HHS Public Access

Author manuscript

Neuron. Author manuscript; available in PMC 2016 August 19.

Published in final edited form as:

Neuron. 2015 August 19; 87(4): 764–780. doi:10.1016/j.neuron.2015.08.007.

The Sorting Receptor SorCS1 Regulates Trafficking of Neurexin and AMPA Receptors

Jeffrey N. Savas[1,5], **Luís F. Ribeiro**[2], **Keimpe D. Wierda**[2], **Rebecca Wright**[3], **Laura A. DeNardo**[3], **Heather C. Rice**[2], **Ingrid Chamma**[4], **Yi-Zhi Wang**[5], **Roland Zemla**[6], **Mathieu Lavallée-Adam**[1], **Kristel M. Vennekens**[2], **Matthew L. O'Sullivan**[3], **Joseph K. Antonios**[3], **Elizabeth A. Hall**[5], **Olivier Thoumine**[4], **Alan D. Attie**[7], **Anirvan Ghosh**[3,8], **John R. Yates III**[1,*], and **Joris de Wit**[2,*]

[1]Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA 92037, USA [2]VIB Center for the Biology of Disease, 3000 Leuven, Belgium; KU Leuven, Center for Human Genetics, 3000 Leuven, Belgium [3]Neurobiology Section, Division of Biology, University of California San Diego, La Jolla, CA 92093, USA [4]CNRS University of Bordeaux, Interdisciplinary Institute for Neuroscience, UMR 5297, F-33000 Bordeaux, France [5]Northwestern University Feinberg School of Medicine, Department of Neurology, Chicago, IL 60611, USA [6]New York University School of Medicine, New York, New York 10016, USA [7]Department of Biochemistry, University of Wisconsin, Madison, WI 53706, USA [8]Neuroscience Discovery, F. Hoffman-La Roche, 4070 Basel, Switzerland

Abstract

The formation, function, and plasticity of synapses require dynamic changes in synaptic receptor composition. Here we identify the sorting receptor SorCS1 as a key regulator of synaptic receptor trafficking. Four independent proteomic analyses identify the synaptic adhesion molecule neurexin and the AMPA glutamate receptor (AMPAR) as major proteins sorted by SorCS1. SorCS1 localizes to early and recycling endosomes and regulates neurexin and AMPAR surface trafficking. Surface proteome analysis of *SorCS1*-deficient neurons shows decreased surface levels of these, and additional, receptors. Quantitative in vivo analysis of *SorCS1* knockout synaptic proteomes identifies SorCS1 as a global trafficking regulator and reveals decreased levels of receptors regulating adhesion and neurotransmission, including neurexins and AMPARs. Consequently, glutamatergic transmission at *SorCS1*–deficient synapses is reduced due to impaired AMPAR surface expression. *SORCS1* mutations have been associated with autism and Alzheimer's disease, suggesting that perturbed receptor trafficking contributes to defects in synaptic composition and function underlying synaptopathies.

Introduction

Proper formation and function of synapses requires the coordinated assembly of large and heterogeneous protein complexes on the pre- and postsynaptic side. The composition of

*Correspondence: jyates@scripps.edu; joris.dewit@cme.vib-kuleuven.be.

these complexes varies with synaptic neurotransmitter type and developmental stage; changes upon activity-induced changes in synaptic strength, and is affected in synaptopathies (Cajigas et al., 2010; Grant, 2012, 2013). Neurons must exert strict control over the local organization of the synaptic machinery in order to allow dynamic changes in synaptic protein content while maintaining normal function. However, the mechanisms that control synaptic proteome composition in development and plasticity are not well understood.

Receptors are core components of the synaptic proteome. Their dynamic trafficking is a key feature underlying the function and plasticity of synapses (Choquet and Triller, 2013). The transport, synaptic insertion and removal of AMPA glutamate receptors (AMPARs) for example are tightly regulated in order to control synaptic efficacy (Anggono and Huganir, 2012; Carroll et al., 2001). An elaborate array of AMPAR-interacting proteins controls AMPAR trafficking and function (Schwenk et al., 2014; Schwenk et al., 2012; Shanks et al., 2012). Adhesion molecules are another key class of synaptic receptors (Giagtzoglou et al., 2009; Missler et al., 2012). Neurexin (Nrxn) presynaptic adhesion molecules play a central role in the formation, maturation, and plasticity of synapses (Krueger et al., 2012; Sudhof, 2008). Loss of all three α-Nrxn genes severely impairs neurotransmitter release (Missler et al., 2003). *NRXNs* are linked to autism and schizophrenia (Reichelt et al., 2012; Sudhof, 2008), indicating that Nrxns are essential for normal circuit function. Nrxns interact with distinct postsynaptic adhesion molecules, including neuroligins (Nlgns), LRRTMs, and secreted cerebellin in complex with GluD2 (de Wit et al., 2009; Dean et al., 2003; Graf et al., 2004; Ichtchenko et al., 1995; Ko et al., 2009; Scheiffele et al., 2000; Siddiqui et al., 2010; Uemura et al., 2010). Some adhesion molecules, such as the LRRTMs, interact with AMPARs (de Wit et al., 2009; Schwenk et al., 2012). Furthermore, presynaptic Nrxn regulates postsynaptic AMPAR trafficking via a *trans*-synaptic interaction with Nlgns and LRRTMs (Aoto et al., 2013). Adhesion molecules and neurotransmitter receptors are thus key components of the synaptic machinery with closely linked function. However, in contrast to AMPARs, little is known about the mechanisms regulating adhesion molecule trafficking.

As core synaptic receptors, adhesion molecules and AMPARs might be co-regulated and share trafficking mechanisms. To test this idea, we took Nrxn as a central synaptic component, attempted to identify Nrxn-interacting proteins involved in trafficking, and then determined if that mechanism is shared more broadly among other synaptic receptors. In a screen for Nrxn-interacting proteins we identified the VPS10P sorting receptor SorCS1. Vacuolar protein sorting 10 (VPS10P) receptor family proteins are important regulators of intracellular trafficking (Hermey, 2009; Willnow et al., 2008). The mammalian VPS10P receptors Sortilin and SorLA sort cargo proteins in Golgi to endosome trafficking pathways (Nielsen et al., 2007; Nielsen et al., 2001; Nykjaer and Willnow, 2012). Less is known about the sorting function and endogenous cargo of the three remaining mammalian VPS10P receptors, SorCS (sortilin-related CNS expressed) 1, -2, and -3 (Hermey et al., 2004; Hermey et al., 1999; Rezgaoui et al., 2001). The three SorCS genes are expressed in complementary patterns in the brain (Hermey et al., 2004; Oetjen et al., 2014), SorCS3 localizes to synapses (Breiderhoff et al., 2013), and SORCS genes have been associated with

autism, schizophrenia, bipolar disorder, ADHD, and late-onset Alzheimer's disease (Christoforou et al., 2011; Grupe et al., 2006; Laumet et al., 2010; Li et al., 2008; Liang et al., 2009; Lionel et al., 2011; Ollila et al., 2009; Reitz et al., 2011; Sanders et al., 2012). Their prominent association with synaptopathies suggests that SorCS receptors might play a role in the trafficking of synaptic components.

Here we identify Nrxn and AMPARs as key synaptic proteins sorted by SorCS1. SorCS1 localizes to early and recycling endosomal compartments and regulates surface trafficking of Nrxn and AMPARs. SorCS1 interactome analysis reveals close association with several synaptic proteins, including Nrxn, Nlgn, and AMPARs. Quantitative surface proteome analysis reveals reduced surface expression of these proteins in *SorCS1*-deficient neurons. In vivo quantitative analysis of *SorCS1* knockout synaptic proteomes shows decreased abundance of receptors regulating adhesion and synaptic transmission, including Nrxns and AMPARs. Loss of *SorCS1* reduces both glutamatergic and GABAergic synaptic transmission, due to impaired surface expression of AMPARs at excitatory synapses and a decrease in the density of inhibitory synapses, respectively. Together, our results identify SorCS1 as a major trafficking regulator of receptors that are essential for synaptic function.

Results

Identification of SorCS1 as a neurexin-binding protein

To discover Nrxn-interacting proteins, we used a combination of affinity chromatography with Nrxn ectodomains and mass spectrometric (MS) analysis (Savas et al., 2014) (Fig. 1A). We purified recombinant Nrxn1β-Fc proteins lacking or containing splice site 4 (Nrxn1β −or +SS4) (Fig. S1A), the best characterized Nrxn splice site in terms of protein interactions (Baudouin and Scheiffele, 2010), and used these to identify interacting proteins in detergent-solubilized rat brain homogenate. We identified Nlgn1-3 as Nrxn-binding proteins with a preference for Nrxn1β(−SS4)-Fc (Fig. 1B) (Boucard et al., 2005), and LRRTM1 as a Nrxn1β(−SS4)-Fc-specific interactor (Ko et al., 2009; Siddiqui et al., 2010) (Table S1), validating our approach.

Strikingly, the two most abundant Nrxn1β-interacting proteins identified in this experiment were the VPS10P receptor proteins SorCS1 and SorCS2 (Fig. 1B). The identification of SorCS1 and -2 sorting receptors as candidate Nrxn interactors was particularly interesting, as these proteins have not previously been identified as Nrxn-binding proteins and might be involved in regulating Nrxn trafficking. SorCS1 and -2 are members of the VPS10P family, which regulates intracellular trafficking and neuronal function (Hermey, 2009; Willnow et al., 2008), but the endogenous cargo and trafficking function of SorCS proteins are not well understood. To determine whether Nrxn also interacts with SorCS at synapses, we repeated our affinity chromatography experiment using synaptosome extracts and again identified Nlgn1-3 and SorCS1 and -2 as the most abundant Nrxn1β-interacting proteins (Fig. 1C, D, and Table S1). The other mammalian VPS10P receptors Sortilin and SorLA were not identified in these experiments (Table S1). The interaction of Nrxn1β with SorCS was lost at high detergent concentrations, while the interaction with Nlgns was not (Fig. S1B), suggesting that the Nrxn1β-SorCS complex is less stable than Nrxn1β-Nlgn. Finally, affinity purifications with an antibody against the Nrxn1/2/3 cytoplasmic domain independently

identified SorCS1 (Fig. S1C). Together, these experiments suggest that SorCS1 is a novel endogenous Nrxn binding partner.

To verify the MS results, we carried out a series of binding assays. We expressed SorCS1cβ-myc in HEK293T cells and applied Nrxn1β(− or +SS4)-Fc to assess binding. Both Nrxn1β-Fc proteins bound to SorCS1 and the positive control Nlgn1, whereas Fc alone showed no detectable binding (Fig. 1E). Rather than labelling the cell surface, Nrxn1β-Fc clustered in large intracellular puncta (Fig. 1E), suggesting internalization of Nrxn1β by SorCS1. In reciprocal experiments, SorCS1-ecto-His strongly bound to the surface of cells expressing Nrxn1β-CFP, but not to cells expressing GFP (Fig. S1D). Complementary pulldown assays with transfected HEK cell lysates confirmed the SorCS1-Nrxn1β interaction (Fig. S1E, S1F). Direct binding assays demonstrated that SorCS1-ecto-His coprecipitated with Nrxn1β-Fc, but not with the control proteins LPHN3-Fc (O'Sullivan et al., 2012) or Fc alone (Fig. 1F), showing a direct interaction between the SorCS1 and Nrxn1β ectodomains.

To estimate the affinity of the interaction, we measured SorCS1-Nrxn1β cell surface binding. Scatchard analysis indicated a Kd=2.22 ± 0.87 nM after subtracting non-specific binding to control cells (Fig. 1G), demonstrating an apparent high affinity binding of SorCS1 to cell surface Nrxn1β. To identify the domain in SorCS1 required for Nrxn1β binding, we analyzed Nrxn1β-Fc binding to full-length (FL) SorCS1cβ and to SorCS1 mutants lacking the pro-peptide domain (ΔPRO), the canonical ligand-binding VPS10P domain (ΔVPS), the polycystic kidney disease domain (ΔPKD), or the leucine-rich domain (ΔLRD) (Fig. 1H). Surface expression was confirmed for all SorCS1 mutants (Fig. S1G). Deletion of the VPS10P domain abolished SorCS1 binding to Nrxn1β in both pulldown (Fig. 1I) and cell surface binding assays (Fig. 1J). Together, these experiments show that the SorCS1-Nrxn1β interaction is direct, of high affinity, and requires the VPS10P domain.

