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DSCAM deficiency leads to premature spine maturation and autism-like behaviors

Abbreviated title: Spine and behavioral deficits by loss of DSCAM

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Abstract

Mutations in some cell adhesion molecules (CAMs) cause abnormal synapse formation and maturation, and serve as one of the potential mechanisms of autism spectrum disorders (ASDs). Recently, DSCAM (Down syndrome cell adhesion molecule) was found to be a high-risk gene for autism. However, it is still unclear how DSCAM contributes to ASD. Here, we show that DSCAM expression was downregulated following synapse maturation, and that DSCAM deficiency caused accelerated dendritic spine maturation during early postnatal development. Mechanistically, the extracellular domain (ECD) of DSCAM interacts with neuroligin1 (NLGN1) to block the NLGN1-neurexin1β (NRXN1β) interaction. DSCAM-ECD was able to rescue spine overmaturation in DSCAM knockdown neurons. Precocious spines in DSCAM-deficient mice showed increased glutamatergic transmission in the developing cortex and induced autism-like behaviors such as social novelty deficits and repetitive behaviors. Thus, DSCAM might be a repressor that prevents premature spine maturation and excessive glutamatergic transmission, and its deficiency could lead to autism-like behaviors. Our study provides new insight into the potential pathophysiological mechanisms of ASDs.

Keywords: Autism, dendritic spine, DSCAM, NLGN1

Significance statement:

DSCAM is not only associated with Down syndrome but is also a strong autism risk
gene based on large-scale sequencing analysis. However, it remains unknown exactly how DSCAM contributes to autism. In mice, either neuron- and astrocyte-specific or pyramidal neuron-specific DSCAM deficiencies resulted in autism-like behaviors and enhanced spatial memory. In addition, DSCAM knockout or knockdown in pyramidal neurons led to increased dendritic spine maturation. Mechanistically, the extracellular domain of DSCAM binds to NLGN1 and inhibits NLGN1-NRXN1β interaction, which can rescue abnormal spine maturation induced by DSCAM deficiency. Our research demonstrates that DSCAM negatively modulates spine maturation, and that DSCAM deficiency leads to excessive spine maturation and autism-like behaviors, thus providing new insight into a potential pathophysiological mechanism of autism.
Introduction

Proper synapse connections are crucial for the precise assembly of neural circuitry and for normal brain function. Dysregulation of synapse formation and maturation is associated with a variety of neurodevelopmental disorders, including autism spectrum disorders (ASDs) (Forrest et al., 2018). Cell adhesion molecules (CAMs), which are cell surface proteins that mediate cell-cell interactions, play an essential role in neural development, especially in synaptic development and function (Dalva et al., 2007). ASDs manifest with deficits in social interaction and communication, as well as in increased repetitive or restrictive behaviors (Huguet et al., 2013; Huguet et al., 2016). However, the etiology underlying ASDs at the molecular, cellular and system levels remains elusive. ASDs are highly heritable diseases (Bailey et al., 1995; Autism Genome Project et al., 2007; Rosenberg et al., 2009). Many genes have been identified as risk factors for ASDs (Bourgeron, 2015). A prominent group among these genes is the set of genes that encode CAMs, such as neuroligins (NLGNs) and neurexin1 (NRXN1). At the cellular and molecular levels, dysfunction of neuronal CAMs is regarded as one of the potential mechanisms of ASDs (Chen et al., 2015). Mice with mutations of these CAMs display autism-like behaviors and deficits in synapse formation and maturation (Sudhof, 2008). However, it remains unknown how these ASDs-related CAMs impact synapse development. Recently, DSCAM (Down syndrome cell adhesion molecule) was found to be one of the genetic risk factors for autism in a large-scale genome-wide association study (GWAS) (Satterstrom et al., 2016).
Further research, including whole-genome sequencing (De Rubeis et al., 2014; Yuen et al., 2017), exome sequencing (Iossifov et al., 2014; Ronemus et al., 2014), and targeted sequencing (Stessman et al., 2017), has shown that DSCAM is a strong autism risk gene. In some ASD patients, DSCAM mutations resulted in premature termination (Iossifov et al., 2014; Wang et al., 2016; Stessman et al., 2017; Yuen et al., 2017), suggesting that DSCAM loss-of-function variants may cause ASD.

DSCAM is a cell adhesion protein that is highly expressed in the developing nervous system and that plays a vital role in neural development (Yamakawa et al., 1998; Agarwala et al., 2001a; Agarwala et al., 2001b; Fuerst et al., 2008; Fuerst et al., 2009). In Drosophila, alternative splicing generates thousands of dscam isoforms, which all regulate dendritic self-avoidance and mosaic tiling (Schmucker et al., 2000; Hughes et al., 2007; Matthews et al., 2007; Hattori et al., 2008). Due to the lack of extensive alternative splicing in vertebrates, DSCAM has only two paralogs, DSCAM and DSCAM-like-1 (DSCAML1) (Agarwala et al., 2001b). In vertebrates, DSCAM paralogs play a similar regulatory role in neurite arborization, dendritic self-avoidance and mosaic tiling (Fuerst et al., 2008; Fuerst et al., 2009). Moreover, DSCAM plays a critical role in synapse development. In Drosophila, dscam was reported to regulate precise synaptic targeting (Millard et al., 2010), and dysregulated dscam levels resulted in altered presynaptic size (Kim et al., 2013; Sterne et al., 2015). Additionally, dscam is involved in mediating de novo and learning-related synapse formation in diverse biological models, including chicks and Aplysia (Yamagata and Sanes, 2008; Li et al., 2009). Disruption of glutamatergic transmission and plasticity has been...
previously documented in DSCAM$^{2L}$-deficient intracortical circuits of the motor cortex, as well as in spinal interneuronal circuits (Thiry et al., 2016; Laflamme et al., 2019). Despite DSCAM’s abundance in the brain and its modulation of diverse physiological functions, little is known about how it regulates synapse development in mammals. Therefore, we hypothesized that DSCAM mediates synapse development and that its deficiency can cause autism.

Here, we examined the expression pattern of DSCAM during postnatal synapse formation and maturation, observed the effects of DSCAM deficiency on dendritic spine development, and investigated its potential as an underlying mechanism of ASD. Furthermore, we generated DSCAM-deficient mice to validate whether the deletion of DSCAM in neurons would result in abnormal spine maturation and altered synaptic transmission, as well as autism-like behaviors. These findings describe a novel pathophysiological role of DSCAM in ASDs.

**Materials and Methods**

**Animals**

Floxed DSCAM mouse (C57BL/6) was a kind gift from Dr. Jane Y Wu (Institute of Biophysics, Chinese Academy of Science). GFAP::Cre (Jackson Laboratory) transgenic (tg) mouse was purchased from Jackson Laboratory, and NEX-Cre mouse was a kind gift from Dr. Yu-Qiang Ding (Institutes of Brain Science, Fudan University). Primers for genotyping are listed below. Behavior tests were performed with 2–3-month male mice. All mice were housed in a constant temperature and humidity...
chamber at 23 °C, and sufficient food and water were administered daily. No more than 5 adult mice per cage were subjected to a 12-h light/dark cycle under standard conditions. All the mice were guaranteed to be hygienic. The animal experiments were carried out following the "Guidelines for the Care and Use of Laboratory Animals" promulgated by Nanchang University. Primers for mice genotyping are as below.

| Primers | Sequence (5’→3’) | Size |
|---------|------------------|------|
| PP 1 ACCTCCCACAAACAAACCAG | Wt = 396bp, Floxed = 504bp |
| TGCAGAAGAGTCAAGGCACA | |
| PP 2 AGACAGGAGGGTACAGAGGA | Wt = 457bp, Floxed = 565bp |
| GTTGTGACCTTTAGGGTTATG | |
| PP 3 GCTTGCTCATGTGAGCTGGA | Floxed = 420bp |
| GGCTGGACGTAAACTCCTC | |
| PP 4 TCTGAGGCGGAAAGAACCAG | Floxed = 306bp |
| GCAGCTGAGGCAAGTCTCCTG | |
| PP 5 GCTTGCTCATGTGAGCTGGA | Wt = 446bp |
| GCAGCTGAGGCAAGTCTCCTG | |
| PP 6 (GFAP-Cre) ACTCCTTCATAAAGCCCT | Tg = 190bp |
| ATCACCTCGTTGACATCGACCG | |
| PP 7 (NEX-Cre) GAGTCCTGGAATCAGTCTTTTTC | Cre = 550bp |
| ATC ACTCGTTGCATCGACCG | Wt = 770bp |
Quantitative real-time PCR (qRT-PCR) analysis

Cortical total RNA was purified from WT mice with Trizol reagent (Invitrogen, NY, USA). 500 ng of total RNA were reverse transcribed to cDNA with oligo (dT) primers. cDNAs were used as the template in qPCR in a 20 μL reaction system containing SYBR GreenER qPCR mix (Invitrogen, NY, USA) with gene-specific primers as below. GAPDH was used as a reference in each sample.

