# HUMAN ANTIBODIES TO THE GLUCAGON RECEPTOR

## FIELD OF THE INVENTION

**[0001]** The present invention is related to human antibodies and antigen-binding fragments of human antibodies that specifically bind the glucagon receptor, and therapeutic methods of using those antibodies.

## STATEMENT OF RELATED ART

**[0002]** Glucagon is a 29 amino acid hormone produced by the alpha cells of pancreatic islets. Glucagon maintains normal levels of glucose in animals, including humans, by counter-balancing the effects of insulin. It is an imbalance of glucagon and insulin that may play an important role in several diseases, such as diabetes mellitus and diabetic ketoacidosis. In particular, studies have shown that higher basal glucagon levels and lack of suppression of postprandial glucagon secretion contribute to diabetic conditions in humans (Muller *et al.*, N Eng J Med 283: 109-115 (1970)).

**[0003]** It is believed that glucagon's effects on elevating blood glucose levels are mediated in part by the activation of certain cellular pathways following the binding of glucagon (GCG) to its receptor (designated GCGR). GCGR is a member of the secretin subfamily (family B) of G-protein-coupled receptors and is predominantly expressed in the liver. The binding of glucagon to its receptor triggers a G-protein signal transduction cascade, activating intracellular cyclic AMP and leading to an increase in glucose output through de novo synthesis (gluconeogenesis) and glycogen breakdown (glycogenolysis) (Wakelam *et al.*, Nature, (1986) 323:68-71; Unson *et al.*, Peptides, (1989), 10:1171-1177; and Pittner and Fain, Biochem. J. (1991), 277:371-378).

**[0004]** The rat glucagon receptor was first isolated and purified by Jelinek *et al* (Jelinek, L.J. *et al.* (1993) *Science* 259(5101): 1614-1616). Subsequently, the rat sequence was used to identify and clone the 477 amino acid human glucagon receptor sequence (Lok, S. *et al.* (1994) *Gene* 140:203-209; MacNeil, D.J. *et al.* (1994) *Biochem. and Biophys. Res. Comm).* U.S. patent No. 5,776,725 discloses an isolated nucleic acid sequence encoding a human or rat glucagon receptor.

**[0005]** Targeting glucagon production or function with a glucagon receptor antagonist, such as an anti-GCGR antibody, may be one method of controlling and lowering blood glucose, and as such, may prove useful for treating diseases such as diabetes mellitus or diabetic ketoacidosis. Furthermore, by lowering glucose levels, it may be possible to prevent or ameliorate certain of the long-term complications associated with elevated glucose levels in diabetic patients.

**[0006]** Early studies demonstrated that polyclonal antibodies to the rat glucagon receptor were able to block glucagon binding (Unson, C.G. (1996) *PNAS* 93(1):310-315). Monoclonal antibodies to the human glucagon receptor were described by Buggy *et al.* (Buggy, J.J. *et al.* (1995) *J. Biol. Chem.* 270(13): 7474; Buggy, J.J. *et al.* (1996) *Horm Metab Res.* 28(5):215-9).

The antibody described by Buggy *et al.* competed with glucagon for the hormone binding site of the receptor and recognized both the human and rat glucagon receptors, but not the mouse receptor. Wright *et al.* disclose a monoclonal antibody raised in a mouse against the human glucagon receptor and conducted detailed protein structure determination of the monoclonal antibody to the receptor (Wright, L.M. (2000) *Acta Crystallographica Section D*. 56(5): 573-580). Other antibodies to the glucagon receptor are described in US patents 5,770,445 and 7,947,809; European patent application EP2074149A2; EP patent EP0658200B1; US patent publications 2009/0041784; 2009/0252727; and 2011/0223160; and PCT publication WO2008/036341.

## BRIEF SUMMARY OF THE INVENTION

**[0007]** In a first aspect, the invention provides fully human monoclonal antibodies (mAbs) and antigen-binding fragments thereof that bind to the human glucagon receptor (hGCGR) and inhibit or block its activity, for example, block the binding of glucagon to its receptor, thereby blocking the elevation of blood glucose levels. The antibodies or antigen binding fragments thereof may be useful for lowering blood glucose levels in a subject that suffers from a disease characterized by increased blood glucose levels, such as diabetes mellitus. The antibodies may also be used to treat a wide range of conditions and disorders in which blocking the interaction of glucagon with the glucagon receptor is desired, thereby having a beneficial effect. The antibodies may ultimately be used to prevent the long-term complications associated with elevated blood glucose levels in diabetic patients, or to ameliorate at least one symptom associated with elevated blood glucose levels in diabetic patients.

**[0008]** The antibodies of the invention can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to affect functionality, *e.g.,* to eliminate residual effector functions (Reddy *et al.,* 2000, J. Immunol. 164:1925-1933).

**[0009]** In one embodiment, the invention provides an isolated human antibody or antigen-binding fragment thereof that specifically binds human glucagon receptor (hGCGR), wherein the antibody binds an ectodomain and/or an extracellular (EC) loop of human GCGR, wherein the ectodomain is the N-terminal domain of GCGR and wherein the EC loop is one or more of EC1, EC2 and EC3.

**[0010]** In one embodiment, the invention provides an antibody or fragment thereof, which binds the N-terminal domain comprising amino acid residues ranging from about amino acid residue number 27 to about amino acid residue 144 of SEQ ID NO: 153, or binds an EC loop of hGCGR, wherein the EC loop is one or more of EC1, EC2, and EC3, wherein EC1 comprises amino acid residues ranging from about amino acid residue 194 to about amino acid residue 226 of SEQ ID NO: 153; EC2 comprises amino acid residues ranging from about amino acid residue 285 to about amino acid residue 305 of SEQ ID NO: 153; and EC3 comprises amino acid residues ranging from about amino acid residue 369 to about amino acid residue 384 of

SEQ ID NO: 153.

**[0011]** In one embodiment, the human antibody or antigen-binding fragment of a human antibody that binds hGCGR comprises a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In certain embodiments, the antibody or antigen-binding fragment of an antibody that binds hGCGR comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 34, 70, 86, 110 and 126, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

**[0012]** In one embodiment, the human antibody or antigen-binding fragment of a human antibody that binds hGCGR comprises a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In certain embodiments, the antibody or antigen-binding fragment of an antibody that binds hGCGR comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 42, 78, 88, 118 and 128, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

**[0013]** In certain embodiments, the human antibody or fragment thereof that binds hGCGR comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NO: 2/10, 18/26, 34/42, 50/58, 66/68, 70/78, 86/88, 90/98, 106/108, 110/118, 126/128, 130/138, and 146/148. In certain embodiments, the HCVR/LCVR amino acid sequence pair is selected from the group consisting of SEQ ID NO: 34/42, 70/78, 86/88, 110/118 and 126/128.

**[0014]** In a related embodiment, the invention includes an antibody or antigen-binding fragment of an antibody which specifically binds hGCGR, wherein the antibody or fragment thereof comprises the heavy and light chain CDR domains contained within heavy and light chain sequence pairs selected from the group consisting of SEQ ID NO: 2/10, 18/26, 34/42, 50/58, 66/68, 70/78, 86/88, 90/98, 106/108, 110/118, 126/128, 130/138 and 146/148. Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See, e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA*

*86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

**[0015]** In certain embodiments, the present invention provides an isolated human antibody or an antigen-binding fragment thereof that binds specifically to hGCGR, wherein the antibody comprises a HCVR comprising the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within the HCVR sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146; and a LCVR comprising the three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within the LCVR sequences selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148.

**[0016]** In one embodiment, the present invention provides an isolated human antibody or antigen-binding fragment of a human antibody that binds hGCGR, comprising a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 24, 40, 56, 76, 96, 116 and 136, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 84, 104, 124 and 144, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

**[0017]** In one embodiment, the invention provides an antibody or fragment thereof that further comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 20, 36, 52, 72, 92, 112 and 132, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 22, 38, 54, 74, 94, 114 and 134, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 28, 44, 60, 80, 100, 120 and 140, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 30, 46, 62, 82, 102, 122 and 142, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

**[0018]** In one embodiment, the antibody or antigen-binding fragment of an antibody comprises:

(a) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 24, 40, 56, 76, 96, 116 and 136; and

(b) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 84, 104, 124 and 144.

**[0019]** In one embodiment, the antibody or antigen-binding fragment of the antibody further comprises:

(c) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 20, 36, 52, 72, 92, 112 and 132;

(d) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 22, 38, 54, 74, 94, 114 and 134;

(e) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 28, 44, 60, 80, 100, 120 and 140; and

(f) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 30, 46, 62, 82, 102, 122 and 142.

**[0020]** In one embodiment, the antibody or antigen-binding fragment thereof comprises a HCVR comprising a HCDR1 domain having an amino acid sequence selected from one of SEQ ID NO: 4, 20, 36, 52, 72, 92, 112 and 132; a HCDR2 domain having an amino acid sequence selected from one of SEQ ID NO: 6, 22, 38, 54, 74, 94, 114 and 134; a HCDR3 domain having an amino acid sequence selected from one of SEQ ID NOs: 8, 24, 40, 56, 76, 96, 116 and 136; and a LCVR comprising a LCDR1 domain having an amino acid sequence selected from one of SEQ ID NO: 12, 28, 44, 60, 80, 100, 120 and 140; a LCDR2 domain having an amino acid sequence selected from one of SEQ ID NO: 14, 30, 46, 62, 82, 102, 122 and 142; and a LCDR3 domain having an amino acid sequence selected from one of SEQ ID NO: 16, 32, 48, 64, 84, 104, 124 and 144.

**[0021]** In certain embodiments, the human antibody or antigen-binding fragment of a human antibody that binds to human GCGR comprises a HCDR3/LCDR3 amino acid sequence pair selected from the group consisting of SEQ ID NO: 8/16, 24/32, 40/48, 56/64, 76/84, 96/104, 116/124 and 136/144. Non-limiting examples of anti-GCGR antibodies having these HCDR3/LCDR3 pairs are the antibodies designated H4H1345N, H4H1617N, H4H1765N, H4H1321B and H4H1321P, H4H1327B and H4H1327P, H4H1328B and H4H1328P, H4H1331B and H4H1331P, H4H1339B and H4H1339P, respectively.

**[0022]** In one embodiment, the human antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences of SEQ ID NO: 4, 6 and 8, respectively and LCDR1, LCDR2 and LCDR3 amino acid sequences of SEQ ID NO: 12, 14 and 16, respectively.

**[0023]** In one embodiment, the human antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences of SEQ ID NO: 20, 22 and 24, respectively and LCDR1, LCDR2 and LCDR3 amino acid sequences of SEQ ID NO: 28, 30 and 32, respectively.

**[0024]** In one embodiment, the human antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences of SEQ ID NO: 36, 38 and 40, respectively and LCDR1, LCDR2 and LCDR3 amino acid sequences of SEQ ID NO: 44, 46 and 48, respectively.

**[0025]** In one embodiment, the human antibody or antigen binding fragment thereof comprises

the HCDR1, HCDR2 and HCDR3 amino acid sequences of SEQ ID NO: 52, 54 and 56, respectively and LCDR1, LCDR2 and LCDR3 amino acid sequences of SEQ ID NO: 60, 62 and 64, respectively.

**[0026]** In one embodiment, the human antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences of SEQ ID NO: 72, 74 and 76, respectively and LCDR1, LCDR2 and LCDR3 amino acid sequences of SEQ ID NO: 80, 82 and 84, respectively.

**[0027]** In one embodiment, the human antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences of SEQ ID NO: 92, 94 and 96, respectively and LCDR1, LCDR2 and LCDR3 amino acid sequences of SEQ ID NO: 100, 102 and 104, respectively.

**[0028]** In one embodiment, the human antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences of SEQ ID NO: 112, 114 and 116, respectively and LCDR1, LCDR2 and LCDR3 amino acid sequences of SEQ ID NO: 120, 122 and 124, respectively.

**[0029]** In one embodiment, the human antibody or antigen binding fragment thereof comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences of SEQ ID NO: 132, 134 and 136, respectively and LCDR1, LCDR2 and LCDR3 amino acid sequences of SEQ ID NO: 140, 142 and 144, respectively.

**[0030]** In one embodiment, the anti-hGCGR antibody or antigen binding fragment thereof comprises a HCDR1 sequence comprising the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8$ (SEQ ID NO: 202), wherein $X^1$ is Gly, $X^2$ is Phe, $X^3$ is Thr, $X^4$ is Phe or Ser, $X^5$ is Ser, $X^6$ is Ser or Asn, $X^7$ is Tyr or Phe, and $X^8$ is Asp, Leu, or Gly; a HCDR2 sequence comprising the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8$ (SEQ ID NO: 203), wherein $X^1$ is Ile, $X^2$ is Ser, Gln, Asp, or Trp, $X^3$ is Ser, Glu, Thr, or Phe , $X^4$ is Asp or Ala, $X^5$ is Gly or Glu, $X^6$ is Arg, Ile, or absent, $X^7$ is Asp or Glu, and $X^8$ is Lys or Thr; a HCDR3 sequence comprising the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} - X^{13} - X^{14} - X^{15} - X^{16} - X^{17} - X^{18} - X^{19} - X^{20} - X^{21}$ (SEQ ID NO: 204), wherein $X^1$ is Ala or Thr, $X^2$ is Lys or Arg, $X^3$ is Glu, $X^4$ is Met, Pro, Gly, or Asp, $X^5$ is Val, Ser, Lys, Arg, or absent, $X^6$ is Tyr, His, Asn, or absent, $X^7$ is Tyr, $X^8$ is Asp or Glu, $X^9$ is Ile, $X^{10}$ is Leu, $X^{11}$ is Thr, $X^{12}$ is Gly, $X^{13}$ is Tyr, Asp, or His, $X^{14}$ is His, Asp, Tyr, or absent, $X^{15}$ is Asn, Tyr, His, or absent, $X^{16}$ is Tyr, $X^{17}$ is Tyr or His, $X^{18}$ is Gly or Ala, $X^{19}$ is Met, $X^{20}$ is Asp and $X^{21}$ is Val or Ile; a LCDR1 sequence comprising the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6$ (SEQ ID NO: 205), wherein $X^1$ is Gln, $X^2$ is Gly or Ala, $X^3$ is Ile, $X^4$ is Asn or Arg, $X^5$ is Asn, and $X^6$ is Tyr or Asp; a LCDR2 sequence comprising the formula $X^1 - X^2 - X^3$ (SEQ ID NO: 206), wherein $X^1$ is Thr or Ala, $X^2$ is Ala or Thr, and $X^3$ is Ser or Phe; and a LCDR3 sequence comprising the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9$ (SEQ ID NO: 207), wherein $X^1$ is Gln or Leu, $X^2$ is Gln, $X^3$ is Tyr, His, or Asp, $X^4$ is Asn or Tyr, $X^5$ is Thr or Ser, $X^6$ is Tyr, Asn, or His, $X^7$ is Pro, $X^8$ is Leu, Phe, Arg, or absent and $X^9$ is Thr.

**[0031]** In one embodiment, the antibody or antigen-binding fragment binds human, monkey, mouse and rat GCGR.

**[0032]** In one embodiment, the antibody or antigen-binding fragment binds human, monkey and mouse GCGR, but does not bind rat GCGR.

**[0033]** In one embodiment, the antibody or antigen-binding fragment binds human, monkey and rat GCGR, but does not bind mouse GCGR.

**[0034]** In one embodiment, the antibody or antigen-binding fragment binds human and monkey GCGR, but does not bind rat or mouse GCGR.

**[0035]** In one embodiment, the antibody or antigen-binding fragment binds human GCGR, but does not bind monkey, mouse or rat GCGR.

**[0036]** In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that neutralizes hGCGR activity, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148; (iii) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 24, 40, 56, 76, 96, 116 and 136, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 84, 104, 124 and 144, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 20, 36, 52, 72, 92, 112 and 132, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 22, 38, 54, 74, 94, 114 and 134, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 28, 44, 60, 80, 100, 120 and 140, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 30, 46, 62, 82, 102, 122 and 142, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) binds any one or more of human, monkey, mouse or rat GCGR; (vi) may or may not block GCGR activity in at least one species other than human; (v) demonstrates a $K_D$ ranging from about $10^{-8}$ to about $10^{-12}$; (vi) lowers blood glucose levels by at least about 25% to about 75% in a mammal experiencing elevated blood glucose levels; (vii) may or may not lower triglyceride levels to

levels observed in a normal mammal; or (viii) demonstrates no adverse effect on blood levels of LDL, HDL, or total cholesterol in a mammal.

**[0037]** In another related embodiment, the invention provides an antibody or antigen-binding fragment thereof that competes for specific binding to hGCGR with an antibody or antigen-binding fragment comprising heavy and light chain CDR domains contained within heavy and light chain sequence pairs selected from the group consisting of SEQ ID NO: 2/10, 18/26, 34/42, 50/58, 66/68, 70/78, 86/88, 90/98, 106/108, 110/118, 126/128, 130/138 and 146/148.

**[0038]** In another related embodiment, the invention provides an antibody or antigen-binding fragment thereof that binds the same epitope on hGCGR that is recognized by an antibody comprising heavy and light chain sequence pairs selected from the group consisting of SEQ ID NO: 2/10, 18/26, 34/42, 50/58, 66/68, 70/78, 86/88, 90/98, 106/108, 110/118, 126/128, 130/138 and 146/148.

**[0039]** In one embodiment, the invention provides for an anti-hGCGR antibody having one or more of the following characteristics:

a) capable of reducing blood glucose levels by about 25% to about 75% for a period of at least 7 days, when administered at a dose ranging from about 1 mg/kg to about 30 mg/kg;
b) capable of resulting in at least a 10% reduction in body weight when administered to a mammal in need of such therapy;
c) capable of reducing blood ketone levels by about 25% to 75% when administered at a dose ranging from about 1 mg/kg to about 30 mg/kg; or
d) capable of reducing blood glucose levels by about 20% to 40% without causing a significant elevation in blood lipids or cholesterol when administered with an antibody specific for proprotein convertase subtilisin/kexin (PCSK)-9, and sustaining lowered blood glucose levels for at least 7 days post treatment.

**[0040]** In a second aspect, the invention provides nucleic acid molecules encoding anti-hGCGR antibodies or fragments thereof. Recombinant expression vectors carrying the nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the invention, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

**[0041]** In one embodiment, the invention provides an antibody or fragment thereof comprising a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 17, 33, 49, 65, 69, 85, 89, 105, 109, 125, 129 and 145, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In one embodiment, the HCVR is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 33, 69, 85, 109 and 125.

**[0042]** In one embodiment, the antibody or fragment thereof further comprises a LCVR

encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, 25, 41, 57, 67, 77, 87, 97, 107, 117, 127, 137 and 147, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In one embodiment, the LCVR is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 41, 77, 87, 117 and 127.

**[0043]** In one embodiment, the invention also provides an antibody or antigen-binding fragment of an antibody comprising a HCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 7, 23, 39, 55, 75, 95, 115 and 135, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 15, 31, 47, 63, 83, 103, 123 and 143, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

**[0044]** In one embodiment, the invention provides an antibody or fragment thereof further comprising a HCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 3, 19, 35, 51, 71, 91, 111 and 131, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 5, 21, 37, 53, 73, 93, 113 and 133, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 11, 27, 43, 59, 79, 99, 119 and 139, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 13, 29, 45, 61, 81, 101, 121 and 141, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

**[0045]** In a third aspect, the invention features a human anti-hGCGR antibody or antigen-binding fragment of an antibody comprising a HCVR encoded by nucleotide sequence segments derived from $V_H$, $D_H$ and $J_H$ germline sequences, and a LCVR encoded by nucleotide sequence segments derived from $V_K$ and $J_K$ germline sequences, with combinations as shown in Table 2.

**[0046]** The invention encompasses anti-hGCGR antibodies having a modified glycosylation pattern. In some applications, modification to remove undesirable glycosylation sites may be useful, or e.g., removal of a fucose moiety to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield *et al.* (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

**[0047]** In a fourth aspect, the invention features a pharmaceutical composition comprising a

recombinant human antibody or fragment thereof, which specifically binds hGCGR, and a pharmaceutically acceptable carrier or diluent.

**[0048]** In one embodiment, the invention features a composition, which is a combination of an antibody or antigen-binding fragment of an antibody of the invention, and a second therapeutic agent. The second therapeutic agent may be any agent that is advantageously combined with the antibody or fragment thereof of the invention.

**[0049]** In one embodiment, the second therapeutic agent may be an agent capable of lowering blood glucose or reducing at least one symptom in a patient suffering from a disease or condition characterized by high blood glucose levels, such as diabetes mellitus.

**[0050]** In certain embodiments, the second therapeutic agent may be an agent that helps to counteract or reduce any possible side effect(s) associated with the antibody or antigen-binding fragment of an antibody of the invention, if such side effect(s) should occur. For example, in the event that any of the anti-hGCGR antibodies increases lipid or cholesterol levels, it may be beneficial to administer a second agent that is effective to lower lipid or cholesterol levels.

**[0051]** The second therapeutic agent may be a small molecule drug, a protein/polypeptide, an antibody, a nucleic acid molecule, such as an anti-sense molecule, or a siRNA. The second therapeutic agent may be synthetic or naturally derived.

**[0052]** In one embodiment, the second therapeutic agent may be a glucagon antagonist, or a second glucagon receptor antagonist, such as another antibody to the glucagon receptor, which is different than the antibodies described herein. It will also be appreciated that the antibodies and pharmaceutically acceptable compositions of the present invention can be employed in combination therapies, that is, the antibodies and pharmaceutically acceptable compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an antibody may be administered concurrently with another agent used to treat the same disorder), or they may achieve different effects (e.g., control of any adverse effects). As used herein, additional therapeutic agents that are normally administered to treat or prevent a particular disease, or condition, are appropriate for the disease, or condition, being treated.

**[0053]** In one embodiment, the anti-hGCGR antibodies of the invention may be used in combination with one or more of the following type 2 diabetes treatments currently available. These include biguanide (metformin), sulfonylureas (such as glyburide, glipizide), peroxisome proliferator-activated receptor (PPAR) gamma agonists (pioglitazone, rosiglitazone); and alpha glucosidase inhibitors (acarbose, voglibose). Additional treatments include injectable treatments such as EXENATIDE® (glucagon-like peptide 1), and SYMLIN® (pramlintide).

**[0054]** In certain embodiments, the composition may include a second agent selected from the group consisting of non-sulfonylurea secretagogues, insulin, insulin analogs, exendin-4 polypeptides, beta 3 adrenoceptor agonists, PPAR agonists, dipeptidyl peptidase IV inhibitors, statins and statin-containing combinations, inhibitors of cholesterol uptake and/or bile acid re-absorption, LDL-cholesterol antagonists, cholesteryl ester transfer protein antagonists, endothelin receptor antagonists, growth hormone antagonists, insulin sensitizers, amylin mimetics or agonists, cannabinoid receptor antagonists, glucagon-like peptide-1 agonists, melanocortins, melanin-concentrating hormone receptor agonists, SNRIs, a fibroblast growth factor 21 (FGF21) mimetic (See, for example, US20110002845 and US20080261236), a fibroblast growth factor receptor 1c (FGFR1c) agonist (See, for example, US20110150901), an inhibitor of advanced glycation endproduct formation, such as, but not limited to, aminoguanidine, and protein tyrosine phosphatase inhibitors.

**[0055]** In certain embodiments, the composition may include a second agent to help lower lipid or cholesterol levels and may include an agent such as a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor (for example, a statin such as atorvastatin, (LIPITOR®), fluvastatin (LESCOL®), lovastatin (MEVACOR®), pitavastatin (LIVALO®), pravastatin (PRAVACHOL®), rosuvastatin (CRESTOR®) and simvastatin (ZOCOR®) and the like.

**[0056]** In certain embodiments, it may be beneficial to administer the antibodies of the invention in combination with any one or more of the following: (1) niacin, which increases lipoprotein catabolism; (2) fibrates or amphipathic carboxylic acids, which reduce low-density lipoprotein (LDL) level, improve high-density lipoprotein (HDL) and TG levels, and reduce the number of non-fatal heart attacks; and (3) activators of the LXR transcription factor that plays a role in cholesterol elimination such as 22-hydroxycholesterol, or fixed combinations such as VYTORIN®) (ezetimibe plus simvastatin); a statin with a bile resin (*e.g.*, cholestyramine, colestipol, colesevelam), a fixed combination of niacin plus a statin (*e.g.*, niacin with lovastatin); or with other lipid lowering agents such as omega-3-fatty acid ethyl esters (for example, omacor). Furthermore, the second therapeutic agent can be one or more other inhibitors of glucagon or GCGR, as well as inhibitors of other molecules, such as angiopoietin-like protein 3 (ANGPTL3), angiopoietin-like protein 4 (ANGPTL4), angiopoietin-like protein 5 (ANGPTL5), angiopoietin-like protein 6 (ANGPTL6), which are involved in lipid metabolism, in particular, cholesterol and/or triglyceride homeostasis. Inhibitors of these molecules include small molecules and antibodies that specifically bind to these molecules and block their activity.

