SorCS1 binds the insulin receptor to enhance insulin sensitivity.

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1 **Type II diabetes mellitus (T2DM) has reached endemic levels and comprises a substantial burden**
2 **to the patient and the society. Along with lifestyle factors, a number of genetic loci predisposing to**
3 **T2DM have been suggested including *SORCS1* that encodes a type-1 transmembrane receptor.**
4 **Here we establish SorCS1 as a high-affinity binding partner for the insulin receptor (IR) that**
5 **increases insulin affinity, Akt activation, and peripheral glucose uptake. Mice lacking full-length**
6 **SorCS1 develop age-dependent insulin resistance characterized by increased plasma glucose and**
7 **insulin levels and a blunted response to exogenous insulin. SorCS1 exists in three forms; as a**
8 **transmembrane monomer and dimer, and as a truncated soluble form (sol-SorCS1) produced in**
9 **adipose tissue and is present in plasma. Whereas dimeric SorCS1 engages the proform of the**
10 **insulin receptor (proIR) during biosynthesis and supports its maturation, the monomeric form**
11 **stabilizes the mature IR at the plasma membrane. In its soluble form, SorCS1 positively correlates**
12 **with body mass index and inversely with plasma glucose in diabetic patients, and in mouse models**
13 **of insulin resistance, overexpression or exogenous administration of the monomeric soluble**
14 **receptor domain restores insulin sensitivity. We conclude that SorCS1 is a critical regulator of**
15 **peripheral insulin sensitivity operating in both a cell autonomous and endocrine manner, and we**
16 **propose sol-SorCS1 as a novel adipokine.**

The mechanisms controlling insulin sensitivity and glucose metabolism are fundamental to all vertebrates. Skeletal muscle is essential to acutely buffer high plasma glucose and reduced insulin sensitivity is affected very early on in the development of type 2 diabetes^{1,2}. The downstream signaling pathways of IR in skeletal muscle (e.g. Akt and mTOR) are well described, but whether insulin sensitivity may be dynamically regulated at the level of the plasma membrane through interaction with co-receptors or other membrane proteins is unknown³⁻⁵. The existence of such a molecular thermostat to adapt insulin sensitivity to the metabolic state would transform our conceptual understanding on how key metabolic processes can be controlled.

SorCS1 is a member of the Vps10p-domain family of sorting receptors that also comprises sortilin, SorLA, SorCS2, and SorCS3^{6,7}. Several studies have identified *SORCS1* as a risk locus for type 2 diabetes mellitus that is conserved in women⁸⁻¹⁰, and in female rats¹¹ and mice¹². Recently, SorCS1 was shown to facilitate the biogenesis of insulin containing secretory granules, but a function outside the β -cells was not studied¹³. The structurally related receptor sortilin can function in the secretory pathway by assisting hepatic lipoprotein export¹⁴⁻¹⁶, while in neurons it physically interacts with tropomyosin receptor kinases (Trks) to enable their surface exposure and signaling capacity¹⁷. We hypothesized that SorCS1 likewise may engage a tyrosine kinase receptor to promote its activity. Given the genetic association with T2DM we here asked whether this tyrosine kinase receptor could be IR.

SorCS1 exists in several isoforms comprising an identical extracellular domain encoded by exons 1-24 but with at least four distinct sorting competent cytoplasmic tails (a, b, c, and d)^{18,19} resulting from alternative splicing from exons 25 to 28 (exon 25 also encodes the transmembrane domain) (**Extended Data Fig. 1**). Regardless the isoform, the extracellular domain (sol-SorCS1) can be efficiently liberated from the plasma membrane by ADAM17/TACE mediated cleavage²⁰ but the biological

relevance of this is unknown. To investigate the function specifically of the full-length receptor variants we generated a mouse model (*Sorcs1*^{Δ25-28}), in which exons 25-28 had been deleted leaving the first 24 exons intact to allow production of only sol-SorCS1 (**Extended Data Fig. 1**). RT-PCR for *Sorcs1* in brain, adipose tissue and muscle extracts confirmed that exons 25-28 had been deleted whereas the extracellular domain encoded by exons 1-24 were transcribed.

