The Alzheimer’s gene SORL1 is a regulator of endosomal traffic and recycling in human neurons

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Abstract

Background Loss of the Sortilin-related receptor 1 (SORL1) gene seems to act as a causal event for Alzheimer’s disease (AD). Recent studies have established that loss of SORL1, as well as mutations in autosomal dominant AD genes APP and PSEN1/2, pathogenically converge by swelling early endosomes, AD’s cytopathological hallmark. Acting together with the retromer trafficking complex, SORL1 has been shown to regulate the recycling of the amyloid precursor protein (APP) out of the endosome, contributing to endosomal swelling and to APP misprocessing. We hypothesized that SORL1 plays a broader role in neuronal endosomal recycling and used human induced pluripotent stem cell-derived neurons (hiPSC-Ns) to test this hypothesis. We examined endosomal recycling of three transmembrane proteins linked to AD pathophysiology: APP, the BDNF receptor Tropomyosin-related kinase B (TRKB), and the glutamate receptor subunit AMPA1 (GLUA1).

Methods We used isogenic hiPSCs engineered to have SORL1 depleted or to have enhanced SORL1 expression. We differentiated neurons from these cell lines and mapped the trafficking of APP, TRKB and GLUA1 within the endosomal network using confocal microscopy. We also performed cell surface recycling and lysosomal degradation assays to assess the functionality of the endosomal network in both SORL1-depleted and -overexpressing neurons. The functional impact of GLUA1 recycling was determined by measuring synaptic activity. Finally, we analyzed alterations in gene expression in SORL1-depleted neurons using RNA sequencing.

Results We find that as with APP, endosomal trafficking of GLUA1 and TRKB is impaired by loss of SORL1. We show that trafficking of all three cargoes to late endosomes and lysosomes is affected by manipulating SORL1 expression. We also show that depletion of SORL1 significantly impacts the endosomal recycling pathway for APP and GLUA1 at the level of the recycling endosome and trafficking to the cell surface. This has a functional effect on neuronal activity as shown by multielectrode array (MEA). Conversely, increased SORL1 expression enhances endosomal recycling for APP and GLUA1. Our unbiased transcriptomic data further support SORL1’s role in endosomal recycling. We observed altered expression networks that regulate cell surface trafficking and neurotrophic signaling in SORL1-depleted neurons.

Conclusion Collectively, and together with other recent observations, these findings suggest that one role for SORL1 is to contribute to endosomal degradation and recycling pathways in neurons, a conclusion that has both pathogenic and therapeutic implications for Alzheimer’s disease.

Keywords Human induced pluripotent stem cells · Neurons · Alzheimer’s disease · Endosomal network · SORL1

Abbreviations

AD Alzheimer’s disease
SORL1 Sortilin-related receptor 1
SORLA Sortilin-related receptor with A-type repeats

APP Amyloid precursor protein
TRKB Tropomyosin-related kinase B
GLUA1 Glutamate receptor subunit AMPA1
AMPA α-Amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid
OE Overexpression
KO Knockout
WT Wild type
LAMP1 Lysosome-associated membrane glycoprotein 1
M6PR Mannose-6-phosphate receptor
Background

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia. The underlying contributors to AD pathology encompass several biological pathways, including endosomal function, amyloid precursor protein (APP) processing, immune function, synaptic function, and lipid metabolism [40]. Among these, endosomal dysfunction in neurons is emerging as a potential causal mechanism[84]. Mutations in the amyloid precursor protein (APP) and the two presenilins (PSEN1 and PSEN2) lead to early-onset autosomal dominant AD. When these mutations are modeled in human neurons and other systems, they cause endosomal swelling, indicative of traffic jams, a phenotype that is a cytopathological hallmark of AD [9, 12, 44]. Recent genetic studies have identified a fourth gene, the trafficking receptor ‘sortilin related receptor 1’ (SORL1), which, when harboring frame-shift mutations leading to premature stop codons, is described as causal for AD [33, 66, 73]. Interestingly, SORL1 is also linked to the more common, late-onset form of AD [45, 70] and its expression is lost in sporadic AD brains [4, 21, 59, 89]. When modeled in human neurons, SORL1 depletion phenocopies APP and PSEN mutations by causing endosomal swelling [37, 42].

The SORL1 gene codes for the protein SORLA, an endosomal sorting protein that is also an adaptor molecule for the retromer trafficking complex [25, 70, 83]. Retromer recycles cargo out of the early endosome, either from the endosome to the trans-Golgi network or with greater importance for cargo out of the early endosome, either from the endosome to the trans-Golgi network or with greater importance for neurons, back to the cell surface [25, 77]. To date, the best evidence for SORL1’s role in retromer-dependent endosomal recycling comes from studies investigating APP trafficking [25, 75, 93]. Our previous work in human neurons demonstrated that SORL1 depletion retains APP in early endosomes, which may contribute to endosomal swelling by blocking recycling [42].

Retromer-dependent trafficking in neurons, however, also recycles cargo other than APP. For example, retromer is required for the normal recycling of glutamate receptors, a trafficking event that mediates synaptic plasticity and synaptic health, and this dependency occurs independent of retromer’s role in APP recycling [61, 88]. Neurotrophin receptors are also trafficked through the endosomal system, in a retromer-dependent manner, and are important for synaptic health [41, 62, 71].

Here, we used human induced pluripotent stem cell-derived neurons (hiPSC-Ns) to test the hypothesis that SORL1 plays a broader role in neuronal endosomal recycling. We use our previously described SORL1-depleted hiPSC lines to generate hiPSC-Ns, which model the loss of SORL1 expression that occurs in AD [42]. Furthermore, we used previously established cell lines engineered to overexpress SORL1 two- to threefold over wild-type levels [96] to test the effects of enhanced SORL1 expression in hiPSC-Ns on these trafficking pathways. Importantly, all cell lines are isogenic. We map the trafficking effects these manipulations have on three specific proteins known to sort through the endosome recycling pathway: APP, the GLUA1 subunit of the AMPA receptor, and neurotrophin receptor TRKB, all of which are implicated in AD [18, 19, 26, 53, 91, 95].

Finally, we performed RNA sequencing on the SORL1-depleted cell lines to explore an unbiased transcriptomics analysis induced by SORL1 depletion. The results generally confirmed our hypothesis, showing that one role for SORL1 is to contribute to endosomal degradation and recycling pathways in neurons, a conclusion that has both pathogenic and therapeutic implications.