**[0057]** In certain embodiments, it may be beneficial to administer the anti-GCGR antibodies of the invention in combination with a nucleic acid that inhibits the activity of hPCSK9, such as an antisense molecule, a double stranded RNA, or a siRNA molecule. Exemplary nucleic acid molecules that inhibit the activity of PCSK9 are described in US2011/0065644, US2011/0039914,US2008/0015162 and US2007/0173473.

**[0058]** In certain embodiments, it may be beneficial to administer the anti-hGCGR antibodies of

the invention in combination with an antibody that specifically binds to and inhibits the activity of hPCSK9, thereby inhibiting its activity, wherein such antibody acts to lower lipid or cholesterol levels. Exemplary anti-hPCSK9 antibodies are described in US2010/0166768. The isolated antibody that specifically binds to human PCSK9, or an antigen-binding fragment thereof, may be administered at a dose ranging from about 0.01 mg/kg to about 30 mg/kg. It may be administered as a single dose or as multiple doses. The anti-hPCSK9 antibody may be administered concurrently with the anti-GCGR antibody, or it may be administered prior to or after the anti-GCGR antibody.

**[0059]** In one embodiment, the second therapeutic agent to be used in combination with an antibody of the invention comprises an isolated antibody that specifically binds to human PCSK9, or an antigen-binding fragment thereof, wherein the anti-hPCSK9 antibody comprises the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within any one of the HCVR sequences selected from the group consisting of SEQ ID NOs: 173 and 177; and the three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the LCVR sequences selected from the group consisting of SEQ ID NOs: 175 and 185.

**[0060]** In one embodiment, the isolated antibody that specifically binds to human PCSK9, or antigen-binding fragment thereof, comprises a heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NO: 173 and 177.

**[0061]** In one embodiment, the isolated antibody that specifically binds to human PCSK9, or antigen-binding fragment thereof, comprises a light chain variable region (LCVR) selected from the group consisting of SEQ ID NO: 175 and 185.

**[0062]** In one embodiment, the isolated antibody that specifically binds to human PCSK9, or antigen-binding fragment thereof, comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 173 and 177 and a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 175 and 185.

**[0063]** In one embodiment, the isolated antibody that specifically binds to human PCSK9, or antigen-binding fragment thereof, comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR), wherein the HCVR/ LCVR sequence pairs are selected from the group consisting of SEQ ID NO: 173/175; and SEQ ID NO: 177/185.

**[0064]** In one embodiment, the isolated antibody that specifically binds to human PCSK9, or antigen-binding fragment thereof, comprises: a HCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 161 and 179; a HCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 163 and 181; a HCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 165 and 183; a LCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 167 and 187; a LCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 169 and 189 and a LCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 171 and 191.

**[0065]** In certain embodiments, the hPCSK9 antibodies to be used in combination with the anti-GCGR antibodies of the invention are encoded by nucleic acid molecules as described herein. For example, in one embodiment, the invention provides an anti-hPCSK9 antibody or fragment thereof comprising a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 172 and 176, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof, and a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 174 and 184, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

**[0066]** In one embodiment, the invention provides an anti-hPCSK9 antibody to be used in combination with the anti-GCGR antibodies of the invention, wherein the anti-PCSK9 antibody or fragment thereof comprises a HCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 160 and 178, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 162 and 180, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 164 and 182, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 166 and 186, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 168 and 188, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 170 and 190, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

**[0067]** When multiple therapeutics are co-administered, dosages may be adjusted accordingly, as is recognized in the pertinent art.

**[0068]** In a fifth aspect, the invention features methods for inhibiting hGCGR activity using the anti-hGCGR antibody or antigen-binding portion of the antibody of the invention, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising the antibody or antigen-binding fragment thereof. The antibodies of the invention may be used to treat any condition or disorder, which is improved, ameliorated, inhibited or prevented by removal, inhibition or reduction of hGCGR activity. It is envisioned that the antibodies of the invention may be used alone, or as adjunct therapy with other agents or methods known to be standard care for treating patients suffering from diseases

or conditions characterized in part by elevated blood glucose or ketone levels, such as, but not limited to, diabetes. Such standard therapy may include fluid administration, or administration of any other pharmaceutical agents useful for lowering blood glucose, ketones, or lipids, or for weight reduction.

**[0069]** The anti-hGCGR antibodies of the invention may function to block the interaction between glucagon and its receptor, thereby inhibiting the glucose elevating effects of glucagon. The use of glucagon receptor antagonists, such as the antibodies described herein, may be an effective means of achieving normal levels of glucose, thereby ameliorating, or preventing one or more symptoms of, or long term complications associated with, for example, diabetes. The use of glucagon receptor antagonists, such as the antibodies described herein, may also be an effective means of achieving normal levels of glucose in non-diabetic patients, who experience hyperglycemia as a result of conditions or disorders not related to diabetes, such as perioperative hyperglycemia (hyperglycemia observed in patients just prior to surgery, or after surgery). In certain embodiments, methods of lowering blood glucose levels or ketone levels in diabetic ketoacidosis are envisioned using the antibodies of the invention. In certain embodiments, methods of treating patients to achieve a reduction in body weight, or to prevent weight gain, or to maintain a normal body weight, are also envisioned using the antibodies of the invention.

**[0070]** The antibodies of the present invention may also be useful for ameliorating conditions such as, for example, impaired glucose tolerance, obesity, or for treating diabetic conditions, or for preventing or reducing the severity of any one or more of the long-term complications associated with diabetes, such as nephropathy, neuropathy, retinopathy, cataracts, stroke, atherosclerosis, impaired wound healing and other complications associated with diabetes, known to those skilled in the art.

**[0071]** Other conditions or disorders treatable by the therapeutic methods of the invention include diabetic ketoacidosis, hyperglycemia (including perioperative hyperglycemia, hyperglycemia in the intensive care unit patient, and hyperosmolar hyperglycemia syndrome), hyperinsulinemia, the metabolic syndrome, insulin resistance syndrome, impaired fasting glucose, or hyperglycemia associated with hypercholesterolemia, hypertriglyceridemia, hyperlipidemia, and general dyslipidemias. In certain embodiments, the invention provides for an isolated antibody or antigen-binding fragment thereof specific for GCGR, s described herein, for use in lowering blood glucose or ketone levels, or for treating a patient having a disease or condition associated with, or characterized in part by high blood glucose or ketone levels, wherein the condition or disease is selected from diabetes, impaired glucose tolerance, obesity, nephropathy, neuropathy, retinopathy, cataracts, stroke, atherosclerosis, impaired wound healing, diabetic ketoacidosis, hyperglycemia, hyperglycemic hyperosmolar syndrome, perioperative hyperglycemia, hyperglycemia in the intensive care unit patient, hyperinsulinemia, the metabolic syndrome, insulin resistance syndrome and impaired fasting glucose. In certain

embodiments, use of the isolated antibody or antigen-binding fragment of the invention is contemplated for preparation of a medicament for lowering blood glucose or ketone levels, or for treating a patient having a disease or condition associated with, or characterized in part by high blood glucose or ketone levels, or for ameliorating at least one symptom of such disease or condition, wherein the condition or disease is selected from any of the above-noted diseases or conditions.

**[0072]** The antibodies may also be useful for treating patients with inoperable glucagonoma (pancreatic endocrine tumor with or without necrolytic migratory erythema and hyperglycemia).

**[0073]** Other embodiments will become apparent from a review of the ensuing detailed description.

## BRIEF DESCRIPTION OF THE FIGURES

**[0074]** Fig. 1 shows the percent change in blood glucose levels in C57BL6 mice after administration of H4H1327P (anti-GCGR antibody), and/or H1H316P (anti-PCSK9 antibody) when given alone or in combination. Control (X with solid line); H4H1327P at 3 mg/kg (■ with solid line); H4H1327P at 10 mg/kg (▲with solid line); H1H316P at 10 mg/kg (▼ with solid line); H4H1327P at 3 mg/kg + H1H316P at 10 mg/kg (◆ with dashed line); H4H1327P at 10 mg/kg + H1H316P at 10 mg/kg (O with dashed lines).

**[0075]** Fig. 2 shows plasma LDL-C levels in C57BL6 mice after administration of H4H1327P, and/or H1H316P when given alone or in combination. Control (X with solid line); H4H1327P at 3 mg/kg (■ with solid line); H4H1327P at 10 mg/kg (▲with solid line); H1H316P at 10 mg/kg (▼ with solid line); H4H1327P at 3 mg/kg + H1H316P at 10 mg/kg (◆ with dashed line); H4H1327P at 10 mg/kg + H1H316P at 10 mg/kg (O with dashed lines).

**[0076]** Fig. 3 shows plasma HDL-C levels in C57BL6 mice after administration of H4H1327P, and/or H1H316P when given alone or in combination. Control (X with solid line); H4H1327P at 3 mg/kg (■ with solid line); H4H1327P at 10 mg/kg (▲with solid line); H1H316P at 10 mg/kg (▼ with solid line); H4H1327P at 3 mg/kg + H1H316P at 10 mg/kg (◆ with dashed line); H4H1327P at 10 mg/kg + H1H316P at 10 mg/kg (O with dashed lines).

**[0077]** Fig. 4 shows total plasma cholesterol levels in C57BL6 mice after administration of H4H1327P, and/or H1H316P when given alone or in combination. Control (X with solid line); H4H1327P at 3 mg/kg (■ with solid line); H4H1327P at 10 mg/kg (▲with solid line); H1H316P at 10 mg/kg (▼ with solid line); H4H1327P at 3 mg/kg + H1H316P at 10 mg/kg (◆ with dashed line); H4H1327P at 10 mg/kg + H1H316P at 10 mg/kg (O with dashed lines).

**[0078]** Fig. 5 shows plasma triglyceride levels in C57BL6 mice after administration of H4H1327P, and/or H1H316P when given alone or in combination. Control (X with solid line); H4H1327P at 3 mg/kg (■ with solid line); H4H1327P at 10 mg/kg (▲with solid line); H1H316P at 10 mg/kg (▼ with solid line); H4H1327P at 3 mg/kg + H1H316P at 10 mg/kg (◆ with dashed line); H4H1327P at 10 mg/kg + H1H316P at 10 mg/kg (O with dashed lines).

**[0079]** Fig. 6 shows hepatic triglyceride levels in C57BL6 mice after administration of

H4H1327P, and/or H1H316P when given alone or in combination. Control (X); H4H1327P at 3 mg/kg (■); H4H1327P at 10 mg/kg (▲); H1H316P at 10 mg/kg (▼); H4H1327P at 3 mg/kg + H1H316P at 10 mg/kg (◆); H4H1327P at 10 mg/kg + H1H316P at 10 mg/kg (○).

## DETAILED DESCRIPTION

**[0080]** Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

**[0081]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are now described.

### Definitions

**[0082]** The "glucagon receptor", also referred to herein as "GCGR", belongs to the G protein-coupled receptor class 2 family and consists of a long amino terminal extracellular domain (See SEQ ID NO: 158 for DNA encoding the N-terminal extracellular domain and SEQ ID NO: 159 for the amino acid sequence of the N-terminal extracellular domain), seven transmembrane segments, and an intracellular C-terminal domain (Jelinek *et al.*, Science 259: 1614-1616 (1993), Segre *et al.*, Trends Endocrinol. Metab 4:309-314 (1993)). Glucagon receptors are notably expressed on the surface of hepatocytes where they bind to glucagon and transduce the signal provided thereby into the cell. Accordingly, the term "glucagon receptor" also refers to one or more receptors that interact specifically with glucagon to result in a biological signal. DNA sequences encoding glucagon receptors of rat and human origin have been isolated and disclosed in the art (EP0658200B1). The murine and cynomolgus monkey homologues have also been isolated and sequenced (Burcelin, *et al.*, Gene 164 (1995) 305-310); McNally *et al.*, Peptides 25 (2004) 1171-1178). As used herein, "glucagon receptor" and "GCGR" are used interchangeably. The expression "GCGR", "hGCGR" or fragments thereof, as used herein, refers to the human GCGR protein or fragment thereof, unless specified as being from a non-human species, *e.g.* "mouse GCGR", "rat GCGR", or "monkey GCGR". Moreover, "GCGR," or "hGCGR", as used herein, refers to human GCGR having the nucleic acid sequence shown in SEQ ID NO: 157 and the amino acid sequence of SEQ ID NO: 153, or a biologically active fragment thereof. There are a variety of sequences related to the GCGR gene having the following Genbank Accession Numbers: NP_000151.1 (human), NP_742089.1 (rat), XP_001111894.1 (rhesus monkey), and NP_032127.2 (mouse). Other sequences disclosed

herein include human GCGR (SEQ ID NO: 153), mouse GCGR (SEQ ID NO: 154), Cynomolgus monkey (SEQ ID NO: 155), rat GCGR (SEQ ID NO: 156). In certain embodiments, fusion proteins useful in the invention may include SEQ ID NO: 149 (hGCGR-hFc, residues 27-144 of NP_000151.1 fused to the Fc region of human IgG) SEQ ID NO:150 (hGCGR-hFc, residues 27-144 of NP_000151.1 fused to the Fc region of human IgG), SEQ ID NO:151 (hGCGR-mmH, residues 27-144 of NP_000151.1 fused to a myc-myc-his tag), and SEQ ID NO:152 (MfGCGR-hFc, containing the N-terminal sequence of Mf, cynomolgus monkey, which is identical to residues 27-144 of GCGR of the rhesus monkey, *Macaca mulatta,* having accession number XP_001111894.1, and which is fused to the Fc region of human IgG).

**[0083]** The term "human proprotein convertase subtilisin/kexin type 9" or "hPCSK9", as used herein, refers to hPCSK9 encoded by the nucleic acid sequence shown in SEQ ID NO:192 and having the amino acid sequence of SEQ ID NO:193, or a biologically active fragment thereof.

**[0084]** The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds (*i.e.*, "full antibody molecules"), as well as multimers thereof (*e.g.* IgM) or antigen-binding fragments thereof. Each heavy chain is comprised of a heavy chain variable region ("HCVR" or "$V_H$") and a heavy chain constant region (comprised of domains $C_H1$, $C_H2$ and $C_H3$). Each light chain is comprised of a light chain variable region ("LCVR or "$V_L$") and a light chain constant region ($C_L$). The $V_H$ and $V_L$ regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each $V_H$ and $V_L$ is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In certain embodiments of the invention, the FRs of the anti-GCGR antibody (or antigen binding fragment thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

**[0085]** Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding. Padlan *et al.* (1995 FASEB J. 9:133-139) analyzed the contact regions between antibodies and their antigens, based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact the antigen. Padlan also found many antibodies in which one or two CDRs had no amino acids in contact with an antigen (see also, Vajdos *et al.* 2002 J Mol Biol 320:415-428).

**[0086]** CDR residues not contacting antigen can be identified based on previous studies (for example residues H60-H65 in CDRH2 are often not required), from regions of Kabat CDRs lying outside Chothia CDRs, by molecular modeling and/or empirically. If a CDR or residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding

position in another human antibody sequence or a consensus of such sequences. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically. Empirical substitutions can be conservative or non-conservative substitutions.

**[0087]** The fully-human anti-GCGR antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the $V_H$ and/or $V_L$ domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

**[0088]** The present invention also includes anti-hGCGR antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more

conservative substitutions. For example, the present invention includes anti-hGCGR antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

**[0089]** The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human mAbs of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.,* mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include mAbs in which CDR sequences derived from the germline of another mammalian species (*e.g.,* mouse), have been grafted onto human FR sequences. The anti-human GCGR antibodies of the invention may be designated as "anti-hGCGR" or "anti-GCGR".

**[0090]** The term "specifically binds," or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by an equilibrium dissociation constant of at least about $1x10^{-6}$ M or less (*e.g.*, a smaller $K_D$ denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. An isolated antibody that specifically binds hGCGR may, however, exhibit cross-reactivity to other antigens such as GCGR molecules from other species. Moreover, multi-specific antibodies that bind to hGCGR and one or more additional antigens or a bi-specific that binds to two different regions of hGCGR are nonetheless considered antibodies that "specifically bind" hGCGR, as used herein.

**[0091]** The term "high affinity" antibody refers to those mAbs having a binding affinity to hGCGR, expressed as $K_D$, of at least $10^{-9}$ M; preferably $10^{-10}$ M; more preferably $10^{-11}$ M, even more preferably $10^{-12}$ M, as measured by surface plasmon resonance, *e.g.,* BIACORE™ or solution-affinity ELISA.

**[0092]** By the term "slow off rate", "Koff" or "kd" is meant an antibody that dissociates from hGCGR with a rate constant of $1 \times 10^{-3}$ $s^{-1}$ or less, preferably $1 \times 10^{-4}$ $s^{-1}$ or less, as determined by surface plasmon resonance, *e.g.*, BIACORE™.

**[0093]** The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding portion" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to hGCGR.

**[0094]** The specific embodiments, antibody or antibody fragments of the invention may be conjugated to a therapeutic moiety ("immunoconjugate"), such as a second GCGR antagonist,

or to biguanide (metformin), a sulfonylurea (such as glyburide, glipizide), a PPAR gamma agonist (such as pioglitazone, or rosiglitazone), an alpha glucosidase inhibitor (such as acarbose, or voglibose), EXENATIDE® (glucagon-like peptide 1), SYMLIN® (pramlintide), a chemotherapeutic agent, a radioisotope, or any other therapeutic moiety useful for treating a disease or condition caused in part by unwanted glucagon activity.

**[0095]** An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies (Abs) having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds hGCGR, or a fragment thereof, is substantially free of Abs that specifically bind antigens other than hGCGR).

**[0096]** A "blocking antibody" or a "neutralizing antibody", as used herein (or an "antibody that neutralizes GCGR activity"), is intended to refer to an antibody whose binding to hGCGR results in inhibition of at least one biological activity of GCGR. For example, an antibody of the invention may aid in preventing the increase in blood glucose levels associated with elevation of glucagon levels. Alternatively, an antibody of the invention may demonstrate the ability to block cAMP production in response to glucagon. This inhibition of the biological activity of GCGR can be assessed by measuring one or more indicators of GCGR biological activity by one or more of several standard *in vitro* or *in vivo* assays known in the art (see examples below).

**[0097]** The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

**[0098]** The term "$K_D$ ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

**[0099]** The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. The term "epitope" also refers to a site on an antigen to which B and/or T cells respond. It also refers to a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

**[0100]** The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is

nucleotide sequence identity in at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or GAP, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

**[0101]** As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 90% sequence identity, even more preferably at least 95%, 98% or 99% sequence identity. Preferably, residue positions, which are not identical, differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, *e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443 45. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

**[0102]** Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or

recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215: 403 410 and (1997) Nucleic Acids Res. 25:3389 402.

**[0103]** In specific embodiments, the antibody or antibody fragment for use in the method of the invention may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for epitopes of more than one target polypeptide. An exemplary bi-specific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) $C_H3$ domain and a second Ig $C_H3$ domain, wherein the first and second Ig $C_H3$ domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bi-specific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig $C_H3$ domain binds Protein A and the second Ig $C_H3$ domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second $C_H3$ may further comprise an Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second $C_H3$ include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 mAbs; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 mAbs; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 mAbs. Variations on the bi-specific antibody format described above are contemplated within the scope of the present invention.

**[0104]** By the phrase "therapeutically effective amount" is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

"Normal glucose levels" refers to mean plasma glucose values in humans of less than about 80 mg/dL for fasting levels, and about less than 110-120 mg/dL for post prandial levels. Plasma glucose may be determined in accordance with Etgen *et al.*, (Metabolism 2000; 49(5): 684-688) or calculated from a conversion of whole blood glucose concentration in accordance with D'Orazio *et al.*, (Clin. Chem. Lab. Med. 2006; 44(12): 1486-1490). "Cholesterol normalization" or "normal cholesterol levels" refers to a total cholesterol level in a human of about less than 200 mg/dL, with a range of about 200-240 mg/dL considered borderline high. From the total normal cholesterol, a mean LDL value in humans of about 100 to about 129 mg/dL is

considered normal and an HDL value above 45 mg/dL is considered normal. The normal triglyceride level in humans is less than 150 mg/dL. The normal total/HDL ratio is below 4.5, and the normal LDL/HDL ratio is less than 3. These values may be determined in accordance with standard laboratory practice (see also, Friedewald, WT, Clin. Chem (1972), 18:499-502; Chen, Y. *et al.* Lipids Health Dis. (2010); 9:52; Keevil, JG, *et al.*, Circulation (2007), 115:1363-1370; and Bairaktari, E. *et al.*, Clin. Biochem. (2000), 33:549-555). In certain embodiments of the invention, the anti-GCGR antibodies may be useful to lower blood glucose levels to within the normal range. In certain embodiments of the invention, the anti-GCGR antibodies may be useful to increase the level of HDL-C. In certain embodiments of the invention, the anti-GCGR antibodies may be useful to decrease the level of triglycerides.

**General Description**

**[0105]** Since glucagon exerts its physiological effects by signaling through the glucagon receptor, the glucagon receptor may be a potential therapeutic target for diabetes and other glucagon related metabolic disorders. The use of glucagon receptor antagonists, such as the antibodies described herein, may be an effective means of achieving normal levels of glucose, thereby ameliorating, or preventing one or more symptoms or long term complications associated with diabetes. The antibodies of the present invention may also be useful for ameliorating conditions associated with, for example, impaired glucose tolerance, for treating obesity, for preventing weight gain, for treating metabolic syndrome, or for treating diabetic conditions, including diabetic ketoacidosis, or for preventing and/or lowering the risk of developing any one or more of the complications associated with diabetes, such as nephropathy, neuropathy, retinopathy, cataracts, stroke, atherosclerosis, impaired wound healing and other complications associated with diabetes, known to those skilled in the art.

**[0106]** The use of the anti-hGCGR antibodies, as described herein, may also be useful for treating other conditions, including hyperglycemia, hyperglycemic hyperosmolar syndrome (Stoner, G.D., American Family Physician, (2005), 71(9):1723-1730; Diabetes Spectrum, Umpierrez, G.E., (2002), 15(1):28-36; Nugent, B.W., Emergency Medicine Clinics of North America, (2005), 23:629-648), perioperative hyperglycemia (Frisch, A. *et al.* Diabetes Care, (2010), 33(8):1783-1788; Hanazaki, K. *et al.* World J Gastroenterol, (2009), 15(33): 4122-4125; Smiley, D.D. *et al.* Southern Medical Journal, (2006), 99(6):580-589; Hermanides, J. *et al.*, The Netherlands J. of Med. 67(6):226-229; Maerz, L.L. *et al.*, Current Opinion in Critical Care, (2011), 17:370-375), hyperglycemia in intensive care unit patients (Gunst, J. *et al.* , Seminars in Dialysis, (2010), 23(2):157-162; Losser, M-R., Critical care, (2010), 14:231), hyperinsulinemia, and insulin resistance syndrome and glucagonoma (pancreatic endocrine tumor with or without necrolytic migratory erythema and hyperglycemia) (See for example, Boden, G. *et al.* , N Engl J Med (1986); 314:1686-1689).

**[0107]** In certain embodiments, the antibodies of the invention were obtained from mice

immunized with a primary immunogen, followed by immunization with a secondary immunogen. The immunogen may be a cell line expressing the GCGR protein, or a biologically active fragment thereof, or DNA encoding the GCGR protein or active fragment thereof, or the GCGR protein or active fragment thereof. For example, in certain embodiments, the primary immunogen may be a cell line engineered using standard procedures known in the art to over-express full-length hGCGR (*e.g.* the mouse MG87 cell line). Alternatively, DNA immunization may be performed using DNA encoding full-length hGCGR (*e.g.* hGCGR constructs derived from accession number NP_000151.1), or DNA encoding a biologically active fragment thereof, for example, DNA encoding the N-terminal domain of GCGR (see, for example, SEQ ID NO: 158, which encodes SEQ ID NO: 159), or a soluble N-terminal protein, including that of SEQ ID NO: 159, or the amino acids spanning residues 27-144 of SEQ ID NO: 153. The secondary immunogen may be a GCGR protein, or biologically active fragment thereof, or a fusion protein, such as hGCGR-mmH (REGN547, SEQ ID NO: 151) or hGCGR-hFc (REGN315, SEQ ID NO: 150; REGN316, SEQ ID NO: 149).

**[0108]** In certain embodiments of the present invention, the N-terminal domain, having the amino acid sequence shown in SEQ ID NO: 159 (without the signal sequence), or any one or more of the ectodomains of GCGR, *e.g.* any one or more of the extracellular regions (or fragments thereof) may be used to prepare antibodies that bind GCGR and inhibit its function, *e.g.* its ability to bind glucagon, which would result in lowering of blood glucose levels.

**[0109]** The full-length amino acid sequence of human GCGR is shown as SEQ ID NO: 153. The signal peptide spans amino acid residues 1-26 of SEQ ID NO: 153; the N-terminal domain spans residues 27-144 of SEQ ID NO: 153; extracellular region 1 (EC1) spans amino acid residues 194-226 of SEQ ID NO: 153; extracellular region 2 (EC2) spans amino acid residues 285-305 of SEQ ID NO: 153; and extracellular region 3 (EC3) spans amino acid residues 369-384 of SEQ ID NO:153.

