

## 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.

17 The mechanisms controlling insulin sensitivity and glucose metabolism are fundamental to all  
18 vertebrates. Skeletal muscle is essential to acutely buffer high plasma glucose and reduced insulin  
19 sensitivity is affected very early on in the development of type 2 diabetes<sup>1,2</sup>. The downstream signaling  
20 pathways of IR in skeletal muscle (e.g. Akt and mTOR) are well described, but whether insulin sensitivity  
21 may be dynamically regulated at the level of the plasma membrane through interaction with co-receptors  
22 or other membrane proteins is unknown<sup>3-5</sup>. The existence of such a molecular thermostat to adapt insulin  
23 sensitivity to the metabolic state would transform our conceptual understanding on how key metabolic  
24 processes can be controlled.

25 SorCS1 is a member of the Vps10p-domain family of sorting receptors that also comprises  
26 sortilin, SorLA, SorCS2, and SorCS3<sup>6,7</sup>. Several studies have identified *SORCS1* as a risk locus for type  
27 2 diabetes mellitus that is conserved in women<sup>8-10</sup>, and in female rats<sup>11</sup> and mice<sup>12</sup>. Recently, SorCS1  
28 was shown to facilitate the biogenesis of insulin containing secretory granules, but a function outside the  
29 β-cells was not studied<sup>13</sup>. The structurally related receptor sortilin can function in the secretory pathway  
30 by assisting hepatic lipoprotein export<sup>14-16</sup>, while in neurons it physically interacts with tropomyosin  
31 receptor kinases (Trks) to enable their surface exposure and signaling capacity<sup>17</sup>. We hypothesized that  
32 SorCS1 likewise may engage a tyrosine kinase receptor to promote its activity. Given the genetic  
33 association with T2DM we here asked whether this tyrosine kinase receptor could be IR.

34 SorCS1 exists in several isoforms comprising an identical extracellular domain encoded by  
35 exons 1-24 but with at least four distinct sorting competent cytoplasmic tails (a, b, c, and d)<sup>18,19</sup> resulting  
36 from alternative splicing from exons 25 to 28 (exon 25 also encodes the transmembrane domain)  
37 (**Extended Data Fig. 1**). Regardless the isoform, the extracellular domain (sol-SorCS1) can be efficiently  
38 liberated from the plasma membrane by ADAM17/TACE mediated cleavage<sup>20</sup> but the biological

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

44 Starting from 23 weeks of age, female *Sorcs1*<sup>Δ25-28</sup> mice fed a normal chow developed  
45 hyperglycemia. This was paralleled by elevated plasma insulin levels (**Fig. 1a-b**). The hyperinsulinemia  
46 progressively increased as animals aged, reaching 4.6-fold higher insulin levels in 50 weeks old  
47 compared 10 weeks old mice, and twice the concentration in wildtype controls at 50 weeks. Consistent  
48 with genetic studies in mice, rats and humans<sup>8-12</sup>, the glycemic phenotype was present only in female  
49 *Sorcs1*<sup>Δ25-28</sup> mice inasmuch plasma glucose in the male transgenics remained normal up till 60 weeks of  
50 age (**Extended Data Fig. 2**). An oral glucose tolerance test (OGTT) confirmed that glucose handling  
51 was impaired in the females. Hence, in 14 months old *Sorcs1*<sup>Δ25-28</sup> mice plasma glucose peaked at 15.7  
52 mM compared to 12.6 mM for their littermate controls and it returned slower to baseline, corresponding  
53 to a 45% increase in the area under the curve (282 vs. 506 mM\*min) (**Fig. 1 c-d**).

