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

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

4 **Abbreviated title:** Spine and behavioral deficits by loss of DSCAM

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25

26 **Conflict of interest:**

27 The authors declare no conflict of interest.

28

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46 **Abstract**

47 Mutations in some cell adhesion molecules (CAMs) cause abnormal synapse
48 formation and maturation, and serve as one of the potential mechanisms of autism
49 spectrum disorders (ASDs). Recently, *DSCAM* (Down syndrome cell adhesion
50 molecule) was found to be a high-risk gene for autism. However, it is still unclear how
51 *DSCAM* contributes to ASD. Here, we show that *DSCAM* expression was
52 downregulated following synapse maturation, and that *DSCAM* deficiency caused
53 accelerated dendritic spine maturation during early postnatal development.
54 Mechanistically, the extracellular domain (ECD) of *DSCAM* interacts with neuroligin1
55 (NLGN1) to block the NLGN1-neurexin1 β (NRXN1 β) interaction. *DSCAM*-ECD was
56 able to rescue spine overmaturation in *DSCAM* knockdown neurons. Precocious
57 spines in *DSCAM*-deficient mice showed increased glutamatergic transmission in the
58 developing cortex and induced autism-like behaviors such as social novelty deficits
59 and repetitive behaviors. Thus, *DSCAM* might be a repressor that prevents premature
60 spine maturation and excessive glutamatergic transmission, and its deficiency could
61 lead to autism-like behaviors. Our study provides new insight into the potential
62 pathophysiological mechanisms of ASDs.

63

64 *Keywords:* Autism, dendritic spine, *DSCAM*, NLGN1

65

66 **Significance statement:**

67 *DSCAM* is not only associated with Down syndrome but is also a strong autism risk

68 gene based on large-scale sequencing analysis. However, it remains unknown
69 exactly how DSCAM contributes to autism. In mice, either neuron- and
70 astrocyte-specific or pyramidal neuron-specific DSCAM deficiencies resulted in
71 autism-like behaviors and enhanced spatial memory. In addition, DSCAM knockout or
72 knockdown in pyramidal neurons led to increased dendritic spine maturation.
73 Mechanistically, the extracellular domain of DSCAM binds to NLGN1 and inhibits
74 NLGN1-NRXN1 β interaction, which can rescue abnormal spine maturation induced
75 by DSCAM deficiency. Our research demonstrates that DSCAM negatively modulates
76 spine maturation, and that DSCAM deficiency leads to excessive spine maturation
77 and autism-like behaviors, thus providing new insight into a potential
78 pathophysiological mechanism of autism.

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91 **Introduction**

92 Proper synapse connections are crucial for the precise assembly of neural circuitry
93 and for normal brain function. Dysregulation of synapse formation and maturation is
94 associated with a variety of neurodevelopmental disorders, including autism spectrum
95 disorders (ASDs) (Forrest et al., 2018). Cell adhesion molecules (CAMs), which are
96 cell surface proteins that mediate cell-cell interactions, play an essential role in neural
97 development, especially in synaptic development and function (Dalva et al., 2007).
98 ASDs manifest with deficits in social interaction and communication, as well as in
99 increased repetitive or restrictive behaviors (Huguet et al., 2013; Huguet et al., 2016).
100 However, the etiology underlying ASDs at the molecular, cellular and system levels
101 remains elusive. ASDs are highly heritable diseases (Bailey et al., 1995; Autism
102 Genome Project et al., 2007; Rosenberg et al., 2009). Many genes have been
103 identified as risk factors for ASDs (Bourgeron, 2015). A prominent group among these
104 genes is the set of genes that encode CAMs, such as neuroligins (NLGNs) and
105 neurexin1 (NRXN1). At the cellular and molecular levels, dysfunction of neuronal
106 CAMs is regarded as one of the potential mechanisms of ASDs (Chen et al., 2015).
107 Mice with mutations of these CAMs display autism-like behaviors and deficits in
108 synapse formation and maturation (Sudhof, 2008). However, it remains unknown how
109 these ASDs-related CAMs impact synapse development. Recently, *DSCAM* (Down
110 syndrome cell adhesion molecule) was found to be one of the genetic risk factors for
111 autism in a large-scale genome-wide association study (GWAS) (Satterstrom et al.,

2020). 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

134 previously documented in DSCAM^{2J}-deficient intracortical circuits of the motor cortex,
 135 as well as in spinal interneuronal circuits (Thiry et al., 2016; Laflamme et al., 2019).
 136 Despite DSCAM's abundance in the brain and its modulation of diverse physiological
 137 functions, little is known about how it regulates synapse development in mammals.
 138 Therefore, we hypothesized that DSCAM mediates synapse development and that its
 139 deficiency can cause autism.
 140 Here, we examined the expression pattern of DSCAM during postnatal synapse
 141 formation and maturation, observed the effects of DSCAM deficiency on dendritic
 142 spine development, and investigated its potential as an underlying mechanism of ASD.
 143 Furthermore, we generated DSCAM-deficient mice to validate whether the deletion of
 144 DSCAM in neurons would result in abnormal spine maturation and altered synaptic
 145 transmission, as well as autism-like behaviors. These findings describe a novel
 146 pathophysiological role of DSCAM in ASDs.

147

148 **Materials and Methods**

149 **Animals**

150 Floxed DSCAM mouse (C57BL/6) was a kind gift from Dr. Jane Y Wu (Institute of
 151 Biophysics, Chinese Academy of Science). *GFAP::Cre* (Jackson Laboratory)
 152 transgenic (tg) mouse was purchased from Jackson Laboratory, and *NEX-Cre* mouse
 153 was a kind gift from Dr. Yu-Qiang Ding (Institutes of Brain Science, Fudan University).
 154 Primers for genotyping are listed below. Behavior tests were performed with
 155 2~3-month male mice. All mice were housed in a constant temperature and humidity

156 chamber at 23 °C, and sufficient food and water were administered daily. No more
 157 than 5 adult mice per cage were subjected to a 12-h light/dark cycle under standard
 158 conditions. All the mice were guaranteed to be hygienic. The animal experiments
 159 were carried out following the "Guidelines for the Care and Use of Laboratory
 160 Animals" promulgated by Nanchang University. Primers for mice genotyping are as
 161 below.

Primers	Sequence (5'→3')	Size
PP 1	ACCTCCCACAAACAAACCAG	Wt = 396bp, Floxed = 504bp
	TGCAGAAGAGTCAAGGCACA	
PP 2	AGACAGGAGGGTACAGAGGA	Wt = 457bp, Floxed = 565bp
	GTTGTGACTTTAGGGTTATG	
PP 3	GCTTGCTCATGTGAGCTGGA	Floxed = 420bp
	GGCTGGACGTAACTCCTC	
PP 4	TCTGAGGCGGAAAGAACCAG	Floxed = 306bp
	GCAGCTGAGAGCAAGTCTCACTG	
PP 5	GCTTGCTCATGTGAGCTGGA	Wt = 446bp
	GCAGCTGAGAGCAAGTCTCACTG	
PP 6 (GFAP-Cre)	ACTCCTTCATAA AGCCCT	Tg = 190bp
	ATCACTCGTTGCATCGACCG	
PP 7 (NEX-Cre)	GAGTCCTGGAATCAGTCTTTTTC	Cre = 550bp
	ATC ACTCGTTGCATCGACCG	Wt = 770bp

	CCGCATAACCAGTGA AACAG	
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163 **Quantitative real-time PCR (qRT-PCR) analysis**

164 Cortical total RNA was purified from WT mice with Trizol reagent (Invitrogen, NY,

165 USA). 500 ng of total RNA were reverse transcribed to cDNA with oligo (dT) primers.

166 cDNAs were used as the template in qPCR in a 20 μ L reaction system containing

167 SYBR GreenER qPCR mix (Invitrogen, NY, USA) with gene-specific primers as below.

168 GAPDH was used as a reference in each sample.

Primers	Sequence (5'→3')
NLGN1-F	TGATGGGAGTGTCTTGGCAAGC
NLGN1-R	CCGTAGTTTCCTTTGGCAGCCT
NLGN2-F	CGATGTCATGCTCAGCGCAGTA
NLGN2-R	CCACACTACCTCTTCAAAGCGG
NLGN3-F	ATCGGTGCATCCTGTGTCAGTC
NLGN3-R	CACTGGTTGGTAGTTCACAGCC
NLGN4-F	GTGGTGATGACCTACTGGACGA
NLGN4-R	GCAGATAGAGCTGGTCTTTGGG
SALM2-F	GGCATCCGTATGTACCAAGTGC
SALM2-R	GCCAGGTCATTCACTAGGAAGG
SALM4-F	AGGCATCCGCATGTACCAGATC
SALM4-R	TCAGCAGGAAGGAGCGACTGTC