**[0110]** In certain embodiments, antibodies that bind specifically to GCGR may be prepared using fragments of the above-noted extracellular regions, or peptides that extend beyond the designated regions by about 5 to about 20 amino acid residues from either, or both, the N or C terminal ends of the regions described herein. In certain embodiments, any combination of the above-noted regions or fragments thereof may be used in the preparation of GCGR specific antibodies. In certain embodiments, any one or more of the above-noted regions of GCGR, or fragments thereof may be used for preparing monospecific, bispecific, or multispecific antibodies.

**Antigen-Binding Fragments of Antibodies**

**[0111]** Unless specifically indicated otherwise, the term "antibody," as used herein, shall be understood to encompass antibody molecules comprising two immunoglobulin heavy chains and two immunoglobulin light chains (*i.e.*, "full antibody molecules") as well as antigen-binding

fragments thereof. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding portion" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to hGCGR. An antibody fragment may include a Fab fragment, a $F(ab')_2$ fragment, a Fv fragment, a dAb fragment, a fragment containing a CDR, or an isolated CDR. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and (optionally) constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

**[0112]** Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

**[0113]** An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR, which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a $V_H$ domain associated with a $V_L$ domain, the $V_H$ and $V_L$ domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain $V_H$ - $V_H$, $V_H$ - $V_L$ or $V_L$ - $V_L$ dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric $V_H$ or $V_L$ domain.

**[0114]** In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an

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

**[0115]** As with full antibody molecules, antigen-binding fragments may be mono-specific or multi-specific (*e.g.*, bi-specific). A multi-specific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multi-specific antibody format, including the exemplary bi-specific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

**Preparation of Human Antibodies**

**[0116]** Methods for generating human antibodies in transgenic mice are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to human GCGR.

**[0117]** Using VELOCIMMUNE™ technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to GCGR are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

**[0118]** Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines,

and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

**[0119]** Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

**[0120]** In general, the antibodies of the instant invention possess very high affinities, typically possessing $K_D$ of from about $10^{-12}$ through about $10^{-9}$ M, when measured by binding to antigen either immobilized on solid phase or in solution phase. The mouse constant regions are replaced with desired human constant regions to generate the fully human antibodies of the invention. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

**Bioequivalents**

**[0121]** The anti-GCGR antibodies and antibody fragments of the present invention encompass proteins having amino acid sequences that vary from those of the described antibodies, but that retain the ability to bind human GCGR. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the anti-GCGR antibody-encoding DNA sequences of the present invention encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an anti-GCGR antibody or antibody fragment that is essentially bioequivalent to an anti-GCGR antibody or antibody fragment of the invention.

**[0122]** Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single does or multiple dose. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered

bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

**[0123]** In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

**[0124]** In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

**[0125]** In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

**[0126]** Bioequivalence may be demonstrated by *in vivo* and/or *in vitro* methods. Bioequivalence measures include, *e.g.*, (a) an *in vivo* test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an *in vitro* test that has been correlated with and is reasonably predictive of human *in vivo* bioavailability data; (c) an *in vivo* test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

**[0127]** Bioequivalent variants of anti-GCGR antibodies of the invention may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antibodies may include anti-GCGR antibody variants comprising amino acid changes, which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations which eliminate or remove glycosylation.

**Biological Characteristics of the Antibodies**

**[0128]** In general, the antibodies of the present invention may function by binding to at least one of the extracellular regions of hGCGR. In certain embodiments, the antibodies of the present invention may bind to an epitope located in at least the N-terminal region, or to an epitope located in at least one of the extracellular (EC) loops of hGCGR.

**[0129]** In certain embodiments, the antibodies of the present invention may function by blocking or inhibiting GCGR activity by binding to the extracellular N-terminal region, the amino acid

sequence of which is shown in SEQ ID NO: 159, and which is encoded by the nucleic acid sequence shown in SEQ ID NO: 158.

**[0130]** In certain embodiments, the antibodies of the present invention may function by blocking or inhibiting GCGR activity by binding to at least one of the EC loops or loop segments within the whole receptor. In one embodiment, the antibodies of the invention may bind to an epitope located in EC1, which is located between about amino acid residue194 to about amino acid residue 226 of SEQ ID NO: 153. Alternatively, or additionally, the antibodies of the invention may bind to an epitope found in EC2, which is located between about amino acid residue 285 to about amino acid residue 302 of SEQ ID NO: 153. Alternatively, or additionally, the antibodies of the invention may bind to an epitope found in EC3, which is located between about amino acid residue 369 to about amino acid residue 384 of SEQ ID NO: 153.

**[0131]** In certain embodiments, the antibodies of the present invention may be bi-specific antibodies. The bi-specific antibodies of the invention may bind one epitope in EC1 and may also bind one epitope in a region of hGCGR other than EC1. In certain embodiments, the bi-specific antibodies of the invention may bind one epitope in EC1 and may also bind one epitope in EC2 or EC3, or in the N-terminal region, or in any other region within EC1, EC2, or EC3 of hGCGR, or any combination thereof. In certain embodiments, the bi-specific antibodies of the invention may bind to two different sites within the same extracellular region.

**[0132]** More specifically, the anti-GCGR antibodies of the invention may exhibit one or more *of* the following characteristics: (1) ability to bind to a human GCGR or a fragment thereof and to a non-human (*e.g.*, mouse, monkey, rat, rabbit, dog, pig, *etc.)* GCGR or fragment thereof; (2) ability to bind to a human GCGR or fragment thereof, but not to a non-human (*e.g.*, mouse, monkey, rat, rabbit, dog, pig, etc.*)* GCGR or fragment thereof; (3) ability to bind to a human GCGR or fragment thereof and to a non-human primate (*e.g.* monkey) GCGR or fragment thereof, but not to a mouse, rat, rabbit, dog or pig GCGR or GCGR fragment; (4) ability to bind to a human GCGR or fragment thereof and to a non-human primate (*e.g.* monkey) GCGR or a fragment thereof, and to a mouse GCGR or a fragment thereof, but not to a rat GCGR; (5) ability to bind to a human GCGR or fragment thereof and to a non-human primate (*e.g.* monkey) GCGR or a fragment thereof, and to a rat GCGR or a fragment thereof, but not to a mouse GCGR; 6) blocks glucagon binding to GCGR; 7) blocks glucagon induced cAMP production; 8) demonstrates the ability to lower blood glucose levels in humans suffering from diabetes and in animal models of diabetes; 9) may or may not lower triglyceride levels to the levels observed in normal mammals; or 10) does not adversely affect plasma lipid levels.

**[0133]** Certain anti-GCGR antibodies of the present invention are able to inhibit or attenuate GCGR activity in an *in vitro* or *in vivo* assay. The ability of the antibodies of the invention to bind to and inhibit binding of glucagon to GCGR may be measured using any standard method known to those skilled in the art, including binding assays, reporter bioassays, such as a luciferase reporter assay.

**[0134]** Non-limiting, exemplary *in vitro* assays for measuring GCGR activity are illustrated in Examples 4 and 5, herein. In Example 4, the binding affinities and kinetic constants of human anti-hGCGR antibodies were determined by surface plasmon resonance and the measurements were conducted on a T100 Biacore instrument. In Example 5, a bioassay was developed in HEK293 cell lines expressing full length human, monkey and mouse GCGR along with a luciferase reporter in order to detect activation through Gαs, and subsequent elevation of cAMP levels and transcriptional activation. Examples 6, 7, 8, 9 and 10 demonstrate the *in vivo* effects of the antibodies on lowering of blood glucose levels, blood ketone levels, and on weight loss, in various animal models.

**[0135]** The present invention also includes anti-GCGR antibodies and antigen binding fragments thereof which bind to at least one biologically active fragment of any of the following proteins, or peptides: SEQ ID NO: 153 (full length hGCGR), residue numbers 27-144 of SEQ ID NO: 153 (N-terminal domain of hGCGR); residues 194-226 of SEQ ID NO: 153; residues 285-305 of SEQ ID NO: 153; residues 369-384 of SEQ ID NO: 153. Any of the GCGR peptides described herein, or fragments thereof, may be used to generate anti-GCGR antibodies.

**[0136]** The peptides may be modified to include addition or substitution of certain residues for tagging or for purposes of conjugation to carrier molecules, such as, KLH. For example, a cysteine may be added at either the N terminal or C terminal end of a peptide, or a linker sequence may be added to prepare the peptide for conjugation to, for example, KLH for immunization. The antibodies specific for GCGR may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface.

**[0137]** In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that specifically binds hGCGR and neutralizes hGCGR activity, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130, and 146; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138, and 148; (iii) comprises any one or more of the heavy chain CDR1 sequences selected from the group consisting of 4, 20, 36, 52, 72, 92, 112 and 132; any one or more of the heavy chain CDR2 sequences selected from the group consisting of 6, 22, 38, 54, 74, 94, 114 and 134; any one or more of the heavy chain CDR3 sequences selected from the group consisting of 8, 24, 40, 56, 76, 96, 116 and 136; any one or more of the light chain CDR1 sequences selected from the group consisting of 12, 28, 44, 60, 80, 100, 120 and 140; any one or more of the light chain CDR2 sequences selected

from the group consisting of 14, 30, 46, 62, 82, 102, 122 and 142; any one or more of the light chain CDR3 sequences selected from the group consisting of 16, 32, 48, 64, 84, 104, 124 and 144; and combinations thereof; (iv) demonstrates binding specificity for any one or more of the following: the N-terminal region of GCGR comprising amino acid residues 27-144 of SEQ ID NO: 153, or for any one or more of the extracellular loops of GCGR, including, for example, EC1, EC2, or EC3, wherein EC1 comprises amino acid residues ranging from about residue 194 to about residue 226 of SEQ ID NO: 153, and wherein EC2 comprises amino acid residues ranging from about residue 285 to about residue 305 of SEQ ID NO: 153; and wherein EC3 comprises amino acid residues ranging from about residue 369 to about residue 384 of SEQ ID NO: 153; (v) binds any one or more of human, monkey, mouse or rat GCGR; (vi) blocks binding of glucagon to GCGR; vi) blocks glucagon induced cAMP production; vii) demonstrates the ability to lower blood glucose levels or blood ketone levels in humans suffering from diabetes or in animal models of diabetes; viii) may or may not lower triglyceride levels to the levels observed in normal mammals; or ix) does not adversely affect plasma lipid levels.

**Epitope Mapping and Related Technologies**

**[0138]** To screen for antibodies that bind to a particular epitope, a routine cross-blocking assay such as that described Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY) can be performed. Other methods include alanine scanning mutants, peptide blots (Reineke (2004) Methods Mol Biol 248:443-63), or peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Protein Science 9: 487-496).

**[0139]** The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

**[0140]** Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (See US 2004/0101920). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the anti-GCGR antibodies of the invention into groups of antibodies

binding different epitopes.

**[0141]** In certain embodiments, the anti-GCGR antibody or antigen-binding fragment of an antibody binds an epitope within at least one of the extracellular regions of GCGR, or to a fragment thereof, wherein the extracellular region is the N-terminal domain, or one of the EC loops, including EC1, EC2, or EC3, as described previously.

**[0142]** In one embodiment, the antibody binds an epitope within the N-terminal region of GCGR, or a fragment thereof, comprising an amino acid sequence ranging from about amino acid residue 27-144 of SEQ ID NO: 153. In one embodiment, the antibody binds an epitope within EC1, or a fragment thereof, comprising an amino acid sequence ranging from about amino acid residue 194-226 of SEQ ID NO: 153. In one embodiment, the antibody binds an epitope within EC2, or a fragment thereof, comprising an amino acid sequence ranging from amino acid residue 285-305 of SEQ ID NO: 153. In one embodiment, the antibody binds an epitope within EC3, or a fragment thereof, comprising an amino acid sequence ranging from about amino acid residue 369-384 of SEQ ID NO: 153.

**[0143]** In certain embodiments, the antibody or antibody fragment binds an epitope which includes more than one of the enumerated epitopes of GCGR within the N-terminal domain, or within EC1, EC2, or EC3, and/or within two or three different extracellular regions (for example, epitopes within the N-terminal region, EC1, EC2 and EC3 loops, or within EC1, EC2, and EC3, or within the N-terminal region, EC2 and EC3 loops, or within the N-terminal region, EC1 and EC3 loops.

**[0144]** In certain embodiments, the antibody is a bi-specific antibody that binds one epitope within one extracellular region of GCGR and another epitope within a different extracellular region of GCGR, including the N-terminal domain, or EC1, EC2, or EC3.

**[0145]** In one embodiment, the antibody is a bi-specific antibody that binds one epitope in the N-terminal region of hGCGR and another epitope in EC1 of hGCGR. In one embodiment, the antibody is a bi-specific antibody that binds one epitope in the N-terminal region of hGCGR and another epitope in EC1 of hGCGR. In one embodiment, the antibody is a bi-specific antibody that binds one epitope in the N-terminal region of hGCGR and another epitope in EC2 of hGCGR. In one embodiment, the antibody is a bi-specific antibody that binds one epitope in the N-terminal region of hGCGR and another epitope in EC3 of hGCGR. In one embodiment, the antibody is a bi-specific antibody that binds one epitope in EC1 of hGCGR and another epitope in EC2 of hGCGR. In one embodiment, the antibody is a bi-specific antibody that binds one epitope in EC1 of hGCGR and another epitope in EC3 of hGCGR. In one embodiment, the antibody is a bi-specific antibody that binds one epitope in EC2 of hGCGR and another epitope in EC3 of hGCGR.

**[0146]** In one embodiment, the antibody is a bi-specific antibody that binds one epitope in the N terminal domain of hGCGR, wherein the one epitope ranges from about residue 27 to about residue 144 of SEQ ID NO: 153 and a second epitope in EC1 of hGCGR, wherein the second

epitope ranges from about residue number 194 to about residue number 226 of SEQ ID NO: 153. In one embodiment, the antibody is a bi-specific antibody that binds one epitope in the N terminal domain of hGCGR within the residues noted above, and a second epitope in EC2 of hGCGR, wherein the second epitope ranges from about residue number 285 to about residue number 305 of SEQ ID NO:153. In one embodiment, the antibody is a bi-specific antibody that binds one epitope in the N terminal domain of hGCGR within the residues noted above, and a second epitope in EC3 of hGCGR, wherein the second epitope ranges from about residue number 369 to about residue number 384 of SEQ ID NO: 153.

**[0147]** In one embodiment, the antibody is a bi-specific antibody that binds one epitope in EC1 of hGCGR from about residue 194 to about residue 226 of SEQ ID NO: 153 and a second epitope in EC2 of GCGR from about residue 285 to about residue 305 of SEQ ID NO: 153. In one embodiment, the antibody is a bi-specific antibody that binds one epitope in EC1 from about residue 194 to about residue 226 of SEQ ID NO: 153 and a second epitope in EC3 of GCGR from about residue 369 to about residue 384 of SEQ ID NO:153. In one embodiment, the antibody is a bi-specific antibody that binds one epitope in EC2 from about residue 285 to about residue 305 of SEQ ID NO:153 and a second epitope in EC3 of GCGR from about residue 369 to about residue 384 of SEQ ID NO:153.

**[0148]** The present invention includes anti-GCGR antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.*, H4H1345N, H4H1617N, H4H1765N, H4H1321B and H4H1321P, H4H1327B and H4H1327P, H4H1328B and H4H1328P, H4H1331B and H4H1331P, H4H1339B and H4H1339P). Likewise, the present invention also includes anti-GCGR antibodies that compete for binding to GCGR or a GCGR fragment with any of the specific exemplary antibodies described herein.

**[0149]** One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-GCGR antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-GCGR antibody of the invention, the reference antibody is allowed to bind to a GCGR protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the GCGR molecule is assessed. If the test antibody is able to bind to GCGR following saturation binding with the reference anti-GCGR antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-GCGR antibody. On the other hand, if the test antibody is not able to bind to the GCGR molecule following saturation binding with the reference anti-GCGR antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti- GCGR antibody of the invention.

**[0150]** To determine if an antibody competes for binding with a reference anti-GCGR antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a GCGR molecule under saturating conditions followed by assessment of binding of the test antibody to the GCGR molecule. In a second

orientation, the test antibody is allowed to bind to a GCGR molecule under saturating conditions followed by assessment of binding of the reference antibody to the GCGR molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the GCGR molecule, then it is concluded that the test antibody and the reference antibody compete for binding to GCGR. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

**[0151]** Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.*, Junghans *et al.*, Cancer Res. 1990 50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

**[0152]** Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

**Species Selectivity and Species Cross-Reactivity**

**[0153]** According to certain embodiments of the invention, the anti-GCGR antibodies bind to human GCGR but not to GCGR from other species. Alternatively, the anti-GCGR antibodies of the invention, in certain embodiments, bind to human GCGR and to GCGR from one or more non-human species. For example, the anti-GCGR antibodies of the invention may bind to human GCGR and may bind or not bind, as the case may be, to one or more of mouse, rat, guinea pig, hamster, gerbil, pig, cat, dog, rabbit, goat, sheep, cow, horse, camel, cynomolgus, marmoset, rhesus or chimpanzee GCGR.

**Immunoconjugates**

**[0154]** The invention encompasses a human anti-GCGR monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as an agent that is capable of reducing blood glucose levels, or a radioisotope, or a chemotherapeutic agent. The type of therapeutic moiety that may be conjugated to the anti-GCGR antibody will take into account the condition to be

treated and the desired therapeutic effect to be achieved. For example, for treating diabetes, or any other condition whereby it is desirable to lower blood glucose, and/or to maintain normal blood glucose levels, an agent such as biguanide (*e.g.* metformin), a sulfonylurea (*e.g.* glyburide, glipizide), a PPAR gamma agonist (*e.g.* pioglitazone, rosiglitazone); an alpha glucosidase inhibitor (*e.g.* acarbose, voglibose), an inhibitor of advanced glycation endproduct formation (*e.g.* aminoguanidine), or a second GCGR inhibitor may be conjugated to the GCGR antibody. Alternatively, if the desired therapeutic effect is to treat the sequelae or symptoms associated with diabetes, or any other condition resulting from high, or uncontrolled blood glucose levels, it may be advantageous to conjugate an agent appropriate to treat the sequelae or symptoms of the condition Examples of suitable agents for forming immunoconjugates are known in the art, see for example, WO 05/103081.

**Multi-specific Antibodies**

**[0155]** The antibodies of the present invention may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, *e.g.*, Tutt *et al.*, 1991, J. Immunol. 147:60-69; Kufer *et al.*, 2004, Trends Biotechnol. 22:238-244. The anti-GCGR antibodies of the present invention can be linked to or co-expressed with another functional molecule, *e.g.*, another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bi-specific or a multi-specific antibody with a second binding specificity. For example, the present invention includes bi-specific antibodies wherein one arm of an immunoglobulin is specific for human GCGR or a fragment thereof, and the other arm of the immunoglobulin is specific for a second therapeutic target or is conjugated to a therapeutic moiety. In certain embodiments of the invention, one arm of an immunoglobulin is specific for an epitope on the N-terminal domain of hGCGR or a fragment thereof, and the other arm of the immunoglobulin is specific for an epitope on one of the EC loops of hGCGR, or a fragment thereof. In certain embodiments, one arm of an immunoglobulin is specific for one EC loop, or a fragment thereof, and the second arm is specific for a second EC loop, or a fragment thereof. In certain embodiments, one arm of an immunoglobulin is specific for one epitope on one EC loop of hGCGR and the other arm is specific for a second epitope on the same EC loop of hGCGR.

**[0156]** An exemplary bi-specific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) $C_H3$ domain and a second Ig $C_H3$ domain, wherein the first and second Ig $C_H3$ domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bi-specific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference.

In one embodiment, the first Ig $C_H3$ domain binds Protein A and the second Ig $C_H3$ domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second $C_H3$ may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second $C_H3$ include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bi-specific antibody format described above are contemplated within the scope of the present invention.

**Therapeutic Administration and Formulations**

**[0157]** The invention provides therapeutic compositions comprising the anti-GCGR antibodies or antigen-binding fragments thereof of the present invention. The administration of therapeutic compositions in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell *et al.* "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

**[0158]** The dose of antibody may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When the antibody of the present invention is used for lowering blood glucose levels associated with GCGR activity in various conditions and diseases, such as diabetes, in an adult patient, it is advantageous to intravenously administer the antibody of the present invention normally at a single dose of about 0.01 to about 30 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. In certain embodiments, the antibody or antigen-binding fragment thereof of the invention can be administered as an initial dose of at least about 0.1 mg to about 800 mg, about 1 to about 500 mg, about 5 to about 300 mg, or about 10 to about 200 mg, to about 100 mg, or to about 50 mg. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the antibody or antigen-binding fragment thereof in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent

doses are separated by at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks.

**[0159]** Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu *et al.* (1987) J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

**[0160]** The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see, for example, Langer (1990) Science 249:1527-1533).

**[0161]** In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose.

**[0162]** The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (*e.g.*, propylene glycol, polyethylene glycol), a nonionic surfactant [*e.g.*, polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

**[0163]** A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the

cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

**[0164]** Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but certainly are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Burghdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but certainly are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK ™ Autoinjector (Amgen, Thousands Oaks, CA), the PENLET ™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.) and the HUMIRA ™ Pen (Abbott Labs, Abbott Park, IL), to name only a few.

**[0165]** Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

**Therapeutic Uses of the Antibodies**

**[0166]** Due to their interaction with the glucagon receptor, the present antibodies are useful for lowering blood glucose levels and also for the treatment of a wide range of conditions and disorders in which blocking the interaction of glucagon with its receptor is beneficial. These disorders and conditions may be selected from any glucagon related metabolic disorder, which involves glucagon receptor signaling that results in the pathophysiology of the disorder, or in the homeostatic response to the disorder. Thus, the antibodies may find use for example to prevent, treat, or alleviate, diseases or conditions or associated symptoms or sequelae, of the endocrine system, the central nervous system, the peripheral nervous system, the cardiovascular system, the pulmonary system, and the gastrointestinal system, while reducing

and or eliminating one or more of the unwanted side effects associated with the current treatments. Glucagon related metabolic disorders include, but are not limited to, type 1 and type 2 diabetes, diabetic ketoacidosis, hyperglycemia, hyperglycemic hyperosmolar syndrome, perioperative hyperglycemia, hyperglycemia in the intensive care unit patient, hyperinsulinemia, postprandial hyperglycemia, impaired fasting glucose (IFG), metabolic syndrome, hyper-/hypokalemia, poor LDL/HDL ratio, eating disorders, weight gain, obesity as a consequence of diabetes, pediatric diabetes, gestational diabetes, diabetic late complications, micro-/macroalbuminuria, nephropathy, retinopathy, neuropathy, diabetic foot ulcers, wound healing, impaired glucose tolerance (IGT), insulin resistance syndromes, syndrome X, glucagonomas, gastrointestinal disorders, obesity, diabetes as a consequence of obesity, etc. The present invention further provides; a method of treating conditions resulting from excessive glucagon in a mammal; a method of inhibiting the glucagon receptor in a mammal; a method of inhibiting a glucagon receptor mediated cellular response in a mammal, or a method of reducing the glycemic level in a mammal comprising administering to a mammal in need of such treatment a glucagon receptor-inhibiting amount of an anti-GCGR antibody or a biologically active fragment thereof.

**[0167]** The present antibodies are effective in lowering blood glucose, both in the fasting and the postprandial stage. In certain embodiments of the invention, the present antibodies are used for the preparation of a pharmaceutical composition for the treatment of type 2 diabetes. In yet a further embodiment of the invention the present antibodies are used for the preparation of a pharmaceutical composition for the delaying or prevention of the progression from impaired glucose tolerance to type 2 diabetes. In yet another embodiment of the invention the present antibodies are used for the preparation of a pharmaceutical composition for the delaying or prevention of the progression from non-insulin requiring diabetes to insulin requiring diabetes. In a further embodiment of the invention the present antibodies are used for the preparation of a pharmaceutical composition for the treatment of type 1 diabetes. Such treatment is normally accompanied by insulin therapy.

**Combination Therapies**

**[0168]** Combination therapies may include an anti-hGCGR antibody of the invention and any additional therapeutic agent that may be advantageously combined with an antibody of the invention, or with a biologically active fragment of an antibody of the invention.

**[0169]** For example, a second therapeutic agent may be employed to aid in further lowering of glucose levels, or to reduce at least one symptom in a patient suffering from a disease or condition characterized by high blood glucose levels, such as diabetes mellitus. Such a second agent may be selected from, for example, a glucagon antagonist or another GCGR antagonist (*e.g.* an anti-glucagon or anti-GCGR antibody or small molecule inhibitor of glucagon or GCGR), or may include other therapeutic moieties useful for treating diabetes, or other diseases or

conditions associated with, or resulting from elevated blood glucose levels, or impaired glucose metabolism, or agents useful for treating any long term complications associated with elevated and/or uncontrolled blood glucose levels. These agents include biguanides, which decrease glucose production in the liver and increase sensitivity to insulin (*e.g.* metformin), or sulfonylureas, which stimulate insulin production (*e.g.* glyburide, glipizide). Additional treatments directed at maintaining glucose homeostasis including PPAR gamma agonists, which act as insulin sensitizers (*e.g.* pioglitazone, rosiglitazone); and alpha glucosidase inhibitors, which slow starch absorption and glucose production (*e.g.* acarbose, voglibose). Additional treatments include injectable treatments such as Exenatide® (glucagon-like peptide 1), and Symlin® (pramlintide).

