

Altered Expression of Cadherin-8 and Cadherin-11 in Neural Circuit Development: Implications for Autism

1 Jeannine A. Frei^{1,*}, Robert F. Niescier¹, Morgan S. Bridi¹, Madel Durens¹, Jonathan E.
2 Nestor¹, Xiaobing Yuan², Derek M. Dykxhoorn³, Michael W. Nestor¹, Shiyong Huang¹, Gene
3 J. Blatt¹, and Yu-Chih Lin^{1,*}

4

5 ¹Program in Neuroscience, Hussman Institute for Autism, Baltimore, MD, USA

6 ²East China Normal University, Shanghai, China

7 ³John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine,
8 Miami, FL, USA

9

10 ***Correspondence:**

11 Jeannine A. Frei

12 jfrei@hussmanautism.org

13 Yu-Chih Lin

14 yclin@hussmanautism.org

15

16 **Keywords:** cadherin-8, cadherin-11, Cdh11 knockout mice, autism spectrum disorder, neuroligin-
17 1, dendrite, synapse, induced pluripotent stem cells

18 Number of words: 7894

19 Number of figures: 6

20

21 **Abstract**

22 Autism spectrum disorder (ASD) is a neurological condition characterized by difficulties in social
23 interaction, communication, and behavior. The classical type II cadherins cadherin-8 (Cdh8,
24 CDH8) and cadherin-11 (Cdh11, CDH11) have been implicated as autism risk gene candidates.
25 To explore the role of cadherins in the etiology of autism, we investigated their expression patterns
26 during mouse brain development and analyzed their functions using *Cdh11* knockout mice.
27 Expression of cadherin-8 and cadherin-11 was developmentally regulated and enriched in cortex,
28 hippocampus, and thalamus/striatum during the peak of dendrite formation and synaptogenesis.
29 Cadherin-8 preferentially localized to excitatory synapses where it interacted with neuroligin-1.
30 Levels of cadherin-8, neuroligin-1, and PSD-95 were all significantly increased in *Cdh11* knockout
31 brains. Additionally, *Cdh11*^{-/-} hippocampal neurons exhibited increased dendritic complexity
32 along with altered neuronal and synaptic activity. Similar to the expression profiles in *Cdh11*
33 knockout mice, induced pluripotent stem cell (iPSC)-derived cortical neural precursor cells
34 (NPCs) and cortical organoids generated from individuals with autism showed elevated CDH8
35 expression levels while CDH11 expression levels were decreased. Together, these results strongly
36 suggest that cadherin-8 and cadherin-11 are involved in regulating the development of neuronal
37 circuitry and that alterations in the expression levels of cadherin-8 and cadherin-11 may contribute
38 to the etiology of autism.

39 Introduction

40 Autism is a neurodevelopmental condition characterized by marked qualitative changes in social
41 interaction, communication, and behavior (American Psychiatric Association, 2013). Current
42 estimates indicate that 1 in 59 children in the United States are affected (Baio et al., 2018). Autism
43 is characterized by both phenotypic and genetic heterogeneity. This genetic complexity is
44 illustrated by the fact that no single gene associated with the condition contributes to more than
45 1% of the autism cases (Huguet, Ey, & Bourgeron, 2013; Jeste & Geschwind, 2014). Nevertheless,
46 many of the genes implicated in the condition encode for synaptic cell adhesion molecules,
47 scaffolding proteins and cytoskeletal regulators (Betancur, Sakurai, & Buxbaum, 2009;
48 Bourgeron, 2015; Lin, Frei, Kilander, Shen, & Blatt, 2016). These autism risk genes converge into
49 distinct cellular pathways that appear to be commonly affected, including neurite outgrowth,
50 dendritic spine stability, synaptogenesis, and synaptic function (Betancur et al., 2009; Bourgeron,
51 2015; Chen, Yu, Fu, & Li, 2014; Hussman et al., 2011; Joensuu, Lanoue, & Hotulainen, 2017; Lin
52 et al., 2016).

