NEURAL REGENERATION RESEARCH
www.nrronline.org

Research Article

# *Sorl1* knockout inhibits expression of brain-derived neurotrophic factor: involvement in the development of late-onset Alzheimer's disease

**Mingri Zhao[2, #], Xun Chen[1, #], Jiangfeng Liu[1], Yanjin Feng[1], Chen Wang[1], Ting Xu[1], Wanxi Liu[2], Xionghao Liu[2], Mujun Liu[3, *], Deren Hou[1, *]**

**https://doi.org/10.4103/1673-5374.387975**

**Date of submission: April 19, 2023**

**Date of decision: July 27, 2023**

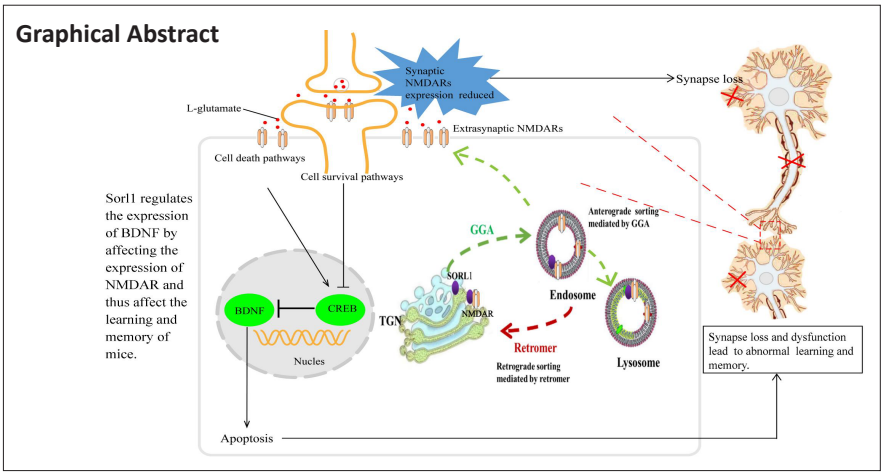
**Date of acceptance: September 15, 2023**

**Date of web publication: November 13, 2023**

***From the Contents***



**Graphical Abstract**




## Abstract

Sortilin-related receptor 1 (*SORL1*) is a critical gene associated with late-onset Alzheimer's disease. SORL1 contributes to the development and progression of this neurodegenerative condition by affecting the transport and metabolism of intracellular β-amyloid precursor protein. To better understand the underlying mechanisms of *SORL1* in the pathogenesis of late-onset Alzheimer's disease, in this study, we established a mouse model of *Sorl1* gene knockout using clustered regularly interspaced short palindromic repeats-associated protein 9 technology. We found that *Sorl1*-knockout mice displayed deficits in learning and memory. Furthermore, the expression of brain-derived neurotrophic factor was significantly downregulated in the hippocampus and cortex, and amyloid β-protein deposits were observed in the brains of *Sorl1*-knockout mice. *In vitro*, hippocampal neuronal cell synapses from homozygous *Sorl1*-knockout mice were impaired. The expression of synaptic proteins, including Drebrin and NR2B, was significantly reduced, and also their colocalization. Additionally, by knocking out the *Sorl1* gene in N2a cells, we found that expression of the N-methyl-D-aspartate receptor, NR2B, and cyclic adenosine monophosphate-response element binding protein was also inhibited. These findings suggest that *SORL1* participates in the pathogenesis of late-onset Alzheimer's disease by regulating the N-methyl-D-aspartate receptor NR2B/cyclic adenosine monophosphate-response element binding protein signaling axis.

**Key Words:** brain-derived neurotrophic factor; late-onset Alzheimer's disease; N-methyl-D-aspartate receptor; sortilin-related receptor 1; synapse

## Introduction

Alzheimer's disease (AD) is a degenerative central nervous system disease characterized by progressive cognitive impairment and behavioral changes, concealed in onset, and affected by genetic and environmental factors. AD consists of familial AD and late-onset AD (LOAD). Clinically, 90% of patients with AD have LOAD (Rostagno, 2022). AD is the main and most common senile dementia. With aging of the population, the prevalence of AD has increased substantially. According to the 2018 "World Alzheimer Report", a new case of dementia occurs every 3 seconds worldwide. Approximately 50 million people were affected by dementia worldwide in 2018. By 2050, the number of people with dementia is expected to reach 152 million (Weidner and Barbarino, 2019). Despite this, the mechanism behind the pathogenesis of AD remains unclear. Moreover, drugs designed to target the characteristic pathological accumulation of amyloid-β (Aβ) peptide have failed to show promising results for years. Therefore, it is crucial to further explore the underlying mechanisms of AD (Asher and Priefer, 2022; Passeri et al., 2022; Abbott, 2023).

The pathogenesis of LOAD is still unknown, but is generally believed to result from the interplay of environmental and polygenic factors. In recent years, a genome-wide association study (GWAS) and our previous case-control study of over 200 patients with AD identified sortilin-related receptor 1 (*Sorl1*) as a high-risk susceptibility gene for LOAD (Feng et al., 2015; Karch and Goate, 2015). The *Sorl1* gene encodes a membrane protein of approximately 250 kDa that is expressed in the central and peripheral nervous systems. SORL1 belongs to the low-density lipoprotein receptor (LDLR) family and the vacuolar protein sorting domain receptor family (Malik and Willnow, 2020). SORL1 primarily exists in the early nuclear endosome and trans-Golgi network. It interacts with distinct cytosolic adaptors for anterograde and retrograde amyloid precursor protein (APP) transport between the trans-Golgi network and early endosomes. Consequently, SORL1 restricts the delivery of APP precursor to endocytic compartments, favoring amyloidogenic breakdown and reduced Aβ deposition (Caglayan et al., 2014; Mishra et al., 2022). A study in human induced pluripotent stem cells showed that *Sorl1* knockout leads to endosome enlargement, which is a typical phenotype of early AD cytopathology. This pathological change is not affected by inhibition of β-secretase (Knupp et al., 2020). Instead, SORL1 participates in the occurrence and development of AD by affecting the metabolism of APP and the intracellular transport of APP, tau, and other related proteins (Knupp et

[1]Department of Neurology, The Third Xiangya Hospital of Central South University, Changsha, Hunan Province, China; [2]Center for Medical Genetics & Hunan Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, Hunan Province, China; [3]Department of Cell Biology, School of Life Sciences, Central South University, Changsha, Hunan Province, China

***Correspondence to:** Deren Hou, PhD, hou0718@126.com; Mujun Liu, PhD, liumujun03@csu.edu.cn.

https://orcid.org/0000-0002-2015-6525 (Deren Hou); https://orcid.org/0000-0002-1558-1448 (Mujun Liu); https://orcid.org/0000-0002-8260-7935 (Mingri Zhao)

#Both authors contributed equally to this work.

***Funding:*** *This study was supported by the Community Development Office of Hunan Provincial Science and Technology Department, China, Nos. 2020SK53613 (to DH), 21JJ31006 (to DH); the Fundamental Research Funds of Central South University, Nos. CX20220375 (to TX), 2023zzts215 (to MZ).*

***How to cite this article:*** *Zhao M, Chen X, Liu J, Feng Y, Wang C, Xu T, Liu W, Liu X, Liu M, Hou D (2024) Sorl1 knockout inhibits expression of brain-derived neurotrophic factor: involvement in the development of late-onset Alzheimer's disease. Neural Regen Res 19(7):1602-1607.*

al., 2020). In addition to the classical pathological features of Aβ deposition and neurofibrillary tangles, patients with AD also show pathological manifestation of neuronal loss in the frontal temporal lobe and hippocampus (Trejo-Lopez et al., 2022). Some studies have shown that expression of brain-derived neurotrophic factor (BDNF) is markedly reduced in patients with AD (Alfonsetti et al., 2023; Lazaldin et al., 2023). Several methods, including aerobic exercise, can increase BDNF expression and substantially improve cognitive behavioral impairments of AD (Gao et al., 2022; Baranowski et al., 2023). Normal expression and transport of BDNF play a vital role in learning and memory (Tanila, 2017). Several studies have demonstrated that *Sorl1* may be a downstream target of BDNF. BDNF can significantly increase *Sorl1* gene transcription through the extracellular signal-regulated kinase pathway, with more than a 10-fold increase in a BDNF-treated group compared with the control group (Rohe et al., 2009). In a BDNF knockout ($BDNF^{-/-}$) mouse model, significant downregulation of SORL1 expression and activity was observed, and decreased BDNF expression can reduce neuroprotective effects. In addition, by affecting the transport and metabolism of APP, BDNF is involved in the occurrence and development of AD through the regulation of SORL1 expression. Rohe et al., (2009) have shown that SORL1 knockout leads to a decrease in the effect of BDNF on Aβ deposition, suggesting that BDNF function in improving the cognitive and behavioral impairments of mice depends on SORL1. As an intracellular sorting receptor-related protein, SORL1 may regulate the expression and transport of BDNF.