Starting from 23 weeks of age, female *Sorcs1*^{Δ25-28} mice fed a normal chow developed hyperglycemia. This was paralleled by elevated plasma insulin levels (**Fig. 1a-b**). The hyperinsulinemia progressively increased as animals aged, reaching 4.6-fold higher insulin levels in 50 weeks old compared 10 weeks old mice, and twice the concentration in wildtype controls at 50 weeks. Consistent with genetic studies in mice, rats and humans⁸⁻¹², the glycemic phenotype was present only in female *Sorcs1*^{Δ25-28} mice inasmuch plasma glucose in the male transgenics remained normal up till 60 weeks of age (**Extended Data Fig. 2**). An oral glucose tolerance test (OGTT) confirmed that glucose handling was impaired in the females. Hence, in 14 months old *Sorcs1*^{Δ25-28} mice plasma glucose peaked at 15.7 mM compared to 12.6 mM for their littermate controls and it returned slower to baseline, corresponding to a 45% increase in the area under the curve (282 vs. 506 mM*min) (**Fig. 1 c-d**).

The perturbed glucose homeostasis was most pronounced when comparing littermates from heterozygous breeding on a mixed C57BL/6BomTac x 129sv genetic background (**Fig. 1a-d**). However, reduced peripheral insulin sensitivity was also evident in 47 weeks old C57BL/6BomTac congenic female mutant mice when subjected to hyperinsulinemic euglycemic clamp^{21,22} (**Fig. 1e-f and Extended Figs. 3a-b**). At the beginning of the experiment, plasma glucose did not differ between genotypes and neither did peripheral glucose uptake nor hepatic glucose production. However, when continuously infusing a similar concentration of insulin (**Extended Data Fig. 3c**) we observed a 37% weaker glucose-

suppressing effect at euglycemic levels in the *Sorcs1*^{Δ25-28} mutants compared to wildtype mice (**Fig. 1e**). This difference was solely accounted for by reduced peripheral glucose uptake since hepatic glucose production had been fully shutdown (**Fig. 1f and Extended Fig. 3b**).

To assess peripheral glucose uptake, we examined the role of SorCS1 on IR signaling in muscle extracts, in primary differentiated myocytes, and in the myocyte-derived cell line C2C12. In 19 weeks old normoglycemic female mice administration of an insulin bolus potently stimulated IR phosphorylation (pIR β) in skeletal muscle of both *Sorcs1*^{Δ25-28} mutant animals and their littermate controls. In marked contrast, at 50 weeks of age, when *Sorcs1*^{Δ25-28} mice were diabetic, pIR was now substantially compromised (**Fig. 2a**). A blunted insulin response was also present in primary *Sorcs1*^{Δ25-28} myocyte cultures, as more than 10-fold higher insulin concentrations were required to activate IR in the mutant cultures compared to those from wildtype mice (**Fig. 2b**). Finally, we studied insulin stimulated AKT signaling in C2C12 cells after CRISPR/Cas9 knockout of *Sorcs1*. Corroborating the data in the primary myocytes, C2C12 knockout cells were less insulin responsive as the EC50 curve for pAKT was right-shifted approximately five-fold (**Fig. 2c**). In the mutant *Sorcs1*^{Δ25-28} myocyte cultures we observed a band with a molecular weight smaller than that of the IR β -chain (**Fig. 2b**). Surface biotinylation experiments revealed that this band was restricted to the interior of the cells indicating a degradation product of proIR or IR. Possibly as a consequence, IR β expression at the plasma membrane was reduced by up to 60% in the *Sorcs1*^{Δ25-28} cultures (**Fig. 2d**). Impaired N-glycosylation of proIR prevents two-chain processing and leads to degradation of the pro-receptor²³. In the mutant myocytes we observed in addition to mature proIR a species of lower molecular weight (**Fig. 2e**). Treatment with PNGase F to remove N-linked oligosaccharides identified this protein as immature non-glycosylated

proIR. Taken together the data argue that intracellular SorCS1 facilitates glycosylation and maturation of proIR and prevents its degradation.

We next asked whether surface exposed SorCS1 may also control IR activity. To this end, IR was overexpressed in HEK 293 cells alone or in combination with SorCS1-b, an isoform that is enriched at the plasma membrane and incapable of endocytosis and sorting²⁴. Co-expression with SorCS1 increased insulin affinity to IR by approximately five-fold (**Fig. 3a**). To assess whether a physical interaction between SorCS1 and IR could be involved we employed surface plasmon resonance (SPR) analysis using the extracellular domains of SorCS1 (sol-SorCS1) and IR. The SPR analysis showed that sol-SorCS1 binds to immobilized IR with an estimated affinity of 5 nM (**Fig. 3b**). Importantly, binding of insulin to IR did not inhibit its interaction with SorCS1, indicating formation of a trimeric complex (**Fig. 3c**). No binding was observed for insulin, pro-insulin, glucagon, or glucagon-like peptide 1 (GLP-1) to SorCS1 (**Extended Data Fig. 4a**). To demonstrate that SorCS1, IR, and insulin can physically interact at the plasma membrane we performed immunoprecipitation (IP) experiments in HEK293 cells co-transfected with SorCS1 and IR in the absence or presence of insulin (**Fig. 3d**). Antibodies against IR co-precipitated SorCS1 and vice versa and this interaction was unaffected by preincubation with 100 nM insulin. Notably, IP of SorCS1 also co-precipitated proIR, which supports its role in facilitating N-linked glycosylation of the receptor in the biosynthetic pathway.