SorCS1 localizes to endosomal compartments and excitatory synapses

The identification of SorCS1 as a Nrxn interactor suggests that SorCS1 may localize to synapses. Punctate SorCS1 immunoreactivity has been reported to localize to neuronal cell bodies and dendrites (Hermey et al., 2001), but the nature of these SorCS1-positive compartments is unclear. To gain insight into the subcellular localization of SorCS1, we first labeled HeLa cells expressing HA-SorCS1cβ with a panel of antibodies for intracellular compartments. SorCS1 displayed a punctate distribution throughout the cytoplasm, where it partially colocalized with the early endosome marker EEA1 (Fig. 2A). Robust colocalization was observed with Alexa 568-transferrin (Tf; 30 min uptake) (Fig. 2B), and with the transferrin receptor (TfR; Fig. S2A). SorCS1 also colocalized with the recycling endosome marker Rab11 and the *trans* Golgi network marker TGN-46, but displayed little overlap with the late endosomal marker CD63 or the lysosomal marker LAMP1 (Fig. 2C, S2B-D). These observations suggest that HA-SorCS1cβ localizes to endosomal recycling compartments in HeLa cells, consistent with a role in regulating protein trafficking.

To determine the subcellular localization of SorCS1 in neurons, we tested a wide panel of commercially and custom-made SorCS1 antibodies, but found that none were suitable for the immunocytochemical detection of SorCS1 (data not shown). We therefore expressed epitope-tagged SorCS1cβ in cultured hippocampal neurons. SorCS1 displayed a punctate

distribution in cell body and dendrites. Quantification of the intensity of SorCS1 fluorescence in axons and dendrites showed that SorCS1 predominantly localizes to the somatodendritic compartment, comparable to the somatodendritic marker TfR-GFP (Farias et al., 2012) (Fig. 2D-G). In dendrites, SorCS1 puncta colocalized with EEA1, Alexa 568-Tf (60 min uptake), (Fig. 2H, I, K) and TfR (Fig. 2K, S2E), but showed less overlap with TGN-46, CD-63, or LAMP1 (Fig. 2K, S2F-H). Co-expression of SorCS1 and Rab-GFP constructs to label different endosomal compartments showed that SorCS1 displayed the highest overlap with the early and recycling endosome markers Rab4, Rab5, and Rab11, but not with the late endosome marker Rab7 (Fig. 2L, S2J-M). These results indicate that SorCS1 localizes to endosomal compartments in dendrites and in dendritic spines.

We next compared the subcellular distribution of SorCS1 to synaptic markers. SorCS2 and SorCS3 have been reported to localize to the postsynaptic density (Bayes et al., 2012; Breiderhoff et al., 2013), suggesting that SorCS1 might also localize to synapses. SorCS1 could be observed colocalizing with PSD95 in the heads of dendritic spines (Fig. 2J, K), and was juxtaposed to VGluT1 (Fig. 2K, S2I). Subcellular fractionation (Fig. S2N) using a verified SorCS1 antibody (Fig. S2O-Q) showed that SorCS1 was present in purified postsynaptic density fractions of rat brain homogenate, which also contained the excitatory postsynaptic marker PSD95, but lacked the presynaptic marker synaptophysin. Little SorCS1 was detected in the presynaptic fraction. Together, these data indicate that SorCS1 predominantly localizes to early and recycling endosomes in neurons, consistent with a role in sorting cargo proteins. In addition, SorCS1 can be detected at the postsynaptic density of glutamatergic synapses.

SorCS1 regulates the cell surface distribution of neurexin

The endosomal localization of SorCS1 is consistent with a role in the intracellular sorting of cargo proteins and the regulation of receptor surface distribution (Yap and Winckler, 2012). If SorCS1 regulates the cell surface distribution of Nrxn, SorCS1 and Nrxn would be expected to colocalize. SorCS1 localizes to the somatodendritic compartment (Fig. 2D-G), whereas Nrxn predominantly functions presynaptically. However, several studies have reported additional dendritic localization of Nrxn (Barker et al., 2008; Berninghausen et al., 2007; Fairless et al., 2008; Taniguchi et al., 2007). We first assessed SorCS1 and Nrxn localization and found that both proteins colocalized in endosomes in HeLa cells and in dendrites (Fig. S3A-C), corroborating previous results demonstrating localization of Nrxn1β in dendritic endosomes (Taniguchi et al., 2007). Subcellular fractionation further indicated that there is a pool of endogenous Nrxn in the somatodendritic compartment (Fig. S2N), confirming previous observations (Berninghausen et al., 2007). Their endosomal colocalization suggests that SorCS1 and Nrxn might preferentially interact in *cis*, rather than in *trans* like other Nrxn interactions. To test whether SorCS1 and Nrxn interact in *cis* or in *trans*, we performed antibody-mediated aggregation experiments and found that clustering of SorCS1 resulted in VPS10P domain-dependent coaggregation of Nrxn1β or vice versa (Fig. S3D). Coculture assays to test whether SorCS1 might also interact with Nrxn in *trans* did not support a SorCS1-Nrxn *trans* interaction (Fig. S3E-G), indicating a preferential *cis* interaction of SorCS1 and Nrxn.

To determine whether SorCS1 regulates surface levels of Nrxn in neurons, we first overexpressed SorCS1cβ-myc (FL or ΔVPS) in hippocampal neurons. These neurons express *SorCS2* and *SorCS3*, but express *SorCS1* at very low levels or not at all (Hermey et al., 2004; Oetjen et al., 2014). We tested multiple Nrxn antibodies, but none of these proved suitable for the detection of endogenous cell surface Nrxn (data not shown). We therefore expressed Nrxn1β-SS4) tagged with an extracellular epitope (SEP-Nrxn1β) (Fu and Huang, 2010) to analyze Nrxn surface levels. Overexpression of SorCS1 FL, but not of SorCS1 ΔVPS, significantly decreased the ratio of surface/total SEP-Nrxn1β in dendrites compared to control cells (Fig. 3A, B). We next tested the effect of loss of *SorCS1* on dendritic Nrxn surface levels. We cultured cortical neurons, which strongly express *SorCS1* (Hermey et al., 2004; Oetjen et al., 2014), from *SorCS1*$^{flox/flox}$ mice (Lane et al., 2010) and expressed Cre recombinase in these cells to reduce *SorCS1* levels. Compared to control cells, the surface SEP-Nrxn1β ratio was significantly increased in *SorCS1* KO dendrites (Fig. 3C, D). This increase in surface/total SEP-Nrxn1β ratio was due to both an increase in SEP-Nrxn1β dendritic surface intensity (Fig. 3E), as well as a small decrease in SEP-Nrxn1β total intensity in *SorCS1* KO dendrites (Fig. 3F). These results indicate that overexpression of SorCS1 decreases, whereas loss of *SorCS1* increases, the dendritic surface levels of Nrxn.

Although Nrxn is present on the dendritic surface (Fairless et al., 2008; Taniguchi et al., 2007), the majority of Nrxn is expressed on the axonal surface (Fairless et al., 2008), which is functionally the most important site for Nrxns. To determine whether loss of *SorCS1* might also affect axonal Nrxn surface levels, we analyzed the SEP-Nrxn1β surface/total ratio in *SorCS1*-deficient axons. We observed strong SEP-Nrxn1β surface expression in control axons (Fig. 3G) and found that the surface SEP-Nrxn1β ratio was significantly decreased in *SorCS1* KO axons (Fig. 3G, H). This decrease underestimates the extent of downregulation of axonal Nrxn surface expression in *SorCS1* KO neurons, because we observed both a robust decrease in SEP-Nrxn1β axonal surface intensity (Fig. 3I), as well as a decrease in SEP-Nrxn1β total intensity (Fig. 3J). Together, these results show that SorCS1 controls Nrxn surface distribution in neurons in a compartment-specific manner: SorCS1 overexpression removes Nrxn from the dendritic surface, whereas *SorCS1* KO results in Nrxn accumulation on the dendritic surface and a strong loss of Nrxn from the axonal surface.

Since Nrxn is lost from the axonal surface in the absence of *SorCS1* (Fig. 3G-J), and total Nrxn intensity is decreased in *SorCS1* KO neurons (Fig. 3F, J), we asked whether loss of *SorCS1* affects total Nrxn protein levels. We analyzed whole cell lysates of DIV14 *SorCS1*$^{flox/flox}$ cortical neurons infected with a lentiviral vector encoding Cre recombinase (LV-Cre) by Western blot, and found a significant decrease in total Nrxn1β levels compared to control neurons (Fig. 3K, L). To test whether Nrxn is degraded following loss of *SorCS1*, we quantified the overlap of SEP-Nrxn1β with the lysosomal marker LAMP1 in *SorCS1* KO neurons and found a significantly increased overlap compared to control cells (Fig. S3H, I). These observations suggest that Nrxn undergoes lysosomal degradation in the absence of SorCS1-mediated sorting. Taken together, these results demonstrate that in the absence of SorCS1, Nrxn is mis-sorted to the dendritic surface, lost from the axonal surface, and ultimately degraded.

SorCS1 interactome analysis identifies neurexins and other synaptic receptors

As a sorting receptor, SorCS1 might regulate the surface trafficking of additional receptors besides Nrxn. To determine whether SorCS1 interacts with additional receptors, we characterized the molecular composition of the SorCS1 complex in the brain. We first performed affinity chromatography using recombinant SorCS1-ecto-His as bait and detergent-solubilized synaptosome extract as prey, followed by MS analysis ('SorCS1-ecto MS'; Fig. 4A). Coomassie staining showed an enrichment of proteins in the SorCS1-ecto-His pulldown lanes, which was absent when the SorCS1-ecto-His bait or the anti-His antibody were omitted (Fig. 4A). Both Nrxn1 and Nrxn2 were detected by SorCS1-ecto MS (Fig. 4C, S4A, and Table S2), in line with our results on the SorCS1-Nrxn interaction (Fig. 1).

In addition to Nrxns, we identified multiple neuronal surface receptors in the SorCS1 ectodomain interactome, several of which were synaptic proteins. These included the synaptic adhesion molecules Nlgn1 and -3 (Fig. 4C, 4D, S4A, and Table S2). Another synaptic protein identified was the AMPA glutamate receptor subunit Gria2/GluA2 (Fig. 4C, S4A, and Table S2). Various other receptors were also present in the SorCS1 ectodomain interactome, including several members of the Plexin semaphorin receptor family, the amyloid precursor protein (APP) (Lane et al., 2010), and Ntrk2/TrkB, the receptor for the neurotrophin BDNF (Table S2). The results from the SorCS1 ectodomain interactome analysis suggest that SorCS1 interacts with multiple receptors and may regulate key synaptic functions.

In a complementary approach to also identify proteins interacting with the cytoplasmic tail of SorCS1, we affinity purified SorCS1 complexes from rat brain extract using two independent antibodies against SorCS1 (Fig. S2O-Q) and a rabbit IgG control antibody, followed by LC-MS/MS analysis ('SorCS1 AP-MS'; Fig. 4B). SorCS2 and SorCS3 were not detected in these samples (Table S3), further demonstrating specificity of the antibodies used. Proteomic analysis of affinity purified SorCS1 complexes revealed a prominent presence of adaptor protein (AP)-2 complex subunits (Fig. S4B, Table S3), confirming previous findings (Nielsen et al., 2008). The AP-2 complex is important for the internalization of cargo proteins in clathrin-mediated endocytosis (Bonifacino and Traub, 2003). In addition to AP-2, the plasma membrane clathrin coat components Eps15 and epsins were also present in the SorCS1 complex (Fig. S4B, Table S3), indicating that the SorCS1 cytoplasmic domain couples to the endocytic machinery. Confirming the SorCS1-ecto MS results, we again identified peptides for the synaptic adhesion molecules Nrxn2 and Nlgn3, and the AMPAR subunits Gria2/GluA2 and -3 in the affinity purified SorCS1 complex (Fig. 4C, S4B, Table S3). Together, these results show that SorCS1 complexes captured from brain membrane extracts contain synaptic adhesion molecules and AMPA glutamate receptors.

Multiple neuronal receptors depend on SorCS1 for surface trafficking

The presence of additional neuronal receptors besides Nrxn in the SorCS1 interactome suggests that SorCS1 may more broadly regulate receptor surface trafficking. To determine whether multiple receptors depend on SorCS1 for their surface trafficking, we performed a

global, quantitative analysis of the surface proteome in control and *SorCS1*-deficient neurons using stable isotope labeling by amino acids in culture (SILAC). Cortical and hippocampal neurons were cultured from *SorCS1*$^{flox/flox}$ mice and divided into two sets. One set of control neurons was cultured in media containing stable heavy arginine and lysine isotopes and mock-infected ("heavy"); the other set of neurons was cultured in normal media and infected with LV-Cre ("light") to reduce SorCS1 levels (*SorCS1* cKO). The two sets of neurons were then surface biotinylated, lysed, mixed 1:1 and precipitated with neutravidin beads, followed by MS analysis ('*SorCS1* cKO surface SILAC analysis'; Fig. 5A and S5A). We performed two independent experiments and quantified between 1999 and 2151 proteins from 7869 and 10146 peptides, respectively (Fig. 5B and S5B-D). The corrected ratios ("light"/"heavy") of the quantified peptides show a normal distribution with a tail indicating peptides with a reduced ratio in *SorCS1* cKO cultures (Fig. 5B), indicating a specific downregulation of surface proteins in *SorCS1* KO cultures.