| Primers | Sequence (5'→3') |
|---------|-----------------|
| NLGN1-F | TGATGGGAGTGCTTTGGCAAGC |
| NLGN1-R | CCGTAGTTTCTTTGCGACGCT |
| NLGN2-F | CGATGTGTCATGCTACGCAGTA |
| NLGN2-R | CCACACTACCTCTCTAAAGCGG |
| NLGN3-F | ATCGGTGACATCTGTGTACGT |
| NLGN3-R | CACTGGTTGGTAGTCACAGCC |
| NLGN4-F | GTGATGATGACCTACTGGACGA |
| NLGN4-R | GCAGATAGGCTGGTCTTTGG |
| SALM2-F | GCACGTCCTGATGCAAGTC |
| SALM2-R | GCCAGGTCATTCACTGGAAAGG |
| SALM4-F | AGGCATCCGCATGTACCAGATC |
| SALM4-R | TCAGCAGGAAGGAGCGACTGTC |
| Primer Name  | Forward Sequence            | Reverse Sequence            |
|-------------|-----------------------------|----------------------------|
| MDGA1-F     | ACCAGTGCCCTGACCTCAGCATA     |                            |
| MDGA1-R     | TTGCCACGAACTTCGCACTGGA      |                            |
| MDGA2-F     | CAAGAGAAGCCTTGGTGCAGCT     |                            |
| MDGA2-R     | CAGCACTCGTATTGGATAGGCTC    |                            |
| NRXN1-F     | ACCGTGCCCTTGAATCCTTTGC     |                            |
| NRXN1-R     | GTCGTAGCTCAAAACGTTGCC      |                            |
| CNTNAP2-F   | GTGATGAGACAGGATAACAGCGG    |                            |
| CNTNAP2-R   | AGTGGTCCTGCCATCAGGAT       |                            |
| CNTN4-F     | CTCCAGCAGAATCCGCCTAAAG     |                            |
| CNTN4-R     | CTCCATTCTGCTCTCGTGAC       |                            |
| CNTN6-F     | CTCTGGTGTCCGGACTCGATT      |                            |
| CNTN6-R     | GGTGTCAAGTCTGCTGCTCTCAA   |                            |
| DSCAM-F     | CATCCGCATGTACGCAAAAC       |                            |
| DSCAM-R     | GAGATGAGGTGGTGTCAGTG      |                            |
| β-catenin-F | GTTCGCCCTTATTGGGACTGCC     |                            |
| β-catenin-R | ATAGCACCCCTGTTCCGCAAAG     |                            |
| N-cadherin-F | CCTCCAGAGTTACTGCTGAC       |                            |
| N-cadherin-R | CCACACTGATTCTGTATGCG      |                            |
| NrCAM-F     | GCAGAGTGAAACATGACCACACC   |                            |
| NrCAM-R     | GTGTAGGTTCCGACATCGCAT     |                            |
| NCAM-F      | GGTTCGGAGATGGTGCTGCT      |                            |
Mouse brain subcellular fractionation was performed as described previously with modifications (Wang et al., 2018). The cerebral cortices from adult mice were homogenized in 10 volumes (vol) of HEPES-buffered sucrose (0.32 M sucrose, 4 mM HEPES/NaOH, pH 7.4) with a glass-Teflon homogenizer. The homogenate was centrifuged at 1,000 g for 10 min to remove the nuclear fraction and unbroken cells. The supernatant (S1) was then centrifuged at 10,000 g for 15 min to yield the crude fraction.
synaptosomal fraction (P1) and the supernatant (S2). The P1 pellet was resuspended in 10 vol of HEPES-buffered sucrose and then centrifuged at 10,000 g for another 15 min. The resulting pellet (P2) was lysed by hypo-osmotic shock in water, rapidly adjusted to 4 mM HEPES, and mixed continuously for 30 min (on ice). The lysate was then centrifuged at 25,000 g for 20 min to yield the supernatant (S3, crude synaptic vesicle fraction) and the pellet (P3, lysed synaptosomal membrane fraction). The P3 pellet was resuspended in HEPES-buffered sucrose, carefully layered on top of a discontinuous gradient containing 0.8-1.0-1.2 M sucrose (top to bottom), and centrifuged at 150,000 g for 2 h. Optional step. The S3 was centrifuged at 165,000 g for 2 h to get the synaptic vesicle protein (SV). The sucrose gradient yielded a floating myelin fraction (G1), a light membrane fraction at the 0.8 M/1.0 M sucrose interface (G2), a synaptosomal plasma membrane (SPM) fraction at the 1.0 M/1.2 M sucrose interface (G3), and a mitochondrial fraction as the pellet (G4). The G3 layer was collected, and an equal volume of HEPES-buffered sucrose was added to centrifuge at 20,000 g for 15 min in order to obtain SPM. The SPM was resuspended with 1% Triton X-100 in 50 mM HEPES/NaOH (pH 8) on ice for 15 min and then centrifuged at 36,000 g for 15 min to yield the supernatant (presynaptic membrane protein, Pre) and the pellet (postsynaptic density, PSD), which was solubilized in 2%SDS PBS buffer at RT.

Plasmids and shRNA construction

The constructs expressing full-length mouse DSCAM with a C-terminal His-tag and
human DSCAM were purchased from Addgene. To generate the construct expressing secretable ECD of DSCAM (FLAG-ECD), mouse DSCAM cDNA encoding 24-1594 amino acids without signal peptide was amplified by PCR and subcloned into PLP2 (new prolactin leader peptide)-FLAG-pCMV6-XL4 downstream of an artificial signal peptide sequence and a FLAG epitope. The constructs of mouse NRXNs (1α-3α and 1β) and NLGNs (1-4) were purchased from Origene. Myc-tagged secretable NRXN1β-ECD was subcloned from full length NRXN1β. For gene knockdown by RNA interference (RNAi), pSUPER vector (OligoEngine) – based small hairpin RNAs (shRNAs) of mouse DSCAM (sh-DSCAM) and DSCAM scramble (sh-control) were constructed. The target sequence of sh-DSCAM was obtained from a previous report: 5′-GTGGGAGAGGAAGTGATAT-3′ (Ly et al., 2008). The sh-control sequence was 5′-AGAGTGGACGTCGATGATTAT 3′. The authenticity of all constructs was verified by DNA sequencing and western blotting analysis.

Cell culture and transfection

Human embryonic kidney (HEK) 293 cells and COS-7 cells (African green monkey kidney fibroblast cells) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco). Transient transfection was performed using polyethylenimine (PEI) (Sigma, 408727), as described before (Chen et al., 2021). Briefly, cells were cultured in 100 mm dishes and at ~70% confluence, they were incubated with precipitates formed by 5 μg of plasmid DNA and 280 μL of 0.05% PEI (wt/vol). Cells were harvested 24~48 h
Cultures of primary hippocampal neurons were prepared from embryonic day (E) 18 Sprague–Dawley rats as described previously (Chen et al., 2021). Briefly, hippocampi were isolated and kept separate from one another in HBSS on ice. Following digestion in 0.25% trypsin plus 0.1 mg/mL DNase I (one hippocampus in 1 mL) at 37 °C for 20 min, dissociated cells were resuspended in plating media (DMEM supplemented with 10% FBS) and plated at a density of $1 \times 10^5$ or $2 \times 10^5$ per well onto poly-D-lysine–coated 20-mm coverslips (WHB) in 12-well plates (Corning). Cells were incubated for 4 h before replacing with maintenance medium [neurobasal medium (Gibco) supplemented with 2% B-27 supplement (Gibco), 1% GlutaMax (Gibco), and 1% penicillin/streptomycin (Gibco)]. Neurons were maintained at 37 °C in 5% CO₂, with half of the medium changed every 2~3 days. For transfection in neurons, calcium phosphate precipitation was performed as described previously. Briefly, the neurons were serum-starved with pre-heated DMEM for 2 h at 37 °C in 10% CO₂. For each well of 12-well plate, 1~6 μg DNA in 1~6 μL was mixed with 5 μL 2.5 M CaCl₂ in ddH₂O (total volume 50 μL), and further mixed with 50 μL of HEPES-buffered saline containing (in millimoles): 274 NaCl, 10 KCl, 1.4 Na₂HPO₄, 15 glucose, and 42 HEPES, pH 7.05. Resulting DNA-calcium phosphate precipitates were added into neurons. Morphology was studied 3–7 d later.

Cell surface binding assay

For obtaining purified ECD, constructs expressing secretable ECD were transfected.
into HEK293 cells. After 24 h, the medium was changed to conditional medium (CM) containing 0.5% fetal bovine serum for another 24 h. Then the CM were collected for immunoprecipitation with the tag antibody and Protein A/G beads. Beads were washed with tris-buffered saline (TBS) (50mM Tris HCl, 150mM NaCl, pH 8.0) buffer three times, then five packed beads volumes of 0.1M glycine HCl buffer (pH 3.0) were added. Samples were incubated with gentle shaking for 5 min at room temperature. They were then centrifuged at 2500 rpm for 5 min to harvest the supernatant. Afterwards, 10 μl of 0.5M Tris HCl, pH 8.0, with 1.5M NaCl, was added.

For surface binding assay, HEK293 or COS-7 cells were transfected with indicated constructs. After 24~48 h, purified protein or CM were added to the transfected cells for 1 h-incubation at 4 °C, and then the cells were subjected to immunostaining with indicated antibodies.

**Cell aggregation assay**

As described previously (Boucard et al., 2012), HEK293 cells were transfected with indicated constructs, respectively. After 48 h, the cells were trypsinized and the green cells were then mixed with red cells to incubate with gentle agitation at room temperature with DMEM supplemented with 10% FBS, 50 mM HEPES-NaOH, pH 7.4, 10 mM CaCl$_2$, and 10 mM MgCl$_2$. After 0.5-1 h, the cell mixtures were transferred gently into a 12-well plate and imaged by fluorescence microscopy. The resulting images were analyzed by counting the number of aggregation particles in the field by ImageJ. Cell aggregation particles were defined as four or more clustered cells with at
least one red and one green cell.

**Time-lapse imaging and analysis of dendritic spines**

Live imaging of cultured neurons was performed as described previously with modifications (Li et al., 2017). Cultured rat hippocampal neurons were transfected by calcium phosphate precipitation at DIV9 and subjected to live imaging at DIV15. Z-stack images of secondary dendrites from transfected neurons were imaged every minute for 30 min, using an Olympus FV1000 confocal microscope with a 40 × (NA1.35) objective for time-lapse imaging. Images were collapsed into 2D projections and analyzed with ImageJ software. Stable spines were defined as protrusions with stable morphology during the entire imaging session; newborn spines were those with emerging protrusions after imaging, regardless of the time they emerged and whether they persisted during the entire imaging session; eliminated spines were spines that were present at the beginning of imaging but then disappeared during the imaging session.

**Immunoprecipitation**

Immunoprecipitation was performed as described previously (Chen et al., 2021). Transfected HEK293 cells were lysed in IP buffer containing (in millimoles): 20 Tris, pH7.6, 50 NaCl, 1 EDTA, 1 NaF, 0.5% Nonidet P-40 (vol/vol), with protease and phosphatase inhibitors. Samples were centrifuged at 12,000 × g for 20 min at 4 °C to remove debris. Lysates (1~2 mg) or CM were incubated with corresponding antibody
(1~2 μg) at 4 °C for either 3-4 h or overnight and then incubated with 10~15 μL Protein A/G magnetic agarose beads (Pierce) at 4 °C for 1 h. Samples were washed with IP buffer and resuspended in SDS sample buffer. Then the samples were subjected to WB.