MDGA1-F	ACCAGTGCCTGACCTCAGCATA
MDGA1-R	TTGCCACGAACTTCGCACTGGA
MDGA2-F	CAAGAGAAGCCTTGGTGCAGCT
MDGA2-R	CAGCACTCGTATTGGATAGGCTC
NRXN1-F	ACCGTGCCTTAGCAATCCTTGC
NRXN1-R	GTCGTAGCTCAAAACCGTTGCC
CNTNAP2-F	GTGATGAGACAGGATACAGCGG
CNTNAP2-R	AGTGGTCCACTGCCATCAGGAT
CNTN4-F	CTCCAGCAGAATCCGCACTAAG
CNTN4-R	CTCCATTCTGGTACTCTCGTGAC
CNTN6-F	CTCTGTTGCTGCGGACCTGATT
CNTN6-R	GTTGTCAGGTCCTGGTCTCCAA
DSCAM-F	CATCCGCATGTACGCCAAGAAC
DSCAM-R	GAGATGAGGTGGGTCCAAGTG
β -catenin-F	GTTGCGCTTCATTATGGACTGCC
β -catenin-R	ATAGCACCCCTGTTCCCGCAAAG
N-cadherin-F	CCTCCAGAGTTTACTGCCATGAC
N-cadherin-R	CCACCACTGATTCTGTATGCCG
NrCAM-F	GCAGAGTGAAACATGACCACACC
NrCAM-R	GTGTAGGTTCCGCCATCGTCAT
NCAM-F	GGTCCGAGATGGTCAGTTGCT

NCAM-R	CAAGGACTCCTGTCCAATACGG
LRRTM2-F	GCCATCGACTTGACAGTGTTCCG
LRRTM2-R	AACGGTCGTGAGGGATTTCAGG
LRRTM1-F	CGGCTTGTTCAAGCTCACAGAG
LRRTM1-R	CCACAATGGCAACCTTATTCCGC
SYNCAM-F	GCTTCTGCTGTTGCTCTTCTCC
SYNCAM-R	GACTTGGCAACTGATGGTCGCA
GAPDH-F	CATCACTGCCACCCAGAAGACTG
GAPDH-R	ATGCCAGTGAGCTTCCCGTTCAG
PSD-95-F	TCAGACGGTCACGATCATCGCT
PSD-95-R	GTTGCTTCGCAGAGATGCAGTC
SYN-F	TATGCCACTGCTGAGCCCTTCA
SYN-R	ATGGCAATCTGCTCAAGCATAGC

169

170 **Subcellular fractionation**

171 Mouse brain subcellular fractionation was performed as described previously with
172 modifications (Wang et al., 2018). The cerebral cortices from adult mice were
173 homogenized in 10 volumes (vol) of HEPES-buffered sucrose (0.32 M sucrose, 4 mM
174 HEPES/NaOH, pH 7.4) with a glass-Teflon homogenizer. The homogenate was
175 centrifuged at 1,000 g for 10 min to remove the nuclear fraction and unbroken cells.
176 The supernatant (S1) was then centrifuged at 10,000 g for 15 min to yield the crude

177 synaptosomal fraction (P1) and the supernatant (S2). The P1 pellet was
178 resuspended in 10 vol of HEPES-buffered sucrose and then centrifuged at 10,000 g
179 for another 15 min. The resulting pellet (P2) was lysed by hypo-osmotic shock in
180 water, rapidly adjusted to 4 mM HEPES, and mixed continuously for 30 min (on ice).
181 The lysate was then centrifuged at 25,000 g for 20 min to yield the supernatant (S3,
182 crude synaptic vesicle fraction) and the pellet (P3, lysed synaptosomal membrane
183 fraction). The P3 pellet was resuspended in HEPES-buffered sucrose, carefully
184 layered on top of a discontinuous gradient containing 0.8-1.0-1.2 M sucrose (top to
185 bottom), and centrifuged at 150,000 g for 2 h. Optional step. The S3 was centrifuged
186 at 165,000 g for 2 h to get the synaptic vesicle protein (SV). The sucrose gradient
187 yielded a floating myelin fraction (G1), a light membrane fraction at the 0.8 M/1.0 M
188 sucrose interface (G2), a synaptosomal plasma membrane (SPM) fraction at the 1.0
189 M/1.2 M sucrose interface (G3), and a mitochondrial fraction as the pellet (G4). The
190 G3 layer was collected, and an equal volume of HEPES-buffered sucrose was added
191 to centrifuge at 20,000 g for 15 min in order to obtain SPM. The SPM was
192 resuspended with 1% Triton X-100 in 50 mM HEPES/NaOH (pH 8) on ice for 15 min
193 and then centrifuged at 36,000 g for 15 min to yield the supernatant (presynaptic
194 membrane protein, Pre) and the pellet (postsynaptic density, PSD), which was
195 solubilized in 2%SDS PBS buffer at RT.

196

197 **Plasmids and shRNA construction**

198 The constructs expressing full-length mouse DSCAM with a C-terminal His-tag and

199 human DSCAM were purchased from Addgene. To generate the construct
200 expressing secretable ECD of DSCAM (FLAG-ECD), mouse DSCAM cDNA
201 encoding 24-1594 amino acids without signal peptide was amplified by PCR and
202 subcloned into PLP2 (new prolactin leader peptide)-FLAG-pCMV6-XL4 downstream
203 of an artificial signal peptide sequence and a FLAG epitope. The constructs of mouse
204 NRXNs (1 α -3 α and 1 β) and NLGNs (1-4) were purchased from Origene. Myc-tagged
205 secretable NRXN1 β -ECD was subcloned from full length NRXN1 β . For gene
206 knockdown by RNA interference (RNAi), pSUPER vector (OligoEngine) – based
207 small hairpin RNAs (shRNAs) of mouse *DSCAM* (sh-DSCAM) and *DSCAM* scramble
208 (sh-control) were constructed. The target sequence of sh-DSCAM was obtained from
209 a previous report: 5'-GTGGGAGAGGAAGTGATAT-3' (Ly et al., 2008). The sh-control
210 sequence was 5'-AGAGTGGACGTCGATGATTAT 3'. The authenticity of all
211 constructs was verified by DNA sequencing and western blotting analysis.

212

213 **Cell culture and transfection**

214 Human embryonic kidney (HEK) 293 cells and COS-7 cells (African green monkey
215 kidney fibroblast cells) were cultured in Dulbecco's modified Eagle's medium (DMEM)
216 (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco). Transient
217 transfection was performed using polyethylenimine (PEI) (Sigma, 408727), as
218 described before (Chen et al., 2021). Briefly, cells were cultured in 100 mm dishes and
219 at ~70% confluence, they were incubated with precipitates formed by 5 μ g of plasmid
220 DNA and 280 μ L of 0.05% PEI (wt/vol). Cells were harvested 24~48 h

221 post-transfection.

222 Cultures of primary hippocampal neurons were prepared from embryonic day (E) 18

223 Sprague–Dawley rats as described previously (Chen et al., 2021). Briefly, hippocampi

224 were isolated and kept separate from one another in HBSS on ice. Following

225 digestion in 0.25% trypsin plus 0.1 mg/mL DNase I (one hippocampus in 1 mL) at

226 37 °C for 20 min, dissociated cells were resuspended in plating media (DMEM

227 supplemented with 10% FBS) and plated at a density of 1×10^5 or 2×10^5 per well

228 onto poly-D-lysine–coated 20-mm coverslips (WHB) in 12-well plates (Corning). Cells

229 were incubated for 4 h before replacing with maintenance medium [neurobasal

230 medium (Gibco) supplemented with 2% B-27 supplement (Gibco), 1% GlutaMax

231 (Gibco), and 1% penicillin/streptomycin (Gibco)]. Neurons were maintained at 37 °C in

232 5% CO₂, with half of the medium changed every 2–3 days. For transfection in neurons,

233 calcium phosphate precipitation was performed as described previously. Briefly, the

234 neurons were serum-starved with pre-heated DMEM for 2 h at 37 °C in 10% CO₂. For

235 each well of 12-well plate, 1–6 µg DNA in 1–6 µL was mixed with 5 µL 2.5 M CaCl₂ in

236 ddH₂O (total volume 50 µL), and further mixed with 50 µL of HEPES-buffered saline

237 containing (in millimoles): 274 NaCl, 10 KCl, 1.4 Na₂HPO₄, 15 glucose, and 42

238 HEPES, pH 7.05. Resulting DNA-calcium phosphate precipitates were added into

239 neurons. Morphology was studied 3–7 d later.

240

241 **Cell surface binding assay**

242 For obtaining purified ECD, constructs expressing secretable ECD were transfected

243 into HEK293 cells. After 24 h, the medium was changed to conditional medium (CM)
244 containing 0.5% fetal bovine serum for another 24 h. Then the CM were collected for
245 immunoprecipitation with the tag antibody and Protein A/G beads. Beads were
246 washed with tris-buffered saline (TBS) (50mM Tris HCl, 150mM NaCl, pH 8.0) buffer
247 three times, then five packed beads volumes of 0.1M glycine HCl buffer (pH 3.0) were
248 added. Samples were incubated with gentle shaking for 5 min at room temperature.
249 They were then centrifuged at 2500 rpm for 5 min to harvest the supernatant.
250 Afterwards, 10 μ l of 0.5M Tris HCl, pH 8.0, with 1.5M NaCl, was added.
251 For surface binding assay, HEK293 or COS-7 cells were transfected with indicated
252 constructs. After 24~48 h, purified protein or CM were added to the transfected cells
253 for 1 h-incubation at 4 °C, and then the cells were subjected to immunostaining with
254 indicated antibodies.

255

256 **Cell aggregation assay**

257 As described previously (Boucard et al., 2012), HEK293 cells were transfected with
258 indicated constructs, respectively. After 48 h, the cells were trypsinized and the green
259 cells were then mixed with red cells to incubate with gentle agitation at room
260 temperature with DMEM supplemented with 10% FBS, 50 mM HEPES-NaOH, pH 7.4,
261 10 mM CaCl_2 , and 10 mM MgCl_2 . After 0.5-1 h, the cell mixtures were transferred
262 gently into a 12-well plate and imaged by fluorescence microscopy. The resulting
263 images were analyzed by counting the number of aggregation particles in the field by
264 ImageJ. Cell aggregation particles were defined as four or more clustered cells with at

265 least one red and one green cell.