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

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

64 To assess peripheral glucose uptake, we examined the role of SorCS1 on IR signaling in  
65 muscle extracts, in primary differentiated myocytes, and in the myocyte-derived cell line C2C12. In 19  
66 weeks old normoglycemic female mice administration of an insulin bolus potently stimulated IR  
67 phosphorylation (pIR $\beta$ ) in skeletal muscle of both *Sorcs1*<sup>Δ25-28</sup> mutant animals and their littermate  
68 controls. In marked contrast, at 50 weeks of age, when *Sorcs1*<sup>Δ25-28</sup> mice were diabetic, pIR was now  
69 substantially compromised (**Fig. 2a**). A blunted insulin response was also present in primary *Sorcs1*<sup>Δ25-</sup>  
70 <sup>28</sup> myocyte cultures, as more than 10-fold higher insulin concentrations were required to activate IR in  
71 the mutant cultures compared to those from wildtype mice (**Fig. 2b**). Finally, we studied insulin  
72 stimulated AKT signaling in C2C12 cells after CRISPR/Cas9 knockout of *Sorcs1*. Corroborating the data  
73 in the primary myocytes, C2C12 knockout cells were less insulin responsive as the EC50 curve for pAKT  
74 was right-shifted approximately five-fold (**Fig. 2c**). In the mutant *Sorcs1*<sup>Δ25-28</sup> myocyte cultures we  
75 observed a band with a molecular weight smaller than that of the IR $\beta$ -chain (**Fig. 2b**). Surface  
76 biotinylation experiments revealed that this band was restricted to the interior of the cells indicating a  
77 degradation product of proIR or IR. Possibly as a consequence, IR $\beta$  expression at the plasma membrane  
78 was reduced by up to 60% in the *Sorcs1*<sup>Δ25-28</sup> cultures (**Fig. 2d**). Impaired N-glycosylation of proIR  
79 prevents two-chain processing and leads to degradation of the pro-receptor<sup>23</sup>. In the mutant myocytes we  
80 observed in addition to mature proIR a species of lower molecular weight (**Fig. 2e**). Treatment with  
81 PNGase F to remove N-linked oligosaccharides identified this protein as immature non-glycosylated

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

84 We next asked whether surface exposed SorCS1 may also control IR activity. To this end,  
85 IR was overexpressed in HEK 293 cells alone or in combination with SorCS1-b, an isoform that is  
86 enriched at the plasma membrane and incapable of endocytosis and sorting<sup>24</sup>. Co-expression with SorCS1  
87 increased insulin affinity to IR by approximately five-fold (**Fig. 3a**). To assess whether a physical  
88 interaction between SorCS1 and IR could be involved we employed surface plasmon resonance (SPR)  
89 analysis using the extracellular domains of SorCS1 (sol-SorCS1) and IR. The SPR analysis showed that  
90 sol-SorCS1 binds to immobilized IR with an estimated affinity of 5 nM (**Fig. 3b**). Importantly, binding  
91 of insulin to IR did not inhibit its interaction with SorCS1, indicating formation of a trimeric complex  
92 (**Fig. 3c**). No binding was observed for insulin, pro-insulin, glucagon, or glucagon-like peptide 1 (GLP-  
93 1) to SorCS1 (**Extended Data Fig. 4a**). To demonstrate that SorCS1, IR, and insulin can physically  
94 interact at the plasma membrane we performed immunoprecipitation (IP) experiments in HEK293 cells  
95 co-transfected with SorCS1 and IR in the absence or presence of insulin (**Fig. 3d**). Antibodies against IR  
96 co-precipitated SorCS1 and vice versa and this interaction was unaffected by preincubation with 100 nM  
97 insulin. Notably, IP of SorCS1 also co-precipitated proIR, which supports its role in facilitating N-linked  
98 glycosylation of the receptor in the biosynthetic pathway.

99 SorCS1 exists as a non-covalent dimer and in a monomeric form, the conformational state  
100 of which is regulated by the level of glycosylation<sup>25</sup>. While unglycosylated receptor is a dimer,  
101 maturation with N-linked oligosaccharides converts it into a monomer<sup>25</sup>. We asked whether the  
102 stoichiometric state may regulate the preference of SorCS1 for proIR or IR. To elucidate this, HEK293  
103 cells expressing endogenous IR were co-transfected with two SorCS1 constructs where the cytoplasmic  
104 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) assay<sup>26</sup>. 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

127 the plasma membrane whereas the dimeric and not fully glycosylated receptor binds proIR in the  
128 secretory pathway<sup>25</sup>.