**Western blotting**

For protein expression detection, tissues were homogenized in PBS plus protease and phosphatase inhibitors. Then the homogenates were lysed in equal volume of 2× RIPA buffer [0.2% SDS (wt/vol), 1% sodium deoxycholate (wt/vol) and 2% Nonidet P-40 (vol/vol) in PBS] plus protease and phosphatase inhibitors. Lysates were centrifuged at 12,000 × g for 20 min at 4 °C to remove debris. The supernatants were subjected to Bradford assay (Pierce) to measure protein concentration and diluted in SDS sample buffer. Protein samples (10~20 μg) were resolved by SDS-PAGE and transferred to PVDF membrane (Millipore). The membrane was immunoblotted with primary and secondary antibodies, and immunoreactive bands were visualized by enhanced chemiluminescence under the gel documentation system (Bio-Rad). Densitometric quantification of protein band intensity was performed using ImageJ. Antibodies were diluted with primary antibody dilution buffer (TBS + 1% TritonX-100 + 5% BSA) for western blotting (WB). Antibodies used in this manuscript are as below.

| Target protein | Host species | Source and Cat#       | Dilution |
|----------------|--------------|-----------------------|----------|
| DSCAM          | Goat         | Millipore (AF3666)    | 1:500    |
| Antibody | Species | Source          | Dilution |
|----------|---------|----------------|----------|
| PSD-95   | Mouse   | Millipore (MAB1598) | 1:1000   |
| β-Actin  | Rabbit  | Millipore (MABT523) | 1:2000   |
| FLAG     | Mouse   | Sigma (1804)     | 1:2000   |
| NeuN     | Mouse   | Millipore (MAB377) | 1:1000   |
| GFP      | Mouse   | Santa Cruz (SC-9996) | 1:1000   |
| PV       | Mouse   | Swant (235)      | 1:10000  |
| His      | Rabbit  | Santa Cruz (SC-8036) | 1:1000   |
| myc      | Mouse   | Santa Cruz (SC-40) | 1:500    |
| NLGN1    | Mouse   | Neuromap (75-160) | 1:500    |
| NLGN1    | Sheep   | ThermoFisher (PA5-48050) | 1:500   |
| Synaptophysin | Rabbit | Proteintech (17785-1-AP) | 1:1000   |
| Tublin   | Rabbit  | Santa Cruz (sc-23948) | 1:2000   |
| Synapsin I | Mouse  | Cell signaling (D12G5) | 1:200    |

**Immunostaining**

Immunostaining of cultured neurons was performed as described previously with modifications (Chen et al., 2021). Primary neurons were fixed with 4% paraformaldehyde (PFA) / 4% sucrose (wt/vol) for 15 min. After washing three times with PBS, neurons were incubated with primary antibody diluted in GDB buffer (30 mM phosphate buffer, pH 7.4, containing 0.2% gelatin, 0.6% Triton X-100, and 0.9 M NaCl) at 4 °C overnight. After washing three times with washing buffer (20 mM...
phosphate buffer and 0.5 M NaCl), neurons were incubated with the corresponding Alexa Fluor-conjugated secondary antibodies (diluted in GDB buffer) at room temperature for 1 h. The images were obtained by FSX100 (Olympus).

Immunostaining of brain slices was performed as described previously (Wang et al., 2018). Mice were deeply anesthetized with isoflurane and perfused with PBS followed by 4% paraformaldehyde (PFA). Brains were postfixed in 4% PFA at 4 °C overnight and dehydrated using 30% sucrose at 4 °C for 2 d. Brain samples were rapidly frozen in OCT, cut into 40-μm sections and mounted on Super Frost Plus slides (Fisher).

Sections were blocked and permeabilized in PBS containing 0.5% Triton X-100 and 5% goat serum for 2 h at room temperature. Sections were incubated at 4 °C overnight with primary antibodies in PBS containing 5% goat serum and 2% BSA. Sections were washed with PBS and then incubated at room temperature for 1-2 h with secondary antibodies. The images were obtained by FSX100 (Olympus).

Golgi Staining.

Golgi staining was performed using the FD Rapid GolgiStain Kit following the manufacturer's protocol (FD Rapid GolgiStainTM Kit, cat: PK401). Brain tissues were incubated in mixed solutions A and B for 2 weeks in the dark at room temperature and solution C for 3 d. Tissues were cut into slices with 80-100μm thickness, stained with solutions D and E, dehydrated in gradient ethanol, cleared with xylene, and mounted on slides for imaging. Images of neurons in sensory cortex L2/3 were taken and imported into ImageJ with NeuronJ plugin for analysis. Dendrites were reconstructed.
and analyzed using the ImageJ with Sholl Analysis plugin, with 10-μm incremental increases in concentric circular diameter from the soma. The images were obtained by FSX100 (Olympus).

**Electrophysiological Analysis.**

Brain slices recording was performed as described previously (Wang et al., 2018). Male mice at P12, P21 or P42 were anesthetized with isoflurane (RWD Life Science). Brains were quickly removed to ice-cold oxygenated (95% O₂ / 5% CO₂) cutting solution containing: 120 mM Choline Chloride, 2.5 mM KCl, 7 mM MgCl₂, 0.5 mM CaCl₂, 1.25 mM NaH₂PO₄, 26 mM NaHCO₃, and 25 mM glucose. Lamellar 300 μm slices of the cortex were cut using VT-1000S Vibratome (Leica Microsystems). The slices were recovered in oxygenated artificial cerebrospinal fluid (ACSF) for 30 min at 32 °C and maintained at room temperature (25 ± 1 °C) for an additional 1 h before recording. The ACSF containing: 124 mM NaCl, 2.5 mM KCl, 2 mM MgSO₄, 2.5 mM CaCl₂, 1.25 mM NaH₂PO₄, 26 mM NaHCO₃, and 10 mM glucose. All solutions were saturated with 95% O₂ / 5% CO₂ (vol/vol).

Slices were placed in the recording chamber, which was superfused (2 mL/min) with ACSF at 32~34 °C. Pyramidal neurons from sensory cortex L2/3 were visualized with infrared optics using an upright microscope equipped with a 40× water-immersion lens (FN-S2N, Nikon) and an infrared CCD camera (IR-1000, DAGE-MTI). Pipettes were pulled by a micropipette puller (P-1000; Sutter Instrument) with a resistance of 3~5 MΩ. The recording was performed with the MultiClamp 700B amplifier and 1550A.
For mEPSCs recording, pyramidal neurons were held at -70 mV in the presence of 20 μM bicuculline (Tocris Bioscience), with the pipette solution containing: 125 mM K-gluconate, 5 mM KCl, 10 mM HEPES, 0.2 mM EGTA, 1 mM MgCl₂, 4 mM Mg-ATP, 0.3 mM Na-GTP, and 10 mM phosphocreatine (pH 7.35, 290~295 mOsm). mEPSCs were recorded in the presence of 1 μM TTX.

For mIPSCs recording, pyramidal neurons were held at -70 mV in the presence of 20 μM CNQX (Sigma) and 100 μM DL-AP5 (Tocris Bioscience), with the pipette solution containing: 130 mM CsCl, 5 mM Cs-methanesulfonate, 10 mM HEPES, 0.2 mM EGTA, 1 mM MgCl₂, 4 mM Mg-ATP, 0.3 mM Na-GTP, 10 mM phosphocreatine and 5 mM QX-314 (pH 7.35, 290~295 mOsm). mIPSCs were recorded in the presence of 1 μM TTX.

Electron microscopy studies were performed as described previously with modifications (Yang et al., 2017). Briefly, anesthetized mice were perfused transcardially with PBS and followed by PBS containing 2% glutaraldehyde and 4% paraformaldehyde. Brains were postfixed at 4°C overnight. Ultrathin sections (70 nm) of the sensory cortex L2/3 region were cut and stained with 2% uranyl acetate (vol/vol)
and Reynolds lead citrate, and they were analyzed with a Hitachi H-7650 transmission electron microscope. Symmetric and asymmetric synapses were visually confirmed and manually counted by investigators unaware of genotypes. Also, synaptic ultrastructural specializations like area, thickness and length of PSD, and presynaptic vesicle numbers were analyzed by investigators blind to genotypes.

Behavioral Analysis.

Behavioral analysis was carried out with 8- to 10-week-old male mice by investigators unaware of genotypes. Mice were handled for 3 days, and 10 minutes for each day for each one mouse before behavioral tests.

For OFT, mice were placed in a chamber (50 × 50 cm). An overhead camera and tracking software (Med Associates) were used to monitor the mouse movement for 10 min. Total distance and time traveled in the center (15 × 15 cm) were measured. The chamber was cleaned with 75% ethanol and wiped with paper towels after each trial.

During a 10-min open-field test period, the amount of time spent grooming and the circling behavior were measured manually.

EPM was performed as described previously (Chanques et al., 2018). The platform was elevated 74 cm above the floor. It consisted of two closed arms (35 × 6 × 22 cm), two open arms (35 × 6 cm), and a central zone (6 × 6 cm). Mice were placed on the central zone and faced an open arm. Mice could freely explore the platform for 10 min.

The total time spent in the open arms and the entries to open arms were recorded by the monitoring software (Med Associates Inc., Fairfax, VT). The apparatus was
cleaned with 75% ethanol after each trial.

Three-chamber social preference test was performed as described previously with minor modifications (Moy et al., 2004). The apparatus was a transparent Plexiglas rectangular box (40 x 72 cm) with three equal transparent partitions (40 x 24 cm), including left, right and center chambers. Two wire cups were placed in the left and right chambers for each one. Firstly, the experimental mouse was placed in the center and allowed to freely explore the three chambers for 10-min. After that, an age- and gender-matched stranger C57BL/6J mouse (S1) was placed in one of the two wire cages and on the other side were the empty wire cages (E). Then, the test mouse was placed in the center for another 10-min session. The test mouse would now choose between S1 and E. After that, a second age- and gender-matched C57BL/6J stranger mouse (S2) was placed in another wire cage. Finally, the test mouse was placed in the center for the last 10-min session. Thus, the test mouse would now choose between an already familiar mouse (S1) and a new stranger mouse (S2). The mouse's movement was recorded by a video-tracking system (Med Associates Inc., Fairfax, VT). Time spent in each chamber and sniffing time within a 1 cm distance to each wire cage (interaction time) were measured. Social preference index was calculated as described (Chiang et al., 2018): [interaction time (S1) - interaction time (E)] / [interaction time (S1) + interaction time (E)] for each genotype; [interaction time (S2) - interaction time (S1)] / [interaction time (S2) + interaction time (S1)] for each genotype.