Methods

Cell lines

Cell lines generated by CRISPR/Cas9 gene-editing technology

The generation of the cell lines used in this paper is described in our previously published work [42] and consists of four clones: two wild-type clones, designated clone A6 and clone A7, and two SORLIKO clones, designated clone E1 and clone E4. Cell lines were generated from our previously published and characterized CV background human induced pluripotent stem cell line [96]. This cell line is male and has a APOE ε3/ε4 genotype [47]. All four clones were shown to have normal karyotypes and are routinely tested for mycoplasma (MycoAlert). The clones used in the experiments in this work are listed in the figure legends.

CRISPR/Cas9 gRNA, ssODN, and primer sequences: gRNA: ATTGAACGACATGAAACCCTC ssODN: GGG AATTGATCCCTATGACAAAAACCAAACCATCTCAT TGAACGACATGAAACCCTCTGGTACATCCAGGCAGTC TTC CGA AGTACAGATTTTCTCTCCAGTCCCGGAAAAC CAGGAAG, Forward primer: cctactcatgcgaagtagtaac, Reverse primer: cctctacaattctgtgtatgc, PCR amplifies 458 bp sequence. These sequences have been previously published in [42].

SORL1 overexpression cell lines: isogenic cell lines with overexpression of SORL1 were generated as previously described [96]. These lines are generated from the CV parental line, the same parental line as the SORLIKO cell lines were made from. Briefly, stable integration of SORL1 cDNA into the genome was achieved using piggybac
transposon system (Systems Biosciences). Vector alone (WT) or vector with SORL1 cDNA (SORL1OE) constructs were introduced into iPSCs by electroporation and stable cell lines were selected with puromycin (2 µg/ml) treatment. We obtained one SORL1OE stable cell line and one vector alone stable cell line. For all overexpression experiments, SORL1OE cells were compared to the vector alone controls.

**hiPSC neuronal differentiation**

hiPSCs were differentiated to neurons using dual-SMAD inhibition [10, 79]. Briefly, hiPSCs were plated on Matrigel coated 6-well plates at a density of 3.5 million cells per well and fed with Basal Neural Maintenance Media (1:1 DMEM/F12 + glutamine media/neurobasal media, 0.5% N2 supplement, 1% B27 supplement, 0.5% GlutaMax, 0.5% insulin-transferrin-selenium, 0.5% NEAA, 0.2% β-mercaptoethanol; Gibco, Waltham, MA) + 10 mM SB-431542 + 0.5 mM LDN-193189 (Biogens, Westlake Village, CA). Cells were fed daily for 7 days. On day 8, cells were incubated with Versene, gently dissociated using cell scrapers, and passaged at a ratio of 1:3. On day 9, media was switched to Basal Neural Maintenance Media and fed daily. On day 13, media was switched to Neural Differentiation Media with 20 ng/ml FGF (R&D Systems, Minneapolis, MN) and fed daily. On day 16, cells were passaged again at a ratio of 1:3. Cells were fed until approximately day 23. At this time, cells were FACs sorted to obtain the CD184/CD24-positive and CD44/CD271-negative neural precursor cell (NPC) population. Following sorting, NPCs were expanded for neural differentiation. For cortical neuronal differentiation, NPCs were plated out in 10 cm cell culture dishes at a density of 6 million cells/10 cm plate. After 24 h, cells were switched to Neural Differentiation media (DMEM-F12 + glutamine, 0.5% N2 supplement, 1% B27 supplement, 0.5% GluMax) + 0.02 µg/ml brain-derived neurotrophic factor (PeproTech, Rocky Hill, NJ) + 0.02 µg/ml glial-cell-derived neurotrophic factor (PeproTech) + 0.5 mM dbcAMP (Sigma Aldrich, St Louis, MO). Media was refreshed twice a week for 3 weeks. After 3 weeks, neurons were selected for CD184/CD44/CD271-negative population by MACS sorting and plated for experiments. The data presented in this study represent 2–3 neuronal differentiations.

**Purification of neurons**

Following 3 weeks of differentiation, neurons were dissociated with accutase and resuspended in Magnet Activated Cell Sorting (MACS) buffer (PBS + 0.5% bovine serum albumin [Sigma Aldrich, St Louis, MO] + 2 mM ethylenediaminetetraacetic acid [Thermo Fisher Scientific, Waltham, MA]). Following a modification of [97], cells were incubated with PE-conjugated mouse anti-Human CD44 and mouse anti-Human CD184 antibodies (BD Biosciences, San Jose, CA) at a concentration of 5 µl/10 million cells. Following antibody incubation, cells were washed with MACS buffer and incubated with anti-PE magnetic beads (BD Biosciences, San Jose, CA) at a concentration of 25 µl/10 million cells. bead-antibody complexes were pulled down using a rare-earth magnet, supernatants were selected, washed, and plated at an appropriate density.

**DQ Red BSA assay for visualization of lysosomal degradation**

Lysosomal proteolytic degradation was evaluated using DQ Red BSA (#D-12051; Thermo Fisher Scientific), a fluorogenic substrate for lysosomal proteases, that generates fluorescence only when enzymatically cleaved in intracellular lysosomal compartments. hiPSC-derived neurons were seeded at a density of 400,000 cells/well of a Matrigel coated 48-well plate. After 24 h, cells were washed once with DPBS, treated with complete media containing either 10 µg/ml DQ Red BSA or vehicle (PBS) and incubated for 6 h or 24 h [16, 72, 90] at 37 °C in a 5% CO₂ incubator as described in [54]. At the end of 6 or 24 h, cells were washed with PBS, fixed with 4% PFA and immunocytochemistry was performed as described in “Methods”. Cells were imaged using a Leica SP8 confocal microscope and all image processing was completed with ImageJ software. Cell bodies were identified by MAP2 labeling, and fluorescence intensity of DQ Red BSA was measured in regions of the images containing the MAP2 label.

**Immunocytochemistry**

For immunocytochemistry, cells were fixed with 4% PFA for 20 min. Fixed cells were washed three times with PBST (PBS with 0.05% tween 20), permeabilized with Triton X-100 in PBS for 15 min, washed twice again with PBST, blocked with 5% BSA in PBS at room temperature for 1 h and incubated with appropriate primary antibodies overnight at 4 °C. The next day, cells were incubated with appropriate secondary antibodies and 1 µg/ml DAPI for 1 h at RT, washed three times with PBST and mounted on glass slides with Prolong Gold Antifade mountant (#P36930; Thermo Fisher Scientific).