Manual inspection of the raw MS1 m/z spectra indicated reduced surface levels of Nrxn1 and Nlgn3, whereas the surface abundance of the cell adhesion molecule Ncam1 was not affected (Fig. 5C). We then graphed the "light"/"heavy" (*SorCS1* cKO/control) ratios for each experiment on a logarithmic scale, with the lowest ratios indicating the most strongly decreased proteins in *SorCS1*-deficient neurons. Our global surface proteome analysis also identified Nrxn1 and Nrxn2 as among the proteins with the strongest decrease in surface expression following loss of *SorCS1* (Fig. 5D and Table S4). In addition to Nrxn, the surface levels of Nlgn3 and the AMPAR subunit Gria2/GluA2, proteins that were also identified in the SorCS1 interactome analysis (Fig. 4 and Table S2, S3), were decreased (Fig. 5D). Additional receptors from the SorCS1 interactome analysis, such as APP and Plexin, also showed reduced surface expression in *SorCS1* KO neurons (Table S4). Thus, multiple neuronal receptors, including the Nrxn and Nlgn synaptic adhesion molecules and AMPARs, depend on SorCS1 for normal surface expression. These results identify SorCS1 as a global regulator of receptor surface trafficking and indicate that loss of *SorCS1* results in altered surface proteome composition.

In vivo loss of SorCS1 decreases synaptic abundance of adhesion and glutamate receptors

Many of the neuronal receptors depending on SorCS1 for their surface expression are synaptic proteins. To determine whether SorCS1 regulates receptor abundance at synapses in vivo, we performed a quantitative analysis of synaptic proteomes from *SorCS1*$^{flox/flox}$ mice crossed with *NEX-Cre* transgenic mice (Goebbels et al., 2006) to specifically decrease *SorCS1* expression in principal cortical and hippocampal neurons. Cortex and hippocampus were dissected from mature *NEX-Cre:SorCS1*$^{flox/flox}$ mice and *SorCS1*$^{flox/flox}$ control mice, homogenized, and mixed 1:1 with an internal standard consisting of cortex and hippocampus dissected from non-transgenic mice that were metabolically labeled in vivo with the stable isotope ^{15}N (Fig. 6A and Experimental Procedures). We then performed LC-MS/MS analysis of each ^{14}N biological replicate with the common ^{15}N internal standard simultaneously to obtain relative ^{14}N/^{15}N peptide ratios (SorCS1 cKO ^{15}N in vivo synapse quantitation; Fig. 6A and S6A, B). We quantified a total of 5882 proteins and found that 95% of the synaptic proteome was not affected by loss of *SorCS1*. We identified 299

significantly regulated proteins (5.1% of total proteins; ANOVA *P* value ≤0.05 and ≥20% change in expression), of which 191 proteins were down- and 108 were upregulated (Table S5).

Manual inspection of the raw MS1 m/z spectra indicated downregulated levels of Nrxn1 in *NEX-Cre:SorCS1*$^{flox/flox}$ synaptosomes compared to ^{15}N controls, whereas the abundance of Ncam1 was not altered (Fig. 6B, S6A). Overall, Nrxn1 was among the most significantly downregulated proteins in *SorCS1*-deficient synaptosomes (1.5-fold downregulation; Fig. 6C, S6C, D, and Table S5). To verify the quantitative MS results, we analyzed Nrxn protein levels in *SorCS1*$^{flox/flox}$ and *NEX-Cre:SorCS1*$^{flox/flox}$ synaptosomes by Western blot and found that loss of *SorCS1* significantly decreased Nrxn levels (Fig. 6E and S6E).

In addition to Nrxn, several other synaptic adhesion molecules were detected among the significantly downregulated proteins in *NEX-Cre:SorCS1*$^{flox/flox}$ synaptosomes, including the Contactin-4 (Cntn4; 2-fold downregulation) and leucine-rich repeat (LRR)-containing synaptic adhesion molecules such as LRRC4B/NGL-3 (1.5-fold downregulation). Besides adhesion molecules, ionotropic glutamate receptor abundance was also significantly decreased in *SorCS1* KO synaptosomes. All four AMPAR subunits (Gria1/GluA1, -2, -3, and -4) and the NMDA receptor subunits Grin2a/GluN2A and -2B were downregulated (all 1.2-fold downregulation (Fig. 6C, S6D, and Table S5). Taken together, the manual inspection of the downregulated proteins suggests that loss of *SorCS1* decreases the abundance of adhesion proteins and glutamate receptors.

We next performed Gene Ontology (GO) analysis to identify over-represented biological processes and classes of proteins in our dataset in an unbiased way. We compared the significantly downregulated proteins to the total collection of quantified proteins and found that cell-cell signaling, synaptic transmission, cell adhesion and cell communication were the four most significantly over-represented biological processes (Fig. 6D, S6F, H). In contrast, GO analysis of upregulated proteins did not reveal such significant enrichment of any process (Fig. S6G). Grouping of significantly altered proteins by protein class showed that receptors and ion channels together represented roughly half of all downregulated proteins (Fig. S6I), indicating that loss of *SorCS1* affects surface protein trafficking at synapses in vivo. Taken together, loss of *SorCS1* in principal glutamatergic neurons of hippocampus and cortex decreases the synaptic abundance of receptors regulating cell adhesion and synaptic transmission.

Importantly, Nrxns and AMPARs were detected in four independent, unbiased proteomic approaches assessing SorCS1 function (Fig. 6F): SorCS1-ecto MS and AP-MS (Fig. 4); *SorCS1* cKO surface SILAC analysis (Fig. 5); and SorCS1 cKO ^{15}N in vivo synapse quantitation (Fig. 6). Specifically, Gria2/GluA2 was significantly identified with all four approaches (joint probability of such event occurring by chance = 0.0009; see Experimental Procedures). In addition, three more proteins were significantly detected using three of these methods: Nrxn1 (SorCS1 cKO ^{15}N in vivo synapse quantitation, *SorCS1* cKO surface SILAC, and SorCS1-ecto MS; joint probability = 0.0023), Nrxn2 (*SorCS1* cKO surface SILAC, SorCS1-ecto MS, and SorCS1 AP-MS; joint probability = 0.0107), and Gria3/GluA3 (SorCS1 cKO ^{15}N in vivo synapse quantitation, *SorCS1* cKO surface SILAC, and

SorCS1 AP-MS; joint probability = 0.0015). Together, the combined results from four independent approaches indicate that Nrxns and AMPARs represent key proteins depending on SorCS1 for their surface and synaptic abundance.

SorCS1 is required for basal glutamatergic and GABAergic synaptic transmission—In the final series of experiments, we asked how an altered abundance of synaptic receptors in the absence of *SorCS1* affects synaptic transmission. To assess basal synaptic transmission in cortical slices, we recorded spontaneous miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs, respectively) from somatosensory layer 5 pyramidal neurons, which normally express *SorCS1* (Hermey et al., 2004; Oetjen et al., 2014) (Allen Brain Atlas). Recordings were performed in P18-21 acute slices from control *SorCS1*$^{flox/flox}$ animals and *SorCS1*$^{flox/flox}$ mice crossed with *Emx1-Cre* mice to decrease *SorCS1* expression in hippocampal and cortical principal neurons (Guo et al., 2000). The frequency of mEPSCs and mIPSCs was strongly decreased in *Emx1-Cre:SorCS1*$^{flox/flox}$ cortical neurons compared to controls, whereas amplitude and decay time were not affected (Fig. 7A-C, S7A-C). We next cultured *SorCS1*$^{flox/flox}$ cortical neurons and electroporated them with Cre or GFP control plasmids. We recorded mEPSCs and mIPSCs from DIV12-16 neurons and found a similar decrease in the frequency of these events in *SorCS1*$^{flox/flox}$ neurons expressing Cre (+ Cre) compared to control *SorCS1*$^{flox/flox}$ neurons (+ EGFP) (Fig. 7D, E). Amplitude, decay time and membrane potential were not affected by loss of *SorCS1* (Fig. 7F, S7D-G). To test whether the effects of loss of *SorCS1* on spontaneous synaptic transmission are cell-autonomous, we recorded mPSCs from neighboring non-electroporated *SorCS1*$^{flox/flox}$ neurons (− Cre) in Cre-electroporated cultures. We found no change in the frequency, amplitude, or decay kinetics of mEPSCs and mIPSCs in - Cre *SorCS1*$^{flox/flox}$ neurons (Fig. 7E, F, S7D-G), indicating that the effects of loss of *SorCS1* on mPSC frequency are cell-autonomous. Together, these results show that the tone of spontaneous glutamatergic and GABAergic transmission is reduced in the absence of *SorCS1*.

A decrease in mPSC frequency could be due to a reduced probability of spontaneous release or a decrease in synapse density. Since our data indicate that the decrease in mPSC frequency occurs only in *SorCS1*-deficient neurons and not in neighboring non-electroporated cells in the same culture, we focused on postsynaptic mechanisms. To examine synapse number in *SorCS1* KO neurons, we quantified excitatory and inhibitory synapse density in DIV14 *SorCS1*$^{flox/flox}$ cortical neurons electroporated with Cre or control plasmids. Loss of *SorCS1* did not affect the density of puncta positive for the excitatory synaptic markers PSD95 and VGluT1 (Fig. 7G, H), but caused a significant decrease in the density of puncta positive for the inhibitory synaptic markers Gephyrin/VGAT (Fig. 7I, J). These effects were specific for cortical neurons, since loss of *SorCS1* in hippocampal neurons, which normally express *SorCS1* at very low levels or not at all (Hermey et al., 2004; Oetjen et al., 2014) (Allen Brain Atlas), did not affect synapse density or mEPSC frequency (Fig. S7H-N). However, SorCS1 overexpression in hippocampal neurons increased functional excitatory synapse density, but did not affect inhibitory synapse density (Fig. S7O-V). These results show that loss of *SorCS1* differentially affects excitatory and

inhibitory synapses: excitatory synapse density is unaltered in *SorCS1* KO neurons, whereas inhibitory synapse density is decreased.

The decreased inhibitory synapse density in *SorCS1* KO neurons can account for the reduced mIPSC frequency in these cells, but the decrease in mEPSC frequency must be due to another mechanism. Given that AMPARs were consistently identified as regulated by SorCS1 (Fig. 6F), we asked whether a loss of AMPAR surface expression at synapses might explain the decreased mEPSC frequency in the absence of *SorCS1*. Cortical DIV14 $SorCS1^{flox/flox}$ neurons electroporated with Cre or GFP were fixed and immunostained under non-permeabilizing conditions for surface GluA1- and GluA2-containing AMPARs. We found a significant decrease in overall GluA1 and GluA2 surface levels in *SorCS1* KO dendrites (Fig. 7K,L, N, O). To determine whether synaptic surface AMPAR levels were also reduced in *SorCS1* KO neurons, we quantified the GluA1 and GluA2 surface puncta colocalizing with the synaptic markers PSD95 and VGluT1. Synaptic GluA1 and GluA2 surface levels were also significantly decreased in *SorCS1* KO neurons compared to control cells (Fig. 7K, M, N, P). In contrast, dendritic and synaptic surface levels of the β2/3 subunit of the inhibitory $GABA_A$ receptor were not altered in *SorCS1* KO neurons (Fig. 7Q-S). Taken together, these results indicate that SorCS1 is required to maintain surface and synaptic abundance of AMPARs, and that loss of *SorCS1* leads to a decrease in synaptic and extrasynaptic AMPAR surface levels.

The decreased mEPSC frequency in *SorCS1* KO neurons suggest that a population of synapses lacks AMPAR surface expression in the absence of *SorCS1*. To test whether the fraction of AMPAR-lacking synapses is increased in *SorCS1* KO neurons, we labeled surface GluA1 and the NMDA receptor subunit NR1 under non-permeabilizing conditions, followed by a permeabilization step to label VGluT1 (Fig. 7T). Quantification of the density of VGluT1-positive synapses containing surface NR1 but lacking surface GluA1 revealed a significant increase in the fraction of AMPAR-lacking synapses in *SorCS1* KO neurons (Fig. 7U). Together, these results show that loss of *SorCS1* differentially affects excitatory and inhibitory synapses: excitatory synapse density is unaffected in *SorCS1* KO neurons, but the fraction of synapses lacking functional AMPARs is increased. The density of inhibitory synapses on the other hand is decreased in *SorCS1* KO neurons, but $GABA_AR$ surface expression at remaining inhibitory synapses is not impaired.