SorCS1 exists as a non-covalent dimer and in a monomeric form, the conformational state of which is regulated by the level of glycosylation²⁵. While unglycosylated receptor is a dimer, maturation with N-linked oligosaccharides converts it into a monomer²⁵. We asked whether the stoichiometric state may regulate the preference of SorCS1 for proIR or IR. To elucidate this, HEK293 cells expressing endogenous IR were co-transfected with two SorCS1 constructs where the cytoplasmic tail had been substituted by a FLAG- and Myc-tag, respectively. The cells were then subjected to

105 sequential co-immunoprecipitation, first with anti-FLAG and after elution from the beads subsequently
 106 with anti-Myc (**Fig. 3e, Extended Data Fig. 4b**). Immunoprecipitation with anti-FLAG-coated beads
 107 precipitates receptor populations that comprise a mixture of monomers (SorCS1-FLAG) and dimers
 108 (SorCS1-FLAG/FLAG and SorCS1-FLAG/Myc). After elution from the beads followed by the second
 109 round of IP using anti-Myc antibodies only dimeric SorCS1 will be left (SorCS1-FLAG/Myc) (**Fig. 3e**).
 110 Anti-FLAG immunoprecipitated in addition to the Myc-tagged SorCS1 both proIR and mature IR.
 111 However, following the successive co-IP with anti-Myc to select for dimeric SorCS1, only proIR was
 112 now co-immunoprecipitated. We conclude that dimeric SorCS1 is specific for proIR whereas monomeric
 113 SorCS1 can bind mature IR. To explore the spatial distribution of these interactions, we used a
 114 bimolecular fluorescence complementation (BiFC) assay²⁶. HEK293-IR cells were transfected with two
 115 human SorCS1 constructs each containing one-half of the Venus fluorescent protein. When SorCS1
 116 dimerizes the two Venus fragments unite to elicit a green fluorescent signal that echoes the subcellular
 117 distribution of dimeric SorCS1. First, we stained the cells with an antibody that recognizes both
 118 monomeric and dimeric SorCS1 and found that only monomeric SorCS1 is present at the plasma
 119 membrane as the BiFC-positive signal was restricted to intracellular compartments (**Fig. 3f**). We next
 120 combined the BiFC signal with proximity ligation assay (PLA) for SorCS1 and IR. In this assay a red
 121 fluorescent signal will appear when the receptors are in close proximity (<30 nm). In unpermeabilized
 122 cells we observed a strong PLA signal at the plasma membrane that was BiFC-negative, indicating
 123 binding of monomeric SorCS1 to mature IR (**Fig. 3g**). In contrast, in permeabilized cells a strong PLA
 124 signal was present in BiFC-positive intracellular structures compatible with the Golgi-compartment and
 125 the endoplasmatic reticulum that is dispersed throughout the cytosol (**Fig. 3h**). Because these
 126 compartments are enriched in proIR, the combined data suggest that monomeric SorCS1 engages IR at

the plasma membrane whereas the dimeric and not fully glycosylated receptor binds proIR in the secretory pathway²⁵.

