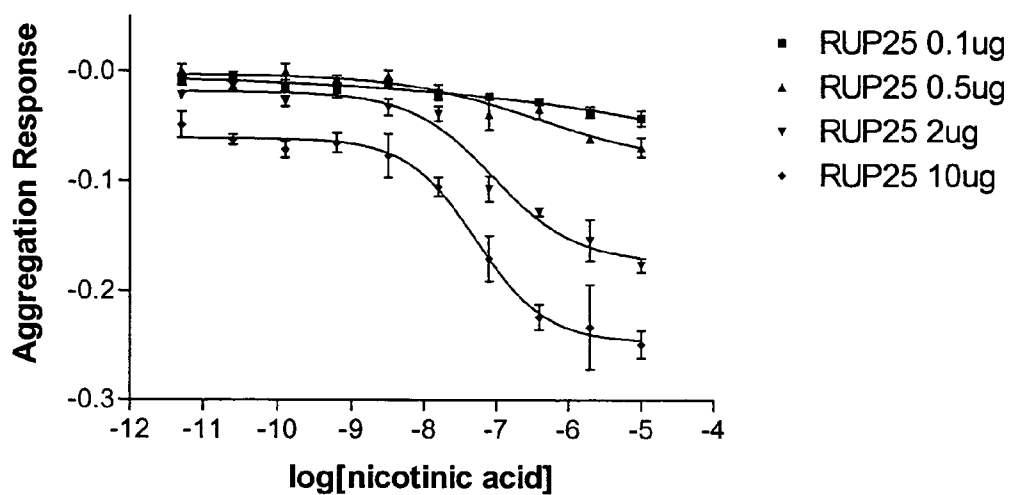


Figure 15B

**Nicotinic Acid Control Cells**

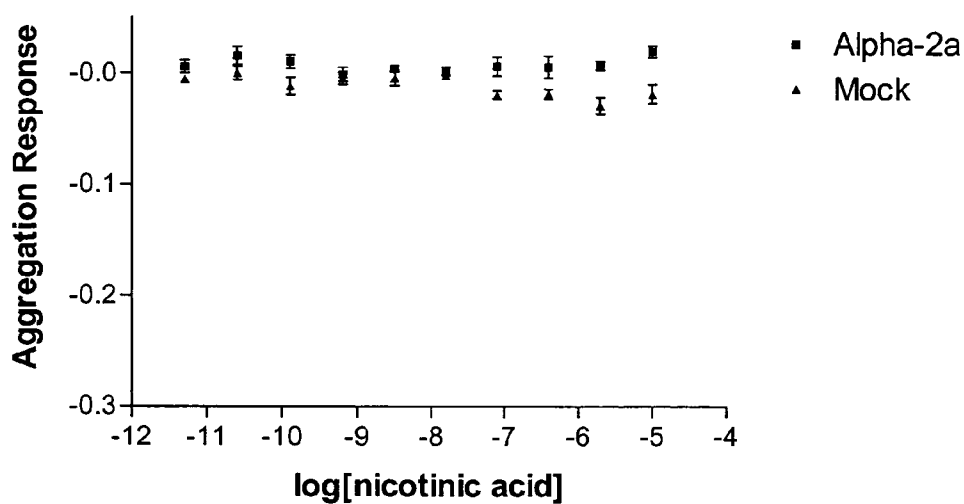


Figure 16

Nicotinic Acid Induced Inositol Phosphate Accumulation in 293 Cells Co-Expressing hRUP25 and GqΔGi

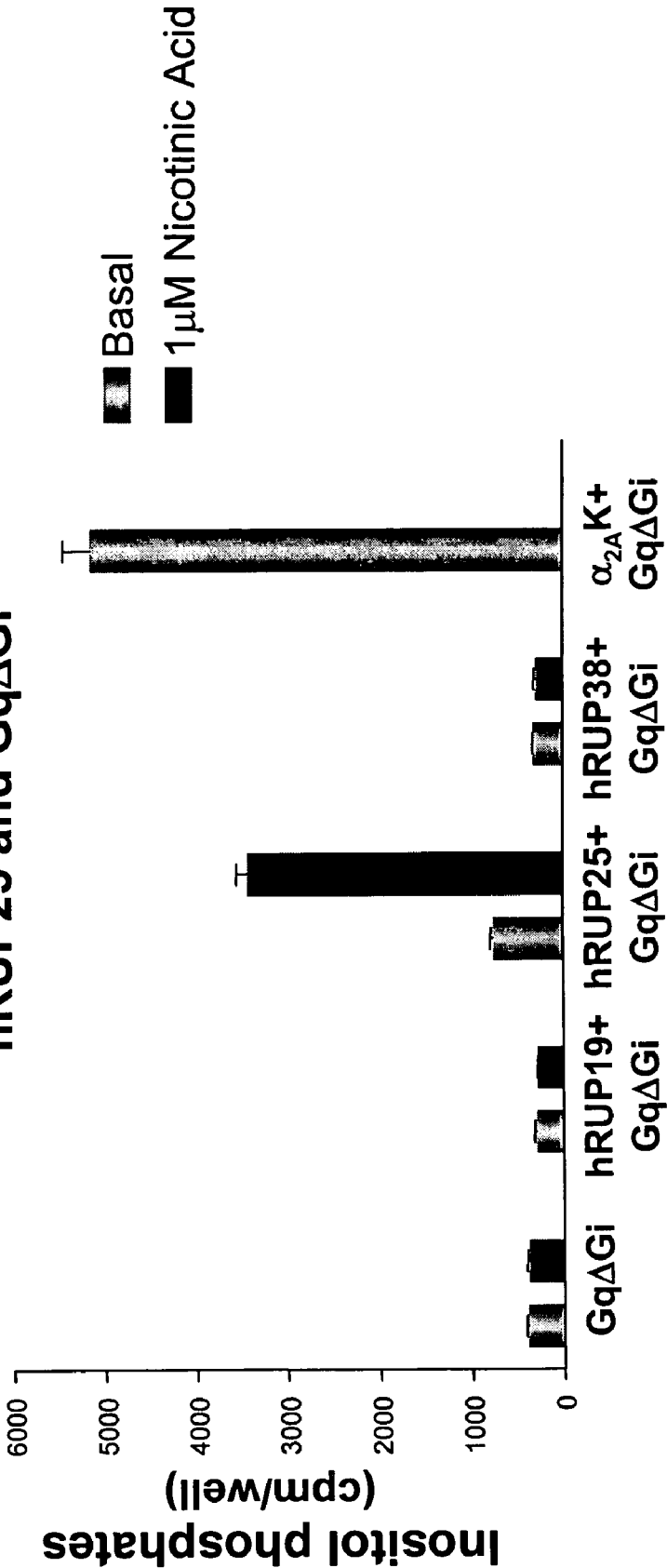


**Figure 17**  
**Saturation Binding of [<sup>3</sup>H]Nicotinic Acid to Membranes from Cells**  
**Expressing Either hRUP25, hRUP38, hRUP19 or Vector Alone**

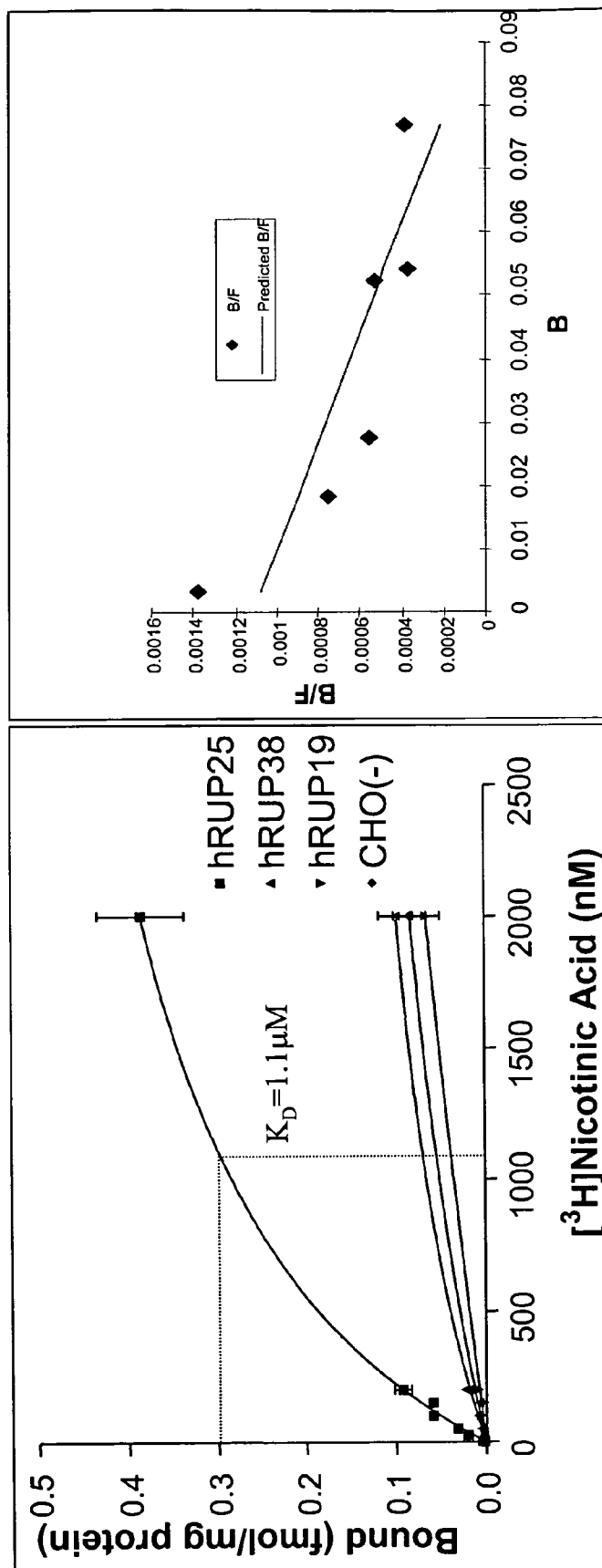
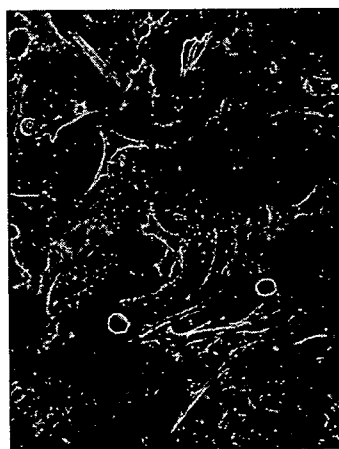


**Figure 18A**  
**hRUP25-CHO Stable Clone Identified by Anti-HA**  
**Immunofluorescence Staining**

Clone #46



Mock

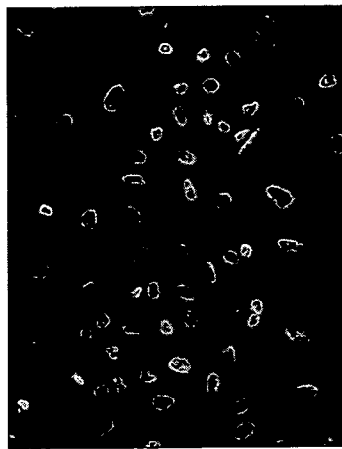
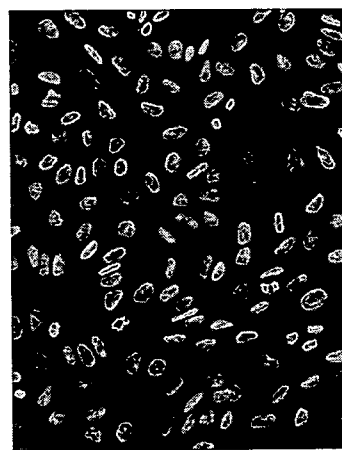


**Figure 18B**  
**Nicotinic Acid and Nicotine Induced Inhibition of**  
**Forskolin Stimulated cAMP Accumulation in**  
**hRUP25-CHO Stable Cell Line #46**

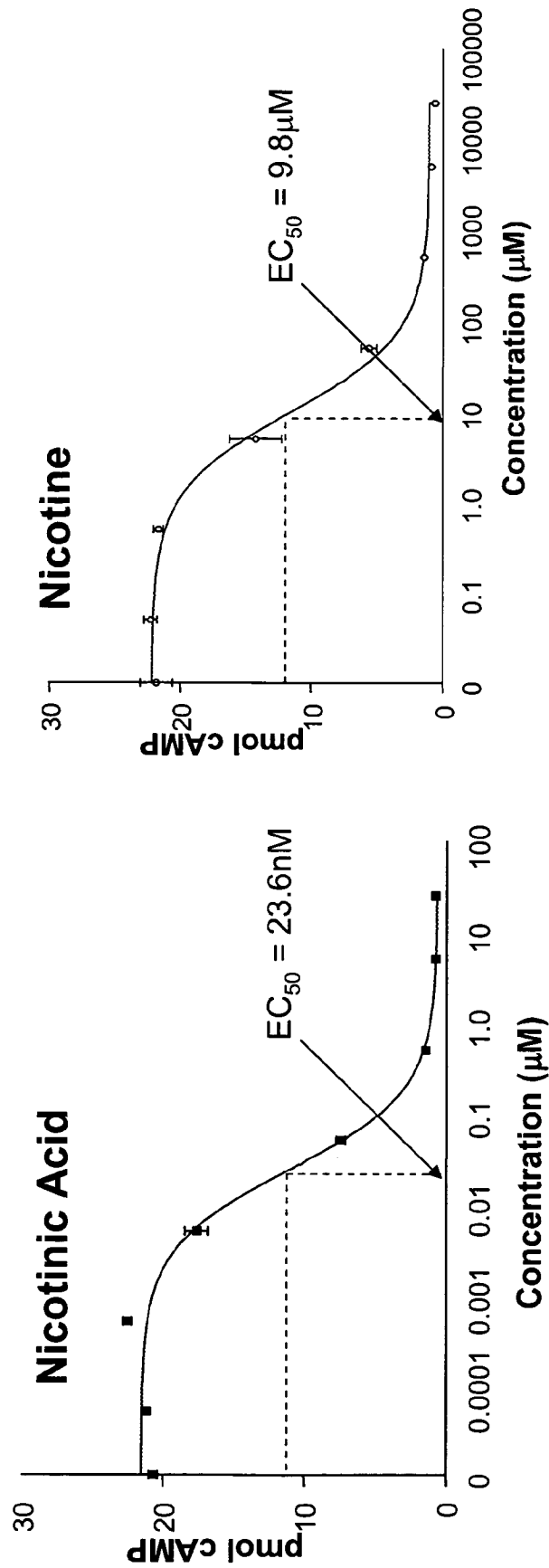


**Figure 19**  
**hRUP25 and mRUP25 Inhibit TSHR Induced cAMP**  
**Accumulation Following Activation by Nicotinic Acid**

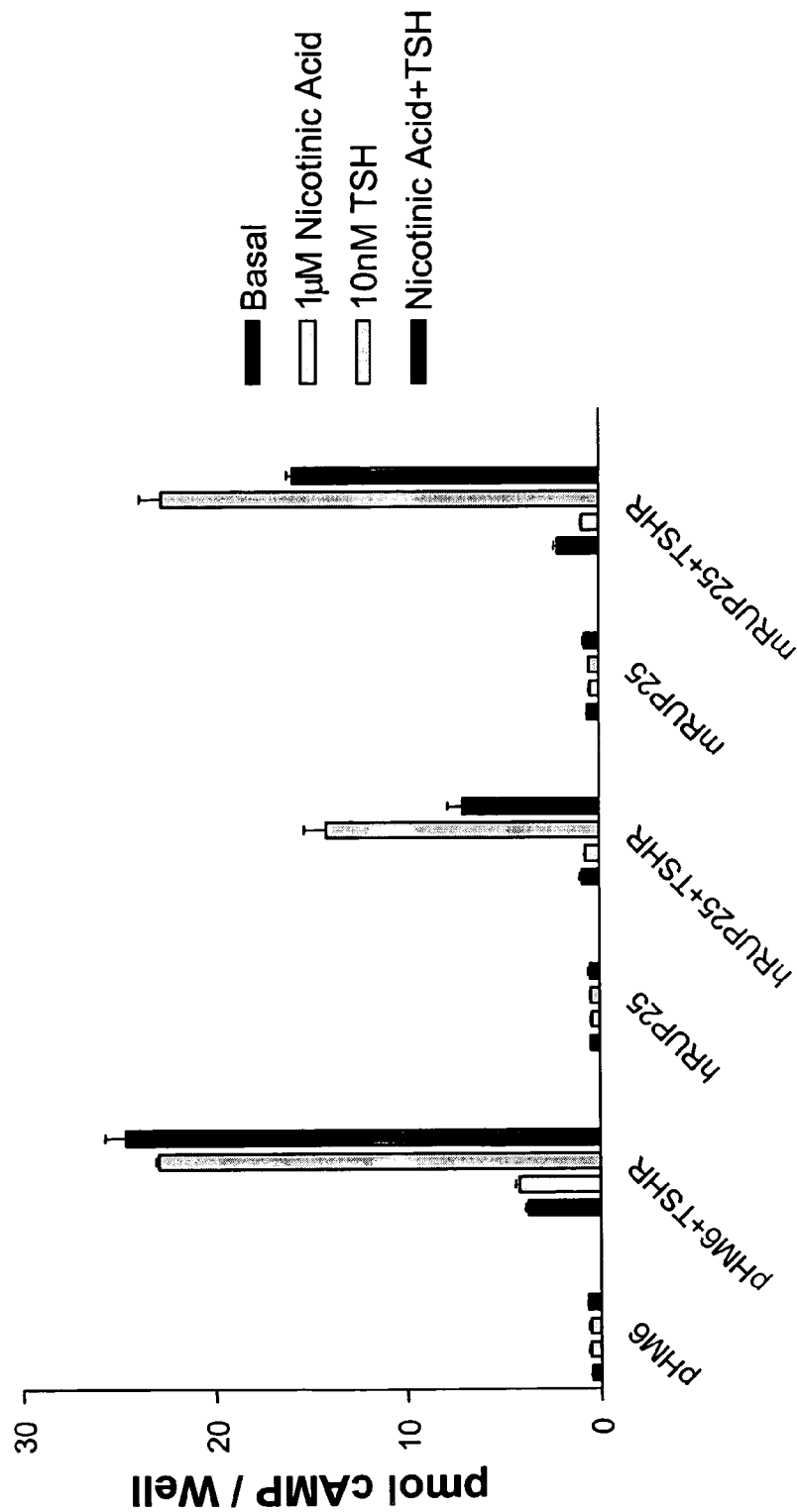


**Figure 20**  
**hRUP25 and mRUP25 Bind to Nicotinic Acid**  
**Specifically and with High Affinity**

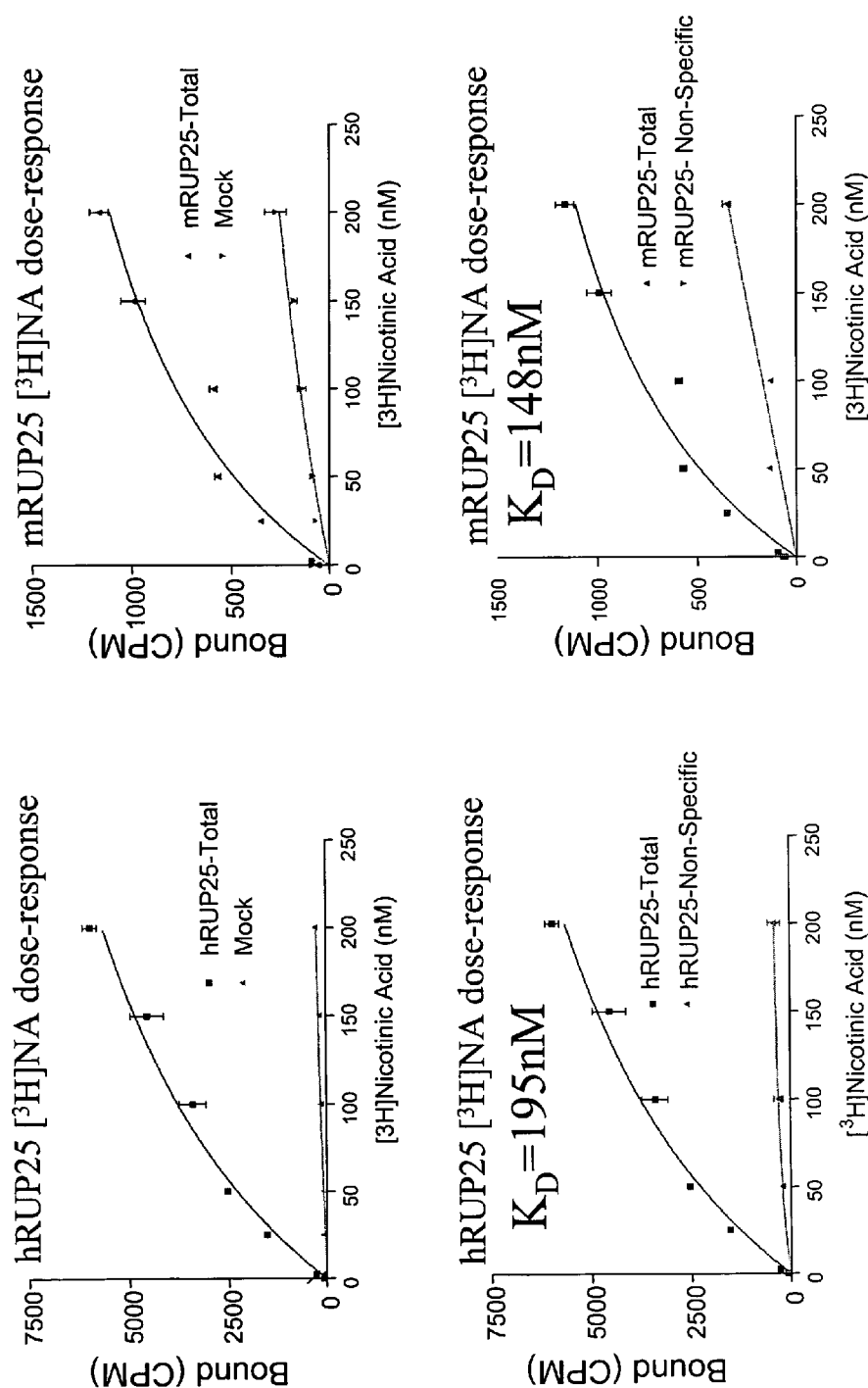


**Figure 21**  
**The Rank Order of Potency of Compounds on hRUP25 Closely Matches**  
**That of the Pharmacologically Defined Nicotinic Acid Receptor**

| Compound                           | EC 50 ( $\mu M$ ) |         |         |                           |
|------------------------------------|-------------------|---------|---------|---------------------------|
|                                    | Adipocytes*       | Spleen* | hRUP25† | hRUP25 (K <sub>d</sub> )‡ |
| Nicotinic acid                     | 1.42              | 0.703   | 0.04    | 0.14                      |
| Pyridazine-4-carboxylic acid       | 3.76              | 3.14    | N.D.    | 2.19                      |
| Acipimox                           | 10.3              | 6.56    | 2       | 2.68                      |
| 3-Pyridine-acetic acid             | 16.4              | 21.8    | 3       | 1.64                      |
| Pyrazine-2-carboxylic acid         | 26                | 22      | 4       | 4.14                      |
| 5-Methylnicotinic acid             | 30.2              | 30.0    | 7       | 3.58                      |
| 5-Methylpyrazine-2-carboxylic acid | 52.0              | 14.5    | 7       | 7.36                      |
| 6-Methylnicotinic acid             | 72.6              | 53.7    | 34      | 21.95                     |
| Nicotinic acid-1-oxide             | 80.4              | 73.7    | 120     | 55.25                     |
| 2-Hydroxynicotinic acid            | 132               | N.D.    | 130     | 145.4                     |
| Furane-3-carboxylic acid           | 142               | N.D.    | 110     | 130.6                     |
| Nicotinamide                       | >1000             | >1000   | >1000   | 128.2                     |
| N.D., not determined.              |                   |         |         |                           |

\* From Lorenzen, A. *et al. Mol. Pharmacol.* 59 (2):349-357, 2001.

† Arena data, inhibition of forskolin-induced cAMP production in hRUP25-CHO stable line #46.

‡ Arena data, [<sup>3</sup>H]nicotinic acid radioligand binding assay on membranes derived from hRUP25-CHO stable line #46.

**Figure 22A**  
**Nicotinic Acid and Related Compounds Inhibit**  
**Isoproterenol Induced Lipolysis in Rat Epididymal Fat**  
**Derived Adipocytes**

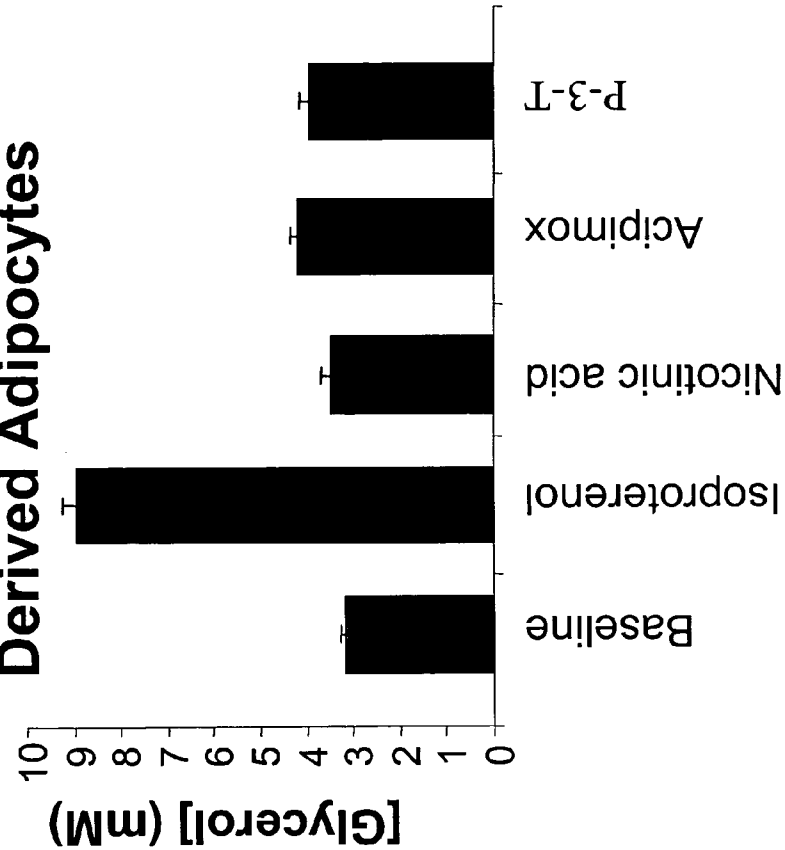


**Figure 22B**  
**Nicotinic Acid Dose-Dependent Inhibition of**  
**Isoproterenol Induced-Lipolysis in Rat Epididymal Fat**  
**Derived Adipocytes**

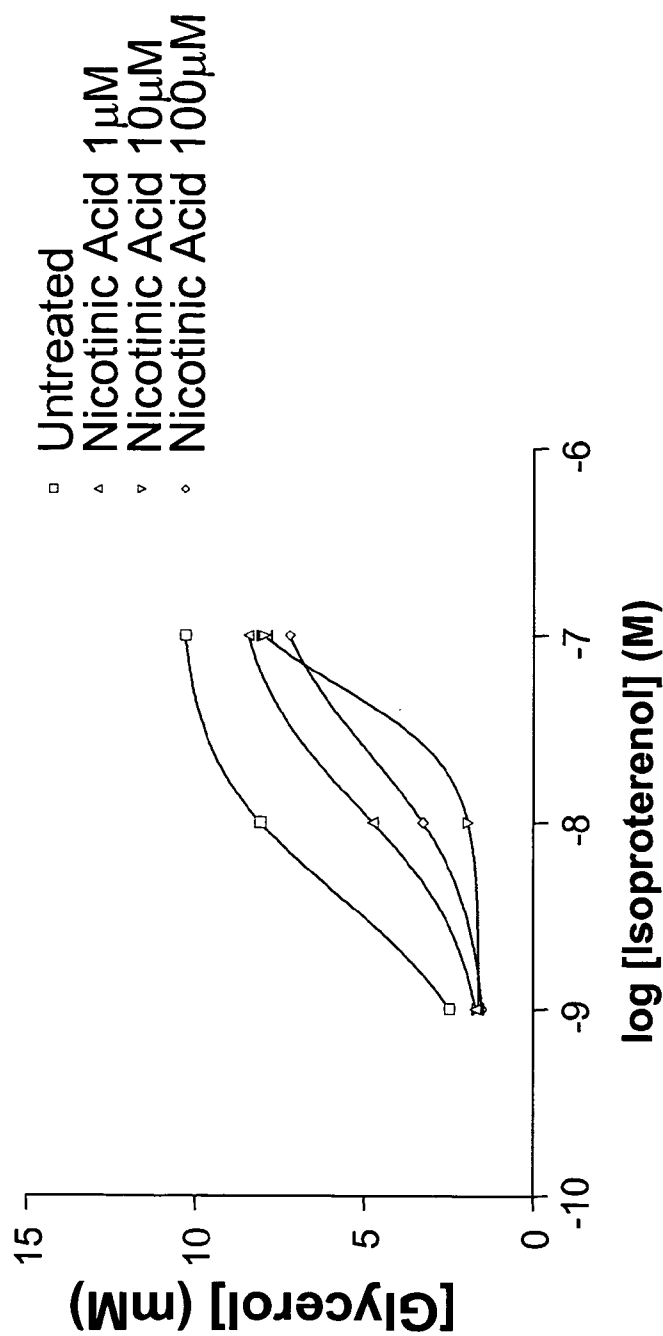


**Figure 23**  
**Dose-Dependent Inhibition of Isoproterenol Induced Lipolysis in Human Subcutaneous-Derived Primary Adipocytes by Nicotinic Acid and P-3-T**

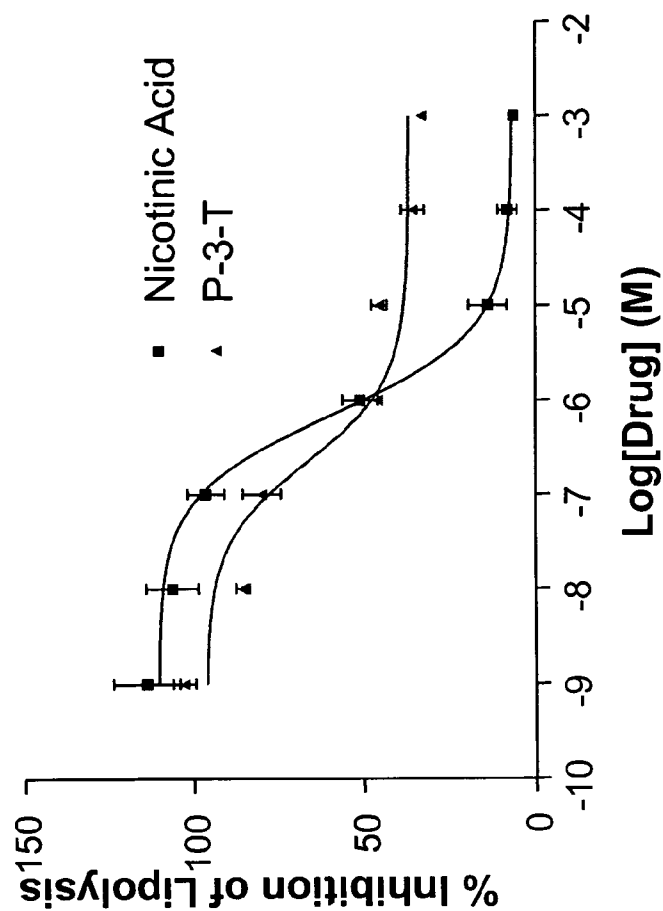


Figure 24

Screening Data for Nicotinic Acid and

1-Isopropyl-1H-Benzotriazole-5-Carboxylic Acid in cAMP Assays

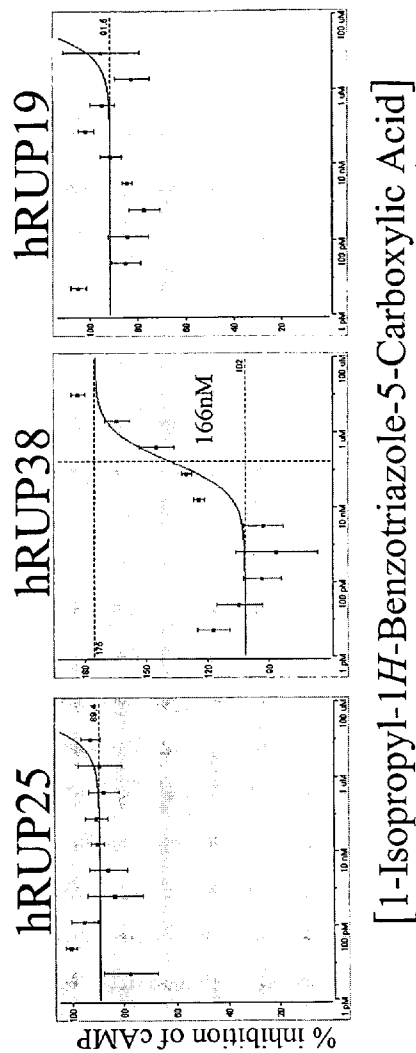
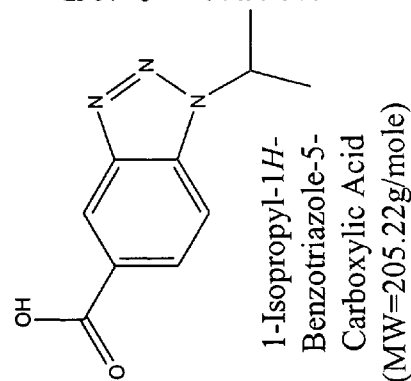
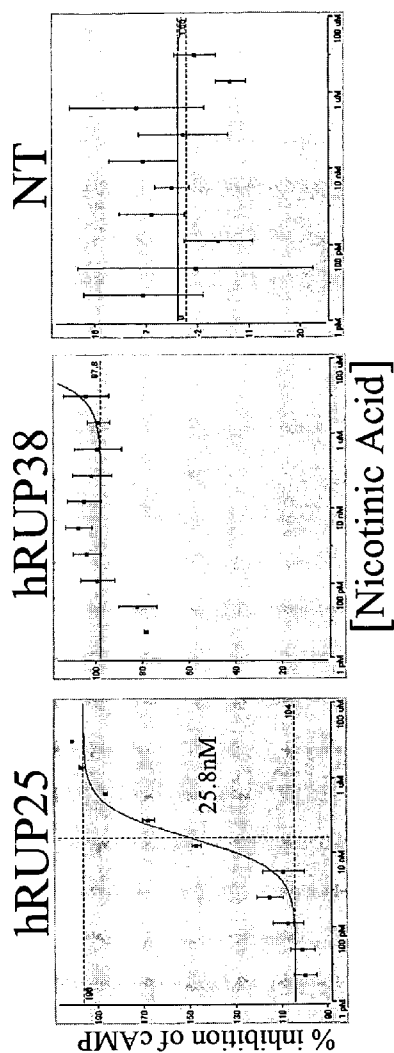
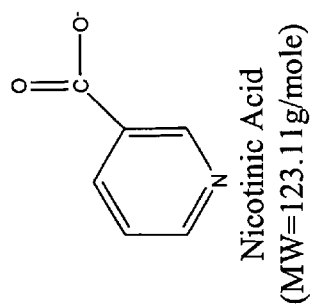


**Figure 25**  
**Inhibition of Isoproterenol Stimulated Lipolysis in**  
**Human Subcutaneous Adipocytes**

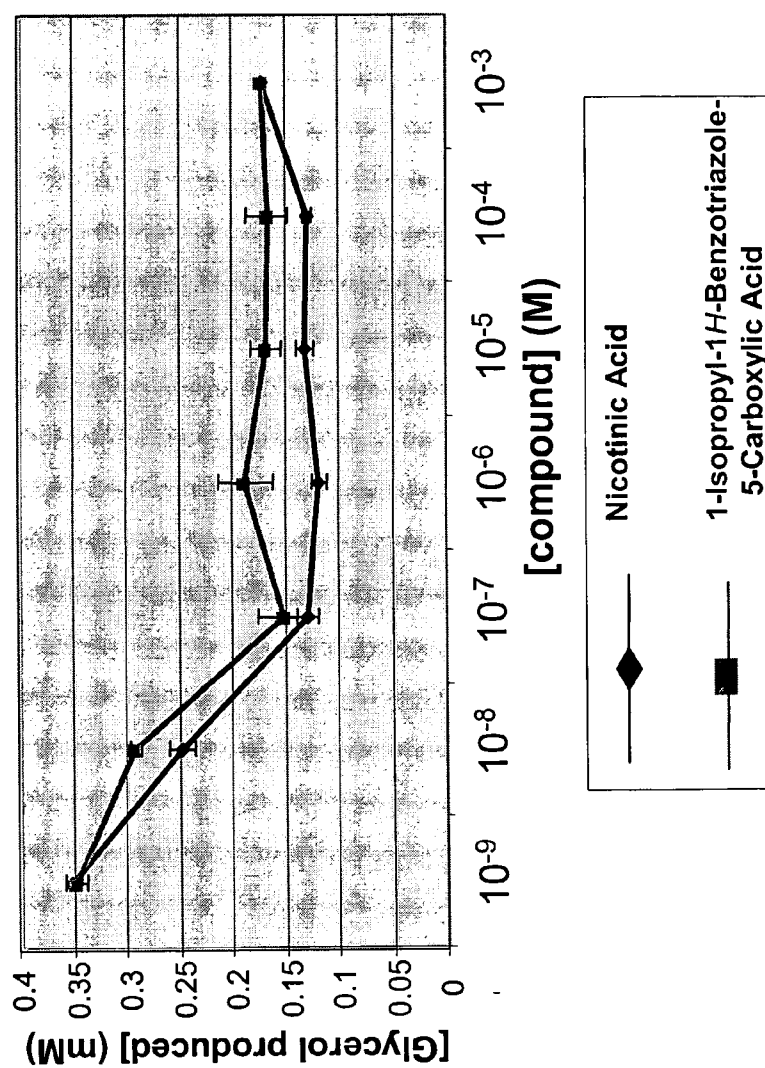


Figure 26

Inhibition of Forskolin Stimulated  
cAMP Accumulation in  
hRUP38-CHO Stable Cell Line by  
3-(5-Bromo-2-Ethoxy-Phenyl)-Acrylic  
Acid

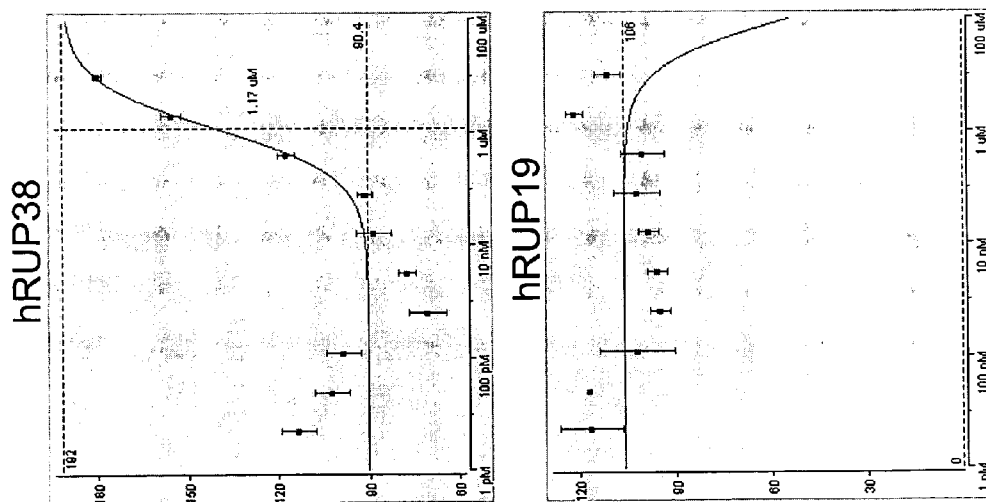


Figure 27

RT-PCR Indicates that hRUP19 is Selectively Expressed in Human Fat Cells

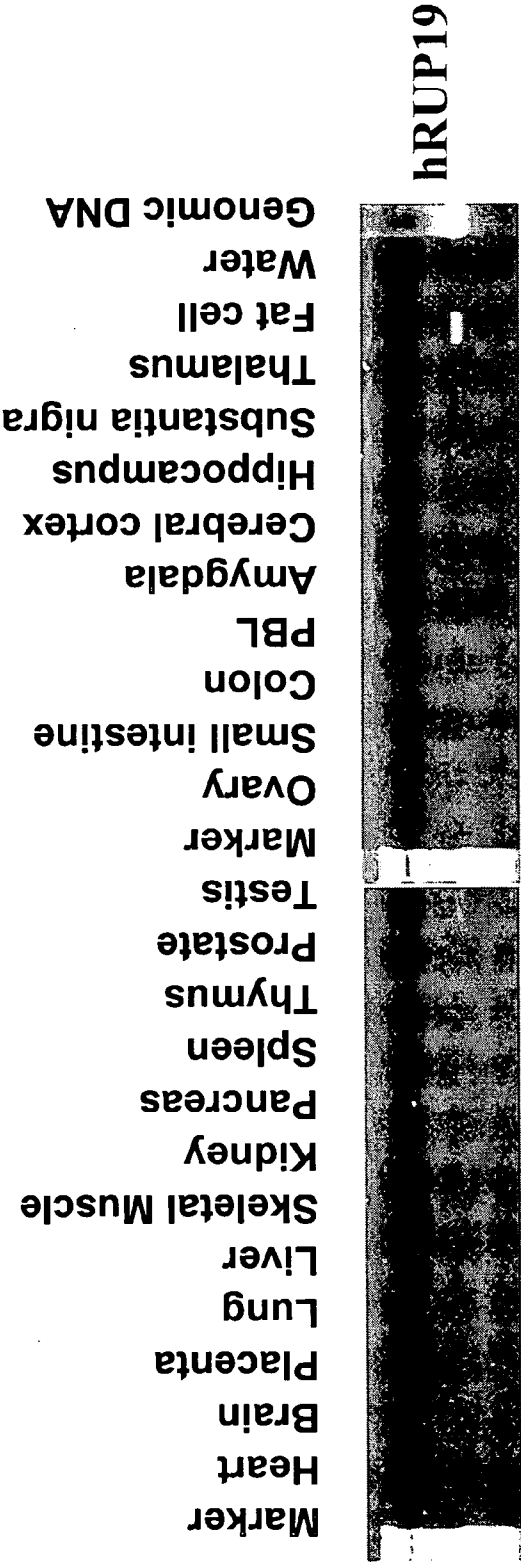
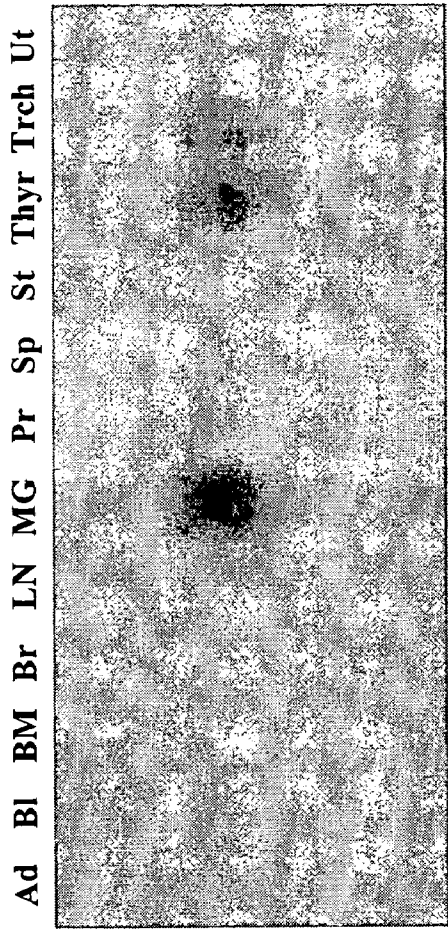


Figure 28

RNA Blot of hRUP19 Expression

in Selected Tissues



Strong mammary gland expression probably due to fat cell-specific expression of this receptor.

Figure 29

RUP19 Expression is Induced During Adipocyte Differentiation

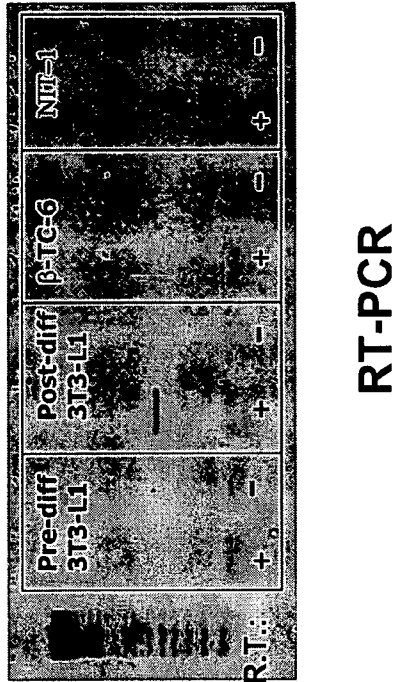
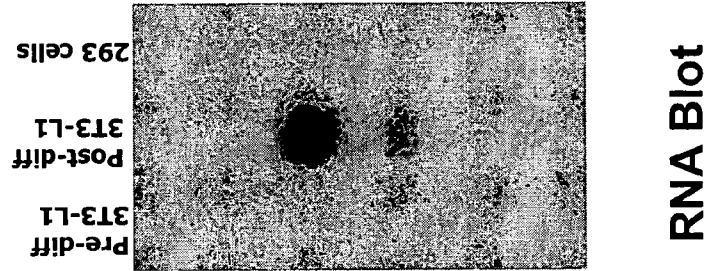


Figure 30

CART-Activated hRUP19 Inhibits cAMP  
Production in Membranes of Transfected 293  
Cells

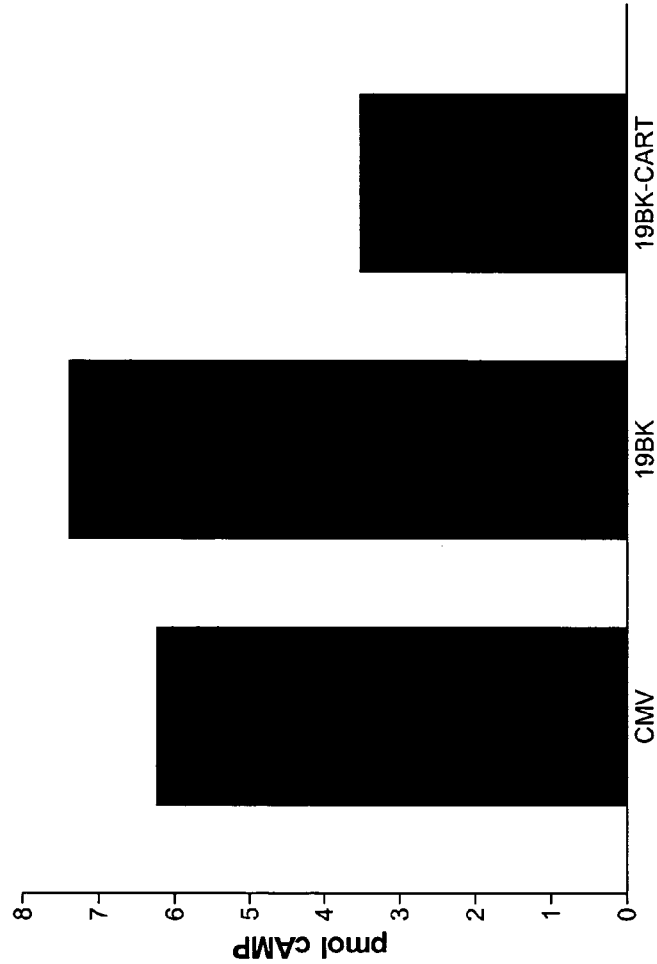
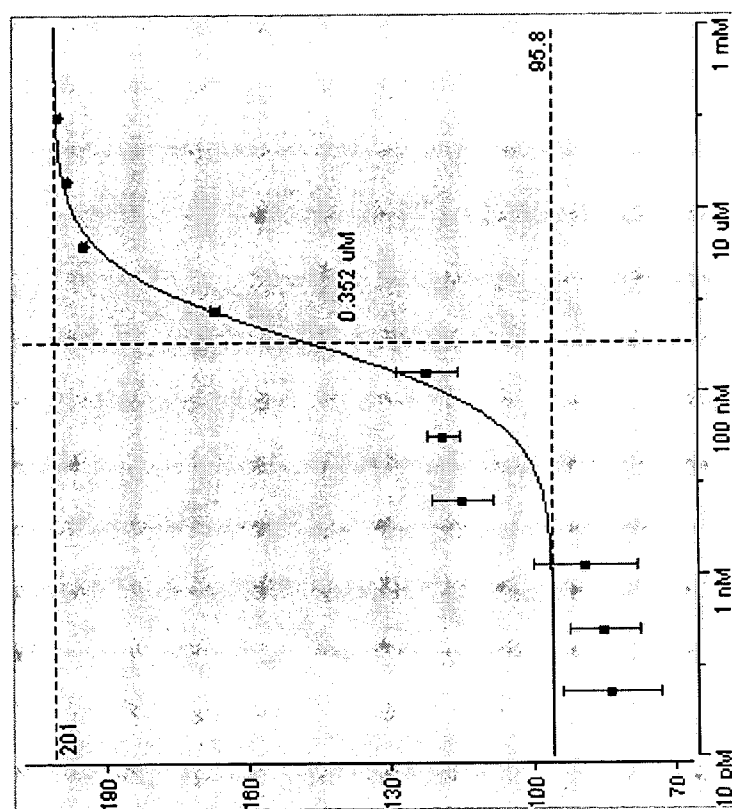


**Figure 31**  
**Inhibition of Forskolin Stimulated cAMP Accumulation**  
**in hRUP25-CHO Stable Cell Line by**  
**(5-Hydroxy-1-methyl-3-propyl-1H-pyrazol-4-yl)-pyridin-3-yl-methanone**



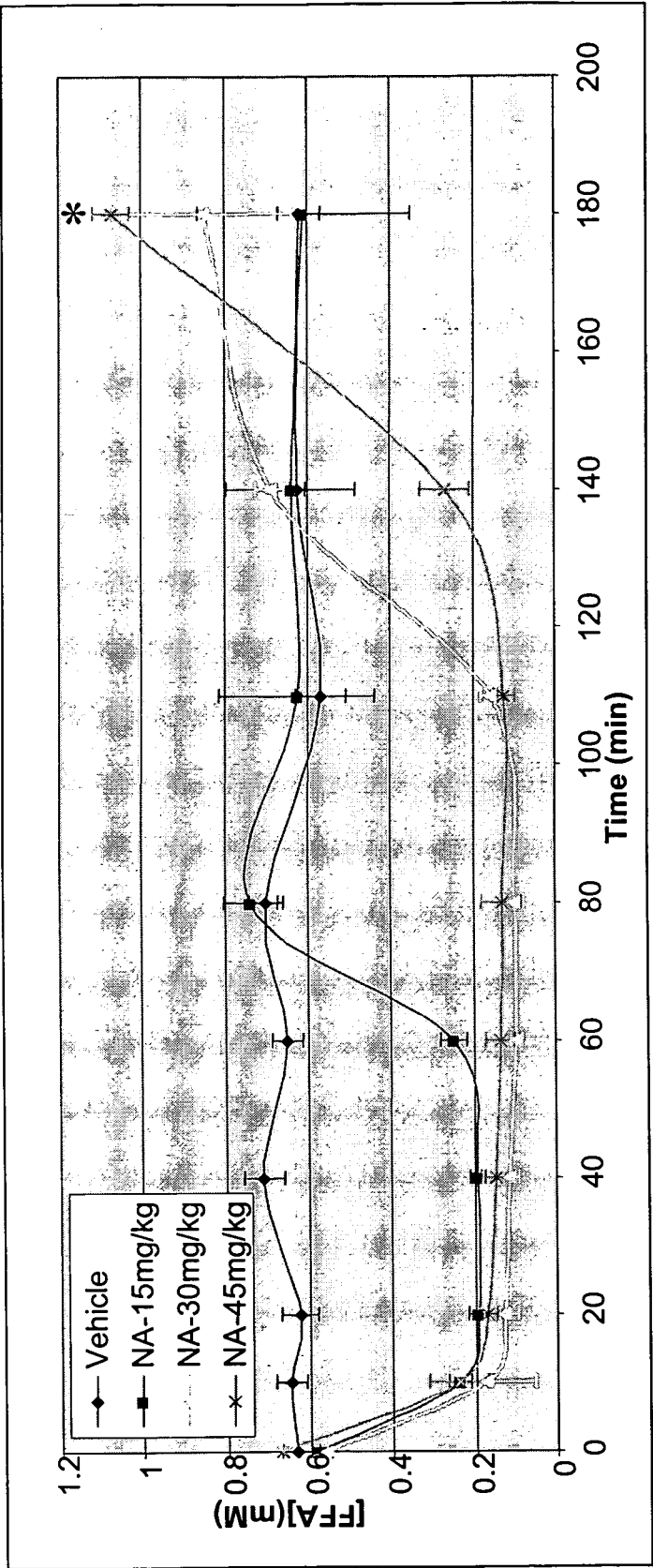
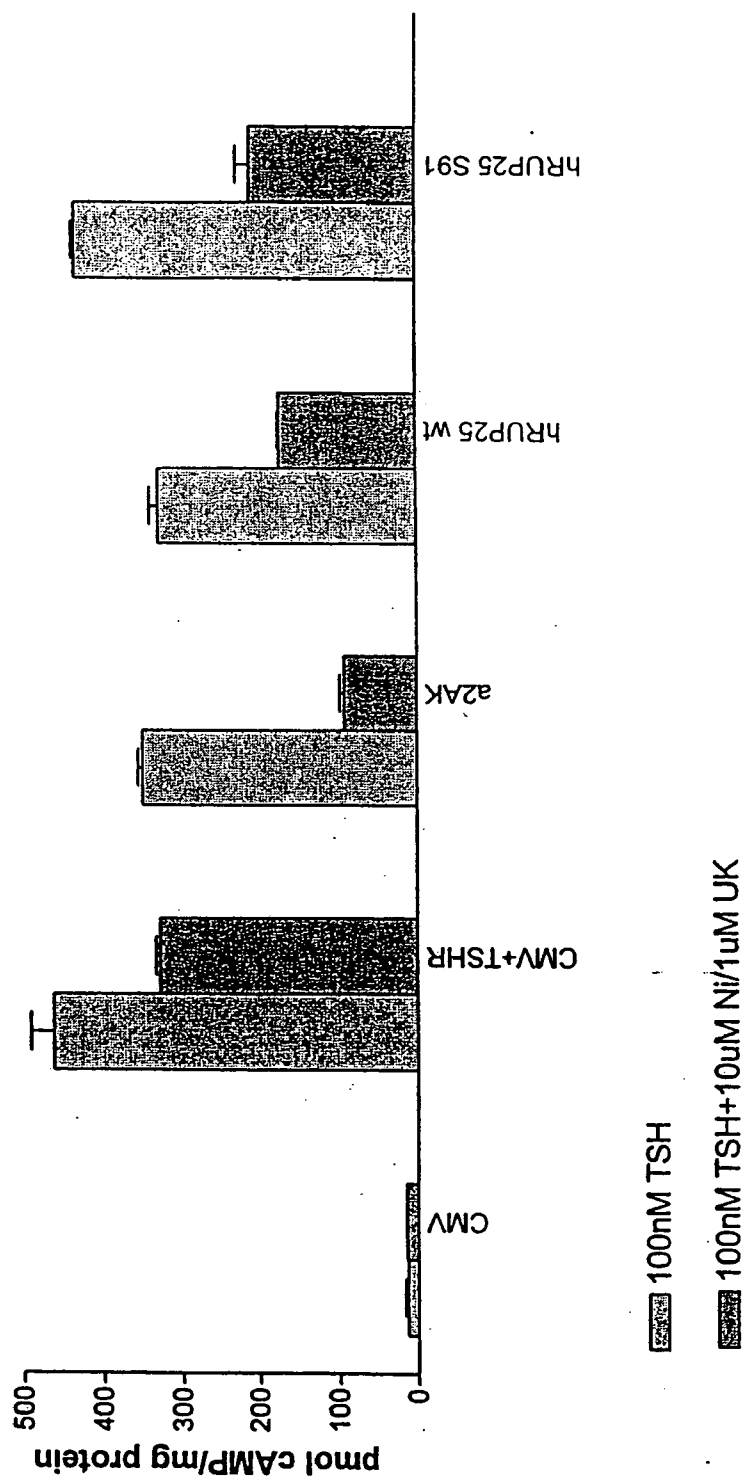


**Figure 32**  
**Measurement of Plasma Free Fatty Acids (FFA) in Rats Administered Niacin**

**Figure 33**  
**Cyclase Assay on 293 Cells**  
**TSHR Co-Transfection with hRUP25 S91**



## HUMAN G PROTEIN-COUPLED RECEPTORS AND MODULATORS THEREOF FOR THE TREATMENT OF METABOLIC-RELATED DISORDERS

[0001] The present application is a Continuation of U.S. Utility patent application Ser. No. 10/314,048, filed Dec. 6, 2002, which is a Continuation-In-Part of U.S. Utility patent application Ser. No. 10/096,511, filed Mar. 12, 2002, (now abandoned), which is a Continuation of U.S. Utility patent application Ser. No. 09/995,543, filed Nov. 27, 2001 (now abandoned) and claims benefit of priority to U.S. Provisional Patent Application Ser. No. 60/399,917, filed Jul. 29, 2002, Ser. No. 60/404,761, filed Aug. 19, 2002 and Ser. No. 60/410,747, filed Sep. 13, 2002, the disclosure of each of which is hereby incorporated by reference in its entirety.

### FIELD OF THE INVENTION

[0002] The present invention relates to methods of identifying whether a candidate compound is a modulator of a G protein-coupled receptor (GPCR). In preferred embodiments, the GPCR is human. In other preferred embodiments, the GPCR is coupled to  $G_i$  and lowers the level of intracellular cAMP. In other preferred embodiments, the GPCR is expressed endogenously by adipocytes. In further preferred embodiments, the GPCR inhibits intracellular lipolysis. In other further preferred embodiments, the GPCR is a nicotinic acid receptor. The present invention also relates to methods of using a modulator of said GPCR. Preferred modulator is agonist. Agonists of the invention are useful as therapeutic agents for the prevention or treatment of metabolic-related disorders, including dyslipidemia, atherosclerosis, coronary heart disease, stroke, insulin resistance and type 2 diabetes.

### BACKGROUND OF THE INVENTION

[0003] A. Nicotinic Acid as an Antilipolytic Agent

[0004] Atherosclerosis and stroke are the numbers one and number three leading causes of death of both men and women in the United States. [See, e.g., *Nature Medicine*, Special Focus on Atherosclerosis, (2002) 8:1209-1262; the disclosure of which is hereby incorporated by reference in its entirety.] Type 2 diabetes is a public health problem that is serious, widespread and increasing [Brownlee M, *Nature* (2001) 414:813-20 and references therein; Zimmet P et al., *Nature* (2001) 414:782-7 and references therein; Sirtell AR et al., *Nature* (2001) 414:799-806 and references therein; the disclosure of each of which is hereby incorporated by reference in its entirety]. Elevated levels of low density lipoprotein (LDL) cholesterol or low levels of high density lipoprotein (HDL) cholesterol are, independently, risk factors for atherosclerosis and associated cardiovascular pathologies. In addition, high levels of plasma free fatty acids are associated with insulin resistance and type 2 diabetes. One strategy for decreasing LDL-cholesterol, increasing HDL-cholesterol, and decreasing plasma free fatty acids is to inhibit lipolysis in adipose tissue. This approach involves regulation of hormone sensitive lipase, which is the rate-limiting enzyme in lipolysis. Lipolytic agents increase cellular levels of cAMP, which leads to activation of hormone sensitive lipase within adipocytes. Agents that lower intracellular cAMP levels, by contrast, would be antilipolytic.

[0005] It is also worth noting in passing that an increase in cellular levels of cAMP down-regulates the secretion of

adiponectin from adipocytes [Delporte, M L et al. *Biochem J* (2002) 367:677-85; the disclosure of which is incorporated by reference in its entirety]. Reduced levels of plasma adiponectin have been associated with metabolic-related disorders, including atherosclerosis, coronary heart disease, stroke, insulin resistance and type 2 diabetes [Matsuda, M et al. *J Biol Chem* (2002) 277:37487-91 and reviewed therein; the disclosure of which is hereby incorporated by reference in its entirety]. [Also see: Yamauchi T et al., *Nat Med* (2002) 8:1288-95; and Tomas E et al., *Proc Natl Acad Sci USA* (2002) November 27; the disclosure of each of which is hereby incorporated by reference in its entirety.] Globular adiponectin protected ob/ob mice from diabetes and apoE deficient mice from atherosclerosis [Yamauchi, T et al. *J Biol Chem* (2002) November; the disclosure of which is hereby incorporated by reference in its entirety]. [Also see Okamoto, Y et al. *Circulation* (2002) 26:2767-70; the disclosure of which is hereby incorporated by reference in its entirety.] There is evidence that the regulation of human serum adiponectin levels through modulation of adipocyte intracellular cAMP level is independent of adipocyte lipolysis [Staiger H et al., *Horm Metab Res* (2002) 34:601-3; the disclosure of which is hereby incorporated by reference in its entirety].

[0006] Nicotinic acid (niacin, pyridine-3-carboxylic acid) is a water-soluble vitamin required by the human body for health, growth and reproduction; a part of the Vitamin B complex. Nicotinic acid is also one of the oldest used drugs for the treatment of dyslipidemia. It is a valuable drug in that it favorably affects virtually all of the lipid parameters listed above [Goodman and Gilman's *Pharmacological Basis of Therapeutics*, editors Harmon J G and Limbird L E, Chapter 36, Mahley R W and Bersot T P (2001) pages 971-1002]. The benefits of nicotinic acid in the treatment or prevention of atherosclerotic cardiovascular disease have been documented in six major clinical trials [Guyton J R (1998) *Am J Cardiol* 82:18U-23U]. Structure and synthesis of analogs or derivatives of nicotinic acid are discussed throughout the Merck Index, *An Encyclopedia of Chemicals, Drugs, and Biologicals*, Tenth Edition (1983), which is incorporated herein by reference in its entirety.

[0007] Nicotinic acid and currently existing analogs thereof inhibit the production and release of free fatty acids from adipose tissue, likely via an inhibition of adenylyl cyclase, a decrease in intracellular cAMP levels, and a concomitant decrease in hormone sensitive lipase activity. Agonists that down-regulate hormone sensitive lipase activity leading to a decrease in plasma free fatty acid levels are likely to have therapeutic value. The consequence of decreasing plasma free fatty acids is two-fold. First, it will ultimately lower LDL-cholesterol and raise HDL-cholesterol levels, independent risk factors, thereby reducing the risk of mortality due to cardiovascular incidence subsequent to atheroma formation. Second, it will provide an increase in insulin sensitivity in individuals with insulin resistance or type 2 diabetes. Unfortunately, the use of nicotinic acid as a therapeutic is partially limited by a number of associated, adverse side-effects. These include flushing, free fatty acid rebound, and liver toxicity.

[0008] Agonists of antilipolytic GPCRs having limited tissue distribution beyond adipose may be especially valuable in view of the diminished opportunity for potentially undesirable side-effects.

[0009] The rational development of novel, nicotinic acid receptor agonists that have fewer side-effects is an area of active investigation, but to date it has been hindered by the inability to molecularly identify the nicotinic acid receptor. Recent work suggests that nicotinic acid may act through a specific GPCR [Lorenzen A, et al. (2001) *Molecular Pharmacology* 59:349-357 and reviewed therein; the disclosure of which is hereby incorporated by reference in its entirety]. Furthermore, it is important to consider that other receptors of the same class may exist on the surface of adipocytes and similarly decrease hormone sensitive lipase activity through a reduction in the level of intracellular cAMP but without the elicitation of adverse effects such as flushing, thereby representing promising novel therapeutic targets.

#### [0010] B. G Protein-Coupled Receptors

[0011] Although a number of receptor classes exist in humans, by far the most abundant and therapeutically relevant is represented by the G protein-coupled receptor (GPCR) class. It is estimated that there are some 30,000-40,000 genes within the human genome, and of these, approximately 2% are estimated to code for GPCRs. Receptors, including GPCRs, for which the endogenous ligand has been identified, are referred to as "known" receptors, while receptors for which the endogenous ligand has not been identified are referred to as "orphan" receptors.

[0012] GPCRs represent an important area for the development of pharmaceutical products: from approximately 20 of the 100 known GPCRs, approximately 60% of all prescription pharmaceuticals have been developed. For example, in 1999, of the top 100 brand name prescription drugs, the following drugs interact with GPCRs (the primary diseases and/or disorders treated related to the drug is indicated in parentheses):

|                                  |                             |                                   |
|----------------------------------|-----------------------------|-----------------------------------|
| Claritin ® (allergies)           | Prozac ® (depression)       | Vasotec ® (hypertension)          |
| Paxil ® (depression)             | Zoloft ® (depression)       | Zyprexa ® (psychotic disorder)    |
| Cozaar ® (hypertension)          | Imitrex ® (migraine)        | Zantac ® (reflux)                 |
| Propulsid ® (reflux disease)     | Risperdal ® (schizophrenia) | Serevent ® (asthma)               |
| Pepcid ® (reflux)                | Gaster ® (ulcers)           | Atrovent ® (bronchospasm)         |
| Effexor ® (depression)           | Depakote ® (epilepsy)       | Cardura ® (prostatic yper trophy) |
| Allegra ® (allergies)            | Lupron ® (prostate cancer)  | Zoladex ® (prostate cancer)       |
| Diprivan ® (anesthesia)          | BuSpar ® (anxiety)          | Ventolin ® (bronchospasm)         |
| Hytrin ® (hypertension)          | Wellbutrin ® (depression)   | Zyrtec ® (rhinitis)               |
| Plavix ® (MI/stroke)             | Toprol-XL ® (hypertension)  | Tenormin ® (angina)               |
| Xalatan ® (glaucoma)             | Singulair ® (asthma)        | Diovan ® (hypertension)           |
| Harnal ® (prostatic hyperplasia) |                             |                                   |

(Med Ad News 1999 Data).

[0013] GPCRs share a common structural motif, having seven sequences of between 22 to 24 hydrophobic amino acids that form seven alpha helices, each of which spans the membrane (each span is identified by number, i.e., transmembrane-1 (TM-1), transmembrane-2 (TM-2), etc.). The transmembrane helices are joined by strands of amino acids between transmembrane-2 and transmembrane-3, transmembrane-4 and transmembrane-5, and transmembrane-6 and transmembrane-7 on the exterior, or "extracellular" side, of the cell membrane (these are referred to as "extracellular" regions 1, 2 and 3 (EC-1, EC-2 and EC-3), respectively). The transmembrane helices are also joined by strands of amino acids between transmembrane-1 and transmembrane-2, transmembrane-3 and transmembrane-4, and transmem-

brane-5 and transmembrane-6 on the interior, or "intracellular" side, of the cell membrane (these are referred to as "intracellular" regions 1, 2 and 3 (IC-1, IC-2 and IC-3), respectively). The "carboxy" ("C") terminus of the receptor lies in the intracellular space within the cell, and the "amino" ("N") terminus of the receptor lies in the extracellular space outside of the cell.

[0014] Generally, when a ligand binds with the receptor (often referred to as "activation" of the receptor), there is a change in the conformation of the receptor that facilitates coupling between the intracellular region and an intracellular "G-protein." It has been reported that GPCRs are "promiscuous" with respect to G proteins, i.e., that a GPCR can interact with more than one G protein. See, Kenakin, T., 43 *Life Sciences* 1095 (1988). Although other G proteins exist, currently, Gq, Gs, Gi, Gz and Go are G proteins that have been identified. Ligand-activated GPCR coupling with the G-protein initiates a signaling cascade process (referred to as "signal transduction"). Under normal conditions, signal transduction ultimately results in cellular activation or cellular inhibition. Although not wishing to be bound to theory, it is thought that the IC-3 loop as well as the carboxy terminus of the receptor interact with the G protein.

[0015] Gi-coupled GPCRs lower intracellular cAMP levels. The Melanophore technology (see *infra*) is useful for identifying Gi-coupled GPCRs.

[0016] Under physiological conditions, GPCRs exist in the cell membrane in equilibrium between two different conformations: an "inactive" state and an "active" state. A receptor in an inactive state is unable to link to the intracellular signaling transduction pathway to initiate signal transduction leading to a biological response. Changing the

receptor conformation to the active state allows linkage to the transduction pathway (via the G-protein) and produces a biological response.

[0017] A receptor may be stabilized in an active state by a ligand or a compound such as a drug. Recent discoveries, including but not exclusively limited to modifications to the amino acid sequence of the receptor, provide means other than ligands or drugs to promote and stabilize the receptor in the active state conformation. These means effectively stabilize the receptor in an active state by simulating the effect of a ligand binding to the receptor. Stabilization by such ligand-independent means is termed "constitutive receptor activation."

## SUMMARY OF THE INVENTION

[0018] RUP25, RUP38, RUP19 and RUP11 belong to a sub-family of human GPCRs on the basis of homology at the nucleotide level. See, Tables B and C, *infra*. Polynucleotide sequence and polypeptide sequence for human (h), rat (r), or mouse (m) RUP25, RUP38, RUP19, or RUP11 is provided in the Sequence Listing (also see, Tables E and F *infra* for corresponding SEQ. ID. NOs.).

[0019] Agonist engagement of Gi-coupled GPCRs is known to lead to lowered levels of intracellular cAMP. Lower levels of cAMP in adipocytes lead to diminished hormone sensitive lipase activity. (See, *supra*.) The present invention is based in part on the discovery by Applicant that GPCRs RUP25, RUP38, and RUP19 are coupled to Gi and expressed endogenously by adipocytes. RUP38 and RUP19 are further shown by Applicant to have limited tissue distribution beyond adipose. RUP11 is also disclosed to be coupled to Gi.

[0020] Applicant discloses herein that RUP25 is a nicotinic acid and an antilipolytic GPCR. Applicant further discloses that RUP38 and RUP19 are antilipolytic GPCRs. RUP11 is also disclosed to be antilipolytic. The present invention is directed in part to methods of identifying whether a candidate compound is a modulator of RUP25, RUP38, RUP19 or RUP11. The present invention also relates to methods of using said modulator of RUP25, RUP38, RUP19 or RUP11. Preferred said modulator is an agonist. Agonists of RUP25, RUP38, RUP19 or RUP11 are useful as therapeutic agents for the prevention or treatment of metabolic-related disorders, including dyslipidemia, atherosclerosis, coronary heart disease, stroke, insulin resistance, and type 2 diabetes.

[0021] Nicotinic acid is disclosed by Applicant to be an agonist for RUP25 but not for RUP38 or RUP19. (–)-Nicotine is also disclosed to be an agonist for RUP25. Exposure of cells expressing RUP25 to nicotinic acid is shown by Applicant to lower the level of intracellular cAMP. Exposure of isolated rat epididymal adipocyte RUP25 to nicotinic acid is shown by Applicant to inhibit lipolysis. Exposure of RUP25 within adipocyte primary cultures derived from human subcutaneous fat to nicotinic acid is shown by Applicant also to inhibit lipolysis. In vivo administration of nicotinic acid to rats is shown by Applicant to lower plasma free fatty acids (FFA).

[0022] Applicant has identified (5-Hydroxy-1-methyl-3-propyl-1H-pyrazol-4-yl)-pyridin-3-yl-methanone to be an agonist for RUP25 but not for RUP38. Exposure of cells expressing RUP25 to (5-hydroxy-1-methyl-3-propyl-1H-pyrazol-4-yl)-pyridin-3-yl-methanone is shown by Applicant to lower the level of intracellular cAMP.

[0023] Applicant has identified 1-Isopropyl-1H-benzotriazole-5-carboxylic acid to be an agonist for hRUP38 but not for RUP25. Exposure of cells expressing RUP38 to 1-Isopropyl-1H-benzotriazole-5-carboxylic acid is shown by Applicant to lower the level of intracellular cAMP. Exposure of RUP38 within adipocyte primary cultures derived from human subcutaneous fat to 1-Isopropyl-1H-benzotriazole-5-carboxylic acid is shown by Applicant to inhibit lipolysis.

[0024] Applicant has identified 3-(5-Bromo-2-ethoxy-phenyl)-acrylic acid to also be an agonist for RUP38 but not for either RUP25 or RUP19. Exposure of cells expressing

RUP38 to 3-(5-Bromo-2-ethoxy-phenyl)-acrylic acid is shown by Applicant to lower the level of intracellular cAMP.

[0025] Applicant, *supra*, provides direct in vitro evidence for RUP25 and RUP38 being antilipolytic and direct in vivo evidence in the rat for RUP25 being antilipolytic. Applicant also notes illustrative clinical evidence that nicotinic acid receptor is antilipolytic. Said evidence is consistent with the disclosure by Applicant in the present application that RUP25 is a nicotinic acid and an antilipolytic GPCR. Said evidence is consistent with the disclosure by Applicant in the present application that RUP38 and RUP19 are antilipolytic GPCRs. Said evidence is consistent with the disclosure by Applicant in the present application that RUP11 is an antilipolytic GPCR.

[0026] RUP38, RUP19 and RUP11 are further disclosed herein as being antilipolytic GPCRs responsive to agonists other than nicotinic acid. The failure of nicotinic acid to serve as an agonist for RUP38, RUP19 or RUP11 indicates that RUP38, RUP19 and RUP11 represent novel antilipolytic pathways not engaged by conventional nicotinic acid therapy.

[0027] See, Table M (Example 24) for a brief Summary and other additional Examples, *infra*.

[0028] In a first aspect, the invention features a method of identifying whether a candidate compound is a modulator of a nicotinic acid GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

[0029] (a) SEQ. ID. NO.:36 (hRUP25);

[0030] (b) SEQ. ID. NO.:137 (mRUP25); and

[0031] (c) SEQ. ID. NO.:139 (rRUP25);

[0032] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

[0033] comprising the steps of:

[0034] (a') contacting the candidate compound with the receptor;

[0035] (b') determining whether the receptor functionality is modulated;

[0036] wherein a change in receptor functionality is indicative of the candidate compound being a modulator of a nicotinic acid GPCR.

[0037] In some embodiments, said nicotinic acid GPCR is endogenous.

[0038] In some preferred embodiments, said nicotinic acid GPCR is recombinant.

[0039] Preferred said identified modulator binds to said GPCR.

[0040] In some embodiments, said contacting is carried out in the presence of a known ligand of the GPCR. In some embodiments, said known ligand is an agonist of the GPCR. In some embodiments, said agonist is nicotinic acid or an analog or derivative thereof. In some embodiments, said agonist is (–)-nicotine or an analog or derivative thereof.

[0041] The invention also relates to a method of identifying whether a candidate compound is a modulator of lipolysis, comprising the steps of:

[0042] (a) contacting the candidate compound with a GPCR comprising an amino acid sequence selected from the group consisting of:

[0043] (i) SEQ. ID. NO.:36 (hRUP25);

[0044] (ii) SEQ. ID. NO.:137 (mRUP25); and

[0045] (iii) SEQ. ID. NO.:139 (rRUP25);

[0046] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence; and

[0047] (b) determining whether the receptor functionality is modulated; wherein a change in receptor functionality is indicative of the candidate compound being a modulator of lipolysis.

[0048] In some embodiments, said GPCR is endogenous.

[0049] In some preferred embodiments, said GPCR is recombinant.

[0050] Preferred said identified modulator binds to said GPCR.

[0051] In some embodiments, said contacting is carried out in the presence of a known ligand of the GPCR. In some embodiments, said known ligand is an agonist of the GPCR. In some embodiments, said agonist is nicotinic acid or an analog or derivative thereof. In some embodiments, said agonist is (–)-nicotine or an analog or derivative thereof.

[0052] The invention also relates to a method of determining whether a candidate compound is a modulator of a nicotinic acid GPCR,

[0053] comprising the steps of:

[0054] (a) culturing nicotinic acid GPCR-expressing host cells under conditions that would allow expression of a recombinant nicotinic acid GPCR, said host cells being transfected with a polynucleotide encoding said recombinant nicotinic acid GPCR comprising an amino acid sequence selected from the group consisting of:

[0055] (i) SEQ. ID. NO.:36 (hRUP25);

[0056] (ii) SEQ. ID. NO.:137 (mRUP25); and

[0057] (iii) SEQ. ID. NO.:139 (rRUP25);

[0058] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

[0059] (b) contacting the nicotinic acid GPCR-expressing host cells of step (a) with the candidate compound;

[0060] (c) contacting control host cells with the candidate compound of step (b), wherein said control host cells do not express recombinant nicotinic acid GPCR protein;

[0061] (d) measuring the modulating effect of the candidate compound which interacts with the recombinant nicotinic acid GPCR from the host cells of step (a) and control host cells of step (c); and

[0062] (e) comparing the modulating effect of the test compound on the host cells and control host cells.

[0063] The invention also relates to a method of determining whether a candidate compound is a modulator of a nicotinic acid GPCR, comprising the steps of:

[0064] (a) culturing nicotinic acid GPCR-expressing host cells under conditions that would allow expression of a recombinant nicotinic acid GPCR, said host cells being transfected with a polynucleotide encoding said recombinant nicotinic acid GPCR comprising an amino acid sequence selected from the group consisting of:

[0065] (i) SEQ. ID. NO.:36 (hRUP25);

[0066] (ii) SEQ. ID. NO.:137 (mRUP25); and

[0067] (iii) SEQ. ID. NO.:139 (rRUP25);

[0068] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

[0069] (b) contacting a first population of nicotinic acid GPCR-expressing cells of step (a) with a known ligand of said nicotinic receptor GPCR;

[0070] (c) contacting a second population of nicotinic acid GPCR-expressing cells of step (a) with the candidate compound and with the known nicotinic acid GPCR ligand;

[0071] (d) contacting control host cells with the candidate compound of step (c), wherein said control host cells do not express recombinant nicotinic acid GPCR protein;

[0072] (e) measuring the modulating effect of the candidate compound, which interacts with recombinant nicotinic acid GPCR, in the presence and absence of the known nicotinic acid GPCR ligand, from the cells of step (b), step (c) and step (d); and

[0073] (f) comparing the modulating effect of the candidate compound as determined from step (b), step (c) and step (d).

[0074] In some embodiments, said ligand is an agonist of said nicotinic acid GPCR. In a particular embodiment, said agonist is nicotinic acid or an analog or derivative thereof. In other particular embodiment, said agonist is (–)-nicotine or an analog or derivative thereof.

[0075] The invention also relates to a method of determining whether a candidate compound is a modulator of a nicotinic acid GPCR, comprising the steps of:

[0076] (a) culturing nicotinic acid GPCR-expressing host cells under conditions that would allow expression of a recombinant nicotinic acid GPCR, said host cells being transfected with a polynucleotide encoding said recombinant nicotinic acid GPCR comprising an amino acid sequence selected from the group consisting of:

[0077] (i) SEQ. ID. NO.:36 (hRUP25);

[0078] (ii) SEQ. ID. NO.:137 (mRUP25); and

[0079] (iii) SEQ. ID. NO.:139 (rRUP25);

[0080] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

- [0081] (b) contacting a first population of the nicotinic acid GPCR-expressing host cells of step (a) with the candidate compound;
- [0082] (c) not contacting a second population of the nicotinic acid GPCR-expressing cells of step (a) with the candidate compound of step (b);
- [0083] (d) contacting control host cells to the candidate compound of step (b), wherein said control host cells do not express recombinant nicotinic acid GPCR protein;
- [0084] (e) measuring the modulating effect of the candidate compound, which interacts with recombinant nicotinic acid GPCR protein, from the cells of step (b) and step (c) and from the cells of step (d); and
- [0085] (f) comparing the modulating effect of the candidate compound as determined from step (b) and step (c) and from step (d).
- [0086] In some embodiments, the nicotinic acid GPCR has an amino acid sequence selected from the group consisting of:

[0087] SEQ. ID. NO.:36 (hRUP25);

[0088] (a) SEQ. ID. NO.:137 (mRUP25); and

[0089] (b) SEQ. ID. NO.:139 (rRUP25);

[0090] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence.

[0091] In some embodiments, the nicotinic acid GPCR comprises a biologically active fragment of said amino acid sequence.

[0092] In some embodiments, said biologically active mutant is CART or EFA. In preferred embodiments, said CART mutant has the amino acid sequence of SEQ. ID. NO.:36 further substituted at amino acid position 230 with lysine in place of isoleucine. In preferred embodiments, said EFA mutant has the amino acid sequence of SEQ. ID. NO.:159.

[0093] In preferred embodiments, said G protein is Gi.

[0094] In other preferred embodiments, said determining is through the use of a Melanophore assay.

[0095] In other preferred embodiments, said determining is through the measurement of the level of a second messenger selected from the group consisting of cyclic AMP (cAMP), cyclic GMP (cGMP), inositol triphosphate (IP<sub>3</sub>), diacylglycerol (DAG), and Ca<sup>2+</sup>. In further preferred embodiments, said second messenger is cAMP. In more preferred embodiments, the level of the cAMP is reduced. In some embodiments, said measurement of cAMP is carried out with membrane comprising said GPCR.

[0096] In other preferred embodiments, said determining is through the measurement of an activity up-regulated or down-regulated by a reduction in intracellular cAMP level. In further preferred embodiments, said down-regulated activity is intracellular lipolysis. In other further preferred embodiments, said down-regulated activity is hormone sensitive lipase activity. In other further preferred embodiments, said up-regulated activity is adiponectin secretion.

[0097] In other preferred embodiments, said determining is through CRE-reporter assay. In preferred embodiments, said reporter is luciferase. In some embodiments, said reporter is  $\beta$ -galactosidase.

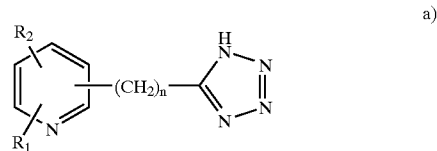
[0098] In other embodiments, said recombinant host cell further comprises promiscuous G alpha 15/16 or chimeric Gq/Gi alpha subunit and said determining is through measurement of intracellular Ca<sup>2+</sup>. In preferred embodiments, said Ca<sup>2+</sup> measurement is carried out by FLIPR.

[0099] In other embodiments, said recombinant host cell further comprises promiscuous G alpha 15/16 or chimeric Gq/Gi alpha subunit and said determining is through measurement of intracellular IP<sub>3</sub>.

[0100] In other preferred embodiments, said determining is through the measurement of GTP $\gamma$ S binding to membrane comprising said GPCR. In further preferred embodiments, said GTP $\gamma$ S is labeled with [<sup>35</sup>S].

[0101] In other preferred embodiments, said method further comprises the step of comparing the modulation of the receptor caused by the candidate compound to a second modulation of the receptor caused by contacting the receptor with a known modulator of the receptor. In some preferred embodiments, said known modulator is an agonist. In some preferred embodiments, said agonist is nicotinic acid or an analog or derivative thereof. In some preferred embodiments, said agonist is (-)-nicotine or an analog or derivative thereof.

[0102] In a second aspect, the invention features a modulator of a nicotinic acid GPCR identified according to the method of the first aspect, provided that the modulator is not identical to a compound having a formula selected from the group consisting of:



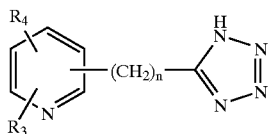
[0103] wherein:

[0104] R<sub>1</sub> is selected from the group consisting of halogen, hydroxyl, acetylamino, amino, alkoxy, carboalkoxy, alkylthio, monoalkylamino, dialkylamino, N-alkylcarbonyl, N,N-dialkylcarbonyl, alkylsulfonyl, said alkyl groups containing from 1 to 4 carbons, trifluoromethyl, trifluoromethoxy, trifluoromethylthio, methoxymethyl, carboxy, carbonyl, alkanoyloxy containing up to 4 carbon atoms, phenyl, p-chlorophenyl, p-methylphenyl and p-aminophenyl;

[0105] R<sub>2</sub> is selected from the group consisting of halogen, alkanoyloxy containing from 1-4 carbon atoms, carboalkoxy containing from 2 to 5 carbon atoms, carbonyl, N-alkyl carbonyl and N,N-dialkylcarbonyl wherein said alkyl groups contain from 1-4 carbon atoms and trifluoromethyl;

[0106] n is a whole number from 0 to 4; and

[0107] N-oxides thereof;

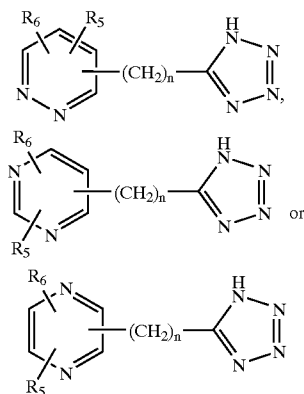


b)

[0108] R<sub>3</sub> and R<sub>4</sub> are hydrogen, alkyl containing from 1 to 4 carbon atoms or cycloalkyl containing from 3 to 7 carbon atoms;

[0109] n is a whole number from 0 to 4; and

[0110] N-oxides thereof;



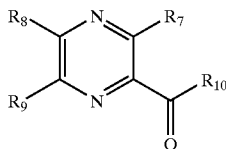
c)

[0111] wherein:

[0112] R<sub>5</sub> and R<sub>6</sub> are each selected from the group consisting of H, halogen, hydroxyl, amino, alkoxy, alkylthio, monoalkylamino, dialkylamino, N-alkylcarbonyl, N,N-dialkylcarbonyl, alkylsulfonyl, alkylsulfonyl, said alkyl groups containing from 1 to 4 carbons, trifluoromethyl, trifluoromethoxy, trifluoromethylthio, carboxy, carbonyl, alkanoyloxy containing up to 4 carbon atoms, phenyl, p-chlorophenyl, p-methylphenyl and p-aminophenyl;

[0113] n is a whole number from 0 to 4; and

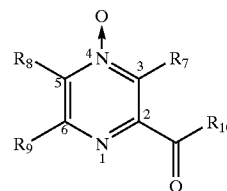
[0114] N-oxides thereof;



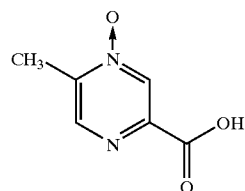
d)

[0115] wherein:

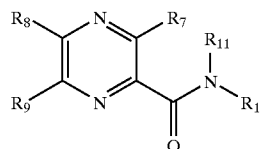
[0116] at least one of R<sub>7</sub>, R<sub>8</sub> and R<sub>9</sub> is C<sub>1-6</sub> alkyl and the others are hydrogen atoms; R<sub>10</sub> is hydroxy or C<sub>1-6</sub> alkoxy, or a salt of the compounds wherein R<sub>4</sub> is hydroxy with a pharmaceutically acceptable base; and a 4-N-oxide thereof. The position of the N-oxide is designated by the following numbering and a structure for a 4-N-oxide has the following structure:



[0117] One particular 4-N-oxide is 5-Methylpyrazine-2-carboxylic acid-4-oxide (Acipimox™) and has the structure:



e)

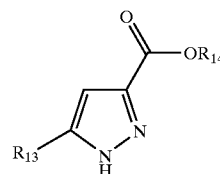


[0118] wherein:

[0119] at least one of R<sub>7</sub>, R<sub>8</sub> and R<sub>9</sub> is C<sub>1-4</sub> alkyl and the others are hydrogen atoms; each of R<sub>11</sub> and R<sub>12</sub>, which may be the same or different, is hydrogen or

[0120] C<sub>1-4</sub> alkyl; and a 4-N-oxide thereof; the position of the N-oxide is the same as described above herein;

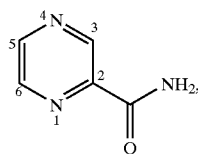
(f)



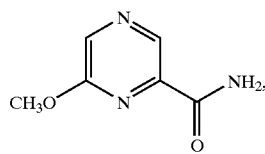
[0121] wherein:

[0122] at least one of R<sub>13</sub> represents an alkyl group of 7-11 carbon atoms and R<sub>14</sub> represents H or a lower alkyl group of up to two carbon atoms, and a pharmaceutically acceptable carrier;

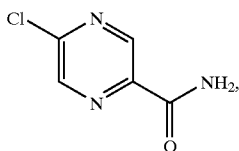
[0123] (g) Pyrazine-2-carboxylic acid amide and has the structure:



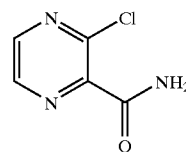
[0128] 6-methoxy-pyrazine-2-carboxylic acid amide and has the structure:



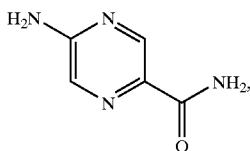
[0124] 5-chloro-pyrazine-2-carboxylic acid amide and has the structure:



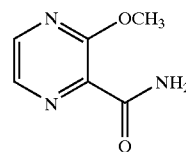
[0129] 3-chloro-pyrazine-2-carboxylic acid amide and has the structure:



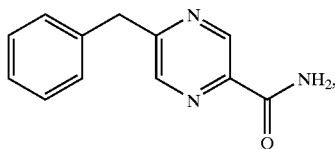
[0125] 5-amino-pyrazine-2-carboxylic acid amide and has the structure:



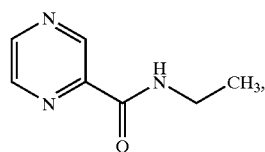
[0130] 3-methoxy-pyrazine-2-carboxylic acid amide and has the structure:



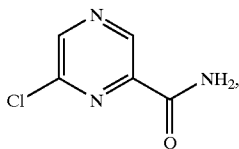
[0126] 5-benzyl-pyrazine-2-carboxylic acid amide and has the structure:



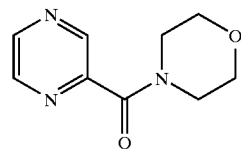
[0131] pyrazine-2-carboxylic acid ethylamide and has the structure:



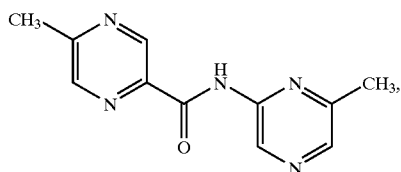
[0127] 6-chloro-pyrazine-2-carboxylic acid amide and has the structure:



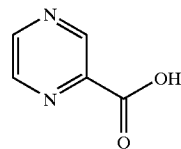
[0132] morpholin-4-yl-pyrazine-2-ylmethanone and has the structure:



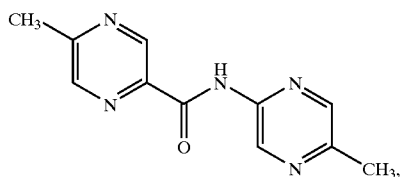
[0133] 5-methyl-pyrazine-2-carboxylic acid (6-methyl-pyrazin-2-yl)-amide and has the structure:



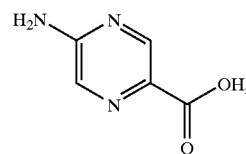
[0138] pyrazine-2-carboxylic acid and has the structure:



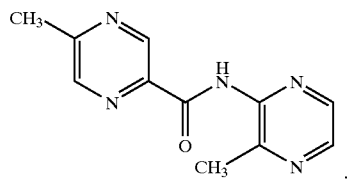
[0134] 5-methyl-pyrazine-2-carboxylic acid (5-methyl-pyrazin-2-yl)-amide and has the structure:



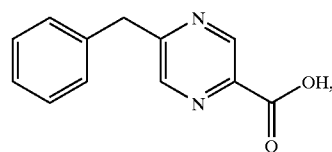
[0139] 5-amino-pyrazine-2-carboxylic acid and has the structure:



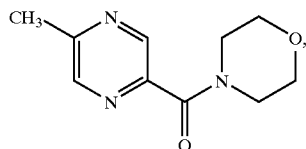
[0135] 5-methyl-pyrazine-2-carboxylic acid (3-methyl-pyrazin-2-yl)-amide and has the structure:



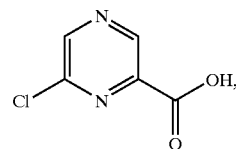
[0140] 5-benzyl-pyrazine-2-carboxylic acid and has the structure:



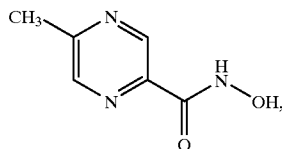
[0136] (5-methyl-pyrazin-2-yl)-morpholin-4-yl-methanone and has the structure:



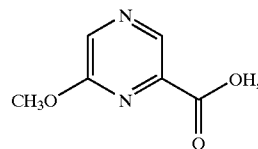
[0141] 6-chloro-pyrazine-2-carboxylic acid and has the structure:



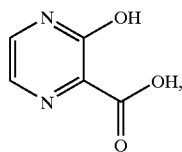
[0137] 5-methyl-pyrazine-2-carboxylic acid hydroxyamide and has the structure:



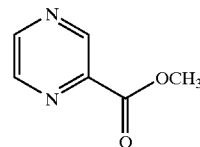
[0142] 6-methoxy-pyrazine-2-carboxylic acid and has the structure:



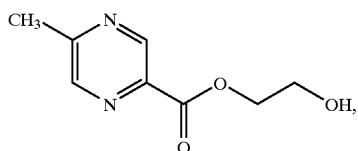
[0143] 3-hydroxy-pyrazine-2-carboxylic acid and has the structure:



[0148] pyrazine-2-carboxylic acid methyl ester and has the structure:

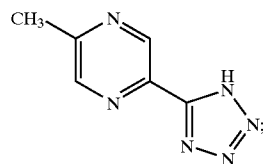


[0144] 5-methyl-pyrazine-2-carboxylic acid 2-hydroxy-ethyl ester and has the structure:

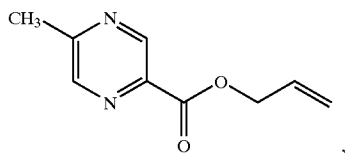


[0149] and

[0150] 2-methyl-5-(1H-tetrazol-5-yl)-pyrazine and has the structure:

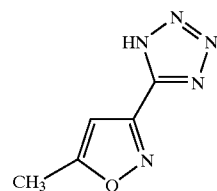


[0145] 5-methyl-pyrazine-2-carboxylic acid allyl ester and has the structure:

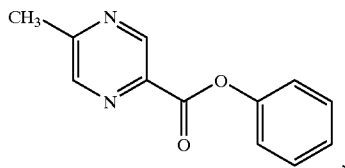


[0151] and 4-N-oxides thereof as described above herein;

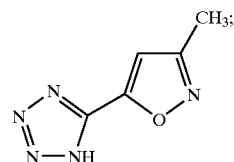
[0152] (h) 5-(3-(5-Methyl)isoxazolyl)tetrazole and has the structure:



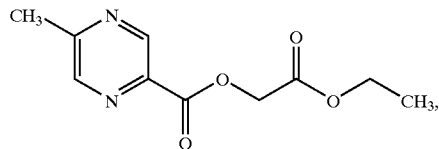
[0146] 5-methyl-pyrazine-2-carboxylic acid phenyl ester and has the structure:



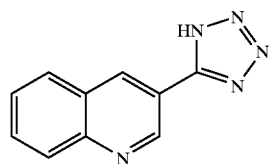
[0153] (i) 5-(5-(3-Methyl)isoxazolyl)tetrazole and has the structure:



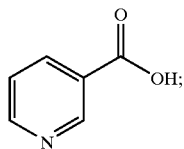
[0147] 5-methyl-pyrazine-2-carboxylic acid ethoxycarbonylmethyl ester and has the structure:



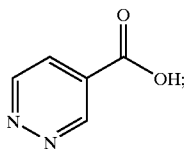
[0154] (j) 5-(3-Quinolyl)tetrazole and has the structure:



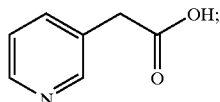
[0155] (k) Nicotinic acid and has the structure:



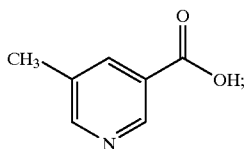
[0156] (l) Pyridazine-4-carboxylic acid and has the structure:



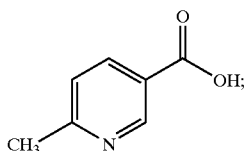
[0157] (m) 3-Pyridine acetic acid and has the structure:



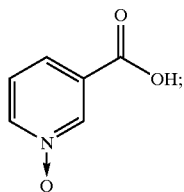
[0158] (n) 5-Methylnicotinic acid and has the structure:



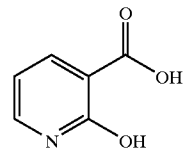
[0159] (o) 6-Methylnicotinic acid and has the structure:



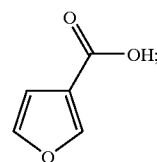
[0160] (p) Nicotinic acid-1-oxide and has the structure:



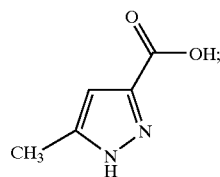
[0161] (q) 2-Hydroxynicotinic acid and has the structure:



[0162] (r) Furane-3-carboxylic acid and has the structure:

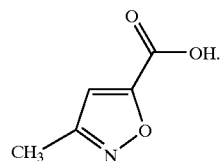


[0163] (s) 5-Methylpyrazole-3-carboxylic acid and has the structure:



[0164] and

[0165] t) 3-Methylisoxazole-5-carboxylic acid and has the structure:



[0166] In preferred embodiments, said modulator is selected from the group consisting of agonist, partial agonist, inverse agonist and antagonist. More preferably, said modulator is an agonist. In some embodiments, said modulator is a partial agonist.