Discussion

Here we identify the sorting receptor SorCS1 as a key regulator of synaptic receptor trafficking. Our proteomic analyses identify the synaptic adhesion molecule Nrxn and the AMPA glutamate receptor as the major proteins sorted by SorCS1. SorCS1 localizes to early and recycling endosomes and regulates Nrxn and AMPAR surface expression in neurons. SorCS1 is found in a molecular complex with Nrxn, other synaptic adhesion molecules, and AMPARs. In cultured neurons, SorCS1 maintains surface levels of these receptors. In vivo, SorCS1 maintains the synaptic abundance of receptors regulating cell adhesion and synaptic transmission, including Nrxns and AMPARs. Loss of *SorCS1* decreases both glutamatergic and GABAergic synaptic transmission due to impaired AMPAR surface levels at excitatory synapses and a decrease in inhibitory synapse density, respectively. Together, our results

show that SorCS1 regulates the trafficking of neuronal receptors that are essential for synaptic function.

Regulation of synaptic receptor trafficking by SorCS1

Our findings show that the surface and synaptic levels of multiple receptors are affected in the absence of *SorCS1*. How can the loss of a sorting protein affect the expression of many surface receptors? Our MS analysis of affinity purified SorCS1 complexes identified AP-2 complex subunits, in agreement with results from a previous yeast three-hybrid analysis (Nielsen et al., 2008). The AP-2 complex plays an important role in the sorting of transmembrane receptors from the cell surface to endosomes (Bonifacino and Traub, 2003; Traub, 2009), which regulate many key processes in neurons including the cell surface distribution of receptors (Yap and Winckler, 2012). In addition to the AP-2 complex, SorCS1 also interacts with the retromer protein Vps35 (Lane et al., 2010; Lane et al., 2013). The retromer complex (Burd and Cullen, 2014) is emerging as a major regulator of endosomal sorting and regulates retrieval of transmembrane cargo proteins from endosomes to the TGN for recycling (Bonifacino and Hurley, 2008; Seaman, 2012), as well as surface delivery of receptors from endosomes (Choy et al., 2014; Small and Petsko, 2015). Knockdown of Vps35 in HeLa cells downregulates the surface expression of 152 membrane proteins (Steinberg et al., 2013). In these cells, retromer function is required to maintain surface expression and prevent lysosomal degradation of a broad array of receptors. The changes in surface proteome composition following Vps35 knockdown in HeLa cells are reminiscent of the changes in receptor surface expression we observe in *SorCS1* KO neurons. By interacting with the AP-2 and retromer complexes, SorCS1 links to major intracellular sorting pathways regulating endocytosis and recycling of cargo, thereby acting as a hub in the control of receptor surface expression in neurons. In addition to direct effects of SorCS1 on receptor trafficking, some of the changes in protein levels following *SorCS1* loss-of-function are likely indirect. These might represent proteins that depend on downregulated receptors for their stabilization, or represent compensatory changes.

Four independent SorCS1 proteomic approaches identified Nrxn and AMPARs as the major proteins sorted by SorCS1, and two quantitative proteomic analyses indicated that their surface and synaptic levels were decreased. Our data indicates that Nrxn undergoes a SorCS1-mediated sorting step in the somatodendritic compartment, and that this sorting step is required to maintain accurate Nrxn levels on the axon surface and in the neuron. Interestingly, the targeting of the axonal receptors L1/NgCAM and the Nrxn family member CASPR2 to axons depends on endocytosis in the somatodendritic compartment. Upon interference with endocytosis, these proteins are mis-sorted to the somatodendritic surface and end up being degraded in lysosomes (Bel et al., 2009; Wisco et al., 2003; Yap et al., 2008). Similarly, axonal trafficking of Nrxn may depend on SorCS1-mediated endocytosis and endosomal sorting in the somatodendritic domain, followed by transport of Nrxn into axons. Thus, SorCS1 could mediate sorting of cargo proteins in endosomes to regulate their recycling back to the cell surface and prevent cargo from entering lysosomal degradation pathways. Future work will delineate the exact mechanisms by which SorCS1 regulates the intracellular trafficking of different cargo proteins.

SorCS1 and synaptic function

Loss of *SorCS1* reduced spontaneous glutamatergic and GABAergic synaptic transmission. The decrease in mPSC frequency in *SorCS1* KO neurons is cell-autonomous and did not occur in neighboring non-transfected cells, indicating a postsynaptic origin of these defects. We do not exclude the possibility that there may be additional presynaptic defects in *SorCS1* neurons that we were unable to resolve in our current experiments. Several proteins involved in regulating presynaptic function are also downregulated in *SorCS1* KO synaptosomes (Table S5), but these do not seem to be involved in the phenotype we observe here. Instead, the decreased glutamatergic transmission in *SorCS1* KO neurons could be attributed to impaired surface expression of AMPARs. Our data indicates an increased fraction of excitatory synapses lacking functional AMPARs following loss of *SorCS1*, whereas the remaining synapses appear to have normal AMPAR content, resulting in decreased mEPSC frequency. Similar impairments in AMPAR surface levels have been observed following loss of the intracellular trafficking regulators Vps35, the sorting nexin SNX27, and Neurobeachin (Choy et al., 2014; Hussain et al., 2014; Loo et al., 2014; Nair et al., 2013; Wang et al., 2013), and loss of Neurobeachin similarly results in the silencing of a population of excitatory synapses (Nair et al., 2013). SorCS1, Vps35 and SNX27 are all linked to retromer function, highlighting the importance of this complex in regulating synaptic AMPAR trafficking. Our results suggest that the main role of SorCS1-mediated sorting of AMPARs is in controlling surface delivery, similar to what has been proposed for Vps35 (Choy et al., 2014).

Impaired trafficking of synaptic adhesion molecules could also contribute to the reduction in AMPAR synaptic surface levels in *SorCS1*-deficient neurons. Nlgn3, which was strongly downregulated on the surface of *SorCS1* KO neurons, regulates AMPAR trafficking (Chanda et al., 2015). The interaction of Nrxn with Nlgn1 and LRRTM2 also controls AMPAR trafficking (Aoto et al., 2013), indicating that synaptic adhesion complexes regulate postsynaptic AMPAR levels. Thus, impaired trafficking of adhesion molecules in the absence of *SorCS1* could affect AMPAR surface stabilization at synapses, emphasizing the closely linked function of these two classes of receptors. The decreased inhibitory synaptic transmission in *SorCS1* KO neurons on the other hand was not caused by impaired $GABA_A$ receptor trafficking, but could be attributed to a decrease in inhibitory synapse density. This decrease might reflect a compensatory response to reduced glutamatergic synaptic transmission in *SorCS1* KO neurons, as overexpression of SorCS1 in hippocampal neurons did not affect inhibitory synapse density.

SorCS1 and synaptopathies

SORCS genes have been associated with a range of synaptopathies, including Alzheimer's disease and autism (Christoforou et al., 2011; Grupe et al., 2006; Laumet et al., 2010; Li et al., 2008; Liang et al., 2009; Lionel et al., 2011; Ollila et al., 2009; Reitz et al., 2011; Sanders et al., 2012). Impaired neuronal receptor trafficking resulting from *SORCS1* mutations could contribute to defects in synaptic proteome composition and function underlying synaptopathies. A SorCS1 cargo protein directly relevant for disease is APP, which accumulates in endosomes in the absence of *SorCS1*, increasing levels of Alzheimer's Aβ peptide (Lane et al., 2010; Lane et al., 2013; Reitz et al., 2011) that is detrimental to

synapse function (Mucke and Selkoe, 2012). Loss of *SorCS1* also affects trafficking of the synaptic organizing proteins Nlgns and Nrxns, which regulate many aspects of synapse development and function (Krueger et al., 2012), and have been linked to autism (Baudouin et al., 2012; Morrow et al., 2008; Tabuchi et al., 2007) and schizophrenia (Etherton et al., 2009; Rujescu et al., 2009). In addition to SorCS1's synaptic function, SorCS2 has recently been shown to act as a coreceptor of the p75 neurotrophin receptor in mediating proneurotrophin-induced growth cone collapse (Anastasia et al., 2013; Deinhardt et al., 2011; Glerup et al., 2014). These findings suggest that other SorCS family members could affect neuronal morphology and wiring in different ways.

Interestingly, our functional analysis indicates that the impact of loss of *SorCS1* on excitatory synapses is relatively subtle; increasing the fraction of functional AMPAR-lacking 'silent' synapses, thereby dampening synaptic transmission but not abolishing it. The persistence of immature, AMPAR-lacking synapses could affect the plasticity and activity-dependent postnatal refinement of cortical circuits, and has been observed in a model of Fragile X mental retardation (Harlow et al., 2010). In the long-term, impaired activity might lead to synaptic loss and eventual neuronal degeneration. Impaired intracellular trafficking is emerging as a common theme in neurodegenerative diseases (Small and Petsko, 2015), underscoring the importance of regulation of receptor trafficking for the maintenance of synaptic composition and function.

Experimental Procedures

Please see online Supplemental Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Christopher Parkhurst, Patrik Verstreken, Rose Goodchild, Matthew Holt, Kevin Lűthy and Peter Penzes for comments on the manuscript; Casper Hoogenraad, Wim Annaert and Ragna Sannerud for reagents and advice; Davide Comoletti for providing Nrxn-ecto-Fc stable cell lines; and Sung Kyu Park, Merve Oney, Christine Wu, Mohit Patel, Margaret Butko, Jacqueline Benthuysen and Max Caccese for technical assistance; Yates, Ghosh, and De Wit lab members for comments. This work was supported by NIH awards F32AG039127 and 4 R00DC013805-02 (JNS); Conseil Régional Aquitaine (IC/OT), Fondation pour la Recherche Médicale (OT), and Agence Nationale de la Recherche (OT); NIH grants R01MH068578 and R01NS067216 (AG); NIH grants P41 GM103533 and R01 MH067880 (JRY); and a NARSAD Young Investigator Award from the Brain and Behavior Research Foundation, ERC Starting Grant (#311083) and FWO Odysseus Grant (JdW). The RAW files, search/quantitative results, and complete parameter files for all mass spectrometry experiments will be publicly available at http://fields.scripps.edu/published/SorCS_NRXN upon publication.

References

Anastasia A, Deinhardt K, Chao MV, Will NE, Irmady K, Lee FS, Hempstead BL, Bracken C. Val66Met polymorphism of BDNF alters prodomain structure to induce neuronal growth cone retraction. Nat Commun. 2013; 4:2490. [PubMed: 24048383]

Anggono V, Huganir RL. Regulation of AMPA receptor trafficking and synaptic plasticity. Curr Opin Neurobiol. 2012; 22:461–469. [PubMed: 22217700]

Aoto J, Martinelli DC, Malenka RC, Tabuchi K, Sudhof TC. Presynaptic neurexin-3 alternative splicing trans-synaptically controls postsynaptic AMPA receptor trafficking. Cell. 2013; 154:75–88. [PubMed: 23827676]

Barker AJ, Koch SM, Reed J, Barres BA, Ullian EM. Developmental control of synaptic receptivity. J Neurosci. 2008; 28:8150–8160. [PubMed: 18701677]

Baudouin S, Scheiffele P. SnapShot: Neuroligin-neurexin complexes. Cell. 2010; 141:908, e901. 908. [PubMed: 20510934]

Baudouin SJ, Gaudias J, Gerharz S, Hatstatt L, Zhou K, Punnakkal P, Tanaka KF, Spooren W, Hen R, De Zeeuw CI, et al. Shared synaptic pathophysiology in syndromic and nonsyndromic rodent models of autism. Science. 2012; 338:128–132. [PubMed: 22983708]

Bayes A, Collins MO, Croning MD, van de Lagemaat LN, Choudhary JS, Grant SG. Comparative study of human and mouse postsynaptic proteomes finds high compositional conservation and abundance differences for key synaptic proteins. PLoS One. 2012; 7:e46683. [PubMed: 23071613]

Bel C, Oguievetskaia K, Pitaval C, Goutebroze L, Faivre-Sarrailh C. Axonal targeting of Caspr2 in hippocampal neurons via selective somatodendritic endocytosis. J Cell Sci. 2009; 122:3403–3413. [PubMed: 19706678]

Berninghausen O, Rahman MA, Silva JP, Davletov B, Hopkins C, Ushkaryov YA. Neurexin Ibeta and neuroligin are localized on opposite membranes in mature central synapses. J Neurochem. 2007; 103:1855–1863. [PubMed: 17868325]

Bonifacino JS, Hurley JH. Retromer. Curr Opin Cell Biol. 2008; 20:427–436. [PubMed: 18472259]

Bonifacino JS, Traub LM. Signals for sorting of transmembrane proteins to endosomes and lysosomes. Annu Rev Biochem. 2003; 72:395–447. [PubMed: 12651740]