**Colocalization analysis**

To investigate colocalization with endo-lysosomal compartments, hiPSC-derived neurons were labeled with markers specific for each intracellular compartment (EEA1 for early endosomes, Rab7 for late endosomes, LAMP1 for lysosomes and Rab11 for recycling endosomes) using Immunocytochemistry. A minimum of 10 fields of confocal z-stack...
images were captured under blinded conditions using a Yokogawa W1 spinning disk confocal microscope (Nikon) and a 100X plan apochromat oil immersion objective. Median filtering was used to remove noise from images and Otsu thresholding was applied to all images. Colocalization was quantified using the JACOP plugin [7] in Image J software [74] and presented as Mander’s correlation coefficient [23, 51]. For all imaging experiments, the data were analyzed in a blinded manner.

Cell Surface Staining: cell surface expression of GLUA1 and APP was determined using immunocytochemistry and confocal microscopy. To label proteins at the cell surface, cells were fixed with 4% PFA, washed and treated with primary and secondary antibodies as described in “Immunocytochemistry” section of “Methods”. Permeabilization with 0.1% Triton X-100 was not performed for this experiment. To label total protein levels, cells were fixed with 4% PFA, washed, permeabilized with 0.1% Triton X-100 and treated with primary and secondary antibodies as described in “Immunocytochemistry” section of “Methods”. Analysis of fluorescence intensity was done using Image J software. Cell surface expression was represented as ratio of fluorescence intensity measured under non-permeabilized conditions and fluorescence intensity measured under permeabilized conditions. For all imaging experiments, the data were analyzed in a blinded manner.

Multielectrode (MEA) assay

hiPSC-derived neural progenitor cells were differentiated into neurons, and neurons were purified as previously described in “Methods”. Purified neurons were mixed with unpurified neurons in a ratio of 5:1, and this mixture was plated onto a Matrigel coated 48-well MEA plate (Axion Biosystems; # M768-tMEA-48 W) at a cell density of 8000 cells/µl (total number of cells/well = 50,000). MEA-plated neurons were initially cultured in neural differentiation media. Media was gradually switched to BrainPhys media (Stem cell technologies; # 05790) by replacing half of a well’s media twice a week. BrainPhys media was supplemented with B27, N2, BDNF, GDNF, and dbcAMP.

Multielectrode (MEA) analysis

Electrical signals from neurons in the MEA plates were recorded twice a week using Axion Biosystems Maestro Pro system. Signals were recorded at a sampling frequency of 12.5 kHz with a 3 kHz Kaiser Window low-pass filter and 200 Hz high-pass filter. Spikes were detected using Axion Axis Navigator recording software with the adaptive threshold method. Recordings were analyzed using the Axion Neural Metric Tool. Firing rate data were limited to active electrodes that detected a minimum of five spikes a minute. Firing rate data from all active electrodes in a well were averaged for plotting and statistical testing.

Antibodies

The following primary antibodies were used: Early endosome antigen 1 (EEA1) at 1:500 (#610456; BD Biosciences); amyloid precursor protein (APP) at 1:500 (#ab32136; Abcam); microtubule-associated protein 2 (MAP2) at 1:1000 (ab92434; Abcam); Ras-related protein Rab-7a (Rab7) at 1:1000 (ab50533; Abcam); Ras-related protein Rab-11 (Rab11) at 1:250 (#610656; BD Biosciences); Lysosome-associated membrane protein-1 (LAMP1) at 1:250 (#sc 2011; Santa Cruz); Tropomyosin receptor kinase B (TRKB; # ab18987; abcam) at 1:1000, GLUA1(# MAB2263; Milipore sigma) at 1:500 and VPS35 (Abcam; #ab97545) at 1:500. DAPI was used at a final concentration of 0.1 µg/ml (Alfa Aesar).

Transferrin recycling assay

To measure recycling pathway function, we utilized transferrin recycling assay as previously described [68]. Purified neurons were seeded at 400,000 cells/well of a 24-well plate containing Matrigel coated 12 mm glass coverslip/well. After 5 DIV, cells were washed once with DMEM-F12 medium and incubated with starving medium (DMEM-F12 medium + 25 mM HEPES + 0.5% BSA) for 30 min at 37 °C in a 5% CO2 incubator to remove any residual transferrin. Thereafter, cells were pulsed with either 100 µg/ml transferrin from human serum conjugated with Alexa Fluor™ 647(#T23366; Thermo Fisher Scientific) or vehicle (PBS) in ‘starving medium’. At the end of 10 min, cells were washed twice with ice-cold PBS to remove any external transferrin and stop internalization of transferrin and washed once with acid stripping buffer (25 mM citric acid + 24.5 mM sodium citrate + 280 mM sucrose + 0.01 mM Deferoxamine) to remove any membrane bound transferrin. Next, cells were either fixed in 4% PFA or ‘Chase medium’ (DMEM-F12 + 50 µM Deferoxamine + 20 mM HEPES + 500 µg/ml Holo-transferrin) was added for different time points. Immunocytochemistry was done using MAP2 antibody to label neurons, confocal images were captured using Leica SP8 confocal microscope under blinded conditions. Fluorescence intensity of transferrin was measured using ImageJ software. Recycling function was presented as transferrin fluorescence intensity as a percentage of the fluorescence intensity measured at time zero.

Measurement of lysosome and recycling endosome size

Immunocytochemistry using antibodies for LAMP1 and MAP2 or RAB11 and MAP2 was performed as described
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above. Using a Leica SP8 confocal microscope with an apochromatic 63X oil immersion lens, z-stack images were obtained under blinded conditions. For the LAMP1 analysis, 17–34 fields were analyzed for a total of 45–76 cells analyzed. For the Rab11 analysis, 15–30 fields were analyzed for a total of 59–124 cells analyzed. Vesicle size measurements were performed using Cell Profiler software as previously described [42, 57]. Briefly, the vesicle channel was masked using the MAP2 channel and automated segmentation algorithms were used to identify individual puncta. The pixel area of each puncta was measured and is presented as mean area of all puncta per field.

Statistical analysis

For all imaging experiments, data were collected and analyzed in a blinded manner. Data were assessed for significance using parametric two-tailed unpaired Student’s t-tests or two-way ANOVA tests. Data are represented as mean ± standard deviation to show the spread of the data. Significance was defined as a value of \( p \leq 0.05 \). All statistical analyses were completed using GraphPad Prism software. Statistical details of individual experiments, including biological and technical replicate information, can be found in the figure legends. All the raw statistical data for the experiments in this paper, including means, difference between the means ± SEM, and 95% confidence intervals are presented in Supplemental Table 1.