129 Both humans and mice express SorCS1 in skeletal muscle and adipose tissue (**Figs. 4a and**  
130 **Extended Data Fig. 1c**). We previously reported that up to 95% of SorCS1 is shed per hour from the  
131 plasma membrane by ADAM17/TACE mediated cleavage<sup>20,24</sup>. Given SorCS1 and IR can bind each other  
132 through an interaction in their extracellular domains (cf. **Fig. 3b and c**), we hypothesized that soluble  
133 SorCS1 could have a glycemic regulatory function. To address this, we studied IR signaling in  
134 differentiated myocyte cultures from *Sorcs1*<sup>A25-28</sup> mice after insulin stimulation in the presence of  
135 increasing concentrations of exogenous sol-SorCS1 (**Fig. 4b**). Sol-SorCS1 augmented insulin induced  
136 phosphorylation of IR and Akt and did so in a concentration dependent manner. No effect was observed  
137 in the absence of insulin demonstrating that sol-SorCS1 is not an insulin mimetic. To establish a function  
138 of sol-SorCS1 *in vivo*, we first compared the plasma concentration in a cohort of T2DM patients with  
139 normoglycemic healthy individuals. In the diabetic group, but not among healthy individuals, plasma  
140 glucose inversely correlated with sol-SorCS1 (**Fig. 4c**). Since differentiated 3T3-L1 adipocytes potently  
141 liberates sol-SorCS1 (**Fig. 4d**) and human and murine adipose tissue express SorCS1 (**Fig. 4a, Extended**  
142 **data Fig. 5**) alongside ADAM17/TACE<sup>20</sup>, we examined, whether body mass index (BMI) associates  
143 with the plasma concentration of sol-SorCS1. We found that BMI positively correlates with measures of  
144 sol-SorCS1 both in diabetic individuals and healthy controls (**Extended Data Fig. 6**). The data suggested  
145 that sol-SorCS1 may act as an adipokine to counteract the increased plasma glucose levels that commonly  
146 follows obesity.

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

149 galactosidase) as a negative control into the tail vein of obese leptin receptor knockout mice (*db/db*) that  
150 develop severe T2DM. One week later, plasma glucose was increased by 55% and insulin by 65% in the  
151 LacZ group due to the gradual worsening of the diabetic phenotype (**Fig. 4e-f**). Remarkably, in the AV-  
152 sol-SorCS1 treated mice, plasma glucose remained unchanged and this despite a decrease in plasma  
153 insulin. In an intraperitoneal glucose tolerance test (IPGTT), AV-sol-SorCS1 mice responded markedly  
154 stronger with lower plasma glucose levels at all timepoints corresponding to a reduction in the area under  
155 the curve (AUC) by 79.5% (**Fig. 4g-h**). The treated animals also exhibited superior ability in mobilizing  
156 insulin compared to the control group (**Fig. 4i**). A similar glucose and insulin reducing effect was  
157 obtained in adenovirus treated male mice with dietary induced T2DM (**Extended Data Fig. 7**). Next we  
158 performed hyperinsulinemic euglycemic clamp<sup>22</sup>. AV-sol-SorCS1 mice showed lower basal plasma  
159 glucose and increased capacity for glucose uptake compared to AV-LacZ treated animals (**Fig. 4j and**  
160 **k**). Glucose tracer studies revealed that this effect was accounted for by increased insulin sensitivity in  
161 peripheral tissues (**Fig. 4l, Extended Fig. 8**). Notably, three weeks post injection, glycated hemoglobin  
162 (HbA1c) had increased considerably in the AV-LacZ group but it stayed unchanged in the AV-sol-  
163 SorCS1 group indicating a long-lasting beneficial effect of the soluble receptor in reducing plasma  
164 glucose (**Fig. 4m**).

