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(54) Title: SORCS1-LIKE AGENT FOR USE IN THE TREATMENT OF INSULIN RESISTANCE AND DISEASES RELATED THERETO

(57) Abstract: The present invention relates to Sor CS1-like agents, including Sor CS1, nucleic acid molecule encoding expression of Sor CS1 and fragments thereof, as well as vectors containing said nucleic acid and to cells expressing Sor CS1 and said fragments, for use in the treatment of insulin resistance.



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SorCS1-like agent for use in the treatment of insulin resistance and diseases related thereto

5 All patent and non-patent references cited in the application, or in the present application, are hereby incorporated by reference in their entirety.

Field of invention

10 The present invention relates to the use of SorCS1-like agents, such as SorCS1 and fragments and variants thereof, for the preparation of a medicament for the treatment, reduction or delay of insulin resistance in patients. The invention furthermore relates to the use of SorCS1-like agents, such as SorCS1 and fragments and variants thereof for sensitizing insulin receptors. The invention also relates to the use of the SorCS1 knockout mouse as an animal model of insulin resistance.

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Background of the invention

The insulin resistance syndrome.

20 The prevalence of metabolic disturbance, collectively known as metabolic syndrome or insulin resistance syndrome, has reached an epidemic proportion in industrialized countries. The insulin resistance syndrome refers to a constellation of findings, including glucose intolerance, obesity, an altered lipid profile (dyslipidemia) and hypertension, that promote the development of type 2 diabetes, cardiovascular disease, cancer, polycystic ovarian disease, and other disorders. In all of these disorders, a central component of the pathophysiology is insulin resistance. The underlying causes of this syndrome are overweight/obesity, physical inactivity and a series of currently not yet well-defined genetic polymorphisms (reviewed in 1+2). Lifestyle interventions and pharmacological treatment of the pathologies of the syndrome are only partially efficient and new therapeutic approaches are urgently needed.

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Insulin and insulin resistance.

35 Insulin is a hormone produced by β -cells in the islets of Langerhans in the pancreas. Insulin release is stimulated as blood glucose levels rise and glucose is removed from the blood by insulin dependent stimulation of glucose transporters located in the cell membranes of target tissue, in particular in adipose tissue, skeletal muscle

and liver. Insulin exerts its biological effects by binding to and activating the membrane-bound insulin receptor (IR), thereby initiating a cascade of intracellular signaling events, which regulates multiple biological processes such as glucose and lipid metabolism, gene expression, protein synthesis, and non-metabolic processes such as cell growth and differentiation. The diverse effects of IR activation are mediated through a multicomponent signaling complex that assembles upon binding of insulin. Thus, the intrinsic protein-tyrosine kinase activity of IR results in autophosphorylation of several tyrosine residues followed by recruitment and phosphorylation of several intracellular protein substrates including IR substrate (IRS) proteins and Src-homology-2 containing (Shc) proteins. This initiates the activation of two main signalling pathways: the phosphatidylinositol 3-kinase (PI3K)-AKT/protein kinase B (PKB) pathway, which is responsible for most of the metabolic actions, including translocation of the glucose transporter GLUT4 to the cell membrane and stimulation of glycogen synthesis, and the Ras-mitogen-activated protein kinase (MAPK) pathway, which regulates expression of some genes and cooperates with the (PI3K)-AKT pathway to control cell growth and differentiation (reviewed in 3-8).

The ability of insulin to stimulate glucose disposal vary continuously throughout a population of apparently healthy persons, and a difference of $\geq 600\%$ exists between the most insulin-sensitive and the most insulin resistance persons. However, the third of the population that is the most insulin resistant is at a much greater risk of developing several abnormalities and clinical syndromes, including type 2 diabetes, cardio vascular diseases, hypertension, stroke, non-alcoholic fatty liver, polycystic ovary disease, and certain forms of cancer (reviewed in 9)

Individuals are said to be 'insulin resistant', because their tissues behave as if there was insufficient insulin in the bloodstream as reflected by decreased insulin response and glucose uptake in liver, adipose tissue, and skeletal muscle. The first response to insulin resistance is a compensatory production and secretion of insulin to compensate for the body's decreased sensitivity, leading to hyperinsulinaemia. Thus, high insulin levels and a decreased responsiveness of tissue to the clearance of glucose from the bloodstream characterize insulin resistance. Insulin resistance is the primary event leading to a series of metabolic changes including compensatory hyperinsulinemia, dyslipidemia, decompensation of pancreatic beta-cells, and hyperglycemia (reviewed in 6-8).

Type 2 diabetes and insulin resistance.

Type-2 diabetes (non-insulin-dependent diabetes) is a complex and heterogeneous disorder associated with an increased risk for mortality as well as morbidity. The incidence is steadily increasing and the disease presently affects more than 150 million people worldwide making it a major public concern. The disorder is a prototypic complex polygenic disease with a strong heritable component but is also heavily influenced by environmental factors such as e.g. obesity. The pathogenesis of type 2 diabetes involves progressive development of insulin resistance in liver and peripheral tissue accompanied by defective insulin secretion from pancreatic beta cells leading to overt hyperglycaemia (an abnormally high amount of glucose levels in blood). The first response to insulin resistance is a compensatory production and secretion of insulin to compensate for the body's decreased sensitivity, leading to hyperinsulinaemia and rendering the individual prediabetic. However, when the pancreas of an insulin resistant individual is unable to produce sufficient hormone to compensate for the increased demand, the β -cell mass will ultimately be exhausted and degenerate leading to hyperglycemia and overt type-2 diabetes (reviewed in 4 and 5). Thus, type 2 diabetes only develops in subjects that are unable to sustain the β -cell compensatory insulin response. These subjects have "susceptible" as opposed to "robust" islets - a condition determined by genetic and/or acquired factors, ex obesity (fig.14).

Identification of a peptide/protein that could restore glucose metabolism and treat insulin resistance hold great promise as new therapeutic targets in the potentially combined treatment of type 2 diabetes, metabolic syndrome and other diseases characterised by insulin resistance.

The SorCS1 receptor.

SorCS1 is one of five members of the mammalian Vps10p-domain (Vps10p-D) receptor family, which also comprises Sortilin, SorLA, SorCS2, and SorCS3. They are all type-1 transmembrane receptors sharing the characteristic structural feature of an N-terminal Vps10p-D with high homology to Vps10p, a sorting protein in yeast (10). At present the physiological function(s) of the receptor family is unclear, but recent findings indicate that both Sortilin and SorLA play a crucial role as regulators of neuronal survival and death (11,12). Interestingly, Sortilin has also been associated with insulin-regulated glucose up-take as it may facilitate

translocation of the glucose transporter GLUT4 from an intracellular compartment to the plasma membrane (13,14).

SorCS1 is unique among the Vps10p-D receptors as it exists in several distinct splice variants, denoted SorCS1- a, b, c, c+, and d, that encode identical extracellular and transmembrane parts, and cytoplasmic domains that differ in length and sequence (10, 11). The present inventors, and others have found that SorCS1, in addition to in the nervous system, is expressed in adipose tissue, skeletal muscle and β -cells of the pancreas; all tissues involved in glucose metabolism. Moreover, each splice variant exhibit a distinct tissue distribution as well as subcellular expression pattern suggesting that the tail-variants might be implicated in different biological activities (15-17).

Summary of the invention

The present inventors have studied the effect of SorCS1 and its different splice variants on the treatment of insulin resistance in mice, and the effect of SorCS1 and the different splice variants on insulin receptor expression using cell studies, and consequently in a main aspect the present invention relates to a SorCS1-like agent for use in treating insulin resistance and/or a disease associated with insulin resistance in an individual, wherein said agent is capable of binding to the insulin receptor (IR) at a SorCS1 binding site and being capable of sensitization of an insulin receptor.

SorCS1 is one of five members of the mammalian Vps10p-domain (Vps10p-D) receptor family, which also comprises Sortilin, SorLA, SorCS2, and SorCS3 (Fig.1). Murine SorCS1 is unique among the Vps10p-D receptors as it exists in several distinct splice variants, denoted mSorCS1- a, b, c, c+, and d (Fig.2)

In brief, the investors have demonstrated that in knockout mice lacking all splice variants of mSorCS1 the old male mice are hyperinsulimic but prediabetic, whereas old SorCS1 knockout female mice are hyperglycaemic and hyperinsulimic, thus both becoming diabetic with age, as a consequence of insulin resistance in the transgenic mice. Furthermore, the investors have shown that murine SorCS1 binds

the insulin receptor and that mSorCS1 regulates the expression of the insulin receptor.

5 Furthermore, the invention relates to a nucleic acid sequence encoding a polypeptide as defined above for use in the treatment of insulin resistance or diseases associated with insulin resistance in an individual, as well as a vector, a host cell and a packaging cell line comprising the nucleic acid for treatment purposes.

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In a further aspect the invention relates to a pharmaceutical composition comprising one or more of the agent as defined above; or the isolated nucleic acid sequence as defined above; or the the expression vector as defined above; or a composition of host cells as defined above; or a packaging cell line as defined above, or a combination thereof.

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Furthermore, in another aspect the present invention relates to a method of treatment of insulin resistance or diseases associated with insulin resistance, said method comprising administering to an individual in need thereof a therapeutically effective amount of the agent as defined above; or the isolated nucleic acid sequence as defined above; or the the expression vector as defined above; or a composition of host cells as defined above; or a packaging cell line as defined above, or a combination thereof.

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25 In another aspect, the present invention relates to a method of upregulating an insulin receptor or a fragment or variant thereof, in a patient in need thereof, said method comprising administering to an individual in need thereof a therapeutically effective amount of the agent as defined above; or the isolated nucleic acid sequence as defined above; or the the expression vector as defined above; or a composition of host cells as defined above; or a packaging cell line as defined above, or a combination thereof.

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In another aspect, the present invention relates to a method of sensitizing an insulin receptor, said method comprising administering a Vps10p-domain receptor selected from the group consisting of:

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- a) SorCS1
- b) SorCS2
- c) SorCS3
- d) Sortilin and
- e) SorLA,

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thus being useful in a method of treatment of insulin resistance or diseases associated with insulin resistance.

10 The diseases associated with insulin resistance are in particular selected from the group consisting of insulin resistance syndrome, Type 2 diabetes mellitus, impaired glucose tolerance, the metabolic syndrome, hyperglycemia, hyperinsulinemia, arteriosclerosis, hypercholesterolemia, hypertriglyceridemia, hyperlipidemia, dyslipidemia, obesity, central obesity, polycystic ovarian syndrome, hypercoagulability, hypertension, microalbuminuria, insulin resistance syndrome
15 (IRS), Type 2 diabetes mellitus, impaired glucose tolerance, the metabolic syndrome, hyperglycemia, and hyperinsulinemia.

In yet another aspect the present invention relates to a kit in parts comprising:

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- a pharmaceutical composition as defined herein,
- a medical instrument or other means for administering said pharmaceutical composition,
- instructions on how to use the kit in parts.

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In a further aspect, the present invention relates to a transgenic knock-out mouse in which the endogenous Vps10p-domain receptor SorCS1 genes have been disrupted to abolish expression of a functional SorCS1 receptor, and wherein said mouse exhibits a reduced response to insulin relative to a non-transgenic control mouse.

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In a further important aspect, the invention relates to a method for screening for the ability of the SorCS1-like agent to reduce blood glucose levels, said method comprising the steps of:

- a) providing a first and a second transgenic mouse of claim 69;
- b) administering to said first transgenic mouse a candidate agent, and

- 5 c) administering to said second transgenic mouse a physiological solution, and
- d) taking blood samples from the mouse of b) and c) respectively, at predetermined time intervals, such as at 15 minutes, 30 minutes, 60 minutes, 2 and 4 hours, subsequent to administration of said agent, and
- 10 e) comparing blood glucose levels in the samples of d); wherein a reduction in blood glucose level of said first transgenic mouse administered said candidate agent relative to said second transgenic mouse not administered said candidate agent indicates that the candidate agent reduces blood glucose levels.

15 In a further important aspect, the invention relates to a method for screening for the ability of the SorCS1-like agent to reduce blood glucose levels, said method comprising the steps of:

- a) providing a first and a second wild-type mouse; and
- b) administering to said first mouse the agent of claim 1, and
- c) administering to said second mouse a physiological solution, and
- 20 d) taking blood samples from the two mice of b) and c) respectively, at predetermined time intervals, such as at 15 minutes, 30 minutes, 60 minutes, 2 and 4 hours, subsequent to administration of said agent, and
- 25 e) comparing plasma glucose levels in the samples of d); wherein a reduction in blood glucose level of said first wild-type mouse administered said agent relative to said second wild type mouse not administered said candidate agent, indicates that the agent reduces blood glucose levels.

30 In a further aspect the invention relates to a transgenic mouse capable of encoding soluble and/or full length SorCS1 in a tissue specific manner, upon activation of expression.

In yet a further aspect the invention relates to the use of an agent capable of enhancing binding activity between SorCS1 or a fragment or variant thereof, and an

insulin receptor for the treatment of insulin resistans and/or diseases associated with insulin resistance.

Brief description of the drawings

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Fig. 1. The Vps10p-domain receptor family. Their structural organization is indicated.

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Fig. 2. Splice variants of mSorCS1. A) Organization of the murine SorCS1 gene leading to the generation of different cytoplasmic tails. B) Amino acid sequences of the mSorCS1 cytoplasmic domains.

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Fig. 3. Expression of the different mSorCS1 splice variants. Fragments obtained by RT-PCR on mRNA from different tissue with specific primer pairs used to identify the extracellular part of SorCS1 (extra) or each of the five tail variants (a, b, c, c⁺, and d).

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Fig. 4. Generation of the mSorCS1 knockout mouse. A) Strategy used to generate mSorCS1 knockout mice by homologous recombination in embryonic stem cells. B) Analysis of mSorCS1 mRNA expression, showing lack of transcription of all mSorCS1 splice variant. C) Western blot analysis of cortex showing lack of mSorCS1 protein in the mSorCS1 knockout (KO) mice.

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Fig. 5 Average blood glucose in A) male and B) female mice at different age. Animals were fasted overnight (16h). Blood samples were obtained by retroorbital bleeding and plasma glucose was measured immediately on an automatic monitor.

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Fig. 6. Plasma insulin levels in female wild-type and SorCS1 knockout mice from 10 to 50 weeks of age. Animals were fasted overnight (16h). Blood samples were obtained by retroorbital bleeding and plasma insulin levels were determined using an ultrasensitive mouse insulin enzyme-linked immunosorbent assay kit.

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Fig. 7. Glucose tolerance test in SorCS1 knockout mice and wild type littermates. Mice 59 weeks of age were fasted overnight (16 h) and injected intraperitoneally with a bolus of glucose (2 mg/g body weight) in sterile saline. Blood

samples were obtained by retroorbital bleeding at times 0, 15, 30, 60, and 120 min after injection, and plasma A) glucose and B) insulin levels were measured. Data are means $\pm$ SEM for four mice in each group.

- 5 **Fig. 8. Elevated levels of fasting plasma glucose and insulin in wild type mice on Western type diet.** Female A) + C) and male B) + D) wild type and SorCS1 knockout mice were fed a high calorie Western type diet (WD) from 10 weeks of age to 50 weeks of age. At 50 weeks of age the animals were fasted overnight (16h), blood samples were obtained by retroorbital bleeding and plasma glucose A) + B) and plasma insulin levels C) + D) were measured. Data are means $\pm$ SEM for 4 to 10 mice in each group.

Fig. 9. Abdominal adipose tissue in wild-type and knockout mice fed a western type diet.

- 15 Female A) and male B) wild type and SorCS1 knockout mice were fed a high calorie Western type diet (WD) from 10 weeks of age to 50 weeks of age. At the end of the study the animals were killed and the abdominal fat (adipose tissue) was separated and weighed. Data are means $\pm$ SEM for 4 to 10 mice in each group.
- 20 **Fig. 10. Expression of IR, phosphorylated IR (pY-IR) and Glut4 in muscle and adipose tissue.** Female SorCS1 knockout (-/-) mice and wild-type (+/+) control mice 50 weeks of age were fasted overnight, injected intraperitoneally with insulin (10 units/kg body weight) in sterile saline, and killed 15 min later. A) Adipose and B) muscle tissue (100 μ g) were analysed by western blotting with anti-IR, anti-IR-pY, anti-Glut, and anti-actin as a loading control.
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- Fig. 11. Physical interaction between SorCS1 and insulin receptor.** A) CHO cells transfected with the indicated receptors (only transiently transfected with IR_A and IR_B) were stimulated with insulin and immunoprecipitated with IR, and analysed by western blotting using α -SorCS1-leu and α -IR, respectively. B) Surface plasmon resonance experiment (BIAcore) showing the direct interaction of soluble full-length extracellular part of SorCS1 with immobilized soluble insulin receptor (IR). The K_d is estimated to approximately 5 nM.
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Fig. 12. Insulin receptor expression in CHO cells transfected with SorCS1. Cell lysat from CHO cells and CHO cells stably expressing mSorCS1-A, mSorCS1-B, mSorCS1-C, mSorCS1-D, and msol.SorCS1 (the extracellular part of SorCS1) were subjected to SDS–PAGE and Western blot analysis using anti-IR, anti-SorCS1-leu and anti-actin as a loading control.

Fig.13: Expression of IR and SorCS1 on the cell membrane. CHO cells and CHO cells stably expressing mSorCS1-B and mSorCS1-C were subjected to surface biotinylation followed by SDS–PAGE and Western blot analysis using anti-IR, anti-SorCS1-leu and anti-actin as a loading control. The Bio lanes contain biotinylated surface proteins, the Intra lanes contain intracellular proteins, and cell lysates (lysate) were used as input control.

Fig. 14: Development stages towards type 2 diabetes in human. Increases in blood glucose concentration during the development of T2D are illustrated on the graph (black line) showing the change from normal to pre-diabetic, before the onset of frank diabetes. Furthermore, the level of insulin during development of T2D is revealed on the same graph (dashed line), showing an increase of insulin during the pre-diabetic state as compensation to insulin resistance and a severe decline in insulin release at onset of frank diabetes as a consequence of β cell failure.

Fig. 15: Insulin immunostaining of pancreatic islets in wild-type and knockout mice 20 days of age. Pancreata were removed, fixed in paraformaldehyde, cryosectioned, and immunostained with anti-insulin antibody. Representative images are shown.

Figure 16: Alignment of SorCS1

Sequence alignment of SorCS1 from Human (homo sapiens), Chimpanzee (Pan troglodytes), Cow (Bos Taurus), Mouse (Mus musculus), Rat (Rattus norvegicus), Dog (Canis lupus familiaris) and Chicken (Gallus gallus) origin. The sequence identity is as demonstrated in table 2.

Table 2: Sequence identity to human SorCS1

Species	Protein (% identity)	DNA (% identity)
Human	100	100

Chimpanzee	99.6	99.4
Dog	97.6	92.5
Cow	92.9	89.8
Mouse	93.2	87.7
Rat	93.2	88.0
Chicken	85.3	79.7

Fig. 17: Decreased plasma glucose levels in female wild-type and SorCS1 knockout mice after hepatic overexpression of soluble SorCS1.

5 SorCS1 knockout or wild-type female mice were injected with an adenovirus for hepatic expression of soluble human SorCS1 or with a control virus encoding LacZ. At the day of injection (0d) as well as 7 days after virus administration (7d) plasma glucose was determined in mice fasted 16 hrs. The figure shows relative plasma glucose after normalization to the values obtained at day 0. (n=4). The figure shows
10 that overexpression of soluble SorCS1 (the extracellular domain) reduces plasma glucose in both wild-type and SorCS1 knockout mice.

Fig. 18: Expression of IR, phosphorylated IR, and Glut4 in muscle and adipose tissue from SorCS1 knockout female mice over-expressing soluble SorCS1.

15 Female SorCS1 knockout (-/-) mice 40 weeks of age were injected with an adenoviral vector expressing either human soluble SorCS1 or LacZ as a control. Twelve days after virus injection, the mice were fasted overnight, injected intraperitoneally with insulin (10 units/kg body weight) in sterile saline, and killed 15 min later. 50 µg lysates from muscle (A) and adipose tissue (B) were analysed by
20 western blotting with anti-IR, anti-IR-pY, and anti-Glut4. The figure shows that treatment with SorCS1-encoding virus increases IR expression, IR phosphorylation as well as Glut4 expression.

Fig. 19: Decreased plasma glucose and insulin levels in diabetic db/db female mice over-expressing soluble SorCS1.

25 Obese type-2 diabetic female db/db mice 10 weeks of age were injected with adenovirus expressing either human soluble SorCS1 or LacZ as a control. At day 0 (d0) prior to virus infection and 7 days after (d7), blood samples from mice fasted overnight (16 hrs) were obtained by retroorbital bleeding and blood glucose (A) and
30 plasma insulin (B) levels were measured. Data are means $\pm$ SEM for 5 mice in each group and are presented as relative values compared to day 0. Mice treated with

SorCS1 virus, but not LacZ virus, exhibit increased insulin sensitivity as reflected by reduced plasma glucose and insulin levels. The increase in both plasma glucose and insulin from day 0 to 7 in the LacZ group reflects that the animals are in the process of developing diabetes.

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Fig. 20: Glucose tolerance test in diabetic db/db female mice with over-expression of soluble SorCS1.

Fasted female db/db mice were 3 days post-infection with adenovira expressing either soluble SorCS1 or LacZ fasted injected intraperitoneally with a bolus of glucose (2mg/g body weight) in sterile saline. Blood samples were obtained by retroorbital bleeding at times 0, 15, 30, 90, and 150 min after injection, and blood glucose levels were measured. Values are means $\pm$ SEM for 5 mice in each group. The experiment shows that baseline blood glucose is restored at 150 min in mice that received the sol-SorCS1 virus, whereas hyperglycemia is maintained in mice treated with LacZ-virus.

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Fig. 21: Plasma glucose and insulin levels in diabetic db/db male mice over-expressing soluble SorCS1.

Obese male db/db mice 6 weeks of age were injected with adenovirus expressing either human soluble SorCS1 or LacZ as a control. At day 0 and 7, mice were fasted overnight (16h), blood samples were obtained by retroorbital bleeding and blood glucose (A) and plasma insulin (B) levels were measured. Data are means $\pm$ SEM for 5 mice in each group and are presented as relative change to day 0. The figure show that treatment with SorCS1 virus increases glucose sensitivity as plasma glucose decreases while insulin levels are similar to that of mice receiving LacZ virus. The increase in plasma insulin from day 0 to 7 in both groups reflects that the animals are in the process of developing diabetes. During the course of the experiment they can still compensate a reduction in insulin sensitivity by increasing insulin production.

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Fig. 22: Subcellular localization of Glut4 in muscle tissue from db/db male mice over-expressing soluble SorCS1.

Light microsomes isolated by subcellular fractionation from muscle tissue of five db/db male mice after over-expression of soluble SorCS1 or lacZ were fractionated in a 0.8 M to 1.6 M sucrose velocity gradient. Gradient fractions were subjected to

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gel electrophoresis and blotted with a Glut4 antibody, thus identifying the location of Glut4 in the different fractions. The experiment shows that SorCS1 expression changes the subcellular localization of Glut4, in line an important role of SorCS1 in regulating glucose uptake.

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Fig. 23: Analysis of SorCS1/IR contact sequences by SPOT analysiss.

A) Consecutive 16-mer amino acid peptides overlapping by three residues of the human insulin receptor were spotted on to filters. The filters were subsequently incubated with the radiolabelled extracellular domains of murine SorCS1, and binding was detected by autoradiography. Possible SorCS1 binding sites in the insulin receptor are indicated. B)

Consecutive 16-mer amino acid peptides overlapping by three residues of human SorCS1-a were spotted on to filters and probed for insulin receptor binding using his-tagged soluble receptor. Peptides capable of SorCS1-a binding were visualized by Western blotting using an antibody against the histidine tag. Possible binding sequences in SorCS1-a are indicated.

Fig. 24: Gene expression profiling of adipose tissue from SorCS1 knockout mice by PCR arrays.

The gene expression in SorCS1 knockout adipose tissue as compared to wild-type adipose tissue was examined for A) 84 genes related to the mouse insulin signalling pathway and B) 84 genes related to mouse lipoprotein signalling & cholesterol metabolism. Genes in the SorCS1 knockouts that are either 3 times higher or lower than that of wild-type mice are listed and their putative functions indicated.

Detailed description on the invention

Definitions

5 Unless specifically indicated otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this invention belongs. For purposes of the present invention, the following terms are defined.

10 Adjuvant: Any substance whose admixture with an administered immunogenic determinant / antigen increases or otherwise modifies the immune response to said determinant.

15 Affinity: The interaction of most ligands with their binding sites can be characterized in terms of a binding affinity. In general, high affinity ligand binding results from greater intermolecular force between the ligand and its receptor while low affinity ligand binding involves less intermolecular force between the ligand and its receptor. In general, high affinity binding involves a longer residence time for the ligand at its receptor binding site than is the case for low affinity binding. High affinity binding of ligands to receptors is often physiologically important when some of the binding
20 energy can be used to cause a conformational change in the receptor, resulting in altered behavior of an associated ion channel or enzyme.

A ligand that can bind to a receptor, alter the function of the receptor and trigger a physiological response is called an agonist for that receptor. Agonist binding to a
25 receptor can be characterized both in terms of how much physiological response can be triggered and the concentration of the agonist that is required to produce the physiological response. High affinity ligand binding implies that a relatively low concentration of a ligand is adequate to maximally occupy a ligand binding site and trigger a physiological response. Low affinity binding implies that a relatively high
30 concentration of a ligand is required before the binding site is maximally occupied and the maximum physiological response to the ligand is achieved. Ligand binding is often characterized in terms of the concentration of ligand at which half of the receptor binding sites are occupied, known as the dissociation constant (K_d). Affinity is also the strength of binding between receptors and their ligands, for example
35 between an antibody and its antigen.

Alcohol: A class of organic compounds containing one or more hydroxyl groups (OH). In this context a saturated or unsaturated, branched or unbranched hydrocarbon group sitting as a substituent on a larger molecule.

- 5 Alicyclic group: the term "alicyclic group" means a cyclic hydrocarbon group having properties resembling those of aliphatic groups.

10 Aliphatic group: in the context of the present invention, the term "aliphatic group" means a saturated or unsaturated linear or branched hydrocarbon group. This term is used to encompass alkyl, alkenyl, and alkynyl groups, for example.

15 Alkyl group: the term "alkyl group" means a saturated linear or branched hydrocarbon group including, for example, methyl, ethyl, isopropyl, t-butyl, heptyl, dodecyl, octadecyl, amyl, 2-ethylhexyl, and the like.

20 Alkenyl group: the term "alkenyl group" means an unsaturated, linear or branched hydrocarbon group with one or more carbon-carbon double bonds, such as a vinyl group.

25 Alkynyl group: the term "alkynyl group" means an unsaturated, linear or branched hydrocarbon group with one or more carbon-carbon triple bonds.

30 Amphiphil: substance containing both polar, water-soluble and nonpolar, water-insoluble groups.

25 Agonist: An agonist is a compound capable of increasing or effecting the activity of a receptor. Specifically, a Vps10p-domain receptor agonist is a compound capable of binding to one or more of binding sites of a Vps10p-domain receptor thereby inducing the same physiological response as a given endogenous agonist ligand compound.

30 Antagonist: An antagonist is in this case synonymous with an inhibitor. An antagonist is a compound capable of decreasing the activity of an effector such as a receptor. Specifically, a Vps10p-domain receptor antagonist is a compound capable of binding to one or more of binding sites of Vps10p-domain receptor thereby
35 inhibiting binding of another ligand thus inhibiting a physiological response.

Antibody: The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (*i.e.*, "antigen-binding portion") or single chain thereof.

- 5 Polyclonal antibody: Polyclonal antibodies are a mixture of antibody molecules recognising a specific given antigen, hence polyclonal antibodies may recognise different epitopes within said antigen.

- 10 Aromatic group: the term "aromatic group" or "aryl group" means a mono- or polycyclic aromatic hydrocarbon group.

- 15 Binding: The term "binding" refers to a condition of proximity between chemical entities or compounds, or portions thereof. The binding may be non-covalent- wherein the juxtaposition is energetically favoured by hydrogen bonding or van der Waals or electrostatic interactions-or it may be covalent. The agents according to the invention are capable of binding to the insulin receptor. An assay for binding may be the Biocore assay discussed in relation to Figure 11B as well as the co.IP assay discussed in relation to Figure 11A.

- 20 Binding site: The term "binding site" or "binding pocket", as used herein, refers to a region of a molecule or molecular complex that, as a result of its shape, favourably associates with another molecule, molecular complex, chemical entity or compound. As used herein, the pocket comprises at least a deep cavity and, optionally a shallow cavity.

- 25 Bioreactive agent or biologically active or biological activity: The terms are as used herein refers to effect of any a substance which may be used in connection with an application that is therapeutic or otherwise useful according to this invention. The biological activity refers to the biological effect in vitro and/or in vivo. In the present context the biological activity of an agent according to this invention is the capability of binding to the insulin receptor and/or enhancing binding of a SorCS1-like agent to the insulin receptor, and in a more preferred embodiment the biological activity includes sensitization of the insulin receptor. The bioactive agents may be neutral, positively or negatively charged. Suitable bioactive agents include, for example, 30 prodrugs, diagnostic agents, therapeutic agents, pharmaceutical agents, drugs, 35

oxygen delivery agents, blood substitutes, synthetic organic molecules, polypeptides, peptides, vitamins, steroids, steroid analogues and genetic determinants, including nucleosides, nucleotides and polynucleotides.

- 5 Cationic group: A chemical group capable of functioning as a proton donor when a compound comprising the chemical group is dissolved in a solvent, preferably when dissolved in water.

- 10 Complex: As used herein the term "complex" refers to the combination of a molecule or a protein, conservative analogues or truncations thereof bound to a chemical entity.

- 15 Cyclic group: the term "cyclic group" means a closed ring hydrocarbon group that is classified as an alicyclic group, aromatic group, or heterocyclic group.

- 20 Cycloalkenyl: means a monovalent unsaturated carbocyclic radical consisting of one, two or three rings, of three to eight carbons per ring, which can optionally be substituted with one or two substituents selected from the group consisting of hydroxy, cyano, lower alkenyl, lower alkoxy, lower haloalkoxy, alkenylthio, halo, haloalkenyl, hydroxyalkenyl, nitro, alkoxy carbonenyl, amino, alkenylamino, alkenylsulfonyl, arylsulfonyl, alkenylaminosulfonyl, arylaminosulfonyl, alkylsulfonylamino, arylsulfonylamino, alkenylaminocarbonyl, arylaminocarbonyl, alkenylcarbonylamino and arylcarbonylamino.

- 25 Cycloalkyl: means a monovalent saturated carbocyclic radical consisting of one, two or three rings, of three to eight carbons per ring, which can optionally be substituted with one or two substituents selected from the group consisting of hydroxy, cyano, lower alkyl, lower alkoxy, lower haloalkoxy, alkylthio, halo, haloalkyl, hydroxyalkyl, nitro, alkoxy carbonyl, amino, alkylamino, alkylsulfonyl, arylsulfonyl, alkylamino-sulfonyl, arylaminosulfonyl, alkylsulfonylamino, arylsulfonylamino, alkylaminocarbonyl, arylaminocarbonyl, alkylcarbonylamino and arylcarbonylamino.
- 30

- 35 Electrostatic interaction: The term "electrostatic interaction" as used herein refers to any interaction occurring between charged components, molecules or ions, due to attractive forces when components of opposite electric charge are attracted to each

other. Examples include, but are not limited to: ionic interactions, covalent interactions, interactions between a ion and a dipole (ion and polar molecule), interactions between two dipoles (partial charges of polar molecules), hydrogen bonds and London dispersion bonds (induced dipoles of polarizable molecules).

5 Thus, for example, "ionic interaction" or "electrostatic interaction" refers to the attraction between a first, positively charged molecule and a second, negatively charged molecule. Ionic or electrostatic interactions include, for example, the attraction between a negatively charged bioactive agent.

10 Form a ring: means that the atoms mentioned are connected through a bond when the ring structure is formed.

Fragments: The polypeptide fragments according to the present invention, including any functional equivalents thereof, may in one embodiment comprise less than 500
15 amino acid residues, such as less than 450 amino acid residues, for example less than 400 amino acid residues, such as less than 350 amino acid residues, for example less than 300 amino acid residues, for example less than 250 amino acid residues, such as less than 240 amino acid residues, for example less than 225 amino acid residues, such as less than 200 amino acid residues, for example less
20 than 180 amino acid residues, such as less than 160 amino acid residues, for example less than 150 amino acid residues, such as less than 140 amino acid residues, for example less than 130 amino acid residues, such as less than 120 amino acid residues, for example less than 110 amino acid residues, such as less than 100 amino acid residues, for example less than 90 amino acid residues, such
25 as less than 85 amino acid residues, for example less than 80 amino acid residues, such as less than 75 amino acid residues, for example less than 70 amino acid residues, such as less than 65 amino acid residues, for example less than 60 amino acid residues, such as less than 55 amino acid residues, for example less than 50 amino acid residues, such as less than 45 amino acid residues, for example less
30 than 40 amino acid residues, such as 35 amino acid residues, for example 30 amino acid residues, such as 25 amino acid residues, such as 20 amino acid residues, for example 15 amino acid residues, such as 10 amino acid residues, for example 5 contiguous amino acid residues of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,
35 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40,

41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51 or a variant thereof being at least 70% identical to said sequences. Also, the polypeptide fragments according to the present invention, including any functional equivalents thereof, may in one embodiment comprise more than 5 amino acid residues, such as more than 10 amino acid residues, for example more than 15 amino acid residues, such as more than 20 amino acid residues, for example more than 25 amino acid residues, for example more than 50 amino acid residues, such as more than 75 amino acid residues, for example more than 100 amino acid residues, such as more than 125 amino acid residues, for example more than 150 amino acid residues, such as more than 175 amino acid residues, for example more than 200 amino acid residues, such as more than 225 amino acid residues, for example more than 250 amino acid residues, such as more than 275 amino acid residues, for example more than 300 amino acid residues, such as more than 325 amino acid residues, for example more than 350 amino acid residues, such as more than 375 amino acid residues, for example more than 400 amino acid residues, such as more than 425 amino acid residues, for example more than 450 amino acid residues, such as more than 475 amino acid residues, for example more than 500 amino acid residues, such as more than 525 amino acid residues, for example more than 550 amino acid residues, such as more than 575 amino acid residues, for example more than 600 amino acid residues, such as 625 amino acid residues, for example 650 amino acid residues, such as 675 amino acid residues, such as 700 amino acid residues of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51 or a variant thereof being at least 70% identical to said sequences.

Functional equivalency: "Functional equivalency" as used in the present invention is, according to one preferred embodiment, established by means of reference to the corresponding functionality of a predetermined fragment of the sequence.

30

Functional equivalents or variants of a SorCS1 polypeptide, or a fragment thereof will be understood to exhibit amino acid sequences gradually differing from the preferred predetermined SorCS1 polypeptide or the SorCS1 fragment sequence respectively, as the number and scope of insertions, deletions and substitutions including conservative substitutions increase, while retaining the biological activity of

35

a SorCS1 polypeptide in this context. This difference is measured as a reduction in homology between the preferred predetermined sequence and the fragment or functional equivalent.

- 5 A functional variant obtained by substitution may well exhibit some form or degree of native SorCS1 activity, and yet be less homologous, if residues containing functionally similar amino acid side chains are substituted. Functionally similar in this respect refers to dominant characteristics of the side chains such as hydrophobic, basic, neutral or acidic, or the presence or absence of steric bulk. Accordingly, in
10 one embodiment of the invention, the degree of identity is not a principal measure of a fragment being a variant or functional equivalent of a preferred predetermined fragment according to the present invention.

Gene "silencing": a process leading to reduced expression of endogenous genes.

- 15 Gene silencing is preferably the result of post-transcriptional reduction of gene expression.

- Group: (Moiety / substitution) as is well understood in this technical area, a large degree of substitution is not only tolerated, but is often advisable. Substitution is
20 anticipated on the materials of the present invention. As a means of simplifying the discussion and recitation of certain terminology used throughout this application, the terms "group" and "moiety" are used to differentiate between chemical species that allow for substitution or that may be substituted and those that do not allow or may not be so substituted. Thus, when the term "group" is used to describe a chemical
25 substituent, the described chemical material includes the unsubstituted group and that group with O, N, or S atoms, for example, in the chain as well as carbonyl groups or other conventional substitution. Where the term "moiety" is used to describe a chemical compound or substituent, only an unsubstituted chemical material is intended to be included. For example, the phrase "alkyl group" is
30 intended to include not only pure open chain saturated hydrocarbon alkyl substituents, such as methyl, ethyl, propyl, t-butyl, and the like, but also alkyl substituents bearing further substituents known in the art, such as hydroxy, alkoxy, alkylsulfonyl, halogen atoms, cyano, nitro, amino, carboxyl, etc. Thus, "alkyl group" includes ether groups, haloalkyls, nitroalkyls, carboxyalkyls, hydroxyalkyls,
35 sulfoalkyls, etc. On the other hand, the phrase "alkyl moiety" is limited to the

inclusion of only pure open chain saturated hydrocarbon alkyl substituents, such as methyl, ethyl, propyl, t-butyl, and the like. The same definitions apply to "alkenyl group" and "alkenyl moiety"; to "alkynyl group" and "alkynyl moiety"; to "cyclic group" and "cyclic moiety"; to "alicyclic group" and "alicyclic moiety"; to "aromatic group" or "aryl group" and to "aromatic moiety" or "aryl moiety"; as well as to "heterocyclic group" and "heterocyclic moiety".

Heterocyclic group: the term "heterocyclic group" means a closed ring hydrocarbon in which one or more of the atoms in the ring is an element other than carbon (e.g., nitrogen, oxygen, sulphur, etc.).

Heterocyclyl means a monovalent saturated cyclic radical, consisting of one to two rings, of three to eight atoms per ring, incorporating one or two ring heteroatoms (chosen from N, O or S(O)₀₋₂, and which can optionally be substituted with one or two substituents selected from the group consisting of hydroxyl, oxo, cyano, lower alkyl, lower alkoxy, lower haloalkoxy, alkylthio, halo, haloalkyl, hydroxyalkyl, nitro, alkoxycarbonyl, amino, alkylamino, alkylsulfonyl, arylsulfonyl, alkylaminosulfonyl, arylaminosulfonyl, alkylsulfonylamino, arylsulfonylamino, alkylaminocarbonyl, arylaminocarbonyl, alkylcarbonylamino, or arylcarbonylamino.