The Barnes circular maze was performed as described previously with modifications...
Rosenfeld and Ferguson, 2014). The Barnes circular maze is a planar, round white Plexiglas platform (75 cm diameter), 1 m above the floor, with 18 evenly spaced holes (7 cm diameter). A black escape box was placed under one hole. During the trial, a 500-lux light was turned on. After each trial, the platform and the escape box were cleaned thoroughly with 75% ethanol. The test consists of a 4-day training trial and a probe trial. One day before the training trial, test mice were habituated in the target box for 3 min. The training trial was repeated for 4 consecutive days, 3 times each day with 20 min intervals. At the beginning of the training trial, a test mouse was placed in a cylindrical holding chamber (8 cm diameter) located in the maze center. After 15 s of holding time, the mouse was allowed to search for the target hole for 3 min. If the mouse failed to find the target hole in 3 min, it was gently guided into the target hole by the investigator's hands. When the mouse entered the escape box, the light was turned off and the mouse remained undisturbed for 1 min. The mouse's movement was recorded, and the number of errors and the latency to find the target hole was measured during the training trials by video tracking software. On day 5, the probe trial was performed with each mouse. The escape box was removed, and the test mouse was allowed to find the target hole freely for 90 s. During the probe trial, the total distance traveled and the latency to find the target hole were measured. % correct pokes and % time in the target area were also measured.

All behavioral results were analyzed by investigators blind to genotypes.

Statistical Analysis.
Statistical analysis was done by the GraphPad Prism version 7.0 (GraphPad Software). Before being analyzed, all data in our study were checked by the D’Agostino-Pearson omnibus normality test to prove they came from a Gaussian distribution. Two-way ANOVA that analyzes more than two parameters was used in behavioral and body weight studies. All statistical analysis were presented as mean ± SEM and analyzed by two-tailed Student’s t-test or one-way ANOVA, including behavioral tests, morphological analysis, electrophysiological studies and western blotting. For the data that did not conform to the Gaussian distribution, we used Mann-Whitney U test or Kruskal-Wallis test for the statistical analysis. Otherwise, Values of p < 0.05 were considered statistically significant. Statistical significance was set at * p < 0.05, ** p < 0.01, and *** p < 0.001.

Results

DSCAM expression is downregulated during synapse maturation

CAMs are critical for synapse formation and maturation, and their expression is regulated during this developmental stage (Foldy et al., 2016). To characterize the expression patterns of CAMs during synapse development, we performed qRT-PCR with mRNA extracted from mouse cortex on postnatal days 0 (P0) to 60 (P60) (Fig.1A). As shown in Fig. 1B, the expression of most CAMs, such as NLGNs, increased gradually during this stage, consistent with synapsin I and postsynaptic density (PSD)-95 expression patterns. Intriguingly, unlike synapsin I and PSD-95, the expression of DSCAM increased from P0 to P14, but dramatically decreased

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afterward (Fig. 1B). When investigated in vitro, DSCAM protein levels in primary cortical neurons similarly increased from DIV (days in vitro) 3 to DIV12; this phenomenon was also followed by a decrease in DSCAM protein levels (Fig. 1C-1D).

Synapse formation commences in the first postnatal week and peaks at P14, and synapse maturation continues thereafter (Farhy-Tselnicker and Allen, 2018). In parallel, the expression of DSCAM in the brain gradually increased from embryonic day 12.5 (E12.5) until it reached its peak at P12, and then decreased until P60 (Fig. 1E-1F). This expression pattern of DSCAM suggests that its level is downregulated during synapse maturation compared to other CAMs. We also examined the subcellular distribution of DSCAM in mouse brains. PSD fractionation assays revealed that DSCAM was localized to the PSD area (Fig. 1G). To confirm the distribution of DSCAM in neurons, we cotransfected His-tagged full-length DSCAM plus GFP into cultured neurons at DIV9 and stained them with anti-His antibody at DIV17. As shown in Fig. 1H-1J, DSCAM was mainly distributed and clustered in GFP-labeled dendrites and dendritic spines. Dendritic spines are morphologically divided into mature and immature spines. Mature spines are mushroom-like, with a width of spine head to neck ratio ≥ 1.5; immature spines have a spine head to neck ratio < 1.5. Interestingly, the fluorescence intensity and size of DSCAM-His puncta were markedly increased in dendritic shafts and in immature spines, compared to those in mature spines. Furthermore, costaining with PSD-95 demonstrated the colocalization of DSCAM-His with PSD-95 (Fig. 1K). Taken together, these results provide an indication of how DSCAM functions in spine maturation.
**DSCAM deficiency results in increased spine maturation in the sensory cortex**

As DSCAM expression was downregulated during the spine maturation period, we were curious as to whether DSCAM deficiency would affect spine density and/or maturation *in vivo*. A previous study reported that DSCAM-null C57BL/6 mice died within 24 h of birth (Amano et al., 2009), so we generated DSCAM floxed mice (DSCAM f/f) (Fig. 2A-2B) and crossed them with a hGFAP::Cre line, where Cre is expressed in neural progenitor cells at E13.5 and in all forebrain neurons and astrocytes of mice (Zhuo et al., 2001). Compared to that of control mice (DSCAM f/f), the expression of DSCAM in the brain was abated by approximately 60% in resulting hGFAP::Cre; DSCAM f/f (hereafter referred to as GFAP-DSCAM f/f) mice (Fig. 2C), in which DSCAM was ablated in neurons and astrocytes. Next, we examined whether DSCAM deficiency could alter neural development. As shown in Fig. 2D-2F, GFAP-DSCAM f/f mice, while displaying a similar body weight to that of control mice and showing no global morphological deficits, ultimately displayed increased brain weight. The density of NeuN+ neurons was decreased in layer 1 (L1) and increased in L2/3 in the cortex of GFAP-DSCAM f/f mice (Fig. 2G). However, the total number of NeuN+ neurons in the cortex (Fig. 2G) and hippocampus (Fig. 2H) remained within normal ranges. The number of PV+ interneurons in the cortex was also unchanged (Fig. 2I).

Neurons in the sensory cortex L2/3 receive multiple inputs and are highly plastic; they are involved in context-specific sensory information processing and in controlling the...
gain of cortical output (Feldman and Brecht, 2005; Feldman, 2009; Petersen and Crochet, 2013). We used Golgi staining to examine dendritic spine development from P12 to P42 in sensory cortex L2/3 of GFAP-DSCAM f/f mice. As shown in Fig. 3A-3D, the densities of both total and mature spines were greater in GFAP-DSCAM f/f mice than in control mice from P12 to P42, while the number of immature spines was greater at P12 and P21, but not P42. Surprisingly, the width of spine heads was also greater at P21 and P42 (Fig. 3E) in GFAP-DSCAM f/f mice. Similar results were observed in sensory cortex L5 (Fig. 3J-3N). As with a previous study (Maynard and Stein, 2012), the dendritic lengths and branches in sensory cortex L2/3 (Fig. 3F-3H) and L5 (Fig. 3O-3Q) were increased in GFAP-DSCAM f/f mice. Dendritic complexity was increased in GFAP-DSCAM f/f mice at P21, but it returned to normal levels by P42 (Fig. 3I and 3R). These results suggest that DSCAM deficiency in forebrain neurons and astrocytes increases mature spine densities and sizes in the sensory cortex.

The size or volume of spine heads is strongly correlated with the PSD area (Harris et al., 1992; Arellano et al., 2007). A larger spine volume indicates a larger PSD area and a stronger synaptic strength (Holtmaat and Svoboda, 2009). To confirm this relationship, we performed electron microscopy (EM) analysis and found that the number of asymmetric (excitatory) synapses was increased in sensory cortex L2/3 in GFAP-DSCAM f/f mice, while the number of symmetric (inhibitory) synapses was unchanged (Fig. 4A-4B). The presynaptic vesicle number was similar in control and GFAP-DSCAM f/f mice (Fig. 4C). However, the area, length, and thickness of the PSD
Glutamatergic transmission is elevated in the L2/3 sensory cortex of DSCAM-deficient mice

Dendritic spine density and morphology are critical for synaptic transmission strength and synaptic stability (Alvarez and Sabatini, 2007; Yuste, 2013). An increase in the number of matured spines corresponds to an increase in glutamatergic transmission.

We examined whether DSCAM deficiency alters neurotransmission in the cortex during development by recording miniature excitatory postsynaptic currents (mEPSCs) and miniature inhibitory postsynaptic currents (mIPSCs) in sensory cortex L2/3 of GFAP-DSCAM f/f mice at P12, P21, and P42. Notably, GFAP-DSCAM f/f mice displayed an increase in mEPSC frequency at all three time points, but mEPSC amplitudes were not changed (Fig. 5A-5C). Neither mIPSC frequencies nor amplitudes were altered in GFAP-DSCAM f/f mice (Fig. 5D-5F). Together, these results suggest that DSCAM deficiency increases glutamatergic transmission in sensory cortex L2/3.

Knockdown of DSCAM in primary neurons disrupts spine dynamics and maturation

Since DSCAM deficiency results in increased spine maturation, we investigated whether DSCAM modulates spine dynamics by knocking down DSCAM in neurons
via small hairpin RNA (sh-RNA). Based on a previous report (Ly et al., 2008), we constructed sh-RNA for mouse or rat DSCAM (sh-DSCAM) and scrambled sh-RNA for use as a control (sh-control). We then confirmed the knockdown efficiency of sh-DSCAM in HEK293T cells transfected with mouse DSCAM-His (Fig. 6A).

Furthermore, we observed and analyzed dendritic spine density in sh-DSCAM-transfected neurons. Compared to that in neurons transfected with sh-control, the total spine density was increased in neurons transfected with sh-DSCAM (Fig. 6B-6C). To exclude the off-target effects of sh-DSCAM, we overexpressed human DSCAM (hDSCAM), which can not be recognized by sh-DSCAM (Fig. 6A), in sh-DSCAM transfected neurons, and we found that hDSCAM overexpression rescued the spine deficits in DSCAM knockdown neurons (Fig. 6B-6C), thus confirming the effects of DSCAM knockdown on spine development.

Furthermore, the neurons were stained with anti-PSD-95 antibody to quantify the number and size of PSD-95 puncta. Compared with nontransfected neurons in the same coverslip or sh-control transfected neurons, neurons transfected with sh-DSCAM displayed increased numbers and sizes of PSD-95 puncta (Fig. 6D-6F).

These results indicate that knockdown of DSCAM in cultured neurons increases dendritic spine maturation.