*Sorl1* knockout ($Sorl1^{-/-}$) mice showed a significant reduction in BDNF protein expression (Rohe et al., 2009), although the mechanism remains largely unknown. The purpose of this study was to clarify the regulatory role of SORL1 on BDNF and the development of AD, providing new ideas for further understanding on the pathogenesis of AD and identify new therapeutic targets.

## Methods

### Animals

Two-month-old male and female C57BL/6 mice (16–20 g) were purchased from Cavens Biotechnology Company (Changzhou, Jiangsu, China; license No. SCXK (Su) 2021-0013). $Sorl1^{-/-}$ mice were generated by our team using clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9) technology (Modell et al., 2022; **Additional file 1**). All mice were raised in a specific pathogen-free mouse feeding room. All experiments were designed and reported according to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (Percie du Sert et al., 2020). All procedures regarding the care and use of animals were approved by the Institutional Animal Care and Use Committee of the Central South University of China (ethics approval No. 2019-S212) on March 11, 2019. Behavioral tests were conducted when the mice reached 11 months of age.

### Morris water maze

The Morris water maze consisted of a circular stainless steel pool (120 cm in diameter and 50 cm high, with the bottom 30 cm above floor level), filled with water (made opaque with milk), and a white platform (12 cm in diameter and 28 cm high) submerged 1–2 cm below the water surface. The water was maintained at 22 ± 2°C, and the platform was placed in the center of the target quadrant. The experimental environment was kept quiet and dark, and the water maze test was conducted at the same time every day. Tests comprised of an oriented navigation and spatial probe test. A computerized tracking system (SMART V3.0, RWD, Shenzhen, China) recorded the swimming location. The oriented navigation training was performed for 4 days, and each mouse was trained four times from four different quadrants every day. The spatial probe test was completed at 1 day after the oriented navigation training (Zhu et al., 2022).

### Step-through test

The apparatus (RWD, 63018) was designed to have a dark and light compartment. These two compartments were connected via a door, with the electrical stimulus only available in the dark compartment. Prior to the experiment, a mouse was placed inside the apparatus and allowed to freely explore for 3 minutes to acclimatize to the surroundings. During the experiment, the mouse was placed in the light room facing the hole, and the automatic recorder (SMART V3.0, RWD) started simultaneously. When the animal entered the dark room through the door, they received an electric shock (current 0.3 mA, voltage 30 V) through a copper grid placed at the bottom of the dark room. The number of times the mouse entered the dark compartment (number of errors), as well as the time it took for the mouse to enter the dark compartment (latency), were recorded within 30 seconds (Gong et al., 2020).

### Shuttle box test

The mouse was placed in a small chamber within the shuttle box (YLS-17B, YiYanKeJi, Jinan, Shandong, China). An optic-acoustic stimulus was administered after a 5-second safety period. If the mouse was still on the same side after the stimulus, it was given an electrical stimulus (current 0.3 mA) for 5 seconds. When the mouse escaped from the electric shock (by the round hole to the opposite side), it was given an optic-acoustic stimulus after a 5-second safety period, followed by an electrical stimulus for 5 seconds. The mouse would repeat this training for 3 minutes.

When an optic-acousto signal was present, even in the absence of an electric shock, the mouse could actively avoid the opposite chamber to form a memory. Within 10 seconds of the occurrence of optic-acousto stimulation, that is before the electric shock, the mouse escaped to a safe area as an active avoidance response; after the electric shock, it escaped to the safe area as a conditioned avoidance response. The number of electric shocks the mouse received (passive avoidance), and the number of times the mouse escaped as a result of optic-acousto stimulation (conditioned avoidance) were recorded (Gong et al., 2020).

### Immunohistochemical staining

Five mice were randomly selected from each group and anesthetized by inhalation of 5% isoflurane (Yuyan Instruments, Shanghai, China). Perfusion was then conducted with phosphate-buffered saline (PBS) and 4% paraformaldehyde. Intact brains were fixed in 4% paraformaldehyde for 24 hours, dehydrated, and embedded in paraffin. Brains embedded in paraffin were cut into 7-μm thick sections and used for immunohistochemical staining. Paraffin sections, including the mouse cortex and hippocampus, were collected and incubated overnight at 4°C with primary antibodies against BDNF (rabbit, 1:200, Abcam, Cambridge, MA, USA, Cat# ab108319, RRID: AB_10862052), Aβ (rabbit, 1:100, Thermo Fisher Scientific, Waltham, MA, USA, Cat# 700254, RRID: AB_2532306), and N-methyl-D-aspartate receptor subunit 2B (NR2B; rabbit, 1:200, Abcam, Cat# ab65783, RRID: AB_1658870). Slices were then incubated in the dark for 60 minutes with the corresponding secondary antibodies (rabbit anti-mouse IgG, 1:200, Abcam Cat# ab6728, RRID: AB_955440; or mouse anti-rabbit IgG, 1:200 Abcam, Cat# ab99797, RRID: AB_10710682). Following staining, the sections were counterstained with 3,3-N-diaminobenzidine (Beyotime, Nanjing, Jiangsu, China, Cat# P0203) and nuclei stained with hematoxylin (Beyotime, Cat# C0105S). Sections were imaged using a Nikon fluorescent microscope (Nikon Eclipse 80i Advanced Research Microscope, Nikon, Tokyo, Japan, RRID:SCR_015572). The average optical density value of the protein was measured and analyzed using ImageJ software (version 1.45, National Institutes of Health, Bethesda, MD, USA) (Schneider et al., 2012).

### N2a cell culture and transfection

The N2a cell line (CLS Cat# 400394/p451_Neuro-2A, RRID:CVCL 0470) was purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle medium/Opti-minimum essential medium (DMEM/Opti-MEM [1:1]; Gibco, Waltham, MA, USA, Cat# 31985070) supplemented with 5% fetal bovine serum (Gibco, Cat# 10099141C), 100 U/mL penicillin, and 100 μg/mL streptomycin (Thermo Fisher Scientific, Cat#10378016) at 37°C in a 5% $CO_2$ incubator. According to the manufacturer's protocol, 2.5 μg control plasmid (GenePharma, Suzhou, Jiangsu, China) and 2.5 μg short hairpin (sh)RNA-*Sorl1* (GenePharma) transfections were performed with Lipofectamine 2000 (Thermo Fisher Scientific, Cat# 11668500). Briefly, the plasmids were mixed with dilute Lipofectamine 2000 transfection reagent and Opti-MEM reduced serum medium, incubated for 5 minutes, and then added to the cells for 48 hours.

### Neuronal culture

Primary mouse hippocampal neuronal cultures were generated from newborn mice (Liao et al., 2022). The mice were anesthetized with isoflurane and then quickly euthanized by cervical dislocation. Hippocampal tissue was dissected from the brain in a precooled Hanks' balanced salt solution and digested at 37°C for 30 minutes in prewarmed Hanks' balanced salt solution containing 0.025% trypsin (Gibco, Cat# R001100). Neurons were then plated onto poly-D-lysine (Cat# A3890401, Thermo Fisher Scientific)-coated coverslips (100,000 cells/mL for 12-well plates). Cultured cells were maintained at 37°C in 5% $CO_2$ in neurobasal medium containing 1× B27 supplement (Thermo Fisher Scientific, Cat#17504044), 1× GlutaMAX (Thermo Fisher Scientific, Cat#35050061), 100 U/mL penicillin, and 100 μg/mL streptomycin solution (Thermo Fisher Scientific, Cat#10378016). After 18 days *in vitro*, cultured neurons were used for immunofluorescence staining and observation.