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

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

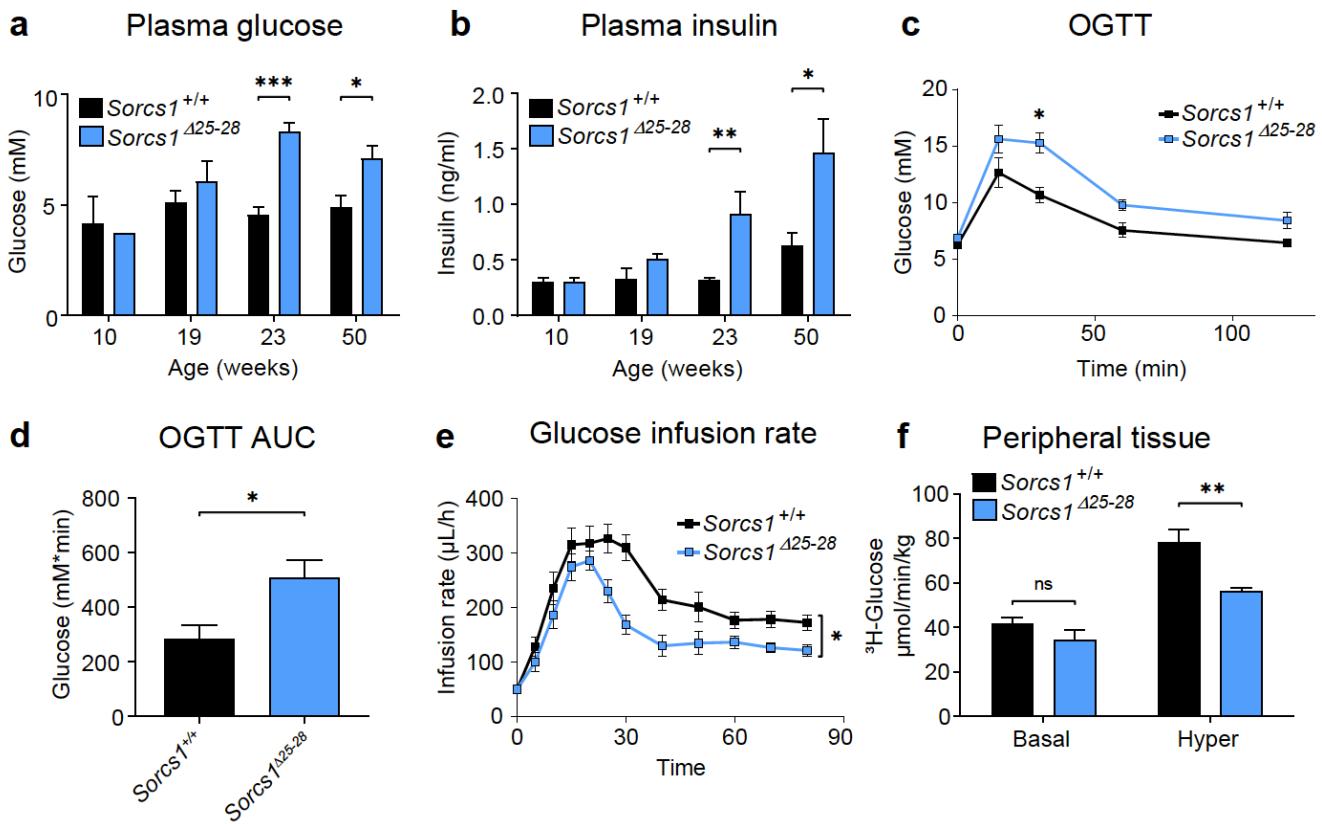
173 In conclusion, we have demonstrated that full-length SorCS1, expressed in peripheral  
174 tissues augments insulin sensitivity by engaging proIR and IR; the dimeric SorCS1 binds intracellular  
175 proIR and the monomeric receptor the surface exposed mature IR. As a soluble protein, monomeric  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

180

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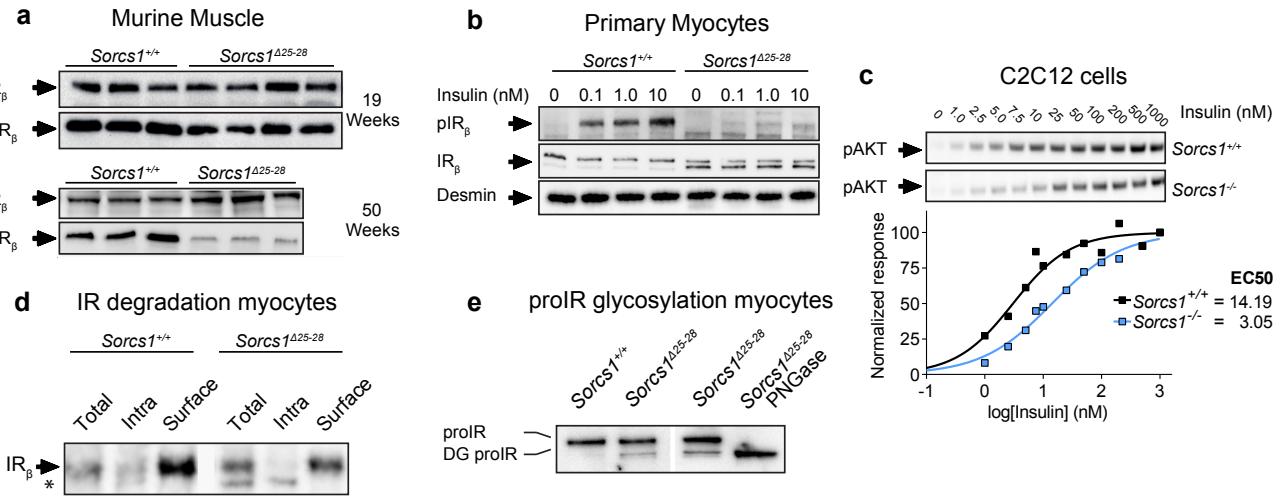
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189 **Figure 1:** Insulin resistance and T2DM in *Sorcs1*<sup>Δ25-28</sup> mice.