266

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

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

280

281 **Immunoprecipitation**

282 Immunoprecipitation was performed as described previously (Chen et al., 2021).
283 Transfected HEK293 cells were lysed in IP buffer containing (in millimoles): 20 Tris,
284 pH7.6, 50 NaCl, 1 EDTA, 1 NaF, 0.5% Nonidet P-40 (vol/vol), with protease and
285 phosphatase inhibitors. Samples were centrifuged at 12,000 × g for 20 min at 4 °C to
286 remove debris. Lysates (1~2 mg) or CM were incubated with corresponding antibody

287 (1~2 μ g) at 4 °C for either 3-4 h or overnight and then incubated with 10~15 μ L Protein
 288 A/G magnetic agarose beads (Pierce) at 4 °C for 1 h. Samples were washed with IP
 289 buffer and resuspended in SDS sample buffer. Then the samples were subjected to
 290 WB.

291

292 **Western blotting**

293 For protein expression detection, tissues were homogenized in PBS plus protease
 294 and phosphatase inhibitors. Then the homogenates were lysed in equal volume of 2
 295 $\times$ RIPA buffer [0.2% SDS (wt/vol), 1% sodium deoxycholate (wt/vol) and 2% Nonidet
 296 P-40 (vol/vol) in PBS] plus protease and phosphatase inhibitors. Lysates were
 297 centrifuged at 12,000 $\times$ g for 20 min at 4 °C to remove debris. The supernatants were
 298 subjected to Bradford assay (Pierce) to measure protein concentration and diluted in
 299 SDS sample buffer. Protein samples (10~20 μ g) were resolved by SDS-PAGE and
 300 transferred to PVDF membrane (Millipore). The membrane was immunoblotted with
 301 primary and secondary antibodies, and immunoreactive bands were visualized by
 302 enhanced chemiluminescence under the gel documentation system (Bio-Rad).
 303 Densitometric quantification of protein band intensity was performed using ImageJ.
 304 Antibodies were diluted with primary antibody dilution buffer (TBS + 1% TritonX-100 +
 305 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

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

306

307 **Immunostaining**

308 Immunostaining of cultured neurons was performed as described previously with
309 modifications (Chen et al., 2021). Primary neurons were fixed with 4%
310 paraformaldehyde (PFA) / 4% sucrose (wt/vol) for 15 min. After washing three times
311 with PBS, neurons were incubated with primary antibody diluted in GDB buffer (30
312 mM phosphate buffer, pH 7.4, containing 0.2% gelatin, 0.6% Triton X-100, and 0.9 M
313 NaCl) at 4 °C overnight. After washing three times with washing buffer (20 mM

314 phosphate buffer and 0.5 M NaCl), neurons were incubated with the corresponding
315 Alexa Fluor-conjugated secondary antibodies (diluted in GDB buffer) at room
316 temperature for 1 h. The images were obtained by FSX100 (Olympus).
317 Immunostaining of brain slices was performed as described previously (Wang et al.,
318 2018). Mice were deeply anesthetized with isoflurane and perfused with PBS followed
319 by 4% paraformaldehyde (PFA). Brains were postfixed in 4% PFA at 4 °C overnight
320 and dehydrated using 30% sucrose at 4 °C for 2 d. Brain samples were rapidly frozen
321 in OCT, cut into 40-µm sections and mounted on Super Frost Plus slides (Fisher).
322 Sections were blocked and permeabilized in PBS containing 0.5% Triton X-100 and 5%
323 goat serum for 2 h at room temperature. Sections were incubated at 4 °C overnight
324 with primary antibodies in PBS containing 5% goat serum and 2% BSA. Sections
325 were washed with PBS and then incubated at room temperature for 1-2 h with
326 secondary antibodies. The images were obtained by FSX100 (Olympus).

327

328 **Golgi Staining.**

329 Golgi staining was performed using the FD Rapid GolgiStain Kit following the
330 manufacturer's protocol (FD Rapid GolgiStain™ Kit, cat: PK401). Brain tissues were
331 incubated in mixed solutions A and B for 2 weeks in the dark at room temperature and
332 solution C for 3 d. Tissues were cut into slices with 80-100µm thickness, stained with
333 solutions D and E, dehydrated in gradient ethanol, cleared with xylene, and mounted
334 on slides for imaging. Images of neurons in sensory cortex L2/3 were taken and
335 imported into ImageJ with NeuronJ plugin for analysis. Dendrites were reconstructed

336 and analyzed using the ImageJ with Sholl Analysis plugin, with 10- μ m incremental
337 increases in concentric circular diameter from the soma. The images were obtained
338 by FSX100 (Olympus).

339

340 **Electrophysiological Analysis.**

341 Brain slices recording was performed as described previously (Wang et al., 2018).

342 Male mice at P12, P21 or P42 were anesthetized with isoflurane (RWD Life Science).

343 Brains were quickly removed to ice-cold oxygenated (95% O₂ / 5% CO₂) cutting

344 solution containing: 120 mM Choline Chloride, 2.5 mM KCl, 7 mM MgCl₂, 0.5 mM

345 CaCl₂, 1.25 mM NaH₂PO₄, 26 mM NaHCO₃, and 25 mM glucose. Lamellar 300 μ m

346 slices of the cortex were cut using VT-1000S Vibratome (Leica Microsystems). The

347 slices were recovered in oxygenated artificial cerebrospinal fluid (ACSF) for 30 min at

348 32 °C and maintained at room temperature (25 $\pm$ 1 °C) for an additional 1 h before

349 recording. The ACSF containing: 124 mM NaCl, 2.5 mM KCl, 2 mM MgSO₄, 2.5 mM

350 CaCl₂, 1.25 mM NaH₂PO₄, 26 mM NaHCO₃, and 10 mM glucose. All solutions were

351 saturated with 95% O₂ / 5% CO₂ (vol/vol).

352 Slices were placed in the recording chamber, which was superfused (2 mL/min) with

353 ACSF at 32~34 °C. Pyramidal neurons from sensory cortex L2/3 were visualized with

354 infrared optics using an upright microscope equipped with a 40 $\times$ water-immersion

355 lens (FN-S2N, Nikon) and an infrared CCD camera (IR-1000, DAGE-MTI). Pipettes

356 were pulled by a micropipette puller (P-1000; Sutter Instrument) with a resistance of

357 3~5 M Ω . The recording was performed with the MultiClamp 700B amplifier and 1550A

358 digitizer (Molecular Device). Series resistance was below 20 M Ω and monitored
359 throughout the experiments.

360 For mEPSCs recording, pyramidal neurons were held at -70 mV in the presence of 20
361 μ M bicuculline (Tocris Bioscience), with the pipette solution containing: 125 mM
362 K-gluconate, 5 mM KCl, 10 mM HEPES, 0.2 mM EGTA, 1 mM MgCl₂, 4 mM Mg-ATP,
363 0.3 mM Na-GTP, and 10 mM phosphocreatine (pH 7.35, 290~295 mOsm). mEPSCs
364 were recorded in the presence of 1 μ M TTX.

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

370 and filled with the solution contained (in millimoles): 125 Cs-methanesulfonate, 5 CsCl,
371 10 HEPES, 0.2 EGTA, 1 MgCl₂, 4 Mg-ATP, 0.3 Na-GTP, 10 phosphocreatine, and 5
372 QX314 (pH 7.4, 285 mOsm). All the chemicals were purchased from Sigma.

373

374 **Electron microscopy.**

375 Electron microscopy studies were performed as described previously with
376 modifications (Yang et al., 2017). Briefly, anesthetized mice were perfused
377 transcardially with PBS and followed by PBS containing 2% glutaraldehyde and 4%
378 paraformaldehyde. Brains were postfixed at 4°C overnight. Ultrathin sections (70 nm)
379 of the sensory cortex L2/3 region were cut and stained with 2% uranyl acetate (vol/vol)

380 and Reynolds lead citrate, and they were analyzed with a Hitachi H-7650 transmission
381 electron microscope. Symmetric and asymmetric synapses were visually confirmed
382 and manually counted by investigators unaware of genotypes. Also, synaptic
383 ultrastructural specializations like area, thickness and length of PSD, and presynaptic
384 vesicle numbers were analyzed by investigators blind to genotypes.

385

386 **Behavioral Analysis.**

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

390 For OFT, mice were placed in a chamber (50 × 50 cm). An overhead camera and
391 tracking software (Med Associates) were used to monitor the mouse movement for 10
392 min. Total distance and time traveled in the center (15 × 15 cm) were measured. The
393 chamber was cleaned with 75% ethanol and wiped with paper towels after each trial.
394 During a 10-min open-field test period, the amount of time spent grooming and the
395 circling behavior were measured manually.

396 EPM was performed as described previously (Chanques et al., 2018). The platform
397 was elevated 74 cm above the floor. It consisted of two closed arms (35 × 6 × 22 cm),
398 two open arms (35 × 6 cm), and a central zone (6 × 6 cm). Mice were placed on the
399 central zone and faced an open arm. Mice could freely explore the platform for 10 min.
400 The total time spent in the open arms and the entries to open arms were recorded by
401 the monitoring software (Med Associates Inc., Farifax, VT). The apparatus was

402 cleaned with 75% ethanol after each trial.