RNA-sequencing analysis

RNA extraction

RNA was collected from 3 separate differentiations including a combination of two WT clones and two SORL1KO clones. Each sample includes 2–3 technical replicates. RNA was collected from 2 million purified neurons for each sample. Purification of total RNA was completed using the PureLink RNA Mini Kit (Thermo Fisher 12183018A). Assessment of purified RNA was completed using a NanoDrop. Final RNA quantification was completed using the Quant-iT RNA assay (Invitrogen) and RNA integrity analysis was completed using a fragment analyzer (Advanced Analytical).

Library preparation and sequencing

Library preparation was completed using the TruSeq Stranded mRNA kit (Illumina RS-122–2103) per manufacturer instructions. Sequencing was performed on a NovaSeq 6000 instrument.

Data analysis

Raw reads were aligned to GRCh38 with reference transcriptome GENCODE release 29 using STAR v2.6.1d [20]. Gene-level expression quantification is generated by RSEM v1.3.1 [48]. Genes with fewer than 20 normalized reads across all samples were omitted from further analysis. We did observe variation in the transcriptome based on differentiation (Supplemental Figure 3A); however, this was corrected for using the sva package [46].

To identify differentially expressed genes (DEGs), we used DESeq [3]. Briefly, we fit two models: a null model where gene expression only depends on batch effects (i.e., differentiation), and an alternative model where gene expression depends on both genotype (SORL1KO vs. WT) and batch effects. Chi-squared tests were performed to compare both fits, and we declare a gene as differentially expressed only when the alternative model fits the expression data better. DEGs are defined as genes with false discovery rate less than 0.05 and fold change greater than 1.5. The top gene ontology package [2] and the SynGO synaptic gene ontology annotations [43] were used to identify GO terms that were enriched. GO terms were tested according to the Fisher’s exact test. Finally, we mapped DEGs onto receptor–ligand interaction diagrams generated by Ramilowski et al. [67] using the igraph plugin [13]. To compare the amount of downregulated vs. upregulated genes, we used a 2-sample test for equality of proportions with continuity correction in R.

Results

SORL1 depletion increases neuronal cargo localization in early endosomes

Using CRISPR/Cas9 genome-editing techniques, we previously generated hiPSC-derived neurons (hiPSC-Ns) deficient in SORL1 expression due to indels introduced in exon 6. We demonstrated that loss of SORL1 expression in these neurons leads to enlarged early endosomes and an increased localization of APP within early endosomes, indicative of endosomal traffic jams [42]. We utilized these same cell lines (hereafter referred to as SORL1KO and their guide-matched isogenic wild-type clones referred to as WT) to examine localization of the BDNF receptor TRKB and the GLUA1 subunit of the neuronal AMPA receptor. TRKB has been shown to bind to SORLA and this interaction mediates trafficking of TRKB to synaptic plasma membranes [71]. GLUA1 is trafficked via the retromer complex, of which SORLA is an adaptor protein [25, 88] and both of these cargoes are important in maintaining healthy neuronal function. Since we previously observed an increase in APP
localization in early endosomes, resulting in a decrease in localization in downstream vesicles such as Ras-related protein (Rab)7 + late endosomes with SORL1 depletion [42], we performed an immunocytochemical analysis of both TRKB and GLUA1 localization with the early endosome marker EEA1. Similar to our previous observations for APP, we documented significantly increased localization of both TRKB (Fig. 1A) and GLUA1 (Fig. 1B) in early endosomes in SORL1KO neurons as compared to isogenic WT control neurons. Accumulation of neuronal cargo in early endosomes is indicative of endosomal traffic jams, which are thought to impact the transit of cellular cargo through other arms of the endo-lysosomal network. Due to SORLA’s role as an adaptor protein for the retromer complex, we also examined whether SORL1 depletion led to changes in retromer subunit localization. We observed that VPS35, a core subunit of the retromer cargo recognition complex is also mis-localized to early endosomes in the absence of SORLA activity, similar to what we observed for APP, TRKB, and GLUR1 (Supplemental Figure 1).

**Modulating SORL1 expression impacts cargo trafficking throughout the endo-lysosomal system**

The early endosome serves as a hub in which internalized cargo can be retrogradely transported to the trans-Golgi, recycled back to the cell surface or degraded as endosomes mature into late endosomes and lysosomes [56]. We have previously observed that APP localization within the trans-Golgi network was decreased in SORL1KO neurons [42]. Here, we tested whether trafficking to the degradative arm of the endo-lysosomal network was affected in our SORL1KO neurons. Trafficking of substrates out of the early endosome to late endosomes and, subsequently, lysosomes is important for protein degradation and SORLA has been previously implicated in promoting Aβ degradation via lysosomes [8]. We treated SORL1-deficient neurons with DQ Red BSA, a proteolysis sensitive fluorogenic substrate that generates fluorescence only when enzymatically cleaved in intracellular lysosomal compartments. Since substrate degradation primarily occurs in lysosomes, altered fluorescence intensity of this reagent is a readout of altered lysosomal degradation [54]. We treated neurons with DQ Red BSA for 6 and 24 h and analyzed fluorescence intensity using confocal microscopy. Consistent with loss of SORL1 leading to endosomal traffic jams, we observed a significant reduction of DQ Red BSA fluorescence intensity at both time points in SORL1KO neurons compared to isogenic WT controls (Fig. 2A). We next performed immunocytochemical staining to quantify the colocalization of our selected neuronal cargo with Rab7, a marker of late endosomes, and LAMP1 (Lysosomal Associated Membrane Protein 1), a lysosome marker. We show a

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**Fig. 1** Loss of SORL1 expression leads to increased TRKB and GLUA1 localization in early endosomes. Representative immunofluorescent images of WT and SORL1KO neurons showing increased colocalization of A TRKB (green) and B GLUA1 (green) with EEA1 (red). All neurons were immunolabeled with MAP2 (far-red) and counterstained with DAPI (blue). Scale bar: 10 μm. In all cases, quantification of colocalization was represented as Mander’s correlation co-efficient (MCC). 1 WT and 2 SORL1KO isogenic clones were used for these experiments and 10 images per clone per genotype were analyzed. Data represented as mean ± SD. Data were analyzed using parametric two-tailed unpaired t test. Significance was defined as a value of *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.
significant decrease in colocalization of TRKB (Fig. 2B) and GLUA1 (Fig. 2C) with Rab7. This result is consistent with our previous observation for APP [41]. We analyzed colocalization of these cargo with LAMP1 and we observed a significant decrease with APP (Fig. 2D) and TRKB (Fig. 2E) and a trend of a decrease with GLUA1 (Fig. 2F). These data indicate some fluidity in the network but suggest that trafficking of APP, TRKB and GLUA1 to late endosomes/lysosomes is all decreased by SORL1KO, although GLUA1 may be more likely to be trafficked to cell surface pathways or utilizes other adaptor proteins for late endosome to lysosomal trafficking. These changes in localization are not due to changes in expression of cargo. We have previously shown that APP levels do not change in SORL1KO neurons [42] and also show here that protein expression of TRKB and GLUA1 are not different (Supplemental Figure 3).