[0167] In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $1000 \mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP25 polypeptide having the amino acid sequence of SEQ. ID. NO.:36. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $900 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $800 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $700 \mu M$  in said assay. In

some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[0168] In some embodiments, said modulator is selective for the GPCR.

[0169] In some embodiments, said modulator is antilipolytic.

[0170] In some embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0171] In highly less preferred embodiments, said modulator is an antibody or derivative thereof.

[0172] In a third aspect, the invention features the method of the first aspect, wherein said candidate compound is an agonist of hRUP38 GPCR comprising the amino acid sequence of SEQ. ID. NO.:135 and wherein said method further comprises the step of comparing the modulation of hRUP25 GPCR comprising the amino acid sequence of SEQ. ID. NO.:36 caused by said agonist to a second modulation of hRUP25 GPCR comprising a variant of said amino acid sequence caused by contacting the variant hRUP25 GPCR with said agonist.

[0173] In preferred embodiments, said variant amino acid sequence is identical to the amino acid sequence of SEQ. ID. NO.:36, further comprising a single amino acid substitution selected from the group consisting of:

[0174] (a) A for V at amino acid position 27 of SEQ. ID. NO.:36;

[0175] (b) V for L at amino acid position 83 of SEQ. ID. NO.:36;

[0176] (c) Y for N at amino acid position 86 of SEQ. ID. NO.:36;

[0177] (d) S for W at amino acid position 91 of SEQ. ID. NO.:36;

[0178] (e) N for K at amino acid position 94 of SEQ. ID. NO.:36;

[0179] (f) V for M at amino acid position 103 of SEQ. ID. NO.:36;

[0180] (g) F for L at amino acid position 107 of SEQ. ID. NO.:36;

[0181] (h) W for R at amino acid position 142 of SEQ. ID. NO.:36;

[0182] (i) V for I at amino acid position 156 of SEQ. ID. NO.:36;

[0183] (j) L for M at amino acid position 167 of SEQ. ID. NO.:36;

[0184] (k) L for P at amino acid position 168 of SEQ. ID. NO.:36;

[0185] (l) P for G at amino acid position 173 of SEQ. ID. NO.:36;

[0186] (m) V for L at amino acid position 176 of SEQ. ID. NO.:36;

[0187] (n) I for S at amino acid position 178 of SEQ. ID. NO.:36;

[0188] (o) R for Q at amino acid position 187 of SEQ. ID. NO.:36;

[0189] (p) L for F at amino acid position 198 of SEQ. ID. NO.:36; and

[0190] (q) N for P at amino acid position 363 of SEQ. ID. NO.:36.

[0191] In particularly preferred embodiments, said method is used to identify whether said substituted amino acid additionally found at the identical position within SEQ. ID. NO.:135 is necessary for modulation of said hRUP38 GPCR by said agonist, comprising the steps of:

[0192] (a) determining the level of modulation of said hRUP25 GPCR by said agonist; and

[0193] (b) determining the level of modulation of said variant hRUP25 GPCR by said agonist;

[0194] wherein if said level of modulation for (b) is greater than said level of modulation for (a), then said substituted amino acid is necessary for modulation of said hRUP38 GPCR by said agonist.

[0195] In a fourth aspect, the invention features a method of modulating the activity of a nicotinic acid GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

[0196] (a) SEQ. D. NO.:36 (hRUP25);

[0197] (b) SEQ. ID. NO.:137 (mRUP25); and

[0198] (c) SEQ. ID. NO.:139 (rRUP25);

[0199] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

[0200] comprising the step of contacting the receptor with the modulator of the second aspect.

[0201] In some embodiments, the nicotinic acid GPCR has an amino acid sequence selected from the group consisting of:

[0202] (a) SEQ. ID. NO.:36 (hRUP25);

[0203] (b) SEQ. ID. NO.:137 (mRUP25); and

[0204] (c) SEQ. ID. NO.:139 (rRUP25);

[0205] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence.

[0206] In some embodiments, the nicotinic acid GPCR comprises an active fragment of said amino acid sequence.

[0207] In some embodiments, the nicotinic acid GPCR is endogenous.

[0208] In some embodiments, the nicotinic acid GPCR is recombinant.

[0209] In some embodiments, said biologically active mutant is CART or EFA. In preferred embodiments, said CART mutant has the amino acid sequence of SEQ. ID. NO.:36 further substituted at amino acid position 230 with lysine in place of isoleucine. In preferred embodiments, said EFA mutant has the amino acid sequence of SEQ. ID. NO.:159.

[0210] In preferred embodiments, said G protein is Gi.

[0211] In some preferred embodiments, said modulator is an agonist.

[0212] In preferred embodiments, said modulator is selective for the GPCR.

[0213] In other preferred embodiments, said contacting comprises administration of the modulator to a membrane comprising the receptor.

[0214] In other preferred embodiments, said contacting comprises administration of the modulator to a cell or tissue comprising the receptor.

[0215] In other preferred embodiments, said contacting comprises administration of the modulator to an individual comprising the receptor. In more preferred embodiments, said individual is a mammal. In other more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[0216] In some preferred embodiments, said modulator is selective for the GPCR.

[0217] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral

bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0218] In some preferred embodiments, said modulator is antipolytic.

[0219] In some preferred embodiments, said modulator is an agonist.

[0220] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP25 polypeptide having the amino acid sequence of SEQ. ID. NO.:36. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[0221] In some preferred embodiments, said administration is oral.

[0222] In preferred embodiments, said modulator is an agonist and said individual is in need of prevention of or treatment for a metabolic-related disorder selected from the group consisting of:

[0223] (a) dyslipidemia;

[0224] (b) atherosclerosis;

[0225] (c) coronary heart disease;

[0226] (d) stroke;

[0227] (e) insulin resistance; and

[0228] (f) type 2 diabetes.

[0229] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0230] In other embodiments, said modulator is an inverse agonist and said metabolic-related disorder relates to a low level of plasma free fatty acids.

[0231] In other preferred embodiments, said modulator is an agonist and said individual is in need of a change in lipid metabolism selected from the group consisting of:

- [0232] (a) a decrease in the level of plasma triglycerides;
- [0233] (b) a decrease in the level of plasma free fatty acids;
- [0234] (c) a decrease in the level of plasma cholesterol;
- [0235] (d) a decrease in the level of LDL-cholesterol;
- [0236] (e) an increase in the level of HDL-cholesterol;
- [0237] (f) a decrease in the total cholesterol/HDL-cholesterol ratio; and
- [0238] (g) an increase in the level of plasma adiponectin.

[0239] In other preferred embodiments, said needed change in lipid metabolism is a decrease in the postprandial increase in plasma free fatty acids due to a high fat meal or an inhibition of the progression from impaired glucose tolerance to insulin resistance.

[0240] In some embodiments, the modulator is an inverse agonist and the needed change in lipid metabolism is an increase in the level of plasma free fatty acids.

[0241] In other preferred embodiments, said modulator is an agonist and said individual is a mouse genetically predisposed to a metabolic-related disorder selected from the group consisting of:

- [0242] (a) dyslipidemia;
- [0243] (b) atherosclerosis;
- [0244] (c) coronary heart disease;
- [0245] (d) stroke;
- [0246] (e) insulin resistance; and
- [0247] (f) type 2 diabetes.

[0248] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure.

[0249] In further preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0250] In further preferred embodiments, said method is used to identify whether said agonist has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

- [0251] (a) dyslipidemia;
- [0252] (b) atherosclerosis;
- [0253] (c) coronary heart disease;

[0254] (d) stroke;

[0255] (e) insulin resistance; and

[0256] (f) type 2 diabetes;

[0257] comprising the steps of:

[0258] (a') administering or not administering said agonist to the mouse; and

[0259] (b') determining whether the disorder is prevented, delayed, or made less severe on administering said agonist compared to not administering said agonist;

[0260] wherein said determination is indicative of said agonist having therapeutic efficacy.

[0261] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0262] In other preferred embodiments, said modulator is an agonist and said individual is a rat genetically predisposed to a metabolic-related disorder selected from the group consisting of:

- [0263] (a) dyslipidemia;
- [0264] (b) atherosclerosis;
- [0265] (c) coronary heart disease;
- [0266] (d) stroke;
- [0267] (e) insulin resistance; and
- [0268] (f) type 2 diabetes.

[0269] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In further preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0270] In further preferred embodiments, said method is used to identify whether said agonist has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

- [0271] (a) dyslipidemia;
- [0272] (b) atherosclerosis;
- [0273] (c) coronary heart disease;
- [0274] (d) stroke;
- [0275] (e) insulin resistance; and
- [0276] (f) type 2 diabetes;

[0277] comprising the steps of:

[0278] (a') administering or not administering said agonist to the rat; and

[0279] (b') determining whether the disorder is prevented, delayed, or made less severe on administering said agonist compared to not administering said agonist;

[0280] wherein said determination is indicative of said agonist having therapeutic efficacy.

[0281] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0282] In a fifth aspect, the invention features a method of preventing or treating a disorder of lipid metabolism in an individual comprising contacting a therapeutically effective amount of the modulator of the second aspect with a nicotinic acid GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

[0283] (a) SEQ. ID. NO.:36 (hRUP25);

[0284] (b) SEQ. ID. NO.:137 (mRUP25); and

[0285] (c) SEQ. ID. NO.:139 (rRUP25);

[0286] or an allelic variant or biologically active fragment of said amino acid sequence.

[0287] In some preferred embodiments, said modulator is selective for the GPCR.

[0288] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0289] In some preferred embodiments, said modulator is antilipolytic.

[0290] In some preferred embodiments, said modulator is an agonist.

[0291] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP25 polypeptide having the amino acid sequence of SEQ. ID. NO.:36. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than

550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[0292] In some preferred embodiments, said contacting comprises oral administration of said modulator to said individual.

[0293] In preferred embodiment, said modulator is an agonist and said disorder of lipid metabolism is selected from the group consisting of:

[0294] (a) elevated level of plasma triglycerides;

[0295] (b) elevated level of plasma free fatty acids;

[0296] (c) elevated level of plasma cholesterol;

[0297] (d) elevated level of LDL-cholesterol;

[0298] (e) reduced level of HDL-cholesterol;

[0299] (f) elevated total cholesterol/HDL-cholesterol ratio; and

[0300] (g) reduced level of plasma adiponectin.

[0301] In a sixth aspect, the invention features a method of preventing or treating a metabolic-related disorder in an individual comprising contacting a therapeutically effective amount of the modulator of the second aspect with a nicotinic acid GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

[0302] (a) SEQ. ID. NO.:36 (hRUP25);

[0303] (b) SEQ. ID. NO.:137 (mRUP25); and

[0304] (c) SEQ. ID. NO.:139 (rRUP25);

[0305] or an allelic variant or biologically active fragment of said amino acid sequence.

[0306] In some preferred embodiments, said modulator is selective for the GPCR.

[0307] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least

30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0308] In some preferred embodiments, said modulator is antilipolytic.

[0309] In some preferred embodiments, said modulator is an agonist.

[0310] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP25 polypeptide having the amino acid sequence of SEQ. ID. NO.:36. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

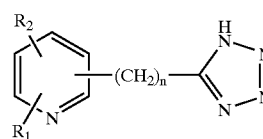
[0311] In some preferred embodiments, said contacting comprises oral administration of said modulator to said individual.

[0312] In preferred embodiment, said modulator is an agonist and said metabolic-related disorder is selected from the group consisting of:

- [0313] (a) dyslipidemia;
- [0314] (b) atherosclerosis;
- [0315] (c) coronary heart disease;
- [0316] (d) stroke;
- [0317] (e) insulin resistance; and
- [0318] (f) type 2 diabetes.

[0319] In a seventh aspect, the invention features a method of preparing a composition which comprises identifying a modulator of a nicotinic acid GPCR and then admixing a carrier and the modulator, wherein the modulator is identi-

fiable by the method of the first aspect and provided that the modulator is not identical to a compound having a formula selected from the group consisting of:



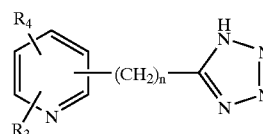
[0320] wherein:

[0321]  $R_1$  is selected from the group consisting of halogen, hydroxyl, acetylamino, amino, alkoxy, carboalkoxy, alkylthio, monoalkylamino, dialkylamino, N-alkylcarbonyl, N,N-dialkylcarbonyl, alkylsulfonyl, said alkyl groups containing from 1 to 4 carbons, trifluoromethyl, trifluoromethoxy, trifluoromethylthio, methoxymethyl, carboxy, carbonyl, alkanoyloxy containing up to 4 carbon atoms, phenyl, p-chlorophenyl, p-methylphenyl and p-aminophenyl;

[0322]  $R_2$  is selected from the group consisting of halogen, alkanoyloxy containing from 14 carbon atoms, carboalkoxy containing from 2 to 5 carbon atoms, carbonyl, N-alkyl carbonyl and N,N-dialkylcarbonyl wherein said alkyl groups contain from 1-4 carbon atoms and trifluoromethyl;

[0323]  $n$  is a whole number from 0 to 4; and

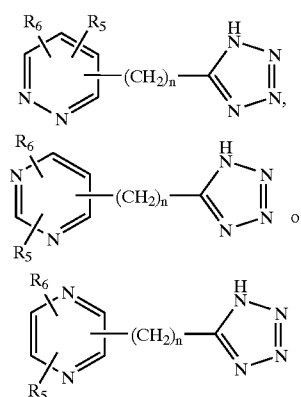
[0324] N-oxides thereof;



[0325]  $R_3$  and  $R_4$  are hydrogen, alkyl containing from 1 to 4 carbon atoms or cycloalkyl containing from 3 to 7 carbon atoms;

[0326]  $n$  is a whole number from 0 to 4; and

[0327] N-oxides thereof;

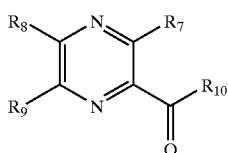


[0328] wherein:

[0329]  $R_5$  and  $R_6$  are each selected from the group consisting of H, halogen, hydroxyl, amino, alkoxy, alkylthio, monoalkylamino, dialkylamino, N-alkylcarbamyl, N,N-dialkylcarbamyl, alkylsulfoxy, alkylsulfonyl, said alkyl groups containing from 1 to 4 carbons, trifluoromethyl, trifluoromethoxy, trifluoromethylthio, carboxy, carbamyl, alkanoyloxy containing up to 4 carbon atoms, phenyl, p-chlorophenyl, p-methylphenyl and p-aminophenyl;

[0330]  $n$  is a whole number from 0 to 4; and

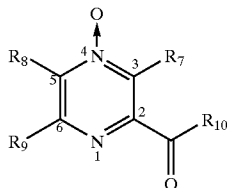
[0331] N-oxides thereof;



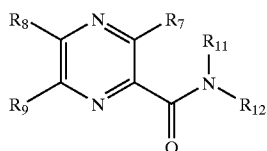
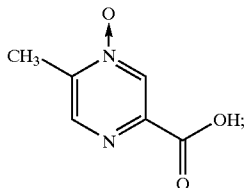
d)

[0332] wherein:

[0333] at least one of  $R_7$ ,  $R_8$  and  $R_9$  is  $C_{1-4}$  alkyl and the others are hydrogen atoms;  $R_{10}$  is hydroxy or  $C_{1-6}$  alkoxy, or a salt of the compounds wherein  $R_4$  is hydroxy with a pharmaceutically acceptable base; and a 4-N-oxide thereof. The position of the N-oxide is designated by the following numbering and a structure for a 4-N-oxide has the following structure:



[0334] One particular 4-N-oxide is 5-Methylpyrazine-2-carboxylic acid-4-oxide (Acipimox<sup>TM</sup>) and has the structure:



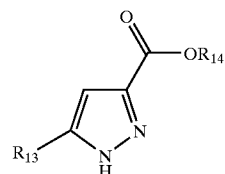
e)

[0335] wherein:

[0336] at least one of  $R_7$ ,  $R_8$  and  $R_9$  is  $C_{1-4}$  alkyl and the others are hydrogen atoms; each of  $R_{11}$

and  $R_{12}$ , which may be the same or different, is hydrogen or  $C_{1-6}$  alkyl; and a 4-N-oxide thereof; the position of the N-oxide is the same as described above herein;

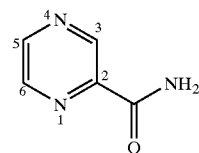
(f)



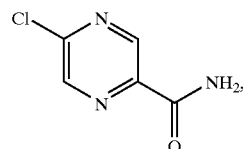
[0337] wherein:

[0338] at least one of  $R_{13}$  represents an alkyl group of 7-11 carbon atoms and  $R_{14}$  represents H or a lower alkyl group of up to two carbon atoms, and a pharmaceutically acceptable carrier;

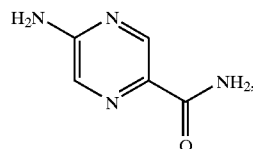
[0339] (g) Pyrazine-2-carboxylic acid amide and has the structure:



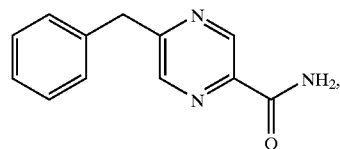
[0340] 5-chloro-pyrazine-2-carboxylic acid amide and has the structure:



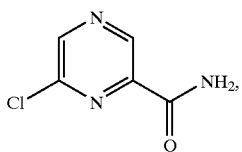
[0341] 5-amino-pyrazine-2-carboxylic acid amide and has the structure:



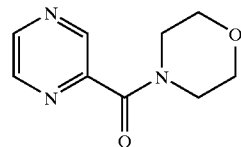
[0342] 5-benzyl-pyrazine-2-carboxylic acid amide and has the structure:



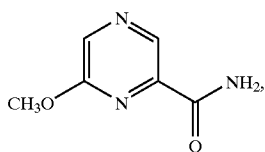
[0343] 6-chloro-pyrazine-2-carboxylic acid amide and has the structure:



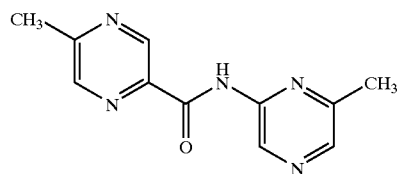
[0348] morpholin-4-yl-pyrazine-2-ylmethanone and has the structure:



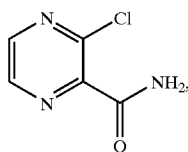
[0344] 6-methoxy-pyrazine-2-carboxylic acid amide and has the structure:



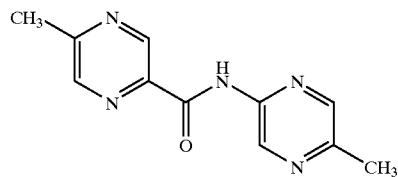
[0349] 5-methyl-pyrazine-2-carboxylic acid (6-methyl-pyrazin-2-yl)-amide and has the structure:



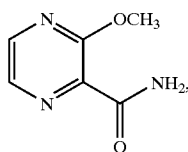
[0345] 3-chloro-pyrazine-2-carboxylic acid amide and has the structure:



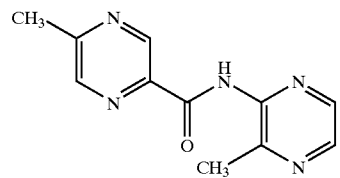
[0350] 5-methyl-pyrazine-2-carboxylic acid (5-methyl-pyrazin-2-yl)-amide and has the structure:



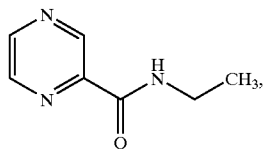
[0346] 3-methoxy-pyrazine-2-carboxylic acid amide and has the structure:



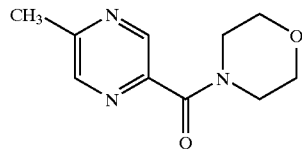
[0351] 5-methyl-pyrazine-2-carboxylic acid (3-methyl-pyrazin-2-yl)-amide and has the structure:



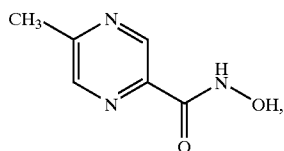
[0347] pyrazine-2-carboxylic acid ethylamide and has the structure:



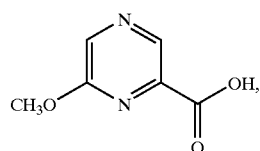
[0352] (5-methyl-pyrazin-2-yl)-morpholin-4-yl-methanone and has the structure:



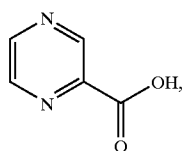
[0353] 5-methyl-pyrazine-2-carboxylic acid hydroxyamide and has the structure:



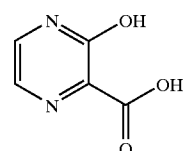
[0358] 6-methoxy-pyrazine-2-carboxylic acid and has the structure:



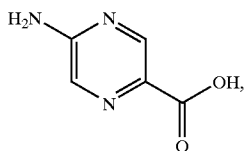
[0354] pyrazine-2-carboxylic acid and has the structure:



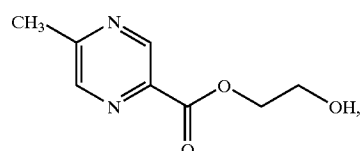
[0359] 3-hydroxy-pyrazine-2-carboxylic acid and has the structure:



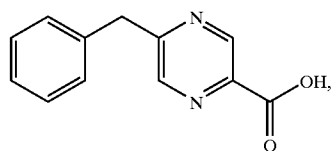
[0355] 5-amino-pyrazine-2-carboxylic acid and has the structure:



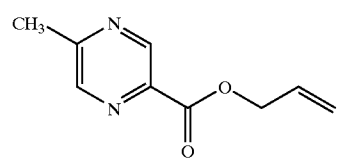
[0360] 5-methyl-pyrazine-2-carboxylic acid 2-hydroxy-ethyl ester and has the structure:



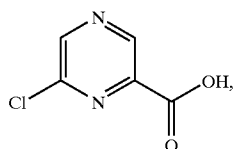
[0356] 5-benzyl-pyrazine-2-carboxylic acid and has the structure:



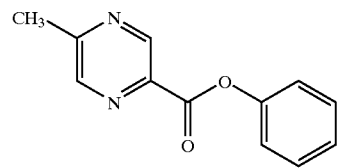
[0361] 5-methyl-pyrazine-2-carboxylic acid allyl ester and has the structure:



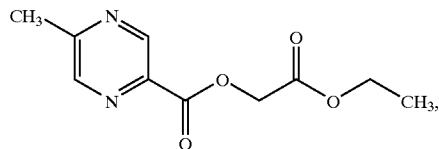
[0357] 6-chloro-pyrazine-2-carboxylic acid and has the structure:



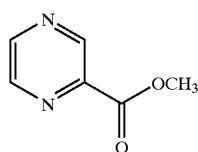
[0362] 5-methyl-pyrazine-2-carboxylic acid phenyl ester and has the structure:



[0363] 5-methyl-pyrazine-2-carboxylic acid ethoxycarbonylmethyl ester and has the structure:

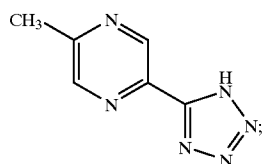


[0364] pyrazine-2-carboxylic acid methyl ester and has the structure:



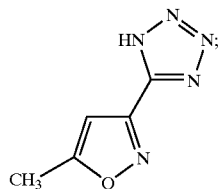
[0365] and

[0366] 2-methyl-5-(1H-tetrazol-5-yl)-pyrazine and has the structure:

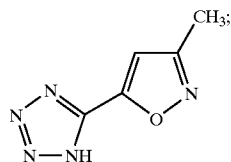


[0367] and 4-N-oxides thereof as described above herein;

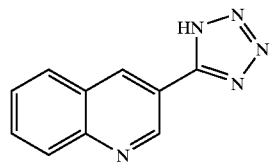
[0368] (h) 5-(3-(5-Methyl)isoxazolyl)tetrazole and has the structure:



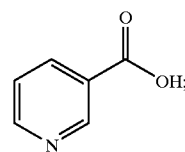
[0369] (i) 5-(5-(3-Methyl)isoxazolyl)tetrazole and has the structure:



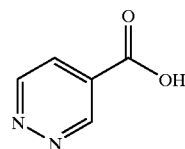
[0370] (j) 5-(3-Quinolyl)tetrazole and has the structure:



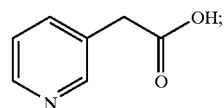
[0371] (k) Nicotinic acid and has the structure:



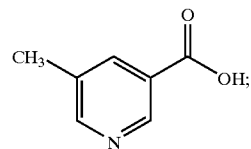
[0372] (l) Pyridazine-4-carboxylic acid and has the structure:



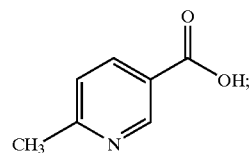
[0373] (m) 3-Pyridine acetic acid and has the structure:



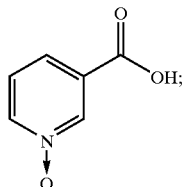
[0374] (n) 5-Methylnicotinic acid and has the structure:



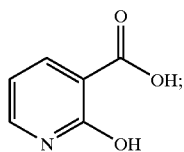
[0375] (o) 6-Methylnicotinic acid and has the structure:



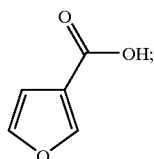
[0376] (p) Nicotinic acid-1-oxide and has the structure:



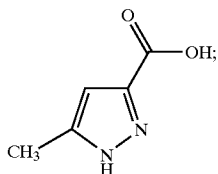
[0377] (q) 2-Hydroxynicotinic acid and has the structure:



[0378] (r) Furane-3-carboxylic acid and has the structure:

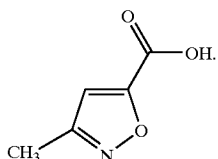


[0379] (s) 5-Methylpyrazole-3-carboxylic acid and has the structure:



[0380] and

[0381] t) 3-Methylisoxazole-5-carboxylic acid and has the structure:



[0382] In preferred embodiments, said modulator is selected from the group consisting of agonist, partial ago-

nist, inverse agonist and antagonist. More preferably, said modulator is an agonist. In some embodiments, said modulator is a partial agonist.

[0383] In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $1000 \mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP25 polypeptide having the amino acid sequence of SEQ. ID. NO.:36. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $900 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $800 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $700 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $600 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $550 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $500 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $450 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $400 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $350 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $300 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $250 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $200 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $150 \mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $100 \mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of  $600 \mu M$  to  $1000 \mu M$ .

[0384] In some embodiments, said modulator is selective for the GPCR.

[0385] In some embodiments, said modulator is antilipolytic.

[0386] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to either intraperitoneal or intravenous administration. In some preferred embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to either intraperitoneal or intravenous administration.

[0387] In an eighth aspect, the invention features a pharmaceutical or physiologically acceptable composition comprising, consisting essentially of, or consisting of the modulator of the second aspect. In preferred embodiments, said modulator is an agonist.

[0388] In some preferred embodiments, said modulator is selective for the GPCR.

[0389] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavail-

ability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0390] In some preferred embodiments, said modulator is antilipolytic.

[0391] In some preferred embodiments, said modulator is an agonist.

[0392] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP25 polypeptide having the amino acid sequence of SEQ. ID. NO.:36. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[0393] In a ninth aspect, the invention features a method of changing lipid metabolism comprising providing or administering to an individual in need of said change said pharmaceutical or physiologically acceptable composition of the eighth aspect, said needed change in lipid metabolism selected from the group consisting of:

- [0394] (a) a decrease in the level of plasma triglycerides;
- [0395] (b) a decrease in the level of plasma free fatty acids;
- [0396] (c) a decrease in the level of plasma cholesterol;
- [0397] (d) a decrease in the level of LDL-cholesterol;
- [0398] (e) an increase in the level of HDL-cholesterol;

[0399] (f) a decrease in the total cholesterol/HDL-cholesterol ratio; and

[0400] (g) an increase in the level of plasma adiponectin.

[0401] In preferred embodiments, a therapeutically effective amount of said pharmaceutical or physiologically acceptable composition is provided or administered to said individual.

[0402] In some preferred embodiments, said providing or administering of said pharmaceutical or physiologically acceptable composition is oral.

[0403] In other preferred embodiments, said needed change in lipid metabolism is a decrease in the postprandial increase in plasma free fatty acids due to a high fat meal or an inhibition of the progression from impaired glucose tolerance to insulin resistance.

[0404] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[0405] In a tenth aspect, the invention features a method of preventing or treating a metabolic-related disorder comprising providing or administering to an individual in need of said treatment said pharmaceutical or physiologically acceptable composition of the eighth aspect, said metabolic-related disorder selected from the group consisting of:

- [0406] (a) dyslipidemia;
- [0407] (b) atherosclerosis;
- [0408] (c) coronary heart disease;
- [0409] (d) stroke;
- [0410] (e) insulin resistance; and
- [0411] (f) type 2 diabetes.

[0412] In preferred embodiments, a therapeutically effective amount of said pharmaceutical or physiologically acceptable composition is provided or administered to said individual.

[0413] In some preferred embodiments, said providing or administering of said pharmaceutical or physiologically acceptable composition is oral.

[0414] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, stroke, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0415] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[0416] In an eleventh aspect, the invention features a method of using the modulator of the second aspect for the preparation of a medicament for the treatment of a disorder in lipid metabolism in an individual.

[0417] In some preferred embodiments, said modulator is selective for the GPCR.

[0418] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0419] In some preferred embodiments, said modulator is antilipolytic.

[0420] In some preferred embodiments, said modulator is an agonist.

[0421] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP25 polypeptide having the amino acid sequence of SEQ. ID. NO.:36. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[0422] In some preferred embodiments, said treatment comprises oral administration of said medicament to said individual.

[0423] In preferred embodiments, said modulator is an agonist and said disorder in lipid metabolism is selected from the group consisting of:

[0424] (a) elevated level of plasma triglycerides;

[0425] (b) elevated level of plasma free fatty acids;

[0426] (c) elevated level of plasma cholesterol;

[0427] (d) elevated level of LDL-cholesterol;

[0428] (e) reduced level of HDL-cholesterol;

[0429] (f) elevated total cholesterol/HDL-cholesterol ratio; and

[0430] (g) reduced level of plasma adiponectin.

[0431] In other preferred embodiments, said disorder in lipid metabolism is an elevated postprandial increase in plasma free fatty acids due to a high fat meal or a progression from impaired glucose tolerance to insulin resistance.

[0432] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[0433] In a twelfth aspect, the invention features a method of using the modulator of the second aspect for the preparation of a medicament for the treatment of a metabolic-related disorder in an individual.

[0434] In some preferred embodiments, said modulator is selective for the GPCR.

[0435] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0436] In some preferred embodiments, said modulator is antilipolytic.

[0437] In some preferred embodiments, said modulator is an agonist.

[0438] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP25 polypeptide having the amino acid sequence of SEQ. ID. NO.:36. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator

is an agonist with an  $EC_{50}$  of less than  $350\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $300\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $250\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $200\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $150\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $100\ \mu\text{M}$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of  $600\ \mu\text{M}$  to  $1000\ \mu\text{M}$ .

[0439] In some preferred embodiments, said treatment comprises oral administration of said medicament to said individual.

[0440] In preferred embodiments, said modulator is an agonist and said metabolic-related disorder is selected from the group consisting of:

- [0441] (a) dyslipidemia;
- [0442] (b) atherosclerosis;
- [0443] (c) coronary heart disease;
- [0444] (d) stroke;
- [0445] (e) insulin resistance; and
- [0446] (f) type 2 diabetes.

[0447] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0448] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[0449] In a thirteenth aspect, the invention features a method of identifying whether a candidate compound binds to a nicotinic acid GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

- [0450] (a) SEQ. ID. NO.:36 (hRUP25);
- [0451] (b) SEQ. ID. NO.:137 (mRUP25); and
- [0452] (c) SEQ. ID. NO.:139 (rRUP25);

[0453] or an allelic variant or a biologically active fragment of said amino acid sequence;

[0454] comprising the steps of:

- [0455] (a') contacting the receptor with a labeled reference compound known to bind to the GPCR in the presence or absence of the candidate compound; and
- [0456] (b') determining whether the binding of said labeled reference compound to the receptor is inhibited in the presence of the candidate compound;

[0457] wherein said inhibition is indicative of the candidate compound binding to a nicotinic acid GPCR.

[0458] In some embodiments, the nicotinic acid GPCR comprises a biologically active fragment of said amino acid sequence.

[0459] In some embodiments, the nicotinic acid GPCR is endogenous.

[0460] In preferred embodiments, the nicotinic acid GPCR is recombinant.

[0461] In preferred embodiments, said G protein is  $G_i$ .

[0462] In some preferred embodiments, said reference compound is nicotinic acid.

[0463] In some preferred embodiments, said reference compound is (-)-nicotine.

[0464] In some preferred embodiments, said reference compound is the modulator of the second aspect.

[0465] In other embodiments, said reference compound is an antibody specific for the GPCR, or a derivative thereof.

[0466] In preferred embodiments, said reference compound comprises a label selected from the group consisting of:

- [0467] (a) radioisotope;
- [0468] (b) enzyme; and
- [0469] (c) fluorophore.

[0470] In some preferred embodiments, said label is  $^3\text{H}$ .

[0471] In other embodiments, said method further comprises the step of comparing the level of inhibition of binding of a labeled first reference compound by the candidate compound to a second level of inhibition of binding of said labeled first reference compound by a second reference compound known to bind to the GPCR.

[0472] In a fourteenth aspect, the invention features a method of making a mouse genetically predisposed to a metabolic-related disorder selected from the group consisting of:

- [0473] (a) dyslipidemia;
- [0474] (b) atherosclerosis;
- [0475] (c) coronary heart disease;
- [0476] (d) stroke;
- [0477] (e) insulin resistance; and
- [0478] (f) type 2 diabetes;

[0479] comprising the step of knocking out the gene encoding the nicotinic acid mRUP25 GPCR polypeptide of SEQ. ID. NO.:137.

[0480] In some preferred embodiments, said knocking out the gene encoding the nicotinic acid mRUP25 GPCR polypeptide of SEQ. ID. NO.:137 is essentially restricted to adipocytes.

[0481] In a fifteenth aspect, the invention features the knockout mouse according to the method of the fourteenth aspect.

[0482] In a sixteenth aspect, the invention features a method of using the knockout mouse of the fifteenth aspect to identify whether a candidate compound has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

- [0483] (a) dyslipidemia;
- [0484] (b) atherosclerosis;
- [0485] (c) coronary heart disease;
- [0486] (d) stroke;
- [0487] (e) insulin resistance; and
- [0488] (f) type 2 diabetes;

[0489] comprising the steps of:

- [0490] (a') administering or not administering the compound to the mouse; and
- [0491] (b') determining whether the disorder is prevented, delayed, or made less severe on administering the compound compared to not administering the compound; wherein said determination is indicative of the compound having therapeutic efficacy.

[0492] In a seventeenth aspect, the invention features a method of making a rat genetically predisposed to a metabolic-related disorder selected from the group consisting of:

- [0493] (a) dyslipidemia;
- [0494] (b) atherosclerosis;
- [0495] (c) coronary heart disease;
- [0496] (d) stroke;
- [0497] (e) insulin resistance; and
- [0498] (f) type 2 diabetes;

[0499] comprising the step of knocking out the gene encoding the nicotinic acid rRUP25 GPCR polypeptide of SEQ. ID. NO.:139.

[0500] In some preferred embodiments, said knocking out the gene encoding the nicotinic acid rRUP25 GPCR polypeptide of SEQ. ID. NO.:139 is essentially restricted to adipocytes.

[0501] In an eighteenth aspect, the invention features the knockout rat according to the method of the seventeenth aspect.

[0502] In a nineteenth aspect, the invention features a method of using the knockout rat of the eighteenth aspect to identify whether a candidate compound has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

- [0503] (a) dyslipidemia;
- [0504] (b) atherosclerosis;
- [0505] (c) coronary heart disease;
- [0506] (d) stroke;
- [0507] (e) insulin resistance; and
- [0508] (f) type 2 diabetes;

[0509] comprising the steps of:

- [0510] (a') administering or not administering the compound to the rat; and
- [0511] (b') determining whether the disorder is prevented, delayed, or made less severe on administering the compound compared to not administering the compound; wherein said determination is indicative of the compound having therapeutic efficacy.

[0512] In a twentieth aspect, the invention features an isolated, purified or recombinant RUP25 polynucleotide selected from the group consisting of:

- [0513] (a) a polynucleotide comprising a contiguous span of at least 75 nucleotides of SEQ. ID. NO.:35, SEQ. ID. NO.:136 or SEQ. ID. NO.:138, or an allelic variant of said polynucleotide;
- [0514] (b) a polynucleotide comprising a contiguous span of at least 150 nucleotides of SEQ. ID. NO.:35, SEQ. ID. NO.:136 or SEQ. ID. NO.:138, or an allelic variant of said polynucleotide;
- [0515] (c) a polynucleotide comprising a contiguous span of at least 250 nucleotides of SEQ. ID. NO.:35, SEQ. ID. NO.:136 or SEQ. ID. NO.:138, or an allelic variant of said polynucleotide;
- [0516] (d) a polynucleotide comprising a contiguous span of at least 350 nucleotides of SEQ. ID. NO.:35, SEQ. ID. NO.:136 or SEQ. ID. NO.:138, or an allelic variant of said polynucleotide;
- [0517] (e) a polynucleotide comprising a contiguous span of at least 500 nucleotides of SEQ. ID. NO.:35, SEQ. ID. NO.:136 or SEQ. ID. NO.:138, or an allelic variant of said polynucleotide;
- [0518] (f) a polynucleotide comprising a contiguous span of at least 750 nucleotides of SEQ. ID. NO.:35, SEQ. ID. NO.:136 or SEQ. ID. NO.:138, or an allelic variant of said polynucleotide;
- [0519] (g) a polynucleotide comprising a contiguous span of at least 1000 nucleotides of SEQ. ID. NO.:35, SEQ. ID. NO.:136 or SEQ. ID. NO.:138, or an allelic variant of said polynucleotide;
- [0520] (h) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 20 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139 or an allelic variant of said polypeptide;
- [0521] (i) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 30 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139 or an allelic variant of said polypeptide;
- [0522] (j) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 40 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139 or an allelic variant of said polypeptide;
- [0523] (k) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 50 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139 or an allelic variant of said polypeptide;

- [0524] (l) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 75 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139 or an allelic variant of said polypeptide;
- [0525] (m) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 100 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139 or an allelic variant of said polypeptide;
- [0526] (n) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 150 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139 or an allelic variant of said polypeptide;
- [0527] (o) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 200 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139 or an allelic variant of said polypeptide;
- [0528] (p) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 250 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139 or an allelic variant of said polypeptide; and
- [0529] (q) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 300 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139 or an allelic variant of said polypeptide.
- [0530] The invention also relates to an isolated, purified or recombinant RUP25 polynucleotide wherein said polynucleotide is selected from the group consisting of:
- [0531] (a) a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ. ID. NO.:35, SEQ. ID. NO.:136 and SEQ. ID. NO.:138 or an allelic variant of said polynucleotide;
- [0532] (b) a polynucleotide selected from the group consisting of the polynucleotide of SEQ. ID. NO.:35, the polynucleotide of SEQ. ID. NO.:136 and the polynucleotide of SEQ. ID. NO.:138, or an allelic variant of said polynucleotide;
- [0533] (c) a polynucleotide comprising a nucleotide sequence encoding a polypeptide having an amino acid sequence selected from the group consisting of SEQ. ID. NO.:36, SEQ. ID. NO.:137 and SEQ. ID. NO.:139 or an allelic variant of said polypeptide; and
- [0534] (d) a polynucleotide encoding a polypeptide having an amino acid sequence selected from the group consisting of SEQ. ID. NO.:36, SEQ. ID. NO.:137 and SEQ. ID. NO.:139 or an allelic variant of said polypeptide.
- [0535] In preferred embodiments, said isolated, purified or recombinant polynucleotide comprises at least 8 contiguous nucleotides of a polynucleotide of the present invention. In other preferred embodiments, said isolated, purified or recombinant polynucleotide comprises at least 10, 12, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 contiguous nucleotides of

a polynucleotide of the present invention. Preferably said polynucleotide encodes full-length RUP25 polypeptide or a biologically active fragment thereof.

[0536] The polynucleotides of the present invention include genomic polynucleotides comprising RUP25 polynucleotides of the invention.

[0537] The present invention also relates to a polynucleotide encoding a fusion protein, wherein said fusion protein comprises an RUP25 polypeptide of the invention fused to a heterologous polypeptide. In a preferred embodiment, said polypeptide of the invention is constitutively active and said heterologous polypeptide is a G protein. In other embodiments, said heterologous polypeptide provides an antigenic epitope. In a preferred embodiment, said heterologous polypeptide provides a hemagglutinin (HA) antigenic epitope. Methods relating to a polynucleotide encoding a fusion protein are well known to those of ordinary skill in the art.

[0538] The polynucleotides of the present invention also include variant polynucleotides at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to an RUP25 polynucleotide of the invention. In a particularly preferred embodiment, polynucleotide sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST"), which is well known in the art [See, e.g., Karlin and Altschul, Proc Natl Acad Sci USA (1990) 87:2264-8; Altschul et al., J Mol Biol (1990) 215:403-410; Altschul et al., Nature Genetics (1993) 3:266-72; and Altschul et al., Nucleic Acids Res (1997) 25:3389-3402; the disclosures of which are incorporated by reference in their entirety].

[0539] In further preferred embodiments, the invention features the complement of said polynucleotide.

[0540] In a twenty-first aspect, the invention features an isolated, purified or recombinant RUP25 polypeptide selected from the group consisting of:

- [0541] (a) a polypeptide comprising a contiguous span of at least 20 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;
- [0542] (b) a polypeptide comprising a contiguous span of at least 30 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;
- [0543] (c) a polypeptide comprising a contiguous span of at least 40 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;
- [0544] (d) a polypeptide comprising a contiguous span of at least 50 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;
- [0545] (e) a polypeptide comprising a contiguous span of at least 75 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;
- [0546] (f) a polypeptide comprising a contiguous span of at least 100 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;
- [0547] (g) a polypeptide comprising a contiguous span of at least 150 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;
- [0548] (h) a polypeptide comprising a contiguous span of at least 200 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;

- [0549] (i) a polypeptide comprising a contiguous span of at least 250 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139; and
- [0550] (j) a polypeptide comprising a contiguous span of at least 300 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;
- [0551] or an allelic variant of said polypeptide.
- [0552] The invention also relates to an isolated, purified or recombinant RUP25 polypeptide wherein said polypeptide is selected from the group consisting of:
- [0553] (a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ. ID. NO.:36, SEQ. ID. NO.:137 and SEQ. ID. NO.:139; and
- [0554] (b) a polypeptide selected from the group consisting of the polypeptide of SEQ. ID. NO.:36, the polypeptide of SEQ. ID. NO.:137 and the polypeptide of SEQ. ID. NO.:139;
- [0555] or an allelic variant, a biologically active mutant, or a biologically active fragment of said polypeptide.
- [0556] In preferred embodiments, said isolated, purified or recombinant polypeptide comprises at least 6 contiguous amino acids of an RUP25 polypeptide of the invention. In further embodiments, said isolated, purified or recombinant polypeptide comprises at least 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, 125, 150, 175, 200, 225, 250, 275 or 300 contiguous amino acids of a polypeptide of the present invention. Preferably, said polypeptide is full-length RUP25 polypeptide or an active fragment thereof.
- [0557] The present invention also relates to a fusion protein, wherein said fusion protein comprises an RUP25 polypeptide of the invention fused to a heterologous polypeptide. In a preferred embodiment, said polypeptide of the invention is constitutively active and said heterologous polypeptide is a G protein. In other preferred embodiments, said heterologous polypeptide provides an antigenic epitope. In particularly preferred embodiment, said heterologous polypeptide provides a hemagglutinin (HA) antigenic epitope. Methods relating to a fusion protein are well known to those of ordinary skill in the art.
- [0558] The polypeptides of the present invention also include variant polypeptides at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an RUP25 polypeptide of the invention. In a particularly preferred embodiments, polypeptide sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST"), which is well known in the art [See, e.g., Karlin and Altschul, *Proc Natl Acad Sci USA* (1990) 87:2264-8; Altschul et al., *J Mol Biol* (1990) 215:403-410; Altschul et al., *Nature Genetics* (1993) 3:266-72; and Altschul et al., *Nucleic Acids Res* (1997) 25:3389-3402; the disclosures of which are incorporated by reference in their entirety].
- [0559] In an twenty-second aspect, the invention features a composition comprising, consisting essentially of, or consisting of the RUP25 polypeptide of the twenty-first aspect.
- [0560] In a twenty-third aspect, the invention features a recombinant vector, said vector comprising, consisting essentially of, or consisting of the polynucleotide of the twentieth aspect. In some preferred embodiments, said vec-

tor is a targeting vector used in a method of inactivating a gene encoding a nicotinic acid GPCR of the invention. In other preferred embodiments, said vector is used in a method of transient or stable transfection.

[0561] In particularly preferred embodiment, said vector is an expression vector for the expression of a nicotinic acid GPCR in a recombinant host cell wherein said expression vector comprises, consists essentially of, or consists of the polynucleotide of the twentieth aspect.

[0562] Although a variety of expression vectors are available to those in the art, for purposes of utilization for both the endogenous and non-endogenous human, mouse and rat GPCRs, it is most preferred that the vector utilized be pCMV. In some alternative embodiments as relates to said human, mouse and rat nicotinic acid GPCRs, it is preferred that the vector utilized be an adenoviral expression vector.

[0563] In a twenty-fourth aspect, the invention features a prokaryotic or eukaryotic host cell comprising, consisting essentially of, or consisting of the recombinant vector of the twenty-third aspect. In some preferred embodiments, said host cell is a eukaryotic embryonic stem cell wherein said vector of the twenty-third aspect has been used in a method to inactivate a gene encoding a nicotinic acid GPCR of the invention within said cell. In some other preferred embodiments, said host cell is a eukaryotic embryonic somatic cell wherein said vector of the twenty-third aspect has been used in a method to inactivate a gene encoding a nicotinic acid GPCR of the invention within said cell. In other preferred embodiments, said host cell is prokaryotic and has been transformed using the vector of the twenty-third aspect. In further preferred embodiments, said host cell is eukaryotic and has been transiently transfected using the vector of the twenty-third aspect. In other further preferred embodiments, said host cell is eukaryotic and has been stably transfected using the vector of the twenty-third aspect.

[0564] In particularly preferred embodiment, said host cell expresses a recombinant nicotinic acid GPCR wherein said host cell comprises, consists essentially of, or consists of the expression vector of the twenty-third aspect.

[0565] A further embodiment includes a prokaryotic or eukaryotic host cell recombinant for the polynucleotide of the twentieth aspect.

[0566] In some embodiments the host cell is eukaryotic, more preferably, mammalian, and more preferably selected from the group consisting of 293, 293T, CHO, and COS-7 cells. In other embodiments, the host cell is eukaryotic, more preferably melanophore.

[0567] In a twenty-fifth aspect, the invention features a process for the expression of a nicotinic acid GPCR in a recombinant host cell comprising the steps of:

[0568] (a) transfecting the expression vector of the twenty-third aspect into a suitable host cell; and

[0569] (b) culturing the host cells under conditions which allow expression of the nicotinic acid GPCR protein from the expression vectors.

[0570] In a twenty-sixth aspect, the invention features an antibody that specifically binds to the polypeptide of the twenty-first aspect. In some preferred embodiments, the antibody is monoclonal. In some embodiments, the antibody is polyclonal.

[0571] In a twenty-seventh aspect, the invention features a method of binding the polypeptide of the twenty-first aspect to the antibody of the twenty-sixth aspect, comprising contacting said antibody with said polypeptide under conditions in which said antibody can specifically bind to said polypeptide.

[0572] In a twenty-eighth aspect, the invention features a method of detecting a nicotinic acid GPCR polypeptide in a biological sample obtained from an individual comprising the steps of:

[0573] (a) obtaining said biological sample from said individual;

[0574] (b) contacting said biological sample with the antibody of the twenty-sixth aspect; and

[0575] (c) detecting the presence or absence of binding of said antibody to said biological sample;

[0576] wherein a detection of said binding is indicative of the receptor polypeptide being expressed in said biological sample.

[0577] In preferred embodiments, said detecting is through the use of an enzyme-labeled secondary reagent. In other preferred embodiments, said detecting is through the use of a fluorophore-labeled secondary reagent. In other preferred embodiments, said detecting is through the use of a radioisotope-labeled secondary reagent. In other embodiments, the antibody is directly labeled with enzyme, fluorophore or radioisotope.

[0578] In other preferred embodiments, said biological sample is taken from adipose, skin or blood.

[0579] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[0580] In further embodiments, said individual has a disorder of lipid metabolism selected from the group consisting of:

[0581] (a) elevated level of plasma triglycerides;

[0582] (b) elevated level of plasma free fatty acids;

[0583] (c) elevated level of plasma cholesterol;

[0584] (d) elevated level of LDL-cholesterol;

[0585] (e) reduced level of HDL-cholesterol;

[0586] (f) elevated total cholesterol/HDL-cholesterol ratio; and

[0587] (g) reduced level of plasma adiponectin.

[0588] In other preferred embodiments, said disorder in lipid metabolism is an elevated postprandial increase in plasma free fatty acids due to a high fat meal or a progression from impaired glucose tolerance to insulin resistance.

[0589] In further embodiments, said individual has a metabolic-related disorder selected from the group consisting of:

[0590] (a) dyslipidemia;

[0591] (b) atherosclerosis;

[0592] (c) coronary heart disease;

[0593] (d) stroke;

[0594] (e) insulin resistance; and

[0595] (f) type 2 diabetes.

[0596] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0597] In other embodiments, said method further comprises the step of comparing the level of detection of said binding for a first individual to the level of detection of said binding for a second individual.

[0598] In a twenty-ninth aspect, the invention features a method of detecting expression of a gene encoding a nicotinic acid GPCR in a biological sample obtained from an individual comprising the steps of:

[0599] (a) obtaining said biological sample from said individual;

[0600] (b) contacting said biological sample with the complementary polynucleotide of the twentieth aspect, optionally labeled, under conditions permissive for hybridization; and

[0601] (c) detecting the presence or absence of said hybridization between said complementary polynucleotide and an RNA species within said sample;

[0602] wherein a detection of said hybridization is indicative of expression of said GPCR gene in said biological sample.

[0603] In preferred embodiments, the biological sample is taken from adipose, skin or blood.

[0604] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[0605] In preferred embodiments, said individual has a disorder of lipid metabolism selected from the group consisting of:

[0606] (a) elevated level of plasma triglycerides;

[0607] (b) elevated level of plasma free fatty acids;

[0608] (c) elevated level of plasma cholesterol;

[0609] (d) elevated level of LDL-cholesterol;

[0610] (e) reduced level of HDL-cholesterol;

[0611] (f) elevated total cholesterol/HDL-cholesterol ratio; and

[0612] (g) reduced level of plasma adiponectin.

[0613] In other preferred embodiments, said disorder in lipid metabolism is an elevated postprandial increase in plasma free fatty acids due to a high fat meal or a progression from impaired glucose tolerance to insulin resistance.

[0614] In other preferred embodiments, said individual has a metabolic-related disorder selected from the group consisting of:

- [0615] (a) dyslipidemia;
- [0616] (b) atherosclerosis;
- [0617] (c) coronary heart disease;
- [0618] (d) stroke;
- [0619] (e) insulin resistance; and
- [0620] (f) type 2 diabetes.

[0621] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0622] In other embodiments, said method further comprises the step of comparing the level of detection of said hybridization for a first individual to the level of detection of said hybridization for a second individual.

[0623] In some preferred embodiments, said complementary polynucleotide is a primer and said hybridization is detected by detecting the presence of an amplification product comprising the sequence of said primer. In more preferred embodiments, said method is RT-PCR.

[0624] In a thirtieth aspect, the invention features a GPCR Fusion Protein construct comprising a constitutively active GPCR and a G protein, said receptor comprising an amino acid sequence selected from the group consisting of:

- [0625] (a) SEQ. ID. NO.:36 (hRUP25);
- [0626] (b) SEQ. ID. NO.:137 (mRUP25); and
- [0627] (c) SEQ. ID. NO.:139 (rRUP25);

[0628] or an allelic variant or a biologically active fragment of said amino acid sequence.

[0629] The invention also relates to a GPCR Fusion Protein construct wherein the isoleucine at amino acid position 230 of SEQ. ID. NO.:36 is substituted by lysine.

[0630] In a thirty-first aspect, the invention features a method of binding a known ligand of RUP25 nicotinic acid GPCR to a polypeptide selected from the group consisting of:

- [0631] (a) a polypeptide comprising a contiguous span of at least 6 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;
- [0632] (b) a polypeptide comprising a contiguous span of at least 10 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;
- [0633] (c) a polypeptide comprising a contiguous span of at least 15 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;
- [0634] (d) a polypeptide comprising a contiguous span of at least 20 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;

[0635] (e) a polypeptide comprising a contiguous span of at least 25 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;

[0636] (f) a polypeptide comprising a contiguous span of at least 30 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;

[0637] (g) a polypeptide comprising a contiguous span of at least 35 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;

[0638] (h) a polypeptide comprising a contiguous span of at least 40 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;

[0639] (i) a polypeptide comprising a contiguous span of at least 45 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139; and

[0640] (j) a polypeptide comprising a contiguous span of at least 50 amino acids of SEQ. ID. NO.:36, SEQ. ID. NO.:137 or SEQ. ID. NO.:139;

[0641] or an allelic variant of said polypeptide;

[0642] comprising the step of contacting said known ligand with said polypeptide under conditions which allow said binding to occur.

[0643] In some embodiments, said known ligand is a modulator of the nicotinic acid GPCR. In some embodiments, said known ligand is an agonist of the nicotinic acid GPCR. In other embodiments, said agonist is nicotinic acid or an analog or derivative thereof. In other embodiments, said agonist is (-)-nicotine or an analog or derivative thereof. In some embodiments, said known ligand is the modulator of the second aspect. In some embodiments, said known ligand is an antibody specific for the GPCR, or a derivative thereof.

[0644] In other preferred embodiments, said method is used to identify whether a candidate compound inhibits said binding of said known ligand to said polypeptide, comprising the steps of:

[0645] (a) contacting said polypeptide with said known ligand, optionally labeled, in the presence or absence of said candidate compound;

[0646] (b) detecting the complex between said known ligand and said polypeptide; and

[0647] (c) determining whether less of said complex is formed in the presence of the compound than in the absence of the compound;

[0648] wherein said determination is indicative of the candidate compound being an inhibitor of said binding of said known ligand to said polypeptide.

[0649] In some embodiments, said known ligand is a known modulator of the nicotinic acid GPCR. In some embodiments, said known ligand is a modulator of the nicotinic acid GPCR. In some embodiments, said known ligand is an agonist of the nicotinic acid GPCR. In some embodiments, said agonist is nicotinic acid or an analog or derivative thereof. In other embodiments, said agonist is (-)-nicotine or an analog or derivative thereof. In some embodiments, said known ligand is the modulator of the

second aspect. In some embodiments, said known ligand is an antibody specific for the GPCR, or a derivative thereof.

[0650] In other preferred embodiments, said method is used to identify whether a candidate compound is an inhibitor of said binding of said known ligand to said polypeptide, comprising the steps of:

[0651] (a) contacting said polypeptide with said known ligand, optionally labeled, in the presence separately of a plurality of concentrations of said candidate compound for a time sufficient to allow equilibration of binding;

[0652] (b) measuring unbound ligand and bound ligand; and

[0653] (c) determining  $K_i$  for the candidate compound;

[0654] wherein a  $K_i$  value of less than 50  $\mu$ M is indicative of the candidate compound being an inhibitor of said binding of said known ligand to said polypeptide. Preferably said  $K_i$  value is less than 25  $\mu$ M, 10  $\mu$ M, 5  $\mu$ M, 1  $\mu$ M, 750 nM, 500 nM, 400 nM, 300 nM, 250 nM, 200 nM, 150 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM or 10 nM. In preferred embodiments,  $K_i$  determination is made through nonlinear curve fitting with the program SCTFIT [De Lean et al. (1982) Mol Pharmacol 21:5-16; cited in Lorenzen et al. (2001) Mol Pharmacol 59:349-357, the disclosures of which are incorporated by reference herein in their entireties].

[0655] In some embodiments, said known ligand is a modulator of the nicotinic acid GPCR. In some embodiments, said known ligand is an agonist of the nicotinic acid GPCR. In other embodiments, said agonist is nicotinic acid or an analog or derivative thereof. In other embodiments, said agonist is (-)-nicotine or an analog or derivative thereof. In some embodiments, said known ligand is the modulator of the second aspect. In some embodiments, said known ligand is an antibody specific for the GPCR, or a derivative thereof.

[0656] In a thirty-second aspect, the invention features a method of binding an optionally labeled affinity reagent specific for a nicotinic acid GPCR to said receptor in a biological sample, said receptor comprising an amino acid sequence selected from the group consisting of:

[0657] (a) SEQ. ID. NO.:36 (hRUP25);

[0658] (b) SEQ. ID. NO.:137 (mRUP25); and

[0659] (c) SEQ. ID. NO.:139 (rRUP25);

[0660] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence, comprising the steps of:

[0661] (a') obtaining said biological sample;

[0662] (b') contacting the affinity reagent with said receptor in said biological sample; and

[0663] (c') detecting the complex of said affinity reagent with said receptor.

[0664] In some embodiments, the nicotinic acid GPCR has an amino acid sequence selected from the group consisting of:

[0665] (a) SEQ. ID. NO.:36 (hRUP25);

[0666] (b) SEQ. ID. NO.:137 (mRUP25); and

[0667] (c) SEQ. ID. NO.:139 (rRUP25);

[0668] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence.

[0669] In some embodiments, the nicotinic acid GPCR comprises an active fragment of said amino acid sequence.

[0670] In some embodiments, the nicotinic acid GPCR is endogenous.

[0671] In some embodiments, the nicotinic acid GPCR is recombinant.

[0672] In some embodiments, said biologically active mutant is CART or EFA. In preferred embodiments, said CART mutant has the amino acid sequence of SEQ. ID. NO.:36 further substituted at amino acid position 230 with lysine in place of isoleucine. In preferred embodiments, said EFA mutant has the amino acid sequence of SEQ. ID. NO.:159.

[0673] In preferred embodiments, said G protein is  $G_i$ .

[0674] In some embodiments, said affinity reagent is a modulator of the GPCR. In some embodiments, said affinity reagent is an agonist of the GPCR. In some embodiments, said affinity reagent is nicotinic acid or an analog or derivative thereof. In some embodiments, said affinity reagent is (-)-nicotine or an analog or derivative thereof. In some embodiments, said affinity reagent is the modulator of the second aspect. In some embodiments, said known ligand is an antibody specific for the GPCR, or a derivative thereof.

[0675] In further preferred embodiments, said affinity reagent comprises a label selected from the group consisting of:

[0676] (a) radioisotope;

[0677] (b) enzyme; and

[0678] (c) fluorophore.

[0679] In preferred embodiments, said radioisotope is  $^3H$ .

[0680] In a thirty-third aspect, the invention features the method of the thirty-second aspect further comprising the step of comparing the level of detection of said complex in a first biological sample to a second level of detection of said complex in a second biological sample.

[0681] In a thirty-fourth aspect, the invention features the method of the thirty-third aspect wherein the relationship between said first and second biological samples is selected from the group consisting of:

[0682] (a) said second biological sample is a replicate of said first biological sample;

[0683] (b) said first biological sample was obtained prior to an experimental intervention whereas said second biological sample was obtained after the experimental intervention, from the same individual;

[0684] (c) said second biological sample was obtained at a different time point after an experimental intervention than was said first biological sample, from the same individual;

- [0685] (d) said second biological sample corresponds to a different subcellular compartment than does said first biological sample;
- [0686] (e) said second biological sample represents a different cell type than does said first biological sample;
- [0687] (f) said second biological sample corresponds to a different tissue than does said first biological sample;
- [0688] (g) said second biological sample was obtained from a different individual than was said first biological sample;
- [0689] (h) said second biological sample was obtained at a different point in time than was said first biological sample, from the same individual;
- [0690] (i) said first biological samples was obtained from a normal individual, whereas said second biological sample was obtained from an individual having a metabolic-related disorder;
- [0691] (o) said first biological sample was obtained from a normal individual, whereas said second biological sample was obtained from an individual having a disorder in lipid metabolism;
- [0692] (k) said first biological sample was obtained before a therapeutic intervention whereas said second biological sample was obtained after the therapeutic intervention, from the same individual;
- [0693] (l) said second biological sample was obtained at a different time point after therapeutic intervention than was said first biological sample, from the same individual; and
- [0694] (m) said first biological sample was not exposed to a compound, whereas said second biological sample was exposed to said compound.
- [0695] In a thirty-fifth aspect, the invention features a method of identifying whether a candidate compound is a modulator of an antilipolytic GPCR, said receptor comprising the amino acid sequence of SEQ. ID. NO.:135 (hRUP38);
- [0696] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;
- [0697] comprising the steps of:
- [0698] (a) contacting the candidate compound with the receptor;
- [0699] (b) determining whether the receptor functionality is modulated;
- [0700] wherein a change in receptor functionality is indicative of the candidate compound being a modulator of an antilipolytic GPCR.
- [0701] In some embodiments, said antilipolytic GPCR is endogenous.
- [0702] In some preferred embodiments, said antilipolytic GPCR is recombinant.
- [0703] Preferred said identified modulator binds to said GPCR.
- [0704] In some embodiments, said contacting is carried out in the presence of a known ligand of the GPCR. In some embodiments, said known ligand is an agonist of the GPCR.
- [0705] The invention also relates to a method of identifying whether a candidate compound is a modulator of lipolysis, comprising the steps of:
- [0706] (a) contacting the candidate compound with a GPCR comprising the amino acid sequence of SEQ. ID. NO.:135 (hRUP38);
- [0707] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence; and
- [0708] (b) determining whether the receptor functionality is modulated;
- [0709] wherein a change in receptor functionality is indicative of the candidate compound being a modulator of lipolysis.
- [0710] In some embodiments, said GPCR is endogenous.
- [0711] In some preferred embodiments, said GPCR is recombinant.
- [0712] Preferred said identified modulator binds to said GPCR.
- [0713] In some embodiments, said contacting is carried out in the presence of a known ligand of the GPCR. In some embodiments, said known ligand is an agonist of the GPCR.
- [0714] The invention also relates to a method of determining whether a candidate compound is a modulator of an antilipolytic GPCR,
- [0715] comprising the steps of:
- [0716] (a) culturing antilipolytic GPCR-expressing host cells under conditions that would allow expression of a recombinant antilipolytic GPCR, said host cells being transfected with a polynucleotide encoding said recombinant antilipolytic GPCR comprising the amino acid sequence of SEQ. ID. NO.:135 (hRUP38); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;
- [0717] (b) contacting the antilipolytic GPCR-expressing host cells of step (a) with the candidate compound;
- [0718] (c) contacting control host cells with the candidate compound of step (b), wherein said control host cells do not express recombinant antilipolytic GPCR protein;
- [0719] (d) measuring the modulating effect of the candidate compound which interacts with the recombinant antilipolytic GPCR from the host cells of step (a) and control host cells of step (c); and
- [0720] (e) comparing the modulating effect of the test compound on the host cells and control host cells.

[0721] The invention also relates to a method of determining whether a candidate compound is a modulator of an antilipolytic GPCR, comprising the steps of:

[0722] (a) culturing antilipolytic GPCR-expressing host cells under conditions that would allow expression of a recombinant antilipolytic GPCR, said host cells being transfected with a polynucleotide encoding said recombinant antilipolytic GPCR comprising the amino acid sequence of SEQ. ID. NO.:135 (hRUP38); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

[0723] (b) contacting a first population of antilipolytic GPCR-expressing cells of step (a) with a known ligand of said antilipolytic GPCR;

[0724] (c) contacting a second population of antilipolytic GPCR-expressing cells of step (a) with the candidate compound and with the known antilipolytic GPCR ligand;

[0725] (d) contacting control host cells with the candidate compound of step (c), wherein said control host cells do not express recombinant antilipolytic GPCR protein;

[0726] (e) measuring the modulating effect of the candidate compound, which interacts with recombinant antilipolytic GPCR, in the presence and absence of the known antilipolytic GPCR ligand, from the cells of step (b), step (c) and step (d); and

[0727] (f) comparing the modulating effect of the candidate compound as determined from step (b), step (c) and step (d).

[0728] In some embodiments, said known ligand is an agonist of the GPCR.

[0729] The invention also relates to a method of determining whether a candidate compound is a modulator of an antilipolytic GPCR,

[0730] comprising the steps of:

[0731] (a) culturing antilipolytic GPCR-expressing host cells under conditions that would allow expression of a recombinant antilipolytic GPCR, said host cells being transfected with a polynucleotide encoding said recombinant antilipolytic GPCR comprising the amino acid sequence of SEQ. ID. NO.:135 (hRUP38); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

[0732] (b) contacting a first population of the antilipolytic GPCR-expressing host cells of step (a) with the candidate compound;

[0733] (c) not contacting a second population of the antilipolytic GPCR-expressing cells of step (a) with the candidate compound of step (b);

[0734] (d) contacting control host cells to the candidate compound of step (b), wherein said control host cells do not express recombinant antilipolytic GPCR protein;

[0735] (e) measuring the modulating effect of the candidate compound, which interacts with recombinant antilipolytic GPCR protein, from the cells of step (b) and step (c) and from the cells of step (d); and

[0736] (f) comparing the modulating effect of the candidate compound as determined from step (b) and step (c) and from step (d).

[0737] In some embodiments, the antilipolytic GPCR has the amino acid sequence of SEQ. ID. NO.:135 (hRUP38); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence.

[0738] In some embodiments, the antilipolytic GPCR comprises a biologically active fragment of said amino acid sequence.

[0739] In some embodiments, said biologically active mutant is CART or EFA. In preferred embodiments, said CART mutant has the amino acid sequence of SEQ. ID. NO.:135 further substituted at amino acid position 230 with lysine in place of isoleucine.

[0740] In preferred embodiments, said G protein is Gi.

[0741] In other preferred embodiments, said determining is through the use of a Melanophore assay.

[0742] In other preferred embodiments, said determining is through the measurement of the level of a second messenger selected from the group consisting of cyclic AMP (cAMP), cyclic GMP (cGMP), inositol triphosphate (IP<sub>3</sub>), diacylglycerol (DAG), and Ca<sup>2+</sup>. In further preferred embodiments, said second messenger is cAMP. In more preferred embodiments, the level of the cAMP is reduced. In some embodiments, said measurement of cAMP is carried out with membrane comprising said GPCR.

[0743] In other preferred embodiments, said determining is through the measurement of an activity up-regulated or down-regulated by a reduction in intracellular cAMP level. In further preferred embodiments, said down-regulated activity is intracellular lipolysis. In other further preferred embodiments, said down-regulated activity is hormone sensitive lipase activity. In other further preferred embodiments, said up-regulated activity is adiponectin secretion.

[0744] In other preferred embodiments, said determining is through CRE-reporter assay. In preferred embodiments, said reporter is luciferase. In some embodiments, said reporter is  $\beta$ -galactosidase.

[0745] In other embodiments, said recombinant host cell further comprises promiscuous G alpha 15/16 or chimeric Gq/Gi alpha subunit and said determining is through measurement of intracellular Ca<sup>2+</sup>. In preferred embodiments, said Ca<sup>2+</sup> measurement is carried out by FLIPR.

[0746] In other embodiments, said recombinant host cell further comprises promiscuous G alpha 15/16 or chimeric Gq/Gi alpha subunit and said determining is through measurement of intracellular IP<sub>3</sub>.

[0747] In other preferred embodiments, said determining is through the measurement of GTP $\gamma$ S binding to membrane comprising said GPCR. In further preferred embodiments, said GTP $\gamma$ S is labeled with [<sup>35</sup>S].

[0748] In other preferred embodiments, said method further comprises the step of comparing the modulation of the receptor caused by the candidate compound to a second modulation of the receptor caused by contacting the receptor with a known modulator of the receptor. In some preferred embodiments, said known modulator is an agonist.

[0749] In a thirty-sixth aspect, the invention features a modulator of an antilipolytic GPCR identified according to the method of the thirty-fifth aspect.

[0750] In some preferred embodiments, said modulator is selected from the group consisting of agonist, partial agonist, inverse agonist and antagonist. More preferably, said modulator is an agonist. In some embodiments, said modulator is a partial agonist.

[0751] In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $1000\ \mu\text{M}$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP38 polypeptide having the amino acid sequence of SEQ. ID. NO.:135. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $900\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $800\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $700\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $600\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $550\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $500\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $450\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $400\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $350\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $300\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $250\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $200\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $150\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $100\ \mu\text{M}$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of  $600\ \mu\text{M}$  to  $1000\ \mu\text{M}$ .

[0752] In some embodiments, said modulator is selective for the GPCR.

[0753] In some embodiments, said modulator is antilipolytic.

[0754] In some embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some preferred embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to

intravenous administration. In some preferred embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0755] In highly less preferred embodiments, said modulator is an antibody or derivative thereof.

[0756] In a thirty-seventh aspect, the invention features the method of the thirty-fifth aspect, wherein said candidate compound is an agonist of hRUP25 GPCR comprising the amino acid sequence of SEQ. ID. NO.:36 and wherein said method further comprises the step of comparing the modulation of hRUP38 GPCR comprising the amino acid sequence of SEQ. ID. NO.:135 caused by said agonist to a second modulation of hRUP38 GPCR comprising a variant of said amino acid sequence caused by contacting the variant hRUP38 GPCR with said agonist.

[0757] In preferred embodiments, said variant amino acid sequence is identical to hRUP38 polypeptide of SEQ. ID. NO.:135, further comprising a single amino acid substitution selected from the group consisting of:

[0758] (a) V for A at amino acid position 27 of SEQ. ID. NO.:135;

[0759] (b) L for V at amino acid position 83 of SEQ. ID. NO.:135;

[0760] (c) N for Y at amino acid position 86 of SEQ. ID. NO.:135;

[0761] (d) W for S at amino acid position 91 of SEQ. ID. NO.:135;

[0762] (e) K for N at amino acid position 94 of SEQ. ID. NO.:135;

[0763] (f) M for V at amino acid position 103 of SEQ. ID. NO.:135;

[0764] (g) L for F at amino acid position 107 of SEQ. ID. NO.:135;

[0765] (h) R for W at amino acid position 142 of SEQ. ID. NO.:135;

[0766] (i) I for V at amino acid position 156 of SEQ. ID. NO.:135;

[0767] (j) M for L at amino acid position 167 of SEQ. ID. NO.:135;

[0768] (k) P for L at amino acid position 168 of SEQ. ID. NO.:135;

[0769] (l) G for P at amino acid position 173 of SEQ. ID. NO.:135;

[0770] (m) L for V at amino acid position 176 of SEQ. ID. NO.:135;

[0771] (n) S for 1 at amino acid position 178 of SEQ. ID. NO.:135;

[0772] (o) Q for R at amino acid position 187 of SEQ. ID. NO.:135;

[0773] (p) F for L at amino acid position 198 of SEQ. ID. NO.:135; and

[0774] (q) P for N at amino acid position 363 of SEQ. ID. NO.:135.

[0775] In particularly preferred embodiments, said method is used to identify whether said substituted amino acid additionally found at the identical position within SEQ. ID. NO.:36 is necessary for modulation of said hRUP25 GPCR by said agonist, comprising the steps of:

[0776] (a) determining the level of modulation of said hRUP38 GPCR by said agonist; and

[0777] (b) determining the level of modulation of said variant hRUP38 GPCR by said agonist;

[0778] wherein if said level of modulation for (b) is greater than said level of modulation for (a), then said substituted amino acid is necessary for modulation of said hRUP25 GPCR by said agonist.

[0779] In a thirty-eighth aspect, the invention features a method of modulating the activity of an antilipolytic GPCR, said receptor comprising the amino acid sequence of SEQ. ID. NO.:135 (hRUP38); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

[0780] comprising the step of contacting the receptor with the modulator of the thirty-sixth aspect.

[0781] In some embodiments, the antilipolytic GPCR has the amino acid sequence of SEQ. ID. NO.:135 (hRUP38); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence.

[0782] In some embodiments, the antilipolytic GPCR comprises an active fragment of said amino acid sequence.

[0783] In some embodiments, the antilipolytic GPCR is endogenous.

[0784] In some embodiments, the antilipolytic GPCR is recombinant.

[0785] In some embodiments, said biologically active mutant is CART or EFA. In preferred embodiments, said CART mutant has the amino acid sequence of SEQ. ID. NO.:135 further substituted at amino acid position 230 with lysine in place of isoleucine.

[0786] In preferred embodiments, said G protein is Gi.

[0787] In some preferred embodiments, said modulator is an agonist.

[0788] In preferred embodiments, said modulator is selective for the GPCR.

[0789] In other preferred embodiments, said contacting comprises administration of the modulator to a membrane comprising the receptor.

[0790] In other preferred embodiments, said contacting comprises administration of the modulator to a cell or tissue comprising the receptor.

[0791] In other preferred embodiments, said contacting comprises administration of the modulator to an individual comprising the receptor. In more preferred embodiments, said individual is a mammal. In other more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[0792] In some preferred embodiments, said modulator is selective for the GPCR.

[0793] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0794] In some preferred embodiments, said modulator is antilipolytic.

[0795] In some preferred embodiments, said modulator is an agonist.

[0796] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTPyS binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP38 polypeptide having the amino acid sequence of SEQ. ID. NO.:135. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[0797] In some preferred embodiments, said administration is oral.

[0798] In preferred embodiments, said modulator is an agonist and said individual is in need of prevention of or treatment for a metabolic-related disorder selected from the group consisting of:

[0799] (a) dyslipidemia;

[0800] (b) atherosclerosis;

[0801] (c) coronary heart disease;

[0802] (d) stroke;

[0803] (e) insulin resistance; and

[0804] (f) type 2 diabetes.

[0805] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0806] In other embodiments, said modulator is an inverse agonist and said metabolic-related disorder relates to a low level of plasma free fatty acids.

[0807] In other preferred embodiments, said modulator is an agonist and said individual is in need of a change in lipid metabolism selected from the group consisting of:

[0808] (a) a decrease in the level of plasma triglycerides;

[0809] (b) a decrease in the level of plasma free fatty acids;

[0810] (c) a decrease in the level of plasma cholesterol;

[0811] (d) a decrease in the level of LDL-cholesterol;

[0812] (e) an increase in the level of HDL-cholesterol;

[0813] (f) a decrease in the total cholesterol/HDL-cholesterol ratio; and

[0814] (g) an increase in the level of plasma adiponectin.

[0815] In other preferred embodiments, said needed change in lipid metabolism is a decrease in the postprandial increase in plasma free fatty acids due to a high fat meal or an inhibition of the progression from impaired glucose tolerance to insulin resistance.

[0816] In some embodiments, the modulator is an inverse agonist and the needed change in lipid metabolism is an increase in the level of plasma free fatty acids.

[0817] In other preferred embodiments, said modulator is an agonist and said individual is a mouse genetically predisposed to a metabolic-related disorder selected from the group consisting of:

[0818] (a) dyslipidemia;

[0819] (b) atherosclerosis;

[0820] (c) coronary heart disease;

[0821] (d) stroke;

[0822] (e) insulin resistance; and

[0823] (f) type 2 diabetes.

[0824] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease

includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure.

[0825] In further preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0826] In further preferred embodiments, said method is used to identify whether said agonist has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

[0827] (a) dyslipidemia;

[0828] (b) atherosclerosis;

[0829] (c) coronary heart disease;

[0830] (d) stroke;

[0831] (e) insulin resistance; and

[0832] (f) type 2 diabetes;

[0833] comprising the steps of:

[0834] (a') administering or not administering said agonist to the mouse; and

[0835] (b') determining whether the disorder is prevented, delayed, or made less severe on administering said agonist compared to not administering said agonist;

[0836] wherein said determination is indicative of said agonist having therapeutic efficacy.

[0837] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0838] In other preferred embodiments, said modulator is an agonist and said individual is a rat genetically predisposed to a metabolic-related disorder selected from the group consisting of:

[0839] (a) dyslipidemia;

[0840] (b) atherosclerosis;

[0841] (c) coronary heart disease;

[0842] (d) stroke;

[0843] (e) insulin resistance; and

[0844] (f) type 2 diabetes.

[0845] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In further preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0846] In further preferred embodiments, said method is used to identify whether said agonist has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

- [0847] (a) dyslipidemia;
- [0848] (b) atherosclerosis;
- [0849] (c) coronary heart disease;
- [0850] (d) stroke;
- [0851] (e) insulin resistance; and
- [0852] (f) type 2 diabetes;

[0853] comprising the steps of:

- [0854] (a') administering or not administering said agonist to the rat; and
- [0855] (b') determining whether the disorder is prevented, delayed, or made less severe on administering said agonist compared to not administering said agonist;

[0856] wherein said determination is indicative of said agonist having therapeutic efficacy.

[0857] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0858] In a thirty-ninth aspect, the invention features a method of preventing or treating a disorder of lipid metabolism in an individual comprising contacting a therapeutically effective amount of the modulator of the thirty-sixth aspect with an antilipolytic GPCR, said receptor comprising the amino acid sequence of SEQ. ID. NO.:135 (hRUP38);

[0859] or an allelic variant or biologically active fragment of said amino acid sequence.

[0860] In some preferred embodiments, said modulator is selective for the GPCR.

[0861] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0862] In some preferred embodiments, said modulator is antilipolytic.

[0863] In some preferred embodiments, said modulator is an agonist.

[0864] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably trans-

fected CHO cells expressing recombinant hRUP38 polypeptide having the amino acid sequence of SEQ. ID. NO.:135. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[0865] In some preferred embodiments, said contacting comprises oral administration of said modulator to said individual.

[0866] In preferred embodiment, said modulator is an agonist and said disorder of lipid metabolism is selected from the group consisting of:

- [0867] (a) elevated level of plasma triglycerides;
- [0868] (b) elevated level of plasma free fatty acids;
- [0869] (c) elevated level of plasma cholesterol;
- [0870] (d) elevated level of LDL-cholesterol;
- [0871] (e) reduced level of HDL-cholesterol;
- [0872] (f) elevated total cholesterol/HDL-cholesterol ratio; and
- [0873] (g) reduced level of plasma adiponectin.

[0874] In a fortieth aspect, the invention features a method of preventing or treating a metabolic-related disorder in an individual comprising contacting a therapeutically effective amount of the modulator of the thirty-sixth aspect with an antilipolytic GPCR, said receptor comprising the amino acid sequence of SEQ. ID. NO.:135 (hRUP38);

[0875] or an allelic variant or biologically active fragment of said amino acid sequence.

[0876] In some preferred embodiments, said modulator is selective for the GPCR.

[0877] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodi-

ments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0878] In some preferred embodiments, said modulator is antilipolytic.

[0879] In some preferred embodiments, said modulator is an agonist.

[0880] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP38 polypeptide having the amino acid sequence of SEQ. ID. NO.:135. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[0881] In some preferred embodiments, said contacting comprises oral administration of said modulator to said individual.

[0882] In preferred embodiment, said modulator is an agonist and said metabolic-related disorder is selected from the group consisting of:

[0883] (a) dyslipidemia;

[0884] (b) atherosclerosis;

[0885] (c) coronary heart disease;

[0886] (d) stroke;

[0887] (e) insulin resistance; and

[0888] (f) type 2 diabetes.

[0889] In a forty-first aspect, the invention features a method of preparing a composition which comprises identifying a modulator of an antilipolytic GPCR and then admixing a carrier and the modulator, wherein the modulator is identifiable by the method of the thirty-fifth aspect.

[0890] In some preferred embodiments, said modulator is selected from the group consisting of agonist, partial agonist, inverse agonist and antagonist. More preferably, said modulator is an agonist. In some embodiments, said modulator is a partial agonist.

[0891] In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP38 polypeptide having the amino acid sequence of SEQ. ID. NO.:135. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[0892] In some embodiments, said modulator is selective for the GPCR.

[0893] In some embodiments, said modulator is antilipolytic.

[0894] In some embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to either intraperitoneal or intravenous administration. In some preferred embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to either intraperitoneal or intravenous administration.

[0895] In a forty-second aspect, the invention features a pharmaceutical or physiologically acceptable composition comprising, consisting essentially of, or consisting of the modulator of the thirty-sixth aspect. In preferred embodiments, said modulator is an agonist.

[0896] In some preferred embodiments, said modulator is selective for the GPCR.

[0897] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%,

at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0898] In some preferred embodiments, said modulator is antilipolytic.

[0899] In some preferred embodiments, said modulator is an agonist.

[0900] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP38 polypeptide having the amino acid sequence of SEQ. ID. NO.:135. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[0901] In a forty-third aspect, the invention features a method of changing lipid metabolism comprising providing or administering to an individual in need of said change said pharmaceutical or physiologically acceptable composition of the forty-second aspect, said needed change in lipid metabolism selected from the group consisting of:

- [0902] (a) a decrease in the level of plasma triglycerides;
- [0903] (b) a decrease in the level of plasma free fatty acids;
- [0904] (c) a decrease in the level of plasma cholesterol;
- [0905] (d) a decrease in the level of LDL-cholesterol;

[0906] (e) an increase in the level of HDL-cholesterol;

[0907] (f) a decrease in the total cholesterol/HDL-cholesterol ratio; and

[0908] (g) an increase in the level of plasma adiponectin.

[0909] In preferred embodiments, a therapeutically effective amount of said pharmaceutical or physiologically acceptable composition is provided or administered to said individual.

[0910] In some preferred embodiments, said providing or administering of said pharmaceutical or physiologically acceptable composition is oral.

[0911] In other preferred embodiments, said needed change in lipid metabolism is a decrease in the postprandial increase in plasma free fatty acids due to a high fat meal or an inhibition of the progression from impaired glucose tolerance to insulin resistance.

[0912] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[0913] In a forty-fourth aspect, the invention features a method of preventing or treating a metabolic-related disorder comprising providing or administering to an individual in need of said treatment said pharmaceutical or physiologically acceptable composition of the forty-second aspect, said metabolic-related disorder selected from the group consisting of:

- [0914] (a) dyslipidemia;
- [0915] (b) atherosclerosis;
- [0916] (c) coronary heart disease;
- [0917] (d) stroke;
- [0918] (e) insulin resistance; and
- [0919] (f) type 2 diabetes.

[0920] In preferred embodiments, a therapeutically effective amount of said pharmaceutical or physiologically acceptable composition is provided or administered to said individual.

[0921] In some preferred embodiments, said providing or administering of said pharmaceutical or physiologically acceptable composition is oral.

[0922] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, stroke, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0923] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[0924] In an forty-fifth aspect, the invention features a method of using the modulator of the thirty-sixth aspect for the preparation of a medicament for the treatment of a disorder in lipid metabolism in an individual.

[0925] In some preferred embodiments, said modulator is selective for the GPCR.

[0926] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0927] In some preferred embodiments, said modulator is antilipolytic.

[0928] In some preferred embodiments, said modulator is an agonist.

[0929] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP38 polypeptide having the amino acid sequence of SEQ. ID. NO.:135. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[0930] In some preferred embodiments, said treatment comprises oral administration of said medicament to said individual.

[0931] In preferred embodiments, said modulator is an agonist and said disorder in lipid metabolism is selected from the group consisting of:

- [0932] (a) elevated level of plasma triglycerides;
- [0933] (b) elevated level of plasma free fatty acids;
- [0934] (c) elevated level of plasma cholesterol;
- [0935] (d) elevated level of LDL-cholesterol;
- [0936] (e) reduced level of HDL-cholesterol;
- [0937] (f) elevated total cholesterol/HDL-cholesterol ratio; and
- [0938] (g) reduced level of plasma adiponectin.

[0939] In other preferred embodiments, said disorder in lipid metabolism is an elevated postprandial increase in plasma free fatty acids due to a high fat meal or a progression from impaired glucose tolerance to insulin resistance.

[0940] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[0941] In a forty-sixth aspect, the invention features a method of using the modulator of the thirty-sixth aspect for the preparation of a medicament for the treatment of a metabolic-related disorder in an individual.

[0942] In some preferred embodiments, said modulator is selective for the GPCR.

[0943] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[0944] In some preferred embodiments, said modulator is antilipolytic.

[0945] In some preferred embodiments, said modulator is an agonist.

[0946] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP38 polypeptide having the amino acid sequence of SEQ. ID. NO.:135. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an

EC<sub>50</sub> of less than 450  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 400  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 350  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 300  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 250  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 200  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 150  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 100  $\mu$ M in said assay. In some preferred embodiments, said modulator is an agonist with an EC<sub>50</sub> in said assay of less than a value selected from the interval of 600  $\mu$ M to 1000  $\mu$ M.

[0947] In some preferred embodiments, said treatment comprises oral administration of said medicament to said individual.

[0948] In preferred embodiments, said modulator is an agonist and said metabolic-related disorder is selected from the group consisting of:

- [0949] (a) dyslipidemia;
- [0950] (b) atherosclerosis;
- [0951] (c) coronary heart disease;
- [0952] (d) stroke;
- [0953] (e) insulin resistance; and
- [0954] (f) type 2 diabetes.

[0955] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[0956] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[0957] In a forty-seventh aspect, the invention features a method of identifying whether a candidate compound is binds to an antilipolytic GPCR, said receptor comprising the amino acid sequence of SEQ. ID. NO.:135 (hRUP38); or an allelic variant or a biologically active fragment of said amino acid sequence; comprising the steps of:

- [0958] (a) contacting the receptor with a labeled reference compound known to bind to the GPCR in the presence or absence of the candidate compound; and
- [0959] (b) determining whether the binding of said labeled reference compound to the receptor is inhibited in the presence of the candidate compound;
- [0960] wherein said inhibition is indicative of the candidate compound binding to an antilipolytic GPCR.

[0961] In some embodiments, the antilipolytic GPCR comprises a biologically active fragment of said amino acid sequence.

[0962] In some embodiments, the antilipolytic GPCR is endogenous.

[0963] In some embodiments, the antilipolytic GPCR is recombinant.

[0964] In preferred embodiments, said G protein is Gi.

[0965] In some preferred embodiments, said reference compound is the modulator of the thirty-sixth aspect.

[0966] In other embodiments, said reference compound is an antibody specific for the GPCR, or a derivative thereof.

[0967] In preferred embodiments, said reference compound comprises a label selected from the group consisting of:

- [0968] (a) radioisotope;
- [0969] (b) enzyme; and
- [0970] (c) fluorophore.

[0971] In some preferred embodiments, said label is <sup>3</sup>H.

[0972] In other embodiments, said method further comprises the step of comparing the level of inhibition of binding of a labeled first reference compound by the candidate compound to a second level of inhibition of binding of said labeled first reference compound by a second reference compound known to bind to the GPCR.

[0973] In a forty-eighth aspect, the invention features a method of making a transgenic mouse, comprising the step of engineering said mouse to carry as part of its own genetic material the gene encoding the human antilipolytic GPCR polypeptide of SEQ. ID. NO.:135 (hRUP38).

[0974] In some preferred embodiments, expression of said gene is placed under the control of an essentially adipocyte specific promoter.

[0975] In a forty-ninth aspect, the invention features the transgenic mouse according to the method of the forty-eighth aspect.

[0976] In a fiftieth aspect, the invention features a method of using the transgenic mouse of the forty-ninth aspect to identify whether an agonist of said human receptor has therapeutic efficacy for the treatment of a disorder of lipid metabolism selected from the group consisting of:

- [0977] (a) elevated level of plasma triglycerides;
- [0978] (b) elevated level of plasma free fatty acids;
- [0979] (c) elevated level of plasma cholesterol;
- [0980] (d) elevated level of LDL-cholesterol;
- [0981] (e) reduced level of HDL-cholesterol;
- [0982] (f) elevated total cholesterol/HDL-cholesterol ratio; and
- [0983] (g) reduced level of plasma adiponectin;

[0984] comprising the steps of:

- [0985] (a') administering or not administering the agonist to the mouse; and

[0986] (b') determining whether on administering the agonist there is a change selected from the group consisting of:

[0987] (i) a decrease in the level of plasma triglycerides;

[0988] (ii) a decrease in the level of plasma free fatty acids;

[0989] (iii) a decrease in the level of plasma cholesterol;

[0990] (iv) a decrease in the level of LDL-cholesterol;

[0991] (v) an increase in the level of HDL-cholesterol;

[0992] (vi) a decrease in the total cholesterol/HDL-cholesterol ratio; and

[0993] (vii) an increase in the level of plasma adiponectin;

[0994] wherein said change is indicative of the agonist having therapeutic efficacy.

[0995] In a fifty-first aspect, the invention features a method of using the transgenic mouse of the forty-ninth aspect to identify whether an agonist of said human receptor has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

[0996] (a) dyslipidemia;

[0997] (b) atherosclerosis;

[0998] (c) coronary heart disease;

[0999] (d) stroke;

[1000] (e) insulin resistance; and

[1001] (f) type 2 diabetes;

[1002] comprising the steps of:

[1003] (a') administering or not administering the agonist to the mouse; and

[1004] (b') determining whether on administering the agonist there is a change selected from the group consisting of:

[1005] (i) a decrease in the level of plasma triglycerides;

[1006] (ii) a decrease in the level of plasma free fatty acids;

[1007] (iii) a decrease in the level of plasma cholesterol;

[1008] (iv) a decrease in the level of LDL-cholesterol;

[1009] (v) an increase in the level of HDL-cholesterol;

[1010] (vi) a decrease in the total cholesterol/HDL-cholesterol ratio; and

[1011] (vii) an increase in the level of plasma adiponectin;

[1012] wherein said change is indicative of the agonist having therapeutic efficacy.

[1013] In a fifty-second aspect, the invention features a method of making a transgenic rat, comprising the step of engineering said rat to carry as part of its own genetic material the gene encoding the human antilipolytic GPCR polypeptide of SEQ. ID. NO.:135 (hRUP38).

[1014] In some preferred embodiments, expression of said gene is placed under the control of an essentially adipocyte specific promoter.

[1015] In a fifty-third aspect, the invention features the transgenic rat according to the method of the fifty-second aspect.

[1016] In a fifty-fourth aspect, the invention features a method of using the transgenic rat of the fifty-third aspect to identify whether an agonist of said human receptor has therapeutic efficacy for the treatment of a disorder of lipid metabolism selected from the group consisting of:

[1017] (a) elevated level of plasma triglycerides;

[1018] (b) elevated level of plasma free fatty acids;

[1019] (c) elevated level of plasma cholesterol;

[1020] (d) elevated level of LDL-cholesterol;

[1021] (e) reduced level of HDL-cholesterol;

[1022] (f) elevated total cholesterol/HDL-cholesterol ratio; and

[1023] (g) reduced level of plasma adiponectin;

[1024] comprising the steps of:

[1025] (a') administering or not administering the agonist to the rat; and

[1026] (b') determining whether on administering the agonist there is a change selected from the group consisting of:

[1027] (i) a decrease in the level of plasma triglycerides;

[1028] (ii) a decrease in the level of plasma free fatty acids;

[1029] (iii) a decrease in the level of plasma cholesterol;

[1030] (iv) a decrease in the level of LDL-cholesterol;

[1031] (v) an increase in the level of HDL-cholesterol;

[1032] (vi) a decrease in the total cholesterol/HDL-cholesterol ratio; and

[1033] (vii) an increase in the level of plasma adiponectin;

[1034] wherein said change is indicative of the agonist having therapeutic efficacy.