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

423 The Barnes circular maze was performed as described previously with modifications

424 (Rosenfeld and Ferguson, 2014). The Barnes circular maze is a planar, round white
425 Plexiglas platform (75 cm diameter), 1 m above the floor, with 18 evenly spaced holes
426 (7 cm diameter). A black escape box was placed under one hole. During the trial, a
427 500-lux light was turned on. After each trial, the platform and the escape box were
428 cleaned thoroughly with 75% ethanol. The test consists of a 4-day training trial and a
429 probe trial. One day before the training trial, test mice were habituated in the target
430 box for 3 min. The training trial was repeated for 4 consecutive days, 3 times each day
431 with 20 min intervals. At the beginning of the training trial, a test mouse was placed in
432 a cylindrical holding chamber (8 cm diameter) located in the maze center. After 15 s of
433 holding time, the mouse was allowed to search for the target hole for 3 min. If the
434 mouse failed to find the target hole in 3 min, it was gently guided into the target hole
435 by the investigator's hands. When the mouse entered the escape box, the light was
436 turned off and the mouse remained undisturbed for 1 min. The mouse's movement
437 was recorded, and the number of errors and the latency to find the target hole was
438 measured during the training trials by video tracking software. On day 5, the probe
439 trial was performed with each mouse. The escape box was removed, and the test
440 mouse was allowed to find the target hole freely for 90 s. During the probe trial, the
441 total distance traveled and the latency to find the target hole were measured. %
442 correct pokes and % time in the target area were also measured.

443 All behavioral results were analyzed by investigators blind to genotypes.

444

445 **Statistical Analysis.**

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

457

458 **Results**

459 **DSCAM expression is downregulated during synapse maturation**

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

468 afterward (Fig. 1B). When investigated *in vitro*, DSCAM protein levels in primary
469 cortical neurons similarly increased from DIV (days *in vitro*) 3 to DIV12; this
470 phenomenon was also followed by a decrease in DSCAM protein levels (Fig. 1C-1D).
471 Synapse formation commences in the first postnatal week and peaks at P14, and
472 synapse maturation continues thereafter (Farhy-Tselnicker and Allen, 2018). In
473 parallel, the expression of DSCAM in the brain gradually increased from embryonic
474 day 12.5 (E12.5) until it reached its peak at P12, and then decreased until P60 (Fig.
475 1E-1F). This expression pattern of DSCAM suggests that its level is downregulated
476 during synapse maturation compared to other CAMs. We also examined the
477 subcellular distribution of DSCAM in mouse brains. PSD fractionation assays
478 revealed that DSCAM was localized to the PSD area (Fig. 1G). To confirm the
479 distribution of DSCAM in neurons, we cotransfected His-tagged full-length DSCAM
480 plus GFP into cultured neurons at DIV9 and stained them with anti-His antibody at
481 DIV17. As shown in Fig. 1H-1J, DSCAM was mainly distributed and clustered in
482 GFP-labeled dendrites and dendritic spines. Dendritic spines are morphologically
483 divided into mature and immature spines. Mature spines are mushroom-like, with a
484 width of spine head to neck ratio ≥ 1.5 ; immature spines have a spine head to neck
485 ratio < 1.5 . Interestingly, the fluorescence intensity and size of DSCAM-His puncta
486 were markedly increased in dendritic shafts and in immature spines, compared to
487 those in mature spines. Furthermore, costaining with PSD-95 demonstrated the
488 colocalization of DSCAM-His with PSD-95 (Fig. 1K). Taken together, these results
489 provide an indication of how DSCAM functions in spine maturation.

490

491 **DSCAM deficiency results in increased spine maturation in the sensory cortex**

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

510 Neurons in the sensory cortex L2/3 receive multiple inputs and are highly plastic; they
511 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

534 were all greater in *GFAP-DSCAM* f/f mice (Fig. 4D-4F). Taken together, these results
535 indicate that DSCAM deficiency increases spine maturation in the sensory cortex.

536

537 **Glutamatergic transmission is elevated in the L2/3 sensory cortex of**
538 **DSCAM-deficient mice**

539 Dendritic spine density and morphology are critical for synaptic transmission strength
540 and synaptic stability (Alvarez and Sabatini, 2007; Yuste, 2013). An increase in the
541 number of matured spines corresponds to an increase in glutamatergic transmission.
542 We examined whether DSCAM deficiency alters neurotransmission in the cortex
543 during development by recording miniature excitatory postsynaptic currents (mEPSCs)
544 and miniature inhibitory postsynaptic currents (mIPSCs) in sensory cortex L2/3 of
545 *GFAP-DSCAM* f/f mice at P12, P21, and P42. Notably, *GFAP-DSCAM* f/f mice
546 displayed an increase in mEPSC frequency at all three time points, but mEPSC
547 amplitudes were not changed (Fig. 5A-5C). Neither mIPSC frequencies nor
548 amplitudes were altered in *GFAP-DSCAM* f/f mice (Fig. 5D-5F). Together, these
549 results suggest that DSCAM deficiency increases glutamatergic transmission in
550 sensory cortex L2/3.

551

552 **Knockdown of DSCAM in primary neurons disrupts spine dynamics and**
553 **maturation**

554 Since DSCAM deficiency results in increased spine maturation, we investigated
555 whether DSCAM modulates spine dynamics by knocking down DSCAM in neurons

556 via small hairpin RNA (sh-RNA). Based on a previous report (Ly et al., 2008), we
557 constructed sh-RNA for mouse or rat DSCAM (sh-DSCAM) and scrambled sh-RNA
558 for use as a control (sh-control). We then confirmed the knockdown efficiency of
559 sh-DSCAM in HEK293T cells transfected with mouse DSCAM-His (Fig. 6A).
560 Furthermore, we observed and analyzed dendritic spine density in
561 sh-DSCAM-transfected neurons. Compared to that in neurons transfected with
562 sh-control, the total spine density was increased in neurons transfected with
563 sh-DSCAM (Fig. 6B-6C). To exclude the off-target effects of sh-DSCAM, we
564 overexpressed human DSCAM (hDSCAM), which can not be recognized by
565 sh-DSCAM (Fig. 6A), in sh-DSCAM transfected neurons, and we found that hDSCAM
566 overexpression rescued the spine deficits in DSCAM knockdown neurons (Fig.
567 6B-6C), thus confirming the effects of DSCAM knockdown on spine development.
568 Furthermore, the neurons were stained with anti-PSD-95 antibody to quantify the
569 number and size of PSD-95 puncta. Compared with nontransfected neurons in the
570 same coverslip or sh-control transfected neurons, neurons transfected with
571 sh-DSCAM displayed increased numbers and sizes of PSD-95 puncta (Fig. 6D-6F).
572 These results indicate that knockdown of DSCAM in cultured neurons increases
573 dendritic spine maturation.

574 Because enhanced spine formation or stabilization leads to increased spine
575 maturation, we performed time-lapse imaging in cultured neurons to observe the
576 dynamic spine maturation process. Cultured cortical neurons were cotransfected with
577 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.

589

590 **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 (NRXN1 α -3 α and 1 β) 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

622 the synaptic area. The protein level of NLGN1 was comparable in the brain between
 623 control and DSCAM-deficient mice (Fig. 7H-7I). However, the level of NLGN1 in the
 624 synaptosomal plasma membrane (SPM) fraction was increased in *GFAP*-DSCAM f/f
 625 mice (Fig. 7J-7K), suggesting that DSCAM deficiency can induce NLGN1 enrichment
 626 in the synaptic membrane.

627

628 **DSCAM inhibits the NLGN1-NRXN1 β interaction**

629 Postsynaptic NLGN1 is involved in synapse maturation by interacting *in trans* with
 630 presynaptic NRXN1 β (Sudhof, 2008). We examined whether DSCAM could regulate
 631 the NLGN1-NRXN1 β interaction by cotransfecting NLGN1, NRXN1 β and DSCAM into
 632 HEK293T cells. As shown in Fig. 8A-8B, NRXN1 β was co-IPed with NLGN1 in the
 633 absence of DSCAM-His. However, NRXN1 β co-IP with NLGN1 was decreased in the
 634 presence of increased DSCAM-His, suggesting that DSCAM inhibits the
 635 NLGN1-NRXN1 β interaction. Since NLGN1 binds *in trans* with NRXN1 β , we
 636 performed cell surface binding assays to detect whether DSCAM could inhibit this
 637 trans-cell interaction. COS-7 cells were cotransfected with NLGN1 and varied
 638 amounts of DSCAM-His. After transfection, the cells were treated with purified
 639 NRXN1 β -ECD-myc protein for 1 h and then fixed for immunostaining with anti-NLGN1,
 640 anti-myc and anti-His antibodies. As shown in Fig. 8C-8D, the red fluorescence, which
 641 indicates cell surface binding of NRXN1 β -ECD-myc, was decreased when
 642 DSCAM-His expression was increased. Moreover, we also used cell aggregation
 643 assays to confirm the inhibition of NLGN1-NRXN1 β trans-cell interactions by DSCAM

644 (Fig. 8E). Cells clustered when HEK293T cells cotransfected with NLGN1 and GFP
 645 were mixed with another set of HEK293T cells cotransfected with NRXN1 β and RFP
 646 (Fig. 8F). DSCAM-His coexpression with NLGN1 in GFP+ cells decreased the
 647 number of aggregates in a dose-dependent manner (Fig. 8F-8G). Taken together,
 648 these results suggest that DSCAM inhibits the NLGN1-NRXN1 β trans-cell interaction.
 649 As DSCAM inhibited the NLGN1-NRXN1 β trans-cell interaction through ECD binding
 650 to NLGN1, we contemplated whether DSCAM-ECD alone could block the
 651 NLGN1-NRXN1 β *in trans* interaction. Using cell aggregation assays, we added CM
 652 containing FLAG-ECD to mixtures of HEK293T cells expressing either NLGN1 or
 653 NRXN1 β . As shown in Fig. 8H-8I, DSCAM-ECD reduced the number of
 654 NLGN1-NRXN1 β cell aggregates in a dose-dependent manner. Next, we theorized
 655 that DSCAM-ECD could rescue spine overmaturation caused by DSCAM deficiency.
 656 We treated DSCAM knockdown neurons with CM containing FLAG-ECD and found
 657 that FLAG-ECD rescued the increased total spine densities induced by DSCAM
 658 knockdown (Fig. 8J-8K). Together, these results suggest that DSCAM may block
 659 NLGN1-NRXN1 β interactions to inhibit premature spine maturation.