53 One large group of synaptic cell adhesion molecules, the cadherin superfamily, has been
54 widely associated with neurodevelopmental disorders, including autism (Lin et al., 2016; Redies,
55 Hertel, & Hübner, 2012). The cadherin family comprises more than one hundred members, which
56 are further divided into subfamilies, including classical type I and II cadherins, clustered and non-
57 clustered protocadherins, and atypical FAT cadherins (Hirano & Takeichi, 2012; Hulpiau & van
58 Roy, 2009). Multiple studies have identified cadherins across all subfamilies as candidate risk
59 genes for autism (Camacho et al., 2012; Chapman et al., 2011; Crepel et al., 2014; Cukier et al.,
60 2014; Depienne et al., 2009; Girirajan et al., 2013; Hussman et al., 2011; Kenny et al., 2014;
61 Marshall et al., 2008; Morrow et al., 2008; Neale et al., 2012; O'Roak et al., 2012; Pagnamenta et
62 al., 2011; Sanders et al., 2011; van Harssel et al., 2013; K. Wang et al., 2009; Willemsen et al.,
63 2010). The most-well studied classical type I cadherin, N-cadherin, is broadly expressed in the
64 central nervous system and has been implicated in multiple processes during nervous system
65 development (Arikkath & Reichardt, 2008; Friedman, Benson, & Huntley, 2015; Hirano &
66 Takeichi, 2012; Seong, Yuan, & Arikkath, 2015; Takeichi & Abe, 2005). In contrast to classical
67 type I cadherins, the expression profile of classical type II cadherins is more restricted to specific
68 brain circuits and subcellular compartments (Inoue, Tanaka, Suzuki, & Takeichi, 1998; Korematsu
69 & Redies, 1997; Suzuki, Inoue, Kimura, Tanaka, & Takeichi, 1997; C. Wang, Pan, Wang, Blatt,
70 & Yuan, 2019). The differential expression patterns of classical type II cadherins allow them to
71 confer sophisticated synaptic specificity (Basu, Taylor, & Williams, 2015).

72 The aim of the current study was to understand the involvement of classical type II
73 cadherins in autism and to decipher potential cellular mechanisms mediated by these cadherins.
74 We focused our study on the classical type II cadherins cadherin-8 (Cdh8, CDH8) and cadherin-
75 11 (Cdh11, CDH11) as these specific cadherins have been identified as autism risk genes in a
76 genome-wide association study and by whole exome sequencing (Hussman et al., 2011, Cukier et
77 al., 2014). In addition, other studies have identified rare mutations and SNP variants in *CDH8* and
78 *CDH11* genes, respectively, in individuals with autism (Pagnamenta et al., 2011, 2008, Crepel et
79 al., 2014). Here, we first examined the expression profiles and binding partners of cadherin-8 and
80 cadherin-11 in developing mouse brains. The results indicated that these two cadherins engage in
81 different cellular pathways. We further used the *Cdh11* knockout mouse to identify potential
82 mechanisms altered in autism as levels of these two cadherins were differentially altered in human
83 induced pluripotent stem cell (hiPSC)-derived neural precursor cells (NPCs) and organoids

Cadherins in Development and Autism

84 generated from individuals with autism. These data suggested that a shift in the balance between
85 cadherin-8 and cadherin-11 causes altered neural circuit formation that may drive aspects of autism
86 pathophysiology.

87 **Materials and Methods**

88 **Animals**

89 C57BL/6 mice were purchased from the animal facility of the University of Maryland School of
90 Medicine Program in Comparative Medicine (Baltimore, MD, USA). *Cdh11^{tm1Mta}/HensJ* mice
91 were purchased from the Jackson Lab (Horikawa, Radice, Takeichi, & Chisaka, 1999). Mice were
92 housed and cared for by the AAALAC accredited program of the University of Maryland School
93 of Medicine. Female mice were group-housed and male mice were singly housed with *ad libitum*
94 food and water accessibility under a standard 12-hour light/dark cycle. Neonatal mice of both sexes
95 were euthanized for the preparation of neuronal and glial cultures. To match the mixed-gender
96 condition in cultures animals of both sexes were used for biochemistry. All experiments were
97 reviewed and approved by the Institutional Care and Use Committees (IACUC) of the University
98 of Maryland School of Medicine and the Hussman Institute for Autism, and were performed in
99 accordance with the animal care guidelines of the National Institute of Health.