Because enhanced spine formation or stabilization leads to increased spine maturation, we performed time-lapse imaging in cultured neurons to observe the dynamic spine maturation process. Cultured cortical neurons were cotransfected with GFP and sh-DSCAM or sh-control at DIV9 and imaged at DIV15~17. The same
secondary dendritic branch was imaged every minute for 1 h, and the percentages of stable, new, and eliminated spines were analyzed. As shown in Fig. 6G-6J, neurons transfected with sh-DSCAM exhibited an increase in stable spines and a decrease in eliminated spines, but they showed no change in the number of new spines during the imaging period compared with neurons transfected with sh-control. In other words, spines were more often stabilized and less often eliminated due to the knockdown of DSCAM. Notably, there was a positive correlation between the percentage of stable spines and spine density and a negative correlation between the percentage of eliminated spines and spine density (Fig. 6K-6M). Taken together, these live imaging data demonstrated that DSCAM knockdown promotes spine stabilization in neurons, thus accelerating spine maturation.

**DSCAM interacts with NLGN1**

DSCAM, a single transmembrane protein, belongs to the Ig superfamily of CAMs that plays a critical role in synapse formation and maturation (Sudhof, 2008, 2017). To explore the underlying mechanisms of DSCAM in spine maturation, we examined whether DSCAM interacts with other CAMs. NLGNs, a class of transmembrane proteins that interact in trans with presynaptic NRXNs, are classic postsynaptic CAMs and are essential for synaptogenesis and synapse maturation (Varoqueaux et al., 2006; Chubykin et al., 2007; Sudhof, 2008; Bemben et al., 2015; Sudhof, 2017). We cotransfected DSCAM-His with NLGNs (NLGN1-4) or NRXNs (NRXN1α-3α and 1β) into HEK293T cells and performed coimmunoprecipitation (co-IP) assays. As shown
in Fig. 7A, NLGN1-4 were co-IPed with DSCAM-His, but NRXNs were not. Conversely, DSCAM-His was also co-IPed with NLGNs (Fig. 7B). Moreover, the FLAG-tagged extracellular domain (ECD) of DSCAM (FLAG-ECD) and NLGNs were cotransfected into HEK293T cells for co-IP assay, and FLAG-ECD was co-IPed with NLGNs (Fig. 7C). These results suggest that DSCAM could interact with NLGN1-4 through its ECD.

To detect whether DSCAM-ECD binds to cell surface NLGNs, we performed a cell surface binding assay. First, we overexpressed FLAG-ECD in HEK293T cells. To confirm whether FLAG-ECD could be secreted into the medium, we collected conditioned medium (CM) and added anti-FLAG antibody to enrich the secretable FLAG-ECD. As shown in Fig. 7D, FLAG-ECD was detected in IPed CM, as well as in cell lysates. Next, we harvested CM with FLAG-ECD or control and added them to fixed HEK293T cells cotransfected with NLGNs and GFP. After incubation, the cells were subjected to immunostaining with anti-FLAG antibody. As shown in Fig. 7E, FLAG-ECD bound to the surface of cells transfected with NLGNs. In fact, more FLAG-ECD was enriched on the cell surface that expressed NLGN1 than on the cell surfaces that expressed NLGN2-4 (Fig. 7F). Altogether, these results suggest that DSCAM interacts with NLGN1 through their ECDs.

NLGN1 is strongly expressed in excitatory synapses and is implicated in ASDs (Varoqueaux et al., 2006; Sudhof, 2008; Nakanishi et al., 2017; Sudhof, 2017). Next, we focused on the DSCAM-NLGN1 interaction. The endogenous interaction of DSCAM and NLGN1 was confirmed by co-IP with mouse brain lysates (Fig. 7G). We then examined whether DSCAM can regulate NLGN1 expression and distribution in
the synaptic area. The protein level of NLGN1 was comparable in the brain between control and DSCAM-deficient mice (Fig. 7H-7I). However, the level of NLGN1 in the synaptosomal plasma membrane (SPM) fraction was increased in GFAP-DSCAM f/f mice (Fig. 7J-7K), suggesting that DSCAM deficiency can induce NLGN1 enrichment in the synaptic membrane.

**DSCAM inhibits the NLGN1-NRXN1β interaction**

Postsynaptic NLGN1 is involved in synapse maturation by interacting *in trans* with presynaptic NRXN1β (Sudhof, 2008). We examined whether DSCAM could regulate the NLGN1-NRXN1β interaction by cotransfecting NLGN1, NRXN1β and DSCAM into HEK293T cells. As shown in Fig. 8A-8B, NRXN1β was co-IPed with NLGN1 in the absence of DSCAM-His. However, NRXN1β co-IP with NLGN1 was decreased in the presence of increased DSCAM-His, suggesting that DSCAM inhibits the NLGN1-NRXN1β interaction. Since NLGN1 binds *in trans* with NRXN1β, we performed cell surface binding assays to detect whether DSCAM could inhibit this trans-cell interaction. COS-7 cells were cotransfected with NLGN1 and varied amounts of DSCAM-His. After transfection, the cells were treated with purified NRXN1β-ECD-myc protein for 1 h and then fixed for immunostaining with anti-NLGN1, anti-myc and anti-His antibodies. As shown in Fig. 8C-8D, the red fluorescence, which indicates cell surface binding of NRXN1β-ECD-myc, was decreased when DSCAM-His expression was increased. Moreover, we also used cell aggregation assays to confirm the inhibition of NLGN1-NRXN1β trans-cell interactions by DSCAM.
(Fig. 8E). Cells clustered when HEK293T cells cotransfected with NLGN1 and GFP were mixed with another set of HEK293T cells cotransfected with NRXN1β and RFP (Fig. 8F). DSCAM-His coexpression with NLGN1 in GFP+ cells decreased the number of aggregates in a dose-dependent manner (Fig. 8F-8G). Taken together, these results suggest that DSCAM inhibits the NLGN1-NRXN1β trans-cell interaction.

As DSCAM inhibited the NLGN1-NRXN1β trans-cell interaction through ECD binding to NLGN1, we contemplated whether DSCAM-ECD alone could block the NLGN1-NRXN1β in trans interaction. Using cell aggregation assays, we added CM containing FLAG-ECD to mixtures of HEK293T cells expressing either NLGN1 or NRXN1β. As shown in Fig. 8H-8I, DSCAM-ECD reduced the number of NLGN1-NRXN1β cell aggregates in a dose-dependent manner. Next, we theorized that DSCAM-ECD could rescue spine overmaturation caused by DSCAM deficiency. We treated DSCAM knockdown neurons with CM containing FLAG-ECD and found that FLAG-ECD rescued the increased total spine densities induced by DSCAM knockdown (Fig. 8J-8K). Together, these results suggest that DSCAM may block NLGN1-NRXN1β interactions to inhibit premature spine maturation.

**DSCAM deficiency induces autism-like behaviors and enhances spatial memory in mice**

Given that DSCAM is one of 102 ASD predominant genes and that its absence accelerated spine maturation and elevated glutamatergic transmission, we next examined the behaviors of DSCAM-deficient mice. First, we conducted autism-associated
behavioral tests in GFAP-DSCAM f/f and control mice. As shown in Fig. 9A-9B, GFAP-DSCAM f/f mice displayed increased amounts of circling and self-grooming than control mice did.

Along with repetitive behaviors, profound social impairment is a core symptom of ASD. We therefore conducted three-chamber social interaction tests (Fig. 9C-9I) for social recognition and social interaction (Moy et al., 2004). In the social preference test, both GFAP-DSCAM f/f and control mice preferred to explore a stranger mouse (stranger1 mouse, S1) than an inanimate object (empty cage, E), as measured by duration spent in each chamber and duration of sniffing (interaction time) (Fig. 9C-9E). Moreover, the social interaction index was indistinguishable between the two genotypes, suggesting normal social preference in GFAP-DSCAM f/f mice (Fig. 9F). Next, we compared social novelty preference. After the E was replaced with another stranger mouse (stranger2 mouse, S2), control mice spent more time in the S2 chamber and interacted more with S2 than they did with the familiar mouse (S1) (Fig. 9G-9H). In contrast, GFAP-DSCAM f/f mice spent less time in the S2 chamber than in the S1 chamber (Fig. 9G). Additionally, GFAP-DSCAM f/f mice did not differentiate between S1 and S2 in terms of sniffing duration (Fig. 9H). Social novelty index score was decreased in GFAP-DSCAM f/f mice (Fig. 9I), suggesting an impairment in social novelty but not sociability.

We also conducted the Barnes circular mazes test to examine the impact of DSCAM deficiency on spatial learning and memory (Fig. 9J-9O). In the 4-day training session, escape latency and number of errors were similar in both genotype (Fig. 9J-9K),
suggesting normal spatial learning for GFAP-DSCAM f/f mice. However, at test, 
GFAP-DSCAM f/f mice displayed reduced latency to reach target and gained 
numbers of correct pokes, but they showed no difference in travel distance and time 
on target, compared with the controls (Fig. 9L-9O), which suggests that DSCAM 
deletion may enhance spatial memory. Taken together, the data indicate that 
GFAP-mediated DSCAM deficiency increases stereotyped behaviors, impairs social 
novelty, and enhances spatial memory.

Moreover, GFAP-DSCAM f/f mice exhibited increased distance traveled and velocity 
in the open field test (OFT) but decreased time in the center (Fig. 9P-9S). In the 
elevated plus maze (EPM), GFAP-DSCAM f/f mice entered the open arms less and 
spent less time in them (Fig. 9T-9W), suggesting increased anxiety-like behaviors due 
to DSCAM deletion in neurons and astrocytes.

DSCAM deficiency in pyramidal neurons induces premature spine maturation 
and autism-like behaviors

DSCAM was primarily expressed in neurons, as examined by western blotting with 
isolated neurons and astrocytes (Fig. 10A), and its expression in cultured neurons 
was negatively regulated during the postnatal development period (Fig. 1C-1D). We 
therefore investigated whether the spine and behavioral deficits in GFAP-DSCAM f/f 
mice could result from DSCAM deficiency in pyramidal neurons. To this end, we 
crossed DSCAM f/f mice with a NEX-Cre line, in which Cre is expressed in pyramidal 
eurons of the neocortex and hippocampus. We found that the DSCAM level was
reduced to 50% in the cortex of the resultant NEX-DSCAM f/f mice (Fig. 10B-10C). Furthermore, the sensory cortex L2/3 of NEX-DSCAM f/f mice at P42 was subjected to Golgi staining. The densities of total spines and mature spines were markedly increased, while the density of immature spines remained unchanged (Fig. 10D-10G). Additionally, the width of spine head increased (Fig. 10H). These results suggest that DSCAM deficiency in pyramidal neurons also lead to spine overmaturation. Next, we examined the behaviors of NEX-DSCAM f/f mice. In the OFT, the duration spent in the center and the velocity were similar between NEX-DSCAM f/f and control mice (Fig. 10I-10L). In the EPM, the NEX-DSCAM f/f mice entered open and closed arms at similar rates as the control mice did, but they reduced the duration spent in open arms (Fig. 10M-10P), suggesting that DSCAM deficiency in pyramidal neurons do not induce anxiety-like behaviors. In addition, NEX-DSCAM f/f mice were subjected to tests for autism-associated behaviors. NEX-DSCAM f/f mice exhibited stereotyped behaviors (Fig. 11A-11B), social novelty deficits (Fig. 11C-11I) and enhanced spatial memory (Fig. 11J-11O). Taken together, these results demonstrate that DSCAM deficiency in pyramidal neurons also induces autism-like behaviors and enhanced spatial memory. Therefore, we propose our DSCAM model of synapse maturation. In control mice, DSCAM was localized to immature spines and interacted with NLGN1 to inhibit the NLGN1-NRXN1β interaction, thus preventing spine overmaturation. In DSCAM-deficient mice, this inhibition of the NLGN1-NRXN1β interaction was removed, and resulting in premature spine maturation, which ultimately led to
autism-like behaviors and enhanced spatial memory (Fig. 11P).