Heteroaryl means a monovalent aromatic cyclic radical having one to three rings, of four to eight atoms per ring, incorporating one or two heteroatoms (chosen from nitrogen, oxygen, or sulphur) within the ring which can optionally be substituted with one or two substituents selected from the group consisting of hydroxy, cyano, lower alkyl, lower alkoxy, lower haloalkoxy, alkylthio, halo, haloalkyl, hydroxyalkyl, nitro, alkoxycarbonyl, amino, alkylamino, alkylsulfonyl, arylsulfonyl, alkylaminosulfonyl, arylaminosulfonyl, alkylsulfonylamino, arylsulfonylamino, alkylaminocarbonyl, arylaminocarbonyl, alkylcarbonylamino and arylcarbonylamino.

Homology: The homology between amino acid sequences may be calculated using well known scoring matrices such as any one of BLOSUM 30, BLOSUM 40, BLOSUM 45, BLOSUM 50, BLOSUM 55, BLOSUM 60, BLOSUM 62, BLOSUM 65, BLOSUM 70, BLOSUM 75, BLOSUM 80, BLOSUM 85, and BLOSUM 90.

Fragments sharing homology with fragments of SEQ ID NOs: SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27,

- 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51, respectively, are to be considered as falling within the scope of the present invention when they are preferably at least about 60 percent homologous, for example at least 65 percent homologous, for example at least 70 percent homologous, for example at least 75 percent homologous, for example at least 80 percent homologous, for example at least 85 percent homologous, for example at least 90 percent homologous, for example at least 92 percent homologous, such as at least 94 percent homologous, for example at least 95 percent homologous, such as at least 96 percent homologous, for example at least 97 percent homologous, such as at least 98 percent homologous, for example at least 99 percent homologous with said predetermined fragment sequences, respectively. According to one embodiment of the invention, the homology percentages refer to identity percentages.
- 15 A further suitably adaptable method for determining structure and function relationships of peptide fragments is described in US 6,013,478, which is herein incorporated by reference. Also, methods of assaying the binding of an amino acid sequence to a receptor moiety are known to the skilled artisan.
- 20 In addition to conservative substitutions introduced into any position of a preferred predetermined SorCS1 polypeptide, or a fragment thereof, it may also be desirable to introduce non-conservative substitutions in any one or more positions of such a SorCS1 polypeptide, or a fragment thereof.
- 25 A non-conservative substitution leading to the formation of a functionally equivalent fragment of a SorCS1 polypeptide, or a fragment thereof would for example i) differ substantially in polarity, for example a residue with a non-polar side chain (Ala, Leu, Pro, Trp, Val, Ile, Leu, Phe or Met) substituted for a residue with a polar side chain such as Gly, Ser, Thr, Cys, Tyr, Asn, or Gln or a charged amino acid such as Asp, Glu, Arg, or Lys, or substituting a charged or a polar residue for a non-polar one; and/or ii) differ substantially in its effect on polypeptide backbone orientation such as substitution of or for Pro or Gly by another residue; and/or iii) differ substantially in electric charge, for example substitution of a negatively charged residue such as Glu or Asp for a positively charged residue such as Lys, His or Arg (and vice versa); and/or iv) differ substantially in steric bulk, for example substitution of a bulky

residue such as His, Trp, Phe or Tyr for one having a minor side chain, e.g. Ala, Gly or Ser (and vice versa).

5 Variants obtained by substitution of amino acids may in one preferred embodiment be made based upon the hydrophobicity and hydrophilicity values and the relative similarity of the amino acid side-chain substituents, including charge, size, and the like. Exemplary amino acid substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine;
10 glutamine and asparagine; and valine, leucine and isoleucine.

In addition to the variants described herein, sterically similar variants may be formulated to mimic the key portions of the variant structure and that such compounds may also be used in the same manner as the variants of the invention.
15 This may be achieved by techniques of modelling and chemical designing known to those of skill in the art. It will be understood that all such sterically similar constructs fall within the scope of the present invention.

In a further embodiment the present invention relates to functional variants
20 comprising substituted amino acids having hydrophilic values or hydropathic indices that are within ± 4.9 , for example within ± 4.7 , such as within ± 4.5 , for example within ± 4.3 , such as within ± 4.1 , for example within ± 3.9 , such as within ± 3.7 , for example within ± 3.5 , such as within ± 3.3 , for example within ± 3.1 , such as within ± 2.9 , for example within ± 2.7 , such as within ± 2.5 , for example within ± 2.3 , such as within ± 2.1 , for example within ± 2.0 , such as within ± 1.8 , for
25 example within ± 1.6 , such as within ± 1.5 , for example within ± 1.4 , such as within ± 1.3 for example within ± 1.2 , such as within ± 1.1 , for example within ± 1.0 , such as within ± 0.9 , for example within ± 0.8 , such as within ± 0.7 , for example within ± 0.6 , such as within ± 0.5 , for example within ± 0.4 , such as
30 within ± 0.3 , for example within ± 0.25 , such as within ± 0.2 of the value of the amino acid it has substituted.

The importance of the hydrophilic and hydropathic amino acid indices in conferring interactive biologic function on a protein is well understood in the art (Kyte & Doo-

little, 1982 and Hopp, U.S. Pat. No. 4,554,101, each incorporated herein by reference).

5 The amino acid hydropathic index values as used herein are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5) (Kyte & Doolittle, 1982).

10

The amino acid hydrophilicity values are: arginine (+3.0); lysine (+3.0); aspartate (+3.0+-1); glutamate (+3.0+-1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5+-1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8);
15 tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4) (U.S. 4,554,101).

In addition to the peptidyl compounds described herein, sterically similar compounds may be formulated to mimic the key portions of the peptide structure and that such compounds may also be used in the same manner as the peptides of the invention.
20 This may be achieved by techniques of modelling and chemical designing known to those of skill in the art. For example, esterification and other alkylations may be employed to modify the amino terminus of, e.g., a di-arginine peptide backbone, to mimic a tetra peptide structure. It will be understood that all such sterically similar constructs fall within the scope of the present invention.

25

Peptides with N-terminal alkylations and C-terminal esterifications are also encompassed within the present invention. Functional equivalents also comprise glycosylated and covalent or aggregative conjugates formed with the same or other SorCS1 polypeptides, or fragment thereof, including dimers or unrelated chemical
30 moieties. Such functional equivalents are prepared by linkage of functionalities to groups which are found in fragment including at any one or both of the N- and C-termini, by means known in the art.

Functional equivalents may thus comprise fragments conjugated to aliphatic or acyl
35 esters or amides of the carboxyl terminus, alkylamines or residues containing

carboxyl side chains, e.g., conjugates to alkylamines at aspartic acid residues; O-acyl derivatives of hydroxyl group-containing residues and N-acyl derivatives of the amino terminal amino acid or amino-group containing residues, e.g. conjugates with fMet-Leu-Phe or immunogenic proteins. Derivatives of the acyl groups are selected
5 from the group of alkyl-moieties (including C3 to C10 normal alkyl), thereby forming alkanoyl species, and carbocyclic or heterocyclic compounds, thereby forming aroyl species. The reactive groups preferably are difunctional compounds known per se for use in cross-linking proteins to insoluble matrices through reactive side groups.

10 Covalent or aggregative functional equivalents and derivatives thereof are useful as reagents in immunoassays or for affinity purification procedures. For example, a fragment of a SorCS1 polypeptide according to the present invention may be insolubilized by covalent bonding to cyanogen bromide-activated Sepharose by methods known per se or adsorbed to polyolefin surfaces, either with or without
15 glutaraldehyde cross-linking, for use in an assay or purification of anti-SorCS1 activity modulator antibodies or cell surface receptors. Fragments may also be labelled with a detectable group, e.g., radioiodinated by the chloramine T procedure, covalently bound to rare earth chelates or conjugated to another fluorescent moiety for use in e.g. diagnostic assays.

20 Mutagenesis of a preferred predetermined SorCS1 polypeptide, or a fragment thereof, can be conducted by making amino acid insertions, usually on the order of about from 1 to 10 amino acid residues, preferably from about 1 to 5 amino acid residues, or deletions of from about from 1 to 10 residues, such as from about 2 to 5
25 residues.

In one embodiment the ligand of binding site 1, 2 or 3 is an oligopeptide synthesised by automated synthesis. Any of the commercially available solid-phase techniques may be employed, such as the Merrifield solid phase synthesis method, in which
30 amino acids are sequentially added to a growing amino acid chain (see Merrifield, J. Am. Chem. Soc. 85:2149-2146, 1963).

Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied Biosystems, Inc. of Foster City, Calif., and may generally
35 be operated according to the manufacturer's instructions. Solid phase synthesis will

enable the incorporation of desirable amino acid substitutions into any fragment of SorCS1 according to the present invention. It will be understood that substitutions, deletions, insertions or any subcombination thereof may be combined to arrive at a final sequence of a functional equivalent. Insertions shall be understood to include
5 amino-terminal and/or carboxyl-terminal fusions, e.g. with a hydrophobic or immunogenic protein or a carrier such as any polypeptide or scaffold structure capable as serving as a carrier.

Oligomers including dimers including homodimers and heterodimers of fragments of
10 sortilin inhibitors according to the invention are also provided and fall under the scope of the invention. SorCS1 polypeptides and fragments, functional equivalents and variants thereof can be produced as homodimers or heterodimers with other amino acid sequences or with native sortilin inhibitor sequences. Heterodimers include dimers containing immunoreactive sortilin inhibiting fragments as well as
15 sortilin inhibiting fragments that need not have or exert any biological activity.

SorCS1 polypeptides, or fragments and variants thereof may be synthesised both in vitro and in vivo. Methods for in vitro synthesis are well known, and methods being suitable or suitably adaptable to the synthesis in vivo of sortilin inhibitors are also
20 described in the prior art. When synthesized in vivo, a host cell is transformed with vectors containing DNA encoding a sortilin peptide inhibitor or a fragment thereof. A vector is defined as a replicable nucleic acid construct. Vectors are used to mediate expression of SorCS1 polypeptides, and/or fragments and variants. An expression vector is a replicable DNA construct in which a nucleic acid sequence encoding the predetermined sortilin inhibiting fragment, or any functional equivalent thereof that
25 can be expressed in vivo, is operably linked to suitable control sequences capable of effecting the expression of the fragment or equivalent in a suitable host. Such control sequences are well known in the art. Both prokaryotic and eukaryotic cells may be used for synthesising ligands.

Cultures of cells derived from multicellular organisms however represent preferred
30 host cells. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of useful host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and WI38, BHK, COS-7, 293 and MDCK cell lines. Preferred host cells are eukaryotic cells known to synthesize
35 endogenous sortilin inhibitors. Cultures of such host cells may be isolated and used

as a source of the fragment, or used in therapeutic methods of treatment, including therapeutic methods aimed at promoting or inhibiting a growth state, or diagnostic methods carried out on the human or animal body.

5 Hydrophobic bond: The term "hydrogen bond" as used herein refers to an attractive force, or bridge, which may occur between a hydrogen atom which is bonded covalently to an electronegative atom, for example, oxygen, sulphur, or nitrogen, and another electronegative atom. The hydrogen bond may occur between a hydrogen atom in a first molecule and an electronegative atom in a second molecule
10 (inter-molecular hydrogen bonding). Also, the hydrogen bond may occur between a hydrogen atom and an electronegative atom which are both contained in a single molecule (intra-molecular hydrogen bonding).

15 Hydrophobic interaction: The term "hydrophobic interaction" as used herein refers to any interaction occurring between essentially non-polar (hydrophobic) components located within attraction range of one another in a polar environment (e.g. water). As used herein, attraction range is on the scale of from 0.1 up to 2 nm. A particular type of hydrophobic interaction is exerted by "Van der Waal's forces", i.e. the attractive forces between non-polar molecules that are accounted for by quantum mechanics.
20 Van der Waal's forces are generally associated with momentary dipole moments which are induced by neighbouring molecules and which involve changes in electron distribution.

25 Insulin: Insulin is a hormone that is produced by the beta cells of the pancreas. The insulin produced is released into the blood stream and is transported throughout the body. Insulin is an important hormone that has many actions within the body. Most of the actions of insulin are directed at metabolism (control) of carbohydrates (sugars and starches), lipids (fats), and proteins. Insulin also is important in regulating the cells of the body including their growth.

30 Insulin resistance: Insulin resistance (IR) is a condition in which the cells of the body become resistant to the effects of insulin, that is, the normal response to a given amount of insulin is reduced. As a result, higher levels of insulin are needed in order for insulin to have its effects. Insulin resistance precedes the development of type 2
35 diabetes, sometimes by several years. In individuals who will ultimately develop type

2 diabetes, it is believed that blood glucose and insulin levels are normal for many years; then at some point in time, insulin resistance develops. Accordingly, the treatment of the cause of insulin resistance is preferred over treatment of the symptoms of diabetes. The present invention is primarily aimed at providing a medicament for use in the treatment of insulin resistance.

In vitro/in vivo: the terms are used in their normal meaning.

Ligand: a substance, compound or biomolecule such as a protein including receptors, that is able to bind to and form a complex with (a second) biomolecule to serve a biological purpose. In a narrower sense, it is a signal triggering molecule binding to a site on a target protein, by intermolecular forces such as ionic bonds, hydrogen bonds and Van der Waals forces. The docking (association) is usually reversible (dissociation). Actual irreversible covalent binding between a ligand and its target molecule is rare in biological systems. As opposed to the meaning in metalorganic and inorganic chemistry, it is irrelevant, whether or not the ligand actually binds at a metal site, as it is the case in hemoglobin. Ligand binding to receptors may alter the chemical conformation, i.e. the three dimensional shape of the receptor protein. The conformational state of a receptor protein determines the functional state of a receptor. The tendency or strength of binding is called affinity. Ligands include substrates, inhibitors, activators, non-self receptors, co-receptors and neurotransmitters.

Pharmaceutical agent: The terms "pharmaceutical agent" or "drug" or "medicament" refer to any therapeutic or prophylactic use of an agent according to the invention, which agent may be used in the treatment (including the prevention, diagnosis, alleviation, or cure) of a malady, affliction, condition, disease or injury in a patient. Therapeutically useful genetic determinants, peptides, polypeptides and polynucleotides may be included within the meaning of the term pharmaceutical or drug. As defined herein, a "therapeutic agent", "pharmaceutical agent" or "drug" or "medicament" is a type of bioactive agent.

Pharmaceutical composition: or drug, medicament or agent refers to any chemical or biological material, compound, or composition capable of inducing a desired therapeutic effect when properly administered to a patient. Some drugs are sold in

an inactive form that is converted in vivo into a metabolite with pharmaceutical activity. For purposes of the present invention, the terms "pharmaceutical composition" and "medicament" preferably encompass an active agent as such or an inactive drug and the active metabolite.

5

Polypeptide: The term "polypeptide" as used herein refers to a molecule comprising at least two amino acids. The amino acids may be natural or synthetic.

"Oligopeptides" are defined herein as being polypeptides of length not more than 100 amino acids. The term "polypeptide" is also intended to include proteins, i.e. functional biomolecules comprising at least one polypeptide; when comprising at least two polypeptides, these may form complexes, be covalently linked or may be non-covalently linked. The polypeptides in a protein can be glycosylated and/or lipidated and/or comprise prosthetic groups.

10

Polynucleotide: "Polynucleotide" as used herein refers to a molecule comprising at least two nucleic acids. The nucleic acids may be naturally occurring or modified, such as locked nucleic acids (LNA), or peptide nucleic acids (PNA). Polynucleotide as used herein generally pertains to

15

- 20 i) a polynucleotide comprising a predetermined coding sequence, or
 - ii) a polynucleotide encoding a predetermined amino acid sequence, or
 - iii) a polynucleotide encoding a fragment of a polypeptide encoded by polynucleotides (i) or (ii), wherein said fragment has at least one predetermined activity as specified herein; and
25
- iv) a polynucleotide the complementary strand of which hybridizes under stringent conditions with a polynucleotide as defined in any one of (i), (ii) and (iii), and encodes a polypeptide, or a fragment thereof, having at least one predetermined activity as specified herein; and
30
- v) a polynucleotide comprising a nucleotide sequence which is degenerate to the nucleotide sequence of polynucleotides (iii) or (iv);
35

or the complementary strand of such a polynucleotide.

5 Prediabetes: Prediabetes refers to the intermediate metabolic states between normal and diabetic glucose homeostasis. It comprises of two distinct states, those of impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) or a combination of both but by itself is not diabetes. Thus, it is a condition in which blood glucose level is higher than normal, but not high enough to be classified as type 2 diabetes.

10 Purified antibody: The term a "purified antibody" is an antibody at least 60 weight percent of which is free from the polypeptides and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation comprises antibody in an amount of at least 75 weight percent, more preferably at least 90 weight percent, and most preferably at least 99 weight percent.

15 Root mean square deviation: The term "root mean square deviation" (rmsd) is used as a mean of comparing two closely related structures and relates to a deviation in the distance between related atoms of the two structures after structurally minimizing this distance in an alignment. Related proteins with closely related
20 structures will be characterized by relatively low RMSD values whereas larger differences will result in an increase of the RMSD value.

25 Sensitization of the insulin receptor: the term "sensitization of the insulin receptor" is used to explain that the agents according to the invention are preferred to be able to stabilise the insulin receptor, and preferably also to increase the amount of insulin
30 receptors. Sensitization of the insulin receptor may be measured by administering an agent according to the invention and then performing a glucose tolerance test, as discussed in relation to Figure 20. Furthermore, the sensitization may be assessed by assessing the amount of insulin receptors before and after administration of the agent according to the invention, whereby an increase is indicative of sensitization
35 of the insulin receptor. Also, the sensitization may be assessed by assessing the amount of activated insulin receptors, ie. phosphorylated insulin receptors. Furthermore, the sensitization may also be assessed by measuring the affinity between insulin and the insulin receptor, in that an increase in affinity is an indication of sensitization.

Sequence identity: Sequence identity is determined in one embodiment by utilising fragments of SorCS1 polypeptides comprising at least 25 contiguous amino acids and having an amino acid sequence which is at least 80%, such as 85%, for example 90%, such as 95%, for example 99% identical to the amino acid sequence of any of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51 respectively, wherein the percent identity is determined with the algorithm GAP, BESTFIT, or FASTA in the Wisconsin Genetics Software Package Release 7.0, using default gap weights.

The following terms are used to describe the sequence relationships between two or more polynucleotides: "predetermined sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity".

Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2: 482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48: 443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. (U.S.A.) 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

As applied to polypeptides, a degree of identity of amino acid sequences is a function of the number of identical amino acids at positions shared by the amino acid sequences. A degree of homology or similarity of amino acid sequences is a function of the number of amino acids, i.e. structurally related, at positions shared by the amino acid sequences.

An "unrelated" or "non-homologous" sequence shares less than 40% identity, though preferably less than 25% identity, with one of the SorCS1 polypeptide sequences of the present invention. The term "substantial identity" means that two

peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity or more (e.g., 99 percent sequence identity). Preferably, residue
5 positions which are not identical differ by conservative amino acid substitutions.

Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino
10 acids having aliphatic-hydroxyl side chains is serine and threonine, a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulphur-containing side chains is cysteine and
15 methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

Additionally, variants are also determined based on a predetermined number of
20 conservative amino acid substitutions as defined herein below. Conservative amino acid substitution as used herein relates to the substitution of one amino acid (within a predetermined group of amino acids) for another amino acid (within the same group), wherein the amino acids exhibit similar or substantially similar characteristics.

25 Within the meaning of the term “conservative amino acid substitution” as applied herein, one amino acid may be substituted for another within the groups of amino acids indicated herein below:

- 30 i) Amino acids having polar side chains (Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, Tyr, and Cys,)
- ii) Amino acids having non-polar side chains (Gly, Ala, Val, Leu, Ile, Phe, Trp, Pro, and Met)
- iii) Amino acids having aliphatic side chains (Gly, Ala Val, Leu, Ile)
- 35 iv) Amino acids having cyclic side chains (Phe, Tyr, Trp, His, Pro)

- v) Amino acids having aromatic side chains (Phe, Tyr, Trp)
- vi) Amino acids having acidic side chains (Asp, Glu)
- vii) Amino acids having basic side chains (Lys, Arg, His)
- viii) Amino acids having amide side chains (Asn, Gln)
- 5 ix) Amino acids having hydroxy side chains (Ser, Thr)
- x) Amino acids having sulphur-containing side chains (Cys, Met),
- xi) Neutral, weakly hydrophobic amino acids (Pro, Ala, Gly, Ser, Thr)
- xii) Hydrophilic, acidic amino acids (Gln, Asn, Glu, Asp), and
- xiii) Hydrophobic amino acids (Leu, Ile, Val)

10

Accordingly, a variant or a fragment thereof according to the invention may comprise, within the same variant of the sequence or fragments thereof, or among different variants of the sequence or fragments thereof, at least one substitution, such as a plurality of substitutions introduced independently of one another.

15

It is clear from the above outline that the same variant or fragment thereof may comprise more than one conservative amino acid substitution from more than one group of conservative amino acids as defined herein above.

- 20 The addition or deletion of at least one amino acid may be an addition or deletion of from preferably 2 to 250 amino acids, such as from 10 to 20 amino acids, for example from 20 to 30 amino acids, such as from 40 to 50 amino acids. However, additions or deletions of more than 50 amino acids, such as additions from 50 to 100 amino acids, addition of 100 to 150 amino acids, addition of 150-250 amino acids,
- 25 are also comprised within the present invention. The deletion and/or the addition may - independently of one another - be a deletion and/or an addition within a sequence and/or at the end of a sequence.

- 30 SorCS1-like agent: The expression "a SorCS1-like agent" as used herein refers to any agent capable of structurally imitating the Vps10p-domain receptor SorCS1 thus having the same or similar biological effect on the insulin receptor, as the effect demonstrated herein by the present inventors. Accordingly, as SorCS1-like agent may be a peptide, a polypeptide, a small organic molecule, siRNA, siDNA, nucleic

acid molecules encoding a polypeptide. In a preferred embodiment, the SorCS1-like agent is a SorCS1 fragment, preferably human soluble SorCS1 (SEQ ID NO: 15) or a precursor thereof.

5 Substituted lower alkyl means a lower alkyl having one to three substituents selected from the group consisting of hydroxyl, alkoxy, amino, amido, carboxyl, acyl, halogen, cyano, nitro and thiol.

10 Treatment: The term "treatment" as used herein refers to a method involving therapy including surgery of a clinical condition in an individual including a human or animal body. The therapy may be ameliorating, curative or prophylactic, i.e. reducing mental and behavioural symptoms.

15 Variants: The term "variants" as used herein refers to amino acid sequence variants said variants preferably having at least 60% identity, for example at least 63% identity, such as at least 66% identity, for example at least 70% sequence identity, for example at least 72% sequence identity, for example at least 75% sequence identity, for example at least 80% sequence identity, such as at least 85% sequence identity, for example at least 90% sequence identity, such as at least 91% sequence
20 identity, for example at least 91% sequence identity, such as at least 92% sequence identity, for example at least 93% sequence identity, such as at least 94% sequence identity, for example at least 95% sequence identity, such as at least 96% sequence identity, for example at least 97% sequence identity, such as at least 98% sequence identity, for example 99% sequence identity with any of the predetermined
25 sequences.

The variants preferably include the fragments shown to bind to the insulin receptor, see for example Figure 23B and/or parts of the SorCS1 sequence conserved from one species to other, see for example Figure 13.

30 Up-regulation of expression: a process leading to increased expression of genes, preferably of endogenous genes.

Insulin resistance
35

Insulin resistance (IR) is a condition in which the cells of the body become resistant to the effects of insulin, that is, the normal response to a given amount of insulin is reduced. As a result, higher levels of insulin are needed in order for insulin to have its effects. The resistance is seen with both the body's own insulin (endogenous) and if insulin is given through injection (exogenous).

There are probably several causes of insulin resistance and as described herein above there is a strong genetic factor. Drug-induced IR may be a further cause. In addition, insulin resistance is seen often in the following conditions: the metabolic syndrome, obesity, pregnancy, infection or severe illness and stress during steroid use. Treatments of insulin resistance in these conditions are aspects of the present invention.

The relationship between insulin resistance and diabetes is as follows. Type 2 diabetes, is the type of diabetes that normally occurs later in life. Insulin resistance precedes the development of type 2 diabetes, sometimes by several years. In individuals who will ultimately develop type 2 diabetes, it is believed that blood glucose and insulin levels are normal for many years; then at some point in time, insulin resistance develops most likely caused by overweight/obesity, physical inactivity and/or a series of currently not yet well-defined genetic polymorphisms. Accordingly, the treatment of the cause of insulin resistance is preferred over treatment of the symptoms of diabetes. The present invention is primarily aimed at providing a medicament for use in the treatment of insulin resistance.

As is well known by those skilled in the art, one of the actions of insulin is to cause the cells of the body, particularly hepatocytes and other cells of the liver, the muscle and fat cells, to remove and use glucose from the blood. In this way insulin controls the blood glucose level. Insulin has this effect on the cells by binding to insulin receptors on the cell surface and to allow influx of glucose into the cells, to be used as energy by the cell. With insulin resistance, the cells do not react appropriately to the insulin (they are resistant), and a signal is sent to the pancreas that more insulin needs to be produced, which in turn results in increased level of insulin in the blood resulting in an even stronger signal through the insulin receptors. In this manner the insulin resistance of the cells increases over time. As long as the pancreas is able to produce enough insulin to overcome this resistance, blood glucose levels remain

normal. When the pancreas can no longer produce enough insulin, the blood glucose levels begin to rise, initially after meals when glucose levels are at their highest and more insulin is needed, but eventually in the fasting state as well. At this point, insulin resistance has resulted in a number of medical conditions, including

5 type 2 diabetes, fatty liver, atherosclerosis wherein the latter in turn may result in coronary artery disease (angina pectoris and heart attack), stroke and peripheral vascular disease. A further medical condition associated with insulin resistance includes skin lesions, acanthosis nigricans (a cosmetic condition involving darkening of the skin in areas where there are creases such as the neck and arm pits). Further

10 conditions associated with IR are skin tags, reproductive abnormalities in women, polycystic ovary disease, hyperandrogenism, high male hormone levels and growth abnormalities.

Growth abnormalities as a result of insulin resistance are caused by the high levels

15 of circulating insulin that may be present in the blood. While insulin's effects on glucose metabolism may be impaired, it's effects on other mechanisms may be intact (or at least less impaired). Insulin, which is an anabolic, can exert effects on growth, through a mediator known as insulin-like growth factor -1. Patients may have actual linear growth and a noticeable coarsening of features. The increase

20 incidence of skin tags mentioned above may be through this mechanism as well.

The ability of insulin to stimulate glucose disposal vary continuously throughout a population of apparently healthy persons, and a difference of $\geq 600\%$ exists between the most insulin-sensitive and the most insulin resistance persons.

25 Approximately 50% of this variability can be attributed to adiposity (25%) and physical fitness (25%), with the remaining 50% likely of genetic origin. The third of the population that is the most insulin resistant is at a much greater risk of developing several abnormalities and clinical syndromes, including type 2 diabetes, cardio vascular diseases, hypertension, stroke, non-alcoholic fatty liver, polycystic

30 ovary disease, and certain forms of cancer

Insulin resistance can be diagnosed by a physician who can identify individuals that are likely to have insulin resistance with a detailed patient history, patient physical examination, and laboratory testing utilizing the risk factors. Tests for diagnosing IR includes but are not limited to euglycemic insulin clamping and intravenous

tolerance testing. However, these are expensive or complicated and are not necessary for managing patients.

5 In general clinical practice, glucose levels in conjunction with fasting insulin levels can give the physician a clue as to whether insulin resistance is present or not in patients without diabetes.

10 Insulin resistance can be treated by attempting to reduce the need for insulin, in combination with increasing the sensitivity of the cells to the action of insulin can be increased.

To decrease the need for insulin the individual suffering from insulin resistance can alter his/her diet, and particularly the intake of carbohydrates through the diet.

15 As described herein, the present invention addresses methods for sensitizing the cells (insulin receptors) to increase the action of insulin.

20 In one aspect the agent according to the present invention may be used to treat diseases and disorders associated with insulin resistance wherein said diseases and disorders are selected from the group consisting of insulin resistance syndrome, Type 2 diabetes mellitus, impaired glucose tolerance, the metabolic syndrome, hyperglycemia, hyperinsulinemia, arteriosclerosis, hypercholesterolemia, hypertriglyceridemia, hyperlipidemia, dyslipidemia, obesity, central obesity, polycystic ovarian syndrome, hypercoagulability, hypertension, microalbuminuria, 25 and any combinations thereof.

Other conditions treatable by the present invention include but are not limited to insulin resistance syndrome (IRS), Type 2 diabetes mellitus, impaired glucose tolerance, the metabolic syndrome, hyperglycemia, and hyperinsulinemia.

30

Agent of the invention

35 The present inventors have found that SorCS1 physically interacts with the insulin receptor (Fig.11), and have furthermore shown by cell biological experiments that the expression of the insulin receptor is elevated in cells stably over-expressing

soluble SorCS1 or the different SorCS1 splice variants (Fig.12), and the elevated amount of insulin receptor is still located on the cell surface (Fig.13).

Also, the inventors have found that overexpression of soluble SorCS1 in SorCS1 knockout mice as well as administration of SorCS1 decreases the plasma glucose level (Fig. 17) and increases expression and phosphorylation of the insulin receptor as well as the glucose transporter type 4 (Glut4) protein (Fig. 18). Moreover, overexpression of soluble SorCS1 in type 2 diabetic female mice decreases the plasma glucose and insulin levels (Fig. 19) and changes the subcellular localization of Glut4, which may consequently regulate glucose uptake.

Therefore, in a main aspect, the present invention relates to a SorCS1-like agent for use in the treatment of insulin resistance and/or a disease associated with insulin resistance in an individual, wherein said agent is capable of binding to the insulin receptor (IR) at a SorCS1 binding site and being capable of sensitization of an insulin receptor. The insulin receptor may be any insulin receptor, but preferably the insulin receptor is a human insulin receptor having the sequence of SEQ ID NO: 56.

The present inventors have found that SorCS1 binds to the insulin receptor through at least one binding site, and that one or more of the following parts of SorCS1 takes part in the binding:

SEQ ID NO: 1 aa 103-124

SEQ ID NO: 1 aa 125-143

SEQ ID NO: 1 aa 144-162

SEQ ID NO: 1 aa 197-218

SEQ ID NO: 1 aa 391-409

SEQ ID NO: 1 aa 661-684

SEQ ID NO: 1 aa 763-783

SEQ ID NO: 1 aa 859-876

Accordingly, the SorCS1-like agent preferably binds to a binding site on the insulin receptor, which binding site is characterised in that one or more of the shown parts of SorCS1 bind(s) to said binding site.

In a preferred embodiment the binding site on the insulin receptor comprises one or more of the sequences defined as follows:

- SEQ ID NO: 56 aa 100-120
- 5 SEQ ID NO: 56 aa 127-150
- SEQ ID NO: 56 aa 284-310
- SEQ ID NO: 56 aa 362-379
- SEQ ID NO: 56 aa 593-610
- SEQ ID NO: 56 aa 629-652
- 10 SEQ ID NO: 56 aa 749-772.

In one preferred embodiment, the agent as defined herein above is selected from the group consisting of

- a) an isolated SorCS1 polypeptide selected from the group consisting of
 - 15 i) an amino acid sequence consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51;
 - ii) a biologically active sequence variant of the amino acid sequence of a) wherein the variant has at least 70% sequence identity to said SEQ ID NO: 1; or
 - 20 iii) a biologically active fragment of any of i or ii wherein said fragment comprises at least 5 contiguous amino acids of any of a) through b), and having at least 70% sequence identity to
 - 25 SEQ ID NO: 1 in a range of overlap of at least 5 amino acids wherein the biological activity is sensitization of an insulin receptor,

or a pharmaceutically acceptable salt thereof.

30

In one embodiment, the polypeptide is a naturally occurring allelic variant of the sequence selected from the group consisting of SEQ ID NO: SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50

and 51, and preferably the polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 5, 10, 15, 21, 27, 33, 37, 39, 43 and 47.

5 In a further embodiment the polypeptide is a variant polypeptide described therein, wherein any amino acid specified in the selected sequence is altered to provide a conservative substitution as defined above. Accordingly, the polypeptide preferably has at least 70%, e.g. 75%, such as 80%, e.g. 85%, such as 90%, e.g. 95%, such as 98%, e.g. 99% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
10 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51.

In one embodiment the polypeptide is glycosylated, such as a polypeptide being selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 6, 7, 8, 9, 11, 12, 13,
15 14, wherein the polypeptide may be glycosylated in one or more of the following amino acid residue positions 184, 352, 433, 765, 776, 816, 847, 908 and 929, and/or wherein the polypeptide is selected from the group consisting of SEQ ID NOs: 16, 17, 18, 19, 20, 22, 26, 28, 29, 30, 31 and 32, wherein the polypeptide may be glycosylated in one or more of the following amino acid residue positions 184,
20 352, 433, 765, 776, 816, 847, 908 and 929, and in another embodiment the glycosylated fragment has the sequence selected from the group consisting of SEQ ID NO: 5, 10 and 15, or the glycosylated polypeptide fragment has the sequence selected from the group consisting of SEQ ID NO: 21, 27 and 33.

25

In some embodiments, however, it is preferred that the polypeptide is deglycosylated.

30 The SorCS1-like agent may comprise a soluble fragment of a polypeptide as defined herein or a fragment of a variant, and accordingly, in one embodiment the polypeptide is a soluble polypeptide being a fragment of the sequences selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 6, 7, 8, 9, 11, 12, 13, 14, or the polypeptide is a soluble polypeptide being a fragment of the sequences of claim 15.

35

It is preferred that the polypeptide is capable of forming at least one intramolecular cystine bridge, and more preferably that the polypeptide as defined herein above comprises a dimer of said polypeptide linked through at least one intermolecular cystine bridge.

5

In one embodiment the polypeptide according to the present invention further comprises an affinity tag, such as a polyhis tag, a GST tag, a HA tag, a Flag tag, a C-myc tag, a HSV tag, a V5 tag, a maltose binding protein tag, a cellulose binding domain tag.

10

Nucleic acid, vectors and host cells

In one aspect, the invention relates to a nucleic acid sequence capable of encoding the polypeptide as defined herein above, wherein the encoded polypeptide has at least 70%%, e.g. 75%, such as 80%, e.g. 85%, such as 90%, e.g. 95%, such as 98%, e.g. 99% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51 or to a fragment thereof.

20

In a preferred aspect the invention relates to a vector, said vector comprising at least one nucleic acid molecule as defined herein above, for use in the treatment of insulin resistance or diseases associated with insulin resistance in an individual.

25 The vector of the invention may further comprise a promoter which may be operably linked to the nucleic acid molecule of the invention.

The promoter may be, but is not limited to the group consisting of: CMV, human UbiC, RSV, Tet-regulatable promoter, Mo-MLV-LTR, Mx1, EF-1alpha, PDGF beta and CaMK II.

30

The vector of the invention may be selected from the group consisting of vectors derived from the Retroviridae family including lentivirus, HIV, SIV, FIV, EAIIV, CIV.

Other vectors of the invention are selected from the group consisting of alphavirus, adenovirus, adeno associated virus, baculovirus, HSV, coronavirus, Bovine papilloma virus, Mo-MLV, preferably adeno associated virus.

- 5 In another preferred embodiment, the invention also relates to a host cell comprising the nucleic acid as described above, and even more preferred an isolated host cell of the invention is transformed or transduced with at least one vector as defined herein above, for use in the treatment of insulin resistance or diseases associated with insulin resistance in an individual.

10

The isolated host may be selected from the group consisting of *Saccharomyces cerevisiae*, *E. coli*, *Aspergillus* and Sf9 insect cells and of mammalian cells selected from the group consisting of human, feline, porcine, simian, canine, murine and rat cells, wherein the mammalian cell may be selected from, but is not limited to the group consisting of muscle cells, hepatocytes, adipocytes and cells of the pancreas such as α cells, β cells and δ cells.

15

In one embodiment the isolated host cell is selected from the group consisting of CHO, CHO-K1, HEI193T, HEK293, COS, PC12, HiB5, RN33b and BHK cells.

20

In another aspect the invention relates to a packaging cell line as defined herein above, wherein said packaging cell line is capable of producing an infective virus particle for use in the treatment of insulin resistance or diseases associated with insulin resistance in an individual, said virus particle comprising a Retroviridae derived genome comprising a 5' retroviral LTR, a tRNA binding site, a packaging signal, a promoter operably linked to a polynucleotide sequence encoding the polypeptide as defined herein above, an origin of second strand DNA synthesis, and a 3' retroviral LTR.

25

- 30 In one embodiment the genome of the packaging cell line is lentivirally derived and the LTRs are lentiviral.

As discussed above the SorCS1-like agent is any agent having the biological activity of SorCS1 in relation to the insulin receptor, ie. an agent which is capable a binding

to the insulin receptor, and more preferably the agent is also capable of sensitizing the insulin receptor whereby it is possible to lower the blood glucose concentration in the individual being administered with the SorCS1-like agent by treating insulin resistance and diseases associated with insulin resistance. The agent may be any
5 type of compound, such as polypeptides, antibodies as well as small organic molecules, wherein the antibody may be selected from the group consisting of: polyclonal antibodies, monoclonal antibodies, humanised antibodies, single chain antibodies, recombinant antibodies directed towards the insulin receptor.

10 Furthermore, as discussed herein administration of nucleic acids either naked, or in host cells or packaging cells, wherein the nucleic acid is capable of encoding the polypeptide as discussed herein, for the treatment of insulin resistance and diseases associated with insulin resistance is also an aspect of the invention.

15 **Antibodies**

As mentioned above, the agent of the present invention may be an antibody, in particularly an antibody directed against the insulin receptor, or more preferred against one or more of the binding sites on the insulin receptor shown in Figure 23A.

20 Antibodies may furthermore be used as research tool for screening various aspects of the invention. Such methods are well known by those skilled in the art but is nevertheless described in further detail below. The antibodies for used for research tools are both antibodies directed towards the insulin receptor as well as directed
25 towards the Vps10p-domain receptor, including SorCS1.