### Immunofluorescence and image analysis

Primary hippocampal neurons were fixed with 4% paraformaldehyde for 20 minutes. After washing with Dulbecco's PBS (DPBS), the cells were permeabilized for 15 minutes with 0.1% PBST (DPBS containing 0.1% Triton X-100) and blocked for 30 minutes with 5% bovine serum albumin dissolved in DPBS. Cells were then incubated overnight at 4°C with primary antibody (NR2B, 1:200; or developmentally regulated brain protein [Drebrin], mouse, 1:200, Abcam, Cat# ab12350, RRID: AB_299034), and washed five times in DPBS. Secondary antibodies (goat anti-rabbit IgG-AlexaFluor 488, 1:200, Cell Signaling Technology, Danvers, MA, USA, Cat# 4412, RRID: AB_1904025; or goat anti-mouse IgG H&L [Cy3®, 1:200, Abcam, Cat# ab97035, RRID: AB_10680176]) were diluted with 5% bovine serum albumin and incubated at room temperature (25°C) for 1 hour. Subsequently, the cells were washed five times in DPBS. Images were acquired with a LAS X SP-5 confocal microscope (Leica DM IRB, Wetzlar, Germany). The colocalized NR2B/Drebrin area was quantified with ImageJ software.

### Reverse transcription-quantitative polymerase chain reaction

N2a cells were lysed with TRIzol® reagent (Sigma-Aldrich, St. Louis, MO, USA, Cat# T9424) according to the manufacturer's instructions. Complementary DNA was synthesized from 1 μg total RNA using a reverse transcription kit (Vazyme, Nanjing, China, Cat# R223). Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis was performed using SYBR Green Master Mix (Vazyme, Cat# Q711). Briefly, reverse transcription was carried out at 37°C for 15 minutes, 85°C for 5 seconds, and then samples were kept at 4°C. Complementary DNA was amplified under the following reaction conditions: 95°C for 30 seconds, 40 cycles at 95°C for 10 seconds,

60°C for 20 seconds, and 70°C for 20 seconds. Sequences of target genes and reference genes are shown in **Additional Table 1**. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an internal control, and relative expression was calculated by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

### Western blotting

Total protein lysates of N2a cells or hippocampal tissue samples were prepared using radio immunoprecipitation assay (RIPA) lysis buffer (Thermo Fisher Scientific, Cat# 89900) in the presence of a protease inhibitor and PhosStop (Selleck, Houston, TX, USA). Protein concentration was quantified using a bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Cat# 23225). Proteins in lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride membranes (Thermo Fisher Scientific, Cat#88518), and immunoblotted overnight at 4°C with the corresponding antibodies: SORL1 (rabbit, 1:1000, Abcam, Cat# ab190684), Drebrin (1:1000), postsynaptic density protein 95 (PSD95; mouse, 1:1000, Abcam, Cat# ab238135, RRID: AB_2895158), BDNF (1:1000), NR2B (1:1000), Aβ (1:1000), β-actin (mouse, 1:5000, Thermo Fisher Scientific, Cat# MA5-15452, RRID: AB_11001306), tubulin (mouse, 1:5000, Cell Signaling Technology, Cat# 5335, RRID: AB_10544694), vinculin (rabbit, 1:2000, Cell Signaling Technology, Cat# 13901, RRID: AB_2728768]), and cyclic adenosine monophosphate-response element binding protein (CREB; rabbit, 1:1000, Cell Signaling Technology, Cat# 9197, RRID: AB_331277). Membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibodies (rabbit anti-mouse IgG, 1:5000; or mouse anti-rabbit IgG, 1:5000) at room temperature (25°C) for 1 hour. Blots were developed using the SuperSignal West Pico Substrate chemiluminescence kit (Thermo Fisher Scientific, Cat# 34580) and Gene Genius Bio-Imaging System (Bio-Rad, Hercules, CA, USA). The optical density of protein bands was analyzed using ImageJ software (Gao et al., 2020).

### Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.0.2 (GraphPad Software, Inc., San Diego, CA, USA, www.graphpad.com). Student's *t*-test was used to compare the mean values of two groups. Three or more means were analyzed by one-way analysis of variance (ANOVA) and Bonferroni's *post hoc* test. The data are expressed as mean ± standard error of the mean (SEM). Statistically significant values were considered $P < 0.05$.

## Results

### *Sorl1*$^{-/-}$ mice have learning and memory impairments

*Sorl1*$^{-/-}$ mice were identified by Sanger sequencing (Crossley et al., 2020) and western blot assay (**Additional Figure 1**). An oriented navigation trial was performed to evaluate spatial learning and memory abilities of the mice (Gong et al., 2020; Zhu et al., 2022). In each group, latency decreased with an increase in training time. Compared with wild-type mice (*Sorl1*$^{WT}$), latency was longer in *Sorl1*$^{-/-}$ mice. However, there was no significant difference ($P > 0.05$) in latency between *Sorl1* heterozygote (*Sorl1*$^{+/-}$) and *Sorl1*$^{WT}$ mice (**Figure 1A**). In the spatial probe test, time spent in the target quadrant and number of crossing platforms are used as indicators of spatial learning and memory abilities in mice.

Better cognitive and memory function can be revealed by more time spent in the target quadrant and more frequent crossings of the central square area (Gong et al., 2020; Zhu et al., 2022). In the spatial probe test, compared with the *Sorl1*$^{WT}$ group, latency in the target quadrant and number of crossings in the central square area were shorter and less in the *Sorl1*$^{-/-}$ group (**Figure 1B**). *Sorl1*$^{-/-}$ mice also had no sense of direction on the escape platform, tended to swim freely in the maze, and were even unable to find the platform location (**Figure 1C**). In the step-through test, compared with the *Sorl1*$^{WT}$ group, the *Sorl1*$^{-/-}$ group exerted a significant decrease in latency and increase in number of mistakes (**Figure 2A** and **B**). In the shuttle box test, compared with the *Sorl1*$^{WT}$ group, the *Sorl1*$^{-/-}$ group exhibited a significantly lower number of passive avoidance responses and higher number of conditioned avoidance responses (**Figure 2C** and **D**). These results indicate a decline in the cognitive memory ability of *Sorl1*$^{-/-}$ mice.

### Aβ deposition and BDNF expression downregulation in the cortex and hippocampus of *Sorl1*$^{-/-}$ mice

*Sorl1* knockout may lead to AD pathology by affecting the transport of APP in endosomes/lysosomes without altering its process or expression. This suggests that the pathology of AD caused by *Sorl1* knockout does not only depend on classical Aβ deposition. At an early stage of AD, SORL1 protein dysfunction may affect the transcription and expression of downstream molecules (Caglayan et al., 2014). We found Aβ deposition in the cortex and hippocampus of *Sorl1*$^{-/-}$ mice (**Figure 3A–D**), along with reduced BDNF expression (**Figure 3A**, **B**, **E**, and **F**). Therefore, the occurrence of AD caused by SORL1 is related to Aβ deposition and reduced expression of BDNF. Our findings suggest that SORL1 might affect learning and memory formation by regulating the expression of BDNF.

### SORL1 regulates the expression of BNDF by mediating NMDAR expression

BDNF plays a critical role in synaptic plasticity and neuronal survival (Rohe et al., 2009). In *Sorl1*$^{-/-}$ mice, Aβ deposits affect BDNF expression (Zhu et al., 2022). A previous study found that BDNF can reduce Aβ deposition by

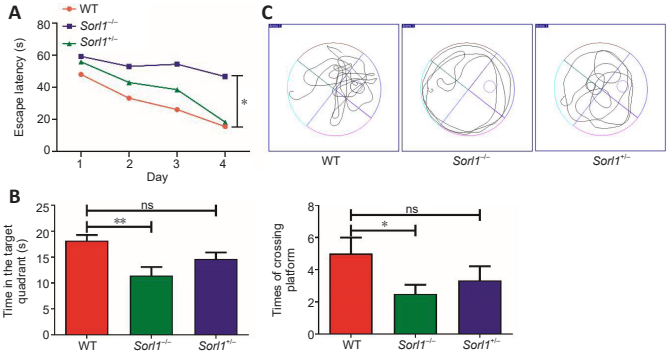



**Figure 1 | The performance of *Sorl1*$^{-/-}$ mice in the Morris water test.**
(A) Latency in the oriented navigation trial. (B) Time spent in the target quadrant and number of times crossing the platform. Data are expressed as mean ± SEM ($n = 5$). *$P < 0.05$, **$P < 0.01$ (one-way analysis of variance and Bonferroni's *post hoc* test). (C) Schematic of mouse movement in the spatial probe test. ns: Not significant; *Sorl1*: sortilin related receptor 1; *Sorl1*$^{-/-}$: *Sorl1* homozygous knockout mice; *Sorl1*$^{+/-}$: *Sorl1* heterozygous knockout mice; WT: wild type.