Discussion

In this study, we provided evidence that DSCAM serves as a repressor for the NLGN1-NRXN1β interaction, and that its deficiency leads to premature spine maturation and excessive glutamatergic transmission, inducing autism-like behaviors. The major findings of this paper are as follows. First, DSCAM was downregulated following synapse maturation, and knockdown of DSCAM in primary neurons increased spine maturation. Second, in vivo, GFAP-mediated DSCAM deficiency in mice led to spine overmaturation and increased glutamatergic transmission. Third, DSCAM interacted with NLGN1 through its ECD and blocked the NLGN1-NRXN1β interaction to suppress spine overmaturation. Finally, DSCAM-deficient mice exhibited autism-like behaviors, including impaired social novelty, increased circling and grooming time, and improved spatial memory.

Dendritic spines are small protrusions from the dendrites of excitatory neurons and play critical roles in learning, memory, and cognition (Yuste and Bonhoeffer, 2004; Berry and Nedivi, 2017). Dysregulation of spine development has been implicated in mental disorders including autism, depression and schizophrenia (Forrest et al., 2018). Previous studies have shown that DSCAM acts as a crucial factor or regulator in synapse development and synaptic plasticity (Yamagata and Sanes, 2008; Li et al., 2009; Maynard and Stein, 2012; Thiry et al., 2016; Laflamme et al., 2019), indicating its role in spine development. Here, we found an increase in the density of matured...
spines and in the volume of spine heads in GFAP-DSCAM f/f and NEX-DSCAM f/f mice. Due to potential differences in staining efficiency between the two batches of Golgi staining, we observed a small difference in the spine density data for the control mice between Fig. 3B-3D and Fig. 11E-11G. We further confirmed the spine deficits in cultured neurons. Live imaging results showed that increased spine density could be attributed to enhanced stability. These results suggest that DSCAM can downregulate spine maturation in the developing sensory cortex.

Several recent studies reported that DSCAM-ICD plays a critical role in synapse development and neuronal migration (Sachse et al., 2019; Arimura et al., 2020). However, we found that DSCAM interacted with NLGN1 through its ECD to disrupt the NLGN1-NRXN1β interaction. NLGN1 plays an essential role in spine maturation (Varoqueaux et al., 2006; Chubykin et al., 2007; Bemben et al., 2015). DSCAM-ECD also rescued abnormal spine maturation in DSCAM knockdown neurons. Therefore, DSCAM might modulate spine maturation through its ECD. Differences in spine densities have previously been reported in patients with neuropsychiatric disorders, such as increased spine densities in L2 of the cortex and L5 of the temporal lobe in ASD patients (Hutsler and Zhang, 2010). In addition, increased spines were also observed in Fragile-X syndrome patients and corresponding mouse models (Comery et al., 1997; Irwin et al., 2000). Thus, dendritic spine malformation may lead to neuronal dysfunction during neural development, and DSCAM may serve as a modulator in spine remodeling.

Disruption of the excitatory/inhibitory (E/I) balance has been implicated in various
neuropsychiatric disorders, including ASD (Rubenstein and Merzenich, 2003; Nelson and Valakh, 2015). Elevation of the E/I ratio in the mouse prefrontal cortex leads to social deficits (Yizhar et al., 2011). In a similar manner, we found that glutamatergic transmission increased in GFAP-DSCAM f/f mice during postnatal development while GABAergic transmission was unchanged, which might result in elevation of the E/I ratio and lead to social novelty deficits (Nelson and Valakh, 2015). Social interaction deficits were found in these DSCAM-deficient mice. Although some ASD mouse models exhibit a reduced E/I ratio, such as mice with autism-associated mutations in NRXNs or SHANKs (Etherton et al., 2009; Jiang and Ehlers, 2013), an enhanced E/I ratio can also be observed in many other ASD mouse models, like TSC1-, MDGA2-, FMRP- or CUL3- deficient mice (Bateup et al., 2013; Zhang et al., 2014; Connor et al., 2016; Dong et al., 2020). Precocious spine maturation in hippocampal neurons dramatically elevated the E/I ratio and impaired the cognitive development in SynGAP mutant mice (Clement et al., 2012). In our autistic model of DSCAM-deficient mice, early spine maturation in DSCAM-deficient neurons contributed to increased excitatory synaptic transmission, which might impair the E/I balance and lead to autism-like behaviors. In addition, DSCAM deficiency increased only mEPSCs frequency, not amplitude, which may account for the normal surface distribution of active AMPARs or NMDARs.

Previous studies have indicated higher cognitive function in patients with macrocephaly and ASD (Courchesne and Pierce, 2005; Sacco et al., 2007). Increased head circumferences have been shown in ASD patients with special
DSCAM-null mice (DSCAMdel17 or DSCAM2J) similarly exhibited ventricular dilation with a dome-shaped head (Xu et al., 2011; Lemieux et al., 2016). Intriguingly, increased spatial memory was observed in both GFAP-DSCAM f/f and NEX-DSCAM f/f mice along with increased brain weight in this study, which implies that brain enlargement might be one factor explaining the enhanced spatial memory in DSCAM-deficient mice. Relatedly, it was previously theorized that mushroom spines (matured spines) can act as memory spines (Bourne and Harris, 2007). More mature spines might be another explanation for the increased spatial memory. Our live imaging results found a positive correlation between the percentage of stable spines and the spine density, which may also contribute to the enhanced spatial memory demonstrated by DSCAM-deficient mice. It has also been reported that NLGN3-R451C mice (an ASD-associated mutation) displayed enhanced spatial learning in the Morris water maze in addition to autism-like behaviors (Tabuchi et al., 2007). Taken together, our results reveal the regulatory functions of DSCAM in spine maturation and its underlying molecular mechanisms in the nervous system. DSCAM-deficient mice with increased spatial memory might belong to the subset of autism mouse models with high functions.

DSCAM is located on chromosome 21 in humans; an extra copy of chromosome 21 causes Down syndrome (DS) (Saito et al., 2000). Upregulated DSCAM expression is found in the brains of DS patients, DS mouse models, and AD mouse models (Saito et al., 2000; Amano et al., 2004; Jia et al., 2011), causing pathological changes in the brain and finally leading to impairment in cognitive functions. Elevated levels of
DSCAM are also found in post-mortem brains from bipolar subjects (Amano et al., 2008). Likewise, DSCAM expression is higher in intractable epilepsy (IE) patients than in controls (Shen et al., 2011). However, DSCAM deficiency has been found to disrupt dendritic self-avoidance and tiling in the mouse retina and cause abnormal neuronal migration (Fuerst et al., 2008; Fuerst et al., 2009; Sachse et al., 2019; Arimura et al., 2020). Additionally, premature termination mutations of DSCAM are observed in some ASDs patients (Iossifov et al., 2014; Wang et al., 2016; Stessman et al., 2017; Yuen et al., 2017). Our results showed that loss of DSCAM in pyramidal neurons induced autism-like behaviors in mice. Together, all these findings suggest that dysregulated DSCAM expression alters spine maturation, resulting in abnormal synaptic transmission, which may explain how gene-dosage imbalance of DSCAM could potentially contribute to the pathogenesis of neurological DSCAM-related disorders.

Author contributions

EF and PC conceived and designed the research project. PC, ZL, QZ, DL and H-FJ performed experiments and collected data. PC, ZL, QZ and TZ analyzed data. LS, JL and LZ provided experimental material and contributed to data analysis and discussion. PC, ZL and EF prepared manuscript figures. EF, PC, LZ, B-ML and B-XP wrote the manuscript. XL, SZ and SW provided technical and intellectual support. All authors provided critical reviews of results and approved the manuscript.
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Figure legends

**Fig 1. Downregulated expression of DSCAM during synapse maturation.**

A-B The mRNA levels of selected CAMs during postnatal synapse development. WT mice cortex was isolated from P0 to P60 for mRNA extraction and qRT-PCR analysis (A). Hot map analysis of qRT-PCR results for selected CAMs (B). N = 3 mice for each age. C-D DSCAM protein level in cortical neurons from DIV3 to DIV21. Primary cortical neurons were lysed at indicated DIV for western blotting (WB) (C). Quantitative analysis of relative protein levels in C, the relative protein levels of all three proteins were set to "1" (D). Data were from three independent experiments and shown as mean ± SEM. E-F DSCAM expression during brain development. Whole-brain homogenates from WT mice at different (indicated) stages from E12.5 to P60 were subjected to WB with the anti-DSCAM antibody (E). Quantitative analysis of data in E (F). N = 3 mice for each age. G Distribution of DSCAM in PSD fraction. Subcellular fractions of the WT brains were blotted with anti-DSCAM, anti-PSD-95 (a PSD marker) and anti-synaptophysin (a presynaptic marker). S1, supernatant 1; P1, pelleted nuclear fraction; S2, supernatant 2; P2, washed crude synaptosomal fraction; S3, crude synaptic vesicle fraction; SV, synaptic vesicle fraction; P3, lysed synaptosomal membrane fraction; SPM, synaptosomal membrane fraction; Pre, presynaptic fraction. H-K Distribution of DSCAM in neurons. Representative images of DSCAM-His distribution in dendrites (H) and in PSD (K). Cortical neurons were isolated from P0 pups, cotransfected with DSCAM-His and GFP at DIV9 and fixed for
staining with anti-His and anti-PSD-95 antibodies at DIV17. In H: arrow, mature (M) dendritic spine; arrowhead, immature spine (IM) dendritic spine. Scale bar, 32 μm (upper panel) and 10 μm (lower panel). Quantitative analysis of DSCAM-His fluorescence intensity (I) and puncta size (J) in H. N = 11 neurons, * p < 0.05, ** p < 0.01, and *** p < 0.001; one-way ANOVA. In K, arrowheads indicate DSCAM-His that was colocalized with PSD-95.