190 **a**, Plasma glucose and, **b**, insulin concentrations in aging female *Sorcs1*<sup>Δ25-28</sup> mice (wildtype n=4,  
191 *Sorcs1*<sup>Δ25-28</sup> n=9). **c**, Impaired glucose tolerance in 50 weeks old female *Sorcs1*<sup>Δ25-28</sup> mice. **d**, AUC of the  
192 data in **c** calculated minus baselines at t=0 (wildtype n=7, *Sorcs1*<sup>Δ25-28</sup> n=10). **e**, In hyperinsulinemic  
193 euglycemic clamp, glucose infusion rate (GIR) is lower in *Sorcs1*<sup>Δ25-28</sup> female mice at steady state (t=60-  
194 90 min). **f**, The insulin resistance is peripheral (wildtype n=7, *Sorcs1*<sup>Δ25-28</sup> n=6). Data are presented as  
195 mean ± SEM, \* = P<0.05, \*\* = P<0.01.

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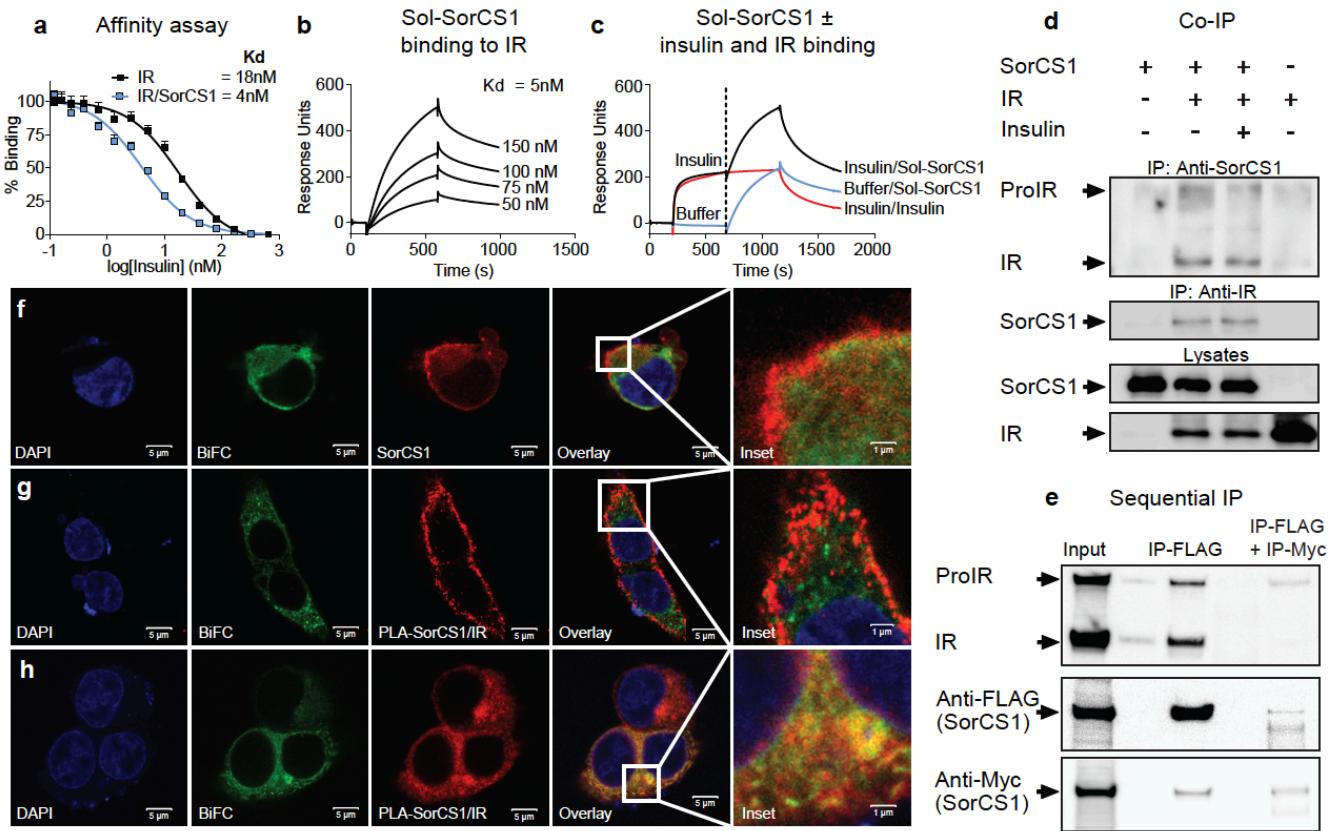
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199 **Figure 2:** Impaired insulin receptor signaling in *Sorcs1*<sup>Δ25-28</sup> muscle.