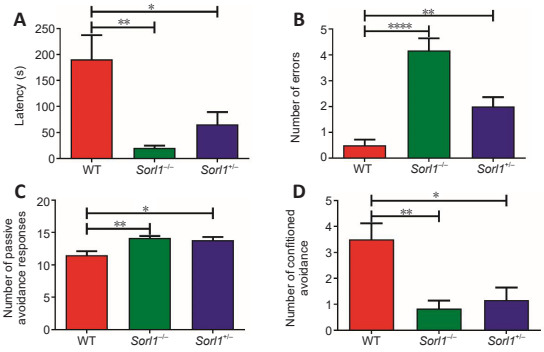



**Figure 2 | Performance of *Sorl1*$^{-/-}$ mice in the step-through and shuttle box tests.**
(A, B) Latency and number of errors in the step-through test. (C, D) Number of passive avoidance responses and conditioned avoidance responses in the shuttle box test. Data are expressed as mean ± SEM ($n = 5$). *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$ (one-way analysis of variance and Bonferroni's *post hoc* test). *Sorl1*: Sortilin related receptor 1; *Sorl1*$^{-/-}$: *Sorl1* homozygous knockout mice; *Sorl1*$^{+/-}$: *Sorl1* heterozygous knockout mice; WT: wild type.

increasing SORL1 expression, with BDNF being an upstream transcriptional regulator of SORL1 (Tanila, 2017). However, our study found that SORL1 also regulates BDNF expression. In N2a cells, after shRNA interference with *Sorl1* (**Additional Figure 2** and **Additional Table 1**), BDNF expression was significantly downregulated (**Figure 4A** and **B**). A previous study found that NMDA receptors (NMDAR) can regulate BDNF expression by turning CREB on/off (Lian et al., 2021). In the shRNA-*Sorl1* cell model, we found reduced levels of NR2B and CREB expression (**Figure 4A**, **C**, and **D**). In the hippocampus and cortex of *Sorl1*$^{-/-}$ mice, NR2B expression was also significantly downregulated (**Figure 4E–G**). Together, this suggests that SORL1 may regulate BDNF expression by controlling NR2B expression, and participating in the occurrence and development of AD.

### *Sorl1* knockout affects synapses of mouse hippocampal neurons

NMDAR are ionotropic glutamate receptors located in the postsynaptic membrane of neurons. They play a crucial role in maintaining synaptic plasticity and neuronal survival (Yu et al., 2023). To investigate the effects of NR2B expression in tissue and neurons of *Sorl1*$^{-/-}$ mice, immunocytochemistry and western blot assays were used to detect the expression of synaptic-associated proteins (e.g., PSD95, DRP, and NR2B) in hippocampal neurons and tissue from *Sorl1*$^{-/-}$ mice.

Compared with *Sorl1*$^{WT}$ mice, the number of normal neurons was significantly reduced in *Sorl1*$^{-/-}$ mice (**Figure 5A**). Some of the neurons in *Sorl1*$^{-/-}$ mice lacked neurofilaments, and the expression of NR2B and DRP was significantly reduced, resulting in decreased colocalization (**Figure 5B** and **C**). These results suggest that loss of SORL1 can lead to reduced NMDAR expression and neuronal damage.

Western blot analysis of mouse hippocampal tissue revealed a significant reduction in protein expression of NR2B, DRP, PSD95, and BDNF (**Figure 5D–H**). These findings suggest that SORL1 knockout may alter the expression of NMDA receptors, as well as the expression of synaptic-related proteins, leading to neuronal damage and abnormal function, thereby causing memory impairment in mice.

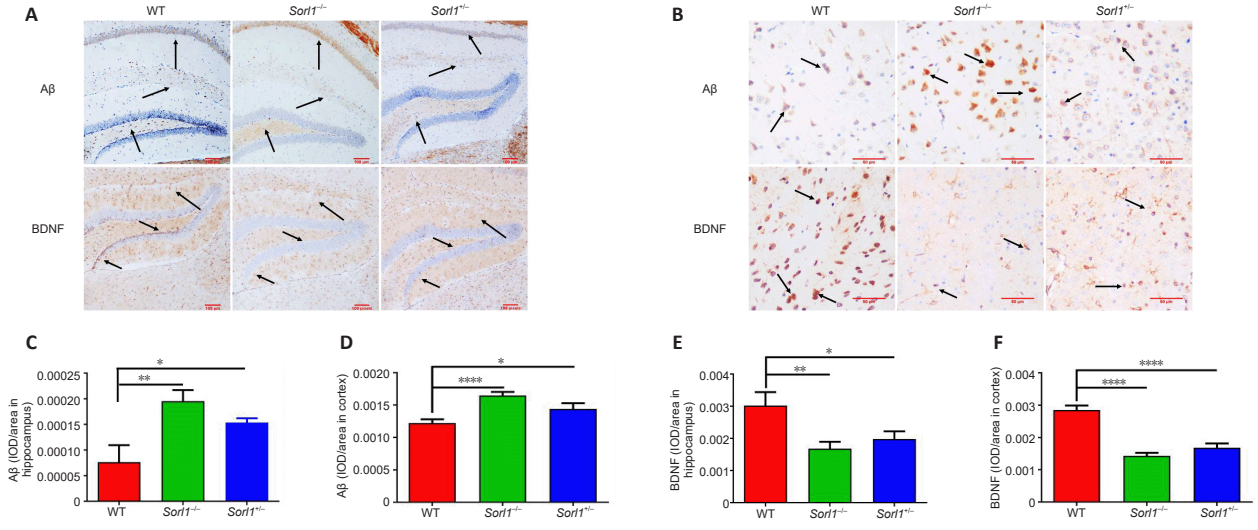



**Figure 3** | **Immunohistochemical detection of Aβ and BDNF in the cortex and hippocampus of *Sorl1*$^{-/-}$ mice.**
(A) Aβ and BDNF immunoreactivities (arrows) in the mouse hippocampus. Scale bar: 100 μm. (B) Aβ and BDNF immunoreactivities (arrows) in the cortex. Scale bar: 50 μm. Compared with the WT group, *Sorl1*$^{-/-}$ mice had more Aβ deposition and a greater reduction in BDNF expression in both the hippocampus (A) and cortex (B). (C, D) Quantitative results of Aβ immunreactivity. (E, F) Quantitative results of BDNF immunreactivity. Data are expressed as mean ± SEM ($n$ = 5). *$P$ < 0.05, **$P$ < 0.01, ****$P$ < 0.0001 (one-way analysis of variance and Bonferroni's *post hoc* test). Aβ: Amyloid-β; BDNF: brain-derived neurotrophic factor; IOD: integrated optical density; *Sorl1*: sortilin related receptor 1; *Sorl1*$^{-/-}$: *Sorl1* homozygous knockout mice; *Sorl1*$^{+/-}$: *Sorl1* heterozygous knockout mice; WT: wild type.