We next utilized previously generated cell lines that overexpress SORL1 cDNA using the piggybac transposon system [96] to test whether increased SORL1 expression may enhance the trafficking pathways that are impaired in the SORL1KO neurons. Importantly, the SORL1 overexpressing (SORL1OE) cell line and control were generated in the same genetic background as our SORL1KO and isogenic WT cell lines. Interestingly, while there was no effect of SORL1 overexpression on DQ Red BSA fluorescence at the earlier time point (6 h), we did see a significant enhancement of DQ RED BSA trafficking at the 24-h time point (Fig. 3A). In accordance, we observed significantly increased localization of our studied cargo with late endosomal and lysosomal markers in the SORL1OE neurons (Fig. 3B–G).

Lysosome size can influence lysosome function and is altered in AD [17, 38]. Similarly, location and number of lysosomes within neurons can alter degradative activity [11, 24, 28, 94] and in some cases, altered lysosomal distribution may represent an early neuropathological defect [98]. Recently, loss of SORL1 in hiPSC neurons was shown to contribute to lysosome dysfunction as indicated by both increased lysosome size and number as well as decreased cathepsin-D activity [37]. Therefore, we first analyzed LAMP1-immunopositive puncta and also documented a significant increase in lysosome size and number in our SORL1KO neurons (Supplemental Figure 2A). Interestingly, although the number of lysosomes marked by LAMP1 puncta is increased in SORL1KO neurons, we did not observe a significant change in LAMP1 protein expression (Supplemental Figure 3). This may be partially explained by differences in autophagy in SORL1 KO neurons which we did not examine in this study but that has been previously reported [37] and further underscores the dynamic complexity of the endo-lysosomal network.

Next, we analyzed colocalization of Cathepsin-D and LAMP1 to determine if loss of SORL1 expression leads to altered Cathepsin-D trafficking in neurons. Retromer trafficking is required to deliver one of the most abundant lysosomal proteases, pro-cathepsin D, to lysosomes via the mannose-6-phosphate receptor (M6PR) [64, 76]. The SORLA protein has a GGA-binding motif within its cytoplasmic domain similar to that of M6PR [39, 86], and mis-trafficking of Cathepsin-D to lysosomes could affect the maturation and degradative capacity of these organelles. Therefore, we analyzed colocalization of Cathepsin-D and LAMP1 to determine if loss of SORL1 expression leads to altered Cathepsin-D trafficking in neurons. However, we did not observe a change in Cathepsin-D colocalization between WT and SORL1KO (Supplemental Figure 2).

Taken together, our data suggest that SORL1 loss in neurons reduces trafficking of cargo out of the early endosome to the late endosome and lysosome, contributing to lysosome stress as evidenced by an increase in size and number in these conditions while the decreased cathepsin-D activity observed upon SORL1 loss [37] may not be due to impairment of lysosomal trafficking of the enzyme.

Loss of SORL1 impacts the endosomal recycling pathway

Another route out of the early endosome is via the endocytic recycling complex (ERC) which can send cargo either to the cell surface or to the trans-Golgi network [29, 50, 52, 55]. To directly examine if SORL1 expression alters recycling function, we performed a transferrin recycling assay using confocal microscopy. Transferrin can be recycled via a fast pathway within approximately 5–10 min after being internalized or via a slower pathway involving the ERC over longer periods of time [60, 85]. We examined the fluorescence intensity of Alexa Fluor 647-conjugated transferrin over a 40-min time course in WT and SORL1KO neurons and observed that a higher percentage of intracellular fluorescent transferrin persisted in SORL1KO neurons at both early and later time points as compared to WT neurons, indicating reduced recycling pathway function (Fig. 4A). Cargo destined for the cell surface can transit the ERC via Rab11 + recycling endosomes [69]. Altered size of recycling endosomes can be indicative of dysfunctional recycling of cargo through these compartments. We tested whether loss of SORL1 expression affected the size of Rab11 + recycling endosomes. Interestingly, we observed a significant increase in the size of Rab11 + recycling endosomes, although there was no change in Rab11 protein expression in the SORL1KO neurons (Fig. 4B, Supplemental Figure 3), suggesting that this endosomal compartment is also under stress. To test if increased size is due to abnormal cargo trafficking through recycling endosomes, we assessed colocalization of APP, TRKB and GLUA1 with Rab11 and observed increased colocalization of all three cargoes with Rab11+ structures in SORL1KO neurons compared to WT neurons (Fig. 4C–E).
Fig. 2 Loss of SORL1 expression impairs trafficking to late endosomes and lysosomes. A SORL1 KO neurons show reduced lysosomal proteolytic activity as measured by DQ Red BSA. Representative immunofluorescent images of WT and SORL1 KO neurons showing double immunolabeling for MAP2 (green) and DQ Red BSA (red). Scale bar: 10 μm. Quantification of fluorescence intensity of DQ Red BSA using ImageJ software. B–F SORL1 KO neurons show reduced colocalization of cargo with late endosomes and lysosomes. Representative immunofluorescent images of WT and SORL1 KO neurons showing reduced colocalization of B TRKB (green) and C GLUA1 (green) with Rab7-positive late endosomes (red) in SORL1 KO neurons. Representative immunofluorescent images of WT and SORL1 KO neurons showing reduced colocalization of D APP (green) and E TRKB (green) and F GLUA1 (green) with LAMP1-positive lysosomes (red) in SORL1 KO neurons. Scale bar: 10 μm. In all cases, quantification of colocalization was represented as Mander’s correlation coefficient (MCC). 1 WT and 2 SORL1 isogenic clones were used for these experiments and 10 images per clone per genotype were analyzed. Data represented as mean ± SD. Data were analyzed using parametric two-tailed unpaired t test. Significance was defined as a value of *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

Together, these data demonstrate that loss of SORL1 impacts neuronal recycling endosome pathways by causing traffic jams in the recycling endosomes, similar to the effect that SORL1 loss has on early endosomes.