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



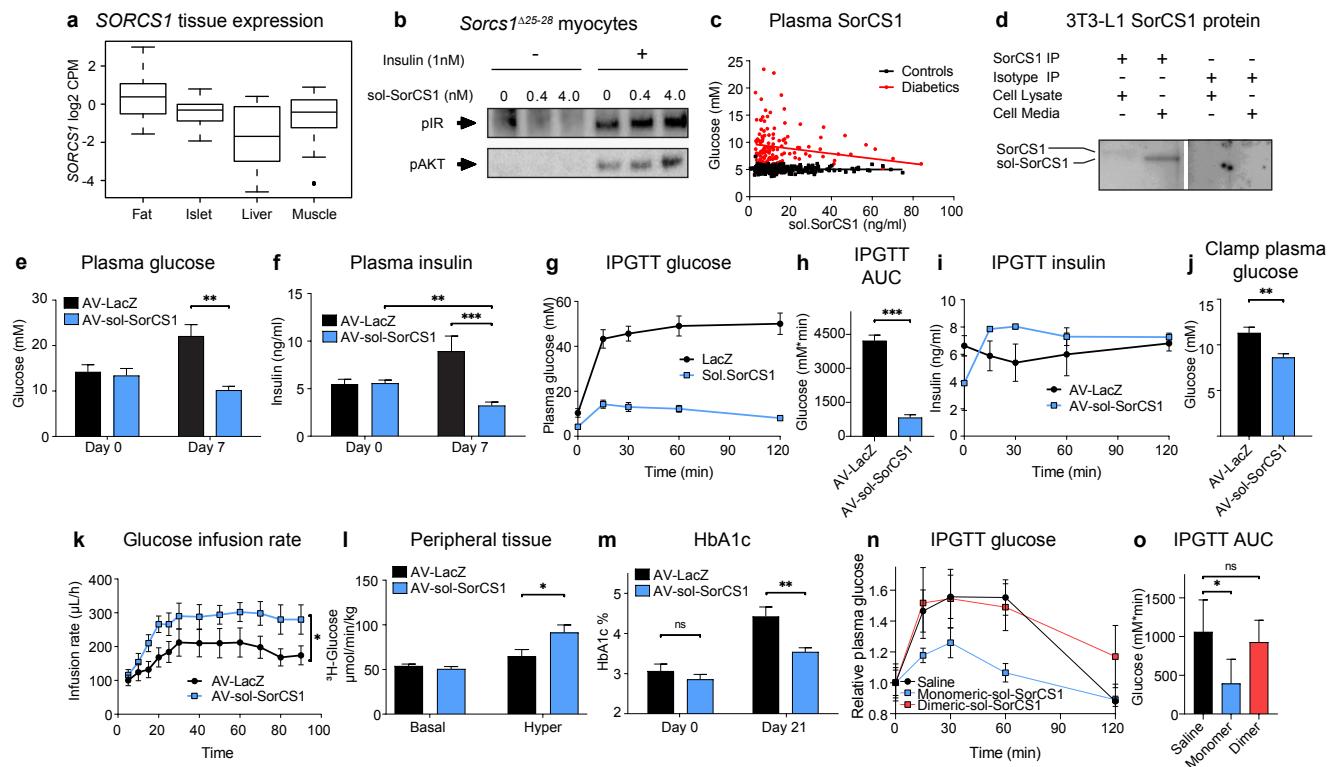
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209 **Figure 3:** SorCS1 and IR physically interact.