[1035] In a fifty-fifth aspect, the invention features a method of using the transgenic rat of the fifty-third aspect to

identify whether an agonist of said human receptor has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

- [1036] (a) dyslipidemia;
  - [1037] (b) atherosclerosis;
  - [1038] (c) coronary heart disease;
  - [1039] (d) stroke;
  - [1040] (e) insulin resistance; and
  - [1041] (f) type 2 diabetes;
- [1042] comprising the steps of:
- [1043] (a') administering or not administering the agonist to the rat; and
  - [1044] (b') determining whether on administering the agonist there is a change selected from the group consisting of:
    - [1045] (i) a decrease in the level of plasma triglycerides;
    - [1046] (ii) a decrease in the level of plasma free fatty acids;
    - [1047] (iii) a decrease in the level of plasma cholesterol;
    - [1048] (iv) a decrease in the level of LDL-cholesterol;
    - [1049] (v) an increase in the level of HDL-cholesterol;
    - [1050] (vi) a decrease in the total cholesterol/HDL-cholesterol ratio; and
    - [1051] (vii) an increase in the level of plasma adiponectin;
- [1052] wherein said change is indicative of the agonist having therapeutic efficacy.
- [1053] In a fifty-sixth aspect, the invention features an isolated, purified or recombinant RUP38 polynucleotide selected from the group consisting of:

- [1054] (a) a polynucleotide comprising a contiguous span of at least 75 nucleotides of SEQ. ID. NO.:134, or an allelic variant of said polynucleotide;
- [1055] (b) a polynucleotide comprising a contiguous span of at least 150 nucleotides of SEQ. ID. NO.:134, or an allelic variant of said polynucleotide;
- [1056] (c) a polynucleotide comprising a contiguous span of at least 250 nucleotides of SEQ. ID. NO.:134, or an allelic variant of said polynucleotide;
- [1057] (d) a polynucleotide comprising a contiguous span of at least 350 nucleotides of SEQ. ID. NO.:134, or an allelic variant of said polynucleotide;
- [1058] (e) a polynucleotide comprising a contiguous span of at least 500 nucleotides of SEQ. ID. NO.:134, or an allelic variant of said polynucleotide;
- [1059] (f) a polynucleotide comprising a contiguous span of at least 750 nucleotides of SEQ. ID. NO.:134, or an allelic variant of said polynucleotide;

- [1060] (g) a polynucleotide comprising a contiguous span of at least 1000 nucleotides of SEQ. ID. NO.:134, or an allelic variant of said polynucleotide;
  - [1061] (h) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 20 amino acids of SEQ. ID. NO.:135 or an allelic variant of said polypeptide;
  - [1062] (i) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 30 amino acids of SEQ. ID. NO.:135, or an allelic variant of said polypeptide;
  - [1063] (j) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 40 amino acids of SEQ. ID. NO.:135, or an allelic variant of said polypeptide;
  - [1064] (k) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 50 amino acids of SEQ. ID. NO.:135 or an allelic variant of said polypeptide;
  - [1065] (l) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 75 amino acids of SEQ. ID. NO.:135 or an allelic variant of said polypeptide;
  - [1066] (m) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 100 amino acids of SEQ. ID. NO.:135 or an allelic variant of said polypeptide;
  - [1067] (n) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 150 amino acids of SEQ. ID. NO.:135 or an allelic variant of said polypeptide;
  - [1068] (o) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 200 amino acids of SEQ. ID. NO.:135 or an allelic variant of said polypeptide;
  - [1069] (p) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 250 amino acids of SEQ. ID. NO.:135 or an allelic variant of said polypeptide; and
  - [1070] (q) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 300 amino acids of SEQ. ID. NO.:135 or an allelic variant of said polypeptide.
- [1071] The invention also relates to an isolated, purified or recombinant RUP25 polynucleotide wherein said polynucleotide is selected from the group consisting of:
- [1072] (a) a polynucleotide comprising the nucleotide sequence of SEQ. ID. NO.:134 or an allelic variant of said nucleotide sequence;
  - [1073] (b) the polynucleotide of SEQ. ID. NO.:134, or an allelic variant of said polynucleotide;
  - [1074] (c) a polynucleotide comprising a nucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ. ID. NO.:135 or an allelic variant of said amino acid sequence; and
  - [1075] (d) a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ. ID. NO.:135, or an allelic variant of said polypeptide.

[1076] In preferred embodiments, said isolated, purified or recombinant polynucleotide comprises at least 8 contiguous nucleotides of a polynucleotide of the present invention. In other preferred embodiments, said isolated, purified or recombinant polynucleotide comprises at least 10, 12, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 contiguous nucleotides of a polynucleotide of the present invention. Preferably said polynucleotide encodes full-length RUP38 polypeptide or a biologically active fragment thereof.

[1077] The polynucleotides of the present invention include genomic polynucleotides comprising RUP38 polynucleotides of the invention.

[1078] The present invention also relates to a polynucleotide encoding a fusion protein, wherein said fusion protein comprises an RUP38 polypeptide of the invention fused to a heterologous polypeptide. In a preferred embodiment, said polypeptide of the invention is constitutively active and said heterologous polypeptide is a G protein. In other embodiments, said heterologous polypeptide provides an antigenic epitope. In a preferred embodiment, said heterologous polypeptide provides a hemagglutinin (HA) antigenic epitope. Methods relating to a polynucleotide encoding a fusion protein are well known to those of ordinary skill in the art.

[1079] The polynucleotides of the present invention also include variant polynucleotides at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to an RUP38 polynucleotide of the invention. In a particularly preferred embodiments, polynucleotide sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST"), which is well known in the art [See, e.g., Karlin and Altschul, Proc Natl Acad Sci USA (1990) 87:2264-8; Altschul et al., J Mol Biol (1990) 215:403-410; Altschul et al., Nature Genetics (1993) 3:266-72; and Altschul et al., Nucleic Acids Res (1997) 25:3389-3402; the disclosures of which are incorporated by reference in their entirety].

[1080] In further preferred embodiments, the invention features the complement of said polynucleotide.

[1081] In a fifty-seventh aspect, the invention features an isolated, purified or recombinant RUP38 polypeptide selected from the group consisting of:

[1082] (a) a polypeptide comprising a contiguous span of at least 20 amino acids of SEQ. ID. NO.:135 or an allelic variant of said contiguous span of amino acids;

[1083] (b) a polypeptide comprising a contiguous span of at least 30 amino acids of SEQ. ID. NO.:135 or an allelic variant of said contiguous span of amino acids;

[1084] (c) a polypeptide comprising a contiguous span of at least 40 amino acids of SEQ. ID. NO.:135 or an allelic variant of said contiguous span of amino acids;

[1085] (d) a polypeptide comprising a contiguous span of at least 50 amino acids of SEQ. ID. NO.:135 or an allelic variant of said contiguous span of amino acids;

[1086] (e) a polypeptide comprising a contiguous span of at least 75 amino acids of SEQ. ID. NO.:135 or an allelic variant of said contiguous span of amino acids;

[1087] (f) a polypeptide comprising a contiguous span of at least 100 amino acids of SEQ. ID. NO.:135 or an allelic variant of said contiguous span of amino acids;

[1088] (g) a polypeptide comprising a contiguous span of at least 150 amino acids of SEQ. ID. NO.:135 or an allelic variant of said contiguous span of amino acids;

[1089] (h) a polypeptide comprising a contiguous span of at least 200 amino acids of SEQ. ID. NO.:135 or an allelic variant of said contiguous span of amino acids;

[1090] (i) a polypeptide comprising a contiguous span of at least 250 amino acids of SEQ. ID. NO.:135 or an allelic variant of said contiguous span of amino acids; and

[1091] (j) a polypeptide comprising a contiguous span of at least 300 amino acids of SEQ. ID. NO.:135 or an allelic variant of said contiguous span of amino acids.

[1092] The invention also relates to an isolated, purified or recombinant RUP38 polypeptide wherein said polypeptide is selected from the group consisting of:

[1093] (a) a polypeptide comprising the amino acid sequence of SEQ. ID. NO.:135 or an allelic variant or a biologically active mutant of said amino acid sequence; and

[1094] (b) the polypeptide having the amino acid sequence of SEQ. ID. NO.:135 or an allelic variant or a biologically active mutant of said amino acid sequence;

[1095] or a biologically active fragment of said polypeptide.

[1096] In preferred embodiments, said isolated, purified or recombinant polypeptide comprises at least 6 contiguous amino acids of an RUP38 polypeptide of the invention. In further embodiments, said isolated, purified or recombinant polypeptide comprises at least 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, 125, 150, 175, 200, 225, 250, 275 or 300 contiguous amino acids of a polypeptide of the present invention. Preferably, said polypeptide is full-length RUP38 polypeptide or an active fragment thereof.

[1097] The present invention also relates to a fusion protein, wherein said fusion protein comprises an RUP38 polypeptide of the invention fused to a heterologous polypeptide. In a preferred embodiment, said polypeptide of the invention is constitutively active and said heterologous polypeptide is a G protein. In other preferred embodiments, said heterologous polypeptide provides an antigenic epitope. In particularly preferred embodiment, said heterologous polypeptide provides a hemagglutinin (HA) antigenic epitope. Methods relating to a fusion protein are well known to those of ordinary skill in the art.

[1098] The polypeptides of the present invention also include variant polypeptides at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an RUP38 polypeptide of the invention. In a particularly preferred embodiments, polypeptide sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST"), which is well known in the art [See, e.g., Karlin and Altschul, *Proc Natl Acad Sci USA* (1990) 87:2264-8; Altschul et al., *J Mol Biol* (1990) 215:403-410; Altschul et al., *Nature Genetics* (1993) 3:266-72; and Altschul et al., *Nucleic Acids Res* (1997) 25:3389-3402; the disclosures of which are incorporated by reference in their entirety].

[1099] In an fifty-eighth aspect, the invention features a composition comprising, consisting essentially of, or consisting of the RUP38 polypeptide of the fifty-seventh aspect.

[1100] In a fifty-ninth aspect, the invention features a recombinant vector, said vector comprising, consisting essentially of, or consisting of the polynucleotide of the fifty-sixth aspect. In some embodiments, said vector is a targeting vector used in a method of inactivating a gene encoding an antilipolytic GPCR of the invention. In some preferred embodiments, said vector is used in a method of transient or stable transfection. In other preferred embodiments, said vector is used in a method of transgenic expression of an antilipolytic GPCR.

[1101] In particularly preferred embodiment, said vector is an expression vector for the expression of a an antilipolytic GPCR in a recombinant host cell wherein said expression vector comprises, consists essentially of, or consists of the polynucleotide of the fifty-sixth aspect. Although a variety of expression vectors are available to those in the art, for purposes of utilization for both the endogenous and non-endogenous human, mouse and rat GPCRs, it is most preferred that the vector utilized be pCMV. In some alternative embodiments as relates to said human, mouse and rat antilipolytic GPCRs, it is preferred that the vector utilized be an adenoviral expression vector.

[1102] In a sixtieth aspect, the invention features a prokaryotic or eukaryotic host cell comprising, consisting essentially of, or consisting of the recombinant vector of the fifty-ninth aspect. In some embodiments, said host cell is a eukaryotic embryonic stem cell wherein said vector of the fifty-ninth aspect has been used in a method to inactivate a gene encoding an antilipolytic GPCR of the invention within said cell. In some embodiments, said host cell is a eukaryotic embryonic somatic cell wherein said vector of the fifty-ninth aspect has been used in a method to inactivate a gene encoding an antilipolytic GPCR of the invention within said cell. In some preferred embodiments, said host cell is derived from a mouse or rat made transgenic for a human RUP38 antilipolytic GPCR of the invention. In some preferred embodiments, said host cell is prokaryotic and has been transformed using the vector of the fifty-ninth aspect. In further preferred embodiments, said host cell is eukaryotic and has been transiently transfected using the vector of the fifty-ninth aspect. In other further preferred embodiments, said host cell is eukaryotic and has been stably transfected using the vector of the fifty-ninth aspect.

[1103] In particularly preferred embodiment, said host cell expresses a recombinant antilipolytic GPCR wherein said host cell comprises, consists essentially of, or consists of the expression vector of the fifty-ninth aspect.

[1104] A further embodiment includes a prokaryotic or eukaryotic host cell recombinant for the polynucleotide of the fifty-sixth aspect.

[1105] In some embodiments the host cell is eukaryotic, more preferably, mammalian, and more preferably selected from the group consisting of 293, 293T, CHO, and COS-7 cells. In other embodiments, the host cell is eukaryotic, more preferably melanophore.

[1106] In a sixty-first aspect, the invention features a process for the expression of an antilipolytic GPCR in a recombinant host cell comprising the steps of:

[1107] (a) transfecting the expression vector of the fifty-ninth aspect into a suitable host cell; and

[1108] (b) culturing the host cells under conditions which allow expression of the antilipolytic GPCR protein from the expression vectors.

[1109] In a sixty-second aspect, the invention features an antibody that specifically binds to the polypeptide of the fifty-seventh aspect. In some preferred embodiments, the antibody is monoclonal. In some embodiments, the antibody is polyclonal.

[1110] In a sixty-third aspect, the invention features a method of binding the polypeptide of the fifty-seventh aspect to the antibody of the sixty-second aspect, comprising contacting said antibody with said polypeptide under conditions in which said antibody can specifically bind to said polypeptide.

[1111] In a sixty-fourth aspect, the invention features a method of detecting an antilipolytic GPCR polypeptide in a biological sample obtained from an individual comprising the steps of:

[1112] (a) obtaining said biological sample from said individual;

[1113] (b) contacting said biological sample with the antibody of the sixty-second aspect; and

[1114] (c) detecting the presence or absence of binding of said antibody to said biological sample;

[1115] wherein a detection of said binding is indicative of the receptor polypeptide being expressed in said biological sample.

[1116] In preferred embodiments, said detecting is through the use of an enzyme-labeled secondary reagent. In other preferred embodiments, said detecting is through the use of a fluorophore-labeled secondary reagent. In other preferred embodiments, said detecting is through the use of a radioisotope-labeled secondary reagent. In other embodiments, the antibody is directly labeled with enzyme, fluorophore or radioisotope.

[1117] In other preferred embodiments, said biological sample is taken from adipose, skin or blood.

[1118] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1119] In further embodiments, said individual has a disorder of lipid metabolism selected from the group consisting of:

- [1120] (a) elevated level of plasma triglycerides;
- [1121] (b) elevated level of plasma free fatty acids;
- [1122] (c) elevated level of plasma cholesterol;
- [1123] (d) elevated level of LDL-cholesterol;
- [1124] (e) reduced level of HDL-cholesterol;
- [1125] (f) elevated total cholesterol/HDL-cholesterol ratio; and
- [1126] (g) reduced level of plasma adiponectin.

[1127] In other preferred embodiments, said disorder in lipid metabolism is an elevated postprandial increase in plasma free fatty acids due to a high fat meal or a progression from impaired glucose tolerance to insulin resistance.

[1128] In further embodiments, said individual has a metabolic-related disorder selected from the group consisting of:

- [1129] (a) dyslipidemia;
- [1130] (b) atherosclerosis;
- [1131] (c) coronary heart disease;
- [1132] (d) stroke;
- [1133] (e) insulin resistance; and
- [1134] (f) type 2 diabetes.

[1135] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1136] In other embodiments, said method further comprises the step of comparing the level of detection of said binding for a first individual to the level of detection of said binding for a second individual.

[1137] In a sixty-fifth aspect, the invention features a method of detecting expression of a gene encoding an antilipolytic GPCR in a biological sample obtained from an individual comprising the steps of:

- [1138] (a) obtaining said biological sample from said individual;
- [1139] (b) contacting said biological sample with the complementary polynucleotide of the fifty-sixth aspect, optionally labeled, under conditions permissive for hybridization; and
- [1140] (c) detecting the presence or absence of said hybridization between said complementary polynucleotide and an RNA species within said sample;

[1141] wherein a detection of said hybridization is indicative of expression of said GPCR gene in said biological sample.

[1142] In preferred embodiments, the biological sample is taken from adipose, skin or blood.

[1143] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1144] In preferred embodiments, said individual has a disorder of lipid metabolism selected from the group consisting of:

- [1145] (a) elevated level of plasma triglycerides;
- [1146] (b) elevated level of plasma free fatty acids;
- [1147] (c) elevated level of plasma cholesterol;
- [1148] (d) elevated level of LDL-cholesterol;
- [1149] (e) reduced level of HDL-cholesterol;
- [1150] (f) elevated total cholesterol/HDL-cholesterol ratio; and
- [1151] (g) reduced level of plasma adiponectin.

[1152] In other preferred embodiments, said disorder in lipid metabolism is an elevated postprandial increase in plasma free fatty acids due to a high fat meal or a progression from impaired glucose tolerance to insulin resistance.

[1153] In other preferred embodiments, said individual has a metabolic-related disorder selected from the group consisting of:

- [1154] (a) dyslipidemia;
- [1155] (b) atherosclerosis;
- [1156] (c) coronary heart disease;
- [1157] (d) stroke;
- [1158] (e) insulin resistance; and
- [1159] (f) type 2 diabetes.

[1160] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1161] In other embodiments, said method further comprises the step of comparing the level of detection of said hybridization for a first individual to the level of detection of said hybridization for a second individual.

[1162] In some preferred embodiments, said complementary polynucleotide is a primer and said hybridization is detected by detecting the presence of an amplification product comprising the sequence of said primer. In more preferred embodiments, said method is RT-PCR.

[1163] In a sixty-sixth aspect, the invention features a GPCR Fusion Protein construct comprising a constitutively active GPCR and a G protein, said receptor comprising the amino acid sequence of SEQ. ID. NO.:135 (hRUP38) or an allelic variant or a biologically active fragment of said amino acid sequence.

[1164] The invention also relates to a GPCR Fusion Protein construct wherein the isoleucine at amino acid position 230 of SEQ. ID. NO.:135 is substituted by lysine.

[1165] In a sixty-seventh aspect, the invention features a method of binding a known ligand of RUP38 antilipolytic GPCR to a polypeptide selected from the group consisting of:

- [1166] (a) a polypeptide comprising a contiguous span of at least 6 amino acids of SEQ. ID. NO.:135;
- [1167] (b) a polypeptide comprising a contiguous span of at least 10 amino acids of SEQ. ID. NO.:135;
- [1168] (c) a polypeptide comprising a contiguous span of at least 15 amino acids of SEQ. ID. NO.:135;
- [1169] (d) a polypeptide comprising a contiguous span of at least 20 amino acids of SEQ. ID. NO.:135;
- [1170] (e) a polypeptide comprising a contiguous span of at least 25 amino acids of SEQ. ID. NO.:135;
- [1171] (f) a polypeptide comprising a contiguous span of at least 30 amino acids of SEQ. ID. NO.:135;
- [1172] (g) a polypeptide comprising a contiguous span of at least 35 amino acids of SEQ. ID. NO.:135;
- [1173] (h) a polypeptide comprising a contiguous span of at least 40 amino acids of SEQ. ID. NO.:135;
- [1174] (i) a polypeptide comprising a contiguous span of at least 45 amino acids of SEQ. ID. NO.:135; and
- [1175] (j) a polypeptide comprising a contiguous span of at least 50 amino acids of SEQ. ID. NO.:135; or an allelic variant of said polypeptide;

[1176] comprising the step of contacting said known ligand with said polypeptide under conditions which allow said binding to occur.

[1177] In some embodiments, said known ligand is a modulator of the antilipolytic GPCR. In some embodiments, said known modulator is an agonist of the antilipolytic GPCR. In some embodiments, said known ligand is the modulator of the thirty-sixth aspect. In some embodiments, said known ligand is an antibody specific for the GPCR, or a derivative thereof.

[1178] In other preferred embodiments, said method is used to identify whether a candidate compound inhibits said binding of said known ligand to said polypeptide, comprising the steps of:

- [1179] (a) contacting said polypeptide with said known ligand, optionally labeled, in the presence or absence of said candidate compound;
- [1180] (b) detecting the complex between said known ligand and said polypeptide; and
- [1181] (c) determining whether less of said complex is formed in the presence of the compound than in the absence of the compound;

[1182] wherein said determination is indicative of the candidate compound being an inhibitor of said binding of said known ligand to said polypeptide.

[1183] In some embodiments, said known ligand is a modulator of the antilipolytic GPCR. In some embodiments, said known modulator is an agonist. In some embodiments, said known ligand is the modulator of the thirty-sixth aspect. In some embodiments, said known ligand is an antibody specific for the GPCR, or a derivative thereof.

[1184] In other preferred embodiments, said method is used to identify whether a candidate compound is an inhibitor of said binding of said known ligand to said polypeptide, comprising the steps of:

- [1185] (a) contacting said polypeptide with said known ligand, optionally labeled, in the presence separately of a plurality of concentrations of said candidate compound for a time sufficient to allow equilibration of binding;
- [1186] (b) measuring unbound ligand and bound ligand; and
- [1187] (c) determining  $K_i$  for the candidate compound;

[1188] wherein a  $K_i$  value of less than 50  $\mu$ M is indicative of the candidate compound being an inhibitor of said binding of said known ligand to said polypeptide. Preferably said  $K_i$  value is less than 25  $\mu$ M, 10  $\mu$ M, 5  $\mu$ M, 1  $\mu$ M, 750 nM, 500 nM, 400 nM, 300 nM, 250 nM, 200 nM, 150 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM or 10 nM. In preferred embodiments,  $K_i$  determination is made through nonlinear curve fitting with the program SCTFIT [De Lean et al. (1982) Mol Pharmacol 21:5-16; cited in Lorenzen et al. (2001) Mol Pharmacol 59:349-357, the disclosures of which are incorporated by reference herein in their entireties].

[1189] In some embodiments, said known ligand is a modulator of the antilipolytic GPCR. In some embodiments, said known modulator is an agonist. In some embodiments, said known ligand is the modulator of the thirty-sixth aspect. In some embodiments, said known ligand is an antibody specific for the GPCR, or a derivative thereof.

[1190] In a sixty-eighth aspect, the invention features a method of binding an optionally labeled affinity reagent specific for an antilipolytic GPCR to said receptor in a biological sample, said receptor comprising the amino acid sequence of SEQ. ID. NO.:135 (hRUP38); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence, comprising the steps of:

- [1191] (a) obtaining said biological sample;
- [1192] (b) contacting the affinity reagent with said receptor in said biological sample; and
- [1193] (c) detecting the complex of said affinity reagent with said receptor.

[1194] In some embodiments, the antilipolytic GPCR has the amino acid sequence of SEQ. ID. NO.:135 (hRUP38); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence.

[1195] In some embodiments, the antilipolytic GPCR comprises a biologically active fragment of said amino acid sequence.

[1196] In some embodiments, the antilipolytic GPCR is endogenous.

[1197] In some embodiments, the antilipolytic GPCR is recombinant.

[1198] In some embodiments, said biologically active mutant is CART or EFA. In preferred embodiments, said CART mutant has the amino acid sequence of SEQ. ID. NO.:135 further substituted at amino acid position 230 with lysine in place of isoleucine.

[1199] In preferred embodiments, said G protein is Gi.

[1200] In some embodiments, said affinity reagent is a modulator of the GPCR. In some embodiments, said affinity reagent is an agonist of the GPCR. In some embodiments, said affinity reagent is the modulator of the thirty-sixth aspect. In some embodiments, said affinity reagent is an antibody specific for the GPCR, or a derivative thereof.

[1201] In further preferred embodiments, said affinity reagent comprises a label selected from the group consisting of:

[1202] (a) radioisotope;

[1203] (b) enzyme; and

[1204] (c) fluorophore.

[1205] In preferred embodiments, said radioisotope is  $^3\text{H}$ .

[1206] In a sixty-ninth aspect, the invention features the method of the sixty-eighth aspect further comprising the step of comparing the level of detection of said complex in a first biological sample to a second level of detection of said complex in a second biological sample.

[1207] In a seventieth aspect, the invention features the method of the sixty-ninth aspect wherein the relationship between said first and second biological samples is selected from the group consisting of:

[1208] (a) said second biological sample is a replicate of said first biological sample;

[1209] (b) said first biological sample was obtained prior to an experimental intervention whereas said second biological sample was obtained after the experimental intervention, from the same individual;

[1210] (c) said second biological sample was obtained at a different time point after an experimental intervention than was said first biological sample, from the same individual;

[1211] (d) said second biological sample corresponds to a different subcellular compartment than does said first biological sample;

[1212] (e) said second biological sample represents a different cell type than does said first biological sample;

[1213] (f) said second biological sample corresponds to a different tissue than does said first biological sample;

[1214] (g) said second biological sample was obtained from a different individual than was said first biological sample;

[1215] (h) said second biological sample was obtained at a different point in time than was said first biological sample, from the same individual;

[1216] (i) said first biological samples was obtained from a normal individual, whereas said second biological sample was obtained from an individual having a metabolic-related disorder;

[1217] (j) said first biological sample was obtained from a normal individual, whereas said second biological sample was obtained from an individual having a disorder in lipid metabolism;

[1218] (k) said first biological sample was obtained before a therapeutic intervention whereas said second biological sample was obtained after the therapeutic intervention, from the same individual;

[1219] (l) said second biological sample was obtained at a different time point after therapeutic intervention than was said first biological sample, from the same individual; and

[1220] (m) said first biological sample was not exposed to a compound, whereas said second biological sample was exposed to said compound.

[1221] In a seventy-first aspect, the invention features a method of identifying whether a candidate compound is a modulator of an antilipolytic GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

[1222] (a) SEQ. ID. NO.:24 (hRUP19);

[1223] (b) SEQ. ID. NO.:151 (mRUP19); and

[1224] (c) SEQ. ID. NO.:157 (rRUP19);

[1225] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

[1226] comprising the steps of:

[1227] (a') contacting the candidate compound with the receptor;

[1228] (b') determining whether the receptor functionality is modulated;

[1229] wherein a change in receptor functionality is indicative of the candidate compound being a modulator of an antilipolytic GPCR.

[1230] In some embodiments, said antilipolytic GPCR is endogenous.

[1231] In some preferred embodiments, said antilipolytic GPCR is recombinant.

[1232] Preferred said identified modulator binds to said GPCR.

[1233] In some embodiments, said contacting is carried out in the presence of a known ligand of the GPCR. In some embodiments, said known ligand is an agonist of the GPCR.

[1234] The invention also relates to a method of identifying whether a candidate compound is a modulator of lipolysis, comprising the steps of:

- [1235] (a) contacting the candidate compound with a GPCR comprising an amino acid sequence selected from the group consisting of:
- [1236] (i) SEQ. ID. NO.:24 (hRUP19);
- [1237] (ii) SEQ. ID. NO.:151 (mRUP19); and
- [1238] (iii) SEQ. ID. NO.:157 (rRUP19);
- [1239] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence; and
- [1240] (b) determining whether the receptor functionality is modulated;
- [1241] wherein a change in receptor functionality is indicative of the candidate compound being a modulator of lipolysis.
- [1242] In some embodiments, said GPCR is endogenous.
- [1243] In some preferred embodiments, said GPCR is recombinant.
- [1244] Preferred said identified modulator binds to said GPCR.
- [1245] In some embodiments, said contacting is carried out in the presence of a known ligand of the GPCR. In some embodiments, said known ligand is an agonist of the GPCR.
- [1246] The invention also relates to a method of determining whether a candidate compound is a modulator of an antilipolytic GPCR, comprising the steps of:
- [1247] (a) culturing antilipolytic GPCR-expressing host cells under conditions that would allow expression of a recombinant antilipolytic GPCR, said host cells being transfected with a polynucleotide encoding said recombinant antilipolytic GPCR comprising an amino acid sequence selected from the group consisting of:
- [1248] (i) SEQ. ID. NO.:24 (hRUP19);
- [1249] (ii) SEQ. ID. NO.:151 (mRUP19); and
- [1250] (iii) SEQ. ID. NO.:157 (rRUP19);
- [1251] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;
- [1252] (b) contacting the antilipolytic GPCR-expressing host cells of step (a) with the candidate compound;
- [1253] (c) contacting control host cells with the candidate compound of step (b), wherein said control host cells do not express recombinant antilipolytic GPCR protein;
- [1254] (d) measuring the modulating effect of the candidate compound which interacts with the recombinant antilipolytic GPCR from the host cells of step (a) and control host cells of step (c); and
- [1255] (e) comparing the modulating effect of the test compound on the host cells and control host cells.
- [1256] The invention also relates to a method of determining whether a candidate compound is a modulator of an antilipolytic GPCR, comprising the steps of:
- [1257] (a) culturing antilipolytic GPCR-expressing host cells under conditions that would allow expression of a recombinant antilipolytic GPCR, said host cells being transfected with a polynucleotide encoding said recombinant antilipolytic GPCR comprising an amino acid sequence selected from the group consisting of:
- [1258] (i) SEQ. ID. NO.:24 (hRUP19);
- [1259] (ii) SEQ. ID. NO.:151 (mRUP19); and
- [1260] (iii) SEQ. ID. NO.:157 (rRUP19);
- [1261] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;
- [1262] (b) contacting a first population of antilipolytic GPCR-expressing cells of step (a) with a known ligand of said antilipolytic GPCR;
- [1263] (c) contacting a second population of antilipolytic GPCR-expressing cells of step (a) with the candidate compound and with the known antilipolytic GPCR ligand;
- [1264] (d) contacting control host cells with the candidate compound of step (c), wherein said control host cells do not express recombinant antilipolytic GPCR protein;
- [1265] (e) measuring the modulating effect of the candidate compound, which interacts with recombinant antilipolytic GPCR, in the presence and absence of the known antilipolytic GPCR ligand, from the cells of step (b), step (c) and step (d); and
- [1266] (f) comparing the modulating effect of the candidate compound as determined from step (b), step (c) and step (d).
- [1267] In some embodiments, said ligand is an agonist of the GPCR.
- [1268] The invention also relates to a method of determining whether a candidate compound is a modulator of an antilipolytic GPCR, comprising the steps of:
- [1269] (a) culturing antilipolytic GPCR-expressing host cells under conditions that would allow expression of a recombinant antilipolytic GPCR, said host cells being transfected with a polynucleotide encoding said recombinant antilipolytic GPCR comprising an amino acid sequence selected from the group consisting of:
- [1270] (i) SEQ. ID. NO.:24 (hRUP19);
- [1271] (ii) SEQ. ID. NO.:151 (mRUP19); and
- [1272] (iii) SEQ. ID. NO.:157 (rRUP19);
- [1273] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;
- [1274] (b) contacting a first population of the antilipolytic GPCR-expressing host cells of step (a) with the candidate compound;

- [1275] (c) not contacting a second population of the antilipolytic GPCR-expressing cells of step (a) with the candidate compound of step (b);
- [1276] (d) contacting control host cells to the candidate compound of step (b), wherein said control host cells do not express recombinant antilipolytic GPCR protein;
- [1277] (e) measuring the modulating effect of the candidate compound, which interacts with recombinant antilipolytic GPCR protein, from the cells of step (b) and step (c) and from the cells of step (d); and
- [1278] (f) comparing the modulating effect of the candidate compound as determined from step (b) and step (c) and from step (d).
- [1279] In some embodiments, the antilipolytic GPCR has an amino acid sequence selected from the group consisting of:
- [1280] (a) SEQ. ID. NO.:24 (hRUP19);
- [1281] (b) SEQ. ID. NO.:151 (mRUP19); and
- [1282] (c) SEQ. ID. NO.:157 (rRUP19);
- [1283] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence.
- [1284] In some embodiments, the antilipolytic GPCR comprises a biologically active fragment of said amino acid sequence.
- [1285] In some embodiments, said biologically active mutant is CART or EFA. In preferred embodiments, said CART mutant has the amino acid sequence of SEQ. ID. NO.:24 further substituted at amino acid position 219 with lysine in place of threonine.
- [1286] In preferred embodiments, said G protein is Gi.
- [1287] In other preferred embodiments, said determining is through the use of a Melanophore assay.
- [1288] In other preferred embodiments, said determining is through the measurement of the level of a second messenger selected from the group consisting of cyclic AMP (cAMP), cyclic GMP (cGMP), inositol triphosphate (IP<sub>3</sub>), diacylglycerol (DAG), and Ca<sup>2+</sup>. In further preferred embodiments, said second messenger is cAMP. In more preferred embodiments, the level of the cAMP is reduced. In some embodiments, said measurement of cAMP is carried out with membrane comprising said GPCR.
- [1289] In other preferred embodiments, said determining is through the measurement of an activity up-regulated or down-regulated by a reduction in intracellular cAMP level. In further preferred embodiments, said down-regulated activity is intracellular lipolysis. In other further preferred embodiments, said down-regulated activity is hormone sensitive lipase activity. In other further preferred embodiments, said up-regulated activity is adiponectin secretion.
- [1290] In other preferred embodiments, said determining is through CRE-reporter assay. In preferred embodiments, said reporter is luciferase. In some embodiments, said reporter is  $\beta$ -galactosidase.
- [1291] In other preferred embodiments, said recombinant host cell further comprises promiscuous G alpha 15/16 or chimeric Gq/Gi alpha subunit and said determining is through measurement of intracellular Ca<sup>2+</sup>. In preferred embodiments, said Ca<sup>2+</sup> measurement is carried out by FLIPR.
- [1292] In other preferred embodiments, said recombinant host cell further comprises promiscuous G alpha 15/16 or chimeric Gq/Gi alpha subunit and said determining is through measurement of intracellular IP<sub>3</sub>.
- [1293] In other preferred embodiments, said determining is through the measurement of GTP $\gamma$ S binding to membrane comprising said GPCR. In further preferred embodiments, said GTP $\gamma$ S is labeled with [<sup>35</sup>S].
- [1294] In other preferred embodiments, said method further comprises the step of comparing the modulation of the receptor caused by the candidate compound to a second modulation of the receptor caused by contacting the receptor with a known modulator of the receptor. In some preferred embodiments, said known modulator is an agonist.
- [1295] In a seventy-second aspect, the invention features a modulator of an antilipolytic GPCR identified according to the method of the seventy-first aspect.
- [1296] In some preferred embodiments, said modulator is selected from the group consisting of agonist, partial agonist, inverse agonist and antagonist. More preferably, said modulator is an agonist. In some embodiments, said modulator is a partial agonist.
- [1297] In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 1000  $\mu$ M in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP19 polypeptide having the amino acid sequence of SEQ. ID. NO.:24. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 900  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 800  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 700  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 600  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 550  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 500  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 450  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 400  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 350  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 300  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 250  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 200  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 150  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 100  $\mu$ M in said assay. In some preferred embodiments, said modulator is an agonist with an EC<sub>50</sub> in said assay of less than a value selected from the interval of 600  $\mu$ M to 1000  $\mu$ M.

[1298] In some embodiments, said modulator is selective for the GPCR.

[1299] In some embodiments, said modulator is antipolytic.

[1300] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some preferred embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some preferred embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[1301] In highly less preferred embodiments, said modulator is an antibody or derivative thereof.

[1302] In a seventy-third aspect, the invention features a method of modulating the activity of an antipolytic GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

[1303] (a) SEQ. ID. NO.:24 (hRUP19);

[1304] (b) SEQ. ID. NO.:151 (mRUP19); and

[1305] (c) SEQ. ID. NO.:157 (rRUP19);

[1306] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

[1307] comprising the step of contacting the receptor with the modulator of the seventy-second aspect.

[1308] In some embodiments, the antipolytic GPCR has an amino acid sequence selected from the group consisting of:

[1309] (a) SEQ. ID. NO.:24 (hRUP19);

[1310] (b) SEQ. ID. NO.:151 (mRUP19); and

[1311] (c) SEQ. ID. NO.:157 (rRUP19);

[1312] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence.

[1313] In some embodiments, the antipolytic GPCR comprises an active fragment of said amino acid sequence.

[1314] In some embodiments, the antipolytic GPCR is recombinant.

[1315] In some embodiments, the antipolytic GPCR is endogenous.

[1316] In some embodiments, said biologically active mutant is CART or EFA. In preferred embodiments, said CART mutant has the amino acid sequence of SEQ. ID. NO.:24 further substituted at amino acid position 219 with lysine in place of threonine.

[1317] In preferred embodiments, said G protein is Gi.

[1318] In some preferred embodiments, said modulator is an agonist.

[1319] In preferred embodiments, said modulator is selective for the GPCR.

[1320] In other preferred embodiments, said contacting comprises administration of the modulator to a membrane comprising the receptor.

[1321] In other preferred embodiments, said contacting comprises administration of the modulator to a cell or tissue comprising the receptor.

[1322] In other preferred embodiments, said contacting comprises administration of the modulator to an individual comprising the receptor. In more preferred embodiments, said individual is a mammal. In other more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1323] In some preferred embodiments, said modulator is selective for the GPCR.

[1324] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[1325] In some preferred embodiments, said modulator is antipolytic.

[1326] In some preferred embodiments, said modulator is an agonist.

[1327] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP19 polypeptide having the amino acid sequence of SEQ. ID. NO.:24. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay.

In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[1328] In some preferred embodiments, said administration is oral.

[1329] In preferred embodiments, said modulator is an agonist and said individual is in need of prevention of or treatment for a metabolic-related disorder selected from the group consisting of:

- [1330] (a) dyslipidemia;
- [1331] (b) atherosclerosis;
- [1332] (c) coronary heart disease;
- [1333] (d) stroke;
- [1334] (e) insulin resistance; and
- [1335] (f) type 2 diabetes.

[1336] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1337] In other embodiments, said modulator is an inverse agonist and said metabolic-related disorder relates to a low level of plasma free fatty acids.

[1338] In other preferred embodiments, said modulator is an agonist and said individual is in need of a change in lipid metabolism selected from the group consisting of:

- [1339] (a) a decrease in the level of plasma triglycerides;
- [1340] (b) a decrease in the level of plasma free fatty acids;
- [1341] (c) a decrease in the level of plasma cholesterol;
- [1342] (d) a decrease in the level of LDL-cholesterol;
- [1343] (e) an increase in the level of HDL-cholesterol;
- [1344] (f) a decrease in the total cholesterol/HDL-cholesterol ratio; and
- [1345] (g) an increase in the level of plasma adiponectin.

[1346] In other preferred embodiments, said needed change in lipid metabolism is a decrease in the postprandial increase in plasma free fatty acids due to a high fat meal or

an inhibition of the progression from impaired glucose tolerance to insulin resistance.

[1347] In some embodiments, the modulator is an inverse agonist and the needed change in lipid metabolism is an increase in the level of plasma free fatty acids.

[1348] In other preferred embodiments, said modulator is an agonist and said individual is a mouse genetically predisposed to a metabolic-related disorder selected from the group consisting of:

- [1349] (a) dyslipidemia;
- [1350] (b) atherosclerosis;
- [1351] (c) coronary heart disease;
- [1352] (d) stroke;
- [1353] (e) insulin resistance; and
- [1354] (f) type 2 diabetes.

[1355] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure.

[1356] In further preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1357] In further preferred embodiments, said method is used to identify whether said agonist has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

- [1358] (a) dyslipidemia;
- [1359] (b) atherosclerosis;
- [1360] (c) coronary heart disease;
- [1361] (d) stroke;
- [1362] (e) insulin resistance; and
- [1363] (f) type 2 diabetes;

[1364] comprising the steps of:

- [1365] (a') administering or not administering said agonist to the mouse; and
- [1366] (b') determining whether the disorder is prevented, delayed, or made less severe on administering said agonist compared to not administering said agonist; wherein said determination is indicative of said agonist having therapeutic efficacy.

[1367] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1368] In other preferred embodiments, said modulator is an agonist and said individual is a rat genetically predisposed to a metabolic-related disorder selected from the group consisting of:

- [1369] (a) dyslipidemia;
- [1370] (b) atherosclerosis;

[1371] (c) coronary heart disease;

[1372] (d) stroke;

[1373] (e) insulin resistance; and

[1374] (f) type 2 diabetes.

[1375] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In further preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1376] In further preferred embodiments, said method is used to identify whether said agonist has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

[1377] (a) dyslipidemia;

[1378] (b) atherosclerosis;

[1379] (c) coronary heart disease;

[1380] (d) stroke;

[1381] (e) insulin resistance; and

[1382] (f) type 2 diabetes;

[1383] comprising the steps of:

[1384] (a') administering or not administering said agonist to the rat; and

[1385] (b') determining whether the disorder is prevented, delayed, or made less severe on administering said agonist compared to not administering said agonist; wherein said determination is indicative of said agonist having therapeutic efficacy.

[1386] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1387] In a seventy-fourth aspect, the invention features a method of preventing or treating a disorder of lipid metabolism in an individual comprising contacting a therapeutically effective amount of the modulator of the seventy-second aspect with an antilipolytic GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

[1388] (a) SEQ. ID. NO.:24 (hRUP19);

[1389] (b) SEQ. ID. NO.:151 (mRUP19); and

[1390] (c) SEQ. ID. NO.:157 (rRUP19);

[1391] or an allelic variant or biologically active fragment of said amino acid sequence.

[1392] In some preferred embodiments, said modulator is selective for the GPCR.

[1393] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[1394] In some preferred embodiments, said modulator is antilipolytic.

[1395] In some preferred embodiments, said modulator is an agonist.

[1396] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP19 polypeptide having the amino acid sequence of SEQ. ID. NO.:24. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[1397] In some preferred embodiments, said contacting comprises oral administration of said modulator to said individual.

[1398] In preferred embodiment, said modulator is an agonist and said disorder of lipid metabolism is selected from the group consisting of:

[1399] (a) elevated level of plasma triglycerides;

[1400] (b) elevated level of plasma free fatty acids;

[1401] (c) elevated level of plasma cholesterol;

[1402] (d) elevated level of LDL-cholesterol;

[1403] (e) reduced level of HDL-cholesterol;

[1404] (f) elevated total cholesterol/HDL-cholesterol ratio; and

[1405] (g) reduced level of plasma adiponectin.

[1406] In a seventy-fifth aspect, the invention features a method of preventing or treating a metabolic-related disorder in an individual comprising contacting a therapeutically effective amount of the modulator of the seventy-second aspect with an antilipolytic GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

[1407] (a) SEQ. ID. NO.:24 (hRUP19);

[1408] (b) SEQ. ID. NO.:151 (mRUP19); and

[1409] (c) SEQ. ID. NO.:157 (rRUP19);

[1410] or an allelic variant or biologically active fragment of said amino acid sequence.

[1411] In some preferred embodiments, said modulator is selective for the GPCR.

[1412] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[1413] In some preferred embodiments, said modulator is antilipolytic.

[1414] In some preferred embodiments, said modulator is an agonist.

[1415] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP19 polypeptide having the amino acid sequence of SEQ. ID. NO.:24. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodi-

ments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[1416] In some preferred embodiments, said contacting comprises oral administration of said modulator to said individual.

[1417] In preferred embodiment, said modulator is an agonist and said metabolic-related disorder is selected from the group consisting of:

[1418] (a) dyslipidemia;

[1419] (b) atherosclerosis;

[1420] (c) coronary heart disease;

[1421] (d) stroke;

[1422] (e) insulin resistance; and

[1423] (f) type 2 diabetes.

[1424] In a seventy-sixth aspect, the invention features a method of preparing a composition which comprises identifying a modulator of a antilipolytic GPCR and then admixing a carrier and the modulator, wherein the modulator is identifiable by the method of the seventy-first aspect.

[1425] In some preferred embodiments, said modulator is selected from the group consisting of agonist, partial agonist, inverse agonist and antagonist. More preferably, said modulator is an agonist. In some embodiments, said modulator is a partial agonist.

[1426] In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP19 polypeptide having the amino acid sequence of SEQ. ID. NO.:24. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodi-

ments, said modulator is an agonist with an  $EC_{50}$  of less than  $100\ \mu\text{M}$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of  $600\ \mu\text{M}$  to  $1000\ \mu\text{M}$ .

[1427] In some embodiments, said modulator is selective for the GPCR.

[1428] In some embodiments, said modulator is antilipolytic.

[1429] In some embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to either intraperitoneal or intravenous administration. In some preferred embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to either intraperitoneal or intravenous administration.

[1430] In a seventy-seventh aspect, the invention features a pharmaceutical or physiologically acceptable composition comprising, consisting essentially of, or consisting of the modulator of the seventy-second aspect. In preferred embodiments, said modulator is an agonist.

[1431] In some preferred embodiments, said modulator is selective for the GPCR.

[1432] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[1433] In some preferred embodiments, said modulator is antilipolytic.

[1434] In some preferred embodiments, said modulator is an agonist.

[1435] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $1000\ \mu\text{M}$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP19 polypeptide having the amino acid sequence of SEQ. ID. NO.:24. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $900\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $800\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $700\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $600\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $550\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $500\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $450\ \mu\text{M}$  in said assay. In some embodi-

ments, said modulator is an agonist with an  $EC_{50}$  of less than  $400\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $350\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $300\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $250\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $200\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $150\ \mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than  $100\ \mu\text{M}$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of  $600\ \mu\text{M}$  to  $1000\ \mu\text{M}$ .

[1436] In a seventy-eighth aspect, the invention features a method of changing lipid metabolism comprising providing or administering to an individual in need of said change said pharmaceutical or physiologically acceptable composition of the seventy-seventh aspect, said needed change in lipid metabolism selected from the group consisting of:

- [1437] (a) a decrease in the level of plasma triglycerides;
- [1438] (b) a decrease in the level of plasma free fatty acids;
- [1439] (c) a decrease in the level of plasma cholesterol;
- [1440] (d) a decrease in the level of LDL-cholesterol;
- [1441] (e) an increase in the level of HDL-cholesterol;
- [1442] (f) a decrease in the total cholesterol/HDL-cholesterol ratio; and
- [1443] (g) an increase in the level of plasma adiponectin.

[1444] In preferred embodiments, a therapeutically effective amount of said pharmaceutical or physiologically acceptable composition is provided or administered to said individual.

[1445] In some preferred embodiments, said providing or administering of said pharmaceutical or physiologically acceptable composition is oral.

[1446] In other preferred embodiments, said needed change in lipid metabolism is a decrease in the postprandial increase in plasma free fatty acids due to a high fat meal or an inhibition of the progression from impaired glucose tolerance to insulin resistance.

[1447] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog; rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1448] In a seventy-ninth aspect, the invention features a method of preventing or treating a metabolic-related disorder comprising providing or administering to an individual in need of said treatment said pharmaceutical or physiologically acceptable composition of the seventy-seventh aspect, said metabolic-related disorder selected from the group consisting of:

- [1449] (a) dyslipidemia;
- [1450] (b) atherosclerosis;
- [1451] (c) coronary heart disease;
- [1452] (d) stroke;
- [1453] (e) insulin resistance; and
- [1454] (f) type 2 diabetes.

[1455] In preferred embodiments, a therapeutically effective amount of said pharmaceutical or physiologically acceptable composition is provided or administered to said individual.

[1456] In some preferred embodiments, said providing or administering of said pharmaceutical or physiologically acceptable composition is oral.

[1457] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, stroke, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1458] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1459] In an eightieth aspect, the invention features a method of using the modulator of the seventy-second aspect for the preparation of a medicament for the treatment of a disorder in lipid metabolism in an individual.

[1460] In some preferred embodiments, said modulator is selective for the GPCR.

[1461] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[1462] In some preferred embodiments, said modulator is antilipolytic.

[1463] In some preferred embodiments, said modulator is an agonist.

[1464] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu$ M in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP19 polypeptide having the amino acid sequence of SEQ. ID. NO.:24. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu$ M in said assay. In some embodi-

ments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu$ M in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu$ M to 1000  $\mu$ M.

[1465] In some preferred embodiments, said treatment comprises oral administration of said medicament to said individual.

[1466] In preferred embodiments, said modulator is an agonist and said disorder in lipid metabolism is selected from the group consisting of:

- [1467] (a) elevated level of plasma triglycerides;
- [1468] (b) elevated level of plasma free fatty acids;
- [1469] (c) elevated level of plasma cholesterol;
- [1470] (d) elevated level of LDL-cholesterol;
- [1471] (e) reduced level of HDL-cholesterol;
- [1472] (f) elevated total cholesterol/HDL-cholesterol ratio; and
- [1473] (g) reduced level of plasma adiponectin.

[1474] In other preferred embodiments, said disorder in lipid metabolism is an elevated postprandial increase in plasma free fatty acids due to a high fat meal or a progression from impaired glucose tolerance to insulin resistance.

[1475] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1476] In an eighty-first aspect, the invention features a method of using the modulator of the seventy-second aspect for the preparation of a medicament for the treatment of a metabolic-related disorder in an individual.

[1477] In some preferred embodiments, said modulator is selective for the GPCR.

[1478] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least

15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[1479] In some preferred embodiments, said modulator is antilipolytic.

[1480] In some preferred embodiments, said modulator is an agonist.

[1481] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP19 polypeptide having the amino acid sequence of SEQ. ID. NO.:24. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[1482] In some preferred embodiments, said treatment comprises oral administration of said medicament to said individual.

[1483] In preferred embodiments, said modulator is an agonist and said metabolic-related disorder is selected from the group consisting of:

- [1484] (a) dyslipidemia;
- [1485] (b) atherosclerosis;
- [1486] (c) coronary heart disease;
- [1487] (d) stroke;
- [1488] (e) insulin resistance; and
- [1489] (f) type 2 diabetes.

[1490] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1491] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1492] In an eighty-second aspect, the invention features a method of identifying whether a candidate compound binds to an antilipolytic GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

[1493] (a) SEQ. ID. NO.:24 (hRUP19);

[1494] (b) SEQ. ID. NO.:151 (mRUP19); and

[1495] (c) SEQ. ID. NO.:157 (rRUP19);

[1496] or an allelic variant or a biologically active fragment of said amino acid sequence; comprising the steps of:

[1497] (a') contacting the receptor with a labeled reference compound known to bind to the GPCR in the presence or absence of the candidate compound; and

[1498] (b') determining whether the binding of said labeled reference compound to the receptor is inhibited in the presence of the candidate compound;

[1499] wherein said inhibition is indicative of the candidate compound binding to an antilipolytic GPCR.

[1500] In some embodiments, the antilipolytic GPCR comprises a biologically active fragment of said amino acid sequence.

[1501] In some embodiments, the antilipolytic GPCR is endogenous.

[1502] In some embodiments, the antilipolytic GPCR is recombinant.

[1503] In preferred embodiments, said G protein is Gi.

[1504] In some preferred embodiments, said reference compound is the modulator of the seventy-second aspect.

[1505] In other embodiments, said reference compound is an antibody specific for the GPCR, or a derivative thereof.

[1506] In preferred embodiments, said reference compound comprises a label selected from the group consisting of:

[1507] (a) radioisotope;

[1508] (b) enzyme; and

[1509] (c) fluorophore.

[1510] In some preferred embodiments, said label is  $^3H$ .

[1511] In other embodiments, said method further comprises the step of comparing the level of inhibition of binding of a labeled first reference compound by the candidate compound to a second level of inhibition of binding of said labeled first reference compound by a second reference compound known to bind to the GPCR.

[1512] In an eighty-third aspect, the invention features a method of making a mouse genetically predisposed to a metabolic-related disorder selected from the group consisting of:

- [1513] (a) dyslipidemia;
- [1514] (b) atherosclerosis;
- [1515] (c) coronary heart disease;
- [1516] (d) stroke;
- [1517] (e) insulin resistance; and
- [1518] (f) type 2 diabetes;

[1519] comprising the step of knocking out the gene encoding the antilipolytic mRUP19 GPCR polypeptide of SEQ. ID. NO.:151.

[1520] In some preferred embodiments, said knocking out the gene encoding the antilipolytic mRUP19 GPCR polypeptide of SEQ. ID. NO.:151 is essentially restricted to adipocytes.

[1521] In an eighty-fourth aspect, the invention features the knockout mouse according to the method of the eighty-third aspect.

[1522] In an eighty-fifth aspect, the invention features a method of using the knockout mouse of the eighty-fourth aspect to identify whether a candidate compound has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

- [1523] (a) dyslipidemia;
- [1524] (b) atherosclerosis;
- [1525] (c) coronary heart disease;
- [1526] (d) stroke;
- [1527] (e) insulin resistance; and
- [1528] (f) type 2 diabetes;

[1529] comprising the steps of:

- [1530] (a') administering or not administering the compound to the mouse; and
- [1531] (b') determining whether the disorder is prevented, delayed, or made less severe on administering the compound compared to not administering the compound;

[1532] wherein said determination is indicative of the compound having therapeutic efficacy.

[1533] In an eighty-sixth aspect, the invention features a method of making a rat genetically predisposed to a metabolic-related disorder selected from the group consisting of:

- [1534] (a) dyslipidemia;
- [1535] (b) atherosclerosis;
- [1536] (c) coronary heart disease;
- [1537] (d) stroke;
- [1538] (e) insulin resistance; and
- [1539] (f) type 2 diabetes;

[1540] comprising the step of knocking out the gene encoding the antilipolytic rRUP19 GPCR polypeptide of SEQ. ID. NO.:157.

[1541] In some preferred embodiments, said knocking out the gene encoding the antilipolytic rRUP19 GPCR polypeptide of SEQ. ID. NO.:157 is essentially restricted to adipocytes.

[1542] In an eighty-seventh aspect, the invention features the knockout rat according to the method of the eighty-sixth aspect.

[1543] In an eighty-eighth aspect, the invention features a method of using the knockout rat of the eighty-seventh aspect to identify whether a candidate compound has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

- [1544] (a) dyslipidemia;
- [1545] (b) atherosclerosis;
- [1546] (c) coronary heart disease;
- [1547] (d) stroke;
- [1548] (e) insulin resistance; and
- [1549] (f) type 2 diabetes;

[1550] comprising the steps of:

- [1551] (a') administering or not administering the compound to the rat; and
- [1552] (b') determining whether the disorder is prevented, delayed, or made less severe on administering the compound compared to not administering the compound;

[1553] wherein said determination is indicative of the compound having therapeutic efficacy.

[1554] In an eighty-ninth aspect, the invention features an isolated, purified or recombinant RUP19 polynucleotide selected from the group consisting of:

- [1555] (a) a polynucleotide comprising a contiguous span of at least 75 nucleotides of SEQ. ID. NO.:23, SEQ. ID. NO.:150 or SEQ. ID. NO.:156, or an allelic variant of said polynucleotide;
- [1556] (b) a polynucleotide comprising a contiguous span of at least 150 nucleotides of SEQ. ID. NO.:23, SEQ. ID. NO.:150 or SEQ. ID. NO.:156, or an allelic variant of said polynucleotide;
- [1557] (c) a polynucleotide comprising a contiguous span of at least 250 nucleotides of SEQ. ID. NO.:23, SEQ. ID. NO.:150 or SEQ. ID. NO.:156, or an allelic variant of said polynucleotide;
- [1558] (d) a polynucleotide comprising a contiguous span of at least 350 nucleotides of SEQ. ID. NO.:23, SEQ. ID. NO.:150 or SEQ. ID. NO.:156, or an allelic variant of said polynucleotide;
- [1559] (e) a polynucleotide comprising a contiguous span of at least 500 nucleotides of SEQ. ID. NO.:23, SEQ. ID. NO.:150 or SEQ. ID. NO.:156, or an allelic variant of said polynucleotide;

- [1560] (f) a polynucleotide comprising a contiguous span of at least 750 nucleotides of SEQ. ID. NOs.:23, SEQ. ID. NO.:150 or 156, or an allelic variant of said polynucleotide;
- [1561] (g) a polynucleotide comprising a contiguous span of at least 1000 nucleotides of SEQ. ID. NO.:23, SEQ. ID. NO.:150 or SEQ. ID. NO.:156, or an allelic variant of said polynucleotide;
- [1562] (h) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 20 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157 or an allelic variant of said polypeptide;
- [1563] (i) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 30 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157 or an allelic variant of said polypeptide;
- [1564] (j) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 40 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157 or an allelic variant of said polypeptide;
- [1565] (k) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 50 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157 or an allelic variant of said polypeptide;
- [1566] (l) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 75 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157 or an allelic variant of said polypeptide;
- [1567] (m) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 100 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157 or an allelic variant of said polypeptide;
- [1568] (n) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 150 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157 or an allelic variant of said polypeptide;
- [1569] (o) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 200 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157 or an allelic variant of said polypeptide;
- [1570] (p) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 250 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157 or an allelic variant of said polypeptide; and
- [1571] (q) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 300 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157 or an allelic variant of said polypeptide.
- [1572] The invention also relates to an isolated, purified or recombinant RUP19 polynucleotide wherein said polynucleotide is selected from the group consisting of:
- [1573] (a) a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ. ID. NO.:23, SEQ. ID. NO.:150 and SEQ. ID. NO.:156 or an allelic variant of said polynucleotide;
- [1574] (b) a polynucleotide selected from the group consisting of the polynucleotide of SEQ. ID. NO.:23, the polynucleotide of SEQ. ID. NO.:150 and the polynucleotide of SEQ. ID. NO.:156, or an allelic variant of said polynucleotide;
- [1575] (c) a polynucleotide comprising a nucleotide sequence encoding a polypeptide having an amino acid sequence selected from the group consisting of SEQ. ID. NO.:24, SEQ. ID. NO.:151 and SEQ. ID. NO.:157 or an allelic variant of said polypeptide; and
- [1576] (d) a polynucleotide encoding a polypeptide having an amino acid sequence selected from the group consisting of SEQ. ID. NO.:24, SEQ. ID. NO.:151 and SEQ. ID. NO.:157 or an allelic variant of said polypeptide.
- [1577] In preferred embodiments, said isolated, purified or recombinant polynucleotide comprises at least 8 contiguous nucleotides of a polynucleotide of the present invention. In other preferred embodiments, said isolated, purified or recombinant polynucleotide comprises at least 10, 12, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 contiguous nucleotides of a polynucleotide of the present invention. Preferably said polynucleotide encodes full-length RUP19 polypeptide or a biologically active fragment thereof.
- [1578] The polynucleotides of the present invention include genomic polynucleotides comprising RUP19 polynucleotides of the invention.
- [1579] The present invention also relates to a polynucleotide encoding a fusion protein, wherein said fusion protein comprises an RUP19 polypeptide of the invention fused to a heterologous polypeptide. In a preferred embodiment, said polypeptide of the invention is constitutively active and said heterologous polypeptide is a G protein. In other embodiments, said heterologous polypeptide provides an antigenic epitope. In a preferred embodiment, said heterologous polypeptide provides a hemagglutinin (HA) antigenic epitope. Methods relating to a polynucleotide encoding a fusion protein are well known to those of ordinary skill in the art.
- [1580] The polynucleotides of the present invention also include variant polynucleotides at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to an RUP19 polynucleotide of the invention. In a particularly preferred embodiment, polynucleotide sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST"), which is well known in the art [See, e.g., Karlin and Altschul, Proc Natl Acad Sci USA (1990) 87:2264-8; Altschul et al., J Mol Biol (1990) 215:403-410; Altschul et al., Nature Genetics (1993) 3:266-72; and Altschul et al., Nucleic Acids Res (1997) 25:3389-3402; the disclosures of which are incorporated by reference in their entirety].

[1581] In further preferred embodiments, the invention features the complement of said polynucleotide.

[1582] In a nintieth aspect, the invention features an isolated, purified or recombinant RUP19 polypeptide selected from the group consisting of:

[1583] (a) a polypeptide comprising a contiguous span of at least 20 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;

[1584] (b) a polypeptide comprising a contiguous span of at least 30 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;

[1585] (c) a polypeptide comprising a contiguous span of at least 40 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;

[1586] (d) a polypeptide comprising a contiguous span of at least 50 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;

[1587] (e) a polypeptide comprising a contiguous span of at least 75 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;

[1588] (f) a polypeptide comprising a contiguous span of at least 100 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;

[1589] (g) a polypeptide comprising a contiguous span of at least 150 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;

[1590] (h) a polypeptide comprising a contiguous span of at least 200 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;

[1591] (i) a polypeptide comprising a contiguous span of at least 250 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157; and

[1592] (j) a polypeptide comprising a contiguous span of at least 300 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;

[1593] or an allelic variant of said polypeptide.

[1594] The invention also relates to an isolated, purified or recombinant RUP19 polypeptide wherein said polypeptide is selected from the group consisting of:

[1595] (a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ. ID. NO.:24, SEQ. ID. NO.:151 and SEQ. ID. NO.:157; and

[1596] (b) a polypeptide selected from the group consisting of the polypeptide of SEQ. ID. NO.:24, the polypeptide of SEQ. ID. NO.: 151 and the polypeptide of SEQ. ID. NO.:157;

[1597] or an allelic variant, a biologically active mutant, or a biologically active fragment of said polypeptide.

[1598] In preferred embodiments, said isolated, purified or recombinant polypeptide comprises at least 6 contiguous amino acids of an RUP19 polypeptide of the invention. In further embodiments, said isolated, purified or recombinant polypeptide comprises at least 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, 125, 150, 175, 200, 225, 250, 275 or 300 contiguous amino acids of a polypeptide of the present

invention. Preferably, said polypeptide is full-length RUP19 polypeptide or an active fragment thereof.

[1599] The present invention also relates to a fusion protein, wherein said fusion protein comprises an RUP19 polypeptide of the invention fused to a heterologous polypeptide. In a preferred embodiment, said polypeptide of the invention is constitutively active and said heterologous polypeptide is a G protein. In other preferred embodiments, said heterologous polypeptide provides an antigenic epitope. In particularly preferred embodiment, said heterologous polypeptide provides a hemagglutinin (HA) antigenic epitope. Methods relating to a fusion protein are well known to those of ordinary skill in the art.

[1600] The polypeptides of the present invention also include variant polypeptides at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an RUP19 polypeptide of the invention. In a particularly preferred embodiments, polypeptide sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST"), which is well known in the art [See, e.g., Karlin and Altschul, *Proc Natl Acad Sci USA* (1990) 87:2264-8; Altschul et al., *J Mol Biol* (1990) 215:403-410; Altschul et al., *Nature Genetics* (1993) 3:266-72; and Altschul et al., *Nucleic Acids Res* (1997) 25:3389-3402; the disclosures of which are incorporated by reference in their entirety].

[1601] In an ninety-first aspect, the invention features a composition comprising, consisting essentially of, or consisting of the RUP19 polypeptide of the nintieth aspect.

[1602] In a ninety-second aspect, the invention features a recombinant vector, said vector comprising, consisting essentially of, or consisting of the polynucleotide of the eighty-ninth aspect. In preferred embodiments, said vector is a targeting vector used in a method of inactivating a gene encoding an antilipolytic GPCR of the invention. In other preferred embodiments, said vector is used in a method of transient or stable transfection.

[1603] In particularly preferred embodiment, said vector is an expression vector for the expression of a antilipolytic GPCR in a recombinant host cell wherein said expression vector comprises, consists essentially of, or consists of the polynucleotide of the eighty-ninth aspect.

[1604] Although a variety of expression vectors are available to those in the art, for purposes of utilization for both the endogenous and non-endogenous human, mouse and rat GPCRs, it is most preferred that the vector utilized be pCMV. In some alternative embodiments as relates to said human, mouse and rat antilipolytic GPCRs, it is preferred that the vector utilized be an adenoviral expression vector.

[1605] In a ninety-third aspect, the invention features a prokaryotic or eukaryotic host cell comprising, consisting essentially of, or consisting of the recombinant vector of the ninety-second aspect. In some preferred embodiments, said host cell is a eukaryotic embryonic stem cell wherein said vector of the ninety-second aspect has been used in a method to inactivate a gene encoding an antilipolytic GPCR of the invention within said cell. In some other preferred embodiments, said host cell is a eukaryotic embryonic somatic cell wherein said vector of the ninety-second aspect has been used in a method to inactivate a gene encoding an antilipolytic GPCR of the invention within said cell. In other preferred embodiments, said host cell is prokaryotic and has

been transformed using the vector of the ninety-second aspect. In further preferred embodiments, said host cell is eukaryotic and has been transiently transfected using the vector of the ninety-second aspect. In other further preferred embodiments, said host cell is eukaryotic and has been stably transfected using the vector of the ninety-second aspect.

[1606] In particularly preferred embodiment, said host cell expresses a recombinant antilipolytic GPCR wherein said host cell comprises, consists essentially of, or consists of the expression vector of the ninety-second aspect.

[1607] A further embodiment includes a prokaryotic or eukaryotic host cell recombinant for the polynucleotide of the eighty-ninth aspect.

[1608] In some embodiments the host cell is eukaryotic, more preferably, mammalian, and more preferably selected from the group consisting of 293, 293T, CHO, and COS-7 cells. In other embodiments, the host cell is eukaryotic, more preferably melanophore.

[1609] In a ninety-fourth aspect, the invention features a process for the expression of an antilipolytic GPCR in a recombinant host cell comprising the steps of:

[1610] (a) transfecting the expression vector of the ninety-second aspect into a suitable host cell; and

[1611] (b) culturing the host cells under conditions which allow expression of the antilipolytic GPCR protein from the expression vectors.

[1612] In a ninety-fifth aspect, the invention features an antibody that specifically binds to the polypeptide of the nintieth aspect. In some preferred embodiments, the antibody is monoclonal. In some embodiments, the antibody is polyclonal.

[1613] In a ninety-sixth aspect, the invention features a method of binding the polypeptide of the nintieth aspect to the antibody of the ninty-fifth aspect, comprising contacting said antibody with said polypeptide under conditions in which said antibody can specifically bind to said polypeptide.

[1614] In a ninety-seventh aspect, the invention features a method of detecting an antilipolytic GPCR polypeptide in a biological sample obtained from an individual comprising the steps of:

[1615] (a) obtaining said biological sample from said individual;

[1616] (b) contacting said biological sample with the antibody of the ninety-fifth aspect; and

[1617] (c) detecting the presence or absence of binding of said antibody to said biological sample;

[1618] wherein a detection of said binding is indicative of the receptor polypeptide being expressed in said biological sample.

[1619] In preferred embodiments, said detecting is through the use of an enzyme-labeled secondary reagent. In other preferred embodiments, said detecting is through the use of a fluorophore-labeled secondary reagent. In other preferred embodiments, said detecting is through the use of

a radioisotope-labeled secondary reagent. In other embodiments, the antibody is directly labeled with enzyme, fluorophore or radioisotope.

[1620] In other preferred embodiments, said biological sample is taken from adipose, skin or blood.

[1621] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1622] In further embodiments, said individual has a disorder of lipid metabolism selected from the group consisting of:

[1623] (a) elevated level of plasma triglycerides;

[1624] (b) elevated level of plasma free fatty acids;

[1625] (c) elevated level of plasma cholesterol;

[1626] (d) elevated level of LDL-cholesterol;

[1627] (e) reduced level of HDL-cholesterol;

[1628] (f) elevated total cholesterol/HDL-cholesterol ratio; and

[1629] (g) reduced level of plasma adiponectin.

[1630] In other preferred embodiments, said disorder in lipid metabolism is an elevated postprandial increase in plasma free fatty acids due to a high fat meal or a progression from impaired glucose tolerance to insulin resistance.

[1631] In further embodiments, said individual has a metabolic-related disorder selected from the group consisting of:

[1632] (a) dyslipidemia;

[1633] (b) atherosclerosis;

[1634] (c) coronary heart disease;

[1635] (d) stroke;

[1636] (e) insulin resistance; and

[1637] (f) type 2 diabetes.

[1638] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1639] In other embodiments, said method further comprises the step of comparing the level of detection of said binding for a first individual to the level of detection of said binding for a second individual.

[1640] In a ninety-eighth aspect, the invention features a method of detecting expression of a gene encoding antilipolytic GPCR in a biological sample obtained from an individual comprising the steps of:

[1641] (a) obtaining said biological sample from said individual;

[1642] (b) contacting said biological sample with the complementary polynucleotide of the eighty-ninth

aspect, optionally labeled, under conditions permissive for hybridization; and

[1643] (c) detecting the presence or absence of said hybridization between said complementary polynucleotide and an RNA species within said sample;

[1644] wherein a detection of said hybridization is indicative of expression of said GPCR gene in said biological sample.

[1645] Methods of labeling a nucleic acid probe are well known to those of ordinary skill in the art.

[1646] In preferred embodiments, the biological sample is taken from adipose, skin or blood.

[1647] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1648] In preferred embodiments, said individual has a disorder of lipid metabolism selected from the group consisting of:

- [1649] (a) elevated level of plasma triglycerides;
- [1650] (b) elevated level of plasma free fatty acids;
- [1651] (c) elevated level of plasma cholesterol;
- [1652] (d) elevated level of LDL-cholesterol;
- [1653] (e) reduced level of HDL-cholesterol;
- [1654] (f) elevated total cholesterol/HDL-cholesterol ratio; and
- [1655] (g) reduced level of plasma adiponectin.

[1656] In other preferred embodiments, said disorder in lipid metabolism is an elevated postprandial increase in plasma free fatty acids due to a high fat meal or a progression from impaired glucose tolerance to insulin resistance.

[1657] In other preferred embodiments, said individual has a metabolic-related disorder selected from the group consisting of:

- [1658] (a) dyslipidemia;
- [1659] (b) atherosclerosis;
- [1660] (c) coronary heart disease;
- [1661] (d) stroke;
- [1662] (e) insulin resistance; and
- [1663] (f) type 2 diabetes.

[1664] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1665] In other embodiments, said method further comprises the step of comparing the level of detection of said hybridization for a first individual to the level of detection of said hybridization for a second individual.

[1666] In some preferred embodiments, said complementary polynucleotide is a primer and said hybridization is detected by detecting the presence of an amplification product comprising the sequence of said primer. In more preferred embodiments, said method is RT-PCR.

[1667] In a ninety-ninths aspect, the invention features a GPCR Fusion Protein construct comprising a constitutively active GPCR and a G protein, said receptor comprising an amino acid sequence selected from the group consisting of:

- [1668] (a) SEQ. ID. NO.:24 (hRUP19);
- [1669] (b) SEQ. ID. NO.:151 (mRUP19); and
- [1670] (c) SEQ. ID. NO.:157 (rRUP19);

[1671] or an allelic variant or a biologically active fragment of said amino acid sequence.

[1672] The invention also relates to a GPCR Fusion Protein construct wherein the threonine at amino acid position 219 of SEQ. ID. NO.:24 is substituted by lysine.

[1673] In a one hundredth aspect, the invention features a method of binding a known ligand of RUP19 antilipolytic GPCR to a polypeptide selected from the group consisting of:

- [1674] (a) a polypeptide comprising a contiguous span of at least 6 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;
- [1675] (b) a polypeptide comprising a contiguous span of at least 10 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;
- [1676] (c) a polypeptide comprising a contiguous span of at least 15 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;
- [1677] (d) a polypeptide comprising a contiguous span of at least 20 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;
- [1678] (e) a polypeptide comprising a contiguous span of at least 25 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;
- [1679] (f) a polypeptide comprising a contiguous span of at least 30 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;
- [1680] (g) a polypeptide comprising a contiguous span of at least 35 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;
- [1681] (h) a polypeptide comprising a contiguous span of at least 40 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;
- [1682] (i) a polypeptide comprising a contiguous span of at least 45 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157; and
- [1683] (j) a polypeptide comprising a contiguous span of at least 50 amino acids of SEQ. ID. NO.:24, SEQ. ID. NO.:151 or SEQ. ID. NO.:157;

[1684] or an allelic variant of said polypeptide;

[1685] comprising the step of contacting said known ligand with said polypeptide under conditions which allow said binding to occur.

[1686] In some embodiments, said known ligand is a modulator of the GPCR. In some embodiments, said known ligand is an agonist of the GPCR. In some embodiments, said known ligand is the modulator of the seventy-second aspect. In some embodiments, said known ligand is an antibody specific for the GPCR, or a derivative thereof.

[1687] In other preferred embodiments, said method is used to identify whether a candidate compound inhibits said binding of said known ligand to said polypeptide, comprising the steps of:

[1688] (a) contacting said polypeptide with said known ligand, optionally labeled, in the presence or absence of said candidate compound;

[1689] (b) detecting the complex between said known ligand and said polypeptide; and

[1690] (c) determining whether less of said complex is formed in the presence of the compound than in the absence of the compound;

[1691] wherein said determination is indicative of the candidate compound being an inhibitor of said binding of said known ligand to said polypeptide.

[1692] In some embodiments, said known ligand is a modulator of the GPCR. In some embodiments, said known ligand is an agonist of the GPCR. In some embodiments, said known ligand is the modulator of the seventy-second aspect. In some embodiments, said known ligand is an antibody specific for the GPCR, or a derivative thereof.

[1693] In other preferred embodiments, said method is used to identify whether a candidate compound is an inhibitor of said binding of said known ligand to said polypeptide, comprising the steps of:

[1694] (a) contacting said polypeptide with said known ligand, optionally labeled, in the presence separately of a plurality of concentrations of said candidate compound for a time sufficient to allow equilibration of binding;

[1695] (b) measuring unbound ligand and bound ligand; and

[1696] (c) determining  $K_i$  for the candidate compound;

[1697] wherein a  $K_i$  value of less than 50  $\mu\text{M}$  is indicative of the candidate compound being an inhibitor of said binding of said known ligand to said polypeptide. Preferably said  $K_i$  value is less than 25  $\mu\text{M}$ , 10  $\mu\text{M}$ , 5  $\mu\text{M}$ , 1  $\mu\text{M}$ , 750 nM, 500 nM, 400 nM, 300 nM, 250 nM, 200 nM, 150 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM or 10 nM. In preferred embodiments,  $K_i$  determination is made through nonlinear curve fitting with the program SCFIT [De Lean et al. (1982) Mol Pharmacol 21:5-16; cited in Lorenzen et al. (2001) Mol Pharmacol 59:349-357, the disclosures of which are incorporated by reference herein in their entireties].

[1698] In some embodiments, said known ligand is a modulator of the GPCR. In some embodiments, said known ligand is an agonist of the GPCR. In some embodiments, said known ligand is the modulator of the seventy-second aspect. In some embodiments, said known ligand is an antibody specific for the GPCR, or a derivative thereof.

[1699] In a one hundred first aspect, the invention features a method of binding an optionally labeled affinity reagent specific for an antipolytic GPCR to said receptor in a biological sample, said receptor comprising an amino acid sequence selected from the group consisting of:

[1700] (a) SEQ. ID. NO.:24 (hRUP19);

[1701] (b) SEQ. ID. NO.:151 (mRUP19); and

[1702] (c) SEQ. ID. NO.:157 (rRUP19);

[1703] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence, comprising the steps of:

[1704] (a') obtaining said biological sample;

[1705] (b') contacting the affinity reagent with said receptor in said biological sample; and

[1706] (c') detecting the complex of said affinity reagent with said receptor.

[1707] In some embodiments, the antipolytic GPCR has an amino acid sequence selected from the group consisting of:

[1708] (a) SEQ. ID. NO.:24 (hRUP19);

[1709] (b) SEQ. ID. NO.:151 (mRUP19); and

[1710] (c) SEQ. ID. NO.:157 (rRUP19);

[1711] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence.

[1712] In some embodiments, the antipolytic GPCR comprises an active fragment of said amino acid sequence.

[1713] In some embodiments, the antipolytic GPCR is endogenous.

[1714] In some embodiments, the antipolytic GPCR is recombinant.

[1715] In some embodiments, said biologically active mutant is CART or EFA. In preferred embodiments, said CART mutant has the amino acid sequence of SEQ. ID. NO.:24 further substituted at amino acid position 219 with lysine in place of threonine.

[1716] In preferred embodiments, said G protein is  $G_i$ .

[1717] In some embodiments, said affinity reagent is a modulator of the GPCR. In some embodiments, said affinity reagent is an agonist of the GPCR. In some embodiments, said affinity reagent is the modulator of the seventy-second aspect. In some embodiments, said affinity reagent is an antibody specific for the GPCR, or a derivative thereof.

[1718] In further preferred embodiments, said affinity reagent comprises a label selected from the group consisting of:

[1719] (a) radioisotope;

[1720] (b) enzyme; and

[1721] (c) fluorophore.

[1722] In preferred embodiments, said radioisotope is  $^3\text{H}$ .

[1723] In a one hundred second aspect, the invention features the method of the one hundred first aspect further

comprising the step of comparing the level of detection of said complex in a first biological sample to a second level of detection of said complex in a second biological sample.

[1724] In a one hundred third aspect, the invention features the method of the one hundred second aspect wherein the relationship between said first and second biological samples is selected from the group consisting of:

[1725] (a) said second biological sample is a replicate of said first biological sample;

[1726] (b) said first biological sample was obtained prior to an experimental intervention whereas said second biological sample was obtained after the experimental intervention, from the same individual;

[1727] (c) said second biological sample was obtained at a different time point after an experimental intervention than was said first biological sample, from the same individual;

[1728] (d) said second biological sample corresponds to a different subcellular compartment than does said first biological sample;

[1729] (e) said second biological sample represents a different cell type than does said first biological sample;

[1730] (f) said second biological sample corresponds to a different tissue than does said first biological sample;

[1731] (g) said second biological sample was obtained from a different individual than was said first biological sample;

[1732] (h) said second biological sample was obtained at a different point in time than was said first biological sample, from the same individual;

[1733] (i) said first biological samples was obtained from a normal individual, whereas said second biological sample was obtained from an individual having a metabolic-related disorder;

[1734] (j) said first biological sample was obtained from a normal individual, whereas said second biological sample was obtained from an individual having a disorder in lipid metabolism;

[1735] (k) said first biological sample was obtained before a therapeutic intervention whereas said second biological sample was obtained after the therapeutic intervention, from the same individual;

[1736] (l) said second biological sample was obtained at a different time point after therapeutic intervention than was said first biological sample, from the same individual; and

[1737] (m) said first biological sample was not exposed to a compound, whereas said second biological sample was exposed to said compound.

[1738] In a one hundred fourth aspect, the invention features a method of identifying whether a candidate compound is a modulator of an antilipolytic GPCR, said receptor comprising the amino acid sequence of SEQ. ID. NO.:8 (hRUP11);

[1739] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

[1740] comprising the steps of:

[1741] (a) contacting the candidate compound with the receptor;

[1742] (b) determining whether the receptor functionality is modulated;

[1743] wherein a change in receptor functionality is indicative of the candidate compound being a modulator of an antilipolytic GPCR.

[1744] In some embodiments, said antilipolytic GPCR is endogenous.

[1745] In some preferred embodiments, said antilipolytic GPCR is recombinant.

[1746] Preferred said identified modulator binds to said GPCR.

[1747] In some embodiments, said contacting is carried out in the presence of a known ligand of the GPCR. In some embodiments, said known ligand is an agonist of the GPCR.

[1748] The invention also relates to a method of identifying whether a candidate compound is a modulator of lipolysis, comprising the steps of:

[1749] (a) contacting the candidate compound with a GPCR comprising the amino acid sequence of SEQ. ID. NO.:8 (hRUP11);

[1750] or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence; and

[1751] (b) determining whether the receptor functionality is modulated;

[1752] wherein a change in receptor functionality is indicative of the candidate compound being a modulator of lipolysis.

[1753] In some embodiments, said GPCR is endogenous.

[1754] In some preferred embodiments, said GPCR is recombinant.

[1755] Preferred said identified modulator binds to said GPCR.

[1756] In some embodiments, said contacting is carried out in the presence of a known ligand of the GPCR. In some embodiments, said known ligand is an agonist of the GPCR.

[1757] The invention also relates to a method of determining whether a candidate compound is a modulator of an antilipolytic GPCR,

[1758] comprising the steps of:

[1759] (a) culturing antilipolytic GPCR-expressing host cells under conditions that would allow expression of a recombinant antilipolytic GPCR, said host cells being transfected with a polynucleotide encoding said recombinant antilipolytic GPCR comprising the amino acid sequence of SEQ. ID. NO.:8 (hRUP11); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

- [1760] (b) contacting the antipolytic GPCR-expressing host cells of step (a) with the candidate compound;
- [1761] (c) contacting control host cells with the candidate compound of step (b), wherein said control host cells do not express recombinant antipolytic GPCR protein;
- [1762] (d) measuring the modulating effect of the candidate compound which interacts with the recombinant antipolytic GPCR from the host cells of step (a) and control host cells of step (c); and
- [1763] (e) comparing the modulating effect of the test compound on the host cells and control host cells.
- [1764] The invention also relates to a method of determining whether a candidate compound is a modulator of an antipolytic GPCR, comprising the steps of:
- [1765] (a) culturing antipolytic GPCR-expressing host cells under conditions that would allow expression of a recombinant antipolytic GPCR, said host cells being transfected with a polynucleotide encoding said recombinant antipolytic GPCR comprising the amino acid sequence of SEQ. ID. NO.:8 (hRUP11); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;
- [1766] (b) contacting a first population of antipolytic GPCR-expressing cells of step (a) with a known ligand of said antipolytic GPCR;
- [1767] (c) contacting a second population of antipolytic GPCR-expressing cells of step (a) with the candidate compound and with the known antipolytic GPCR ligand;
- [1768] (d) contacting control host cells with the candidate compound of step (c), wherein said control host cells do not express recombinant antipolytic GPCR protein;
- [1769] (e) measuring the modulating effect of the candidate compound, which interacts with recombinant antipolytic GPCR, in the presence and absence of the known antipolytic GPCR ligand, from the cells of step (b), step (c) and step (d); and
- [1770] (f) comparing the modulating effect of the candidate compound as determined from step (b), step (c) and step (d).
- [1771] In some embodiments, said known ligand is an agonist of the GPCR.
- [1772] The invention also relates to a method of determining whether a candidate compound is a modulator of an antipolytic GPCR, comprising the steps of:
- [1773] (a) culturing antipolytic GPCR-expressing host cells under conditions that would allow expression of a recombinant antipolytic GPCR, said host cells being transfected with a polynucleotide encoding said recombinant antipolytic GPCR comprising the amino acid sequence of SEQ. ID. NO.:8 (hRUP11); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;
- [1774] (b) contacting a first population of the antipolytic GPCR-expressing host cells of step (a) with the candidate compound;
- [1775] (c) not contacting a second population of the antipolytic GPCR-expressing cells of step (a) with the candidate compound of step (b);
- [1776] (d) contacting control host cells to the candidate compound of step (b), wherein said control host cells do not express recombinant antipolytic GPCR protein;
- [1777] (e) measuring the modulating effect of the candidate compound, which interacts with recombinant antipolytic GPCR protein, from the cells of step (b) and step (c) and from the cells of step (d); and
- [1778] (f) comparing the modulating effect of the candidate compound as determined from step (b) and step (c) and from step (d).
- [1779] In some embodiments, the antipolytic GPCR has the amino acid sequence of SEQ. ID. NO.:8 (hRUP11); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence.
- [1780] In some embodiments, the antipolytic GPCR comprises a biologically active fragment of said amino acid sequence.
- [1781] In some embodiments, said biologically active mutant is CART or EFA. In preferred embodiments, said CART mutant has the amino acid sequence of SEQ. ID. NO.:8 further substituted at amino acid position 294 with lysine in place of methionine.
- [1782] In preferred embodiments, said G protein is Gi.
- [1783] In other preferred embodiments, said determining is through the use of a Melanophore assay.
- [1784] In other preferred embodiments, said determining is through the measurement of the level of a second messenger selected from the group consisting of cyclic AMP (cAMP), cyclic GMP (cGMP), inositol triphosphate (IP<sub>3</sub>), diacylglycerol (DAG), and Ca<sup>2+</sup>. In further preferred embodiments, said second messenger is cAMP. In more preferred embodiments, the level of the cAMP is reduced. In some embodiments, said measurement of cAMP is carried out with membrane comprising said GPCR.
- [1785] In other preferred embodiments, said determining is through the measurement of an activity up-regulated or down-regulated by a reduction in intracellular cAMP level. In further preferred embodiments, said down-regulated activity is intracellular lipolysis. In other further preferred embodiments, said down-regulated activity is hormone sensitive lipase activity. In other further preferred embodiments, said up-regulated activity is adiponectin secretion.
- [1786] In other preferred embodiments, said determining is through CRE-reporter assay. In preferred embodiments, said reporter is luciferase. In some embodiments, said reporter is  $\beta$ -galactosidase.
- [1787] In other preferred embodiments, said recombinant host cell further comprises promiscuous G alpha 15/16 or chimeric Gq/Gi alpha subunit and said determining is through measurement of intracellular Ca<sup>2+</sup>. In preferred embodiments, said Ca<sup>2+</sup> measurement is carried out by FLIPR.
- [1788] In other preferred embodiments, said recombinant host cell further comprises promiscuous G alpha 15/16 or chimeric Gq/Gi alpha subunit and said determining is through measurement of intracellular IP<sub>3</sub>.

[1789] In other preferred embodiments, said determining is through the measurement of GTP $\gamma$ S binding to membrane comprising said GPCR. In further preferred embodiments, said GTP $\gamma$ S is labeled with [ $^{35}$ S].

[1790] In other preferred embodiments, said method further comprises the step of comparing the modulation of the receptor caused by the candidate compound to a second modulation of the receptor caused by contacting the receptor with a known modulator of the receptor. In some preferred embodiments, said known modulator is an agonist.

[1791] In a one hundred fifth aspect, the invention features a modulator of an antilipolytic GPCR identified according to the method of the one hundred fourth aspect.

[1792] In some preferred embodiments, said modulator is selected from the group consisting of agonist, partial agonist, inverse agonist and antagonist. More preferably, said modulator is an agonist. In some embodiments, said modulator is a partial agonist.

[1793] In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 1000  $\mu$ M in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP11 polypeptide having the amino acid sequence of SEQ. ID. NO.:8. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 900  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 800  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 700  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 600  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 550  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 500  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 450  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 400  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 350  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 300  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 250  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 200  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 150  $\mu$ M in said assay. In some embodiments, said modulator is an agonist with an EC<sub>50</sub> of less than 100  $\mu$ M in said assay. In some preferred embodiments, said modulator is an agonist with an EC<sub>50</sub> in said assay of less than a value selected from the interval of 600  $\mu$ M to 1000  $\mu$ M.

[1794] In some embodiments, said modulator is selective for the GPCR.

[1795] In some embodiments, said modulator is antilipolytic.

[1796] In some embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some preferred embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%,

at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some preferred embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[1797] In highly less preferred embodiments, said modulator is an antibody or derivative thereof.

[1798] In a one hundred sixth aspect, the invention features a method of modulating the activity of an antilipolytic GPCR, said receptor comprising the amino acid sequence of SEQ. ID. NO.:8 (hRUP11); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

[1799] comprising the step of contacting the receptor with the modulator of the one hundred fifth aspect.

[1800] In some embodiments, the antilipolytic GPCR has the amino acid sequence of SEQ. ID. NO.:8 (hRUP11); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence.

[1801] In some embodiments, the antilipolytic GPCR comprises an active fragment of said amino acid sequence.

[1802] In some embodiments, the antilipolytic GPCR is endogenous.

[1803] In some embodiments, the antilipolytic GPCR is recombinant.

[1804] In some embodiments, said biologically active mutant is CART or EFA. In preferred embodiments, said CART mutant has the amino acid sequence of SEQ. ID. NO.:8 further substituted at amino acid position 294 with lysine in place of methionine.

[1805] In preferred embodiments, said G protein is Gi.

[1806] In some preferred embodiments, said modulator is an agonist.

[1807] In preferred embodiments, said modulator is selective for the GPCR.

[1808] In other preferred embodiments, said contacting comprises administration of the modulator to a membrane comprising the receptor.

[1809] In other preferred embodiments, said contacting comprises administration of the modulator to a cell or tissue comprising the receptor.

[1810] In other preferred embodiments, said contacting comprises administration of the modulator to an individual comprising the receptor. In more preferred embodiments, said individual is a mammal. In other more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1811] In some preferred embodiments, said modulator is selective for the GPCR.

[1812] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15%

relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[1813] In some preferred embodiments, said modulator is antilipolytic.

[1814] In some preferred embodiments, said modulator is an agonist.

[1815] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP11 polypeptide having the amino acid sequence of SEQ. ID. NO.:8. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[1816] In some preferred embodiments, said administration is oral.

[1817] In preferred embodiments, said modulator is an agonist and said individual is in need of prevention of or treatment for a metabolic-related disorder selected from the group consisting of:

- [1818] (a) dyslipidemia;
- [1819] (b) atherosclerosis;
- [1820] (c) coronary heart disease;
- [1821] (d) stroke;
- [1822] (e) insulin resistance; and
- [1823] (f) type 2 diabetes.

[1824] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease,

hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1825] In other embodiments, said modulator is an inverse agonist and said metabolic-related disorder relates to a low level of plasma free fatty acids.

[1826] In other preferred embodiments, said modulator is an agonist and said individual is in need of a change in lipid metabolism selected from the group consisting of:

- [1827] (a) a decrease in the level of plasma triglycerides;
- [1828] (b) a decrease in the level of plasma free fatty acids;
- [1829] (c) a decrease in the level of plasma cholesterol;
- [1830] (d) a decrease in the level of LDL-cholesterol;
- [1831] (e) an increase in the level of HDL-cholesterol;
- [1832] (f) a decrease in the total cholesterol/HDL-cholesterol ratio; and
- [1833] (g) an increase in the level of plasma adiponectin.

[1834] In other preferred embodiments, said needed change in lipid metabolism is a decrease in the postprandial increase in plasma free fatty acids due to a high fat meal or an inhibition of the progression from impaired glucose tolerance to insulin resistance.

[1835] In some embodiments, the modulator is an inverse agonist and the needed change in lipid metabolism is an increase in the level of plasma free fatty acids.

[1836] In other preferred embodiments, said modulator is an agonist and said individual is a mouse genetically predisposed to a metabolic-related disorder selected from the group consisting of:

- [1837] (a) dyslipidemia;
- [1838] (b) atherosclerosis;
- [1839] (c) coronary heart disease;
- [1840] (d) stroke;
- [1841] (e) insulin resistance; and
- [1842] (f) type 2 diabetes.

[1843] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure.

[1844] In further preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1845] In further preferred embodiments, said method is used to identify whether said agonist has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

- [1846] (a) dyslipidemia;
  - [1847] (b) atherosclerosis;
  - [1848] (c) coronary heart disease;
  - [1849] (d) stroke;
  - [1850] (e) insulin resistance; and
  - [1851] (f) type 2 diabetes;
- [1852] comprising the steps of:
- [1853] (a') administering or not administering said agonist to the mouse; and
  - [1854] (b') determining whether the disorder is prevented, delayed, or made less severe on administering said agonist compared to not administering said agonist;
- [1855] wherein said determination is indicative of said agonist having therapeutic efficacy.
- [1856] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.
- [1857] In other preferred embodiments, said modulator is an agonist and said individual is a rat genetically predisposed to a metabolic-related disorder selected from the group consisting of:
- [1858] (a) dyslipidemia;
  - [1859] (b) atherosclerosis;
  - [1860] (c) coronary heart disease;
  - [1861] (d) stroke;
  - [1862] (e) insulin resistance; and
  - [1863] (f) type 2 diabetes.
- [1864] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In further preferred embodiments, said metabolic-related disorder is hyperlipidemia.
- [1865] In further preferred embodiments, said method is used to identify whether said agonist has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:
- [1866] (a) dyslipidemia;
  - [1867] (b) atherosclerosis;
  - [1868] (c) coronary heart disease;
  - [1869] (d) stroke;
  - [1870] (e) insulin resistance; and
  - [1871] (f) type 2 diabetes;

- [1872] comprising the steps of:
  - [1873] (a') administering or not administering said agonist to the rat; and
  - [1874] (b') determining whether the disorder is prevented, delayed, or made less severe on administering said agonist compared to not administering said agonist;
- [1875] wherein said determination is indicative of said agonist having therapeutic efficacy.
- [1876] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.
- [1877] In a one hundred seventh aspect, the invention features a method of preventing or treating a disorder of lipid metabolism in an individual comprising contacting a therapeutically effective amount of the modulator of the one hundred fifth aspect with an antilipolytic GPCR, said receptor comprising the amino acid sequence of SEQ. ID. NO.:8 (hRUP11);
- [1878] or an allelic variant or biologically active fragment of said amino acid sequence.
- [1879] In some preferred embodiments, said modulator is selective for the GPCR.
- [1880] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.
- [1881] In some preferred embodiments, said modulator is antilipolytic.
- [1882] In some preferred embodiments, said modulator is an agonist.
- [1883] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTPyS binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP11 polypeptide having the amino acid sequence of SEQ. ID. NO.:8. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than

550  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 500  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 450  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 400  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 350  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 300  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 250  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 200  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 150  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 100  $\mu\text{M}$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  in said assay of less than a value selected from the interval of 600  $\mu\text{M}$  to 1000  $\mu\text{M}$ .

[1884] In some preferred embodiments, said contacting comprises oral administration of said modulator to said individual.

[1885] In preferred embodiment, said modulator is an agonist and said disorder of lipid metabolism is selected from the group consisting of:

- [1886] (a) elevated level of plasma triglycerides;
- [1887] (b) elevated level of plasma free fatty acids;
- [1888] (c) elevated level of plasma cholesterol;
- [1889] (d) elevated level of LDL-cholesterol;
- [1890] (e) reduced level of HDL-cholesterol;
- [1891] (f) elevated total cholesterol/HDL-cholesterol ratio; and
- [1892] (g) reduced level of plasma adiponectin.

[1893] In a one hundred eighth aspect, the invention features a method of preventing or treating a metabolic-related disorder in an individual comprising contacting a therapeutically effective amount of the modulator of the one hundred fifth aspect with an antilipolytic GPCR, said receptor comprising the amino acid sequence of SEQ. ID. NO.:8 (hRUP11);

[1894] or an allelic variant or biologically active fragment of said amino acid sequence.

[1895] In some preferred embodiments, said modulator is selective for the GPCR.

[1896] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[1897] In some preferred embodiments, said modulator is antilipolytic.

[1898] In some preferred embodiments, said modulator is an agonist.

[1899] In some preferred embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 1000  $\mu\text{M}$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP11 polypeptide having the amino acid sequence of SEQ. ID. NO.:8. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 900  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 800  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 700  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 600  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 550  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 500  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 450  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 400  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 350  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 300  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 250  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 200  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 150  $\mu\text{M}$  in said assay. In some embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  of less than 100  $\mu\text{M}$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $\text{EC}_{50}$  in said assay of less than a value selected from the interval of 600  $\mu\text{M}$  to 1000  $\mu\text{M}$ .

[1900] In some preferred embodiments, said contacting comprises oral administration of said modulator to said individual.

[1901] In preferred embodiment, said modulator is an agonist and said metabolic-related disorder is selected from the group consisting of:

- [1902] (a) dyslipidemia;
- [1903] (b) atherosclerosis;
- [1904] (c) coronary heart disease;
- [1905] (d) stroke;
- [1906] (e) insulin resistance; and
- [1907] (f) type 2 diabetes.

[1908] In a one hundred ninth aspect, the invention features a method of preparing a composition which comprises identifying a modulator of an antilipolytic GPCR and then admixing a carrier and the modulator, wherein the modulator is identifiable by the method of the one hundred fourth aspect.

[1909] In some preferred embodiments, said modulator is selected from the group consisting of agonist, partial agonist, inverse agonist and antagonist. More preferably, said modulator is an agonist. In some embodiments, said modulator is a partial agonist.

[1910] In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP11 polypeptide having the amino acid sequence of SEQ. ID. NO.:8. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[1911] In some embodiments, said modulator is selective for the GPCR.

[1912] In some embodiments, said modulator is antipolytic.