Both humans and mice express SorCS1 in skeletal muscle and adipose tissue (**Figs. 4a and Extended Data Fig. 1c**). We previously reported that up to 95% of SorCS1 is shed per hour from the plasma membrane by ADAM17/TACE mediated cleavage^{20,24}. Given SorCS1 and IR can bind each other through an interaction in their extracellular domains (cf. **Fig. 3b and c**), we hypothesized that soluble SorCS1 could have a glycemic regulatory function. To address this, we studied IR signaling in differentiated myocyte cultures from *Sorcs1*⁴²⁵⁻²⁸ mice after insulin stimulation in the presence of increasing concentrations of exogenous sol-SorCS1 (**Fig. 4b**). Sol-SorCS1 augmented insulin induced phosphorylation of IR and Akt and did so in a concentration dependent manner. No effect was observed in the absence of insulin demonstrating that sol-SorCS1 is not an insulin mimetic. To establish a function of sol-SorCS1 *in vivo*, we first compared the plasma concentration in a cohort of T2DM patients with normoglycemic healthy individuals. In the diabetic group, but not among healthy individuals, plasma glucose inversely correlated with sol-SorCS1 (**Fig. 4c**). Since differentiated 3T3-L1 adipocytes potentially liberates sol-SorCS1 (**Fig. 4d**) and human and murine adipose tissue express SorCS1 (**Fig. 4a, Extended data Fig. 5**) alongside ADAM17/TACE²⁰, we examined, whether body mass index (BMI) associates with the plasma concentration of sol-SorCS1. We found that BMI positively correlates with measures of sol-SorCS1 both in diabetic individuals and healthy controls (**Extended Data Fig. 6**). The data suggested that sol-SorCS1 may act as an adipokine to counteract the increased plasma glucose levels that commonly follows obesity.

To establish a glucose lowering and insulin sensitizing effect of sol-SorCS1 *in vivo*, we injected an adenovirus encoding human sol-SorCS1 (AV-sol-SorCS1) or AV-LacZ (encoding β -

galactosidase) as a negative control into the tail vein of obese leptin receptor knockout mice (*db/db*) that develop severe T2DM. One week later, plasma glucose was increased by 55% and insulin by 65% in the LacZ group due to the gradual worsening of the diabetic phenotype (**Fig. 4e-f**). Remarkably, in the AV-sol-SorCS1 treated mice, plasma glucose remained unchanged and this despite a decrease in plasma insulin. In an intraperitoneal glucose tolerance test (IPGTT), AV-sol-SorCS1 mice responded markedly stronger with lower plasma glucose levels at all timepoints corresponding to a reduction in the area under the curve (AUC) by 79.5% (**Fig. 4g-h**). The treated animals also exhibited superior ability in mobilizing insulin compared to the control group (**Fig. 4i**). A similar glucose and insulin reducing effect was obtained in adenovirus treated male mice with dietary induced T2DM (**Extended Data Fig. 7**). Next we performed hyperinsulinemic euglycemic clamp²². AV-sol-SorCS1 mice showed lower basal plasma glucose and increased capacity for glucose uptake compared to AV-LacZ treated animals (**Fig. 4j and k**). Glucose tracer studies revealed that this effect was accounted for by increased insulin sensitivity in peripheral tissues (**Fig. 4l, Extended Fig. 8**). Notably, three weeks post injection, glycated hemoglobin (HbA1c) had increased considerably in the AV-LacZ group but it stayed unchanged in the AV-sol-SorCS1 group indicating a long-lasting beneficial effect of the soluble receptor in reducing plasma glucose (**Fig. 4m**).

In cells, full-length dimeric SorCS1 binds exclusively to the proIRs whereas monomeric SorCS1 recognizes mature IR (**Fig 3e-h**). Hence, we speculated that the active form of sol-SorCS1 liberated by ADAM17 mediated cleavage is the monomer. To demonstrate this, we purified monomeric and dimeric sol-SorCS1 from transfected CHO cells and injected *db/db* mice with each form intraperitoneally 10 hrs and 1 hr prior to glucose tolerance test. Intriguingly, monomeric sol-SorCS1, but not the dimeric receptor, profoundly improved glucose tolerance (**Fig. 4n**). Hence, the AUC for plasma

glucose was reduced by approximately 60% (from 1060 to 393 mM*min, $P < 0.05$) in animals treated with monomeric sol-SorCS1 (**Fig. 4o**).

In conclusion, we have demonstrated that full-length SorCS1, expressed in peripheral tissues augments insulin sensitivity by engaging proIR and IR; the dimeric SorCS1 binds intracellular proIR and the monomeric receptor the surface exposed mature IR. As a soluble protein, monomeric SorCS1 can target tissues remote from where it is being produced (**Extended Data Fig. 9**). Given SorCS1 is expressed in adipose tissue, that it can be shed from adipocytes, and that its plasma concentration positively correlates with BMI, we propose sol-SorCS1 as a novel adipokine to enhance insulin sensitivity.