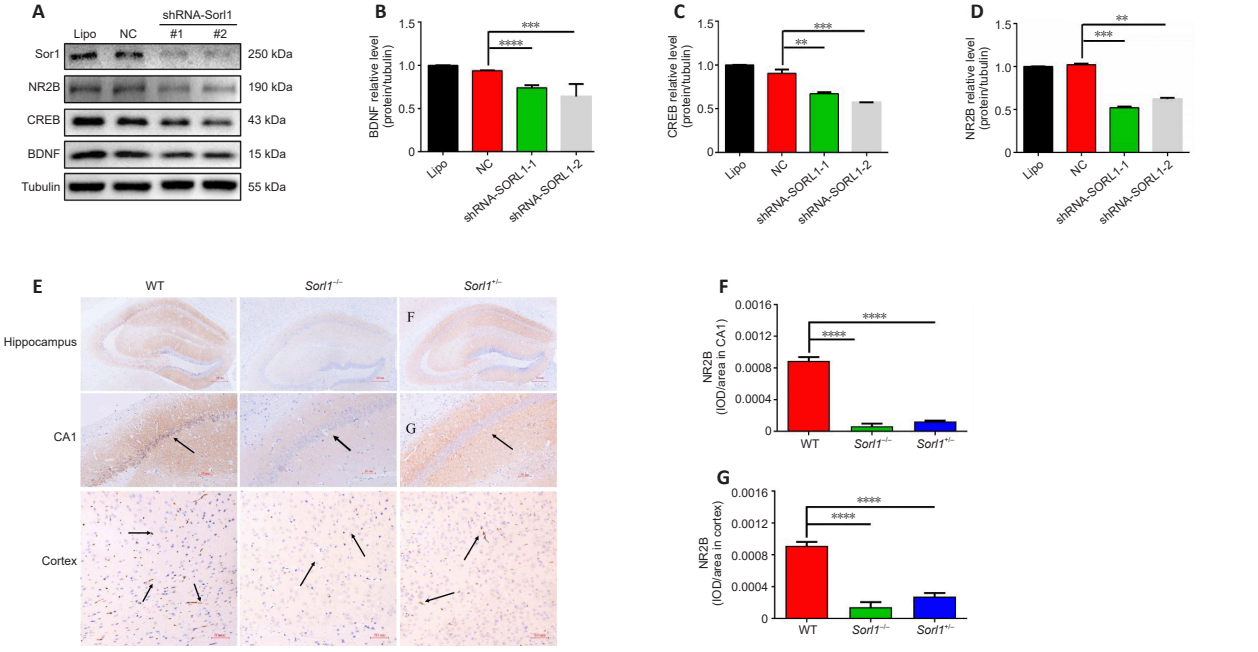



**Figure 4** | **SORL1 affects BNDF expression by regulating NMDAR expression.**
(A) Western blot detection of NR2B, CREB, and BDNF expression after shRNA interference of SORL1 in N2a cells ($n$ > 3). (B–D) Statistical results of BDNF, CREB, and NR2B and expression in A. (E) Immunohistochemical detection of NR2B immunreactivity in the mouse hippocampus, hippocampal CA1 region, and cortex ($n$ = 5). Compared with the WT group, the hippocampus and cortex of *Sorl1*$^{-/-}$ mice showed reduced NR2B immunreactivity. Scale bar: 200 μm (upper), 50 μm (middle and lower). (F) Quantitative results of NR2B immunreactivity in the hippocampus CA1 region. (G) Quantitative results of NR2B expression in the cortex. Data are expressed as mean ± SEM ($n$ = 5). **$P$ < 0.01, ***$P$ < 0.001, ****$P$ < 0.0001 (one-way analysis of variance and Bonferroni's *post hoc* test). BDNF: Brain-derived neurotrophic factor; CA1: cornu ammonis 1; CREB: cyclic adenosine monophosphate-response element binding protein; IOD: integrated optical density; Lipo: lipofectamine 2000 transfection reagent control group; NC: normal plasmid control group; NR2B: N-methyl-D-aspartate receptor subunit 2B; shRNA: short hairpin RNA; SORL1: Sortilin related receptor 1; *Sorl1*$^{-/-}$: *Sorl1* homozygous knockout mice; *Sorl1*$^{+/-}$: *Sorl1* heterozygous knockout mice; WT: wild type.

## Discussion

Here, we observed that *Sorl1*$^{-/-}$ mice displayed impairments in learning and cognitive memory. SORL1 exerts its effects on BDNF expression by inhibiting transcription/translation of CREB. Abnormal disruption of the SORL1-NMDAR-CREB-BDNF signaling pathway impairs memory formation and maintenance.

SORL1 mRNA levels are reduced in patients with AD. GWAS also suggest that the SORL1 gene and single nucleotide polymorphisms are associated with risk of AD (Campion et al., 2019; Katsumata et al., 2022). SORL1 acts as a sorting protein-related receptor and shuttles between the Golgi matrix, endoplasmic reticulum, and cytoplasm (Caglayan et al., 2014). Therefore, SORL1 is involved in the transport and metabolism of various substances within cells. As a LDLR family protein, SORL1 regulates the transport and metabolism of Aβ (Mishra et al., 2022).

Studies have demonstrated that SORL1 plays a crucial role in the processing and degradation of the APP protein. When γ- and β-secretases cleave the APP protein into toxic Aβ fragments, the presence of SORL1 can mitigate γ-secretase activity, and thus reduce the formation of harmful Aβ (Dumanis et al., 2015; Jensen et al., 2023). However, the absence of SORL1 in a human pluripotent stem cell model leads to enlarged endosomes, impedance of normal APP transport within cells, and induction of AD-related pathologies (Knupp et al., 2020). As a highly penetrant gene associated with AD, SORL1 may contribute to disease progression in multiple ways.

In the early stages of AD, pathological changes involving neuronal loss in the frontal and temporal lobes and hippocampus (such as nerve injury, synaptic loss, and synaptic dysfunction) were found before the appearance of classical pathological features of Aβ deposition. These pathological changes are closely related to BDNF, which plays a critical role in learning and memory

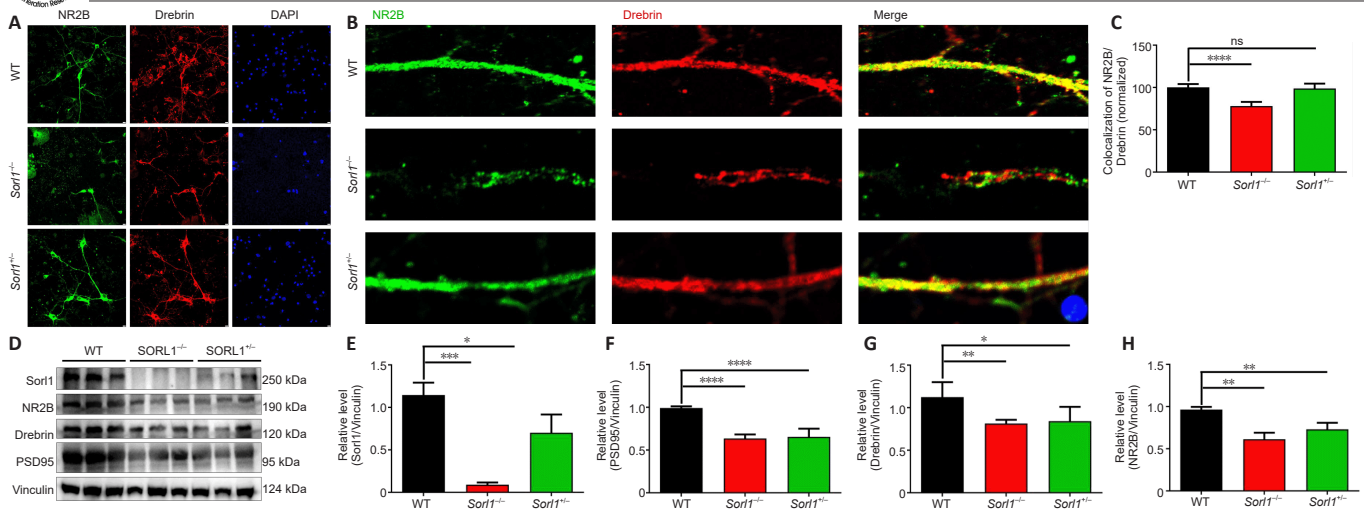



**Figure 5** | **SORL1 knockout affects NR2B, Drebrin, and PSD95 expression in the hippocampus.**
(A, B) Colocalization of NR2B (green, AlexaFluor 488) with Drebrin (red, Cy3) ($n$ = 5). Compared with the WT group, protein expression of NR2B and Drebrin was significantly reduced, and colocalization decreased, in neuronal cells of *Sorl1*$^{-/-}$ mice. Scale bar: 25 μm. (C) Quantitative analysis of the percentage of NR2B colocalized with Drebrin. (D) Western blot detection of Sorl1, PSD95, Drebrin, and NR2B expression in the hippocampus ($n$ > 3). (E–H) Statistical results of Sorl1, PSD95, Drebrin, and NR2B expression in D. Data are expressed as mean ± SEM ($n$ = 5). **$P$ < 0.01, ***$P$ < 0.001, ****$P$ < 0.0001 (one-way analysis of variance and Bonferroni's *post hoc* test). BDNF: Brain-derived neurotrophic factor; CA1: cornu ammonis 1; DAPI: 4′,6-diamidino-2-phenylindole; Drebrin: developmentally regulated brain protein; Lipo: lipofectamine 2000 transfection reagent control group; NC: normal plasmid control group; NR2B: N-methyl-D-aspartate receptor subunit 2B; ns: not significant; PSD95: postsynaptic density protein 95; shRNA: short hairpin RNA; SORL1: Sortilin related receptor 1; *Sorl1*$^{-/-}$: *Sorl1* homozygous knockout mice; *Sorl1*$^{+/-}$: *Sorl1* heterozygous knockout mice; WT: wild type.

formation. Reduced BDNF expression is a critical factor contributing to AD-related dementia. Compared with normal control individuals, expression of BDNF was significantly reduced in the peripheral blood of patients with AD (Lu et al., 2013; Mecca et al., 2020; Perdigão et al., 2020; O'Dell et al., 2021). Rohe et al. (2009) found that BDNF reduces Aβ deposition by promoting the expression of SORL1. However, exogenous application of BDNF did not reduce Aβ deposition in *Sorl1*$^{-/-}$ mice. This suggests that SORL1 plays an important role in the function of BDNF; specifically, *Sorl1* knockout reduces the function of BDNF in improving cognitive and behavioral impairments in mice (Peng et al., 2009; Knupp et al., 2020).