**[0170]** In certain other embodiments, the composition may include a second agent selected from the group consisting of non-sulfonylurea secretagogues, insulin, insulin analogs, exendin-4 polypeptides, beta 3 adrenoceptor agonists, PPAR agonists, dipeptidyl peptidase IV inhibitors, statins and statin-containing combinations, cholesterol absorption inhibitors, LDL-cholesterol antagonists, cholesteryl ester transfer protein antagonists, endothelin receptor antagonists, growth hormone antagonists, insulin sensitizers, amylin mimetics or agonists, cannabinoid receptor antagonists, glucagon-like peptide-1 agonists, melanocortins, melanin-concentrating hormone receptor agonists, SNRIs, and protein tyrosine phosphatase inhibitors.

**[0171]** In certain other embodiments, combination therapy may include administration of a second agent to counteract any potential side effect(s) resulting from administration of an antibody of the invention, if such side effect(s) occur. For example, in the event that any of the anti-GCGR antibodies increases lipid or cholesterol levels, it may be beneficial to administer a second agent to lower lipid or cholesterol levels, using an agent such as a HMG-CoA reductase inhibitor (for example, a statin such as atorvastatin, (LIPITOR®), fluvastatin (LESCOL®), lovastatin (MEVACOR®), pitavastatin (LIVALO®), pravastatin (PRAVACHOL®), rosuvastatin (CRESTOR®) and simvastatin (ZOCOR®). Alternatively, the antibodies of the invention may be combined with an agent such as VYTORIN®, which is a preparation of a statin and another agent—such as ezetimibe/simvastatin.

**[0172]** In certain embodiments, it may be beneficial to administer the antibodies of the invention in combination with any one or more of the following: (1) niacin, which increases lipoprotein catabolism; (2) fibrates or amphipathic carboxylic acids, which reduce low-density lipoprotein (LDL) level, improve high-density lipoprotein (HDL) and TG levels, and reduce the number of non-fatal heart attacks; and (3) activators of the LXR transcription factor that plays a role in cholesterol elimination such as 22-hydroxycholesterol, or a statin with a bile resin (*e.g.*, cholestyramine, colestipol, colesevelam), a fixed combination of niacin plus a statin (*e.g.*, niacin with lovastatin); or with other lipid lowering agents such as omega-3-fatty acid ethyl esters (for example, omacor).

**[0173]** Furthermore, the second therapeutic agent can be one or more other inhibitors of

glucagon or GCGR, as well as inhibitors of other molecules, such as angiopoietin-like protein 3 (ANGPTL3), angiopoietin-like protein 4 (ANGPTL4), angiopoietin-like protein 5 (ANGPTL5), angiopoietin-like protein 6 (ANGPTL6), which are involved in lipid metabolism, in particular, cholesterol and/or triglyceride homeostasis. Inhibitors of these molecules include small molecules and antibodies that specifically bind to these molecules and block their activity.

**[0174]** In certain embodiments, it may be beneficial to administer the antibodies of the invention in combination with an antibody that acts to lower lipid or cholesterol levels, such as, but not limited to, for example, any anti-PCSK9 (proprotein convertase subtilisin/kexin type 9) antibody, such as those described in US2010/0166768. Other anti-PCSK9 antibodies are described in US2010/0040611, US2010/0041102, US2010/0040610, US2010/0113575, US2009/0232795, US2009/0246192, US2010/0233177, US2009/0142352, US2009/0326202, US2010/0068199, US2011/0033465, US2011/0027287, US2010/0150937, US2010/0136028 and WO2009/055783.

**[0175]** In certain embodiments, it may be beneficial to administer the anti-GCGR antibodies of the invention in combination with a nucleic acid that inhibits the activity of PCSK9 (proprotein convertase subtilisin/kexin type 9), such as an antisense molecule, a double stranded RNA, or a siRNA molecule. Exemplary nucleic acid molecules that inhibit the activity of PCSK9 are described in US2011/0065644, US2011/0039914, US2008/0015162 and US2007/0173473.

**[0176]** The additional therapeutically active component(s) may be administered prior to, concurrent with, or after the administration of the anti-GCGR antibody of the present invention. For purposes of the present disclosure, such administration regimens are considered the administration of an anti-GCGR antibody "in combination with" a second therapeutically active component.

**Diagnostic Uses of the Antibodies**

**[0177]** The anti-GCGR antibodies of the present invention may also be used to detect and/or measure GCGR in a sample, *e.g.*, for diagnostic purposes. For example, an anti-GCGR antibody, or fragment thereof, may be used to diagnose a condition or disease characterized by aberrant expression (*e.g.*, over-expression, under-expression, lack of expression, etc.) of GCGR. Exemplary diagnostic assays for GCGR may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-GCGR antibody of the invention, wherein the anti-GCGR antibody is labeled with a detectable label or reporter molecule or used as a capture ligand to selectively isolate GCGR protein from patient samples. Alternatively, an unlabeled anti-GCGR antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as $^{3}H$, $^{14}C$, $^{32}P$, $^{35}S$, or $^{125}I$; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, β-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or

measure GCGR in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

**[0178]** Samples that can be used in GCGR diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient, which contains detectable quantities of GCGR protein, or fragments thereof, under normal or pathological conditions. Generally, levels of GCGR in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with a disease or condition associated with abnormal GCGR levels or activity) will be measured to initially establish a baseline, or standard, level of GCGR. This baseline level of GCGR can then be compared against the levels of GCGR measured in samples obtained from individuals suspected of having a GCGR related disease or condition, or symptoms associated with such disease or condition.

## EXAMPLES

**[0179]** The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

### Example 1. Generation of Human Antibodies to Human GCGR

**[0180]** An immunogen comprising any one of the following can be used to generate antibodies to hGCGR. For example, cells expressing hGCGR were used in certain embodiments as an immunogen to generate antibodies to hGCGR. Additionally, DNA encoding hGCGR was used in certain embodiments as an immunogen to prepare the antibodies of the invention. Furthermore, in certain embodiments, peptides comprising amino acid sequences from the N-terminal domain of hGCGR were utilized as an immunogen to generate antibodies to human GCGR. In addition, in certain embodiments, peptides comprising amino acid sequences from any of the extracellular loop regions EC1, EC2, or EC3, of hGCGR may be utilized as an immunogen to generate antibodies to human GCGR. The cells, DNA, or peptides that were used as immunogens, as noted above, were administered directly, with an adjuvant to stimulate the immune response, to a VELOCIMMUNE® mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions. The antibody immune response was monitored by a GCGR-specific immunoassay. When a desired immune response was achieved splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce GCGR-specific antibodies. Using this technique, and the various

immunogens described above, several anti-GCGR chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains) were obtained; certain exemplary antibodies generated in this manner were designated as H4H1345N, H4H1617N and H4H1765N.

**[0181]** Anti-GCGR antibodies were also isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in U.S. 2007/0280945A1. Using this method, several fully human anti-GCGR antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H4H1321B, H4H1321P, H4H1327B, H4H1327P, H4H1328B, H4H1328P, H4H1331B, H4H1331P, H4H1339B and H4H1339P.

**[0182]** The biological properties of the exemplary anti-GCGR antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

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

**[0183]** Table 1 sets forth the heavy and light chain variable region amino acid sequence pairs of selected anti-GCGR antibodies and their corresponding antibody identifiers. Antibodies having the same numerical antibody designation, but differing by a letter suffix of N, B or P refer to antibodies having heavy and light chains with identical CDR sequences but with sequence variations in regions that fall outside of the CDR sequences (*i.e.*, in the framework regions). Thus, N, B and P variants of a particular antibody have identical CDR sequences within their heavy and light chain variable regions but differ from one another within their framework regions.

**Table 1**

| | SEQ ID NOs: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Antibody Designation** | **HCVR** | **HCDR1** | **HCDR2** | **HCDR3** | **LCVR** | **LCDR1** | **LCDR2** | **LCDR3** |
| H4H1345N | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 |
| H4H1617N | 18 | 20 | 22 | 24 | 26 | 28 | 30 | 32 |
| H4H1765N | 34 | 36 | 38 | 40 | 42 | 44 | 46 | 48 |
| H4H1321B | 50 | 52 | 54 | 56 | 58 | 60 | 62 | 64 |
| H4H1321P | 66 | 52 | 54 | 56 | 68 | 60 | 62 | 64 |
| H4H1327B | 70 | 72 | 74 | 76 | 78 | 80 | 82 | 84 |
| H4H1327P | 86 | 72 | 74 | 76 | 88 | 80 | 82 | 84 |
| H4H1328B | 90 | 92 | 94 | 96 | 98 | 100 | 102 | 104 |
| H4H1328P | 106 | 92 | 94 | 96 | 108 | 100 | 102 | 104 |
| H4H1331B | 110 | 112 | 114 | 116 | 118 | 120 | 122 | 124 |
| H4H1331P | 126 | 112 | 114 | 116 | 128 | 120 | 122 | 124 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| H4H1339B | 130 | 132 | 134 | 136 | 138 | 140 | 142 | 144 |
| H4H1339P | 146 | 132 | 134 | 136 | 148 | 140 | 142 | 144 |

**Example 3. Variable Gene Utilization Analysis**

**[0184]** To analyze the structure of antibodies produced, the nucleic acids encoding antibody variable regions were cloned and sequenced. From the nucleic acid sequence and predicted amino acid sequence of the antibodies, gene usage was identified for each Heavy Chain Variable Region (HCVR) and Light Chain Variable Region (LCVR). Table 2 sets forth the gene usage for selected antibodies in accordance with the invention.

**Table 2**

| Antibody | Antibody Identifier | HCVR | | | LCVR | |
|---|---|---|---|---|---|---|
| | HCVR/LCVR SEQ ID NOs | $V_H$ | $D_H$ | $J_H$ | $V_K$ | $J_K$ |
| H4H1617N | 18/26 | V1-24 | D3-9 | J6 | V2-28 | J1 |
| H4H1345N | 2/10 | V1-24 | D3-9 | J6 | V2-28 | J1 |
| H4H1765N | 34/42 | V3-48 | D6-6 | J6 | V2-28 | J1 |
| H4H1321P | 66/68 | V3-30 | D3-9 | J6 | V1-16 | J4 |
| H4H1327P | 86/88 | V3-7 | D3-9 | J6 | V1-17 | J3 |
| H4H1328P | 106/108 | V3-13 | D3-9 | J6 | V1-17 | J4 |
| H4H1331P | 126/128 | V3-33 | D3-9 | J6 | V1-17 | J1/J4 |
| H4H1339P | 146/148 | V3-13 | D3-9 | J6 | V1-6 | J1 |

**Example 4. Antibody Binding to Soluble GCGR as Determined by Surface Plasmon Resonance**

**[0185]** Binding affinities and kinetic constants of human monoclonal anti-hGCGR antibodies binding to human and monkey soluble recombinant hGCGR ectodomain (hGCGR and MfGCGR, respectively) were determined by surface plasmon resonance at both 25°C and 37°C. Measurements were conducted on a T100 Biacore instrument. Antibodies were captured onto the Biacore sensor chip surface via a covalently-linked anti-human kappa antibody capture surface, and the soluble GCGR proteins were applied to the surface either in a monovalent (hGCGR expressed with a myc-myc-hexa-histidine C-terminal tag) or bivalent (hGCGR and MfGCGR expressed with an N-terminal Fc fusion) format. The amino acid sequence identifiers of the reagents used in this example are shown in Table 3.

Table 3

| Description | Construct | SEQ ID NO: |
|---|---|---|
| anti-GCGR positive control hIgG4(S108P) | 150kDa, dimer | (See Yan, Hai *et al.* WO2008/036341) |
| mfGCGR-N-terminal hFc | 160k | 152 |
| hGCGR-mFc | 80654.42 Da, dimer | 149 |
| hGCGR-mmh | 18,965Da, monomer | 151 |

**[0186]** The soluble GCGR was applied to the flow cell in separate injections at multiple concentrations ranging from 3.1 nM to 50 nM, and kinetic association ($k_a$) and dissociation ($k_d$) rate constants were determined by fitting the data to a 1:1 binding model using Scrubber v2.0a curve fitting software. Binding dissociation equilibrium constants and dissociative half-lives were calculated from the kinetic rate constants as: $K_D = k_d / k_a$; $t_{1/2} = (\ln 2/k_d)$.

**Table 4a: Biacore data for binding at 25°C**

| Antibody Designation | Antigen tested | ka | kd | $K_D$ | T1/2 (min) |
|---|---|---|---|---|---|
| H4H1321P | hGCGR-mmh | 1.06E+06 | 3.74E-03 | 3.54E-09 | 3 |
| | hGCGR-hFc | 1.20E+06 | 2.85E-04 | 2.38E-10 | 41 |
| | mfGCGR-hFc | 1.76E+06 | 4.18E-05 | 2.38E-11 | 276 |
| H4H1327P | hGCGR-mmh | 9.52E+05 | 3.52E-04 | 3.69E-10 | 33 |
| | hGCGR-hFc | 1.21E+06 | 4.10E-05 | 3.38E-11 | 282 |
| | mfGCGR-hFc | 1.60E+06 | 1.44E-05 | 9.02E-12 | 802 |
| H4H1328P | hGCGR-mmh | 1.03E+06 | 2.12E-03 | 2.06E-09 | 5 |
| | hGCGR-hFc | 1.13E+06 | 2.26E-04 | 2.01E-10 | 51 |
| | mfGCGR-hFc | 1.60E+06 | 8.46E-05 | 5.29E-11 | 137 |
| H4H1331P | hGCGR-mmh | 6.57E+05 | 1.11E-04 | 1.70E-10 | 104 |
| | hGCGR-hFc | 7.60E+05 | 1.54E-05 | 2.02E-11 | 751 |
| | mfGCGR-hFc | 1.17E+06 | 8.12E-06 | 6.90E-12 | 1423 |
| H4H1339P | hGCGR-mmh | 6.45E+05 | 5.32E-04 | 8.25E-10 | 22 |
| | hGCGR-hFc | 1.00E+06 | 6.20E-05 | 6.18E-11 | 186 |
| | mfGCGR-hFc | 1.26E+06 | 2.28E-05 | 1.82E-11 | 506 |
| H4H1345N | hGCGR-mmh | 7.98E+05 | 3.44E-04 | 4.31E-10 | 34 |
| | hGCGR-hFc | 7.90E+05 | 5.72E-05 | 7.24E-11 | 202 |
| | mfGCGR-hFc | 9.53E+05 | 2.42E-05 | 2.54E-11 | 477 |
| H4H1617N | hGCGR-mmh | 1.07E+06 | 1.99E-04 | 1.87E-10 | 58 |
| | hGCGR-hFc | 8.18E+05 | 3.18E-05 | 3.89E-11 | 363 |
| | mfGCGR-hFc | 1.26E+06 | 1.38E-05 | 1.10E-11 | 835 |
| H4H1765N | hGCGR-mmh | 3.26E+05 | 3.05E-05 | 9.30E-11 | 379 |
| | hGCGR-hFc | 4.10E+05 | 4.95E-06 | 1.22E-11 | 2331 |
| | mfGCGR-hFc | 6.43E+05 | 1.00E-06 | 1.56E-12 | 11550 |
| Isotype-matched comparator antibody | hGCGR-mmh | 6.67E+05 | 1.68E-04 | 2.52E-10 | 69 |
| | hGCGR-hFc | 8.21E+05 | 2.04E-05 | 2.49E-11 | 565 |
| | mfGCGR-hFc | 1.23E+06 | 6.09E-06 | 4.95E-12 | 1897 |

Table 4b: Biacore data for binding at 37°C

| Antibody Designation | Antigen tested | ka | kd | $K_D$ | T1/2 (min) |
|---|---|---|---|---|---|
| H4H1321P | hGCGR-mmh | 1.58E+06 | 2.02E-02 | 1.28E-08 | 1 |
| | hGCGR-hFc | 1.41E+06 | 1.09E-04 | 7.70E-11 | 106 |
| | mfGCGR-hFc | 2.19E+06 | 7.59E-05 | 3.47E-11 | 152 |
| H4H1327P | hGCGR-mmh | 1.48E+06 | 2.00E-03 | 1.35E-09 | 6 |
| | hGCGR-hFc | 1.48E+06 | 2.32E-04 | 1.57E-10 | 50 |
| | mfGCGR-hFc | 2.22E+06 | 7.94E-05 | 3.57E-11 | 145 |
| H4H1328P | hGCGR-mmh | 1.61E+06 | 1.08E-02 | 6.67E-09 | 1 |
| | hGCGR-hFc | 1.55E+06 | 1.92E-04 | 1.24E-10 | 60 |
| | mfGCGR-hFc | 2.03E+06 | 7.17E-05 | 3.53E-11 | 161 |
| H4H1331P | hGCGR-mmh | 9.73E+05 | 5.19E-04 | 5.33E-10 | 22 |
| | hGCGR-hFc | 1.17E+06 | 9.12E-05 | 7.79E-11 | 127 |
| | mfGCGR-hFc | 1.60E+06 | 4.12E-05 | 2.57E-11 | 281 |
| H4H1339P | hGCGR-mmh | 8.76E+05 | 4.30E-03 | 4.91E-09 | 3 |
| | hGCGR-hFc | 1.17E+06 | 3.71E-04 | 3.18E-10 | 31 |
| | mfGCGR-hFc | 1.69E+06 | 1.07E-04 | 6.31E-11 | 108 |
| H4H1345N | hGCGR-mmh | 9.28E+05 | 1.97E-03 | 2.12E-09 | 6 |
| | hGCGR-hFc | 9.52E+05 | 3.09E-04 | 3.24E-10 | 37 |
| | mfGCGR-hFc | 1.27E+06 | 1.28E-04 | 1.01E-10 | 91 |
| H4H1617N | hGCGR-mmh | 1.20E+06 | 1.13E-03 | 9.43E-10 | 10 |
| | hGCGR-hFc | 1.18E+06 | 2.14E-04 | 1.81E-10 | 54 |
| | mfGCGR-hFc | 1.49E+06 | 8.72E-05 | 5.86E-11 | 133 |
| H4H1765N | hGCGR-mmh | 4.41E+05 | 1.11E-04 | 2.52E-10 | 104 |
| | hGCGR-hFc | 6.64E+05 | 3.57E-05 | 5.37E-11 | 324 |
| | mfGCGR-hFc | 9.04E+05 | 1.48E-05 | 1.64E-11 | 778 |
| Isotype-matched comparator antibody | hGCGR-mmh | 8.73E+05 | 1.46E-03 | 1.68E-09 | 8 |
| | hGCGR-hFc | 1.15E+06 | 1.82E-04 | 1.59E-10 | 63 |
| | mfGCGR-hFc | 1.66E+06 | 6.27E-05 | 3.77E-11 | 184 |

**[0187]** As shown in Tables 4a and 4b, the exemplary antibodies exhibited high affinity binding to both human and monkey GCGR soluble proteins. A significant increase in binding affinity (5-fold to 15-fold) was observed when flowing the bivalent hGCGR in comparison to monovalent hGCGR. The antibodies consistently bound with higher affinity (3-fold to 10-fold) to the monkey variant, MfGCGR, compared to hGCGR.

**Example 5. Bioassay to measure the Effects of Anti-GCGR Antibodies on GCGR Activation**

**[0188]** GCGR is a G-protein coupled receptor and its ligand, glucagon (GCG), stimulates adenylyl cyclase activity through Gαs and phosphoinositol turnover through Gq (Jiang and Zhang, (2003), Am J Physiol Endocrinol Metab 284: E671-E678). A bioassay was developed to

detect activation through Gαs, subsequent elevation of cAMP levels and transcriptional activation. HEK293 cell lines were generated to stably express full-lengths of human GCGR (GenBank accession number NP_000151.1; SEQ ID NO: 153), monkey (*Macaca fascicularis*) GCGR (SEQ ID NO: 155), and mouse GCGR (NP_032127.2; SEQ ID NO: 154.) along with a luciferase reporter assay. The stable cell lines were isolated and maintained in 10% FBS, DMEM, NEAA, Pen/Strep, and 500mg/ml G418. For rat GCGR, the HEK293 cell line expressing the reporter gene [CRE (4X)-luciferase-IRES-GFP] was transiently transfected with full-length rat GCGR (NP_742089.1; SEQ ID NO: 156) using Lipofectamine2000 (Invitrogen).

**[0189]** For the bioassay, 293/GCGR cells were seeded onto 96-well assay plates at 20,000 cells/well in low serum media, 0.1%FBS and OPTIMEM, and incubated at 37°C and 5% $CO_2$ overnight. Next day, GCG was serially diluted at 1:3 and added to cells starting from 100nM to 0.002nM including no GCG control for dose response. For inhibition, antibodies were serially diluted at 1:3 and added to cells starting from 200 to 0.003 (for hGCGR cells) or 100nM to 0.002nM (for monkey, mouse and rat GCGR cells) including no antibody control with constant concentration of 100pM GCG. Luciferase activity was detected after 5.5 hrs of incubation in 37°C and 5% $CO_2$.

**[0190]** EC50 values for stimulation of each reporter cell-line by 100 pM GCG are shown in Table 5a. The results of the IC50 values for antibodies blocking stimulation of cells by 100 pM GCG are shown in Table 5b, including the results for two control antibodies, Control mAb1 (positive control expressed as hIgG4 isotype; for example, see WO2008/036341 for the antibody designated as "A-9" having the HCVR of SEQ ID NO: 275, HCDR1 of SEQ ID NO: 102, HCDR2 of SEQ ID NO: 128, and HCDR3 of SEQ ID NO: 169 and the LCVR of SEQ ID NO: 229, LCDR1 of SEQ ID NO: 14, LCDR2 of SEQ ID NO: 50 and the LCDR3 of SEQ ID NO: 74) and Control mAb2 (an isotype-matched negative control).

**[0191]** Regarding the inhibition of human GCGR by anti-GCGR antibodies, the activation of GCGR by GCG was shown to stimulate luciferase activity with an EC50 of 113 pM and all antibodies except Control mAb2 (isotype matched negative control) blocked the activation of GCG at 100 pM and decreased the luciferase activity.

**[0192]** With respect to the inhibition of monkey GCGR by anti-GCGR antibodies, the activation of GCGR by GCG was shown to stimulate luciferase activity with an EC50 of 36 pM and all antibodies except Control mAb2 (isotype matched negative control) blocked the activation of GCG at 100 pM and decreased the luciferase activity. H4H1765N showed a partial inhibition of GCG at highest concentration of antibody tested, 100 nM.

**[0193]** Regarding the inhibition of mouse GCGR by anti-GCGR antibodies, the activation of GCGR by GCG was shown to stimulate luciferase activity with an EC50 of 83 pM and all antibodies except H4H1345N, H4H1617N, H4H1765N and Control mAb2 (isotype matched negative control) blocked the activation of GCG at 100 pM and decreased the luciferase activity.

**[0194]** With respect to the inhibition of rat GCGR by anti-GCGR antibodies, the activation of GCGR by GCG was shown to stimulate luciferase activity with an EC50 of 252 pM and all antibodies except H4H1765N and Control mAb2 (isotype matched negative control) blocked the activation of GCG at 100 pM and decreased the luciferase activity.

**Table 5a**

| Cell lines | hGCGR | mfGCGR | mGCGR | rat GCGR |
|---|---|---|---|---|
| EC50 (pM) | 113 | 36 | 83 | 252 |
| Constant GCG (pM) | 100 | | | |

**Table 5b**

| Antibody Designation | IC50 (nM) | | | |
|---|---|---|---|---|
| | hGCGR | mfGCGR | mGCGR | rat GCGR |
| H4H1321P | 0.27 | 4.03 | 1.13 | 1.21 |
| H4H1327P | 0.39 | 2.56 | 1.04 | 0.88 |
| H4H1328P | 0.24 | 2.73 | 1.26 | 0.93 |
| H4H1331P | 0.66 | 8.29 | 1.62 | 3.87 |
| H4H1339P | 0.46 | 2.85 | 1.60 | 0.97 |
| H4H1345N | 2.22 | 3.86 | Not Blocked | 8.07 |
| H4H1617N | 1.25 | 4.24 | Not Blocked | 3.66 |
| H4H1765N | 12.78 | 75.16 | Not Blocked | Not Blocked |
| Control mAb1 Positive control | 0.30 | 2.38 | 1.69 | 0.69 |
| Control mAb2 Negative control | Not Blocked | Not Blocked | Not Blocked | Not Blocked |

**[0195]** In summary, eight anti-hGCGR fully-human antibodies were tested and demonstrated blocking of activation of human GCGR by 100 pM GCG in a reporter cell line that exhibited an EC50 of 113 pM when stimulated by GCG alone. In the monkey GCGR reporter cell line, seven out of eight tested antibodies fully inhibited activation by 100 pM GCG. H4H1765N did not fully inhibit monkey GCGR at the highest concentration of antibody tested, 100 nM. Five out of eight of the antibodies fully inhibited the activation by 100 pM GCG in the mouse GCGR reporter cell line, and seven out of eight antibodies inhibited the activation by 100 pM GCG in the rat GCGR-transfected reporter cells.