It is an aspect of the present invention to provide antibodies or functional equivalents thereof specifically recognising and binding epitopes of the Vps10p-domain receptors and insulin receptors.

30 The antibody or functional equivalent thereof may be any antibody known in the art, for example a polyclonal or a monoclonal antibody derived from a mammal or a synthetic antibody, such as a single chain antibody or hybrids comprising antibody fragments. Furthermore, the antibody may be mixtures of monoclonal antibodies or

artificial polyclonal antibodies. In addition functional equivalents of antibodies may be antibody fragments, in particular epitope binding fragments. Furthermore, antibodies or functional equivalent thereof may be a small molecule mimicking an antibody. Naturally occurring antibodies are immunoglobulin molecules consisting of heavy and light chains. In preferred embodiments of the invention, the antibody is a monoclonal antibody.

The antibodies according to the present invention may also be recombinant antibodies. Recombinant antibodies are antibodies or fragments thereof or functional equivalents thereof produced using recombinant technology. For example recombinant antibodies may be produced using a synthetic library or by phage display. Recombinant antibodies may be produced according to any conventional method for example the methods outlined in "Recombinant Antibodies", Frank Breitling, Stefan Dübel, Jossey-Bass, September 1999.

The antibodies according to the present invention may also be bispecific antibodies, i.e. antibodies specifically recognising two different epitopes. Bispecific antibodies may in general be prepared starting from monoclonal antibodies, or from recombinant antibodies, for example by fusing two hybridoma's in order to combine their specificity, by Chemical crosslinking or using recombinant technologies. Antibodies according to the present invention may also be tri-specific antibodies.

Functional equivalents of antibodies may in one preferred embodiment be a fragment of an antibody, preferably an antigen binding fragment or a variable region. Examples of antibody fragments useful with the present invention include Fab, Fab', F(ab')₂ and Fv fragments. Papain digestion of antibodies produces two identical antigen binding fragments, called the Fab fragment, each with a single antigen binding site, and a residual "Fc" fragment, so-called for its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen binding fragments which are capable of cross-linking antigen, and a residual other fragment (which is termed pFc'). Additional fragments can include diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. As used herein, "functional fragment" with respect to antibodies, refers to Fv, F(ab) and F(ab')₂ fragments.

Preferred antibody fragments retain some or essential all the ability of an antibody to selectively binding with its antigen or receptor. Some preferred fragments are defined as follows:

5

(1) Fab is the fragment that contains a monovalent antigen-binding fragment of an antibody molecule. A Fab fragment can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain.

10

(2) Fab' is the fragment of an antibody molecule and can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain. Two Fab' fragments are obtained per antibody molecule. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region.

15

(3) (Fab')₂ is the fragment of an antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction. F(ab')₂ is a dimer of two Fab' fragments held together by two disulfide bonds.

20

(4) Fv is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, non-covalent association (V_H-V_L dimer). It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

25

30

In one embodiment of the present invention the antibody is a single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule. Such single chain

35

antibodies are also referred to as "single-chain Fv" or "scFv" antibody fragments. Generally, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains that enables the scFv to form the desired structure for antigen binding.

5

Procedures for making antibodies

Polyclonal and monoclonal antibodies directed against a specific antigen, or epitope of an antigen, can be produced according to standard procedures (see e.g. Antibodies – A laboratory Manual by Ed Harlow and David Lane, Cold Spring Harbor Laboratory 1998, ISBN 0-87969-314-2). The procedure for subsequent generation of humanized antibodies or fragments thereof has also been described (e.g. A. M. Scott et al, Cancer Research 60:3254-3261, 2000; A. Nissim and Y. Chernajovsky, Handb. Exp. Pharmacol. 181:3-18, 2008; A. Mountain and J. R. Adair, Biotechnol. Genet. Eng. Rev. 10:1-142, 1992).

15

Humanised antibody framework

It is not always desirable to use non-human antibodies for human therapy, since the non-human "foreign" epitopes may elicit immune response in the individual to be treated. To eliminate or minimize the problems associated with non-human antibodies, it is desirable to engineer chimeric antibody derivatives, i.e., "humanized" antibody molecules that combine the non-human Fab variable region binding determinants with a human constant region (Fc). Such antibodies are characterized by equivalent antigen specificity and affinity of the monoclonal and polyclonal antibodies described above, and are less immunogenic when administered to humans, and therefore more likely to be tolerated by the individual to be treated.

20

25

Accordingly, in one embodiment the binding polypeptide has a binding domain carried on a humanised antibody framework, also called a humanised antibody.

30

Human Antibodies

Human monoclonal antibodies of the invention can be produced by a variety of techniques, including conventional monoclonal antibody methodology, e.g., the standard somatic cell hybridization technique of Kohler and Milstein, *Nature* 256:495 (1975). Although somatic cell hybridization procedures are preferred, in principle,

35

other techniques for producing monoclonal antibody can be employed, *e.g.*, viral or oncogenic transformation of B-lymphocytes or phage display techniques using libraries of human antibody genes.

5

To generate fully human monoclonal antibodies to the epitopes of interest to the present invention, transgenic or transchromosomal mice containing human immunoglobulin genes (*e.g.*, HCo12, HCo7 or KM mice) can be immunized with an enriched preparation of the antigen and/or cells expressing the epitopes of the
10 receptor targets of the present invention, as described, for example, by Lonberg *et al.* (1994), *supra*; Fishwild *et al.* (1996), *supra*, and WO 98/24884. Alternatively, mice can be immunized with DNA encoding the CaOU-1 epitope. Preferably, the mice will be 6-16 weeks of age upon the first infusion.

15

Monovalent antibodies

The monospecific binding polypeptide may be monovalent, i.e. having only one binding domain.

20

For a monovalent antibody, the immunoglobulin constant domain amino acid residue sequences comprise the structural portions of an antibody molecule known in the art as CH1, CH2, CH3 and CH4. Preferred are those binding polypeptides which are known in the art as C_L. Preferred C_L polypeptides are selected from the group consisting of C_{kappa} and C_{lambda}.

25

Furthermore, insofar as the constant domain can be either a heavy or light chain constant domain (C_H or C_L, respectively), a variety of monovalent binding polypeptide compositions are contemplated by the present invention. For example, light chain constant domains are capable of disulfide bridging to either another light
30 chain constant domain, or to a heavy chain constant domain. In contrast, a heavy chain constant domain can form two independent disulfide bridges, allowing for the possibility of bridging to both another heavy chain and to a light chain, or to form polymers of heavy chains.

35

Thus, in another embodiment, the invention contemplates an isolated monovalent

binding polypeptide wherein the constant chain domain C has a cysteine residue capable of forming at least one disulfide bridge, and where at least two monovalent polypeptides are covalently linked by said disulfide bridge.

- 5 In preferred embodiments, the constant chain domain C can be either C_L or C_H. Where C is C_L, the C_L polypeptide is preferably selected from the group consisting of C_{kappa} and C_{lambda}.

- 10 In another embodiment, the invention contemplates a binding polypeptide composition comprising a monovalent polypeptide as above except where C is C_L having a cysteine residue capable of forming a disulfide bridge, such that the composition contains two monovalent polypeptides covalently linked by said disulfide bridge.

15 *Multispecificity, including bispecificity*

In a preferred embodiment the present invention relates to multispecific binding polypeptides, which have affinity for and are capable of binding at least two different entities. Multispecific binding polypeptides can include bispecific binding polypeptides.

- 20 In one embodiment the multispecific molecule is a bispecific antibody (BsAb), which carries at least two different binding domains, where preferably at least one of which is of antibody origin.

- 25 A bispecific molecule of the invention can also be a single chain bispecific molecule, such as a single chain bispecific antibody, a single chain bispecific molecule comprising one single chain antibody and a binding domain, or a single chain bispecific molecule comprising two binding domains. Multispecific molecules can also be single chain molecules or may comprise at least two single chain molecules.

- 30 The multispecific, including bispecific, antibodies may be produced by any suitable manner known to the person skilled in the art.

- 35 The traditional approach to generate bispecific whole antibodies was to fuse two hybridoma cell lines each producing an antibody having the desired specificity.

Because of the random association of immunoglobulin heavy and light chains, these hybrid hybridomas produce a mixture of up to 10 different heavy and light chain combinations, only one of which is the bispecific antibody. Therefore, these bispecific antibodies have to be purified with cumbersome procedures, which considerably decrease the yield of the desired product.

By using a bispecific or multispecific binding polypeptide according to the invention the invention offers several advantages as compared to monospecific/monovalent binding polypeptides.

While human monoclonal antibodies are preferred, other antibodies which can be employed in the bispecific or multispecific molecules of the invention are murine, chimeric and humanized monoclonal antibodies. Such murine, chimeric and humanized monoclonal antibodies can be prepared by methods known in the art.

The inventors of this application have raised antibodies against several parts of the Vps10p-domain receptors. The present invention is directed to antibodies against the unifying feature of this receptor family – the Vps10p domain. The below sequence alignment of the Vps10p-domain demonstrate the conservation within this receptor family.

Table 1: Antibodies against Vps10p-domain receptors

Receptor	Name	Antigen	Species	Western	IH/IC	Ref.
SorLA	SORLA goat	extracellular domain	goat	X	X	Schmidt et. al., J.Biol.Chem. 282:32956-67, 2007
	Hale SORLA	Cytoplasmic domain	rabbit	X		
	SORLA LA	Complement type repeat	rabbit	X		
	Sol SORLA	extracellular domain	rabbit	X	X	Andersen et al., PNAS 103:13461-6, 2005
	SORLA tail	Cytoplasmic domain	rabbit	X		
	SORLA VPS	VPS10p domain	rabbit	X		
	#606870	Peptide seq.	rabbit	X		

		in Vps10p-domain				
	#642739	C-terminal	rabbit	X		
	#643739	Cytoplasmic tail	rabbit	X		
	20C11	Extracellular domain	mouse	X	X	
	AG4	Extracellular domain	mouse	X		
Sortilin	#5264	Extracellular domain	rabbit	X	X	Munck Petersen et al, EMBO J. 18 :595-604, 1999
	#5448	Cytoplasmic domain	rabbit	X	X	Jansen et al, Nature Neurosci. 10 :1449-1457, 2007
	#5287	Cytoplasmic domain	rabbit	X		
	CP 96 334 SR 96 204	propeptide	Rabbit	X		Munck Petersen et al, EMBO J. 18 :595-604, 1999
	#5438	Vps10p	rabbit	X		
	Sortilin goat /Laika	Extracellular domain	goat	X		
	F9	Extracellular domain	mouse	X	X	
	F11	Extracellular domain	mouse	X	X	
	AF2934	Extracellular domain	goat	X	X	R&D Systems, Jansen et al, Nature Neurosci. 10 :1449-1457, 2007
	AF3154	Extracellular domain	goat	X	X	R&D Systems; Jansen et al, Nature Neurosci. 10 :1449-1457, 2007
	anti-NTR3	Extracellular domain	mouse	X	X	BD Transduction Laboratories,
	ANT-009	Extracellular domain	mouse	X	X	Alomone Labs ;

						Nykjaer et al, Nature 427 :8 43-848, 2004
SorCS1	AF3457	Extracellular domain	goat	X	X	BD Transduction Laboratories
	SorCS1 goat	Extracellular domain	goat	X		
	L-SorCS1	Extracellular domain	rabbit	X	X	Hermey et al, J.Biol.Chem. 279:50221- 50229, 2003
	Leu- SorCS1	Leucine-rich domain	rabbit	X	X	Hermey et al, J.Biol.Chem. 279:50221- 50229, 2003
	#5466	Extracellular domain	rabbit	X	X	
	1D	Extracellular domain	mouse	X		
	4H	Extracellular domain	mouse	X		
	6B	Extracellular domain	mouse	X		
	4A	Extracellular domain	mouse	X		
SorCS2	AF4237	Extracellular domain	sheep	X		BD Transduction Laboratories
	SorCS2 goat	Extracellular domain	goat	X	X	
	#5422	Extracellular domain	rabbit	X	X	Hermey et al, Biochem. J., 395:285-93, 2006
	#5431	28 C- terminal amino acids	rabbit	X	X	
	SorCS2- prp	propeptide	rabbit	X		Schousboe Sjoegaard, Dissertation, Aarhus University, 2005
	M1	Extracellular domain	mouse		X	Roland Holst, Master of Science Thesis, Aarhus University, 2006
	M3	Extracellular	mouse		X	Roland Holst,

		domain				Master of Science Thesis, Aarhus University, 2006
	M4	Extracellular domain	mouse		X	Roland Holst, Master of Science Thesis, Aarhus University, 2006
	M7	Extracellular domain	mouse		X	Roland Holst, Master of Science Thesis, Aarhus University, 2006
	M9	Extracellular domain	mouse		X	Roland Holst, Master of Science Thesis, Aarhus University, 2006
	M10	Extracellular domain	mouse		X	Roland Holst, Master of Science Thesis, Aarhus University, 2006
	M13	Extracellular domain	mouse	X		Roland Holst, Master of Science Thesis, Aarhus University, 2006
	M15	Extracellular domain	mouse		X	Roland Holst, Master of Science Thesis, Aarhus University, 2006
	M18	Extracellular domain	mouse	X	X	Roland Holst, Master of Science Thesis,

						Aarhus University, 2006
	M19	Extracellular domain	mouse	X	X	Roland Holst, Master of Science Thesis, Aarhus University, 2006
	S21	Extracellular domain	mouse	X		Roland Holst, Master of Science Thesis, Aarhus University, 2006
	SorCS2-GST-73aa	Extracellular domain	rabbit	X		
	SorCS2-GST-100aa	Extracellular domain	rabbit	X		
	SorCS2-GST-172aa	Extracellular domain	rabbit	X		
SorCS3	SorCS3-N	extracellular domain	rabbit	X		
	SorCS3-C	15 C-terminal aa	rabbit	X		
	Sort3 N Term #5389	N-terminal domain	rabbit	X	X	Westergaard et al, FEBS Lett. 579:1172-6, 2005
	#5432	Extracellular domain	rabbit	X	X	
	MAB3067	Extracellular domain	mouse	X		BD Transduction Laboratories
	MAB30671	Extracellular domain	mouse	X		BD Transduction Laboratories
	AF3326	Extracellular domain	goat	X		BD Transduction Laboratories
	SorCS3 goat	Extracellular domain	goat	X		

Successful clinical use of antibodies

A number of therapeutic antibodies are in clinical use. Examples include Genentech's Rituxan, an antibody directed against the CD20 receptor (used in rheumatoid arthritis), Johnson & Johnson's Remicade, an antibody directed against TNF alpha receptor (in Psoriasis), Roche's Avastin, an anti-VEGF antibody used for treatment of colorectal and lung cancer, as well as Herceptin, an antibody against the receptor HRE2 used in breast cancer therapy.

Assessing binding to a receptor is routine work for the person skilled in the biotechnical field. In this regard it has to be mentioned that the Vps10p-domain receptor family were known at the priority date of this invention and binding assays involving.

In one embodiment, the agent of the present invention is an anti-Insulin Receptor polyclonal or monoclonal antibody.

15

Transgenic SorCS1 mice

The present inventors have found that SorCS1 is expressed in adipose tissue, skeletal muscle and β -cells of the pancreas; all tissues involved in glucose metabolism (Fig.3). In order to examine the function of SorCS1 and its different splice variants the investors generated a conditional knockout mouse using a new developed targeting strategy based on FLP recombination and an insertion technique called 'recombinase-mediated cassette exchange'. The model was used to generate a 'full' knockout mouse lacking all splice forms of SorCS1 whereby expression of all the splice variants have been disrupted (Fig.4). SorCS1 mice deficient in all splice variants ('full' knockout) show no gross abnormalities or signs of changed behaviour, they are fertile and they exhibit a normal life span (unpublished). However, the SorCS1 knockout mouse was phenotypically characterized with respect to glucose metabolism and development of type-2 diabetes, and preliminary results support an important role of the receptor in development of diabetes. Whereas blood glucose levels in fasting male mice at 17 and 50 weeks of age were similar to that of control littermates, female mice at the age of 50 weeks showed a dramatic elevation in blood glucose as compared to age-matched control mice (Fig.5). However, both genders exhibited elevated levels of insulin at 50 weeks of age (Fig.6 female only). In agreement, their pancreatic islets

were up to 3-fold enlarged as determined by immunostaining for a β -cell marker (Fig. 15). The results indicate that old SorCS1 knockout male mice are hyperinsulimic but prediabetic, whereas old SorCS1 knockout female mice are hyperglycaemic and hyperinsulimic, thus becoming diabetic.

5

Furthermore, the inventors have also found that both male and female SorCS1 knockout mice have normal body weight. The absence of obesity in the knockout mice makes it possible to dissociate the effect of obesity on the prediabetic or diabetic phenotype, which complicates analysis in several existing animal models of diabetes. However, because obesity is a significant risk factor for type-2 diabetes, SorCS1 deficient animals were also fed a high calorie Western type diet to study the impact of obesity on disease progression. Physiological measurements revealed increases in plasma glucose and insulin levels and in abdominal fat for the wild type mice on high calorie diet compared to wild type mice on normal diet (fig.8+9). In contrast, the SorCS1 knockout mice showed no significant changes on Western type diet compared to normal diet, thus showing no aggravation of the diabetic status. The lower amount of abdominal fat in the knockout mice on western diet compared to the wild type mice confirm the insulin resistance of the knockout mice as it leads to reduced uptake of glucose in the adipose tissue and thereby less production of abdominal fat.

Accordingly, the present inventors have shown that the SorCS1 knockout mouse is a unique animal model for studying insulin resistance and diseases related to insulin resistance, in particularly diabetes because the SorCS1 knockout mouse develops the symptoms normally related to insulin resistance and diabetes, including the late symptoms of diabetes, such as neuropathic symptoms.

In addition, the inventors have shown that 50 weeks old male and female SorCS1^{-/-} mice exhibit elevated amount of phosphorylated IR as compared to age-matched controls (Fig.10) suggesting that SorCS1 may partake in insulin signalling in peripheral tissues. Alternatively, a signal derived from SorCS1 that convert on the insulin signalling pathways may be missing, resulting in compensatory upregulation of phosphorylated IR . Since SorCS1 is also engaged in cellular sorting, the receptor may also regulate the subcellular distribution of IR.

Therefore, one important aspect of the present invention relates to a transgenic knock-out mouse in which the endogenous Vps10p-domain receptor SorCS1 genes have been disrupted to abolish expression of a functional SorCS1 receptor, and
5 wherein said mouse exhibits a reduced response to insulin relative to a non-transgenic control mouse.

In a further embodiment the invention relates to the transgenic mouse as defined herein above, wherein said disruption comprises a deletion of the SorCS1 receptor
10 gene nucleotide sequences encoding the start codon or a region of the mouse SorCS1 receptor from the extracellular domain, transmembrane domain, or the cytoplasmic domain.

In one aspect, the invention relates to a transgenic mouse capable of encoding
15 soluble and/or full length SorCS1 in a tissue specific manner, upon activation of expression. The procedure for preparing said mouse is described in example 12. The tissue to be specifically activated may be selected from, but is not limited to, the group consisting of liver, muscle, pancreas and adipose tissue.

20 **Methods of screening for agents of the invention**

The present invention provides specific targets and methods for screening and evaluating further candidate agents including SorCS1 peptide and polypeptide fragments and mutant and variants thereof.

25 While the screening of a large number of peptides for a certain physiological activity may be a laborious undertaking, the exact disclosures of the assay herein to be carried out enables the skilled person to reproduce the present invention without undue burden of experimentation and without needing inventive skill.

30 For this purpose screening libraries of candidate agents are readily available for purchase on the market. Whether a library is a peptide library or a chemical library does not have any impact in the present situation since screening of chemical libraries is also routine work. In fact screening of chemical libraries is a service
35 offered by commercial companies, and it is clear from their presentation material

(See e.g. <http://www.analyticon.com/>) that they do not consider the screening work as such to be inventive.

Initially in the process of screening for SorCS1-like agents it is relevant to perform binding studies as discussed herein, in particularly in relation to the Figures and the Examples to verify that the agent binds to the insulin receptor. Furthermore, it may be relevant to show that the agent in fact also sensitizes the insulin receptor. As discussed above, this may be done indirectly by showing that administration of the SorCS1-like agent in fact reduces the blood glucose concentration by for example performing a glucose tolerance test (GTT), and preferably also showing that the insulin concentration is lowered, if it initially was increased. Furthermore, sensitisation of the insulin receptor may also be measured by measuring the amount of insulin receptor, since administration of soluble SorCS1 leads to an increase of insulin receptors.

Accordingly, in one embodiment the present invention relates to a method for screening for the ability of the SorCS1-like agent as defined herein above to reduce blood glucose levels, said method comprising the steps of

- a) providing a first and a second transgenic mouse of claim 69;
- b) administering to said first transgenic mouse a candidate agent, and
- c) administering to said second transgenic mouse a physiological solution, and
- d) taking blood samples from the mouse of b) and c) respectively, at predetermined time intervals, such as at 15 minutes, 30 minutes, 60 minutes, 2 and 4 hours, subsequent to administration of said agent, and
- e) comparing blood glucose levels in the samples of d); wherein a reduction in blood glucose level of said first transgenic mouse administered said candidate agent relative to said second transgenic mouse not administered said candidate agent indicates that the candidate agent reduces blood glucose levels.

In another embodiment, the invention relates to a method for screening for the ability of the SorCS1-like agent of the invention to reduce blood glucose levels, said method comprising the steps of

- a) providing a first and a second wild-type mouse; and
- 5 f) administering to said first mouse the agent of claim 1, and
- g) administering to said second mouse a physiological solution, and
- h) taking blood samples from the two mice of b) and c) respectively, at predetermined time intervals, such as at 15 minutes, 30 minutes, 60 minutes, 2 and 4 hours, subsequent to administration of said agent, and
- 10 i) comparing plasma glucose levels in the samples of d); wherein a reduction in blood glucose level of said first wild-type mouse administered said agent relative to said second wild type mouse not administered said candidate agent, indicates that the agent reduces blood glucose levels.
- 15

Pharmaceutical composition and administration forms

The present invention also encompass pharmaceutical compositions comprising the agent as defined herein. In the present context the term agent and compound is
20 considered synonyms when discussin the pharmaceutical composition.

The main routes of drug delivery according to this invention are parenteral, oral or enteral, and topical in order to introduce the agent into the blood stream to ultimately target the sites of the insulin receptors.

25

The agent may be administered to cross any mucosal membrane of an animal to which the biologically active substance is to be given, e.g. in the nose, vagina, eye, mouth, genital tract, lungs, gastrointestinal tract, or rectum, preferably the mucosa of the nose, or mouth.

30

The agents may be administered orally or enterally

Compounds of the invention may also be administered parenterally, that is by intravenous, intramuscular, subcutaneous intranasal, intrarectal, intravaginal or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred. Appropriate dosage forms for such administration may be prepared by conventional techniques. The compounds may also be administered by inhalation, which is by intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques.

The compounds according to the invention may be administered with at least one other compound, such as for example insulin. The compounds may be administered simultaneously, either as separate formulations or combined in a unit dosage form, or administered sequentially.

In one embodiment of the present invention, the dosage of the active ingredient of the pharmaceutical composition as defined herein above, is between 10 µg to 500 mg per kg body mass, such as between 20 µg and 400 mg, e.g. between 30 µg and 300 mg, such as between 40 µg and 200 mg, e.g. between 50 µg and 100 mg, such as between 60 µg and 90 µg, e.g. between 70 µg and 80 µg.

Furthermore, the dosage may be administered as a bolus administration or as a continuous administration. In relation to bolus administration the pharmaceutical composition may be administered at intervals of 30 minutes to 24 hours, such as at intervals of 1 to 6 hours. When the administration is continuous it is administered over an interval of time that normally is from 6 hours to 7 days. However, normally the dosage will be administered as a bolus 1-3 times per day.

In one important aspect of the present invention the the duration of the treatment is life long.

Formulations

Whilst it is possible for the compounds or salts of the present invention to be administered as the raw chemical, it is preferred to present them in the form of a

pharmaceutical formulation. Accordingly, the present invention further provides a pharmaceutical formulation, for medicinal application, which comprises a compound of the present invention or a pharmaceutically acceptable salt thereof, as herein defined, and a pharmaceutically acceptable carrier therefore.

5

The agents of the present invention may be formulated into a wide variety dosage forms, suitable for the various administration forms discussed above.

10

The pharmaceutical compositions and dosage forms may comprise the agents of the invention or its pharmaceutically acceptable salt or a crystal form thereof as the active component.

Furthermore, the pharmaceutical compositions may comprises pharmaceutically acceptable carriers that can be either solid or liquid.

15

Solid form preparations are normally provided for oral or enteral administration, such as powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, wetting agents, tablet disintegrating agents, or an encapsulating material.

20

Preferably, the composition will be about 0.5% to 75% by weight of a compound or compounds of the invention, with the remainder consisting of suitable pharmaceutical excipients. For oral administration, such excipients include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatin, sucrose, magnesium carbonate, and the like.

25

In powders, the carrier is a finely divided solid which is a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired. Powders and tablets preferably contain from one to about seventy percent of the active compound. Suitable carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin,

30

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tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. The term "preparation" is intended to include the formulation of the active compound with encapsulating material as carrier providing a capsule in which the active component, with or without carriers, is surrounded by a carrier, which is in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be as solid forms suitable for oral administration.

Drops according to the present invention may comprise sterile or non-sterile aqueous or oil solutions or suspensions, and may be prepared by dissolving the active ingredient in a suitable aqueous solution, optionally including a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100°C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container aseptically. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, in addition to the active component, colorants, flavours, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

Other forms suitable for oral administration include liquid form preparations including emulsions, syrups, elixirs, aqueous solutions, aqueous suspensions, toothpaste, gel dentrifice, chewing gum, or solid form preparations which are intended to be converted shortly before use to liquid form preparations. Emulsions may be prepared in solutions in aqueous propylene glycol solutions or may contain emulsifying agents such as lecithin, sorbitan monooleate, or acacia. Aqueous solutions can be prepared by dissolving the active component in water and adding

suitable colorants, flavours, stabilizing and thickening agents. Aqueous suspensions can be prepared by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well known suspending agents. Solid form
5 preparations include solutions, suspensions, and emulsions, and may contain, in addition to the active component, colorants, flavours, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

The compounds of the present invention may be formulated for parenteral
10 administration (e.g., by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, for example solutions in aqueous polyethylene glycol. Examples
15 of oily or nonaqueous carriers, diluents, solvents or vehicles include propylene glycol, polyethylene glycol, vegetable oils (e.g., olive oil), and injectable organic esters (e.g., ethyl oleate), and may contain formulatory agents such as preserving, wetting, emulsifying or suspending, stabilizing and/or dispersing agents.
Alternatively, the active ingredient may be in powder form, obtained by aseptic
20 isolation of sterile solid or by lyophilisation from solution for constitution before use with a suitable vehicle, e.g., sterile, pyrogen-free water.

Oils useful in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils useful in such formulations include peanut,
25 soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

Suitable soaps for use in parenteral formulations include fatty alkali metal,
30 ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides; (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c)
35 nonionic detergents such as, for example, fatty amine oxides, fatty acid

alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-.beta.-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

- 5 The parenteral formulations typically will contain from about 0.5 to about 25% by weight of the active ingredient in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations
- 10 will typically range from about 5 to about 15% by weight. Suitable surfactants include polyethylene sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and
- 15 vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.
- 20 The compounds of the invention can also be delivered topically for transdermal or transmucosal administration. Regions for topical administration include the skin surface and also mucous membrane tissues of the vagina, rectum, nose, mouth, and throat. Compositions for topical administration via the skin and mucous membranes should not give rise to signs of irritation, such as swelling or redness.
- 25 Transdermal administration typically involves the delivery of a pharmaceutical agent for percutaneous passage of the drug into the systemic circulation of the patient. The skin sites include anatomic regions for transdermally administering the drug and include the forearm, abdomen, chest, back, buttock, mastoidal area, and the like.
- 30 The topical composition may include a pharmaceutically acceptable carrier adapted for topical administration. Thus, the composition may take the form of a suspension, solution, ointment, lotion, sexual lubricant, cream, foam, aerosol, spray, suppository, implant, inhalant, tablet, such as a sublingual tablet, capsule, dry powder, syrup, balm or lozenge, for example. Methods for preparing such compositions are well
- 35 known in the pharmaceutical industry.

The compounds of the present invention may be formulated for topical administration to the epidermis as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or
5 oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or colouring agents. Formulations suitable for topical administration in the mouth include lozenges comprising active agents in a flavoured
10 base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Creams, ointments or pastes according to the present invention are semi-solid
15 formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy base. The base may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic
20 soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives or a fatty acid such as steric or oleic acid together with an alcohol such as propylene glycol or a macrogel. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surfactant such as a sorbitan ester or a polyoxyethylene derivative thereof.
25 Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as siliceous silicas, and other ingredients such as lanolin, may also be included.

Lotions according to the present invention include those suitable for application to
30 the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

35

Transdermal delivery may be accomplished by exposing a source of the complex to a patient's skin for an extended period of time. Transdermal patches have the added advantage of providing controlled delivery of a pharmaceutical agent-chemical modifier complex to the body. See Transdermal Drug Delivery: Developmental
5 Issues and Research Initiatives, Hadgraft and Guy (eds.), Marcel Dekker, Inc., (1989); Controlled Drug Delivery: Fundamentals and Applications, Robinson and Lee (eds.), Marcel Dekker Inc., (1987); and Transdermal Delivery of Drugs, Vols. 1-3, Kydonieus and Berner (eds.), CRC Press, (1987). Such dosage forms can be made by dissolving, dispersing, or otherwise incorporating the pharmaceutical
10 agent-chemical modifier complex in a proper medium, such as an elastomeric matrix material. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate-controlling membrane or dispersing the compound in a polymer matrix or gel.

15 For example, a simple adhesive patch can be prepared from a backing material and an acrylate adhesive. The pharmaceutical agent-chemical modifier complex and any enhancer are formulated into the adhesive casting solution and allowed to mix thoroughly. The solution is cast directly onto the backing material and the casting
20 solvent is evaporated in an oven, leaving an adhesive film. The release liner can be attached to complete the system.

Foam matrix patches are similar in design and components to the liquid reservoir system, except that the gelled pharmaceutical agent-chemical modifier solution is
25 constrained in a thin foam layer, typically a polyurethane. This foam layer is situated between the backing and the membrane which have been heat sealed at the periphery of the patch.

For passive delivery systems, the rate of release is typically controlled by a
30 membrane placed between the reservoir and the skin, by diffusion from a monolithic device, or by the skin itself serving as a rate-controlling barrier in the delivery system. See U.S. Pat. Nos. 4,816,258; 4,927,408; 4,904,475; 4,588,580, 4,788,062; and the like. The rate of drug delivery will be dependent, in part, upon the nature of the membrane. For example, the rate of drug delivery across membranes within the
35 body is generally higher than across dermal barriers. The rate at which the complex

is delivered from the device to the membrane is most advantageously controlled by the use of rate-limiting membranes which are placed between the reservoir and the skin. Assuming that the skin is sufficiently permeable to the complex (i.e., absorption through the skin is greater than the rate of passage through the membrane), the
5 membrane will serve to control the dosage rate experienced by the patient.

Suitable permeable membrane materials may be selected based on the desired degree of permeability, the nature of the complex, and the mechanical considerations related to constructing the device. Exemplary permeable membrane
10 materials include a wide variety of natural and synthetic polymers, such as polydimethylsiloxanes (silicone rubbers), ethylenevinylacetate copolymer (EVA), polyurethanes, polyurethane-polyether copolymers, polyethylenes, polyamides, polyvinylchlorides (PVC), polypropylenes, polycarbonates, polytetrafluoroethylenes (PTFE), cellulosic materials, e.g., cellulose triacetate and cellulose nitrate/acetate,
15 and hydrogels, e.g., 2-hydroxyethylmethacrylate (HEMA).

The compounds of the present invention may also be formulated for administration as suppositories. A low melting wax, such as a mixture of fatty acid glycerides or cocoa butter is first melted and the active component is dispersed homogeneously,
20 for example, by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and to solidify.

The active compound may be formulated into a suppository comprising, for example, about 0.5% to about 50% of a compound of the invention, disposed in a
25 polyethylene glycol (PEG) carrier (e.g., PEG 1000 [96%] and PEG 4000 [4%]).

The compounds of the present invention may be formulated for vaginal administration. Pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to
30 be appropriate.

The compounds of the present invention may be formulated for nasal administration. The solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The formulations may be
35 provided in a single or multidose form. In the latter case of a dropper or pipette this

may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray this may be achieved for example by means of a metering atomizing spray pump.

- 5 The compounds of the present invention may be formulated for aerosol administration, particularly to the respiratory tract and including intranasal administration. The compound will generally have a small particle size for example of the order of 5 microns or less. Such a particle size may be obtained by means known in the art, for example by micronization. The active ingredient is provided in a
- 10 pressurized pack with a suitable propellant such as a chlorofluorocarbon (CFC) for example dichlorodifluoromethane, trichlorofluoromethane, or dichlorotetrafluoroethane, carbon dioxide or other suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by a metered valve. Alternatively the active ingredients may be provided
- 15 in a form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidone (PVP). The powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form for example in capsules or cartridges of e.g., gelatin or blister packs from
- 20 which the powder may be administered by means of an inhaler.

When desired, formulations can be prepared with enteric coatings adapted for sustained or controlled release administration of the active ingredient.

- 25 The pharmaceutical preparations are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a
- 30 capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

Pharmaceutically acceptable salts

- Pharmaceutically acceptable salts of the instant compounds, where they can be
- 35 prepared, are also intended to be covered by this invention. These salts will be ones

which are acceptable in their application to a pharmaceutical use. By that it is meant that the salt will retain the biological activity of the parent compound and the salt will not have untoward or deleterious effects in its application and use in treating diseases.

5

Pharmaceutically acceptable salts are prepared in a standard manner. If the parent compound is a base it is treated with an excess of an organic or inorganic acid in a suitable solvent. If the parent compound is an acid, it is treated with an inorganic or organic base in a suitable solvent.

10

The compounds of the invention may be administered in the form of an alkali metal or earth alkali metal salt thereof, concurrently, simultaneously, or together with a pharmaceutically acceptable carrier or diluent, especially and preferably in the form of a pharmaceutical composition thereof, whether by oral, rectal, or parenteral (including subcutaneous) route, in an effective amount.

15

Examples of pharmaceutically acceptable acid addition salts for use in the present inventive pharmaceutical composition include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulfuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, p-toluenesulphonic acids, and arylsulphonic, for example.

20

In one embodiment the pharmaceutical composition as defined herein above comprises a pharmaceutically acceptable carrier.

25

In one embodiment of the present invention the pH of the pharmaceutical composition as defined herein above is between pH 4 and pH 9.

30

Kit of parts

In one aspect the present invention relates to a kit in parts comprising:

- a pharmaceutical composition as defined herein above
- a medical instrument or other means for administering the medicament
- instructions on how to use the kit in parts.

35

- optionally a second active ingredient as defined herein above

In a further embodiment the instrument as defined herein above is also called insulin pen described in US Patents Nos. 5,462,535, US 5,999,323 and US 5,984,906.

- 5 The second ingredient may be any suitable active ingredient normally administered to individuals suffering from insulin resistance and diseases associated with insulin resistance such as insulin. By sensitizing the insulin receptor due to administration of a pharmaceutical composition as defined herein it is believed that the need for insulin is reduced.

10

Treatments

- As discussed above the present invention also relates to the treatment of insulin resistance or diseases associated with insulin resistance, said method comprising
15 administering to an individual in need thereof a therapeutically effective amount of the agent as defined above; or the isolated nucleic acid sequence as defined above; or the the expression vector as defined above; or a composition of host cells as defined above; or a packaging cell line as defined above, or a combination thereof.

- 20 The diseases associated with insulin resistance are in particular selected from the group consisting of insulin resistance syndrome, Type 2 diabetes mellitus, impaired glucose tolerance, the metabolic syndrome, hyperglycemia, hyperinsulinemia, arteriosclerosis, hypercholesterolemia, hypertriglyceridemia, hyperlipidemia, dyslipidemia, obesity, central obesity, polycystic ovarian syndrome,
25 hypercoagulability, hypertension, microalbuminuria, insulin resistance syndrome (IRS), Type 2 diabetes mellitus, impaired glucose tolerance, the metabolic syndrome, hyperglycemia, and hyperinsulinemia.

- The present invention have found that administration of a SorCS1-like agent
30 sensitizes the insulin receptor, in that it stabilises the insulin receptor, increases the amount of insulin receptors, and/or increases the amount of activated insulin receptors (phosphorylated insulin receptors are measured). Therefore, in another aspect, the present invention relates to a method of sensitizing an insulin receptor, said method comprising administering a Vps10p-domain receptor selected from the
35 group consisting of:

- f) SorCS1
- g) SorCS2
- h) SorCS3
- i) Sortilin and
- 5 j) SorLA,

thus being useful in a method of treatment of insulin resistance or diseases associated with insulin resistance.

Furthermore, the inventors have found that when administering a SorCS1-like agent
 10 then the insulin receptors may be upregulated, and accordingly, the present invention relates to a method of upregulating an insulin receptor or a fragment or variant thereof, in a patient in need thereof, said method comprising administering to an individual in need thereof a therapeutically effective amount of the agent as defined above; or the isolated nucleic acid sequence as defined above; or the the
 15 expression vector as defined above; or a composition of host cells as defined above; or a packaging cell line as defined above, or a combination thereof.

Furthermore, it has been found that a SorCS1-like agent increases the insulin sensitivity, and accordingly the present invention also relates to a method for
 20 increasing insulin sensitivity comprising administering to an individual in need thereof a therapeutically effective amount of the agent as defined above; or the isolated nucleic acid sequence as defined above; or the the expression vector as defined above; or a composition of host cells as defined above; or a packaging cell line as defined above, or a combination thereof. This individual is typically an
 25 individual suffering from any of the diseases mentioned above, more likely an individual suffering from diabetes type 1 or type 2.

Detailed Description of the drawings

30 **Figure 1: The Vps10p-domain receptor family.** Their structural organization is indicated.