In the *Sorl1*$^{-/-}$ mouse, we found significantly decreased BDNF expression and significantly increased Aβ deposition, indicating that SORL1 (as an intracellular sorting receptor-related protein) may regulate the expression and transport of BDNF. Furthermore, using a shRNA-*Sorl1* N2a cell model, we found that BDNF expression was reduced and protein expression of the upstream transcriptional regulator, CREB, and NR2B were significantly downregulated.

CREB is an important transcription factor that regulates BDNF. Some studies have shown that activation of CREB transcriptional activity can significantly increase BDNF expression and play an important role in maintaining cognitive functions such as nerve cell growth, synaptic plasticity, and dendritic regeneration (Amidfar et al., 2020; Sharma and Singh, 2020).

Activation of CREB is related to the balance of synaptic/extrasynaptic NMDAR. The NMDAR is a tetramer formed by multiple NR2/NR3 subunits and NR1. Like α-amino-3-hydroxy-5-methyl-4-isoxazolyl propionic acid (AMPA) receptors, NMDAR is an ionic glutamate receptor subtype located in the postsynaptic membrane of neurons. NMDAR are crucial for maintaining synaptic plasticity and neuronal survival, and essential for the formation of learning and memory. It has been found that synaptic NMDAR activation can promote the expression of CREB and BDNF, which in turn plays an important role in maintaining cell growth and differentiation, synaptic plasticity, and synaptic long-term potentiation. Reduced synaptic NMDAR expression can lead to an overload of $Ca^{2+}$ influx, dephosphorylation of CREB, and inefficient transcription of BDNF, resulting in learning and memory impairments (Qiu et al., 2020; Lian et al., 2021; Yu et al., 2023). A study found that expression of the NMDAR subunit, N2B, in rats decreased markedly after microwave irradiation. Rat spatial learning and memory ability also decreased (Wang et al., 2015). Zhu et al. (2018) found that treatment with xanthoceraside in AD transgenic mice increased expression levels of NR2B and reduced learning and memory impairments. Further, Zhu's study suggested that NR2B plays a crucial role in enhancing learning and memory. Our study found that NR2B expression was significantly decreased in neurons of the hippocampal CA1 region and cortex in *Sorl1*$^{-/-}$ mice. In mouse hippocampal neurons, protein expression and colocalization of NR2B and Drebrin were reduced, with similar results observed in western blotting of mouse hippocampal tissue. Downregulated expression of NR2B was also observed in shRNA-*Sorl1* N2a cells. SORL1 participates in the progression of AD through the SORL1-NMDAR-CREB-BDNF axis.

In conclusion, our study found that *Sorl1*$^{-/-}$ mice exhibited learning and memory impairments. *Sorl1* participates in the pathogenesis of AD through multiple pathways. In addition to its impact on APP transport and metabolism, regulating the NMDAR-CREB-BDNF signaling axis is also an important mechanism in the occurrence and development of AD.

SORL1 knockout can lead to abnormalities in NMDAR expression and distribution, reduced BDNF and synaptic protein expression, and neuronal cell damage, which may be key factors in mouse memory disorders. Therefore, when BDNF is used to treat AD, attention should be paid to whether the patient has *Sorl1* gene mutations and whether *Sorl1* can be normally expressed. Moreover, how SORL1 regulates the transport and expression of NMDAR, as well as its effects on the expression, transport, and metabolism of BDNF, still needs further study.

**Author contributions:** *DH, ML, and XL conceived and designed the study. MZ and XC performed the study. JL and TX analyzed the data. YF, CW and WL provided reagents/materials/analysis tools. MZ wrote the paper with support from ML and DH. All authors have read and approved the final manuscript.*
**Conflicts of interest:** *The authors declare no conflict of interest.*
**Author statement:** *This paper has been posted as a preprint on Research Square with doi: https://doi.org/10.21203/rs.3.rs-750372/v1, which is available from: https://www.researchsquare.com/article/rs-750372/v1.pdf.*
**Data availability statement:** *All data generated or analyzed during this study are included in this published article and its Additional files.*

**Open peer reviewer:** *Jose Miguel Laffita Mesa, Karolinska Universitetssjukhuset, Sweden.*
**Additional files:**
***Additional file 1:*** *Construction and identification of Sorl1 KO mice.*
***Additional Table 1:*** *Primers, shRNA, and guide RNA used in the study.*
***Additional Figure 1:*** *Construction and identification of Sorl1 knockout mice.*
***Additional Figure 2:*** *shRNA interference with Sorl1 in N2a cells.*

## References

Abbott A (2023) Conquering Alzheimer's: a look at the therapies of the future. Nature 616:26-28.

Alfonsetti M, d'Angelo M, Castelli V (2023) Neurotrophic factor-based pharmacological approaches in neurological disorders. Neural Regen Res 18:1220-1228.

Amidfar M, de Oliveira J, Kucharska E, Budni J, Kim YK (2020) The role of CREB and BDNF in neurobiology and treatment of Alzheimer's disease. Life Sci 257:118020.

Asher S, Priefer R (2022) Alzheimer's disease failed clinical trials. Life Sci 306:120861.

Baranowski BJ, Mohammad A, Finch MS, Brown A, Dhaliwal R, Marko DM, LeBlanc PJ, McCormick CM, Fajardo VA, MacPherson REK (2023) Exercise training and BDNF injections alter amyloid precursor protein (APP) processing enzymes and improve cognition. J Appl Physiol (1985) 135:121-135.

Caglayan S, Takagi-Niidome S, Liao F, Carlo AS, Schmidt V, Burgert T, Kitago Y, Füchtbauer EM, Füchtbauer A, Holtzman DM, Takagi J, Willnow TE (2014) Lysosomal sorting of amyloid-β by the SORLA receptor is impaired by a familial Alzheimer's disease mutation. Sci Transl Med 6:223ra220.

Campion D, Charbonnier C, Nicolas G (2019) SORL1 genetic variants and Alzheimer disease risk: a literature review and meta-analysis of sequencing data. Acta Neuropathol 138:173-186.

Crossley BM, Bai J, Glaser A, Maes R, Porter E, Killian ML, Clement T, Toohey-Kurth K (2020) Guidelines for Sanger sequencing and molecular assay monitoring. J Vet Diagn Invest 32:767-775.

Dumanis SB, Burgert T, Caglayan S, Füchtbauer A, Füchtbauer EM, Schmidt V, Willnow TE (2015) Distinct functions for anterograde and retrograde sorting of SORLA in amyloidogenic processes in the brain. J Neurosci 35:12703-12713.

Feng X, Hou D, Deng Y, Li W, Tian M, Yu Z (2015) SORL1 gene polymorphism association with late-onset Alzheimer's disease. Neurosci Lett 584:382-389.

Gao D, Barber PR, Chacko JV, Kader Sagar MA, Rueden CT, Grislis AR, Hiner MC, Eliceiri KW (2020) FLIMJ: an open-source ImageJ toolkit for fluorescence lifetime image data analysis. PLoS One 15:e0238327.

Gao L, Zhang Y, Sterling K, Song W (2022) Brain-derived neurotrophic factor in Alzheimer's disease and its pharmaceutical potential. Transl Neurodegener 11:4.

Gong Y, Chen J, Jin Y, Wang C, Zheng M, He L (2020) GW9508 ameliorates cognitive impairment via the cAMP-CREB and JNK pathways in APPswe/PS1dE9 mouse model of Alzheimer's disease. Neuropharmacology 164:107899.

Jensen AMG, Kitago Y, Fazeli E, Vægter CB, Small SA, Petsko GA, Andersen OM (2023) Dimerization of the Alzheimer's disease pathogenic receptor SORLA regulates its association with retromer. Proc Natl Acad Sci U S A 120:e2212180120.

Karch CM, Goate AM (2015) Alzheimer's disease risk genes and mechanisms of disease pathogenesis. Biol Psychiatry 77:43-51.