210 **a**, SorCS1 overexpression in HEK293 cells increases insulin affinity to IR. **b**, SPR analysis demonstrates  
211 dose-dependent binding of sol-SorCS to IR. **c**, Complex formation of SorCS1, IR, and insulin determined  
212 by SPR. At 1200 sec, the additive signals for Sol-SorCS1 (*blue*) and insulin (*red*) equals the response  
213 units obtained when IR is incubated with both proteins simultaneously (*black*). **d**, Co-  
214 immunoprecipitation between SorCS1 and proIR/ IR. The interaction is independent on the presence of  
215 saturating concentrations of insulin (100 nM). **e**, Binding of dimeric SorCS1 is restricted to proIR, but  
216 monomeric SorCS1 can bind mature IR. **f**, SorCS1 dimers that are BiFC-positive (*green*) are confined  
217 to cytosolic compartments, whereas monomeric and BiFC-negative SorCS1 (*red*), is present at the cell  
218 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.

221



222

223 **Figure 4:** Sol-SorCS1 is an insulin sensitizer.

224 **a**, mRNA expression of SorCS1 in human tissues (n=12/group). **b**, Insulin-induced activation of IR and  
225 AKT in *Sorcs1*<sup>A25-28</sup> primary myocytes correlates with sol-SorCS1 concentration. **c**, In diabetic patients  
226 sol-SorCS1 in plasma negatively correlates with fasting plasma glucose ( $\beta$ -value -2.6E-3, P<0.001). **d**,  
227 Expression of full-length SorCS1 and shedding of its soluble form in 3T3-L1 adipocytes. **e**, Treatment  
228 with AV-sol-SorCS1, but not AV-LacZ, reduces plasma glucose levels and, **f**, plasma insulin in 7 weeks  
229 old *db/db* mice. **g**, Enhanced glucose uptake determined by IPGTT in AV-sol-SorCS1 treated *db/db* mice  
230 shown in real-time and, **h**, as AUC (n=4/group). **i**, Plasma insulin in the IPGTT shown in g-h. **j**, Lower  
231 fasting plasma glucose but, **k**, higher infusion rate in euglycemic hyperinsulinemic clamp of AV-sol-  
232 SorCS1 treated *db/db* mice (n=5/group). **l**, The higher infusion rate is accounted for by increased  
233 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, \* =  $P < 0.05$ , \*\* =  $P < 0.01$ , \*\*\* =  $P < 0.001$ .