Figure 2: Splice variants of mSorCS1. A) Organization of the murine SorCS1 gene leading to the generation of different cytoplasmic tails. The black boxes
 35 represent exon 23 and 24 with typical splice sites. In the composite internal/terminal

exon 25 (grey) and 26 (white), the dotted line indicates a potential splice site. The alternative used terminal exons 25, 26, 27, and 28 are shown in white. B) Amino acid sequences of the mSorCS1 cytoplasmic domains. Dileucine motifs are underlined, SH3 domain-binding motifs are overlined, SH2 domain-binding motifs are underlined by dashed lines, and YXXØ motifs are overlined by dashed lines.

Figure 3: Expression of the different mSorCS1 splice variants. Expression of the extracellular part of SorCS1 (SorCS1.ex) and the five tail splice variants SorCS1-a, -b, -c, c⁺ and -d were determined in tissue from adult mice by reverse transcription-PCR (RT-PCR) with specific primer pairs. The SorCS1.ex specific primers are spanning the exon 21 to 24 junctions giving a 390 bp product. The SorCS1-a specific primers are spanning the exon 21 to 25 junctions giving a 586 bp product. The SorCS1-b specific primers are spanning the exon 21 to 25 junctions and the exon 25 to 27 junctions giving a 621bp product. The SorCS1-c specific primers are spanning the exon 21 to 26 junctions giving a 626bp product. The SorCS1-d specific primers are spanning the exon 21 to 25 junctions and the exon 25 to 28 junction giving a 636bp product. Total RNA preparations were made from hippocampus, liver, adipose tissue (fat), muscle, pancreas and testis isolated from wild type and hippocampus and liver from SorCS1-KO mice of about 8 weeks of age using the Versagene Total RNA purification Kit (Gentra Systems). Briefly, tissues were surgically removed and frozen on dry ice. Frozen tissue samples were disrupted and homogenized for up to 60 sec using a rotor stator (Ultra-Turrax, IKA-Werke) in 800 µl lysis buffer containing 5 mM Tris (2-carboxyethyl) phosphine (TCEP) and the total RNA was purified according to the manufacturers protocol for the kit. RT-PCR were performed with 0.75 µg to 1 µg total RNA from each sample using the TITANIUM One-step RT-PCR kit (Clontech). All reactions were performed in 50 µl volume containing 1 x One-step buffer (40 mM tricine, 20 mM KCl, 3 mM MgCl₂, 3.75µg/µl BSA), 0.2 mM of each dNTP, 25 µl Thermostabilizing reagent, 10 µl GC-melt, 20 µM Oligo(dT)primer, 20 units Recombinant RNase inhibitor, 1 x RT-TITANIUM™ Taq enzyme mix (all supplied with the kit) and 45 µM of each primer. PCR conditions were: 50°C for 1 hour, 94°C for 5 min, 35 cycles at 94°C for 30 sec, 64°C for 30 sec, 68°C for 1 min, and 68°C for 2 min.

Figure 4: Generation of the mSorCS1 knockout mouse. A) Strategy used to generate mSorCS1 knockout mice by homologous recombination in embryonic stem

cells. A schematic representation of the wild-type murine SorCS1 locus (top), the targeting vector (middle), and the homologous recombinant genome (bottom) are shown. B) Analysis of mSorCS1 mRNA expression, showing lack of transcription of all mSorCS1 splice variant. Fragments are obtained by RT-PCR on mRNA from hippocampus of wild-type (WT) and SorCS1 knockout (KO) mice using specific primer pairs to identify the extracellular part of SorCS1 (ext) or each of the five tail variants (a, b, c, c⁺, and d) (see fig.3). C) Western blot analysis of cortex showing lack of mSorCS1 protein in the mSorCS1 knockout (KO) mice. Proteins were extracted as lysates from cortex obtained at E14.5. The tissue was dissolved in 100 μ l TNE-buffer (10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% nonidet P-40 (Sigma Aldrich) pH. 8) containing protease inhibitors (CompleteMini) by vigorous vortexing. After freezing ON at 20°C, the lysates were vortexed and centrifuged 10 min at 1000 x g. The lysates (supernatant) were transferred to a new tube and Bio-Rad Protein Assay measured the protein concentration. Lysates (200 μ g) were resolved on SDS-PAGE and transferred to nitrocellulose. The blot was then probed with a rabbit polyclonal antibody against the leucine-rich part of SorCS1 (α -hSorCS1-leu). Arrow indicates band of SorCS1. Neo; neomycin, TK; thymidine kinase, FRT/F3; Flp recombinase target sites.

Figure 5: Average blood glucose in A) male and B) female mice at different age. Animals were fasted overnight (16h). Wild type (wt) and SorCS1 knockout (KO) mice were anesthetized with diethyl ether, blood samples were obtained by retroorbital bleeding and plasma glucose was measured immediately on an automatic monitor (Ascensia Contour from Bayer). Statistically significant increases in blood glucose levels from knockout relative to wild type mice are indicated with stars. Error bars indicate SEM.

Figure 6: Plasma insulin levels in female wild-type and SorCS1 knockout mice from 10 to 50 weeks of age. Animals were fasted overnight (16h). Mice were anesthetized with diethyl ether, blood samples were obtained by retroorbital bleeding and plasma insulin levels were determined using an ultrasensitive mouse insulin enzyme-linked immunosorbent assay kit (DRG Diagnostics, Marburg, Germany). Data are means $\pm$ SEM for 4 to 10 mice in each group. Statistically significant increases in blood glucose levels from knockout relative to wild type mice are indicated with stars.

Figure 7: Glucose tolerance test in SorCS1 knockout mice and wild type littermates. Female wild type (wt) and SorCS1 knockout (KO) mice 59 weeks of age were fasted overnight (16 h) and injected intraperitoneally with a bolus of D-glucose (Sigma) (2 mg/g body weight) in sterile saline. Mice were anesthetized with diethyl ether, blood samples were obtained by retroorbital bleeding at times 0, 15, 30, 60, and 120 min after injection, and plasma A) glucose and B) insulin levels were measured. Plasma glucose was measured immediately after sampling on an automatic monitor (Ascensia Contour from Bayer). Insulin levels were determined using an ultrasensitive mouse insulin enzyme-linked immunosorbent assay kit (DRG Diagnostics, Marburg, Germany). Data are means $\pm$ SEM for four mice in each group.

Figure 8: Elevated plasma glucose- and insulin levels in wild type mice on Western type diet. Female A) + C) and male B) + D) wild type (wt) and SorCS1 knockout (KO) mice were fed a high calorie Western type diet (WD) (24%protein, 41%carbonhydrate, 24%fat) (Research Diets. D12451) from 10 weeks of age to 50 weeks of age. At 50 weeks of age the animals were fasted overnight (16h), anesthetized with diethyl ether and blood samples were obtained by retroorbital bleeding. Plasma glucose levels A) + B) were measured immediately after sampling on an automatic monitor (Ascensia Contour from Bayer), whereas plasma insulin levels C) + D) were determined using an ultrasensitive mouse insulin enzyme-linked immunosorbent assay kit (DRG Diagnostics, Marburg, Germany). Data are means $\pm$ SEM for 4 to 10 mice in each group.

Figure 9: Abdominal adipose tissue in wild-type and knockout mice on western type diet.

Female A) and male B) wild type and SorCS1 knockout mice were fed a high calorie Western type diet (WD) (24%protein, 41%carbonhydrate, 24%fat) (Research Diets. D12451) from 10 weeks of age to 50 weeks of age. At the end of the study the animals were killed and the abdominal fat (adipose tissue) was separated and weighed. Data are means $\pm$ SEM for 4 to 10 mice in each group.

Figure 10: Expression of IR, phosphorylated IR (pY-IR) and Glut4 in muscle and adipose tissue. Female SorCS1 knockout (-/-) mice and wild-type (+/+) control

mice 50 weeks of age were fasted overnight, injected intraperitoneally with insulin (Novorapid, Novo Nordisk A/S) (10 units/kg body weight) in sterile saline, and killed 15 min later. A) Adipose and B) muscle tissue were removed and homogenized in lysis buffer TNE-buffer (10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% nonidet P-40 (Sigma Aldrich) pH. 8) containing protease inhibitors (CompleteMini). The lysates were cleared by centrifugation 10 min at 1000 x g, and protein concentrations were determined by Bio-Rad Protein Assay. Equal amounts of total protein for different samples (100 µg) were separated on a 4-16% SDS-PAGE gel and transferred onto polyvinylidene difluoride (PVDF) membranes (Amersham Pharmacia). Membrane was analysed by western blotting with anti-IR (Santa Cruz Biotechnology, sc-711), anti-IR-pY (R&D systems, AF2507), anti-Glut4 (Abcam, ab654), and anti-β-actin (Sigma, AF5441) as a loading control. Bound antibodies were developed by SuperSignal West Pico reagent (Pierce) and a Fuji film LAS3000.

Figure 11: Physical interaction between SorCS1 and insulin receptor. A) CHO cells transfected with the indicated receptors (only transient transfected with IR_A and IR_B) were stimulated with insulin for 30 min followed by crosslinking with 5 nM DSP (Pierce) and subsequently lysed. The cell lysates was incubated with antibody against IR (Santa Cruz Biotechnology, sc-711) bound to Gammabind beads (GE Healthcare). The precipitated complexes were eluted from the washed beads with SDS loading buffer. The eluate was subjected to SDS-PAGE and Western blot analysis using α-SorCS1-leu and α-IR to reveal the presence of a SorCS1:IR complex. Crude lysates subjected to Western blot analysis using α-SorCS1-leu and α-IR were included to assess the transfection efficiency. B) Surface plasmon resonance experiment (BIAcore) showing the direct interaction of soluble full-length extracellular part of SorCS1 with immobilized soluble insulin receptor (IR) (R&D systems). The soluble SorCS1 concentrations used were 50 nM, 75 nM, and 150 nM. The K_d is estimated to approximately 5 nM.

Figure 12: Insulin receptor expression in CHO cells transfected with SorCS1. Chinese hamster ovary (CHO) cells stably transfected with the four murine SorCS1 splice variants (SorCS1-a,-b,-c,-d) and msol.SorCS1 (the extracellular part of SorCS1) were grown to confluency in serum-free HyQ-CCM5 CHO medium (HyClone) supplemented with antibiotics (50 U/ml penicillin/ 50 µg/ml streptomycin). The cells were washed with PBS and lysed in lysis-buffer (1% Triton X-100, 20 mM

Tris-HCl, 10 mM EDTA, pH 8.0), supplemented with proteinase inhibitors (CompleteMini, Roche Molecular Biochemicals). Aliquots of the lysates, corresponding to 10 µg protein, were dissolved in SDS sample buffer and subjected to reducing SDS-PAGE using 4-16% acrylamide gels. For immunoblotting, proteins were electrophoretically transferred onto polyvinylidene difluoride (PVDF) membranes (Amersham Pharmacia) and probed with anti-IR (Santa Cruz Biotechnology, sc-711), anti-SorCS1-leu and anti-β-actin (Sigma, AF5441) as a loading control. Bound antibodies were developed by SuperSignal West Pico reagent (Pierce) and a Fuji film LAS3000.

Figure 13: Expression of IR and SorCS1 on the cell membrane. Cell surface expression of the insulin receptor and SorCS1 was determined by cell surface biotinylation. CHO cells and CHO cells stably expressing mSorCS1-B and mSorCS1-C were subjected to surface biotinylation using the membrane impermeable biotinylation reagent NHS-SS-biotin (Pierce). Cells were grown to confluency, following which cells were washed with phosphate-buffered saline (PBS). Biotinylation was carried out using 0.5 mg/ml NHS-SS-biotin in PBS for 90 min at 4 °C with gentle shaking. After labeling, cells were washed twice with ice-cold PBS to remove the residual NHS-SS-biotin. Subsequently, cells were solubilized in lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% nonidet P-40 (Sigma Aldrich) pH. 8) containing protease inhibitors (CompleteMini) by gently shaking on ice for approximately 10 min. The lysate were clarified by centrifugation at 14,000xg for 5 min at 4 °C, 20 µl of the cleared lysate was saved (lysate fraction) and the rest of the lysate was incubated overnight with 100 µl of streptavidin-agarose beads (Sigma) at 4 °C with gentle agitation. After incubation, the lysate/beads mixture was separated by centrifugation at 14,000xg for 5 min at 4 °C. The lysate fraction contains the intracellular proteins of the cells (Intra). The beads were washed twice with PBS and the captured biotinylated proteins (Bio) were eluted from the beads with 150 µl of SDS sample buffer. Finally, a portion of the biotinylated (Bio) (30 µl), the intracellular (Intra) (25 µl), and the crude lysates (Lysate) was subjected to SDS-PAGE and Western blot analysis using anti-IR (Santa Cruz Biotechnology, sc-711), anti-IR-pY (R&D systems, AF2507), anti-Glut4 (Abcam, ab654), and anti-β-actin (Sigma, AF5441) as a loading control. Bound antibodies were developed by SuperSignal West Pico reagent (Pierce) and a Fuji film LAS3000.

Figure 14: Development stages towards type 2 diabetes in human. Type 2 diabetes (T2D) develops in response to obesity in subjects that have underlying genetic and acquired predispositions to both insulin resistance and β cell dysfunction. Over time, islet β cell compensation for the insulin resistance fails, resulting in progressive decline in β cell function. As a consequence, subject's progress from normal glucose tolerance to impaired glucose tolerance (pre-diabetes) and finally to established T2D. Increases in blood glucose concentration during the development of T2D are illustrated on the graph (black line) showing the change from normal to pre-diabetic, before the onset of frank diabetes. Furthermore, the level of insulin during development of T2D is revealed on the same graph (dashed line), showing an increase of insulin during the pre-diabetic state as compensation to insulin resistance and a severe decline in insulin release at onset of frank diabetes as a consequence of β cell failure.

Figure 15: Insulin immunostaining of pancreatic islets in wild-type and knockout mice 20 days of age. Pancreata were removed and fixed with 4% paraformaldehyde, freshly prepared in PBS. Samples were embedded in Tissue-Tek (Sakura). Cryosections (10 μ m) were obtained from several positions throughout the pancreas, and stored in -80°C . For immunostaining, the slide were placed in PBS for 2x5 min, blocked in 0.2% hydrogen peroxid (H_2O_2) in methanol for 15 min at -20°C , washed with PBS (1x5min) and PBS + 0.1% TritonX-100 (2x10 min) before preincubation with 10% fetal calf serum (FCS) in PBS for 30 min. Slides were subsequently rinsed in PBS (3x2 min) and incubated overnight at 4°C in primary antibody guinea pig anti-insulin (I-8510, Sigma) diluted in PBS + 10% FCS (1:500). Slides were washed with PBS (3x15 min), incubated with secondary antibody Cy3-conjugated anti-guinea pig (706-165-148, Jackson ImmunoResearch) diluted in PBS + FCS (1:500) in the dark for 1 hr at RT, and subsequently washed in PBS (3x15min) and allowed to air-dry. Finally, the slides were mounted with Vectashield with DAPI (H-1200, Vector Labs) and analysed by confocal scanning laser microscopy (LMS 510, Carl Zeiss).

Figure 16: Alignment of SorCS1

Sequence alignment of SorCS1 from Human (homo sapiens), Chimpanzee (Pan troglodytes), Cow (Bos Taurus), Mouse (Mus musculus), Rat (Rattus norvegicus),

Dog (*Canis lupus familiaris*) and Chicken (*Gallus gallus*) origin. The sequence identity is as demonstrated in table 2.

Table 2: Sequence identity to human SorCS1

Species	Protein (% identity)	DNA (% identity)
Human	100	100
Chimpanzee	99.6	99.4
Dog	97.6	92.5
Cow	92.9	89.8
Mouse	93.2	87.7
Rat	93.2	88.0
Chicken	85.3	79.7

5

Fig. 17: Decreased plasma glucose levels in female wild-type and SorCS1 knockout mice after hepatic overexpression of soluble SorCS1.

Wild-type and SorCS1 knockout female mice were injected with an adenovirus over-expressing soluble SorCS1. The recombinant adenovirus for expression of human soluble SorCS1 (hsol.SorCS1) was generated as follows:

pcDNA3.1/Zeo(-)/hsol.SorCS1 encoding the human soluble SorCS1 cDNA (amino acids 1-1100) was digested with Pme1 and Apa1 and the fragment encoding hsol.SorCS1 inserted into the shuttle plasmid pVQpacAd5CMVK-NpA (ViraQuest Inc, North Liberty, IA). ViraQuest Inc, North Liberty, IA, then used this shuttle plasmid for generation and propagation of adenovirus over-expressing hsol.SorCS1. Female SorCS1 knockout and wild-type mice 40 weeks of age were fasted overnight. In the morning, on day 0, blood samples were obtained by retroorbital bleeding and plasma glucose was measured immediately on an automatic monitor (Ascentia Contour from Bayer). Then, the mice were injected in the tail vein with 2E9 pfu's of an adenoviral vector with either hsol.SorCS1 or LacZ as a negative control (from ViraQuest Inc, North Liberty, IA). On day 7, measurements of plasma glucose were repeated on overnight fasted mice to evaluate the effect of the SorCS1 and LacZ protein. The data are means $\pm$ SEM for 3 mice in each group. Mice with over-expression of soluble SorCS1 exhibited a significant decrease in plasma glucose ($\approx 40\%$) both in SorCS1 knockout mice and wild-type mice. This increase was not seen in the mice that received the control virus LacZ.

Fig. 18: Expression of IR, phosphorylated IR, and Glut4 in muscle and adipose tissue from SorCS1 knockout female mice over-expressing soluble SorCS1.

Female SorCS1 knockout (-/-) mice 40 weeks of age were injected with a adenoviral vector expressing either hsol.SorCS1 or LacZ as a negative control (see detailed protocol in Fig.17). On day 12 after virus injection, the mice were fasted overnight, injected intraperitoneally with insulin (Novorapid, Novo Nordisk A/S) (10 units/kg body weight) in sterile saline, and killed 15 min later. A) Muscle and B) adipose tissue were removed and homogenized in lysis buffer TNE-buffer (10mM Tris-HCl, 150 mM NaCl, 1mM EDTA, 1% nonidet P-40, pH. 8) containing protease inhibitors (Complete Mini, Roche) and phosphatase inhibitors (cocktail 1, Sigma Aldrich). The lysates were cleared by centrifugation 10 min at 10.000 x g, and protein concentration were determined by Bio-Rad Protein Assay. Equal amount of total protein (50 µg) for different samples were separated on a 4-12 % Bis-tris gel (Nupage, Invitrogen) and transferred onto polyvinylidene difluoride (PVDF) membranes (Amersham Pharmacia). Membranes were analysed by western blotting with anti-IR (Santa Cruz Biotechnology, sc-711), anti-IR-pY (R&D systems, AF2507) and anti-Glut4 (Abcam, ab654). Bound antibodies were developed by Super-Signal West Pico reagent (Pierce) and a Fuji film LAS3000. In both A) muscle and B) adipose tissue from SorCS1 knockout mice over-expressing soluble SorCS1 there are elevated amount of IR, phosphorylated IR (IR-pY) and glut4 compared to mice expressing the LacZ control protein, suggesting increased insulin sensitivity in mice which received the hsol.SorCS1 virus.

Fig. 19: Decreased plasma glucose and insulin levels in diabetic db/db female mice over-expressing soluble SorCS1.

To evaluate the effect of soluble SorCS1 in an obese mouse model that spontaneously develops type 2 diabetes we used the db/db mouse strain (BKS.Cg-m⁺/+Lpr^{db}/BomTac from Taconic). These mice lack the leptin receptor consequently the mice become obese and develop insulin resistance and finally severe diabetes at the age of 6-8 weeks. We injected adenovirus expressing either hsol.SorCS1 or LacZ as a control (as described in Fig. 17), to examine the effect on plasma glucose and insulin levels. In detail, db/db female mice 10 weeks of age were fasted overnight. In the morning, on day 0, the mice were anesthetized with diethyl ether and blood samples were obtained by retroorbital bleeding. A) Blood glucose was measured immediately on an automatic monitor (Ascentia Contour from Bayer),

whereas B) plasma insulin levels were determined using an ultrasensitive mouse insulin enzyme-linked immunoabsorbent assay kit (DRG Diagnostics). Thereafter the mice were injected in the tail vein with 2E9 pfu's of an adenoviral vector with either hsol.SorCS1 or LacZ (from ViraQuest Inc, North Liberty, IA) as a negative control virus. On day 7, measurements of blood glucose and plasma insulin were repeated on overnight fasted mice to evaluate the effect of the SorCS1 and LacZ protein. Data are means $\pm$ SEM for 5 mice in each group. On day 7, db/db female mice with over-expression of soluble SorCS1 exhibited a significant decrease in blood glucose ($\approx 35\%$) compared to the mice that received the control LacZ virus. Furthermore, on day 7 there was also a significant decrease in the plasma insulin levels in the db/db female mice over-expressing soluble SorCS1 compared to mice that express the control virus. Thus, over-expression of soluble SorCS1 improves the diabetic status in this type 2 diabetic mouse model.

Fig. 20: Glucose tolerance test in diabetic db/db female mice with over-expression of soluble SorCS1.

Female db/db mice injected with adenoviruses expressing either soluble SorCS1 or LacZ (see fig. 17) where on day 3 fasted over-night (16 hrs). On day 4 the mice were injected intraperitoneally with a bolus of glucose (2mg/g body weight) in sterile saline. The animals were anesthetized with diethyl ether and blood samples were obtained by retroorbital bleeding at times 0, 15, 30, 90, and 150 min after injection. Blood glucose levels were measured immediately after sampling on an automatic monitor (Ascentia Contour from Bayer). Data are means $\pm$ SEM for 5 mice in each group. The results show, that over-expression of soluble SorCS1 renders the mice more sensitive to insulin as the level of blood glucose returns to baseline after 150 min. By contrast, blood glucose in mice expressing LacZ stays elevated during the course of the experiments. In conclusion, db/db female mice with over-expression of soluble SorCS1 are less insulin resistant.

Fig. 21: Plasma glucose and insulin levels in diabetic db/db male mice over-expressing soluble SorCS1.

To evaluate the effect of soluble SorCS1 in an obese mouse model that spontaneously develops type 2 diabetes we used the db/db mouse strain (BKS.Cg-*m+/+Lpr^{db}/BomTac* from Taconic). These mice lack the leptin receptor consequently the mice become obese and develop insulin resistance and finally severe diabetes

at the age of 6-8 weeks. We injected adenovirus expressing either hsol.SorCS1 or LacZ as a control (as described in Fig. 17), to examine the effect on plasma glucose and insulin levels. In detail, db/db male mice 6 weeks of age were fasted overnight. In the morning, on day 0, the mice were anesthetized with diethyl ether and blood samples were obtained by retroorbital bleeding. A) Blood glucose was measured immediately on an automatic monitor (Ascentia Contour from Bayer), whereas B) plasma insulin levels were determined using an ultrasensitive mouse insulin enzyme-linked immunoabsorbent assay kit (DRG Diagnostics). Thereafter the mice were injected in the tail vein with 2E9 pfu's of an adenoviral vector with either hsol.SorCS1 or LacZ (from ViraQuest Inc, North Liberty, IA) as a negative control virus. On day 7, measurements of blood glucose and plasma insulin were repeated on overnight fasted mice to evaluate the effect of the SorCS1 and LacZ protein. Data are means $\pm$ SEM for 5 mice in each group. On day 7, db/db male mice with over-expression of soluble SorCS1 exhibited a significant decrease in blood glucose ($\approx 35\%$) compared to the mice that received the control LacZ virus. Because the decline in glucose levels were not accounted by an increased insulin concentration as compared to LacZ treated animals, we conclude that over-expression of soluble SorCS1 improves the diabetic status in male type 2 diabetic db/db mice.

Fig. 22: Subcellular localization of Glut4 in muscle tissue from db/db male mice over-expressing soluble SorCS1.

To evaluate if over-expression of soluble SorCS1 might change the distribution of Glut4 we conducted subcellular fractionation on muscle tissue from db/db male mice over-expressing soluble SorCS1. In detail, db/db male mice 6 weeks of age were injected in the tail vein with a adenoviral vector expressing either hsol.SorCS1 or LacZ as a control as described in fig. 17. On day 7 after virus injection, the mice were fasted overnight, injected intraperitoneally with insulin (Novorapid, Novo Nordisk A/S) (10 units/kg body weight) in sterile saline, and killed 15 min later. Muscle tissue from 5 mice injected with the same virus was removed, pooled and transferred to 5 ml of HEPES-buffered sucrose (0.25 M sucrose, 1 mM EDTA, 20 mM HEPES-KOH, pH. 7.4), homogenized by 10 strokes up and down using a Teflon pestle, and centrifuged at 1000 x g for 10 min. Thus, heavy mitochondrial, light mitochondrial, and microsomal fraction were obtained by several round of centrifugation. First, the supernatant was centrifuged at 3.000 x g for 10 min, then the resulting supernatant was centrifuged at 16.000 x g for 10 min, and finally the

resulting supernatant was centrifuged a 100.000 x g for 45 min giving a pellet containing the microsomal fraction. The microsomal fractions were resuspended in 0.5 ml HEPES-buffered solution and subjected to sucrose (velocity) gradient centrifugation. The 0.5 ml microsomal samples were loaded onto a 12 ml linear 0.8 M to 1.6 M sucrose gradient in 1 M HEPES, pH 7.2, and centrifuged 18 h in a swinging bucket rotor (SW41 Ti) at 84.000 x g. Each gradient was separated into 24 fractions starting from the top of the tube. Finally, gel electrophoresis and Western blotting analyzed the expression of Glut4 in the different fractions. The result shows that the sedimentation distribution of Glut4 in muscle tissue over-expressing SorCS1 is different from muscle tissue expressing the control protein lacZ. Thus, accumulation of glut4 shifted from fractions 2 - 4 after LacZ treatment to fractions 8-12 in the SorCS1 group. This indicate that over-expression of soluble SorCS1 might change the distribution of Glut4 and thereby modulate glucose uptake.

Fig. 23 A+B: Analysis of SorCS1/IR contact sequences by SPOT analysiss.

Co-immunoprecipitation and BIAcore (surface Plasmon resonance) experiments showed SorCS1 can physically associate with the insulin receptor. We here used SPOT synthesis analysis to identify linear amino acid sequences in either of the two receptors that may partake in the protein-protein interaction. In practice filters were spotted with consecutive 15-mer peptides overlapping by three amino acids from the N- to the C-terminus of SorCS1 (B) and IR (A), and the filters were subsequently probed with (A) ¹²⁵I-labelled soluble human SorCS1 or (B) histidine-tagged insulin receptor (R&D systems, no1544-IR/CF). The filters were then washed and bound proteins visualized. The binding assay was performed directly on the peptide membrane through immunodetection or radiography of bound protein. In detail, the membrane was washed 1 x 10 min in 96% ethanol, followed by 3 x 10 min washing with 1 x TBS (500 mM Tris-HCl, 1500 mM NaCl), pH.8.0 and 3 hrs incubation in membrane blocking buffer (BB; 1 x Blocking buffer (B6429, Sigma), 1 x TBS, 5% sucrose). The blocked IR-membrane was incubated overnight with ¹²⁵I-sol.SorCS1 (400.000 cpm/ml BB) and the SorCS1-membrane with his-IR (10 µg/ml BB). Both membrane were washed 3 x 10 min with 1 x TBS. A) Bound his-IR on the SorCS1 membrane was detected by immunodetection using primary anti-body against the histidine tag, α-histidine (Invitrogen) (Mouse monoclonal) followed by an HRP-tagged secondary anti-mouse antibody (Sigma). Bound antibody was visualized

using the enhanced chemiluminescence (ECL) Western blotting Detection reagent (Amersham biosciences) and a Fuji film LAS1000). Bound radiolabelled SorCS1 to the IR-membrane was detected by radiography using an Fuji image plate, and after 12 hrs exposure subsequently developed using a Fujifilm FLA3000. A signal (SPOT) indicates that the ligand binds to a peptide. Overlapping linear binding epitopes are represented by signals from neighbouring spots. The SPOT's are framed on the membrane figures and key sequences corresponding to the SPOT's are indicated beneath the membrane.

Fig. 24 A+B: Gene expression profiling of adipose tissue from SorCS1 knockout mice by PCR arrays.

Using gene array analysis of adipose tissue from SorCS1 knockout wild-type adipose mice we tested expression of A) 84 genes related to the mouse insulin signalling pathway and B) 84 genes related to mouse lipoprotein signalling & cholesterol metabolism. In practice, first strand cDNA was synthesized from total RNA (Applied Biosystems) from SorCS1 knockout (-/-) and wild-type (+/+) adipose tissue from female mice 50 weeks of age (n = 3). Then superarray of A) Mouse Insulin Signalling Pathway (PAMM-030A RT2 Profiler PCR arrays) or B) the type Mouse Lipoprotein Signalling & Cholesterol Metabolism (PAMM-080-A RT2 Profiler PCR arrays) were processed using an ABI7900 platform (Applied Biosystems) and SYBR Green/Rox PCR (SABiosciences). AROS Applied Biotechnology, Aarhus, Denmark, did the expression analyses. Genes showing an expression more than 3 times up- or down-regulated in the SorCS1 knockout mice when compared to wild-type mice are listed in the upper tables and their known functions are indicated in the table below. Several genes in A and B show changed expression in the SorCS1 knockout mice compared to the wild-type mice indicating that insulin and cholesterol signalling pathways and metabolism are altered in SorCS1 knockout mice.

Examples

Example 1: Expression of mSorCS1 splice variants in tissues from mice

Expression of the extracellular part of SorCS1 (SorCS1.ex) and the five tail splice variants SorCS1-a, -b, -c, c⁺ and -d were determined in various tissues from adult mice (see Fig. 3). The organization of the SorCS1 gene and the amino acid

sequences of the cytoplasmic domains of the splice variants are shown in Figure 2A and 2B, respectively.

Expression of the extracellular part of SorCS1 (SorCS1.ex) and splice variants were determined by reverse transcription-PCR (RT-PCR) with specific primer pairs. The SorCS1.ex specific primers (SEQ ID NO: 57 and SEQ ID NO: 58) are spanning the exon 21 to 24 junctions giving a 390 bp product. The SorCS1-a specific primers (SEQ ID NO: 59 and SEQ ID NO: 60) are spanning the exon 21 to 25 junctions giving a 586 bp product. The SorCS1-b specific primers (SEQ ID NO: 61 and SEQ ID NO: 62) are spanning the exon 21 to 25 junctions and the exon 25 to 27 junctions giving a 621bp product. The SorCS1-c (SEQ ID NO: 63 and SEQ ID NO: 64) specific primers are spanning the exon 21 to 26 junctions giving a 626bp product. The SorCS1-d specific primers (SEQ ID NO: 65 and SEQ ID NO: 66) are spanning the exon 21 to 25 junctions and the exon 25 to 28 junction giving a 636bp product. Total RNA preparations were made from hippocampus, liver, adipose tissue (fat), muscle, pancreas and testis isolated from wild type and hippocampus and liver from SorCS1-KO mice of about 8 weeks of age using the Versagene Total RNA purification Kit (Gentra Systems). Briefly, tissues were surgically removed and frozen on dry ice. Frozen tissue samples were disrupted and homogenized for up to 60 sec using a rotor stator (Ultra-Turrax, IKA-Werke) in 800 µl lysis buffer containing 5 mM Tris (2-carboxyethyl) phosphine (TCEP) and the total RNA was purified according to the manufacturers protocol for the kit. RT-PCR were performed with 0.75 µg to 1 µg total RNA from each sample using the TITANIUM One-step RT-PCR kit (Clontech). All reactions were performed in 50 µl volume containing 1 x One-step buffer (40 mM tricine, 20 mM KCl, 3 mM MgCl₂, 3.75µg/µl BSA), 0.2 mM of each dNTP, 25 µl Thermostabilizing reagent, 10 µl GC-melt, 20 µM Oligo(dT)primer, 20 units Recombinant RNase inhibitor, 1 x RT-TITANIUM™ Taq enzyme mix (all supplied with the kit) and 45 µM of each primer. PCR conditions were: 50°C for 1 hour, 94°C for 5 min, 35 cycles at 94°C for 30 sec, 64°C for 30 sec, 68°C for 1 min, and 68°C for 2 min.

Example 2: Generation of the mSorCS1 knockout mouse

To investigate the function of SorCS1 and its different splice variants, a conditional knockout mouse was generated by homologous recombination in embryonic stem cells. The homologous recombination was initiated by the site-specific FLP recombinase at a FRT-site, which results in "recombinase-mediated cassette

exchange". The recombination event is illustrated in Fig. 4A, where the SorCS1 gene is "exchanged" with the Neo gene thereby generating a full knockout mouse where all SorCS1 splice variants are disrupted.

Expression of SorCS1 was tested in wild-type and SorCS1 knockout mice by RT-PCR on mRNA from hippocampus of wild-type (WT) and SorCS1 knockout (KO) mice using specific primer pairs to identify the extracellular part of SorCS1 (ext) or each of the five tail variants (a, b, c, c⁺, and d). The results shown in Figure 4B reveal that transcription of all mSorCS1 splice variants are disrupted in the SorCS1 knockout mouse.

Western blot analysis of cortex revealed the lack of mSorCS1 protein in the mSorCS1 knockout (KO) mice (Fig. 4C). Proteins were extracted as lysates from cortex obtained at E14.5. The tissue was dissolved in 100 µl TNE-buffer (10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% nonidet P-40 (Sigma Aldrich) pH. 8) containing protease inhibitors (CompleteMini) by vigorous vortexing. After freezing ON at 20°C, the lysates were vortexed and centrifuged 10 min at 1000 x g. The lysates (supernatant) were transferred to a new tube and Bio-Rad Protein Assay measured the protein concentration. Lysates (200µg) were resolved on SDS-PAGE and transferred to nitrocellulose. The blot was then probed with a rabbit polyclonal antibody against the leucine-rich part of SorCS1 (α-hSorCS1-leu).

Example 3: Plasma glucose levels in SorCS1 knockout mice

Type 2 diabetes (T2D) develops in response to obesity in subjects that have underlying genetic and acquired predispositions to both insulin resistance and β cell dysfunction. Over time, islet β cell compensation for the insulin resistance fails, resulting in progressive decline in β cell function. As a consequence, subject's progress from normal glucose tolerance to impaired glucose tolerance (pre-diabetes) and finally to established T2D. Increases in blood glucose concentration during the development of T2D are illustrated on the graph (black line) showing the change from normal to pre-diabetic, before the onset of frank diabetes. Furthermore, the level of insulin during development of T2D is revealed on the same graph (dashed line), showing an increase of insulin during the pre-diabetic state as compensation to insulin resistance and a severe decline in insulin release at onset of frank diabetes as a consequence of β cell failure (Fig. 14)

To examine the SorCS1 knockout mouse with respect to glucose metabolism, the plasma glucose levels were determined in male (Fig. 5A) and female mice (Fig. 5B) at different age. Animals were fasted overnight (16h). Mice were anesthetized with diethyl ether, blood samples were obtained by retroorbital bleeding and plasma glucose was measured immediately on an automatic monitor (Ascensia Contour from Bayer). The results in Figure 5B shows a statistically significant increase in plasma glucose levels of female SorCS1 knockout mice at an age of 23 and 50 weeks relative to wild type mice.

10 **Example 4: Plasma insulin levels in female SorCS1 knockout mice**

To further examine the SorCS1 knockout mouse with respect to glucose metabolism, the plasma insulin levels were determined in female SorCS1 knockout mice from 10 to 20 weeks of age (Fig. 6). Animals were fasted overnight (16h). Mice were anesthetized with diethyl ether, blood samples were obtained by retroorbital bleeding and plasma insulin levels were determined using an ultrasensitive mouse insulin enzyme-linked immunosorbent assay kit (DRG Diagnostics, Marburg, Germany). Data are means $\pm$ SEM for 4 to 10 mice in each group. In agreement with the results shown in Figure 5B, the results in Figure 6 shows a statistically significant increase plasma insulin levels of female SorCS1 knockout mice at an age of 23 and 50 weeks relative to wild type mice.

Insulin levels were further investigated by immunostaining of tissues from wild-type and SorCS1 knockout mice 20 days of age.

Pancreata were removed and fixed with 4% paraformaldehyde, freshly prepared in PBS. Samples were embedded in Tissue-Tek (Sakura). Cryosections (10 μ m) were obtained from several positions throughout the pancreas, and stored in -80°C. For immunostaining, the slide were placed in PBS for 2x5 min, blocked in 0.2% hydrogen peroxid (H₂O₂) in methanol for 15 min at -20°C, washed with PBS (1x5min) and PBS + 0.1% TritonX-100 (2x10 min) before preincubation with 10% fetal calf serum (FCS) in PBS for 30 min. Thereafter rinsed in PBS (3x2 min) and incubated overnight at 4°C in primary antibody guinea pig anti-insulin (I-8510, Sigma) diluted in PBS + 10% FCS (1:500). Slides were washed with PBS (3x15 min), incubated with secondary antibody Cy3-conjugated anti-guinea pig (706-165-148, Jackson ImmunoResearch) diluted in PBS + FCS (1:500) in the dark for 1 hr at RT, and subsequently washed in PBS (3x15min) and allowed to air-dry. Finally, the

slides were mounted with Vectashield with DAPI (H-1200, Vector Labs) and analysed by confocal scanning laser microscopy (LMS 510, Carl Zeiss). This data suggest that the pancreas strives to compensate the decreased insulin sensitivity by increasing the size of beta-cell islets and insulin production.

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Example 5: Glucose tolerance test in female SorCS1 knockout mice

The glucose tolerance of female SorCS1 knockout mice was tested by measuring glucose and insulin levels at different time points after injection with glucose (Fig. 8)

10 Female mice 59 weeks of age were fasted overnight (16 h) and injected intraperitoneally with a bolus of D-glucose (Sigma) (2 mg/g body weight) in sterile saline. Mice were anesthetized with diethyl ether, blood samples were obtained by retroorbital bleeding at times 0, 15, 30, 60, and 120 min after injection, and plasma glucose levels (Fig. 8A) and insulin levels (Fig. 8B) were measured. Plasma glucose

15 levels were measured immediately after sampling on an automatic monitor (Ascensia Contour from Bayer). Insulin levels were determined using an ultrasensitive mouse insulin enzyme-linked immunosorbent assay kit (DRG Diagnostics, Marburg, Germany). The results in Fig. 7B show increased insulin levels in female SorCS1 knockout mice at all time points (0-120 min) after injection.