SORL1 depletion reduces cell surface levels of cargo

Together, our data indicate that SORL1 KO neurons have impaired cargo recycling with increased retention of cargo in recycling endosomes. These observations led us to test whether this cargo was indeed trafficked to the cell surface. A portion of APP has been shown to return to the cell surface via recycling endosomes [15] and SORLA can interact with the sorting nexin SNX27 to return APP to the cell surface [15, 35], although in that study the exact compartment was not described. Furthermore, recycling endosomes are the source for repopulation of AMPA receptors to the synapse during long-term potentiation [61]. We, therefore, examined cell surface levels of APP and GLUA1 using immunofluorescence and confocal microscopy. We documented a significant decrease in cell surface staining of both APP (Fig. 5A) and GLUA1 (Fig. 5B) in SORL1 KO neurons as compared to WT, consistent with our hypothesis that SORLA is involved in regulating traffic from recycling endosomes. Due to the importance of GLUA1 in the formation of functional excitatory synapses, we next analyzed neuronal activity by culturing SORL1 KO and isogenic WT neurons on multi-electrode array (MEA) plates. Interestingly, we observed an early increase in the mean firing rate of SORL1 KO neurons at an early time point (27 days post-plating); however, neuronal firing in SORL1 KO neurons was significantly reduced at a later time point (66 days post-plating) (Fig. 5C) suggesting that synaptic activity may be partially impaired in these cells.

SORL1 overexpression enhances endosomal recycling

Defects in cell surface recycling have severe consequences in neurons, especially as these processes are necessary for healthy neuronal function and enhancing pathways that promote endosomal recycling in neurons may be beneficial. Using our SORL1 OE neurons, we analyzed recycling function using the transferrin recycling assay and observed that SORL1 OE neurons showed significantly faster transferrin recycling (Fig. 6A). We next tested whether colocalization of cargo with recycling endosomes and cell surface recycling was altered between SORL1 OE and WT neurons. Interestingly, the size of Rab11 + recycling endosomes was significantly smaller in SORL1 OE neurons (Fig. 6B) possibly indicating that increased SORL1 expression is clearing cargo more rapidly from this compartment. We observed a significant increase in localization of cargo with Rab11 + recycling endosomes (Fig. 6C–E). While this result was initially surprising, as we also saw increased colocalization with Rab11 + recycling endosomes in our SORL1 KO neurons (Fig. 4C–E), we further documented a significant increase of APP and GLUA1 on the cell surface compared to WT neurons with only endogenous SORL1 expression (Fig. 6F, G), as opposed to decreased APP and GLUA1 localization on the cell surface in SORL1 KO neurons (Fig. 5A, B). These results suggest that cell surface trafficking via a Rab11 pathway is enhanced by increased SORL1 expression and that a crucial action of SORLA is the trafficking out of recycling endosomes. Thus, our data support a critical role for SORLA for trafficking cargo from recycling endosomes to the cell surface. In addition, we show for the first time that SORLA levels may regulate cell surface recycling of AMPA receptor subunits in human neurons.

SORL1 depletion affects gene expression

To determine a more global effect of chronic SORL1 loss in human neurons, we performed bulk RNA sequencing of SORL1 KO neurons compared to WT neurons. Interestingly, we observed that there were significantly more downregulated genes in SORL1 KO neurons than upregulated ones (Supplemental Figure 4B). While none of the cargo we explicitly studied in this work was differentially expressed, GO analysis showed that the top downregulated molecular function pathways in the SORL1 KO cells were related to receptor–ligand activity and extracellular matrix organization (Fig. 7A). The top upregulated molecular function pathways were related to ion channel activity (Fig. 7B). To understand these data in the context of an integrated network, we used an analysis method that infers ligand receptor
KO neurons (Supplemental SORL1 were upregulated in observed that pathways associated with synaptic function Interestingly when we performed enrichment analysis, we decrease of GLUA1 puncta on the cell surface and observed neuronal health and development. Since we documented a may ultimately impact multiple pathways important for presence of endosomal traffic jams in SORL1 KO neurons trophin and growth factor signaling and suggesting that the and fibroblast growth factor/fibroblast growth factor receptor (FGF/FGFR) signaling, indicating alterations in neurotrophin and growth factor signaling and suggesting that the presence of endosomal traffic jams in SORL1KO neurons may ultimately impact multiple pathways important for neuronal health and development. Since we documented a decrease of GLUA1 puncta on the cell surface and observed altered neuronal activity on MEAs, we further mined our RNA-seq data for genes involved in synaptic function. Interestingly when we performed enrichment analysis, we observed that pathways associated with synaptic function were upregulated in SORL1KO neurons (Supplemental Figure 5).

Discussion

Trafficking through the endo-lysosomal network regulates intracellular location of proteins, dictating their homeostasis, function and influence on cellular physiology. Functional studies by our group and others document endosomal abnormalities in hiPSC-derived neuronal models of AD [37, 42, 44]. Emerging from this evidence is the role of SORL1 as an endosomal gene that plays essential roles in mediating cargo trafficking. Recent work has implicated SORL1 as AD’s fourth causal gene [5, 73], and of these genes, it is the only one linked to the common late-onset form of the disease. Understanding SORL1’s function is paramount for understanding AD’s pathogenic mechanisms and for potential therapeutic interventions.

Acting as a adaptor molecule of the retromer trafficking complex, SORLA has already been pathogenically linked to AD by its role in recycling APP out of endosomes [4, 31, 59]. This current work and our previous study [42] shows that SORLI/ SORL1 depletion leads to increased APP localization in early and recycling endosomes. By lengthening the residence time of APP in these endosomal compartments, accelerated amyloidogenic cleavage of APP occurs due to the close proximity of APP and BACE1 [87]. Indeed, loss of SORLI leads to the accumulation of Aβ peptides, an antecedent of ‘amyloid pathology’ [4, 42, 70]. We hypothesized that loss of SORLI in neurons would impact other cargo important for healthy neuronal function. To test this hypothesis, in addition to APP, we examined localization of the neurotrophin receptor TRKB and the GLUA1 subunit of the AMPA receptor and also observed that these proteins are increased in early endosomes (Fig. 1). These cargoes link to another key pathology of AD: neurodegeneration, a slowly progressive process that begins with synaptic dysfunction characterized by glutamate receptor loss, which then progresses to synaptic loss before ultimately, over years, leading to widespread neuronal cell death [78].