[1913] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to either intraperitoneal or intravenous administration. In some preferred embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to either intraperitoneal or intravenous administration.

[1914] In an one hundred tenth aspect, the invention features a pharmaceutical or physiologically acceptable composition comprising, consisting essentially of, or consisting of the modulator of the one hundred fifth aspect. In preferred embodiments, said modulator is an agonist.

[1915] In some preferred embodiments, said modulator is selective for the GPCR.

[1916] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at

least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[1917] In some preferred embodiments, said modulator is antipolytic.

[1918] In some preferred embodiments, said modulator is an agonist.

[1919] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP11 polypeptide having the amino acid sequence of SEQ. ID. NO.:8. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[1920] In a one hundred eleventh aspect, the invention features a method of changing lipid metabolism comprising providing or administering to an individual in need of said change said pharmaceutical or physiologically acceptable composition of the one hundred tenth aspect, said needed change in lipid metabolism selected from the group consisting of:

- [1921] (a) a decrease in the level of plasma triglycerides;
- [1922] (b) a decrease in the level of plasma free fatty acids;
- [1923] (c) a decrease in the level of plasma cholesterol;
- [1924] (d) a decrease in the level of LDL-cholesterol;
- [1925] (e) an increase in the level of HDL-cholesterol;
- [1926] (f) a decrease in the total cholesterol/HDL-cholesterol ratio; and
- [1927] (g) an increase in the level of plasma adiponectin.

[1928] In preferred embodiments, a therapeutically effect amount of said pharmaceutical or physiologically acceptable composition is provided or administered to said individual.

[1929] In some preferred embodiments, said providing or administering of said pharmaceutical or physiologically acceptable composition is oral.

[1930] In other preferred embodiments, said needed change in lipid metabolism is a decrease in the postprandial increase in plasma free fatty acids due to a high fat meal or an inhibition of the progression from impaired glucose tolerance to insulin resistance.

[1931] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1932] In a one hundred twelfth aspect, the invention features a method of preventing or treating a metabolic-related disorder comprising providing or administering to an individual in need of said treatment said pharmaceutical or physiologically acceptable composition of the one hundred tenth aspect, said metabolic-related disorder selected from the group consisting of:

- [1933] (a) dyslipidemia;
- [1934] (b) atherosclerosis;
- [1935] (c) coronary heart disease;
- [1936] (d) stroke;
- [1937] (e) insulin resistance; and
- [1938] (f) type 2 diabetes.

[1939] In preferred embodiments, a therapeutically effect amount of said pharmaceutical or physiologically acceptable composition is provided or administered to said individual.

[1940] In some preferred embodiments, said providing or administering of said pharmaceutical or physiologically acceptable composition is oral.

[1941] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, stroke, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1942] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1943] In an one hundred thirteenth aspect, the invention features a method of using the modulator of the one hundred fifth aspect for the preparation of a medicament for the treatment of a disorder in lipid metabolism in an individual.

[1944] In some preferred embodiments, said modulator is selective for the GPCR.

[1945] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[1946] In some preferred embodiments, said modulator is antilipolytic.

[1947] In some preferred embodiments, said modulator is an agonist.

[1948] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP11 polypeptide having the amino acid sequence of SEQ. ID. NO.:8. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[1949] In some preferred embodiments, said treatment comprises oral administration of said medicament to said individual.

[1950] In preferred embodiments, said modulator is an agonist and said disorder in lipid metabolism is selected from the group consisting of:

- [1951] (a) elevated level of plasma triglycerides;
- [1952] (b) elevated level of plasma free fatty acids;
- [1953] (c) elevated level of plasma cholesterol;
- [1954] (d) elevated level of LDL-cholesterol;

[1955] (e) reduced level of HDL-cholesterol;

[1956] (f) elevated total cholesterol/HDL-cholesterol ratio; and

[1957] (g) reduced level of plasma adiponectin.

[1958] In other preferred embodiments, said disorder in lipid metabolism is an elevated postprandial increase in plasma free fatty acids due to a high fat meal or a progression from impaired glucose tolerance to insulin resistance.

[1959] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1960] In a one hundred fourteenth aspect, the invention features a method of using the modulator of the one hundred fifth aspect for the preparation of a medicament for the treatment of a metabolic-related disorder in an individual.

[1961] In some preferred embodiments, said modulator is selective for the GPCR.

[1962] In some preferred embodiments, said modulator is orally bioavailable. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intraperitoneal administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, or at least 45% relative to intravenous administration. In some embodiments, said oral bioavailability is at least 1%, at least 5%, at least 10%, or at least 15% relative to intravenous administration.

[1963] In some preferred embodiments, said modulator is antilipolytic.

[1964] In some preferred embodiments, said modulator is an agonist.

[1965] In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 1000  $\mu M$  in GTP $\gamma$ S binding assay carried out with membrane from stably transfected CHO cells expressing recombinant hRUP11 polypeptide having the amino acid sequence of SEQ. ID. NO.:8. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 900  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 800  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 700  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 600  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 550  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 500  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 450  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 400  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 350  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 300  $\mu M$  in said assay. In some embodi-

ments, said modulator is an agonist with an  $EC_{50}$  of less than 250  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 200  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 150  $\mu M$  in said assay. In some embodiments, said modulator is an agonist with an  $EC_{50}$  of less than 100  $\mu M$  in said assay. In some preferred embodiments, said modulator is an agonist with an  $EC_{50}$  in said assay of less than a value selected from the interval of 600  $\mu M$  to 1000  $\mu M$ .

[1966] In some preferred embodiments, said treatment comprises oral administration of said medicament to said individual.

[1967] In preferred embodiments, said modulator is an agonist and said metabolic-related disorder is selected from the group consisting of:

[1968] (a) dyslipidemia;

[1969] (b) atherosclerosis;

[1970] (c) coronary heart disease;

[1971] (d) stroke;

[1972] (e) insulin resistance; and

[1973] (f) type 2 diabetes.

[1974] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[1975] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[1976] In a one hundred fifteenth aspect, the invention features a method of identifying whether a candidate compound binds to an antilipolytic GPCR, said receptor comprising the amino acid sequence of SEQ. ID. NO.:8 (hRUP11); or an allelic variant or a biologically active fragment of said amino acid sequence;

[1977] comprising the steps of:

[1978] (a) contacting the receptor with a labeled reference compound known to bind to the GPCR in the presence or absence of the candidate compound; and

[1979] (b) determining whether the binding of said labeled reference compound to the receptor is inhibited in the presence of the candidate compound;

[1980] wherein said inhibition is indicative of the candidate compound binding to an antilipolytic GPCR.

[1981] In some embodiments, the antilipolytic acid GPCR comprises a biologically active fragment of said amino acid sequence.

[1982] In some embodiments, the antilipolytic GPCR is endogenous.

[1983] In some embodiments, the antilipolytic GPCR is recombinant.

[1984] In preferred embodiments, said G protein is Gi.

[1985] In some preferred embodiments, said reference compound is the modulator of the one hundred fifth aspect.

[1986] In other embodiments, said reference compound is an antibody specific for the GPCR, or a derivative thereof.

[1987] In preferred embodiments, said reference compound comprises a label selected from the group consisting of:

[1988] (a) radioisotope;

[1989] (b) enzyme; and

[1990] (c) fluorophore.

[1991] In some preferred embodiments, said label is  $^3\text{H}$ .

[1992] In other embodiments, said method further comprises the step of comparing the level of inhibition of binding of a labeled first reference compound by the candidate compound to a second level of inhibition of binding of said labeled first reference compound by a second reference compound known to bind to the GPCR.

[1993] In a one hundred sixteenth aspect, the invention features a method of making a transgenic mouse, comprising the step of engineering said mouse to carry as part of its own genetic material the gene encoding the human antilipolytic GPCR polypeptide of SEQ. ID. NO.:8 (hRUP11).

[1994] In some preferred embodiments, expression of said gene is placed under the control of an essentially adipocyte specific promoter.

[1995] In a one hundred seventeenth aspect, the invention features the transgenic mouse according to the method of the one hundred sixteenth aspect.

[1996] In a one hundred eighteenth aspect, the invention features a method of using the transgenic mouse of the one hundred seventeenth aspect to identify whether an agonist of said human receptor has therapeutic efficacy for the treatment of a disorder of lipid metabolism selected from the group consisting of:

[1997] (a) elevated level of plasma triglycerides;

[1998] (b) elevated level of plasma free fatty acids;

[1999] (c) elevated level of plasma cholesterol;

[2000] (d) elevated level of LDL-cholesterol;

[2001] (e) reduced level of HDL-cholesterol;

[2002] (f) elevated total cholesterol/HDL-cholesterol ratio; and

[2003] (g) reduced level of plasma adiponectin;

[2004] comprising the steps of:

[2005] (a') administering or not administering the agonist to the mouse; and

[2006] (b') determining whether on administering the agonist there is a change selected from the group consisting of:

[2007] (i) a decrease in the level of plasma triglycerides;

[2008] (ii) a decrease in the level of plasma free fatty acids;

[2009] (iii) a decrease in the level of plasma cholesterol;

[2010] (iv) a decrease in the level of LDL-cholesterol;

[2011] (v) an increase in the level of HDL-cholesterol;

[2012] (vi) a decrease in the total cholesterol/HDL-cholesterol ratio; and

[2013] (vii) an increase in the level of plasma adiponectin;

[2014] wherein said change is indicative of the agonist having therapeutic efficacy.

[2015] In a one hundred nineteenth aspect, the invention features a method of using the transgenic mouse of the one hundred seventeenth aspect to identify whether an agonist of said human receptor has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

[2016] (a) dyslipidemia;

[2017] (b) atherosclerosis;

[2018] (c) coronary heart disease;

[2019] (d) stroke;

[2020] (e) insulin resistance; and

[2021] (f) type 2 diabetes;

[2022] comprising the steps of:

[2023] (a') administering or not administering the agonist to the mouse; and

[2024] (b') determining whether on administering the agonist there is a change selected from the group consisting of:

[2025] (i) a decrease in the level of plasma triglycerides;

[2026] (ii) a decrease in the level of plasma free fatty acids;

[2027] (iii) a decrease in the level of plasma cholesterol;

[2028] (iv) a decrease in the level of LDL-cholesterol;

[2029] (v) an increase in the level of HDL-cholesterol;

[2030] (vi) a decrease in the total cholesterol/HDL-cholesterol ratio; and

[2031] (vii) an increase in the level of plasma adiponectin;

[2032] wherein said change is indicative of the agonist having therapeutic efficacy.

[2033] In a one hundred twentieth aspect, the invention features a method of making a transgenic rat, comprising the step of engineering said rat to carry as part of its own genetic material the gene encoding the human antilipolytic GPCR polypeptide of SEQ. ID. NO.:8 (hRUP11).

[2034] In some preferred embodiments, expression of said gene is placed under the control of an essentially adipocyte specific promoter.

[2035] In a one hundred twenty-first aspect, the invention features the transgenic rat according to the method of the one hundred twentieth aspect.

[2036] In a one hundred twenty-second aspect, the invention features a method of using the transgenic rat of the one hundred twenty-first aspect to identify whether an agonist of said human receptor has therapeutic efficacy for the treatment of a disorder of lipid metabolism selected from the group consisting of:

- [2037] (a) elevated level of plasma triglycerides;
- [2038] (b) elevated level of plasma free fatty acids;
- [2039] (c) elevated level of plasma cholesterol;
- [2040] (d) elevated level of LDL-cholesterol;
- [2041] (e) reduced level of HDL-cholesterol;
- [2042] (f) elevated total cholesterol/HDL-cholesterol ratio; and
- [2043] (g) reduced level of plasma adiponectin;

[2044] comprising the steps of:

- [2045] (a') administering or not administering the agonist to the rat; and
- [2046] (b') determining whether on administering the agonist there is a change selected from the group consisting of:
  - [2047] (i) a decrease in the level of plasma triglycerides;
  - [2048] (ii) a decrease in the level of plasma free fatty acids;
  - [2049] (iii) a decrease in the level of plasma cholesterol;
  - [2050] (iv) a decrease in the level of LDL-cholesterol;
  - [2051] (v) an increase in the level of HDL-cholesterol;
  - [2052] (vi) a decrease in the total cholesterol/HDL-cholesterol ratio; and
  - [2053] (vii) an increase in the level of plasma adiponectin;

[2054] wherein said change is indicative of the agonist having therapeutic efficacy.

[2055] In a one hundred twenty-third aspect, the invention features a method of using the transgenic rat of the one hundred twenty-first aspect to identify whether an agonist of

said human receptor has therapeutic efficacy for the treatment of a metabolic-related disorder selected from the group consisting of:

- [2056] (a) dyslipidemia;
- [2057] (b) atherosclerosis;
- [2058] (c) coronary heart disease;
- [2059] (d) stroke;
- [2060] (e) insulin resistance; and
- [2061] (f) type 2 diabetes;

[2062] comprising the steps of:

- [2063] (a') administering or not administering the agonist to the rat; and
- [2064] (b') determining whether on administering the agonist there is a change selected from the group consisting of:
  - [2065] (i) a decrease in the level of plasma triglycerides;
  - [2066] (ii) a decrease in the level of plasma free fatty acids;
  - [2067] (iii) a decrease in the level of plasma cholesterol;
  - [2068] (iv) a decrease in the level of LDL-cholesterol;
  - [2069] (v) an increase in the level of HDL-cholesterol;
  - [2070] (vi) a decrease in the total cholesterol/HDL-cholesterol ratio; and
  - [2071] (vii) an increase in the level of plasma adiponectin;

[2072] wherein said change is indicative of the agonist having therapeutic efficacy.

[2073] In a one hundred twenty-fourth aspect, the invention features an isolated, purified or recombinant RUP1 polynucleotide selected from the group consisting of:

- [2074] (a) a polynucleotide comprising a contiguous span of at least 75 nucleotides of SEQ.ID. NO.:7, or an allelic variant of said polynucleotide;
- [2075] (b) a polynucleotide comprising a contiguous span of at least 150 nucleotides of SEQ. ID. NO.:7, or an allelic variant of said polynucleotide;
- [2076] (c) a polynucleotide comprising a contiguous span of at least 250 nucleotides of SEQ. ID. NO.:7, or an allelic variant of said polynucleotide;
- [2077] (d) a polynucleotide comprising a contiguous span of at least 350 nucleotides of SEQ. ID. NO.:7, or an allelic variant of said polynucleotide;
- [2078] (e) a polynucleotide comprising a contiguous span of at least 500 nucleotides of SEQ. ID. NO.:7, or an allelic variant of said polynucleotide;
- [2079] (f) a polynucleotide comprising a contiguous span of at least 750 nucleotides of SEQ. ID. NO.:7, or an allelic variant of said polynucleotide;

- [2080] (g) a polynucleotide comprising a contiguous span of at least 1000 nucleotides of SEQ. ID. NO.:7, or an allelic variant of said polynucleotide;
- [2081] (h) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 20 amino acids of SEQ. ID. NO.:8 or an allelic variant of said polypeptide;
- [2082] (i) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 30 amino acids of SEQ. ID. NO.:8, or an allelic variant of said polypeptide;
- [2083] (j) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 40 amino acids of SEQ. ID. NO.:8, or an allelic variant of said polypeptide;
- [2084] (k) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 50 amino acids of SEQ. ID. NO.:8, or an allelic variant of said polypeptide;
- [2085] (l) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 75 amino acids of SEQ. ID. NO.:8, or an allelic variant of said polypeptide;
- [2086] (m) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 100 amino acids of SEQ. ID. NO.:8, or an allelic variant of said polypeptide;
- [2087] (n) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 150 amino acids of SEQ. ID. NO.:8, or an allelic variant of said polypeptide;
- [2088] (o) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 200 amino acids of SEQ. ID. NO.:8, or an allelic variant of said polypeptide;
- [2089] (p) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 250 amino acids of SEQ. ID. NO.:8, or an allelic variant of said polypeptide; and
- [2090] (q) a polynucleotide encoding a polypeptide comprising a contiguous span of at least 300 amino acids of SEQ. ID. NO.:8, or an allelic variant of said polypeptide.
- [2091] The invention also relates to an isolated, purified or recombinant RUP11 polynucleotide wherein said polynucleotide is selected from the group consisting of:
- [2092] (a) a polynucleotide comprising the nucleotide sequence of SEQ. ID. NO.:7 or an allelic variant of said nucleotide sequence;
- [2093] (b) the polynucleotide of SEQ. ID. NO.:7, or an allelic variant of said polynucleotide;
- [2094] (c) a polynucleotide comprising a nucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ. ID. NO.:8 or an allelic variant of said amino acid sequence; and
- [2095] (d) a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ. ID. NO.:8, or an allelic variant of said polypeptide.
- [2096] In preferred embodiments, said isolated, purified or recombinant polynucleotide comprises at least 8 contiguous nucleotides of a polynucleotide of the present invention. In other preferred embodiments, said isolated, purified or recombinant polynucleotide comprises at least 10, 12, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 contiguous nucleotides of a polynucleotide of the present invention. Preferably said polynucleotide encodes full-length RUP11 polypeptide or a biologically active fragment thereof.
- [2097] The polynucleotides of the present invention include genomic polynucleotides comprising RUP11 polynucleotides of the invention.
- [2098] The present invention also relates to a polynucleotide encoding a fusion protein, wherein said fusion protein comprises an RUP11 polypeptide of the invention fused to a heterologous polypeptide. In a preferred embodiment, said polypeptide of the invention is constitutively active and said heterologous polypeptide is a G protein. In other embodiments, said heterologous polypeptide provides an antigenic epitope. In a preferred embodiment, said heterologous polypeptide provides a hemagglutinin (HA) antigenic epitope. Methods relating to a polynucleotide encoding a fusion protein are well known to those of ordinary skill in the art.
- [2099] The polynucleotides of the present invention also include variant polynucleotides at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to an RUP11 polynucleotide of the invention. In a particularly preferred embodiment, polynucleotide sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST"), which is well known in the art [See, e.g., Karlin and Altschul, Proc Natl Acad Sci USA (1990) 87:2264-8; Altschul et al., J Mol Biol (1990) 215:403-410; Altschul et al., Nature Genetics (1993) 3:266-72; and Altschul et al., Nucleic Acids Res (1997) 25:3389-3402; the disclosures of which are incorporated by reference in their entirety].
- [2100] In further preferred embodiments, the invention features the complement of said polynucleotide.
- [2101] In a one hundred twenty-fifth aspect, the invention features an isolated, purified or recombinant RUP11 polypeptide selected from the group consisting of:
- [2102] (a) a polypeptide comprising a contiguous span of at least 20 amino acids of SEQ. ID. NO.:8 or an allelic variant of said contiguous span of amino acids;
- [2103] (b) a polypeptide comprising a contiguous span of at least 30 amino acids of SEQ. ID. NO.:8 or an allelic variant of said contiguous span of amino acids;
- [2104] (c) a polypeptide comprising a contiguous span of at least 40 amino acids of SEQ. ID. NO.:8 or an allelic variant of said contiguous span of amino acids;
- [2105] (d) a polypeptide comprising a contiguous span of at least 50 amino acids of SEQ. ID. NO.:8 or an allelic variant of said contiguous span of amino acids;

- [2106] (e) a polypeptide comprising a contiguous span of at least 75 amino acids of SEQ. ID. NO.:8 or an allelic variant of said contiguous span of amino acids;
- [2107] (f) a polypeptide comprising a contiguous span of at least 100 amino acids of SEQ. ID. NO.:8 or an allelic variant of said contiguous span of amino acids;
- [2108] (g) a polypeptide comprising a contiguous span of at least 150 amino acids of SEQ. ID. NO.:8 or an allelic variant of said contiguous span of amino acids;
- [2109] (h) a polypeptide comprising a contiguous span of at least 200 amino acids of SEQ. ID. NO.:8 or an allelic variant of said contiguous span of amino acids;
- [2110] (i) a polypeptide comprising a contiguous span of at least 250 amino acids of SEQ. ID. NO.:8 or an allelic variant of said contiguous span of amino acids; and
- [2111] (j) a polypeptide comprising a contiguous span of at least 300 amino acids of SEQ. ID. NO.:8 or an allelic variant of said contiguous span of amino acids.
- [2112] The invention also relates to an isolated, purified or recombinant RUP11 polypeptide wherein said polypeptide is selected from the group consisting of:
- [2113] (a) a polypeptide comprising the amino acid sequence of SEQ. ID. NO.:8 or an allelic variant or a biologically active mutant of said amino acid sequence; and
- [2114] (b) the polypeptide having the amino acid sequence of SEQ. ID. NO.:8 or an allelic variant or a biologically active mutant of said amino acid sequence;
- [2115] or a biologically active fragment of said polypeptide.
- [2116] In preferred embodiments, said isolated, purified or recombinant polypeptide comprises at least 6 contiguous amino acids of an RUP11 polypeptide of the invention. In further embodiments, said isolated, purified or recombinant polypeptide comprises at least 10, 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, 125, 150, 175, 200, 225, 250, 275 or 300 contiguous amino acids of a polypeptide of the present invention. Preferably, said polypeptide is full-length RUP11 polypeptide or an active fragment thereof.
- [2117] The present invention also relates to a fusion protein, wherein said fusion protein comprises an RUP11 polypeptide of the invention fused to a heterologous polypeptide. In a preferred embodiment, said polypeptide of the invention is constitutively active and said heterologous polypeptide is a G protein. In other preferred embodiments, said heterologous polypeptide provides an antigenic epitope. In particularly preferred embodiment, said heterologous polypeptide provides a hemagglutinin (HA) antigenic epitope. Methods relating to a fusion protein are well known to those of ordinary skill in the art.
- [2118] The polypeptides of the present invention also include variant polypeptides at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an RUP11 polypeptide of the invention. In a particularly preferred embodiments, polypeptide sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST"), which is well known in the art [See, e.g., Karlin and Altschul, Proc Natl Acad Sci USA (1990) 87:2264-8; Altschul et al., J Mol Biol (1990) 215:403-410; Altschul et al., Nature Genetics (1993) 3:266-72; and Altschul et al., Nucleic Acids Res (1997) 25:3389-3402; the disclosures of which are incorporated by reference in their entirety].
- [2119] In an one hundred twenty-sixth aspect, the invention features a composition comprising, consisting essentially of, or consisting of the RUP11 polypeptide of the one hundred twenty-fifth aspect.
- [2120] In a one hundred twenty-seventh aspect, the invention features a recombinant vector, said vector comprising, consisting essentially of, or consisting of the polynucleotide of the one hundred twenty-fourth aspect. In some embodiments, said vector is a targeting vector used in a method of inactivating a gene encoding an antilipolytic GPCR of the invention. In some preferred embodiments, said vector is used in a method of transient or stable transfection. In other preferred embodiments, said vector is used in a method of transgenic expression.
- [2121] In particularly preferred embodiment, said vector is an expression vector for the expression of a an antilipolytic GPCR in a recombinant host cell wherein said expression vector comprises, consists essentially of, or consists of the polynucleotide of the one hundred twenty-fourth aspect.
- [2122] Although a variety of expression vectors are available to those in the art, for purposes of utilization for both the endogenous and non-endogenous human, mouse and rat GPCRs, it is most preferred that the vector utilized be pCMV. In some alternative embodiments as relates to said human, mouse and rat antilipolytic GPCRs, it is preferred that the vector utilized be an adenoviral expression vector.
- [2123] In a one hundred twenty-eighth aspect, the invention features a prokaryotic or eukaryotic host cell comprising, consisting essentially of, or consisting of the recombinant vector of the one hundred twenty-seventh aspect. In some embodiments, said host cell is a eukaryotic embryonic stem cell wherein said vector of the one hundred twenty-seventh aspect has been used in a method to inactivate a gene encoding an antilipolytic GPCR of the invention within said cell. In some embodiments, said host cell is a eukaryotic embryonic somatic cell wherein said vector of the one hundred twenty-seventh aspect has been used in a method to inactivate a gene encoding an antilipolytic GPCR of the invention within said cell. In some preferred embodiments, said host cell is derived from a mouse or rat made transgenic for a human RUP11 antilipolytic GPCR of the invention. In other preferred embodiments, said host cell is prokaryotic and has been transformed using the vector of the one hundred twenty-seventh aspect. In further preferred embodiments, said host cell is eukaryotic and has been transiently transfected using the vector of the one hundred twenty-seventh aspect. In other further preferred embodiments, said host cell is eukaryotic and has been stably transfected using the vector of the one hundred twenty-seventh aspect.
- [2124] In particularly preferred embodiment, said host cell expresses a recombinant antilipolytic GPCR wherein said

host cell comprises, consists essentially of, or consists of the expression vector of the one hundred twenty-seventh aspect.

[2125] A further embodiment includes a prokaryotic or eukaryotic host cell recombinant for the polynucleotide of the one hundred twenty-fourth aspect.

[2126] In some embodiments the host cell is eukaryotic, more preferably, mammalian, and more preferably selected from the group consisting of 293, 293T, CHO, and COS-7 cells. In other embodiments, the host cell is eukaryotic, more preferably melanophore.

[2127] In a one hundred twenty-ninth aspect, the invention features a process for the expression of an antilipolytic GPCR in a recombinant host cell comprising the steps of:

[2128] (a) transfecting the expression vector of the one hundred twenty-seventh aspect into a suitable host cell; and

[2129] (b) culturing the host cells under conditions which allow expression of the antilipolytic GPCR protein from the expression vectors.

[2130] In a one hundred thirtieth aspect, the invention features an antibody that specifically binds to the polypeptide of the one hundred twenty-fifth aspect. In some preferred embodiments, the antibody is monoclonal. In some embodiments, the antibody is polyclonal.

[2131] In a one hundred thirty-first aspect, the invention features a method of binding the polypeptide of the one hundred twenty-fifth aspect to the antibody of the one hundred thirtieth aspect, comprising contacting said antibody with said polypeptide under conditions in which said antibody can specifically bind to said polypeptide.

[2132] In a one hundred thirty-second aspect, the invention features a method of detecting an antilipolytic GPCR polypeptide in a biological sample obtained from an individual comprising the steps of:

[2133] (a) obtaining said biological sample from said individual;

[2134] (b) contacting said biological sample with the antibody of the one hundred thirtieth aspect; and

[2135] (c) detecting the presence or absence of binding of said antibody to said biological sample;

[2136] wherein a detection of said binding is indicative of the receptor polypeptide being expressed in said biological sample.

[2137] In preferred embodiments, said detecting is through the use of an enzyme-labeled secondary reagent. In other preferred embodiments, said detecting is through the use of a fluorophore-labeled secondary reagent. In other preferred embodiments, said detecting is through the use of a radioisotope-labeled secondary reagent. In other embodiments, the antibody is directly labeled with enzyme, fluorophore or radioisotope.

[2138] In other preferred embodiments, said biological sample is taken from adipose, skin or blood.

[2139] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[2140] In further embodiments, said individual has a disorder of lipid metabolism selected from the group consisting of:

[2141] (a) elevated level of plasma triglycerides;

[2142] (b) elevated level of plasma free fatty acids;

[2143] (c) elevated level of plasma cholesterol;

[2144] (d) elevated level of LDL-cholesterol;

[2145] (e) reduced level of HDL-cholesterol;

[2146] (f) elevated total cholesterol/HDL-cholesterol ratio; and

[2147] (g) reduced level of plasma adiponectin.

[2148] In other preferred embodiments, said disorder in lipid metabolism is an elevated postprandial increase in plasma free fatty acids due to a high fat meal or a progression from impaired glucose tolerance to insulin resistance.

[2149] In further embodiments, said individual has a metabolic-related disorder selected from the group consisting of:

[2150] (a) dyslipidemia;

[2151] (b) atherosclerosis;

[2152] (c) coronary heart disease;

[2153] (d) stroke;

[2154] (e) insulin resistance; and

[2155] (f) type 2 diabetes.

[2156] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[2157] In other embodiments, said method further comprises the step of comparing the level of detection of said binding for a first individual to the level of detection of said binding for a second individual.

[2158] In a one hundred thirty-third aspect, the invention features a method of detecting expression of a gene encoding an antilipolytic GPCR in a biological sample obtained from an individual comprising the steps of:

[2159] (a) obtaining said biological sample from said individual;

[2160] (b) contacting said biological sample with the complementary polynucleotide of the one hundred twenty-fourth aspect, optionally labeled, under conditions permissive for hybridization; and

[2161] (c) detecting the presence or absence of said hybridization between said complementary polynucleotide and an RNA species within said sample;

[2162] wherein a detection of said hybridization is indicative of expression of said GPCR gene in said biological sample.

[2163] In preferred embodiments, the biological sample is taken from adipose, skin or blood.

[2164] In preferred embodiments, said individual is a mammal. In more preferred embodiments, said mammal is a horse, cow, sheep, pig, cat, dog, rabbit, mouse, rat, non-human primate or human. Yet more preferred is mouse, rat or human. Most preferred is human.

[2165] In preferred embodiments, said individual has a disorder of lipid metabolism selected from the group consisting of:

- [2166] (a) elevated level of plasma triglycerides;
- [2167] (b) elevated level of plasma free fatty acids;
- [2168] (c) elevated level of plasma cholesterol;
- [2169] (d) elevated level of LDL-cholesterol;
- [2170] (e) reduced level of HDL-cholesterol;
- [2171] (f) elevated total cholesterol/HDL-cholesterol ratio; and
- [2172] (g) reduced level of plasma adiponectin.

[2173] In other preferred embodiments, said disorder in lipid metabolism is an elevated postprandial increase in plasma free fatty acids due to a high fat meal or a progression from impaired glucose tolerance to insulin resistance.

[2174] In other preferred embodiments, said individual has a metabolic-related disorder selected from the group consisting of:

- [2175] (a) dyslipidemia;
- [2176] (b) atherosclerosis;
- [2177] (c) coronary heart disease;
- [2178] (d) stroke;
- [2179] (e) insulin resistance; and
- [2180] (f) type 2 diabetes.

[2181] In other preferred embodiments, said metabolic-related disorder is selected from the group consisting of obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. In other preferred embodiments, said metabolic-related disorder is hyperlipidemia.

[2182] In other embodiments, said method further comprises the step of comparing the level of detection of said hybridization for a first individual to the level of detection of said hybridization for a second individual.

[2183] In some preferred embodiments, said complementary polynucleotide is a primer and said hybridization is detected by detecting the presence of an amplification product comprising the sequence of said primer. In more preferred embodiments, said method is RT-PCR.

[2184] In a one hundred thirty-fourth aspect, the invention features a GPCR Fusion Protein construct comprising a constitutively active GPCR and a G protein, said receptor comprising the amino acid sequence of SEQ. ID. NO.:8 (hRUP11) or an allelic variant or a biologically active fragment of said amino acid sequence.

[2185] The invention also relates to a GPCR Fusion Protein construct wherein the methionine at amino acid position 294 of SEQ. ID. NO.:8 is substituted by lysine.

[2186] In a one hundred thirty-fifth aspect, the invention features a method of binding a known ligand of RUP11 antilipolytic GPCR to a polypeptide selected from the group consisting of:

- [2187] (a) a polypeptide comprising a contiguous span of at least 6 amino acids of SEQ. ID. NO.:8;
- [2188] (b) a polypeptide comprising a contiguous span of at least 10 amino acids of SEQ. ID. NO.:8;
- [2189] (c) a polypeptide comprising a contiguous span of at least 15 amino acids of SEQ. ID. NO.:8;
- [2190] (d) a polypeptide comprising a contiguous span of at least 20 amino acids of SEQ. ID. NO.:8;
- [2191] (e) a polypeptide comprising a contiguous span of at least 25 amino acids of SEQ. ID. NO.:8;
- [2192] (f) a polypeptide comprising a contiguous span of at least 30 amino acids of SEQ. ID. NO.:8;
- [2193] (g) a polypeptide comprising a contiguous span of at least 35 amino acids of SEQ. ID. NO.:8;
- [2194] (h) a polypeptide comprising a contiguous span of at least 40 amino acids of SEQ. ID. NO.:8;
- [2195] (i) a polypeptide comprising a contiguous span of at least 45 amino acids of SEQ. ID. NO.:8; and
- [2196] (j) a polypeptide comprising a contiguous span of at least 50 amino acids of SEQ. ID. NO.:8; or an allelic variant of said polypeptide;

[2197] comprising the step of contacting said known ligand with said polypeptide under conditions which allow said binding to occur.

[2198] In some embodiments, said known ligand is a modulator of the GPCR. In some embodiments, said known ligand is an agonist of the GPCR. In some embodiments, said known ligand is the modulator of the one hundred fifth aspect. In some embodiments, said known ligand is an antibody specific for the GPCR, or a derivative thereof.

[2199] In other preferred embodiments, said method is used to identify whether a candidate compound inhibits said binding of said known ligand to said polypeptide, comprising the steps of:

- [2200] (a) contacting said polypeptide with said known ligand, optionally labeled, in the presence or absence of said candidate compound;
- [2201] (b) detecting the complex between said known ligand and said polypeptide; and
- [2202] (c) determining whether less of said complex is formed in the presence of the compound than in the absence of the compound;

[2203] wherein said determination is indicative of the candidate compound being an inhibitor of said binding of said known ligand to said polypeptide.

[2204] In some embodiments, said known ligand is a modulator of the GPCR. In some embodiments, said known

ligand is an agonist of the GPCR. In some embodiments, said known ligand is the modulator of the one hundred fifth aspect. In some embodiments, said known ligand is an antibody specific for the GPCR, or a derivative thereof.

[2205] In other preferred embodiments, said method is used to identify whether a candidate compound is an inhibitor of said binding of said known ligand to said polypeptide, comprising the steps of:

[2206] (a) contacting said polypeptide with said known ligand, optionally labeled, in the presence separately of a plurality of concentrations of said candidate compound for a time sufficient to allow equilibration of binding;

[2207] (b) measuring unbound ligand and bound ligand; and

[2208] (c) determining  $K_i$  for the candidate compound;

[2209] wherein a  $K_i$  value of less than 50  $\mu$ M is indicative of the candidate compound being an inhibitor of said binding of said known ligand to said polypeptide. Preferably said  $K_i$  value is less than 25  $\mu$ M, 10  $\mu$ M, 5  $\mu$ M, 1  $\mu$ M, 750 nM, 500 nM, 400 nM, 300 nM, 250 nM, 200 nM, 150 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM or 10 nM. In preferred embodiments,  $K_i$  determination is made through nonlinear curve fitting with the program SCTFIT [De Lean et al. (1982) Mol Pharmacol 21:5-16; cited in Lorenzen et al. (2001) Mol Pharmacol 59:349-357, the disclosures of which are incorporated by reference herein in their entireties].

[2210] In some embodiments, said known ligand is a modulator of the GPCR. In some embodiments, said known ligand is an agonist of the GPCR. In some embodiments, said known ligand is the modulator of the one hundred fifth aspect. In some embodiments, said known ligand is an antibody specific for the GPCR, or a derivative thereof.

[2211] In a one hundred thirty-six aspect, the invention features a method of binding an optionally labeled affinity reagent specific for an antilipolytic GPCR to said receptor in a biological sample, said receptor comprising the amino acid sequence of SEQ. ID. NO.:8 (hRUP11); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence, comprising the steps of:

[2212] (a) obtaining said biological sample;

[2213] (b) contacting the affinity reagent with said receptor in said biological sample; and

[2214] (c) detecting the complex of said affinity reagent with said receptor.

[2215] In some embodiments, the antilipolytic GPCR has the amino acid sequence of SEQ. ID. NO.:8 (hRUP11); or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence.

[2216] In some embodiments, the antilipolytic GPCR comprises a biologically active fragment of said amino acid sequence.

[2217] In some embodiments, the antilipolytic GPCR is endogenous.

[2218] In some embodiments, the antilipolytic GPCR is recombinant.

[2219] In some embodiments, said biologically active mutant is CART or EFA. In preferred embodiments, said CART mutant has the amino acid sequence of SEQ. ID. NO.:8 further substituted at amino acid position 294 with lysine in place of methionine.

[2220] In preferred embodiments, said G protein is Gi.

[2221] In some embodiments, said affinity reagent is a modulator of the GPCR. In some embodiments, said affinity reagent is an agonist of the GPCR. In some embodiments, said affinity reagent is the modulator of the one hundred fifth aspect. In some embodiments, said affinity reagent is an antibody specific for the GPCR, or a derivative thereof.

[2222] In further preferred embodiments, said affinity reagent comprises a label selected from the group consisting of:

[2223] (a) radioisotope;

[2224] (b) enzyme; and

[2225] (c) fluorophore.

[2226] In preferred embodiments, said radioisotope is  $^3\text{H}$ .

[2227] In a one hundred thirty-seventh aspect, the invention features the method of the one hundred thirty-sixth aspect further comprising the step of comparing the level of detection of said complex in a first biological sample to a second level of detection of said complex in a second biological sample.

[2228] In a one hundred thirty-eighth aspect, the invention features the method of the one hundred thirty-seventh aspect wherein the relationship between said first and second biological samples is selected from the group consisting of:

[2229] (a) said second biological sample is a replicate of said first biological sample;

[2230] (b) said first biological sample was obtained prior to an experimental intervention whereas said second biological sample was obtained after the experimental intervention, from the same individual;

[2231] (c) said second biological sample was obtained at a different time point after an experimental intervention than was said first biological sample, from the same individual;

[2232] (d) said second biological sample corresponds to a different subcellular compartment than does said first biological sample;

[2233] (e) said second biological sample represents a different cell type than does said first biological sample;

[2234] (f) said second biological sample corresponds to a different tissue than does said first biological sample;

[2235] (g) said second biological sample was obtained from a different individual than was said first biological sample;

- [2236] (h) said second biological sample was obtained at a different point in time than was said first biological sample, from the same individual;
- [2237] (i) said first biological samples was obtained from a normal individual, whereas said second biological sample was obtained from an individual having a metabolic-related disorder;
- [2238] (j) said first biological sample was obtained from a normal individual, whereas said second biological sample was obtained from an individual having a disorder in lipid metabolism;
- [2239] (k) said first biological sample was obtained before a therapeutic intervention whereas said second biological sample was obtained after the therapeutic intervention, from the same individual;
- [2240] (l) said second biological sample was obtained at a different time point after therapeutic intervention than was said first biological sample, from the same individual; and
- [2241] (m) said first biological sample was not exposed to a compound, whereas said second biological sample was exposed to said compound.
- [2242] In a one hundred thirty-ninth aspect, the invention features an isolated EFA-hRUP25 polynucleotide selected from the group consisting of:
- [2243] (a) a polynucleotide comprising the nucleotide sequence of SEQ. ID. NO.:158;
- [2244] (b) a polynucleotide having the nucleotide sequence of SEQ. ID. NO.:158;
- [2245] (c) a polynucleotide comprising a polynucleotide encoding the polypeptide having the amino acid sequence of SEQ. ID. NO.:159 or a biologically active fragment of said polypeptide; and
- [2246] (d) a polynucleotide encoding the polypeptide having the amino acid sequence of SEQ. ID. NO.:159 or a biologically active fragment of said polypeptide.
- [2247] In a one hundred fortieth aspect, the invention features an isolated EFA-hRUP25 polypeptide selected from the group consisting of:
- [2248] (a) a polypeptide comprising the amino acid sequence of SEQ. ID. NO.:159, or a biologically active fragment of said polypeptide; and
- [2249] (b) a polypeptide having the amino acid sequence of SEQ. ID. NO.:159, or a biologically active fragment of said polypeptide.
- [2250] In an one hundred forty-first aspect, the invention features a composition comprising, consisting essentially of, or consisting of the EFA-hRUP25 polypeptide of the one hundred fortieth aspect.
- [2251] In a one hundred forty-second aspect, the invention features a recombinant vector comprising the polynucleotide of the one hundred thirty-ninth aspect. In some preferred embodiments, said vector is used in a method of transient or stable transfection.
- [2252] In particularly preferred embodiment, said vector is an expression vector for the expression of an EFA-hRUP25 nicotinic acid GPCR in a recombinant host cell wherein said expression vector comprises, consists essentially of, or consists of the polynucleotide of the one hundred thirty-ninth aspect.
- [2253] Although a variety of expression vectors are available to those in the art, it is most preferred that the vector utilized be pCMV. In some alternative embodiments as relates to EFA-hRUP25 nicotinic acid GPCR, it is preferred that the vector utilized be an adenoviral expression vector.
- [2254] In a one hundred forty-third aspect, the invention features a prokaryotic or eukaryotic host cell comprising, consisting essentially of, or consisting of the recombinant vector of the one hundred forty-second aspect. In some embodiments, said host cell is prokaryotic and has been transformed using the vector of the one hundred forty-second aspect. In some embodiments, said host cell is eukaryotic and has been transiently transfected using the vector of the one hundred forty-second aspect. In some preferred embodiments, said host cell is eukaryotic and has been stably transfected using the vector of the one hundred forty-second aspect.
- [2255] In particularly preferred embodiment, said host cell expresses a recombinant EFA-hRUP25 nicotinic acid GPCR wherein said host cell comprises, consists essentially of, or consists of the expression vector of the one hundred forty-second aspect.
- [2256] A further embodiment includes a prokaryotic or eukaryotic host cell recombinant for the polynucleotide of the one hundred thirty-ninth aspect.
- [2257] In some embodiments the host cell is eukaryotic, more preferably, mammalian, and more preferably selected from the group consisting of 293, 293T, CHO, and COS-7 cells. In other embodiments, the Host Cell is eukaryotic, more preferably melanophore.
- [2258] In a one hundred forty-fourth aspect, the invention features a process for the expression of an EFA-hRUP25 nicotinic acid GPCR in a recombinant host cell comprising the steps of:
- [2259] (a) transfecting the expression vector of the one hundred forty-second aspect into a suitable host cell; and
- [2260] (b) culturing the host cells under conditions which allow expression of the EFA-hRUP25 nicotinic acid GPCR protein from the expression vectors.
- [2261] In a one hundred forty-fifth aspect, the invention features a method of making an EFA mutant of an endogenous GPCR polypeptide having constitutive activity, comprising the steps of:
- [2262] (a) introducing 1, 2, 3, 4, or 5 substitutions, insertions, or deletions into the amino acid sequence of the endogenous GPCR polypeptide;
- [2263] (b) measuring the activity of the mutant GPCR of (a) in the absence of agonist and in the presence of a known agonist;
- [2264] (c) measuring the activity of the endogenous GPCR in the absence of agonist and in the presence of said known agonist; and
- [2265] (d) comparing (b) and (c);

[2266] wherein a determination that the agonist screening window of (b) is at least 20% greater than that of (c) identifies the mutant resulting from (a) to be an EFA mutant of the endogenous GPCR.

[2267] In some embodiments, said number of substitutions, insertions, or deletions is 1.

[2268] In some embodiments, said number of substitutions, insertions, or deletions is 1 or 2.

[2269] In some embodiments, said number of substitutions, insertions, or deletions is 1, 2 or 3.

[2270] In some embodiments, said number of substitutions, insertions, or deletions is 1, 2, 3 or 4.

[2271] In more preferred embodiments, said number of substitutions, insertions, or deletions is 1, 2, 3, 4 or 5.

[2272] Applicant reserves the right to exclude any one or more candidate compounds from any of the embodiments of the invention. Applicant also reserves the right to exclude any one or more modulators from any of the embodiments of the invention, including but not limited to nicotinic acid or any analog or derivative thereof. Applicant further reserves the right to exclude any polynucleotide or polypeptide from any of the embodiments of the invention. Applicant additionally reserves the right to exclude any metabolic-related disorder or any disorder of lipid metabolism from any of the embodiments of the invention.

[2273] Throughout this application, various publications, patents and published patent applications are cited. The disclosures of these publications, patents and published patent applications referenced in this application are hereby incorporated by reference in their entirety into the present disclosure. Citation herein by Applicant of a publication, patent, or published patent application is not an admission by Applicant of said publication, patent, or published patent application as prior art.

[2274] Modifications and extension of the disclosed inventions that are within the purview of the skilled artisan are encompassed within the above disclosure and the claims that follow.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[2275] **FIG. 1.** **FIG. 1** depicts second messenger IP<sub>3</sub> production from endogenous version hRUP12 ("hRUP12") as compared with the control ("CMV").

[2276] **FIG. 2.** **FIG. 2** depicts the results of a second messenger cell-based cyclic AMP assay providing comparative results for constitutive signaling of endogenous hRUP13 ("hRUP13") and a control vector ("CMV").

[2277] **FIG. 3.** **FIG. 3** depicts the signal measured comparing CMV, endogenous hRUP13 ("hRUP13 wt") and non-endogenous, constitutively activated hRUP13 ("hRUP13(A268K)"), utilizing 8XCRE-Luc reporter plasmid.

[2278] **FIG. 4.** **FIG. 4** depicts the results of a [<sup>35</sup>S]GTPγS assay providing comparative results for constitutive signaling by hRUP13:Gs Fusion Protein ("hRUP13-Gs") and a control vector ("CMV").

[2279] **FIG. 5.** **FIG. 5** depicts the signal measured comparing CMV, endogenous hRUP14 ("hRUP14 wt") and non-endogenous, constitutively activated hRUP13 ("hRUP14(L246K)"), utilizing 8XCRE-Luc reporter plasmid.

[2280] **FIG. 6.** **FIG. 6** depicts the signal measured comparing CMV, endogenous hRUP15 ("hRUP15 wt") and non-endogenous, constitutively activated hRUP15 ("hRUP15(A398K)"), utilizing 8XCRE-Luc reporter plasmid.

[2281] **FIG. 7.** **FIG. 7** depicts the results of a second messenger cell-based cyclic AMP assay providing comparative results for constitutive signaling of endogenous hRUP15 ("hRUP15 wt"), non-endogenous, constitutively activated version of hRUP15 ("hRUP15(A398K)") and a control vector ("CMV").

[2282] **FIG. 8.** **FIG. 8** depicts the results of a [<sup>35</sup>S]GTPγS assay providing comparative results for constitutive signaling by hRUP15:Gs Fusion Protein ("hRUP15-Gs") and a control vector ("CMV").

[2283] **FIG. 9.** **FIG. 9** depicts second messenger IP<sub>3</sub> production from endogenous version hRUP17 ("hRUP17") as compared with the control ("CMV").

[2284] **FIG. 10.** **FIG. 10** depicts messenger IP<sub>3</sub> production from endogenous version hRUP21 ("hRUP21") as compared with the control ("CMV").

[2285] **FIG. 11.** **FIG. 11** depicts the signal measured comparing CMV, endogenous hRUP23 ("hRUP23 wt") and non-endogenous, constitutively activated hRUP23 ("hRUP23(W275K)"), utilizing 8XCRE-Luc reporter plasmid.

[2286] **FIG. 12.** **FIG. 12** depicts results from a primary screen of several candidate compounds against hRUP13; results for "Compound A" are provided in well A2 and "Compound B" are provided in well G9.

[2287] **FIGS. 13A-C.** **FIGS. 13A and 13B** are histograms representing relative expression levels of hRUP25 (**FIG. 13A**) and hRUP38 (**FIG. 13B**) detected in different human tissues via DNA microarray. The horizontal axis displays the different tissues, identified in vertical text above the bar. The vertical axis indicates level of expression of either hRUP25 (**FIG. 13A**) or hRUP38 (**FIG. 13B**). In **FIG. 13A** and **FIG. 13B**, note the high level of expression in primary adipocytes of hRUP25 and hRUP38, respectively (the signal toward the left of each of the histograms corresponding to primary adipocytes is identified by a vertical arrow above the bar, for ease of reference).

[2288] **FIG. 13C** is a photograph of an ethidium bromide stained gel illustrating the relative expression of hRUP25 and hRUP38 as detected by RT-PCR using cDNA derived from a number of human tissues as template. Note the controls of the far right three lanes.

[2289] **FIGS. 14A-C.** **FIGS. 14A, 14B and 14C** depict melanophores transfected with DNA plasmids expressing hRUP25 (**FIG. 14A**), hRUP38 (**FIG. 14B**) and hRUP19 (**FIG. 14C**) without treatment. These cells are pigment-aggregated because hRUP25 (**FIG. 14A**), hRUP38 (**FIG. 14B**) and hRUP19 (**FIG. 14C**) are Gi-coupled receptors having a high basal level of activity, and therefore driving

the aggregation to a measurable level in the absence of a ligand. hRUP11 is also a Gi-coupled receptor having a high basal level of activity (not shown).

[2290] FIGS. 15A-B. FIGS. 15A and 15B illustrate the dose-dependant, nicotinic acid induced aggregation response of melanophores transfected with increasing amounts of plasmid DNA encoding hRUP25 (FIG. 15A). Cells transfected with 10  $\mu$ g of plasmid DNA encoding hRUP25, respond to nicotinic acid with an EC<sub>50</sub> of about 54 nM.

[2291] As negative controls, FIG. 15B depicts melanophores transfected with either salmon sperm DNA (Mock) or plasmid DNA encoding the  $\alpha_{2A}$ AR. As is evident there is no aggregation response in these cells upon nicotinic acid treatment at doses up to 10  $\mu$ M.

[2292] FIG. 16. FIG. 16 illustrates the nicotinic acid induced-inositol phosphates (IPs) accumulation in HEK293 cells co-expressing hRUP25 and the chimeric G $\alpha$ q-subunit in which the last five amino acids have been replaced with the corresponding amino acids of G $\alpha$ i (Gq $\Delta$ Gi). This construct has been shown to convert the signaling of a Gi-coupled receptor to the Gq pathway (i.e. accumulation of inositol phosphates) in response to receptor activation. Cells transfected with Gq $\Delta$ Gi plus either empty plasmid or the constitutively activated  $\alpha_{2A}$ AR ( $\alpha_{2A}$ K) are non-responsive to nicotinic acid and served as controls for the IP assay. Cells transfected with Gq $\Delta$ Gi plus either hRUP19 or hRUP38 are also unresponsive to nicotinic acid, indicating that nicotinic acid is not an agonist for either hRUP19 or hRUP38.

[2293] FIG. 17. FIG. 17 shows the results from saturation binding of [<sup>3</sup>H]nicotinic acid to membranes from cells expressing either hRUP25, hRUP38, hRUP19 or vector alone [CHO(-)]. Note that only hRUP25 binds nicotinic acid in a specific and high-affinity manner.

[2294] FIGS. 18A-B. FIG. 18A is a set of immunofluorescent photomicrographs illustrating the expression of hemagglutinin (HA)-tagged hRUP25 in a stably transfected line of CHO cells (top; clone #46). No significant labeling is detected in mock stably-transfected CHO cells (Mock). The lower panels identify the nuclear (DAPI) staining of cells in the same field.

[2295] FIG. 18B illustrates nicotinic acid and (-)-nicotine induced-inhibition of forskolin stimulated cAMP accumulation in hRUP25-CHO cell stable line #46 (described in preceding paragraph). The EC<sub>50</sub> for nicotinic acid is 23.6 nM and that for (-)-nicotine is 9.8  $\mu$ M.

[2296] FIG. 19. FIG. 19 indicates that, in response to nicotinic acid, both hRUP25 and the mouse ortholog mRUP25 can inhibit TSHR stimulated cAMP production (in the presence and absence of TSH).

[2297] FIG. 20. FIG. 20 shows the saturation binding curves of [<sup>3</sup>H]nicotinic acid ([<sup>3</sup>H]NA) to membranes prepared from HEK293 cells transiently expressing either hRUP25 or mRUP25. Note the significant binding of [<sup>3</sup>H]NA relative to either that found in membranes derived from mock transfected cells or in the presence of an excess of non-labeled nicotinic acid (200  $\mu$ M).

[2298] FIG. 21. FIG. 21 is a table comparing the rank order of potency of various compounds on hRUP25 and the pharmacologically defined nicotinic acid receptor. The

potencies at hRUP25 derived both by a functional analysis measuring the inhibition of forskolin induced cAMP production and competitive radioligand binding assays, closely match the order of potencies of the pharmacologically defined nicotinic acid receptor.

[2299] FIGS. 22A-B. FIG. 22A depicts nicotinic acid and related compounds inhibiting isoproterenol induced lipolysis in rat epididymal fat derived adipocytes at a concentration of 10  $\mu$ M. P-3-T represents 3-tetrazole-5-pyridine.

[2300] FIG. 22B illustrates a nicotinic acid dose-dependent inhibition of isoproterenol induced-lipolysis in rat epididymal fat derived adipocytes. Note the rightward shift in the dose-response curves with increasing concentrations of nicotinic acid.

[2301] FIG. 23. FIG. 23 illustrates the ability of both nicotinic acid and the related compound P-3-T (3-tetrazole-5-pyridine) to inhibit isoproterenol induced lipolysis in adipocyte primary cultures derived from human subcutaneous fat in a dose-dependant manner. The EC<sub>50</sub> value for nicotinic acid and P-3-T were 716 nM and 218 nM respectively.

[2302] FIG. 24. FIG. 24 presents screening data via adenylyl cyclase assay for hRUP38. Note that nicotinic acid does not activate inhibition of forskolin stimulated cAMP in hRUP38-expressing CHO cells whereas 1-Isopropyl-1H-benzotriazole-5-carboxylic acid does. 1-Isopropyl-1H-benzotriazole-5-carboxylic acid has no effect on CHO cells expressing either hRUP25 or hRUP19. The EC<sub>50</sub> for nicotinic acid is 25.8 nM and that for 1-Isopropyl-1H-benzotriazole-5-carboxylic acid is 166 nM. NT indicates not tested. (Also see the legend to FIG. 18A above for details directed to stable CHO transfectants.) Also see Example 30, infra.

[2303] FIG. 25. Nicotinic acid and 1-Isopropyl-1H-benzotriazole-5-carboxylic acid were separately dose-dependently applied to isoproterenol stimulated (100 nM) primary human adipocytes. FIG. 25 illustrates the ability of 1-Isopropyl-1H-benzotriazole-5-carboxylic acid to inhibit isoproterenol stimulated lipolysis in adipocyte primary cultures derived from human subcutaneous fat in a dose-dependant manner comparable to that of nicotinic acid.

[2304] FIG. 26. FIG. 26 presents screening data via adenylyl cyclase assay for hRUP38. The horizontal axis indicates the concentration of 3-(5-Bromo-2-ethoxy-phenyl)-acrylic acid. The vertical axis indicates "% inhibition of cAMP". Note that a value of 100% on the vertical axis corresponds to the cAMP level of forskolin stimulated cells in the absence of 3-(5-Bromo-2-ethoxy-phenyl)-acrylic acid, whereas a value of 200% on the vertical axis corresponds to the cAMP level of unstimulated cells in the absence of 3-(5-Bromo-2-ethoxy-phenyl)-acrylic acid. Note that 3-(5-Bromo-2-ethoxy-phenyl)-acrylic acid activates inhibition of forskolin stimulated cAMP in hRUP38-expressing CHO cells but has no effect on CHO cells expressing either hRUP25 or hRUP19. The EC<sub>50</sub> for 3-(5-Bromo-2-ethoxy-phenyl)-acrylic acid is 1.17  $\mu$ M. (Also see the legend to FIG. 18A above for details directed to stable CHO transfectants.)

[2305] FIG. 27. FIG. 27 presents an RT-PCR analysis of hRUP19 expression using a panel of human tissues. The analysis indicates that hRUP19 is selectively expressed in fat cells. Low expression is also evident in testis, placenta, kidney and spleen.

[2306] **FIG. 28.** **FIG. 28** presents a Northern blot analysis of hRUP19 expression using a panel of human tissues. The analysis indicates that hRUP19 is strongly expressed in mammary gland, probably due to fat cell-specific expression of hRUP19. Ad, adrenal gland; Bl, bladder, BM, bone marrow; Br, brain (whole); LN, lymph node; MG, mammary gland; Pr, prostate; Sp, spinal cord; St, stomach; Thyr, thyroid; Trch, trachea; Ut, uterus.

[2307] **FIG. 29.** **FIG. 29** presents an analysis of RUP19 expression as a function of adipocyte differentiation. RT-PCR and Northern blot analysis of mRUP19 expression by mouse 3T3 pre-adipocytes and differentiated 3T3 adipocytes was carried out. The analysis indicates that RUP19 expression is induced during adipocyte differentiation. Pre-diff 3T3-L1, mouse 3T3 pre-adipocytes; Post-diff 3T3-L1, differentiated 3T3 adipocytes;  $\beta$ -TC-6, a mouse insulin-producing cell line; NIT-1, a mouse insulin-producing cell line.

[2308] **FIG. 30.** **FIG. 30** presents a CART analysis of signal transduction by hRUP19. The analysis indicates that CART-activated hRUP19 inhibits cAMP production in membranes of transfected 293 cells.

[2309] **FIG. 31.** **FIG. 31** presents screening data via adenyl cyclase assay for hRUP25. The horizontal axis indicates the concentration of (5-hydroxy-1-methyl-3-propyl-1H-pyrazol-4-yl)-pyridin-3-yl-methanone. The vertical axis indicates "% inhibition of cAMP". Note that a value of 100% on the vertical axis corresponds to the cAMP level of forskolin stimulated cells in the absence of (5-hydroxy-1-methyl-3-propyl-1H-pyrazol-4-yl)-pyridin-3-yl-methanone, whereas a value of 200% on the vertical axis corresponds to the cAMP level of unstimulated cells in the absence of (5-hydroxy-1-methyl-3-propyl-1H-pyrazol-4-yl)-pyridin-3-yl-methanone. Note that (5-hydroxy-1-methyl-3-propyl-1H-pyrazol-4-yl)-pyridin-3-yl-methanone activates inhibition of forskolin stimulated cAMP in hRUP25-expressing CHO cells and has an  $EC_{50}$  of 352 nM. (5-Hydroxy-1-methyl-3-propyl-1H-pyrazol-4-yl)-pyridin-3-yl-methanone has no activity on hRUP38-expressing CHO cells up to a concentration of at least 100  $\mu$ M (not shown). Also see Example 29, infra.

[2310] **FIG. 32.** **FIG. 32** presents a time-course analysis of plasma free fatty acids (FFA) concentration in rats administered either vehicle or niacin [NA] at 15 mg/kg, 30 mg/kg, or 45 mg/kg. Also see Example 31, infra.

[2311] **FIG. 33.** **FIG. 33** presents an analysis of the agonist screening window for EFA-hRUP25 GPCR polypeptide of SEQ. ID. NO.:159 ["hRUP25-S91"] relative to that for endogenous hRUP25 GPCR polypeptide of SEQ. ID. NO.:36 ["hRUP25 wt"]. Samples were set up in triplicate. HEK293 cells were transfected with pCMV vector alone ["CMV"], with TSHR alone ["CMV+TSHR"], or were co-transfected with TSHR and either a2AK (a constitutively activated lysine mutant of alpha2A adrenergic receptor) ["a2AK"] or endogenous hRUP25 ["hRUP25 wt"] or EFA-hRUP25 ["hRUP25-S91"]. Niacin ["Ni"] was taken as a known agonist of hRUP25. UK14,304 ["UK"] was taken as a known agonist of a2AK, a positive control for the assay. The level of intracellular cAMP was determined for each sample. Also see Example 32, infra, for more details.

## DETAILED DESCRIPTION

### [2312] Definitions

[2313] The scientific literature that has evolved around receptors has adopted a number of terms to refer to ligands having various effects on receptors. For clarity and consistency, the following definitions will be used throughout this patent document. To the extent that these definitions conflict with other definitions for these terms, the following definitions shall control:

[2314] **ADIPONECTIN.** ADIPONECTIN is a plasma protein secreted by adipocytes and comprised of an N-terminally disposed collagen-like region and a C-terminal globular region. Reduced levels of plasma ADIPONECTIN have been associated with a number of metabolic-related disorders, including atherosclerosis, coronary heart disease, stroke, insulin resistance and type 2 diabetes. The serum ADIPONECTIN level for women has been reported to be higher than that for men, for example 13.5  $\mu$ g ml<sup>-1</sup> versus 7.2  $\mu$ g ml<sup>-1</sup> in one study [Yamamoto Y et al., Clin Sci (Lond) (2002) 103:137-42; the disclosure of which is hereby incorporated by reference in its entirety].

[2315] **AFFINITY REAGENTS** shall mean compounds that specifically and measurably bind to a target molecule. Preferably the target molecule is a GPCR.

[2316] **AGONISTS** shall mean materials (e.g., ligands, candidate compounds) that activate an intracellular response when they bind to the receptor. In some embodiments, AGONISTS are those materials not previously known to activate the intracellular response when they bind to the receptor (e.g. to enhance GTP $\gamma$ S binding to membranes or to lower intracellular cAMP level). In some embodiments, AGONISTS are those materials not previously known to inhibit lipolysis when they bind to the receptor.

[2317] **ALLELIC VARIANT.** See VARIANT.

[2318] **ALLOSTERIC MODULATORS** shall mean materials (e.g., ligands, candidate compounds) that affect the functional activity of the receptor but which do not inhibit the endogenous ligand from binding to the receptor. Allosteric modulators include inverse agonists, partial agonists and agonists.

TABLE A

|               |     |   |
|---------------|-----|---|
| ALANINE       | ALA | A |
| ARGININE      | ARG | R |
| ASPARAGINE    | ASN | N |
| ASPARTIC ACID | ASP | D |
| CYSTEINE      | CYS | C |
| GLUTAMIC ACID | GLU | E |
| GLUTAMINE     | GLN | Q |
| GLYCINE       | GLY | G |
| HISTIDINE     | HIS | H |
| ISOLEUCINE    | ILE | I |
| LEUCINE       | LEU | L |
| LYSINE        | LYS | K |
| METHIONINE    | MET | M |
| PHENYLALANINE | PHE | F |
| PROLINE       | PRO | P |
| SERINE        | SER | S |
| THREONINE     | THR | T |
| TRYPTOPHAN    | TRP | W |
| TYROSINE      | TYR | Y |
| VALINE        | VAL | V |

[2319] ANTAGONISTS shall mean materials (e.g., ligands, candidate compounds) that competitively bind to the receptor at the same site as the agonists but which do not activate an intracellular response, and can thereby inhibit the intracellular responses elicited by agonists. ANTAGONISTS do not diminish the baseline intracellular response in the absence of an agonist. In some embodiments, ANTAGONISTS are those materials not previously known to compete with an agonist to inhibit the cellular response when they bind to the receptor, e.g. wherein the cellular response is GTP $\gamma$ S binding to membranes or to the lowering of intracellular cAMP level.

[2320] ANTIBODIES are intended herein to encompass monoclonal antibodies and polyclonal antibodies. ANTIBODIES are further intended to encompass IgG, IgA, IgD, IgE, and IgM. ANTIBODIES include whole antibodies, including single-chain whole antibodies, and antigen binding fragments thereof, including Fab, Fab', F(ab)<sub>2</sub> and F(ab')<sub>2</sub>. ANTIBODIES may be from any animal origin. Preferably, ANTIBODIES are human, murine, rabbit, goat, guinea pig, hamster, camel, donkey, sheep, horse or chicken. Preferably ANTIBODIES have binding affinities with a dissociation constant or K<sub>d</sub> value less than 5 $\times 10^{-6}$ M, 10 $^{-6}$ M, 5 $\times 10^{-7}$ M, 10 $^{-7}$ M, 5 $\times 10^{-8}$ M, 10 $^{-8}$ M, 5 $\times 10^{-9}$ M,

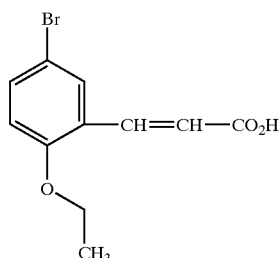
[2321] 10 $^{-9}$ M, 5 $\times 10^{-10}$  M 10 $^{-10}$ M, 5 $\times 10^{-11}$ M, 10 $^{-11}$ M, 5 $\times 10^{-12}$ M, 10 $^{-12}$ M, 5 $\times 10^{-13}$ M, 10 $^{-13}$ M,

[2322] 5 $\times 10^{-14}$ M 10 $^{-14}$ M, 5 $\times 10^{-15}$ M and 10 $^{-15}$ M. ANTIBODIES of the present invention may be prepared by any suitable method known in the art.

[2323] ATHEROSCLEROSIS is intended herein to encompass disorders of large and medium-sized arteries that result in the progressive accumulation within the intima of smooth muscle cells and lipids. Atherosclerosis is the primary cause of heart disease and stroke.

[2324] BIOLOGICALLY ACTIVE FRAGMENT is interchangeable herein with ACTIVE FRAGMENT and shall mean a fragment of full-length polypeptide or full-length amino acid sequence retaining part or all of the functionality of said full-length polypeptide or full-length amino acid sequence. In particular embodiment, a GPCR comprising an active fragment of a full-length GPCR polypeptide or full-length GPCR amino acid sequence retains part or all of the functionality of said GPCR comprising said full-length polypeptide or said full-length amino acid sequence. Said GPCR functionality is understood to include but not intended to be limited to ligand binding, G protein coupling, and ligand-facilitated coupling to G protein. By way of illustration and not limitation, BIOLOGICALLY ACTIVE FRAGMENT is intended herein to encompass full-length GPCR polypeptide absent the N-terminal methionine.

[2325] 3-(5-BROMO-2-ETHOXY-PHENYL)-ACRYLIC ACID shall be understood herein to have the formula:



[2326] and to encompass the E isomer, the Z isomer, and mixtures of E and Z isomers.

[2327] CANDIDATE COMPOUND shall mean a molecule (for example, and not limitation, a chemical compound) that is amenable to a screening technique. Preferably, the phrase "candidate compound" does not include compounds which were publicly known to be compounds selected from the group consisting of inverse agonist, agonist or antagonist to a receptor, as previously determined by an indirect identification process ("indirectly identified compound"); more preferably, not including an indirectly identified compound which has previously been determined to have therapeutic efficacy in at least one mammal; and, most preferably, not including an indirectly identified compound which has previously been determined to have therapeutic utility in humans.

[2328] CHOLESTEROL. Generally, the total cholesterol/HDL-cholesterol (i.e., TC/HDL) ratio represents a useful predictor as to the risk of an individual in developing a more serious condition, such as an HDL-related condition, such as but not limited to atherosclerosis and complications therefrom. The classification of plasma lipid levels is shown in Chart A:

CHART A

| CLASSIFICATION OF PLASMA LIPID LEVELS |               |                 |
|---------------------------------------|---------------|-----------------|
| TOTAL CHOLESTEROL                     | <200 mg/dl    | Desirable       |
|                                       | 200–239 mg/dl | Borderline High |
|                                       | >240 mg/dl    | High            |
| HDL-CHOLESTEROL                       | <40 mg/dl     | Low (Men)       |
|                                       | <50 mg/dl     | Low (Women)     |
|                                       | >60 mg/dl     | High            |

From: 2001 National Cholesterol Education Program Guidelines

[2329] Accordingly, the recommended total cholesterol/HDL-C (i.e., TC/HDL) ratio indicates that a ratio of less than or equal to 3.5 is ideal and a ratio of greater than 4.5 is considered an increased "at risk." The value of determining the TC/HDL ratio is clearly evident in the circumstance where an individual presents with "normal" LDL and total cholesterol but possesses low HDL-cholesterol. Based on LDL and total cholesterol the individual may not qualify for treatment however, factor in the HDL-cholesterol level then a more accurate risk assessment may be obtained. Thus, if the individual's level of HDL-cholesterol is such that the ratio is greater than 4.5 then therapeutic or preventive intervention may be warranted. A physician or care provider may determine the need of prevention or treatment based on a TC/HDL ratio; for example, a TC/HDL ratio of 2.5 or greater, 3.0 or greater, 3.5 or greater, 4.0 or greater, 4.5 or greater, 5.0 or greater, or a TC/HDL ratio of 5.5 or greater.

[2330] CODON shall mean a grouping of three nucleotides (or equivalents to nucleotides) which generally comprise a nucleoside [adenosine (A), guanosine (G), cytidine (C), uridine (U) and thymidine (T)] coupled to a phosphate group and which, when translated, encodes an amino acid.

[2331] COMPOSITION means a material comprising at least one component; a "pharmaceutical composition" is an example of a composition.

[2332] COMPOUND EFFICACY shall mean a measurement of the ability of a compound to inhibit or stimulate receptor functionality; i.e. the ability to activate/inhibit a signal transduction pathway, in contrast to receptor binding affinity. Exemplary means of detecting compound efficacy are disclosed in the Example section of this patent document.

[2333] COMPRISING, CONSISTING ESSENTIALLY OF, and CONSISTING OF are defined herein according to their standard meaning. A defined meaning set forth in the M.P.E.P. controls over a defined meaning in the art and a defined meaning set forth in controlling Federal Circuit case law controls over a meaning set forth in the M.P.E.P.

[2334] CONSTITUTIVELY ACTIVE RECEPTOR shall mean a receptor stabilized in an active state by means other than through binding of the receptor to its ligand or a chemical equivalent thereof. A CONSTITUTIVELY ACTIVE RECEPTOR may be endogenous or non-endogenous.

[2335] CONSTITUTIVELY ACTIVATED RECEPTOR shall mean an endogenous receptor that has been modified so as to be constitutively active. CART is an acronym for Constitutively Activated Receptor Technology and when used herein prefixing or suffixing a GPCR, shall be understood to identify said prefixed or suffixed GPCR as a CONSTITUTIVELY ACTIVATED RECEPTOR.

[2336] CONSTITUTIVE RECEPTOR ACTIVATION shall mean activation of a receptor in the absence of binding to its ligand or a chemical equivalent thereof.

[2337] CONTACT or CONTACTING shall mean bringing at least two moieties together, whether in an in vitro system or an in vivo system.

[2338] CORONARY HEART DISEASE is intended herein to encompass disorders comprising a narrowing of the small blood vessels that supply blood and oxygen to the heart. CORONARY HEART DISEASE usually results from the build up of fatty material and plaque. As the coronary arteries narrow, the flow of blood to the heart can slow or stop. CORONARY HEART DISEASE can cause chest pain (stable angina), shortness of breath, heart attack, or other symptoms. CORONARY HEART DISEASE is intended herein to include coronary artery disease, the most common type of heart disease. Coronary artery disease results from atherosclerosis.

[2339] DECREASE is used to refer to a reduction in a measurable quantity and is used synonymously with the terms "reduce", "diminish", "lower", and "lessen".

[2340] DIABETES as used herein is intended to encompass the usual diagnosis of DIABETES made from any of the methods including, but not limited to, the following list: symptoms of diabetes (e.g., polyuria, polydipsia, polyphagia) plus casual plasma glucose levels of greater than or equal to 200 mg/dl, wherein casual plasma glucose is defined any time of the day regardless of the timing of meal or drink consumption; 8 hour fasting plasma glucose levels of less than or equal to 126 mg/dl; and plasma glucose levels of greater than or equal to 200 mg/dl 2 hours following oral administration of 75 g anhydrous glucose dissolved in water.

[2341] DIRECTLY IDENTIFYING or DIRECTLY IDENTIFIED, in relationship to the phrase "candidate com-

pound", shall mean the screening of a candidate compound against a constitutively activated receptor, preferably a constitutively activated orphan receptor, and most preferably against a constitutively activated G protein-coupled cell surface orphan receptor, and assessing the compound efficacy of such compound. This phrase is, under no circumstances, to be interpreted or understood to be encompassed by or to encompass the phrase "indirectly identifying" or "indirectly identified."

[2342] DISORDERS OF LIPID METABOLISM are intended herein to include, but not be limited to, dyslipidemia.

[2343] DYSLIPIDEMIA is intended herein to encompass disorders comprising any one of elevated level of plasma free fatty acids, elevated level of plasma cholesterol, elevated level of LDL-cholesterol, reduced level of HDL-cholesterol, elevated ratio of total cholesterol to HDL-cholesterol, and elevated level of plasma triglycerides.

[2344] EFA-GPCR shall mean a mutant GPCR polypeptide that consists of 1, 2, 3, 4, or 5 amino acid substitutions, deletions, or insertions relative to the amino acid sequence of an endogenous GPCR polypeptide having constitutive activity, wherein the agonist screening window of the mutant GPCR is expanded by greater than 20%, greater than 25%, greater than 30%, greater than 31%, greater than 32%, greater than 33%, greater than 34%, greater than 35%, greater than 36%, greater than 37%, greater than 38%, greater than 39%, or greater than 40% relative to that of said endogenous GPCR.

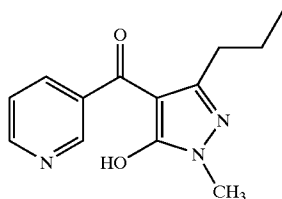
[2345] ENDOGENOUS shall mean a material that a mammal naturally produces. ENDOGENOUS in reference to, for example and not limitation, the term "receptor," shall mean that which is naturally produced by a mammal (for example, and not limitation, a human) or a virus. ENDOGENOUS shall be understood to encompass allelic variants of a gene as well as the allelic polypeptide variants so encoded. By contrast, the term NON-ENDOGENOUS in this context shall mean that which is not naturally produced by a mammal (for example, and not limitation, a human) or a virus. For example, and not limitation, a receptor which is not constitutively active in its endogenous form, but when manipulated becomes constitutively active, is most preferably referred to herein as a "non-endogenous, constitutively activated receptor." Both terms can be utilized to describe both "in vivo" and "in vitro" systems. For example, and not limitation, in a screening approach, the endogenous or non-endogenous receptor may be in reference to an in vitro screening system. As a further example and not limitation, where the genome of a mammal has been manipulated to include a non-endogenous constitutively activated receptor, screening of a candidate compound by means of an in vivo system is viable.

[2346] EXPRESSION VECTOR is defined herein as a DNA sequence that is required for the transcription of cloned DNA and the translation of the transcribed mRNAs in an appropriate host cell recombinant for said EXPRESSION VECTOR. An appropriately constructed EXPRESSION VECTOR should contain an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. By way of illustration and not limitation, pCMV is an expression vector.

[2347] G PROTEIN COUPLED RECEPTOR FUSION PROTEIN and GPCR FUSION PROTEIN, in the context of the invention disclosed herein, each mean a non-endogenous protein comprising an endogenous, constitutively activate GPCR or a non-endogenous, constitutively activated GPCR fused to at least one G protein, most preferably the alpha (α) subunit of such G protein (this being the subunit that binds GTP), with the G protein preferably being of the same type as the G protein that naturally couples with endogenous orphan GPCR. For example, and not limitation, in an endogenous state, if the G protein “G<sub>s</sub>α” is the predominate G protein that couples with the GPCR, a GPCR Fusion Protein based upon the specific GPCR would be a non-endogenous protein comprising the GPCR fused to G<sub>s</sub>α; in some circumstances, as will be set forth below, a non-predominant G protein can be fused to the GPCR. The G protein can be fused directly to the C-terminus of the constitutively active GPCR or there may be spacers between the two.

[2348] HOST CELL shall mean a cell capable of having a Plasmid and/or Vector incorporated therein. In the case of a prokaryotic Host Cell, a Plasmid is typically replicated as an autonomous molecule as the Host Cell replicates (generally, the Plasmid is thereafter isolated for introduction into a eukaryotic Host Cell); in the case of a eukaryotic Host Cell, a Plasmid may be integrated into the cellular DNA of the Host Cell such that when the eukaryotic Host Cell replicates, the Plasmid replicates. In some embodiments the Host Cell is eukaryotic, more preferably, mammalian, and more preferably selected from the group consisting of 293, 293T, CHO, and COS-7 cells. In other embodiments, the Host Cell is eukaryotic, more preferably melanophore.

[2349] (5-HYDROXY-1-METHYL-3-PROPYL-1H-PYRAZOL-4-YL)-PYRIDIN-3-YL-METHANONE shall be understood herein to have the formula:



[2350] IN NEED OF PREVENTION OR TREATMENT as used herein refers to a judgement made by a caregiver (e.g. physician, nurse, nurse practitioner, etc. in the case of humans; veterinarian in the case of animals, including non-human mammals) that an individual or animal requires or will benefit from treatment. This judgement is made based on a variety of factors that are in the realm of a caregiver's expertise, but that include the knowledge that the individual or animal is ill, or will be ill, as the result of a condition that is treatable by the compounds of the invention.

[2351] INDIRECTLY IDENTIFYING or INDIRECTLY IDENTIFIED means the traditional approach to the drug discovery process involving identification of an endogenous ligand specific for an endogenous receptor, screening of candidate compounds against the receptor for determination of those which interfere and/or compete with the ligand-

receptor interaction, and assessing the efficacy of the compound for affecting at least one second messenger pathway associated with the activated receptor.

[2352] INDIVIDUAL as used herein refers to any animal, including mammals, preferably mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, and most preferably humans.

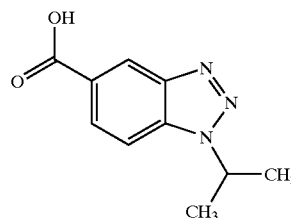
[2353] INHIBIT or INHIBITING, in relationship to the term “response” shall mean that a response is decreased or prevented in the presence of a compound as opposed to in the absence of the compound.

[2354] INSULIN RESISTANCE as used herein is intended to encompass the usual diagnosis of insulin resistance made by any of a number of methods, including but not restricted to: the intravenous glucose tolerance test or measurement of the fasting insulin level. It is well known that there is an excellent correlation between the height of the fasting insulin level and the degree of insulin resistance. Therefore, one could use elevated fasting insulin levels as a surrogate marker for insulin resistance for the purpose of identifying which normal glucose tolerance (NGT) individuals have insulin resistance. A diagnosis of insulin resistance can also be made using the euglycemic glucose clamp test.

[2355] INVERSE AGONISTS shall mean materials (e.g., ligand, candidate compound) that bind either to the endogenous form or to the constitutively activated form of the receptor so as to reduce the baseline intracellular response of the receptor observed in the absence of agonists.

[2356] ISOLATED shall mean that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or DNA or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such a polynucleotide could be part of a vector and/or such a polynucleotide or polypeptide could be part of a composition, and still be isolated in that the vector or composition is not part of its natural environment.

[2357] 1-ISOPROPYL-1H-BENZOTRIAZOLE-5-CARBOXYLIC ACID shall be understood herein to have the formula:



[2358] KNOCKOUT MOUSE/RAT is intended herein to encompass a mouse or rat that has been manipulated by recombinant means such that a single gene of choice has been inactivated or “knocked-out” in a manner that leaves all other genes unaffected.

[2359] KNOWN RECEPTOR shall mean an endogenous receptor for which the endogenous ligand specific for that receptor has been identified.

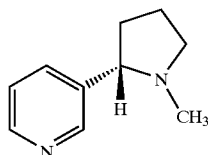
[2360] LIGAND shall mean a molecule specific for a naturally occurring receptor.

[2361] METABOLIC-RELATED DISORDERS are intended herein to include, but not be limited to, dyslipidemia, atherosclerosis, coronary heart disease, stroke, insulin resistance and type 2 diabetes.

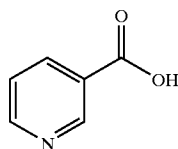
[2362] As used herein, the terms MODULATE or MODIFY are meant to refer to an increase or decrease in the amount, quality, or effect of a particular activity, function or molecule.

[2363] MUTANT or MUTATION in reference to an endogenous receptor's nucleic acid and/or amino acid sequence shall mean a specified change or changes to such endogenous sequences such that a mutated form of an endogenous non-constitutively activated receptor evidences constitutive activation of the receptor. In terms of equivalents to specific sequences, a subsequent mutated form of a human receptor is considered to be equivalent to a first mutation of the human receptor if (a) the level of constitutive activation of the subsequent mutated form of a human receptor is substantially the same as that evidenced by the first mutation of the receptor; and (b) the percent sequence (amino acid and/or nucleic acid) homology between the subsequent mutated form of the receptor and the first mutation of the receptor is at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and most preferably at least 99%. In some embodiments, owing to the fact that some preferred cassettes disclosed herein for achieving constitutive activation include a single amino acid and/or codon change between the endogenous and the non-endogenous forms of the GPCR, it is preferred that the percent sequence homology should be at least 98%.

[2364] (-)-NICOTINE shall be understood herein to have the formula:



[2365] NICOTINIC ACID shall be understood herein to have the formula:



[2366] As used herein, the term NICOTINIC ACID ANALOG OR DERIVATIVE is meant to molecules which bind to nicotinic acid receptors and have substantially similar effects on the receptor. Such analogs and derivatives are well-known to those skilled in the art and include, but are not limited to, Acipimox<sup>TM</sup> and niacinamide.

[2367] NON-ORPHAN RECEPTOR shall mean an endogenous naturally occurring molecule specific for an identified ligand wherein the binding of a ligand to a receptor activates an intracellular signaling pathway.

[2368] ORPHAN RECEPTOR shall mean an endogenous receptor for which the ligand specific for that receptor has not been identified or is not known.

[2369] PARTIAL AGONISTS shall mean materials (e.g., ligands, candidate compounds) that activate the intracellular response when they bind to the receptor to a lesser degree/extent than do full agonists.

[2370] PHARMACEUTICAL COMPOSITION shall mean a composition comprising at least one active ingredient, whereby the composition is amenable to investigation for a specified, efficacious outcome in a mammal (for example, and not limitation, a human). Those of ordinary skill in the art will understand and appreciate the techniques appropriate for determining whether an active ingredient has a desired efficacious outcome based upon the needs of the artisan.

[2371] PLASMID shall mean the combination of a Vector and cDNA. Generally, a Plasmid is introduced into a Host Cell for the purposes of replication and/or expression of the cDNA as a protein.

[2372] POLYNUCLEOTIDES shall mean RNA, DNA, or RNA/DNA hybrid sequences of more than one nucleotide in either single chain or duplex form. The polynucleotides of the invention may be prepared by any known method, including synthetic, recombinant, ex vivo generation, or a combination thereof, as well as utilizing any purification methods known in the art.

[2373] POLYPEPTIDE shall refer to a polymer of amino acids without regard to the length of the polymer. Thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not specify or exclude post-expression modifications of polypeptides. For example, polypeptides that include the covalent attachment of glycosyl groups, acetyl groups, phosphate groups, lipid groups and the like are expressly encompassed by the term POLYPEPTIDE.

[2374] PRIMER is used herein to denote a specific oligonucleotide sequence which is complementary to a target nucleotide sequence and used to hybridize to the target nucleotide sequence. A primer serves as an initiation point for nucleotide polymerization catalyzed by DNA polymerase, RNA polymerase, or reverse transcriptase.

[2375] PURIFIED is used herein to describe a polynucleotide or polynucleotide vector of the invention that has been separated from other compounds including, but not limited to, other nucleic acids, carbohydrates, lipids and proteins (such as the enzymes used in the synthesis of the polynucleotide). A polynucleotide is substantially pure when at least about 50%, 60%, 75%, or 90% of a sample contains a single polynucleotide sequence. A substantially pure polynucleotide typically comprises about 50, 60, 70, 80, 90, 95, 99% weight/weight of a nucleic acid sample. Polynucleotide purity or homogeneity may be indicated by a number of means well known in the art, such as agarose or polyacrylamide gel electrophoresis of a sample, followed by visualizing a single polynucleotide band upon staining the gel.

[2376] Similarly, the term **PURIFIED** is used herein to describe a polypeptide of the invention that has been separated from other compounds including, but not limited to, nucleic acids, lipids, carbohydrates and other proteins. In some preferred embodiments, a polypeptide is substantially pure when at least about 50%, 60%, 75%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 99.5% of the polypeptide molecules of a sample have a single amino acid sequence. In some preferred embodiments, a substantially pure polypeptide typically comprises about 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 99.5% weight/weight of a protein sample. Polypeptide purity or homogeneity is indicated by a number of methods well known in the art, such as agarose or polyacrylamide gel electrophoresis of a sample, followed by visualizing a single polypeptide band upon staining the gel.

[2377] Further, as used herein, the term **PURIFIED** does not require absolute purity; rather, it is intended as a relative definition. Purification of starting material or natural material to at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated.

[2378] **RECEPTOR FUNCTIONALITY** shall refer to the normal operation of a

[2379] receptor to receive a stimulus and moderate an effect in the cell, including, but not limited to regulating gene transcription, regulating the influx or efflux of ions, effecting a catalytic reaction, and/or modulating activity through G-proteins.

[2380] **SECOND MESSENGER** shall mean an intracellular response produced as a result of receptor activation. A second messenger can include, for example, inositol triphosphate (IP<sub>3</sub>), diacylglycerol (DAG), cyclic AMP (cAMP), cyclic GMP (cGMP), and Ca<sup>2+</sup>. Second messenger response can be measured for a determination of receptor activation. In addition, second messenger response can be measured for the direct identification of candidate compounds, including for example, inverse agonists, partial agonists, agonists, and antagonists.

[2381] **SIGNAL TO NOISE RATIO** shall mean the signal generated in response to activation, amplification, or stimulation wherein the signal is above the background noise or the basal level in response to non-activation, non-amplification, or non-stimulation.

[2382] **SPACER** shall mean a translated number of amino acids that are located after the last codon or last amino acid of a gene, for example a GPCR of interest, but before the start codon or beginning regions of the G protein of interest, wherein the translated number amino acids are placed in-frame with the beginnings regions of the G protein of interest. The number of translated amino acids can be one, two, three, four, etc., and up to twelve.

[2383] **STIMULATE** or **STIMULATING**, in relationship to the term "response" shall mean that a response is increased in the presence of a compound as opposed to in the absence of the compound.

[2384] **STROKE** is a cardiovascular disease that affects the blood vessels supplying blood to the brain and is intended herein to include cerebral thrombosis, the most common type of **STROKE**. Cerebral thrombosis occurs when a blood clot (thrombus) forms and blocks blood flow in an artery bringing blood to part of the brain. Blood clots usually form in arteries damaged by atherosclerosis.

[2385] **SUBJECT** shall mean primates, including but not limited to humans and baboons, as well as pet animals such as dogs and cats, laboratory animals such as rats and mice, and farm animals such as horses, sheep, and cows.

[2386] **SUBSTANTIALLY** shall refer to a result which is within 40% of a control result, preferably within 35%, more preferably within 30%, more preferably within 25%, more preferably within 20%, more preferably within 15%, more preferably within 10%, more preferably within 5%, more preferably within 2%, and most preferably within 1% of a control result. For example, in the context of receptor functionality, a test receptor may exhibit substantially similar results to a control receptor if the transduced signal, measured using a method taught herein or similar method known to the art-skilled, is within 40% of the signal produced by a control signal.

[2387] **TRANSGENIC MOUSE/RAT** shall be intended herein to encompass a mouse or rat that has been engineered through recombinant means to carry a foreign gene, or transgene, of choice as part of its own genetic material.

[2388] **VARIANT** as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A variant of a polynucleotide or polypeptide may be a naturally occurring one such as an **ALLELIC VARIANT**, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

[2389] **VECTOR** in reference to cDNA shall mean a circular DNA capable of incorporating at least one cDNA and capable of incorporation into a Host Cell.

[2390] The order of the following sections is set forth for presentational efficiency and is not intended, nor should be construed, as a limitation on the disclosure or the claims to follow.

[2391] A. Introduction

[2392] The traditional study of receptors has always proceeded from the a priori assumption (historically based) that the endogenous ligand must first be identified before discovery could proceed to find antagonists and other molecules that could affect the receptor. Even in cases where an antagonist might have been known first, the search immediately extended to looking for the endogenous ligand. This mode of thinking has persisted in receptor research even after the discovery of constitutively activated receptors. What has not been heretofore recognized is that it is the active state of the receptor that is most useful for discovering agonists, partial agonists, and inverse agonists of the receptor. For those diseases which result from an overly active receptor or an under-active receptor, what is desired in a therapeutic drug is a compound which acts to diminish the active state of a receptor or enhance the activity of the receptor, respectively, not necessarily a drug which is an antagonist to the endogenous ligand. This is because a

compound that reduces or enhances the activity of the active receptor state need not bind at the same site as the endogenous ligand. Thus, as taught by the present invention, in some preferred embodiments, a search for therapeutic compounds should start by screening compounds against the ligand-independent active state.

#### [2393] B. Identification of Human GPCRs

[2394] The efforts of the Human Genome project has led to the identification of a plethora of information regarding nucleic acid sequences located within the human genome; it has been the case in this endeavor that genetic sequence information has been made available without an understanding or recognition as to whether or not any particular genomic sequence does or may contain open-reading frame information that translate human proteins. Several methods of identifying nucleic acid sequences within the human genome are within the purview of those having ordinary skill in the art. For example, and not limitation, a variety of human GPCRs, disclosed herein, were discovered by reviewing the GenBank™ database. Table B, below, lists several endogenous GPCRs that we have discovered, along with other GPCRs that are homologous to the disclosed GPCR.

TABLE B

| Disclosed Human Orphan GPCRs | Accession Number Identified | Open Reading Frame (Base Pairs) | Reference To Homologous GPCR | Per Cent Homology To Designated GPCR |
|------------------------------|-----------------------------|---------------------------------|------------------------------|--------------------------------------|
| hRUP8                        | AL121755                    | 1,152 bp                        | NPY2R                        | 27%                                  |
| hRUP9                        | AC0113375                   | 1,260 bp                        | GAL2R                        | 22%                                  |
| hRUP10                       | AC008745                    | 1,014 bp                        | C5aR                         | 40%                                  |
| hRUP11                       | AC013396                    | 1,272 bp                        | HM74                         | 36%                                  |
| hRUP12                       | AP000808                    | 966 bp                          | Mas1                         | 34%                                  |
| hRUP13                       | AC011780                    | 1,356 bp                        | Fish GPRX-ORYLA              | 43%                                  |
| hRUP14                       | AL137118                    | 1,041 bp                        | CysLT1R                      | 35%                                  |
| hRUP15                       | AL016468                    | 1,527 bp                        | RE2                          | 30%                                  |
| hRUP16                       | AL136106                    | 1,068 bp                        | GLR101                       | 37%                                  |
| hRUP17                       | AC023078                    | 969 bp                          | Mas1                         | 37%                                  |
| hRUP18                       | AC008547                    | 1,305 bp                        | Oxytocin                     | 31%                                  |
| hRUP19                       | AC026331                    | 1,041 bp                        | HM74                         | 52%                                  |
| hRUP20                       | AL161458                    | 1,011 bp                        | GPR34                        | 25%                                  |
| hRUP21                       | AC026756                    | 1,014 bp                        | P2Y1R                        | 37%                                  |
| hRUP22                       | AC027026                    | 993 bp                          | hRUP17 Mas1                  | 67% 37%                              |
| hRUP23                       | AC007104                    | 1,092 bp                        | Rat GPR26                    | 31%                                  |
| hRUP24                       | AL355388                    | 1,125 bp                        | SALPR                        | 44%                                  |
| hRUP25                       | AC026331                    | 1,092 bp                        | HM74                         | 95%                                  |
| hRUP26                       | AC023040                    | 1,044 bp                        | Rabbit 5HT1D                 | 27%                                  |
| hRUP27                       | AC027643                    | 1,020 bp                        | MCH                          | 38%                                  |
| hRUP38                       | AC026331                    | 1,164 bp                        | HM74                         | 100%                                 |

[2395] Such receptors are disclosed, for example, in application Ser. No. 09/714,008, filed Nov. 16, 2000, which is incorporated by reference in its entirety.

[2396] Receptor homology is useful in terms of gaining an appreciation of a role of the receptors within the human body. As the patent document progresses, techniques for mutating these receptors to establish non-endogenous, constitutively activated versions of these receptors will be discussed.

[2397] The techniques disclosed herein have also been applied to other human, orphan GPCRs known to the art, as will be apparent as the patent document progresses.

#### [2398] C. Identification of the Mouse (m) and Rat (r) Orthologs of Human (h) RUP25 and Identification of the Mouse (m) and Rat (r) Orthologs of Human (h) RUP19

TABLE C

| Disclosed Mouse (m) or Rat (r) RUP25 | Accession Number Identified | Open Reading Frame (Base Pairs) | Reference To Orthologous Human GPCR | Per Cent Homology To Designated GPCR |
|--------------------------------------|-----------------------------|---------------------------------|-------------------------------------|--------------------------------------|
| mRUP25                               | AJ300199                    | 1,083 bp                        | hRUP25                              | 83%                                  |
| rRUP25                               | None                        | 1,086 bp                        | hRUP25                              | 71%                                  |
| mRUP19                               | XM_144529                   | 1,032 bp                        | hRUP19                              | 81%                                  |
| rRUP19                               | None                        | 1,056 bp                        | hRUP19                              | 83%                                  |

#### [2399] D. Receptor Screening

[2400] Screening candidate compounds against a non-endogenous, constitutively activated version of the GPCRs disclosed herein allows for the direct identification of candidate compounds which act at the cell surface receptor, without requiring use, or, in some embodiments, of the knowledge of the identity of the receptor's endogenous ligand. Using routine and often commercially available techniques, one can determine areas within the body where the endogenous version of human GPCRs disclosed herein is expressed and/or over-expressed. The expression location of a receptor in a specific tissue provides a scientist with the ability to assign a physiological functional role of the receptor. It is also possible using these techniques to determine related disease/disorder states which are associated with the expression and/or over-expression of the receptor; such an approach is disclosed in this patent document. Furthermore, expression of a receptor in diseased organs can assist one in determining the magnitude of the clinical relevance of the receptor.

[2401] Constitutive activation of the GPCRs disclosed herein is based upon the distance from the proline residue at which is presumed to be located within TM6 of the GPCR; this algorithmic technique is disclosed in co-pending and commonly assigned patent document PCT Application Number PCT/US99/23938, published as WO 00/22129 on Apr. 20, 2000, which, along with the other patent documents listed herein, is incorporated herein by reference in its entirety. The algorithmic technique is not predicated upon traditional sequence "alignment" but rather a specified distance from the aforementioned TM6 proline residue (or, of course, endogenous constitutive substitution for such proline residue). By mutating the amino acid residue located 16 amino acid residues from this residue (presumably located in the IC3 region of the receptor) to, preferably, a lysine residue, constitutive activation of the receptor may be obtained. Other amino acid residues may be useful in the mutation at this position to achieve this objective and will be discussed in detail, below.

#### [2402] E. Disease/Disorder Identification and/or Selection

[2403] As will be set forth in greater detail below, inverse agonists and agonists to the non-endogenous, constitutively activated GPCR can be identified by the methodologies of this invention. Such inverse agonists and agonists are good candidates as lead compounds in drug discovery programs for treating diseases and/or disorders related to this receptor. Because of the ability to directly identify inverse agonists and agonists to the GPCR, thereby allowing for the development of pharmaceutical compositions, a search for diseases and disorders associated with the GPCR is relevant. The expression location of a receptor in a specific tissue

provides a scientist with the ability to assign a physiological function to the receptor. For example, scanning both diseased and normal tissue samples for the presence of the GPCR now becomes more than an academic exercise or one which might be pursued along the path of identifying an endogenous ligand to the specific GPCR. Tissue scans can be conducted across a broad range of healthy and diseased tissues. Such tissue scans provide a potential first step in associating a specific receptor with a disease and/or disorder. Furthermore, expression of a receptor in diseased organs can assist one in determining the magnitude of the clinical relevance of the receptor.