Katsumata Y, Shade LM, Hohman TJ, Schneider JA, Bennett DA, Farfel JM, Kukull WA, Fardo DW, Nelson PT (2022) Multiple gene variants linked to Alzheimer's-type clinical dementia via GWAS are also associated with non-Alzheimer's neuropathologic entities. Neurobiol Dis 174:105880.

Knupp A, Mishra S, Martinez R, Braggin JE, Szabo M, Kinoshita C, Hailey DW, Small SA, Jayadev S, Young JE (2020) Depletion of the AD risk gene SORL1 selectively impairs neuronal endosomal traffic independent of amyloidogenic APP processing. Cell Rep 31:107719.

Lazaldin MAM, Iezhitsa I, Agarwal R, Agarwal P, Ismail NM (2023) Neuroprotective effects of exogenous brain-derived neurotrophic factor on amyloid-beta 1-40-induced retinal degeneration. Neural Regen Res 18:382-388.

Lian WW, Zhou W, Zhang BY, Jia H, Xu LJ, Liu AL, Du GH (2021) DL0410 ameliorates cognitive disorder in SAMP8 mice by promoting mitochondrial dynamics and the NMDAR-CREB-BDNF pathway. Acta Pharmacol Sin 42:1055-1068.

Liao P, Yuan Y, Liu Z, Hou X, Li W, Wen J, Zhang K, Jiao B, Shen L, Jiang H, Guo J, Tang B, Zhang Z, Hu Z, Wang J (2022) Association of variants in the KIF1A gene with amyotrophic lateral sclerosis. Transl Neurodegener 11:46.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-408.

Lu B, Nagappan G, Guan X, Nathan PJ, Wren P (2013) BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. Nat Rev Neurosci 14:401-416.

Malik AR, Willnow TE (2020) VPS10P domain receptors: sorting out brain health and disease. Trends Neurosci 43:870-885.

Mecca AP, Chen MK, O'Dell RS, Naganawa M, Toyonaga T, Godek TA, Harris JE, Bartlett HH, Zhao W, Nabulsi NB, Wyk BCV, Varma P, Arnsten AFT, Huang Y, Carson RE, van Dyck CH (2020) In vivo measurement of widespread synaptic loss in Alzheimer's disease with SV2A PET. Alzheimers Dement 16:974-982.

Mishra S, Knupp A, Szabo MP, Williams CA, Kinoshita C, Hailey DW, Wang Y, Andersen OM, Young JE (2022) The Alzheimer's gene SORL1 is a regulator of endosomal traffic and recycling in human neurons. Cell Mol Life Sci 79:162.

Modell AE, Lim D, Nguyen TM, Sreekanth V, Choudhary A (2022) CRISPR-based therapeutics: current challenges and future applications. Trends Pharmacol Sci 43:151-161.

O'Dell RS, Mecca AP, Chen MK, Naganawa M, Toyonaga T, Lu Y, Godek TA, Harris JE, Bartlett HH, Banks ER, Kominek VL, Zhao W, Nabulsi NB, Ropchan J, Ye Y, Vander Wyk BC, Huang Y, Arnsten AFT, Carson RE, van Dyck CH (2021) Association of Aβ deposition and regional synaptic density in early Alzheimer's disease: a PET imaging study with [(11)C]UCB-J. Alzheimers Res Ther 13:11.

Passeri E, Elkhoury K, Morsink M, Broersen K, Linder M, Tamayol A, Malaplate C, Yen FT, Arab-Tehrany E (2022) Alzheimer's disease: treatment strategies and their limitations. Int J Mol Sci 23:13954.

Peng S, Garzon DJ, Marchese M, Klein W, Ginsberg SD, Francis BM, Mount HT, Mufson EJ, Salehi A, Fahnestock M (2009) Decreased brain-derived neurotrophic factor depends on amyloid aggregation state in transgenic mouse models of Alzheimer's disease. J Neurosci 29:9321-9329.

Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, et al. (2020) The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol 18:e3000410.

Perdigão C, Barata MA, Araújo MN, Mirfakhar FS, Castanheira J, Guimas Almeida C (2020) Intracellular trafficking mechanisms of synaptic dysfunction in Alzheimer's disease. Front Cell Neurosci 14:72.

Qiu LL, Pan W, Luo D, Zhang GF, Zhou ZQ, Sun XY, Yang JJ, Ji MH (2020) Dysregulation of BDNF/TrkB signaling mediated by NMDAR/Ca(2+)/calpain might contribute to postoperative cognitive dysfunction in aging mice. J Neuroinflammation 17:23.

Rohe M, Synowitz M, Glass R, Paul SM, Nykjaer A, Willnow TE (2009) Brain-derived neurotrophic factor reduces amyloidogenic processing through control of SORLA gene expression. J Neurosci 29:15472-15478.

Rostagno AA (2022) Pathogenesis of Alzheimer's disease. Int J Mol Sci 24:107.

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671-675.

Sharma VK, Singh TG (2020) CREB: A multifaceted target for Alzheimer's disease. Curr Alzheimer Res 17:1280-1293.

Tanila H (2017) The role of BDNF in Alzheimer's disease. Neurobiol Dis 97:114-118.

Trejo-Lopez JA, Yachnis AT, Prokop S (2022) Neuropathology of Alzheimer's disease. Neurotherapeutics 19:173-185.

Wang H, Peng R, Zhao L, Wang S, Gao Y, Wang L, Zuo H, Dong J, Xu X, Zhou H, Su Z (2015) The relationship between NMDA receptors and microwave-induced learning and memory impairment: a long-term observation on Wistar rats. Int J Radiat Biol 91:262-269.

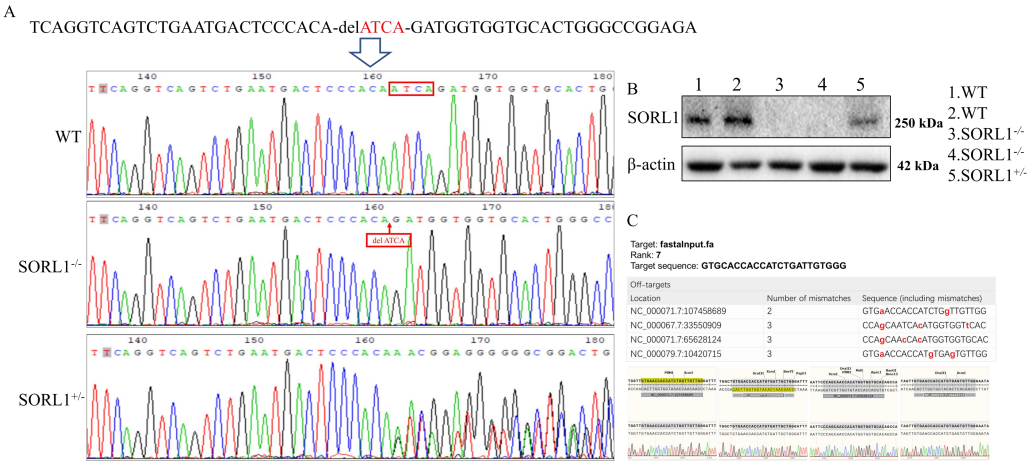
Weidner WS, Barbarino P (2019) P4-443: The state of the art of dementia research: new frontiers. Alzheimers Dement 15:1473.

Yu SP, Jiang MQ, Shim SS, Pourkhodadad S, Wei L (2023) Extrasynaptic NMDA receptors in acute and chronic excitotoxicity: implications for preventive treatments of ischemic stroke and late-onset Alzheimer's disease. Mol Neurodegener 18:43.

Zhu L, Yang L, Zhao X, Liu D, Guo X, Liu P, Chi T, Ji X, Zou L (2018) Xanthoceraside modulates NR2B-containing NMDA receptors at synapses and rescues learning-memory deficits in APP/PS1 transgenic mice. Psychopharmacology (Berl) 235:337-349.

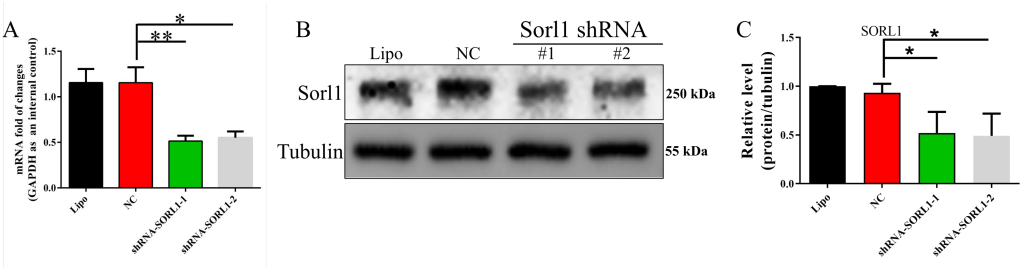
Zhu XC, Liu L, Dai WZ, Ma T (2022) Crry silencing alleviates Alzheimer's disease injury by regulating neuroinflammatory cytokines and the complement system. Neural Regen Res 17:1841-1849.