The early endosome is considered the central station in the sorting and trafficking of cargo throughout the many stations of the endo-lysosomal system. While the early endosome is the station that is affected first and foremost in AD, it is not surprising that a primary dysfunction in this central station will secondarily influence trafficking throughout the system including the recycling and degradative pathways. Indeed, SORLA was shown to traffic Aβ to lysosomes in neuroblastoma cells, a function that is impaired by an AD-associated variant [8]. Our work presented in this study, along with other recent work [37] also supports a role for SORLI in lysosomal trafficking in neurons. We observe a decrease in the pH-sensitive fluorogenic substrate DQ RED BSA in our SORLIKO neurons and decreased localization of APP, TRKB, and GLUA1 in late endosomes and lysosomes in SORLIKO neurons and that this is reversed in SORL1OE neurons (Figs. 2, 3). We interpret our functional and colocalization data to suggest that loss of SORLI expression mainly affects trafficking of cargo to lysosomes, but our data do not rule out a role of SORLI in neuronal lysosome function. Hung et al. reported decreased Cathepsin-D activity in SORLI-deficient neurons, suggesting that SORLI loss directly impacts lysosome function [37]. While we did
Fig. 4 Loss of SORL1 impacts the cell surface recycling pathway. A SORL1 KO neurons show slower rate of transferrin recycling. Quantification of fluorescence intensity of intracellular transferrin at different time points after treating cells with Alexa Fluor 647-conjugated transferrin for 15 min, using ImageJ software. Data represented as percent of time 0 fluorescence intensity. 2 WT and 2 SORL1 KO isogenic clones were used for these experiments. 12 images per clone per genotype were analyzed. B SORL1 KO neurons show larger recycling endosomes. Representative immunofluorescent images of WT and SORL1 KO neurons labeled with antibodies for MAP2 (red) and Rab11 (green). Nuclei were counterstained with DAPI (blue). Scale bar: 5 μm. Quantification of size of Rab11-labeled recycling endosomes using CellProfiler software. 1 WT and 2 SORL1 KO isogenic clones were used for these experiments. 15 images per clone per genotype were analyzed. C APP (green), D TRKB (green) and E GLUA1 (green) with Rab11-positive recycling endosomes (red) in SORL1 KO neurons. Scale bar: 10 μm. In all cases, quantification of colocalization was represented as Mander’s correlation coefficient (MCC). 1 WT and 2 SORL1 KO isogenic clones were used for these experiments. 10 images per clone per genotype were analyzed. Data represented as mean ± SD. Data were analyzed using parametric two-tailed unpaired t test and two-way ANOVA. Significance was defined as a value of *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.
Fig. 5 Loss of SORL1 expression impairs recycling to the cell surface. A–B SORL1 KO neurons show reduced cell surface expression of APP (A) and GLUA1 (B). Representative immunofluorescent images of WT and SORL1 KO neurons labeled with antibodies for APP (A) (red) and GLUA1 (B) (red). Scale bar: 5 μm. Intensity of APP and GLUA1 measured using ImageJ software. Data are presented as a ratio of surface intensity to total intensity. 2 WT and 2 SORL1 KO clones were used in these experiments. 16 images per clone per genotype were analyzed. Data represented as mean ± SD. Normally distributed data were analyzed using parametric two-tailed unpaired t test. Significance was defined as a value of *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. C Multielectrode array (MEA) analysis of WT and SORL1 KO neurons at early (d27) and late (d66) time points of differentiation. 1 WT and 1 SORL1 KO clone was used for these experiments. Data represented as mean ± SD. Data were analyzed using parametric two-tailed unpaired t test. Significance was defined as a value of *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.
**SORL1** enhances endosomal recycling. A SORL1 OE neurons show faster rate of transferrin recycling. Quantification of fluorescence intensity of intracellular transferrin at different time points after treating cells with Alexa Fluor 647-conjugated transferrin for 15 min, using ImageJ software. Data represented as percent of time zero fluorescence intensity. 1 cell line of each genotype (WT vs. SORL1 OE) was used for these experiments. 10 images per genotype were analyzed. B SORL1 OE neurons show reduced size of endosomes. Representative immunofluorescent images of WT and SORL1 OE neurons labeled with Rab11 (green) and MAP2 (red) showing smaller Rab11-positive recycling endosomes in SORL1 OE neurons. Nuclei counterstained with DAPI (blue). Quantification of Rab11+ recycling endosome size performed using CellProfiler software and represented as area of Rab11+ vesicles. Scale bar: 5 μm. 1 cell line of each genotype (WT vs. SORL1 OE) was used for these experiments. 26 images per genotype were analyzed. Representative immunofluorescent images of WT and SORL1 OE neurons showing increased colocalization of C APP (green), D TRKB (green) and E GLUA1 (green) with Rab11 (red)-positive recycling endosomes. SORL1 OE neurons and controls have endogenous GFP expression due to the piggybac vector system. GFP fluorescence is pseudo-colored (Far-red) and was used to outline cell bodies. Quantification of colocalization with Rab11 represented as Mander’s Co-efficient (MCC). Scale bar: 10 μm. 1 cell line of each genotype (WT vs. SORL1 OE) was used for these experiments. 10 images per genotype were analyzed. Representative immunofluorescent images of WT and SORL1 OE neurons showing increased cell surface expression of F APP (red) and G GLUA1 (red) in SORL1 OE neurons. Scale bar: 5 μm. Fluorescence intensity of APP and GLUA1 measured using ImageJ software. Data are presented as a ratio of surface intensity to total intensity. Nuclei counterstained with DAPI. 1 cell line of each genotype (WT vs. SORL1 OE) was used for these experiments. 12–14 images per genotype were analyzed. Data represented as mean ± SD. Data were analyzed using parametric two-tailed unpaired t test and two-way ANOVA. Significance defined as a value of *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

not observe a difference in Cathepsin-D localization to lysosomes, it is important to note that LAMP1 only partially colocalizes with Cathepsin-D in neurons [11]. Furthermore, the loss of proteolytic activity evidenced by decreased intensity of DQ Red BSA in SORL1KO neurons may not be completely due to reduced trafficking to lysosomes but could be a result of abnormal lysosomal function as DQ Red BSA is internalized by a process called macropinocytosis wherein macropinosomes can be directly trafficked to lysosomes [30, 49, 65]. Interestingly, while we did not observe an effect on DQ Red BSA at a shorter time point in SORL1OE neurons, we did see increased fluorescence of this reagent at a 24-h time point (Fig. 3), suggesting that over time, increased SORL1 expression impairs lysosome trafficking and/or function in cortical neurons.