100

101 **Antibodies**

102 The antibodies used in this study are listed in Table 1. The specificity of the antibodies was
103 carefully examined prior to conducting the experiments (Supplementary Figure 1).

104

105 **Plasmids**

106 Myc-flag-tagged full-length *Cdh8* was purchased from Origene (plasmid #MR218916). *Cdh8* was
107 expressed under the CMV promoter in the pCMV6 vector. Flag-tagged full-length *Cdh11* was
108 expressed under the EF-1 α promoter in the pBos vector (gift from Dr. Megan Williams, University
109 of Utah, USA). HA-tagged *Nlgn1* plasmid was a gift from Peter Scheiffele (Addgene plasmid #
110 15260; RRID:Addgene_15260) (Chih, Gollan, & Scheiffele, 2006). *Nlgn1* was expressed under
111 the chicken β -actin promoter in the pCAAGs vector. pLL3.7-GFP was a gift from Luk Parijs
112 (Addgene plasmid # 11795; RRID:Addgene_11795) (Rubinson et al., 2003).

113

114 **Cell cultures and transfection**

115 Glial cell cultures were prepared from postnatal day 0 (P0) C57BL/6 mouse cortices and cultured
116 in Dulbecco's MEM growth medium (Invitrogen Cat#11960044) supplemented with 10% FBS
117 (Millipore Sigma Cat#F4135), 2 mM L-glutamine (Invitrogen Cat#25030081) and 1%
118 penicillin/streptomycin (Invitrogen Cat#15140122). Primary neuronal cultures were prepared
119 from P0 C57BL/6 mouse cortex (3-4 animals per culture) or hippocampus (8-10 animals per
120 culture). Hippocampal cultures from *Cdh11^{tm1Mta}/HensJ* mice were prepared from individual pups
121 at P0. The genotype of each pup was determined after the culture was prepared. Only *Cdh11* wild-
122 type and knockout cultures were used for further experiments. In brief, brain tissue was dissected
123 and meninges were removed. Tissue was digested in papain and cells were dissociated and plated
124 on surfaces coated with 20 μ g/ml poly-D-lysine (Millipore Sigma Cat#P6407). Cortical and
125 hippocampal cultures were maintained in serum-free Neurobasal-A media (Invitrogen
126 Cat#10888022) containing 2 mM L-glutamine (Gibco Cat#25030081), 1% penicillin/streptomycin

Cadherins in Development and Autism

127 (Gibco Cat#15140122) and 2% B27 supplement (Gibco Cat#17504044). For Western blot
128 analysis, glial cells were harvested at 14 days *in vitro* (DIV) and cortical neurons were harvested
129 at different time points: 1, 3, 7 and 14 DIV. Neuro-2A (N2a, mouse neuroblastoma cell line;
130 ATCC) cells were maintained in Dulbecco's MEM growth medium (Invitrogen Cat#11960044)
131 supplemented with 10% FBS (Millipore Sigma Cat#F4135), 2 mM L-glutamine (Gibco
132 Cat#25030081) and 1% penicillin/streptomycin (Gibco Cat#15140122). N2a cells were
133 transfected with full-length plasmids using Lipofectamine 3000 (Invitrogen Cat#L3000015)
134 according to the manufacturer's protocol. Cells were harvested 48 hours post-transfection.

135

136 **Western blot analysis**

137 At the indicated points in development, mice of both sexes were sacrificed and brains were quickly
138 dissected. Either whole brains or different brain areas, including cortex, hippocampus, cerebellum
139 and thalamus/striatum were collected. Brain tissue was snap-frozen in liquid nitrogen. All tissues
140 and cells were lysed in RIPA buffer (Cell Signaling Technologies Cat# 9806S) supplemented with
141 PMSF (Cell Signaling Technologies Cat#8553S) and protease and phosphatase inhibitor cocktail
142 (Thermo Fisher Scientific Cat#78442). Protein concentration was determined using Pierce BCA
143 protein assay kit (Thermo Fisher Scientific Cat#23227) and measured by the Tecan Spark 10M
144 multimode microplate reader. Ten micrograms of protein samples from brain or cell lysates were
145 run on 10% Tris-glycine SDS-PAGE gels and transferred to a PVDF membrane. Membranes were
146 blocked in 5% milk/TBS-T followed by incubation of primary antibodies overnight and secondary
147 HRP-coupled antibodies for one hour at room temperature. Blots were imaged with the ChemiDoc
148 Touch Imaging System (Bio Rad; see Supplementary Figure 2 for full scans of original blots) and
149 band densitometries were analyzed using the Image Lab software (Bio Rad). Intensities were
150 normalized to either GAPDH or β -actin signals. Full scans of all blots