**[2404]** The DNA sequence of the GPCR can be used to make a probe/primer. In some preferred embodiments the DNA sequence is used to make a probe for (a) dot-blot analysis against tissue-mRNA, and/or (b) RT-PCR identification of the expression of the receptor in tissue samples. The presence of a receptor in a tissue source, or a diseased tissue, or the presence of the receptor at elevated concentrations in diseased tissue compared to a normal tissue, can be used to correlate location to function and indicate the receptor's physiological role/function and create a treatment regimen, including but not limited to, a disease associated with that function/role. Receptors can also be localized to regions of organs by this technique. Based on the known or assumed roles/functions of the specific tissues to which the receptor is localized, the putative physiological function of the receptor can be deduced. For example and not limitation, proteins located/expressed in areas of the thalamus are associated with sensorimotor processing and arousal (see, Goodman & Gilman's, *The Pharmacological Basis of Therapeutics*, 9<sup>th</sup> Edition, page 465 (1996)). Proteins expressed in the hippocampus or in Schwann cells are associated with learning and memory, and myelination of peripheral nerves, respectively (see, Kandel, E. et al., *Essentials of Neural Science and Behavior* pages 657, 680 and 28, respectively (1995)).

**[2405]** F. Screening of Candidate Compounds

**[2406]** 1. Generic GPCR Screening Assay Techniques

**[2407]** When a G protein receptor becomes constitutively active, it binds to a G protein (e.g., Gq, Gs, Gi, Gz, Go) and stimulates the binding of GTP to the G protein. The G protein then acts as a GTPase and slowly hydrolyzes the GTP to GDP, whereby the receptor, under normal conditions, becomes deactivated. However, constitutively activated receptors continue to exchange GDP to GTP. A non-hydrolyzable analog of GTP, [<sup>35</sup>S]GTPγS, can be used to monitor enhanced binding to membranes which express constitutively activated receptors. It is reported that [<sup>35</sup>S]GTPγS can be used to monitor G protein coupling to membranes in the absence and presence of ligand. An example of this monitoring, among other examples well-known and available to those in the art, was reported by Traynor and Nahorski in 1995. The preferred use of this assay system is for initial screening of candidate compounds because the system is generically applicable to all G protein-coupled receptors regardless of the particular G protein that interacts with the intracellular domain of the receptor.

**[2408]** 2. Specific GPCR Screening Assay Techniques

**[2409]** Once candidate compounds are identified using the "generic" G protein-coupled receptor assay (i.e., an assay to select compounds that are agonists or inverse agonists), in some embodiments further screening to confirm that the compounds have interacted at the receptor site is preferred.

For example, a compound identified by the "generic" assay may not bind to the receptor, but may instead merely "uncouple" the G protein from the intracellular domain.

**[2410]** a. Gs, Gz and Gi.

**[2411]** Gs stimulates the enzyme adenylyl cyclase. Gi (and Gz and Go), on the other hand, inhibit adenylyl cyclase. Adenylyl cyclase catalyzes the conversion of ATP to cAMP; thus, constitutively activated GPCRs that couple the Gs protein are associated with increased cellular levels of cAMP. On the other hand, constitutively activated GPCRs that couple Gi (or Gz, Go) protein are associated with decreased cellular levels of cAMP. See, generally, "Indirect Mechanisms of Synaptic Transmission," Chpt. 8, *From Neuron To Brain* (3<sup>rd</sup> Ed.) Nichols, J. G. et al eds. Sinauer Associates, Inc. (1992). Thus, assays that detect cAMP can be utilized to determine if a candidate compound is, e.g., an inverse agonist to the receptor (i.e., such a compound would decrease the levels of cAMP). A variety of approaches known in the art for measuring cAMP can be utilized; in some embodiments a preferred approach relies upon the use of anti-cAMP antibodies in an ELISA-based format. Another type of assay that can be utilized is a whole cell second messenger reporter system assay. Promoters on genes drive the expression of the proteins that a particular gene encodes. Cyclic AMP drives gene expression by promoting the binding of a cAMP-responsive DNA binding protein or transcription factor (CREB) that then binds to the promoter at specific sites called cAMP response elements and drives the expression of the gene. Reporter systems can be constructed which have a promoter containing multiple cAMP response elements before the reporter gene, e.g., β-galactosidase or luciferase. Thus, a constitutively activated Gs-linked receptor causes the accumulation of cAMP that then activates the gene and expression of the reporter protein. The reporter protein such as α-galactosidase or luciferase can then be detected using standard biochemical assays (Chen et al. 1995).

**[2412]** b. Go and Gq.

**[2413]** Gq and Go are associated with activation of the enzyme phospholipase C, which in turn hydrolyzes the phospholipid PIP<sub>2</sub>, releasing two intracellular messengers: diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP<sub>3</sub>). Increased accumulation of IP<sub>3</sub> is associated with activation of Gq- and Go-associated receptors. See, generally, "Indirect Mechanisms of Synaptic Transmission," Chpt. 8, *From Neuron To Brain* (3<sup>rd</sup> Ed.) Nichols, J. G. et al eds. Sinauer Associates, Inc. (1992). Assays that detect IP<sub>3</sub> accumulation can be utilized to determine if a candidate compound is, e.g., an inverse agonist to a Gq- or Go-associated receptor (i.e., such a compound would decrease the levels of IP<sub>3</sub>). Gq-associated receptors can also be examined using an AP1 reporter assay in that Gq-dependent phospholipase C causes activation of genes containing AP1 elements; thus, activated Gq-associated receptors will evidence an increase in the expression of such genes, whereby inverse agonists thereto will evidence a decrease in such expression, and agonists will evidence an increase in such expression. Commercially available assays for such detection are available.

**[2414]** 3. GPCR Fusion Protein

**[2415]** The use of an endogenous, constitutively activated GPCR or a non-endogenous, constitutively activated GPCR, for use in screening of candidate compounds for the direct identification of inverse agonists or agonists provides an

interesting screening challenge in that, by definition, the receptor is active even in the absence of an endogenous ligand bound thereto. Thus, in order to differentiate between, e.g., the non-endogenous receptor in the presence of a candidate compound and the non-endogenous receptor in the absence of that compound, with an aim of such a differentiation to allow for an understanding as to whether such compound may be an inverse agonist or agonist or have no affect on such a receptor, in some embodiments it is preferred that an approach be utilized that can enhance such differentiation. In some embodiments, a preferred approach is the use of a GPCR Fusion Protein.

**[2416]** Generally, once it is determined that a non-endogenous GPCR has been constitutively activated using the assay techniques set forth above (as well as others known to the art-skilled), it is possible to determine the predominant G protein that couples with the endogenous GPCR. Coupling of the G protein to the GPCR provides a signaling pathway that can be assessed. In some embodiments it is preferred that screening take place using a mammalian expression system, such a system will be expected to have endogenous G protein therein. Thus, by definition, in such a system, the non-endogenous, constitutively activated GPCR will continuously signal. In some embodiments it is preferred that this signal be enhanced such that in the presence of, e.g., an inverse agonist to the receptor, it is more likely that it will be able to more readily differentiate, particularly in the context of screening, between the receptor when it is contacted with the inverse agonist.

**[2417]** The GPCR Fusion Protein is intended to enhance the efficacy of G protein coupling with the non-endogenous GPCR. The GPCR Fusion Protein is preferred for screening with either an endogenous, constitutively active GPCR or a non-endogenous, constitutively activated GPCR because such an approach increases the signal that is generated in such screening techniques. This is important in facilitating a significant "signal to noise" ratio; such a significant ratio is preferred for the screening of candidate compounds as disclosed herein.

**[2418]** The construction of a construct useful for expression of a GPCR Fusion Protein is within the purview of those having ordinary skill in the art. Commercially available expression vectors and systems offer a variety of approaches that can fit the particular needs of an investigator. Important criteria in the construction of such a GPCR Fusion Protein construct include but are not limited to, that the endogenous GPCR sequence and the G protein sequence both be in-frame (preferably, the sequence for the endogenous GPCR is upstream of the G protein sequence), and that the "stop" codon of the GPCR be deleted or replaced such that upon expression of the GPCR, the G protein can also be expressed. Other embodiments include constructs wherein the endogenous GPCR sequence and the G protein sequence are not in-frame and/or the "stop" codon is not deleted or replaced. The GPCR can be linked directly to the G protein, or there can be spacer residues between the two (preferably, no more than about 12, although this number can be readily ascertained by one of ordinary skill in the art). Based upon convenience it is preferred to use a spacer. In some embodiments it is preferred, that the G protein that couples to the non-endogenous GPCR will have been identified prior to the creation of the GPCR Fusion Protein construct. Because there are only a few G proteins that have been identified, it is preferred that a construct comprising the sequence of the G protein (i.e., a universal G protein construct, see Example 5(a) below) be available for insertion

of an endogenous GPCR sequence therein; this provides for further efficiency in the context of large-scale screening of a variety of different endogenous GPCRs having different sequences.

**[2419]** As noted above, constitutively activated GPCRs that couple to Gi, Gz and Go are expected to inhibit the formation of cAMP making assays based upon these types of GPCRs challenging (i.e., the cAMP signal decreases upon activation thus making the direct identification of, e.g., inverse agonists (which would further decrease this signal), challenging. As will be disclosed herein, it has been ascertained that for these types of receptors, it is possible to create a GPCR Fusion Protein that is not based upon the GPCR's endogenous G protein, in an effort to establish a viable cyclase-based assay. Thus, for example, an endogenous Gi coupled receptor can be fused to a Gs protein—such a fusion construct, upon expression, "drives" or "forces" the endogenous GPCR to couple with, e.g., Gs rather than the "natural" Gi protein, such that a cyclase-based assay can be established. Thus, for Gi, Gz and Go coupled receptors, in some embodiments it is preferred that when a GPCR Fusion Protein is used and the assay is based upon detection of adenylyl cyclase activity, that the fusion construct be established with Gs (or an equivalent G protein that stimulates the formation of the enzyme adenylyl cyclase).

TABLE D

| G protein | Effect of cAMP Production upon Activation of GPCR (i.e., constitutive activation or agonist binding) | Effect of IP <sub>3</sub> Accumulation upon Activation of GPCR (i.e., constitutive activation or agonist binding) | Effect of cAMP Production upon contact with an Inverse Agonist | Effect on IP <sub>3</sub> Accumulation upon contact with an Inverse Agonist |
|-----------|------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------|-----------------------------------------------------------------------------|
|           |                                                                                                      |                                                                                                                   |                                                                |                                                                             |
| Gs        | Increase                                                                                             | N/A                                                                                                               | Decrease                                                       | N/A                                                                         |
| Gi        | Decrease                                                                                             | N/A                                                                                                               | Increase                                                       | N/A                                                                         |
| Gz        | Decrease                                                                                             | N/A                                                                                                               | Increase                                                       | N/A                                                                         |
| Go        | Decrease                                                                                             | Increase                                                                                                          | Increase                                                       | Decrease                                                                    |
| Gq        | N/A                                                                                                  | Increase                                                                                                          | N/A                                                            | Decrease                                                                    |

**[2420]** Equally effective is a G Protein Fusion construct that utilizes a Gq Protein fused with a Gs, Gi, Gz or Go Protein. In some embodiments a preferred fusion construct can be accomplished with a Gq Protein wherein the first six (6) amino acids of the G-protein  $\alpha$ -subunit ("G $\alpha$ q") is deleted and the last five (5) amino acids at the C-terminal end of G $\alpha$ q is replaced with the corresponding amino acids of the G $\alpha$  of the G protein of interest. For example, a fusion construct can have a Gq (6 amino acid deletion) fused with a Gi Protein, resulting in a "Gq/Gi Fusion Construct". This fusion construct will force the endogenous Gi coupled receptor to couple to its non-endogenous G protein, Gq, such that the second messenger, for example, inositol triphosphate or diacylglycerol, can be measured in lieu of cAMP production.

**[2421]** 4. Co-Transfection of a Target Gi Coupled GPCR with a Signal-Enhancer Gs Coupled GPCR (cAMP Based Assays)

**[2422]** A Gi coupled receptor is known to inhibit adenylyl cyclase, and, therefore, decreases the level of cAMP production, which can make the assessment of cAMP levels challenging. In some preferred embodiments, an effective technique in measuring the decrease in production of cAMP

as an indication of constitutive activation of a receptor that predominantly couples  $G_i$  upon activation can be accomplished by co-transfecting a signal enhancer, e.g., a non-endogenous, constitutively activated receptor that predominantly couples with  $G_s$  upon activation (e.g., TSHR-A6231, disclosed below), with the  $G_i$  linked GPCR. As is apparent, constitutive activation of a  $G_s$  coupled receptor can be determined based upon an increase in production of cAMP. Constitutive activation of a  $G_i$  coupled receptor leads to a decrease in production cAMP. Thus, the co-transfection approach is intended to advantageously exploit these "opposite" affects. For example, co-transfection of a non-endogenous, constitutively activated  $G_s$  coupled receptor (the "signal enhancer") with the endogenous  $G_i$  coupled receptor (the "target receptor") provides a baseline cAMP signal (i.e., although the  $G_i$  coupled receptor will decrease cAMP levels, this "decrease" will be relative to the substantial increase in cAMP levels established by constitutively activated  $G_s$  coupled signal enhancer). By then co-transfecting the signal enhancer with a constitutively activated version of the target receptor, cAMP would be expected to further decrease (relative to base line) due to the increased functional activity of the  $G_i$  target (i.e., which decreases cAMP).

[2423] Screening of candidate compounds using a cAMP based assay can then be accomplished, with two 'changes' relative to the use of the endogenous receptor/G-protein fusion: first, relative to the  $G_i$  coupled target receptor, "opposite" effects will result, i.e., an inverse agonist of the  $G_i$  coupled target receptor will increase the measured cAMP signal, while an agonist of the  $G_i$  coupled target receptor will decrease this signal; second, as would be apparent, candidate compounds that are directly identified using this approach should be assessed independently to ensure that these do not target the signal enhancing receptor (this can be done prior to or after screening against the co-transfected receptors).

[2424] G. Medicinal Chemistry

[2425] Candidate Compounds

[2426] Any molecule known in the art can be tested for its ability to modulate (increase or decrease) the activity of a GPCR of the present invention. For identifying a compound that modulates activity, candidate compounds can be directly provided to a cell expressing the receptor.

[2427] This embodiment of the invention is well suited to screen chemical libraries for molecules which modulate, e.g., inhibit, antagonize, or agonize, the amount of, or activity of, a receptor. The chemical libraries can be peptide libraries, peptidomimetic libraries, chemically synthesized libraries, recombinant, e.g., phage display libraries, and in vitro translation-based libraries, other non-peptide synthetic organic libraries, etc. This embodiment of the invention is also well suited to screen endogenous candidate compounds comprising biological materials, including but not limited to plasma and tissue extracts, and to screen libraries of endogenous compounds known to have biological activity.

[2428] In some embodiments direct identification of candidate compounds is conducted in conjunction with compounds generated via combinatorial chemistry techniques, whereby thousands of compounds are randomly prepared for such analysis. The candidate compound may be a member of a chemical library. This may comprise any convenient number of individual members, for example tens to hundreds to thousand to millions of suitable compounds, for example peptides, peptoids and other oligomeric compounds (cyclic or linear), and template-based smaller molecules, for

example benzodiazepines, hydantoins, biaryls, carbocyclic and polycyclic compounds (e.g., naphthalenes, phenothiazines, acridines, steroids etc.), carbohydrate and amino acid derivatives, dihydropyridines, benzhydryls and heterocycles (e.g., trizines, indoles, thiazolidines etc.). The numbers quoted and the types of compounds listed are illustrative, but not limiting. Preferred chemical libraries comprise chemical compounds of low molecular weight and potential therapeutic agents.

[2429] Exemplary chemical libraries are commercially available from several sources (ArQule, Tripos/PanLabs, ChemDesign, Pharmacopoeia). In some cases, these chemical libraries are generated using combinatorial strategies that encode the identity of each member of the library on a substrate to which the member compound is attached, thus allowing direct and immediate identification of a molecule that is an effective modulator. Thus, in many combinatorial approaches, the position on a plate of a compound specifies that compound's composition. Also, in one example, a single plate position may have from 1-20 chemicals that can be screened by administration to a well containing the interactions of interest. Thus, if modulation is detected, smaller and smaller pools of interacting pairs can be assayed for the modulation activity. By such methods, many candidate molecules can be screened.

[2430] Many diversity libraries suitable for use are known in the art and can be used to provide compounds to be tested according to the present invention. Alternatively, libraries can be constructed using standard methods. Further, more general, structurally constrained, organic diversity (e.g., nonpeptide) libraries, can also be used. By way of example, a benzodiazepine library (see e.g., Bunin et al., 1994, Proc. Natl. Acad. Sci. USA 91:4708-4712) may be used.

[2431] In another embodiment of the present invention, combinatorial chemistry can be used to identify modulators of the GPCRs of the present invention. Combinatorial chemistry is capable of creating libraries containing hundreds of thousands of compounds, many of which may be structurally similar. While high throughput screening programs are capable of screening these vast libraries for affinity for known targets, new approaches have been developed that achieve libraries of smaller dimension but which provide maximum chemical diversity. (See e.g., Matter, 1997, Journal of Medicinal Chemistry 40:1219-1229).

[2432] One method of combinatorial chemistry, affinity fingerprinting, has previously been used to test a discrete library of small molecules for binding affinities for a defined panel of proteins. The fingerprints obtained by the screen are used to predict the affinity of the individual library members for other proteins or receptors of interest (in the instant invention, the receptors of the present invention). The fingerprints are compared with fingerprints obtained from other compounds known to react with the protein of interest to predict whether the library compound might similarly react. For example, rather than testing every ligand in a large library for interaction with a complex or protein component, only those ligands having a fingerprint similar to other compounds known to have that activity could be tested. (See, e.g., Kauvar et al., 1995, Chemistry and Biology 2:107-118; Kauvar, 1995, Affinity fingerprinting, Pharmaceutical Manufacturing International. 8:25-28; and Kauvar, Toxic-Chemical Detection by Pattern Recognition in New Frontiers in Agrochemical Immunoassay, D. Kurtz. L. Stanker and J. H. Skerrett. Editors, 1995, AOAC: Washington, D.C., 305-312).

[2433] In some preferred embodiments, the candidate compound is an hydroxypyrazole derivative. In some preferred embodiments, the candidate compound is a benzotriazole carboxylic acid or ester derivative.

[2434] Candidate Compounds Identified as Modulators

[2435] Generally, the results of such screening will be compounds having unique core structures; thereafter, these compounds may be subjected to additional chemical modification around a preferred core structure(s) to further enhance the medicinal properties thereof. Such techniques are known to those in the art and will not be addressed in detail in this patent document.

[2436] H. Pharmaceutical Compositions

[2437] The invention provides methods of treatment (and prevention) by administration to an individual in need of said treatment (or prevention) a therapeutically effect amount of a modulator of the invention [also see, e.g., PCT Application Number PCT/IB02/01461 published as WO 02/066505 on 29 Aug. 2002; the disclosure of each of which is hereby incorporated by reference in its entirety]. In a preferred aspect, the modulator is substantially purified. The individual is preferably an animal including, but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, rabbits, rats, mice, etc., and is preferably a mammal, and most preferably human.

[2438] Modulators of the invention can be administered to non-human animals [see Examples, *infra*] and/or humans, alone or in pharmaceutical or physiologically acceptable compositions where they are mixed with suitable carriers or excipient(s) using techniques well known to those in the art. Suitable pharmaceutically-acceptable carriers are available to those in the art; for example, see Remington's Pharmaceutical Sciences, 16<sup>th</sup> Edition, 1980, Mack Publishing Co., (Oslo et al., eds.).

[2439] The pharmaceutical or physiologically acceptable composition is then provided at therapeutically effect dose. A therapeutically effective dose refers to that amount of a modulator sufficient to result in prevention or amelioration of symptoms or physiological status of metabolic-related disorders or disorders of lipid metabolism as determined illustratively and not by limitation by the methods described herein.

[2440] It is expressly considered that the modulators of the invention may be provided alone or in combination with other pharmaceutically or physiologically acceptable compounds. Other compounds for the treatment of disorders of the invention are currently well known in the art. One aspect of the invention encompasses the use according to embodiments disclosed herein further comprising one or more agents selected from the group consisting of  $\alpha$ -glucosidase inhibitor, aldose reductase inhibitor, biguanide, HMG-CoA reductase inhibitor, squalene synthesis inhibitor, fibrate, LDL catabolism enhancer, angiotensin converting enzyme inhibitor, insulin secretion enhancer and thiazolidinedione. In some embodiments the agent is a  $\alpha$ -glucosidase inhibitor. In some embodiments the  $\alpha$ -glucosidase inhibitor is acarbose, voglibose or miglitol. In some embodiments the  $\alpha$ -glucosidase inhibitor is voglibose. In some embodiments the agent is an aldose reductase inhibitor. In some embodiments the aldose reductase inhibitor is tolurestat; epalrestat; imirestat; zenarestat; zopolrestat; or sorbinil. In some embodiments the agent is a biguanide. In some embodiments the biguanide is phenformin, metformin or buformin. In some

embodiments the agent is a HMG-CoA reductase inhibitor. In some embodiments the HMG-CoA reductase inhibitor is rosuvastatin, pravastatin, simvastatin, lovastatin, atorvastatin, fluvastatin or cerivastatin. In some embodiments the agent is a fibrate. In some embodiments the fibrate is bezafibrate, beclobrate, binifibrate, ciprofibrate, clinofibrate, clofibrate, clofibric acid, etofibrate, fenofibrate, gemfibrozil, nicofibrate, pirifibrate, ronifibrate, simfibrate, or theofibrate. In some embodiments the agent is an angiotensin converting enzyme inhibitor. In some embodiments the angiotensin converting enzyme inhibitor is captopril, enalapril, alacepril, delapril; ramipril, lisinopril, imidapril, benazepril, ceronapril, cilazapril, enalaprilat, fosinopril, moxetopril, perindopril, quinapril, spirapril, temocapril ortrandolapril. In some embodiments the agent is an insulin secretion enhancer. In some embodiments the insulin secretion enhancer is tolbutamide; chlorpropamide; tolazamide; acetohexamide; glycopyramide; glibenclamide; gliclazide; 1-butyl-3-metanilylurea; carbutamide; glibonuride; glipizide; gliquidone; glisoxepid; glybuthiazole; glibuzole; glyhexamide; glymidine; glypinamide; phenbutamide; tolclamide, glimepiride, nateglinide, or mitiglinide. In some embodiments the agent is a thiazolidinedione. In some embodiments the thiazolidinedione is rosiglitazone or pioglitazone. In some embodiments the thiazolidinedione is rosiglitazone. In some embodiments, the agent is human adiponectin or a fragment thereof comprising the globular domain.

[2441] In some embodiments the metabolic disorder is selected from the group consisting of dyslipidemia, atherosclerosis, coronary heart disease, insulin resistance, obesity, impaired glucose tolerance, atheromatous disease, hypertension, stroke, Syndrome X, heart disease and type 2 diabetes. In some embodiments the metabolic disorder is selected from the group consisting of dyslipidemia, atherosclerosis, coronary heart disease, stroke, insulin resistance and type 2 diabetes. In some embodiments, the disorder of lipid metabolism is selected from the group consisting of elevated level of plasma triglycerides, elevated level of plasma free fatty acids, elevated level of plasma cholesterol, elevated level of LDL-cholesterol, reduced level of HDL-cholesterol, elevated total cholesterol/HDL-cholesterol ratio, and reduced level of plasm adiponectin.

[2442] Routes of Administration

[2443] Suitable routes of administration include oral, nasal, rectal, transmucosal, or intestinal administration, parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, intrapulmonary (inhaled) or intraocular injections using methods known in the art. Other particularly preferred routes of administration are aerosol and depot formulation. Sustained release formulations, particularly depot, of the invented medicaments are expressly contemplated.

[2444] Composition/Formulation

[2445] Pharmaceutical or physiologically acceptable compositions and medicaments for use in accordance with the present invention may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries. Proper formulation is dependent upon the route of administration chosen.

[2446] Certain of the medicaments described herein will include a pharmaceutically or physiologically acceptable carrier and at least one modulator of the invention. For

injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer such as a phosphate or bicarbonate buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[2447] Pharmaceutical or physiologically acceptable preparations that can be taken orally include push-fit capsules made of gelatin, as well as soft, sealed capsule made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

[2448] For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[2449] For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs for a nebulizer, with the use of a suitable gaseous propellant, e.g., carbon dioxide. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin, for use in an inhaler or insufflator, may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[2450] The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspension, solutions or emulsions in aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[2451] Pharmaceutical or physiologically acceptable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Aqueous suspension may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[2452] Alternatively, the active ingredient may be in powder or lyophilized form for constitution with a suitable vehicle, such as sterile pyrogen-free water, before use.

[2453] In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[2454] In a particular embodiment, the compounds can be delivered via a controlled release system. In one embodiment, a pump may be used (Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201-240; Buchwald et al., 1980, Surgery 88:507-516; Saudek et al., 1989, N. Engl. J. Med. 321:574-579). In another embodiment, polymeric materials can be used (Medical Applications of Controlled Release, Langer and Wise, eds., CRC Press, Boca Raton, Fla., 1974; Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball, eds., Wiley, New York, 1984; Ranger and Peppas, 1983, Macromol. Sci. Rev. Macromol. Chem. 23:61; Levy et al., 1985, Science 228:190-192; Doring et al., 1989, Ann. Neurol. 25:351-356; Howard et al., 1989, J. Neurosurg. 71:858-863). Other controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533).

[2455] Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days.

[2456] Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for modulator stabilization may be employed.

[2457] The pharmaceutical or physiologically acceptable compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

[2458] Effective Dosage

[2459] Pharmaceutical or physiologically acceptable compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve their intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the subject being treated. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[2460] For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes or encompasses a concentration point or range shown to antiproliferative in an in vitro system. [See Examples, infra, for in vitro assays and in vivo animal models.] Such information can be used to more accurately determine useful doses in humans.

[2461] A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD<sub>50</sub> (the dose lethal to 50% of the test population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the test population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD<sub>50</sub> and ED<sub>50</sub>. Compounds that exhibit high therapeutic indices are preferred.

[2462] The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub>, with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See, e.g., Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1).

[2463] Dosage amount and interval may be adjusted individually to provide plasma levels of the active compound which are sufficient to prevent or treat a disorder of the invention, depending on the particular situation. Dosages necessary to achieve these effects will depend on individual characteristics and route of administration.

[2464] Dosage intervals can also be determined using the value for the minimum effective concentration. Compounds should be administered using a regimen that maintains plasma levels above the minimum effective concentration for 10-90% of the time, preferably between 30-99%, and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

[2465] The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration, and the judgement of the prescribing physician.

[2466] A preferred dosage range for the amount of a modulator of the invention, which can be administered on a daily or regular basis to achieve desired results, including but not limited to reduction of the level of plasma triglycerides, reduction of the level of plasma free fatty acids, elevation of the level of HDL-cholesterol, reduction of the level of LDL-cholesterol, reduction of the level of plasma cholesterol, reduction of the total cholesterol/HDL-cholesterol ratio, or elevation of the level of plasma adiponectin, is 0.1-100 mg/kg body mass. Other preferred dosage range is 0.1-30 mg/kg body mass. Other preferred dosage range is 0.1-10 mg/kg body mass. Other preferred dosage range is 0.1-3.0 mg/kg body mass. Of course, these daily dosages can be delivered or administered in small amounts periodically during the course of a day. It is noted that these dosage ranges are only preferred ranges and are not meant to be limiting to the invention.

#### [2467] I. Methods of Treatment

[2468] The invention is drawn inter alia to methods of preventing or treating disorders of lipid metabolism and metabolic-related disorders comprising providing an individual in need of such treatment with a modulator of the invention. Preferably the modulator is provided to the individual in a pharmaceutical composition that is preferably taken orally. Preferably the individual is a mammal, and most preferably a human. In preferred embodiments, the disorder of lipid metabolism is selected from the group consisting of elevated level of triglycerides, elevated level of plasma free fatty acids, elevated level of plasma cholesterol, elevated level of LDL-cholesterol, reduced level of HDL-cholesterol, elevated total cholesterol/HDL-cholesterol ratio, and reduced level of plasma adiponectin. In preferred embodiments, the metabolic-related disorder is selected from the group consisting of dyslipidemia, atherosclerosis, coronary heart disease, stroke, insulin resistance, and type 2

diabetes. Other metabolic-related disorders to be treated by modulators of the invention include obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other metabolic-related disorder to be treated by modulators of the invention is hyperlipidemia. In other embodiments, the invention provides for a method of using a modulator of the invention as an inhibitor of the progression from impaired glucose tolerance to insulin resistance.

[2469] The invention also features methods of preventing or treating disorders of lipid metabolism or metabolic-related disorders comprising providing an individual in need of such treatment with a modulator identified by assays of the invention. Preferably, the modulator is provided to the individual in a pharmaceutical composition that is preferably taken orally. Preferably the individual is a mammal, and most preferably a human. In preferred embodiments, the disorder of lipid metabolism is selected from the group consisting of elevated level of triglycerides, elevated level of plasma free fatty acids, elevated level of plasma cholesterol, elevated level of LDL-cholesterol, reduced level of HDL-cholesterol, elevated total cholesterol/HDL-cholesterol ratio, and reduced level of plasma adiponectin. In preferred embodiments, the metabolic-related disorder is selected from the group consisting of dyslipidemia, atherosclerosis, coronary heart disease, stroke, insulin resistance, and type 2 diabetes. Other metabolic-related disorders to be treated by modulators of the invention include obesity, impaired glucose tolerance, atheromatous disease, hypertension, Syndrome X, and heart disease. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other metabolic-related disorder to be treated by modulators of the invention is hyperlipidemia. In other embodiments, the invention provides for a method of using a modulator of the invention as an inhibitor of the progression from impaired glucose tolerance to insulin resistance.

#### [2470] J. Other Utility

[2471] Although a preferred use of the non-endogenous versions of the GPCRs disclosed herein may be for the direct identification of candidate compounds as inverse agonists or agonists (preferably for use as pharmaceutical agents), other uses of these versions of GPCRs exist. For example, in vitro and in vivo systems incorporating GPCRs can be utilized to further elucidate and understand the roles these receptors play in the human condition, both normal and diseased, as well as understanding the role of constitutive activation as it applies to understanding the signaling cascade. In some embodiments it is preferred that the endogenous receptors be "orphan receptors", i.e., the endogenous ligand for the receptor has not been identified. In some embodiments, therefore, the modified, non-endogenous GPCRs can be used to understand the role of endogenous receptors in the human body before the endogenous ligand has been identified. Such receptors can be used to further elucidate known receptors and the pathways through which they transduce a signal. The present methods may also be useful in developing treatment regimens for diseases and disorders associated with the tissues in which the receptors are localized. Examples of such diseases and disorders and tissues in which the receptors are localized are set forth supra and infra.

[2472] Agents that modulate (i.e., increase, decrease, or block) nicotinic acid receptor functionality may be identified by contacting a candidate compound with a nicotinic acid receptor and determining the effect of the candidate compound on nicotinic acid receptor functionality. The selectivity of a compound that modulates the functionality of the nicotinic acid receptor can be evaluated by comparing its effects on the nicotinic acid receptor to its effects on other receptors. Following identification of compounds that modulate nicotinic acid receptor functionality, such candidate compounds may be further tested in other assays including, but not limited to, in vivo models, in order to confirm or quantitate their activity. Modulators of nicotinic acid receptor functionality will be therapeutically useful in treatment of diseases and physiological conditions in which normal or aberrant nicotinic acid receptor functionality is involved.

[2473] Agents that modulate (i.e., increase, decrease, or block) antilipolytic receptor functionality may be identified by contacting a candidate compound with an antilipolytic receptor and determining the effect of the candidate compound on antilipolytic receptor functionality. The selectivity of a compound that modulates the functionality of an antilipolytic receptor can be evaluated by comparing its effects on the antilipolytic receptor to its effects on other receptors. Following identification of compounds that modulate antilipolytic receptor functionality, such candidate compounds may be further tested in other assays including, but not limited to, in vivo models, in order to confirm or quantitate their activity. Modulators of antilipolytic receptor functionality will be therapeutically useful in treatment of diseases and physiological conditions in which normal or aberrant antilipolytic receptor functionality is involved.

[2474] Other uses of the disclosed receptors and methods will become apparent to those in the art based upon, inter alia, a review of this patent document.

### EXAMPLES

[2475] The following examples are presented for purposes of elucidation, and not limitation, of the present invention. While specific nucleic acid and amino acid sequences are disclosed herein, those of ordinary skill in the art are credited with the ability to make minor modifications to these sequences while achieving the same or substantially similar results reported below. The traditional approach to application or understanding of sequence cassettes from one sequence to another (e.g. from rat receptor to human receptor or from human receptor A to human receptor B) is generally predicated upon sequence alignment techniques whereby the sequences are aligned in an effort to determine areas of commonality. The mutational approach disclosed herein does not rely upon this approach but is instead based upon an algorithmic approach and a positional distance from a conserved proline residue located within the TM6 region of human GPCRs. Once this approach is secured, those in the art are credited with the ability to make minor modifications thereto to achieve substantially the same results (i.e., constitutive activation) disclosed herein. Such modified approaches are considered within the purview of this disclosure.

[2476] The following Examples are provided for illustrative purposes and not as a means of limitation. One of ordinary skill in the art would be able to design equivalent assays and methods based on the disclosure herein, all of which form part of the present invention.

[2477] Although a variety of expression vectors are available to those in the art, for purposes of utilization for both the endogenous and non-endogenous human, mouse and rat GPCRs, it is most preferred that the vector utilized be pCMV. This vector was deposited with the American Type Culture Collection (ATCC) on Oct. 13, 1998 (10801 University Blvd., Manassas, Va. 20110-2209 USA) under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. The DNA was tested by the ATCC and determined to be viable. The ATCC has assigned the following deposit number to pCMV: ATCC #203351. In some alternative embodiments as relates to said human, mouse and rat GPCRs, it is preferred that the vector utilized be an adenoviral expression vector.

[2478] Recombinant DNA techniques relating to the subject matter of the present invention and well known to those of ordinary skill in the art can be found, e.g. in Maniatis T et al., *Molecular Cloning: A Laboratory Manual* (1989) Cold Spring Harbor Laboratory; U.S. Pat. No. 6,399,373; and PCT Application Number PCT/IB02/01461 published as WO 02/066505 on 29 Aug. 2002; the disclosure of each of which is hereby incorporated by reference in its entirety.

### Example 1

[2479] A. Endogenous Human GPCRs

[2480] Identification of Human GPCRs

[2481] The disclosed endogenous human GPCRs were identified based upon a review of the GenBank™ database information. While searching the database, the following cDNA clones were identified as evidenced below (Table E).

TABLE E

| Disclosed Human Orphan GPCRs | Accession Number Identified | Complete DNA Sequence (Base Pairs) | Open Reading Frame (Base Pairs) | Nucleic Acid SEQ. ID. NO. | Amino Acid SEQ. ID. NO. |
|------------------------------|-----------------------------|------------------------------------|---------------------------------|---------------------------|-------------------------|
| hRUP8                        | AL121755                    | 147,566 bp                         | 1,152 bp                        | 1                         | 2                       |
| hRUP9                        | AC0113375                   | 143,181 bp                         | 1,260 bp                        | 3                         | 4                       |
| bRUP10                       | AC008745                    | 94,194 bp                          | 1,014 bp                        | 5                         | 6                       |
| hRUP11                       | AC013396                    | 155,086 bp                         | 1,272 bp                        | 7                         | 8                       |
| hRUP12                       | AP000808                    | 177,764 bp                         | 966 bp                          | 9                         | 10                      |
| hRUP13                       | AC011780                    | 167,819 bp                         | 1,356 bp                        | 11                        | 12                      |
| bRUP14                       | AL137118                    | 168,297 bp                         | 1,041 bp                        | 13                        | 14                      |
| hRUP15                       | AL016468                    | 138,828 bp                         | 1,527 bp                        | 15                        | 16                      |
| hRUP16                       | AL136106                    | 208,042 bp                         | 1,068 bp                        | 17                        | 18                      |
| hRUP17                       | AC023078                    | 161,735 bp                         | 969 bp                          | 19                        | 20                      |
| hRUP18                       | AC008547                    | 117,304 bp                         | 1,305 bp                        | 21                        | 22                      |
| hRUP19                       | AC026331                    | 145,183 bp                         | 1,041 bp                        | 23                        | 24                      |
| hRUP20                       | AL161458                    | 163,511 bp                         | 1,011 bp                        | 25                        | 26                      |
| hRUP21                       | AC026756                    | 156,534 bp                         | 1,014 bp                        | 27                        | 28                      |
| hRUP22                       | AC027026                    | 151,811 bp                         | 993 bp                          | 29                        | 30                      |
| hRUP23                       | AC007104                    | 200,000 bp                         | 1,092 bp                        | 31                        | 32                      |
| hRUP24                       | AL355388                    | 190,538 bp                         | 1,125 bp                        | 33                        | 34                      |
| hRUP25                       | AC026331                    | 145,183 bp                         | 1,092 bp                        | 35                        | 36                      |
| hRUP26                       | AC023040                    | 178,508 bp                         | 1,044 bp                        | 37                        | 38                      |
| hRUP27                       | AC027643                    | 158,700 bp                         | 1,020 bp                        | 39                        | 40                      |
| hRUP38                       | AC026331                    | 145,183 bp                         | 1,164 bp                        | 134                       | 135                     |

[2482] 1. Full Length Cloning

[2483] a. hRUP8 (Seq. Id. Nos. 1 & 2)

[2484] The disclosed human HRUP8 was identified based upon the use of EST database (dbEST) information. While searching the dbEST, a cDNA clone with accession number AL121755 was identified to encode a novel GPCR. The following PCR primers were used for RT-PCR with human testis Marathon-Ready cDNA (Clontech) as templates:

5'-CTTGCAGACATCACCATGGCAGCC-3' (SEQ.ID.NO.:41; sense) and  
 5'-GTGATGCTCTGAGTACTGGACTGG-3' (SEQ.ID.NO.:42; antisense).

[2485] PCR was performed using Advantage cDNA polymerase (Clontech; manufacturing instructions will be followed) in 50  $\mu$ l reaction by the following cycles: 94° C. for 30 sec; 94° C. for 10 sec; 65° C. for 20 sec, 72° C. for 1.5 min, and 72° C. for 7 min. Cycles 2 through 4 were repeated 35 times.

[2486] A 1.2 kb PCR fragment was isolated and cloned into the pCRII-TOPO vector (Invitrogen) and sequenced using the ABI Big Dye Terminator kit (P.E. Biosystem). See, SEQ.ID.NO.:1. The putative amino acid sequence for hRUP8 is set forth in SEQ.ID.NO.:2.

[2487] b. hRUP9 (Seq. Id. Nos. 3 & 4)

[2488] The disclosed human hRUP9 was identified based upon the use of GeneBank database information. While searching the database, a cDNA clone with Accession Number AC011375 was identified as a human genomic sequence from chromosome 5. The full length hRUP9 was cloned by PCR using primers:

5'-GAAGCTGTGAAGAGTGATGC-3' (SEQ.ID.NO.:43; sense),  
 5'-GTCAGCAATATTGATAAGCAGCAG-3' (SEQ.ID.NO.:44; antisense)

[2489] and human genomic DNA (Promega) as a template. Taq Plus Precision polymerase (Stratagene) was used for the amplification in a 10011 reaction with 5% DMSO by the following cycle with step 2 to step 4 repeated 35 times: 94°

[2493] and human leukocyte Marathon-Ready cDNA (Clontech) as a template. Advantage cDNA polymerase (Clontech) was used for the amplification in a 50  $\mu$ l reaction by the following cycle with step 2 to step 4 repeated 35 times: 94° C. for 30 seconds; 94° C. for 10 seconds; 62° C. for 20 seconds; 72° C. for 1.5 minutes; 72° C. for 7 minutes. A 1.0 Kb PCR fragment was isolated and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator kit (P.E. Biosystems). The nucleic acid sequence of the novel human receptor hRUP10 is set forth in SEQ.ID.NO.:5 and the putative amino acid sequence thereof is set forth in SEQ.ID.NO.:6.

[2494] d. hRUP11 (Seq. Id. Nos. 7 & 8)

[2495] The disclosed human hRUP11 was identified based upon the use of GenBank database information. While

searching the database, a cDNA clone with accession number AC013396 was identified as a human genomic sequence from chromosome 2. The full length hRUP11 was cloned by PCR using primers:

5'-CCAGGATGTTGTGTACCGTGGTGGC-3' (SEQ.ID.NO.:47; sense),  
 5'-CACAGCGCTGCAGCCCTGCAGCTGGC-3' (SEQ.ID.NO.:48; antisense)

C. for 1 minute; 94° C. for 30 seconds; 56° C. for 30 seconds; 72° C. for 2 minutes; 72° C. for 5 minutes.

[2490] A 1.3 Kb PCR fragment was isolated and cloned into the pCRII-TOPO vector (Invitrogen) from 1% agarose gel and completely sequenced using the ABI Big Dye Terminator kit (P.E. Biosystems). See, SEQ.ID.NO.:3. The putative amino acid sequence for hRUP8 is set forth in SEQ.ID.NO.:4. The sequence of hRUP9 clones isolated from human genomic DNA matched with the sequence obtained from data base.

[2491] c. hRUP10 (Seq. Id. Nos. 5 & 6)

[2492] The disclosed human hRUP10 was identified based upon the use of GenBank database information. While searching the database, a cDNA clone with accession number AC008754 was identified as a human genomic sequence from chromosome 19. The full length hRUP10 was cloned by RT-PCR using primers:

5'-CCATGGGGAACGATTCTGTCTAGCTACG-3' (SEQ.ID.NO.:45; sense) and  
 5'-GCTATGCCTGAAGCCAGTCTTGTG-3' (SEQ.ID.NO.:46; antisense)

[2496] and human genomic DNA (Clontech) as a template. TaqPlus Precision DNA polymerase (Stratagene) was used for the amplification in a 50  $\mu$ l reaction by the following cycle with step 2 to step 4 repeated 35 times: 94° C. for 3 minutes; 94° C. for 20 seconds; 67° C. for 20 seconds; 72° C. for 1.5 minutes; 72° C. for 7 minutes. A 1.3 Kb PCR fragment was isolated and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator kit (P.E. Biosystems). The nucleic acid sequence of the novel human receptor hRUP11 is set forth in SEQ.ID.NO.:7 and the putative amino acid sequence thereof is set forth in SEQ.ID.NO.:8.

[2497] e. hRUP12 (Seq. Id. Nos. 9 & 10)

[2498] The disclosed human hRUP12 was identified based upon the use of GenBank database. While searching the database, a cDNA clone with accession number

AP000808 was identified to encode a new GPCR, having significant homology with rat RTA and human mas1 oncogene GPCRs. The full length hRUP12 was cloned by PCR using primers:

5'-CTTCCTCTCGTAGGGATGAACCAGAC-3' (SEQ.ID.NO.:49; sense)

5'-CTCGCACAGGTGGGAAGCACCTGTGG-3' (SEQ.ID.NO.:50; antisense)

[2499] and human genomic DNA (Clontech) as template. TaqPlus Precision DNA polymerase (Stratagene) was used for the amplification by the following cycle with step 2 to step 4 repeated 35 times: 94° C. for 3 min; 94° C. for 20 sec; 65° C. for 20 sec; 72° C. for 2 min and 72° C. for 7 min. A 1.0 kb PCR fragment was isolated and cloned into the pCRII-TOPO vector (Invitrogen) and sequenced using the ABI Big Dye Terminator kit (P.E. Biosystems) (see, SEQ.ID.NO.:9 for nucleic acid sequence and SEQ.ID.NO.:10 for deduced amino acid sequence).

[2500] f. hRUP13 (Seq. Id. Nos. 11 & 12)

[2501] The disclosed human HRUP13 was identified based upon the use of GenBank database. While searching the database, a cDNA clone with accession number AC011780 was identified to encode a new GPCR, having significant homology with GPCR fish GPRX-ORYLA. The full length hRUP13 was cloned by PCR using primers:

5'-GCCTGTGACAGGAGGTACCCTGG-3' (SEQ.ID.NO.:51; sense)

5'-CATATCCCTCCGAGTGTCCAGCGGC-3' (SEQ.ID.NO.:52; antisense)

[2502] and human genomic DNA (Clontech) as template. TaqPlus Precision DNA polymerase (Stratagene) was used for the amplification by the following cycle with step 2 to step 4 repeated 35 times: 94° C. for 3 min; 94° C. for 20 sec; 65° C. for 20 sec; 72° C. for 2 min and 72° C. for 7 min. A 1.35 kb PCR fragment was isolated and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator kit (P.E. Biosystems) (see, SEQ.ID.NO.:11 for nucleic acid sequence and SEQ.ID.NO.:12 for deduced amino acid sequence).

[2503] g. hRUP14 (Seq. Id. Nos. 13 & 14)

[2504] The disclosed human hRUP14 was identified based upon the use of the GenBank database information. While searching the database, a cDNA clone with Accession Number AL137118 was identified as a human genomic sequence from chromosome 13. The full length hRUP14 was cloned by PCR using primers:

5'-GCATGGAGAGAAAATTTATGTCCTTGCAACC-3' (SEQ.ID.NO.:53; sense)

5'-CAAGAACAGGTCTCATCTAAGAGCTCC-3' (SEQ.ID.NO.:54; antisense)

[2505] and human genomic DNA(Promega) as a template. Taq Plus Precision polymerase (Stratagene) and 5% DMSO were used for the amplification by the following cycle with step 2 and step 3 repeated 35 times: 94° C. for 3 minute; 94° C. for 20 seconds; 58° C. for 2 minutes; 72° C. for 10 minutes.

[2506] A 1.1 Kb PCR fragment was isolated and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator kit (P.E. Biosystems) (see, SEQ.ID.NO.:13 for nucleic acid sequence and SEQ.ID.NO.:14 for deduced amino acid sequence). The sequence of hRUP14 clones isolated from human genomic DNA matched with the sequence obtained from database.

[2507] h. hRUP15 (Seq. Id. Nos. 15 & 16)

[2508] The disclosed human hRUP15 was identified based upon the use of the GenBank database information. While searching the database, a cDNA clone with Accession Number AC016468 was identified as a human genomic sequence. The full length hRUP15 was cloned by PCR using primers:

5'-GCTGTTGCCATGACGTCCACCTGCAC-3' (SEQ.ID.NO.:55; sense)  
5'-GGACAGTTCAAGTTTGCCTTAGAAC-3' (SEQ.ID.NO.:56; antisense)

[2509] and human genomic DNA(Promega) as a template. Taq Plus Precision polymerase (Stratagene) was used for the amplification by the following cycle with step 2 to 4 repeated 35 times: 94° C. for 3 minute; 94° C. for 20 seconds; 65° C. for 20 seconds; 72° C. for 2 minutes and 72° C. for 7 minutes.

[2510] A 1.5 Kb PCR fragment was isolated and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator kit (P.E. Biosystems). See, SEQ.ID.NO.:15 for nucleic acid sequence and SEQ.ID.NO.:16 for deduced amino acid sequence. The sequence of hRUP15 clones isolated from human genomic DNA matched with the sequence obtained from database.

[2511] i. hRUP16 (Seq. Id. Nos. 17 & 18)

[2512] The disclosed human hRUP16 was identified based upon the use of the GenBank database information. While searching the database, a cDNA clone with Accession Number AL136106 was identified as a human genomic sequence from chromosome 13. The full length hRUP16 was cloned by PCR using primers:

5'-CTTTCGATACTGCTCCTATGCTC-3' (SEQ.ID.NO.:57; sense, 5' of initiation codon),  
5'-GTAGTCCACTGAAAGTCCAGTGATCC-3' (SEQ.ID.NO.:58; antisense, 3' of stop codon)

[2513] and human skeletal muscle Marathon-Ready cDNA(Clontech) as template. Advantage cDNA polymerase (Clontech) was used for the amplification in a 50 ul reaction by the following cycle with step 2 to 4 repeated 35 times: 94° C. for 30 seconds; 94° C. for 5 seconds; 69° C. for 15 seconds; 72° C. for 1 minute and 72° C. for 5 minutes.

[2514] A 1.1 Kb PCR fragment was isolated and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the T7 Sequenase kit (Amersham). See, SEQ.ID.NO.:17 for nucleic acid sequence and SEQ.ID.NO.:18 for deduced amino acid sequence. The sequence of hRUP16 clones matched with four unordered segments of AL136106, indicating that the hRUP16 cDNA is composed of 4 exons.

[2515] j. hRUP17 (Seq. Id. Nos. 19 & 20)

[2516] The disclosed human hRUP17 was identified based upon the use of the GenBank database information. While searching the database, a cDNA clone with Accession Number AC023078 was identified as a human genomic sequence from chromosome 11. The full length hRUP17 was cloned by PCR using primers:

5'-TTTCTGAGCATGGATCCAACCATCTC-3' (SEQ.ID.NO.:59; sense, containing initiation codon)

5'-CTGTCTGACAGGGCAGAGGCTCTTC-3' (SEQ.ID.NO.:60; antisense, 3' of stop codon)

[2517] and human genomic DNA (Promega) as template. Advantage cDNA polymerase mix (Clontech) was used for the amplification in a 100  $\mu$ l reaction with 5% DMSO by the following cycle with step 2 to 4 repeated 30 times: 94° C. for 1 min; 94° C. for 15 sec; 67° C. for 20 sec; 72° C. for 1 min and 30 sec; and 72° C. for 5 min.

[2518] A 970 bp PCR fragment was isolated from a 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:19 for nucleic acid sequence and SEQ.ID.NO.:20 for deduced amino acid sequence.

[2519] k. hRUP18 (Seq. Id. Nos. 21 & 22)

[2520] The disclosed human hRUP18 was identified based upon the use of the GenBank database information. While searching the database, a cDNA clone with Accession Number AC008547 was identified as a human genomic sequence from chromosome 5. The full length hRUP18 was cloned by PCR using primers:

5'-GGAAGCTCGTATAGACCCAGCGTCGCTCC-3' (SEQ.ID.NO.:61; sense, 5' of the initiation codon),

5'-GGAGGTTGCGCCTTAGCGACAGATGACC-3' (SEQ.ID.NO.:62; antisense, 3' of stop codon)

[2521] and human genomic DNA (Promega) as template. TaqPlus precision DNA polymerase (Stratagene) was used for the amplification in a 100  $\mu$ l reaction with 5% DMSO by the following cycle with step 2 to 4 repeated 35 times: 95° C. for 5 min; 95° C. for 30 sec; 65° C. for 30 sec; 72° C. for 2 min; and 72° C. for 5 min.

[2522] A 1.3 kb PCR fragment was isolated from a 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:21 for nucleic acid sequence and SEQ.ID.NO.:22 for deduced amino acid sequence.

[2523] l. hRUP19 (Seq. Id. Nos. 23 & 24)

[2524] The disclosed human hRUP19 was identified based upon the use of the GenBank database information. While searching the database, a cDNA clone with Accession Number AC026331 was identified as a human genomic sequence from chromosome 12. The full length hRUP19 was cloned by PCR using primers: 5'-CTGCACCCGGACACT-

TGCTCTG-3' (SEQ.ID.NO.:63; sense, 5' of initiation codon), 5'-GTCTGCTTGTTCAGTGCCACTCAAC-3' (SEQ.ID.NO.:64; antisense, containing the stop codon) and human genomic DNA (Promega) as template. TaqPlus Precision DNA polymerase (Stratagene) was used for the amplification with 5% DMSO by the following cycle with step 2 to 4 repeated 35 times: 94° C. for 1 min; 94° C. for 15 sec; 70° C. for 20 sec; 72° C. for 1 min and 30 sec; and 72° C. for 5 min.

[2525] A 1.1 kp PCR fragment was isolated from a 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:23 for nucleic acid sequence and SEQ.ID.NO.:24 for deduced amino acid sequence.

[2526] m. hRUP20 (Seq. Id. Nos. 25 & 26)

[2527] The disclosed human hRUP20 was identified based upon the use of the GenBank database information. While searching the database, a cDNA clone with Accession Num-

ber AL161458 was identified as a human genomic sequence from chromosome 1. The full length hRUP20 was cloned by PCR using primers:

5'-TATCTGCAATTCTATTCTAGCTCCTG-3' (SEQ.ID.NO.:65; sense, 5' of initiation codon),

5'-TGTCCTTAATAAAGTCACATGAATGC-3' (SEQ.ID.NO.:66; antisense, 3' of stop codon)

[2528] and human genomic DNA (Promega) as template. Advantage cDNA polymerase mix (Clontech) was used for the amplification with 5% DMSO by the following cycle with step 2 to 4 repeated 35 times: 94° C. for 1 min; 94° C. for 15 sec; 60° C. for 20 sec; 72° C. for 1 min and 30 sec; and 72° C. for 5 min.

[2529] A 1.0 kp PCR fragment was isolated from a 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:25 for nucleic acid sequence and SEQ.ID.NO.:26 for deduced amino acid sequence.

[2530] n. hRUP21 (Seq. Id. Nos. 27 & 28)

[2531] The disclosed human hRUP21 was identified based upon the use of the GenBank database information. While searching the database, a cDNA clone with Accession Number AC026756 was identified as a human genomic sequence from chromosome 13. The full length hRUP21 was cloned by PCR using primers:

5'-GGAGACAACCATGAATGAGCCAC-3' (SEQ.ID.NO.:67; sense)

5'-TATTTCAAGGGTTGTTTGAGTAAC-3' (SEQ.ID.NO.:68; antisense)

[2532] and human genomic DNA (Promega) as template. Taq Plus Precision polymerase (Stratagene) was used for the amplification in a 100%1 reaction with 5% DMSO by the following cycle with step 2 to 4 repeated 30 times: 94° C. for 1 min; 94° C. for 15 sec; 55° C. for 20 sec; 72° C. for 1 min and 30 sec; and 72° C. for 5 min.

[2533] A 1,014 bp PCR fragment was isolated from a 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:27 for nucleic acid sequence and SEQ.ID.NO.:28 for deduced amino acid sequence.

[2534] o. hRUP22 (Seq. Id. Nos. 29 & 30)

[2535] The disclosed human hRUP22 was identified based upon the use of the GenBank database information. While searching the database, a cDNA clone with Accession Number AC027026 was identified as a human genomic sequence from chromosome 11. The full length hRUP22 was cloned by PCR using primers:

5'-GGCACCAGTGGAGGTTTCTGAGCATG-3' (SEQ.ID.NO.:69; sense, containing initiation codon)

5'-CTGATGGAAGTAGAGGCTGTCCATCTC-3' (SEQ.ID.NO.:70; antisense, 3' of stop codon)

[2536] and human genomic DNA (Promega) as template. TaqPlus Precision DNA polymerase (Stratagene) was used for the amplification in a 100 µl reaction with 5% DMSO by the following cycle with step 2 to 4 repeated 30 times: 94° C., 1 minutes 94° C., 15 seconds 55° C., 20 seconds 72° C., 1.5 minute 72° C., 5 minutes.

[2537] A 970 bp PCR fragment was isolated from a 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye

Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:29 for nucleic acid sequence and SEQ.ID.NO.:30 for deduced amino acid sequence.

[2538] p. hRUP23 (Seq. Id. Nos. 31 & 32)

[2539] The disclosed human hRUP23 was identified based upon the use of the GenBank database information. While searching the database, a cDNA clone with Accession Number AC007104 was identified as a human genomic sequence from chromosome 4. The full length hRUP23 was cloned by PCR using primers:

5'-CCTGGCGAGCCGCTAGCGCCATG-3' (SEQ.ID.NO.:71; sense, ATG as the initiation codon),

5'-ATGAGCCCTGCCAGGCCCTCAGT-3' (SEQ.ID.NO.:72; antisense, TCA as the stop codon)

[2540] and human placenta Marathon-Ready cDNA (Clontech) as template. Advantage cDNA polymerase (Clontech) was used for the amplification in a 50  $\mu$ l reaction by the following cycle with step 2 to 4 repeated 35 times: 95° C. for 30 sec; 95° C. for 15 sec; 66° C. for 20 sec; 72° C. for 1 min and 20 sec; and 72° C. for 5 min.

[2541] A 1.0 kb PCR fragment was isolated and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:31 for nucleic acid sequence and SEQ.ID.NO.:32 for deduced amino acid sequence.

[2542] q. hRUP24 (Seq. Id. Nos. 33 & 34)

[2543] The disclosed human hRUP24 was identified based upon the use of the GenBank database information. While searching the database, a cDNA clone with Accession Number AL355388 was identified as a human genomic sequence from chromosome 1. The full length hRUP24 was cloned by PCR using primers:

5'-CTGCGATGCCACACTCAATACTTCTG-3' (SEQ.ID.NO.:73; sense, 5' of initiation codon),

5'-AAGGATCCTACACTTGGTGGATCTCAG-3' (SEQ.ID.NO.:74; antisense, 3' of stop codon)

5'-AGCCATCCCTGCCAGGAAGCATGG-3' (SEQ.ID.NO.:77; sense, containing initiation codon)

5'-CCAGACTGTGGACTCAAGAACTCTAGG-3' (SEQ.ID.NO.:78; antisense, containing stop codon)

[2544] and human genomic DNA (Promega) as template. Advantage cDNA polymerase mix (Clontech) was used for the amplification with 5% DMSO by the following cycle with step 2 to 4 repeated 35 times: 94° C. for 1 minute; 94° C. for 15 seconds; 56° C. for 20 seconds 72° C. for 1 minute 30 seconds and 72° C. for 5 minutes.

[2545] A 1.2 kb PCR fragment was isolated from a 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:33 for nucleic acid sequence and SEQ.ID.NO.:34 for deduced amino acid sequence.

[2546] r. hRUP25 (Seq. Id. Nos. 35 & 36)

[2547] The disclosed human hRUP25 was identified based upon the use of the GenBank database information. While searching the database, a cDNA clone with Accession Number AC026331 was identified as a human genomic sequence from chromosome 12. The full length hRUP25 was cloned by PCR using primers:

5'-GCTGGAGCATTCACTAGGCGAG-3' (SEQ.ID.NO.:75; sense, 5' of initiation codon),

5'-AGATCCTGGTTCTTGGTGACAATG-3' (SEQ.ID.NO.:76; antisense, 3' of stop codon)

[2548] and human genomic DNA (Promega) as template. Advantage cDNA polymerase mix (Clontech) was used for the amplification with 5% DMSO by the following cycle with step 2 to 4 repeated 35 times: 94° C. for 1 minute; 94° C. for 15 seconds; 56° C. for 20 seconds 72° C. for 1 minute 30 seconds and 72° C. for 5 minutes.

[2549] A 1.2 kb PCR fragment was isolated from a 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:35 for nucleic acid sequence and SEQ.ID.NO.:36 for deduced amino acid sequence.

[2550] s. hRUP26 (Seq. Id. Nos. 37 & 38)

[2551] The disclosed human hRUP26 was identified based upon the use of GenBank database information. While searching the database, a cDNA clone with Accession Number AC023040 was identified as a human genomic sequence from chromosome 2. The full length hRUP26 was cloned by RT-PCR using hRUP26 specific primers:

[2552] and human pancreas Marathon-Ready cDNA (Clontech) as template. Advantage cDNA polymerase mix (Clontech) was used for the amplification in a 100  $\mu$ l reaction with 5% DMSO by the following cycle with step 2 to 4 repeated 35 times: 94° C. for 5 minute; 95° C. for 30 seconds; 65° C. for 30 seconds 72° C. for 2 minute and 72° C. for 5 minutes.

[2553] A 1.1 kb PCR fragment was isolated from 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:37 for nucleic acid sequence and SEQ.ID.NO.:38 for deduced amino acid sequence.

[2554] t. hRUP27 (Seq. Id. Nos. 39 & 40)

[2555] The disclosed human hRUP27 was identified based upon the use of GenBank database information. While searching the database, a cDNA clone with Accession Number AC027643 was identified as a human genomic sequence from chromosome 12. The full length hRUP27 was cloned by PCR using hRUP27 specific primers:

5'-AGTCCACGAACAATGAATCCATTTCATG-3' (SEQ.ID.NO.:79; sense, containing initiation codon),

5'-ATCATGTCTAGACTCATGGTGATCC-3' (SEQ.ID.NO.:80; antisense, 3' of stop codon)

[2556] and the human adult brain Marathon-Ready cDNA (Clontech) as template. Advantage cDNA polymerase mix (Clontech) was used for the amplification in a 50  $\mu$ l reaction with 5% DMSO by the following cycle with step 2 to 4 repeated 35 times: 94° C. for 1 minute; 94° C. for 10 seconds; 58° C. for 20 seconds 72° C. for 1 minute 30 seconds and 72° C. for 5 minutes.

[2557] A 1.1 kb PCR fragment was isolated from 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:35 for nucleic acid sequence and SEQ.ID.NO.:36 for deduced amino acid sequence. The sequence of hRUP27 cDNA clone isolated from human brain was determined to match with five unordered segments of AC027643, indicating that the hRUP27 cDNA is composed of 5 exons.

[2558] a. hRUP38 (Seq. Id. Nos. 134 & 135)

[2559] The disclosed human hRUP38 was identified based upon the use of GenBank database information. While searching the database, a cDNA clone was identified as a human genomic sequence from chromosome 12. The full length hRUP38 was cloned by PCR using hRUP38 specific primers:

5'-GCACTCATGAATCGGCACCA-3' (SEQ.ID.NO.:148; sense, containing initiation codon),

5'-CAGTGACATTACTCGATGCA-3' (SEQ.ID.NO.:149; antisense, 3' of stop codon)

[2560] and human genomic DNA (Promega) as template. Advantage cDNA polymerase mix (Clontech) was used for the amplification in a 50  $\mu$ l reaction with 5% DMSO by the following cycle with step 2 to 4 repeated 35 times: 94° C. for 1 minute; 94° C. for 10 seconds; 60° C. for 20 seconds 72° C. for 1 minute 30 seconds and 72° C. for 5 minutes.

[2561] A 1.2 kb PCR fragment was isolated from 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:134 for nucleic acid sequence and SEQ.ID.NO.:135 for deduced amino acid sequence. The sequence of hRUP38 DNA clone isolated from human genomic DNA was determined to match with one genomic sequence on chromosome 12 and is without introns.

[2562] B. ENDOGENOUS MOUSE AND RAT GPCRS

[2563] 1. Identification of Mouse and Rat GPCRS

[2564] The mouse and rat orthologs of hRUP25 and the mouse ortholog of hRUP19 have been identified and are disclosed below as determined from genomic sequence. The rat ortholog of hRUP19 has also been identified (PCT Application Number PCT/US02/04397, published as WO 02/83736 on Oct. 24, 2002; said disclosure is hereby incorporated by reference in its entirety) and is provided below.

[2565] Evidence to date suggests that there is no mouse or rat ortholog of hRUP38. As the hRUP25 polynucleotide sequence is about 95% identical to hRUP38 polynucleotide sequence, as hRUP25 and hRUP38 are found on the same arm of chromosome 12, and as an hRUP38 ortholog is absent from rodents, one may hypothesize without wishing to be bound by theory that hRUP38 was the product of gene duplication. This event must have happened subsequent to the divergence of human from rodents. Possibly hRUP38 represents a novel antilipolytic regulatory pathway for which there is no counterpart in rodent.

[2566] Evidence to date suggests that there may also be no mouse or rat ortholog of hRUP11.

TABLE F

| Disclosed<br>Mouse (m)<br>and Rat (r)<br>GPCRs | Accession<br>Number<br>Identified | Complete<br>DNA<br>Sequence<br>(Base<br>Pairs) | Open<br>Reading<br>Frame<br>(Base Pairs) | Nucleic<br>Acid<br>SEQ.<br>ID. NO. | Amino<br>Acid<br>SEQ.<br>ID. NO. |
|------------------------------------------------|-----------------------------------|------------------------------------------------|------------------------------------------|------------------------------------|----------------------------------|
| mRUP25                                         | AJ300199                          | —                                              | 1,083 bp                                 | 136                                | 137                              |
| rRUP25                                         | None                              | —                                              | 1,086 bp                                 | 138                                | 139                              |

TABLE F-continued

| Disclosed<br>Mouse (m)<br>and Rat (r)<br>GPCRs | Accession<br>Number<br>Identified | Complete<br>DNA<br>Sequence<br>(Base<br>Pairs) | Open<br>Reading<br>Frame<br>(Base Pairs) | Nucleic<br>Acid<br>SEQ.<br>ID. NO. | Amino<br>Acid<br>SEQ.<br>ID. NO. |
|------------------------------------------------|-----------------------------------|------------------------------------------------|------------------------------------------|------------------------------------|----------------------------------|
| mRUP19                                         | XM_144529                         | —                                              | 1,032 bp                                 | 150                                | 151                              |
| rRUP19                                         | None                              | —                                              | 1,056 bp                                 | 156                                | 157                              |

[2567] 2. Full Length Cloning

[2568] a. mRUP25 (Seq. Id. Nos. 136 & 137)

[2569] In order to clone the open reading frame encoding the mouse RUP25 receptor we applied a PCR based cloning strategy. Primers were designed and synthesized based on the start and stop codon sequence of the mouse PUMA-g sequence, published on Genbank, and used on mouse genomic DNA (Promega). The PCR primers were as follows:

5'-ATGAGCAAGTCAGACCATTTTCTAGTGATA-3' (SEQ. ID. NO.:140; sense)

5'-TTATCTGGCTTCCACATCTCGTTAA-3' (SEQ. ID. NO.:141; antisense)

[2570] Advantage cDNA polymerase mix (Clontech) was used for the amplification with 5% DMSO by the following cycle with step 2 to 4 repeated 35 times: 94° C. for 1 minute; 94° C. for 15 seconds; 56° C. for 20 seconds 72° C. for 1 minute 30 seconds and 72° C. for 5 minutes.

[2571] A 1.2 kb PCR fragment was isolated from a 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems). See, SEQ.ID.NO.:35 for nucleic acid sequence and SEQ.ID.NO.:36 for deduced amino acid sequence.

[2572] b. rRUP25 (Seq. Id. Nos. 138 & 139)

[2573] The rat RUP25 receptor was cloned in an analogous fashion, however this was done assuming the sequence would be similar to the mouse sequence because there is no previously published rat sequence. Again, we applied a PCR based cloning strategy. Primers were designed and synthesized based on the start and stop codon sequence of the mouse PUMA-g sequence, published on Genbank, and used on rat genomic DNA (Promega). The PCR primers were as follows:

5'-ATGAGCAAGTCAGACCATTTTCTAGTGATA-3' (SEQ. ID. NO.:142; sense)

5'-TTATCTGGCTTCCACATCTCGTTAA-3' (SEQ. ID. NO.:143; antisense)

[2574] Cloned Pfu polymerase was used for the amplification by the following cycle with step 2 to 4 repeated 35 times: 94° C. for 1 minute; 94° C. for 30 sec; 55° C. for 1 min; 72° C. for 2 min; and a final extension at 72° C. for 10 minutes.

[2575] A 1.2 kb PCR fragment was isolated from a 1% agarose gel and cloned into the pCRII-TOPO vector (Invitrogen) and 12 clones were completely sequenced using the ABI Big Dye Terminator Kit (P.E. Biosystems).

#### Example 2

#### [2576] PREPARATION OF NON-ENDOGENOUS, CONSTITUTIVELY ACTIVATED GPCRS

[2577] Those skilled in the art are credited with the ability to select techniques for mutation of a nucleic acid sequence. Presented below are approaches utilized to create non-endogenous versions of several of the human GPCRs disclosed above. The mutations disclosed below are based upon an algorithmic approach whereby the 16<sup>th</sup> amino acid (located in the IC3 region of the GPCR) from a conserved proline (or an endogenous, conservative substitution therefor) residue (located in the TM6 region of the GPCR, near the TM6/IC3 interface) is mutated, preferably to an alanine, histimine, arginine or lysine amino acid residue, most preferably to a lysine amino acid residue.

#### [2578] 1. Transformer Site-Directed™ Mutagenesis

[2579] Preparation of non-endogenous human GPCRs may be accomplished on human GPCRs using, inter alia,

Transformer Site-Directed™ Mutagenesis Kit (Clontech) according to the manufacturer instructions. Two mutagenesis primers are utilized, most preferably a lysine mutagenesis oligonucleotide that creates the lysine mutation, and a selection marker oligonucleotide. For convenience, the codon mutation to be incorporated into the human GPCR is also noted, in standard form (Table G):

TABLE G

| Receptor Identifier | Codon Mutation          |
|---------------------|-------------------------|
| hRUP8               | V274K                   |
| hRUP9               | T249K                   |
| hRUP10              | R232K                   |
| hRUP11              | M294K                   |
| hRUP12              | F220K                   |
| hRUP16              | A238K                   |
| hRUP17              | Y215K                   |
| hRUP18              | L294K                   |
| hRUP19              | T219K                   |
| hRUP20              | K248A<br>K248H<br>K248R |

TABLE G-continued

| Receptor Identifier | Codon Mutation |
|---------------------|----------------|
| hRUP21              | R240K          |
| hRUP22              | Y222K          |
| hRUP24              | A245K          |
| hRUP25              | I230K          |
| hRUP26              | V285K          |
| hRUP27              | T248K          |

#### [2580] 2. QuikChange™ Site-Directed™ Mutagenesis

[2581] Preparation of non-endogenous human GPCRs can also be accomplished by using QuikChange™ Site-Directed™ Mutagenesis Kit (Stratagene, according to manufacturer's instructions). Endogenous GPCR is preferably used as a template and two mutagenesis primers utilized, as well as, most preferably, a lysine mutagenesis oligonucleotide and a selection marker oligonucleotide (included in kit). For convenience, the codon mutation incorporated into the novel human GPCR and the respective oligonucleotides are noted, in standard form (Table H):

TABLE H

| Receptor Identifier | Codon Mutation | 5'-3' orientation (sense), (SEQ.ID.NO.)<br>mutation underlined | 5'-3' orientation (antisense)<br>(SEQ.ID.NO.)      | Cycle Conditions<br>Min ('), Sec (")<br>Cycles 2-4 repeated 16 times         |
|---------------------|----------------|----------------------------------------------------------------|----------------------------------------------------|------------------------------------------------------------------------------|
| hRUP13              | A268K          | GGGGAGGGAAGCA<br><u>AAGTGGTCTCCTG</u><br>G (81)                | CCAGGAGAACCACC<br><u>TTTGCTTTCCCTCCC</u><br>C (82) | 98° for 2'<br>98° for 30"<br>56° C. for 30"<br>72° for 11' 40"<br>72° for 5' |
| hRUP14              | L246K          | CAGGAAGGCA <u>AAGA</u><br>CCACCATCATCATC<br>(85)               | GATGATGATGGTGG<br><u>TCTTTGCCTTCCTG</u><br>(86)    | 98° for 2'<br>98° for 30"<br>55° C. for 30"<br>72° for 11' 40"<br>72° for 5' |
| hRUP15              | A398K          | CCAGTGCAAAGCT <u>A</u><br><u>AGAAAGTGATCTTC</u><br>(89)        | GAAGATCACTTTCT<br><u>TAGCTTTGCACTGG</u><br>(90)    | 98° for 2'<br>98° for 30"<br>55° C. for 30"<br>72° for 11' 40"<br>72° for 5' |
| hRUP23              | W275K          | GCCGCCACCGCGCC<br><u>AAGAGGAAGATTGG</u><br>C (93)              | GCCAATCTTCCT <u>CTT</u><br>GGCGCGGTGGCGGC<br>(94)  | 98° for 2'<br>98° for 30"<br>56° C. for 30"<br>72° for 11' 40"<br>72° for 5' |

[2582] The non-endogenous human GPCRs were then sequenced and the derived and verified nucleic acid and amino acid sequences are listed in the accompanying "Sequence Listing" appendix to this patent document, as summarized in Table I below:

TABLE I

| Non Endogenous Human GPCR | Nucleic Acid Sequence Listing | Amino Acid Sequence Listing |
|---------------------------|-------------------------------|-----------------------------|
| hRUP13                    | SEQ. ID. NO.: 83              | SEQ. ID. NO.: 84            |
| hRUP14                    | SEQ. ID. NO.: 87              | SEQ. ID. NO.: 88            |
| hRUP15                    | SEQ. ID. NO.: 91              | SEQ. ID. NO.: 92            |
| hRUP23                    | SEQ. ID. NO.: 95              | SEQ. ID. NO.: 96            |

## Example 3

## [2583] RECEPTOR EXPRESSION

[2584] Although a variety of cells are available to the art for the expression of proteins, it is most preferred that mammalian cells be utilized. The primary reason for this is predicated upon practicalities, i.e., utilization of, e.g., yeast cells for the expression of a GPCR, while possible, introduces into the protocol a non-mammalian cell which may not (indeed, in the case of yeast, does not) include the receptor-coupling, genetic-mechanism and secretory pathways that have evolved for mammalian systems—thus, results obtained in non-mammalian cells, while of potential use, are not as preferred as that obtained from mammalian cells. Of the mammalian cells, COS-7, 293 and 293T cells are particularly preferred, although the specific mammalian cell utilized can be predicated upon the particular needs of the artisan.

## [2585] a. Transient Transfection

[2586] On day one,  $6 \times 10^6$ /10 cm dish of 293 cells well were plated out. On day two, two reaction tubes were prepared (the proportions to follow for each tube are per plate): tube A was prepared by mixing 4  $\mu$ g DNA (e.g., pCMV vector; pCMV vector with receptor cDNA, etc.) in 0.5 ml serum free DMEM (Gibco BRL); tube B was prepared by mixing 24  $\mu$ l lipofectamine (Gibco BRL) in 0.5 ml serum free DMEM. Tubes A and B were admixed by inversions (several times), followed by incubation at room temperature for 30-45 min. The admixture is referred to as the "transfection mixture". Plated 293 cells were washed with 1xPBS, followed by addition of 5 ml serum free DMEM. 1 ml of the transfection mixture was added to the cells, followed by incubation for 4 hrs at 37° C./5% CO<sub>2</sub>. The transfection mixture was removed by aspiration, followed by the addition of 10 ml of DMEM/10% Fetal Bovine Serum. Cells were incubated at 37° C./5% CO<sub>2</sub>. After 48 hr incubation, cells were harvested and utilized for analysis.

## [2587] b. Stable Cell Lines: Gs Fusion Protein

[2588] Approximately  $12 \times 10^6$  293 cells are plated on a 15 cm tissue culture plate. Grown in DME High Glucose Medium containing ten percent fetal bovine serum and one percent sodium pyruvate, L-glutamine, and antibiotics. Twenty-four hours following plating of 293 cells (or to 80% confluency), the cells are transfected using 12  $\mu$ g of DNA. The 12  $\mu$ g of DNA is combined with 60  $\mu$ l of lipofectamine and 2 mL of DME High Glucose Medium without serum. The medium is aspirated from the plates and the cells are washed once with medium without serum. The DNA, lipofectamine, and medium mixture are added to the plate along with 10 mL of medium without serum. Following incubation at 37 degrees Celsius for four to five hours, the medium is aspirated and 25 ml of medium containing serum is added. Twenty-four hours following transfection, the medium is aspirated again, and fresh medium with serum is added.

Forty-eight hours following transfection, the medium is aspirated and medium with serum is added containing geneticin (G418 drug) at a final concentration of 500  $\mu\text{g}/\text{mL}$ . The transfected cells now undergo selection for positively transfected cells containing the G418 resistant gene. The medium is replaced every four to five days as selection occurs. During selection, cells are grown to create stable pools, or split for stable clonal selection.

#### Example 4

##### [2589] ASSAYS FOR DETERMINATION OF CONSTITUTIVE ACTIVITY OF NON-ENDOGENOUS GPCRS

[2590] A variety of approaches are available for assessment of constitutive activity of the non-endogenous human GPCRs. The following are illustrative; those of ordinary skill in the art are credited with the ability to determine those techniques that are preferentially beneficial for the needs of the artisan.

##### [2591] 1. Membrane Binding Assays: [ $^{35}\text{S}$ ]GTP $\gamma$ S Assay

[2592] When a G protein-coupled receptor is in its active state, either as a result of ligand binding or constitutive activation, the receptor couples to a G protein and stimulates the release of GDP and subsequent binding of GTP to the G protein. The alpha subunit of the G protein-receptor complex acts as a GTPase and slowly hydrolyzes the GTP to GDP, at which point the receptor normally is deactivated. Constitutively activated receptors continue to exchange GDP for GTP. The non-hydrolyzable GTP analog, [ $^{35}\text{S}$ ]GTP $\gamma$ S, can be utilized to demonstrate enhanced binding of [ $^{35}\text{S}$ ]GTP $\gamma$ S to membranes expressing constitutively activated receptors. The advantage of using [ $^{35}\text{S}$ ]GTP $\gamma$ S binding to measure constitutive activation is that: (a) it is generically applicable to all G protein-coupled receptors; (b) it is proximal at the membrane surface making it less likely to pick-up molecules which affect the intracellular cascade.

[2593] The assay utilizes the ability of G protein coupled receptors to stimulate [ $^{35}\text{S}$ ]GTP $\gamma$ S binding to membranes expressing the relevant receptors. The assay can, therefore, be used in the direct identification method to screen candidate compounds to known, orphan and constitutively activated G protein-coupled receptors. The assay is generic and has application to drug discovery at all G protein-coupled receptors.

[2594] The [ $^{35}\text{S}$ ]GTP $\gamma$ S assay was incubated in 20 mM HEPES and between 1 and about 20 mM  $\text{MgCl}_2$  (this amount can be adjusted for optimization of results, although 20 mM is preferred) pH 7.4, binding buffer with between about 0.3 and about 1.2 nM [ $^{35}\text{S}$ ]GTP $\gamma$ S (this amount can be adjusted for optimization of results, although 1.2 is preferred) and 12.5 to 75  $\mu\text{g}$  membrane protein (e.g., 293 cells expressing the Gs Fusion Protein; this amount can be adjusted for optimization) and 10  $\mu\text{M}$  GDP (this amount can be changed for optimization) for 1 hour. Wheatgerm agglutinin beads (25  $\mu\text{l}$ ; Amersham) were then added and the mixture incubated for another 30 minutes at room temperature. The tubes were then centrifuged at 1500 $\times$ g for 5 minutes at room temperature and then counted in a scintillation counter.

##### [2595] 2. Adenylyl Cyclase

[2596] A Flash Plate<sup>TM</sup> Adenylyl Cyclase kit (New England Nuclear; Cat. No. SMP004A) designed for cell-

based assays can be modified for use with crude plasma membranes. The Flash Plate wells can contain a scintillant coating which also contains a specific antibody recognizing cAMP. The cAMP generated in the wells can be quantitated by a direct competition for binding of radioactive cAMP tracer to the cAMP antibody. The following serves as a brief protocol for the measurement of changes in cAMP levels in whole cells that express the receptors.

[2597] Transfected cells were harvested approximately twenty four hours after transient transfection. Media is carefully aspirated off and discarded. 10 ml of PBS is gently added to each dish of cells followed by careful aspiration. 1 ml of Sigma cell dissociation buffer and 3 ml of PBS are added to each plate. Cells were pipetted off the plate and the cell suspension was collected into a 50 ml conical centrifuge tube. Cells were then centrifuged at room temperature at 1,100 rpm for 5 min. The cell pellet was carefully resuspended into an appropriate volume of PBS (about 3 ml/plate). The cells were then counted using a hemocytometer and additional PBS was added to give the appropriate number of cells (with a final volume of about 50  $\mu\text{l}$ /well).

[2598] cAMP standards and Detection Buffer (comprising 1  $\mu\text{Ci}$  of tracer [ $^{125}\text{I}$ ] cAMP (50  $\mu\text{l}$ ) to 11 ml Detection Buffer) was prepared and maintained in accordance with the manufacturer's instructions. Assay Buffer was prepared fresh for screening and contained 50  $\mu\text{l}$  of Stimulation Buffer, 3  $\mu\text{l}$  of test compound (12  $\mu\text{M}$  final assay concentration) and 50  $\mu\text{l}$  cells. Assay Buffer was stored on ice until utilized. The assay was initiated by addition of 50  $\mu\text{l}$  of cAMP standards to appropriate wells followed by addition of 50  $\mu\text{l}$  of PBSA to wells H-11 and H12. 50  $\mu\text{l}$  of Stimulation Buffer was added to all wells. DMSO (or selected candidate compounds) was added to appropriate wells using a pin tool capable of dispensing 3  $\mu\text{l}$  of compound solution, with a final assay concentration of 12  $\mu\text{M}$  test compound and 100  $\mu\text{l}$  total assay volume. The cells were then added to the wells and incubated for 60 min at room temperature. 100  $\mu\text{l}$  of Detection Mix containing tracer cAMP was then added to the wells. Plates were then incubated additional 2 hours followed by counting in a Wallac MicroBeta scintillation counter. Values of cAMP/well were then extrapolated from a standard cAMP curve which was contained within each assay plate.

##### [2599] 3. Cell-Based cAMP for Gi Coupled Target GPCRS

[2600] TSHR is a Gs coupled GPCR that causes the accumulation of cAMP upon activation. TSHR will be constitutively activated by mutating amino acid residue 623 (i.e., changing an alanine residue to an isoleucine residue). A Gi coupled receptor is expected to inhibit adenylyl cyclase, and, therefore, decrease the level of cAMP production, which can make assessment of cAMP levels challenging. An effective technique for measuring the decrease in production of cAMP as an indication of constitutive activation of a Gi coupled receptor can be accomplished by co-transfecting, most preferably, non-endogenous, constitutively activated TSHR (TSHR-A623I) (or an endogenous, constitutively active Gs coupled receptor) as a "signal enhancer" with a Gi linked target GPCR to establish a baseline level of cAMP. Upon creating a non-endogenous version of the Gi coupled receptor, this non-endogenous version of the target GPCR is then co-transfected with the signal enhancer, and it is this material that can be used for screening. We will utilize such approach to effectively

generate a signal when a cAMP assay is used; this approach is preferably used in the direct identification of candidate compounds against Gi coupled receptors. It is noted that for a Gi coupled GPCR, when this approach is used, an inverse agonist of the target GPCR will increase the cAMP signal and an agonist will decrease the cAMP signal.

**[2601]** On day one,  $2 \times 10^4$  293 cells/well will be plated out. On day two, two reaction tubes will be prepared (the proportions to follow for each tube are per plate): tube A will be prepared by mixing 2  $\mu$ g DNA of each receptor transfected into the mammalian cells, for a total of 4  $\mu$ g DNA (e.g., pCMV vector; pCMV vector with mutated THSR (TSHR-A623I); TSHR-A623I and GPCR, etc.) in 1.2 ml serum free DMEM (Irvine Scientific, Irvine, Calif.); tube B will be prepared by mixing 120  $\mu$ l lipofectamine (Gibco BRL) in 1.2 ml serum free DMEM. Tubes A and B will then be admixed by inversions (several times), followed by incubation at room temperature for 30-45 min. The admixture is referred to as the "transfection mixture". Plated 293 cells will be washed with 1xPBS, followed by addition of 10 ml serum free DMEM. 2.4 ml of the transfection mixture will then be added to the cells, followed by incubation for 4 hrs at 37° C./5% CO<sub>2</sub>. The transfection mixture will then be removed by aspiration, followed by the addition of 25 ml of DMEM/10% Fetal Bovine Serum. Cells will then be incubated at 37° C./5% CO<sub>2</sub>. After 24 hr incubation, cells will then be harvested and utilized for analysis.

**[2602]** A Flash Plate™ Adenylyl Cyclase kit (New England Nuclear; Cat. No. SMP004A) is designed for cell-based assays, however, can be modified for use with crude plasma membranes depending on the need of the skilled artisan. The Flash Plate wells will contain a scintillant coating which also contains a specific antibody recognizing cAMP. The cAMP generated in the wells can be quantitated by a direct competition for binding of radioactive cAMP tracer to the cAMP antibody. The following serves as a brief protocol for the measurement of changes in cAMP levels in whole cells that express the receptors.

**[2603]** Transfected cells will be harvested approximately twenty four hours after transient transfection. Media will be carefully aspirated off and discarded. 10 ml of PBS will be gently added to each dish of cells followed by careful aspiration. 1 ml of Sigma cell dissociation buffer and 3 ml of PBS will be added to each plate. Cells will be pipetted off the plate and the cell suspension will be collected into a 50 ml conical centrifuge tube. Cells will then be centrifuged at room temperature at 1,100 rpm for 5 min. The cell pellet will be carefully re-suspended into an appropriate volume of PBS (about 3 ml/plate). The cells will then be counted using a hemocytometer and additional PBS is added to give the appropriate number of cells (with a final volume of about 50  $\mu$ l/well).

**[2604]** cAMP standards and Detection Buffer (comprising 1  $\mu$ Ci of tracer [<sup>125</sup>I cAMP (50  $\mu$ l) to 11 ml Detection Buffer) will be prepared and maintained in accordance with the manufacturer's instructions. Assay Buffer should be prepared fresh for screening and contained 5011 of Stimulation Buffer, 3  $\mu$ l of test compound (12  $\mu$ M final assay concentration) and 50  $\mu$ l cells, Assay Buffer can be stored on ice until utilized. The assay can be initiated by addition of 50  $\mu$ l of cAMP standards to appropriate wells followed by addition of 50  $\mu$ l of PBSA to wells H-11 and H12. Fifty  $\mu$ l of

Stimulation Buffer will be added to all wells. Selected compounds (e.g., TSH) will be added to appropriate wells using a pin tool capable of dispensing 3  $\mu$ l of compound solution, with a final assay concentration of 12  $\mu$ M test compound and 100  $\mu$ l total assay volume. The cells will then be added to the wells and incubated for 60 min at room temperature. 100  $\mu$ l of Detection Mix containing tracer cAMP will then be added to the wells. Plates were then incubated additional 2 hours followed by counting in a Wallac MicroBeta scintillation counter. Values of cAMP/well will then be extrapolated from a standard cAMP curve which is contained within each assay plate.

#### **[2605]** 4. Reporter-Based Assays

##### **[2606]** a. CRE-LUC Reporter Assay (Gs-Associated Receptors)

**[2607]** 293 and 293T cells are plated-out on 96 well plates at a density of  $2 \times 10^4$  cells per well and were transfected using Lipofectamine Reagent (BRL) the following day according to manufacturer instructions. A DNA/lipid mixture is prepared for each 6-well transfection as follows: 260 ng of plasmid DNA in 100  $\mu$ l of DMEM were gently mixed with 2  $\mu$ l of lipid in 100  $\mu$ l of DMEM (the 260 ng of plasmid DNA consisted of 200 ng of a 8xCRE-Luc reporter plasmid, 50 ng of pCMV comprising endogenous receptor or non-endogenous receptor or pCMV alone, and 10 ng of a GPRS expression plasmid (GPRS in pcDNA3 (Invitrogen)). The 8xCRE-Luc reporter plasmid was prepared as follows: vector SRIF- $\beta$ -gal was obtained by cloning the rat somatostatin promoter (-71/+51) at BglIV-HindIII site in the p $\beta$ gal-Basic Vector (Clontech). Eight (8) copies of cAMP response element were obtained by PCR from an adenovirus template AdpCF126CCRE8 (see, 7 *Human Gene Therapy* 1883 (1996)) and cloned into the SRIF- $\beta$ -gal vector at the Kpn-BglIV site, resulting in the 8xCRE- $\beta$ -gal reporter vector. The 8xCRE-Luc reporter plasmid was generated by replacing the beta-galactosidase gene in the 8xCRE- $\beta$ -gal reporter vector with the luciferase gene obtained from the pGL3-basic vector (Promega) at the HindIII-BamHI site. Following 30 min. incubation at room temperature, the DNA/lipid mixture was diluted with 400  $\mu$ l of DMEM and 100  $\mu$ l of the diluted mixture was added to each well. 100  $\mu$ l of DMEM with 10% FCS were added to each well after a 4 hr incubation in a cell culture incubator. The following day the transfected cells were changed with 200  $\mu$ l/well of DMEM with 10% FCS. Eight (8) hours later, the wells were changed to 100  $\mu$ l/well of DMEM without phenol red, after one wash with PBS. Luciferase activity were measured the next day using the LucLite™ reporter gene assay kit (Packard) following manufacturer instructions and read on a 1450 MicroBeta™ scintillation and luminescence counter (Wallac).

##### **[2608]** b. AP1 Reporter Assay (Gq-Associated Receptors)

**[2609]** A method to detect Gq stimulation depends on the known property of Gq-dependent phospholipase C to cause the activation of genes containing AP1 elements in their promoter. A Pathdetect™ AP-1 cis-Reporting System (Stratagene, Catalogue # 219073) can be utilized following the protocol set forth above with respect to the CREB reporter assay, except that the components of the calcium phosphate precipitate were 410 ng pAP 1-Luc, 80 ng pCMV-receptor expression plasmid, and 20 ng CMV-SEAP.

[2610] c. SRF-LUC Reporter Assay (Gq-Associated Receptors)

[2611] One method to detect Gq stimulation depends on the known property of Gq-dependent phospholipase C to cause the activation of genes containing serum response factors in their promoter. A Pathdetect™ SRF-Luc-Reporting System (Stratagene) can be utilized to assay for Gq coupled activity in, e.g., COS7 cells. Cells are transfected with the plasmid components of the system and the indicated expression plasmid encoding endogenous or non-endogenous GPCR using a Mammalian Transfection™ Kit (Stratagene, Catalogue #200285) according to the manufacturer's instructions. Briefly, 410 ng SRF-Luc, 80 ng pCMV-receptor expression plasmid and 20 ng CMV-SEAP (secreted alkaline phosphatase expression plasmid; alkaline phosphatase activity is measured in the media of transfected cells to control for variations in transfection efficiency between samples) are combined in a calcium phosphate precipitate as per the manufacturer's instructions. Half of the precipitate is equally distributed over 3 wells in a 96-well plate, kept on the cells in a serum free media for 24 hours. The last 5 hours the cells are incubated with 1  $\mu$ M Angiotensin, where indicated. Cells are then lysed and assayed for luciferase activity using a LucLite™ Kit (Packard, Cat. # 6016911) and "Trilux 1450 Microbeta" liquid scintillation and luminescence counter (Wallac) as per the manufacturer's instructions. The data can be analyzed using GraphPad Prism™ 2.0a (GraphPad Software Inc.).

[2612] d. Intracellular IP<sub>3</sub> Accumulation Assay (Gq-Associated Receptors)

[2613] On day 1, cells comprising the receptors (endogenous and/or non-endogenous) can be plated onto 24 well plates, usually 1×10<sup>5</sup> cells/well (although this number can be optimized). On day 2 cells can be transfected by firstly mixing 0.25  $\mu$ g DNA in 50  $\mu$ l serum free DMEM/well and 2  $\mu$ l lipofectamine in 50  $\mu$ l serumfree DMEM/well. The

solutions are gently mixed and incubated for 15-30 min at room temperature. Cells are washed with 0.5 ml PBS and 400  $\mu$ l of serum free media is mixed with the transfection media and added to the cells. The cells are then incubated for 3-4 hrs at 37° C./5% CO<sub>2</sub> and then the transfection media is removed and replaced with 1 ml/well of regular growth media. On day 3 the cells are labeled with <sup>3</sup>H-myo-inositol. Briefly, the media is removed and the cells are washed with 0.5 ml PBS. Then 0.5 ml inositol-free/serum free media (GIBCO BRL) is added/well with 0.25  $\mu$ Ci of <sup>3</sup>H-myo-inositol/well and the cells are incubated for 16-18 hrs o/n at 37° C./5% CO<sub>2</sub>. On Day 4 the cells are washed with 0.5 ml PBS and 0.45 ml of assay medium is added containing inositol-free/serum free media 10  $\mu$ M pargyline 10 mM lithium chloride or 0.4 ml of assay medium and 5011 of 10× ketanserin (ket) to final concentration of 10  $\mu$ M. The cells are then incubated for 30 min at 37° C. The cells are then washed with 0.5 ml PBS and 200  $\mu$ l of fresh/ice cold stop solution (1M KOH; 18 mM Na-borate; 3.8 mM EDTA) is added/well. The solution is kept on ice for 5-10 min or until cells were lysed and then neutralized by 200  $\mu$ l of fresh/ice cold neutralization sol. (7.5% HCL). The lysate is then transferred into 1.5 ml eppendorf tubes and 1 ml of chloroform/methanol (1:2) is added/tube. The solution is vortexed for 15 sec and the upper phase is applied to a Biorad AG1-X8™ anion exchange resin (100-200 mesh). Firstly, the resin is washed with water at 1:1.25 WN and 0.9 ml of upper phase is loaded onto the column. The column is washed with 10 mls of 5 mM myo-inositol and 10 ml of 5 mM Na-borate/60 mM Na-formate. The inositol tris phosphates are eluted into scintillation vials containing 10 ml of scintillation cocktail with 2 ml of 0.1 M formic acid/1 M ammonium formate. The columns are regenerated by washing with 10 ml of 0.1 M formic acid/3M ammonium formate and rinsed twice with dd H<sub>2</sub>O and stored at 4° C. in water.