660

661 **DSCAM deficiency induces autism-like behaviors and enhances spatial memory** 662 **in mice**

663 Given that *DSCAM* is one of 102 ASD predominant genes and that its absence
 664 accelerated spine maturation and elevated glutamatergic transmission, we next examined
 665 the behaviors of DSCAM-deficient mice. First, we conducted autism-associated

666 behavioral tests in *GFAP-DSCAM* f/f and control mice. As shown in Fig. 9A-9B,
667 *GFAP-DSCAM* f/f mice displayed increased amounts of circling and self-grooming
668 than control mice did.

669 Along with repetitive behaviors, profound social impairment is a core symptom of ASD.

670 We therefore conducted three-chamber social interaction tests (Fig. 9C-9I) for social
671 recognition and social interaction (Moy et al., 2004). In the social preference test, both

672 *GFAP-DSCAM* f/f and control mice preferred to explore a stranger mouse (stranger1
673 mouse, S1) than an inanimate object (empty cage, E), as measured by duration spent
674 in each chamber and duration of sniffing (interaction time) (Fig. 9C-9E). Moreover, the

675 social interaction index was indistinguishable between the two genotypes, suggesting
676 normal social preference in *GFAP-DSCAM* f/f mice (Fig. 9F). Next, we compared

677 social novelty preference. After the E was replaced with another stranger mouse
678 (stranger2 mouse, S2), control mice spent more time in the S2 chamber and
679 interacted more with S2 than they did with the familiar mouse (S1) (Fig. 9G-9H). In

680 contrast, *GFAP-DSCAM* f/f mice spent less time in the S2 chamber than in the S1
681 chamber (Fig. 9G). Additionally, *GFAP-DSCAM* f/f mice did not differentiate between

682 S1 and S2 in terms of sniffing duration (Fig. 9H). Social novelty index score was
683 decreased in *GFAP-DSCAM* f/f mice (Fig. 9I), suggesting an impairment in social
684 novelty but not sociability.

685 We also conducted the Barnes circular mazes test to examine the impact of DSCAM
686 deficiency on spatial learning and memory (Fig. 9J-9O). In the 4-day training session,

687 escape latency and number of errors were similar in both genotype (Fig. 9J-9K),

688 suggesting normal spatial learning for *GFAP*-DSCAM f/f mice. However, at test,
689 *GFAP*-DSCAM f/f mice displayed reduced latency to reach target and greated
690 numbers of correct pokes, but they showed no difference in travel distance and time
691 on target, compared with the controls (Fig. 9L-9O), which suggests that DSCAM
692 deletion may enhance spatial memory. Taken together, the data indicate that
693 *GFAP*-mediated DSCAM deficiency increases stereotyped behaviors, impairs social
694 novelty, and enhances spatial memory.

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

700

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

703 DSCAM was primarily expressed in neurons, as examined by western blotting with
704 isolated neurons and astrocytes (Fig. 10A), and its expression in cultured neurons
705 was negatively regulated during the postnatal development period (Fig. 1C-1D). We
706 therefore investigated whether the spine and behavioral deficits in *GFAP*-DSCAM f/f
707 mice could result from DSCAM deficiency in pyramidal neurons. To this end, we
708 crossed DSCAM f/f mice with a *NEX*-Cre line, in which Cre is expressed in pyramidal
709 neurons of the neocortex and hippocampus. We found that the DSCAM level was

710 reduced to 50% in the cortex of the resultant *NEX-DSCAM* f/f mice (Fig. 10B-10C).
711 Furthermore, the sensory cortex L2/3 of *NEX-DSCAM* f/f mice at P42 was subjected
712 to Golgi staining. The densities of total spines and mature spines were markedly
713 increased, while the density of immature spines remained unchanged (Fig. 10D-10G).
714 Additionally, the width of spine head increased (Fig. 10H). These results suggest that
715 *DSCAM* deficiency in pyramidal neurons also lead to spine overmaturation.
716 Next, we examined the behaviors of *NEX-DSCAM* f/f mice. In the OFT, the duration
717 spent in the center and the velocity were similar between *NEX-DSCAM* f/f and control
718 mice (Fig. 10I-10L). In the EPM, the *NEX-DSCAM* f/f mice entered open and closed
719 arms at similar rates as the control mice did, but they reduced the duration spent in
720 open arms (Fig. 10M-10P), suggesting that *DSCAM* deficiency in pyramidal neurons
721 do not induce anxiety-like behaviors.
722 In addition, *NEX-DSCAM* f/f mice were subjected to tests for autism-associated
723 behaviors. *NEX-DSCAM* f/f mice exhibited stereotyped behaviors (Fig. 11A-11B),
724 social novelty deficits (Fig. 11C-11I) and enhanced spatial memory (Fig. 11J-11O).
725 Taken together, these results demonstrate that *DSCAM* deficiency in pyramidal
726 neurons also induces autism-like behaviors and enhanced spatial memory. Therefore,
727 we propose our *DSCAM* model of synapse maturation. In control mice, *DSCAM* was
728 localized to immature spines and interacted with *NLGN1* to inhibit the
729 *NLGN1-NRXN1 β* interaction, thus preventing spine overmaturation. In
730 *DSCAM*-deficient mice, this inhibition of the *NLGN1-NRXN1 β* interaction was
731 removed, and resulting in premature spine maturation, which ultimately led to

732 autism-like behaviors and enhanced spatial memory (Fig. 11P).

733

734 Discussion

735 In this study, we provided evidence that DSCAM serves as a repressor for the
736 NLGN1-NRXN1 β interaction, and that its deficiency leads to premature spine
737 maturation and excessive glutamatergic transmission, inducing autism-like behaviors.

738 The major findings of this paper are as follows. First, DSCAM was downregulated
739 following synapse maturation, and knockdown of DSCAM in primary neurons
740 increased spine maturation. Second, *in vivo*, GFAP-mediated DSCAM deficiency in
741 mice led to spine overmaturation and increased glutamatergic transmission. Third,
742 DSCAM interacted with NLGN1 through its ECD and blocked the NLGN1-NRXN1 β
743 interaction to suppress spine overmaturation. Finally, DSCAM-deficient mice exhibited
744 autism-like behaviors, including impaired social novelty, increased circling and
745 grooming time, and improved spatial memory.

746 Dendritic spines are small protrusions from the dendrites of excitatory neurons and
747 play critical roles in learning, memory, and cognition (Yuste and Bonhoeffer, 2004;
748 Berry and Nedivi, 2017). Dysregulation of spine development has been implicated in
749 mental disorders including autism, depression and schizophrenia (Forrest et al., 2018).
750 Previous studies have shown that DSCAM acts as a crucial factor or regulator in
751 synapse development and synaptic plasticity (Yamagata and Sanes, 2008; Li et al.,
752 2009; Maynard and Stein, 2012; Thiry et al., 2016; Laflamme et al., 2019), indicating
753 its role in spine development. Here, we found an increase in the density of matured

754 spines and in the volume of spine heads in *GFAP*-DSCAM f/f and *NEX*-DSCAM f/f
755 mice. Due to potential differences in staining efficiency between the two batches of
756 Golgi staining, we observed a small difference in the spine density data for the control
757 mice between Fig. 3B-3D and Fig. 11E-11G. We further confirmed the spine deficits in
758 cultured neurons. Live imaging results showed that increased spine density could be
759 attributed to enhanced stability. These results suggest that DSCAM can downregulate
760 spine maturation in the developing sensory cortex.

761 Several recent studies reported that DSCAM-ICD plays a critical role in synapse
762 development and neuronal migration (Sachse et al., 2019; Arimura et al., 2020).
763 However, we found that DSCAM interacted with NLGN1 through its ECD to disrupt the
764 NLGN1-NRXN1 β interaction. NLGN1 plays an essential role in spine maturation
765 (Varoqueaux et al., 2006; Chubykin et al., 2007; Bembien et al., 2015). DSCAM-ECD
766 also rescued abnormal spine maturation in DSCAM knockdown neurons. Therefore,
767 DSCAM might modulate spine maturation through its ECD. Differences in spine
768 densities have previously been reported in patients with neuropsychiatric disorders,
769 such as increased spine densities in L2 of the cortex and L5 of the temporal lobe in
770 ASD patients (Hutsler and Zhang, 2010). In addition, increased spines were also
771 observed in Fragile-X syndrome patients and corresponding mouse models (Comery et
772 al., 1997; Irwin et al., 2000). Thus, dendritic spine malformation may lead to neuronal
773 dysfunction during neural development, and DSCAM may serve as a modulator in
774 spine remodeling.