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Example 6: Elevated levels of plasma glucose and insulin in wild type mice on Western type diet

Female (Fig. 8A+8C) and male (Fig. 8B+8D) wild type and SorCS1 knockout mice were fed a high calorie Western type diet (WD) (24%protein, 41%carbonhydrate, 24%fat) (Research Diets. D12451) from 10 weeks of age to 50 weeks of age. At 50

25 weeks of age the animals were fasted overnight (16h), anesthetized with diethyl ether and blood samples were obtained by retroorbital bleeding. Plasma glucose levels (Fig. 8A+8B) were measured immediately after sampling on an automatic monitor (Ascensia Contour from Bayer), whereas plasma insulin levels (Fig. 8C+8D)

30 were determined using an ultrasensitive mouse insulin enzyme-linked immunosorbent assay kit (DRG Diagnostics, Marburg, Germany). The results depicted in Figure 6 shows plasma glucose and insulin levels are elevated in wild type mice on Western type diet.

Example 7: Demonstration of increased insulin receptor phosphorylation in SorCS1 knockout mice.

Female SorCS1 knockout (-/-) mice and wild-type (+/+) control mice 40 and 50 weeks of age were fasted overnight, injected intraperitoneally with insulin (10 units/kg body weight) in sterile saline, and killed 15 min later. Muscle tissue were removed and homogenized in lysis buffer TNE-buffer (10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% nonidet P-40 (Sigma Aldrich), pH= 8.0 containing protease (CompleteMini, Roche) and phosphatase inhibitors (Cocktail 1, Sigma Aldrich). The lysates were cleared by centrifugation 10 min at 1000 x g, and protein concentration were determined by Bio-Rad Protein Assay. Equal amounts of total protein from different samples (100 µg) were separated on a 4-16% SDS-PAGE gel and transferred onto polyvinylidene difluoride (PVDF) membranes (Amersham Pharmacia). Membrane were subjected to Western blotting with anti-IR (Santa Cruz Biotechnology, sc-711), anti-IR-pY (R&D, AF 2507), and anti-β-actin (Sigma Aldrich, AF5441) as a loading control. Furthermore, tyrosine phosphorylation of IR were also analysed by immunoprecipitation with an anti-phosphotyrosine antibody (4G10). The immunoprecipitaion was conducted as follows. 100 µg protein from muscle tissue was incubated with Gammabind G-Sepharose beads (Amersham Bioscience) coated with anti-phosphotyrosine (4G10, Upstate/Millipore) overnight at 4°C. The beads were subsequently washed 4 x 5 min, and finally resuspended in reducing sample buffer (20 mM DTE, 2.5% SDS) and boiled. The supernatant of the boiled samples, containing precipitated proteins, were analyzed by Western blotting using anti-IR (Santa Cruz Biotechnology, sc-711). All bound antibodies were developed by Super Signal West Pico reagent (Pierce) and a Fuji film LAS3000. Figure 10 shows an increased expression of insulin receptor and a decreased phosphorylation of the insulin receptor in SorCS1 knockout mice aged 50 weeks suggesting that IR accumulates in a compartment in SorCS1 knockout mice thereby precluding the receptor from phosphorylation and activation. The results suggest that SorCS1 plays a role in insulin signalling and activation of the insulin receptor.

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Example 8: Physical interaction between SorCS1 and insulin receptor

To examine the interaction between SorCS1 an the insulin receptor (IR), Chinese hamster ovary (CHO) cells stably transfected with the four murine SorCS1 splice variants (SorCS1-a,-b,-c,-d) and msol.SorCS1 (the extracellular part of SorCS1) were grown to confluency in serum-free HyQ-CCM5 CHO medium (HyClone)

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supplemented with antibiotics (50 U/ml penicillin/ 50 µg/ml streptomycin). The cells were washed with PBS and lysed in lysis-buffer (1% Triton X-100, 20 mM Tris-HCl, 10 mM EDTA, pH 8.0), supplemented with proteinase inhibitors (CompleteMini, Roche Molecular Biochemicals). Aliquots of the lysates, corresponding to 10 µg protein, were dissolved in SDS sample buffer and subjected to reducing SDS-PAGE using 4-16% acrylamide gels. For immunoblotting, proteins were electrophoretically transferred onto polyvinylidene difluoride (PVDF) membranes (Amersham Pharmacia) and probed with anti-IR (Santa Cruz Biotechnology, sc-711), anti-SorCS1-leu and anti-β-actin (Sigma, AF5441) as a loading control. Bound antibodies were developed by SuperSignal West Pico reagent (Pierce) and a Fuji film LAS3000. Cell lines stably transfected with the different splice variants of SorCS1 showed elevated expression of the IR compared to CHO cells with out SorCS1 expression (Fig.12). To identify the cellular localisation of the elevated amount of IR in the SorCS1 transfected cells surface biotinylation were conducted. CHO cells and CHO cells stably expressing mSorCS1-B and mSorCS1-C were subjected to surface biotinylation using the membrane impermeable biotinylation reagent NHS-SS-biotin (Pierce). Confluent cell monolayers were washed in phosphate-buffered saline (PBS) and biotinylation was carried out using 0.5 mg/ml NHS-SS-biotin in PBS for 90 min at 4 °C with gentle shaking. After labeling, cells were washed twice with ice-cold PBS to remove the residual NHS-SS-biotin. Subsequently, cells were solubilized in lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% nonidet P-40 (Sigma Aldrich) pH. 8) containing protease inhibitors (CompleteMini) by gently shaking on ice for approximately 10 min. The lysate were clarified by centrifugation at 14,000xg for 5 min at 4 °C, 20 µl of the cleared lysate was saved (lysate fraction) and the rest of the lysate was incubated overnight with 100 µl of streptavidin-agarose beads (Sigma) at 4 °C with gentle agitation. After incubation, the lysate/beads mixture was separated by centrifugation at 14,000xg for 5 min at 4 °C. The lysate fraction contains the intracellular proteins of the cells (Intra). The beads were washed twice with PBS and the captured biotinylated proteins (Bio) were eluted from the beads with 150 µl of SDS sample buffer. Finally, a portion of the biotinylated (Bio) (30 µl), the intracellular (Intra) (25 µl), and the crude lysates (Lysate) was subjected to SDS-PAGE and Western blot analysis using anti-IR (Santa Cruz Biotechnology, sc-711), anti-IR-pY (R&D systems, AF2507), anti-Glut4 (Abcam, ab654), and anti-β-actin (Sigma, AF5441) as a loading control. The elevated amount of the insulin receptor in SorCS1-B and SorCS1-C

cells were located on the cell surface (in the Bio fraction), co-localising with a portion of the SorCS1 proteins (Fig. 13), indicating that SorCS1 regulates the expression of IR by physical interaction and/or by lowering the turn over of the IR protein.

5 **Example 9: Demonstration of SorCS1:IR complex formation.**

To examine the potential physical interaction between SorCS1 and IR, CHO cell stably transfected with plasmids encoding SorCS1-B and -C and subsequently transiently transfected with IR_A and IR_B were used for immunoprecipitation. The cells were transfected with plasmids encoding IR_A and IR_B using the HiFect kit (Amamax) according to the supplier's protocol. After two days of growth and at 80% confluency the cells were crosslinked with DSP (Peirce) and subsequently lysed. The cell lysates was incubated with antibody against IR (Santa Cruz Biotechnology, sc-711) bound to Gammabind beads (GE Healthcare). The precipitated complexes were eluted from the washed beads with SDS loading buffer and subjected to SDS-
10 PAGE and Western blot analysis using anti-SorCS1-leu and anti-IR (Santa Cruz Biotechnology, sc-711). Western blot analysis revealed the presence of a SorCS1:IR complex (Fig. 11A). The direct interaction of the extracellular domains of SorCS1 and IR was also demonstrated using surface plasmon resonance (Biacore, Sweden) using CaHBS as standard running buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, 2 mM CaCl₂, 1 mM EGTA, 0.005% tween-20). A biosensor chip from Biacore (CM5, cat.no. BR-1000-14) was activated using the NHS/EDC method as described by the supplier followed by coating with soluble IR (R&D systems, 28-956). Soluble SorCS1 showed strong binding to soluble insulin receptor with a K_d estimated to approximately 5 nM (Fig. 11B).

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Example 10: Analysis of SorCS1/IR contact sites based on SPOT synthesis.

The co-immunoprecipitation and the Biacore experiments showed protein interaction at whole molecular level. However, the SPOT synthesis method is used to identify the protein interaction at amino acid level using a protein-derived scan of overlapping peptides either from SorCS1 or IR, thereby identifying small SorCS1
30 peptide agonists. The SPOT synthesis maps linear epitopes (protein chain involved in interaction) using overlapping peptides derived from the entire primary sequence of either human SorCS1-a (Fig. 23A) or human IR-B (Fig. 23B). In detail, the SorCS1 and IR sequence is fragmented and synthesized on cellulose with short
35 overlapping peptides (15 amino acids in length and shifted by 3 amino acid) from C-

terminus to N-terminus, which is subsequently probed for binding to the respective partner protein, IR-protein (histidine-tagged) (R&D systems, no1544-IR/CF) and soluble mouse SorCS1 protein (I^{125} -tagged). The binding assay is performed directly on the peptide membrane through immunodetection or radiography of bound protein. In detail, the membrane is washed 1 x 10 min in 96% ethanol, followed by 3 x 10 min wash with 1 x TBS (500 mM Tris-HCl, 1500 mM NaCl), pH.8.0 and 3 hrs incubation in membrane blocking buffer (BB; 1 x Blocking buffer (B6429, Sigma), 1 x TBS, 5% sucrose). The blocked IR-membrane is incubated overnight with I^{125} -sol.SorCS1 (400.000 cpm/ml BB) and the SorCS1-membrane with his-IR (10 μ g/ml BB). Both membrane are washed 3 x 10 min with 1 x TBS. In Fig. 23A, the bound protein on the SorCS1 membrane was detected by immunodetection using primary anti-body against the histidine tag, α -histidine (Invitrogen) (Mouse monoclonal) followed by an HRP-tagged secondary anti-mouse antibody (Sigma). Bound antibody was visualized by using the enhanced chemiluminescence (ECL) Western blotting Detection reagent (Amersham biosciences) and a Fuji film LAS1000. In Fig. 23B, the bound protein on the IR-membrane was detected by radiography as the membrane was exposed to a fuji image plate for 12 hrs, which was subsequently developed using a Fujifilm FLA3000. A specific signal (SPOT) indicates that the peptide interacts with the applied ligand. Linear binding epitopes are present in neighbouring peptides on the SPOT membrane and represent the binding site.

Example 11: Binding of specific peptides to either SorCS1 or IR

The SPOT analysis identified synthetic SorCS1 or IR candidate peptides binding to their ligand protein (IR and SorCS1). The binding was further confirmed by surface plasmon resonance (Biacore, Sweden) analysis (Fig. 11B) using CaHBS as standard running buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, 2 mM $CaCl_2$, 1 mM EGTA, 0.005% tween-20). A biosensor chip from Biacore (CM5, cat.no. BR-1000-14) was activated using the NHS/EDC method as described by the supplier followed by coating with soluble IR (R&D systems, 28-956). Full-length SorCS1 was tested for binding to IR by passage over the biosensor chip, showing a positive sigmoid binding curve, indicating direct interaction of soluble full-length extracellular part of SorCS1 with immobilized soluble insulin receptor (IR)

Example 6: Competition studies. The synthetic SorCS1 and IR peptides that bind to the ligands protein are used in competitions studies to establish their influence on

the interaction between SorCS1 and IR in the SorCS1:IR complex. A biosensor chip from Biacore (CM5, cat.no. BR-1000-14) was activated using the NHS/EDC method as described by the supplier followed by coating with soluble IR (R&D systems, 28-956) or soluble SorCS1. The chips were incubated with samples of pure soluble SorCS1 or soluble IR (R&D systems, 28-956) (300nM, 40µl) in the absence of competing peptide to determine the maximal binding capacity set to represent 100% binding (see example 5). In subsequent experiments, a similar amount of soluble SorCS1 or IR are injected to the ligand ship, but in the presence of competing peptides at different concentrations to determine their ability to diminish or destroy the interaction between SorCS1 and IR.

Example 7: Competition Studies

The synthetic SorCS1 and IR peptides that bind to the ligands protein are used in competitions studies in cells to establish their influence on the interaction between SorCS1 and IR in the SorCS1:IR complex. A) CHO cell stably transfected with plasmids encoding the different splice variants of SorCS1 and subsequently transiently transfected with IR_A and IR_B were used for immunoprecipitation. The cells are transfected with plasmids encoding IR using the Hifect kit (Amaza) according to the supplier's protocol. The cells are grown two days in media without or with competing synthetic SorCS1 or IR peptide in different concentrations and at 80% confluency the cells are crosslinked with DSP and subsequently lysed. The cell lysates was incubated with antibody against IR (Santa Cruz Biotechnology, sc-711) bound to Gammabind beads (GE Healthcare). The precipitated complexes are eluted from the washed beads with SDS loading buffer and subjected to SDS-PAGE and Western blot analysis using anti-SorCS1-leu and anti-IR (Santa Cruz Biotechnology, sc-711). Western blot analyses are used to reveal the presence or absence of a SorCS1:IR complex, and thereby establish the ability of the synthetic peptide to diminish or destroy the interaction between SorCS1 and IR. B) Expression of endogene IR in the absence or presence of synthetic SorCS1 peptide(s) is examined in CHO cells and CHO cells stably transfected with the four murine SorCS1 splice variants (SorCS1-a,-b,-c,-d) and msol.SorCS1 (the extracellular part of SorCS1). The cells are grown to 80% confluency in serum-free HyQ-CCM5 CHO medium (HyClone) and in serum-free HyQ-CCM5 CHO medium supplemented with different concentration of synthetic SorCC1 peptide. The cells were washed with PBS and lysed in lysis-buffer and aliquots of the lysates are

subjected to reducing SDS-PAGE and Western blot analysis using anti-SorCS1-leu and anti-IR (Santa Cruz Biotechnology, sc-711). Western blot analyses are used to reveal the influence of the synthetic SorCS1 peptide on the expression of IR in cells without or with stably expression of SorCS1, and thereby establish the ability of the synthetic peptide to diminish or destroy the up-regulation of IR in cells expressing SorCS1.

Example 8: Administering of soluble SorCS1 or SorCS1 peptides for the treatment of insulin resistance.

The soluble domain of mouse SorCS1 peptide(s) able to bind to IR (see example 5) is expressed recombinantly at a large scale in a mammalian cell culture and is subsequently purified by for example immunoaffinity chromatography. The protein/peptid is administered by peritoneal, intravenous, intramuscular or subcutaneous injection to e.g. SorCS1 knockout mice or another diabetic animal model showing insulin resistance (1mg to 1 g/kg body weight each day or every week) in parallel with a wild type reference mouse. Good effect is obtained, and the same methods using human SorCS1 are applied for patient with insulin resistance.

Example 9: The application of DNA encoding soluble SorCS1 or SorCS1 peptides for the treatment of insulin resistance.

Gene Therapy in a clinical setting

Gene therapy is defined as the introduction of exogenous genetic material into cells or tissue in order to cure a disease or to avoid associated symptoms, in this case insulin resistance. The genetic material can be introduced into living cells/patients using different delivery methods/compounds: a) as naked therapeutic genetic molecules (DNA), where the genetic material itself is introduced directly into the tissue/patient (example 9), b) as specialized gene delivery vehicles, where the gene is inserted into different biological entities suited for gene delivery before introduction into the patient (example 10), and c) as virus, where the gene is inserted into a viral vector before introduction into the patient. Proteins might have a short life-time when introduced into the mouse or patient, so an additional treatment is applied were plasmid DNA encoding soluble SorCS1 or SorCS1 peptides are delivered to the SorCS1 knockout mice either by peritoneal injection, oral administration or injection directly into muscle or adipose tissue. The DNA encoding soluble SorCS1 or specific SorCS1 fragments are transcribed into protein in the

organism restoring the level of SorCS1 and thereby treating the insulin resistance. The same method is used in humans lacking SorCS1 or showing insulin resistance treating the symptoms of the patient.

5 **Example 10: The application of gene delivery vehicles containing soluble SorCS1 or specific SorCS1 fragments for the treatment of insulin resistance.**

To overcome any limitations of using plasmid DNA or adenovirus for expression of SorCS1 (soluble or specific fragments) specialized gene delivery vehicles (GDVs) are used which improve delivery efficiency and cell specificity whilst protecting
10 against immune recognition. Several different GDVs will be produced: A) Strains of bacteria with desirable properties are transformed with plasmid cargo containing SorCS1 and amplified to generate GDVs. B) the phagemid, a modified bacterial plasmid with phage sequence within, is used as the cargo of SorCS1 and transformed into bacteria. The bacteria is infected with a replication-defective helper
15 phage that produces essential gene for the packing of the phagemid vector into bacteriophage GDVs. C) Virus surface proteins are produced in cell culture and purified as capsid monomers. The genetic cargo containing SorCS1 is then packaged into a virion as the monomers are transferred to a buffer that promotes assembly of the virion. D) Erythrocytes are harvested from the patient and lysed to
20 produce erythrocyte ghosts. The ghosts are the loaded, through osmotic pressure, with the genetic cargo containing SorCS1 before being reintroduced into the patients. E) Patients-derived primary cells are harvested and stimulated to produce exosomes, which are then purified and loaded, by electroporation, with the genetic cargo containing SorCS1 before being reintroduced into the patient.

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Example 12: The application of adenovirus expressing soluble SorCS1 or specific SorCS1 fragments for the treatment of insulin resistance.

Genetic material expressing soluble SorCS1 or specific peptides of SorCS1 is inserted into an adeno-associated viral vector. The adeno-associated viral vector is
30 chosen because, unlike first-generation adenoviruses that contain a full complement of viral proteins, this vector encodes no viral proteins and has negligible toxicity. Furthermore, this virus gives prolonged and stable transgene (SorCS1) expression, which lower the need of repeated injection of virus to the patient. In detail, a DNA construct encoding either soluble SorCS1 or fragments of SorCS1 is inserted into an
35 adeno-associated viral vector. The adeno-associated virus is together with a helper

plasmid introduced into a cell culture and a large amount of adeno-associated virus produced. Finally, 1×10^{11} adeno-associated virus particles is injected into SorCS1 knockout mice. The viral-expressed SorCS1 cures the insulin resistance of the knockout mice and the same method is used to treat patients with insulin resistance.

5

Example 13: Generation of mouse overexpressing SorCS1

For tissue-specific induction of SorCS1 expression in the mouse, an expression construct containing a CAAG promoter (chicken beta-actin / minimal CMV) upstream of a lox-STOP-lox cassette, followed by the cDNA of full-length SorCS1 (all splice variants) or soluble SorCS1 is introduced by homologous recombination into the ROSA gene locus. To drive expression, the stop cassette is excised by cross-breeding with transgenic mice that express Cre-recombinase in a tissue specific manner. Alternatively, recombinant virus expressing Cre may be subjected to the mice containing the CAAG promoter (chicken beta-actin / minimal CMV), lox-STOP-lox cassette, followed by the cDNA of full-length SorCS1 (all splice variants) or soluble SorCS1 to induce expression of full-length or soluble SorCS1, respectively. Thus, liver expression is achieved by injecting Cre-expressing adenovirus into e.g. the tail vein.

This mouse may be used for, but is not limited to screening purposes for measuring glucose and insulin levels as well as insulin-receptor expression and phosphorylation (e.g. glucose tolerance and insulin strain) prior and subsequent to induction. In addition, the mouse may be crossbred with a SorCS1 knockout mouse, in order to study if SorCS1 overexpression can normalise or improve the phenotype.

Example 13: Decreased plasma glucose levels in mice overexpressing soluble SorCS1.

To examine the use of SorCS1 for treatment of insulin resistance, wild-type and SorCS1 knockout female mice were injected with an adenovirus over-expressing soluble SorCS1. The recombinant adenovirus for expression of human soluble SorCS1 (hsol.SorCS1) was generated as follows: pcDNA3.1/Zeo(-)/hsol.SorCS1 encoding the human soluble SorCS1 cDNA (amino acids 1-1100) was digested with Pme1 and Apa1 and the fragment encoding hsol.SorCS1 inserted into the shuttle plasmid pVQpacAd5CMVK-NpA (ViraQuest Inc, North Liberty, IA). ViraQuest Inc, North Liberty, IA, then used this shuttle plasmid for generation and propagation of adenovirus over-expressing hsol. SorCS1. Female SorCS1 knockout and wild-type

mice 40 weeks of age were fasted overnight. In the morning, on day 0, blood samples were obtained by retroorbital bleeding and plasma glucose was measured immediately on an automatic monitor (Ascentia Contour from Bayer). The mice were subsequently injected in the tail vein with 2E9 pfu's of an adenoviral vector with either hsol.SorCS1 or LacZ as a negative control (from ViraQuest Inc, North Liberty, IA). On day 7, measurements of plasma glucose were repeated on overnight fasted mice to evaluate the effect of the SorCS1 and LacZ protein. As shown in Fig. 17, wild-type and SorCS1 knockout mice, which over-expressed soluble SorCS1 protein, exhibited a significant decrease in plasma glucose levels ($\approx 40\%$) both in. As expected, a significant decrease in glucose levels was not observed in mice that received the LacZ control virus.

Example 14: Expression and phosphorylation of IR and expression of Glut4 in SorCS1 knockout mice over-expressing soluble SorCS1.

To further examine the effect of SorCS1 on insulin resistance, the expression of insulin receptor (IR), phosphorylation of IR and the expression of glucose transporter type 4 (Glut4) was determined in mice overexpressing SorCS1. Female SorCS1 knockout (-/-) mice 40 weeks of age were injected with an adenoviral vector expressing either hsol.SorCS1 or LacZ as a negative control (see example 13). On day 12 after virus injection, the mice were fasted overnight, injected intraperitoneally with insulin (Novorapid, Novo Nordisk A/S) (10 units/kg body weight) in sterile saline, and killed 15 min later. Muscle and adipose tissue were removed and homogenized in lysis buffer TNE-buffer (10mM Tris-HCl, 150 mM NaCl, 1mM EDTA, 1% nonidet P-40, pH. 8) containing protease inhibitors (Complete Mini, Roche) and phosphatase inhibitors (cocktail 1, Sigma Aldrich). The lysates were cleared by centrifugation 10 min at 10.000 x g, and protein concentration were determined by Bio-Rad Protein Assay. Equal amount of total protein (50 μ g) for different samples were separated on a 4-12 % Bis-tris gel (Nupage, Invitrogen) and transferred onto polyvinylidene difluoride (PVDF) membranes (Amersham Pharmacia). Membranes were analysed by western blotting with anti-IR (Santa Cruz Biotechnology, sc-711), anti-IR-pY (R&D systems, AF2507) and anti-Glut4 (Abcam, ab654). Bound antibodies were developed by Super-Signal West Pico reagent (Pierce) and a Fuji film LAS3000. Elevated amounts of IR, phosphorylated IR (IR-pY) and glut4 were observed in both muscle (Fig. 18A) and adipose (Fig. 18B) tissue from SorCS1 knockout mice over-expressing soluble SorCS1 when compared to mice expressing

the LacZ control protein, suggesting increased insulin sensitivity in mice overexpressing SorCS1.

Example 15: Decreased plasma glucose and insulin levels in diabetic db/db female mice over-expressing soluble SorCS1.

To evaluate the effect of soluble SorCS1 in an obese mouse model that spontaneously develops type 2 diabetes we used the db/db mouse strain (BKS.Cg-*m+/+Lpr^{db}/BomTac* from Taconic). These mice lack the leptin receptor consequently the mice become obese and develop insulin resistance and finally severe diabetes at the age of 6-8 weeks. To examine the effect on plasma glucose and insulin levels, mice were injected with adenovirus expressing either hsol.SorCS1 or LacZ as a control (see example 13). In detail, db/db female mice 10 weeks of age were fasted overnight. In the morning, on day 0, the mice were anesthetized with diethyl ether and blood samples were obtained by retroorbital bleeding. Blood glucose was measured immediately on an automatic monitor (Ascentia Contour from Bayer), whereas plasma insulin levels were determined using an ultrasensitive mouse insulin enzyme-linked immunoabsorbent assay kit (DRG Diagnostics). Thereafter the mice were injected in the tail vein with 2E9 pfu's of an adenoviral vector with either hsol.SorCS1 or LacZ (from ViraQuest Inc, North Liberty, IA) as a negative control virus. On day 7, measurements of blood glucose and plasma insulin were repeated on overnight fasted mice to evaluate the effect of the SorCS1 and LacZ protein. Data shown in Fig. 19 are means $\pm$ SEM for 5 mice in each group. On day 7, db/db female mice with over-expression of soluble SorCS1 exhibited a significant decrease in blood glucose ($\approx 35\%$) compared to the mice that received the control LacZ virus (Fig. 19A). Furthermore, on day 7 there was also a significant decrease in the plasma insulin levels in the db/db female mice over-expressing soluble SorCS1 compared to mice that express the control virus (Fig. 19B). Thus, over-expression of soluble SorCS1 improves the diabetic status in the type 2 diabetic (db/db) mouse model.

Example 16: Glucose tolerance test in diabetic db/db female mice with over-expression of soluble SorCS1.

To examine the effect of SorCS1 on glucose tolerance, female db/db mice injected with adenoviruses expressing either soluble SorCS1 or LacZ (see example 13) where on day 3 fasted over-night (16 hrs). On day 4 the mice were injected

intraperitoneally with a bolus of glucose (2mg/g body weight) in sterile saline. The animals were anesthetized with diethyl ether and blood samples were obtained by retroorbital bleeding at times 0, 15, 30, 90, and 150 minutes after injection. Blood glucose levels were measured immediately after sampling on an automatic monitor (Ascentia Contour from Bayer). Data shown in Fig. 20 are means $\pm$ SEM for 5 mice in each group. The results in Fig. 20 show that over-expression of soluble SorCS1 renders the mice more sensitive to insulin as the level of blood glucose becomes normal (i.e. the same as before glucose injection) after 150 min. By contrast, the blood glucose level in mice expressing the control protein LacZ remains elevated during the 150 minutes. In conclusion, these results show that db/db female mice over-expressing soluble SorCS1 are less insulin resistant.

Example 17: Plasma glucose and insulin levels in diabetic db/db male mice over-expressing soluble SorCS1.

To evaluate the effect of soluble SorCS1 in an obese mouse model that spontaneously develops type 2 diabetes, we used the db/db mouse strain (BKS.Cg-*m⁺/+Lpr^{db}/BomTac* from Taconic). Mice were injected with adenovirus expressing either hsol.SorCS1 or LacZ as a control (see example 13), to examine the effect on plasma glucose and insulin levels. In detail, db/db male mice 6 weeks of age were fasted overnight. In the morning, on day 0, the mice were anesthetized with diethyl ether and blood samples were obtained by retroorbital bleeding. Blood glucose was measured immediately on an automatic monitor (Ascentia Contour from Bayer), whereas plasma insulin levels were determined using an ultrasensitive mouse insulin enzyme-linked immunoabsorbent assay kit (DRG Diagnostics). The mice were subsequently injected in the tail vein with 2E9 pfu's of an adenoviral vector with either hsol.SorCS1 or LacZ (from ViraQuest Inc, North Liberty, IA) as a negative control. On day 7, measurements of blood glucose and plasma insulin were repeated on overnight fasted mice to evaluate the effect of the SorCS1 and LacZ protein. The data shown in Fig. 21 are means $\pm$ SEM for 5 mice in each group. On day 7, db/db male mice with over-expression of soluble SorCS1 exhibited a significant decrease in blood glucose ($\approx$ 35%) compared to the mice that received the control LacZ virus (Fig. 21A). Because the decline in glucose levels were not accounted by an increased insulin concentration in mice overexpressing SorCS1 (Fig. 21B), we conclude that over-expression of soluble SorCS1 improves the diabetic status in male type 2 diabetic db/db mice.

Example 18: Subcellular localization of Glut4 in muscle tissue from db/db male mice over-expressing soluble SorCS1.

To evaluate if over-expression of soluble SorCS1 might change the distribution of Glut4 we conducted subcellular fractionation on muscle tissue from db/db male mice over-expressing soluble SorCS1. In detail, db/db male mice 6 weeks of age were injected in the tail vein with an adenoviral vector expressing either hsol.SorCS1 or LacZ as a control (see example 13). On day 7 after virus injection, the mice were fasted overnight, injected intraperitoneally with insulin (Novorapid, Novo Nordisk A/S) (10 units/kg body weight) in sterile saline, and killed 15 min later. Muscle tissue from 5 mice injected with the same virus was removed, pooled and transferred to 5 ml of HEPES-buffered sucrose (0.25 M sucrose, 1 mM EDTA, 20 mM HEPES-KOH, pH. 7.4), homogenized by 10 strokes up and down using a Teflon pestle, and centrifuged at 1000 x g for 10 min. Thus, heavy mitochondrial, light mitochondrial, and microsomal fraction were obtained by several round of centrifugation. First, the supernatant was centrifuged at 3.000 x g for 10 min, then the resulting supernatant was centrifuged at 16.000 x g for 10 min, and finally the resulting supernatant was centrifuged a 100.000 x g for 45 min giving a pellet containing the microsomal fraction. The microsomal fractions were resuspended in 0.5 ml HEPES-buffered solution and subjected to sucrose (velocity) gradient centrifugation. The 0.5 ml microsomal samples were loaded onto a 12 ml linear 0.8 M to 1.6 M sucrose gradient in 1 M HEPES, pH 7.2, and centrifuged 18 h in a swinging bucket rotor (SW41 Ti) at 84.000 x g. Each gradient was separated into 24 fractions starting from the top of the tube. Finally, gel electrophoresis and Western blotting analyzed the expression of Glut4 in the different fractions. The results in Fig. 22 show that the sedimentation distribution of Glut4 in muscle tissue over-expressing SorCS1 (lower panel) is different from muscle tissue expressing the control protein lacZ (upper panel). Thus, over-expression of soluble SorCS1 in db/db male mice may change the distribution of Glut4 and thereby modulate glucose uptake.

Example 19: Gene expression profiling of adipose tissue from SorCS1 knockout mice by PCR arrays.

To examine the gene expression profile of SorCS1 knockout mice, the expression of 84 genes related to the mouse insulin signalling pathway and 84 genes related to mouse lipoprotein signalling & cholesterol metabolism was determined using

microarray analysis. The microarray analysis were performed using RNA from adipose tissue of SorCS1 knockout wild-type adipose mice. In practice, first strand cDNA was synthesized from total RNA (Applied Biosystems) from SorCS1 knockout (-/-) and wild-type (+/+) adipose tissue from female mice 50 weeks of age (n = 3).

5 Then superarray of Mouse Insulin Signalling Pathway (PAMM-030A RT2 Profiler PCR arrays) or B) the type Mouse Lipoprotein Signalling & Cholesterol Metabolism (PAMM-080-A RT2 Profiler PCR arrays) were processed using an ABI7900 platform (Applied Biosystems) and SYBR Green/Rox PCR (SABiosciences). AROS Applied Biotechnology, Aarhus, Denmark, did the expression analyses. Genes showing an

10 expression more than 3 times up- or down-regulated in the SorCS1 knockout mice when compared to wild-type mice are listed in the upper tables and their known functions are indicated in the table below. The data in Fig. 24 shows that the expression of several genes are changed expression in the SorCS1 knockout mice compared to the wild-type mice, indicating that insulin and cholesterol signalling

15 pathways and metabolism are altered in SorCS1 knockout mice.

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Overview of sequences

- SEQ ID NO 1: Homo sapiens preproSorCS1b (Isoform 1)
 SEQ ID NO 2: Homo sapiens preproSorCS1 (Isoform 2)
 SEQ ID NO 3: Homo sapiens preproSorCS1c (Isoform 3)
 5 SEQ ID NO 4: Homo sapiens preproSorCS1a (Isoform 4)
 SEQ ID NO 5: Soluble Homo sapiens preproSorCS1
 SEQ ID NO 6: Homo sapiens proSorCS1b (Isoform 1)
 SEQ ID NO 7: Homo sapiens proSorCS1 (Isoform 2)
 SEQ ID NO 8: Homo sapiens proSorCS1c (Isoform 3)
 10 SEQ ID NO 9: Homo sapiens proSorCS1a (Isoform 4)
 SEQ ID NO 10: Soluble Homo sapiens proSorCS1
 SEQ ID NO 11: Homo sapiens mature SorCS1b (Isoform 1)
 SEQ ID NO 12: Homo sapiens mature SorCS1 (Isoform 2)
 SEQ ID NO 13: Homo sapiens mature SorCS1c (Isoform 3)
 15 SEQ ID NO 14: Homo sapiens mature SorCS1a (Isoform 4)
 SEQ ID NO 15: Soluble Homo sapiens mature SorCS1
 SEQ ID NO 16: Mouse preproSorCS1b (isoform 1)
 SEQ ID NO 17: Mouse preproSorCS1a (isoform 2)
 SEQ ID NO 18: Mouse preproSorCS1c (isoform 3)
 20 SEQ ID NO 19: Mouse preproSorCS1c+ (isoform 4)
 SEQ ID NO 20: Mouse preproSorCS1d
 SEQ ID NO 21: Soluble mouse preproSorCS1
 SEQ ID NO 22: Mouse proSorCS1b (isoform 1)
 SEQ ID NO 23: Mouse proSorCS1a (isoform 2)
 25 SEQ ID NO 24: Mouse proSorCS1c (isoform 3)
 SEQ ID NO 25: Mouse proSorCS1c+ (isoform 4)
 SEQ ID NO 26: Mouse proSorCS1d
 SEQ ID NO 27: Soluble mouse proSorCS1
 SEQ ID NO 28: Mouse mature SorCS1b (isoform 1)
 30 SEQ ID NO 29: Mouse mature SorCS1a (isoform 2)
 SEQ ID NO 30: Mouse mature SorCS1c (isoform 3)
 SEQ ID NO 31: Mouse mature SorCS1c+ (isoform 4)
 SEQ ID NO 32: Mouse mature SorCS1d
 SEQ ID NO 33: Soluble mouse mature SorCS1
 35 SEQ ID NO 34: Chimpanzee preproSorCS1

- SEQ ID NO 35: Chimpanzee proSorCS1
 SEQ ID NO 36: Chimpanzee mature SorCS1
 SEQ ID NO 37: Chimpanzee soluble SorCS1
 SEQ ID NO 38: Dog mature SorCS1
 5 SEQ ID NO 39: Dog soluble SorCS1
 SEQ ID NO 40: Cow preproSorCS1
 SEQ ID NO 41: Cow proSorCS1
 SEQ ID NO 42: Cow mature SorCS1
 SEQ ID NO 43: Cow soluble SorCS1
 10 SEQ ID NO 44: Rat preproSorSC1
 SEQ ID NO 45: Rat proSorCS1
 SEQ ID NO 46: Rat mature SorCS1
 SEQ ID NO 47: Rat soluble SorCS1
 SEQ ID NO 48: Chicken preproSorCS1
 15 SEQ ID NO 49: Chicken proSorCS1
 SEQ ID NO 50: Chicken mature SorCS1
 SEQ ID NO 51: Chicken soluble SorCS1
 SEQ ID NO 52: Homo sapiens Sortilin
 SEQ ID NO 53: Homo sapiens SorLA
 20 SEQ ID NO 54: Homo sapiens SorCS2
 SEQ ID NO 55: Homo sapiens SorCS3
 SEQ ID NO 56: Homo sapiens Human Insulin Receptor (IR)
- SEQ ID NO: 57
 25 SorCS1 (ex24), forward primer
 5'-AAGTCTCTGCTGGGAACGCCATACTGCAAG-3
- SEQ ID NO: 58
 SorCS1 (ex24), reverse primer
 30 5'-GTGGACAAGAACTTGGACGCCAGGCTTCAG-3
- SEQ ID NO: 59
 SorCS1-a (ex25), forward primer
 5'-AAGTCTCTGCTGGGAACGCCATACTGCAAG-3
 35

SEQ ID NO: 60

SorCS1-a (ex25), reverse primer

5'-TATTGCTTCTGAACCTGGCAGAAAGAGGAG-3'

5 SEQ ID NO: 61

SorCS1-b (ex27), forward primer

5'-AAGTCTCTGCTGGGAACGCCATACTGCAAG-3

SEQ ID NO: 62

10 SorCS1-b (ex27), reverse primer

5'-GCTTTGGCGATGAAGGTGGAGTTGCTGGCT-3'

SEQ ID NO: 63

SorCS1-c (ex26), forward primer

15 5'-AAGTCTCTGCTGGGAACGCCATACTGCAAG-3

SEQ ID NO: 64

SorCS1-c (ex26), reverse primer

5'-CAGGGTGAGGGACACTGGGCCTGCTTTCAG-3

20

SEQ ID NO: 65

SorCS1-d (ex28), forward primer

5'-AAGTCTCTGCTGGGAACGCCATACTGCAAG-3

25 SEQ ID NO: 66

SorCS1-d (ex28), reverse primer

5'-CGGATCTCTTGGAAGTGAAGTTACAGATGCTTG-3

Claims

- 5 1. A SorCS1-like agent for use in treating insulin resistance and/or a disease associated with insulin resistance in an individual, wherein said agent is capable of binding to the insulin receptor (IR) at a SorCS1 binding site and being capable of sensitization of an insulin receptor.
- 10 2. The agent of claim 1, wherein the insulin receptor is a human insulin receptor.
- 15 3. The agent of any of the preceding claims, wherein the binding site on the insulin receptor is a binding site for a peptide comprising one or more sequences being defined as follows:
- SEQ ID NO: 1 aa 103-124
 SEQ ID NO: 1 aa 125-143
 SEQ ID NO: 1 aa 144-162
 SEQ ID NO: 1 aa 197-218
20 SEQ ID NO: 1 aa 391-409
 SEQ ID NO: 1 aa 661-684
 SEQ ID NO: 1 aa 763-783
 SEQ ID NO: 1 aa 859-876
- 25 4. The agent of any of the preceding claims, wherein the binding site on the insulin receptor comprises one or more of the sequences defined as follows:
- SEQ ID NO: 56 aa 100-120
 SEQ ID NO: 56 aa 127-150
30 SEQ ID NO: 56 aa 284-310
 SEQ ID NO: 56 aa 362-379
 SEQ ID NO: 56 aa 593-610
 SEQ ID NO: 56 aa 629-652
 SEQ ID NO: 56 aa 749-772.
- 35

5. The agent of any of the preceding claims, wherein said agent is selected from the group consisting of
- j) an isolated SorCS1 polypeptide selected from the group consisting of
 - iv) an amino acid sequence consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51;
 - v) a biologically active sequence variant of the amino acid sequence of a) wherein the variant has at least 70% sequence identity to said SEQ ID NO: 1; or
 - vi) a biologically active fragment of any of i or ii wherein said fragment comprises at least 5 contiguous amino acids of any of a) through b), and having at least 70% sequence identity to SEQ ID NO: 1 in a range of overlap of at least 5 amino acids wherein the biological activity is sensitization of an insulin receptor.
6. The polypeptide of claim any of the preceding claims, wherein the polypeptide is a naturally occurring allelic variant of the sequence selected from the group consisting of SEQ ID NO: SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51.
7. The polypeptide of claim any of the preceding claims, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 5, 10, 15, 21, 27, 33, 37, 39, 43 and 47.
8. The polypeptide of claim any of the preceding claims, wherein the polypeptide is a variant polypeptide described therein, wherein any amino acid specified in the selected sequence is altered to provide a conservative substitution.