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Figure 1: Insulin resistance and T2DM in *Sorcs1*^{Δ25-28} mice.

a, Plasma glucose and, **b**, insulin concentrations in aging female *Sorcs1*^{Δ25-28} mice (wildtype n=4, *Sorcs1*^{Δ25-28} n=9). **c**, Impaired glucose tolerance in 50 weeks old female *Sorcs1*^{Δ25-28} mice. **d**, AUC of the data in **c** calculated minus baselines at t=0 (wildtype n=7, *Sorcs1*^{Δ25-28} n=10). **e**, In hyperinsulinemic euglycemic clamp, glucose infusion rate (GIR) is lower in *Sorcs1*^{Δ25-28} female mice at steady state (t=60-90 min). **f**, The insulin resistance is peripheral (wildtype n=7, *Sorcs1*^{Δ25-28} n=6). Data are presented as mean ± SEM, * = P<0.05, ** = P<0.01.

Figure 2: Impaired insulin receptor signaling in *Sorcs1^{Δ25-28}* muscle.

a, IR phosphorylation is reduced in 50 but not in 19 weeks old female mutant mice, 15 min post insulin injection. **b**, Impaired insulin-induced IR phosphorylation in differentiated primary myocytes from *Sorcs1^{Δ25-28}* mice. Expression of desmin confirms the cultures are equally differentiated. **c**, Reduced phosphorylation of AKT in *Sorcs1^{-/-}* C2C12 cells. **d**, Surface biotinylation of *Sorcs1^{Δ25-28}* primary myocytes shows reduced IR β expression at the plasma membrane and accumulation of an intracellular IR β immunoreactive fragment. Asterisk indicate the low molecular weight band. **e**, PNGase treatment reveals reduced N-linked glycosylation of proIR in *Sorcs1^{Δ25-28}* myocyte cultures. DG proIR designates deglycosylated proIR.

Figure 3: SorCS1 and IR physically interact.

a, SorCS1 overexpression in HEK293 cells increases insulin affinity to IR. **b**, SPR analysis demonstrates dose-dependent binding of sol-SorCS to IR. **c**, Complex formation of SorCS1, IR, and insulin determined by SPR. At 1200 sec, the additive signals for Sol-SorCS1 (*blue*) and insulin (*red*) equals the response units obtained when IR is incubated with both proteins simultaneously (*black*). **d**, Co-immunoprecipitation between SorCS1 and proIR/ IR. The interaction is independent on the presence of saturating concentrations of insulin (100 nM). **e**, Binding of dimeric SorCS1 is restricted to proIR, but monomeric SorCS1 can bind mature IR. **f**, SorCS1 dimers that are BiFC-positive (*green*) are confined to cytosolic compartments, whereas monomeric and BiFC-negative SorCS1 (*red*), is present at the cell surface (**f**, *inset*). **g**, In non-permeabilized HEK293, PLA for SorCS1 and IR (*red*) demonstrates binding

219 between monomeric SorCS1 (BiFC-negative) and IR. **h**, Dimeric SorCS1 (BiFC-positive, *green*)
220 interacts with pro-IR (*red*) in cytosolic compartment.
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Figure 4: Sol-SorCS1 is an insulin sensitizer.

a, mRNA expression of SorCS1 in human tissues (n=12/group). **b**, Insulin-induced activation of IR and AKT in *Sorcs1*^{Δ25-28} primary myocytes correlates with sol-SorCS1 concentration. **c**, In diabetic patients sol-SorCS1 in plasma negatively correlates with fasting plasma glucose (β -value -2.6E-3, $P < 0.001$). **d**, Expression of full-length SorCS1 and shedding of its soluble form in 3T3-L1 adipocytes. **e**, Treatment with AV-sol-SorCS1, but not AV-LacZ, reduces plasma glucose levels and, **f**, plasma insulin in 7 weeks old *db/db* mice. **g**, Enhanced glucose uptake determined by IPGTT in AV-sol-SorCS1 treated *db/db* mice shown in real-time and, **h**, as AUC (n=4/group). **i**, Plasma insulin in the IPGTT shown in g-h. **j**, Lower fasting plasma glucose but, **k**, higher infusion rate in euglycemic hyperinsulinemic clamp of AV-sol-SorCS1 treated *db/db* mice (n=5/group). **l**, The higher infusion rate is accounted for by increased peripheral glucose uptake. **m**, Reduced HbA1c in *db/db* mice 21 days after infection with AV-sol-SorCS1

234 infection. **n**, Intraperitoneal injection of recombinant monomeric, but not dimeric, sol-SorCS1 increases
 235 glucose tolerance (IPGTT) in *db/db* mice. **o**, AUC of the data displayed in panel **n**. Data are presented as
 236 means $\pm$ SEM, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

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Figure 1

Figure 2

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