Boucard AA, Chubykin AA, Comoletti D, Taylor P, Sudhof TC. A splice code for trans-synaptic cell adhesion mediated by binding of neuroligin 1 to alpha- and beta-neurexins. Neuron. 2005; 48:229–236. [PubMed: 16242404]

Breiderhoff T, Christiansen GB, Pallesen LT, Vaegter C, Nykjaer A, Holm MM, Glerup S, Willnow TE. Sortilin-Related Receptor SORCS3 Is a Postsynaptic Modulator of Synaptic Depression and Fear Extinction. PLoS One. 2013; 8:e75006. [PubMed: 24069373]

Burd C, Cullen PJ. Retromer: a master conductor of endosome sorting. Cold Spring Harb Perspect Biol. 2014; 6

Cajigas IJ, Will T, Schuman EM. Protein homeostasis and synaptic plasticity. Embo J. 2010; 29:2746–2752. [PubMed: 20717144]

Carroll RC, Beattie EC, von Zastrow M, Malenka RC. Role of AMPA receptor endocytosis in synaptic plasticity. Nat Rev Neurosci. 2001; 2:315–324. [PubMed: 11331915]

Chanda S, Aoto J, Lee SJ, Wernig M, Sudhof TC. Pathogenic mechanism of an autism-associated neuroligin mutation involves altered AMPA-receptor trafficking. Mol Psychiatry. 2015

Choquet D, Triller A. The dynamic synapse. Neuron. 2013; 80:691–703. [PubMed: 24183020]

Choy RW, Park M, Temkin P, Herring BE, Marley A, Nicoll RA, von Zastrow M. Retromer mediates a discrete route of local membrane delivery to dendrites. Neuron. 2014; 82:55–62. [PubMed: 24698268]

Christoforou A, McGhee KA, Morris SW, Thomson PA, Anderson S, McLean A, Torrance HS, Le Hellard S, Pickard BS, StClair D, et al. Convergence of linkage, association and GWAS findings for a candidate region for bipolar disorder and schizophrenia on chromosome 4p. Mol Psychiatry. 2011; 16:240–242. [PubMed: 20351716]

de Wit J, Sylwestrak E, O'Sullivan ML, Otto S, Tiglio K, Savas JN, Yates JR 3rd, Comoletti D, Taylor P, Ghosh A. LRRTM2 interacts with Neurexin1 and regulates excitatory synapse formation. Neuron. 2009; 64:799–806. [PubMed: 20064388]

Dean C, Scholl FG, Choih J, DeMaria S, Berger J, Isacoff E, Scheiffele P. Neurexin mediates the assembly of presynaptic terminals. Nat Neurosci. 2003; 6:708–716. [PubMed: 12796785]

Deinhardt K, Kim T, Spellman DS, Mains RE, Eipper BA, Neubert TA, Chao MV, Hempstead BL. Neuronal growth cone retraction relies on proneurotrophin receptor signaling through Rac. Sci Signal. 2011; 4:ra82. [PubMed: 22155786]

Etherton MR, Blaiss CA, Powell CM, Sudhof TC. Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. Proc Natl Acad Sci U S A. 2009; 106:17998–18003. [PubMed: 19822762]

Fairless R, Masius H, Rohlmann A, Heupel K, Ahmad M, Reissner C, Dresbach T, Missler M. Polarized targeting of neurexins to synapses is regulated by their C-terminal sequences. J Neurosci. 2008; 28:12969–12981. [PubMed: 19036990]

Farias GG, Cuitino L, Guo X, Ren X, Jarnik M, Mattera R, Bonifacino JS. Signal-mediated, AP-1/clathrin-dependent sorting of transmembrane receptors to the somatodendritic domain of hippocampal neurons. Neuron. 2012; 75:810–823. [PubMed: 22958822]

Fu Y, Huang ZJ. Differential dynamics and activity-dependent regulation of alpha- and beta-neurexins at developing GABAergic synapses. Proc Natl Acad Sci U S A. 2010; 107:22699–22704. [PubMed: 21149722]

Giagtzoglou N, Ly CV, Bellen HJ. Cell adhesion, the backbone of the synapse: “vertebrate” and “invertebrate” perspectives. Cold Spring Harb Perspect Biol. 2009; 1:a003079. [PubMed: 20066100]

Glerup S, Olsen D, Vaegter CB, Gustafsen C, Sjoegaard SS, Hermey G, Kjolby M, Molgaard S, Ulrichsen M, Boggild S, et al. SorCS2 Regulates Dopaminergic Wiring and Is Processed into an Apoptotic Two-Chain Receptor in Peripheral Glia. Neuron. 2014; 82:1074–1087. [PubMed: 24908487]

Goebbels S, Bormuth I, Bode U, Hermanson O, Schwab MH, Nave KA. Genetic targeting of principal neurons in neocortex and hippocampus of NEX-Cre mice. Genesis. 2006; 44:611–621. [PubMed: 17146780]

Graf ER, Zhang X, Jin SX, Linhoff MW, Craig AM. Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. Cell. 2004; 119:1013–1026. [PubMed: 15620359]

Grant SG. Synaptopathies: diseases of the synaptome. Curr Opin Neurobiol. 2012; 22:522–529. [PubMed: 22409856]

Grant SG. SnapShot: Organizational Principles of the Postsynaptic Proteome. Neuron. 2013; 80:534–534. e531. [PubMed: 24139049]

Grupe A, Li Y, Rowland C, Nowotny P, Hinrichs AL, Smemo S, Kauwe JS, Maxwell TJ, Cherny S, Doil L, et al. A scan of chromosome 10 identifies a novel locus showing strong association with late-onset Alzheimer disease. Am J Hum Genet. 2006; 78:78–88. [PubMed: 16385451]

Guo H, Hong S, Jin XL, Chen RS, Avasthi PP, Tu YT, Ivanco TL, Li Y. Specificity and efficiency of Cre-mediated recombination in Emx1-Cre knock-in mice. Biochem Biophys Res Commun. 2000; 273:661–665. [PubMed: 10873661]

Harlow EG, Till SM, Russell TA, Wijetunge LS, Kind P, Contractor A. Critical period plasticity is disrupted in the barrel cortex of FMR1 knockout mice. Neuron. 2010; 65:385–398. [PubMed: 20159451]

Hermey G. The Vps10p-domain receptor family. Cell Mol Life Sci. 2009; 66:2677–2689. [PubMed: 19434368]

Hermey G, Plath N, Hubner CA, Kuhl D, Schaller HC, Hermans-Borgmeyer I. The three sorCS genes are differentially expressed and regulated by synaptic activity. J Neurochem. 2004; 88:1470–1476. [PubMed: 15009648]

Hermey G, Riedel IB, Hampe W, Schaller HC, Hermans-Borgmeyer I. Identification and characterization of SorCS, a third member of a novel receptor family. Biochem Biophys Res Commun. 1999; 266:347–351. [PubMed: 10600506]

Hermey G, Riedel IB, Rezgaoui M, Westergaard UB, Schaller C, Hermans-Borgmeyer I. SorCS1, a member of the novel sorting receptor family, is localized in somata and dendrites of neurons throughout the murine brain. Neurosci Lett. 2001; 313:83–87. [PubMed: 11684345]

Hussain NK, Diering GH, Sole J, Anggono V, Huganir RL. Sorting Nexin 27 regulates basal and activity-dependent trafficking of AMPARs. Proc Natl Acad Sci U S A. 2014; 111:11840–11845. [PubMed: 25071192]

Ichtchenko K, Hata Y, Nguyen T, Ullrich B, Missler M, Moomaw C, Sudhof TC. Neuroligin 1: a splice site-specific ligand for beta-neurexins. Cell. 1995; 81:435–443. [PubMed: 7736595]

Ko J, Fuccillo MV, Malenka RC, Sudhof TC. LRRTM2 functions as a neurexin ligand in promoting excitatory synapse formation. Neuron. 2009; 64:791–798. [PubMed: 20064387]

Krueger DD, Tuffy LP, Papadopoulos T, Brose N. The role of neurexins and neuroligins in the formation, maturation, and function of vertebrate synapses. Curr Opin Neurobiol. 2012; 22:412–422. [PubMed: 22424845]

Lane RF, Raines SM, Steele JW, Ehrlich ME, Lah JA, Small SA, Tanzi RE, Attie AD, Gandy S. Diabetes-associated SorCS1 regulates Alzheimer's amyloid-beta metabolism: evidence for involvement of SorL1 and the retromer complex. J Neurosci. 2010; 30:13110–13115. [PubMed: 20881129]

Lane RF, Steele JW, Cai D, Ehrlich ME, Attie AD, Gandy S. Protein sorting motifs in the cytoplasmic tail of SorCS1 control generation of Alzheimer's amyloid-beta peptide. J Neurosci. 2013; 33:7099–7107. [PubMed: 23595767]

Laumet G, Chouraki V, Grenier-Boley B, Legry V, Heath S, Zelenika D, Fievet N, Hannequin D, Delepine M, Pasquier F, et al. Systematic analysis of candidate genes for Alzheimer's disease in a French, genome-wide association study. J Alzheimers Dis. 2010; 20:1181–1188. [PubMed: 20413850]

Li H, Wetten S, Li L, St Jean PL, Upmanyu R, Surh L, Hosford D, Barnes MR, Briley JD, Borrie M, et al. Candidate single-nucleotide polymorphisms from a genomewide association study of Alzheimer disease. Arch Neurol. 2008; 65:45–53. [PubMed: 17998437]

Liang X, Slifer M, Martin ER, Schnetz-Boutaud N, Bartlett J, Anderson B, Zuchner S, Gwirtsman H, Gilbert JR, Pericak-Vance MA, Haines JL. Genomic convergence to identify candidate genes for Alzheimer disease on chromosome 10. Hum Mutat. 2009; 30:463–471. [PubMed: 19241460]

Lionel AC, Crosbie J, Barbosa N, Goodale T, Thiruvahindrapuram B, Rickaby J, Gazzellone M, Carson AR, Howe JL, Wang Z, et al. Rare copy number variation discovery and cross-disorder comparisons identify risk genes for ADHD. Sci Transl Med. 2011; 3:95ra75.

Loo LS, Tang N, Al-Haddawi M, Dawe GS, Hong W. A role for sorting nexin 27 in AMPA receptor trafficking. Nat Commun. 2014; 5:3176. [PubMed: 24458027]

Missler M, Sudhof TC, Biederer T. Synaptic cell adhesion. Cold Spring Harb Perspect Biol. 2012; 4:a005694. [PubMed: 22278667]

Missler M, Zhang W, Rohlmann A, Kattenstroth G, Hammer RE, Gottmann K, Sudhof TC. Alpha-neurexins couple Ca2+ channels to synaptic vesicle exocytosis. Nature. 2003; 423:939–948. [PubMed: 12827191]

Morrow EM, Yoo SY, Flavell SW, Kim TK, Lin Y, Hill RS, Mukaddes NM, Balkhy S, Gascon G, Hashmi A, et al. Identifying autism loci and genes by tracing recent shared ancestry. Science. 2008; 321:218–223. [PubMed: 18621663]

Mucke L, Selkoe DJ. Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction. Cold Spring Harb Perspect Med. 2012; 2:a006338. [PubMed: 22762015]

Nair R, Lauks J, Jung S, Cooke NE, de Wit H, Brose N, Kilimann MW, Verhage M, Rhee J. Neurobeachin regulates neurotransmitter receptor trafficking to synapses. J Cell Biol. 2013; 200:61–80. [PubMed: 23277425]

Nielsen MS, Gustafsen C, Madsen P, Nyengaard JR, Hermey G, Bakke O, Mari M, Schu P, Pohlmann R, Dennes A, Petersen CM. Sorting by the cytoplasmic domain of the amyloid precursor protein binding receptor SorLA. Mol Cell Biol. 2007; 27:6842–6851. [PubMed: 17646382]

Nielsen MS, Keat SJ, Hamati JW, Madsen P, Gutzmann JJ, Engelsberg A, Pedersen KM, Gustafsen C, Nykjaer A, Gliemann J, et al. Different motifs regulate trafficking of SorCS1 isoforms. Traffic. 2008; 9:980–994. [PubMed: 18315530]

Nielsen MS, Madsen P, Christensen EI, Nykjaer A, Gliemann J, Kasper D, Pohlmann R, Petersen CM. The sortilin cytoplasmic tail conveys Golgi-endosome transport and binds the VHS domain of the GGA2 sorting protein. Embo J. 2001; 20:2180–2190. [PubMed: 11331584]

Nykjaer A, Willnow TE. Sortilin: a receptor to regulate neuronal viability and function. Trends Neurosci. 2012; 35:261–270. [PubMed: 22341525]