775 Disruption of the excitatory/inhibitory (E/I) balance has been implicated in various

776 neuropsychiatric disorders, including ASD (Rubenstein and Merzenich, 2003; Nelson
777 and Valakh, 2015). Elevation of the E/I ratio in the mouse prefrontal cortex leads to
778 social deficits (Yizhar et al., 2011). In a similar manner, we found that glutamatergic
779 transmission increased in *GFAP-DSCAM* f/f mice during postnatal development while
780 GABAergic transmission was unchanged, which might result in elevation of the E/I
781 ratio and lead to social novelty deficits (Nelson and Valakh, 2015). Social interaction
782 deficits were found in these *DSCAM*-deficient mice. Although some ASD mouse
783 models exhibit a reduced E/I ratio, such as mice with autism-associated mutations in
784 *NRXNs* or *SHANKs* (Etherton et al., 2009; Jiang and Ehlers, 2013), an enhanced E/I
785 ratio can also be observed in many other ASD mouse models, like *TSC1*-, *MDGA2*-,
786 *FMRP*- or *CUL3*- deficient mice (Bateup et al., 2013; Zhang et al., 2014; Connor et al.,
787 2016; Dong et al., 2020). Precocious spine maturation in hippocampal neurons
788 dramatically elevated the E/I ratio and impaired the cognitive development in *SynGAP*
789 mutant mice (Clement et al., 2012). In our autistic model of *DSCAM*-deficient mice,
790 early spine maturation in *DSCAM*-deficient neurons contributed to increased
791 excitatory synaptic transmission, which might impair the E/I balance and lead to
792 autism-like behaviors. In addition, *DSCAM* deficiency increased only mEPSCs
793 frequency, not amplitude, which may account for the normal surface distribution of
794 active AMPARs or NMDARs.

795 Previous studies have indicated higher cognitive function in patients with
796 macrocephaly and ASD (Courchesne and Pierce, 2005; Sacco et al., 2007).
797 Increased head circumferences have been shown in ASD patients with special

798 capabilities (Bena et al., 2013). DSCAM-null mice ($DSCAM^{del17}$ or $DSCAM^{2J}$) similarly
799 exhibited ventricular dilation with a dome-shaped head (Xu et al., 2011; Lemieux et al.,
800 2016). Intriguingly, increased spatial memory was observed in both *GFAP*-DSCAM f/f
801 and *NEX*-DSCAM f/f mice along with increased brain weight in this study, which
802 implies that brain enlargement might be one factor explaining the enhanced spatial
803 memory in DSCAM-deficient mice. Relatedly, it was previously theorized that
804 mushroom spines (matured spines) can act as memory spines (Bourne and Harris,
805 2007). More mature spines might be another explanation for the increased spatial
806 memory. Our live imaging results found a positive correlation between the percentage
807 of stable spines and the spine density, which may also contribute to the enhanced
808 spatial memory demonstrated by DSCAM-deficient mice. It has also been reported
809 that NLGN3-R451C mice (an ASD-associated mutation) displayed enhanced spatial
810 learning in the Morris water maze in addition to autism-like behaviors (Tabuchi et al.,
811 2007). Taken together, our results reveal the regulatory functions of DSCAM in spine
812 maturation and its underlying molecular mechanisms in the nervous system.
813 DSCAM-deficient mice with increased spatial memory might belong to the subset of
814 autism mouse models with high functions.

815 *DSCAM* is located on chromosome 21 in humans; an extra copy of chromosome 21
816 causes Down syndrome (DS) (Saito et al., 2000). Upregulated DSCAM expression is
817 found in the brains of DS patients, DS mouse models, and AD mouse models (Saito et
818 al., 2000; Amano et al., 2004; Jia et al., 2011), causing pathological changes in the
819 brain and finally leading to impairment in cognitive functions. Elevated levels of

820 DSCAM are also found in post-mortem brains from bipolar subjects (Amano et al.,
821 2008). Likewise, DSCAM expression is higher in intractable epilepsy (IE) patients
822 than in controls (Shen et al., 2011). However, DSCAM deficiency has been found to
823 disrupt dendritic self-avoidance and tiling in the mouse retina and cause abnormal
824 neuronal migration (Fuerst et al., 2008; Fuerst et al., 2009; Sachse et al., 2019;
825 Arimura et al., 2020). Additionally, premature termination mutations of DSCAM are
826 observed in some ASDs patients (Iossifov et al., 2014; Wang et al., 2016; Stessman
827 et al., 2017; Yuen et al., 2017). Our results showed that loss of DSCAM in pyramidal
828 neurons induced autism-like behaviors in mice. Together, all these findings suggest
829 that dysregulated DSCAM expression alters spine maturation, resulting in abnormal
830 synaptic transmission, which may explain how gene-dosage imbalance of DSCAM
831 could potentially contribute to the pathogenesis of neurological DSCAM-related
832 disorders.

833

834 **Author contributions**

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

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1056

1057 **Figure legends**1058 **Fig 1. Downregulated expression of DSCAM during synapse maturation.**

1059 **A-B** The mRNA levels of selected CAMs during postnatal synapse development. WT
 1060 mice cortex was isolated from P0 to P60 for mRNA extraction and qRT-PCR analysis
 1061 (**A**). Hot map analysis of qRT-PCR results for selected CAMs (**B**). N = 3 mice for each
 1062 age. **C-D** DSCAM protein level in cortical neurons from DIV3 to DIV21. Primary
 1063 cortical neurons were lysed at indicated DIV for western blotting (WB) (**C**).
 1064 Quantitative analysis of relative protein levels in **C**, the relative protein levels of all
 1065 three proteins were set to "1" (**D**). Data were from three independent experiments and
 1066 shown as mean $\pm$ SEM. **E-F** DSCAM expression during brain development.
 1067 Whole-brain homogenates from WT mice at different (indicated) stages from E12.5 to
 1068 P60 were subjected to WB with the anti-DSCAM antibody (**E**). Quantitative analysis of
 1069 data in **E** (**F**). N = 3 mice for each age. **G** Distribution of DSCAM in PSD fraction.
 1070 Subcellular fractions of the WT brains were blotted with anti-DSCAM, anti-PSD-95 (a
 1071 PSD marker) and anti-synaptophysin (a presynaptic marker). S1, supernatant 1; P1,
 1072 pelleted nuclear fraction; S2, supernatant 2; P2, washed crude synaptosomal fraction;
 1073 S3, crude synaptic vesicle fraction; SV, synaptic vesicle fraction; P3, lysed
 1074 synaptosomal membrane fraction; SPM, synaptosomal membrane fraction; Pre,
 1075 presynaptic fraction. **H-K** Distribution of DSCAM in neurons. Representative images
 1076 of DSCAM-His distribution in dendrites (**H**) and in PSD (**K**). Cortical neurons were
 1077 isolated from P0 pups, cotransfected with DSCAM-His and GFP at DIV9 and fixed for

1078 staining with anti-His and anti-PSD-95 antibodies at DIV17. In **H**: arrow, mature (M)
 1079 dendritic spine; arrowhead, immature spine (IM) dendritic spine. Scale bar, 32 μ m
 1080 (upper panel) and 10 μ m (lower panel). Quantitative analysis of DSCAM-His
 1081 fluorescence intensity (**I**) and puncta size (**J**) in **H**. **N** = 11 neurons, * $p < 0.05$, ** $p <$
 1082 0.01, and *** $p < 0.001$; one-way ANOVA. In **K**, arrowheads indicate DSCAM-His that
 1083 was colocalized with PSD-95.

1084

1085 **Fig. 2 Normal gross brain structure of *GFAP-DSCAM* f/f.**

1086 **A** The genomic structure of floxed DSCAM mice. The first loxp (1st loxP) was inserted
 1087 between exon1 and exon2, and the second loxp (2nd loxP) was inserted after the last
 1088 exon (exon33). **B** Genotyping of wild type (wt, m1), *GFAP-DSCAM* f/wt (m2) and
 1089 *GFAP-DSCAM* f/f (m3) mice. Primer pair 1 (PP1) and PP2 were for 1st loxP, PP3 and
 1090 PP4 were for 2nd loxP, PP5 was for wt allele and PP6 for *GFAP-Cre* transgene. **C**
 1091 DSCAM expression is decreased in the brain of *GFAP-DSCAM* f/f mice. Whole brain
 1092 lysates from *GFAP-DSCAM* f/f and control mice were probed with anti-DSCAM
 1093 antibody. Actin served as a loading control. The relative DSCAM level (band intensity
 1094 of DSCAM / actin) was quantified. **N** = 4 mice for control, **n** = 5 mice for
 1095 *GFAP-DSCAM* f/f mice. *** $p < 0.001$, Student's t-test. Data were shown as mean $\pm$
 1096 SEM. **D-F** *GFAP-DSCAM* f/f mice displayed normal body weight and gross brain
 1097 structure, but increased brain weight. Representative appearance and brain images of
 1098 *GFAP-DSCAM* f/f and control mice (**D**). Quantitative analysis of body and brain weight
 1099 for each genotype (**E**). Brain weight: **n** = 5 for control, **n** = 6 for *GFAP-DSCAM* f/f mice.