- 5 9. The polypeptide of claim any of the preceding claims, wherein the polypeptide has at least 70% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51.
- 10 10. The polypeptide of claim any of the preceding claims, wherein the polypeptide has at least 75% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51;.
- 15 11. The polypeptide of claim any of the preceding claims, wherein the polypeptide has at least 80% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 20 47, 48, 49, 50 and 51.
- 25 12. The polypeptide of claim any of the preceding claims, wherein the polypeptide has at least 85% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51.
- 30 13. The polypeptide of claim any of the preceding claims, wherein the polypeptide has at least 90% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51.

14. The polypeptide of claim any of the preceding claims, wherein the polypeptide has at least 95% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51.
15. The polypeptide of claim any of the preceding claims, wherein the polypeptide has at least 98% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51;.
16. The polypeptide of claim any of the preceding claims, wherein the polypeptide has at least 99% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 and 51.
17. The polypeptide of claim any of the preceding claims, wherein the polypeptide is selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 6, 7, 8, 9, 11, 12, 13, 14.
18. The polypeptide of claim any of the preceding claims, wherein the polypeptide is selected from the group consisting of SEQ ID NOs: 16, 17, 18, 19, 20, 22, 26, 28, 29, 30, 31 and 32.
19. The polypeptide according to any of the preceding claims, wherein the polypeptide is glycosylated.

20. The polypeptide according to any of the preceding claims, wherein the polypeptide is glycosylated in amino acid residue positions 184, 352, 433, 765, 776, 816, 847, 908 and 929.
- 5 21. The polypeptide according to any of the preceding claims, wherein the polypeptide is a soluble polypeptide being a fragment of the sequences of claim 17.
- 10 22. The polypeptide according to any of the preceding claims, wherein the glycosylated fragment has the sequence selected from the group consisting of SEQ ID NO: 5, 10 and 15.
- 15 23. The polypeptide according to any of the preceding claims, wherein the polypeptide is glycosylated in amino acid residue positions 184, 352, 433, 775, 815, 846, 907 and 929.
- 20 24. The polypeptide according to any of the preceding claims, wherein the polypeptide is a soluble polypeptide being a fragment of the sequences of claim 18.
- 25 25. The polypeptide according to any of the preceding claims, wherein the glycosylated fragment has the sequence selected from the group consisting of SEQ ID NO: 21, 27 and 33.
26. The polypeptide according to any of the preceding claims, wherein the polypeptide comprises one of more of the following sequences:
- 30 SEQ ID NO: 1 aa 103-124
SEQ ID NO: 1 aa 125-143
SEQ ID NO: 1 aa 144-162
SEQ ID NO: 1 aa 197-218
SEQ ID NO: 1 aa 391-409
SEQ ID NO: 1 aa 661-684
SEQ ID NO: 1 aa 763-783
- 35 SEQ ID NO: 1 aa 859-876

or a variant thereof, wherein the variant has at least 70% sequence identity to said one or more sequences.

5 27. The polypeptide according to any of the preceding claims, wherein the polypeptide is capable of forming at least one intramolecular cystine bridge.

 28. The polypeptide according to any of the preceding claims, comprising a
10 dimer of said protein linked through at least one intermolecular cystine bridge.

 29. The polypeptide according to any of the preceding claims, further
 comprising an affinity tag, such as a polyhis tag, a GST tag, a HA tag, a
15 Flag tag, a C-myc tag, a HSV tag, a V5 tag, a maltose binding protein tag, a cellulose binding domain tag.

 30. The agent according to any of the preceding claims, wherein said agent is
 selected from the group consisting of antibodies, antisense RNA, antisense-
20 DNA, small organic molecules and siRNA.

 31. The agent according to claim 30, wherein said antibody is selected from the
 group consisting of: polyclonal antibodies, monoclonal antibodies,
 humanised antibodies, single chain antibodies, recombinant antibodies.

25 32. The agent according to claim 30 or 31, wherein the antibody is an anti-insulin receptor antibody.

 33. A nucleic acid sequence encoding a polypeptide as defined in any of claims
 5-32 for use in the treatment of insulin resistance or diseases associated
30 with insulin resistance in an individual.

34. A vector comprising at least one nucleic acid molecule as defined in claim 33 for use in the treatment of insulin resistance or diseases associated with insulin resistance in an individual.
- 5 35. The vector of claim 34, further comprising a promoter operably linked to the nucleic acid molecule.
- 10 36. The vector of claim 35, wherein the promoter is selected from the group consisting of: CMV, human UbiC, RSV, Tet-regulatable promoter, Mo-MLV-LTR, Mx1, EF-1alpha, PDGF beta and CaMK II.
- 15 37. The vector of claim 34 to 36, wherein the vector is selected from the group consisting of vectors derived from the Retroviridae family including lentivirus, HIV, SIV, FIV, EAV, CIV.
- 20 38. The vector of claim 34 to 37, wherein the vector is selected from the group consisting of alphavirus, adenovirus, adeno associated virus, baculovirus, HSV, coronavirus, Bovine papilloma virus, Mo-MLV, preferably adeno associated virus.
- 25 39. An isolated host cell transformed or transduced with at least one vector of any of the claims 34 to 38 for use in the treatment of insulin resistance or diseases associated with insulin resistance in an individual.
- 30 40. The isolated host cell of claim 39, said host cell selected from the group consisting of *Saccharomyces cerevisiae*, *E. coli*, *Aspergillus* and Sf9 insect cells.
41. The isolated host cell of claim 40, said host cell selected from the group consisting of mammalian cells selected from the group consisting of human, feline, porcine, simian, canine, murine and rat cells.

42. The isolated host cell of claim 41 wherein said mammalian cell is selected from the group consisting of muscle cells, hepatocytes, adipocytes and cells of the pancreas such as α cells, β cells and δ cells.
- 5 43. The isolated host cell of claim 41 or 42, said host cell selected from the group consisting of CHO, CHO-K1, HEI193T, HEK293, COS, PC12, HiB5, RN33b and BHK cells.
- 10 44. A packaging cell line being capable of producing an infective virus particle for use in the treatment of insulin resistance or diseases associated with insulin resistance in an individual, said virus particle comprising a Retroviridae derived genome comprising a 5' retroviral LTR, a tRNA binding site, a packaging signal, a promoter operably linked to a polynucleotide sequence encoding the polypeptide of any of the claims 4 to 32, an origin of
15 second strand DNA synthesis, and a 3' retroviral LTR.
45. The packaging cell line of claim 44, wherein the genome is lentivirally derived and the LTRs are lentiviral.
- 20 46. A method of treatment of insulin resistance or diseases associated with insulin resistance, said method comprising administering to an individual in need thereof a therapeutically effective amount of:
- 25 a) agent of any of the claims 1 to 32; or
b) the isolated nucleic acid sequence of a claim 33; or
c) the expression vector of any of the claims 34 to 38; or
d) a composition of host cells according to any of the claims 39 to 43; or
e) a packaging cell line according to any of the claims 44 to 45, or
f) a combination of two or more of any of a) through e).
- 30 47. A method of upregulating an insulin receptor or a fragment or variant thereof, in an individual in need thereof, said method comprising administering to a therapeutically effective amount of:
- a) agent of any of the claims 1 to 32; or
b) the isolated nucleic acid sequence of a claim 33; or

- 5
- c) the expression vector of any of the claims 34 to 38; or
 - d) a composition of host cells according to any of the claims 39 to 43; or
 - e) a packaging cell line according to any of the claims 44 to 45, or
 - f) a combination of two or more of any of a) through e).

- 10
48. A pharmaceutical composition comprising a combination one or more of:
- a) agent of any of the claims 1 to 32; or
 - b) the isolated nucleic acid sequence of a claim 33; or
 - c) the expression vector of any of the claims 34 to 38; or
 - d) a composition of host cells according to any of the claims 39 to 43; or
 - e) a packaging cell line according to any of the claims 44 to 45, or
 - f) a combination of two or more of any of a) through e).

15

49. The pharmaceutical composition according claim 48 comprising a second active ingredient.

- 20
50. The pharmaceutical composition according to claims 48 to 49 comprising a pharmaceutically acceptable carrier.

51. The pharmaceutical composition according to any of claims 48 to 50, wherein the pH of the composition is between pH 4 and pH 10.

- 25
52. The pharmaceutical composition according to any of claims 48 to 51, formulated for administration by injection, suppository, oral administration, sublingual tablet or spray, cutaneous administration, inhalation or for local administration using an implantable biocompatible capsule.

- 30
53. The pharmaceutical composition according to claim 52, wherein the injection is intravenous, intramuscular, intraspinal, intraperitoneal, subcutaneous, a bolus or a continuous administration.

- 35
54. The pharmaceutical composition according to any of claims 48 to 53, wherein administration occurs at intervals of 30 minutes to 24 hours.

55. The pharmaceutical composition according to any of claims 48 to 54, wherein administration occurs at intervals of 1 to 6 hours.
- 5 56. The pharmaceutical composition according to any of claims 48 to 55, wherein the duration of the treatment is from 6 to 72 hours.
57. The pharmaceutical composition according to any of claims 48 to 56, wherein the duration of the treatment is life long.
- 10 58. The pharmaceutical composition according to any of claims 48 to 57, wherein the dosage of the active ingredient is between 10 µg to 500 mg per kg body mass.
- 15 59. A method of sensitizing an insulin receptor, said method comprising administering to the insulin receptor or an individual comprising said receptor a Vps10p-domain receptor selected from the group consisting of:
- 20 a) SorCS1
b) SorCS2
c) SorCS3
d) Sortilin and
e) SorLA,
- or a biologically active fragment thereof.
- 25 60. The agent according to any of claims 1-32, the nucleic acid according to claim 33, the vector according to any of claims 34-38, the host cell according to any of claims 39-43, the packaging cell line according to any of claims 44-45, the method of any of claims 46-47, or the pharmaceutical composition of any of claims 48 to 58, wherein the disease associated with
- 30 insulin resistance is selected from the group consisting of insulin resistance syndrome, Type 2 diabetes mellitus, impaired glucose tolerance, the metabolic syndrome, hyperglycemia, hyperinsulinemia, arteriosclerosis, hypercholesterolemia, hypertriglyceridemia, hyperlipidemia, dyslipidemia,

obesity, central obesity, polycystic ovarian syndrome, hypercoagulability, hypertension, microalbuminuria, and any combinations thereof.

5 61. The agent according to claim 60, wherein the disease associated with insulin resistance is selected from the group consisting of insulin resistance syndrome (IRS), Type 2 diabetes mellitus, impaired glucose tolerance, the metabolic syndrome, hyperglycemia, and hyperinsulinemia.

10 62. A kit in parts comprising:

- a pharmaceutical composition as defined in any of claims 48 to 58,
- a medical instrument or other means for administering said pharmaceutical composition,
- 15 - instructions on how to use the kit in parts.

63. The kit in parts according to claim 62, comprising a second active ingredient.

20 64. A transgenic knock-out mouse in which the endogenous Vps10p-domain receptor SorCS1 genes have been disrupted to abolish expression of a functional SorCS1 receptor, and wherein said mouse exhibits a reduced response to insulin relative to a non-transgenic control mouse.

25 65. The transgenic mouse of claim 64, wherein said disruption comprises a deletion of the SorCS1 receptor gene nucleotide sequences encoding the start codon or a region of the mouse SorCS1 receptor from the extracellular domain, transmembrane domain, or the cytoplasmic domain.

30 66. A method for screening for the ability of the SorCS1-like agent of claim 1 to reduce blood glucose levels, said method comprising the steps of

- f) providing a first and a second transgenic mouse of claim 64;
- g) administering to said first transgenic mouse a candidate agent, and

- 5 h) administering to said second transgenic mouse a physiological solution, and
- i) taking blood samples from the mouse of b) and c) respectively, at predetermined time intervals, such as at 15 minutes, 30 minutes, 60 minutes, 2 and 4 hours, subsequent to administration of said agent, and
- 10 j) comparing blood glucose levels in the samples of d); wherein a reduction in blood glucose level of said first transgenic mouse administered said candidate agent relative to said second transgenic mouse not administered said candidate agent indicates that the candidate agent reduces blood glucose levels.

67. A method for screening for the ability of the SorCS1-like agent of claim 1 to reduce blood glucose levels, said method comprising the steps of

- 15 f) providing a first and a second wild-type mouse; and
- g) administering to said first mouse the agent of claim 1, and
- h) administering to said second mouse a physiological solution, and
- 20 i) taking blood samples from the two mice of b) and c) respectively, at predetermined time intervals, such as at 15 minutes, 30 minutes, 60 minutes, 2 and 4 hours, subsequent to administration of said agent, and
- 25 j) comparing plasma glucose levels in the samples of d); wherein a reduction in blood glucose level of said first wild-type mouse administered said agent relative to said second wild type mouse not administered said candidate agent, indicates that the agent reduces blood glucose levels.

68. A transgenic mouse capable of encoding soluble and/or full length SorCS1 in a tissue specific manner, upon activation of expression.

30

69. The mouse of claim 68, wherein said tissue is selected from the group consisting of liver, muscle, pancreas and adipose tissue.

70. Use of an agent capable of enhancing binding activity between SorCS1 or a fragment or variant thereof, and an insulin receptor for the treatment of insulin resistans and/or diseases associated with insulin resistance.

5

71. The agent of claim 70, wherein said agent is selected from the group consisting of peptides, antibodies, antisense RNA, antisense-DNA, small organic molecules and siRNA.

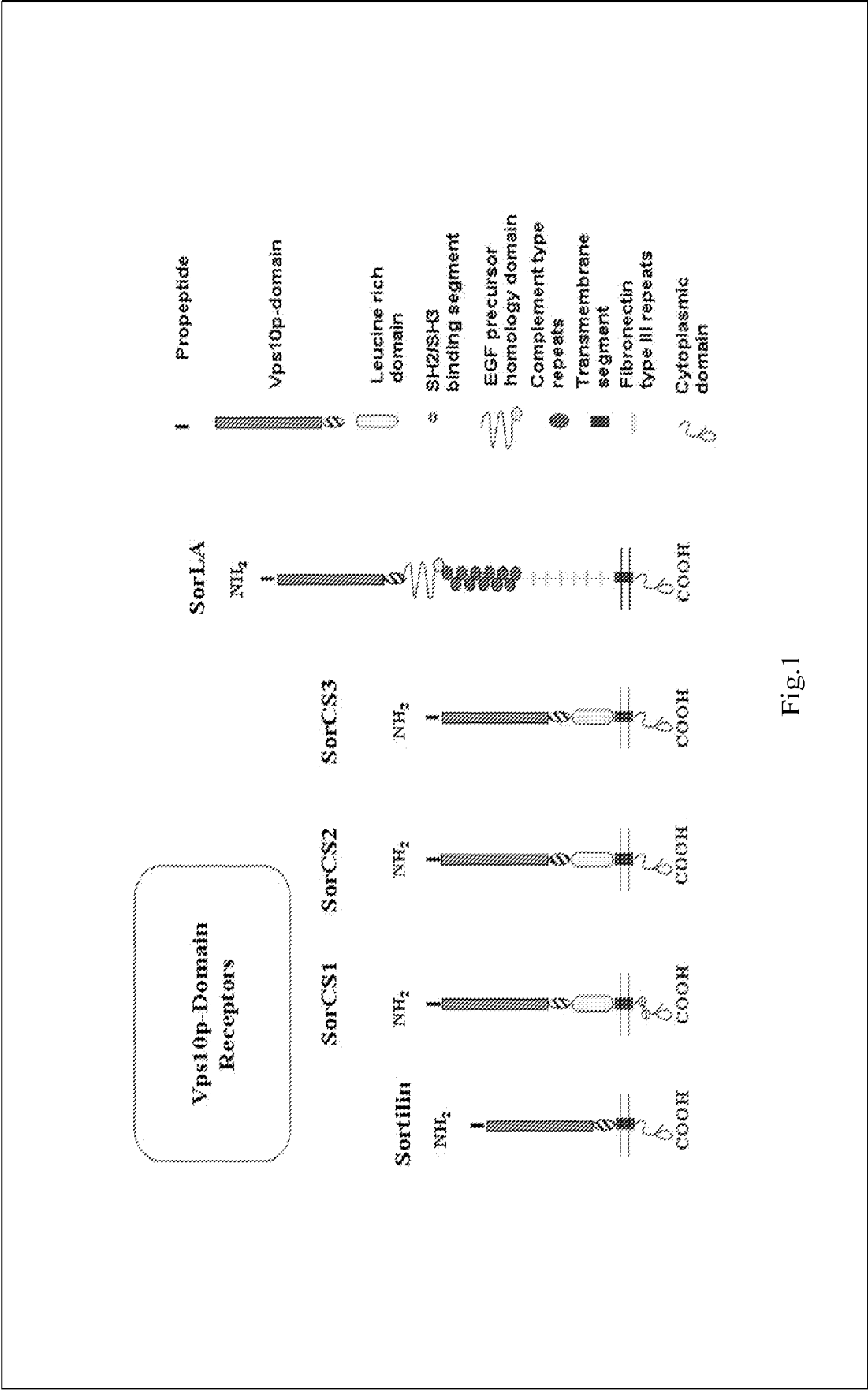


Fig.1

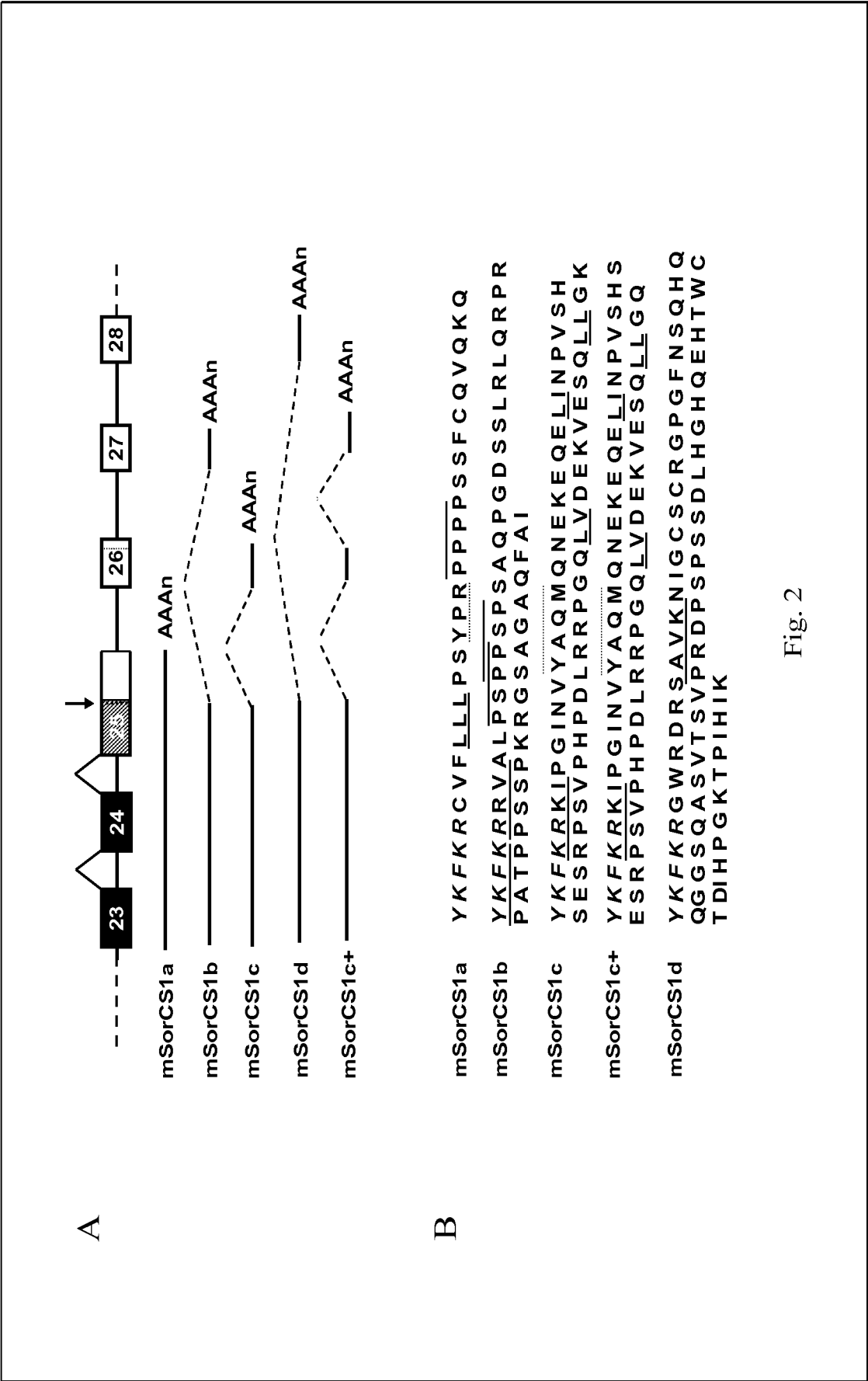


Fig. 2

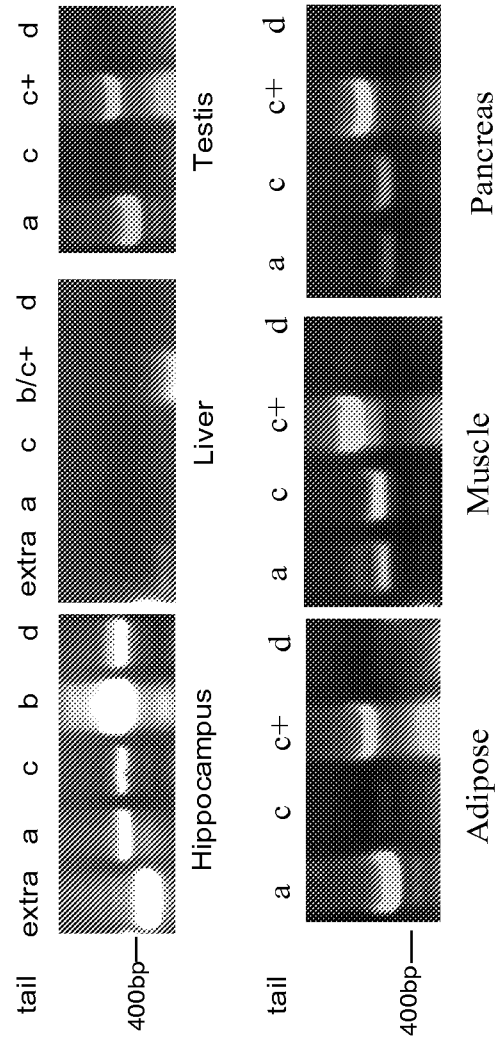


Fig. 3

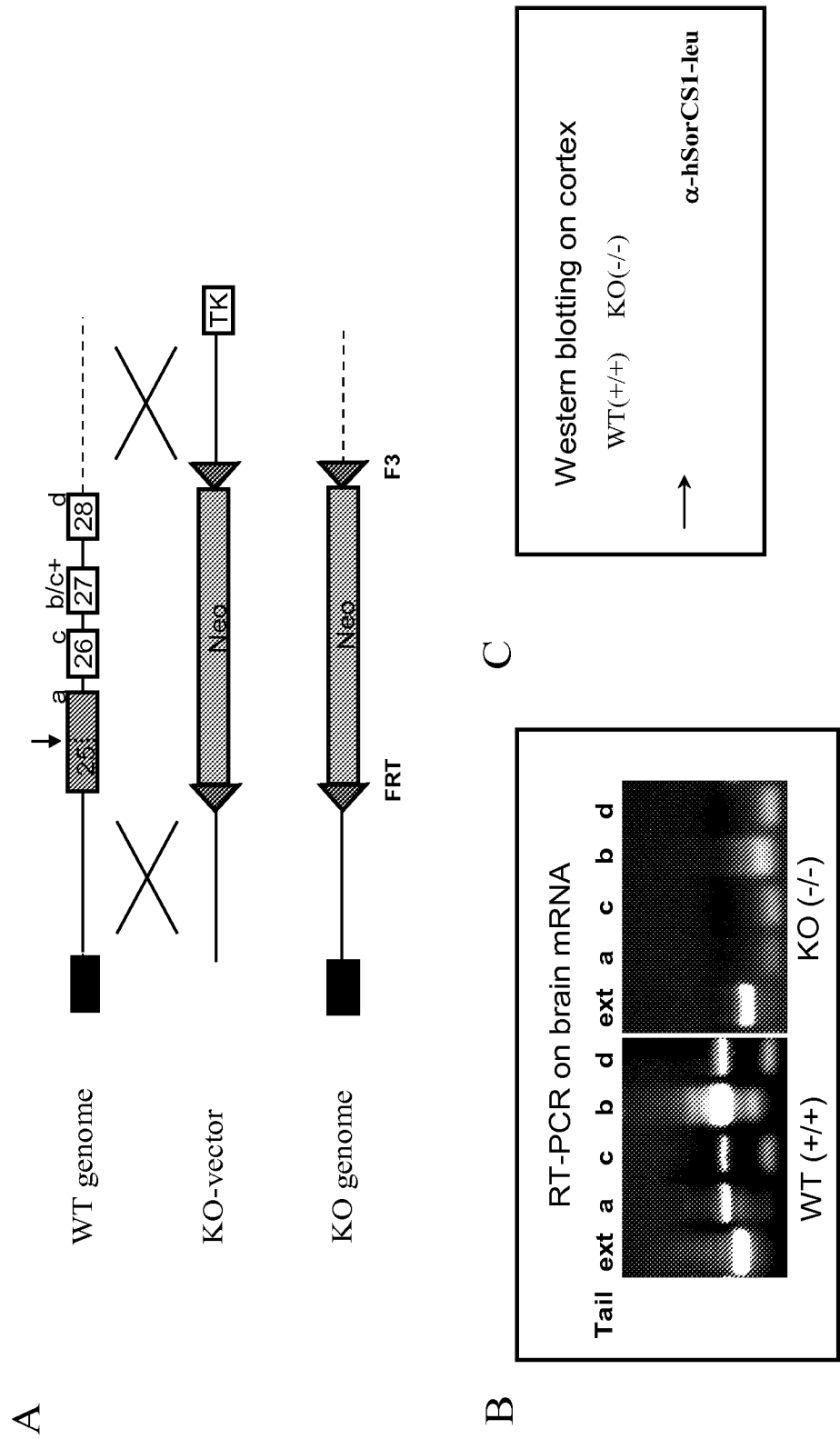


Fig. 4

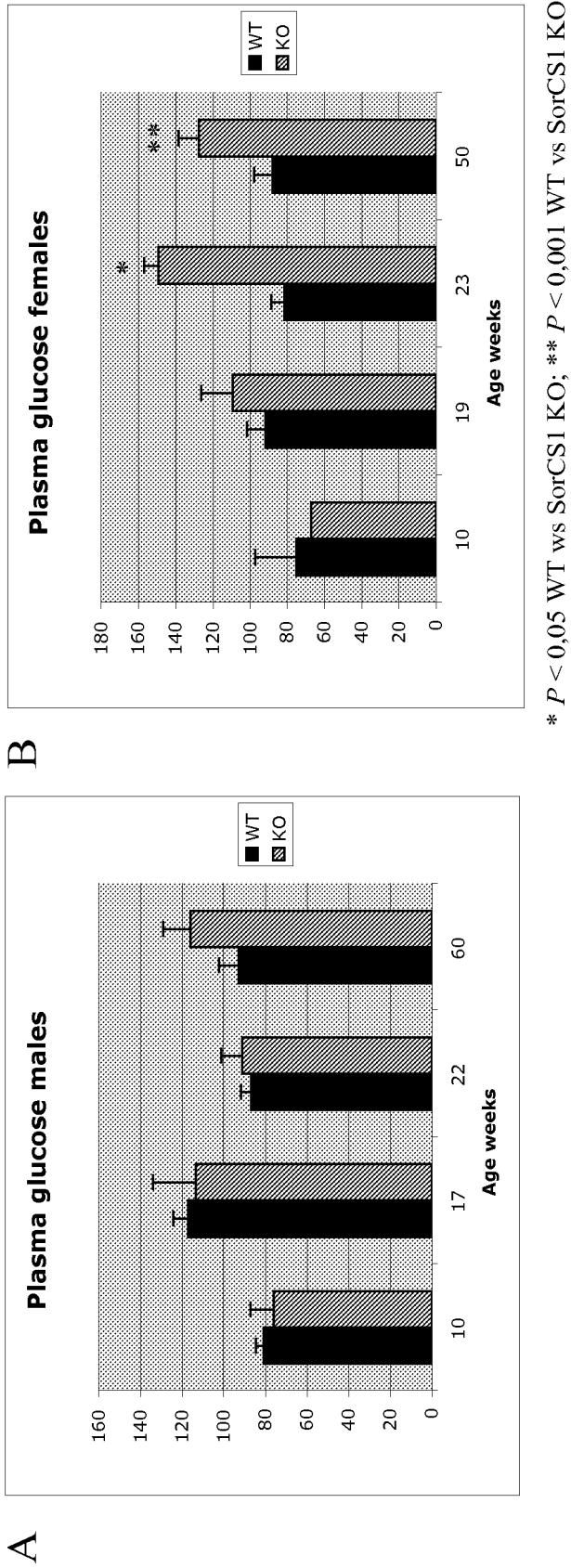
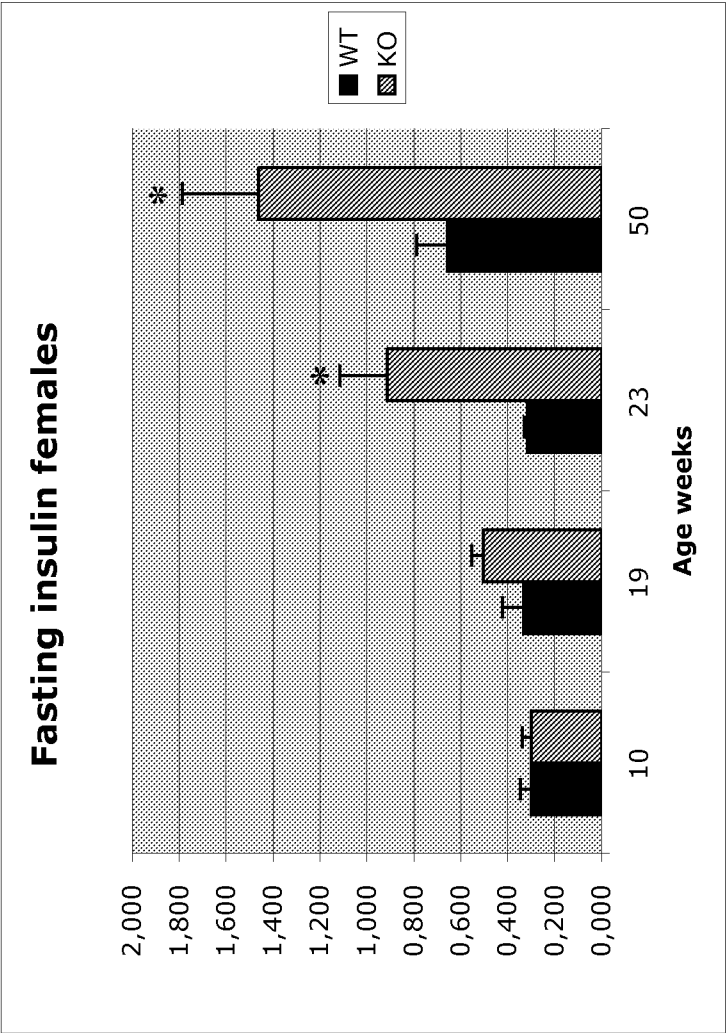
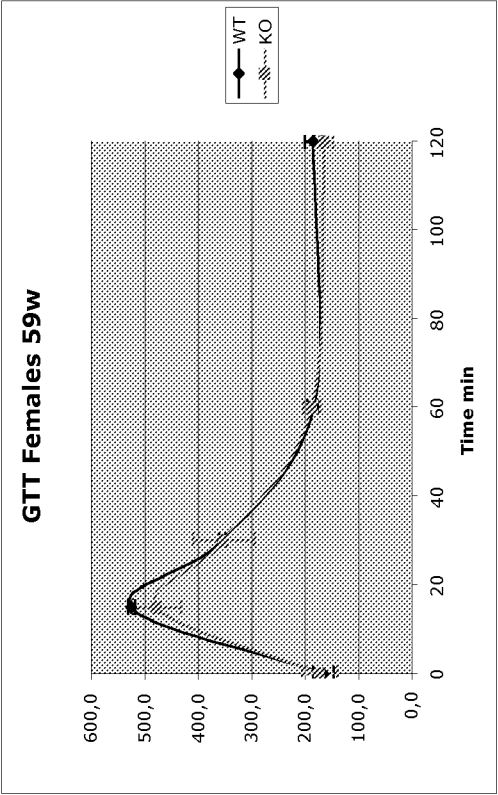


Fig. 5

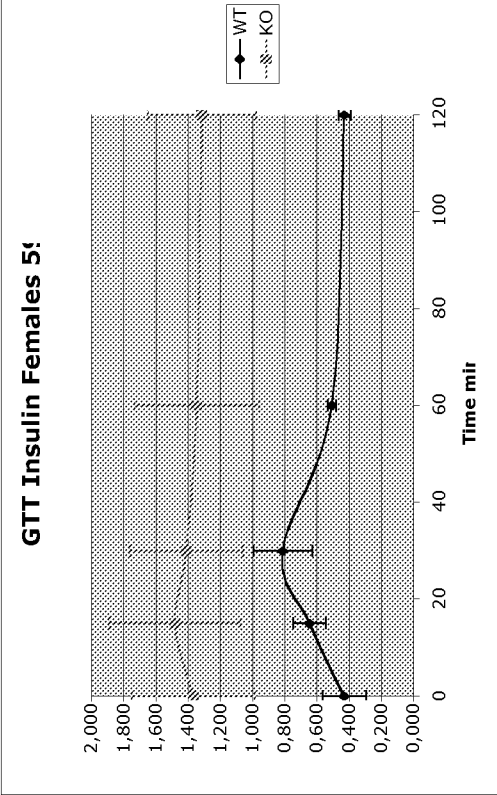


* $P < 0,05$ wt vs SorCS1 KO

Fig. 6



A



B

Fig. 7

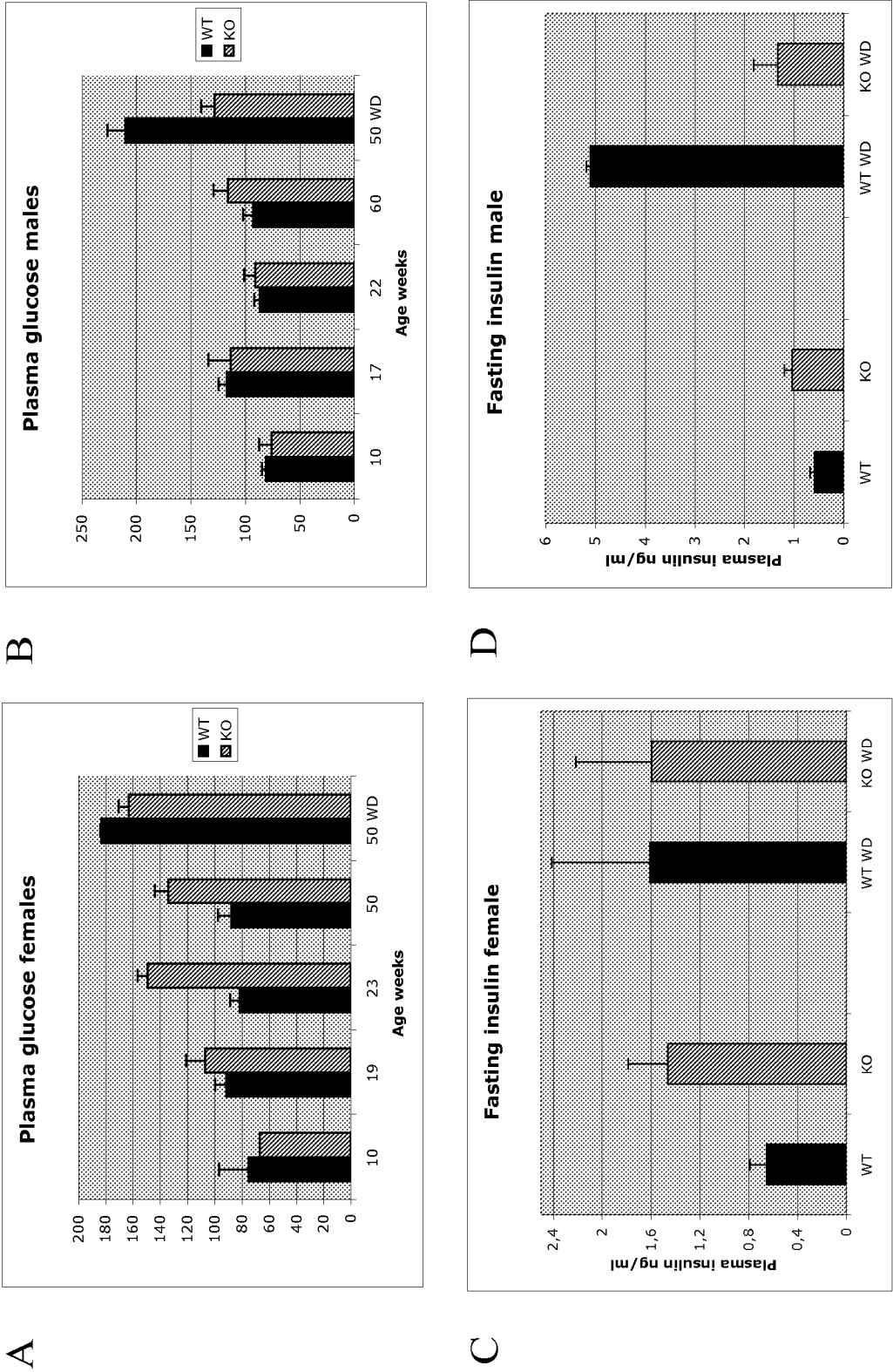
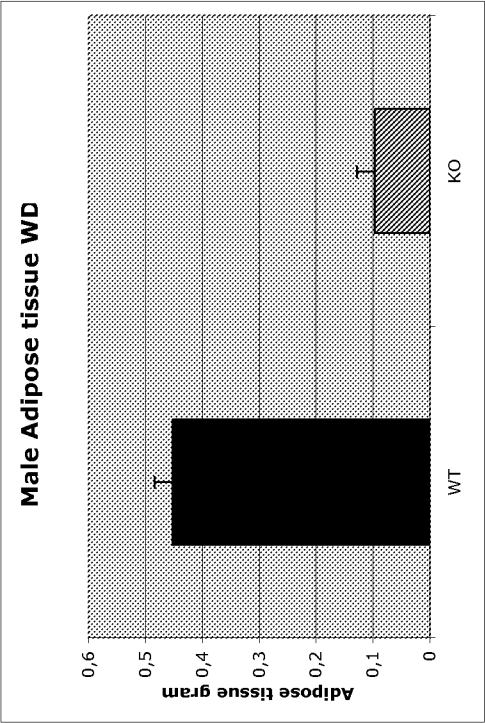