[2614] Exemplary results are presented below in Table J:

TABLE J

| Receptor | Mutation | Assay Utilized (Figure No.) | Signal Generated: CMV      | Signal Generated: Endogenous Version (Relative Light Units) | Signal Generated: Non-Endogenous Version (Relative Light Units) | Difference (≡&lt;) Between 1. CMV v. Wild-type 2. Wild-type v. Mutant |
|----------|----------|-----------------------------|----------------------------|-------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------------|
| hRUP12   | N/A      | IP <sub>3</sub> (FIG. 1)    | 317.03 cpm/mg protein      | 3463.29 cpm/mg protein                                      | —                                                               | 1. 11 Fold<br>←                                                       |
| hRUP13   | N/A      | cAMP (FIG. 2)               | 8.06 pmol/cAMP/mg protein  | 19.10 pmol/cAMP/mg protein                                  | —                                                               | 1. 2.4 Fold<br>←                                                      |
|          | A268K    | 8XCRC-LUC (FIG. 3)          | 3665.43 LCPS               | 83280.17 LCPS                                               | 61713.6 LCPS                                                    | 1. 23 Fold<br>←<br>2. 26% <                                           |
| hRUP14   | L246K    | 8XCRC-LUC (FIG. 5)          | 86.07 LCPS                 | 1962.87 LCPS                                                | 789.73 LCPS                                                     | 1. 23 Fold<br>←<br>2. 60% <                                           |
| hRUP15   | A398K    | 8XCRC-LUC (FIG. 6)          | 86.07 LCPS                 | 18286.77 LCPS                                               | 17034.83 LCPS                                                   | 1. 212 Fold<br>←<br>2. 1% <                                           |
|          | A398K    | cAMP (FIG. 7)               | 15.00 pmol/cAMP/mg protein | 164.4 pmol/cAMP/mg protein                                  | 117.5 pmol/cAMP/mg protein                                      | 1. 11 Fold<br>←<br>2. 29% <                                           |

TABLE J-continued

| Receptor | Mutation | Assay Utilized (Figure No.) | Signal Generated: CMV       | Signal Generated: Endogenous Version (Relative Light Units) | Signal Generated: Non-Endogenous Version (Relative Light Units) | Difference (<math>\Leftarrow</math>) Between: 1. CMV v. Wild-type 2. Wild-type v. Mutant |
|----------|----------|-----------------------------|-----------------------------|-------------------------------------------------------------|-----------------------------------------------------------------|------------------------------------------------------------------------------------------|
| hRUP17   | N/A      | IP <sub>3</sub> (FIG. 9)    | 317.03 cpm/mg protein       | 741.07 cpm/mg protein                                       | —                                                               | 1. 2.3 Fold <math>\Leftarrow</math>                                                      |
| hRUP21   | N/A      | IP <sub>3</sub> (FIG. 10)   | 730.5 cpm/mg protein        | 1421.9 cpm/mg protein                                       | —                                                               | 1. 2 Fold <math>\Leftarrow</math>                                                        |
| hRUP23   | W275K    | 8XCRLUC (FIG. 11)           | 311.73 pmol/cAMP/mg protein | 13756.00 pmol/cAMP/mg protein                               | 9756.87 pmol/cAMP/mg protein                                    | 1. 44 Fold <math>\Leftarrow</math> 2. 30% <                                              |

N/A = not applied

[2615] Exemplary results of GTP $\gamma$ S assay for detecting constitutive activation, as disclosed in Example 4(1) above, was accomplished utilizing G<sub>s</sub>-Fusion Protein Constructs on human hRUP13 and hRUP15. Table K below lists the signals generated from this assay and the difference in signals as indicated:

pcDNA3.1(-) (Invitrogen, cat. no. V795-20) by subcloning using the HindIII restriction site of that vector. The correct orientation for the G<sub>s</sub> $\alpha$  sequence was determined after subcloning into pcDNA3.1(-). The modified pcDNA3.1(-) containing the rat G<sub>s</sub> $\alpha$  gene at HindIII sequence was then verified; this vector was now available as a “universal” G<sub>s</sub> $\alpha$

TABLE K

| Receptor: Gs Fusion Protein | Assay Utilized          | Signal Generated: CMV (cpm bound GTP) | Signal Generated: Fusion Protein (cpm bound GTP) | Signal Generated: CMV + 10 $\mu$ M GDP (cpm bound GTP) | Signal Generated: Fusion Protein + 10 $\mu$ M GDP (cpm bound GTP) | Difference Between: 1. CMV v. Fusion Protein 2. CMV + GDP vs. Fusion + GDP 3. Fusion vs. Fusion + GDP (cpm bound GTP) |
|-----------------------------|-------------------------|---------------------------------------|--------------------------------------------------|--------------------------------------------------------|-------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|
| hRUP13-Gs                   | GTP $\gamma$ S (FIG. 4) | 32494.0                               | 49351.30                                         | 11148.30                                               | 28834.67                                                          | 1. 1.5 Fold <math>\Leftarrow</math> 2. 2.6 Fold <math>\Leftarrow</math> 3. 42% <                                      |
| hRUP15-Gs                   | GTP $\gamma$ S (FIG. 8) | 30131.67                              | 32493.67                                         | 7697.00                                                | 14157.33                                                          | 1. 1.1 Fold <math>\Leftarrow</math> 2. 1.8 Fold <math>\Leftarrow</math> 3. 56% <                                      |

## Example 5

## [2616] FUSION PROTEIN PREPARATION

## [2617] a. GPCR:Gs Fusion Construct

[2618] The design of the constitutively activated GPCR-G protein fusion construct was accomplished as follows: both the 5' and 3' ends of the rat G protein G<sub>s</sub> $\alpha$  (long form; Itoh, H. et al., 83 *PNAS* 3776 (1986)) were engineered to include a HindIII (5'-AAGCTT-3') sequence thereon. Following confirmation of the correct sequence (including the flanking HindIII sequences), the entire sequence was shuttled into

protein vector. The pcDNA3.1(-) vector contains a variety of well-known restriction sites upstream of the HindIII site, thus beneficially providing the ability to insert, upstream of the Gs protein, the coding sequence of an endogenous, constitutively active GPCR. This same approach can be utilized to create other “universal” G protein vectors, and, of course, other commercially available or proprietary vectors known to the artisan can be utilized—the important criteria is that the sequence for the GPCR be upstream and in-frame with that of the G protein.

[2619] hRUP13 couples via Gs. For the following exemplary GPCR Fusion Proteins, fusion to Gs $\alpha$  was accomplished.

[2620] A hRUP13-Gs $\alpha$  Fusion Protein construct was made as follows: primers were designed as follows:

5'-gattc[TCTAGAAT]GGAGTCTCACCCATCCCCAG-3' (SEQ.ID.NO.:97; sense)  
 5'-gattc[GATATC]CGTGACTCCAGCCGGGTGAGGCGGC-3'. (SEQ.ID.NO.:98; antisense)

[2621] Nucleotides in lower caps are included as spacers in the restriction sites (designated in brackets) between the G protein and hRUP13. The sense and anti-sense primers included the restriction sites for XbaI and EcoRV, respectively, such that spacers (attributed to the restriction sites) exist between the G protein and hRUP15.

[2622] PCR was then utilized to secure the respective receptor sequences for fusion within the Gs $\alpha$  universal vector disclosed above, using the following protocol for each: 100 ng cDNA for hRUP15 was added to separate tubes containing 2  $\mu$ l of each primer (sense and anti-sense), 3  $\mu$ L of 10 mM dNTPs, 10  $\mu$ L of 10 $\times$ TaqPlus<sup>TM</sup> Precision buffer, 1  $\mu$ L of TaqPlus<sup>TM</sup> Precision polymerase (Stratagene: #600211), and 80  $\mu$ L of water. Reaction temperatures and cycle times for hRUP15 were as follows with cycle steps 2 through 4 were repeated 35 times: 94° C. for 1 min; 94° C. for 30 seconds; 62° C. for 20 sec; 72° C. 1 min 40 sec; and 72° C. 5 min. PCR product for was run on a 1% agarose gel and then purified (data not shown). The purified product was digested with XbaI and EcoRV and the desired inserts purified and ligated into the Gs universal vector at the respective restriction site. The positive clones was isolated following transformation and determined by restriction enzyme digest; expression using 293 cells was accomplished following the protocol set forth infra. Each positive clone for hRUP15-Gs Fusion Protein was sequenced to verify correctness. (See, SEQ.ID.NO.:99 for nucleic acid sequence and SEQ.ID.NO.:100 for amino acid sequence).

[2623] hRUP15 couples via Gs. For the following exemplary GPCR Fusion Proteins, fusion to Gs $\alpha$  was accomplished.

[2624] A hRUP15-Gs $\alpha$  Fusion Protein construct was made as follows: primers were designed as follows:

5'-TCTAGAATGACGTCCACCTGCACCAACAGC-3' (SEQ.ID.NO.:101; sense)  
 5'-gatattcGCAGGAAAAGTAGCAGAATCGTAGGAAG-3'. (SEQ.ID.NO.:102; antisense)

[2625] Nucleotides in lower caps are included as spacers in the restriction sites between the G protein and hRUP15.

5'-gatcaagcttcCATGGCGTGCTGCGTGCAGGAG-3' (SEQ.ID.NO.:132) and  
 5'-gatcggtatccTTAGAACAGGCCGAGTCCTTCAGGTTTCAGTGCAGGATGGTG-3' (SEQ.ID.NO.:133)

The sense and anti-sense primers included the restriction sites for EcoRV and XbaI, respectively, such that spacers (attributed to the restriction sites) exists between the G protein and hRUP15.

[2626] PCR was then utilized to secure the respective receptor sequences for fusion within the Gs $\alpha$  universal

vector disclosed above, using the following protocol for each: 100 ng cDNA for hRUP15 was added to separate tubes containing 2  $\mu$ l of each primer (sense and anti-sense), 3  $\mu$ L of 10 mM dNTPs, 10  $\mu$ L of 10 $\times$ TaqPlus<sup>TM</sup> Precision buffer, 1  $\mu$ L of TaqPlus<sup>TM</sup> Precision polymerase (Stratagene: #600211), and 80  $\mu$ L of water. Reaction temperatures and cycle times for hRUP15 were as follows with cycle steps 2 through 4 were repeated 35 times: 94° C. for 1 min; 94° C. for 30 seconds; 62° C. for 20 sec; 72° C. for 1 min 40 sec; and 72° C. for 5 min. The PCR product for was run on a 1% agarose gel and then purified (data not shown). The purified product was digested. The purified product was digested with EcoRV and XbaI and the desired inserts purified and ligated into the Gs universal vector at the respective restriction site. The positive clones were isolated following transformation and determined by restriction enzyme digest; expression using 293 cells was accomplished following the protocol set forth infra. Each positive clone for hRUP15-Gs Fusion Protein was sequenced to verify correctness. (See, SEQ.ID.NO.:103 for nucleic acid sequence and SEQ.ID.NO.:104 for amino acid sequence).

[2627] b. Gq(6 Amino Acid Deletion)/Gi Fusion Construct

[2628] The design of a Gq (del)/Gi fusion construct can be accomplished as follows: the N-terminal six (6) amino acids (amino acids 2 through 7, having the sequence of TLESIM (SEQ.ID.NO.:129) G $\alpha$ q-subunit will be deleted and the C-terminal five (5) amino acids, having the sequence EYNLV (SEQ.ID.NO.:130) will be replaced with the corresponding amino acids of the G $\alpha$ i Protein, having the sequence DCGLF (SEQ.ID.NO.:131). This fusion construct will be obtained by PCR using the following primers:

[2629] and Plasmid 63313 which contains the mouse G $\alpha$ q-wild type version with a hemagglutinin tag as template. Nucleotides in lower caps are included as spacers.

[2630] TaqPlus Precision DNA polymerase (Stratagene) will be utilized for the amplification by the following cycles, with steps 2 through 4 repeated 35 times: 95° C. for 2 min;

95° C. for 20 sec; 56° C. for 20 sec; 72° C. for 2 min; and 72° C. for 7 min. The PCR product will be cloned into a pCRII-TOPO vector (Invitrogen) and sequenced using the ABI Big Dye Terminator kit (P.E. Biosystems). Inserts from a TOPO clone containing the sequence of the fusion construct will be shuttled into the expression vector pcDNA3.1(+) at the HindIII/BanHI site by a 2 step cloning process.

#### Example 6

#### [2631] TISSUE DISTRIBUTION OF THE DISCLOSED HUMAN GPCRS

#### [2632] A. RT-PCR

[2633] RT-PCR was applied to confirm the expression and to determine the tissue distribution of several novel human GPCRs. Oligonucleotides utilized were GPCR-specific and

the human multiple tissue cDNA panels (MTC, Clontech) as templates. Taq DNA polymerase (Stratagene) were utilized for the amplification in a 40  $\mu$ l reaction according to the manufacturer's instructions. 20  $\mu$ l of the reaction will be loaded on a 1.5% agarose gel to analyze the RT-PCR products. Table L below lists the receptors, the cycle conditions and the primers utilized.

[2634] By way of illustration, RT-PCR results for hRUP25 and hRUP38 are shown in FIG. 13C. RT-PCR results for hRUP19 are shown in FIG. 27, and RT-PCR results for mRUP19 are shown in FIG. 29. Applicant discloses herein that hRUP25, hRUP38 and hRUP19 are expressed by primary adipocytes and, in the case of hRUP38 and hRUP19, have limited tissue distribution beyond adipose. That hRUP19 has limited tissue distribution is further apparent by Northern blot analysis (FIG. 28).

TABLE L

| Receptor Identifier | Cycle Conditions<br>Min ( ' ), Sec ( " )<br>Cycles 2-4 repeated 30 times                                  | 5' Primer (SEQ.ID.NO.)                       | 3' Primer (SEQ.ID.NO.)                        | DNA Fragment | Tissue Expression                                                                                        |
|---------------------|-----------------------------------------------------------------------------------------------------------|----------------------------------------------|-----------------------------------------------|--------------|----------------------------------------------------------------------------------------------------------|
| hRUP10              | 94° for 30"<br>94° for 10"<br>62° C. for 20"<br>72° for 1'<br>72° for 7'<br>*cycles 2-4 repeated 35 times | CATGTATG<br>CCAGCGTC<br>CTGCTCC<br>(105)     | GCTATGCCT<br>GAAGCCAG<br>TCTTGTG<br>(106)     | 730 bp       | Kidney, leukocyte, liver, placenta and spleen                                                            |
| hRUP11              | 94° for 2'<br>94° for 15"<br>67° C. for 15"<br>72° for 45"<br>72° for 5'                                  | GCACCTGC<br>TCCTGAGC<br>ACCTTCTCC<br>(107)   | CACAGCGC<br>TGCAGCCCT<br>GCAGCTGG<br>C (108)  | 630 bp       | Liver, kidney, pancreas, colon, small intestinal, spleen and prostate                                    |
| hRUP12              | 94° for 2'<br>94° for 15"<br>66° C. for 15"<br>72° for 45"<br>72° for 5'                                  | CCAGTGAT<br>GACTCTGT<br>CCAGCCTG<br>(109)    | CAGACACTT<br>GGCAGGGA<br>CGAGGTG<br>(110)     | 490 bp       | Brain, colon, heart, kidney, leukocyte, pancreas, prostate, small intestinal, spleen, testis, and thymus |
| hRUP13              | 94° for 1'<br>94° for 15"<br>68° C. for 20"<br>72° for 1' 45"<br>72° for 5'                               | CTTGTGGTC<br>TACTGCAG<br>CATGTTCC<br>G (111) | CATATCCCT<br>CCGAGTGTC<br>CAGCGGC<br>(112)    | 700 bp       | Placenta and lung                                                                                        |
| hRUP14              | 94° for 1'<br>94° for 15"<br>68° C. for 20"<br>72° for 1' 45"<br>72° for 5'                               | ATGGATCC<br>TTATCATG<br>GCTTCCTC<br>(113)    | CAAGAACA<br>GGTCTCATC<br>TAAGAGCT<br>CC (114) | 700 bp       | Not yet determined                                                                                       |
| hRUP16              | 94° for 30"<br>94° for 5"<br>69° C. for 15"<br>72° for 30"<br>72° for 5'                                  | CTCTGATG<br>CCATCTGCT<br>GGATTCCT<br>G (115) | GTAGTCCAC<br>TGAAAGTC<br>CAGTGATCC<br>(116)   | 370 bp       | Fetal brain, fetal kidney and fetal skeletal muscle                                                      |
| hRUP18              | 94° for 2'<br>94° for 15"<br>60° C. for 20"                                                               | TGGTGGCG<br>ATGGCCAA<br>CAGCGCTC             | GTTGCGCCT<br>TAGCGACA<br>GATGACC              | 330 bp       | Pancreas                                                                                                 |

TABLE L-continued

| Receptor Identifier | Cycle Conditions<br>Min ( ' ), Sec ( " )<br>Cycles 2-4<br>repeated 30 times                                  | 5' Primer<br>(SEQ.ID.NO.)                     | 3' Primer<br>(SEQ.ID.NO.)                     | DNA Fragment | Tissue Expression                                  |
|---------------------|--------------------------------------------------------------------------------------------------------------|-----------------------------------------------|-----------------------------------------------|--------------|----------------------------------------------------|
|                     | 72° for 1'<br>72° for 5'                                                                                     | (117)                                         | (118)                                         |              |                                                    |
| hRUP19              | 95° for 4'<br>95° for 1'<br>60.5° C. for 30"<br>72° for 1'<br>72° for 7'<br>*cycles 2-4<br>repeated 35 times | GGCCGTGG<br>CTGATTTC<br>TCCTTAT<br>(152)      | AACCGGGT<br>CGCCTTCTT<br>CATCC (153)          | 492 bp       | Adipose, adipocyte                                 |
| hRUP21              | 94° for 1'<br>94° for 15"<br>56° C. for 20"<br>72° for 40"<br>*cycles 2-3<br>repeated 30 times               | TCAACCTG<br>TATAGCAG<br>CATCCTC<br>(119)      | AAGGAGTA<br>GCAGAATG<br>GTTAGCC<br>(120)      |              | Kidney, lung and testis                            |
| hRUP22              | 94° for 30"<br>94° for 15"<br>69° C. for 20"<br>72° for 40"<br>*cycles 2-3<br>repeated 30 times              | GACACCTG<br>TCAGCGGT<br>CGTGTGTG<br>(121)     | CTGATGGA<br>AGTAGAGG<br>CTGTCCATC<br>TC (122) |              | Testis, thymus and spleen                          |
| hRUP23              | 94° for 2'<br>94° for 15"<br>60° C. for 20"<br>72° for 1'<br>72° for 5'                                      | GCGCTGAG<br>CGCAGACC<br>AGTGGCTG<br>(123)     | CACGGTGA<br>CGAAGGGC<br>ACGAGCTC<br>(124)     | 520 bp       | Placenta                                           |
| hRUP25              | 96° for 2'<br>96° for 30"<br>55° C. for 1'<br>72° for 2'<br>72° for 10'                                      | CTGATGGA<br>CAACTATG<br>TGAGGCGT<br>TGG (144) | GCTGAAGC<br>TGCTGCACA<br>AATTTGCAC<br>C (145) | 297 bp       | Adipocyte, spleen, leukocyte, kidney, lung, testis |
| hRUP26              | 94° for 2'<br>94° for 15"<br>650° C. for 20"<br>72° for 1'<br>72° for 5'                                     | AGCCATCC<br>CTGCCAGG<br>AAGCATGG<br>(125)     | CCAGGTAG<br>GTGTGCAG<br>CACAAATGG<br>C (126)  | 470 bp       | Pancreas                                           |
| hRUP27              | 94° for 30"<br>94° for 10"<br>55° C. for 20"<br>72° for 1'<br>72° for 3'<br>*cycles 2-4<br>repeated 35 times | CTGTTCAA<br>CAGGGCTG<br>GTTGGCAA<br>C (127)   | ATCATGTCT<br>AGAGTCAT<br>GGTGATCC<br>(128)    | 890 bp       | Brain                                              |
| hRUP38              | 96° for 2'<br>96° for 30"<br>55° C. for 1'<br>72° for 2'<br>72° for 10'                                      | CTACTATGT<br>GCGGCGTT<br>CA (146)             | CCCTTCTTG<br>GAATGGTT<br>ATT (147)            | 852 bp       | Adipocyte, spleen, lung                            |

**[2635] B. AFFYMETRIX GENECHIP® TECHNOLOGY**

**[2636]** Amino acid sequences were submitted to Affymetrix for the designing and manufacturing of microarray containing oligonucleotides to monitor the expression levels of G protein-coupled receptors (GPCRs) using their GeneChip® Technology. Also present on the microarray were probes for characterized human brain tissues from Harvard Brain Bank or obtained from commercially available sources. RNA samples were amplified, labeled, hybridized to the microarray, and data analyzed according to manufacturer's instructions.

**[2637]** Adipose tissues were monitored for the level of gene expression of each of the GPCRs represented on the microarray. GPCRs were determined to be expressed if the expression index was greater than 100 (based upon and according to manufacturer's instructions). The data was analyzed and had indicated that classification of GPCRs with an expression index greater than 100 was reasonable because a number of known GPCRs had previously been reported to be expressed in neuronal tissues with an expression index greater than 100.

**[2638]** Using the GeneChip, Applicant has discovered hRUP25 and hRUP38 to have high levels of expression in adipocytes, consistent with hRUP25 and hRUP38 playing a role in lipolysis (see, Goodman & Gilman's, *The Pharmacological Basis of Therapeutics*, 9th Edition, page 235 (1996). See **FIGS. 13A and 13B**. **FIG. 13A** is a plot representing the expression level of hRUP25 in various tissues. hRUP25 is highly expressed by primary adipocytes. **FIG. 13B** is a plot representing the expression level of hRUP38 in various tissues. hRUP38 is highly expressed by primary adipocytes.

**[2639]** This patent document discloses the identification of nicotinic acid as a ligand and agonist of human, mouse and rat RUP25. See, Examples infra. The patent document further discloses that nicotinic acid is not an agonist of hRUP38 or hRUP19. In the case of hRUP38, this was an unexpected result, as hRUP25 and hRUP38 are about 95% identical (Table B), although it is not a result without precedent [see, e.g., Yan M et al. *Science* (2000) 290:523-7; the disclosure of which is hereby incorporated by reference in its entirety].

**Example 7**

**[2640]** Protocol: Direct Identification of Inverse Agonists and Agonists

**[2641] A. [<sup>35</sup>S]GTPγS Assay**

**[2642]** Although we have utilized endogenous, constitutively active GPCRs for the direct identification of candidate compounds as, e.g., inverse agonists, for reasons that are not altogether understood, intra-assay variation can become exacerbated. In some embodiments, a GPCR Fusion Protein, as disclosed above, is also utilized with a non-endogenous, constitutively activated GPCR. When such a protein is used, intra-assay variation appears to be substantially stabilized, whereby an effective signal-to-noise ratio is obtained. This has the beneficial result of allowing for a more robust identification of candidate compounds. Thus, in some embodiments it is preferred that for direct identification, a GPCR Fusion Protein be used and that when utilized, the following assay protocols be utilized.

**[2643] 1. Membrane Preparation**

**[2644]** In some embodiments membranes comprising the constitutively active orphan GPCR/Fusion Protein of interest and for use in the direct identification of candidate compounds as inverse agonists or agonists are preferably prepared as follows:

**[2645] a. Materials**

**[2646]** "Membrane Scrape Buffer" is comprised of 20 mM HEPES and 10 mM EDTA, pH 7.4; "Membrane Wash Buffer" is comprised of 20 mM HEPES and 0.1 mM EDTA, pH 7.4; "Binding Buffer" is comprised of 20 mM HEPES, 100 mM NaCl, and 10 mM MgCl<sub>2</sub>, pH 7.4

**[2647] b. Procedure**

**[2648]** All materials will be kept on ice throughout the procedure. Firstly, the media will be aspirated from a confluent monolayer of cells, followed by rinse with 10 ml cold PBS, followed by aspiration. Thereafter, 5 ml of Membrane Scrape Buffer will be added to scrape cells; this will be followed by transfer of cellular extract into 50 ml centrifuge tubes (centrifuged at 20,000 rpm for 17 minutes at 4° C.). Thereafter, the supernatant will be aspirated and the pellet will be resuspended in 30 ml Membrane Wash Buffer followed by centrifuge at 20,000 rpm for 17 minutes at 4° C. The supernatant will then be aspirated and the pellet resuspended in Binding Buffer. This will then be homogenized using a Brinkman Polytron™ homogenizer (15-20 second bursts until the all material is in suspension). This is referred to herein as "Membrane Protein".

**[2649] 2. Bradford Protein Assay**

**[2650]** Following the homogenization, protein concentration of the membranes will be determined using the Bradford Protein Assay (protein can be diluted to about 1.5 mg/ml, aliquoted and frozen (-80° C.) for later use; when frozen, protocol for use will be as follows: on the day of the assay, frozen Membrane Protein is thawed at room temperature, followed by vortex and then homogenized with a Polytron at about 12x1,000 rpm for about 5-10 seconds; it was noted that for multiple preparations, the homogenizer should be thoroughly cleaned between homogenization of different preparations).

**[2651] a. Materials**

**[2652]** Binding Buffer (as per above); Bradford Dye Reagent; Bradford Protein Standard will be utilized, following manufacturer instructions (Biorad, cat. no. 500-0006).

**[2653] b. Procedure**

**[2654]** Duplicate tubes will be prepared, one including the membrane, and one as a control "blank". Each contained 800 μl Binding Buffer. Thereafter, 10 μl of Bradford Protein Standard (1 mg/ml) will be added to each tube, and 10 μl of membrane Protein will then be added to just one tube (not the blank). Thereafter, 200 μl of Bradford Dye Reagent will be added to each tube, followed by vortex of each. After five (5) minutes, the tubes will be re-vortexed and the material therein will be transferred to cuvettes. The cuvettes will then be read using a CECIL 3041 spectrophotometer, at wavelength 595.

**[2655] 3. Direct Identification Assay****[2656] a. Materials**

**[2657]** GDP Buffer consisted of 37.5 ml Binding Buffer and 2 mg GDP (Sigma, cat. no. G-7127), followed by a

series of dilutions in Binding Buffer to obtain 0.2  $\mu$ M GDP (final concentration of GDP in each well was 0.1  $\mu$ M GDP); each well comprising a candidate compound, has a final volume of 200  $\mu$ l consisting of 100  $\mu$ l GDP Buffer (final concentration, 0.1  $\mu$ M GDP), 50  $\mu$ l Membrane Protein in Binding Buffer, and 50  $\mu$ l [ $^{35}$ S]GTP $\gamma$ S (0.6 nM) in Binding Buffer (2.5  $\mu$ l [ $^{35}$ S]GTP $\gamma$ S per 10 ml Binding Buffer).

**[2658]** b. Procedure

**[2659]** Candidate compounds will be preferably screened using a 96-well plate format (these can be frozen at  $-80^{\circ}$  C.). Membrane Protein (or membranes with expression vector excluding the GPCR Fusion Protein, as control), will be homogenized briefly until in suspension. Protein concentration will then be determined using the Bradford Protein Assay set forth above. Membrane Protein (and control) will then be diluted to 0.25 mg/ml in Binding Buffer (final assay concentration, 12.5  $\mu$ g/well). Thereafter, 100  $\mu$ l GDP Buffer was added to each well of a Wallac Scintistrip<sup>TM</sup> (Wallac). A 5  $\mu$ l pin-tool will then be used to transfer 5  $\mu$ l of a candidate compound into such well (i.e., 51  $\mu$ l in total assay volume of 200  $\mu$ l is a 1:40 ratio such that the final screening concentration of the candidate compound is 10  $\mu$ M). Again, to avoid contamination, after each transfer step the pin tool should be rinsed in three reservoirs comprising water (IX), ethanol (IX) and water (2 $\times$ )-excess liquid should be shaken from the tool after each rinse and dried with paper and kimwipes. Thereafter, 50  $\mu$ l of Membrane Protein will be added to each well (a control well comprising membranes without the GPCR Fusion Protein was also utilized), and pre-incubated for 5-10 minutes at room temperature. Thereafter, 50  $\mu$ l of [ $^{35}$ S]GTP $\gamma$ S (0.6 nM) in Binding Buffer will be added to each well, followed by incubation on a shaker for 60 minutes at room temperature (again, in this example, plates were covered with foil). The assay will then be stopped by spinning of the plates at 4000 RPM for 15 minutes at  $22^{\circ}$  C. The plates will then be aspirated with an 8 channel manifold and sealed with plate covers. The plates will then be read on a Wallac 1450 using setting "Prot. #37" (as per manufacturer instructions).

**[2660]** B. Cyclic AMP Assay

**[2661]** Another assay approach to directly identified candidate compound was accomplished by utilizing a cyclase-based assay. In addition to direct identification, this assay approach can be utilized as an independent approach to provide confirmation of the results from the [ $^{35}$ S]GTP $\gamma$ S approach as set forth above.

**[2662]** A modified Flash Plate<sup>TM</sup> Adenylyl Cyclase kit (New England Nuclear; Cat. No. SMP004A) was preferably utilized for direct identification of candidate compounds as inverse agonists and agonists to constitutively activated orphan GPCRs in accordance with the following protocol.

**[2663]** Transfected cells were harvested approximately three days after transfection. Membranes were prepared by homogenization of suspended cells in buffer containing 20 mM HEPES, pH 7.4 and 10 mM MgCl<sub>2</sub>. Homogenization was performed on ice using a Brinkman Polytron<sup>TM</sup> for approximately 10 seconds. The resulting homogenate is centrifuged at 49,000 $\times$ g for 15 minutes at  $4^{\circ}$  C. The resulting pellet was then resuspended in buffer containing 20 mM HEPES, pH 7.4 and 0.1 mM EDTA, homogenized for 10 seconds, followed by centrifugation at 49,000 $\times$ g for 15

minutes at  $4^{\circ}$  C. The resulting pellet was then stored at  $-80^{\circ}$  C. until utilized. On the day of direct identification screening, the membrane pellet was slowly thawed at room temperature, resuspended in buffer containing 20 mM HEPES, pH 7.4 and 10 mM MgCl<sub>2</sub>, to yield a final protein concentration of 0.60 mg/ml (the resuspended membranes are placed on ice until use).

**[2664]** cAMP standards and Detection Buffer (comprising 2  $\mu$ Ci of tracer [ $^{125}$ I] cAMP (100  $\mu$ l) to 11 ml Detection Buffer) were prepared and maintained in accordance with the manufacturer's instructions. Assay Buffer was prepared fresh for screening and contained 20 mM HEPES, pH 7.4, 10 mM MgCl<sub>2</sub>, 20 mM phosphocreatine (Sigma), 0.1 units/ml creatine phosphokinase (Sigma), 50  $\mu$ M GTP (Sigma), and 0.2 mM ATP (Sigma); Assay Buffer was then stored on ice until utilized.

**[2665]** Candidate compounds identified as per above (if frozen, thawed at room temperature) were added, preferably, to 96-well plate wells (3  $\mu$ l/well; 12  $\mu$ M final assay concentration), together with 40  $\mu$ l Membrane Protein (30  $\mu$ g/well) and 50  $\mu$ l of Assay Buffer. This admixture was then incubated for 30 minutes at room temperature, with gentle shaking.

**[2666]** Following the incubation, 10011 of Detection Buffer was added to each well, followed by incubation for 2-24 hours. Plates were then counted in a Wallac Micro-Beta<sup>TM</sup> plate reader using "Prot. #31" (as per manufacturer instructions).

**[2667]** A representative screening assay plate (96 well format) result is presented in **FIG. 12**. Each bar represents the results for a different compound in each well, plus hRUP13-Gs $\alpha$  Fusion Protein construct, as prepared in Example 5(a) above. The representative results presented in **FIG. 12** also provide standard deviations based upon the mean results of each plate ("m") and the mean plus two arbitrary preference for selection of inverse agonists as "leads" from the primary screen involves selection of candidate compounds that that reduce the percent response by at least the mean plate response, minus two standard deviations. Conversely, an arbitrary preference for selection of agonists as "leads" from the primary screen involves selection of candidate compounds that increase the percent response by at least the mean plate response, plus the two standard deviations. Based upon these selection processes, the candidate compounds in the following wells were directly identified as putative inverse agonist (Compound A) and agonist (Compound B) to hRUP13 in wells A2 and G9, respectively. See, **FIG. 12**. It is noted for clarity: these compounds have been directly identified without any knowledge of the endogenous ligand for this GPCR. By focusing on assay techniques that are based upon receptor function, and not compound binding affinity, we are able to ascertain compounds that are able to reduce the functional activity of this receptor (Compound A) as well as increase the functional activity of the receptor (Compound B). Based upon the location of these receptors in, for example, lung tissue (see, for example, hRUP13 and hRUP21 in Example 6), pharmaceutical agents can be developed for potential therapeutic treatment of lung cancer.

## Example 8

**[2668] MELANOPHORE TECHNOLOGY**

**[2669]** Melanophores are skin cells found in lower vertebrates. They contain pigmented organelles termed melanosomes. Melanophores are able to redistribute these melanosomes along a microtubule network upon G-protein coupled receptor (GPCR) activation. The result of this pigment movement is an apparent lightening or darkening of the cells. In melanophores, the decreased levels of intracellular cAMP that result from activation of a Gi-coupled receptor cause melanosomes to migrate to the center of the cell, resulting in a dramatic lightening in color. If cAMP levels are then raised, following activation of a Gs-coupled receptor, the melanosomes are re-dispersed and the cells appear dark again. The increased levels of diacylglycerol that result from activation of Gq-coupled receptors can also induce this re-dispersion. In addition, the technology is also suited to the study of certain receptor tyrosine kinases. The response of the melanophores takes place within minutes of receptor activation and results in a simple, robust color change. The response can be easily detected using a conventional absorbance microplate reader or a modest video imaging system. Unlike other skin cells, the melanophores derive from the neural crest and appear to express a full complement of signaling proteins. In particular, the cells express an extremely wide range of G-proteins and so are able to functionally express almost all GPCRs.

**[2670]** Melanophores can be utilized to identify compounds, including natural ligands, against GPCRs. This method can be conducted by introducing test cells of a pigment cell line capable of dispersing or aggregating their pigment in response to a specific stimulus and expressing an exogenous clone coding for the GPCR. A stimulant, e.g., melatonin, sets an initial state of pigment disposition wherein the pigment is aggregated within the test cells if activation of the GPCR induces pigment dispersion. However, stimulating the cell with a stimulant to set an initial state of pigment disposition wherein the pigment is dispersed if activation of the GPCR induces pigment aggregation. The test cells are then contacted with chemical compounds, and it is determined whether the pigment disposition in the cells changed from the initial state of pigment disposition. Dispersion of pigments cells due to the candidate compound, including but not limited to a ligand, coupling to the GPCR will appear dark on a petri dish, while aggregation of pigments cells will appear light.

**[2671]** Materials and methods will be followed according to the disclosure of U.S. Pat. No. 5,462,856 and U.S. Pat. No. 6,051,386. These patent disclosures are hereby incorporated by reference in their entirety.

**[2672]** Melanophores were transfected by electroporation with plasmids coding for the GPCRs, for example hRUP25, hRUP38, hRUP11 and hRUP19. Pre-screening of the GPCRs in melanophores was performed in the absence of nicotinic acid following the protocol below to determine the G protein coupling. This pre-screen evidenced that hRUP25 (**FIG. 14A**), hRUP38 (**FIG. 14B**) and hRUP19 (**FIG. 14C**) are strongly Gi-coupled. hRUP11 is also strongly Gi-coupled (not shown). Consistent with hRUP19 being Gi-coupled, CART-activated hRUP19 inhibits cAMP production in membranes of transfected 293 cells (**FIG. 30**).

**[2673]** The cells were plated in 96-well plates (one receptor per plate). 48 hours post-transfection, half of the cells on

each plate were treated with 10 nM melatonin. Melatonin activates an endogenous Gi-coupled receptor in the melanophores and causes them to aggregate their pigment. The remaining half of the cells were transferred to serum-free medium 0.7× L-15 (Gibco). After one hour, the cells in serum-free media remained in a pigment-dispersed state while the melatonin-treated cells were in a pigment-aggregated state. At this point, the cells were treated with a dose response of nicotinic acid (Sigma). If the plated GPCRs bound to nicotinic acid, the melanophores would be expected to undergo a color change in response to the compound. If the receptor were either a Gs or Gq coupled receptor, then the melatonin-aggregated melanophores would undergo pigment dispersion. In contrast, if the receptor was a Gi-coupled receptor, then the pigment-dispersed cells would be expected to undergo a dose-dependent pigment aggregation.

**[2674]** Melanophores transfected with hRUP25 were treated with nicotinic acid. Upon this treatment, the cells underwent pigment aggregation in a dose-dependent manner. hRUP25-expressing cells that were pre-aggregated with melatonin did not disperse upon nicotinic acid treatment, which is consistent with the receptor being a Gi-coupled receptor. See, **FIG. 15** and *infra*.

**[2675]** To confirm and extend these results, melanophores were transfected with a range of hRUP25 DNA from 0 to 10  $\mu$ g. As controls, melanophores were also transfected with 10  $\mu$ g of  $\alpha_{2A}$  Adrenergic receptor (a known Gi-coupled receptor) and salmon sperm DNA (Gibco), as a mock transfection. On day 3, the cells were again incubated for 1 hour in serum-free L-15 medium (Gibco) and remained in a pigment-dispersed state. The cells were then treated with a dose response of nicotinic acid. See, **FIG. 15A**. **FIG. 15A** depicts the aggregation response of nicotinic acid at melanophores transfected with various ranges of hRUP25. At 10  $\mu$ g of hRUP25, the EC<sub>50</sub> for nicotinic acid is about 54 nM. Stated differently, at very low concentrations, nicotinic acid evidences binding to hRUP25.

**[2676]** Reference is now made to **FIG. 15B**. In **FIG. 15B**, both the mock transfected and  $\alpha_{2A}$  transfected cells did not respond to nicotinic acid. This data evidences that nicotinic acid binds specifically to the Gi-coupled receptor hRUP25.

**[2677]** The data show that the greater the amount of hRUP25 plasmid DNA transfected, the greater the magnitude of the observed aggregation response. Collectively these data indicate that hRUP25: 1) is a Gi-coupled receptor that 2) binds to nicotinic acid.

**[2678]** As set forth herein, nicotinic acid is a ligand for, and agonist of, human, mouse and rat RUP25. It is further shown that hRUP38, hRUP11, hRUP19, and human, mouse and rat RUP25 are Gi-coupled. Additionally, hRUP38, human and mouse RUP19, hRUP11, and human, mouse, and rat RUP25 may be used in methods described herein to identify antagonists, agonists, inverse agonists, partial agonists, allosteric enhancers, and negative allosteric modulators. As discussed supra, methods of modifying nicotinic acid receptor activity in adipocytes using a modulator of the receptor are set forth. Preferably, the modulator is an agonist.

## Example 9

**[2679] NICOTINIC ACID INDUCED-INOSITOL PHOSPHATES ACCUMULATION IN 293 CELLS CO-EXPRESSING hRUP AND GqΔGi**

**[2680] FIG. 16** illustrates the nicotinic acid induced-inositol phosphates (IPs) accumulation in HEK293 cells co-expressing hRUP25 and the chimeric Gαq-subunit in which the last five amino acids have been replaced with the corresponding amino acids of Gαi (GqΔGi). This construct has been shown to convert the signaling of a Gi-coupled receptor to the Gq pathway (i.e. accumulation of inositol phosphates) in response to receptor activation. Cells transfected with GqΔGi plus either empty plasmid or the constitutively activated α<sub>2A</sub>AR (α<sub>2A</sub>K) are non-responsive to nicotinic acid and served as controls for the IP assay. Cells transfected with GqΔGi plus either hRUP19 or hRUP38 are also unresponsive to nicotinic acid, indicating that nicotinic acid is not an agonist for either hRUP19 or hRUP38.

## Example 10

**[2681] SATURATION BINDING OF [<sup>3</sup>H] NICOTINIC ACID TO MEMBRANES FROM CELLS EXPRESSING EITHER hRUP25, hRUP38, hRUP19 OR VECTOR ALONE**

**[2682] FIG. 17** shows the results from saturation binding of [<sup>3</sup>H] nicotinic acid to membranes from cells expressing either hRUP25, hRUP38, hRUP19 or vector alone [CHO(-)]. Only hRUP25 was found to bind nicotinic acid in a specific and high-affinity manner.

## Example 11

**[2683] NICOTINIC ACID AND (-)-NICOTINE INDUCED-INHIBITION OF FORSKOLIN STIMULATED cAMP ACCUMULATION IN hRUP25-CHO CELL STABLE LINE #46**

**[2684] FIG. 18A** is a set of immunofluorescent photomicrographs illustrating the expression of hemagglutinin(HA)-tagged hRUP25 in a stably transfected line of CHO cells (top; clone #46). No significant labeling is detected in mock stably-transfected CHO cells (Mock). The lower panels identify the nuclear (DAPI) staining of cells in the same field.

**[2685] FIG. 18B** illustrates nicotinic acid and (-)-nicotine induced-inhibition of forskolin stimulated cAMP accumulation in hRUP25-CHO cell stable line #46 (described in preceding paragraph). The EC<sub>50</sub> for nicotinic acid is 23.6 nM and that for (-)-nicotine is 9.8 μM.

## Example 12

**[2686] hRUP25 AND mRUP25 INHIBIT TSHR INDUCED-CAMP ACCUMULATION FOLLOWING ACTIVATION BY NICOTINIC ACID**

**[2687] FIG. 19** indicates that, in response to nicotinic acid, both hRUP25 and the mouse ortholog mRUP25 can inhibit TSHR stimulated cAMP production (in the presence and absence of TSH).

## Example 13

**[2688] hRUP25 AND mRUP25 BIND TO NICOTINIC ACID SPECIFICALLY AND WITH HIGH AFFINITY**

**[2689] FIG. 20** shows the saturation binding curves of [<sup>3</sup>H]nicotinic acid ([<sup>3</sup>H]NA) to membranes prepared from HEK293 cells transiently expressing either hRUP25 or mRUP25. Note the significant binding of [<sup>3</sup>H]NA relative to either that found in membranes derived from mock transfected cells or in the presence of an excess of non-labeled nicotinic acid (200 μM).

**[2690]** Radioligand binding was done as follows. Media was removed from cells grown in culture [either stably or transiently transfected with negative control (empty plasmid) or with the individual receptors hRUP25, mRUP25, rRUP25, hRUP38, hRUP11 or hRUP19] and cells were scraped and homogenized in buffer containing 15 mM HEPES, 5 mM EDTA, 5 mM EGTA, plus protease inhibitors (leupeptin, PMSF and pepstatin). Membranes were harvested following centrifugation at 30,000×g, 4° C. for 30 min. Membranes were then resuspended and re-homogenized in CHAPS binding buffer (50 mM Tris-HCl and 0.02% CHAPS, pH 7.4). Aliquots were taken for protein analysis via the Bradford protein assay and normalized such that each binding reaction contained the same amount of membrane protein (25-50 μg). 50 nM [<sup>3</sup>H]nicotinic acid was added to each sample and either buffer (for total samples) or a desired amount of non-labeled compound (at the same volumes and in the same diluent) was added and the reactions were left at room temperature gently shaking for 1 hr. Free ligand was separated from bound ligand via rapid filtration onto a filter. Appropriate scintillant was added to each sample and counted in an appropriate scintillation counter. Data was analyzed using Excel and PrismGraph. In some cases radioligand binding was performed using a scintillation proximity assay (SPA) in which case the samples did not require filtration or the addition of scintillant.

## Example 14

**[2691] THE RANK ORDER OF POTENCY OF COMPOUNDS ON hRUP25 CLOSELY MATCHES THAT OF THE PHARMACOLOGICALLY DEFINED NICOTINIC ACID RECEPTOR**

**[2692] FIG. 21** is a table comparing the rank order of potency of various compounds on hRUP25 and the pharmacologically defined nicotinic acid receptor. The potencies at hRUP25 derived both by a functional analysis measuring the inhibition of forskolin induced cAMP production and competitive radioligand binding assays, closely match the order of potencies of the pharmacologically defined nicotinic acid receptor.

## Example 15

**[2693] NICOTINIC ACID AND RELATED COMPOUNDS INHIBIT ISOPROTERENOL INDUCED LIPOLYSIS IN RAT EPIDIDYMAL FAT DERIVED ADIPOCYTES**

**[2694] FIG. 22A** depicts nicotinic acid and related compounds inhibiting isoproterenol induced lipolysis in rat epididymal fat derived adipocytes at a concentration of 10 μM. P-3-T represents 3-tetrazole-5-pyridine.

**[2695] FIG. 22B** illustrates a nicotinic acid dose-dependent inhibition of isoproterenol induced-lipolysis in rat epididymal fat derived adipocytes. Note the rightward shift in the dose-response curves with increasing concentrations of nicotinic acid.

[2696] Lipolysis assays were done following the isolation of adipocytes from rat or human. The source of fat from rats was the epididymal fat and from humans was either subcutaneous or omental. Cells were isolated following collagenase digestion and floatation. An elevation of intracellular cAMP levels and concomitant activation of lipolysis via hormone sensitive lipase was accomplished using isoproterenol, forskolin, 3-isobutyl-1-methyl-xanthine (IBMX) or a combination thereof at concentrations and times determined empirically and depending on the source of tissue. Lipolysis was allowed to continue for the desired time in the presence or absence of drug (e.g. nicotinic acid, P-3-T, etc). Data was analyzed using Excel and PrismGraph.

[2697] To show that a modulator of hRUP19 behaves similarly, an analogous assay is set up using said modulator of hRUP19. Preferred said modulator is an agonist.

[2698] To show that a modulator of hRUP38 behaves similarly, an analogous assay is set up using said modulator of hRUP38, wherein the rat is transgenic for hRUP38. Preferred said modulator is an agonist.

[2699] To show that a modulator of hRUP11 behaves similarly, an analogous assay is set up using said modulator of hRUP11, wherein the rat is transgenic for hRUP11. Preferred said modulator is an agonist.

#### Example 16

[2700] DOSE-DEPENDENT INHIBITION OF ISOPROTERENOL INDUCED-LIPOLYSIS IN HUMAN, SUBCUTANEOUS-DERIVED, PRIMARY ADIPOCYTES VIA NICOTINIC ACID AND P-3-T

[2701] FIG. 23 illustrates the ability of both nicotinic acid and the related compound P-3-T (3-tetrazole-5-pyridine) to inhibit isoproterenol induced lipolysis in adipocyte primary cultures derived from human subcutaneous fat in a dose-dependant manner. The EC<sub>50</sub> value for nicotinic acid and P-3-T were 716 nM and 218 nM respectively. (Also see Example 15, supra.)

#### Example 17

##### SCREENING DATA FOR NICOTINIC ACID AND

##### 1-ISOPROPYL-1H-BENZOTRIAZOLE-5-CARBOXYLIC ACID IN cAMP ASSAYS

[2702] FIG. 24 presents screening data via adenylyl cyclase assay for hRUP38. Note that nicotinic acid does not activate inhibition of forskolin stimulated cAMP hRUP38-expressing CHO cells whereas 1-Isopropyl-1H-benzotriazole-5-carboxylic acid does. 1-Isopropyl-1H-benzotriazole-5-carboxylic acid has no effect on CHO cells expressing either hRUP25 or hRUP19.

#### Example 18

[2703] INHIBITION OF ISOPROTERENOL STIMULATED LIPOLYSIS IN HUMAN SUBCUTANEOUS ADIPOCYTES

[2704] Nicotinic acid (an agonist of hRUP25) and 1-Isopropyl-1H-benzotriazole-5-carboxylic acid (an agonist of hRUP38; see Example 17, supra) were separately dose-dependently applied to isoproterenol (100 nM) stimulated primary human adipocytes. FIG. 25 illustrates the ability of

1-Isopropyl-1H-benzotriazole-5-carboxylic acid to inhibit isoproterenol stimulated lipolysis in adipocyte primary cultures derived from human subcutaneous fat in a dose-dependent manner comparable to that of nicotinic acid.

[2705] To show that a modulator of hRUP19 behaves similarly, an analogous assay is set up using said modulator of hRUP19. Preferred said modulator is an agonist.

[2706] To show that a modulator of hRUP11 behaves similarly, an analogous assay is set up using said modulator of hRUP11. Preferred said modulator is an agonist.

#### Example 19

[2707] INHIBITION OF FORSKOLIN STIMULATED cAMP ACCUMULATION IN hRUP38-CHO STABLE CELL LINE BY 3-(BROMO-2-ETHOXY-PHENYL)-ACRYLIC ACID

[2708] FIG. 26 presents screening data via adenylyl cyclase assay for hRUP38. Note that 3-(5-Bromo-2-ethoxy-phenyl)-acrylic acid activates inhibition of forskolin stimulated cAMP in hRUP38-expressing CHO cells but has no effect on CHO cells expressing either hRUP25 or hRUP19. The EC<sub>50</sub> for 3-(5-Bromo-2-ethoxy-phenyl)-acrylic acid is 1.17  $\mu$ M. (Also see the legend to Example 11, supra, for details directed to stable CHO transfectants.)

#### Example 20

##### RT-PCR INDICATES THAT hRUP19 IS SELECTIVELY EXPRESSED IN HUMAN FAT CELLS

[2709] FIG. 27 presents an RT-PCR analysis of hRUP19 expression using a panel of human tissues. The analysis indicates that hRUP19 is selectively expressed in fat cells. Low expression is also evident in testis, placenta, kidney and spleen.

[2710] Oligonucleotides used for PCR had the following sequences: 5'-GGCCGTGGCTGATTTCCTCCTTAT-3' (SEQ. ID. NO.:152; forward primer) and 5'-AACCGGGTCGCCTTCTTCATCC-3' (SEQ. ID. NO.:153; reverse primer). Commercially available human multiple tissue cDNA panels were used as templates [Clontech, MTC panels Human I (#K1420-1) and Human II (K-1421-1), and human fat cell cDNA (#7128-1)]. 1 ng cDNA was used per PCR amplification. PCR was performed using Platinum PCR SuperMix (Life Technologies, Inc.); according to manufacturer instructions. The following cycles were used: 95° C. for 4 min; 95° C. for 1 min; 60.5° C. for 30 sec, 72° C. for 1 min, and 72° C. for 7 min; cycles 2 through 4 were repeated 35 times. The resulting PCR reactions (15  $\mu$ l) were loaded on a 1.5% agarose gel to analyze the RT-PCR products, and a specific 492 base-pair DNA fragment representing hRUP19 was specifically amplified from cDNA of fat cell origin. Low expression was also evident in testis, placenta, kidney, and spleen.

#### Example 21

[2711] NORTHERN BLOT ANALYSIS OF hRUP19 EXPRESSION IN SELECTED TISSUES

[2712] FIG. 28 presents a Northern blot analysis of hRUP19 expression using a panel of human tissues. The analysis indicates that hRUP19 is strongly expressed in

mammary gland, probably due to fat cell-specific expression of hRUP19. Ad, adrenal gland; Bl, bladder; BM, bone marrow; Br, brain (whole); LN, lymph node; MG, mammary gland; Pr, prostate; Sp, spinal cord; St, stomach; Thyr, thyroid; Trch, trachea; Ut, uterus.

[2713] A pre-made blot containing Poly A+ RNA from 12 human tissues was purchased from Clontech (Human 12-Lane II, Cat. # 7784-1). hRUP19 cDNA probe encompassing the coding region was generated by PCR using a plasmid containing hRUP19 cDNA as template. The blot was prehybridized with 20 ml Clontech ExpressHyb solution (Cat. # 8015-2) for 30 minutes in a hybridization oven at 65° C., according to the manufacturer's directions. 25 ng of a random primer-labeled, hRUP19 cDNA probe was added to the prehybridization solution, and the incubation was continued for an additional 2 hr. The membrane was then washed according to the manufacturer's directions (Cat. # 8015-2) and exposed to film for autoradiography over a period of two days. Ad, adrenal gland; Bl, bladder; BM, bone marrow; Br, brain (whole); LN, lymph node; MG, mammary gland; Pr, prostate; Sp, spinal cord; St, stomach; Thyr, thyroid; Trch, trachea; Ut, uterus.

#### Example 22

[2714] RUP19 EXPRESSION IS INDUCED DURING ADIPOCYTE DIFFERENTIATION: CHARACTERIZATION OF mRUP19 EXPRESSION IN MOUSE 3T3 PRE-ADIPOCYTES AND ADIPOCYTES

[2715] FIG. 29 presents an analysis of RUP19 mRNA expression as a function of adipocyte differentiation. The analysis indicates that RUP19 mRNA expression is induced during adipocyte differentiation.

[2716] 3T3 pre-adipocytes were cultured in DMEM containing 10% bovine calf serum. These cells were induced to differentiate into an adipocyte phenotype using a standard protocol [Haraguchi K et al (1996) Biochem Biophys Res Comm 223:193-198; the disclosure of which is hereby incorporated by reference in its entirety]. Briefly, 1 day after confluence, cells were treated with DMEM containing 10% FBS, 10 µg/ml insulin, 0.2 µg/ml dexamethasone, and 0.5 mM isobutylmethylxanthine. After 3 days, the cells were shifted to media supplemented with 10 µg/ml insulin and 10% FBS, and 2 days later, the cells were shifted to media containing 10% FBS alone. After an additional 48 hrs, total RNA was isolated from undifferentiated or differentiated cells using RNAzol according to the manufacturer's directions. Separate populations of undifferentiated and differentiated cells were subjected to staining with Oil Red, to confirm the induction of an adipocyte phenotype with this protocol.

[2717] Northern blot analysis. 10 µg of total RNA from 293 cells, 3T3 preadipocytes and 3T3 adipocytes was subjected to electrophoresis on a 1% agarose/formaldehyde gel and transferred to a nylon membrane using standard protocols. The blot was hybridized to a 361 bp mRUP19 cDNA probe and exposed to film as described in FIG. 28. The mRUP19 cDNA probe corresponds to nucleotides 5-365 of SEQ. ID. NO.:150.

[2718] RT-PCR analysis. To detect mRUP19 mRNA by RT-PCR, the following primers were used: 5'-ACTGTG-GTGGCTGTGGATAGGTA-3' (SEQ. ID. NO.:154; forward

primer) and 5'-GCAGATTGTGAGCTTGCGCTAGAA-3' (SEQ. ID. NO.:155; reverse primer). These are predicted to generate an mRUP19 product of 567 bp. The cDNA templates were prepared using a RETROscript™ First Strand Synthesis Kit for RT-PCR (Ambion, Inc., Cat. # 1710), according to the manufacturer's directions, except that duplicate reactions were done for each input RNA, and in one of these, reverse transcriptase was excluded from the reaction. 3 µl of each reaction was used for PCR. The reaction conditions for the PCR were as follows: 1 cycle @ 94° C./5 min., 25 cycles @ 94° C./30 sec, 59° C./30 sec, 72° C./1 min, and 1 cycle @ 72° C./5 min. The reactions were then analyzed on a 1% agarose gel. Pre-diff 3T3-L1, mouse 3T3 pre-adipocytes; Post-diff 3T3-L1, differentiated 3T3 adipocytes; β-TC-6, a mouse insulin-producing cell line; NIT-1, a mouse insulin-producing cell line.

#### Example 23

[2719] CART-ACTIVATED hRUP19 INHIBITS cAMP PRODUCTION IN MEMBRANES OF TRANSFECTED 293 CELLS

[2720] FIG. 30 presents a CART analysis of signal transduction by hRUP19. The analysis indicates that CART-activated hRUP19 inhibits cAMP production in membranes of transfected 293 cells.

[2721] Membranes were prepared as follows. 15 µg of the following expression plasmids were each introduced into 293 cells (one 15 cm dish per transfection) using Lipofectamine Reagent (Invitrogen, #18324-020) according to the manufacturer's instructions: pCMV-MCS (empty CMV expression plasmid), pCMV-hRUP19, pCMV-hRUP19-CART (same as pCMV-hRUP19, except that codon 219 has been converted from threonine to lysine). After 48 hours, a crude membrane preparation was prepared using standard protocols. Briefly, cells were washed with ice cold PBS, removed from the plate by scraping in the presence of a hypotonic Tris/EDTA buffer, fragmented using a pre-chilled dounce homogenizer, spun at low speed to pellet nuclei and intact cells, and finally, the supernatant is subjected to centrifugation at 20,000 rpm in a Beckman Avanti J-20 centrifuge. The membrane pellet is then resuspended at a protein concentration of 1 mg/ml for use in a membrane cyclase assay. The membrane cyclase assay was carried out as per the manufacturer's recommendation using an Adenylyl Cyclase Activation FlashPlate Assay Kit (Perkin Elmer Life Sciences, Inc., #SMP004B).

#### Example 24

[2722] SUMMARY: hRUP25, mRUP25, rRUP25, hRUP38, hRUP19, mRUP19, rRUP19, AND hRUP11

TABLE M

| Disclosed<br>Nicotinic<br>Acid<br>Receptor<br>Sub-Family<br>GPCRs | Expression<br>by<br>Adipocytes<br>or<br>Adipose | Gi-Coupled<br>(Lowers the<br>Level of<br>Intracellular<br>cAMP) | Shown to<br>Inhibit<br>Intracellular<br>Lipolysis | Agonist                                                           |
|-------------------------------------------------------------------|-------------------------------------------------|-----------------------------------------------------------------|---------------------------------------------------|-------------------------------------------------------------------|
| hRUP25                                                            | yes                                             | yes                                                             | yes                                               | nicotinic acid;<br>(-)-nicotine;<br>see FIG. 21;<br>(5-hydroxy-1- |

TABLE M-continued

| Disclosed<br>Nicotinic<br>Acid<br>Receptor<br>Sub-Family<br>GPCRs | Expression<br>by<br>Adipocytes<br>or<br>Adipose | Gi-Coupled<br>(Lowers the<br>Level of<br>Intracellular<br>cAMP) | Shown to<br>Inhibit<br>Intracellular<br>Lipolysis | Agonist                                                                                                                                                                                                                               |
|-------------------------------------------------------------------|-------------------------------------------------|-----------------------------------------------------------------|---------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| mRUP25                                                            | yes                                             | yes                                                             | n.d.                                              | methyl-<br>3-propyl-1H-<br>pyrazol-4-yl)-<br>pyridin-3-<br>yl-methanone<br>nicotinic acid<br>nicotinic acid<br>1-Isopropyl-<br>1H-<br>benzotriazole-<br>5-carboxylic<br>acid; 3-(5-<br>Bromo-2-<br>ethoxy-<br>phenyl)-acrylic<br>acid |
| rRUP25                                                            | yes                                             | yes                                                             | yes                                               |                                                                                                                                                                                                                                       |
| hRUP38                                                            | yes                                             | yes                                                             | yes                                               |                                                                                                                                                                                                                                       |
| hRUP11                                                            | n.d.                                            | yes                                                             | n.d.                                              | n.d.                                                                                                                                                                                                                                  |
| hRUP19                                                            | yes                                             | yes                                                             | n.d.                                              | n.d.                                                                                                                                                                                                                                  |
| mRUP19                                                            | yes                                             | n.d.                                                            | n.d.                                              | n.d.                                                                                                                                                                                                                                  |
| rRUP19                                                            | n.d.                                            | n.d.                                                            | n.d.                                              | n.d.                                                                                                                                                                                                                                  |

n.d.: not displayed

## Example 25

**[2723] RODENT DIABETES MODELS**

**[2724]** Rodent models of type 2 diabetes associated with obesity and insulin resistance have been developed. Genetic models such as db/db and ob/ob [see Diabetes (1982) 31:1-6] in mice and fa/fa in Zucker rats have been developed for understanding the pathophysiology of disease and for testing candidate therapeutic compounds [Diabetes (1983) 32:830-838; Annu Rep Sankyo Res Lab (1994) 46:1-57]. The homozygous animals, C57 BL/KsJ-db/db mice developed by Jackson Laboratory are obese, hyperglycemic, hyperinsulinemic and insulin resistant [J Clin Invest (1990) 85:962-967], whereas heterozygotes are lean and normoglycemic. In the db/db model, mice progressively develop insulinopenia with age, a feature commonly observed in late stages of human type 2 diabetes when sugar levels are insufficiently controlled. Since this model resembles that of human type 2 diabetes, the compounds of the present invention are tested for activities including, but not limited to, lowering of plasma glucose and triglycerides. Zucker (fa/fa) rats are severely obese, hyperinsulinemic, and insulin resistant [Coleman, Diabetes (1982) 31:1; E Shafir in Diabetes Mellitus, H Rifkin and D Porte, Jr, Eds [Elsevier Science Publishing Co, New York, ed. 4, (1990), pp. 299-340]], and the fa/fa mutation may be the rat equivalent of the murine db mutation [Friedman et al, Cell (1992) 69:217-220; Truett et al, Proc Natl Acad Sci USA (1991) 88:7806]. Tubby (tub/tub) mice are characterized by obesity, moderate insulin resistance and hyperinsulinemia without significant hyperglycemia [Coleman et al, Heredity (1990) 81:424].

**[2725]** The present invention encompasses the use of compounds of the invention for reducing the insulin resistance and hyperglycemia in any or all of the above rodent diabetes models, in humans with type 2 diabetes or other preferred metabolic-related disorders or disorders of lipid

metabolism described previously, or in models based on other mammals. Plasma glucose and insulin levels will be tested, as well as other factors including, but not limited to, plasma free fatty acids and triglycerides.

**[2726] In Vivo Assay for Anti-Hyperglycemic Activity of Compounds of the Invention**

**[2727]** Genetically altered obese diabetic mice (db/db) (male, 7-9 weeks old) are housed (7-9 mice/cage) under standard laboratory conditions at 22° C. and 50% relative humidity, and maintained on a diet of Purina rodent chow and water ad libitum. Prior to treatment, blood is collected from the tail vein of each animal and blood glucose concentrations are determined using One Touch Basic Glucose Monitor System (Lifescan). Mice that have plasma glucose levels between 250 to 500 mg/dl are used. Each treatment group consists of seven mice that are distributed so that the mean glucose levels are equivalent in each group at the start of the study. db/db mice are dosed by micro-osmotic pumps, inserted using isoflurane anesthesia, to provide compounds of the invention, saline, or an irrelevant compound to the mice subcutaneously (s.c.). Blood is sampled from the tail vein at intervals thereafter and analyzed for blood glucose concentrations. Significant differences between groups (comparing compounds of the invention to saline-treated) are evaluated using Student t-test.

**[2728]** The foregoing is provided by way of illustration and not limitation. Other illustrative rodent models for type 2 diabetes have been described [Moller D E, Nature (2001) 414:821-7 and references therein; and Reed M J et al., Diabetes, Obesity and Metabolism (1999) 1:75-86 and reference therein; the disclosure of each of which is hereby incorporated by reference in its entirety].

## Example 26

**[2729] MOUSE ATHEROSCLEROSIS MODEL**

**[2730]** Adiponectin-deficient mice generated through knocking out the adiponectin gene have been shown to be predisposed to atherosclerosis and to be insulin resistant. The mice are also a suitable model for ischemic heart disease [Matsuda, M et al. J Biol Chem (2002) July, and references cited therein, the disclosures of which are incorporated herein by reference in their entirety].

**[2731]** Adiponectin knockout mice are housed (7-9 mice/cage) under standard laboratory conditions at 22° C. and 50% relative humidity. The mice are dosed by micro-osmotic pumps, inserted using isoflurane anesthesia, to provide compounds of the invention, saline, or an irrelevant compound to the mice subcutaneously (s.c.). Neointimal thickening and ischemic heart disease are determined for different groups of mice sacrificed at different time intervals. Significant differences between groups (comparing compounds of the invention to saline-treated) are evaluated using Student t-test.

**[2732]** The foregoing mouse model of atherosclerosis is provided by way of illustration and not limitation. By way of further example, Apolipoprotein E-deficient mice have also been shown to be predisposed to atherosclerosis [Plump A S et al., Cell (1992) 71:343-353; the disclosure of which is hereby incorporated by reference in its entirety].

**[2733]** A preferred model is that of diet-induced atherosclerosis in C57BL/6J mice, an inbred strain known to be

susceptible to diet-induced atherosclerotic lesion formation. This model is well known to those persons of ordinary skill in the art [Kamada N et al., *J Atheroscler Thromb* (2001) 8:1-6; Garber D W et al., *J Lipid Res* (2001) 42:545-52; Smith J D et al., *J Intern Med* (1997) 242:99-109; the disclosure of each of which is hereby incorporated by reference in its entirety].

#### Example 27

##### [2734] TRANSGENIC MOUSE/RAT

##### [2735] hRUP38

[2736] The present invention also provides methods and compositions for the generation of mice and rats that express hRUP38 recombinant human antilipolytic GPCR polypeptide of the invention.

[2737] Methods of making transgenic animals such as mice and rats are well known to those of ordinary skill in the art, and any such method can be used in the present invention. Briefly, transgenic mammals can be produced, e.g., by transfecting a pluripotent stem cell such as an ES cell with a polynucleotide encoding hRUP38 polypeptide of the invention. Successfully transformed ES cells can then be introduced into an early stage embryo that is then implanted into the uterus of a mammal of the same species. In certain cases, the transformed ("transgenic") cells will comprise part of the germ line of the resulting animal and adult animals comprising the transgenic cells in the germ line can then be mated to other animals, thereby eventually producing a population of transgenic animals that have the transgene in each of their cells and that can stably transmit the transgene to each of their offspring. Other methods of introducing the polynucleotide can be used, for example introducing the polynucleotide encoding hRUP38 polypeptide of the invention into a fertilized egg or early stage embryo via microinjection. Alternatively, the transgene may be introduced into an animal by infection of zygotes with a retrovirus containing the transgene [Jaenisch, R, *Proc Natl Acad Sci USA* (1976) 73:1260-4]. Methods of making transgenic mammals are described, e.g., in Wall et al., *J Cell Biochem* (1992) 49:113-20; Hogan et al., in *Manipulating the Mouse Embryo. A Laboratory Manual*. (1986) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; in WO 91/08216; or in U.S. Pat. No. 4,736,866; all of which disclosures are hereby incorporated by reference in their entirety.

[2738] In preferred embodiments, expression of said gene is placed under the control of an essentially adipocyte specific promoter. In further preferred embodiments, said essentially adipocyte specific promoter is adiponectin gene promoter [Das, K et al., *Biochem Biophys Res Commun* (2001) 280:1120-9; Barth, N et al., *Diabetologia* (2002) 45:1425-1433; the disclosures of which are hereby incorporated by reference in its entirety]. In other further preferred embodiments, said essentially adipocyte specific promoter is resistin gene promoter [Hartman, H B et al. *J Biol Chem* (2002) 277:19754-61, which disclosure is hereby incorporated by reference in its entirety]. In other preferred embodiments, said essentially adipocyte specific promoter is aP2 [Felmer, R et al., *J Endocrinol* (2002) 175:487-498; the disclosure of which is hereby incorporated by reference in its entirety]. In other further preferred embodiments, expression of said gene is kept under the control of its endogenous promoter.

##### [2739] hRUP11

[2740] The present invention also provides methods and compositions for the generation of mice and rats that express hRUP11 recombinant human antilipolytic GPCR polypeptide of the invention.

[2741] Methods of making transgenic animals such as mice and rats are well known to those of ordinary skill in the art, and any such method can be used in the present invention. Briefly, transgenic mammals can be produced, e.g., by transfecting a pluripotent stem cell such as an ES cell with a polynucleotide encoding hRUP11 polypeptide of the invention. Successfully transformed ES cells can then be introduced into an early stage embryo that is then implanted into the uterus of a mammal of the same species. In certain cases, the transformed ("transgenic") cells will comprise part of the germ line of the resulting animal and adult animals comprising the transgenic cells in the germ line can then be mated to other animals, thereby eventually producing a population of transgenic animals that have the transgene in each of their cells and that can stably transmit the transgene to each of their offspring. Other methods of introducing the polynucleotide can be used, for example introducing the polynucleotide encoding hRUP11 polypeptide of the invention into a fertilized egg or early stage embryo via microinjection. Alternatively, the transgene may be introduced into an animal by infection of zygotes with a retrovirus containing the transgene [Jaenisch, R, *Proc Natl Acad Sci USA* (1976) 73:1260-4]. Methods of making transgenic mammals are described, e.g., in Wall et al., *J Cell Biochem* (1992) 49:113-20; Hogan et al., in *Manipulating the Mouse Embryo. A Laboratory Manual*. (1986) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; in WO 91/08216; or in U.S. Pat. No. 4,736,866; all of which disclosures are hereby incorporated by reference in their entirety.

[2742] In preferred embodiments, expression of said gene is placed under the control of an adipocyte specific promoter. In further preferred embodiments, said adipocyte specific promoter is adiponectin gene promoter [Das, K et al., *Biochem Biophys Res Commun* (2001) 280:1120-9; Barth, N et al., *Diabetologia* (2002) 45:1425-1433; the disclosures of which are hereby incorporated by reference in its entirety]. In other further preferred embodiments, said adipocyte specific promoter is resistin gene promoter [Hartman, H B et al. *J Biol Chem* (2002) 277:19754-61, which disclosure is hereby incorporated by reference in its entirety]. In other preferred embodiments, said adipocyte specific promoter is aP2 [Felmer, R et al., *J Endocrinol* (2002) 175:487-498; the disclosure of which is hereby incorporated by reference in its entirety]. In other further preferred embodiments, expression of said gene is kept under the control of its endogenous promoter.

#### Example 28

##### [2743] KNOCKOUT MOUSE/RAT

##### [2744] Mouse

##### [2745] RUP25

[2746] A preferred DNA construct will comprise, from 5'-end to 3'-end: (a) a first nucleotide sequence that is comprised in the mRUP25 genomic sequence; (b) a nucleotide sequence comprising a positive selection marker, such

as the marker for neomycin resistance (neo); and (c) a second nucleotide sequence that is comprised in the mRUP25 genomic sequence and is located on the genome downstream of the first mRUP25 nucleotide sequence (a). mRUP25 genomic sequence will be isolated using methods well known to those of ordinary skill in the art (Maniatis T et al., *Molecular Cloning: A Laboratory Manual* (1989) Cold Spring Harbor Laboratory; the disclosure of which is hereby incorporated by reference in its entirety).

[2747] In preferred embodiments, this DNA construct also comprises a negative selection marker located upstream the nucleotide sequence (a) or downstream the nucleotide sequence (c). Preferably, the negative selection marker comprises the thymidine kinase (tk) gene [Thomas et al., *Cell* (1986) 44:419-28], the hygromycin beta gene [Te Riele et al., *Nature* (1990) 348:649-51], the hprt gene [Van der Lugt et al., *Gene* (1991) 105:263-7; Reid et al., *Proc Natl Acad Sci USA* (1990) 87:4299-4303] or the Diphtheria toxin A fragment (Dt-A) gene [Nada et al., *Cell* (1993) 73:1125-35; Yagi et al., *Proc Natl Acad Sci USA* (1990) 87:9918-9922], which disclosures are hereby incorporated by reference in their entireties. Preferably, the positive selection marker is located within an mRUP25 exon sequence so as to interrupt the sequence encoding an mRUP25 polypeptide. These replacement vectors are described, for example, by Thomas et al., *Cell* (1986) 44:419-28; Thomas et al., *Cell* (1987) 51:503-12; Mansour et al., *Nature* (1988) 336:348-52; Kol-ler et al., *Annu Rev Immunol* (1992) 10:705-30; and U.S. Pat. No. 5,631,153; which disclosures are hereby incorporated by reference in their entireties.

[2748] The first and second nucleotide sequences (a) and (c) may be indifferently located within an mRUP25 regulatory sequence, an intronic sequence, an exon sequence or a sequence containing both regulatory and/or intronic and/or exon sequences. The size of the nucleotide sequences (a) and (c) ranges from 1 to 50 kb, preferably from 1 to 10 kb, more preferably from 2 to 6 kb, and most preferably from 2 to 4 kb.

[2749] Methods of making a knockout mouse are well known to those of ordinary skill in the art and have been used to successfully inactivate a wide range of genes.

#### [2750] RUP19

[2751] A preferred DNA construct will comprise, from 5'-end to 3'-end: (a) a first nucleotide sequence that is comprised in the mRUP19 genomic sequence; (b) a nucleotide sequence comprising a positive selection marker, such as the marker for neomycin resistance (neo); and (c) a second nucleotide sequence that is comprised in the mRUP19 genomic sequence and is located on the genome downstream of the first mRUP19 nucleotide sequence (a). mRUP19 genomic sequence will be isolated using methods well known to those of ordinary skill in the art (Maniatis T et al., *Molecular Cloning: A Laboratory Manual* (1989) Cold Spring Harbor Laboratory; the disclosure of which is hereby incorporated by reference in its entirety).

[2752] In preferred embodiments, this DNA construct also comprises a negative selection marker located upstream the nucleotide sequence (a) or downstream the nucleotide sequence (c). Preferably, the negative selection marker comprises the thymidine kinase (tk) gene [Thomas et al., *Cell* (1986) 44:419-28], the hygromycin beta gene [Te Riele et

al., *Nature* (1990) 348:649-51], the hprt gene [Van der Lugt et al., *Gene* (1991) 105:263-7; Reid et al., *Proc Natl Acad Sci USA* (1990) 87:4299-4303] or the Diphtheria toxin A fragment (Dt-A) gene [Nada et al., *Cell* (1993) 73:1125-35; Yagi et al., *Proc Natl Acad Sci USA* (1990) 87:9918-9922], which disclosures are hereby incorporated by reference in their entireties. Preferably, the positive selection marker is located within an mRUP19 exon sequence so as to interrupt the sequence encoding an mRUP19 polypeptide. These replacement vectors are described, for example, by Thomas et al., *Cell* (1986) 44:419-28; Thomas et al., *Cell* (1987) 51:503-12; Mansour et al., *Nature* (1988) 336:348-52; Kol-ler et al., *Annu Rev Immunol* (1992) 10:705-30; and U.S. Pat. No. 5,631,153; which disclosures are hereby incorporated by reference in their entireties.

[2753] The first and second nucleotide sequences (a) and (c) may be indifferently located within an mRUP19 regulatory sequence, an intronic sequence, an exon sequence or a sequence containing both regulatory and/or intronic and/or exon sequences. The size of the nucleotide sequences (a) and (c) ranges from 1 to 50 kb, preferably from 1 to 10 kb, more preferably from 2 to 6 kb, and most preferably from 2 to 4 kb.

[2754] Methods of making a knockout mouse are well known to those of ordinary skill in the art and have been used to successfully inactivate a wide range of genes.

#### [2755] Rat

##### [2756] RUP25

[2757] Gene targeting technology for the rat is less robust than that for the mouse and is an area of active interest. One approach will be to inactivate rRUP25 gene in rat embryonic stem cell (ESC)-like cells and then inject cells with inactivated rRUP25 gene into rat blastocysts generated after fusion of two-cell embryos [Krivokharchenko et al., *Mol Reprod Dev* (2002) 61:460-5].

[2758] An alternative approach will be to inactivate rRUP25 gene in rat ESC-like cells and then transfer the nucleus of the rat ESC-like cells having inactivated rRUP25 gene into enucleated oocytes [Sato K et al., *Hum Cell* (2001) 14:301-4; Wakayama and Yanagimachi, *Semin Cell Dev Biol* (1999) 10:253-8; Hochedlinger and Jaenisch, *Nature* (2002) 415:1035-8; Yanagimachi, *Mol Cell Endocrinol* (2002) 187:241-8; the disclosures of which are incorporated herein by reference in their entireties].

[2759] rRUP25 genomic sequence can be isolated using methods well known to those of ordinary skill in the art (Maniatis T et al., *Molecular Cloning: A Laboratory Manual* (1989) Cold Spring Harbor Laboratory; the disclosure of which is hereby incorporated by reference in its entirety).

##### [2760] RUP19

[2761] Gene targeting technology for the rat is less robust than that for the mouse and is an area of active interest. One approach will be to inactivate rRUP19 gene in rat embryonic stem cell (ESC)-like cells and then inject cells with inactivated rRUP19 gene into rat blastocysts generated after fusion of two-cell embryos [Krivokharchenko et al., *Mol Reprod Dev* (2002) 61:460-5].

[2762] An alternative approach will be to inactivate rRUP19 gene in rat ESC-like cells and then transfer the

nucleus of the rat ESC-like cells having inactivated rRUP25 gene into enucleated oocytes [Sato K et al., *Hum Cell* (2001) 14:301-4; Wakayama and Yanagimachi, *Semin Cell Dev Biol* (1999) 10:253-8; Hochedlinger and Jaenisch, *Nature* (2002) 415:1035-8; Yanagimachi, *Mol Cell Endocrinol* (2002) 187:241-8; the disclosures of which are incorporated herein by reference in their entirety].

[2763] rRUP19 genomic sequence can be isolated using methods well known to those of ordinary skill in the art (Maniatis T et al., *Molecular Cloning: A Laboratory Manual* (1989) Cold Spring Harbor Laboratory; the disclosure of which is hereby incorporated by reference in its entirety).

#### [2764] CRE-LOXP SYSTEM

[2765] These new DNA constructs make use of the site specific recombination system of the P1 phage. The P1 phage possesses a recombinase called Cre that interacts with a 34 base pair loxP site. The loxP site is composed of two palindromic sequences of 13 bp separated by an 8 bp conserved sequence [Hoess R H et al, *Nucleic Acids Res* (1986) 14:2287-300; which disclosure is hereby incorporated by reference in its entirety]. The recombination by the Cre enzyme between two loxP sites having an identical orientation leads to the deletion of the DNA fragment.

[2766] The Cre-loxP system used in combination with a homologous recombination technique has been first described by Gu et al. [Gu H et al., *Cell* (1993) 73:1155-64; Gu H et al., *Science* (1994) 265:103-6; which disclosures are hereby incorporated by reference in their entirety]. Briefly, a nucleotide sequence of interest to be inserted in a targeted location of the genome harbors at least two loxP sites in the same orientation and located at the respective ends of a nucleotide sequence to be excised from the recombinant genome. The excision event requires the presence of the recombinase (Cre) enzyme within the nucleus of the recombinant cell host. The recombinase enzyme may be brought at the desired time either by (a) incubating the recombinant cell hosts in a culture medium containing this enzyme, by injecting the Cre enzyme directly into the desired cell, such as by lipofection of the enzyme into the cells, such as described by Baubonis et al. [Baubonis W and Sauer B, *Nucleic Acids Res* (1993) 21:2025-9; which disclosure is hereby incorporated by reference in its entirety]; (b) transfecting the cell host with a vector comprising the Cre Coding sequence operably linked to a promoter functional in the recombinant cell host, which promoter being optionally inducible, said vector being introduced in the recombinant cell host, such as described by Gu et al. [Gu H et al., *Cell* (1993) 73:1155-64; **which disclosure is hereby incorporated by reference in its entirety**] and Sauer et al. [Sauer B and Henderson N, *Proc Natl Acad Sci USA* (1988) 85:5166-70; which disclosure is hereby incorporated by reference in its entirety]; (c) introducing into the genome of the cell host a polynucleotide comprising the Cre coding sequence operably linked to a promoter functional in the recombinant cell host, which promoter is optionally inducible, and said polynucleotide being inserted in the genome of the cell host either by a random insertion event or an homologous recombination event, such as described by Gu et al. [Gu H et al., *Science* (1994) 265:103-6; which disclosure is hereby incorporated by reference in its entirety].

[2767] Vectors and methods using the Cre-loxP system are describe by Zou et al. (1994), which disclosure is hereby incorporated by reference in its entirety.

[2768] In preferred embodiments of the invention, Cre is introduced into the genome of the cell host by strategy (c) above, wherein said promoter is essentially adipocyte specific and leads to essentially adipocyte specific knockout of (loxP-flanked) mRUP25 or mRUP19 in the mouse or rRUP25 or rRUP19 in the rat. In some embodiments, said essentially adipocyte specific promoter is adiponectin gene promoter [Das, K et al., *Biochem Biophys Res Commun* (2001) 280:1120-9; Barth, N et al., *Diabetologia* (2002) 45:1425-1433; the disclosures of which are hereby incorporated by reference in its entirety]. In some embodiments, said essentially adipocyte specific promoter is resistin gene promoter [Hartman, H B et al. *J Biol Chem* (2002) 277:19754-61, which disclosure is hereby incorporated by reference in its entirety]. In some embodiments, said essentially adipocyte specific promoter is aP2 [Felmer, R et al., *J Endocrinol* (2002) 175:487-498; the disclosure of which is hereby incorporated by reference in its entirety]. Methods of constructing a lineage-specific knockout are well known to persons of ordinary skill in the art, as illustrated but not intended to be limited by: Kuhn R and Torres R M, *Methods Mol Biol* (2002) 180:175-204; Sauer B, *Methods* (1998) 14:381-92; Gutstein D E et al., *Circulation Research* (2001) 88:333; Minamino T et al., *Circulation Research* (2001) 88:587; and Bex A et al., *J Urol* (2002) 168:2641-2644; the disclosure of each of which is hereby incorporated by reference in its entirety.

#### Example 29

##### [2769] FLUOROMETRIC IMAGING PLATE READER (FLIPR) ASSAY FOR THE MEASUREMENT OF INTRACELLULAR CALCIUM CONCENTRATION

[2770] Target Receptor (experimental) and pCMV (negative control) stably transfected cells from respective clonal lines are seeded into poly-D-lysine pretreated 96-well plates (Becton-Dickinson, #356640) at  $5.5 \times 10^4$  cells/well with complete culture medium (DMEM with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate) for assay the next day. To prepare Fluo4- $\mu$ M (Molecular Probe, #F14202) incubation buffer stock, 1 mg Fluo4- $\mu$ M is dissolved in 467  $\mu$ l DMSO and 467  $\mu$ l Fluoronic acid (Molecular Probe, #P3000) to give a 1 mM stock solution that can be stored at  $-20^\circ$  C. for a month. Fluo4- $\mu$ M is a fluorescent calcium indicator dye.

[2771] Candidate compounds are prepared in wash buffer (1 $\times$ HBSS/2.5 mM Probenicid/20 mM HEPES at pH 7.4).

[2772] At the time of assay, culture medium is removed from the wells and the cells are loaded with 100  $\mu$ l of 4  $\mu$ M Fluo4- $\mu$ M/2.5 mM Probenicid (Sigma, #P8761)/20 mM HEPES/complete medium at pH 7.4. Incubation at  $37^\circ$  C./5% CO<sub>2</sub> is allowed to proceed for 60 min.

[2773] After the 1 hr incubation, the Fluo4- $\mu$ M incubation buffer is removed and the cells are washed 2 $\times$  with 100  $\mu$ l wash buffer. In each well is left 100  $\mu$ l wash buffer. The plate is returned to the incubator at  $37^\circ$  C./5% CO<sub>2</sub> for 60 min.

[2774] FLIPR (Fluorometric Imaging Plate Reader; Molecular Device) is programmed to add 50  $\mu$ l candidate compound on the 30<sup>th</sup> second and to record transient changes in intracellular calcium concentration ([Ca<sup>2+</sup>]) evoked by the candidate compound for another 150 seconds. Total fluorescence change counts are used to determine

agonist activity using the FLIPR software. The instrument software normalizes the fluorescent reading to give equivalent initial readings at zero.

[2775] In some embodiments, the cells comprising Target Receptor further comprise promiscuous G alpha 15/16 or the chimeric Gq/Gi alpha unit.

[2776] Although the foregoing provides a FLIPR assay for agonist activity using stably transfected cells, a person of ordinary skill in the art would readily be able to modify the assay in order to characterize antagonist activity. Said person of ordinary skill in the art would also readily appreciate that, alternatively, transiently transfected cells could be used.

#### Example 30

##### [2777] IN VIVO PIG MODEL OF HDL-CHOLESTEROL AND ATHEROSCLEROSIS.

[2778] The utility of a modulator of the present invention as a medical agent in the prevention or treatment of a high total cholesterol/HDL-cholesterol ratio and conditions relating thereto is demonstrated, without limitation, by the activity of the modulator in lowering the ratio of total cholesterol to HDL-cholesterol, in elevating HDL-cholesterol, or in protection from atherosclerosis in an in vivo pig model. Pigs are used as an animal model because they reflect human physiology, especially lipid metabolism, more closely than most other animal models. An illustrative in vivo pig model not intended to be limiting is presented here.

[2779] Yorkshire albino pigs (body weight  $25.5 \pm 4$  kg) are fed a saturated fatty acid rich and cholesterol rich (SFA-CHO) diet during 50 days (1 kg chow  $35 \text{ kg}^{-1}$  pig weight), composed of standard chow supplemented with 2% cholesterol and 20% beef tallow [Royo T et al., *European Journal of Clinical Investigation* (2000) 30:843-52; which disclosure is hereby incorporated by reference in its entirety]. Saturated to unsaturated fatty acid ratio is modified from 0.6 in normal pig chow to 1.12 in the SFA-CHO diet. Animals are divided into two groups, one group (n=8) fed with the SFA-CHO diet and treated with placebo and one group (n=8) fed with the SFA-CHO diet and treated with the modulator ( $3.0 \text{ mg kg}^{-1}$ ). Control animals are fed a standard chow for a period of 50 days. Blood samples are collected at baseline (2 days after the reception of the animals), and 50 days after the initiation of the diet. Blood lipids are analyzed. The animals are sacrificed and necropsied.

[2780] Alternatively, the foregoing analysis comprises a plurality of groups each treated with a different dose of the modulator. Preferred said doses are selected from the group consisting of:  $0.1 \text{ mg kg}^{-1}$ ,  $0.3 \text{ mg kg}^{-1}$ ,  $1.0 \text{ mg kg}^{-1}$ ,  $3.0 \text{ mg kg}^{-1}$ ,  $10 \text{ mg kg}^{-1}$ ,  $30 \text{ mg kg}^{-1}$  and  $100 \text{ mg kg}^{-1}$ . Alternatively, the foregoing analysis is carried out at a plurality of timepoints. Preferred said timepoints are selected from the group consisting of 10 weeks, 20 weeks, 30 weeks, 40 weeks, and 50 weeks.

##### [2781] HDL-Cholesterol

[2782] Blood is collected in trisodium citrate (3.8%, 1:10). Plasma is obtained after centrifugation (1200 g 15 min) and immediately processed. Total cholesterol, HDL-cholesterol, and LDL-cholesterol are measured using the automatic analyzer Kodak Ektachem DT System (Eastman Kodak Company, Rochester, N.Y., USA). Samples with value

parameters above the range are diluted with the solution supplied by the manufacturer and then re-analyzed. The total cholesterol/HDL-cholesterol ratio is determined. Comparison is made of the level of HDL-cholesterol between groups. Comparison is made of the total cholesterol/HDL-cholesterol ratio between groups.

[2783] Elevation of HDL-cholesterol or reduction of the total cholesterol/HDL-cholesterol ratio on administration of the modulator is taken as indicative of the modulator having the aforesaid utility.

##### [2784] Atherosclerosis

[2785] The thoracic and abdominal aortas are removed intact, opened longitudinally along the ventral surface, and fixed in neutral-buffered formalin after excision of samples from standard sites in the thoracic and abdominal aorta for histological examination and lipid composition and synthesis studies. After fixation, the whole aortas are stained with Sudan IV and pinned out flat, and digital images are obtained with a TV camera connected to a computerized image analysis system (Image Pro Plus; Media Cybernetics, Silver Spring, Md.) to determine the percentage of aortic surface involved with atherosclerotic lesions [Gerrity R G et al, *Diabetes* (2001) 50:1654-65; Cornhill J F et al, *Arteriosclerosis, Thrombosis, and Vascular Biology* (1985) 5:415-26; which disclosures are hereby incorporated by reference in their entirety]. Comparison is made between groups of the percentage of aortic surface involved with atherosclerotic lesions.

[2786] Reduction of the percentage of aortic surface involved with atherosclerotic lesions on administration of the modulator is taken as indicative of the modulator having the aforesaid utility.

##### [2787] Plasma Free Fatty Acids

[2788] It would be readily apparent to anyone of ordinary skill in the art that the foregoing in vivo pig model is easily modified in order to address, without limitation, the activity of the modulator in lowering plasma free fatty acids.

#### Example 31

##### [2789] MEASUREMENT OF PLASMA FREE FATTY ACIDS (FFA) IN RATS ADMINISTERED NIACIN.

[2790] Catheters were surgically implanted into the jugular veins of male Sprague Dawley rats. The following day rats were deprived of food and approximately 16 hours later were given interperitoneal (IP) injections of either vehicle, or niacin [NA] at  $15 \text{ mg/kg}$ ,  $30 \text{ mg/kg}$  or  $45 \text{ mg/kg}$  body weight. Blood was drawn ( $\sim 200 \text{ ml}$ ) at the indicated time points and plasma was isolated following centrifugation. Plasma FFA were then measured via the NEFA C kit according to manufacturer specifications (Wako Chemicals USA, Inc). All three concentrations of niacin significantly decrease plasma FFA levels. [See, FIG. 32.] \* Indicates a statistical increase in plasma FFA versus vehicle control indicating a rebound effect in the  $45 \text{ mg/kg}$  niacin treated rats at 3 hr (i.e., plasma FFA levels go beyond basal levels following inhibition).

[2791] By way of illustration and not limitation, said rat model has utility as an in vivo animal model for modulators of RUP25 provided by the invention. By way of illustration and not limitation, said rat model also has utility as an in

vivo animal model for modulators of RUP19 provided by the invention. By way of illustration and not limitation, hRUP38 and hRUP11 transgenic rats provided by the invention have utility as in vivo animal models for modulators of hRUP38 and hRUP11 provided by the invention.

[2792] To show that a modulator of hRUP25 other than niacin behaves similarly, an analogous assay is set up using said modulator of hRUP25. Preferred said modulator is an agonist.

Sense primer: CTATGTGAGGCGTTCAGACTGGAAGTTTG; (SEQ. ID. NO.:160)

Antisense primer: CAAACTTCAGTCTGAACGCCTCACATAG. (SEQ. ID. NO.:161)

[2793] To show that a modulator of hRUP38 behaves similarly, an analogous assay is set up using said modulator of hRUP38, wherein the rat is transgenic for hRUP38. Preferred said modulator is an agonist.

[2794] To show that a modulator of hRUP19 behaves similarly, an analogous assay is set up using said modulator of hRUP19. Preferred said modulator is an agonist.

[2795] To show that a modulator of hRUP11 behaves similarly, an analogous assay is set up using said modulator of hRUP11, wherein the rat is transgenic for hRUP11. Preferred said modulator is an agonist.

### Example 32

#### [2796] PREPARATION OF NON-ENDOGENOUS, ENHANCED-FOR-AGONIST [EFA-] GPCRS

[2797] Constitutive activity of a GPCR reduces the available window for identification of an agonist of the GPCR, where said window is taken here to be the difference in assay readout between the GPCR in the absence of agonist and the GPCR in the presence of a known agonist. A mutant of a constitutively active GPCR that is less constitutively active but comparably or more responsive to said known agonist [Enhanced-for-Agonist GPCR; EFA-GPCR] would have novel utility for the identification of modulators of said GPCR, particularly agonists.

[2798] EFA-GPCR is disclosed herewith as a mutant GPCR polypeptide that consists of 1, 2, 3, 4, or 5 amino acid substitutions, deletions, or insertions relative to the amino acid sequence of an endogenous GPCR polypeptide having constitutive activity, wherein the agonist screening window of the mutant GPCR is expanded by greater than 20%, greater than 25%, greater than 30%, greater than 31%, greater than 32%, greater than 33%, greater than 34%, greater than 35%, greater than 36%, greater than 37%, greater than 38%, greater than 39%, or greater than 40% relative to that of said endogenous GPCR.

[2799] As the GPCRs of the invention are constitutively active to a significant degree, an EFA version of said GPCRs would have novel utility in screening for modulators of said GPCRs, particularly agonists.

[2800] Herewith Applicant discloses EFA-hRUP25 “hRUP25-S91” polynucleotide of SEQ. ID. NO.:158 and the encoded polypeptide of SEQ. ID. NO.:159. EFA-hRUP25 polypeptide “hRUP25-S91” differs from endogenous hRUP25 polypeptide of SEQ. ID. NO.:36 through substitution of the tryptophan at amino acid position 91 with serine. Mutagenesis was performed using QuickChange Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer’s instructions. The mutagenesis primers had the following sequences:

[2801] Activity of EFA-hRUP25 “hRUP25-S91” polypeptide is presented in **FIG. 33**. The window for agonist screening is 44% larger for EFA-hRUP25 (433-210 pmol cAMP/mg protein) relative to that of endogenous hRUP25 (329-174 pmol cAMP/mg protein).  $\{[(433-210=223)/(329-174=155)]=1.44=144\%.$

[2802] The invention relates in part to an isolated EFA-hRUP25 polypeptide comprising the amino acid sequence of SEQ. ID. NO.:159 and to isolated polynucleotide encoding said EFA-hRUP25 polypeptide. A preferred EFA-hRUP25 polynucleotide has the nucleotide sequence of SEQ. ID. NO.:158.

[2803] The invention further relates in part to isolated EFA-hRUP25 polypeptide comprising an amino acid sequence consisting of 1, 2, 3, or 4 amino acid substitutions, deletions, or insertions relative to the amino acid sequence of SEQ. ID. NO.:36 in addition to the substitution of serine for tryptophan at amino acid position 91, as well as to isolated polynucleotide encoding said EFA-hRUP25 polypeptide.

[2804] The invention also relates in part to a method of using a polypeptide comprising an EFA-hRUP25 amino acid sequence to identify a modulator of EFA-hRUP25. The invention also relates to a method of using a polypeptide comprising an EFA-hRUP25 amino acid sequence to identify a modulator of lipolysis. Preferred said modulator of EFA-hRUP25 is an agonist.

[2805] Other embodiments encompass EFA-mRUP25 and EFA-rRUP25 polypeptide and polynucleotide. Also preferred is a method of using EFA-mRUP25 or EFA-rRUP25 to identify a modulator of EFA-mRUP25 or EFA-rRUP25. Also preferred is a method of using EFA-mRUP25 or EFA-rRUP25 to identify a modulator of lipolysis. Preferred said modulator of EFA-mRUP25 or EFA-rRUP25 is an agonist.

[2806] Other embodiments encompass EFA-RUP38 polypeptide and polynucleotide. Also preferred is a method of using EFA-RUP38 to identify a modulator of EFA-RUP38. Also preferred is a method of using EFA-RUP38 to identify a modulator of lipolysis. Preferred said modulator of EFA-RUP38 is an agonist.

[2807] Other embodiments encompass EFA-hRUP19 polypeptide and polynucleotide. Also preferred is a method of using EFA-hRUP19 to identify a modulator of EFA-

hRUP19. Also preferred is a method of using EFA-hRUP19 to identify a modulator of lipolysis. Preferred said modulator of EFA-hRUP19 is an agonist.

[2808] Other embodiments encompass EFA-mRUP19 and EFA-rRUP19 polypeptide and polynucleotide. Also preferred is a method of using EFA-mRUP19 or EFA-rRUP19 to identify a modulator of EFA-mRUP19 or EFA-rRUP19. Also preferred is a method of using EFA-mRUP19 or EFA-rRUP19 to identify a modulator of lipolysis. Preferred said modulator of EFA-mRUP19 or EFA-rRUP19 is an agonist.

[2809] Other embodiments encompass EFA-RUP11 polypeptide and polynucleotide. Also preferred is a method of using EFA-RUP11 to identify a modulator of EFA-RUP11. Also preferred is a method of using EFA-RUP11 to identify a modulator of lipolysis. Preferred said modulator of RUP11 is an agonist.

[2810] The invention also provides for a method of making an EFA mutant of an endogenous GPCR polypeptide having constitutive activity, comprising the steps of:

[2811] (a) introducing 1, 2, 3, 4, or 5 substitutions, insertions, or deletions into the amino acid sequence of the endogenous GPCR polypeptide;

[2812] (b) measuring the activity of the mutant GPCR of (a) in the absence of agonist and in the presence of a known agonist;

[2813] (c) measuring the activity of the endogenous GPCR in the absence of agonist and in the presence of said known agonist; and

[2814] (d) comparing (b) and (c);

[2815] wherein a determination that the agonist screening window of (b) is greater than 20%, greater than 25%, greater than 30%, greater than 31%, greater than 32%, greater than 33%, greater than 34%, greater than 35%, greater than 36%, greater than 37%, greater than 38%, greater than 39%, or greater than 40% than that of (c) identifies the mutant resulting from (a) to be an EFA mutant of the endogenous GPCR.

[2816] In some embodiments, the agonist screening window of (b) is greater than 20% than that of (c).

[2817] In some preferred embodiments, said known agonist is nicotinic acid.