O'Sullivan ML, de Wit J, Savas JN, Comoletti D, Otto-Hitt S, Yates JR 3rd, Ghosh A. FLRT Proteins Are Endogenous Latrophilin Ligands and Regulate Excitatory Synapse Development. Neuron. 2012; 73:903–910. [PubMed: 22405201]

Oetjen S, Mahlke C, Hermans-Borgmeyer I, Hermey G. Spatio-temporal expression analysis of the growth factor receptor SorCS3. J Comp Neurol. 2014

Ollila HM, Soronen P, Silander K, Palo OM, Kieseppa T, Kaunisto MA, Lonnqvist J, Peltonen L, Partonen T, Paunio T. Findings from bipolar disorder genome-wide association studies replicate in a Finnish bipolar family-cohort. Mol Psychiatry. 2009; 14:351–353. [PubMed: 19308021]

Reichelt AC, Rodgers RJ, Clapcote SJ. The role of neurexins in schizophrenia and autistic spectrum disorder. Neuropharmacology. 2012; 62:1519–1526. [PubMed: 21262241]

Reitz C, Tokuhiro S, Clark LN, Conrad C, Vonsattel JP, Hazrati LN, Palotas A, Lantigua R, Medrano M, I ZJ-V, et al. SORCS1 alters amyloid precursor protein processing and variants may increase Alzheimer's disease risk. Ann Neurol. 2011; 69:47–64. [PubMed: 21280075]

Rezgaoui M, Hermey G, Riedel IB, Hampe W, Schaller HC, Hermans-Borgmeyer I. Identification of SorCS2, a novel member of the VPS10 domain containing receptor family, prominently expressed in the developing mouse brain. Mech Dev. 2001; 100:335–338. [PubMed: 11165493]

Rujescu D, Ingason A, Cichon S, Pietilainen OP, Barnes MR, Toulopoulou T, Picchioni M, Vassos E, Ettinger U, Bramon E, et al. Disruption of the neurexin 1 gene is associated with schizophrenia. Hum Mol Genet. 2009; 18:988–996. [PubMed: 18945720]

Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, Ercan-Sencicek AG, DiLullo NM, Parikshak NN, Stein JL, et al. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature. 2012; 485:237–241. [PubMed: 22495306]

Savas JN, De Wit J, Comoletti D, Zemla R, Ghosh A, Yates JR 3rd. Ecto-Fc MS identifies ligand-receptor interactions through extracellular domain Fc fusion protein baits and shotgun proteomic analysis. Nat Protoc. 2014; 9:2061–2074. [PubMed: 25101821]

Scheiffele P, Fan J, Choih J, Fetter R, Serafini T. Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. Cell. 2000; 101:657–669. [PubMed: 10892652]

Schwenk J, Baehrens D, Haupt A, Bildl W, Boudkkazi S, Roeper J, Fakler B, Schulte U. Regional diversity and developmental dynamics of the AMPA-receptor proteome in the mammalian brain. Neuron. 2014; 84:41–54. [PubMed: 25242221]

Schwenk J, Harmel N, Brechet A, Zolles G, Berkefeld H, Muller CS, Bildl W, Baehrens D, Huber B, Kulik A, et al. High-resolution proteomics unravel architecture and molecular diversity of native AMPA receptor complexes. Neuron. 2012; 74:621–633. [PubMed: 22632720]

Seaman MN. The retromer complex - endosomal protein recycling and beyond. J Cell Sci. 2012; 125:4693–4702. [PubMed: 23148298]

Shanks NF, Savas JN, Maruo T, Cais O, Hirao A, Oe S, Ghosh A, Noda Y, Greger IH, Yates JR 3rd, Nakagawa T. Differences in AMPA and kainate receptor interactomes facilitate identification of AMPA receptor auxiliary subunit GSG1L. Cell Rep. 2012; 1:590–598. [PubMed: 22813734]

Siddiqui TJ, Pancaroglu R, Kang Y, Rooyakkers A, Craig AM. LRRTMs and neuroligins bind neurexins with a differential code to cooperate in glutamate synapse development. J Neurosci. 2010; 30:7495–7506. [PubMed: 20519524]

Small SA, Petsko GA. Retromer in Alzheimer disease, Parkinson disease and other neurological disorders. Nat Rev Neurosci. 2015; 16:126–132. [PubMed: 25669742]

Steinberg F, Gallon M, Winfield M, Thomas EC, Bell AJ, Heesom KJ, Tavare JM, Cullen PJ. A global analysis of SNX27-retromer assembly and cargo specificity reveals a function in glucose and metal ion transport. Nat Cell Biol. 2013; 15:461–471. [PubMed: 23563491]

Sudhof TC. Neuroligins and neurexins link synaptic function to cognitive disease. Nature. 2008; 455:903–911. [PubMed: 18923512]

Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, Sudhof TC. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science. 2007; 318:71–76. [PubMed: 17823315]

Taniguchi H, Gollan L, Scholl FG, Mahadomrongkul V, Dobler E, Limthong N, Peck M, Aoki C, Scheiffele P. Silencing of neuroligin function by postsynaptic neurexins. J Neurosci. 2007; 27:2815–2824. [PubMed: 17360903]

Traub LM. Tickets to ride: selecting cargo for clathrin-regulated internalization. Nat Rev Mol Cell Biol. 2009; 10:583–596. [PubMed: 19696796]

Uemura T, Lee SJ, Yasumura M, Takeuchi T, Yoshida T, Ra M, Taguchi R, Sakimura K, Mishina M. Trans-synaptic interaction of GluRdelta2 and Neurexin through Cbln1 mediates synapse formation in the cerebellum. Cell. 2010; 141:1068–1079. [PubMed: 20537373]

Wang X, Zhao Y, Zhang X, Badie H, Zhou Y, Mu Y, Loo LS, Cai L, Thompson RC, Yang B, et al. Loss of sorting nexin 27 contributes to excitatory synaptic dysfunction by modulating glutamate receptor recycling in Down's syndrome. Nat Med. 2013; 19:473–480. [PubMed: 23524343]

Willnow TE, Petersen CM, Nykjaer A. VPS10P-domain receptors - regulators of neuronal viability and function. Nat Rev Neurosci. 2008; 9:899–909. [PubMed: 19002190]

Wisco D, Anderson ED, Chang MC, Norden C, Boiko T, Folsch H, Winckler B. Uncovering multiple axonal targeting pathways in hippocampal neurons. J Cell Biol. 2003; 162:1317–1328. [PubMed: 14517209]

Yap CC, Winckler B. Harnessing the power of the endosome to regulate neural development. Neuron. 2012; 74:440–451. [PubMed: 22578496]

Yap CC, Wisco D, Kujala P, Lasiecka ZM, Cannon JT, Chang MC, Hirling H, Klumperman J, Winckler B. The somatodendritic endosomal regulator NEEP21 facilitates axonal targeting of L1/NgCAM. The Journal of cell biology. 2008; 180:827–842. [PubMed: 18299352]

Highlights

- Proteomics identifies neurexins and AMPA receptors as key proteins sorted by SorCS1
- SorCS1 regulates surface levels of neurexin and AMPA receptors
- SorCS1 maintains synaptic abundance of adhesion proteins and AMPA receptors in vivo
- Impaired AMPA receptor trafficking in absence SorCS1 reduces synaptic transmission

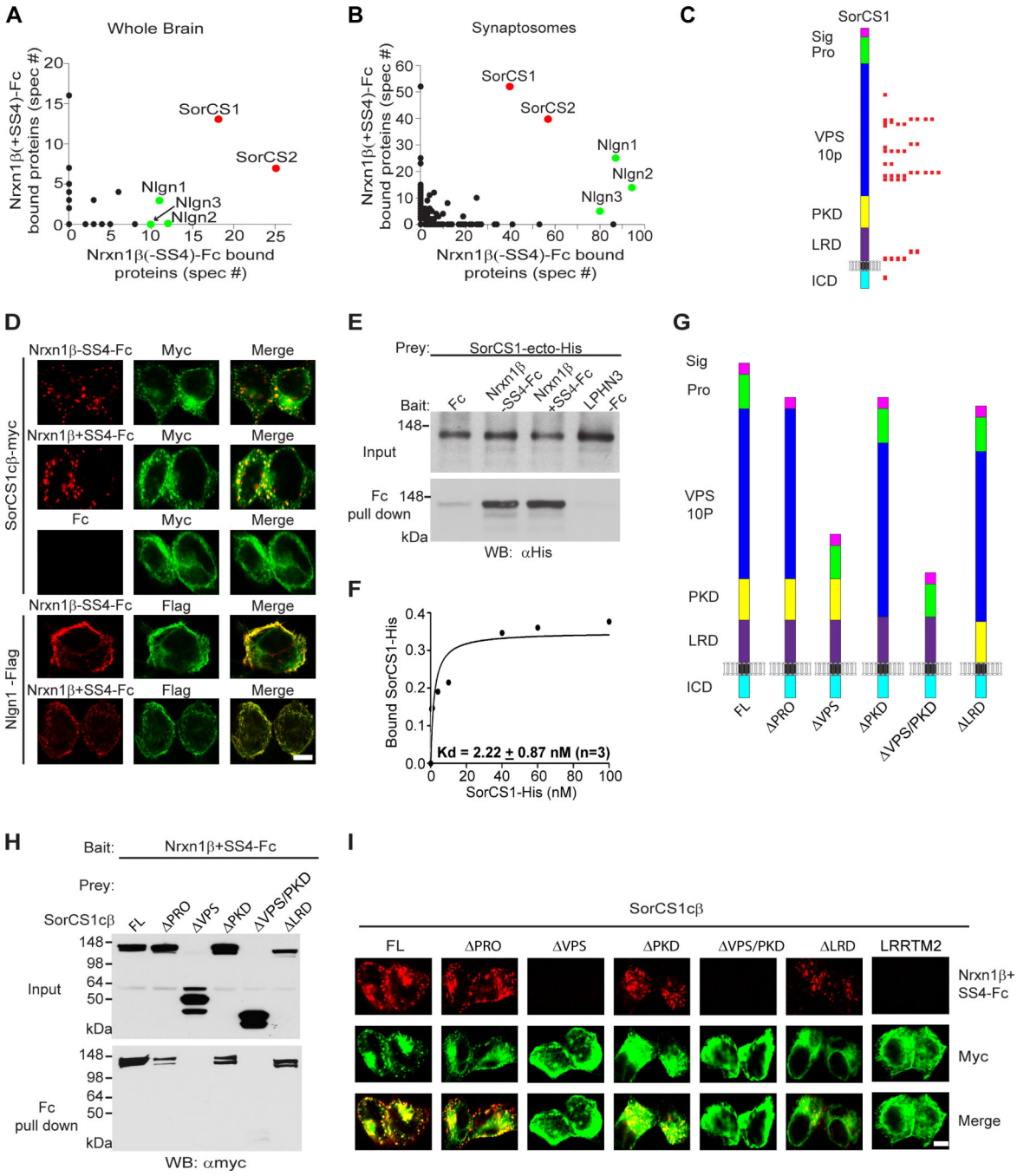


Figure 1. Identification of SorCS1 as a Nrxn-binding protein

(A) Proteomics workflow to identify Nrxn1β-interacting proteins. (B) Frequency of detection of all peptides (total spectra count) for proteins identified in both Nrxn1β(+SS4)-Fc and Nrxn1β(−SS4)-Fc affinity purifications after background (Fc alone) subtraction with whole brain prey extracts. (C) Nrxn1β-Fc affinity purification as in (B) with synaptosome prey extracts. (D) SorCS1 protein domain organization with mapped peptide MS identifications (red) from synaptosome prey experiment with Nrxn1β(+SS4) bait. Sig: signal peptide, Pro: pro-peptide, VPS10P: vacuolar protein sorting 10 protein, PKD: polycystic

kidney disease domain, LRD: leucine-rich domain, ICD: intracellular domain. (E) Surface binding experiments with Nrxn1β-Fc or control Fc on HEK293T cells expressing SorCS1cβ-myc or FLAG-Nlgn1. (F) Direct interaction of SorCS1-ecto-His with Nrxn1β-Fc. Fc proteins were mixed with SorCS1-ecto-His, precipitated and analyzed by Western blot. (G) Estimation of SorCS1/Nrxn1β(−SS4) binding affinity. Representative experiment demonstrating concentration-dependent SorCS1-ecto-His binding to FLAG-Nrxn1β(−SS4)-expressing HEK cells (*n*=3 independent experiments). (H) SorCS1 deletion mutants. (I) Pulldown experiment with Nrxn1β(+SS4)-Fc on HEK cell lysates transfected with SorCS1 deletion constructs. SorCS1/Nrxn1β binding requires the VPS10P domain. (J) Binding of Nrxn1β(+SS4)-Fc to HEK cells expressing SorCS1 deletion constructs or myc-LRRTM2 as a control. Scale bar in E and J 10 μm. See also Figure S1.