Fig. 8

B



A

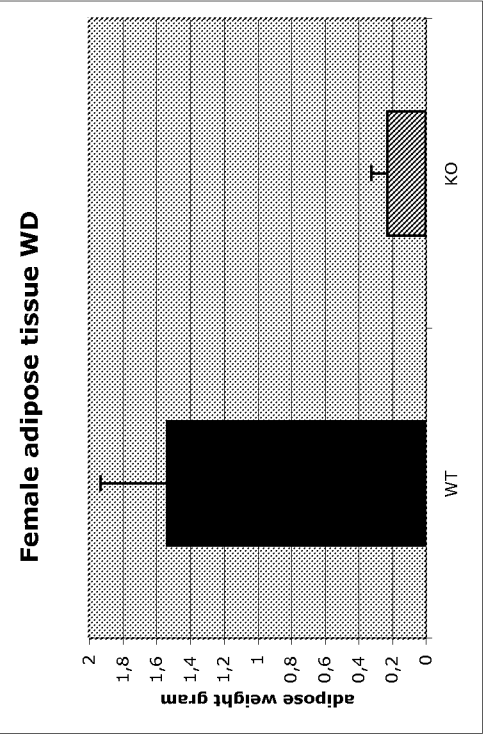


Fig. 9

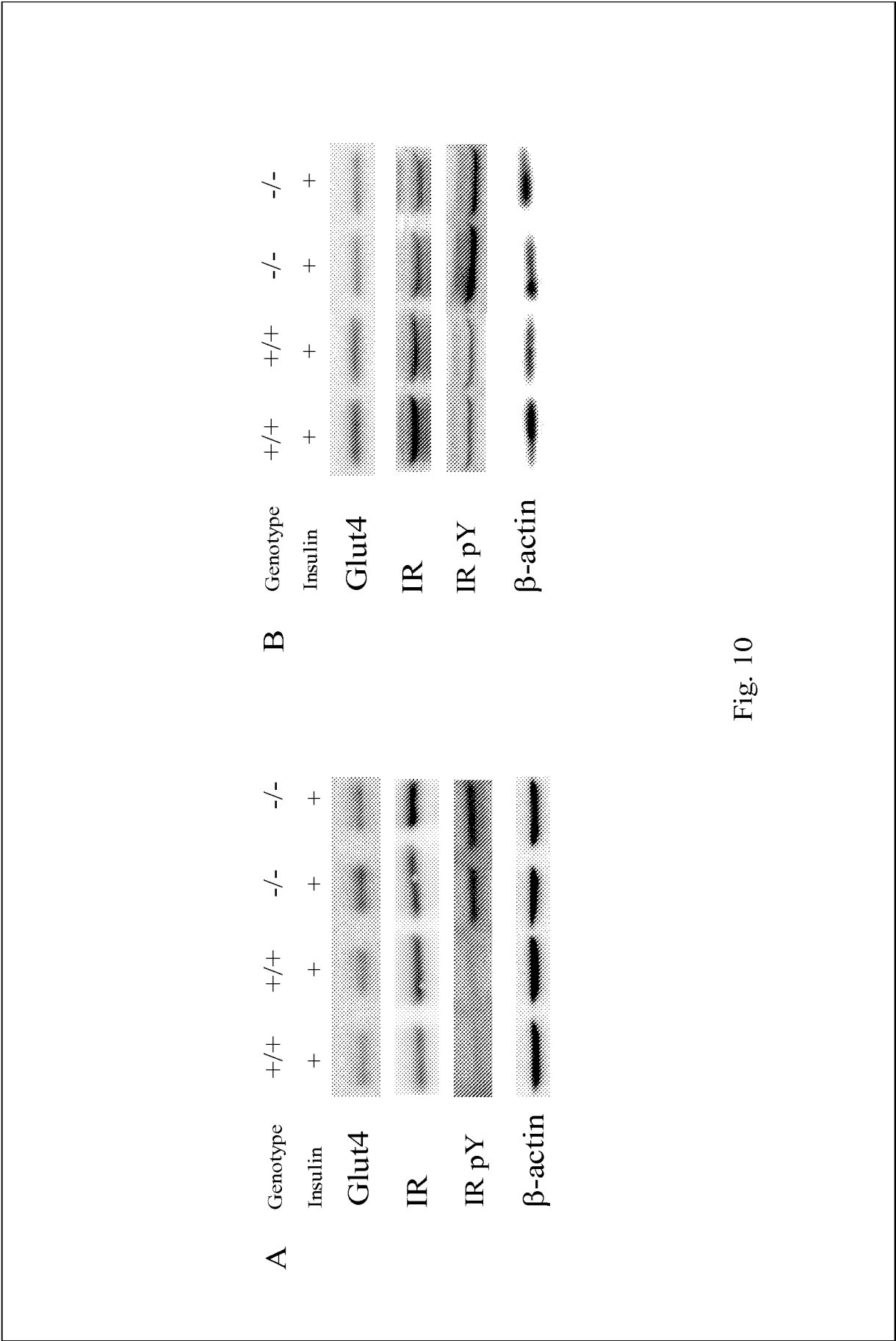


Fig. 10

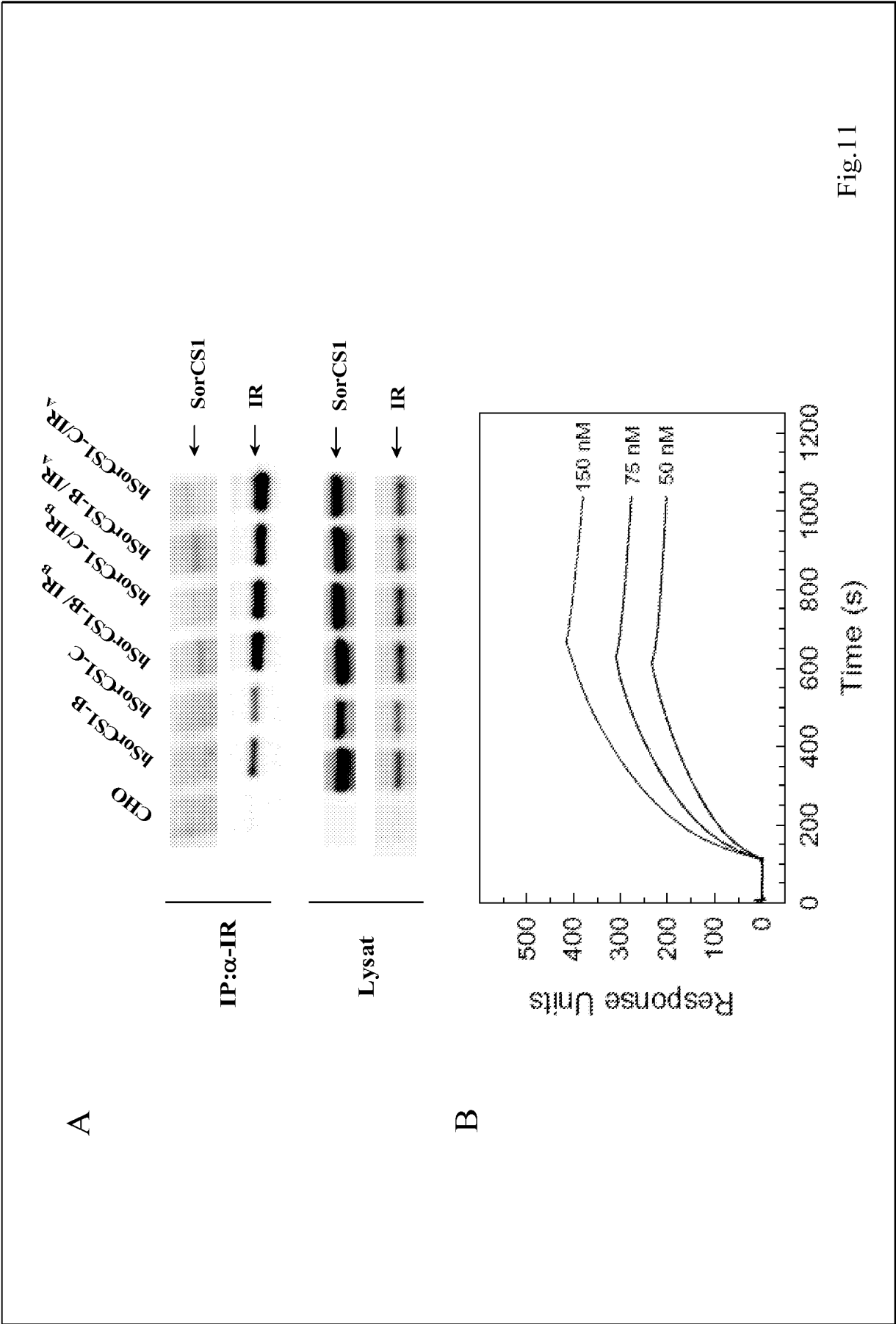


Fig.11

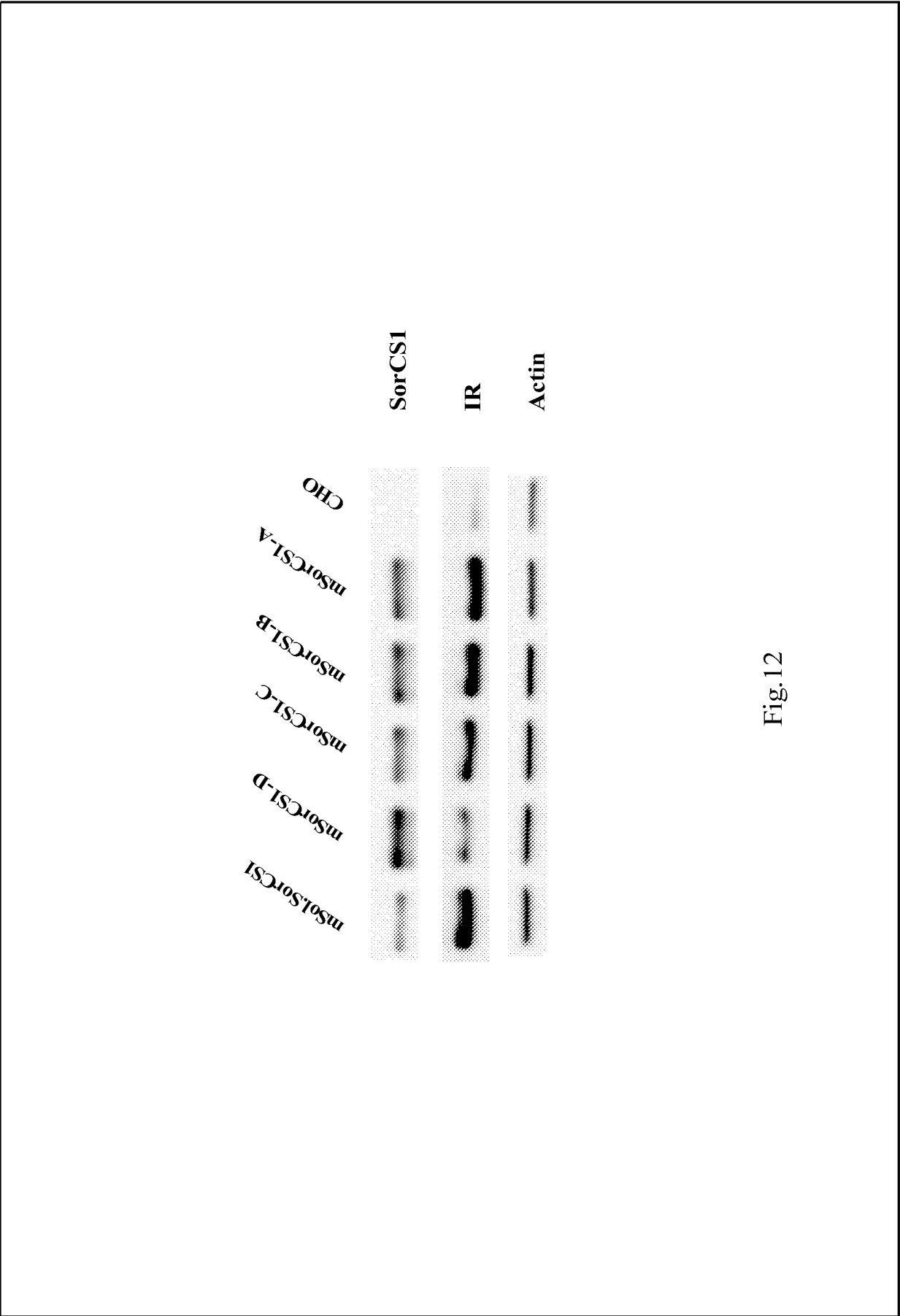


Fig.12

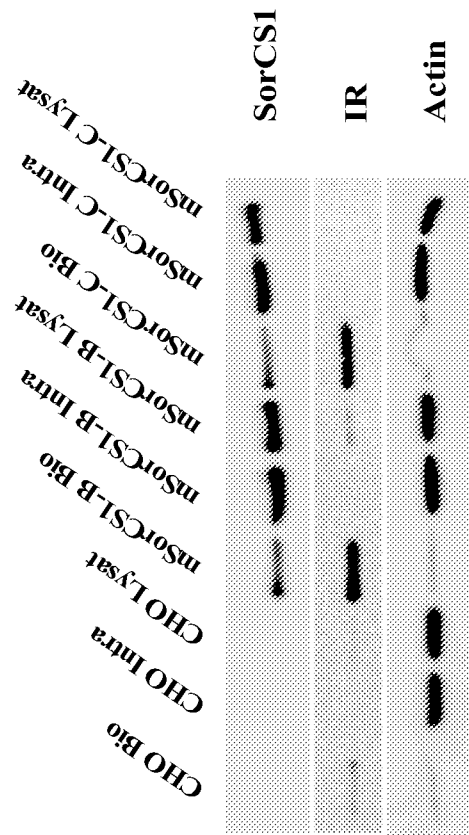
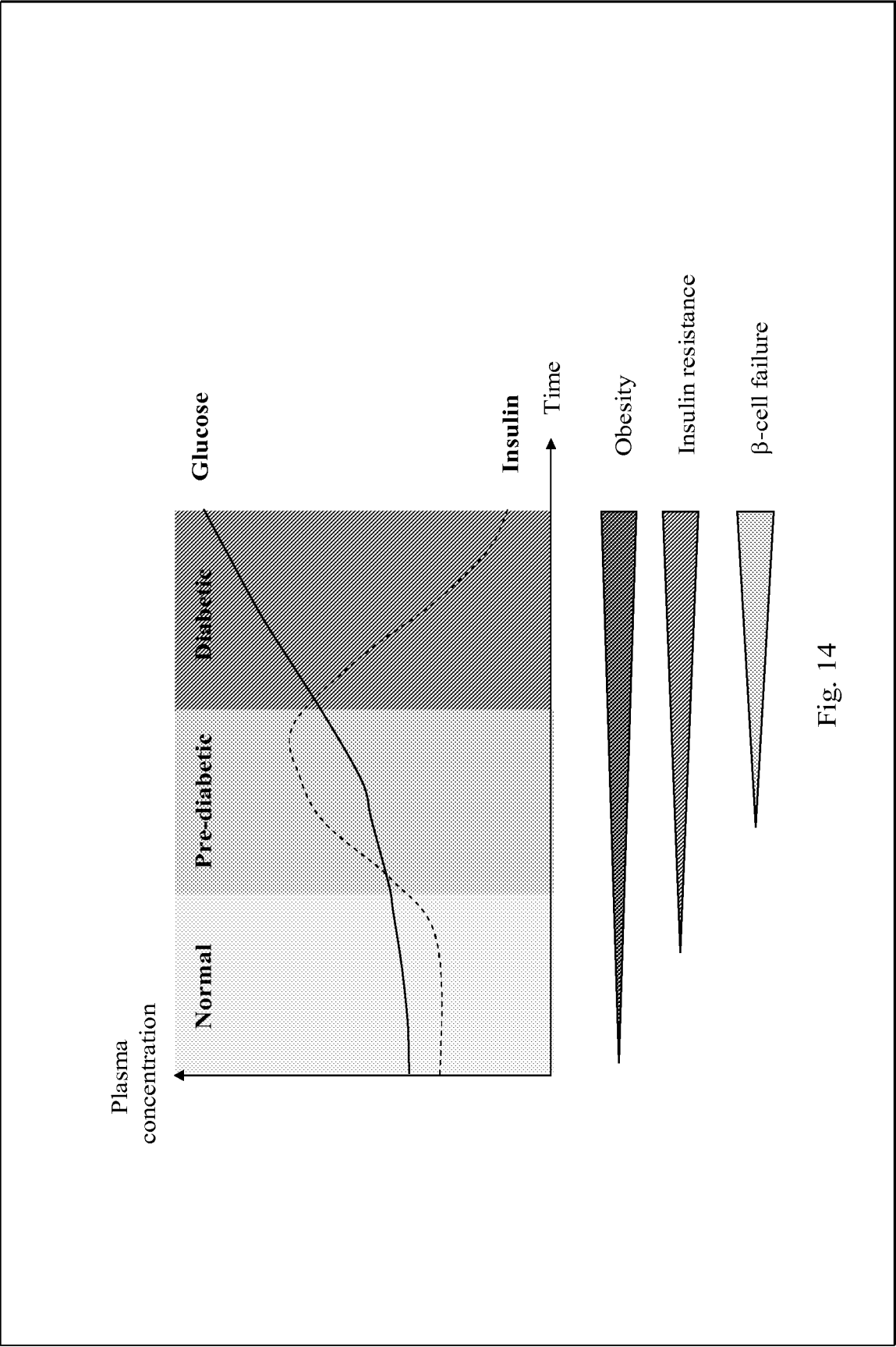


Fig. 13



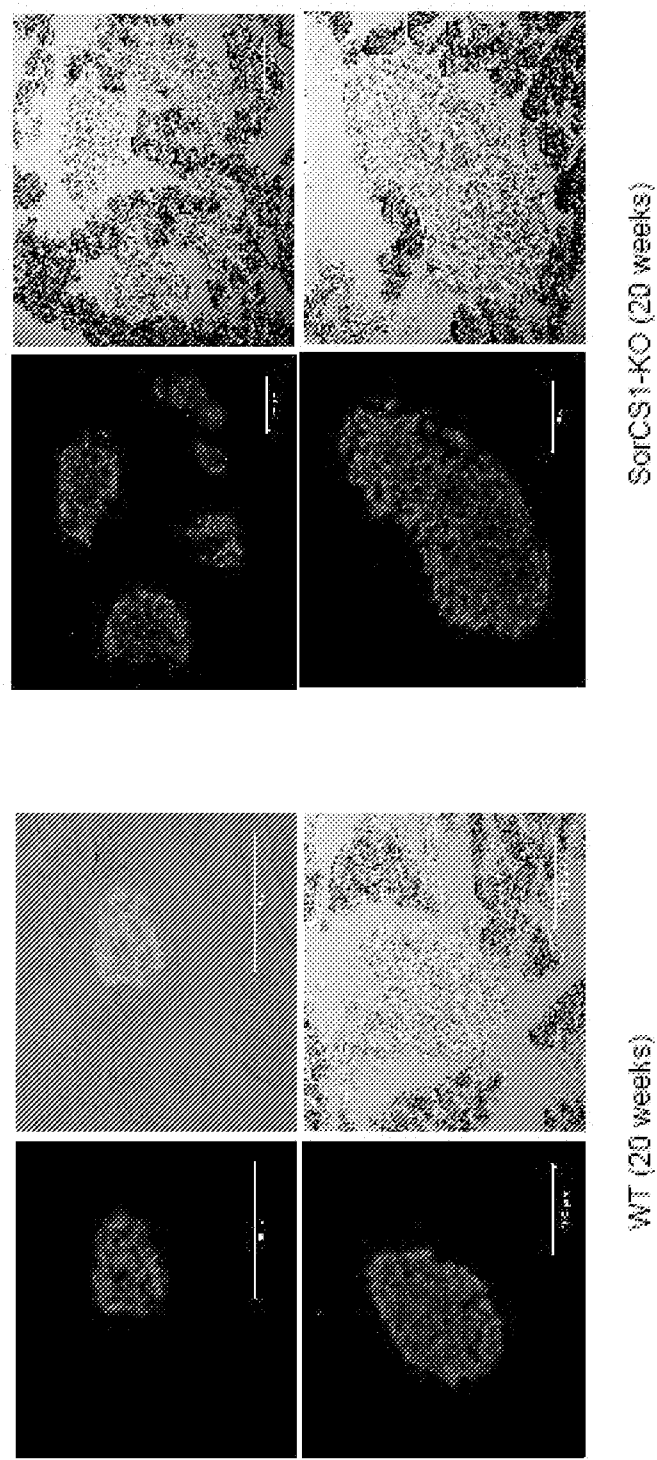


Fig.15

Alignment of the SorCS1 protein from different species

Sequence 1: gj161743975 ref NP_001013049.1	1198 aa	Human (homo sapiens)
Sequence 2: gj114632730 ref XP_508026.2	1361 aa	Chimpanzee (Pan troglodytes)
Sequence 3: gj119917739 ref XP_001253082.	1196 aa	Cow (Bos Taurus)
Sequence 4: gj12025518 ref NP_067352.1	1167 aa	Mouse (Mus musculus)
Sequence 5: gj109460253 ref XP_220080.4	1369 aa	Rat (Rattus norvegicus)
Sequence 6: gj173998824 ref XP_535010.2	997 aa	Dog (Canis lupus familiaris)
Sequence 7: gj118093020 ref XP_421750.2	1258 aa	Chicken (Gallus gallus)

Human (homo sapiens)		
Chimpanzee (Pan troglodytes)		-----MTGRFPPNRKLPVTHRPLREFGVDRPRGQGARTQRWDPFRPG 42
Cow (Bos taurus)		-----
Mouse (Mus musculus)		-----
Rat (Rattus norvegicus)		MRAQVFLQQSGLSEKHTDGIPTVPSINVIPERSKELLVPRGRVLLWLAG 50
Dog (Canis lupus familiaris)		-----
Chicken (Gallus gallus)		-----

Human (homo sapiens)		-----
Chimpanzee (Pan troglodytes)		RRVGGERRGEAAAPARPGSRRLQRPETRARARSASDFSSAPPPRG 92
Cow (Bos taurus)		-----
Mouse (Mus musculus)		-----
Rat (Rattus norvegicus)		TLRSLGRVGDSTARAPLPVHSAARRDAAPGALAEAPALPPASPRRRRW 100
Dog (Canis lupus familiaris)		-----
Chicken (Gallus gallus)		-----

Human (homo sapiens)		-----
Chimpanzee (Pan troglodytes)		GGSLRPDPRSAQAPGRAAATCPAPPPAPTAAQLGWSWGPERRPPTAAS 142
Cow (Bos taurus)		-----
Mouse (Mus musculus)		-----
Rat (Rattus norvegicus)		LAAPSQPARSSQAQPGRAAGTCAPPPRAPPPPNLDGVVVLSAHPAAVSP 150
Dog (Canis lupus familiaris)		-----
Chicken (Gallus gallus)		-----MEKV 4

Fig. 16

Human (homo sapiens)	-----MGKVGAGGGGQARLSALLAGAGLLILCAP 29
Chimpanzee (Pan troglodytes)	AELRAATFVLGPLSPILLFAPAMGKVGAGGGGQARLSALLAGAGLLIVLCAP 192
Cow (Bos taurus)	-----MGKVGAGGVSPAGLSALLAGAGLLIVLCAP 29
Mouse (Mus musculus)	-----MGKVGAGDGSSAGLSALLAGAGLLMLLAP 29
Rat (Rattus norvegicus)	VPRSAACILRFGTSPSLLISAPEMGKVGAGDGSSAALSALLTGAGLLMLLAP 200
Dog (Canis lupus familiaris)	-----
Chicken (Gallus gallus)	AGWYPAWHVALLYWTWLFLEFTFGTGAETCRSCHSQVNPQQQHPQOGLP 54
Human (homo sapiens)	GVCGGGCCPSPHPSSAPRSASTPRGFHQGRPGRAPATPLPLVVRPLFS 79
Chimpanzee (Pan troglodytes)	GVCGGGCCPPPHPSAPRSASTPRGFHQGRPGRAPATPLPLVVRPLFS 242
Cow (Bos taurus)	GSCGGSCCPRRHPSPAAPRWAPTPGGLPHQGRGRAPATPLPOLGRPLFA 79
Mouse (Mus musculus)	GVCSSLSCCPCPPQHPSSTPRRTLTTPRGFPHPGPLGRAPATPPPLFMRPLFA 79
Rat (Rattus norvegicus)	GICSSLSCCPCPPQHPSSTPRWTLTTPRGFSYPGPLGRAPATPPPLFMRPLFA 250
Dog (Canis lupus familiaris)	-----
Chicken (Gallus gallus)	MHLKTRGEKERWRIPEGEQGERGRGAGMDTEDAELQAPFDPALAGTKFS 104
Human (homo sapiens)	-VAPGDRALSILERARGTGASMAVAARSRR-RRSGADQEKAEERGEASRS 127
Chimpanzee (Pan troglodytes)	-VAPGDRALSILERARGTGASMAVAARSRR-RRSGADQEKAEERGEASRS 290
Cow (Bos taurus)	-VAPGDRALSILERAPGTGASVAVAPRSGRK-RRSGEDQEKAEERGEASRS 127
Mouse (Mus musculus)	-VAPGDRALFLERAGGSRVSVATAARSRR-RRSGTEPEKIEPGEASRS 127
Rat (Rattus norvegicus)	-VAPGDRALFLERAGGSRVSVATAARSRR-RRSGMDPEKTEPEGEASRS 298
Dog (Canis lupus familiaris)	-----
Chicken (Gallus gallus)	GWETRWSASEPEKAPGDSRPGAAAAPTGEYPQGGQAGGRRNSARRRRGRS 154
Human (homo sapiens)	PRGVLRDGGQQEPGTRERDPDKATFRFMEELRLTSTTFALTGDSAHNQAM 177
Chimpanzee (Pan troglodytes)	PRGVLRDGGQQEPGTHLERDPDKATFRFMEELRLTSTTFALTGDSAHNQAM 340
Cow (Bos taurus)	PRGVLRDRGQLEPGTHLERDPDKATFRFLEELKLTSTTFALTGDSAHNQAM 177
Mouse (Mus musculus)	RRDMLDGGQQGLGTGARDPGKATFRFMEELRLTSTTFALTGDSAHNQAM 177
Rat (Rattus norvegicus)	RRDMLDGGQQGGPGTGARDPGKATFRFMEELRLTSTTFALTGDSAHNQAM 348
Dog (Canis lupus familiaris)	-----MRKVS-----EIM 8
Chicken (Gallus gallus)	PRGTAGPGGAAG---REPGGGAARFGLLEELRLGSTTFALTGDSAHNQAM 201
	::: * : *

Fig. 16, continued

Human (homo sapiens)	VHSGHNSSVILILTKLYDYNLGSITSSSLWRSTDYGTTEYKLNKVGK 227
Chimpanzee (Pan troglodytes)	VHSGHNSSVILILTKLYDYNLGSITSSSLWRSTDYGTTEYKLNKVGK 390
Cow (Bos taurus)	VHSGHNSSVILILTKLYDYNLGSITSSSLWRSTDYGTTEYKLNKVGK 227
Mouse (Mus musculus)	VHSGHNSSVILILTKLYDYNLGSITSSSLWRSTDYGTTEYKLNKVGK 227
Rat (Rattus norvegicus)	VHSGHNSSVILILTKLYDYNLGSITSSSLWRSTDYGTTEYKLNKVGK 398
Dog (Canis lupus familiaris)	VLRSH--SVILILTKLYDYNLGSITSSSLWRSTDYGTTEYKLNKVGK 56
Chicken (Gallus gallus)	VHSGQNSSVILILTKLYDYNLGSITSSSLWRSTDYGTTEYKLNKVGK 251
	* * : *****:*****:*****:*****:*****:*****:***
Human (homo sapiens)	TILSYLVCPNTNKRKIMLLTDPEIESLLISSDEGATYQKYLNFYIQL 277
Chimpanzee (Pan troglodytes)	TILSYLVCPNTNKRKIMLLTDPEIESLLISSDEGATYQKYLNFYIQL 440
Cow (Bos taurus)	TILSYLVCPNTNKRKIMLLTDPEIESLLISSDEGATYQKYLNFYIQL 277
Mouse (Mus musculus)	TILSYLVCPNTNKRKIMLLTDPEIESLLISSDEGATYQKYLNFYIQL 277
Rat (Rattus norvegicus)	TILSYLVCPNTNKRKIMLLTDPEIESLLISSDEGATYQKYLNFYIQL 448
Dog (Canis lupus familiaris)	TILSYLVCPNTNKRKIMLLTDPEIESLLISSDEGATYQKYLNFYIQL 106
Chicken (Gallus gallus)	TILSYLVCPNTNKRKIMLLTDPEIESLLISSDEGATYQKYLNFYIQL 301
	*****:*****:*****:*****:*****:*****:***
Human (homo sapiens)	LFHPKQEDWILAYSQDQKLYSSAEFGRRWQLIQEGVVPNRFYWSVMGSNK 327
Chimpanzee (Pan troglodytes)	LFHPKQEDWILAYSQDQKLYSSAEFGRRWQLIQEGVVPNRFYWSVMGSNK 490
Cow (Bos taurus)	LFHPKQEDWILAYSQDQKLYSSAEFGRRWQLIQEGVVPNRFYWSVMGSNK 327
Mouse (Mus musculus)	LFHPKQEDWILAYSQDQKLYSSAEFGRRWQLIQEGVVPNRFYWSVMGSNK 327
Rat (Rattus norvegicus)	LFHPKQEDWILAYSQDQKLYSSAEFGRRWQLIQEGVVPNRFYWSVMGSNK 498
Dog (Canis lupus familiaris)	LFHPKQEDWILAYSQDQKLYSSAEFGRRWQLIQEGVVPNRFYWSVMGSNK 156
Chicken (Gallus gallus)	LFHPKQEDWILAYSQDQKLYSSAEFGRRWQLIQEGVVPNRFYWSVMGSNK 351
	*****:*****:*****:*****:*****:*****:***
Human (homo sapiens)	EPDLVHLEARTVDGSHSYLTCRMQNTNANRNPFGYIDPDSLIVQDHY 377
Chimpanzee (Pan troglodytes)	EPDLVHLEARTVDGSHSYLTCRMQNTNANRNPFGYIDPDSLIVQDHY 540
Cow (Bos taurus)	EPDLVHLEARTVDGSHSYLTCRMQNTNANRNPFGYIDPDSLIVQDHY 377
Mouse (Mus musculus)	EPDLVHLEARTVDGSHSYLTCRMQNTNANRNPFGYIDPDSLIVQDHY 377
Rat (Rattus norvegicus)	EPDLVHLEARTVDGSHSYLTCRMQNTNANRNPFGYIDPDSLIVQDHY 548
Dog (Canis lupus familiaris)	EPDLVHLEARTVDGSHSYLTCRMQNTNANRNPFGYIDPDSLIVQDHY 206
Chicken (Gallus gallus)	EPDLVHLEARTVDGSHSYLTCRMQNTNANRNPFGYIDPDSLIVQDHY 401
	*****:*****:*****:*****:*****:*****:***

Fig. 16, continued

Human (homo sapiens)	VFVQLTSGGRPHYYVSYRRNAFAQMKLPKYPALPKDMHVI	427
Chimpanzee (Pan troglodytes)	VFVQLTSGGRPHYYVSYRRNAFAQMKLPKYPALPKDMHVI	590
Cow (Bos taurus)	VFVQLTSGGRPHYYVSYRRNAFAQMKLPKYPALPKDMHVI	427
Mouse (Mus musculus)	VFVQLTSGGRPHYYVSYRRNAFAQMKLPKYPALPKDMHVI	427
Rat (Rattus norvegicus)	VFVQLTSGGRPHYYVSYRRNAFAQMKLPKYPALPKDMHVI	598
Dog (Canis lupus familiaris)	VFVQLTSGGRPHYYVSYRRNAFAQMKLPKYPALPKDMHVI	256
Chicken (Gallus gallus)	VFVQLTSGGRPHYYVSYRRNAFTPIKLPKYSPLPKDMHVI	451
	*****:****:*****:****:*****:*****:*****	
Human (homo sapiens)	QEWNQDNTNLYISDTRGVYFTLALENVQSSRGPEGNIMIDLYEVAGIKG	477
Chimpanzee (Pan troglodytes)	QEWNQDNTNLYISDTRGVYFTLALENVQSSRGPEGNIMIDLYEVAGIKG	640
Cow (Bos taurus)	QEWNQDNTNLYISDTRGVYFTLALENVQSSRGPEGNIMIDLYEVAGIKG	477
Mouse (Mus musculus)	QEWNQDNTNLYISDTRGVYFTLALENVQSSRGPEGNIMIDLYEVAGIKG	477
Rat (Rattus norvegicus)	QEWNQDNTNLYISDTRGVYFTLALENVQSSRGPEGNIMIDLYEVAGIKG	648
Dog (Canis lupus familiaris)	QEWNQDNTNLYISDTRGVYFTLALENVQSSRGPEGNIMIDLYEVAGIKG	306
Chicken (Gallus gallus)	QEWNQDNTNLYISDTRGVYFTLALENVQSSRGPEGNIMIDLYEVAGIKG	501
	*****:****:*****:****:*****:*****:*****	
Human (homo sapiens)	MFLANKKIDNQVKTFTYTKGRDWRLLQAPDTRLRGDPVHCLLPYCSLHL	527
Chimpanzee (Pan troglodytes)	MFLANKKIDNQVKTFTYTKGRDWRLLQAPDTRLRGDPVHCLLPYCSLHL	690
Cow (Bos taurus)	MFLANKKIDNQVKTFTYTKGRDWRLLQAPDTRLRGDPVHCLLPYCSLHL	527
Mouse (Mus musculus)	MFLANKKIDNQVKTFTYTKGRDWRLLQAPDTRLRGDPVHCLLPYCSLHL	527
Rat (Rattus norvegicus)	MFLANKKIDNQVKTFTYTKGRDWRLLQAPDTRLRGDPVHCLLPYCSLHL	698
Dog (Canis lupus familiaris)	MFLANKKIDNQVKTFTYTKGRDWRLLQAPDTRLRGDPVHCLLPYCSLHL	356
Chicken (Gallus gallus)	MFLANKKIDNQVKTFTYTKGRDWRLLQAPDTRLRGDPVHCLLPYCSLHL	551
	*****:****:*****:****:*****:*****:*****	
Human (homo sapiens)	HLKVSSENPYTSIIASKDTAPSIIVASGNIGSELSPTDISMFVSSDAGNT	577
Chimpanzee (Pan troglodytes)	HLKVSSENPYTSIIASKDTAPSIIVASGNIGSELSPTDISMFVSSDAGNT	740
Cow (Bos taurus)	HLKVSSENPYTSIIASKDTAPSIIVASGNIGSELSPTDISMFVSSDAGNT	577
Mouse (Mus musculus)	HLKVSSENPYTSIIASKDTAPSIIVASGNIGSELSPTDISMFVSSDAGNT	577
Rat (Rattus norvegicus)	HLKVSSENPYTSIIASKDTAPSIIVASGNIGSELSPTDISMFVSSDAGNT	748
Dog (Canis lupus familiaris)	HLKVSSENPYTSIIASKDTAPSIIVASGNIGSELSPTDISMFVSSDAGNT	406
Chicken (Gallus gallus)	HLKVSSENPYTSIIASKDTAPSIIVASGNIGSELSPTDISMFVSSDAGNT	601
	*****:****:*****:****:*****:*****:*****	