1100 Data were shown as mean $\pm$ SEM; * $p < 0.05$, ns, $p > 0.05$ (student's t-test).
 1101 Representative Nissle-staining images of brains from each genotype. Scale bar, 1
 1102 mm (F). **G-I** *GFAP-DSCAM* f/f mice displayed normal densities of NeuN+ and PV+
 1103 neurons. Representative NeuN immunostaining images and quantitative analysis of
 1104 NeuN+ cell densities in sensory cortex (G) and hippocampus (H) of each genotype.
 1105 Scale bar, 100 μ m in G and 32 μ m in H. N = 32 slices from 4 control mice, n = 43
 1106 slices from 5 *GFAP-DSCAM* f/f mice. Data were shown as mean $\pm$ SEM. ns, $p > 0.05$,
 1107 Student's t-test. Representative PV immunostaining images and quantitative analysis
 1108 of PV+ cell densities in the sensory cortex of each genotype (I). Scale bar, 100 μ m. N
 1109 = 17 slices from 4 control mice, n = 21 slices from 5 *GFAP-DSCAM* f/f mice. Data
 1110 were shown as mean $\pm$ SEM. ns, $p > 0.05$, Student's t-test.

1111

1112 **Fig 3. Abnormal spine maturation in sensory cortex of *GFAP-DSCAM* f/f mice.**

1113 **A-R** *GFAP-DSCAM* f/f mice displayed increased spine maturation in the sensory
 1114 cortex (A-E and J-N) and increased dendritic arborization at P21 (F-I and O-R).
 1115 Representative Golgi staining images of dendritic spines in sensory cortex from P12,
 1116 P21 and P42 male mice of each genotype (A and J). Scale bar, 10 μ m. Quantitative
 1117 analysis of total (B and K), M (C and L) and IM (D and M) spine density, as well as
 1118 spine head width (E and N). Representative dendrite trace images from Golgi staining
 1119 in sensory cortex L2/3 (F) and L5 (O) of each genotype at P12, P21 and P42. Scale
 1120 bars, 32 μ m. Quantitative analysis of dendritic length (G and P), branches (H and Q),
 1121 and complexity (I and R) in F and O. N = 5 mice for each genotype. Data were shown

1122 as mean $\pm$ SEM. Ns, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; two-way
1123 ANOVA.

1124

1125 **Fig. 4 Increased asymmetric synapse number and PSD size in sensory cortex of**

1126 ***GFAP-DSCAM f/f* mice**

1127 **A-F** *GFAP-DSCAM f/f* mice displayed increased density of asymmetric (AS) synapses
1128 and enlarged PSD area. Representative electron microscopy images of sensory
1129 cortex L2/3 from each genotype (**A**). For each genotype, scale bar was 1 μ m for left
1130 panels and 0.5 μ m for right enlarged insets. Red arrowheads, AS synapses; black
1131 arrowheads, symmetric (SY) synapses. Red box, enlarged insets of AS synapses;
1132 black box, enlarged insets of SY synapses. **B-F** Quantitative analysis of data in **A**. N =
1133 3 for each genotype. Data were shown as mean $\pm$ SEM. ** $p < 0.01$, *** $p < 0.001$,
1134 Student's t-test, two-way ANOVA.

1135

1136 **Fig 5. Increased glutamatergic transmission in sensory cortex L2/3 of**

1137 ***GFAP-DSCAM f/f* mice.**

1138 **A-F** *GFAP-DSCAM f/f* mice displayed increased mEPSC frequency, but normal
1139 mIPSC. The sensory cortical slices of each genotype at P12 (**A** and **D**), P21 (**D** and **E**)
1140 and P42 (**C** and **F**) were collected for whole-cell voltage clamp recording of mEPSC
1141 (**A-C**) and mIPSC (**D-F**). Upper panels, representative traces of mEPSCs (**A-C**) and
1142 mIPSC (**D-F**). Scale bars, 30 pA, 1 s. Lower panels, histogram summary and
1143 cumulative probability plots of mEPSC (**A-C**) and mIPSC (**D-F**) inter-event intervals

1144 and amplitude. P12: n = 34 neurons from 4 control, n = 16 neurons from 5
 1145 GFAP-DSCAM f/f mice; P21: n = 20 neurons from 5 control, n = 22 neurons from 5
 1146 GFAP-DSCAM f/f mice. P42: n = 17 neurons from 4 control, n=17 neuron from 4
 1147 GFAP-DSCAM f/fmice. Data were shown as mean $\pm$ SEM. ** p < 0.01, *** p < 0.001,
 1148 Student's t-test.

1149

1150 **Fig 6. Increased spine maturation and stability in DSCAM knockdown neurons.**

1151 **A** Knockdown of overexpressed mouse DSCAM-His in HEK293T cells. HEK293T
 1152 cells were cotransfected with mouse DSCAM-His, small hairpin RNA (sh-RNA) for
 1153 mouse/rat DSCAM (sh-DSCAM) or control scramble sh-RNA (sh-control), and human
 1154 DSCAM. 24hr after transfection, cells were lysed and subjected to WB with
 1155 anti-DSCAM antibody. Data were from three independent experiments and are shown
 1156 as mean $\pm$ SEM. Ns, p > 0.05, *** p < 0.001; Student's t-test. **B-C** Knockdown of
 1157 DSCAM in cultured cortical neurons increased mature spine density. Representative
 1158 images of pyramidal neuron morphology and spine density in cortical neurons (**B**).
 1159 Neurons were isolated from E18 rat to culture and transfected with GFP plus
 1160 sh-DSCAM or sh-control and hDSCAM or not at DIV9, and they were fixed for staining
 1161 at DIV17. Scale bar, 16 μ m (upper panel) and 10 μ m (lower panel). Statistical analysis
 1162 of total spine density in **B** (**C**). N = 29 neurons for sh-control, n = 79 neurons for
 1163 sh-DSCAM, n = 22 neurons for shDSCAM + hDSCAM. Ns, p > 0.05, *** p < 0.001,
 1164 student's t-test. Data were shown as mean $\pm$ SEM. **D-F** Knockdown of DSCAM in
 1165 cultured cortical neurons increased the density and size of PSD-95 puncta.

1166 Representative images of PSD-95 staining in cortical neurons (**D**). Primary cortical
 1167 neurons were transfected with sh-DSCAM or sh-control plus GFP at DIV9, and they
 1168 were fixed for PSD-95 staining at DIV17. Dotted line box, dendrite of transfected
 1169 neurons (enlarge in middle panels); Solid line box, dendrite of non-transfected
 1170 neurons (enlarge in lower panels). Scale bar, 16 μ m (upper panel) and 10 μ m (lower
 1171 two panels). Quantitative analysis of data in **D** for the density (**E**) and size (**F**) of
 1172 PSD-95 puncta. N = 21 neurons for transfected and non-transfected respectively in
 1173 sh-control, n = 49 neurons for transfected and non-transfected respectively in
 1174 sh-DSCAM. ** p < 0.01, *** p < 0.001, two-way ANOVA. Data were shown as mean $\pm$
 1175 SEM. **G** Representative images of time-lapse imaging from cortical neurons
 1176 transfected with sh-DSCAM or sh-control taken at 5 adjacent time points during the
 1177 60-min live-imaging period. Scale bar, 10 μ m. N = 21 neurons for sh-control, n = 19
 1178 neurons for sh-DSCAM. **H-J** Quantitative analysis for percentages of stable (**H**), new
 1179 (**I**) and eliminated spines (**J**). Ns, p > 0.05, ** p < 0.01, *** p < 0.001, student's t-test.
 1180 Data were shown as mean $\pm$ SEM. **K-M** Linear correlation of spine density and
 1181 percentage of stable (**K**), new (**L**) or eliminated (**M**) spines in DSCAM knockdown
 1182 neurons. R = 0.7595 for stable spines, R = -0.4751 for new spines and R = -0.6434 for
 1183 eliminated spines.

1184

1185 **Fig. 7 Interaction of DSCAM to NLGN1 through its ECD.**

1186 **A-B** DSCAM interacts with NLGNs. HEK293T cells were cotransfected with
 1187 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 μ m. 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 $\pm$ 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

1210 shown as mean $\pm$ SEM. *** $p < 0.001$, two-way ANOVA. **J-K** NLGN1 protein level was
 1211 increased in SPM fraction of GFAP-DSCAM f/f mice. SPM fractions from each
 1212 genotype were probed with anti-DSCAM and anti-NLGN1 antibodies (**J**). Quantitative
 1213 analysis of data in **J** (**K**). $N = 9$ mice for each genotype. Data were shown as mean $\pm$
 1214 SEM. ** $p < 0.01$, *** $p < 0.001$, two-way ANOVA.