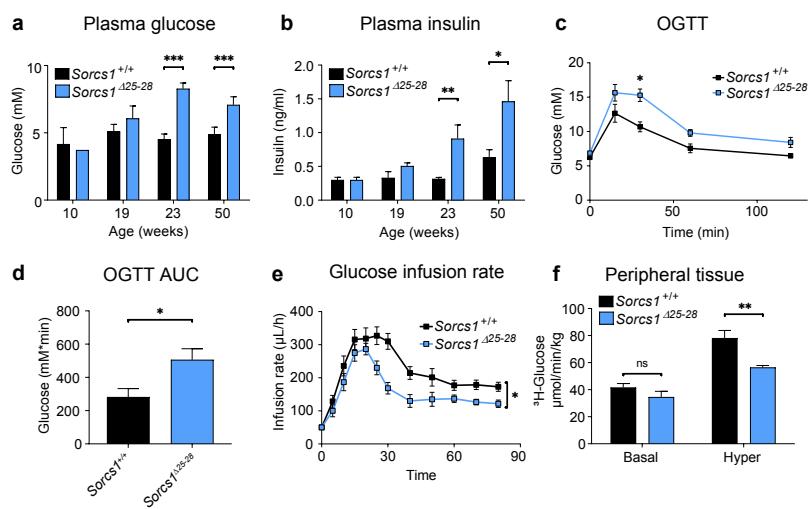
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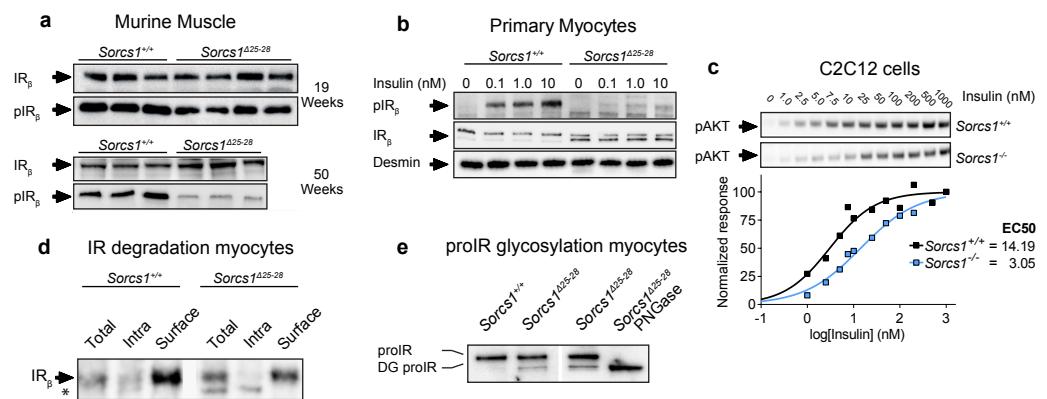
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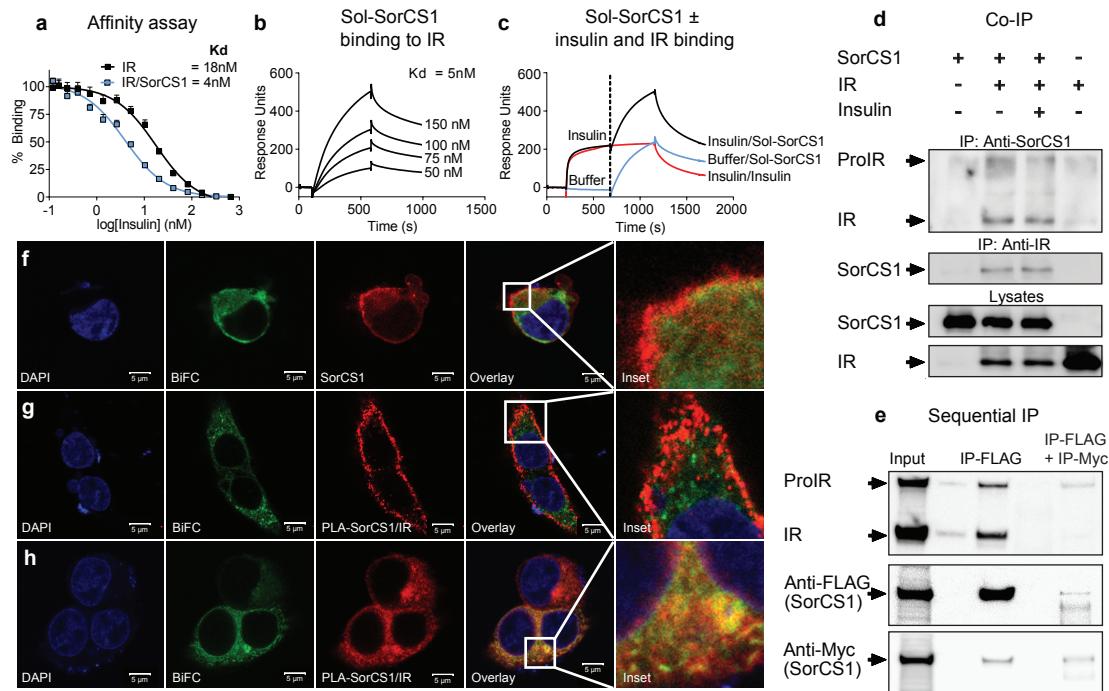
Figure 1



**Figure 2**



**Figure 3**



**Figure 4**