Fig. 16, continued

Human (homo sapiens)	WRQIFEEHSLVLYDQGGLVAMKHTSLPIRHLWLSFDEGRSWSKYSF	627
Chimpanzee (Pan troglodytes)	WRQIFEEHSLVLYDQGGLVAMKHTSLPIRHLWLSFDEGRSWSKYSF	790
Cow (Bos taurus)	WRQIFEEHSLVLYDQGGLVAMKHTSLPIRHLWLSFDEGRSWSKYSF	627
Mouse (Mus musculus)	WRQIFEEHSILYDQGGLVAMKHTSLPIRHLWLSFDEGRSWSKYSF	798
Rat (Rattus norvegicus)	WRQIFEEHSLVLYDQGGLVAMKHTSLPIRHLWLSFDEGRSWSKYSF	627
Dog (Canis lupus familiaris)	WRQIFEEHSLVLYDQGGLVAMKHTSLPIRHLWLSFDEGRSWSKYSF	456
Chicken (Gallus gallus)	WRQIFEEHSLVLYDQGGLVAIKHTSLPIRHLWLSFDEGRSWSKYSF	651
	*****:*****:*****:*****:*****:*****:	
Human (homo sapiens)	IPLFYDGVLGPEGETLIMTVFGHFHRSEWLKVVDYKSIDRRCAEED	677
Chimpanzee (Pan troglodytes)	IPLFYDGVLGPEGETLIMTVFGHFHRSEWLKVVDYKSIDRRCAEED	840
Cow (Bos taurus)	IPLFYDGVLGPEGETLIMTLIDHS -- HSDWLVTALQPCCLFLGNWEWD	675
Mouse (Mus musculus)	IPLFYDGVLGPEGETLIMTVFGHFHRSEWLKVVDYKSIDRRCAEED	677
Rat (Rattus norvegicus)	IPLFYDGVLGPEGETLIMTVFGHFHRSEWLKVVDYKSIDRRCAEED	848
Dog (Canis lupus familiaris)	IPLFYDGVLGPEGETLIMTVFGHFHRSEWLKVVDYKSIDRRCAEED	506
Chicken (Gallus gallus)	IPLFYDGILGPEGETLIMTVFGHFHRSEWLKVVDYKSIDRRCAEED	701
	:*****:*****:..*:~::~:~::**	
Human (homo sapiens)	YRPWQLHSQGEACINGAKRIYKKRKSERKCMQGYAGAMESEPVCCTEAD	727
Chimpanzee (Pan troglodytes)	YRPWQLHSQGEACINGAKRIYKKRKSERKCMQGYAGAMESEPVCCTEAD	890
Cow (Bos taurus)	KIAMPQDQGEACINGAKRIYKKRKSERKCMQGYAGAMESEPVCCTEAD	725
Mouse (Mus musculus)	YRPWQLHSQGEACINGAKRIYKKRKSERKCMQ - KYAGAMESEPVCCTEAD	726
Rat (Rattus norvegicus)	YRPWQLHSQGEACINGAKRIYKKRKSERKCMQGYAGAMESEPVCCTEAD	898
Dog (Canis lupus familiaris)	YRPWQLHSQGEACINGAKRIYKKRKSERKCMQGYAGAMESEPVCCTEAD	556
Chicken (Gallus gallus)	YWTWQLHSQGEACINGAKRIYKKRKSEKKCMQGYAGAMTSEPVCCTDAD	751
	*.:~::~:~::*****:*****:**** *****:***	
Human (homo sapiens)	FDCDYGERHSNGQCLPAFWFNPSLSKDCLSGQSYLNSTGYRKVVSNNC	777
Chimpanzee (Pan troglodytes)	FDCDYGERHSNGQCLPAFWFNPSLSKDCLSGQSYLNSTGYRKVVSNNC	940
Cow (Bos taurus)	FDCDYGERHSNGQCLPAFWFNPSLSKDCLSGQSYLNSTGYRKVVSNNC	775
Mouse (Mus musculus)	FDCDYGERHSNGQCLPAFWFNPSLSKDCLSGQSYLNSTGYRKVVSNNC	776
Rat (Rattus norvegicus)	FDCDYGERHSNGQCLPAFWFNPSLSKDCLSGQSYLNSTGYRKVVSNNC	948
Dog (Canis lupus familiaris)	FDCDYGERHSNGQCLPAFWFNPSLSKDCLSGQSYLNSTGYRKVVSNNC	606
Chicken (Gallus gallus)	FDCDYGERHSNGQCLPAFWFNPSLSKDCLSGQSYLNSTGYRKVVSNNC	801
	*****:*****:*****:*****:*****:*****:	

Fig. 16, continued

[illegible]

Fig. 16, continued

Human (homo sapiens)	1021
Chimpanzee (Pan troglodytes)	1190
Cow (Bos taurus)	1025
Mouse (Mus musculus)	1026
Rat (Rattus norvegicus)	1198
Dog (Canis lupus familiaris)	856
Chicken (Gallus gallus)	1051
Human (homo sapiens)	1077
Chimpanzee (Pan troglodytes)	1240
Cow (Bos taurus)	1076
Mouse (Mus musculus)	1075
Rat (Rattus norvegicus)	1248
Dog (Canis lupus familiaris)	906
Chicken (Gallus gallus)	1100
Human (homo sapiens)	1127
Chimpanzee (Pan troglodytes)	1290
Cow (Bos taurus)	1125
Mouse (Mus musculus)	1126
Rat (Rattus norvegicus)	1298
Dog (Canis lupus familiaris)	956
Chicken (Gallus gallus)	1150
Human (homo sapiens)	1177
Chimpanzee (Pan troglodytes)	1340
Cow (Bos taurus)	1175
Mouse (Mus musculus)	1167
Rat (Rattus norvegicus)	1348
Dog (Canis lupus familiaris)	997
Chicken (Gallus gallus)	1200

Fig. 16, continued

Human (homo sapiens)	GSISIVAENQSTKEIPTVNV-----	1198
Chimpanzee (Pan troglodytes)	GSISIVAENQSTKEIPTVNV-----	1361
Cow (Bos taurus)	GSISIVAENQSTKEIPTVNV-----	1196
Mouse (Mus musculus)	-----	
Rat (Rattus norvegicus)	GSISIVAENQSTKEIPTVNV-----	1369
Dog (Canis lupus familiaris)	-----	
Chicken (Gallus gallus)	EQPQATVQNPRKGNAAKVVWTFDFQKACGSPRGFKPRPGAEEVFPQLLSPR	1250
Human (homo sapiens)	-----	
Chimpanzee (Pan troglodytes)	-----	
Cow (Bos taurus)	-----	
Mouse (Mus musculus)	-----	
Rat (Rattus norvegicus)	-----	
Dog (Canis lupus familiaris)	-----	
Chicken (Gallus gallus)	AQKKSLEPM	1258

Fig. 16, continued

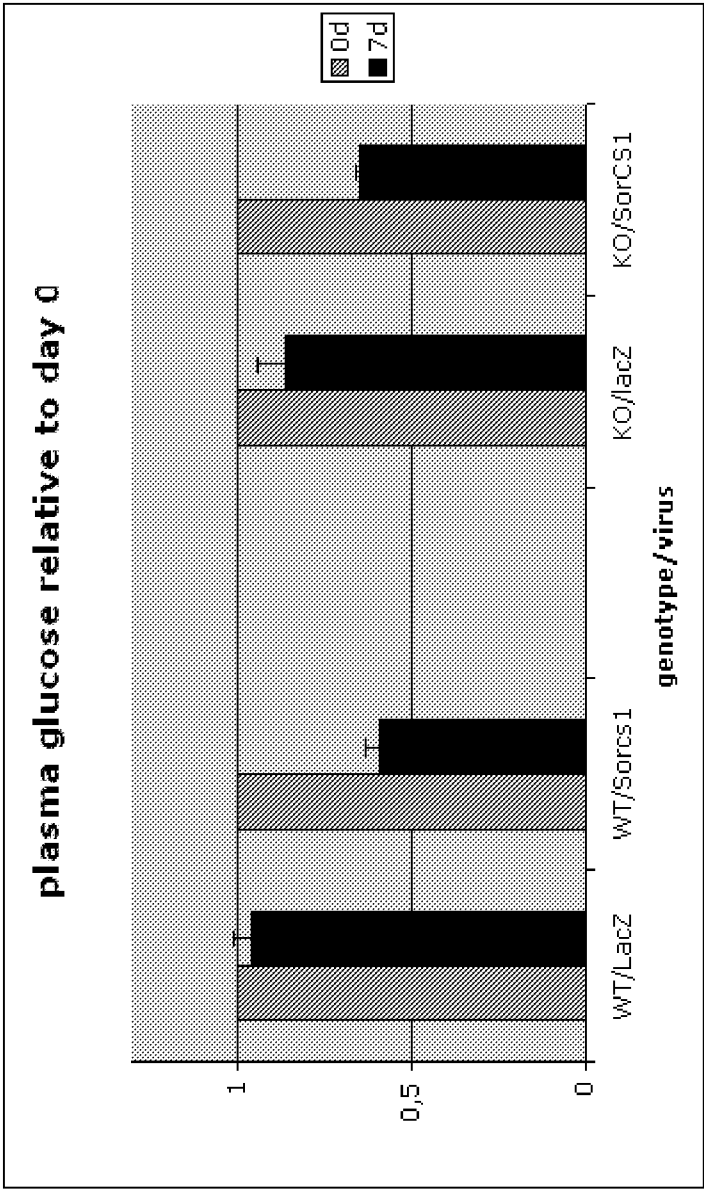


Fig. 17

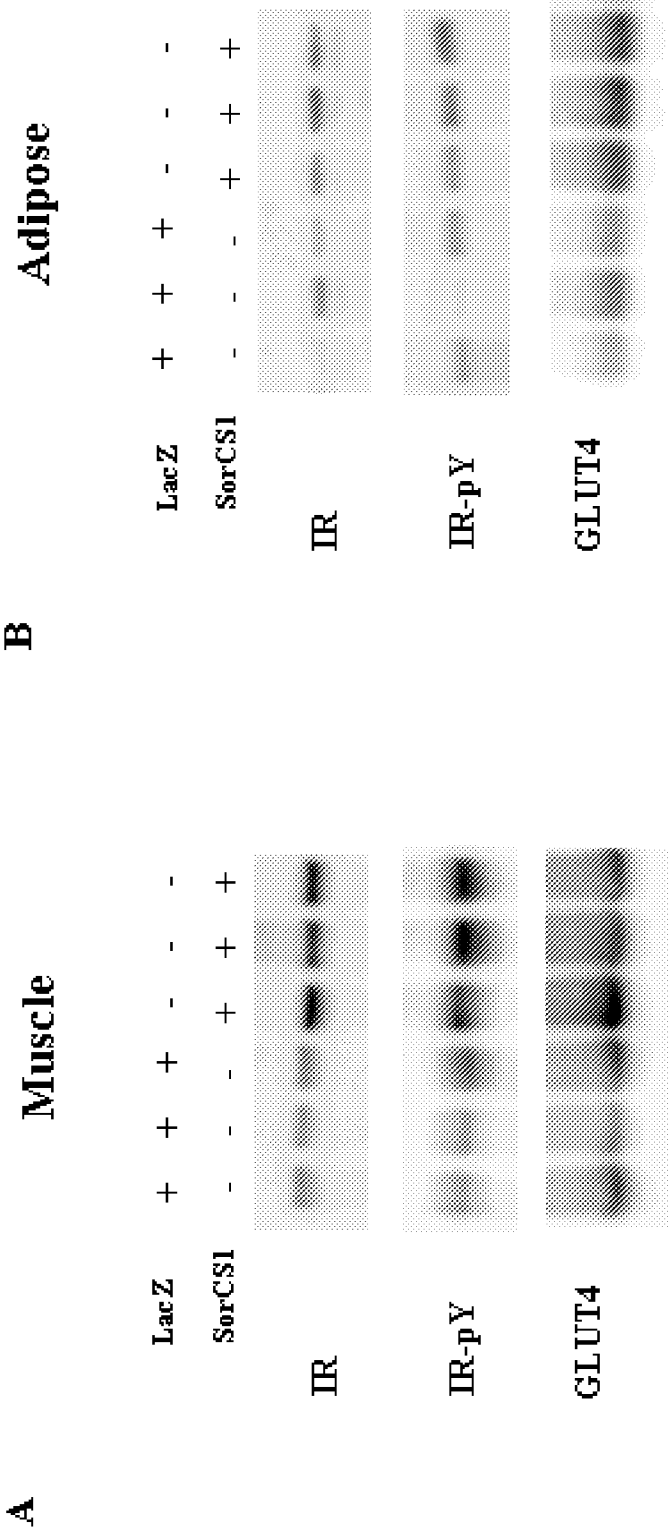
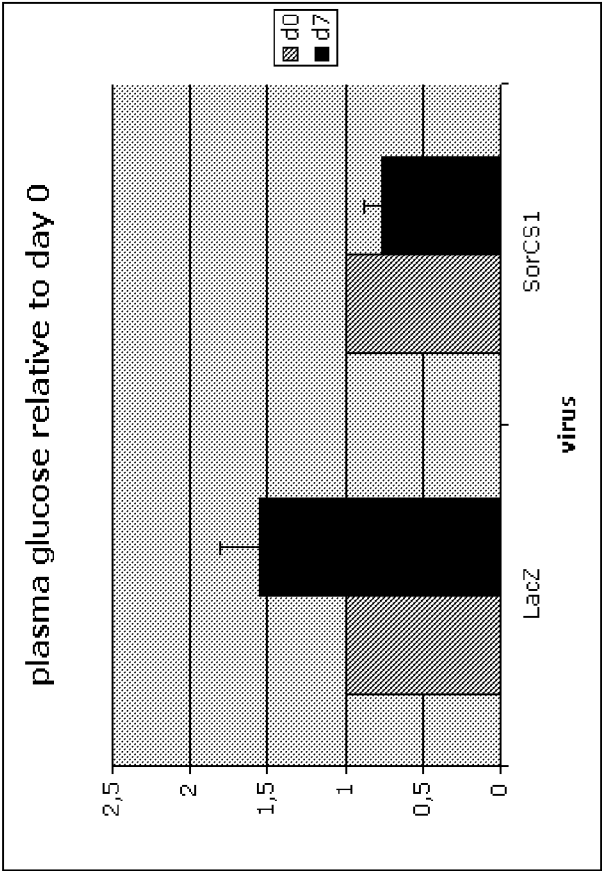


Fig. 18

A



B

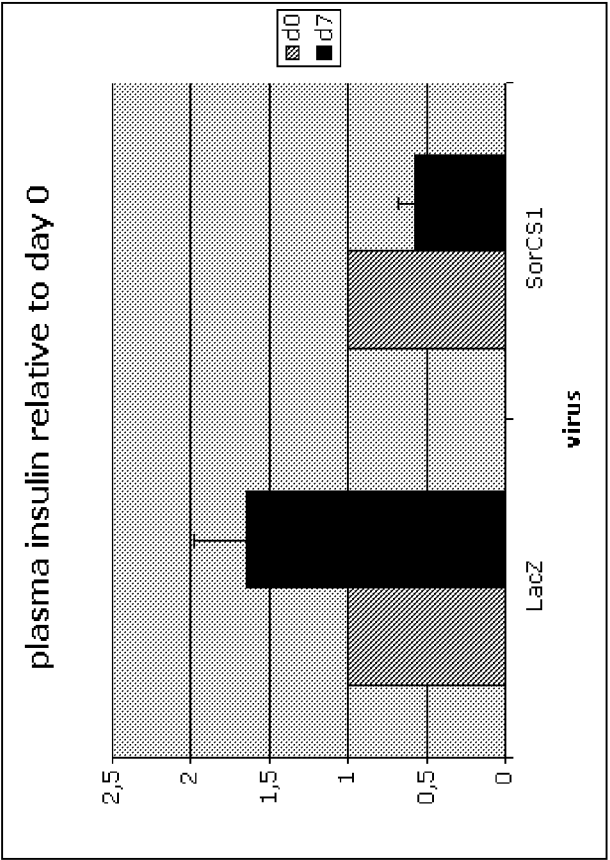


Fig. 19

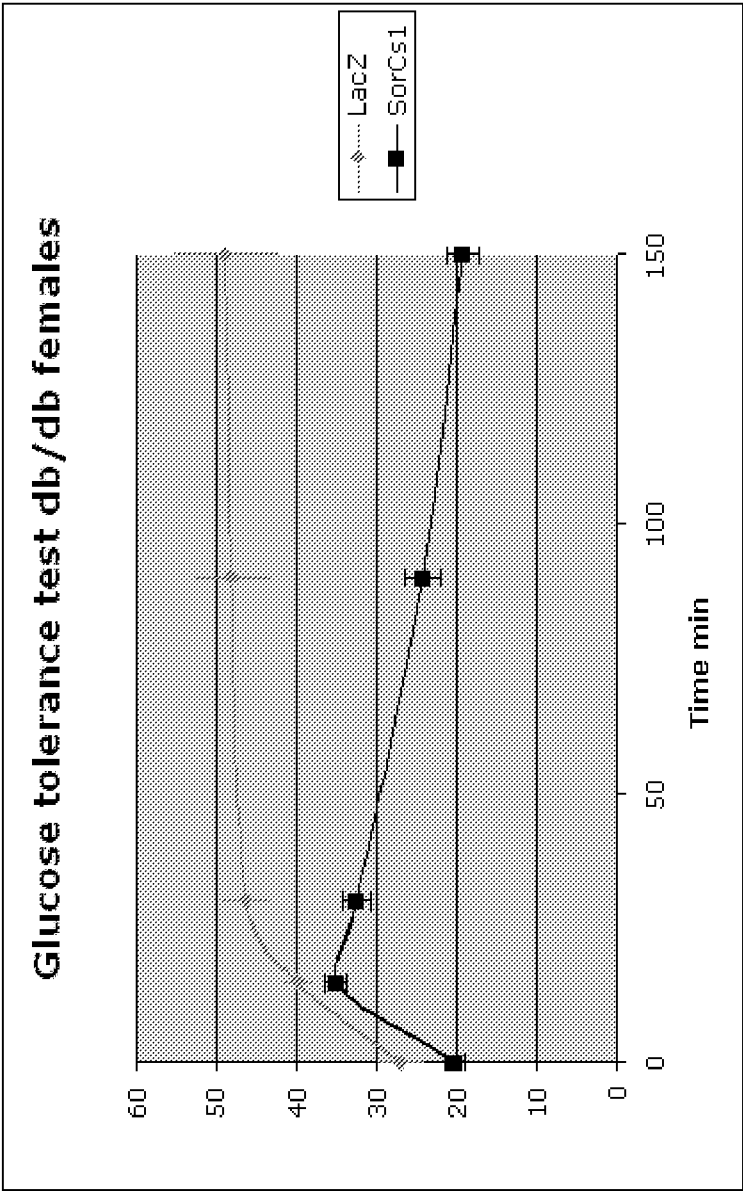
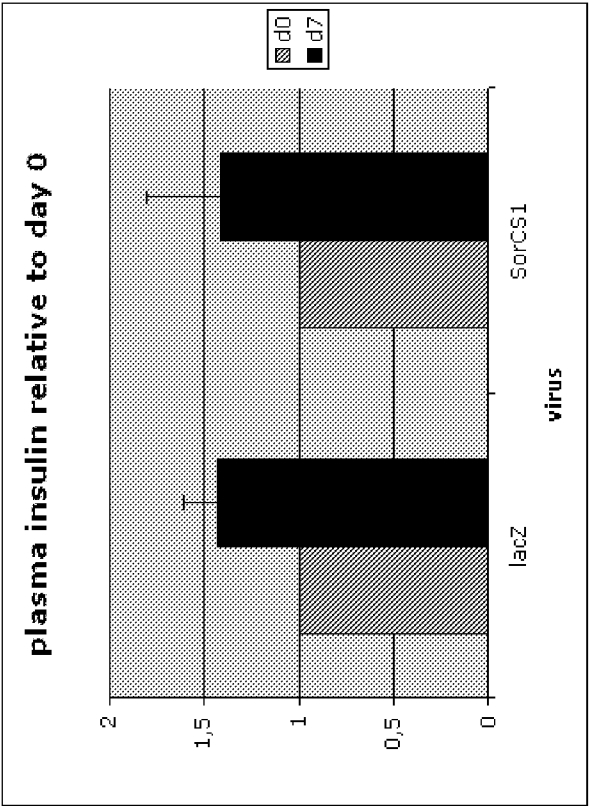


Fig. 20

B



A

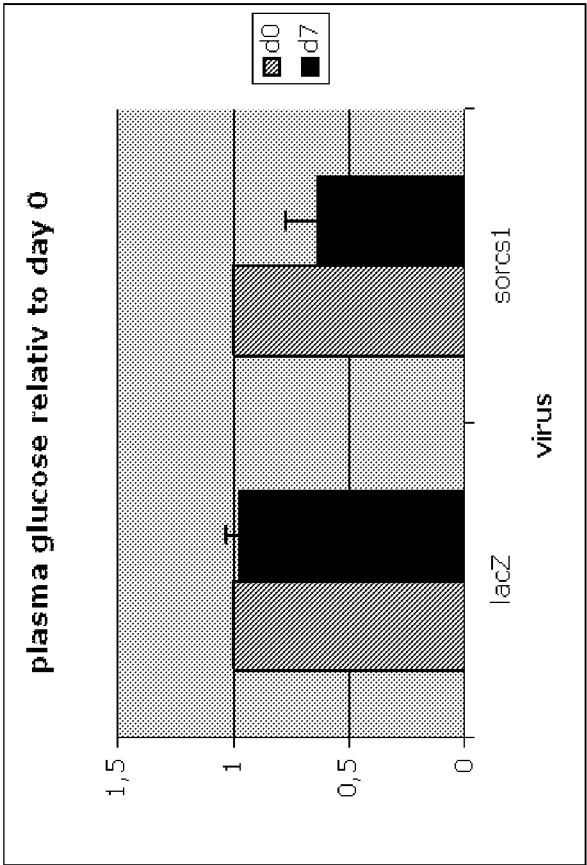


Fig. 21

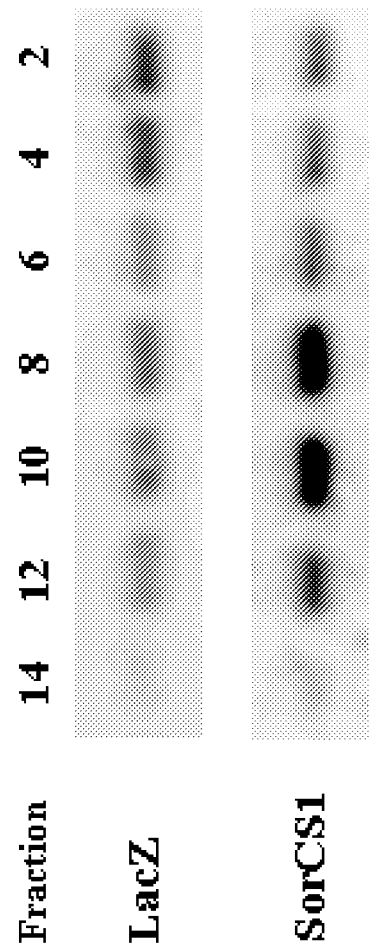


Fig. 22

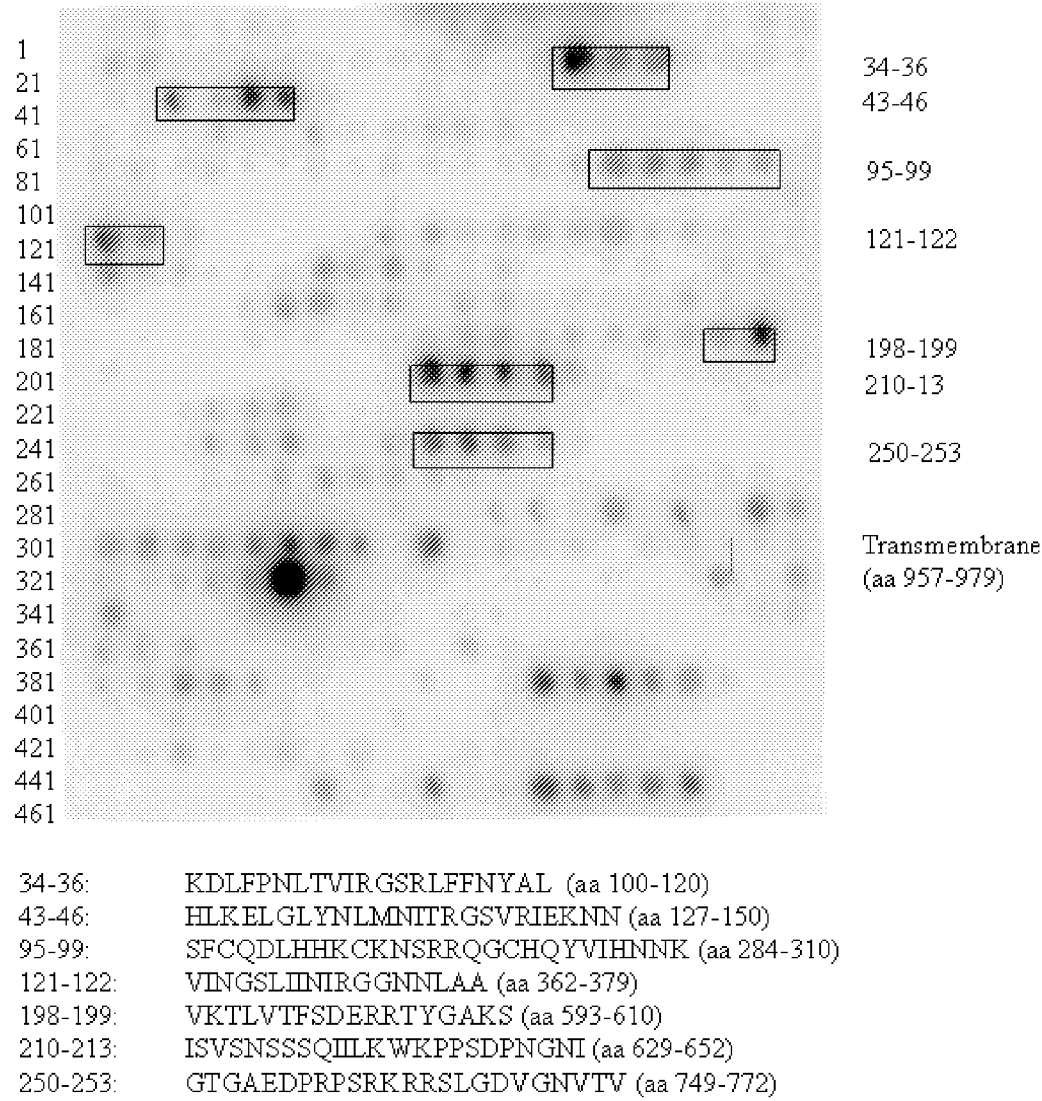


Fig. 23A

Human IR-his on hSorCS1-a

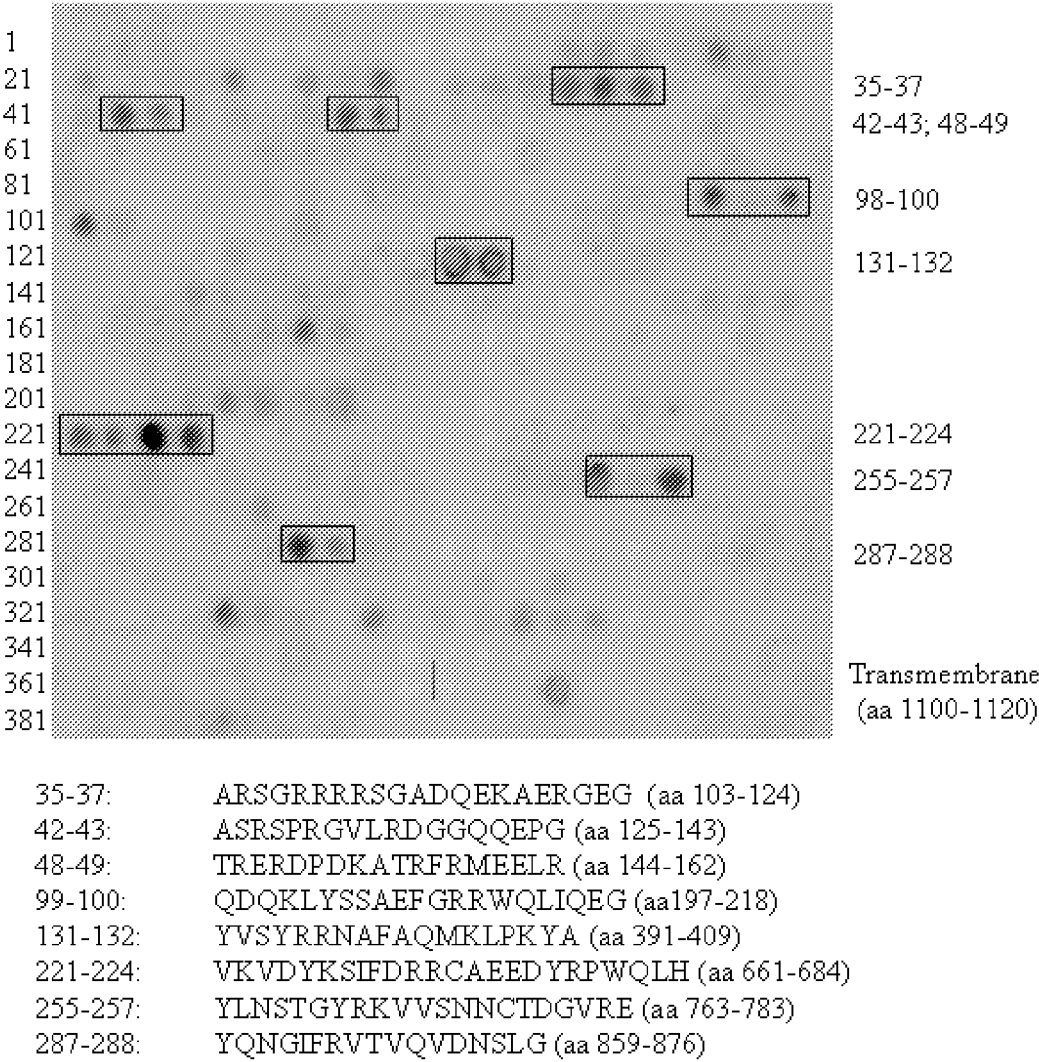


Fig. 23B

Insulin signalling

(adipose tissue SorCS1 KO >< wildtype)

Gene	Fold regulation	p-value
Aebp1 (AEB binding protein 1)	-5.93	0.406
Dok1(docking protein 1)	7.24	0.188
Igf1r (insulin-like growth factor 1 receptor)	3.30	0.00008
Irs2 (Insulin receptor substrate 2)	-2.95	0.100
Pik3cb (phosphoinositide-3-kinase, catalytic, beta polypeptide)	-5.65	0.196
Ucp1 (uncoupling protein 1)	-25.35	0.085

Gene	Function
Aebp1	A transcriptional repressor with carboxypeptidase activity. Binds promoter of adipose P2 gene.
Dok1	A member of the IRS family. Function as a scaffolding protein that recruits signalling molecules (ras-GTP, Nck)
Igf1r	Binds IGF1 with high affinity. Has tyrosine kinase activity. Plays a critical role in transformation events.
Irs2	A cytoplasmic signalling molecule that mediates effects of insulin, insulin-like growth factor 1, and other cytokines by acting as a molecular adaptor between diverse receptor tyrosine kinases and downstream effectors.
Pik3cb	PI3k phosphorylate the inositol ring in inositol lipids. Is a participant in signalling pathways regulating cell growth
Ucp1	Plays a role in heat production by uncoupling oxidative phosphorylation from the respiratory chain

Fig. 24A

**Lipoprotein signalling and Cholesterol metabolism
(Adipose tissue SorCS1 KO >> wildtype)**

Gene	Fold reg.	p-value
Akr1d1 (aldo-keto reductase family 1, member D1)	8.06	0.042
Colec12 (collectin sub-family member 12)	3.12	0.046
Dhcr7 (7-dehydrocholesterol reductase)	3.82	0.040
Hmgcs2 (3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2)	6.01	0.013
Lcat (Lecithin cholesterol acyltransferase)	3.37	0.008
Ldlr (Low density protein receptor)	3.44	0.014
Mvd (mevalonate (diphospho) decarboxylase)	3.48	0.039
Nr1h4 (Nuclear receptor subfamily 1, group H, member 4)	3.49	0.041
Osbpl5 (Oxysterol binding protein-like 5)	3.61	0.048
Prkag2 (Protein kinase, AMP-activated, gamma 2 non-catalytic)	5.27	0.007
Soat1 (Sterol O-acyltransferase 1)	11.75	0.020
Vldlr (very low density lipoprotein receptor)	4.53	0.018

Gene	Function
Akr1d1	Catalysis of the 5-beta-reduction of bile acid intermediates and steroid hormones.
Colec12	A scavenger receptor binding to carbohydrate antigens on microorganisms and oxidized phospholipids of oxidatively damaged or apoptotic cells.
Dhcr7	A penultimate enzyme of sterol biosynthesis that converts 7-dehydro cholesterol to cholesterol
Hmgcs2	Mediates the first reaction in ketogenesis, a metabolic pathway that provides lipid-derived energy during carbohydrate deprivation
Lcat	An extracellular cholesterol esterifying enzyme. The esterification of cholesterol is required for cholesterol transport.
Ldlr	A cell surface protein involved in receptor mediated endocytosis of specific ligands eg LDL
Mvd	Catalyzes the conversion of mevalonate pyrophosphate into isopentenyl pyrophosphate - one of the early steps in cholesterol biosynthesis
Nr1h4	Regulates the expression of various transport proteins and biosynthetic enzymes crucial to the physiological maintenance of cholesterol and bile acid homeostasis.
Osbpl5	An intracellular lipid receptor - a member of the oxysterol-binding protein family.
Prkag2	Subunit of AMPK an important energy-sensing enzyme that monitors cellular energy status and functions by inactivating key enzymes involved in regulating de novo biosynthesis of fatty acid and cholesterol
Soat1	Forms cholesterol esters from cholesterol. Accumulation of cholesterol esters as cytoplasmic lipid droplets is a characteristic feature of early stages of atherosclerotic plaques
Vldlr	Mediates cholesterol uptake possible in collaboration with Ldlr

Fig. 24B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2010/050131

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC:A01K, A61K, A61P, C12N

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

SE, DK, FI, NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, PAJ, WPI data, BIOSIS, EMBASE, MEDLINE, EBI sequence search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Pedersen et al. "From gene to function: conditional knockout of four splice variants of the murine SorCS1 gene" DANISH MEDICALBULLETIN 2007, vol54, no 2; whole document; abstract	64, 65
A	-- Goodarzi et al. "SORCS1: A novel human type 2 diabetes susceptibility gene suggested by the mouse", Diabetes, 2007, vol 56, no 7, pages 1922-1929, ISSN 0012-1797; whole document; abstract	1-71
A	-- Granhall et al. "High-resolution quantitative trait locus analysis reveals multiple diabetes susceptibility loci mapped to intervals < 800 kb in the species-conserved Niddm1i of the GK rat", Genetics, 2006, vol 174, no 3, pages 1565-1572, ISSN 0016- 6731; whole document; abstract	1-71



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

08-09-2010

Date of mailing of the international search report

09-09-2010

Name and mailing address of the ISA/SE

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Magnus Hallberg

Telephone No. + 46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2010/050131

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Clee, et al. "Positional cloning of Sorcs1, a type 2 diabetes quantitative trait locus", Nature Genetics, 2006, vol 38, no 6, pages 688-693, ISSN 1061-4036; whole document; abstract	1-71
X	-- WO 03027228 A2 (INCYTE GENOMICS INC ET AL), 3 April 2003 (2003-04-03); abstract; page 57, line 12 - page 57, line 15; claims 8, 17, 18; -& DATABASE GSP [online] Accession no. ADC42845 retrieved from EBI. SEQ ID NO 5 is 99.9% identical in 990 aa overlap with SEQ ID NO:15.	48-58, 62-63
A	--	1-47, 59-61, 64-71
X	WO 2004022719 A2 (WISCONSIN ALUMNI RES FOUND), 18 March 2004 (2004-03-18); paragraphs [0013], [0024], [0029]	68-69
A	--	1-67, 70-71
E	WO 2010069331 A2 (LUNDBECK & CO AS H ET AL), 24 June 2010 (2010-06-24); claims 1, 35, 71	48-58, 62-65
A	-- WO 2006042002 A2 (UNIV OREGON HEALTH & SCIENCE ET AL), 20 April 2006 (2006-04-20); claims 1-4, 11, 15, 17 -----	1-71

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2010/050131

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 46, 47, 59, 70
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 46, 47, 59 and 70 relate to a method for treatment of the human or animal body by therapy, see PCT rule 39.1(iv). Nevertheless, a search has been made for these claims. The search has been directed to the technical content of the claims
2. ☒ Claims Nos.: 1-5, 30-58, 60-63, 67, 70-71
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 1-5, 30-58, 60-63, and 67 relates to a SorCS1-like agent. The agent i only

.../...
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of: Box No. II

defined by the desirable features that it should be capable of binding to the insulin receptor (IR) at a SorCS1 binding site and that it should be capable of sensitization of an insulin receptor. According to the claims and the definition in the description, the agent could be represented by a peptide, polypeptide, small organic molecule, siRNA, siDNA, antibody and nucleic acids encoding the polypeptide.

The claims cover all products having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In fact, the only "SorCS1-like agent" that have been shown to have an effect for treating insulin resistance, is a full length SorCS1 protein expressed in the animal to be treated. Therefore, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to the desirable features (i.e. that it should be capable of binding to the insulin receptor (IR) at some particular sites and that it should be capable of sensitization the insulin receptor). Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the different SorCS1 polypeptides and variants thereof described in the descriptions, claims and sequence list of the application.

Claims 70-71 relate to use of an agent capable of enhancing binding activity between SorCS1 or a fragment or variant thereof, and an insulin receptor for the treatment of insulin resistance. Again, the agent is only defined by a desirable characteristic and the description does not give any examples of agents which have been shown to possess this characteristic. Therefore, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible, (Article 5 and 6 PCT). Thus the ISA have only been able to search the general aspect of the claims.

Thence it follows that a reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability only can be established for those parts which have been searched.

Continuation of: second sheet

International Patent Classification (IPC)

A61K 38/17 (2006.01)

A01K 67/027 (2006.01)

C12N 15/85 (2006.01)

A61P 3/00 (2006.01)

A61P 3/04 (2006.01)

A61P 3/10 (2006.01)

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Use the application number as username. The password is **WRWUWDSQQX**.

Paper copies can be ordered at a cost of 50 SEK per copy from PRV InterPat (telephone number 08-782 28 85).

Cited literature, if any, will be enclosed in paper form.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/DK2010/050131

WO	03027228 A2	03/04/2003	AU	2002362586 A1	07/04/2003
			CA	2453985 A1	03/04/2003
			EP	1537138 A2	08/06/2005
			JP	2005508631 T	07/04/2005
WO	2004022719 A2	18/03/2004	AU	2003268519 A1	29/03/2004
			CA	2498476 A1	18/03/2004
			EP	1554397 A4	21/06/2006
WO	2010069331 A2	24/06/2010	NONE		
WO	2006042002 A2	20/04/2006	AU	2005294347 A1	20/04/2006
			CA	2583230 A1	20/04/2006
			EP	1807106 A2	18/07/2007
			US	20080206231 A1	28/08/2008