**Example 6. Effect of Anti-GCGR Antibodies in ob/ob Mice**

**[0196]** Selected anti-hGCGR antibodies, all of which cross-react with mouse GCGR, were tested for their ability to reduce blood glucose levels in ob/ob mice, a mouse model of type 2 diabetes. ob/ob mice were put into ten groups of five or six animals. Each group received subcutaneous injections of each antibody at 1 or 10 mg/kg. The control group was injected with a hIgG isotype control antibody, which does not bind to any known mouse proteins. Two or seven days after antibody dosing at 1 or 10 mg/kg, respectively, a few drops of blood obtained by tail bleeds were collected from mice. Specifically, for the group given the antibody designated H4H1327P at 10mg/kg, tail bleeds were collected more frequently at 2, 4, 7, 9, 11, 14, 16, 18, and 21 days after dosing. Blood glucose levels from the tail bleed samples were determined by ACCU-CHEK® Compact Plus (Roche). The percent reduction in blood glucose from the mean blood glucose levels of the control group was calculated for each animal at each time point. The average percent reduction in blood glucose was calculated for each antibody group. Table 6 summarizes the mean blood glucose levels of the control group. Results, expressed as (mean ± SEM) of percent blood glucose reduction, are shown in Tables 7a and 7b.

**Table 6**

| Time | Blood glucose (mg/dL) |
|---|---|
| Day 0 | 197 ± 14 |
| Day 2 | 185 ± 13 |
| Day 4 | 167 ± 6 |
| Day 7 | 202 ± 20 |
| Day 9 | 205 ± 18 |
| Day 11 | 195 ± 23 |
| Day 14 | 229 ± 13 |
| Day 16 | 206 ± 6 |
| Day 18 | 187 ± 11 |
| Day 21 | 209 ± 16 |

**Table 7a**

| Dosage | Time (days) | Blood glucose reduction (%) | | | |
|---|---|---|---|---|---|
| | | Antibody Designation | | | |
| | | H4H1327P | H4H1328P | H4H1331P | H4H1339P |
| 1 mg/kg | 2 | 49 ± 1 | 45 ± 2 | 46 ± 2 | 46 ± 2 |
| 10 mg/kg | 7 | 53 ± 2 | 50 ± 2 | 55 ± 2 | 52 ± 2 |

**Table 7b**

| Antibody Designation | Blood glucose reduction (%) |
|---|---|
| | Time (days) |

| | 2 | 4 | 7 | 9 | 11 | 14 | 16 | 18 | 21 |
|---|---|---|---|---|---|---|---|---|---|
| H4H1327P | 58±2 | 52±2 | 53±2 | 56±2 | 47±3 | 51±3 | 45±4 | 34±5 | -5±17 |

**[0197]** Mice treated with the anti-hGCGR antibodies tested (shown in Tables 7a and 7b) exhibited significant reductions in blood glucose levels compared to mice injected with control antibody.

**Example 7. Effect of Anti-GCGR Antibodies in Transgenic Mice Expressing the Human GCGR Protein**

**[0198]** The effects of anti-hGCGR antibodies on blood glucose and plasma lipid levels were determined in transgenic mice expressing the human GCGR protein ("humanized GCGR mice"). Humanized GCGR mice were generated by replacing the mouse GCGR gene with the human GCGR gene (SEQ ID NO: 157; encoding full-length protein, GenBank accession number NP_000151.1; SEQ ID NO: 153) in C57BL6/129 (F1H4) embryonic stem cells. After germ line transmission was established, heterozygous mice ($GCGR^{hum/+}$) were bred together to generate homozygous mice ($GCGR^{hum/hum}$) on a C57BL6 background. Homozygous humanized GCGR mice were put into ten groups of three or four animals. Each group received subcutaneous injections of each antibody at 3 mg/kg. The Control I group was injected with a hIgG isotype control antibody, which does not bind to any known mouse proteins. The Control II group was injected with an anti-hGCGR hIgG4 antibody, which has been validated to decrease blood glucose levels of humanized GCGR mice. Mice were bled three days after antibody dosing, and blood glucose levels were determined by ACCU-CHEK® Compact Plus (Roche). The percent reduction in blood glucose from the mean blood glucose levels of the Control I group was calculated for each animal. The average percent reduction in blood glucose was calculated for each antibody group. Table 8a summarizes the mean blood glucose levels of the control group. Results, expressed as (mean ± SEM) of percent blood glucose reduction, are shown in Table 8b.

**[0199]** Additionally with the Control I, Control II and H4H1765N groups, mice were bled before and 3 and 8 days after antibody dosing, and plasma lipid levels were determined by ADVIA® 1650 Chemistry System (Siemens). Averages were calculated for each of the measurements of low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total cholesterol (TOTAL-C), triglycerides (TG), nonesterified fatty acids (NEFA) levels for each of the three groups. Results, expressed as (mean ± SEM) of plasma lipid concentrations, are shown in Table 8c.

**[0200]** Mice treated with most of the anti-hGCGR antibodies tested (shown in Table 8b) exhibited significant reductions in blood glucose levels compared to mice receiving control antibody. Mice treated with certain of the anti-hGCGR antibodies tested (shown in Table 8c) exhibited significant reductions in triglyceride levels compared to mice receiving control

antibody. In particular, the lowering of triglyceride levels was observed with two anti-GCGR antibodies, one designated as H4H1765N (data shown below in Table 8c) and the other designated as H4H1327P (data not shown).

**Table 8a**

| Time | Blood glucose (mg/dL) |
|---|---|
| Day 0 | 155 ± 6 |
| Day 1 | 160 ± 5 |
| Day 3 | 155 ± 5 |
| Day 6 | 164 ± 7 |
| Day 8 | 156 ± 7 |
| Day 10 | 154 ± 9 |
| Day 13 | 149 ± 5 |

**Table 8b**

| Antibody Designation | Blood glucose reduction (%) |
|---|---|
| Control II | 37 ± 3 |
| H4H1321P | 32 ± 4 |
| H4H1327P | 40 ± 7 |
| H4H1328P | 31 ± 7 |
| H4H1331P | 33 ± 4 |
| H4H1339P | 31 ± 6 |
| H4H1617N | 15 ± 5 |
| H4H1345N | 14 ± 2 |
| H4H1765N | 32 ± 4 |

**Table 8c**

| Antibody Designation | Time (days) | LDL-C (mg/dL) | HDL-C (mg/dL) | TOTAL-C (mg/dL) | TG (mg/dL) | NEFA (mmol/L) |
|---|---|---|---|---|---|---|
| Control I | Pre | 9.4 ± 1.4 | 47 ± 4 | 97 ± 10 | 98 ± 6 | 0.67 ± 0.06 |
| | 3 | 7.7 ± 1.1 | 45 ± 3 | 95 ± 4 | 80 ± 8 | 0.96 ± 0.03 |
| | 8 | 9.8 ± 2.2 | 46 ± 2 | 99 ± 4 | 120 ± 19 | 0.88 ± 0.05 |
| Control II | Pre | 6.7 ± 0.4 | 37 ± 1 | 76 ± 2 | 69 ± 12 | 0.60 ± 0.09 |
| | 3 | 11.2 ± 1.6 | 51 ± 2 | 101 ± 8 | 58 ± 5 | 0.80 ± 0.11 |
| | 8 | 14.4 ± 2.1 | 57 ± 3 | 114 ± 7 | 74 ± 15 | 0.73 ± 0.04 |
| H4H1765N | Pre | 8.7 ± 0.2 | 39 ± 6 | 79 ± 8 | 94 ± 19 | 0.81 ± 0.12 |
| | 3 | 8.0 ± 1.1 | 46 ± 6 | 91 ± 9 | 68 ± 7 | 0.72 ± 0.06 |
| | 8 | 9.2 ± 1.6 | 46 ± 6 | 92 ± 8 | 75 ± 7 | 0.70 ± 0.05 |

**Example 8: Effect of Combination Therapy with an Anti-GCGR Antibody and an Antibody Specific for PCSK9 (proprotein convertase subtilisin/kexin type 9) on Blood Glucose, Plasma Lipid and Hepatic Triglyceride Levels in Mice**

**Reagents**

**[0201]** The following antibodies were used to study the effect of combination therapy with an anti-GCGR antibody and an antibody specific for PCSK9 on blood glucose levels, plasma lipid levels and hepatic triglyceride levels in C57BL/6 mice: An anti-hIL4R antibody designated

REGN496, which is an hIgG4 isotype control; an anti-GCGR (hIgG4) antibody designated H4H1327P; and an anti-PCSK9 (hIgG1) antibody designated H1H316P. The amino acid sequence identifiers for the HCVR, LCVR, HCDRs, and LCDRs are shown below in Table 9.

**Table 9**

| | SEQ ID NUMBERS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **REGN AB Designation** | **HCVR** | **HCDR1** | **HCDR2** | **HCDR3** | **LCVR** | **LCDR1** | **LCDR2** | **LCDR3** |
| REGN496 | 194 | 195 | 196 | 197 | 198 | 199 | 200 | 201 |
| H4H1327P | 86 | 72 | 74 | 76 | 88 | 80 | 82 | 84 |
| H1H316P | 173 | 161 | 163 | 165 | 175 | 167 | 169 | 171 |

**Experimental Procedure**

**[0202]** The combined effects of H4H1327P, an anti-hGCGR antibody, and H1H316P, an anti-hPCSK9 antibody, on blood glucose, plasma lipid, and hepatic triglyceride (TG) levels were determined in C57BL/6 mice.

**[0203]** H4H1327P cross-reacts with mouse GCGR, and H1H316P cross-reacts with mouse PCSK9. C57BL/6 mice were put into six groups of six animals. Each group received once a week subcutaneous injections of an antibody or a combination of two antibodies. The first group was injected at 10 mg/kg with a hIgG4 isotype control antibody, which does not bind to any known mouse protein. The second and third group received H4H1327P at 3 mg/kg and 10 mg/kg, respectively. The fourth group was injected with 10 mg/kg H1H316P. The fifth group received a combination of 3 mg/kg H4H1327P and 10 mg/kg H1H316P, and the sixth group was injected with a combination of 10 mg/kg H4H1327P and 10 mg/kg H1H316P. Eleven and 19 days after the initial antibody dosing, mice were bled for blood glucose and plasma lipid measurements. At Day 19, liver was harvested for the determination of hepatic TG content. Blood glucose levels were measured with the use of ACCU-CHEK® Compact Plus (Roche). The percent reduction in blood glucose from the mean blood glucose level of the isotype control group was calculated for each animal. The percent reduction and associated error in blood glucose for each treatment group was then calculated by averaging across values for the individual animals in each group. Results, expressed as (mean ± SEM) of percent blood glucose reduction, are shown in Table 10a and in Figure 1.

**[0204]** Plasma lipid levels were determined by ADVIA® 1650 Chemistry System (Siemens). Hepatic TG contents were measured using a colorimetric assay (Teco Diagnostics) after extraction of TG from tissue with chloroform/methanol. Means were calculated for each of the measurements of plasma low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total cholesterol (TOTAL-C), TG and hepatic TG levels for each group. Results, expressed as (mean ± SEM) of plasma and hepatic lipid concentrations, are shown in

Table 10b and in Figure 2 (plasma LDL-C levels); Figure 3 (Plasma HDL-C levels); Figure 4 (Plasma Total-C levels); Figure 5 (Plasma TG levels); and Figure 6 (hepatic TG content).

**Results summary and conclusions:**

**Table 10a**

| Time (days) | Blood glucose reduction (%) | | | | |
|---|---|---|---|---|---|
| | H4H1327P 3mg/kg | H4H1327P 10mg/kg | H1H316P 10mg/kg | H4H1327P 3mg/kg + H1H316P 10mg/kg | H4H1327P 10mg/kg + H1H316P 10mg/kg |
| 11 | 33 ± 7 | 40 ± 5 | 3 ± 3 | 28 ± 7 | 39 ± 2 |
| 19 | 25 ± 4 | 28 ± 4 | - 4 ± 6 | 21 ± 7 | 27 ± 2 |

**Table 10b**

| Antibody | Time (days) | Plasma | | | | Liver |
|---|---|---|---|---|---|---|
| | | LDL-C (mg/dL) | HDL-C (mg/dL) | TOTAL-C (mg/dL) | TG (mg/dL) | TG (mg/g tissue) |
| Control | Pre-dosing | 5.2±0.7 | 37±2 | 65±2 | 101±11 | NA |
| | 11 | 4.4±0.3 | 39±2 | 66±2 | 72±16 | NA |
| | 19 | 6.0±0.3 | 46±1 | 74±1 | 84±6 | 2.4±0.1 |
| H4H1327P 3mg/kg | Pre-dosing | 3.3±0.1 | 35±1 | 58±2 | 109±11 | NA |
| | 11 | 7.4±0.6 | 51±2 | 89±3 | 96±14 | NA |
| | 19 | 7.7±0.2 | 59±4 | 93±5 | 80±8 | 2.4±0.2 |
| H4H1327P 10mg/kg | Pre-dosing | 4.2±0.2 | 39±1 | 67±2 | 120±16 | NA |
| | 11 | 8.3±0.3 | 54±3 | 91±4 | 70±8 | NA |
| | 19 | 10.4±0.8 | 56±1 | 95±3 | 75±5 | 1.9±0.1 |
| H1H316P 10mg/kg | Pre-dosing | 4.0±0.2 | 35±2 | 62±3 | 114±8 | NA |
| | 11 | 3.0±0.3 | 21±3 | 48±5 | 97±8 | NA |
| | 19 | 2.9±0.2 | 30±1 | 50±1 | 91±11 | 2.1±0.3 |
| H4H1327P 10mg/kg + H1H316P 10mg/kg | Pre-dosing | 4.2±0.2 | 36±1 | 64±1 | 124±12 | NA |
| | 11 | 4.3±0.3 | 38±1 | 65±1 | 109±16 | NA |
| | 19 | 5.4±0.5 | 40±2 | 66±3 | 96±11 | 2.1±0.1 |
| H4H1327P 3mg/kg + H1H316P 10mg/kg | Pre-dosing | 4.8±0.7 | 38±2 | 65±4 | 107±18 | NA |
| | 11 | 4.9±0.5 | 41±1 | 67±2 | 89±10 | NA |
| | 19 | 5.3±0.6 | 42±2 | 67±3 | 85±9 | 2.1±0.2 |

NA: Not applicable

**Tabulated Data Summary:**

**[0205]** Mice treated with H4H1327P alone showed significant reductions in blood glucose levels and increases in LDL, HDL, and total cholesterol levels in comparison to mice receiving control mAb. Mice treated with H1H316P alone exhibited significant reductions in cholesterol levels with no change in blood glucose levels. Mice treated with a combination of H4H1327P and H1H316P showed significant reductions in blood glucose levels with no alterations in cholesterol levels in comparison to mice receiving control mAb. Hepatic TG contents were not altered in any of the treatment groups compared to the isotype control group.

**Example 9. The Effect of an Anti-GCGR antibody in a Diet-induced Obesity Mouse Model**

**[0206]** The effects of H4H1327P, an anti-hGCGR antibody that cross-reacts with mouse GCGR, on blood glucose levels and body weights were determined in a diet-induced obesity (DIO) mouse model of type 2 diabetes (T2D).

**[0207]** The DIO model is developed by feeding mice on a high fat (60% kcal) diet (HFD) from 5~6 weeks of age. After approximately 6 weeks on the diet, mice develop metabolic abnormalities including insulin resistance, glucose intolerance, and obesity. The DIO model induces a physiological condition in mice closer to human T2D than that induced by the other two commonly used T2D models, ob/ob and db/db mice, since the latter two models result from mutations in leptin or leptin receptor genes, respectively, which are rarely the cause of T2D in humans.

**[0208]** In this study, eleven week-old male C57BL/6 mice, placed on HFD since 5 weeks of age, were purchased from Taconic farms, Inc. and kept on the diet for another 8 weeks. The mice were divided into 4 groups of 10 animals per group at 19 weeks of age. Each group received weekly (on days 0, 7, 14, and 21) subcutaneous injections of H4H1327P at 3, 10, or 30 mg/kg, or 30 mg/kg of the hIgG4 isotype control, which does not bind to any known mouse protein. Blood glucose levels and body weights were measured periodically, and 6 days after administering the last antibody dose (on day 27), 6 mice/group were sacrificed. For the next 6 weeks, blood glucose and body weights were monitored for the remaining 4 mice/group. The percent reduction in blood glucose levels compared to the mean blood glucose level of the isotype control group was calculated for each animal. The percent reduction and associated error in blood glucose for each treatment group was then calculated by averaging across values for the individual animals in each group. Results, expressed as (mean ± SEM) of percent blood glucose reduction, are shown in Table 11. The percent change in body weight from the baseline (weight at day 0) was calculated for each animal. The percent change and associated error in body weight for each treatment group was then calculated by averaging across values for the individual animals in each group. Results, expressed as (mean ± SEM) of percent body weight change from baseline, are shown in Table 12.

**Results summary and conclusions:**

**[0209]** H4H1327P, at all dosages tested, reduced blood glucose and body weight of DIO mice compared to the isotype control groups. The greatest relative blood glucose reduction (48.5%) occurred in the highest dose (30 mg/kg) group at day 10, and the greatest relative body weight reduction (12.8%) occurred in the highest dose group at day 28. The lowest dose (3 mg/kg) groups achieved mean relative blood glucose lowering and mean body weight lowering values of at least 70% the values exhibited by the highest dose groups through day 28 (one week following the last dose). The observed blood glucose and body weight lowering effects following

the last treatment on day 21 (*i.e.*, on days 28, 47, and 68) persisted longer for the higher H4H1327P dose groups compared to the lower dose groups.

**Table 11**

| Time (days) | Blood glucose reduction (%) | | |
|---|---|---|---|
| | H4H1327P 3mg/kg | H4H1327P 10mg/kg | H4H1327P 30mg/kg |
| 10 | 44.6 ± 2.8 | 44.4 ± 1.8 | 48.5 ± 1.5 |
| 19 | 33.9 ± 2.3 | 39.0 ± 1.9 | 37.5 ± 2.5 |
| 28 | 24.8 ± 1.7 | 28.1 ± 2.8 | 32.4 ± 1.6 |
| 47 | -3.9 ± 7.1 | 21.2 ± 7.0 | 30.0 ± 2.4 |
| 68 | -4.3 ± 7.9 | -1.1 ± 5.8 | -12.9 ± 12.1 |

**Table 12**

| Time (days) | Body weight change from baseline (%) | | | |
|---|---|---|---|---|
| | Isotype control 30mg/kg | H4H1327P 3mg/kg | H4H1327P 10mg/kg | H4H1327P 30mg/kg |
| 10 | 1.8 ± 0.6 | -5.5 ± 0.6 | -6.1 ± 0.6 | -5.8 ± 0.6 |
| 19 | 2.2 ± 0.6 | -7.3 ± 0.6 | -9.6 ± 0.6 | -10.3 ± 0.7 |
| 28 | 1.8 ± 0.4 | -9.2 ± 1.2 | -10.5 ± 0.5 | -12.8 ± 1.0 |
| 47 | 6.6 ± 0.8 | 5.2 ± 1.3 | 2.5 ± 1.4 | -3.7 ± 2.2 |
| 68 | 10.4 ± 1.1 | 9.7 ± 0.7 | 10.6 ± 1.0 | 8.2 ± 1.1 |

**Example 10. The Effect of an Anti-GCGR antibody in a Streptozotocin (STZ)-induced Mouse Model of Diabetic Ketoacidosis**

**[0210]** The effects of H4H1327P, an anti-hGCGR antibody, which cross-reacts with mouse GCGR, on plasma ketone and glucose levels were determined in a streptozotocin (STZ) - induced mouse model of diabetic ketoacidosis (DKA). STZ is a chemical toxic to pancreatic beta cells of mammals which, therefore, destroys this cell type when administered to rodents. A single, high dose (200 mg/kg) injection of STZ to C57BL/6 mice leads to the development of severe hyperglycemia and ketonuria, conditions exhibited in human DKA, in 3 days. Nine-week-old male C57BL/6 mice, purchased from Taconic farms, Inc. were divided into 2 groups of 10 animals, and each group received either intraperitoneal injections of STZ (in citrate buffer) at 200 mg/kg or vehicle (also in citrate buffer). Three days later, severe hyperglycemia (blood glucose levels >400 mg/dL) and ketonuria were confirmed in all STZ treated animals. The next morning, the STZ treated mice were divided into 2 groups of 5 animals, and each group received an intravenous injection of H4H1327P or hIgG4 isotype control at 10 mg/kg. The citrate buffer treated mice were also divided into 2 groups of 5 animals, and each group received intravenous injection of H4H1327P or hIgG4 isotype control at 10 mg/kg. Mice were bled 18 hours before antibody dosing (2.5 days after the STZ administration) and 28 hours after antibody dosing under isoflurane anesthesia for plasma collection. Plasma ketone levels were determined by beta-hydroxybutyrate assay (Biovision), and plasma glucose levels by ADVIA® 1650 Chemistry System (Siemens). Averages were calculated for the measurements of beta-

hydroxybutyrate and glucose levels for each of the four groups. Results, expressed as (mean ± SEM) of plasma beta-hydroxybutyrate and glucose concentrations, are shown in Table 13.

**Results summary and conclusions:**

**[0211]** A reduction (average 67%) in plasma beta-hydroxybutyrate (ketone) concentration was observed in STZ-induced diabetic ketoacidotic mice 28 hours after H4H1327P treatment in comparison to plasma levels 18 hours prior to the treatment, demonstrating that H4H1327P effectively lowered plasma ketone levels in a mouse model of DKA. For the STZ-treated mice dosed with isotype control antibody, a 14% average increase in blood glucose was observed for the serum samples collected 28 hours after control antibody treatment compared to the samples collected 18 hours before antibody treatment, whereas for the H4H1327P dosed mice in the STZ-treated group less than 1% average change in glucose was observed between serum samples collected at these two time points.

**Table 13**

| | Time from antibody treatment (hrs) | Vehicle/ Isotype control | Vehicle/ H4H1327P | STZ/ Isotype control | STZ/ H4H1327P |
|---|---|---|---|---|---|
| Beta-hydroxybutyrate (mM) | - 18 | 1.1±0.6 | 0.6±0.2 | 3.4±0.7 | 3.6±0.2 |
| | 28 | 0.4±0.0 | 0.8±0.4 | 2.4±0.6 | 1.2±0.1 |
| Glucose (mg/dL) | -18 | 214 ± 24 | 250 ± 6 | 610 ± 39 | 601 ± 26 |
| | 28 | 238 ± 16 | 152 ± 5 | 696 ± 66 | 606 ± 59 |

**Example 11. Generation of a Bi-specific Antibody**

**[0212]** Various bi-specific antibodies are generated for use in practicing the methods of the invention. For example, GCGR-specific antibodies are generated in a bi-specific format (a "bi-specific") in which variable regions binding to distinct ectodomain and/or EC loop epitopes on GCGR are linked together to confer dual-loop specificity within a single binding molecule. Appropriately designed bi-specifics may enhance overall glucagon receptor blocking efficacy through increasing both GCGR specificity and binding avidity. Variable regions with specificity for individual ectodomain epitopes (*e.g.*, segments of the N-terminal domain, or of the EC1, EC2, or EC3 GCGR loops) or that can bind to different regions within one ectodomain segment or loop are paired on a structural scaffold that allows each variable region to bind simultaneously to the separate epitopes, or to different regions within one ectodomain or EC loop. In one example for a bi-specific, heavy chain variable regions ($V_H$) from a binder with one ectodomain or loop specificity are recombined with light chain variable regions ($V_L$) from a series of binders with a second ectodomain or EC loop specificity to identify non-cognate $V_L$ partners that can be paired with an original $V_H$ without disrupting the original specificity for that $V_H$. In this way, a single $V_L$ segment (*e.g.*, $V_L1$) can be combined with two different $V_H$ domains (*e.g.*, $V_H1$ and $V_H2$) to generate a bi-specific comprised of two binding "arms" ($V_H1$- $V_L1$ and

$V_H2$- $V_L1$). Use of a single $V_L$ segment reduces the complexity of the system and thereby simplifies and increases efficiency in cloning, expression, and purification processes used to generate the bi-specific (See, for example, USSN13/022759 and US2010/0331527).