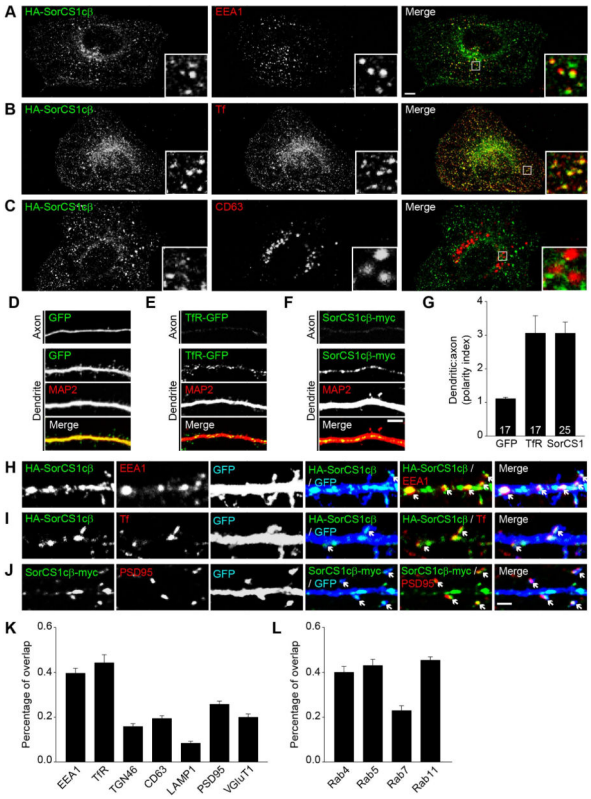


Figure 2. SorCS1 localizes to endosomal compartments and excitatory synapses

(A-C) SorCS1 localization in HeLa cells. (A) HA-SorCS1cβ (green) expressed in HeLa cells colocalizes with the early endosome marker EEA1 (red). (B) HA-SorCS1cβ colocalizes with internalized Alexa 568-transferrin (Tf) (red). (C) HA-SorCS1cβ does not colocalize with the late endosome marker CD63 (red). (D-L) SorCS1 localization in neurons. (D-G) DIV14 hippocampal neurons expressing GFP, TfR-GFP, or SorCS1cβ-myc were immunostained for MAP2 (a marker for dendrites; in red) and ankyrin G (a marker for the axon initial segment; in blue, image not shown) in order to determine SorCS1 somatodendritic *vs.* axonal distribution. Shown are representative images with the axonal and dendritic distribution of GFP (D), TfR-GFP (E), or SorCS1cβ-myc (F) proteins. (G) Quantification of the dendritic *vs.* axonal distribution (D:A – polarity index) of GFP, TfR-GFP and SorCS1cβ. D:A=1, uniform distribution in axons and dendrites; D:A<1, preferential axonal distribution; and D:A>1, preferential dendritic distribution. Number in bars indicates *n* for each condition in three independent experiments. (H) HA-SorCS1cβ (green) coexpressed with GFP (blue) in hippocampal neurons colocalizes with EEA1 (red) in dendrites and spines of hippocampal neurons (arrows). (I) HA-SorCS1cβ colocalizes with Alexa 568-Tf (red) in dendrites and spines (arrows). (J) SorCS1cβ-myc shows a partial overlap with the postsynaptic excitatory marker PSD95 (red) in dendritic spines (arrows). (K, L) Quantification of the colocalization of HA-SorCS1cβ in hippocampal neurons with different markers in three independent experiments (*n*=15 cells). Bar graphs show mean ± SEM. Scale bar in (A-C) 1 μm; in (D-F) 10 μm; in (H-J) 2 μm. See also Figure S2.

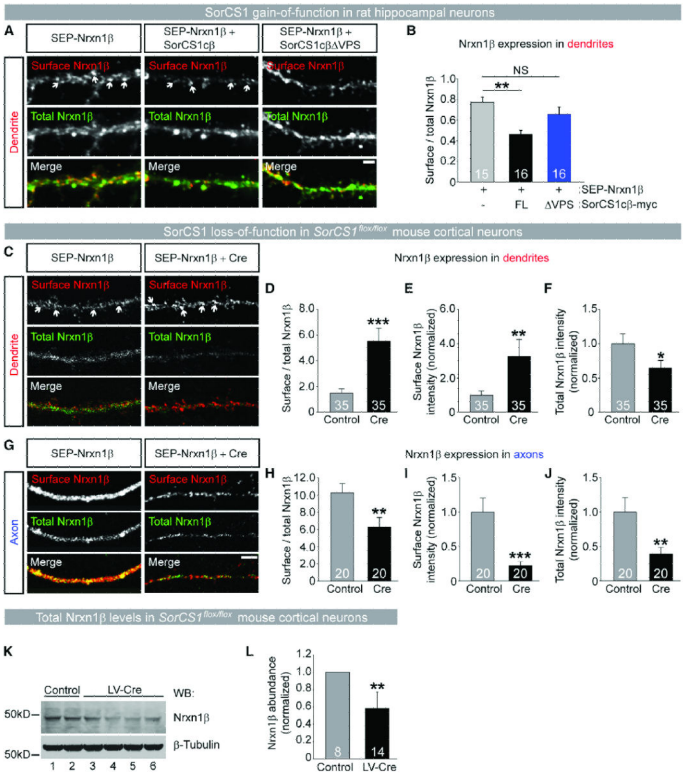
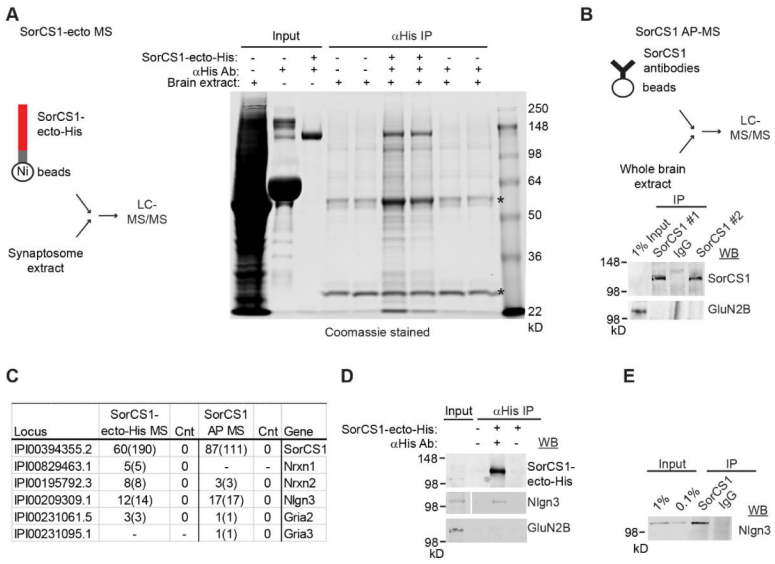


Figure 3. SorCS1 regulates Nrxnsurface distribution in neurons

(A, B) SorCS1 gain-of-function experiments in rat hippocampal neurons. (A) Overexpression of full-length (FL) SorCS1cβ-myc, but not SorCS1β-myc ΔVPS, decreasesdendritic Nrxn1β surface levels. Neurons were co-transfected with SEP-Nrxn1β-SS4) and an empty vector or SorCS1cβ-myc constructs,and immunostained for GFP under non-permeabilizing conditions to label the surface pool of SEP-Nrxn1β. Total SEP-Nrxn1β intensity was acquired relying on endogenous GFP fluorescence. (B) Quantification of the dendritic surface/total intensity ratio of SEP-Nrxn1β. $^{**}P < 0.001$ by t-test with non-parametric Mann-Whitney rank-sum test. (C-L) SorCS1 loss-of-function experiments in *SorCS1*$^{flox/flox}$ neurons. (C) Loss of *SorCS1* increases dendritic Nrxn1β surface levels. *SorCS1*$^{flox/flox}$ cortical neurons were co-electroporated with SEP-Nrxn1β-SS4) and an empty vector (Control) or Cre-myc (Cre), and immunostained for GFP under non-permeabilizing conditions to label the surface pool of SEP-Nrxn1β. (D) Quantification of dendritic/total intensity ratio of SEP-Nrxn1β. (E) Loss of *SorCS1* increases dendritic SEP-Nrxn1β surface intensity. (F) Dendritic SEP-Nrxn1β total intensity is reduced in *SorCS1*-deficient neurons. (G) Loss of *SorCS1* strongly decreases axonal SEP-Nrxn1β surface levels. (H) Quantification of axonal SEP-Nrxn1β surface/total intensity ratio. (I) Loss of *SorCS1* strongly reduces axonal SEP-Nrxn1β surface intensity. (J) Axonal SEP-Nrxn1β intensity is reduced in *SorCS1*-deficient neurons. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ by Mann-Whitney test. Bar graphs in (B, D-F, H -J) show mean ± SEM; number in bars indicates *n* for each condition. Scale bar (A, C, and G) 5 μm. (K) Extracts from DIV14 *SorCS1*$^{flox/flox}$ cortical neurons infected with LV-Cre at DIV2 show a reduction in Nrxn1β levels compared to control cells. $^{**}P < 0.01$ by Student's t-test. Bar graph shows mean ± STD; number in bars indicates *n* for each condition. See also Figure S3.



Locus	SorCS1-ecto-His MS	Cnt	SorCS1 AP MS	Cnt	Gene
IPI00394355.2	60(190)	0	87(111)	0	SorCS1
IPI00829463.1	5(5)	0	-	-	Nrxn1
IPI00195792.3	8(8)	0	3(3)	0	Nrxn2
IPI00209309.1	12(14)	0	17(17)	0	Nlgn3
IPI00231061.5	3(3)	0	1(1)	0	Gria2
IPI00231095.1	-	-	1(1)	0	Gria3

Figure 4. SorCS1 interactome analysis identifies synaptic adhesion molecules and AMPA glutamate receptors

(A) Schematic representation recombinant His-tagged SorCS1-ectodomain (SorCS1-ecto-His) interaction screen. Representative Coomassie-stained gel of proteins bound to bead-coupled SorCS1-ecto-His with synaptosome extracts, with indicated negative control purifications. Asterisks indicate non-specific background bands. (B) SorCS1 complex affinity purification scheme. SorCS1 complexes were immunoprecipitated from whole postnatal rat brain extracts with two independent SorCS1 antibodies and rabbit IgG controls. Western blot analysis of immunoprecipitated SorCS1 complexes with SorCS1 and GluN2B antibodies shows specific SorCS1 enrichment. (C) MS summary table for SorCS1-ecto-His and SorCS1 affinity purifications (SorCS1-ecto-His MS and SorCS1 AP-MS, respectively). Indicated is the number of peptides and spectral counts (in parenthesis) for each protein in both purification schemes with negative controls (see Table S2 and S3 and Experimental Procedures). (D) Western blot validation for the recovery of the bait SorCS1-ecto-His and prey Nlgn3 proteins, but not GluN2B. (E) Western blot validation for the recovery of Nlgn3 in immunoprecipitated SorCS1 complexes. See also Figure S4.

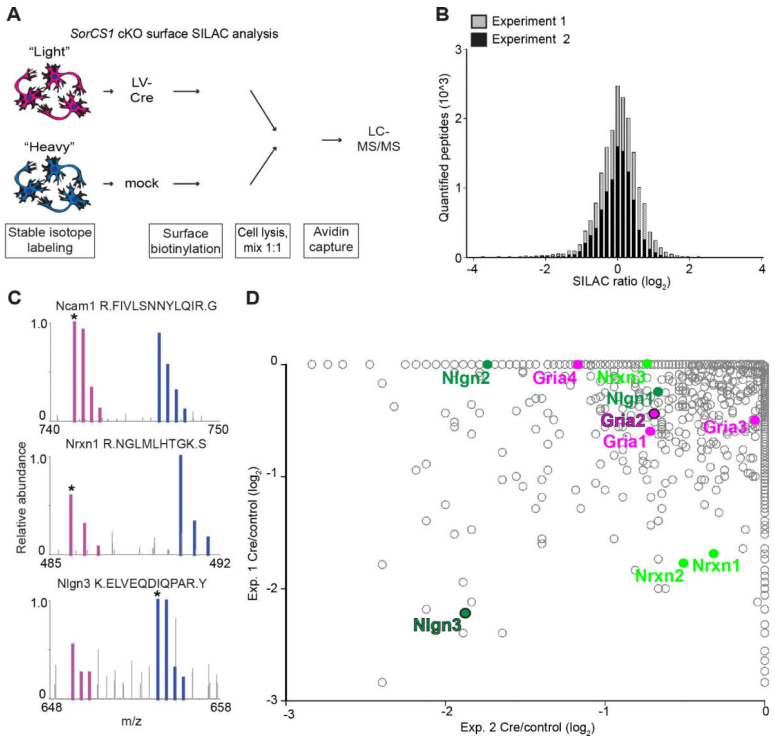


Figure 5. Quantitative surface proteome analysis reveals reduced surface levels of Nrxn, Nlgn, and AMPA receptors in *SorCS1*-deficient neurons

(A) Experimental scheme *SorCS1* cKO surface SILAC analysis. Mock-infected *SorCS1*$^{flox/flox}$ neurons were grown in SILAC media ("Heavy", blue) and biotinylated surface proteins were isolated and quantified compared to LV-Cre-infected *SorCS1*$^{flox/flox}$ neurons (*SorCS1* cKO; "Light", red) using mass spectrometry (see Experimental Procedures). (B) Corrected peptide ratio distribution for two independent biological replicates (experiment 1 and 2) shows normal distributions with tails skewed towards peptides with a reduced SILAC ratio in the SorCS1 cKO condition (dotted blue box). (C) Raw MS1 scans for Nrxn1 and Nlgn3 show reduced intensity for *SorCS1* cKO condition (red) while Ncam1 is unchanged. Starred peaks were identified by MS/MS. (D) Cross-hair scatter plot from two independent experiments shows that Nrxn1/2, Nlgn3, and Gria1/2 are among the most strongly downregulated proteins in *SorCS1*-deficient neurons. Proteins measured as downregulated in one experiment but unquantified in the other are shown on that axis as 0. Outlined proteins were determined as significantly downregulated (See Experimental Procedures), Student's t-test $P < 0.05$; n=1297 proteins. See also Figure S5.