*C-Editors: Zhao LJ, Zhao M; S-Editors: Yu J, Li CH; L-Editors: James R, Song LP; T-Editor: Jia Y*




**Additional Figure 1 Construction and identification of *Sorl1* knockout mice.**

(A) Mouse DNA sequencing genotype identification. (B) Detection of the expression of SORL1 in different groups of mouse brain tissue using Western blotting. (C) Potential off-target events in the CRISPR-Cas. Cas9 gRNA of *Sorl1* sequence is on the top. Chromosomal position, number of nucleotide mismatch and DNA sequence in genome potential off-target sites are shown. The potential off-target sequences were analyzed by Sanger sequencing. del: Delete; SORL1: Sortilin related receptor 1; Sorl1$^{-/-}$: Sorl1 homozygous knockout mice; Sorl1$^{+/-}$: Sorl1 heterozygous knockout mice; WT: wild type.



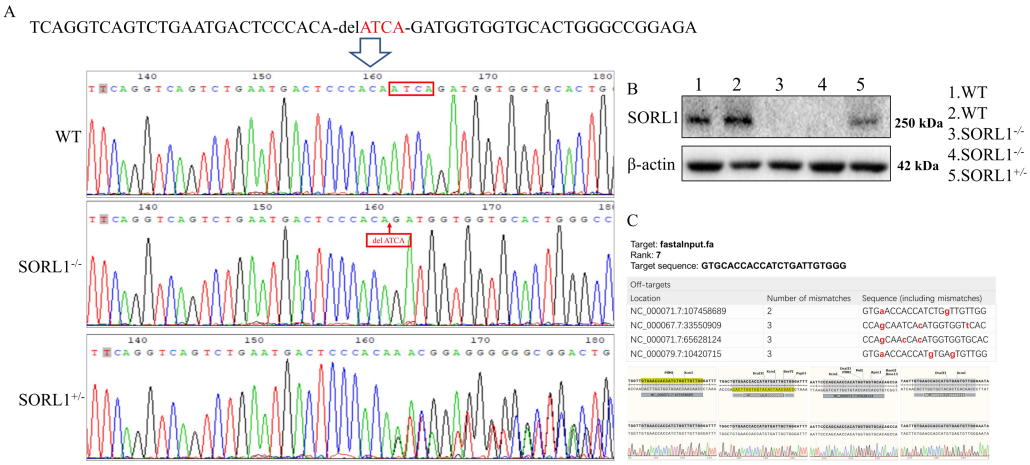

**Additional Figure 2 shRNA interference with *Sorl1* in N2a cells.**

(A) RT-qPCR to detect shRNA interference efficiency. (B, C) Western blot to detect shRNA interference efficiency. Data are expressed as means ± SEM ($n$ = 3). *$P$ < 0.05, **$P$ < 0.01 (one-way ANOVA and Bonferroni's post hoc test). GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; Lipo: lipofectamine 2000 transfection reagent control group; NC: normal plasmid control group; RT-qPCR: reverse transcription-quantitative polymerase chain reaction; shRNA: short hairpin RNA; SORL1: Sortilin related receptor 1; WT: wild type.

**Additional Table 1 Primers, shRNA, and guide RNA used in the study**

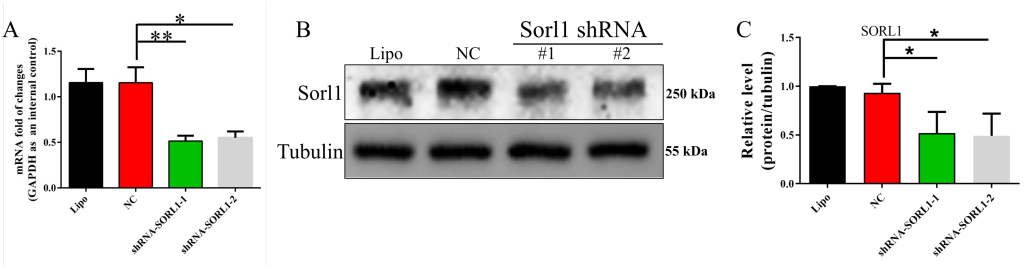
| Name | Sequence (5'-3') | Application |
|---|---|---|
| *Sorl1* | Forward: AGC AGG AGG GAG TCG AGA C | RT-qPCR |
| | Reverse: GTT CCT AGC CGG AGA TCG C | |
| GAPDH | Forward: ATC ATC CCT GCA TCC ACT | RT-qPCR |
| | Reverse: ATC CAC GAC GGA CAC ATT | |
| Negative control | GTT CTC CGA ACG TGT CAC GT | shRNA |
| shRNA-*Sorl1*-#1 | GCA CAA CAC CAA TGA CTT TGT | |
| shRNA-*Sorl1*-#2 | GCT AGC AAC TCT ACA GAA ATA | |
| SORL1 Sanger sequencing | Forward: ATG GCG ACA CGG AGC AGC AGG AG | Sanger sequencing for genotyping |
| | Reverse: AAG ATG TAC CTC TTG TTG TCA GCA GGG CTG | |
| Guide RNA-#1 | GTG CAC CAC CAT CTG ATT GTG GG | CRISPR-Cas9 |

CRISPR-Cas9: Clustered regularly interspaced short palindromic repeats-associated protein 9; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; shRNA: short hairpin RNA; SORL1: Sortilin related receptor 1; RT-qPCR: reverse transcription-quantitative polymerase chain reaction.




**Additional Figure 1 Construction and identification of *Sorl1* knockout mice.**

(A) Mouse DNA sequencing genotype identification. (B) Detection of the expression of SORL1 in different groups of mouse brain tissue using Western blotting. (C) Potential off-target events in the CRISPR-Cas. Cas9 gRNA of *Sorl1* sequence is on the top. Chromosomal position, number of nucleotide mismatch and DNA sequence in genome potential off-target sites are shown. The potential off-target sequences were analyzed by Sanger sequencing. del: Delete; SORL1: Sortilin related receptor 1; Sorl1$^{-/-}$: Sorl1 homozygous knockout mice; Sorl1$^{+/-}$: Sorl1 heterozygous knockout mice; WT: wild type.




**Additional Figure 2 shRNA interference with *Sorl1* in N2a cells.**

(A) RT-qPCR to detect shRNA interference efficiency. (B, C) Western blot to detect shRNA interference efficiency. Data are expressed as means ± SEM ($n$ = 3). *$P$ < 0.05, **$P$ < 0.01 (one-way ANOVA and Bonferroni's post hoc test). GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; Lipo: lipofectamine 2000 transfection reagent control group; NC: normal plasmid control group; RT-qPCR: reverse transcription-quantitative polymerase chain reaction; shRNA: short hairpin RNA; SORL1: Sortilin related receptor 1; WT: wild type.

**Additional Table 1 Primers, shRNA, and guide RNA used in the study**

| Name | Sequence (5'-3') | Application |
|---|---|---|
| *Sorl1* | Forward: AGC AGG AGG GAG TCG AGA C | RT-qPCR |
| | Reverse: GTT CCT AGC CGG AGA TCG C | |
| GAPDH | Forward: ATC ATC CCT GCA TCC ACT | RT-qPCR |
| | Reverse: ATC CAC GAC GGA CAC ATT | |
| Negative control | GTT CTC CGA ACG TGT CAC GT | shRNA |
| shRNA-*Sorl1*-#1 | GCA CAA CAC CAA TGA CTT TGT | |
| shRNA-*Sorl1*-#2 | GCT AGC AAC TCT ACA GAA ATA | |
| SORL1 Sanger sequencing | Forward: ATG GCG ACA CGG AGC AGC AGG AG | Sanger sequencing for genotyping |
| | Reverse: AAG ATG TAC CTC TTG TTG TCA GCA GGG CTG | |
| Guide RNA-#1 | GTG CAC CAC CAT CTG ATT GTG GG | CRISPR-Cas9 |

CRISPR-Cas9: Clustered regularly interspaced short palindromic repeats-associated protein 9; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; shRNA: short hairpin RNA; SORL1: Sortilin related receptor 1; RT-qPCR: reverse transcription-quantitative polymerase chain reaction.