1215

1216 **Fig 8. Inhibition of NLGN1-NRXN1 β interaction by DSCAM.**

1217 **A-B** DSCAM inhibits the NLGN1-NRXN1 β interaction. HEK293T cells were
 1218 cotransfected with different amounts of DSCAM-His and indicated constructs. Cell
 1219 lysates were IPed with anti-FLAG antibody and resulting complexes were blotted with
 1220 anti-GFP, anti-FLAG and anti-His antibodies (**A**). The relative intensity of co-IPed
 1221 NRXN1 β (intensity of co-IPed NRXN1 β / IPed NLGN1) was quantified in **B**. Data were
 1222 from three independent experiments and shown as mean $\pm$ SEM. * $p < 0.05$, ** $p <$
 1223 0.01, one-way ANOVA. **C-G** DSCAM inhibits transcellular NLGN1-NRXN1 β
 1224 interaction in cell surface binding assay (**C-D**) and cell aggregation assay (**E-G**).
 1225 COS-7 cells were cotransfected with NLGN1 and increasing amounts of DSCAM-His
 1226 for 24 h and incubated with purified NRXN1 β -ECD-myc for 1 h before fixation. Fixed
 1227 cells were immuno-stained with anti-NLGN1, anti-myc and anti-His antibodies.
 1228 Representative immunostaining images of cells (**C**). Scale bar, 10 μ m. Quantitative
 1229 analysis of relative red fluorescence intensity (intensity of red fluorescence / green
 1230 fluorescence) in **C** (**D**). Data were from three independent experiments and shown as
 1231 mean $\pm$ SEM. ** $p < 0.01$, *** $p < 0.001$, one-way ANOVA. Schematic illustration of cell

1232 aggregation assay (**E**). HEK293T cells expressing NRXN1 β or empty vector with RFP
 1233 (red cells) were mixed with cells expressing NLGN1 and GFP plus different amounts
 1234 of DSCAM (green cells). After 1 h, cell mixtures were imaged (**F**) and cell aggregates
 1235 were counted (**G**). Scale bar, 200 μ m. N = 15 images for each condition from three
 1236 independent experiments. Data were shown as mean $\pm$ SEM. ** $p < 0.01$, *** $p <$
 1237 0.001, one-way ANOVA. **H-I** DSCAM-ECD disrupts transcellular NLGN1-NRXN1 β
 1238 interaction. Different amounts of CM with FLAG-ECD were added into HEK293T cell
 1239 mixtures of NRXN1 β (red cells) and NLGN1 (green cells). Cell aggregates were
 1240 imaged (**H**) and counted (**I**). Scale bar, 200 μ m. Data were from three independent
 1241 experiments and shown as mean $\pm$ SEM. ** $p < 0.01$, and *** $p < 0.001$, one-way
 1242 ANOVA. **J-K** DSCAM-ECD rescued spine over-maturation in DSCAM knockdown
 1243 neurons. Primary cortical neurons were transfected at DIV9 with sh-DSCAM or
 1244 sh-control. At DIV15, transfected neurons were incubated with CM containing
 1245 FLAG-ECD or mock for 2 days and fixed. Representative images of dendritic spines
 1246 (**J**). Scale bar, 10 μ m. Quantitative analysis of total spine density in **J** (**K**). N = 20
 1247 neurons for sh-control + mock; n = 48 neurons for sh-DSCAM + mock; n = 21 neurons
 1248 for sh-DSCAM + FLAG-ECD. Data were shown as mean $\pm$ SEM. ** $p < 0.01$, and *** p
 1249 < 0.001 , one-way ANOVA.

1250

1251 **Fig 9. Autism-like behaviors and enhanced spatial memory in *GFAP-DSCAM* f/f**
 1252 **mice.**

1253 **A-B** *GFAP-DSCAM* f/f mice displayed increased circling and grooming in OFT. During

1254 10-min OFT, mice circling numbers (**A**) and grooming time (**B**) were counted. For
 1255 circling, n = 14 for control mice, n = 15 for *GFAP-DSCAM* f/f mice; for grooming, n =
 1256 16 for control mice, n = 19 for *GFAP-DSCAM* f/f mice. Data were shown as mean $\pm$
 1257 SEM. ** p < 0.01, Student's t-test. **C-I** *GFAP-DSCAM* f/f mice displayed reduced
 1258 social novelty. Representative heat map for the mice movements in three-chamber
 1259 social interaction test (**C**). S1, stranger1 mouse; S2, stranger2 mouse; E, empty cage;
 1260 C, center chamber. Quantitative analysis of time staying in each chamber (**D** and **G**),
 1261 interaction time (**E** and **H**), and social interaction index (**F** and **I**). Social interaction
 1262 index was calculated as: in **F**, [interaction time (S1) - interaction time (E)] / [interaction
 1263 time (S1) + interaction time (E)] for each genotype; in **I**, [interaction time (S2) -
 1264 interaction time (S1)] / [interaction time (S2) + interaction time (S1)] for each genotype.
 1265 N = 17 mice for control, n = 19 for *GFAP-DSCAM* f/f mice. Data were shown as mean
 1266 $\pm$ SEM. Ns, p > 0.05; *** p < 0.001. Student's t-test for **F** and **I**, two-way ANOVA for **D**,
 1267 **E**, **G** and **H**. **J-K** *GFAP-DSCAM* f/f mice displayed elevated spatial memory in Barnes
 1268 maze. Quantitative analysis of escape latency (**J**) and error numbers (**K**) in a 4-day
 1269 training session. Quantitative analysis of moved distances (**L**), latency to target (**M**),
 1270 time in target (**N**) and correct pokes (**O**) in the test session. N = 14 for control mice, n
 1271 = 20 for *GFAP-DSCAM* f/f mice. Data were shown as mean $\pm$ SEM. Ns, p > 0.05; *p <
 1272 0.05; ** p < 0.01. Student's t-test for **L-O**, two-way ANOVA for **J-K**. **P-S**
 1273 *GFAP-DSCAM* f/f mice displayed anxiety-like behavior in OFT. Male 2-month mice
 1274 were placed in the chambers and movement was monitored for 10 min.
 1275 Representative traces of mice in OFT (**P**). The square with a dotted line indicates the

center area. Quantitative analysis of traveled distance (**Q**), duration in the center (**R**) and velocity (**S**). $N = 19$ for control mice, $n = 21$ for *GFAP-DSCAM* f/f mice. Data were shown as mean $\pm$ SEM. ** $p < 0.01$, Student's t-test for **R** and **S**, Mann-Whitney U test for **Q**. **T-W** *GFAP-DSCAM* f/f mice displayed anxiety-like behavior in EPM. Mice were put in the center of the EPM and they could freely explore for 10 min. The time stayed in and entries into the open arms were recorded. Representative traces of mice in EPM (**T**). Quantitative analysis of entries into the open arms (**U**) and the closed arms (**V**), as well as duration in the open arms (**W**). $N = 15$ for control mice, $n = 17$ for *GFAP-DSCAM* f/f mice. Data were shown as mean $\pm$ SEM. * $p < 0.05$, student's t-test.

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 $\pm$ 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 μ m. 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 $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, Student's t-test. **I-L** *NEX-DSCAM* f/f

1298 mice displayed normal in OFT. Representative traces of mice in OFT (**I**). Quantitative
 1299 analysis of traveled distances (**J**), duration in the center (**K**) and velocity (**L**). N = 11
 1300 for control mice, n = 11 for *NEX-DSCAM* f/f mice. Data were shown as mean $\pm$ SEM.
 1301 Ns, $p > 0.05$; ** $p < 0.01$. Student's t-test for **K** and **L**, Mann-Whitney U test for **J**. **M-P**
 1302 *NEX-DSCAM* f/f mice displayed normal in EPM. Representative traces of mice in
 1303 EPM (**M**). Quantitative analysis of entries into the open arms (**N**) and the closed arms
 1304 (**O**), as well as duration in the open arms (**P**). N = 13 for control mice, n = 10 for
 1305 *NEX-DSCAM* f/f mice. Data were shown as mean $\pm$ SEM. Ns, $p > 0.05$; ** $p < 0.01$.
 1306 Student's t-test.

1307

1308 **Fig 11. Autism-like behaviors and enhanced spatial memory in *NEX-DSCAM* f/f**
 1309 **mice.**

1310 **A-B** *NEX-DSCAM* f/f mice displayed increased circling and grooming in OFT. During
 1311 10-min OFT, mice circling numbers (**A**) and grooming time (**B**) were counted. N = 11
 1312 for control mice, n = 11 for *NEX-DSCAM* f/f mice. Data were shown as mean $\pm$ SEM. *
 1313 $p < 0.05$, Student's t-test. **C-I** *NEX-DSCAM* f/f mice displayed reduced social novelty.
 1314 Representative heat map for the mice movements in three-chamber social interaction
 1315 test (**C**). Quantitative analysis of time staying in each chamber (**D** and **G**), interaction
 1316 time (**E** and **H**), and social interaction index (**F** and **I**). N = 9 mice for control, n = 10 for
 1317 *NEX-DSCAM* f/f mice. Data were shown as mean $\pm$ SEM. Ns, $p > 0.05$; * $p < 0.05$; ** p
 1318 < 0.01 . Student's t-test for **E**, **F**, **H**, and **I**; One-way ANOVA for *NEX-DSCAM* f/f group
 1319 in **D** and control group in **G**; Kruskal-Wallis test for control group in **D** and

1320 *NEX-DSCAM* f/f group in **G**. **J-O** *NEX-DSCAM* f/f mice displayed elevated spatial
1321 memory in Barnes maze. Quantitative analysis of escape latency (**J**) and error
1322 numbers (**K**) in 4-day training session. Quantitative analysis of moved distances (**L**),
1323 latency to target (**M**), time in target (**N**) and correct pokes (**O**) in the test session. N =
1324 13 for control mice, n = 11 for *NEX-DSCAM* f/f mice. Data were shown as mean $\pm$
1325 SEM. Ns, $p > 0.05$; * $p < 0.05$; ** $p < 0.01$. Two-way ANOVA for **J-K**; student's t-test for
1326 **L**, **M** and **O**; Mann-Whitney U test for **N**. **P** Schematic illustration for *DSCAM*
1327 deficiency on spine maturation.

Figure. 1

Figure. 2

Figure. 3

Figure. 4

Figure. 5

Figure 6

Figure 9

Figure. 10

Figure 11