Our experiments also point to SORL1’s role in cell surface recycling. Using a prototypical cargo, transferrin, we demonstrate a reciprocal role between loss and enhancement of SORL1 expression in cell surface recycling. Specifically, we show that SORL1KO neurons have defects in transferrin recycling at both early (10 min) and late (40 min) time points while SORL1OE neurons have faster recycling at these time points (Figs. 4, 6). This suggests that SORL1 functions in both fast and slow endosomal recycling. Our data further implicates the recycling pathway by showing that modulation of SORL1 expression affects recycling endosome size and the amount of cargo (APP, TRKB and GLUA1) localized to recycling endosomes (Figs. 4, 6).

Endocytic recycling comprises returning cargo, primarily membrane proteins, to the cell surface [14]. We studied a canonical SORL1 cargo, APP, and show that loss of SORL1 expression results in reduced cell surface APP while enhanced expression increases cell surface APP (Fig. 5). These results corroborate previous work showing that SORL1 and SNX27 work to return APP to the cell surface [15, 35] in a human model.

We also show that SORL1 plays a role in recycling glutamate receptors in human neurons (Fig. 5). This finding is critically important as recent work indicates that in mouse cortical neurons, SORLA interacts with a neuronal specific retromer subunit, VPS26b, to promote recycling of glutamate receptors [80]. In our cortical neurons when SORL1 is depleted, there is a reduction of GLUA1 subunits on the cell surface and this may result in synaptic impairment. MEA data comparing SORL1KO and isogenic WT neurons shows alterations in weighted mean firing rate as neurons mature (Fig. 5). Interestingly, we observed an increase in neuronal firing at an early time point and a significant decrease in firing at a later time point. SORL1KO mice live to adulthood but have been described to have some deficits in learning and memory that may also be age dependent [27, 32]. Some of these alterations could be explained by compensatory expression changes of ion channels or synaptic genes induced by chronic loss of SORL1 during the course of neuronal differentiation from pluripotent stem cells. Our RNA-seq data do show upregulation of ion channels and channel activity (Fig. 7). Interestingly, when we further interrogated our RNA-seq data for pathways enriched in synaptic function using the SynGo database [43], we observed an upregulation of differentially expressed genes in synaptic pathways (Supplemental Figure 5). This data suggests that SORL1KO neurons may attempt to compensate for altered trafficking of synaptic receptors by upregulating gene expression.

Our unbiased transcriptomic screen further supported that neurotrophic signaling and cell surface recycling pathways are impacted by SORL1 deficiency (Fig. 7). In one study, the SORL1 cytoplasmic tail was observed to translocate to the nucleus and activate transcription in a reporter gene assay [6]. Another possibility is that due to its effects on APP cleavage, SORL1 levels may influence the APP intracellular domain (AICD) which is known to affect gene transcription [58]. Despite this, distinct genes regulated by SORLA are not known. Rather than looking for a direct effect on gene regulation, our goal for the analysis was to determine the global effect of SORL1 loss or overexpression on neuronal...
networks. Indeed, our data does not show that the specific cargo proteins described here are differentially expressed. However, the analysis does indicate that loss of SORL1 in human neurons impacts cell surface networks, including receptor ligand interactions in neurotrophic and growth factor pathways, β-integrin signaling, and ephrin signaling.
The Alzheimer’s gene SORL1 is a regulator of endosomal traffic and recycling in human neurons.

This corroborates previous work and the altered networks we observe impact neuronal health, axonal guidance, and synapse formation [34, 36, 63].

Importantly, enhancing SORL1 expression improves cell surface trafficking of GLUA1 (Fig. 6). Trafficking of glutamate receptors is an event that is critical for preventing synaptic dysfunction and synaptic loss, thus our results link SORL1 to AD’s early-stage neurodegenerative process. Since retromer-dependent glutamate receptor recycling has been shown to occur independent of APP [88], our previous and current results suggest that reduced SORL1 activity can, at least in principle, drive two key AD pathologies, amyloid pathology and synaptic pathology, through parallel mechanisms [84].

We summarize our findings in relation to our previous work [42] in Fig. 8. In general, our results in human neurons corroborate studies in other cellular and animal models that implicate SORL1 as a key player in endosomal trafficking [1, 22, 31, 63, 82]. However, our study...
has certain limitations. Endosomal trafficking, in particular endosomal recycling, is a complex process. Because of its known genetic risk for AD, we specifically focused on the impact of modulating SORL1 expression in human neurons on this pathway. However, many other factors, some of which may be independent of SORL1, may also impact this process. Dissecting these mechanisms in multiple model systems will be important for both basic and preclinical neurobiology. Furthermore, our results encompass only one human genome. Future studies will benefit from looking at SORL1 deficiency or overexpression in multiple human genetic backgrounds. Furthermore, in this work we are describing purely neuronal phenotypes although SORL1 is expressed in other CNS cells. Future work looking at cell autonomous and non-cell autonomous mechanisms of SORL1 depletion or overexpression in human glial or brain organoid models will be informative.

Conclusions

In this work, we report that SORL1 depletion impair endosomal trafficking leading to retaining of cargo in early and recycling endosomes and impacts cell surface recycling and lysosomal trafficking of neuronal cargo. In particular, we demonstrate that SORL1 expression in neurons affects cell surface localization of GLUA1, with an impact on synaptic activity clearly highlighting the importance of SORL1 for the endosomal recycling pathways in neurons. This phenotype may ultimately translate to synaptic dysfunction and neurodegeneration as occurring in AD. Interestingly, increasing SORL1 expression enhances endosomal recycling and increases cell surface GLUA1. While the secondary downstream effects induced by SORL1 depletion in the endolysosomal system are interesting and likely relevant to AD’s ultimate pathogenesis, from a therapeutic perspective it is best to target SORL1’s primary defect, which seems to localize to the endosomal recycling pathway. Interestingly, recent biomarker studies suggest that defects in retromer-dependent endosomal recycling occur in a majority of patients with ‘sporadic’ AD [81], suggesting that the observed SORL1-induced defects may generalize across early- and late-onset forms of the disorder. Collectively, our results support the conclusion that SORL1, and the retromer-dependent pathway in which it functions, is a valid therapeutic target and interventions directed at this pathway may ameliorate endosomal recycling defects that seem to act as, at least, one primary driver of AD.
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The Alzheimer’s gene SORL1 is a regulator of endosomal traffic and recycling in human neurons

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