[2818] Methods of carrying out site-specific mutagenesis are well known to those of ordinary skill in the art. [See, e.g., in Maniatis T et al., *Molecular Cloning: A Laboratory Manual* (1989) Cold Spring Harbor Laboratory; the disclosure of which is hereby incorporated by reference in its entirety]. Many commercial kits for carrying out site-specific mutagenesis are well known to those of ordinary skill

in the art and are readily available. Those skilled in the art are credited with the ability to select techniques for mutation of a nucleic acid sequence.

### Example 33

#### [2819] ORAL BIOAVAILABILITY

[2820] Based upon the in vivo data developed, as for example by way of illustration and not limitation data through the rat model of Example 31 supra, oral bioavailability of a modulator of the invention is determined. The modulator is administered by oral gavage at doses ranging from 0.1 mg kg<sup>-1</sup> to 100 mg kg<sup>-1</sup>. Oral administration of the modulator is shown to reduce the level of plasma free fatty acids. The effect of the modulator is shown to be dose-dependent and comparable to the effect after intraperitoneal administration. The dose of modulator required to achieve half-maximal reduction of plasma free fatty acids through oral administration is compared to the dose of modulator required to achieve half-maximal reduction of plasma free fatty acids through intraperitoneal administration. By way of illustration, if said oral dose is twice said intraperitoneal dose, then the oral bioavailability of the modulator is taken to be 50%. More generally, if said oral dose is  $\theta$  mg kg<sup>-1</sup> and said intraperitoneal dose is  $\rho$  mg kg<sup>-1</sup>, then the oral bioavailability of the modulator as a percentage is taken to be  $[(\rho/\theta) \times 100]$ .

[2821] It is readily envisioned that the reference route of administration may be other than intraperitoneal. In some embodiments, said reference route of administration is intravenous.

[2822] It would be readily apparent to anyone of ordinary skill in the art that the aforesaid determination could be modified to utilize a different in vivo animal model other than normal Sprague Dawley rats. It would also be readily apparent to anyone of ordinary skill in the art that the bioactivity readout in the aforesaid determination could be a parameter other than plasma free fatty acids.

[2823] Alternative, physicochemico analytical approaches for assessing oral bioavailability are well known to those of ordinary skill in the art [see, e.g., without limitation: Wong P C et al., *Cardiovasc Drug Rev* (2002) 20:137-52; and Buchan P et al., *Headache* (2002) Suppl 2:S54-62; the disclosure of each of which is hereby incorporated by reference in its entirety]. By way of further illustration and not limitation, said alternative analytical approaches may comprise liquid chromatography-tandem mass spectrometry [Chavez-Eng C M et al., *J Chromatogr B Analyt Technol Biomed Life Sci* (2002) 767:117-29; Jetter A et al., *Clin Pharmacol Ther* (2002) 71:21-9; Zimmerman J J et al., *J Clin Pharmacol* (1999) 39:1155-61; and Barrish A et al., *Rapid Commun Mass Spectrom* (1996) 10:1033-7; the disclosure of each of which is hereby incorporated by reference in its entirety].

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#### SEQUENCE LISTING

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<212> TYPE: DNA

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&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 1

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&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 2

```

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

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Ser | Val | Asn | Tyr | Leu | Arg | Thr | Val | Ser | Leu | Tyr | Val | Ser | Thr | Asn |
| 130 |     |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Ala | Leu | Leu | Ala | Ile | Ala | Ile | Asp | Arg | Tyr | Leu | Ala | Ile | Val | His | Pro |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
| Leu | Lys | Pro | Arg | Met | Asn | Tyr | Gln | Thr | Ala | Ser | Phe | Leu | Ile | Ala | Leu |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Val | Trp | Met | Val | Ser | Ile | Leu | Ile | Ala | Ile | Pro | Ser | Ala | Tyr | Phe | Ala |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Thr | Glu | Thr | Val | Leu | Phe | Ile | Val | Lys | Ser | Gln | Glu | Lys | Ile | Phe | Cys |
|     | 195 |     |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Gly | Gln | Ile | Trp | Pro | Val | Asp | Gln | Gln | Leu | Tyr | Tyr | Lys | Ser | Tyr | Phe |
| 210 |     |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| Leu | Phe | Ile | Phe | Gly | Val | Glu | Phe | Val | Gly | Pro | Val | Val | Thr | Met | Thr |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Leu | Cys | Tyr | Ala | Arg | Ile | Ser | Arg | Glu | Leu | Trp | Phe | Lys | Ala | Val | Pro |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Gly | Phe | Gln | Thr | Glu | Gln | Ile | Arg | Lys | Arg | Leu | Arg | Cys | Arg | Arg | Lys |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Thr | Val | Leu | Val | Leu | Met | Cys | Ile | Leu | Thr | Ala | Tyr | Val | Leu | Cys | Trp |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     |     | 285 |     |     |
| Ala | Pro | Phe | Tyr | Gly | Phe | Thr | Ile | Val | Arg | Asp | Phe | Phe | Pro | Thr | Val |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| Phe | Val | Lys | Glu | Lys | His | Tyr | Leu | Thr | Ala | Phe | Tyr | Val | Val | Glu | Cys |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
| Ile | Ala | Met | Ser | Asn | Ser | Met | Ile | Asn | Thr | Val | Cys | Phe | Val | Thr | Val |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
| Lys | Asn | Asn | Thr | Met | Lys | Tyr | Phe | Lys | Lys | Met | Met | Leu | Leu | His | Trp |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |
| Arg | Pro | Ser | Gln | Arg | Gly | Ser | Lys | Ser | Ser | Ala | Asp | Leu | Asp | Leu | Arg |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
| Thr | Asn | Gly | Val | Pro | Thr | Thr | Glu | Glu | Val | Asp | Cys | Ile | Arg | Leu | Lys |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |

&lt;210&gt; SEQ ID NO 3

&lt;211&gt; LENGTH: 1260

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 3

|            |            |            |            |            |            |     |
|------------|------------|------------|------------|------------|------------|-----|
| atgctggcag | ctgcctttgc | agactctaac | tccagcagca | tgaatgtgtc | ctttgctcac | 60  |
| ctccactttg | ccggagggta | cctgcctctc | gattcccagg | actggagaac | catcatcccg | 120 |
| gctctcttgg | tggtgtctgt | cctggtgggc | ttcgtgggaa | acctgtgtgt | gattggcatc | 180 |
| ctccttcaca | atgcttggaa | aggaaagcca | tccatgatcc | actccctgat | tctgaatctc | 240 |
| agcctggctg | atctctccct | cctgctgttt | tctgcaceta | tccgagctac | ggcgtactcc | 300 |
| aaaagtgttt | gggactctag | ctggtttgtc | tgcaagtcct | ctgactgggt | tatccacaca | 360 |
| tgcatggcag | ccaagagcct | gacaatcggt | gtggtggcca | aagtatgctt | catgtatgca | 420 |
| agtgaccag  | ccaagcaagt | gagtatccac | aactacacca | tctggtcagt | gctgggtggc | 480 |
| atctggactg | tggttagcct | gttacccttg | ccggaatggt | tctttagcac | catcaggcat | 540 |
| catgaagggt | tggaatgtg  | cctcgtggat | gtaccagctg | tggtgaaga  | gtttatgtcg | 600 |

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atgttttgga agctctaccc actcctggca ttggccttc cattatTTTT tgccagcttt 660
tatttctgga gagcttatga ccaatgtaaa aaacgaggaa ctaagactca aaatcttaga 720
aaccagatac gctcaaaagca agtcacagtg atgtctgtga gcattgccat catctctgct 780
ctcttggtgc tccccgaatg ggtagcttgg ctgtgggtat ggcattctgaa ggctgcaggc 840
ccggcccccac cacaaggttt catagccctg tctcaagtct tgatgttttc catctcttca 900
gcaaatcctc tcatttttct tgtgatgtcg gaagagttca ggaaggctt gaaagggtga 960
tgaaaatgga tgataaccaa aaaacctcca actgtctcag agtctcagga aacaccagct 1020
ggcaactcag agggctcttc tgacaaggtt ccatctccag aatccccagc atccatacca 1080
gaaaaagaga aaccagctc tccctcctct ggcaaaggga aaactgagaa ggcagagatt 1140
cccatccttc ctgacgtaga gcagttttgg catgagaggg acacagtccc ttctgtacag 1200
gacaatgacc ctatcccctg ggaacatgaa gatcaagaga caggggaagg tgtaaataag 1260

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&lt;210&gt; SEQ ID NO 4

&lt;211&gt; LENGTH: 419

&lt;212&gt; TYPE: PR

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 4

```

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

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|             | 245                                                 | 250 | 255 |
|-------------|-----------------------------------------------------|-----|-----|
| Ile Ile Ser | Ala Leu Leu Trp Leu Pro Glu Trp Val Ala Trp Leu Trp |     |     |
|             | 260                                                 | 265 | 270 |
| Val Trp His | Leu Lys Ala Ala Gly Pro Ala Pro Pro Gln Gly Phe Ile |     |     |
|             | 275                                                 | 280 | 285 |
| Ala Leu Ser | Gln Val Leu Met Phe Ser Ile Ser Ser Ala Asn Pro Leu |     |     |
|             | 290                                                 | 295 | 300 |
| Ile Phe Leu | Val Met Ser Glu Glu Phe Arg Glu Gly Leu Lys Gly Val |     |     |
| 305         | 310                                                 | 315 | 320 |
| Trp Lys Trp | Met Ile Thr Lys Lys Pro Pro Thr Val Ser Glu Ser Gln |     |     |
|             | 325                                                 | 330 | 335 |
| Glu Thr Pro | Ala Gly Asn Ser Glu Gly Leu Pro Asp Lys Val Pro Ser |     |     |
|             | 340                                                 | 345 | 350 |
| Pro Glu Ser | Pro Ala Ser Ile Pro Glu Lys Glu Lys Pro Ser Ser Pro |     |     |
|             | 355                                                 | 360 | 365 |
| Ser Ser Gly | Lys Gly Lys Thr Glu Lys Ala Glu Ile Pro Ile Leu Pro |     |     |
|             | 370                                                 | 375 | 380 |
| Asp Val Glu | Gln Phe Trp His Glu Arg Asp Thr Val Pro Ser Val Gln |     |     |
| 385         | 390                                                 | 395 | 400 |
| Asp Asn Asp | Pro Ile Pro Trp Glu His Glu Asp Gln Glu Thr Gly Glu |     |     |
|             | 405                                                 | 410 | 415 |

Gly Val Lys

&lt;210&gt; SEQ ID NO 5

&lt;211&gt; LENGTH: 1014

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 5

```

atggggaacg attctgtcag ctacgagtat ggggattaca gcgacctctc ggaccgccct    60
gtggactgcc tggatggcgc ctgcctggcc atcgaccgcg tgcgcgtggc cccgctccca    120
ctgtatgccg ccatcttcct ggtgggggtg ccgggcaatg ccatggtggc ctgggtggct    180
gggaaggtgg cccgcccggag ggtgggtgcc acctggttgc tccacctggc cgtggcggat    240
ttgctgtgct gtttgtctct gcccatcctg gcagtgccca ttgccctggg aggccactgg    300
ccgtatgggt cagtgggctg tggggcgtg ccctccatca tcctgctgac catgtatgcc    360
agcgtcctgc tcctggcagc tctcagtgcc gacctctgct tcctggctct cgggcctgcc    420
tggtggtcta cggttcagcg ggcgtgcggg gtgcaggtgg cctgtggggc agcctggaca    480
ctggccttgc tgctaccgt gccctccgcc atctaccgcc ggctgcacca ggagcacttc    540
ccagcccggc tgcagtgtgt ggtggactac ggcggctcct ccagcacoga gaatgcggtg    600
actgccatcc ggtttctttt tggcttcctg gggcccctgg tggccgtggc cagctgccac    660
agtgcctcc tgtgtgggc agcccagcgc tgcgggcgcg tgggcacagc cattgtggtg    720
gggttttttg tctgtgggc accctaccac ctgctggggc tgggtctcac tgtggcggcc    780
ccgaactccg cactcctggc cagggccctg cgggctgaac ccctcatcgt gggccttgcc    840
ctcgtcaca gctgcctcaa tcccatgctc ttcctgtatt ttgggagggc tcaactccgc    900
cggtcactgc cagctgcctg tcaactgggc ctgagggagt ccagggcca ggaagaaagt    960
gtggacagca agaaatccac cagccatgac ctggtctcgg agatggaggt gtag          1014

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<210> SEQ ID NO 6
<211> LENGTH: 337
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met Gly Asn Asp Ser Val Ser Tyr Glu Tyr Gly Asp Tyr Ser Asp Leu
1          5          10          15

Ser Asp Arg Pro Val Asp Cys Leu Asp Gly Ala Cys Leu Ala Ile Asp
20          25          30

Pro Leu Arg Val Ala Pro Leu Pro Leu Tyr Ala Ala Ile Phe Leu Val
35          40          45

Gly Val Pro Gly Asn Ala Met Val Ala Trp Val Ala Gly Lys Val Ala
50          55          60

Arg Arg Arg Val Gly Ala Thr Trp Leu Leu His Leu Ala Val Ala Asp
65          70          75          80

Leu Leu Cys Cys Leu Ser Leu Pro Ile Leu Ala Val Pro Ile Ala Arg
85          90          95

Gly Gly His Trp Pro Tyr Gly Ala Val Gly Cys Arg Ala Leu Pro Ser
100         105         110

Ile Ile Leu Leu Thr Met Tyr Ala Ser Val Leu Leu Leu Ala Ala Leu
115         120         125

Ser Ala Asp Leu Cys Phe Leu Ala Leu Gly Pro Ala Trp Trp Ser Thr
130         135         140

Val Gln Arg Ala Cys Gly Val Gln Val Ala Cys Gly Ala Ala Trp Thr
145         150         155         160

Leu Ala Leu Leu Leu Thr Val Pro Ser Ala Ile Tyr Arg Arg Leu His
165         170         175

Gln Glu His Phe Pro Ala Arg Leu Gln Cys Val Val Asp Tyr Gly Gly
180         185         190

Ser Ser Ser Thr Glu Asn Ala Val Thr Ala Ile Arg Phe Leu Phe Gly
195         200         205

Phe Leu Gly Pro Leu Val Ala Val Ala Ser Cys His Ser Ala Leu Leu
210         215         220

Cys Trp Ala Ala Arg Arg Cys Arg Pro Leu Gly Thr Ala Ile Val Val
225         230         235         240

Gly Phe Phe Val Cys Trp Ala Pro Tyr His Leu Leu Gly Leu Val Leu
245         250         255

Thr Val Ala Ala Pro Asn Ser Ala Leu Leu Ala Arg Ala Leu Arg Ala
260         265         270

Glu Pro Leu Ile Val Gly Leu Ala Leu Ala His Ser Cys Leu Asn Pro
275         280         285

Met Leu Phe Leu Tyr Phe Gly Arg Ala Gln Leu Arg Arg Ser Leu Pro
290         295         300

Ala Ala Cys His Trp Ala Leu Arg Glu Ser Gln Gly Gln Asp Glu Ser
305         310         315         320

Val Asp Ser Lys Lys Ser Thr Ser His Asp Leu Val Ser Glu Met Glu
325         330         335

Val

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<210> SEQ ID NO 7
<211> LENGTH: 1272

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-continued

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 7

```

atgttgtgtc accgtggtgg ccagctgata gtgccaatca tcccactttg ccctgagcac    60
tcctgcaggg gtagaagact ccagaacctt ctctcaggcc catggcccaa gcagcccatg    120
gaacttcata acctgagctc tccatctccc tctctctcct cctctgttct cctccctcc    180
ttctctccct caccctcctc tgctccctct gcctttacca ctgtgggggg gtcctctgga    240
gggcccctgc accccacctc ttccctcgtg gtgtctgcct tcctggcacc aatcctggcc    300
ctggagtttg tcctgggcct ggtggggaac agtttgggcc tcttcattct ctgcatccac    360
acgcggccct ggacctcaa cacggtgttc ctggtcagcc tggtgccgc tgacttcctc    420
ctgatcagca acctgcccct ccgcgtggac tactacctcc tccatgagac ctggcgcttt    480
ggggctcgtg ctgcaaagt caacctcttc atgctgtcca ccaaccgcac gggcagcgtt    540
gtcttctca cagccatcgc actcaaccgc tacctgaagg tggtcagcc ccaccacgtg    600
ctgagccgtg cttccgtggg ggcagctgcc cgggtggccg ggggactctg ggtgggcac    660
ctgctcctca acgggcacct gctcctgagc accttctccg gcccctcctg cctcagctac    720
aggggtggga cgaagccctc ggcctcgtc cgctggcacc aggcactgta cctgctggag    780
ttcttctcgc cactggcgct catcctcttt gctattgtga gcattgggct caccatccgg    840
aaccgtggtc tgggcgggca ggcaggcccg cagagggcca tgcgtgtgct ggccatggtg    900
gtggccgtct acaccatctg cttcttgccc agcatcatct ttggcatggc ttccatggtg    960
gctttctggc tgtccgctg ccgatccctg gacctctgca cacagctctt ccatggctcc    1020
ctggccttca cctacctcaa cagtgtcctg gaccccgctc tctactgctt ctctagcccc    1080
aacttcctcc accagagccg ggccttgctg ggcctcacgc ggggccggca gggcccagtg    1140
agcgacgaga gctcctacca accctccagg cagtggcgct accgggaggc ctctaggaag    1200
gcggaggcca tagggaagct gaaagtgcag ggcgaggtct ctctggaaaa ggaaggctcc    1260
tcccagggct ga                                                    1272

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&lt;210&gt; SEQ ID NO 8

&lt;211&gt; LENGTH: 423

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 8

```

Met Leu Cys His Arg Gly Gly Gln Leu Ile Val Pro Ile Ile Pro Leu
 1              5              10              15

Cys Pro Glu His Ser Cys Arg Gly Arg Arg Leu Gln Asn Leu Leu Ser
      20              25              30

Gly Pro Trp Pro Lys Gln Pro Met Glu Leu His Asn Leu Ser Ser Pro
 35              40              45

Ser Pro Ser Leu Ser Ser Ser Val Leu Pro Pro Ser Phe Ser Pro Ser
 50              55              60

Pro Ser Ser Ala Pro Ser Ala Phe Thr Thr Val Gly Gly Ser Ser Gly
 65              70              75              80

Gly Pro Cys His Pro Thr Ser Ser Ser Leu Val Ser Ala Phe Leu Ala
      85              90              95

Pro Ile Leu Ala Leu Glu Phe Val Leu Gly Leu Val Gly Asn Ser Leu

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| 100                                                             | 105 | 110 |
|-----------------------------------------------------------------|-----|-----|
| Ala Leu Phe Ile Phe Cys Ile His Thr Arg Pro Trp Thr Ser Asn Thr |     |     |
| 115                                                             | 120 | 125 |
| Val Phe Leu Val Ser Leu Val Ala Ala Asp Phe Leu Leu Ile Ser Asn |     |     |
| 130                                                             | 135 | 140 |
| Leu Pro Leu Arg Val Asp Tyr Tyr Leu Leu His Glu Thr Trp Arg Phe |     |     |
| 145                                                             | 150 | 155 |
| Gly Ala Ala Ala Cys Lys Val Asn Leu Phe Met Leu Ser Thr Asn Arg |     |     |
| 165                                                             | 170 | 175 |
| Thr Ala Ser Val Val Phe Leu Thr Ala Ile Ala Leu Asn Arg Tyr Leu |     |     |
| 180                                                             | 185 | 190 |
| Lys Val Val Gln Pro His His Val Leu Ser Arg Ala Ser Val Gly Ala |     |     |
| 195                                                             | 200 | 205 |
| Ala Ala Arg Val Ala Gly Gly Leu Trp Val Gly Ile Leu Leu Leu Asn |     |     |
| 210                                                             | 215 | 220 |
| Gly His Leu Leu Leu Ser Thr Phe Ser Gly Pro Ser Cys Leu Ser Tyr |     |     |
| 225                                                             | 230 | 235 |
| Arg Val Gly Thr Lys Pro Ser Ala Ser Leu Arg Trp His Gln Ala Leu |     |     |
| 245                                                             | 250 | 255 |
| Tyr Leu Leu Glu Phe Phe Leu Pro Leu Ala Leu Ile Leu Phe Ala Ile |     |     |
| 260                                                             | 265 | 270 |
| Val Ser Ile Gly Leu Thr Ile Arg Asn Arg Gly Leu Gly Gly Gln Ala |     |     |
| 275                                                             | 280 | 285 |
| Gly Pro Gln Arg Ala Met Arg Val Leu Ala Met Val Val Ala Val Tyr |     |     |
| 290                                                             | 295 | 300 |
| Thr Ile Cys Phe Leu Pro Ser Ile Ile Phe Gly Met Ala Ser Met Val |     |     |
| 305                                                             | 310 | 315 |
| Ala Phe Trp Leu Ser Ala Cys Arg Ser Leu Asp Leu Cys Thr Gln Leu |     |     |
| 325                                                             | 330 | 335 |
| Phe His Gly Ser Leu Ala Phe Thr Tyr Leu Asn Ser Val Leu Asp Pro |     |     |
| 340                                                             | 345 | 350 |
| Val Leu Tyr Cys Phe Ser Ser Pro Asn Phe Leu His Gln Ser Arg Ala |     |     |
| 355                                                             | 360 | 365 |
| Leu Leu Gly Leu Thr Arg Gly Arg Gln Gly Pro Val Ser Asp Glu Ser |     |     |
| 370                                                             | 375 | 380 |
| Ser Tyr Gln Pro Ser Arg Gln Trp Arg Tyr Arg Glu Ala Ser Arg Lys |     |     |
| 385                                                             | 390 | 395 |
| Ala Glu Ala Ile Gly Lys Leu Lys Val Gln Gly Glu Val Ser Leu Glu |     |     |
| 405                                                             | 410 | 415 |
| Lys Glu Gly Ser Ser Gln Gly                                     |     |     |
| 420                                                             |     |     |

&lt;210&gt; SEQ ID NO 9

&lt;211&gt; LENGTH: 966

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 9

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| atgaaccaga ctttgaatat cagtgggacc gtggagtcag ccctaaacta ttccagaggg  | 60  |
| agcacagtgc acacggccta cctggtgctg agctccctgg ccatgttcac ctgctgtgctg | 120 |
| gggatggcag gcaacagcat ggtgatctgg ctgctgggct ttcgaatgca caggaacccc  | 180 |

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ttctgcatct atactcctcaa cctggcggca gccgacctcc tcttcctctt cagcatggct 240
tccacgctca gcctggaaac ccagcccctg gtcaatacca ctgacaaggt ccacgagctg 300
atgaagagac tgatgtactt tgcctacaca gtgggcctga gcctgctgac ggccatcagc 360
acccagcgct gtctctctgt cctcttcctt atctggttca agtgtcaccg gcccaggcac 420
ctgtcagcct ggggtgtgtg cctgctgtgg acaactctgtc tcctgatgaa cgggttgacc 480
tcttccttct gcagcaagtt cttgaaattc aatgaagatc ggtgcttcag ggtggacatg 540
gtccaggccg ccctcatcat ggggggtctta accccagtga tgactctgtc cagcctgacc 600
ctctttgtct ggggtcggag gagctcccag cagtggcggc gccagcccac acggctgttc 660
gtggtggtcc tggcctctgt cctggtgttc ctcatctgtt ccctgcctct gagcatctac 720
tggtttgtgc tctactggtt gagcctgccg cccgagatgc aggtcctgtg cttcagcttg 780
tcacgcctct cctcgtccgt aagcagcagc gccaaacccg tcactctactt cctggtgggc 840
agccggagga gccacaggct gccaccagg tccctgggga ctgtgctcca acaggcgctt 900
cgcgaggagc ccgagctgga aggtggggag acgcccaccg tgggcaccaa tgagatgggg 960
gcttga

```

&lt;210&gt; SEQ ID NO 10

&lt;211&gt; LENGTH: 321

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 10

```

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

Tyr Ser Arg Gly Ser Thr Val His Thr Ala Tyr Leu Val Leu Ser Ser
20          25          30

Leu Ala Met Phe Thr Cys Leu Cys Gly Met Ala Gly Asn Ser Met Val
35          40          45

Ile Trp Leu Leu Gly Phe Arg Met His Arg Asn Pro Phe Cys Ile Tyr
50          55          60

Ile Leu Asn Leu Ala Ala Ala Asp Leu Leu Phe Leu Phe Ser Met Ala
65          70          75          80

Ser Thr Leu Ser Leu Glu Thr Gln Pro Leu Val Asn Thr Thr Asp Lys
85          90          95

Val His Glu Leu Met Lys Arg Leu Met Tyr Phe Ala Tyr Thr Val Gly
100         105         110

Leu Ser Leu Leu Thr Ala Ile Ser Thr Gln Arg Cys Leu Ser Val Leu
115         120         125

Phe Pro Ile Trp Phe Lys Cys His Arg Pro Arg His Leu Ser Ala Trp
130         135         140

Val Cys Gly Leu Leu Trp Thr Leu Cys Leu Leu Met Asn Gly Leu Thr
145         150         155         160

Ser Ser Phe Cys Ser Lys Phe Leu Lys Phe Asn Glu Asp Arg Cys Phe
165         170         175

Arg Val Asp Met Val Gln Ala Ala Leu Ile Met Gly Val Leu Thr Pro
180         185         190

Val Met Thr Leu Ser Ser Leu Thr Leu Phe Val Trp Val Arg Arg Ser
195         200         205

Ser Gln Gln Trp Arg Arg Gln Pro Thr Arg Leu Phe Val Val Val Leu

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-continued

| 210                                                             | 215 | 220     |
|-----------------------------------------------------------------|-----|---------|
| Ala Ser Val Leu Val Phe Leu Ile Cys Ser Leu Pro Leu Ser Ile Tyr |     |         |
| 225                                                             | 230 | 235 240 |
| Trp Phe Val Leu Tyr Trp Leu Ser Leu Pro Pro Glu Met Gln Val Leu |     |         |
|                                                                 | 245 | 250 255 |
| Cys Phe Ser Leu Ser Arg Leu Ser Ser Ser Val Ser Ser Ser Ala Asn |     |         |
|                                                                 | 260 | 265 270 |
| Pro Val Ile Tyr Phe Leu Val Gly Ser Arg Arg Ser His Arg Leu Pro |     |         |
|                                                                 | 275 | 280 285 |
| Thr Arg Ser Leu Gly Thr Val Leu Gln Gln Ala Leu Arg Glu Glu Pro |     |         |
|                                                                 | 290 | 295 300 |
| Glu Leu Glu Gly Gly Glu Thr Pro Thr Val Gly Thr Asn Glu Met Gly |     |         |
| 305                                                             | 310 | 315 320 |

Ala

<210> SEQ ID NO 11  
 <211> LENGTH: 1356  
 <212> TYPE: DNA  
 <213> ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 11

```

atggagtcct caccatcccc ccagtcacatc gggaaactctt ccactttggg gagggtcctt    60
caaacccag gtccctctac tgccagtggg gtcccgagg tggggctacg ggatgttgct    120
tcggaatctg tggccctctt ctcatgctc ctgctggact tgactgctgt ggcctggcaat    180
gccgctgtga tggccgtgat cgccaagacg cctgccctcc gaaaatttgt ctctgtcttc    240
cacctctgcc tgggtggacct gctggctgcc ctgaccctca tgcccctggc catgctctcc    300
agctctgccc tctttgacca cgccctcttt ggggaggtgg cctgccgcct ctacttgttt    360
ctgagcgtgt gctttgtcag cctggccatc ctctcggtgt cagccatcaa tgtggagcgc    420
tactattacg tagtccaccc catgcgctac gaggtgcgca tgacgctggg gctgggtggc    480
tctgtgctgg tgggtgtgtg ggtgaaggcc ttggccatgg cttctgtgcc agtgttgagg    540
agggctctct gggaggaagg agctccaggt gtcccccag gctgttcact ccagtggagc    600
cacagtgcct actgccagct ttttgtgtg gtctttgctg tcctttactt tctgttgccc    660
ctgctcctca tacttgttgt ctactgcagc atgttccgag tggcccgcgt ggcctgcatg    720
cagcacgggc cgctgcccac gtggatggag acaccccggc aacgctccga atctctcagc    780
agccgctcca cgatggctac cagctcgagg gcccccaga ccacccaca ccggacgttt    840
gggggaggga aagcagcagt ggttctcctg gctgtggggg gacagttoct gctctgttg    900
ttgccctact tctctttcca cctctatgtt gccctgagtg ctacgcccac ttcaactggg    960
caggtggaga gtgtggctac ctggattggc tacttttgct tcacttccaa ccctttcttc   1020
tatggatgtc tcaaccggga gatccggggg gagctcagca agcagtttgt ctgcttcttc   1080
aagccagctc cagaggagga gctgaggctg cctagccggg agggctccat tgaggagAAC   1140
ttcctgcagt tccttcaggg gactggctgt cctctgagt cctgggtttc ccgaccccta   1200
cccagcccca agcaggagcc acctgctgtt gactttcgaa tcccaggcca gatagctgag   1260
gagacctctg agttcctgga gcagcaactc accagcgaca tcatcatgtc agacagctac   1320
ctccgtcctg ccgcctcacc ccggctggag tcatga                                1356

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<210> SEQ ID NO 12
<211> LENGTH: 451
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Met Glu Ser Ser Pro Ile Pro Gln Ser Ser Gly Asn Ser Ser Thr Leu
1      5      10      15

Gly Arg Val Pro Gln Thr Pro Gly Pro Ser Thr Ala Ser Gly Val Pro
20     25     30

Glu Val Gly Leu Arg Asp Val Ala Ser Glu Ser Val Ala Leu Phe Phe
35     40     45

Met Leu Leu Leu Asp Leu Thr Ala Val Ala Gly Asn Ala Ala Val Met
50     55     60

Ala Val Ile Ala Lys Thr Pro Ala Leu Arg Lys Phe Val Phe Val Phe
65     70     75     80

His Leu Cys Leu Val Asp Leu Leu Ala Ala Leu Thr Leu Met Pro Leu
85     90     95

Ala Met Leu Ser Ser Ser Ala Leu Phe Asp His Ala Leu Phe Gly Glu
100    105    110

Val Ala Cys Arg Leu Tyr Leu Phe Leu Ser Val Cys Phe Val Ser Leu
115    120    125

Ala Ile Leu Ser Val Ser Ala Ile Asn Val Glu Arg Tyr Tyr Tyr Val
130    135    140

Val His Pro Met Arg Tyr Glu Val Arg Met Thr Leu Gly Leu Val Ala
145    150    155    160

Ser Val Leu Val Gly Val Trp Val Lys Ala Leu Ala Met Ala Ser Val
165    170    175

Pro Val Leu Gly Arg Val Ser Trp Glu Glu Gly Ala Pro Ser Val Pro
180    185    190

Pro Gly Cys Ser Leu Gln Trp Ser His Ser Ala Tyr Cys Gln Leu Phe
195    200    205

Val Val Val Phe Ala Val Leu Tyr Phe Leu Leu Pro Leu Leu Leu Ile
210    215    220

Leu Val Val Tyr Cys Ser Met Phe Arg Val Ala Arg Val Ala Ala Met
225    230    235    240

Gln His Gly Pro Leu Pro Thr Trp Met Glu Thr Pro Arg Gln Arg Ser
245    250    255

Glu Ser Leu Ser Ser Arg Ser Thr Met Val Thr Ser Ser Gly Ala Pro
260    265    270

Gln Thr Thr Pro His Arg Thr Phe Gly Gly Gly Lys Ala Ala Val Val
275    280    285

Leu Leu Ala Val Gly Gly Gln Phe Leu Leu Cys Trp Leu Pro Tyr Phe
290    295    300

Ser Phe His Leu Tyr Val Ala Leu Ser Ala Gln Pro Ile Ser Thr Gly
305    310    315    320

Gln Val Glu Ser Val Val Thr Trp Ile Gly Tyr Phe Cys Phe Thr Ser
325    330    335

Asn Pro Phe Phe Tyr Gly Cys Leu Asn Arg Gln Ile Arg Gly Glu Leu
340    345    350

Ser Lys Gln Phe Val Cys Phe Phe Lys Pro Ala Pro Glu Glu Glu Leu
355    360    365

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Arg Leu Pro Ser Arg Glu Gly Ser Ile Glu Glu Asn Phe Leu Gln Phe  
 370 375 380

Leu Gln Gly Thr Gly Cys Pro Ser Glu Ser Trp Val Ser Arg Pro Leu  
 385 390 395 400

Pro Ser Pro Lys Gln Glu Pro Pro Ala Val Asp Phe Arg Ile Pro Gly  
 405 410 415

Gln Ile Ala Glu Glu Thr Ser Glu Phe Leu Glu Gln Gln Leu Thr Ser  
 420 425 430

Asp Ile Ile Met Ser Asp Ser Tyr Leu Arg Pro Ala Ala Ser Pro Arg  
 435 440 445

Leu Glu Ser  
 450

<210> SEQ ID NO 13  
 <211> LENGTH: 1041  
 <212> TYPE: DNA  
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

```

atggagagaa aatttatgtc cttgcaacca tccatctccg tatcagaaat ggaaccaaatt    60
ggcaccttca gcaataacaa cagcaggaac tgcacaattg aaaacttcaa gagagaatttt    120
ttcccaattg tatatctgat aatatttttc tggggagtct tgggaaatgg gttgtccata    180
tatgttttcc tgcagcctta taagaagtcc acatctgtga acgttttcat gctaaatctg    240
gccatttcag atctcctgtt cataagcacg cttcccttca gggctgacta ttatcttaga    300
ggctccaatt ggatatttgg agacctggcc tgcaggatta tgtcttattc cttgtatgtc    360
aacagtaca gcagtattta tttcctgacc gtgctgagtg ttgtgcgttt cctggcaatg    420
gttcacccct ttcggcttct gcatgtcacc agcatcagga gtgcctggat cctctgtggg    480
atcatatgga tccttatcat ggcttctca ataatgctcc tggacagtgg ctctgagcag    540
aacggcagtg tcacatcatg cttagagctg aatctctata aaattgctaa gctgcagacc    600
atgaactata ttgccttggg ggtgggctgc ctgctgccat tttcacact cagcatctgt    660
tatctgctga tcattcgggt tctgttaaaa gtggaggtcc cagaatcggg gctgcggggt    720
tctcacagga aggcactgac caccatcatc atcaccttga tcattcttct cttgtgtttc    780
ctgccctatc acacactgag gaccgtccac ttgacgacat ggaaagtggg tttatgcaaa    840
gacagactgc ataaagcttt ggttatcaca ctggccttgg cagcagccaa tgcctgcttc    900
aatcctctgc tctattactt tgctggggag aattttaagg acagactaaa gtctgcactc    960
agaaaaggcc atccacagaa ggcaaagaca aagtgtgttt tccctgttag tgtgtggttg   1020
agaaaggaaa caagagtata a                                     1041

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<210> SEQ ID NO 14  
 <211> LENGTH: 346  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Met Glu Arg Lys Phe Met Ser Leu Gln Pro Ser Ile Ser Val Ser Glu  
 1 5 10 15

Met Glu Pro Asn Gly Thr Phe Ser Asn Asn Asn Ser Arg Asn Cys Thr  
 20 25 30

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

&lt;210&gt; SEQ ID NO 15

&lt;211&gt; LENGTH: 1527

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 15

```

atgacgtcca cctgcaccaa cagcacgcgc gagagtaaca gcagccacac gtgcatgccc      60
ctctccaaaa tgcccatcag cctggcccac ggcacatccc gctcaaccgt gctgggttatc      120
ttctctgcgc cctcttttct cggaacata gtgctggcgc tagtggttga gcgcaagccg      180
cagctgctgc aggtgaccaa ccgtttttatc tttaacctcc tcgtcaccga cctgctgcag      240

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atttcgctcg tggccccctg ggtggtggcc acctctgtgc ctctcttctg gcccccaac 300
agccacttct gcacggccct ggttagcctc acccacctgt tcgccttcgc cagcgtcaac 360
accattgtcg tgggtgcagt ggatcgctac ttgtccatca tccaccctct ctccataccg 420
tccaagatga cccagcgccg cggttacctg ctctcttatg gcacctggat tgtggccatc 480
ctgcagagca ctccctccact ctacggctgg ggccaggctg cctttgatga gcgcaatgct 540
ctctgtccca tgatctgggg ggccagcccc agctacacta ttctcagcgt ggtgtccttc 600
atcgtcattc cactgattgt catgattgcc tgctactccg tgggttctctg tgcagcccg 660
aggcagcatg ctctgtctga caatgtcaag agacacagct tggaagtgcg agtcaaggac 720
tgtgtggaga atgaggatga agagggagca gagaagaagg aggagttcca ggatgagagt 780
gagtttcgcc gccagcatga aggtgaggtc aaggccaagg agggcagaat ggaagccaag 840
gacggcagcc tgaaggccaa ggaaggaagc acggggacca gtgagagtag tgtagaggcc 900
aggggcagcg aggaggtcag agagagcagc acggtggcca gcgacggcag catggagggt 960
aaggaaggca gcaccaaagt tgaggagaac agcatgaagg cagacaaggg tcgcacagag 1020
gtcaaccagt gcagcattga ctggggtgaa gatgacatgg agtttggtga agacgacatc 1080
aatttcagtg aggatgacgt cgaggcagtg aacatcccgg agagcctccc acccagtcgt 1140
cgtaacagca acagcaaccc tcctctgccc aggtgctacc agtgcaaagc tgctaaagt 1200
atcttcatca tcattttctc ctatgtgcta tccctggggc cctactgctt tttagcagtc 1260
ctggccgtgt ggggtgagtg cgaaacccag gtacccagtg gggtgatcac cataatcatc 1320
tggtttttct tcctgcagtg ctgcattcac ccctatgtct atggctacat gcacaagacc 1380
attaagaagg aaatccagga catgctgaag aagttcttct gcaaggaaaa gccccgaaa 1440
gaagatagcc acccagacct gcccggaaca gagggtgagg ctgaaggcaa gattgtccct 1500
tcctacgatt ctgctacttt tccttga 1527

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&lt;210&gt; SEQ ID NO 16

&lt;211&gt; LENGTH: 508

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 16

```

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

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Arg Tyr Leu Ser Ile Ile His Pro Leu Ser Tyr Pro Ser Lys Met Thr
 130                135                140

Gln Arg Arg Gly Tyr Leu Leu Leu Tyr Gly Thr Trp Ile Val Ala Ile
145                150                155                160

Leu Gln Ser Thr Pro Pro Leu Tyr Gly Trp Gly Gln Ala Ala Phe Asp
      165                170                175

Glu Arg Asn Ala Leu Cys Ser Met Ile Trp Gly Ala Ser Pro Ser Tyr
      180                185                190

Thr Ile Leu Ser Val Val Ser Phe Ile Val Ile Pro Leu Ile Val Met
      195                200                205

Ile Ala Cys Tyr Ser Val Val Phe Cys Ala Ala Arg Arg Gln His Ala
      210                215                220

Leu Leu Tyr Asn Val Lys Arg His Ser Leu Glu Val Arg Val Lys Asp
225                230                235                240

Cys Val Glu Asn Glu Asp Glu Glu Gly Ala Glu Lys Lys Glu Glu Phe
      245                250                255

Gln Asp Glu Ser Glu Phe Arg Arg Gln His Glu Gly Glu Val Lys Ala
      260                265                270

Lys Glu Gly Arg Met Glu Ala Lys Asp Gly Ser Leu Lys Ala Lys Glu
      275                280                285

Gly Ser Thr Gly Thr Ser Glu Ser Ser Val Glu Ala Arg Gly Ser Glu
      290                295                300

Glu Val Arg Glu Ser Ser Thr Val Ala Ser Asp Gly Ser Met Glu Gly
305                310                315                320

Lys Glu Gly Ser Thr Lys Val Glu Glu Asn Ser Met Lys Ala Asp Lys
      325                330                335

Gly Arg Thr Glu Val Asn Gln Cys Ser Ile Asp Leu Gly Glu Asp Asp
      340                345                350

Met Glu Phe Gly Glu Asp Asp Ile Asn Phe Ser Glu Asp Asp Val Glu
      355                360                365

Ala Val Asn Ile Pro Glu Ser Leu Pro Pro Ser Arg Arg Asn Ser Asn
      370                375                380

Ser Asn Pro Pro Leu Pro Arg Cys Tyr Gln Cys Lys Ala Ala Lys Val
385                390                395                400

Ile Phe Ile Ile Ile Phe Ser Tyr Val Leu Ser Leu Gly Pro Tyr Cys
      405                410                415

Phe Leu Ala Val Leu Ala Val Trp Val Asp Val Glu Thr Gln Val Pro
      420                425                430

Gln Trp Val Ile Thr Ile Ile Ile Trp Leu Phe Phe Leu Gln Cys Cys
      435                440                445

Ile His Pro Tyr Val Tyr Gly Tyr Met His Lys Thr Ile Lys Lys Glu
      450                455                460

Ile Gln Asp Met Leu Lys Lys Phe Phe Cys Lys Glu Lys Pro Pro Lys
465                470                475                480

Glu Asp Ser His Pro Asp Leu Pro Gly Thr Glu Gly Gly Thr Glu Gly
      485                490                495

Lys Ile Val Pro Ser Tyr Asp Ser Ala Thr Phe Pro
      500                505

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&lt;210&gt; SEQ ID NO 17

&lt;211&gt; LENGTH: 1068

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&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 17

```

atgcccttga cggacggcat ttcttcattt gaggacctct tggctaacaa taccctcaga    60
atatttgtct gggttatagc ttccattacc tgctttggaa atctttttgt cattggcatg    120
agatctttca ttaaagctga aaatacaact cacgctatgt ccatcaaaat cctttgttgc    180
gctgattgcc tgatgggtgt ttacttgttc tttgttggca ttttcgatat aaaataccga    240
gggcagtatc agaagtatgc cttgctgtgg atggagagcg tgcagtgccg cctcatgggg    300
ttcctggcca tgctgtccac cgaagtctct gttctgtac tgacctactt gactttggag    360
aagttcctgg tcattgtctt ccccttcagt aacattcgac ctggaaaacg gcagacctca    420
gtcatcctca ttgcatctg gatggcgga tttttaatag ctgtaattcc attttggaat    480
aaggattatt ttgaaaactt ttatgggaaa aatggagtat gtttccact ttattatgac    540
caaacagaag atattggaag caaagggtat tctcttgaa ttttcctagg tgtgaacttg    600
ctggcttttc tcatcattgt gtttctctat attactatgt tctgttccat tcaaaaaacc    660
gccttgcaga ccacagaagt aaggaattgt ttggaagag aggtggctgt tgcaaatcgt    720
ttctttttta tagtgttctc tgatgccatc tgctggattc ctgtatttgt agttaaatac    780
ctttccctct tccgggtgga aataccagac acaatgactt cctggatagt gatttttttc    840
cttcagttta acagtgtttt gaatccaatc ctctatactc tcacaaccaa cttttttaag    900
gacaagtga aacagctgct gcacaaacat cagaggaaat caattttcaa aattaaaaaa    960
aaaagtttat ctacatccat tgtgtggata gaggactcct cttccctgaa acttgggggt    1020
ttgaacaaaa taacacttgg agacagtata atgaaaccag tttcctag    1068

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&lt;210&gt; SEQ ID NO 18

&lt;211&gt; LENGTH: 355

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 18

```

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

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Cys Ile Trp Met Ala Gly Phe Leu Ile Ala Val Ile Pro Phe Trp Asn  
 145 150 155 160  
 Lys Asp Tyr Phe Gly Asn Phe Tyr Gly Lys Asn Gly Val Cys Phe Pro  
 165 170 175  
 Leu Tyr Tyr Asp Gln Thr Glu Asp Ile Gly Ser Lys Gly Tyr Ser Leu  
 180 185 190  
 Gly Ile Phe Leu Gly Val Asn Leu Leu Ala Phe Leu Ile Ile Val Phe  
 195 200 205  
 Ser Tyr Ile Thr Met Phe Cys Ser Ile Gln Lys Thr Ala Leu Gln Thr  
 210 215 220  
 Thr Glu Val Arg Asn Cys Phe Gly Arg Glu Val Ala Val Ala Asn Arg  
 225 230 235 240  
 Phe Phe Phe Ile Val Phe Ser Asp Ala Ile Cys Trp Ile Pro Val Phe  
 245 250 255  
 Val Val Lys Ile Leu Ser Leu Phe Arg Val Glu Ile Pro Asp Thr Met  
 260 265 270  
 Thr Ser Trp Ile Val Ile Phe Phe Leu Pro Val Asn Ser Ala Leu Asn  
 275 280 285  
 Pro Ile Leu Tyr Thr Leu Thr Thr Asn Phe Phe Lys Asp Lys Leu Lys  
 290 295 300  
 Gln Leu Leu His Lys His Gln Arg Lys Ser Ile Phe Lys Ile Lys Lys  
 305 310 315 320  
 Lys Ser Leu Ser Thr Ser Ile Val Trp Ile Glu Asp Ser Ser Ser Leu  
 325 330 335  
 Lys Leu Gly Val Leu Asn Lys Ile Thr Leu Gly Asp Ser Ile Met Lys  
 340 345 350  
 Pro Val Ser  
 355

&lt;210&gt; SEQ ID NO 19

&lt;211&gt; LENGTH: 969

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 19

```

atggatccaa ccattctcaac cttggacaca gaactgacac caatcaacgg aactgaggag    60
actctttgct acaagcagac cttgagcctc acggtgctga cgtgcatcgt ttcccttgtc    120
gggctgacag gaaacgcagt tgtgctctgg ctccctgggt gccgcacgag caggaacgcc    180
ttctccatct acatcctcaa cttggccgca gcagacttcc tcttccctcag cggccgcctt    240
atatattccc tgtaaagctt catcagtatc ccccatacca tctctaaaat cctctatcct    300
gtgatgatgt tttcctactt tgcaggcctg agctttctga gtgccgtgag caccgagcgc    360
tgcctgtccg tcctgtggcc catctggtac cgctgccacc gcccacaca cctgtcagcg    420
gtggtgtgtg tcctgctctg ggccctgtcc ctgctgcgga gcatcctgga gtggatgtta    480
tgtggtcttc tgttcagtgg tgctgattct gcttggtgtc aaacatcaga ttcatcaca    540
gtcgcgtggc tgattttttt atgtgtggtt ctctgtgggt ccagcctggt cctgctgac    600
aggattctct gtggatcccg gaagataccg ctgaccaggc tgtacgtgac catcctgctc    660
acagtactgg tcttcctcct ctgtggcctg ccttttgga ttcagttttt cctattttta    720
tggatccacg tggacaggga agtcttattt tgtcatgttc atctagtttc tattttctctg    780

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tccgctctta acagcagtgc caaccccatc atttacttct tcgtgggctc ctttaggcag      840
cgtaaaata ggcagaacct gaagctggtt ctccagaggg ctctgcagga cgcgtctgag      900
gtggatgaag gtggagggca gcttcctgag gaaatcctgg agctgtcggg aagcagattg      960
gagcagtga                                          969

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<210> SEQ ID NO 20
<211> LENGTH: 322
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 20

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```

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

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Glu Gln

&lt;210&gt; SEQ ID NO 21

&lt;211&gt; LENGTH: 1305

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 21

```

atggaggatc tctttagccc ctcaattctg ccgccggcgc ccaacatttc cgtgcccata 60
ttgtctgggt ggggtctcaa cctgaccttg gggcaaggag cccctgcctc tgggccgccc 120
agccgccgcg tccgcctggt gttcctgggg gtcacacctg tggtagcggt ggcaggcaac 180
accacagtgc tgtgccgcct gtgcggcggc ggcgggcctt ggcggggccc caagcgtcgc 240
aagatggact tcctgctggt gcagctggcc ctggcggacc tgtacgcgtg cgggggcacg 300
gcgctgtcac agctggcctg ggaactgtg ggcgagcccc gcgcggccac gggggacctg 360
gcgtgccgct tcctgcagct gctgcaggca tccgggcggg gcgcctcggc ccacctcgtg 420
gtgctcatcg ccctcgagcg ccggcgcgcg gtgcgtcttc cgcacggcgg gccgctgccc 480
gcgcgtgccc tcgccgcctt gggctggctg ctggcactgc tgcctggcgt gcccccgccc 540
ttcgtggtgc gcggggactc cccctcgccg ctgccgcgcg cgcgcgcgcc aacgtccctg 600
cagccaggcg cgcggggcgc cgcggcgccc tggccggggg agcgtcgtg ccacgggata 660
ttcgcgcccc tgcgcgctg gcacctgcag gtctacgctg tctacgaggc cgtcgcgggc 720
ttcgtcgcgc ctgttacggt cctgggcgtc gcttgcgccc acctactctc cgtctggtgg 780
cggcaccggc cgcaggcccc cgcggctgca gcgcctggt cggcgagccc aggtcgagcc 840
cctgcgcccc gcgcgctgcc ccgcgccaa gtgcagagcc tgaagatgag cctgctgctg 900
gcgctgctgt tcgtgggctg cgagctgccc tactttgccg cccggctggc gcccgctgg 960
tcgtccgggc ccgcgggaga ctgggaggga gagggcctgt cggcggcgtg gcgcgtggtg 1020
gcgatggcca acagcgctct caatcccttc gtctacctct tcttccaggc gggcgactgc 1080
cggctccggc gacagctgcg gaagcggctg ggctctctgt gctgcgcgcc gcagggaggc 1140
gcggaggacg agggagggcc ccggggccac caggcgctct accgccaacg ctggccccac 1200
cctcattatc accatgctcg gcgggaaccg ctggacgagg gcggcttgcg cccacccctt 1260
ccgcgcccc caccctgccc ttgctcctgc gaaagtgcct tctag 1305

```

&lt;210&gt; SEQ ID NO 22

&lt;211&gt; LENGTH: 434

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 22

```

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

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

<210> SEQ ID NO 23  
 <211> LENGTH: 1041  
 <212> TYPE: DNA  
 <213> ORGANISM: Homo sapiens  
 <400> SEQUENCE: 23

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atgtacaacg ggctgtgctg ccgcatcgag ggggacacca tctcccaggt gatgccgccg      60
ctgtcattg tggcctttgt gctggggcga ctaggcaatg gggtcgccct gtgtggtttc      120
tgcttcacac tgaagacctg gaagcccagc actgtttacc ttttcaattt ggcctgtggct      180
gatttcctcc ttatgatctg cctgcctttt cggacagact attacctcag acgtagacac      240
tgggcttttg gggacattcc ctgccgagtg gggctcttca cgttggccat gaacagggcc      300
gggagcatcg tgttccttac ggtggtggct gcggacaggt atttcaaagt ggtccacccc      360
caccacgcgg tgaacactat ctccaccggt gtggcggctg gcacgtctctg caccctgtgg      420
gcccctggta tcctgggaac agtgtatctt ttgctggaga accatctctg cgtgcaagag      480
acggccgtct cctgtgagag cttcatcatg gagtcggcca atggctggca tgacatcatg      540
ttccagctgg agttctttat gcccctcggt atcatcttat tttgctcctt caagattggt      600
tggagcctga ggcggaggca gcagctggcc agacaggctc ggatgaagaa ggcgaccggg      660
ttcatcatgg tgggtggaat tgtgttcacg acatgctacc tgcccagcgt gtctgctaga      720
ctctatttcc tctggacggt gccctcgagt gcctgcgacg cctctgtcca tggggccctg      780
cacataaccc tcagcttcac ctacatgaac agcatgctgg atcccctggt gtattatattt      840
tcaagccctt cctttcccaa attctacaac aagctcaaaa tctgcagtct gaaacccaag      900
cagccaggac actcaaaaac aaaaaggccg gaagagatgc caatttcgaa cctcggtcgc      960
aggagtgtga tcagtgtggc aaatagtttc caaagccagt ctgatgggca atgggatccc     1020
cacattgttg agtggcactg a                                           1041

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&lt;210&gt; SEQ ID NO 24

&lt;211&gt; LENGTH: 346

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 24

```

Met Tyr Asn Gly Ser Cys Cys Arg Ile Glu Gly Asp Thr Ile Ser Gln
 1             5             10             15

Val Met Pro Pro Leu Leu Ile Val Ala Phe Val Leu Gly Ala Leu Gly
 20             25             30

Asn Gly Val Ala Leu Cys Gly Phe Cys Phe His Met Lys Thr Trp Lys
 35             40             45

Pro Ser Thr Val Tyr Leu Phe Asn Leu Ala Val Ala Asp Phe Leu Leu
 50             55             60

Met Ile Cys Leu Pro Phe Arg Thr Asp Tyr Tyr Leu Arg Arg Arg His
 65             70             75             80

Trp Ala Phe Gly Asp Ile Pro Cys Arg Val Gly Leu Phe Thr Leu Ala
 85             90             95

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

Arg Tyr Phe Lys Val Val His Pro His His Ala Val Asn Thr Ile Ser
115             120             125

Thr Arg Val Ala Ala Gly Ile Val Cys Thr Leu Trp Ala Leu Val Ile
130             135             140

Leu Gly Thr Val Tyr Leu Leu Leu Glu Asn His Leu Cys Val Gln Glu
145             150             155             160

Thr Ala Val Ser Cys Glu Ser Phe Ile Met Glu Ser Ala Asn Gly Trp

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|                                                                 | 165 | 170 | 175 |
|-----------------------------------------------------------------|-----|-----|-----|
| His Asp Ile Met Phe Gln Leu Glu Phe Phe Met Pro Leu Gly Ile Ile | 180 | 185 | 190 |
| Leu Phe Cys Ser Phe Lys Ile Val Trp Ser Leu Arg Arg Arg Gln Gln | 195 | 200 | 205 |
| Leu Ala Arg Gln Ala Arg Met Lys Lys Ala Thr Arg Phe Ile Met Val | 210 | 215 | 220 |
| Val Ala Ile Val Phe Ile Thr Cys Tyr Leu Pro Ser Val Ser Ala Arg | 225 | 230 | 240 |
| Leu Tyr Phe Leu Trp Thr Val Pro Ser Ser Ala Cys Asp Pro Ser Val | 245 | 250 | 255 |
| His Gly Ala Leu His Ile Thr Leu Ser Phe Thr Tyr Met Asn Ser Met | 260 | 265 | 270 |
| Leu Asp Pro Leu Val Tyr Tyr Phe Ser Ser Pro Ser Phe Pro Lys Phe | 275 | 280 | 285 |
| Tyr Asn Lys Leu Lys Ile Cys Ser Leu Lys Pro Lys Gln Pro Gly His | 290 | 295 | 300 |
| Ser Lys Thr Gln Arg Pro Glu Glu Met Pro Ile Ser Asn Leu Gly Arg | 305 | 310 | 315 |
| Arg Ser Cys Ile Ser Val Ala Asn Ser Phe Gln Ser Gln Ser Asp Gly | 325 | 330 | 335 |
| Gln Trp Asp Pro His Ile Val Glu Trp His                         | 340 | 345 |     |

&lt;210&gt; SEQ ID NO 25

&lt;211&gt; LENGTH: 1011

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 25

|                                                                    |      |
|--------------------------------------------------------------------|------|
| atgaacaaca atacaacatg tattcaacca tctatgatct cttccatggc tttaccaatc  | 60   |
| atttacatcc tcctttgtat tgttgggtgtt tttggaaaca ctctctctca atggatattt | 120  |
| ttaacaaaaa taggtaaaaa aacatcaacg cacatctacc tgtcacacct tgtgactgca  | 180  |
| aacttacttg tgtgcagtgc catgcctttc atgagtatct atttcctgaa aggtttccaa  | 240  |
| tggaatatac aatctgctca atgcagagtg gtcaattttc tggaactct atccatgcat   | 300  |
| gcaagtatgt ttgtcagtct cttaatttta agttggattg ccataagccg ctatgctacc  | 360  |
| ttaatgcaa aggattcctc gcaagagact acttcatgct atgagaaaat attttatggc   | 420  |
| catttactga aaaaatttcg ccagcccaac tttgctagaa aactatgcat ttacatatgg  | 480  |
| ggagttgtac tgggcataat cattccagtt accgtatact actcagtcac agaggctaca  | 540  |
| gaaggagaag agagcctatg ctacaatcgg cagatggaac taggagccat gatctctcag  | 600  |
| attgcaggtc tcattggaac cacatttatt ggattttcct ttttagtagt actaacatca  | 660  |
| tactactcct ttgtaagcca tctgagaaaa ataagaacct gtacgtccat tatggagaaa  | 720  |
| gatttgactt acagtctctg gaaaagacat cttttgtgca tccagattct actaatagtt  | 780  |
| tgcttccttc cttatagtat ttttaaacc ctttttatg ttctacacca aagagataac    | 840  |
| tgtcagcaat tgaattattt aatagaaaca aaaaacattc tcacctgtct tgcttcggcc  | 900  |
| agaagtagca cagaccccat tatatttctt ttattagata aaacattcaa gaagacacta  | 960  |
| tataatctct ttacaaagtc taattcagca catatgcaat catatggttg a           | 1011 |

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&lt;210&gt; SEQ ID NO 26

&lt;211&gt; LENGTH: 336

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 26

Met Asn Asn Asn Thr Thr Cys Ile Gln Pro Ser Met Ile Ser Ser Met  
1 5 10 15

Ala Leu Pro Ile Ile Tyr Ile Leu Leu Cys Ile Val Gly Val Phe Gly  
20 25 30

Asn Thr Leu Ser Gln Trp Ile Phe Leu Thr Lys Ile Gly Lys Lys Thr  
35 40 45

Ser Thr His Ile Tyr Leu Ser His Leu Val Thr Ala Asn Leu Leu Val  
50 55 60

Cys Ser Ala Met Pro Phe Met Ser Ile Tyr Phe Leu Lys Gly Phe Gln  
65 70 75 80

Trp Glu Tyr Gln Ser Ala Gln Cys Arg Val Val Asn Phe Leu Gly Thr  
85 90 95

Leu Ser Met His Ala Ser Met Phe Val Ser Leu Leu Ile Leu Ser Trp  
100 105 110

Ile Ala Ile Ser Arg Tyr Ala Thr Leu Met Gln Lys Asp Ser Ser Gln  
115 120 125

Glu Thr Thr Ser Cys Tyr Glu Lys Ile Phe Tyr Gly His Leu Leu Lys  
130 135 140

Lys Phe Arg Gln Pro Asn Phe Ala Arg Lys Leu Cys Ile Tyr Ile Trp  
145 150 155 160

Gly Val Val Leu Gly Ile Ile Ile Pro Val Thr Val Tyr Tyr Ser Val  
165 170 175

Ile Glu Ala Thr Glu Gly Glu Glu Ser Leu Cys Tyr Asn Arg Gln Met  
180 185 190

Glu Leu Gly Ala Met Ile Ser Gln Ile Ala Gly Leu Ile Gly Thr Thr  
195 200 205

Phe Ile Gly Phe Ser Phe Leu Val Val Leu Thr Ser Tyr Tyr Ser Phe  
210 215 220

Val Ser His Leu Arg Lys Ile Arg Thr Cys Thr Ser Ile Met Glu Lys  
225 230 235 240

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

Leu Leu Ile Val Cys Phe Leu Pro Tyr Ser Ile Phe Lys Pro Ile Phe  
260 265 270

Tyr Val Leu His Gln Arg Asp Asn Cys Gln Gln Leu Asn Tyr Leu Ile  
275 280 285

Glu Thr Lys Asn Ile Leu Thr Cys Leu Ala Ser Ala Arg Ser Ser Thr  
290 295 300

Asp Pro Ile Ile Phe Leu Leu Leu Asp Lys Thr Phe Lys Lys Thr Leu  
305 310 315 320

Tyr Asn Leu Phe Thr Lys Ser Asn Ser Ala His Met Gln Ser Tyr Gly  
325 330 335

&lt;210&gt; SEQ ID NO 27

&lt;211&gt; LENGTH: 1014

&lt;212&gt; TYPE: DNA

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&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 27

```

atgaatgagc cactagacta tttagcaaat gcttctgatt tccccgatta tgcagctgct    60
tttgaaatt gcactgatga aaacatccca ctcaagatgc actacctccc tgttatttat    120
ggcattatct tcctcgtggg atttcaggc aatgcagtag tgatatccac ttacattttc    180
aaaaatgagac cttggaagag cagcaccatc attatgctga acctggcctg cacagatctg    240
ctgtatctga ccagcctccc ctctctgatt cactactatg ccagtggcga aaactggatc    300
tttgagagatt tcatgtgtaa gtttatccgc ttcagcttcc atttcaacct gtatagcagc    360
atcctcttcc tcacctgttt cagcatcttc cgctactgtg tgatcattca ccaaatgagc    420
tgcttttcca ttcacaaaac tcgatgtgca gttgtagcct gtgctgtggg gtggatcatt    480
tcactggtag ctgtcattcc gatgacctc ttgatcacat caaccaacag gaccaacaga    540
tcagcctgtc tcgacctcac cagttcggat gaactcaata ctattaagtg gtacaacctg    600
attttgactg caactacttt ctgcctcccc ttggtgatag tgacactttg ctataccacg    660
attatccaca ctctgaccca tggactgcaa actgacagct gccttaagca gaaagcacga    720
aggctaacca ttctgtact ccttgcatth tacgtatgtt tttaccctt ccatatcttg    780
agggtcattc ggatcgaatc tcgctgctt tcaatcagtt gttccattga gaatcagatc    840
catgaagctt acatcgtttc tagaccatta gctgctctga acacctttgg taacctgtta    900
ctatatgtgg tggtcagcga caactttcag caggctgtct gctcaacagt gagatgcaaa    960
gtaagcggga accttgagca agcaaagaaa attagttact caaacaaccc ttga    1014

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&lt;210&gt; SEQ ID NO 28

&lt;211&gt; LENGTH: 337

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 28

```

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

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Ser Leu Val Ala Val Ile Pro Met Thr Phe Leu Ile Thr Ser Thr Asn  
165 170 175

Arg Thr Asn Arg Ser Ala Cys Leu Asp Leu Thr Ser Ser Asp Glu Leu  
180 185 190

Asn Thr Ile Lys Trp Tyr Asn Leu Ile Leu Thr Ala Thr Thr Phe Cys  
195 200 205

Leu Pro Leu Val Ile Val Thr Leu Cys Tyr Thr Thr Ile Ile His Thr  
210 215 220

Leu Thr His Gly Leu Gln Thr Asp Ser Cys Leu Lys Gln Lys Ala Arg  
225 230 235 240

Arg Leu Thr Ile Leu Leu Leu Leu Ala Phe Tyr Val Cys Phe Leu Pro  
245 250 255

Phe His Ile Leu Arg Val Ile Arg Ile Glu Ser Arg Leu Leu Ser Ile  
260 265 270

Ser Cys Ser Ile Glu Asn Gln Ile His Glu Ala Tyr Ile Val Ser Arg  
275 280 285

Pro Leu Ala Ala Leu Asn Thr Phe Gly Asn Leu Leu Leu Tyr Val Val  
290 295 300

Val Ser Asp Asn Phe Gln Gln Ala Val Cys Ser Thr Val Arg Cys Lys  
305 310 315 320

Val Ser Gly Asn Leu Glu Gln Ala Lys Lys Ile Ser Tyr Ser Asn Asn  
325 330 335

Pro

<210> SEQ ID NO 29  
<211> LENGTH: 993  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 29

```

atggatccaa ccaccccggc ctggggaaca gaaagtacaa cagtgaatgg aaatgaccaa    60
gcccttcttc tgctttgtgg caaggagacc ctgatcccg tcttctgat ccttttcatt    120
gccctggtcg ggctggtagg aaacgggttt gtgctctggc tcctgggctt ccgcatgcmc    180
aggaacgcct tctctgtcta cgctctcagc ctggccgggg ccgacttctt ctctctctgc    240
ttccagatta taaattgcct ggtgtacctc agtaacttct tctgttccat ctccatcaat    300
ttccctagct tcttcaccac tgtgatgacc tgtgcctacc ttgcaggcct gagcatgctg    360
agcaccgtca gcaccgagcg ctgcctgtcc gtcctgtggc ccatctggta tcgctgccgc    420
cgccccagac acctgtcagc ggtcgtgtgt gtcctgtctt gggccctgtc cctactgtctg    480
agcatcttgg aaggggaagt ctgtggcttc ttatttagtg atgggtgactc tggttggtgt    540
cagacatttg atttcatcac tgcagcgtgg ctgatttttt tattcatggg tctctgtggg    600
tccagtctgg ccctgctggt caggatcttc tgtggctcca ggggtctgcc actgaccagg    660
ctgtacctga ccatcctgct cacagtgtcg gtgttcctoc tctgcggcct gccctttggc    720
attcagtggt tcctaataatt atggatctgg aaggattctg atgtcttatt ttgtcatatt    780
catccagttt cagttgtcct gtcactcttc aacagcagtg ccaaccccat catttacttc    840
ttcgtgggct cttttaggaa gcagtggcgg ctgcagcagc cgatcctcaa gctggctctc    900
cagaggggctc tgcaggacat tgctgaggtg gatcacagtg aaggatgctt ccgtcagggc    960
accccgagga tgtcgagaag cagtctggtg tag                                993

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<210> SEQ ID NO 30  
<211> LENGTH: 330  
<212> TYPE: PRT  
<213> ORGANISM: Homo sapiens  
  
<400> SEQUENCE: 30  
Met Asp Pro Thr Thr Pro Ala Trp Gly Thr Glu Ser Thr Thr Val Asn  
1 5 10 15  
Gly Asn Asp Gln Ala Leu Leu Leu Cys Gly Lys Glu Thr Leu Ile  
20 25 30  
Pro Val Phe Leu Ile Leu Phe Ile Ala Leu Val Gly Leu Val Gly Asn  
35 40 45  
Gly Phe Val Leu Trp Leu Leu Gly Phe Arg Met Arg Arg Asn Ala Phe  
50 55 60  
Ser Val Tyr Val Leu Ser Leu Ala Gly Ala Asp Phe Leu Phe Leu Cys  
65 70 75 80  
Phe Gln Ile Ile Asn Cys Leu Val Tyr Leu Ser Asn Phe Phe Cys Ser  
85 90 95  
Ile Ser Ile Asn Phe Pro Ser Phe Phe Thr Thr Val Met Thr Cys Ala  
100 105 110  
Tyr Leu Ala Gly Leu Ser Met Leu Ser Thr Val Ser Thr Glu Arg Cys  
115 120 125  
Leu Ser Val Leu Trp Pro Ile Trp Tyr Arg Cys Arg Arg Pro Arg His  
130 135 140  
Leu Ser Ala Val Val Cys Val Leu Leu Trp Ala Leu Ser Leu Leu Leu  
145 150 155 160  
Ser Ile Leu Glu Gly Lys Phe Cys Gly Phe Leu Phe Ser Asp Gly Asp  
165 170 175  
Ser Gly Trp Cys Gln Thr Phe Asp Phe Ile Thr Ala Ala Trp Leu Ile  
180 185 190  
Phe Leu Phe Met Val Leu Cys Gly Ser Ser Leu Ala Leu Leu Val Arg  
195 200 205  
Ile Leu Cys Gly Ser Arg Gly Leu Pro Leu Thr Arg Leu Tyr Leu Thr  
210 215 220  
Ile Leu Leu Thr Val Leu Val Phe Leu Leu Cys Gly Leu Pro Phe Gly  
225 230 235 240  
Ile Gln Trp Phe Leu Ile Leu Trp Ile Trp Lys Asp Ser Asp Val Leu  
245 250 255  
Phe Cys His Ile His Pro Val Ser Val Val Leu Ser Ser Leu Asn Ser  
260 265 270  
Ser Ala Asn Pro Ile Ile Tyr Phe Phe Val Gly Ser Phe Arg Lys Gln  
275 280 285  
Trp Arg Leu Gln Gln Pro Ile Leu Lys Leu Ala Leu Gln Arg Ala Leu  
290 295 300  
Gln Asp Ile Ala Glu Val Asp His Ser Glu Gly Cys Phe Arg Gln Gly  
305 310 315 320  
Thr Pro Glu Met Ser Arg Ser Ser Leu Val  
325 330

<210> SEQ ID NO 31  
<211> LENGTH: 1092  
<212> TYPE: DNA

-continued

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 31

```

atgggccccg gcgagggcgt gctggcgggg ctcttggtga tggactggc cgtggcgctg    60
ctatccaacg cactggtgct gctttgttgc gectacagcg ctgagctccg cactcgagcc    120
tcaggcgctc tcctggtgaa tctgtcgctg ggccacctgc tggcggcggc gctggacatg    180
cccttcacgc tgctcggtgt gatgcgcggg cggacaccgt cggcgcccgg cgcattgcaa    240
gtcattggct tcctggacac cttcctggcg tccaacgcgg cgctgagcgt ggcggcgctg    300
agcgcagacc agtggctggc agtgggcttc cactgcgct acgccggacg cctgcgaccg    360
cgctatgccg gctgctgct gggctgtgcc tggggacagt cgctggcctt ctcaggcgct    420
gcacttggct gctcgtgct tggctacagc agcgccctcg cgtcctgttc gctgcgcctg    480
ccgcccagac ctgagcgtcc gcgcttcgca gccttcaccg ccacgctcca tgcctggggc    540
ttcgtgctgc cgctggcggt gctctgcctc acctcgctcc aggtgcaccg ggtggcacgc    600
agccactgcc agcgcattga caccgtcacc atgaaggcgc tcgcgctgct cgcgcacctg    660
caccaccagt tgcggcagcg ctgcctcatc cagcagaagc ggcgccgcca ccgcgccacc    720
aggaagattg gcattgctat tgcgaccttc ctcactctgt ttgccccgta tgtcatgacc    780
aggctggcgg agctcgtgcc cttcgtcacc gtgaacgccc agtggggcat cctcagcaag    840
tgcctgacct acagcaaggc ggtggccgac ccgttcacgt actctctgct ccgccggccg    900
ttccgccaag tcctggccgg catggtgcac cggctgctga agagaacccc gcgccagca    960
tccaccatg acagctctct gcatgtggcc ggcatggtgc accagctgct gaagagaacc   1020
ccgcgcccag cgtccacca caacggctct gtggacacag agaatgattc ctgcctgcag   1080
cagacacact ga                                     1092

```

&lt;210&gt; SEQ ID NO 32

&lt;211&gt; LENGTH: 363

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 32

```

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

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Ser Trp Leu Gly Tyr Ser Ser Ala Phe Ala Ser Cys Ser Leu Arg Leu  
 145 150 155 160  
 Pro Pro Glu Pro Glu Arg Pro Arg Phe Ala Ala Phe Thr Ala Thr Leu  
 165 170 175  
 His Ala Val Gly Phe Val Leu Pro Leu Ala Val Leu Cys Leu Thr Ser  
 180 185 190  
 Leu Gln Val His Arg Val Ala Arg Ser His Cys Gln Arg Met Asp Thr  
 195 200 205  
 Val Thr Met Lys Ala Leu Ala Leu Leu Ala Asp Leu His Pro Ser Val  
 210 215 220  
 Arg Gln Arg Cys Leu Ile Gln Gln Lys Arg Arg Arg His Arg Ala Thr  
 225 230 235 240  
 Arg Lys Ile Gly Ile Ala Ile Ala Thr Phe Leu Ile Cys Phe Ala Pro  
 245 250 255  
 Tyr Val Met Thr Arg Leu Ala Glu Leu Val Pro Phe Val Thr Val Asn  
 260 265 270  
 Ala Gln Trp Gly Ile Leu Ser Lys Cys Leu Thr Tyr Ser Lys Ala Val  
 275 280 285  
 Ala Asp Pro Phe Thr Tyr Ser Leu Leu Arg Arg Pro Phe Arg Gln Val  
 290 295 300  
 Leu Ala Gly Met Val His Arg Leu Leu Lys Arg Thr Pro Arg Pro Ala  
 305 310 315 320  
 Ser Thr His Asp Ser Ser Leu Asp Val Ala Gly Met Val His Gln Leu  
 325 330 335  
 Leu Lys Arg Thr Pro Arg Pro Ala Ser Thr His Asn Gly Ser Val Asp  
 340 345 350  
 Thr Glu Asn Asp Ser Cys Leu Gln Gln Thr His  
 355 360

&lt;210&gt; SEQ ID NO 33

&lt;211&gt; LENGTH: 1125

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 33

```

atgccacac tcaactctc tgccctctcca cccacattct tctgggccaa tgcctccgga      60
ggcagtgtgc tgagtgtcga tgatgtccg atgcctgtca aattcctagc cctgaggctc      120
atggttgccc tggcctatgg gcttgtggg gccattggct tgctgggaaa tttggcggtg      180
ctgtgggtac tgagtaactg tgcccggaga gccctggcc caccttcaga caccttcgtc      240
ttcaacctgg ctctggcgga cctgggactg gcaactcactc tccccttttg ggcagccgag      300
tcggcactgg actttcactg gcccttcgga ggtgccctct gcaagatggt tctgacggcc      360
actgtcctca acgtctatgc cagcatcttc ctcatcacag cgctgagcgt tgctcgctac      420
tgggtggtgg ccatggctgc ggggccaggc acccacctct cactottctg ggcccgaata      480
gccaccctgg cagtgtgggc ggcggctgcc ctggtgacgg tgcccacagc tgtcttcggg      540
gtggagggtg aggtgtgtgg tgtgcgcctt tgccctgtgc gtttccccag cagggtactg      600
ctgggggcct accagctgca gaggggtgtg ctggctttca tggtgccctt gggcgctcatc      660
accaccagct acctgtgtgt gctggccttc ctgcagcggc ggcaacggcg gcggcaggac      720
agcagggtcg tggcccgcctc tgtccgcctc ctggtggctt ccttcttctc ctgctgggtt      780

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-continued

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```

cccaaccatg tggctactct ctgggggtgc ctggtgaagt ttgacctggt gccctggaac      840
agtactttct atactatcca gacgtatgtc ttccctgtca ctacttgctt ggcacacagc      900
aatagctgcc tcaaccctgt gctgtactgt ctcctgaggg gggagccccg gcaggctctg      960
gcaggcacct tcagggatct gcggtcgagg ctgtggcccc agggcggagg ctgggtgcaa     1020
cagggtggccc taaagcaggt aggcaggcgg tgggtcgcaa gcaacccccg ggagagccgc     1080
ccttctaccc tgctcaccaa cctggacaga gggacaccgg ggtga                       1125

```

&lt;210&gt; SEQ ID NO 34

&lt;211&gt; LENGTH: 374

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 34

```

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

```

-continued

| 290                                                                | 295 | 300 |      |
|--------------------------------------------------------------------|-----|-----|------|
| Asn Pro Val Leu Tyr Cys Leu Leu Arg Arg Glu Pro Arg Gln Ala Leu    |     |     |      |
| 305                                                                | 310 | 315 | 320  |
| Ala Gly Thr Phe Arg Asp Leu Arg Ser Arg Leu Trp Pro Gln Gly Gly    |     |     |      |
|                                                                    | 325 | 330 | 335  |
| Gly Trp Val Gln Gln Val Ala Leu Lys Gln Val Gly Arg Arg Trp Val    |     |     |      |
|                                                                    | 340 | 345 | 350  |
| Ala Ser Asn Pro Arg Glu Ser Arg Pro Ser Thr Leu Leu Thr Asn Leu    |     |     |      |
|                                                                    | 355 | 360 | 365  |
| Asp Arg Gly Thr Pro Gly                                            |     |     |      |
| 370                                                                |     |     |      |
| <210> SEQ ID NO 35                                                 |     |     |      |
| <211> LENGTH: 1092                                                 |     |     |      |
| <212> TYPE: DNA                                                    |     |     |      |
| <213> ORGANISM: Homo sapiens                                       |     |     |      |
| <400> SEQUENCE: 35                                                 |     |     |      |
| atgaatcggc accatctgca ggatcacttt ctggaaatag acaagaagaa ctgctgtgtg  |     |     | 60   |
| ttccgagatg acttcattgt caagtggttg ccgccggtgt tggggctgga gtttatcttc  |     |     | 120  |
| gggcttcttg gcaatggcct tgccctgtgg attttctggt tccacctcaa gtcctggaaa  |     |     | 180  |
| tccagccgga ttttctgttt caacctggca gtggctgact ttctactgat catctgcctg  |     |     | 240  |
| cccttcctga tggacaacta tgtgaggcgt tgggactgga agtttgggga catcccttgc  |     |     | 300  |
| cggctgatgc tcttcatggt ggctatgaac cgccagggca gcatcatctt cctcacggtg  |     |     | 360  |
| gtggcggtag acaggtatatt ccgggtggtc catccccacc acgccctgaa caagatctcc |     |     | 420  |
| aatcggacag cagccatcat ctcttgctt ctgtggggca tcaactattgg cctgacagtc  |     |     | 480  |
| cacctcctga agaagaagat gccgatccag aatggcggtg caaatttgtg cagcagcttc  |     |     | 540  |
| agcatctgcc ataccttcca gtggcacgaa gccatgttcc tcctggagtt ctctctgccc  |     |     | 600  |
| ctgggcatca tcctgttctg ctcagccaga attatctgga gcctgcggca gagacaaatg  |     |     | 660  |
| gaccggcatg ccaagatcaa gagagccatc accttcatca tgggtgtggc catcgtcttt  |     |     | 720  |
| gtcatctgct tccttcccag cgtggttgtg cggatccgca tcttctggct cctgcacact  |     |     | 780  |
| tcgggcacgc agaattgtga agtgtaccgc tcggtggacc tggcgttctt tactactctc  |     |     | 840  |
| agcttcacct acatgaacag catgctggac cccgtggtgt actacttctc cagcccatcc  |     |     | 900  |
| tttcccaact tcttctccac ttgatcaac cgctgcctcc agaggaagat gacaggtgag   |     |     | 960  |
| ccagataata accgcagcac gagcgtcgag ctacacaggg accccaacaa aaccagaggc  |     |     | 1020 |
| gctccagagg cgtaaatggc caactccggt gagccatgga gccctcttta tctgggccca  |     |     | 1080 |
| acctctcctt aa                                                      |     |     | 1092 |

<210> SEQ ID NO 36  
 <211> LENGTH: 363  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo sapiens  
 <400> SEQUENCE: 36

|                                                                 |    |    |    |
|-----------------------------------------------------------------|----|----|----|
| Met Asn Arg His His Leu Gln Asp His Phe Leu Glu Ile Asp Lys Lys |    |    |    |
| 1                                                               | 5  | 10 | 15 |
| Asn Cys Cys Val Phe Arg Asp Asp Phe Ile Val Lys Val Leu Pro Pro |    |    |    |
| 20                                                              | 25 | 30 |    |

-continued

---

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

&lt;210&gt; SEQ ID NO 37

&lt;211&gt; LENGTH: 1044

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 37

atgggggatg agctggcacc ttgccctgtg ggcactacag cttggccggc cctgatccag 60

ctcatcagca agacaccctg catgcccacaa gcagccagca acatttcctt gggcctgggg 120

## -continued

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```

gacctcaggg tgcccagctc catgctgtac tggcttttcc ttccctcaag cctgctggct 180
gcagccacac tggctgtcag cccctgtgtg ctggtgacca tcctgcggaa ccaacggctg 240
cgacaggagc cccactacct gctcccggct aacatcctgc tctcagacct ggcctacatt 300
ctctccaca tgctcatctc ctccagcagc ctgggtggct gggagctggg ccgcatggcc 360
tgtggcattc tcaactgatgc tgtcttcgcc gcctgcacca gcaccatcct gtccttcacc 420
gccattgtgc tgcacaccta cctggcagtc atccatccac tgcgctacct ctccctcatg 480
tcccctgggg ctgcctggaa ggcagtggcc ctcatctggc tgggtggcctg ctgcttcccc 540
acattcctta tttggctcag caagtggcag gatgcccagc tggaggagca aggagcttca 600
tacatcctac caccaagcat gggcaccag ccgggatgtg gcctcctggg cattgttacc 660
tacacctcca ttctgtgcgt tctgttcctc tgcacagctc tcattgccaa ctgtttctgg 720
aggatctatg cagaggccaa gacttcaggc atctgggggc agggctattc ccgggccagg 780
ggcacctgc tgatccactc agtgctgac acattgtacg tgagcacagg ggtggtgttc 840
tcccctggaca tgggtgctgac caggtaccac cacattgact ctgggactca cacatggctc 900
ctggcagcta acagtgaggt actcatgatg cttccccgtg ccatgctccc atacctgtac 960
ctgctccgct accggcagct gttgggcatg gtccggggcc acctcccatc caggaggcac 1020
caggccatct ttaccatttc ctacg 1044

```

&lt;210&gt; SEQ ID NO 38

&lt;211&gt; LENGTH: 347

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 38

```

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

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Gln Leu Glu Glu Gln Gly Ala Ser Tyr Ile Leu Pro Pro Ser Met Gly  
195 200 205

Thr Gln Pro Gly Cys Gly Leu Leu Val Ile Val Thr Tyr Thr Ser Ile  
210 215 220

Leu Cys Val Leu Phe Leu Cys Thr Ala Leu Ile Ala Asn Cys Phe Trp  
225 230 235 240

Arg Ile Tyr Ala Glu Ala Lys Thr Ser Gly Ile Trp Gly Gln Gly Tyr  
245 250 255

Ser Arg Ala Arg Gly Thr Leu Leu Ile His Ser Val Leu Ile Thr Leu  
260 265 270

Tyr Val Ser Thr Gly Val Val Phe Ser Leu Asp Met Val Leu Thr Arg  
275 280 285

Tyr His His Ile Asp Ser Gly Thr His Thr Trp Leu Leu Ala Ala Asn  
290 295 300

Ser Glu Val Leu Met Met Leu Pro Arg Ala Met Leu Pro Tyr Leu Tyr  
305 310 315 320

Leu Leu Arg Tyr Arg Gln Leu Leu Gly Met Val Arg Gly His Leu Pro  
325 330 335

Ser Arg Arg His Gln Ala Ile Phe Thr Ile Ser  
340 345

&lt;210&gt; SEQ ID NO 39

&lt;211&gt; LENGTH: 1023

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 39

```

atgaatccat ttcattgcac ttgttgaac acctctgccg aacttttaaa caaatcctgg      60
aataaagagt ttgcttatca aactgccagt gtggttagata cagtcaccc ccttccatg      120
attgggatta tctgttcaac agggctggtt ggcaacatcc tcattgtatt cactataata      180
agatccagga aaaaaacagt ccctgacatc tatactctgca acctggctgt ggctgatttg      240
gtccacatag ttggaatgcc ttttcttatt caccaatggg cccgaggggg agagtgggtg      300
tttggggggc ctctctgcac catcatcaca tccctggata cttgtaacca attgcctgt      360
agtgccatca tgactgtaat gagtgtggac aggtactttg ccctcgcca accatttcga      420
ctgacacggt ggagaacaag gtacaagacc atccggatca atttgggcct ttgggcagct      480
tcctttatcc tggcattgcc tgtctgggtc tactcgaagg tcatcaaatt taaagacggt      540
gttgagagtt gtgcttttga ttgacatcc cctgacgatg tactctggta tacactttat      600
ttgacgataa caactttttt ttccctcta cccttgattt tgggtgtgta tattttaatt      660
ttatgctata cttgggagat gtatcaacag aataaggatg ccagatgctg caatcccagt      720
gtacaaaaac agagagtgat gaagttgaca aagatggtgc tgggtgctggt gtagtcttt      780
atcctgagtg ctgccctta tcatgtgata caactggtga acttacagat ggaacagccc      840
acactggcct tctatgtggg ttattacctc tccatctgtc tcagctatgc cagcagcagc      900
attaaccctt ttctctacat cctgctgagt ggaaatttcc agaaacgtct gcctcaaatc      960
caaagaagag cgactgagaa ggaaatcaac aatatgggaa acactctgaa atcacacttt     1020
tag                                                                1023

```

&lt;210&gt; SEQ ID NO 40

-continued

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<211> LENGTH: 340
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 40
Met Asn Pro Phe His Ala Ser Cys Trp Asn Thr Ser Ala Glu Leu Leu
 1             5             10             15
Asn Lys Ser Trp Asn Lys Glu Phe Ala Tyr Gln Thr Ala Ser Val Val
 20             25             30
Asp Thr Val Ile Leu Pro Ser Met Ile Gly Ile Ile Cys Ser Thr Gly
 35             40             45
Leu Val Gly Asn Ile Leu Ile Val Phe Thr Ile Ile Arg Ser Arg Lys
 50             55             60
Lys Thr Val Pro Asp Ile Tyr Ile Cys Asn Leu Ala Val Ala Asp Leu
 65             70             75             80
Val His Ile Val Gly Met Pro Phe Leu Ile His Gln Trp Ala Arg Gly
 85             90             95
Gly Glu Trp Val Phe Gly Gly Pro Leu Cys Thr Ile Ile Thr Ser Leu
100            105            110
Asp Thr Cys Asn Gln Phe Ala Cys Ser Ala Ile Met Thr Val Met Ser
115            120            125
Val Asp Arg Tyr Phe Ala Leu Val Gln Pro Phe Arg Leu Thr Arg Trp
130            135            140
Arg Thr Arg Tyr Lys Thr Ile Arg Ile Asn Leu Gly Leu Trp Ala Ala
145            150            155            160
Ser Phe Ile Leu Ala Leu Pro Val Trp Val Tyr Ser Lys Val Ile Lys
165            170            175
Phe Lys Asp Gly Val Glu Ser Cys Ala Phe Asp Leu Thr Ser Pro Asp
180            185            190
Asp Val Leu Trp Tyr Thr Leu Tyr Leu Thr Ile Thr Thr Phe Phe Phe
195            200            205
Pro Leu Pro Leu Ile Leu Val Cys Tyr Ile Leu Ile Leu Cys Tyr Thr
210            215            220
Trp Glu Met Tyr Gln Gln Asn Lys Asp Ala Arg Cys Cys Asn Pro Ser
225            230            235            240
Val Pro Lys Gln Arg Val Met Lys Leu Thr Lys Met Val Leu Val Leu
245            250            255
Val Val Val Phe Ile Leu Ser Ala Ala Pro Tyr His Val Ile Gln Leu
260            265            270
Val Asn Leu Gln Met Glu Gln Pro Thr Leu Ala Phe Tyr Val Gly Tyr
275            280            285
Tyr Leu Ser Ile Cys Leu Ser Tyr Ala Ser Ser Ser Ile Asn Pro Phe
290            295            300
Leu Tyr Ile Leu Leu Ser Gly Asn Phe Gln Lys Arg Leu Pro Gln Ile
305            310            315            320
Gln Arg Arg Ala Thr Glu Lys Glu Ile Asn Asn Met Gly Asn Thr Leu
325            330            335
Lys Ser His Phe
340

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<210> SEQ ID NO 41
<211> LENGTH: 24
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 41

cttgacagaca tcacccatggc agcc

24

<210> SEQ ID NO 42  
<211> LENGTH: 24  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 42

gtgatgctct gagtactgga ctgg

24

<210> SEQ ID NO 43  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 43

gaagctgtga agagtgatgc

20

<210> SEQ ID NO 44  
<211> LENGTH: 24  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 44

gtcagcaata ttgataagca gcag

24

<210> SEQ ID NO 45  
<211> LENGTH: 27  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 45

ccatggggaa cgattctgtc agctacg

27

<210> SEQ ID NO 46  
<211> LENGTH: 24  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 46

gctatgcctg aagccagtct tgtg

24

<210> SEQ ID NO 47  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 47

ccaggatggt gtgtcaccgt ggtggc 26

<210> SEQ ID NO 48  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 48

cacagcgctg cagccctgca gctggc 26

<210> SEQ ID NO 49  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 49

cttcctctcg tagggatgaa ccagac 26

<210> SEQ ID NO 50  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 50

ctcgcacagg tgggaagcac ctgtgg 26

<210> SEQ ID NO 51  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 51

gcctgtgaca ggaggtaccc tgg 23

<210> SEQ ID NO 52  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 52

catatccctc cgagtgtcca gcggc 25

<210> SEQ ID NO 53  
<211> LENGTH: 31  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 53

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gcatggagag aaaatttatg tccttgcaac c

31

<210> SEQ ID NO 54  
<211> LENGTH: 27  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<223> OTHER INFORMATION: Novel Sequence  
  
<400> SEQUENCE: 54

caagaacagg tctcatctaa gagctcc

27

<210> SEQ ID NO 55  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence  
  
<400> SEQUENCE: 55

gctgttgcca tgacgtccac ctgcac

26

<210> SEQ ID NO 56  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence  
  
<400> SEQUENCE: 56

ggacagttca aggtttgcct tagaac

26

<210> SEQ ID NO 57  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence  
  
<400> SEQUENCE: 57

ctttcgatac tgctcctatg ctc

23

<210> SEQ ID NO 58  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence  
  
<400> SEQUENCE: 58

gtagtccact gaaagtcag tgatcc

26

<210> SEQ ID NO 59  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence  
  
<400> SEQUENCE: 59

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tttctgagca tggatccaac catctc 26

<210> SEQ ID NO 60  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 60

ctgtctgaca gggcagaggc tcttc 25

<210> SEQ ID NO 61  
<211> LENGTH: 28  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 61

ggaactcgta tagaccacgc gtcgtcc 28

<210> SEQ ID NO 62  
<211> LENGTH: 28  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 62

ggaggttgcg ccttagcgac agatgacc 28

<210> SEQ ID NO 63  
<211> LENGTH: 22  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 63

ctgcaccgg acattgctc tg 22

<210> SEQ ID NO 64  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 64

gtctgcttgt tcagtccac tcaac 25

<210> SEQ ID NO 65  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 65

tatctgcaat tctattctag ctctg 26

<210> SEQ ID NO 66

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<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 66

tgtccctaataaagtcacatgaatgc

26

<210> SEQ ID NO 67  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 67

ggagacaaccatgaatgagcac

23

<210> SEQ ID NO 68  
<211> LENGTH: 24  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 68

tatttcaagggtgttttgagtaac

24

<210> SEQ ID NO 69  
<211> LENGTH: 27  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 69

ggcaccagtgagggttttctgagcatg

27

<210> SEQ ID NO 70  
<211> LENGTH: 27  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 70

ctgatggaagtagaggctgtccatctc

27

<210> SEQ ID NO 71  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 71

cctggcgagcgcctagcgccatg

23

<210> SEQ ID NO 72  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:

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<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 72

atgagccctg ccaggccctc agt 23

<210> SEQ ID NO 73

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 73

ctgcatgcc cacactcaat acttctg 27

<210> SEQ ID NO 74

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 74

aaggatccta cacttggtgg atctcag 27

<210> SEQ ID NO 75

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 75

gctggagcat tcactaggcg ag 22

<210> SEQ ID NO 76

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 76

agatcctggt tcttggtgac aatg 24

<210> SEQ ID NO 77

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 77

agccatccct gccaggaagc atgg 24

<210> SEQ ID NO 78

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 78

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|                                                                                                                                                                                     |     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| ccagactgtg gactcaagaa ctctagg                                                                                                                                                       | 27  |
| <210> SEQ ID NO 79<br><211> LENGTH: 28<br><212> TYPE: DNA<br><213> ORGANISM: Artificial Sequence<br><220> FEATURE:<br><223> OTHER INFORMATION: Novel Sequence<br><400> SEQUENCE: 79 |     |
| agtcacgaa caatgaatcc atttcatg                                                                                                                                                       | 28  |
| <210> SEQ ID NO 80<br><211> LENGTH: 25<br><212> TYPE: DNA<br><213> ORGANISM: Artificial Sequence<br><220> FEATURE:<br><223> OTHER INFORMATION: Novel Sequence<br><400> SEQUENCE: 80 |     |
| atcatgtcta gactcatggt gatcc                                                                                                                                                         | 25  |
| <210> SEQ ID NO 81<br><211> LENGTH: 30<br><212> TYPE: DNA<br><213> ORGANISM: Artificial Sequence<br><220> FEATURE:<br><223> OTHER INFORMATION: Novel Sequence<br><400> SEQUENCE: 81 |     |
| ggggagggaa agcaaagggtg gtccctcctgg                                                                                                                                                  | 30  |
| <210> SEQ ID NO 82<br><211> LENGTH: 30<br><212> TYPE: DNA<br><213> ORGANISM: Artificial Sequence<br><220> FEATURE:<br><223> OTHER INFORMATION: Novel Sequence<br><400> SEQUENCE: 82 |     |
| ccaggagaac cacctttgct ttccctcccc                                                                                                                                                    | 30  |
| <210> SEQ ID NO 83<br><211> LENGTH: 1356<br><212> TYPE: DNA<br><213> ORGANISM: Homo sapiens<br><400> SEQUENCE: 83                                                                   |     |
| atggagtcct caccatccc ccagtcacga ggaactctt ccactttggg gagggtcct                                                                                                                      | 60  |
| caaaacccag gtccctctac tgccagtggg gtcccgagg tggggctacg ggatgttgct                                                                                                                    | 120 |
| tcggaatctg tggccctctt cttcatgctc ctgctggact tgactgctgt ggctggcaat                                                                                                                   | 180 |
| gccgctgtga tggccgtgat cgccaagacg cctgccctcc gaaaatttgt cttcgtcttc                                                                                                                   | 240 |
| cacctctgcc tgggtggacct gctggctgcc ctgaccctca tgcccctggc catgctctcc                                                                                                                  | 300 |
| agctctgccc tctttgacca cgccctcttt ggggaggtgg cctgccgcct ctacttgttt                                                                                                                   | 360 |
| ctgagcgtgt gctttgtcag cctggccatc ctctcgggtg cagccatcaa tgtggagcgc                                                                                                                   | 420 |
| tactattacg tagtccaccc catgcgctac gaggtgcgca tgacgctggg gctggtggcc                                                                                                                   | 480 |
| tctgtgctgg tgggtgtgtg ggtgaaggcc ttggccatgg cttctgtgcc agtgttgga                                                                                                                    | 540 |

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aggggtctcct gggaggaagg agctcccagt gtccccccag gctgttcact ccagtggagc   600
cacagtgcct actgccagct ttttggtgtg gtctttgctg tcctttactt tctgttgccc   660
ctgctcctca tacttgttgt ctactgcagc atgttccgag tggcccgcggt ggctgccatg   720
cagcacgggc cgctgcccac gtggatggag acaccccggc aacgctccga atctctcagc   780
agccgctcca cgatgggtcac cagctcgggg gccccccaga cccccccaca ccggacgttt   840
gggggagggg aagcaaagggt ggttctcctg gctgtggggg gacagttcct gctctgttgg   900
ttgccctact tctctttcca cctctatgtt gccctgagtg ctcagcccat ttcaactggg   960
caggtggaga gtgtgggtcac ctggattggc tacttttgct tcacttccaa ccctttcttc  1020
tatggatgtc tcaaccggca gatccggggg gagctcagca agcagtttgt ctgcttcttc  1080
aagccagctc cagaggagga gctgaggctg cctagccggg agggctccat tgaggagAAC  1140
ttcctgcagt tccttcaggg gactggctgt ccttctgagt cctgggtttc ccgacccta  1200
cccagcccca agcaggagcc acctgctgtt gactttcga tcccaggcca gatagctgag  1260
gagacctctg agttcctgga gcagcaactc accagcgaca tcatcatgtc agacagctac  1320
ctccgtcctg ccgcctcacc ccggtggag tcatga                               1356