**[0213]** Alternatively, antibodies that bind both GCGR and a second target, such as, but not limited to, for example, human proprotein convertase subtilisin/kexin type 9 (hPCSK9), may be prepared in a bi-specific format using techniques described herein, or other techniques known to those skilled in the art. Antibody variable regions binding to distinct GCGR regions that are extracellularly exposed are linked together with variable regions that bind to relevant sites on, for example, PCSK9, to confer dual-antigen specificity within a single binding molecule. Appropriately designed bi-specifics of this nature serve a dual function. For example, in the case of a bi-specific antibody that binds both GCGR and PCSK9, one may be able to lower blood glucose by virtue of one arm of the bi-specific antibody binding GCGR, while at the same time lowering plasma lipids, by virtue of the second arm of the antibody binding PCSK9. Variable regions with specificity for individual ectodomain epitopes of GCGR, are combined with a variable region with specificity for PCSK9 and are paired on a structural scaffold that allows each variable region to bind to the separate antigens.

**[0214]** The bi-specific binders are tested for binding and functional blocking of the target antigens, for example, GCGR and/or PCSK9, in any of the assays described above for antibodies. For example, standard methods to measure soluble protein binding are used to assess the bispecific-PCSK9 interaction, such as Biacore, ELISA, size exclusion chromatography, multi-angle laser light scattering, direct scanning calorimetry, and other methods. Binding of bi-specific antibodies to cells expressing GCGR is determined through flow cytometry using a fluorescently labeled secondary antibody recognizing either or both of the two target antigens bound to cells. Cross-reactivity to the different GCGR ectodomains or loops within and between different species variants is assessed using an ELISA binding assay in which synthetic peptides representing the different ectodomains or loops are coated onto the wells of microtiter plates, and binding of a bi-specific is determined through use of a secondary detection antibody. Binding experiments with loop peptides can also be conducted using surface plasmon resonance experiments, in which real-time binding interaction of peptide to antibody is measured by flowing a peptide or bi-specific across a sensor surface on which bi-specific or peptide, respectively, is captured. Functional *in vitro* blocking of the GCGR receptor by a bi-specific is determined using any bioassay such as that described herein, or by *in vivo* determination of blood glucose levels in appropriate animal models, such as those described herein. Functional *in vitro* blocking of PCSK9 by a bi-specific is determined using any bioassay such as that described in WO2010/077854, or in US2010/0166768, or by *in vivo* determination of plasma lipid levels in appropriate animal models, such as those described herein.

What is claimed is:

1. An isolated human antibody or antigen-binding fragment thereof that specifically binds human glucagon receptor (hGCGR), wherein the antibody binds an ectodomain and/or an extracellular (EC) loop of human GCGR, wherein the ectodomain is the N-terminal domain of GCGR and wherein the EC loop is one or more of EC1, EC2 and EC3.

2. An antibody or fragment thereof according to claim 1, wherein the N-terminal domain comprises amino acid residues ranging from about amino acid residue number 27 to about amino acid residue 144 of SEQ ID NO: 153; EC1 comprises amino acid residues ranging from about amino acid residue 194 to about amino acid residue 226 of SEQ ID NO: 153; EC2 comprises amino acid residues ranging from about amino acid residue 285 to about amino acid residue 305 of SEQ ID NO: 153; and EC3 comprises amino acid residues ranging from about amino acid residue 369 to about amino acid residue 384 of SEQ ID NO: 153.

3. An antibody or fragment thereof according to claim 1 or 2, comprising the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR), wherein the HCVR has an amino acid sequence selected from one of SEQ ID NOs: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146; and the CDRs of a light chain variable region (LCVR), wherein the LCVR has an amino acid sequence selected from one of SEQ ID NOs: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148.

4. An antibody or fragment thereof according to any one of claims 1 through 3, wherein the HCVR comprises a HCDR1 domain having an amino acid sequence selected from one of SEQ ID NO: 4, 20, 36, 52, 72, 92, 112 and 132; a HCDR2 domain having an amino acid sequence selected from one of SEQ ID NO: 6, 22, 38, 54, 74, 94, 114 and 134; a HCDR3 domain having an amino acid sequence selected from one of SEQ ID NOs: 8, 24, 40, 56, 76, 96, 116 and 136; and

a LCVR comprising a LCDR1 domain having an amino acid sequence selected from one of SEQ ID NO: 12, 28, 44, 60, 80, 100, 120 and 140; a LCDR2 domain having an amino acid sequence selected from one of SEQ ID NO: 14, 30, 46, 62, 82, 102, 122 and 142; and a LCDR3 domain having an amino acid sequence selected from one of SEQ ID NO: 16, 32, 48, 64, 84, 104, 124 and 144.

5. An antibody or antigen-binding fragment thereof according to any one of claims 1 through 4, comprising: (a) a HCVR having an amino acid sequence selected from one of SEQ ID NO: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146; and (b) a LCVR having an

amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148.

6. An antibody or antigen-binding fragment thereof according to any of claims 1 through 5, comprising a HCVR/LCVR sequence pair selected from the group consisting of SEQ ID NO: 2/10, 18/26, 34/42, 50/58, 66/68, 70/78, 86/88, 90/98, 106/108, 110/118, 126/128, 130/138, and 146/148.

7. An antibody or antigen-binding fragment thereof that competes for specific binding to hGCGR with the antibody or antigen-binding fragment thereof according to any one of claims 1 through 6.

8. An antibody or antigen-binding fragment thereof that binds the same epitope on hGCGR as the antibody or antigen-binding fragment thereof according to any one of claims 1 through 6.

9. An isolated nucleic acid molecule encoding the antibody or antigen-binding fragment of an antibody of any of claims 1 through 8.

10. An expression vector comprising the nucleic acid molecule of claim 9.

11. A method of producing an anti-human GCGR antibody or antigen-binding fragment thereof comprising the steps of introducing the expression vector of claim 10 into an isolated host cell, growing the cell under conditions permitting production of the antibody or fragment thereof, and recovering the antibody so produced.

12. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof according to any one of claims 1 through 8 and a pharmaceutically acceptable carrier or diluent.

13. A method for lowering blood glucose or ketone levels, or for treating a condition or disease associated with, or characterized in part by high blood glucose or ketone levels, or at least one symptom or complication associated with the condition or disease, the method comprising administering the antibody or antigen-binding fragment according to any one of claims 1 through 8, or the pharmaceutical composition of claim 12, to a patient in need thereof, such that blood glucose or ketone levels are lowered or that the condition or disease is mediated, or at least one symptom or complication associated with the condition or disease is alleviated or reduced in severity.

14. The method according to claim 13, wherein the condition or disease is selected from the group consisting of diabetes, impaired glucose tolerance, obesity, nephropathy, neuropathy, retinopathy, cataracts, stroke, atherosclerosis, impaired wound healing, diabetic ketoacidosis, hyperglycemia, hyperglycemic hyperosmolar syndrome, perioperative hyperglycemia, hyperglycemia in the intensive care unit patient, hyperinsulinemia, the metabolic syndrome, insulin resistance syndrome and impaired fasting glucose.

15. The method according to either one of claims 13 or 14, wherein the antibody or antigen-binding fragment is administered to the patient in combination with a second therapeutic agent.

16. The method according to claim 15, wherein the second therapeutic agent is selected from the group consisting of insulin, a biguanide (metformin), a sulfonylurea (such as glyburide, glipizide), a PPAR gamma agonist (pioglitazone, rosiglitazone), an alpha glucosidase inhibitor (acarbose, voglibose), EXENATIDE® (glucagon-like peptide 1), SYMLIN® (pramlintide), a glucagon antagonist, and a second GCGR antagonist.

17. The method according to claim 15, wherein the second therapeutic agent is a 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) inhibitor.

18. The method according to claim 17, wherein the HMG-CoA reductase inhibitor is a statin selected from the group consisting of atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin and simvastatin.

19. The method according to claim 15, wherein the second therapeutic agent is an isolated antibody, or an antigen-binding fragment thereof, that specifically binds to human proprotein convertase subtilisin/kexin type 9 (PCSK9).

20. A pharmaceutical composition comprising an antibody or antigen-binding fragment thereof according to any one of claims 1 through 8, and a second therapeutic agent comprising an isolated antibody, or an antigen-binding fragment thereof, that specifically binds to human PCSK9, and a pharmaceutically acceptable carrier or diluent.

21. The isolated antibody or antigen-binding fragment thereof according to any one of claims 1 through 8, or the pharmaceutical composition of either of claim 12 or 20, for use in lowering blood glucose or ketone levels, or for treating a patient having a disease or condition associated with, or characterized in part by high blood glucose or ketone levels, wherein the condition or disease is selected from the group consisting of diabetes, impaired glucose tolerance, obesity, nephropathy, neuropathy, retinopathy, cataracts, stroke, atherosclerosis, impaired wound healing, diabetic ketoacidosis, hyperglycemia, hyperglycemic hyperosmolar

syndrome, perioperative hyperglycemia, hyperglycemia in the intensive care unit patient, hyperinsulinemia, the metabolic syndrome, insulin resistance syndrome and impaired fasting glucose.

22. Use of the isolated antibody or antigen-binding fragment thereof according to any one of claims 1 through 8, or the pharmaceutical composition of either of claim 12 or 20, in the manufacture of a medicament for lowering blood glucose or ketone levels, or for treating a patient having a disease or condition associated with, or characterized in part by high blood glucose or ketone levels, wherein the condition or disease is selected from the group consisting of diabetes, impaired glucose tolerance, obesity, nephropathy, neuropathy, retinopathy, cataracts, stroke, atherosclerosis, impaired wound healing, diabetic ketoacidosis, hyperglycemia, hyperglycemic hyperosmolar syndrome, perioperative hyperglycemia, hyperglycemia in the intensive care unit patient, hyperinsulinemia, the metabolic syndrome, insulin resistance syndrome and impaired fasting glucose.

SEQUENCE LISTING

<110> Regeneron Pharmaceuticals, Inc.

<120> Human Antibodies to the Glucagon Receptor

<130> 1400A-WO

<140> To be assigned
<141> Filed herewith

<150> 61/416,409
<151> 2010-11-23

<150> 61/481,958
<151> 2011-05-03

<150> 61/551,032
<151> 2011-10-25

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            20                  25                  30
Ser Met His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

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Ala Gly Phe Asp Pro Glu Glu Gly Lys Ile Ile Tyr Ala Gln Lys Phe
    50                  55                  60
Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
65                  70                  75                  80
Met Glu Leu Ser Ser Leu Arg Ser Gly Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Thr Ser Asp Ile Leu Thr Gly Tyr Tyr Arg Asp Tyr Tyr Gly Leu
            100                 105                 110
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tacctgcaga agccagggca gtctccacaa ctcctgatct atttgggttc taatcgggcc 180
tccggggtcc ctgacaggtt cagtggcagt ggatcaggca cagattttac actgaaaatc 240
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Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
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Lys Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser

```
        35                  40                  45
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
    50                  55                  60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Thr
                85                  90                  95
Leu Gln Thr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105                 110
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<400> 13
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<210> 14
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cctggaaaag ggcttgagtg gatgggaggt tttgatcctg aagaaggtga aataatctac 180
gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaga cacagcctac 240
atggagctga gcagcctgag atctggggac acggccgttt attactgtgc aacaagcgat 300
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Ser Val Lys Val Ser Cys Lys Val Ser Gly His Ile Leu Thr Asp Leu
20 25 30
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Gly Phe Asp Pro Glu Glu Gly Glu Ile Ile Tyr Ala Gln Lys Phe

```
    50                  55                  60
Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
65                  70                  75                  80
Met Glu Leu Ser Ser Leu Arg Ser Gly Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Thr Ser Asp Ile Leu Thr Gly Tyr Tyr Arg Asp Tyr Tyr Gly Leu
            100                 105                 110
Asp Val Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
        115                 120                 125
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<400> 22
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1 5 10 15
Asp Val

<210> 25
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tacctgcaga agccagggca gtctccacaa ctcctgatct atttgggttc taatcgggcc 180
tccggggtcc ctgacaggtt cagtggcagt ggatcaggca cagattttac actgaaaatc 240
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Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30
Lys Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

```
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
    50                  55                  60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Thr
                85                  90                  95
Leu Gln Thr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105                 110


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<210> 31
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<400> 32
Met Gln Thr Leu Gln Thr Pro Arg Thr
1 5

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ccagggaagg ggctggagtg ggtttcatac attagtagag gtggtagtct gatacactac 180
acagactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ttcactgtat 240
ctgcaaatga acagcctgag agccgaggac acggctgttt attactgtgt gagagaccca 300
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accgtctcct ca 372

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20 25 30
Glu Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Tyr Ile Ser Arg Gly Gly Ser Leu Ile His Tyr Thr Asp Ser Val
50 55 60

```
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Val Arg Asp Pro Ala Ala Arg Tyr His Tyr Tyr Tyr His Gly Met Asp
            100                 105                 110
Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
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<210> 41
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tatctgcaga agccagggca gtctccacag ctcctgatct atttgggttc tagtcgggcc 180
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Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Asn
20 25 30
Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Ser Arg Ala Ser Gly Val Pro

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    50                  55                  60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ile Leu Lys Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
                85                  90                  95
Leu Gln Thr Pro Trp Thr Phe Gly Arg Gly Thr Lys Val Glu Ile Lys
            100                 105                 110


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<210> 47
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<400> 48
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gtagactccg tgaagggccg attcaccatc tccagagaca actccaagaa cacgctttat 240
ctgcaaatga acagcctgag agctgaggac acggctgttt attactgtgc gaaagagatg 300
gtgtattacg atattttgac tggttatcat aactactacg gtatggacgt ctggggccaa 360
gggaccacgg tcaccgtctc ctca 384

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

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65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Lys Glu Met Val Tyr Tyr Asp Ile Leu Thr Gly Tyr His Asn Tyr
            100                 105                 110
Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115                 120                 125


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<210> 55
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 1 5 10 15
Tyr Gly Met Asp Val
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<210> 57
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gggaaagccc ctaagtccct gatccatact gcatccagtt tgcaaagtgg ggtcccatca 180
aagttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240
gaagattttg caacttatta ctgccaacag tataatactt accctctcac tttcggcgga 300
gggaccaaag tggagatcaa acga 324

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<211> 108
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<220>
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Asn Asn Tyr
 20 25 30
Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Ala Pro Lys Ser Leu Ile
 35 40 45
His Thr Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Lys Phe Ser Gly

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    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Thr Tyr Pro Leu
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
            100                 105
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<210> 61
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<400> 61
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<210> 62
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<400> 62
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<210> 63

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Gln Gln Tyr Asn Thr Tyr Pro Leu Thr
1 5

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

```
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Lys Glu Met Val Tyr Tyr Asp Ile Leu Thr Gly Tyr His Asn Tyr
            100                 105                 110
Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser
        115                 120                 125
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<210> 67
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 67
gacatccaga tgacccagtc tccatcctca ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgtc gggcgagtca gggcattaac aattatttag cctggtttca gcagaaacca 120
gggaaagccc ctaagtccct gatccatact gcatccagtt tgcaaagtgg ggtcccatca 180
aagttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240
gaagattttg caacttatta ctgccaacag tataatactt accctctcac tttcggcgga 300
gggaccaagg tggagatcaa a 321

<210> 68
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 68
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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1               5                  10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Asn Asn Tyr
            20                  25                  30
Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Ala Pro Lys Ser Leu Ile
        35                  40                  45
His Thr Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Lys Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Thr Tyr Pro Leu
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
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<210> 69
<211> 384
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 69
caggtgcagc tggtgcagtc tgggggaggc ttggtccagc ctggggggtc cctgagactc 60
tcctgtgcag cctccggatt cacctttagt aactatttga tgaactgggt ccgccaggct 120
ccagggaagg ggctggagtg gctggccaac atacaggaag atggaattga gaaatactat 180
gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctcactgtat 240
ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagagagccc 300
tcccattacg atattttgac tggttatgac tactattacg gtatggacgt ctggggccaa 360
gggaccacgg tcaccgtctc ctca 384

<210> 70
<211> 128
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 70
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30
Leu Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45
Ala Asn Ile Gln Glu Asp Gly Ile Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Glu Pro Ser His Tyr Asp Ile Leu Thr Gly Tyr Asp Tyr Tyr
100 105 110
Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125

<210> 71
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 71
ggattcacct ttagtaacta tttg 24

<210> 72
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 72
Gly Phe Thr Phe Ser Asn Tyr Leu
1 5

<210> 73
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 73
atacaggaag atggaattga gaaa 24

<210> 74
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 74
Ile Gln Glu Asp Gly Ile Glu Lys
1 5

<210> 75
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 75
gcgagagagc cctcccatta cgatattttg actggttatg actactatta cggtatggac 60
gtc 63

<210> 76
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 76
Ala Arg Glu Pro Ser His Tyr Asp Ile Leu Thr Gly Tyr Asp Tyr Tyr
1 5 10 15
Tyr Gly Met Asp Val
20

<210> 77
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 77
gacatccagt tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgcc gggcaagtca gggcattaga aatgatttag gctggtatca gcagaaacca 120
gggaaagccc ctaagcgcct gatctatgct gcatccagtt tgcaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacagaa ttcattctca cagtcagcag cctgcagcct 240
gaagactttg caacttatta ctgtctacag tataatagta acccattcac tttcggccct 300
gggaccaagg tggagatcaa acga 324

<210> 78
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 78
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
20 25 30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Glu Phe Ile Leu Thr Val Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Tyr Asn Ser Asn Pro Phe
85 90 95
Thr Phe Gly Pro Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 79
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 79
cagggcatta gaaatgat 18

<210> 80
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 80
Gln Gly Ile Arg Asn Asp
1 5

<210> 81
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 81
gctgcatcc 9

<210> 82
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 82
Ala Ala Ser
1

<210> 83
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 83
ctacagtata atagtaaccc attcact 27

<210> 84
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 84
Leu Gln Tyr Asn Ser Asn Pro Phe Thr
1 5

<210> 85
<211> 381
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 85
gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggtc cctgagactc 60
tcctgtgcag cctccggatt cacctttagt aactatttga tgaactgggt ccgccaggct 120
ccagggaagg ggctggagtg gctggccaac atacaggaag atggaattga gaaatactat 180
gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctcactgtat 240
ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagagagccc 300
tcccattacg atattttgac tggttatgac tactattacg gtatggacgt ctggggccaa 360
gggaccacgg tcaccgtctc c 381

<210> 86
<211> 127
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 86
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20                  25                  30
Leu Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45
Ala Asn Ile Gln Glu Asp Gly Ile Glu Lys Tyr Tyr Val Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Glu Pro Ser His Tyr Asp Ile Leu Thr Gly Tyr Asp Tyr Tyr
            100                 105                 110
Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser
        115                 120                 125


<210> 87
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 87
gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgcc gggcaagtca gggcattaga aatgatttag gctggtatca gcagaaacca 120
gggaaagccc ctaagcgcct gatctatgct gcatccagtt tgcaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacagaa ttcattctca cagtcagcag cctgcagcct 240
gaagactttg caacttatta ctgtctacag tataatagta acccattcac tttcggccct 300
gggaccaaag tggatatcaa a 321

<210> 88
<211> 107
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic

<400> 88
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1               5                  10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
            20                  25                  30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
        35                  40                  45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Glu Phe Ile Leu Thr Val Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Tyr Asn Ser Asn Pro Phe
                85                  90                  95
Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
            100                 105


<210> 89
<211> 381
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 89
gaggtgcagc tggtgcagtc tgggggagcc ttggtacagc ctgggggtc cctgagactc 60
tcctgtacag cctctggttt caccttcagt aactacgaca tgcactgggt ccgccaaact 120
acaggaaaag gtctggagtg gatctcagct attgatactg ctggtgacac atactatcca 180
ggctccgtga agggccgatt caccgtctcc agagaaaatg ccaagaactc cttttatctt 240
caaatgaaca gcctgagagc cggggacacg gctgtgtatt actgtgcaag ggaggggaag 300
tattacgata ttttgactgg tgactaccac tactacggta tggacgtctg gggccaaggg 360
accacggtca ccgtctcctc a                                          381

<210> 90
<211> 127
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 90
Glu Val Gln Leu Val Gln Ser Gly Gly Ala Leu Val Gln Pro Gly Gly
 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20                  25                  30
Asp Met His Trp Val Arg Gln Thr Thr Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45
Ser Ala Ile Asp Thr Ala Gly Asp Thr Tyr Tyr Pro Gly Ser Val Lys
    50                  55                  60
Gly Arg Phe Thr Val Ser Arg Glu Asn Ala Lys Asn Ser Phe Tyr Leu
65                  70                  75                  80
Gln Met Asn Ser Leu Arg Ala Gly Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95
Arg Glu Gly Lys Tyr Tyr Asp Ile Leu Thr Gly Asp Tyr His Tyr Tyr

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            100                 105                 110
Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115                 120                 125


<210> 91
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 91
ggtttcacct tcagtaacta cgac                                          24

<210> 92
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 92
Gly Phe Thr Phe Ser Asn Tyr Asp
 1               5


<210> 93
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 93
attgatactg ctggtgacac a                                             21

<210> 94
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 94
Ile Asp Thr Ala Gly Asp Thr
 1               5


<210> 95
<211> 63
<212> DNA
<213> Artificial Sequence
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<220>
<223> Synthetic

<400> 95
gcaagggagg ggaagtatta cgatattttg actggtgact accactacta cggtatggac 60
gtc 63

<210> 96
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 96
Ala Arg Glu Gly Lys Tyr Tyr Asp Ile Leu Thr Gly Asp Tyr His Tyr
 1               5                  10                  15
Tyr Gly Met Asp Val
            20

<210> 97
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 97
gccatccgga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgtc gggcaagtca gggcattaga aatgatttag gctggtatca gcagaaacca 120
gggaaagccc ctaagcgact gatctatgct acatccagtt tgcaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacagaa ttcactctca caatcagcag cctgcagcct 240
gaagattttg caacttatta ctgtctacag cataatagtt acccgctcac tttcggcgga 300
gggaccaagg tggaaatcaa acga 324

<210> 98
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 98
Ala Ile Arg Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1               5                  10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
            20                  25                  30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
        35                  40                  45
Tyr Ala Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln His Asn Ser Tyr Pro Leu

85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 99
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 99
cagggcatta gaaatgat 18

<210> 100
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 100
Gln Gly Ile Arg Asn Asp
1 5

<210> 101
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 101
gctacatcc 9

<210> 102
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 102
Ala Thr Ser
1

<210> 103
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 103
ctacagcata atagttaccc gctcact 27

<210> 104
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 104
Leu Gln His Asn Ser Tyr Pro Leu Thr
1 5

<210> 105
<211> 378
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 105
gaggtgcagc tggtggagtc tgggggagcc ttggtacagc ctgggggtc cctgagactc 60
tcctgtacag cctctggttt caccttcagt aactacgaca tgcactgggt ccgccaaact 120
acaggaaaag gtctggagtg gatctcagct attgatactg ctggtgacac atactatcca 180
ggctccgtga agggccgatt caccgtctcc agagaaaatg ccaagaactc cttttatctt 240
caaatgaaca gcctgagagc cggggacacg gctgtgtatt actgtgcaag ggaggggaag 300
tattacgata ttttgactgg tgactaccac tactacggta tggacgtctg gggccaaggg 360
accacggtca ccgtctcc 378

<210> 106
<211> 126
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 106
Glu Val Gln Leu Val Glu Ser Gly Gly Ala Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30
Asp Met His Trp Val Arg Gln Thr Thr Gly Lys Gly Leu Glu Trp Ile
35 40 45
Ser Ala Ile Asp Thr Ala Gly Asp Thr Tyr Tyr Pro Gly Ser Val Lys
50 55 60
Gly Arg Phe Thr Val Ser Arg Glu Asn Ala Lys Asn Ser Phe Tyr Leu
65 70 75 80
Gln Met Asn Ser Leu Arg Ala Gly Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Arg Glu Gly Lys Tyr Tyr Asp Ile Leu Thr Gly Asp Tyr His Tyr Tyr

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            100                 105                 110
Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser
        115                 120                 125
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<210> 107
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 107
gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgtc gggcaagtca gggcattaga aatgatttag gctggtatca gcagaaacca 120
gggaaagccc ctaagcgact gatctatgct acatccagtt tgcaaagtgg ggtcccatca 180
aggttcagcg gcagtggatc tgggacagaa ttcactctca caatcagcag cctgcagcct 240
gaagattttg caacttatta ctgtctacag cataatagtt acccgctcac tttcggcgga 300
gggaccaagc tggagatcaa a 321

<210> 108
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 108
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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1               5                  10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
            20                  25                  30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
        35                  40                  45
Tyr Ala Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln His Asn Ser Tyr Pro Leu
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105
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<210> 109
<211> 372
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 109
caggtgcagc tggtgcagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc 60
tcctgtgcag cgtctgggtt cacctttagt aactttggca tgcactgggt ccgccaggct 120
ccaggcaagg ggctggagtg ggtggcagtt atatggtttg atgaaattga taaatactat 180

gcagactccg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240
ccgcaaatga acagcctgag agccgaagac acggctgtgt attactgtgc gcgagaagat 300
tacgatattt tgactggtta ctattacgct atggacgtct ggggccaagg gaccacggtc 360
accgtctcct ca 372

<210> 110
<211> 124
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 110
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Phe
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Val Ile Trp Phe Asp Glu Ile Asp Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Pro Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Glu Asp Tyr Asp Ile Leu Thr Gly Tyr Tyr Tyr Ala Met Asp
100 105 110
Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 111
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 111
gggttcacct ttagtaactt tggc 24

<210> 112
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 112
Gly Phe Thr Phe Ser Asn Phe Gly
1 5

<210> 113
<211> 24

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 113
atatggtttg atgaaattga taaa 24

<210> 114
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 114
Ile Trp Phe Asp Glu Ile Asp Lys
1 5

<210> 115
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 115
gcgcgagaag attacgatat tttgactggt tactattacg ctatggacgt c 51

<210> 116
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 116
Ala Arg Glu Asp Tyr Asp Ile Leu Thr Gly Tyr Tyr Tyr Ala Met Asp
1 5 10 15
Val

<210> 117
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 117
gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60

atcacttgcc gggcaagtca gggcattaga aatgatttag gctggtatca gcagaaacca 120
gggaaagccc ctaagcgcct aatctatgct gcatcccgtt tgcaaagtgg ggtcccatcg 180
aggttcagcg gcagtggatc tgggacagaa ttcactctca caatcagcag cctgcagcct 240
gaagattttg gaacttatta ctgtctacag cataatagtc accccacctt cggccaaggg 300
accaaggtgg agatcaaacg a 321

<210> 118
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 118
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
20 25 30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45
Tyr Ala Ala Ser Arg Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Gly Thr Tyr Tyr Cys Leu Gln His Asn Ser His Pro Thr
85 90 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 119
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 119
cagggcatta gaaatgat 18

<210> 120
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 120
Gln Gly Ile Arg Asn Asp
1 5

<210> 121
<211> 9
<212> DNA

<213> Artificial Sequence

<220>
<223> Synthetic

<400> 121
gctgcatcc 9

<210> 122
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 122
Ala Ala Ser
1

<210> 123
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 123
ctacagcata atagtcaccc cacc 24

<210> 124
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 124
Leu Gln His Asn Ser His Pro Thr
1 5

<210> 125
<211> 369
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 125
caggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc 60
tcctgtgcag cgtctgggtt cacctttagt aactttggca tgcactgggt ccgccaggct 120
ccaggcaagg ggctggagtg ggtggcagtt atatggtttg atgaaattga taaatactat 180
gcagactccg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240

ccgcaaatga acagcctgag agccgaagac acggctgtgt attactgtgc gcgagaagat 300
tacgatattt tgactggtta ctattacgct atggacgtct ggggccaagg gaccacggtc 360
accgtctcc 369

<210> 126
<211> 123
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 126
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Phe
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Val Ile Trp Phe Asp Glu Ile Asp Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Pro Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Glu Asp Tyr Asp Ile Leu Thr Gly Tyr Tyr Tyr Ala Met Asp
100 105 110
Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser
115 120

<210> 127
<211> 318
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 127
gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgcc gggcaagtca gggcattaga aatgatttag gctggtatca gcagaaacca 120
gggaaagccc ctaagcgcct aatctatgct gcatcccgtt tgcaaagtgg ggtcccatcg 180
aggttcagcg gcagtggatc tgggacagaa ttcactctca caatcagcag cctgcagcct 240
gaagattttg gaacttatta ctgtctacag cataatagtc accccacctt cggccaaggg 300
accaaggtgg agatcaaa 318

<210> 128
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 128
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