**Fig. 2 Normal gross brain structure of GFAP-DSCAM f/f.**

A. The genomic structure of floxed DSCAM mice. The first loxp (1st loxP) was inserted between exon1 and exon2, and the second loxp (2nd loxP) was inserted after the last exon (exon33). B. Genotyping of wild type (wt, m1), GFAP-DSCAM f/wt (m2) and GFAP-DSCAM f/f (m3) mice. Primer pair 1 (PP1) and PP2 were for 1st loxP, PP3 and PP4 were for 2nd loxP, PP5 was for wt allele and PP6 for GFAP-Cre transgene. C. DSCAM expression is decreased in the brain of GFAP-DSCAM f/f mice. Whole brain lysates from GFAP-DSCAM f/f and control mice were probed with anti-DSCAM antibody. Actin served as a loading control. The relative DSCAM level (band intensity of DSCAM / actin) was quantified. N = 4 mice for control, n = 5 mice for GFAP-DSCAM f/f mice. *** p < 0.001, Student’s t-test. Data were shown as mean ± SEM. D-F. GFAP-DSCAM f/f mice displayed normal body weight and gross brain structure, but increased brain weight. Representative appearance and brain images of GFAP-DSCAM f/f and control mice (D). Quantitative analysis of body and brain weight for each genotype (E). Brain weight: n = 5 for control, n = 6 for GFAP-DSCAM f/f mice.
Data were shown as mean ± SEM; * p < 0.05, ns, p > 0.05 (student’s t-test).

Representative Nissl-staining images of brains from each genotype. Scale bar, 1 mm (F). G-I GFAP-DSCAM f/f mice displayed normal densities of NeuN+ and PV+ neurons. Representative NeuN immunostaining images and quantitative analysis of NeuN+ cell densities in sensory cortex (G) and hippocampus (H) of each genotype.

Scale bar, 100 μm in G and 32 μm in H. N = 32 slices from 4 control mice, n = 43 slices from 5 GFAP-DSCAM f/f mice. Data were shown as mean ± SEM. ns, p > 0.05, Student’s t-test. Representative PV immunostaining images and quantitative analysis of PV+ cell densities in the sensory cortex of each genotype (I). Scale bar, 100 μm. N = 17 slices from 4 control mice, n = 21 slices from 5 GFAP-DSCAM f/f mice. Data were shown as mean ± SEM. ns, p > 0.05, Student’s t-test.

Fig 3. Abnormal spine maturation in sensory cortex of GFAP-DSCAM f/f mice.

A-R GFAP-DSCAM f/f mice displayed increased spine maturation in the sensory cortex (A-E and J-N) and increased dendritic arborization at P21 (F-I and O-R).

Representative Golgi staining images of dendritic spines in sensory cortex from P12, P21 and P42 male mice of each genotype (A and J). Scale bar, 10 μm. Quantitative analysis of total (B and K), M (C and L) and IM (D and M) spine density, as well as spine head width (E and N). Representative dendrite trace images from Golgi staining in sensory cortex L2/3 (F) and L5 (O) of each genotype at P12, P21 and P42. Scale bars, 32 μm. Quantitative analysis of dendritic length (G and P), branches (H and Q), and complexity (I and R) in F and O. N = 5 mice for each genotype. Data were shown
Fig. 4 Increased asymmetric synapse number and PSD size in sensory cortex of GFAP-DSCAM f/f mice

A-F GFAP-DSCAM f/f mice displayed increased density of asymmetric (AS) synapses and enlarged PSD area. Representative electron microscopy images of sensory cortex L2/3 from each genotype (A). For each genotype, scale bar was 1 μm for left panels and 0.5 μm for right enlarged insets. Red arrowheads, AS synapses; black arrowheads, symmetric (SY) synapses. Red box, enlarged insets of AS synapses; black box, enlarged insets of SY synapses.

Fig 5. Increased glutamatergic transmission in sensory cortex L2/3 of GFAP-DSCAM f/f mice.

A-F GFAP-DSCAM f/f mice displayed increased mEPSC frequency, but normal mIPSC. The sensory cortical slices of each genotype at P12 (A and D), P21 (D and E) and P42 (C and F) were collected for whole-cell voltage clamp recording of mEPSC (A-C) and mIPSC (D-F). Upper panels, representative traces of mEPSCs (A-C) and mIPSC (D-F). Scale bars: 30 pA, 1 s. Lower panels, histogram summary and cumulative probability plots of mEPSC (A-C) and mIPSC (D-F) inter-event intervals as mean ± SEM. Ns, p > 0.05, * p < 0.05, ** p < 0.01, and *** p < 0.001; two-way ANOVA.
P12: n = 34 neurons from 4 control, n = 16 neurons from 5 GFAP-DSCAM floxed/floxed (f/f) mice; P21: n = 20 neurons from 5 control, n = 22 neurons from 5 GFAP-DSCAM floxed/floxed mice; P42: n = 17 neurons from 4 control, n = 17 neurons from 4 GFAP-DSCAM floxed/floxed mice. Data were shown as mean ± SEM. ** p < 0.01, *** p < 0.001, Student’s t-test.

Fig 6. Increased spine maturation and stability in DSCAM knockdown neurons.

A Knockdown of overexpressed mouse DSCAM-His in HEK293T cells. HEK293T cells were cotransfected with mouse DSCAM-His, small hairpin RNA (sh-RNA) for mouse/rat DSCAM (sh-DSCAM) or control scramble sh-RNA (sh-control), and human DSCAM. 24hr after transfection, cells were lysed and subjected to WB with anti-DSCAM antibody. Data were from three independent experiments and are shown as mean ± SEM. Ns, p > 0.05, *** p < 0.001; Student’s t-test. B-C Knockdown of DSCAM in cultured cortical neurons increased mature spine density. Representative images of pyramidal neuron morphology and spine density in cortical neurons (B).

Neurons were isolated from E18 rat to culture and transfected with GFP plus sh-DSCAM or sh-control and hDSCAM or not at DIV9, and they were fixed for staining at DIV17. Scale bar, 16 μm (upper panel) and 10 μm (lower panel). Statistical analysis of total spine density in B (C). N = 29 neurons for sh-control, n = 79 neurons for sh-DSCAM, n = 22 neurons for sh-DSCAM + hDSCAM. Ns, p > 0.05, *** p < 0.001, student’s t-test. Data were shown as mean ± SEM. D-F Knockdown of DSCAM in cultured cortical neurons increased the density and size of PSD-95 puncta.
Representative images of PSD-95 staining in cortical neurons (D). Primary cortical neurons were transfected with sh-DSCAM or sh-control plus GFP at DIV9, and they were fixed for PSD-95 staining at DIV17. Dotted line box, dendrite of transfected neurons (enlarge in middle panels); Solid line box, dendrite of non-transfected neurons (enlarge in lower panels). Scale bar, 16 μm (upper panel) and 10 μm (lower two panels). Quantitative analysis of data in D for the density (E) and size (F) of PSD-95 puncta. N = 21 neurons for transfected and non-transfected respectively in sh-control, n = 49 neurons for transfected and non-transfected respectively in sh-DSCAM. ** p < 0.01, *** p < 0.001, two-way ANOVA. Data were shown as mean ± SEM. G Representative images of time-lapse imaging from cortical neurons transfected with sh-DSCAM or sh-control taken at 5 adjacent time points during the 60-min live-imaging period. Scale bar, 10 μm. N = 21 neurons for sh-control, n = 19 neurons for sh-DSCAM. H-J Quantitative analysis for percentages of stable (H), new (I) and eliminated spines (J). Ns, p > 0.05, ** p < 0.01, *** p < 0.001, student’s t-test. Data were shown as mean ± SEM. K-M Linear correlation of spine density and percentage of stable (K), new (L) or eliminated (M) spines in DSCAM knockdown neurons. R = 0.7595 for stable spines, R = -0.4751 for new spines and R = -0.6434 for eliminated spines.

Fig. 7 Interaction of DSCAM to NLGN1 through its ECD.

A-B DSCAM interacts with NLGNs. HEK293T cells were cotransfected with DSCAM-His and indicated constructs for co-IP assays with anti-His (A) or anti-GFP.
antibody (B). The inputs and resulting co-IP complexes were blotted with anti-GFP and anti-His antibodies. C DSCAM-ECD interacts with NLGNs. HEK293T cells were cotransfected with FLAG-tagged ECD of DSCAM (FLAG-ECD) or empty control (Mock) and indicated constructs for co-IP assay with anti-GFP antibody. D FLAG-ECD was secreted to the medium. HEK293T cells were transfected with FLAG-ECD or empty control. After 24 h, the medium was changed to conditional medium (CM) containing 0.5% fetal bovine serum for another 24 h. The CM was collected to IP with anti-FLAG antibody to enrich secretable FLAG-ECD. The resulting IP complexes and cell lysates were probed with anti-FLAG antibody. E-F FLAG-ECD binds to the cell surface of cells expressing NLGNs. HEK293T cells were cotransfected with NLGN1-4 or empty vector plus GFP for 24 h and fixed. Before the immunostaining with anti-FLAG antibody, the fixed cells were incubated with CM containing FLAG-ECD for 4 h. Representative immunostaining images of cells treated with FLAG-ECD (E). Scale bar, 8 μm. Quantitative analysis of relative red fluorescence intensity (intensity of red fluorescence / green fluorescence) in E (F). Data were from three independent experiments and shown as mean ± SEM. *** p < 0.001, one-way ANOVA. G Endogenous DSCAM interacts with NLGN1 in vivo. WT brain lysates were IPed with anti-DSCAM antibody and resulting complexes were blotted with anti-DSCAM and anti-NLGN1 antibodies. H-I NLGN1 protein level was not altered in the GFAP-DSCAM f/f mice. Whole-brain homogenates from GFAP-DSCAM f/f and control mice were subjected to WB with indicated antibodies (H). Quantitative analysis of data in H (I). N = 3 mice for each genotype. Data were
shown as mean ± SEM. *** p < 0.001, two-way ANOVA. J-K NLGN1 protein level was increased in SPM fraction of GFAP-DSCAM f/f mice. SPM fractions from each genotype were probed with anti-DSCAM and anti-NLGN1 antibodies (J). Quantitative analysis of data in J (K). N = 9 mice for each genotype. Data were shown as mean ± SEM. ** p < 0.01, *** p < 0.001, two-way ANOVA.