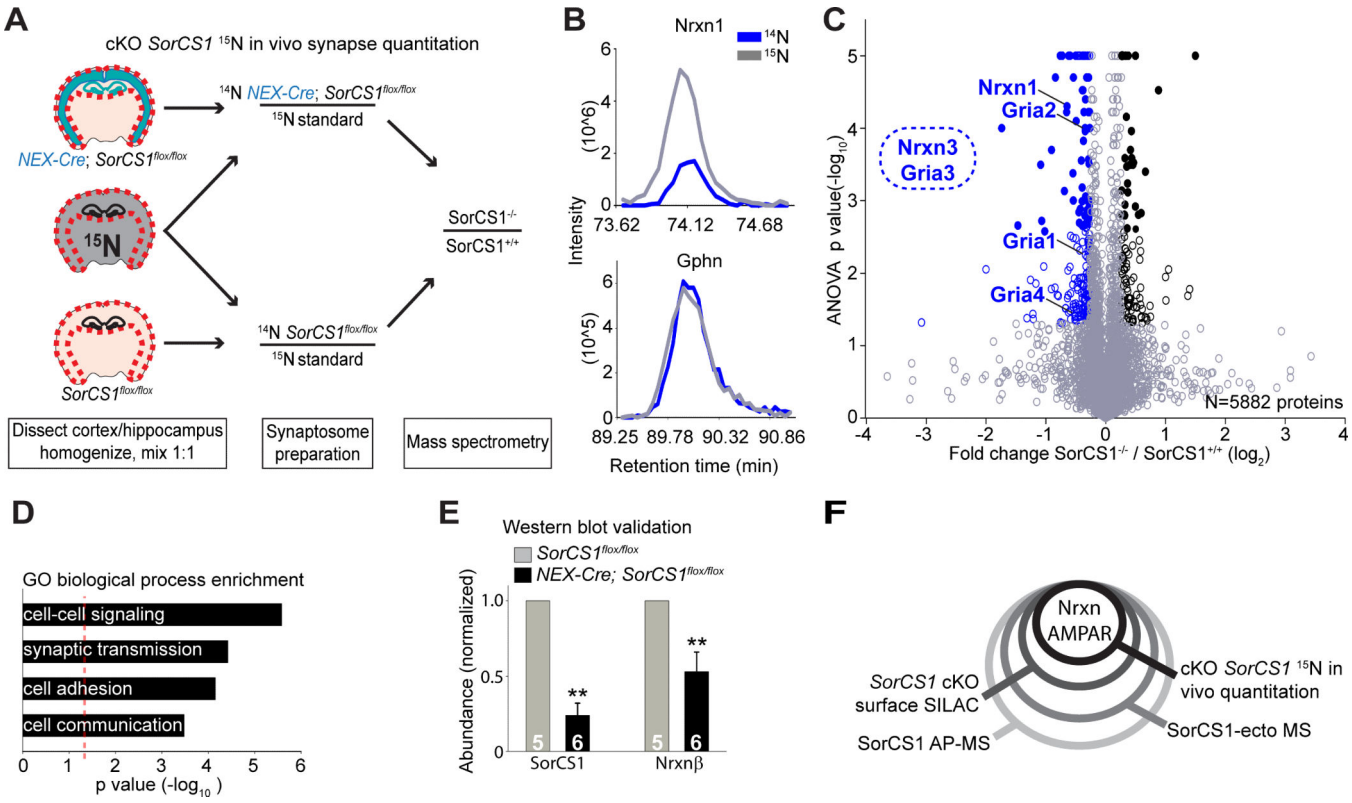


Figure 6. In vivo loss of SorCS1 decreases synaptic abundance of adhesion and glutamate receptors

(A) Quantitative in vivo proteomic scheme to identify synaptic proteins regulated by SorCS1. *NEX-Cre:SorCS1*$^{flox/flox}$ (*n*=6) or *SorCS1*$^{flox/flox}$ (*n*=5) brain region homogenates were mixed 1:1 with ^{15}N internal standard. Synaptosomes were prepared simultaneously and analyzed by mass spectrometry (see Experimental Procedures). (B) MS1 reconstructed chromatograms of representative peptides. Elution profile MS1 traces are shown for a representative peptide from Nrxn and Ncam1. Internal standard ^{15}N signal in gray, *NEX-Cre:SorCS1*$^{flox/flox}$ ^{14}N signal in blue. (C) Proteomic summary volcano plot, x-axis = $\log_2$ *SorCS1*$^{-/-}$ / *SorCS1*$^{+/+}$; y-axis = -$\log_{10}$ T-test *P* value. *N*=5882 total proteins represented: 299 (5.1%) regulated proteins (*P* value ≤0.05 and ≥20% altered expression: blue and black open circles); 104 most confident proteins (*P* value ≤0.05 and ≥20% altered expression and Benjamini-Hochberg correction ≤0.05: blue and black filled circles). *P* values = 0 were graphed as 0.00001. Nrxn3 and Gria3 were below the limit of detection in *SorCS1*$^{-/-}$ and were considered singletons (dotted oval). (D) Panther GO biological process analysis of significantly downregulated proteins graphed in rank order by decreasing *P* value (154 of 191 downregulated proteins mapped, *P* value < 0.05). Dotted red line indicates significance cut-off (*P* < 0.05). (E) Western blot validation of SorCS1 and Nrxnβ levels relative to β-tubulin in synaptosome extracts used in quantitative mass spectrometry screen. ***P* < 0.005 by Student's t-test. Bar graph shows mean ± STD; number in bars indicates *n* for each condition. (F) Venn diagram: four independent proteomics approaches significantly identify Nrxns and AMPARs as key proteins regulated by SorCS1 (See Experimental Procedures for details). See also Figure S6.

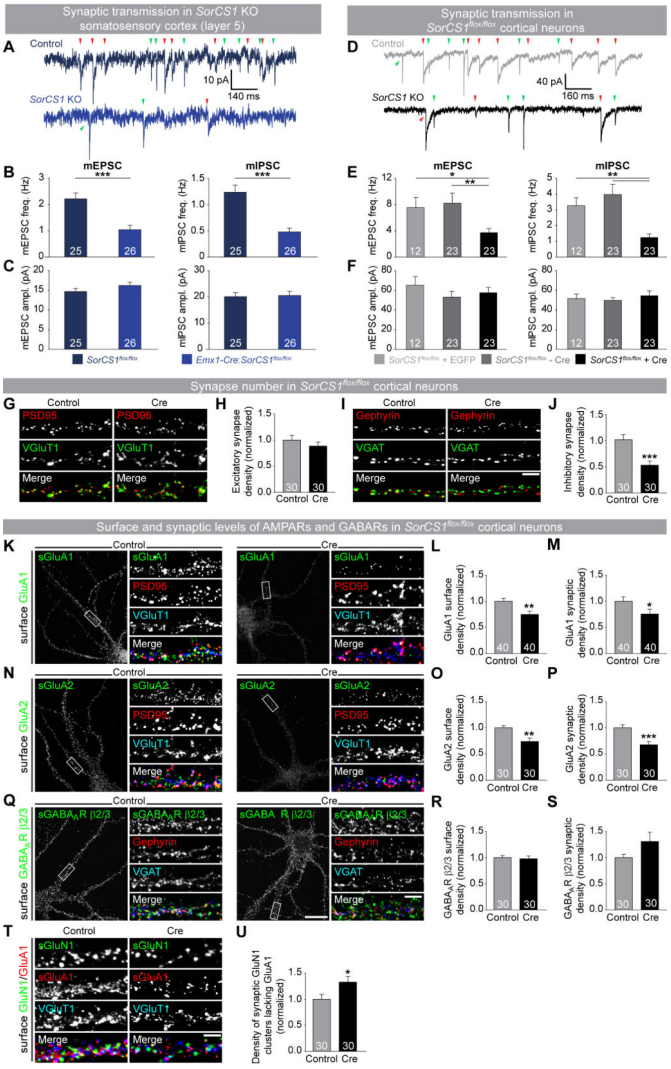


Figure 7. SorCS1 is required for basal glutamatergic and GABAergic synaptic transmission

(A-C) Analysis of synaptic transmission in layer 5 cortical neurons (somatosensory cortex) in acute slices of *SorCS1*$^{flox/flox}$ (Control) and *Emx1-Cre:SorCS1*$^{flox/flox}$ (*SorCS1* KO) mice. (A) Example traces. Segregation of mEPSCs (green arrowheads) and mIPSCs (red arrowheads) was based on difference in decay time kinetics. (B, C) mEPSC and mIPSC frequency, but not amplitude, are decreased in *SorCS1* KO layer 5 neurons. (D-F) Analysis of synaptic transmission in *SorCS1*$^{flox/flox}$ dissociated cortical neurons electroporated with EGFP (Control) or Cre (*SorCS1* KO). (D) Example traces. (E, F) Reduced mEPSC and mIPSC frequency, but not amplitude, in *SorCS1*$^{flox/flox}$ neurons expressing Cre (+ Cre) compared to neighboring non-electroporated neurons (− Cre) or control neurons (+ EGFP). (G-J) Analysis of synapse number in *SorCS1*$^{flox/flox}$ cortical neurons. (G, H) The density of VGluT1/PSD95-positive puncta per length of dendrite is not changed in DIV14 *SorCS1*$^{flox/flox}$ cortical neurons electroporated with Cre compared to EGFP-electroporated cells (Control). (I, J) The density of Gephyrin/VGAT-positive puncta is decreased in *SorCS1* KO neurons. (J). ***$P < 0.001$ Mann-Whitney test. (K-U) Analysis of surface expression of AMPARs and GABARs in *SorCS1*$^{flox/flox}$ cortical neurons. (K) *SorCS1*$^{flox/flox}$ cortical

neurons were electroporated with EGFP (Control) or Cre (Cre) plasmids and immunostained on DIV14 for surface GluA1 under non-permeabilizing conditions using antibodies against extracellular epitopes. After permeabilization neurons were stained for GFP, PSD-95 and VGluT1. (L) Quantification of total cell surface density of GluA1 shows decreased surface expression in *SorCS1* KO neurons. (M) Decreased synaptic surface levels of GluA1 in *SorCS1* KO neurons. (N-P) Decreased cell surface and synaptic surface levels of GluA2 in *SorCS1* KO neurons. (Q) *SorCS1*$^{flox/flox}$ cortical neurons were immunostained for surface $GABA_AR$ β2/3 under non-permeabilizing conditions, and for GFP, Gephyrin and VGAT following permeabilization. (R) $GABA_AR$ β2/3 (R) surface expression per length of dendrite and (S) $GABA_AR$ β2/3 synaptic surface expression are not affected in *SorCS1* KO neurons. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 Mann-Whitney test. (T) DIV14 *SorCS1*$^{flox/flox}$ cortical neurons electroporated with EGFP (Control) and Cre (Cre) surface-labeled for GluN1 and GluA1. (U) Quantification of the density of VGluT1-positive synapses containing surface GluN1 but lacking surface GluA1 normalized to controls. The fraction of AMPAR-lacking synapses is increased in *SorCS1* KO neurons. **P* < 0.05 by Mann-Whitney test. Bar graphs show mean ± SEM; number in bars indicates number of cells analyzed for each condition in 3-4 independent experiments. Scale bars (G, I, K, N, Q, T) 5 μm; whole cell panels in (K, N, Q) 20 μm. See also Figure S7.