```
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Lys Asn Asp
            20                  25                  30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
        35                  40                  45
Tyr Ala Ala Ser Arg Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Gly Thr Tyr Tyr Cys Leu Gln His Asn Ser His Pro Thr
                85                  90                  95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
```

<210> 129
<211> 381
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 129
gaggtgcagc tggtggagtc gggggggaggc atggtacagc ctgggggtc cctgagactc 60
tcctgtgcag cctctggatt cacctccagt aactacgaca tgcactgggt ccgccaagct 120
acaggaaaag gtctggagtg ggtctcaagt attgatactg ctggggacac ttactatcca 180
gactccgtga agggccgctt tatcatctcc agagaaaatg ccaaaaactc cctgtatctt 240
caaatgaata gcctgagagc cggggacacg gctgtgtatt actgtacaag ggagccccga 300
aattacgaaa ttttgactgg tcactaccac taccacggta tggacatctg gggccaaggg 360
accacggtca ccgtctcctc a 381

<210> 130
<211> 127
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 130
```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Met Val Gln Pro Gly Gly
 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Ser Ser Asn Tyr
            20                  25                  30
Asp Met His Trp Val Arg Gln Ala Thr Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ser Ser Ile Asp Thr Ala Gly Asp Thr Tyr Tyr Pro Asp Ser Val Lys
    50                  55                  60
Gly Arg Phe Ile Ile Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr Leu
65                  70                  75                  80
Gln Met Asn Ser Leu Arg Ala Gly Asp Thr Ala Val Tyr Tyr Cys Thr
                85                  90                  95
Arg Glu Pro Arg Asn Tyr Glu Ile Leu Thr Gly His Tyr His Tyr His
            100                 105                 110
Gly Met Asp Ile Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115                 120                 125
```

<210> 131
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 131
ggattcacct ccagtaacta cgac 24

<210> 132
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 132
Gly Phe Thr Ser Ser Asn Tyr Asp
1 5

<210> 133
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 133
attgatactg ctggggacac t 21

<210> 134
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 134
Ile Asp Thr Ala Gly Asp Thr
1 5

<210> 135
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 135
acaagggagc cccgaaatta cgaaattttg actggtcact accactacca cggtatggac 60

atc 63

<210> 136
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 136
Thr Arg Glu Pro Arg Asn Tyr Glu Ile Leu Thr Gly His Tyr His Tyr
1 5 10 15
His Gly Met Asp Ile
20

<210> 137
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 137
gacatccaga tgacccagtc gccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgcc gggcaagtca ggccattaga aatgatttag gctggtatca gcagaaacca 120
gggaaagccc ctaaactcct gatctatact gcattcagtt tacagagtgg ggtcccatca 180
aggttcagcg gcagtaaatc tggcacagac ttcactctca ccatcagcag cctgcagcct 240
gaagattttg cgacttatta ctgtctgcag gattacacta atcctcggac gttcggccaa 300
gggaccaagg tggagatcaa acga 324

<210> 138
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 138
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ala Ile Arg Asn Asp
20 25 30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Thr Ala Phe Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Lys Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Tyr Thr Asn Pro Arg
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 139
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 139
caggccatta gaaatgat 18

<210> 140
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 140
Gln Ala Ile Arg Asn Asp
1 5

<210> 141
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 141
actgcattc 9

<210> 142
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 142
Thr Ala Phe
1

<210> 143
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 143
ctgcaggatt acactaatcc tcggacg 27

<210> 144
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 144
Leu Gln Asp Tyr Thr Asn Pro Arg Thr
 1               5


<210> 145
<211> 378
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 145
gaggtgcagc tggtggagtc gggggggaggc atggtacagc ctgggggtc cctgagactc 60
tcctgtgcag cctctggatt cacctccagt aactacgaca tgcactgggt ccgccaagct 120
acaggaaaag gtctggagtg ggtctcaagt attgatactg ctggggacac ttactatcca 180
gactccgtga agggccgctt tatcatctcc agagaaaatg ccaaaaactc cctgtatctt 240
caaatgaata gcctgagagc cggggacacg gctgtgtatt actgtacaag ggagccccga 300
aattacgaaa ttttgactgg tcactaccac taccacggta tggacatctg gggccaaggg 360
accacggtca ccgtctcc                                              378

<210> 146
<211> 126
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 146
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Met Val Gln Pro Gly Gly
 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Ser Ser Asn Tyr
            20                  25                  30
Asp Met His Trp Val Arg Gln Ala Thr Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ser Ser Ile Asp Thr Ala Gly Asp Thr Tyr Tyr Pro Asp Ser Val Lys
    50                  55                  60
Gly Arg Phe Ile Ile Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr Leu
65                  70                  75                  80
Gln Met Asn Ser Leu Arg Ala Gly Asp Thr Ala Val Tyr Tyr Cys Thr
                85                  90                  95
Arg Glu Pro Arg Asn Tyr Glu Ile Leu Thr Gly His Tyr His Tyr His
            100                 105                 110
Gly Met Asp Ile Trp Gly Gln Gly Thr Thr Val Thr Val Ser
        115                 120                 125

<210> 147
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 147
gccatccaga tgacccagtc gccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgcc gggcaagtca ggccattaga aatgatttag gctggtatca gcagaaacca 120
gggaaagccc ctaaactcct gatctatact gcattcagtt tacagagtgg ggtcccatca 180
aggttcagcg gcagtaaatc tggcacagac ttcactctca ccatcagcag cctgcagcct 240
gaagattttg cgacttatta ctgtctgcag gattacacta atcctcggac gttcggccaa 300
gggaccaagg tggaaatcaa a 321

<210> 148
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 148
Ala Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1               5                  10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ala Ile Arg Asn Asp
            20                  25                  30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45
Tyr Thr Ala Phe Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Lys Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Tyr Thr Asn Pro Arg
                85                  90                  95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105


<210> 149
<211> 345
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 149
Gln Val Met Asp Phe Leu Phe Glu Lys Trp Lys Leu Tyr Gly Asp Gln
 1               5                  10                  15
Cys His His Asn Leu Ser Leu Leu Pro Pro Pro Thr Glu Leu Val Cys
            20                  25                  30
Asn Arg Thr Phe Asp Lys Tyr Ser Cys Trp Pro Asp Thr Pro Ala Asn
        35                  40                  45
Thr Thr Ala Asn Ile Ser Cys Pro Trp Tyr Leu Pro Trp His His Lys
    50                  55                  60

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

<210> 150
<211> 348
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

```
<400> 150
Gln Val Met Asp Phe Leu Phe Glu Lys Trp Lys Leu Tyr Gly Asp Gln
 1                  5                  10                  15
Cys His His Asn Leu Ser Leu Leu Pro Pro Pro Thr Glu Leu Val Cys
            20                  25                  30
Asn Arg Thr Phe Asp Lys Tyr Ser Cys Trp Pro Asp Thr Pro Ala Asn
        35                  40                  45
Thr Thr Ala Asn Ile Ser Cys Pro Trp Tyr Leu Pro Trp His His Lys
    50                  55                  60
Val Gln His Arg Phe Val Phe Lys Arg Cys Gly Pro Asp Gly Gln Trp
65                  70                  75                  80
Val Arg Gly Pro Arg Gly Gln Pro Trp Arg Asp Ala Ser Gln Cys Gln
```

```
                85                  90                  95
Met Asp Gly Glu Glu Ile Glu Val Gln Lys Glu Val Ala Lys Met Tyr
            100                 105                 110
Ser Ser Phe Gln Val Met Gly Pro Gly Asp Lys Thr His Thr Cys Pro
        115                 120                 125
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
    130                 135                 140
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
145                 150                 155                 160
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
                165                 170                 175
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
            180                 185                 190
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
        195                 200                 205
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
    210                 215                 220
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
225                 230                 235                 240
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
                245                 250                 255
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
            260                 265                 270
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
        275                 280                 285
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
    290                 295                 300
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
305                 310                 315                 320
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
                325                 330                 335
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
            340                 345
```

<210> 151
<211> 146
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 151

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

```
Ser Ser Phe Gln Val Met Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
        115                 120                 125
Gly Gly Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu His His His His
    130                 135                 140
His His
145
```

<210> 152
<211> 351
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 152

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

```
                325                 330                 335
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
            340                 345                 350


<210> 153
<211> 477
<212> PRT
<213> Homo sapiens

<400> 153
Met Pro Pro Cys Gln Pro Gln Arg Pro Leu Leu Leu Leu Leu Leu Leu
 1               5                  10                  15
Leu Ala Cys Gln Pro Gln Val Pro Ser Ala Gln Val Met Asp Phe Leu
            20                  25                  30
Phe Glu Lys Trp Lys Leu Tyr Gly Asp Gln Cys His His Asn Leu Ser
        35                  40                  45
Leu Leu Pro Pro Pro Thr Glu Leu Val Cys Asn Arg Thr Phe Asp Lys
    50                  55                  60
Tyr Ser Cys Trp Pro Asp Thr Pro Ala Asn Thr Thr Ala Asn Ile Ser
65                  70                  75                  80
Cys Pro Trp Tyr Leu Pro Trp His His Lys Val Gln His Arg Phe Val
                85                  90                  95
Phe Lys Arg Cys Gly Pro Asp Gly Gln Trp Val Arg Gly Pro Arg Gly
            100                 105                 110
Gln Pro Trp Arg Asp Ala Ser Gln Cys Gln Met Asp Gly Glu Glu Ile
        115                 120                 125
Glu Val Gln Lys Glu Val Ala Lys Met Tyr Ser Ser Phe Gln Val Met
    130                 135                 140
Tyr Thr Val Gly Tyr Ser Leu Ser Leu Gly Ala Leu Leu Leu Ala Leu
145                 150                 155                 160
Ala Ile Leu Gly Gly Leu Ser Lys Leu His Cys Thr Arg Asn Ala Ile
                165                 170                 175
His Ala Asn Leu Phe Ala Ser Phe Val Leu Lys Ala Ser Ser Val Leu
            180                 185                 190
Val Ile Asp Gly Leu Leu Arg Thr Arg Tyr Ser Gln Lys Ile Gly Asp
        195                 200                 205
Asp Leu Ser Val Ser Thr Trp Leu Ser Asp Gly Ala Val Ala Gly Cys
    210                 215                 220
Arg Val Ala Ala Val Phe Met Gln Tyr Gly Ile Val Ala Asn Tyr Cys
225                 230                 235                 240
Trp Leu Leu Val Glu Gly Leu Tyr Leu His Asn Leu Leu Gly Leu Ala
                245                 250                 255
Thr Leu Pro Glu Arg Ser Phe Phe Ser Leu Tyr Leu Gly Ile Gly Trp
            260                 265                 270
Gly Ala Pro Met Leu Phe Val Val Pro Trp Ala Val Val Lys Cys Leu
        275                 280                 285
Phe Glu Asn Val Gln Cys Trp Thr Ser Asn Asp Asn Met Gly Phe Trp
    290                 295                 300
Trp Ile Leu Arg Phe Pro Val Phe Leu Ala Ile Leu Ile Asn Phe Phe
305                 310                 315                 320
Ile Phe Val Arg Ile Val Gln Leu Leu Val Ala Lys Leu Arg Ala Arg
                325                 330                 335
Gln Met His His Thr Asp Tyr Lys Phe Arg Leu Ala Lys Ser Thr Leu
            340                 345                 350
Thr Leu Ile Pro Leu Leu Gly Val His Glu Val Val Phe Ala Phe Val
        355                 360                 365
Thr Asp Glu His Ala Gln Gly Thr Leu Arg Ser Ala Lys Leu Phe Phe
```

```
    370                 375                 380
Asp Leu Phe Leu Ser Ser Phe Gln Gly Leu Leu Val Ala Val Leu Tyr
385                 390                 395                 400
Cys Phe Leu Asn Lys Glu Val Gln Ser Glu Leu Arg Arg Arg Trp His
                405                 410                 415
Arg Trp Arg Leu Gly Lys Val Leu Trp Glu Glu Arg Asn Thr Ser Asn
            420                 425                 430
His Arg Ala Ser Ser Ser Pro Gly His Gly Pro Pro Ser Lys Glu Leu
        435                 440                 445
Gln Phe Gly Arg Gly Gly Gly Ser Gln Asp Ser Ser Ala Glu Thr Pro
    450                 455                 460
Leu Ala Gly Gly Leu Pro Arg Leu Ala Glu Ser Pro Phe
465                 470                 475
```

<210> 154
<211> 485
<212> PRT
<213> Mus musculus

<400> 154

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

```
    290                 295                 300
Trp Trp Ile Leu Arg Ile Pro Val Phe Leu Ala Leu Leu Ile Asn Phe
305                 310                 315                 320
Phe Ile Phe Val His Ile Ile His Leu Leu Val Ala Lys Leu Arg Ala
                325                 330                 335
His Gln Met His Tyr Ala Asp Tyr Lys Phe Arg Leu Ala Arg Ser Thr
            340                 345                 350
Leu Thr Leu Ile Pro Leu Leu Gly Val His Glu Val Val Phe Ala Phe
        355                 360                 365
Val Thr Asp Glu His Ala Gln Gly Thr Leu Arg Ser Thr Lys Leu Phe
    370                 375                 380
Phe Asp Leu Phe Leu Ser Ser Phe Gln Gly Leu Leu Val Ala Val Leu
385                 390                 395                 400
Tyr Cys Phe Leu Asn Lys Glu Val Gln Ala Glu Leu Met Arg Arg Trp
                405                 410                 415
Arg Gln Trp Gln Glu Gly Lys Ala Leu Gln Glu Glu Arg Leu Ala Ser
            420                 425                 430
Ser His Gly Ser His Met Ala Pro Ala Gly Pro Cys His Gly Asp Pro
        435                 440                 445
Cys Glu Lys Leu Gln Leu Met Ser Ala Gly Ser Ser Ser Gly Thr Gly
    450                 455                 460
Cys Val Pro Ser Met Glu Thr Ser Leu Ala Ser Ser Leu Pro Arg Leu
465                 470                 475                 480
Ala Asp Ser Pro Thr
                485
```

<210> 155
<211> 491
<212> PRT
<213> Macaca fascicularis

<400> 155

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

```
        195                 200                 205
Asp Leu Ser Val Ser Ile Trp Leu Ser Asp Gly Ala Val Ala Gly Cys
    210                 215                 220
Arg Val Ala Ala Val Phe Met Gln Tyr Gly Val Val Ala Asn Tyr Cys
225                 230                 235                 240
Trp Leu Leu Val Glu Gly Leu Tyr Leu His Asn Leu Leu Gly Leu Ala
                245                 250                 255
Thr Leu Pro Glu Arg Ser Phe Phe Ser Leu Tyr Leu Gly Ile Gly Trp
            260                 265                 270
Gly Ala Pro Met Leu Phe Ile Ile Pro Trp Val Val Val Arg Cys Leu
        275                 280                 285
Phe Glu Asn Ile Gln Cys Trp Thr Ser Asn Asp Asn Met Gly Phe Trp
    290                 295                 300
Trp Ile Leu Arg Phe Pro Val Phe Leu Ala Ile Leu Ile Asn Phe Phe
305                 310                 315                 320
Ile Phe Ile Arg Ile Val His Leu Leu Val Ala Lys Leu Arg Ala Arg
                325                 330                 335
Glu Met His His Thr Asp Tyr Lys Phe Arg Leu Ala Lys Ser Thr Leu
            340                 345                 350
Thr Leu Ile Pro Leu Leu Gly Val His Glu Val Val Phe Ala Phe Val
        355                 360                 365
Thr Asp Glu His Ala Gln Gly Thr Leu Arg Phe Ala Lys Leu Phe Phe
    370                 375                 380
Asp Leu Phe Leu Ser Ser Phe Gln Gly Leu Leu Val Ala Val Leu Tyr
385                 390                 395                 400
Cys Phe Leu Asn Lys Glu Val Gln Ser Glu Leu Arg Arg His Trp His
                405                 410                 415
Arg Trp Arg Leu Gly Lys Val Leu Gln Glu Glu Arg Gly Thr Ser Asn
            420                 425                 430
His Lys Ala Pro Ser Ala Pro Gly Gln Gly Leu Pro Gly Lys Lys Leu
        435                 440                 445
Gln Ser Gly Arg Asp Gly Gly Ser Gln Asp Ser Ser Ala Glu Ile Pro
    450                 455                 460
Leu Ala Gly Gly Leu Pro Arg Leu Ala Glu Ser Pro Phe Ser Thr Leu
465                 470                 475                 480
Leu Gly Pro Gln Leu Gly Leu Asp Ser Gly Thr
                485                 490
```

<210> 156
<211> 485
<212> PRT
<213> Rattus norvegicus

<400> 156

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

```
            100                 105                 110
Gly Gln Ser Trp Arg Asp Ala Ser Gln Cys Gln Met Asp Asp Asp Glu
        115                 120                 125
Ile Glu Val Gln Lys Gly Val Ala Lys Met Tyr Ser Ser Tyr Gln Val
    130                 135                 140
Met Tyr Thr Val Gly Tyr Ser Leu Ser Leu Gly Ala Leu Leu Leu Ala
145                 150                 155                 160
Leu Val Ile Leu Leu Gly Leu Arg Lys Leu His Cys Thr Arg Asn Tyr
                165                 170                 175
Ile His Gly Asn Leu Phe Ala Ser Phe Val Leu Lys Ala Gly Ser Val
            180                 185                 190
Leu Val Ile Asp Trp Leu Leu Lys Thr Arg Tyr Ser Gln Lys Ile Gly
        195                 200                 205
Asp Asp Leu Ser Val Ser Val Trp Leu Ser Asp Gly Ala Val Ala Gly
    210                 215                 220
Cys Arg Val Ala Thr Val Ile Met Gln Tyr Gly Ile Ile Ala Asn Tyr
225                 230                 235                 240
Cys Trp Leu Leu Val Glu Gly Val Tyr Leu Tyr Ser Leu Leu Ser Ile
                245                 250                 255
Thr Thr Phe Ser Glu Lys Ser Phe Phe Ser Leu Tyr Leu Cys Ile Gly
            260                 265                 270
Trp Gly Ser Pro Leu Leu Phe Val Ile Pro Trp Val Val Val Lys Cys
        275                 280                 285
Leu Phe Glu Asn Val Gln Cys Trp Thr Ser Asn Asp Asn Met Gly Phe
    290                 295                 300
Trp Trp Ile Leu Arg Ile Pro Val Leu Leu Ala Ile Leu Ile Asn Phe
305                 310                 315                 320
Phe Ile Phe Val Arg Ile Ile His Leu Leu Val Ala Lys Leu Arg Ala
                325                 330                 335
His Gln Met His Tyr Ala Asp Tyr Lys Phe Arg Leu Ala Arg Ser Thr
            340                 345                 350
Leu Thr Leu Ile Pro Leu Leu Gly Val His Glu Val Val Phe Ala Phe
        355                 360                 365
Val Thr Asp Glu His Ala Gln Gly Thr Leu Arg Ser Thr Lys Leu Phe
    370                 375                 380
Phe Asp Leu Phe Phe Ser Ser Phe Gln Gly Leu Leu Val Ala Val Leu
385                 390                 395                 400
Tyr Cys Phe Leu Asn Lys Glu Val Gln Ala Glu Leu Leu Arg Arg Trp
                405                 410                 415
Arg Arg Trp Gln Glu Gly Lys Ala Leu Gln Glu Glu Arg Met Ala Ser
            420                 425                 430
Ser His Gly Ser His Met Ala Pro Ala Gly Thr Cys His Gly Asp Pro
        435                 440                 445
Cys Glu Lys Leu Gln Leu Met Ser Ala Gly Ser Ser Ser Gly Thr Gly
    450                 455                 460
Cys Glu Pro Ser Ala Lys Thr Ser Leu Ala Ser Ser Leu Pro Arg Leu
465                 470                 475                 480
Ala Asp Ser Pro Thr
                485
```

<210> 157
<211> 1434
<212> DNA
<213> Homo sapiens

<400> 157
atgcccccct gccagccaca gcgacccctg ctgctgttgc tgctgctgct ggcctgccag 60

ccacaggtcc cctccgctca ggtgatggac ttcctgtttg agaagtggaa gctctacggt 120
gaccagtgtc accacaacct gagcctgctg ccccctccca cggagctggt gtgcaacaga 180
accttcgaca agtattcctg ctggccggac acccccgcca ataccacggc caacatctcc 240
tgcccctggt acctgccttg gcaccacaaa gtgcaacacc gcttcgtgtt caagagatgc 300
gggcccgacg gtcagtgggt gcgtggaccc cgggggcagc cttggcgtga tgcctcccag 360
tgccagatgg atggcgagga gattgaggtc cagaaggagg tggccaagat gtacagcagc 420
ttccaggtga tgtacacagt gggctacagc ctgtccctgg gggccctgct cctcgccttg 480
gccatcctgg ggggcctcag caagctgcac tgcacccgca atgccatcca cgcgaatctg 540
tttgcgtcct tcgtgctgaa agccagctcc gtgctggtca ttgatgggct gctcaggacc 600
cgctacagcc agaaaattgg cgacgacctc agtgtcagca cctggctcag tgatggagcg 660
gtggctggct gccgtgtggc cgcggtgttc atgcaatatg gcatcgtggc caactactgc 720
tggctgctgg tggagggcct gtacctgcac aacctgctgg gcctggccac cctccccgag 780
aggagcttct tcagcctcta cctgggcatc ggctggggtg cccccatgct gttcgtcgtc 840
ccctgggcag tggtcaagtg tctgttcgag aacgtccagt gctggaccag caatgacaac 900
atgggcttct ggtggatcct gcggttcccc gtcttcctgg ccatcctgat caacttcttc 960
atcttcgtcc gcatcgttca gctgctcgtg gccaagctgc gggcacggca gatgcaccac
1020
acagactaca agttccggct ggccaagtcc acgctgaccc tcatccctct gctgggcgtc
1080
cacgaagtgg tctttgcctt cgtgacggac gagcacgccc agggcaccct gcgctccgcc
1140
aagctcttct tcgacctctt cctcagctcc ttccagggcc tgctggtggc tgtcctctac
1200
tgcttcctca acaaggaggt gcagtcggag ctgcggcggc gttggcaccg ctggcgcctg
1260
ggcaaagtgc tatgggagga gcggaacacc agcaaccaca gggcctcatc ttcgcccggc
1320
cacggccctc ccagcaagga gctgcagttt gggaggggtg gtggcagcca ggattcatct
1380
gcggagaccc ccttggctgg tggcctccct agattggctg agagcccctt ctga
1434

<210> 158
<211> 354
<212> DNA
<213> Homo sapiens

<400> 158
caggtgatgg acttcctgtt tgagaagtgg aagctctacg gtgaccagtg tcaccacaac 60
ctgagcctgc tgcccccctcc cacggagctg gtgtgcaaca gaaccttcga caagtattcc 120
tgctggccgg acacccccgc caataccacg gccaacatct cctgcccctg gtacctgcct 180
tggcaccaca aagtgcaaca ccgcttcgtg ttcaagagat gcgggcccga cggtcagtgg 240
gtgcgtggac cccgggggca gccttggcgt gatgcctccc agtgccagat ggatggcgag 300
gagattgagg tccagaagga ggtggccaag atgtacagca gcttccaggt gatg 354

<210> 159
<211> 118
<212> PRT
<213> Homo sapiens

<400> 159
Gln Val Met Asp Phe Leu Phe Glu Lys Trp Lys Leu Tyr Gly Asp Gln
 1               5                  10                  15
Cys His His Asn Leu Ser Leu Leu Pro Pro Pro Thr Glu Leu Val Cys
            20                  25                  30
Asn Arg Thr Phe Asp Lys Tyr Ser Cys Trp Pro Asp Thr Pro Ala Asn
        35                  40                  45
Thr Thr Ala Asn Ile Ser Cys Pro Trp Tyr Leu Pro Trp His His Lys