Fig 8. Inhibition of NLGN1-NRXN1β interaction by DSCAM.

A-B DSCAM inhibits the NLGN1-NRXN1β interaction. HEK293T cells were cotransfected with different amounts of DSCAM-His and indicated constructs. Cell lysates were IPed with anti-FLAG antibody and resulting complexes were blotted with anti-GFP, anti-FLAG and anti-His antibodies (A). The relative intensity of co-IPed NRXN1β (intensity of co-IPed NRXN1β / IPed NLGN1) was quantified in B. Data were from three independent experiments and shown as mean ± SEM. * p < 0.05, ** p < 0.01, one-way ANOVA. C-G DSCAM inhibits transcellular NLGN1-NRXN1β interaction in cell surface binding assay (C-D) and cell aggregation assay (E-G). COS-7 cells were cotransfected with NLGN1 and increasing amounts of DSCAM-His for 24 h and incubated with purified NRXN1β-ECD-myc for 1 h before fixation. Fixed cells were immuno-stained with anti-NLG1, anti-myc and anti-His antibodies. Representative immunostaining images of cells (C). Scale bar, 10 μm. Quantitative analysis of relative red fluorescence intensity (intensity of red fluorescence / green fluorescence) in C (D). Data were from three independent experiments and shown as mean ± SEM. ** p < 0.01, *** p < 0.001, one-way ANOVA. Schematic illustration of cell
aggregation assay (E). HEK293T cells expressing NRXN1β or empty vector with RFP (red cells) were mixed with cells expressing NLGN1 and GFP plus different amounts of DSCAM (green cells). After 1 h, cell mixtures were imaged (F) and cell aggregates were counted (G). Scale bar, 200 μm. N = 15 images for each condition from three independent experiments. Data were shown as mean ± SEM. ** p < 0.01, *** p < 0.001, one-way ANOVA.

H-I DSCAM-ECD disrupts transcellular NLGN1-NRXN1β interaction. Different amounts of CM with FLAG-ECD were added into HEK293T cell mixtures of NRXN1β (red cells) and NLGN1 (green cells). Cell aggregates were imaged (H) and counted (I). Scale bar, 200 μm. Data were from three independent experiments and shown as mean ± SEM. ** p < 0.01, and *** p < 0.001, one-way ANOVA.

J-K DSCAM-ECD rescued spine over-maturation in DSCAM knockdown neurons. Primary cortical neurons were transfected at DIV9 with sh-DSCAM or sh-control. At DIV15, transfected neurons were incubated with CM containing FLAG-ECD or mock for 2 days and fixed. Representative images of dendritic spines (J). Scale bar, 10 μm. Quantitative analysis of total spine density in J (K). N = 20 neurons for sh-control + mock; n = 48 neurons for sh-DSCAM + mock; n = 21 neurons for sh-DSCAM + FLAG-ECD. Data were shown as mean ± SEM. ** p < 0.01, and *** p < 0.001, one-way ANOVA.

Fig 9. Autism-like behaviors and enhanced spatial memory in GFAP-DSCAM f/f mice.

A-B GFAP-DSCAM f/f mice displayed increased circling and grooming in OFT. During
10-min OFT, mice circling numbers (A) and grooming time (B) were counted. For circling, \( n = 14 \) for control mice, \( n = 15 \) for \( GFAP\)-DSCAM f/f mice; for grooming, \( n = 16 \) for control mice, \( n = 19 \) for \( GFAP\)-DSCAM f/f mice. Data were shown as mean ± SEM. ** \( p < 0.01 \), Student’s t-test. C-I \( GFAP\)-DSCAM f/f mice displayed reduced social novelty. Representative heat map for the mice movements in three-chamber social interaction test (C). S1, stranger1 mouse; S2, stranger2 mouse; E, empty cage; C, center chamber. Quantitative analysis of time staying in each chamber (D and G), interaction time (E and H), and social interaction index (F and I). Social interaction index was calculated as: in F, \([\text{interaction time (S1)} - \text{interaction time (E)}] / [\text{interaction time (S1)} + \text{interaction time (E)}]\) for each genotype; in I, \([\text{interaction time (S2)} - \text{interaction time (S1)}] / [\text{interaction time (S2)} + \text{interaction time (S1)}]\) for each genotype. N = 17 mice for control, \( n = 19 \) for \( GFAP\)-DSCAM f/f mice. Data were shown as mean ± SEM. Ns, \( p > 0.05 \); *** \( p < 0.001 \). Student’s t-test for F and I, two-way ANOVA for D, E, G and H. J-K \( GFAP\)-DSCAM f/f mice displayed elevated spatial memory in Barnes maze. Quantitative analysis of escape latency (J) and error numbers (K) in a 4-day training session. Quantitative analysis of moved distances (L), latency to target (M), time in target (N) and correct pokes (O) in the test session. N = 14 for control mice, \( n = 20 \) for \( GFAP\)-DSCAM f/f mice. Data were shown as mean ± SEM. Ns, \( p > 0.05 \); *\( p < 0.05 \); ** \( p < 0.01 \). Student’s t-test for L-O, two-way ANOVA for J-K. P-S \( GFAP\)-DSCAM f/f mice displayed anxiety-like behavior in OFT. Male 2-month mice were placed in the chambers and movement was monitored for 10 min. Representative traces of mice in OFT (P). The square with a dotted line indicates the
Fig. 10 Spine overmaturation in NEX-DSCAM f/f mice.

A DSCAM is expressed in neurons. Whole brain lysates, as well as cell lysates of cultured neurons and astrocytes from mice were probed with anti-DSCAM antibody.

B-C DSCAM expression is reduced in NEX-DSCAM f/f mice. Lysates of isolated sensory cortex from each genotype were probed with anti-DSCAM antibody (B).

Quantitative analysis of relative DSCAM intensity in B (C). N = 3 mice for each genotype. Data were shown as mean ± SEM. ** p < 0.01, student’s test.

D-H NEX-DSCAM f/f mice displayed increased spine maturation in the sensory cortex L2/3.

Representative Golgi staining images of dendritic spines in sensory cortex (D). Scale bar, 10 μm. Quantitative analysis of total I, M (F) and IM (G) spine density, as well as spine head width (H). N = 3 mice for each genotype. Data were shown as mean ± SEM. * p < 0.05, ** p < 0.01, and *** p < 0.001, Student’s t-test.
mice displayed normal in OFT. Representative traces of mice in OFT (I). Quantitative analysis of traveled distances (J), duration in the center (K) and velocity (L). N = 11 for control mice, n = 11 for NEX-DSCAM f/f mice. Data were shown as mean ± SEM. Ns, p > 0.05; ** p < 0.01. Student’s t-test for K and L, Mann-Whitney U test for J. M-P

NEX-DSCAM f/f mice displayed normal in EPM. Representative traces of mice in EPM (M). Quantitative analysis of entries into the open arms (N) and the closed arms (O), as well as duration in the open arms (P). N = 13 for control mice, n = 10 for NEX-DSCAM f/f mice. Data were shown as mean ± SEM. Ns, p > 0.05; ** p < 0.01. Student’s t-test.

Fig 11. Autism-like behaviors and enhanced spatial memory in NEX-DSCAM f/f mice.

A-B NEX-DSCAM f/f mice displayed increased circling and grooming in OFT. During 10-min OFT, mice circling numbers (A) and grooming time (B) were counted. N = 11 for control mice, n = 11 for NEX-DSCAM f/f mice. Data were shown as mean ± SEM. * p < 0.05, Student’s t-test.

C-I NEX-DSCAM f/f mice displayed reduced social novelty. Representative heat map for the mice movements in three-chamber social interaction test (C). Quantitative analysis of time staying in each chamber (D and G), interaction time (E and H), and social interaction index (F and I). N = 9 mice for control, n = 10 for NEX-DSCAM f/f mice. Data were shown as mean ± SEM. Ns, p > 0.05; * p < 0.05; ** p < 0.01. Student’s t-test for E, F, H, and I; One-way ANOVA for NEX-DSCAM f/f group in D and control group in G; Kruskal-Wallis test for control group in D and
NEX-DSCAM f/f group in G. J-O NEX-DSCAM f/f mice displayed elevated spatial memory in Barnes maze. Quantitative analysis of escape latency (J) and error numbers (K) in 4-day training session. Quantitative analysis of moved distances (L), latency to target (M), time in target (N) and correct pokes (O) in the test session. N = 13 for control mice, n = 11 for NEX-DSCAM f/f mice. Data were shown as mean ± SEM. Ns, p > 0.05; * p < 0.05; ** p < 0.01. Two-way ANOVA for J-K; student's t-test for L, M and O; Mann-Whitney U test for N. P Schematic illustration for DSCAM deficiency on spine maturation.
Figure 1

A. Diagram showing the process from brain to mRNA, cDNA, and qRT-PCR.

B. Heatmap with gene expression levels over days in vitro (DIV).

C. Western blot showing protein levels over DIV.

D. Graph showing relative protein levels of DSCAM, Synapsin I, PSD-95, and Actin.

E. Immunoblot for DSCAM and Actin.

F. Graph showing relative DSCAM levels.

G. Western blot for DSCAM, PSD-95, and Synaptophysin.

H. Confocal images of GFP, DSCAM-HA, and Merge.

I. Graph showing puncta area (um²).

J. Graph showing fluorescence intensity (AU).

K. Confocal images of PSD-95 and Merge.
Figure 3

A B C D

E F G H

I J K L

M N O P

Q R
Figure. 4

A control

GFAP-DSCAM fit

B

C

D

E

F

Graphs showing statistical analysis of synaptic numbers, spine numbers, PSD area, and PSD length with significant differences indicated by asterisks (**, ***) and non-significant differences marked as ns (not significant).
Figure 5
Figure 6

A

DSCAM-His

DSCAM

Actin

Relative DSCAM level

ns

1.0

0.5

0.0

B

sh-control

sh-DSCAM

sh-DSCAM + hDSCAM

C

Total spine density (10 μm)

*** ns

0

2

4

6

D

GFP

PSD-95

Merge

E

sh-control

sh-DSCAM

F

GFP

PSD-95

Merge

G

Time (Min)

0

15

30

45

90

H

I

J

K

L

M

Stable spines (%)

New spines (%)

Eliminated spines (%)

Stable spines (%)

New spines (%)

Eliminated spines (%)

R = 0.7995

R = -0.4751

R = -0.6434
Figure 10

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P