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&lt;210&gt; SEQ ID NO 84

&lt;211&gt; LENGTH: 451

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 84

```

Met Glu Ser Ser Pro Ile Pro Gln Ser Ser Gly Asn Ser Ser Thr Leu
 1             5             10             15

Gly Arg Val Pro Gln Thr Pro Gly Pro Ser Thr Ala Ser Gly Val Pro
          20             25             30

Glu Val Gly Leu Arg Asp Val Ala Ser Glu Ser Val Ala Leu Phe Phe
          35             40             45

Met Leu Leu Leu Asp Leu Thr Ala Val Ala Gly Asn Ala Ala Val Met
          50             55             60

Ala Val Ile Ala Lys Thr Pro Ala Leu Arg Lys Phe Val Phe Val Phe
          65             70             75             80

His Leu Cys Leu Val Asp Leu Leu Ala Ala Leu Thr Leu Met Pro Leu
          85             90             95

Ala Met Leu Ser Ser Ser Ala Leu Phe Asp His Ala Leu Phe Gly Glu
          100            105            110

Val Ala Cys Arg Leu Tyr Leu Phe Leu Ser Val Cys Phe Val Ser Leu
          115            120            125

Ala Ile Leu Ser Val Ser Ala Ile Asn Val Glu Arg Tyr Tyr Tyr Val
          130            135            140

Val His Pro Met Arg Tyr Glu Val Arg Met Thr Leu Gly Leu Val Ala
          145            150            155            160

Ser Val Leu Val Gly Val Trp Val Lys Ala Leu Ala Met Ala Ser Val
          165            170            175

Pro Val Leu Gly Arg Val Ser Trp Glu Glu Gly Ala Pro Ser Val Pro
          180            185            190

Pro Gly Cys Ser Leu Gln Trp Ser His Ser Ala Tyr Cys Gln Leu Phe
          195            200            205

Val Val Val Phe Ala Val Leu Tyr Phe Leu Leu Pro Leu Leu Leu Ile

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| 210                                                             | 215 | 220     |
|-----------------------------------------------------------------|-----|---------|
| Leu Val Val Tyr Cys Ser Met Phe Arg Val Ala Arg Val Ala Ala Met |     |         |
| 225                                                             | 230 | 235 240 |
| Gln His Gly Pro Leu Pro Thr Trp Met Glu Thr Pro Arg Gln Arg Ser |     |         |
|                                                                 | 245 | 250 255 |
| Glu Ser Leu Ser Ser Arg Ser Thr Met Val Thr Ser Ser Gly Ala Pro |     |         |
|                                                                 | 260 | 265 270 |
| Gln Thr Thr Pro His Arg Thr Phe Gly Gly Gly Lys Ala Lys Val Val |     |         |
|                                                                 | 275 | 280 285 |
| Leu Leu Ala Val Gly Gly Gln Phe Leu Leu Cys Trp Leu Pro Tyr Phe |     |         |
|                                                                 | 290 | 295 300 |
| Ser Phe His Leu Tyr Val Ala Leu Ser Ala Gln Pro Ile Ser Thr Gly |     |         |
| 305                                                             | 310 | 315 320 |
| Gln Val Glu Ser Val Val Thr Trp Ile Gly Tyr Phe Cys Phe Thr Ser |     |         |
|                                                                 | 325 | 330 335 |
| Asn Pro Phe Phe Tyr Gly Cys Leu Asn Arg Gln Ile Arg Gly Glu Leu |     |         |
|                                                                 | 340 | 345 350 |
| Ser Lys Gln Phe Val Cys Phe Phe Lys Pro Ala Pro Glu Glu Glu Leu |     |         |
|                                                                 | 355 | 360 365 |
| Arg Leu Pro Ser Arg Glu Gly Ser Ile Glu Glu Asn Phe Leu Gln Phe |     |         |
|                                                                 | 370 | 375 380 |
| Leu Gln Gly Thr Gly Cys Pro Ser Glu Ser Trp Val Ser Arg Pro Leu |     |         |
| 385                                                             | 390 | 395 400 |
| Pro Ser Pro Lys Gln Glu Pro Pro Ala Val Asp Phe Arg Ile Pro Gly |     |         |
|                                                                 | 405 | 410 415 |
| Gln Ile Ala Glu Glu Thr Ser Glu Phe Leu Glu Gln Gln Leu Thr Ser |     |         |
|                                                                 | 420 | 425 430 |
| Asp Ile Ile Met Ser Asp Ser Tyr Leu Arg Pro Ala Ala Ser Pro Arg |     |         |
|                                                                 | 435 | 440 445 |
| Leu Glu Ser                                                     |     |         |
| 450                                                             |     |         |

<210> SEQ ID NO 85  
 <211> LENGTH: 28  
 <212> TYPE: DNA  
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 85

caggaaggca aagaccacca tcatcatc

28

<210> SEQ ID NO 86  
 <211> LENGTH: 28  
 <212> TYPE: DNA  
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 86

gatgatgatg gtggtctttg ccttcctg

28

<210> SEQ ID NO 87  
 <211> LENGTH: 1041  
 <212> TYPE: DNA  
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 87

atggagagaa aatttatgtc cttgcaacca tccatctccg tatcagaaat ggaaccaa

60

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ggcaccttca gcaataacaa cagcaggaac tgcacaattg aaaacttcaa gagagaattt 120
ttcccaattg tatatctgat aatatttttc tggggagtct tgggaaatgg gttgtccata 180
tatgttttcc tgcagcctta taagaagtcc acatctgtga acgttttcat gctaaatctg 240
gccatttcag atctcctgtt cataagcacg cttcccttca gggctgacta ttatcttaga 300
ggctccaatt ggatatttgg agacctggcc tgcaggatta tgtcttattc cttgtatgtc 360
aacatgtaca gcagtattta tttcctgacc gtgctgagtg ttgtgcgttt cctggcaatg 420
gttcacccct ttcggcttct gcatgtcacc agcatcagga gtgcctggat cctctgtggg 480
atcatatgga tccttatcat ggcttctca ataatgctcc tggacagtgg ctctgagcag 540
aacggcagtg tcacatcatg cttagagctg aatctctata aaattgctaa gctgcagacc 600
atgaactata ttgccttggt ggtgggctgc ctgctgccat ttttcacact cagcatctgt 660
tatctgtctga tcattcgggt tctgttaaaa gtggaggtcc cagaatcggg gctgcggggt 720
tctcacagga aggcaaagac caccatcatc atcaccttga tcatcttctt cttgtgtttc 780
ctgccctatc acacactgag gaccgtccac ttgacgacat ggaagtgagg tttatgcaaa 840
gacagactgc ataaagcttt ggttatcaca ctggccttgg cagcagccaa tgcctgcttc 900
aatcctctgc tctattactt tgctggggag aattttaagg acagactaaa gtctgcactc 960
agaaaaggcc atccacagaa ggcaaagaca aagtgtgttt tccctgttag tgtgtggttg 1020
agaaaggaaa caagagtata a 1041

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&lt;210&gt; SEQ ID NO 88

&lt;211&gt; LENGTH: 346

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 88

```

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

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Gly Ser Glu Gln Asn Gly Ser Val Thr Ser Cys Leu Glu Leu Asn Leu  
                   180                  185                  190

Tyr Lys Ile Ala Lys Leu Gln Thr Met Asn Tyr Ile Ala Leu Val Val  
                   195                  200                  205

Gly Cys Leu Leu Pro Phe Phe Thr Leu Ser Ile Cys Tyr Leu Leu Ile  
                   210                  215                  220

Ile Arg Val Leu Leu Lys Val Glu Val Pro Glu Ser Gly Leu Arg Val  
                   225                  230                  235                  240

Ser His Arg Lys Ala Lys Thr Thr Ile Ile Ile Thr Leu Ile Ile Phe  
                   245                  250                  255

Phe Leu Cys Phe Leu Pro Tyr His Thr Leu Arg Thr Val His Leu Thr  
                   260                  265                  270

Thr Trp Lys Val Gly Leu Cys Lys Asp Arg Leu His Lys Ala Leu Val  
                   275                  280                  285

Ile Thr Leu Ala Leu Ala Ala Ala Asn Ala Cys Phe Asn Pro Leu Leu  
                   290                  295                  300

Tyr Tyr Phe Ala Gly Glu Asn Phe Lys Asp Arg Leu Lys Ser Ala Leu  
                   305                  310                  315                  320

Arg Lys Gly His Pro Gln Lys Ala Lys Thr Lys Cys Val Phe Pro Val  
                   325                  330                  335

Ser Val Trp Leu Arg Lys Glu Thr Arg Val  
                   340                  345

<210> SEQ ID NO 89  
 <211> LENGTH: 28  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 89

ccagtgcaaa gctaagaaag tgatcttc

28

<210> SEQ ID NO 90  
 <211> LENGTH: 28  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Novel Sequence  
 <220> FEATURE:  
 <221> NAME/KEY: misc\_feature  
 <223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 90

gaagatcact ttcttagctt tgcaactgg

28

<210> SEQ ID NO 91  
 <211> LENGTH: 1527  
 <212> TYPE: DNA  
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 91

atgacgtcca cctgcaccaa cagcacgcgc gagagtaaca gcagccacac gtgcatgccc 60

ctctccaaaa tgcccatcag cctggcccac ggcacatccc gctcaaccgt gctggttata 120

ttctctgcgc cctcttttcgt cggcaacata gtgctggcgc tagtggttga gcgcaagccg 180

cagctgctgc aggtgaccaa ccgtttttatc ttttaacctcc tcgtcaccga cctgctgcag 240

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atttcgctcg tggcccccctg ggtggtggcc acctctgtgc ctctcttctg gccctcaac 300
agccacttct gcacggccct ggtagcctc acccacctgt tcgccttcgc cagcgtaac 360
accattgtcg tgggtgcagt ggatcgctac ttgtccatca tccaccctct ctccctaccg 420
tccaagatga cccagcgccg cggttacctg ctctcttatg gcacctggat tgtggccatc 480
ctgcagagca ctctccact ctacggctgg ggccaggctg cctttgatga gcgcaatgct 540
ctctgtccca tgatctgggg ggccagcccc agctacacta ttctcagcgt ggtgtccttc 600
atcgtcattc cactgattgt catgattgcc tgctactccg tgggtttctg tgcagcccg 660
aggcagcatg ctctgtgta caatgtcaag agacacagct tggaagtgcg agtcaaggac 720
tgtgtggaga atgagatga agaggagca gagaagaagg aggagtcca ggatgagagt 780
gagtttcgcc gccagcatga aggtgaggtc aaggccaagg agggcagaat ggaagccaag 840
gacggcagcc tgaaggccaa ggaaggaagc acggggacca gtgagagtag tgtagaggcc 900
aggggcagcg aggaggtcag agagagcagc acggtggcca gcgacggcag catggagggt 960
aaggaaggca gcaccaaagt tgaggagaac agcatgaagg cagacaaggg tcgcacagag 1020
gtcaaccagt gcagcattga cttgggtgaa gatgacatgg agtttgggtga agacgacatc 1080
aatttcagtg aggatgacgt cgaggcagtg aacatccccg agagcctccc acccagtcgt 1140
cgtaacagca acagcaaccc tcctctgccc aggtgctacc agtgcaaagc taagaaagtg 1200
atcttcatca tcattttctc ctatgtgcta tccctggggc cctactgctt tttagcagtc 1260
ctggccgtgt ggggtgagtgt cgaaaccag gtaccccatg gggatgacac cataatcatc 1320
tggtttttct tcctgcagtg ctgcatccac ccctatgtct atggctacat gcacaagacc 1380
attaagaagg aaatccagga catgctgaag aagttcttct gcaaggaaaa gccccgaaa 1440
gaagatagcc acccagacct gcccggaaca gaggtggga ctgaaggcaa gattgtccct 1500
tcctacgatt ctgtacttt tccttga 1527

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&lt;210&gt; SEQ ID NO 92

&lt;211&gt; LENGTH: 508

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 92

```

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

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Tyr | Leu | Ser | Ile | Ile | His | Pro | Leu | Ser | Tyr | Pro | Ser | Lys | Met | Thr |
| 130 |     |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Gln | Arg | Arg | Gly | Tyr | Leu | Leu | Leu | Tyr | Gly | Thr | Trp | Ile | Val | Ala | Ile |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
| Leu | Gln | Ser | Thr | Pro | Pro | Leu | Tyr | Gly | Trp | Gly | Gln | Ala | Ala | Phe | Asp |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Glu | Arg | Asn | Ala | Leu | Cys | Ser | Met | Ile | Trp | Gly | Ala | Ser | Pro | Ser | Tyr |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Thr | Ile | Leu | Ser | Val | Val | Ser | Phe | Ile | Val | Ile | Pro | Leu | Ile | Val | Met |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Ile | Ala | Cys | Tyr | Ser | Val | Val | Phe | Cys | Ala | Ala | Arg | Arg | Gln | His | Ala |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| Leu | Leu | Tyr | Asn | Val | Lys | Arg | His | Ser | Leu | Glu | Val | Arg | Val | Lys | Asp |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Cys | Val | Glu | Asn | Glu | Asp | Glu | Glu | Gly | Ala | Glu | Lys | Lys | Glu | Glu | Phe |
|     |     |     | 245 |     |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Gln | Asp | Glu | Ser | Glu | Phe | Arg | Arg | Gln | His | Glu | Gly | Glu | Val | Lys | Ala |
|     |     | 260 |     |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Lys | Glu | Gly | Arg | Met | Glu | Ala | Lys | Asp | Gly | Ser | Leu | Lys | Ala | Lys | Glu |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| Gly | Ser | Thr | Gly | Thr | Ser | Glu | Ser | Ser | Val | Glu | Ala | Arg | Gly | Ser | Glu |
|     |     | 290 |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| Glu | Val | Arg | Glu | Ser | Ser | Thr | Val | Ala | Ser | Asp | Gly | Ser | Met | Glu | Gly |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
| Lys | Glu | Gly | Ser | Thr | Lys | Val | Glu | Glu | Asn | Ser | Met | Lys | Ala | Asp | Lys |
|     |     |     | 325 |     |     |     |     |     | 330 |     |     |     |     | 335 |     |
| Gly | Arg | Thr | Glu | Val | Asn | Gln | Cys | Ser | Ile | Asp | Leu | Gly | Glu | Asp | Asp |
|     |     | 340 |     |     |     |     | 345 |     |     |     |     |     | 350 |     |     |
| Met | Glu | Phe | Gly | Glu | Asp | Asp | Ile | Asn | Phe | Ser | Glu | Asp | Asp | Val | Glu |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
| Ala | Val | Asn | Ile | Pro | Glu | Ser | Leu | Pro | Pro | Ser | Arg | Arg | Asn | Ser | Asn |
|     |     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |
| Ser | Asn | Pro | Pro | Leu | Pro | Arg | Cys | Tyr | Gln | Cys | Lys | Ala | Lys | Lys | Val |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |
| Ile | Phe | Ile | Ile | Ile | Phe | Ser | Tyr | Val | Leu | Ser | Leu | Gly | Pro | Tyr | Cys |
|     |     |     | 405 |     |     |     |     |     | 410 |     |     |     |     | 415 |     |
| Phe | Leu | Ala | Val | Leu | Ala | Val | Trp | Val | Asp | Val | Glu | Thr | Gln | Val | Pro |
|     |     | 420 |     |     |     |     |     | 425 |     |     |     |     | 430 |     |     |
| Gln | Trp | Val | Ile | Thr | Ile | Ile | Ile | Trp | Leu | Phe | Phe | Leu | Gln | Cys | Cys |
|     |     | 435 |     |     |     |     | 440 |     |     |     |     |     | 445 |     |     |
| Ile | His | Pro | Tyr | Val | Tyr | Gly | Tyr | Met | His | Lys | Thr | Ile | Lys | Lys | Glu |
|     | 450 |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |
| Ile | Gln | Asp | Met | Leu | Lys | Lys | Phe | Phe | Cys | Lys | Glu | Lys | Pro | Pro | Lys |
| 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |
| Glu | Asp | Ser | His | Pro | Asp | Leu | Pro | Gly | Thr | Glu | Gly | Gly | Thr | Glu | Gly |
|     |     |     | 485 |     |     |     |     |     | 490 |     |     |     |     | 495 |     |
| Lys | Ile | Val | Pro | Ser | Tyr | Asp | Ser | Ala | Thr | Phe | Pro |     |     |     |     |
|     |     | 500 |     |     |     |     |     | 505 |     |     |     |     |     |     |     |

&lt;210&gt; SEQ ID NO 93

&lt;211&gt; LENGTH: 29

&lt;212&gt; TYPE: DNA

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<213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 93

gccgccaccg cgccaagagg aagattggc 29

<210> SEQ ID NO 94  
 <211> LENGTH: 29  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 94

gccaatcttc ctcttggcgc ggtggcggc 29

<210> SEQ ID NO 95  
 <211> LENGTH: 1092  
 <212> TYPE: DNA  
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 95

atggggcccg gcgaggcgct gctggcgggt ctcttggtga tggtagctggc cgtggcgctg 60  
 ctatccaacg cactgggtgct gctttgttgc gcctacagcg ctgagctccg cactcgagcc 120  
 tcaggcgctcc tcctgggtgaa tctgtcgctg ggccacctgc tggctggcggc gctggacatg 180  
 cccttcacgc tgctcggtgt gatgcgcggg cggacaccgt cggcgcccgg cgcattgcaa 240  
 gtcattggct tcctggacac ctctctggcg tccaacgcgg cgctgagcgt ggcggcgctg 300  
 agcgcagacc agtggctggc agtgggcttc cactgcgct acgcccggacg cctgcgaccg 360  
 cgctatgcg gcctgctgct gggctgtgcc tggggacagt cgctggcctt ctcaggcgct 420  
 gcacttggct gctcgtggct tggctacagc agcgccttcg cgtcctgttc gctgcgcctg 480  
 ccgcccagc ctgagcgtcc gcgcttcgca gccttcaccg ccacgctcca tgcctgtggc 540  
 ttctgtgctgc cgctggcggt gctctgcctc acctcgctcc aggtgcaccg ggtggcacgc 600  
 agccactgcc agcgcattga caccgtcacc atgaaggcgc tcgcgctgct cgcgcacctg 660  
 caccaccagt tgccggcagc ctgcctcacc cagcagaagc ggcgccgcca ccgcgccacc 720  
 aggaagattg gattgctat tgcgacctc ctcatctgct ttgccccgta tgcattgacc 780  
 aggtggcgg agctcgtgcc ctctgtcacc gtgaacgccc agaagggcat cctcagcaag 840  
 tgcctgacct acagcaaggc ggtggccgac ccgttcacgt actctctgct ccgccggccg 900  
 ttccgccaa tcctggccgg catggtgcac cggctgctga agagaacccc gcgccagca 960  
 tccaccatg acagctctct gcatgtggcc ggcattgtgc accagctgct gaagagaacc 1020  
 ccgcgccag cgtccacca caacggctct gtggacacag agaattgatt ctgcctgcag 1080  
 cagacacact ga 1092

<210> SEQ ID NO 96  
 <211> LENGTH: 363  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 96

Met Gly Pro Gly Glu Ala Leu Leu Ala Gly Leu Leu Val Met Val Leu  
 1 5 10 15

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

<210> SEQ ID NO 97  
 <211> LENGTH: 34  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Novel Sequence  
 <400> SEQUENCE: 97

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gatctctaga atggagtcct caccatccc ccag 34

<210> SEQ ID NO 98  
<211> LENGTH: 36  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence  
  
<400> SEQUENCE: 98

gacgatatc cgtgactcca gccgggtga ggcggc 36

<210> SEQ ID NO 99  
<211> LENGTH: 2610  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens and Rat  
  
<400> SEQUENCE: 99

atggagtcct caccatccc ccagtcata ggaactctt ccactttggg gagggtcctt 60  
caaacccag gtccctctac tgccagtggg gtcccgagg tggggctacg ggatgttgc 120  
tcggaatctg tggccctctt ctctatgctc ctgctggact tgactgctgt ggctggcaat 180  
gccgctgtga tggccgtgat cgccaagacg cctgccctcc gaaaatttgt ctctgtcttc 240  
cacctctgcc tgggtggacct gctggctgcc ctgacctca tgccctggc catgctctcc 300  
agctctgcc tctttgacca cgccctcttt ggggaggtgg cctgccgcct ctacttgttt 360  
ctgagcgtgt gctttgtcag cctggccatc ctctcggtgt cagccatcaa tgtggagcgc 420  
tactattacg tagtccacc catgcgtac gaggtgcga tgacgctggg gctgggtggc 480  
tctgtgctgg tgggtgtgtg ggtgaaggcc ttggccatgg cttctgtgcc agtgttgga 540  
agggctcct gggaggaagg agctccagc gtcccccag gctgttact ccagtggagc 600  
cacagtgcct actgccagct ttttgtgtg gtctttgtc tcctttactt tctgttgccc 660  
ctgctcctca tacttgttgt ctactgcagc atgttccgag tggcccgct ggctgccatg 720  
cagcacgggc cgtgccccac gtggatggag acacccggc aacgctccga atctctcagc 780  
agcgcctcca cgtatgtcac cagctcggg gcccccaga cccccaca ccggacgttt 840  
gggggagga aagcagcagt ggttctcctg gctgtggggg gacagttcct gctctgttg 900  
ttgccctact tctctttcca cctctatgtt gccctgagt ctcagcccat ttcaactggg 960  
caggtggaga gtgtggtcac ctggattggc tacttttgc tcaactcaa cctttcttc 1020  
tatggatgtc tcaaccggca gatccgggg gagctcagca agcagttgt ctgcttcttc 1080  
aagccagctc cagaggagga gctgaggctg cctagccggg agggctccat tgaggagaac 1140  
ttcctgcagt tccttcagg gactggctgt cctctgagt cctgggttc ccgacctca 1200  
cccagccca agcaggagcc acctgtgtt gactttcga tccaggcca gatagctgag 1260  
gagacctctg agttcctgga gcagcaactc accagcgaca tcatcatgtc agacagctac 1320  
ctcgtcctg ccgcctcacc ccggctggg tcagcgatat ctgcagaatt ccaccacact 1380  
ggactagtgg atccgagctc ggtaccaagc ttgggctgca ggtcgatggg ctgcctcggc 1440  
aacagtaaga ccgaggacca gcgcaacgag gagaaggcgc agcgcgaggc caacaaaaag 1500  
atcgagaagc agctcgagaa ggacaagcag gtctaccggg ccacgcaccg cctgctgctg 1560  
ctgggtgctg gagagtctgg caaagcacc attgtgaagc agatgaggat cctacatgtt 1620

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aatgggttta acggagaggg cggcgaagag gacccgcagg ctgcaaggag caacagcgat 1680
ggtgagaagg ccaccaaagt gcaggacatc aaaaacaacc tgaaggaggc cattgaaacc 1740
attgtggccg ccatgagcaa cctggtgccc cccgtggagc tggccaaccc tgagaaccag 1800
ttcagagtgg actacattct gagcgtgatg aacgtgcaa actttgactt cccacctgaa 1860
ttctatgagc atgccaaggc tctgtgggag gatgagggag ttcgtgcctg ctacgagcgc 1920
tccaacgagt accagctgat cgactgtgcc cagtacttcc tggacaagat tgatgtgatc 1980
aagcaggccg actacgtgcc aagtgaccag gacctgcttc gctgccgcgt cctgacctct 2040
ggaatctttg agaccaagtt ccagtggtgac aaagtcaact tccacatggt cgatgtgggc 2100
ggccagcgcg atgaacgccg caagtggatc cagtgcctca atgatgtgac tgccatcatc 2160
ttcgtggtgg ccagcagcag ctacaacatg gtcacccggg aggacaacca gaccaaccgt 2220
ctgcaggagg ctctgaacct cttcaagagc atctggaaca acagatggct gcgtaccatc 2280
tctgtgatcc tcttcctcaa caagcaagat ctgcttgctg agaaggctct cgctgggaaa 2340
tcgaagattg aggactactt tccagagttc gctcgctaca ccactcctga ggatgcgact 2400
cccagccccg gagaggaccc acgctgacc cgggccaaagt acttcatccg ggatgagttt 2460
ctgagaatca gcactgctag tggagatgga cgctcactact gctaccctca ctttacctgc 2520
gccgtggaca ctgagaacat ccgccgtgtc ttcaacgact gccgtgacat catccagcgc 2580
atgcaccttc gccaatacga gctgctctaa 2610

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&lt;210&gt; SEQ ID NO 100

&lt;211&gt; LENGTH: 869

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens and Rat

&lt;400&gt; SEQUENCE: 100

```

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

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Val | Leu | Gly | Arg | Val | Ser | Trp | Glu | Glu | Gly | Ala | Pro | Ser | Val | Pro | 180 | 185 | 190 |     |
| Pro | Gly | Cys | Ser | Leu | Gln | Trp | Ser | His | Ser | Ala | Tyr | Cys | Gln | Leu | Phe | 195 | 200 | 205 |     |
| Val | Val | Val | Phe | Ala | Val | Leu | Tyr | Phe | Leu | Leu | Pro | Leu | Leu | Leu | Ile | 210 | 215 | 220 |     |
| Leu | Val | Val | Tyr | Cys | Ser | Met | Phe | Arg | Val | Ala | Arg | Val | Ala | Ala | Met | 225 | 230 | 235 | 240 |
| Gln | His | Gly | Pro | Leu | Pro | Thr | Trp | Met | Glu | Thr | Pro | Arg | Gln | Arg | Ser | 245 | 250 | 255 |     |
| Glu | Ser | Leu | Ser | Ser | Arg | Ser | Thr | Met | Val | Thr | Ser | Ser | Gly | Ala | Pro | 260 | 265 | 270 |     |
| Gln | Thr | Thr | Pro | His | Arg | Thr | Phe | Gly | Gly | Gly | Lys | Ala | Ala | Val | Val | 275 | 280 | 285 |     |
| Leu | Leu | Ala | Val | Gly | Gly | Gln | Phe | Leu | Leu | Cys | Trp | Leu | Pro | Tyr | Phe | 290 | 295 | 300 |     |
| Ser | Phe | His | Leu | Tyr | Val | Ala | Leu | Ser | Ala | Gln | Pro | Ile | Ser | Thr | Gly | 305 | 310 | 315 | 320 |
| Gln | Val | Glu | Ser | Val | Val | Thr | Trp | Ile | Gly | Tyr | Phe | Cys | Phe | Thr | Ser | 325 | 330 | 335 |     |
| Asn | Pro | Phe | Phe | Tyr | Gly | Cys | Leu | Asn | Arg | Gln | Ile | Arg | Gly | Glu | Leu | 340 | 345 | 350 |     |
| Ser | Lys | Gln | Phe | Val | Cys | Phe | Phe | Lys | Pro | Ala | Pro | Glu | Glu | Glu | Leu | 355 | 360 | 365 |     |
| Arg | Leu | Pro | Ser | Arg | Glu | Gly | Ser | Ile | Glu | Glu | Asn | Phe | Leu | Gln | Phe | 370 | 375 | 380 |     |
| Leu | Gln | Gly | Thr | Gly | Cys | Pro | Ser | Glu | Ser | Trp | Val | Ser | Arg | Pro | Leu | 385 | 390 | 395 | 400 |
| Pro | Ser | Pro | Lys | Gln | Glu | Pro | Pro | Ala | Val | Asp | Phe | Arg | Ile | Pro | Gly | 405 | 410 | 415 |     |
| Gln | Ile | Ala | Glu | Glu | Thr | Ser | Glu | Phe | Leu | Glu | Gln | Gln | Leu | Thr | Ser | 420 | 425 | 430 |     |
| Asp | Ile | Ile | Met | Ser | Asp | Ser | Tyr | Leu | Arg | Pro | Ala | Ala | Ser | Pro | Arg | 435 | 440 | 445 |     |
| Leu | Glu | Ser | Ala | Ile | Ser | Ala | Glu | Phe | His | His | Thr | Gly | Leu | Val | Asp | 450 | 455 | 460 |     |
| Pro | Ser | Ser | Val | Pro | Ser | Leu | Gly | Cys | Arg | Ser | Met | Gly | Cys | Leu | Gly | 465 | 470 | 475 | 480 |
| Asn | Ser | Lys | Thr | Glu | Asp | Gln | Arg | Asn | Glu | Glu | Lys | Ala | Gln | Arg | Glu | 485 | 490 | 495 |     |
| Ala | Asn | Lys | Lys | Ile | Glu | Lys | Gln | Leu | Gln | Lys | Asp | Lys | Gln | Val | Tyr | 500 | 505 | 510 |     |
| Arg | Ala | Thr | His | Arg | Leu | Leu | Leu | Leu | Gly | Ala | Gly | Glu | Ser | Gly | Lys | 515 | 520 | 525 |     |
| Ser | Thr | Ile | Val | Lys | Gln | Met | Arg | Ile | Leu | His | Val | Asn | Gly | Phe | Asn | 530 | 535 | 540 |     |
| Gly | Glu | Gly | Gly | Glu | Glu | Asp | Pro | Gln | Ala | Ala | Arg | Ser | Asn | Ser | Asp | 545 | 550 | 555 | 560 |
| Gly | Glu | Lys | Ala | Thr | Lys | Val | Gln | Asp | Ile | Lys | Asn | Asn | Leu | Lys | Glu | 565 | 570 | 575 |     |
| Ala | Ile | Glu | Thr | Ile | Val | Ala | Ala | Met | Ser | Asn | Leu | Val | Pro | Pro | Val |     |     |     |     |

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| 580 |     |     |     |     | 585 |     |     |     |     | 590 |     |     |     |     |     |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Glu | Leu | Ala | Asn | Pro | Glu | Asn | Gln | Phe | Arg | Val | Asp | Tyr | Ile | Leu | Ser |  |
| 595 |     |     |     |     | 600 |     |     |     |     | 605 |     |     |     |     |     |  |
| Val | Met | Asn | Val | Pro | Asn | Phe | Asp | Phe | Pro | Pro | Glu | Phe | Tyr | Glu | His |  |
| 610 |     |     |     |     | 615 |     |     |     |     | 620 |     |     |     |     |     |  |
| Ala | Lys | Ala | Leu | Trp | Glu | Asp | Glu | Gly | Val | Arg | Ala | Cys | Tyr | Glu | Arg |  |
| 625 |     |     |     |     | 630 |     |     |     |     | 635 |     |     |     |     | 640 |  |
| Ser | Asn | Glu | Tyr | Gln | Leu | Ile | Asp | Cys | Ala | Gln | Tyr | Phe | Leu | Asp | Lys |  |
| 645 |     |     |     |     | 650 |     |     |     |     | 655 |     |     |     |     |     |  |
| Ile | Asp | Val | Ile | Lys | Gln | Ala | Asp | Tyr | Val | Pro | Ser | Asp | Gln | Asp | Leu |  |
| 660 |     |     |     |     | 665 |     |     |     |     | 670 |     |     |     |     |     |  |
| Leu | Arg | Cys | Arg | Val | Leu | Thr | Ser | Gly | Ile | Phe | Glu | Thr | Lys | Phe | Gln |  |
| 675 |     |     |     |     | 680 |     |     |     |     | 685 |     |     |     |     |     |  |
| Val | Asp | Lys | Val | Asn | Phe | His | Met | Phe | Asp | Val | Gly | Gly | Gln | Arg | Asp |  |
| 690 |     |     |     |     | 695 |     |     |     |     | 700 |     |     |     |     |     |  |
| Glu | Arg | Arg | Lys | Trp | Ile | Gln | Cys | Phe | Asn | Asp | Val | Thr | Ala | Ile | Ile |  |
| 705 |     |     |     |     | 710 |     |     |     |     | 715 |     |     |     |     | 720 |  |
| Phe | Val | Val | Ala | Ser | Ser | Ser | Tyr | Asn | Met | Val | Ile | Arg | Glu | Asp | Asn |  |
| 725 |     |     |     |     | 730 |     |     |     |     | 735 |     |     |     |     |     |  |
| Gln | Thr | Asn | Arg | Leu | Gln | Glu | Ala | Leu | Asn | Leu | Phe | Lys | Ser | Ile | Trp |  |
| 740 |     |     |     |     | 745 |     |     |     |     | 750 |     |     |     |     |     |  |
| Asn | Asn | Arg | Trp | Leu | Arg | Thr | Ile | Ser | Val | Ile | Leu | Phe | Leu | Asn | Lys |  |
| 755 |     |     |     |     | 760 |     |     |     |     | 765 |     |     |     |     |     |  |
| Gln | Asp | Leu | Leu | Ala | Glu | Lys | Val | Leu | Ala | Gly | Lys | Ser | Lys | Ile | Glu |  |
| 770 |     |     |     |     | 775 |     |     |     |     | 780 |     |     |     |     |     |  |
| Asp | Tyr | Phe | Pro | Glu | Phe | Ala | Arg | Tyr | Thr | Thr | Pro | Glu | Asp | Ala | Thr |  |
| 785 |     |     |     |     | 790 |     |     |     |     | 795 |     |     |     |     | 800 |  |
| Pro | Glu | Pro | Gly | Glu | Asp | Pro | Arg | Val | Thr | Arg | Ala | Lys | Tyr | Phe | Ile |  |
| 805 |     |     |     |     | 810 |     |     |     |     | 815 |     |     |     |     |     |  |
| Arg | Asp | Glu | Phe | Leu | Arg | Ile | Ser | Thr | Ala | Ser | Gly | Asp | Gly | Arg | His |  |
| 820 |     |     |     |     | 825 |     |     |     |     | 830 |     |     |     |     |     |  |
| Tyr | Cys | Tyr | Pro | His | Phe | Thr | Cys | Ala | Val | Asp | Thr | Glu | Asn | Ile | Arg |  |
| 835 |     |     |     |     | 840 |     |     |     |     | 845 |     |     |     |     |     |  |
| Arg | Val | Phe | Asn | Asp | Cys | Arg | Asp | Ile | Ile | Gln | Arg | Met | His | Leu | Arg |  |
| 850 |     |     |     |     | 855 |     |     |     |     | 860 |     |     |     |     |     |  |
| Gln | Tyr | Glu | Leu | Leu |     |     |     |     |     |     |     |     |     |     |     |  |
| 865 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |

<210> SEQ ID NO 101  
 <211> LENGTH: 30  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 101

tctagaatga cgtccacctg caccaacagc

30

<210> SEQ ID NO 102  
 <211> LENGTH: 34  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Novel Sequence

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&lt;400&gt; SEQUENCE: 102

gatatcgtag gaaaagtagc agaatcgtag gaag 34

&lt;210&gt; SEQ ID NO 103

&lt;211&gt; LENGTH: 2781

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo Sapiens and Rat

&lt;400&gt; SEQUENCE: 103

atgacgtcca cctgcaccaa cagcacgcgc gagagtaaca gcagccacac gtgcatgccc 60  
ctctccaaaa tgcccatcag cctggccccc ggcatcatcc gctcaaccgt gctgggtatc 120  
ttcctcgccg cctcttttct cggcaacata gtgctggcgc tagtggtgca gcgcaagccg 180  
cagctgctgc aggtgaccaa ccgtttttatc tttaacctcc tcgtcaccga cctgctgcag 240  
atttcgctcg tggccccctg ggtgggtggc acctctgtgc ctctcttctg gccctcaac 300  
agccacttct gcacggccct ggttagcttc acccacctgt tcgccttcgc cagcgtcaac 360  
accattgtcg tgggtgtcagt ggatcgctac ttgtccatca tccacctctc ctctaccg 420  
tccaagatga cccagcgccg cggttacctg ctctctatg gcacctgat tgtggccatc 480  
ctgcagagca ctctccact ctacggctgg ggccaggctg cctttgatga gcgcaatgct 540  
ctctgctcca tgatctgggg ggccagcccc agctacacta ttctcagcgt ggtgtccttc 600  
atcgtcattc cactgattgt catgattgcc tgctactccg tgggtgtctg tgcagcccg 660  
aggcagcatg ctctgctgta caatgtcaag agacacagct tggagtgcg agtcaaggac 720  
tgtgtggaga atgaggatga agagggagca gagaagaagg aggagtcca ggatgagagt 780  
gagtttcgcc gccagcatga aggtgaggtc aaggccaagg agggcagaat ggaagccaag 840  
gacggcagcc tgaagcccaa ggaagggaagc acggggacca gtgagagtag tgtagaggcc 900  
aggggcagcg agggagtcag agagagcagc acgggtggcca gcgacggcag catggagggt 960  
aaggaaggca gcaccaaagt tgaggagaac agcatgaagg cagacaaggg tcgcacagag 1020  
gtcaaccagt gcagcattga ctgggtgaa gatgacatgg agtttggtga agacgacatc 1080  
aatttcagtg aggatgacgt cgaggcagtg aacatcccgg agagcctccc acccagtcgt 1140  
cgtaacagca acagcaaccc tcctctgccc aggtgctacc agtgcaaagc tgctaaagt 1200  
atcttcatca tcattttctc ctatgtgcta tccctggggc cctactgctt ttagcagtc 1260  
ctggccgtgt ggggtgatgt cgaaaccag gtacccaggt ggggtatcac cataatcatc 1320  
tggcttttct tcctgcagtg ctgcatccac ccctatgtct atggctacat gcacaagacc 1380  
attaagaagg aaatccagga catgtgaag aagttcttct gcaaggaaaa gccccgaaa 1440  
gaagatagcc acccagacct gcccggaaca gaggggtggga ctgaaggcaa gattgtccct 1500  
tcctacgatt ctgctacttt tcctgcgata tctgcagaat tccaccacac tggactagt 1560  
gatccgagct cggtagcaag ctggggtgc aggtcgatgg gctgcctcgg caacagtaag 1620  
accgaggacc agcgcaacga ggagaaggcg cagcgcgagg ccaacaaaaa gatcgagaag 1680  
cagctgcaga aggacaagca ggtctaccgg gccacgcacc gcctgctgct gctgggtgct 1740  
ggagagtctg gcaaaagcac cattgtgaag cagatgagga tcctacatgt taatgggttt 1800  
aacggagagg gcggcgaaga ggaccgcag gctgcaagga gcaacagcga tggtgagaag 1860  
gccaccaaag tcgagacat caaaaacaac ctgaaggagg ccattgaaac cattgtggcc 1920

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gccatgagca acctggtgcc ccccgaggag ctggccaacc ctgagaacca gttcagagtg 1980
gactacattc tgagcgtgat gaacgtgcc aactttgact tcccacctga attctatgag 2040
catgccaaag ctctgtggga ggatgaggga gttcgtgcct gctacgagcg ctccaacgag 2100
taccagctga tcgactgtgc ccagtacttc ctggacaaga ttgatgtgat caagcaggcc 2160
gactacgtgc caagtgacca ggacctgctt cgctgccgcy tcctgacctc tggaatcttt 2220
gagaccaagt tccaggtgga caaagtcaac ttccacatgt tcgatgtggg cggccagcgc 2280
gatgaacgcc gcaagtggat ccagtgttc aatgatgtga ctgccatcat ctctgtggtg 2340
gccagcagca gctacaacat ggtcatccgg gaggacaacc agaccaaccg tctgcaggag 2400
gctctgaacc tcttcaagag catctggaac aacagatggc tgctaccat ctctgtgatc 2460
ctcttctca acaagcaaga tctgcttgct gagaaggtcc tcgctgggaa atcgaagatt 2520
gaggactact ttccagagtt cgctcgctac accactcctg aggatgcgac tcccagagccc 2580
ggagaggacc cagcgtgac cggggccaag tacttcatcc gggatgagtt tctgagaatc 2640
agcactgcta gtggagatgg acgtcactac tgctacctc actttacctg cgccgtggac 2700
actgagaaca tccgcctgtg cttcaacgac tgccgtgaca tcattccagcg catgcatctt 2760
cgccaatacg agctgctcta a 2781

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&lt;210&gt; SEQ ID NO 104

&lt;211&gt; LENGTH: 926

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens and Rat

&lt;400&gt; SEQUENCE: 104

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Met Thr Ser Thr Cys Thr Asn Ser Thr Arg Glu Ser Asn Ser Ser His
1          5          10          15

Thr Cys Met Pro Leu Ser Lys Met Pro Ile Ser Leu Ala His Gly Ile
20        25        30

Ile Arg Ser Thr Val Leu Val Ile Phe Leu Ala Ala Ser Phe Val Gly
35        40        45

Asn Ile Val Leu Ala Leu Val Leu Gln Arg Lys Pro Gln Leu Leu Gln
50        55        60

Val Thr Asn Arg Phe Ile Phe Asn Leu Leu Val Thr Asp Leu Leu Gln
65        70        75        80

Ile Ser Leu Val Ala Pro Trp Val Val Ala Thr Ser Val Pro Leu Phe
85        90        95

Trp Pro Leu Asn Ser His Phe Cys Thr Ala Leu Val Ser Leu Thr His
100       105       110

Leu Phe Ala Phe Ala Ser Val Asn Thr Ile Val Val Val Ser Val Asp
115       120       125

Arg Tyr Leu Ser Ile Ile His Pro Leu Ser Tyr Pro Ser Lys Met Thr
130       135       140

Gln Arg Arg Gly Tyr Leu Leu Leu Tyr Gly Thr Trp Ile Val Ala Ile
145       150       155       160

Leu Gln Ser Thr Pro Pro Leu Tyr Gly Trp Gly Gln Ala Ala Phe Asp
165       170       175

Glu Arg Asn Ala Leu Cys Ser Met Ile Trp Gly Ala Ser Pro Ser Tyr
180       185       190

Thr Ile Leu Ser Val Val Ser Phe Ile Val Ile Pro Leu Ile Val Met
195       200       205

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

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Gln | Ala | Ala | Arg | Ser | Asn | Ser | Asp | Gly | Glu | Lys | Ala | Thr | Lys | Val |
| 610 |     |     |     |     |     | 615 |     |     |     |     | 620 |     |     |     |     |
| Gln | Asp | Ile | Lys | Asn | Asn | Leu | Lys | Glu | Ala | Ile | Glu | Thr | Ile | Val | Ala |
| 625 |     |     |     | 630 |     |     |     | 635 |     |     |     |     |     | 640 |     |
| Ala | Met | Ser | Asn | Leu | Val | Pro | Pro | Val | Glu | Leu | Ala | Asn | Pro | Glu | Asn |
|     |     |     | 645 |     |     |     |     | 650 |     |     |     |     |     | 655 |     |
| Gln | Phe | Arg | Val | Asp | Tyr | Ile | Leu | Ser | Val | Met | Asn | Val | Pro | Asn | Phe |
|     |     | 660 |     |     |     |     |     | 665 |     |     |     |     | 670 |     |     |
| Asp | Phe | Pro | Pro | Glu | Phe | Tyr | Glu | His | Ala | Lys | Ala | Leu | Trp | Glu | Asp |
|     |     | 675 |     |     |     |     | 680 |     |     |     |     | 685 |     |     |     |
| Glu | Gly | Val | Arg | Ala | Cys | Tyr | Glu | Arg | Ser | Asn | Glu | Tyr | Gln | Leu | Ile |
|     | 690 |     |     |     |     | 695 |     |     |     |     | 700 |     |     |     |     |
| Asp | Cys | Ala | Gln | Tyr | Phe | Leu | Asp | Lys | Ile | Asp | Val | Ile | Lys | Gln | Ala |
| 705 |     |     |     |     | 710 |     |     |     |     | 715 |     |     |     |     | 720 |
| Asp | Tyr | Val | Pro | Ser | Asp | Gln | Asp | Leu | Leu | Arg | Cys | Arg | Val | Leu | Thr |
|     |     |     | 725 |     |     |     |     | 730 |     |     |     |     |     | 735 |     |
| Ser | Gly | Ile | Phe | Glu | Thr | Lys | Phe | Gln | Val | Asp | Lys | Val | Asn | Phe | His |
|     |     |     | 740 |     |     |     |     | 745 |     |     |     |     | 750 |     |     |
| Met | Phe | Asp | Val | Gly | Gly | Gln | Arg | Asp | Glu | Arg | Arg | Lys | Trp | Ile | Gln |
|     |     | 755 |     |     |     |     | 760 |     |     |     |     | 765 |     |     |     |
| Cys | Phe | Asn | Asp | Val | Thr | Ala | Ile | Ile | Phe | Val | Val | Ala | Ser | Ser | Ser |
|     |     | 770 |     |     |     |     | 775 |     |     |     |     | 780 |     |     |     |
| Tyr | Asn | Met | Val | Ile | Arg | Glu | Asp | Asn | Gln | Thr | Asn | Arg | Leu | Gln | Glu |
| 785 |     |     |     |     | 790 |     |     |     |     | 795 |     |     |     |     | 800 |
| Ala | Leu | Asn | Leu | Phe | Lys | Ser | Ile | Trp | Asn | Asn | Arg | Trp | Leu | Arg | Thr |
|     |     |     | 805 |     |     |     |     | 810 |     |     |     |     |     | 815 |     |
| Ile | Ser | Val | Ile | Leu | Phe | Leu | Asn | Lys | Gln | Asp | Leu | Leu | Ala | Glu | Lys |
|     |     |     | 820 |     |     |     |     | 825 |     |     |     |     | 830 |     |     |
| Val | Leu | Ala | Gly | Lys | Ser | Lys | Ile | Glu | Asp | Tyr | Phe | Pro | Glu | Phe | Ala |
|     |     | 835 |     |     |     |     | 840 |     |     |     |     | 845 |     |     |     |
| Arg | Tyr | Thr | Thr | Pro | Glu | Asp | Ala | Thr | Pro | Glu | Pro | Gly | Glu | Asp | Pro |
|     |     | 850 |     |     |     | 855 |     |     |     |     |     | 860 |     |     |     |
| Arg | Val | Thr | Arg | Ala | Lys | Tyr | Phe | Ile | Arg | Asp | Glu | Phe | Leu | Arg | Ile |
| 865 |     |     |     |     | 870 |     |     |     |     | 875 |     |     |     |     | 880 |
| Ser | Thr | Ala | Ser | Gly | Asp | Gly | Arg | His | Tyr | Cys | Tyr | Pro | His | Phe | Thr |
|     |     |     | 885 |     |     |     |     | 890 |     |     |     |     |     | 895 |     |
| Cys | Ala | Val | Asp | Thr | Glu | Asn | Ile | Arg | Arg | Val | Phe | Asn | Asp | Cys | Arg |
|     |     | 900 |     |     |     |     |     | 905 |     |     |     |     | 910 |     |     |
| Asp | Ile | Ile | Gln | Arg | Met | His | Leu | Arg | Gln | Tyr | Glu | Leu | Leu |     |     |
|     | 915 |     |     |     |     |     | 920 |     |     |     |     | 925 |     |     |     |

<210> SEQ ID NO 105  
 <211> LENGTH: 23  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 105

catgtatgcc agcgtcctgc tcc

23

<210> SEQ ID NO 106  
 <211> LENGTH: 24  
 <212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 106

gctatgcctg aagccagtct tgtg 24

<210> SEQ ID NO 107  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 107

gcacctgctc ctgagcacct tctcc 25

<210> SEQ ID NO 108  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<221> NAME/KEY: misc\_feature  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 108

cacagcgctg cagccctgca gctggc 26

<210> SEQ ID NO 109  
<211> LENGTH: 24  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 109

ccagtgatga ctctgtccag cctg 24

<210> SEQ ID NO 110  
<211> LENGTH: 24  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 110

cagacacttg gcagggacga ggtg 24

<210> SEQ ID NO 111  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 111

cttgtgtct actgcagcat gttccg 26

<210> SEQ ID NO 112  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

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&lt;400&gt; SEQUENCE: 112

catatccctc cgagtgtcca gcggc

25

&lt;210&gt; SEQ ID NO 113

&lt;211&gt; LENGTH: 24

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Novel Sequence

&lt;400&gt; SEQUENCE: 113

atggatcctt atcatggctt cctc

24

&lt;210&gt; SEQ ID NO 114

&lt;211&gt; LENGTH: 27

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Novel Sequence

&lt;400&gt; SEQUENCE: 114

caagaacagg tctcatctaa gagctcc

27

&lt;210&gt; SEQ ID NO 115

&lt;211&gt; LENGTH: 26

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Novel Sequence

&lt;400&gt; SEQUENCE: 115

ctctgatgcc atctgctgga ttctg

26

&lt;210&gt; SEQ ID NO 116

&lt;211&gt; LENGTH: 26

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Novel Sequence

&lt;400&gt; SEQUENCE: 116

gtagtcact gaaagtcag tgatcc

26

&lt;210&gt; SEQ ID NO 117

&lt;211&gt; LENGTH: 24

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Novel Sequence

&lt;400&gt; SEQUENCE: 117

tgggtggcgat ggccaacagc gctc

24

&lt;210&gt; SEQ ID NO 118

&lt;211&gt; LENGTH: 24

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Novel Sequence

&lt;400&gt; SEQUENCE: 118

gttgcgcctt agcgacagat gacc

24

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<210> SEQ ID NO 119  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 119

tcaacctgta tagcagcatc ctc 23

<210> SEQ ID NO 120  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 120

aaggagtagc agaattggta gcc 23

<210> SEQ ID NO 121  
<211> LENGTH: 24  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 121

gacacctgtc agcggtcgtg tgtg 24

<210> SEQ ID NO 122  
<211> LENGTH: 27  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 122

ctgatggaag tagaggctgt ccatctc 27

<210> SEQ ID NO 123  
<211> LENGTH: 24  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 123

gcgctgagcg cagaccagtg gctg 24

<210> SEQ ID NO 124  
<211> LENGTH: 24  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 124

cacggtgacg aagggcacga gctc 24

<210> SEQ ID NO 125  
<211> LENGTH: 24

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<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 125

agccatccct gccaggaagc atgg

24

<210> SEQ ID NO 126  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 126

ccaggtaggt gtgcagcaca atggc

25

<210> SEQ ID NO 127  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 127

ctgttcaaca gggctggttg gcaac

25

<210> SEQ ID NO 128  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 128

atcatgtcta gactcatggt gatcc

25

<210> SEQ ID NO 129  
<211> LENGTH: 6  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 129

Thr Leu Glu Ser Ile Met  
1 5

<210> SEQ ID NO 130  
<211> LENGTH: 5  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 130

Glu Tyr Asn Leu Val  
1 5

<210> SEQ ID NO 131  
<211> LENGTH: 5  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 131

Asp Cys Gly Leu Phe  
1 5

<210> SEQ ID NO 132  
<211> LENGTH: 36  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 132

Gly Ala Thr Cys Ala Ala Gly Cys Thr Thr Cys Cys Ala Thr Gly Gly  
1 5 10 15  
Cys Gly Thr Gly Cys Thr Gly Cys Cys Thr Gly Ala Gly Cys Gly Ala  
20 25 30  
Gly Gly Ala Gly  
35

<210> SEQ ID NO 133  
<211> LENGTH: 53  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 133

Gly Ala Thr Cys Gly Gly Ala Thr Cys Cys Thr Thr Ala Gly Ala Ala  
1 5 10 15  
Cys Ala Gly Gly Cys Cys Gly Cys Ala Gly Thr Cys Cys Thr Thr Cys  
20 25 30  
Ala Gly Gly Thr Thr Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly Ala  
35 40 45  
Thr Gly Gly Thr Gly  
50

<210> SEQ ID NO 134  
<211> LENGTH: 1164  
<212> TYPE: DNA  
<213> ORGANISM: Homo Sapien

<400> SEQUENCE: 134

atgaatcggc accatctgca ggatcacttt ctggaaatag acaagaagaa ctgctgtgtg 60  
ttccgagatg acttcattgc caagggtgtg ccgccggtgt tggggctgga gtttatcttt 120  
gggcttctgg gcaatggcct tgccctgtgg attttctgtt tccacctcaa gtccctggaaa 180  
tccagccgga ttttctgtt caacctggca gtagctgact ttctactgat catctgcctg 240  
ccgttcgtga tggactacta tgtgcggcgt tcagactgga actttgggga catcccttgc 300  
cggctggtgc tcttcatgtt tgccatgaac cgccagggca gcatcatott cctcaccgtg 360  
gtggcggtag acaggtatgtt ccgggtgtgc catccccacc acgccctgaa caagatctcc 420  
aattggacag cagccatcat ctcttgcctt ctgtggggca tcaactgttg cctaacagtc 480  
cacctcctga agaagaagtt gctgatccag aatggccctg caaatgtgtg catcagcttc 540  
agcatctgcc ataccttccg gtggcacgaa gctatgttcc tcctggagtt cctcctgccc 600

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ctgggcatca tcctgttctg ctcagccaga attatctgga gcctgcggca gagacaaatg    660
gaccggcatg ccaagatcaa gagagccatc accttcatca tgggtgtggc catcgtcttt    720
gtcatctgct tccttccag cgtggttggt cggatccgca tcttctggct cctgcacact    780
tcgggcacgc agaattgtga agtgtaccgc tcggtggacc tggcgttctt tatcactctc    840
agcttcacct acatgaacag catgctggac cccgtggtgt actacttctc cagcccatcc    900
tttcccaact tcttctccac ttgatcaac cgctgcctcc agaggaagat gacaggtgag    960
ccagataata accgcagcac gagcgtcgag ctcacagggg accccaacaa aaccagaggc   1020
gtccagagg cgtaaatggc caactccggt gagccatgga gccctcttta tctgggcca    1080
acctcaaata accattccaa gaaggacat tgtcaccaag aaccagcatc tctggagaaa   1140
cagttgggct gttgcatcga gtaa                                           1164

```

&lt;210&gt; SEQ ID NO 135

&lt;211&gt; LENGTH: 387

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 135

```

Met Asn Arg His His Leu Gln Asp His Phe Leu Glu Ile Asp Lys Lys
 1              5              10              15

Asn Cys Cys Val Phe Arg Asp Asp Phe Ile Ala Lys Val Leu Pro Pro
      20              25              30

Val Leu Gly Leu Glu Phe Ile Phe Gly Leu Leu Gly Asn Gly Leu Ala
      35              40              45

Leu Trp Ile Phe Cys Phe His Leu Lys Ser Trp Lys Ser Ser Arg Ile
 50              55              60

Phe Leu Phe Asn Leu Ala Val Ala Asp Phe Leu Leu Ile Ile Cys Leu
65              70              75              80

Pro Phe Val Met Asp Tyr Tyr Val Arg Arg Ser Asp Trp Asn Phe Gly
      85              90              95

Asp Ile Pro Cys Arg Leu Val Leu Phe Met Phe Ala Met Asn Arg Gln
      100             105             110

Gly Ser Ile Ile Phe Leu Thr Val Val Ala Val Asp Arg Tyr Phe Arg
      115             120             125

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

Ala Ile Ile Ser Cys Leu Leu Trp Gly Ile Thr Val Gly Leu Thr Val
      145             150             155             160

His Leu Leu Lys Lys Lys Leu Leu Ile Gln Asn Gly Pro Ala Asn Val
      165             170             175

Cys Ile Ser Phe Ser Ile Cys His Thr Phe Arg Trp His Glu Ala Met
      180             185             190

Phe Leu Leu Glu Phe Leu Leu Pro Leu Gly Ile Ile Leu Phe Cys Ser
      195             200             205

Ala Arg Ile Ile Trp Ser Leu Arg Gln Arg Gln Met Asp Arg His Ala
      210             215             220

Lys Ile Lys Arg Ala Ile Thr Phe Ile Met Val Val Ala Ile Val Phe
      225             230             235             240

Val Ile Cys Phe Leu Pro Ser Val Val Val Arg Ile Arg Ile Phe Trp
      245             250             255

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Leu Leu His Thr Ser Gly Thr Gln Asn Cys Glu Val Tyr Arg Ser Val  
                   260                  265                  270  
 Asp Leu Ala Phe Phe Ile Thr Leu Ser Phe Thr Tyr Met Asn Ser Met  
                   275                  280                  285  
 Leu Asp Pro Val Val Tyr Tyr Phe Ser Ser Pro Ser Phe Pro Asn Phe  
                   290                  295                  300  
 Phe Ser Thr Leu Ile Asn Arg Cys Leu Gln Arg Lys Met Thr Gly Glu  
                   305                  310                  315                  320  
 Pro Asp Asn Asn Arg Ser Thr Ser Val Glu Leu Thr Gly Asp Pro Asn  
                   325                  330                  335  
 Lys Thr Arg Gly Ala Pro Glu Ala Leu Met Ala Asn Ser Gly Glu Pro  
                   340                  345                  350  
 Trp Ser Pro Ser Tyr Leu Gly Pro Thr Ser Asn Asn His Ser Lys Lys  
                   355                  360                  365  
 Gly His Cys His Gln Glu Pro Ala Ser Leu Glu Lys Gln Leu Gly Cys  
                   370                  375                  380  
 Cys Ile Glu  
 385

<210> SEQ ID NO 136  
 <211> LENGTH: 1083  
 <212> TYPE: DNA  
 <213> ORGANISM: Mouse

<400> SEQUENCE: 136

```

atgagcaagt cagaccattt tctagtata aacggcaaga actgctgtgt gttccgagat    60
gaaaacatcg ccaaggtctt gccaccgttg ttggggcttg aatttgtgtt cggactcctg    120
ggcaatggcc ttgccttggt gatatttctgt ttccacctca agtcctggaa atccagccgg    180
atatttctgt tcaacttggc cgtggctgac ttctcctga tcacttgcct gccgttcctg    240
acggacaact atgtccataa ctgggactgg aggttcggag gcatcccttg ccgtgtgatg    300
ctcttcatgt tggctatgaa ccgacagggc agcatcatct tcctcaccgt ggtggctgtg    360
gaccgctact tccgggtggt ccatccacac cacttcttga acaagatctc caaccggacg    420
gcggccatca tttcttgctt cttgtgggtg ctacccatcg gcctgactgt ccacctctc    480
tatacaaaca tgatgaccaa aaatggcgag gcatatctgt gtagcagctt cagcatctgt    540
tacaacttca ggtggcacga tgctatgttc ctcttggaat tcttcttgcc cctggccatc    600
atcttgttct gctcaggcag gatcatctgg agcctgagga agagacagat ggacagacat    660
gccaagatca agagggccat caacttcacg atggtggtgg ctattgtatt catcatttgc    720
ttcttacctc gtgtggctgt gcgcatccgc atcttctggc ttctctacaa atataacgta    780
cgcaactgtg acatctactc ctcggtggac ctggctttct ttaccacct tagctttacc    840
tacatgaaca gcatgctgga ccctgtggtc tactatttct ccagcccatc ttccccaac    900
ttcttctcca cgtgtatcaa ccgctgcctt cgaaagaaaa cattgggtga acccgataat    960
aaccgaagca ctagtgtgga gctcacgggg gaccccgca caaccagaag tattccaggg   1020
gcgctaattg ctgacccag tgagccaggc agcccccctt atctggcttc cacatctcgt   1080
taa                                                1083
  
```

<210> SEQ ID NO 137

-continued

&lt;211&gt; LENGTH: 360

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Mouse

&lt;400&gt; SEQUENCE: 137

```

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

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<210> SEQ ID NO 138  
 <211> LENGTH: 1086  
 <212> TYPE: DNA  
 <213> ORGANISM: Rat

<400> SEQUENCE: 138

```

atgagcaagt cagaccattt tctagtata aacggcaaga actgctgtgt gttccgagat    60
gaaaacatcg ccaaggctct gccgccgggtg ttggggctgg agtttgtgtt tggactcctg   120
ggtaatggcc ttgccttgtg gatcttctgt ttccatctca aatcctggaa atccagccgg   180
atcttcttgt tcaacctggc cgtggctgac tttctcctga tcatttgctt gccgttcttg   240
acggacaact atgtccagaa ctgggactgg aggttcggga gcaccccttg ccgcgtgatg   300
ctcttcatgt tggccatgaa ccgacagggc agcatcatct tcctcacggg ggtggctgtg   360
gacaggtaact tcagggtggt ccaccgcac cacttcctga acaagatctc caaccggacg   420
gcggccatca tctcttgctt cctgtggggc atcaccatcg ggcttgaca gtccaccttc   480
ctctacacgg acatgatgac ccgaaacggc gatgcaaacc tgtgcagcag ttttagcatc   540
tgctacactt tcagggtgga cgatgcaatg ttctctctgg aattcttcct gcccttgggc   600
atcatcctgt tctgctctgg caggatcatt tggagcctaa ggcagagaca gatggacagg   660
cacgtcaaga tcaagagggc catcaacttc atcatggtgg ttgccattgt gtttgtcatc   720
tgcttctctc ccagtgtggc cgtgaggatc cgcattctct ggctcctcta caaacacaac   780
gtgaggaact gtgacatcta ctctctgtg gacttggcct tcttcaccac ccttagcttt   840
acctacatga acagcatgct cgaccgggtg gtctactatt tctccagccc atctttcccc   900
aacttcttct ccacgtgcat caaccgttgc cttcgaagga aaaccttggg cgaaccagat   960
aataaccgga gcacgagtgt ggagctcacg ggggacccca gcacaatcag aagtattcca  1020
ggggcattaa tgactgacct cagtgagcca ggcagccccc cttatctggc ttccacatct  1080
cgtaa                                           1086
  
```

<210> SEQ ID NO 139  
 <211> LENGTH: 361  
 <212> TYPE: PRT  
 <213> ORGANISM: Rat

<400> SEQUENCE: 139

```

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

Ser Cys Phe Leu Trp Gly Ile Thr Ile Gly Pro Gly Gln Ser Thr Phe  
145 150 155 160

Leu Tyr Thr Asp Met Met Thr Arg Asn Gly Asp Ala Asn Leu Cys Ser  
165 170 175

Ser Phe Ser Ile Cys Tyr Thr Phe Arg Trp His Asp Ala Met Phe Leu  
180 185 190

Leu Glu Phe Phe Leu Pro Leu Gly Ile Ile Leu Phe Cys Ser Gly Arg  
195 200 205

Ile Ile Trp Ser Leu Arg Gln Arg Gln Met Asp Arg His Val Lys Ile  
210 215 220

Lys Arg Ala Ile Asn Phe Ile Met Val Val Ala Ile Val Phe Val Ile  
225 230 235 240

Cys Phe Leu Pro Ser Val Ala Val Arg Ile Arg Ile Phe Trp Leu Leu  
245 250 255

Tyr Lys His Asn Val Arg Asn Cys Asp Ile Tyr Ser Ser Val Asp Leu  
260 265 270

Ala Phe Phe Thr Thr Leu Ser Phe Thr Tyr Met Asn Ser Met Leu Asp  
275 280 285

Pro Val Val Tyr Tyr Phe Ser Ser Pro Ser Phe Pro Asn Phe Phe Ser  
290 295 300

Thr Cys Ile Asn Arg Cys Leu Arg Arg Lys Thr Leu Gly Glu Pro Asp  
305 310 315 320

Asn Asn Arg Ser Thr Ser Val Glu Leu Thr Gly Asp Pro Ser Thr Ile  
325 330 335

Arg Ser Ile Pro Gly Ala Leu Met Thr Asp Pro Ser Glu Pro Gly Ser  
340 345 350

Pro Pro Tyr Leu Ala Ser Thr Ser Arg  
355 360

<210> SEQ ID NO 140  
<211> LENGTH: 30  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 140

atgagcaagt cagaccattt tctagtgata

30

<210> SEQ ID NO 141  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:  
<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 141

ttatctggct tccacatctc gttaa

25

<210> SEQ ID NO 142  
<211> LENGTH: 30  
<212> TYPE: DNA  
<213> ORGANISM: Artificial  
<220> FEATURE:

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<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 142

atgagcaagt cagaccattt tctagtgata

30

<210> SEQ ID NO 143

<211> LENGTH: 25

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 143

ttatctggct tccacatctc gttaa

25

<210> SEQ ID NO 144

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 144

ctgatggaca actatgtgag gcgttgg

27

<210> SEQ ID NO 145

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 145

gctgaagctg ctgcacaaat ttgcacc

27

<210> SEQ ID NO 146

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 146

ctactatgtg cggcgttca

19

<210> SEQ ID NO 147

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 147

cccttcttgg aatggttatt t

21

<210> SEQ ID NO 148

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 148

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gcactcatga atcggcacca 20

<210> SEQ ID NO 149  
 <211> LENGTH: 20  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 149

cagtgacatt actcgatgca 20

<210> SEQ ID NO 150  
 <211> LENGTH: 1032  
 <212> TYPE: DNA  
 <213> ORGANISM: Mouse

<400> SEQUENCE: 150

```

atggacaacg ggtcgtgctg tctcatcgag ggggagccca tctcccaagt gatgcctcct    60
ctactcatcc tggctcttcgt gcttggcgcc ctgggcaacg gcatagccct gtgcggcttc    120
tgctttcaca tgaagacctg gaagtcaagc actatattacc ttttcaactt ggctgtggcc    180
gattttctcc tcatgatctg cttacccctt cggacagact actacctcag acgcagacac    240
tggatttttg gagatatcgc ctgtcgcctg gtcctcttca agctggccat gaatagggcc    300
gggagcattg tcttcctcac tgtgtgggct gtggataggt atttcaaagt ggtccacccc    360
caccatattg tgaatgccat ctccaaccgg actgccgccc ccaccgctg tgcctctggt    420
actttgggtc tcttggggac tgtgtatctt ctgatggaga gtcacctgtg tgtgcagggg    480
acactgtcgt cctgtgagag cttcatcatg gagtcagcca acgggtggca cgatgtcatg    540
ttccagctgg agttcttctt gcccctgaca atcatcttgt tctgctcggg caacgttggt    600
tggagcctga gacggaggca gcagctgacc agacaggctc ggatgaggag gggcaccggg    660
ttcatcatgg tgggtggctt tgtgttcac acgtgttacc tgcccagcgt gctggctagg    720
ctctacttcc tctggacggt gcccactagt gcctgtgacc cctctgtcca cacagccctc    780
cacgtcacc tgaagcttca ctacctgaac agtatgctgg atcccctgt atattacttc    840
tcaagcccct cgctcccaa attctacacc aagctcaca tctgcagcct gaagcccaaa    900
cgccaggac gcacgaagac gcggagggtc gaagagatgc caatttcgaa cctctgcagt    960
aagagctcca tcgatggggc aaatcggtcc cagaggccat ctgacgggca gtgggatctc   1020
caagtgtggt ga                                     1032

```

<210> SEQ ID NO 151  
 <211> LENGTH: 343  
 <212> TYPE: PRT  
 <213> ORGANISM: Mouse

<400> SEQUENCE: 151

```

Met Asp Asn Gly Ser Cys Cys Leu Ile Glu Gly Glu Pro Ile Ser Gln
 1             5             10             15

Val Met Pro Pro Leu Leu Ile Leu Val Phe Val Leu Gly Ala Leu Gly
      20             25             30

Asn Gly Ile Ala Leu Cys Gly Phe Cys Phe His Met Lys Thr Trp Lys
      35             40             45

Ser Ser Thr Ile Tyr Leu Phe Asn Leu Ala Val Ala Asp Phe Leu Leu

```

-continued

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

<210> SEQ ID NO 152  
 <211> LENGTH: 24  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 152

ggcgcgtggct gatttcctcc ttat

24

<210> SEQ ID NO 153  
 <211> LENGTH: 22  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Novel Sequence

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&lt;400&gt; SEQUENCE: 153

aacgggtcg ccttcttcat cc 22

&lt;210&gt; SEQ ID NO 154

&lt;211&gt; LENGTH: 23

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Novel Sequence

&lt;400&gt; SEQUENCE: 154

actgtggtgg ctgtggatag gta 23

&lt;210&gt; SEQ ID NO 155

&lt;211&gt; LENGTH: 24

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Novel Sequence

&lt;400&gt; SEQUENCE: 155

gcagattgtg agcttggcgt agaa 24

&lt;210&gt; SEQ ID NO 156

&lt;211&gt; LENGTH: 1056

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Rat

&lt;400&gt; SEQUENCE: 156

atgtctctcc tctctccgag tgctatggac aacgggtcgt gctgtctcat cgagggggaa 60

cccatcacc aggtaatgcc acctttactc atcctggcct tcctgcttgg agccctgggc 120

aacggcctag ccctgtgtgg tttctgcttt cacatgaaga cctggaagtc gagcactatt 180

taccttttca acttggtgtg agccgatttt ctctcatga tctgcctacc ccttcggaca 240

gactactacc tcagacgtag gcattggatt ttgggggata ttccctgccg cctgggtctc 300

ttcatgctgg ccatgaatag ggccggaagc attgtcttcc tcaactgtgg ggccgtggac 360

aggatattca aagtgttcca cccccaccat atggtgaacg ccatctccaa tcggactgca 420

gctgccatcg tctgtgtcct ctggactttg gtcactcttg ggactgtgta tcttctgatg 480

gagagtcacc tgtgtgtgcg ggggatggg tcactcttgg agagcttcat catggagtca 540

gccaacgggt ggcacgatat catgttccag ctggagttct tcctgcccct gaccatcatc 600

ttgttctgct ctttcaaagt tgtttgagc ctgagacaga ggcaacagct gaccagacag 660

gctcggatga ggaggccac ccggttcac atggtgggtg cttccgtgtt catcacgtgt 720

tacctgcccc gcgtgttggc gaggtcttac ttctcttgga cggtgccctc cagtgttgt 780

gacccctctg tccacatagc tctccatgac accctgagtc tcacctacct gaacagcatg 840

ctggaccctc ttgtgtacta cttttcaagc cctcgttcc ccaaattota cgccaagctc 900

aaaaaccgca gcttgaaacc cagacgcccc ggacgctcgc aggcacggag gtcggaagag 960

atgccaattt cgaatctctg tcgtaagagt tccaccgatg tggtaaatag ttcccagagg 1020

ccgtctgacg ggcagtgggg tctccaagtg tgttga 1056

&lt;210&gt; SEQ ID NO 157

&lt;211&gt; LENGTH: 351

&lt;212&gt; TYPE: PRT

-continued

&lt;213&gt; ORGANISM: Rat

&lt;400&gt; SEQUENCE: 157

```

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

```

&lt;210&gt; SEQ ID NO 158

&lt;211&gt; LENGTH: 1092

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

-continued

&lt;400&gt; SEQUENCE: 158

```

atgaatcggc accatctgca ggatcacttt ctggaaatag acaagaagaa ctgctgtgtg      60
ttccgagatg acttcattgt caaggtgttg ccgccggtgt tggggctgga gtttatcttc      120
gggcttctgg gcaatggcct tgccctgtgg attttctgtt tccacctcaa gtccctggaaa      180
tccagccgga ttttcctgtt caacctggca gtggctgact ttctactgat catctgcctg      240
cccttcctga tggacaacta tgtgaggcgt tgggactgga agtttgggga catcccttgc      300
cggtgatgc tcttcattgt ggctatgaac cgccagggca gcatcatctt cctcacggtg      360
gtggcggtag acaggtattt ccgggtggtc catccccacc acgccctgaa caagatctcc      420
aatcggacag cagccatcat ctcttgctt ctgtggggca tcactattgg cctgacagtc      480
cacctcctga agaagaagat gccgatccag aatggcggtg caaatgtgtg cagcagcttc      540
agcatctgcc ataccttcca gtggcacgaa gccatgttcc tcctggagtt ctctctgccc      600
ctgggcatca tcctgttctg ctacagccaga attatctgga gcctgcggca gagacaaatg      660
gaccggcatg ccaagatcaa gagagccatc accttcatca tgggtgtggc catcgtcttt      720
gtcatctgct tccttccag cgtggttgtg cggatccgca tcttctggct cctgcacact      780
tcgggcacgc agaattgtga agtgtaccgc tcggtggacc tggcgttctt tatcactctc      840
agcttcacct acatgaacag catgctggac ccggtggtgt actactcttc cagcccatcc      900
tttcccaact tcttctccac ttgatcaac cgctgcctcc agaggaagat gacaggtgag      960
ccagataata accgcagcac gagcgtcgag ctacagggg accccaacaa aaccagaggc     1020
gctccagagg cgtaaatggc caactccggt gagccatgga gcccctctta tctggggcca     1080
acctctcctt aa                                                                1092

```

&lt;210&gt; SEQ ID NO 159

&lt;211&gt; LENGTH: 363

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 159

```

Met Asn Arg His His Leu Gln Asp His Phe Leu Glu Ile Asp Lys Lys
 1             5             10             15

Asn Cys Cys Val Phe Arg Asp Asp Phe Ile Val Lys Val Leu Pro Pro
 20             25             30

Val Leu Gly Leu Glu Phe Ile Phe Gly Leu Leu Gly Asn Gly Leu Ala
 35             40             45

Leu Trp Ile Phe Cys Phe His Leu Lys Ser Trp Lys Ser Ser Arg Ile
 50             55             60

Phe Leu Phe Asn Leu Ala Val Ala Asp Phe Leu Leu Ile Ile Cys Leu
 65             70             75             80

Pro Phe Leu Met Asp Asn Tyr Val Arg Arg Ser Asp Trp Lys Phe Gly
 85             90             95

Asp Ile Pro Cys Arg Leu Met Leu Phe Met Leu Ala Met Asn Arg Gln
100            105            110

Gly Ser Ile Ile Phe Leu Thr Val Val Ala Val Asp Arg Tyr Phe Arg
115            120            125

Val Val His Pro His His Ala Leu Asn Lys Ile Ser Asn Arg Thr Ala
130            135            140

Ala Ile Ile Ser Cys Leu Leu Trp Gly Ile Thr Ile Gly Leu Thr Val

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-continued

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|                                                                 |     |     |     |
|-----------------------------------------------------------------|-----|-----|-----|
| 145                                                             | 150 | 155 | 160 |
| His Leu Leu Lys Lys Lys Met Pro Ile Gln Asn Gly Gly Ala Asn Leu |     |     |     |
|                                                                 | 165 | 170 | 175 |
| Cys Ser Ser Phe Ser Ile Cys His Thr Phe Gln Trp His Glu Ala Met |     |     |     |
|                                                                 | 180 | 185 | 190 |
| Phe Leu Leu Glu Phe Phe Leu Pro Leu Gly Ile Ile Leu Phe Cys Ser |     |     |     |
|                                                                 | 195 | 200 | 205 |
| Ala Arg Ile Ile Trp Ser Leu Arg Gln Arg Gln Met Asp Arg His Ala |     |     |     |
|                                                                 | 210 | 215 | 220 |
| Lys Ile Lys Arg Ala Ile Thr Phe Ile Met Val Val Ala Ile Val Phe |     |     |     |
|                                                                 | 225 | 230 | 235 |
| Val Ile Cys Phe Leu Pro Ser Val Val Val Arg Ile Arg Ile Phe Trp |     |     |     |
|                                                                 | 245 | 250 | 255 |
| Leu Leu His Thr Ser Gly Thr Gln Asn Cys Glu Val Tyr Arg Ser Val |     |     |     |
|                                                                 | 260 | 265 | 270 |
| Asp Leu Ala Phe Phe Ile Thr Leu Ser Phe Thr Tyr Met Asn Ser Met |     |     |     |
|                                                                 | 275 | 280 | 285 |
| Leu Asp Pro Val Val Tyr Tyr Phe Ser Ser Pro Ser Phe Pro Asn Phe |     |     |     |
|                                                                 | 290 | 295 | 300 |
| Phe Ser Thr Leu Ile Asn Arg Cys Leu Gln Arg Lys Met Thr Gly Glu |     |     |     |
|                                                                 | 305 | 310 | 315 |
| Pro Asp Asn Asn Arg Ser Thr Ser Val Glu Leu Thr Gly Asp Pro Asn |     |     |     |
|                                                                 | 325 | 330 | 335 |
| Lys Thr Arg Gly Ala Pro Glu Ala Leu Met Ala Asn Ser Gly Glu Pro |     |     |     |
|                                                                 | 340 | 345 | 350 |
| Trp Ser Pro Ser Tyr Leu Gly Pro Thr Ser Pro                     |     |     |     |
|                                                                 | 355 | 360 |     |

<210> SEQ ID NO 160  
 <211> LENGTH: 29  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 160

ctatgtgagg cggtcagact ggaagtttg

29

<210> SEQ ID NO 161  
 <211> LENGTH: 29  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Novel Sequence

<400> SEQUENCE: 161

caaacttcca gtctgaacgc ctcacatag

29

What is claimed is:

1. A method of identifying whether a candidate compound is a modulator of a nicotinic acid GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

- (a) SEQ. ID. NO.:36 (hRUP25);
- (b) SEQ. ID. NO.:137 (mRUP25); and
- (c) SEQ. ID. NO.:139 (rRUP25);

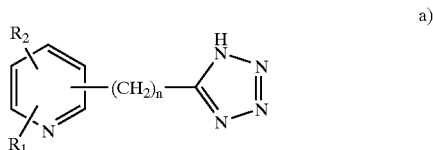
or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

comprising the steps of:

- (a') contacting the candidate compound with the receptor; and
- (b') determining whether the receptor functionality is modulated;

wherein a change in receptor functionality is indicative of the candidate compound being a modulator of a nicotinic acid GPCR.

2. A modulator of a nicotinic acid GPCR (RUP25) identified according to the method of claim 1, provided that the modulator is not identical to a compound selected from the group consisting of:



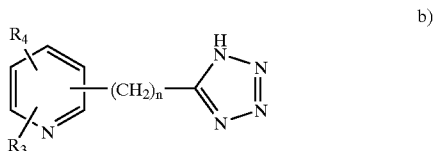
wherein:

$R_1$  is selected from the group consisting of halogen, hydroxyl, acetylamino, amino, alkoxy, carboalkoxy, alkylthio, monoalkylamino, dialkylamino, N-alkylcarbamyl, N,N-dialkylcarbamyl, alkylsulfonyl, said alkyl groups containing from 1 to 4 carbons, trifluoromethyl, trifluoromethoxy, trifluoromethylthio, methoxymethyl, carboxy, carbamyl, alkanoyloxy containing up to 4 carbon atoms, phenyl, p-chlorophenyl, p-methylphenyl and p-aminophenyl;

$R_2$  is selected from the group consisting of halogen, alkanoyloxy containing from 14 carbon atoms, carboalkoxy containing from 2 to 5 carbon atoms, carbamyl, N-alkyl carbamyl and N,N-dialkylcarbamyl wherein said alkyl groups contain from 1-4 carbon atoms and trifluoromethyl;

$n$  is a whole number from 0 to 4; and

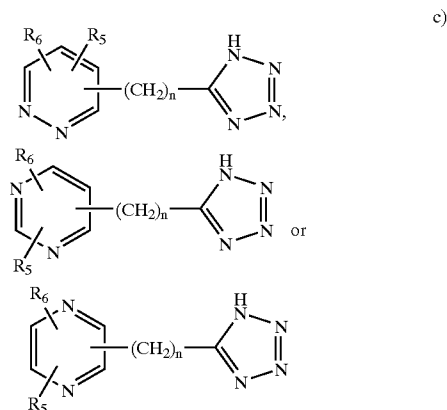
N-oxides thereof;



$R_3$  and  $R_4$  are hydrogen, alkyl containing from 1 to 4 carbon atoms or cycloalkyl containing from 3 to 7 carbon atoms;

$n$  is a whole number from 0 to 4; and

N-oxides thereof.



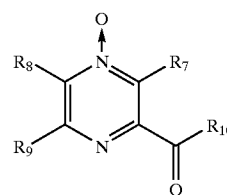
wherein:

$R_5$  and  $R_6$  are each selected from the group consisting of H, halogen, hydroxyl, amino, alkoxy, alkylthio, monoalkylamino, dialkylamino, N-alkylcarbamyl, N,N-dialkylcarbamyl, alkylsulfonyl, said alkyl groups containing from 1 to 4 carbons, trifluoromethyl, trifluoromethoxy, trifluoromethylthio, carboxy, carbamyl, alkanoyloxy containing up to 4 carbon atoms, phenyl, p-chlorophenyl, p-methylphenyl and p-aminophenyl;

$n$  is a whole number from 0 to 4; and

N-oxides thereof;

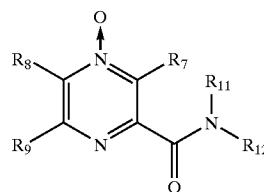
d)



wherein:

at least one of  $R_7$ ,  $R_8$  and  $R_9$  is  $C_{1-4}$  alkyl and the others are hydrogen atoms;  $R_{10}$  is hydroxy or  $C_1$  alkoxy, or a salt of the compounds wherein  $R_4$  is hydroxy with a pharmaceutically acceptable base;

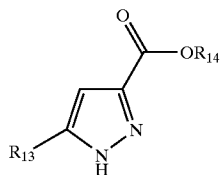
e)



wherein:

at least one of  $R_7$ ,  $R_8$  and  $R_9$  is  $C_{1-4}$  alkyl and the others are hydrogen atoms; each of  $R_{11}$  and  $R_{12}$ , which may be the same or different, is hydrogen or  $C_{1-4}$  alkyl;

f)



wherein:

at least one of  $R_{13}$  represents an alkyl group of 7-11 carbon atoms and  $R_{14}$  represents H or a lower alkyl group of up to two carbon atoms, and a pharmaceutically acceptable carrier;

g) pyrazine-2-carboxylic acid amide, 5-chloro-pyrazine-2-carboxylic acid amide, 5-amino-pyrazine-2-carboxylic acid amide, 5-benzyl-pyrazine-2-carboxylic acid amide, 6-chloro-pyrazine-2-carboxylic acid amide, 6-methoxy-pyrazine-2-carboxylic acid amide, 3-chloro-pyrazine-2-carboxylic acid amide, 3-methoxy-pyrazine-2-carboxylic acid amide, pyrazine-2-carboxylic acid ethylamide, morpholin-4-yl-pyrazine-2-ylmethanone, 5-methyl-pyrazine-2-carboxylic acid (6-methyl-pyrazin-2-yl)-amide, 5-methyl-pyrazine-2-carboxylic acid (5-methyl-pyrazin-2-yl)-amide, 5-methyl-pyrazine-2-carboxylic acid (3-methyl-pyrazin-2-yl)-amide, (5-methyl-pyrazin-2-yl)-morpholin-4-ylmethanone, 5-methyl-pyrazine-2-carboxylic acid hydroxyamide, pyrazine-2-carboxylic acid, 5-amino-pyrazine-2-carboxylic acid, 5-benzyl-pyrazine-2-carboxylic acid, 6-chloro-pyrazine-2-carboxylic acid, 6-methoxy-pyrazine-2-carboxylic acid, 3-hydroxy-pyrazine-2-carboxylic acid, 5-methyl-pyrazine-2-carboxylic acid 2-hydroxy-ethyl ester, 5-methyl-pyrazine-2-carboxylic acid allyl ester, 5-methyl-pyrazine-2-carboxylic acid phenyl ester, 5-methyl-pyrazine-2-carboxylic acid ethoxycarbonylmethyl ester, pyrazine-2-carboxylic acid methyl ester or 2-methyl-5-(1H-tetrazol-5-yl)-pyrazine; and 4-N-oxides thereof;

h) 5-(3-(5-Methyl)isoxazolyl)tetrazole;

i) 5-(5-(3-Methyl)isoxazolyl)tetrazole;

j) 5-(3-Quinolyl)tetrazole;

k) Nicotinic acid;

l) Pyridazine-4-carboxylic acid;

m) 3-pyridine acetic acid;

n) 5-Methylnicotinic acid;

o) 6-Methylnicotinic acid;

p) Nicotinic acid-1-oxide;

q) 2-Hydroxynicotinic acid;

r) Furane-3-carboxylic acid;

s) 5-Methylpyrazole-3-carboxylic acid; and

t) 3-Methylisoxazole-5-carboxylic acid.

3. A method of modulating the activity of a nicotinic acid GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

(a) SEQ. ID. NO.:36 (hRUP25);

(b) SEQ. ID. NO.:137 (mRUP25); and

(c) SEQ. ID. NO.:139 (rRUP25);

or an allelic variant, a biologically active mutant, or a biologically active fragment of said amino acid sequence;

comprising the step of contacting the receptor with the modulator of claim 2.

4. A method of preventing or treating a disorder of lipid metabolism in an individual comprising contacting a therapeutically effective amount of the modulator of claim 2 with a nicotinic acid GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

(a) SEQ. ID. NO.:36 (hRUP25);

(b) SEQ. ID. NO.:137 (mRUP25); and

(c) SEQ. ID. NO.:139 (rRUP25);

or an allelic variant or biologically active fragment of said amino acid sequence.

5. A method of preventing or treating a metabolic-related disorder in an individual comprising contacting a therapeutically effective amount of the modulator of claim 2 with a nicotinic acid GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

(a) SEQ. ID. NO.:36 (hRUP25);

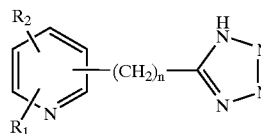
(b) SEQ. ID. NO.:137 (mRUP25); and

(c) SEQ. ID. NO.:139 (rRUP25);

or an allelic variant or biologically active fragment of said amino acid sequence.

6. A method of preparing a composition which comprises identifying a modulator of a nicotinic acid GPCR and then admixing a carrier and the modulator, wherein the modulator is identifiable by the method of claim 1 and provided that the modulator is not identical to a compound selected from the group consisting of:

a)



a)

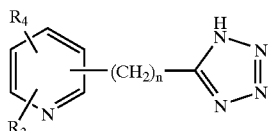
wherein:

$R_1$  is selected from the group consisting of halogen, hydroxyl, acetylamino, amino, alkoxy, carboalkoxy, alkylthio, monoalkylamino, dialkylamino, N-alkyl-carbamyl, N,N-dialkylcarbamyl, alkylsulfonyl, said alkyl groups containing from 1 to 4 carbons, trifluoromethyl, trifluoromethoxy, trifluoromethylthio, methoxymethyl, carboxy, carbamyl, alkanoyloxy containing up to 4 carbon atoms, phenyl, p-chlorophenyl, p-methylphenyl and p-aminophenyl;

R<sub>2</sub> is selected from the group consisting of halogen, alkanoyloxy containing from 1-4 carbon atoms, carboalkoxy containing from 2 to 5 carbon atoms, carbamyl, N-alkyl carbamyl and N,N-dialkylcarbamyl wherein said alkyl groups contain from 1-4 carbon atoms and trifluoromethyl;

n is a whole number from 0 to 4; and

N-oxides thereof;

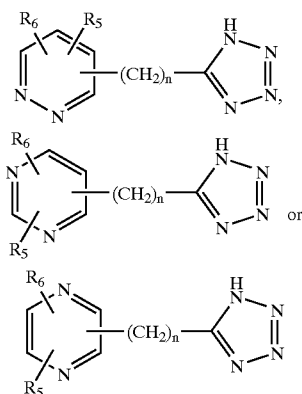


b)

R<sub>3</sub> and R<sub>4</sub> are hydrogen, alkyl containing from 1 to 4 carbon atoms or cycloalkyl containing from 3 to 7 carbon atoms;

n is a whole number from 0 to 4; and

N-oxides thereof.



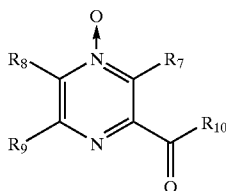
c)

wherein:

R<sub>5</sub> and R<sub>6</sub> are each selected from the group consisting of H, halogen, hydroxyl, amino, alkoxy, alkylthio, monoalkylamino, dialkylamino, N-alkylcarbamyl, N,N-dialkylcarbamyl, alkylsulfoxy, alkylsulfony, said alkyl groups containing from 1 to 4 carbons, trifluoromethyl, trifluoromethoxy, trifluoromethylthio, carboxy, carbamyl, alkanoyloxy containing up to 4 carbon atoms, phenyl, p-chlorophenyl, p-methylphenyl and p-aminophenyl;

n is a whole number from 0 to 4; and

N-oxides thereof;

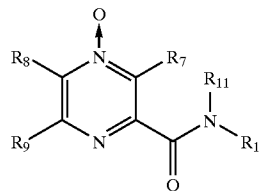


d)

wherein:

at least one of R<sub>7</sub>, R<sub>8</sub> and R<sub>9</sub> is C<sub>1-4</sub> alkyl and the others are hydrogen atoms; R<sub>10</sub> is hydroxy or C<sub>1-4</sub> alkoxy, or a salt of the compounds wherein R<sub>4</sub> is hydroxy with a pharmaceutically acceptable base;

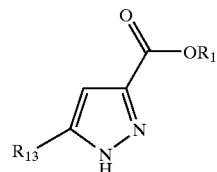
e)



wherein:

at least one of R<sub>7</sub>, R<sub>8</sub> and R<sub>9</sub> is C<sub>1-6</sub> alkyl and the others are hydrogen atoms; each of R<sub>11</sub> and R<sub>12</sub>, which may be the same or different, is hydrogen or C<sub>1-6</sub> alkyl;

f)



wherein:

at least one of R<sub>13</sub> represents an alkyl group of 7-11 carbon atoms and R<sub>14</sub> represents H or a lower alkyl group of up to two carbon atoms, and a pharmaceutically acceptable carrier;

g) pyrazine-2-carboxylic acid amide, 5-chloro-pyrazine-2-carboxylic acid amide, 5-amino-pyrazine-2-carboxylic acid amide, 5-benzyl-pyrazine-2-carboxylic acid amide, 6-chloro-pyrazine-2-carboxylic acid amide, 6-methoxy-pyrazine-2-carboxylic acid amide, 3-chloro-pyrazine-2-carboxylic acid amide, 3-methoxy-pyrazine-2-carboxylic acid amide, pyrazine-2-carboxylic acid ethylamide, morpholin-4-yl-pyrazine-2-ylmethanone, 5-methyl-pyrazine-2-carboxylic acid (6-methyl-pyrazin-2-yl)-amide, 5-methyl-pyrazine-2-carboxylic acid (5-methyl-pyrazin-2-yl)-amide, 5-methyl-pyrazine-2-carboxylic acid (3-methyl-pyrazin-2-yl)-amide, (5-methyl-pyrazin-2-yl)-morpholin-4-yl-methanone, 5-methyl-pyrazine-2-carboxylic acid hydroxyamide, pyrazine-2-carboxylic acid, 5-amino-pyrazine-2-carboxylic acid, 5-benzyl-pyrazine-2-carboxylic acid, 6-chloro-pyrazine-2-carboxylic acid, 6-methoxy-pyrazine-2-carboxylic acid, 3-hydroxy-pyrazine-2-carboxylic acid, 5-methyl-pyrazine-2-carboxylic acid 2-hydroxy-ethyl ester, 5-methyl-pyrazine-2-carboxylic acid allyl ester, 5-methyl-pyrazine-2-carboxylic acid phenyl ester, 5-methyl-pyrazine-2-carboxylic acid ethoxycarbonylmethyl ester, pyrazine-2-carboxylic acid methyl ester or 2-methyl-5-(1H-tetrazol-5-yl)-pyrazine; and 4-N-oxides thereof;

h) 5-(3-(5-Methyl)isoxazolyl)tetrazole;

i) 5-(5-(3-Methyl)isoxazolyl)tetrazole;

j) 5-(3-Quinolyl)tetrazole;

- k) Nicotinic acid;
- l) Pyridazine-4-carboxylic acid;
- m) 3-pyridine acetic acid;
- n) 5-Methylnicotinic acid;
- o) 6-Methylnicotinic acid;
- p) Nicotinic acid-1-oxide;
- q) 2-Hydroxynicotinic acid;
- r) Furane-3-carboxylic acid;
- s) 5-Methylpyrazole-3-carboxylic acid; and
- t) 3-Methylisoxazole-5-carboxylic acid.

7. A pharmaceutical or physiologically acceptable composition comprising, consisting essentially of, or consisting of the modulator of claim 2.

8. A method of changing lipid metabolism comprising providing or administering to an individual in need of said change said pharmaceutical or physiologically acceptable composition of claim 7.

9. A method of preventing or treating a metabolic-related disorder comprising providing or administering to an individual in need of said prevention or treatment said pharmaceutical or physiologically acceptable composition of claim 7.

10. A method of using the modulator of claim 2 for the preparation of a medicament for the treatment of a disorder in lipid metabolism in an individual.

11. A method of using the modulator of claim 2 for the preparation of a medicament for the treatment of a metabolic-related disorder in an individual.

12. A method of identifying whether a candidate compound binds to a nicotinic acid GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

- (a) SEQ. ID. NO.:36 (hRUP25);
- (b) SEQ. ID. NO.:137 (mRUP25); and
- (c) SEQ. ID. NO.:139 (rRUP25);

or an allelic variant or a biologically active fragment of said amino acid sequence;

comprising the steps of:

- (a') contacting the receptor with a labeled reference compound known to bind to the GPCR in the presence or absence of the candidate compound; and
- (b') determining whether the binding of said labeled reference compound to the receptor is inhibited in the presence of the candidate compound;

wherein said inhibition is indicative of the candidate compound binding to a nicotinic acid GPCR.

13. A method of making a mouse genetically predisposed to a metabolic-related disorder or a disorder of lipid metabolism comprising the step of knocking out the gene encoding the polypeptide of SEQ. ID. NO.:137 (mRUP25) or the polypeptide of SEQ. ID. NO.:151 (mRUP19).

14. A knockout mouse according to the method of claim 13.

15. A method of using the knockout mouse of claim 14 to identify whether a candidate compound has therapeutic efficacy for the prevention or treatment of said metabolic-related disorder or said disorder of lipid metabolism.

16. A method of making a rat genetically predisposed to a metabolic-related disorder or a disorder of lipid metabolism comprising the step of knocking out the gene encoding the polypeptide of SEQ. ID. NO.:139 (rRUP25) or the polypeptide of SEQ. ID. NO.:157 (rRUP19).

17. A knockout rat according to the method of claim 16.

18. A method of using the knockout rat of claim 17 to identify whether a candidate compound has therapeutic efficacy for the prevention or treatment of said metabolic-related disorder or said disorder of lipid metabolism.

19. An isolated EFA-hRUP25 polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ. ID. NO.:158;
- (b) a polynucleotide having the nucleotide sequence of SEQ. ID. NO.:158;
- (c) a polynucleotide comprising a polynucleotide encoding the polypeptide having the amino acid sequence of SEQ. ID. NO.:159 or a biologically active fragment of said polypeptide; and
- (d) a polynucleotide encoding the polypeptide having the amino acid sequence of SEQ. ID. NO.:159 or a biologically active fragment of said polypeptide.

20. An isolated EFA-hRUP25 polypeptide selected from the group consisting of:

- (a) a polypeptide comprising the amino acid sequence of SEQ. ID. NO.:159, or a biologically active fragment of said polypeptide; and
- (b) a polypeptide having the amino acid sequence of SEQ. ID. NO.:159, or a biologically active fragment of said polypeptide.

21. A recombinant vector comprising the polynucleotide of claim 19.

22. A host cell comprising the recombinant vector of claim 21.

\* \* \* \* \*