```
    50                  55                  60
Val Gln His Arg Phe Val Phe Lys Arg Cys Gly Pro Asp Gly Gln Trp
65                  70                  75                  80
Val Arg Gly Pro Arg Gly Gln Pro Trp Arg Asp Ala Ser Gln Cys Gln
                85                  90                  95
Met Asp Gly Glu Glu Ile Glu Val Gln Lys Glu Val Ala Lys Met Tyr
            100                 105                 110
Ser Ser Phe Gln Val Met
        115
```

<210> 160
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 160
ggattcacct ttaacaacta tgcc 24

<210> 161
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 161
```
Gly Phe Thr Phe Asn Asn Tyr Ala
 1               5
```

<210> 162
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 162
attagtggta gcggtggtac taca 24

<210> 163
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 163
```
Ile Ser Gly Ser Gly Gly Thr Thr
 1               5
```

<210> 164
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 164
gcgaaagatt ctaactgggg aaatttcgat ctc 33

<210> 165
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 165
Ala Lys Asp Ser Asn Trp Gly Asn Phe Asp Leu
1 5 10

<210> 166
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 166
cagagtgttt tatacaggtc caacaatagg aacttc 36

<210> 167
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 167
Gln Ser Val Leu Tyr Arg Ser Asn Asn Arg Asn Phe
1 5 10

<210> 168
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 168

tgggcatct 9

<210> 169
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 169
Trp Ala Ser
1

<210> 170
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 170
caacaatatt atactactcc gtacact 27

<210> 171
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 171
Gln Gln Tyr Tyr Thr Thr Pro Tyr Thr
1 5

<210> 172
<211> 354
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 172
gaggtgcagc tggtggagtc tgggggaggc ttggtacagc ctgggggtc cctgagactc 60
tcctgtgcag cctctggatt cacctttaac aactatgcca tgaactgggt ccgccaggct 120
ccaggaaagg gactggactg ggtctcaact attagtggta gcggtggtac tacaaactac 180
gcagactccg tgaagggccg tttcattatt tcccgagaca gttccaaaca cacgctgtat 240
ctgcaaatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagattct 300
aactggggaa atttcgatct ctggggccgt ggcaccctgg tcactgtctc ctca 354

<210> 173
<211> 118
<212> PRT

<213> Artificial Sequence

<220>
<223> Synthetic

<400> 173
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Asn Tyr
            20                  25                  30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Asp Trp Val
        35                  40                  45
Ser Thr Ile Ser Gly Ser Gly Gly Thr Thr Asn Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Ile Ile Ser Arg Asp Ser Ser Lys His Thr Leu Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Lys Asp Ser Asn Trp Gly Asn Phe Asp Leu Trp Gly Arg Gly Thr
            100                 105                 110
Leu Val Thr Val Ser Ser
        115

<210> 174
<211> 339
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 174
gacatcgtga tgacccagtc tccagactcc ctggctgtgt ctctgggcga gagggccacc 60
atcaactgca agtccagcca gagtgtttta tacaggtcca acaataggaa cttcttaggt 120
tggtaccagc agaaaccagg gcagcctcct aatctactca tttactgggc atctacccgg 180
gaatccgggg tccctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc 240
atcagcagcc tgcaggctga agatgtggca gtttattact gtcaacaata ttatactact 300
ccgtacactt ttggccaggg gaccaagctg gagatcaaa                        339

<210> 175
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 175
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
 1               5                  10                  15
Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Arg
            20                  25                  30
Ser Asn Asn Arg Asn Phe Leu Gly Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45
Pro Pro Asn Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60
Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr

65 70 75 80
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95
Tyr Tyr Thr Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
100 105 110
Lys

<210> 176
<211> 381
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 176
gagatgcaac tggtggagtc tgggggaggc ttggtccagc ctgggggtc cctgagactc 60
tcctgtgcag cctctggatt cacctttagt agtcactgga tgaagtgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtggccaac ataaaccaag atggaagtga gaaatactat 180
gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctcactgttt 240
ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagagatatt 300
gtactaatgg tctatgatat ggactactac tactacggta tggacgtctg gggccaaggg 360
accacggtca ccgtctcctc a 381

<210> 177
<211> 127
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 177
Glu Met Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
20 25 30
Trp Met Lys Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Asn Ile Asn Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Phe
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asp Ile Val Leu Met Val Tyr Asp Met Asp Tyr Tyr Tyr Tyr
100 105 110
Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125

<210> 178
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 178
ggattcacct ttagtagtca ctgg 24

<210> 179
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 179
Gly Phe Thr Phe Ser Ser His Trp
1 5

<210> 180
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 180
ataaaccaag atggaagtga gaaa 24

<210> 181
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 181
Ile Asn Gln Asp Gly Ser Glu Lys
1 5

<210> 182
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 182
gcgagagata ttgtactaat ggtctatgat atggactact actactacgg tatggacgtc 60

<210> 183
<211> 20
<212> PRT

<213> Artificial Sequence

<220>
<223> Synthetic

<400> 183
Ala Arg Asp Ile Val Leu Met Val Tyr Asp Met Asp Tyr Tyr Tyr Tyr
 1               5                  10                  15
Gly Met Asp Val
            20

<210> 184
<211> 336
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 184
gatattgtga tgactcagtc tccactctcc ctgcccgtca cccctggaga gccggcctcc 60
atctcctgca ggtctagtca gagcctcctg catagtaatg gaaacaacta tttggattgg 120
tacctgcaga agccagggca gtctccacag ctcctgatct atttgggttc taatcgggcc 180
tccggggtcc ctgacaggtt cagtggcagt ggatcaggca cagattttac actgaaaatc 240
agcagagtgg aggctgagga tgttggggtt tattactgca tgcaaactct acaaactccg 300
ctcactttcg gcggagggac caaggtggag atcaaa                          336

<210> 185
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 185
Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
 1               5                  10                  15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
            20                  25                  30
Asn Gly Asn Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
    50                  55                  60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Thr
                85                  90                  95
Leu Gln Thr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

<210> 186
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 186
cagagcctcc tgcatagtaa tggaaacaac tat 33

<210> 187
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 187
Gln Ser Leu Leu His Ser Asn Gly Asn Asn Tyr
1 5 10

<210> 188
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 188
ttgggttct 9

<210> 189
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 189
Leu Gly Ser
1

<210> 190
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 190
atgcaaactc tacaaactcc gctcact 27

<210> 191
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 191
Met Gln Thr Leu Gln Thr Pro Leu Thr
1 5

<210> 192
<211> 2076
<212> DNA
<213> Homo sapiens

<400> 192
atgggcaccg tcagctccag gcggtcctgg tggccgctgc cactgctgct gctgctgctg 60
ctgctcctgg gtcccgcggg cgcccgtgcg caggaggacg aggacggcga ctacgaggag 120
ctggtgctag ccttgcgttc cgaggaggac ggcctggccg aagcacccga gcacggaacc 180
acagccacct tccaccgctg cgccaaggat ccgtggaggt tgcctggcac ctacgtggtg 240
gtgctgaagg aggagaccca cctctcgcag tcagagcgca ctgcccgccg cctgcaggcc 300
caggctgccc gccggggata cctcaccaag atcctgcatg tcttccatgg ccttcttcct 360
ggcttcctgg tgaagatgag tggcgacctg ctggagctgg ccttgaagtt gccccatgtc 420
gactacatcg aggaggactc ctctgtcttt gcccagagca tcccgtggaa cctggagcgg 480
attacccctc cacggtaccg ggcggatgaa taccagcccc ccgacggagg cagcctggtg 540
gaggtgtatc tcctagacac cagcatacag agtgaccacc gggaaatcga gggcagggtc 600
atggtcaccg acttcgagaa tgtgcccgag gaggacggga cccgcttcca cagacaggcc 660
agcaagtgtg acagtcatgg cacccacctg gcaggggtgg tcagcggccg ggatgccggc 720
gtggccaagg gtgccagcat gcgcagcctg cgcgtgctca actgccaagg gaagggcacg 780
gttagcggca ccctcatagg cctggagttt attcggaaaa gccagctggt ccagcctgtg 840
gggccactgg tggtgctgct gcccctggcg ggtgggtaca gccgcgtcct caacgccgcc 900
tgccagcgcc tggcgaggge tggggtcgtg ctggtcaccg ctgccggcaa cttccgggac 960
gatgcctgcc tctactcccc agcctcagct cccgaggtca tcacagttgg ggccaccaat
1020
gcccaggacc agccggtgac cctggggact ttggggacca actttggccg ctgtgtggac
1080
ctctttgccc caggggagga catcattggt gcctccagcg actgcagcac ctgctttgtg
1140
tcacagagtg ggacatcaca ggctgctgcc cacgtggctg gcattgcagc catgatgctg
1200
tctgccgagc cggagctcac cctggccgag ttgaggcaga gactgatcca cttctctgcc
1260
aaagatgtca tcaatgaggc ctggttccct gaggaccagc gggtactgac ccccaacctg
1320
gtggccgccc tgcccccccag cacccatggg gcaggttggc agctgttttg caggactgtg
1380
tggtcagcac actcggggcc tacacggatg gccacagcca tcgcccgctg cgccccagat
1440
gaggagctgc tgagctgctc cagtttctcc aggagtggga agcggcgggg cgagcgcatg
1500
gaggcccaag ggggcaagct ggtctgccgg gcccacaacg cttttggggg tgagggtgtc
1560
tacgccattg ccaggtgctg cctgctaccc caggccaact gcagcgtcca cacagctcca
1620
ccagctgagg ccagcatggg gacccgtgtc cactgccacc aacagggcca cgtcctcaca
1680
ggctgcagct cccactggga ggtggaggac cttggcaccc acaagccgcc tgtgctgagg
1740

ccacgaggtc agcccaacca gtgcgtgggc cacagggagg ccagcatcca cgcttcctgc
1800
tgccatgccc caggtctgga atgcaaagtc aaggagcatg gaatcccggc ccctcaggag
1860
caggtgaccg tggcctgcga ggagggctgg accctgactg gctgcagtgc cctccctggg
1920
acctcccacg tcctgggggc ctacgccgta gacaacacgt gtgtagtcag gagccgggac
1980
gtcagcacta caggcagcac cagcgaagag gccgtgacag ccgttgccat ctgctgccgg
2040
agccggcacc tggcgcaggc ctcccaggag ctccag
2076

<210> 193
<211> 692
<212> PRT
<213> Homo sapiens

<400> 193
Met Gly Thr Val Ser Ser Arg Arg Ser Trp Trp Pro Leu Pro Leu Leu
 1               5                  10                  15
Leu Leu Leu Leu Leu Leu Leu Gly Pro Ala Gly Ala Arg Ala Gln Glu
            20                  25                  30
Asp Glu Asp Gly Asp Tyr Glu Glu Leu Val Leu Ala Leu Arg Ser Glu
        35                  40                  45
Glu Asp Gly Leu Ala Glu Ala Pro Glu His Gly Thr Thr Ala Thr Phe
    50                  55                  60
His Arg Cys Ala Lys Asp Pro Trp Arg Leu Pro Gly Thr Tyr Val Val
65                  70                  75                  80
Val Leu Lys Glu Glu Thr His Leu Ser Gln Ser Glu Arg Thr Ala Arg
                85                  90                  95
Arg Leu Gln Ala Gln Ala Ala Arg Arg Gly Tyr Leu Thr Lys Ile Leu
            100                 105                 110
His Val Phe His Gly Leu Leu Pro Gly Phe Leu Val Lys Met Ser Gly
        115                 120                 125
Asp Leu Leu Glu Leu Ala Leu Lys Leu Pro His Val Asp Tyr Ile Glu
    130                 135                 140
Glu Asp Ser Ser Val Phe Ala Gln Ser Ile Pro Trp Asn Leu Glu Arg
145                 150                 155                 160
Ile Thr Pro Pro Arg Tyr Arg Ala Asp Glu Tyr Gln Pro Pro Asp Gly
                165                 170                 175
Gly Ser Leu Val Glu Val Tyr Leu Leu Asp Thr Ser Ile Gln Ser Asp
            180                 185                 190
His Arg Glu Ile Glu Gly Arg Val Met Val Thr Asp Phe Glu Asn Val
        195                 200                 205
Pro Glu Glu Asp Gly Thr Arg Phe His Arg Gln Ala Ser Lys Cys Asp
    210                 215                 220
Ser His Gly Thr His Leu Ala Gly Val Val Ser Gly Arg Asp Ala Gly
225                 230                 235                 240
Val Ala Lys Gly Ala Ser Met Arg Ser Leu Arg Val Leu Asn Cys Gln
                245                 250                 255
Gly Lys Gly Thr Val Ser Gly Thr Leu Ile Gly Leu Glu Phe Ile Arg
            260                 265                 270
Lys Ser Gln Leu Val Gln Pro Val Gly Pro Leu Val Val Leu Leu Pro
        275                 280                 285
Leu Ala Gly Gly Tyr Ser Arg Val Leu Asn Ala Ala Cys Gln Arg Leu
    290                 295                 300
Ala Arg Ala Gly Val Val Leu Val Thr Ala Ala Gly Asn Phe Arg Asp

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305                 310                 315                 320
Asp Ala Cys Leu Tyr Ser Pro Ala Ser Ala Pro Glu Val Ile Thr Val
                325                 330                 335
Gly Ala Thr Asn Ala Gln Asp Gln Pro Val Thr Leu Gly Thr Leu Gly
            340                 345                 350
Thr Asn Phe Gly Arg Cys Val Asp Leu Phe Ala Pro Gly Glu Asp Ile
        355                 360                 365
Ile Gly Ala Ser Ser Asp Cys Ser Thr Cys Phe Val Ser Gln Ser Gly
    370                 375                 380
Thr Ser Gln Ala Ala Ala His Val Ala Gly Ile Ala Ala Met Met Leu
385                 390                 395                 400
Ser Ala Glu Pro Glu Leu Thr Leu Ala Glu Leu Arg Gln Arg Leu Ile
                405                 410                 415
His Phe Ser Ala Lys Asp Val Ile Asn Glu Ala Trp Phe Pro Glu Asp
            420                 425                 430
Gln Arg Val Leu Thr Pro Asn Leu Val Ala Ala Leu Pro Pro Ser Thr
        435                 440                 445
His Gly Ala Gly Trp Gln Leu Phe Cys Arg Thr Val Trp Ser Ala His
    450                 455                 460
Ser Gly Pro Thr Arg Met Ala Thr Ala Ile Ala Arg Cys Ala Pro Asp
465                 470                 475                 480
Glu Glu Leu Leu Ser Cys Ser Ser Phe Ser Arg Ser Gly Lys Arg Arg
                485                 490                 495
Gly Glu Arg Met Glu Ala Gln Gly Gly Lys Leu Val Cys Arg Ala His
            500                 505                 510
Asn Ala Phe Gly Gly Glu Gly Val Tyr Ala Ile Ala Arg Cys Cys Leu
        515                 520                 525
Leu Pro Gln Ala Asn Cys Ser Val His Thr Ala Pro Pro Ala Glu Ala
    530                 535                 540
Ser Met Gly Thr Arg Val His Cys His Gln Gln Gly His Val Leu Thr
545                 550                 555                 560
Gly Cys Ser Ser His Trp Glu Val Glu Asp Leu Gly Thr His Lys Pro
                565                 570                 575
Pro Val Leu Arg Pro Arg Gly Gln Pro Asn Gln Cys Val Gly His Arg
            580                 585                 590
Glu Ala Ser Ile His Ala Ser Cys Cys His Ala Pro Gly Leu Glu Cys
        595                 600                 605
Lys Val Lys Glu His Gly Ile Pro Ala Pro Gln Glu Gln Val Thr Val
    610                 615                 620
Ala Cys Glu Glu Gly Trp Thr Leu Thr Gly Cys Ser Ala Leu Pro Gly
625                 630                 635                 640
Thr Ser His Val Leu Gly Ala Tyr Ala Val Asp Asn Thr Cys Val Val
                645                 650                 655
Arg Ser Arg Asp Val Ser Thr Thr Gly Ser Thr Ser Glu Glu Ala Val
            660                 665                 670
Thr Ala Val Ala Ile Cys Cys Arg Ser Arg His Leu Ala Gln Ala Ser
        675                 680                 685
Gln Glu Leu Gln
    690
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<210> 194
<211> 115
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 194
Glu Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val His Pro Gly Gly
 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Arg Asn
            20                  25                  30
Ala Met Phe Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ser Gly Ile Gly Thr Gly Gly Ala Thr Asn Tyr Ala Asp Ser Val Lys
    50                  55                  60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65                  70                  75                  80
Gln Met Asn Ser Leu Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys Ala
                85                  90                  95
Arg Gly Arg Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
            100                 105                 110
Val Ser Ser
        115


<210> 195
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 195
Gly Phe Thr Phe Ser Arg Asn Ala
 1               5


<210> 196
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 196
Ile Gly Thr Gly Gly Ala Thr
 1               5


<210> 197
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 197
Ala Arg Gly Arg Tyr Tyr Phe Asp Tyr
 1               5

<210> 198
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 198
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1               5                  10                  15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
            20                  25                  30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
        35                  40                  45
Ile Phe Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65                  70                  75                  80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
                85                  90                  95
Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105


<210> 199
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 199
Gln Ser Val Ser Ser Ser Tyr
 1               5


<210> 200
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 200
Gly Ala Ser
 1


<210> 201
<211> 10
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic

<400> 201
Gln Gln Tyr Gly Ser Ser Pro Pro Trp Thr
 1               5                  10


<210> 202
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> VARIANT
<222> (1)...(1)
<223> Xaa = Gly

<220>
<221> VARIANT
<222> (2)...(2)
<223> Xaa = Phe

<220>
<221> VARIANT
<222> (3)...(3)
<223> Xaa = Thr

<220>
<221> VARIANT
<222> (4)...(4)
<223> Xaa = Phe or Ser


<220>
<221> VARIANT
<222> (5)...(5)
<223> Xaa = Ser


<220>
<221> VARIANT
<222> (6)...(6)
<223> Xaa = Ser or Asn


<220>
<221> VARIANT
<222> (7)...(7)
<223> Xaa = Tyr or Phe


<220>
<221> VARIANT
<222> (8)...(8)
<223> Xaa = Asp, Leu, or Gly

<400> 202
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1               5


<210> 203
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> VARIANT
<222> (1)...(1)
<223> Xaa = Ile

<220>
<221> VARIANT
<222> (2)...(2)
<223> Xaa = Ser, Gln, Asp, or Trp

<220>
<221> VARIANT
<222> (3)...(3)
<223> Xaa = Ser, Glu, Thr, or Phe

<220>
<221> VARIANT
<222> (4)...(4)
<223> Xaa = Asp or Ala


<220>
<221> VARIANT
<222> (5)...(5)
<223> Xaa = Gly or Glu


<220>
<221> VARIANT
<222> (6)...(6)
<223> Xaa = Arg, Ile, or absent


<220>
<221> VARIANT
<222> (7)...(7)
<223> Xaa = Asp or Glu


<220>
<221> VARIANT
<222> (8)...(8)
<223> Xaa = Lys or Thr

<400> 203
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1               5

<210> 204
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> VARIANT
<222> (1)...(1)
<223> Xaa = Ala or Thr

<220>
<221> VARIANT
<222> (2)...(2)
<223> Xaa = Lys or Arg

<220>
<221> VARIANT
<222> (3)...(3)
<223> Xaa = Glu

<220>
<221> VARIANT
<222> (4)...(4)
<223> Xaa = Met, Pro, Gly, or Asp

<220>
<221> VARIANT
<222> (5)...(5)
<223> Xaa = Val, Ser, Lys, Arg, or absent

<220>
<221> VARIANT
<222> (6)...(6)
<223> Xaa = Tyr, His, Asn, or absent

<220>
<221> VARIANT
<222> (7)...(7)
<223> Xaa = Tyr

<220>
<221> VARIANT
<222> (8)...(8)
<223> Xaa = Asp or Glu

<220>
<221> VARIANT
<222> (9)...(9)
<223> Xaa = Ile

<220>
<221> VARIANT
<222> (10)...(10)
<223> Xaa = Leu

<220>
<221> VARIANT
<222> (11)...(11)
<223> Xaa = Thr

<220>
<221> VARIANT
<222> (12)...(12)
<223> Xaa = Gly

<220>
<221> VARIANT
<222> (13)...(13)
<223> Xaa = Tyr, Asp, or His

<220>
<221> VARIANT
<222> (14)...(14)
<223> Xaa = His, Asp, Tyr, or absent

<220>
<221> VARIANT
<222> (15)...(15)
<223> Xaa = Asn, Tyr, His, or absent

<220>
<221> VARIANT
<222> (16)...(16)
<223> Xaa = Tyr

<220>
<221> VARIANT
<222> (17)...(17)
<223> Xaa = Tyr or His

<220>
<221> VARIANT
<222> (18)...(18)
<223> Xaa = Gly or Ala

<220>
<221> VARIANT
<222> (19)...(19)
<223> Xaa = Met

<220>
<221> VARIANT
<222> (20)...(20)
<223> Xaa = Asp

<220>
<221> VARIANT
<222> (21)...(21)
<223> Xaa = Val or Ile

<400> 204
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1               5                  10                  15
Xaa Xaa Xaa Xaa Xaa
            20

<210> 205
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> VARIANT
<222> (1)...(1)
<223> Xaa = Gln

<220>
<221> VARIANT
<222> (2)...(2)
<223> Xaa = Gly or Ala

<220>
<221> VARIANT
<222> (3)...(3)
<223> Xaa = Ile

<220>
<221> VARIANT
<222> (4)...(4)
<223> Xaa = Asn or Arg

<220>
<221> VARIANT
<222> (5)...(5)

<223> Xaa = Asn

<220>
<221> VARIANT
<222> (6)...(6)
<223> Xaa = Tyr or Asp

<400> 205
Xaa Xaa Xaa Xaa Xaa Xaa
 1               5

<210> 206
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> VARIANT
<222> (1)...(1)
<223> Xaa = Thr or Ala

<220>
<221> VARIANT
<222> (2)...(2)
<223> Xaa = Ala or Thr

<220>
<221> VARIANT
<222> (3)...(3)
<223> Xaa = Ser or Phe

<400> 206
Xaa Xaa Xaa
 1

<210> 207
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> VARIANT
<222> (1)...(1)
<223> Xaa = Gln or Leu

<220>
<221> VARIANT
<222> (2)...(2)
<223> Xaa = Gln

<220>
<221> VARIANT
<222> (3)...(3)
<223> Xaa = Tyr, His, or Asp

<220>
<221> VARIANT
<222> (4)...(4)
<223> Xaa = Asn or Tyr

<220>
<221> VARIANT
<222> (5)...(5)
<223> Xaa = Thr or Ser

<220>
<221> VARIANT
<222> (6)...(6)
<223> Xaa = Tyr, Asn, or His

<220>
<221> VARIANT
<222> (7)...(7)
<223> Xaa = Pro

<220>
<221> VARIANT
<222> (8)...(8)
<223> Xaa = Leu, Phe, Arg, or absent

<220>
<221> VARIANT
<222> (9)...(9)
<223> Xaa = Thr

<400> 207
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1               5