151

152 **Synaptic fractionation**

153 Synaptic plasma membrane (SPM) and postsynaptic density (PSD) fractions were prepared
154 according to Bermejo et al. (Bermejo, Milenkovic, Salahpour, & Ramsey, 2014). In brief, brains
155 of P21 mice were quickly removed and forebrain was dissected on ice. Brain tissues were
156 homogenized in 0.32 M sucrose in 4 mM HEPES (pH 7.4) at 900 rpm with 12 strokes using a
157 glass-Teflon tissue homogenizer. Removal of the nuclear fraction (P1) was achieved by low speed
158 centrifugation at 900 x g for 10 minutes. The supernatant (S1) was collected and centrifuged at
159 10,000 x g for 15 minutes to yield crude synaptosomal fraction (P2) and cytosol/light membranes
160 in the supernatant (S2). P2 pellet was lysed in ddH₂O by hypo-osmotic shock and centrifuged at
161 25,000 x g for 20 minutes to obtain pelleted synaptosomes (P3) and vesicular fraction (S3). The
162 vesicular fraction was pelleted by ultracentrifugation at 165,000 x g for 2 hours. Synaptosomes
163 (P3) were layered on top of a discontinuous sucrose gradient. Ultracentrifugation of the gradient
164 at 150,000 x g for 2 hours yielded the SPM fraction. SPM was collected and pelleted by
165 ultracentrifugation at 200,000 x g for 30 minutes. To prepare PSD fraction, SPM was incubated in
166 0.5% Triton-X100 for 15 minutes followed by centrifugation at 32,000 x g for 30 minutes. All
167 centrifugation steps were performed at 4°C. Fractions were analyzed by Western blot and
168 intensities of cadherin-8, cadherin-11 and PSD-95 positive signals in SPM and PSD fractions were
169 normalized to the total protein input.

170

171 **Co-immunoprecipitation**

172 P14 forebrain and transfected and untransfected N2a cells were homogenized in lysis buffer
173 containing 20 mM Tris, pH 7.5, 150 mM NaCl and 1% Triton X-100 supplemented with PMSF
174 (Cell Signaling Technologies Cat#8553S) and protease and phosphatase inhibitor cocktail
175 (Thermo Fisher Scientific Cat#78442). Protein extract (0.5 mg, standardized to 1 mg/ml) was
176 precleared with 50 μ l Protein G Sepharose 4 Fast Flow (Millipore Sigma Cat#GE17-0618-01) or
177 rProtein A Sepharose Fast Flow (Millipore Sigma Cat#GE17-1279-01) for 1 hour at 4° C.
178 Precleared supernatant was incubated with 2 μ g anti-cadherin-8 (DSHB Cat#CAD8-1), 7 μ g anti-
179 cadherin-11 (Thermo Fisher Scientific Cat#32-1700) or 4 μ g anti-HA antibodies (Millipore Sigma
180 Cat#H6908), and mouse or rabbit IgGs for 2 hours at 4° C. Samples were precipitated with 50 μ l
181 of pre-equilibrated Protein G Sepharose 4 Fast Flow or rProtein A Sepharose Fast Flow for 1 hour
182 at 4°C with gentle mixing. Immunoprecipitates were washed three times in lysis buffer and eluted
183 by boiling in 50 μ l sample buffer. Overexpressed myc-flag-tagged cadherin-8 was
184 immunoprecipitated using the Pierce c-My-tag IP/Co-IP kit according to the manufacturer's
185 protocol (Thermo Fisher Cat#23620). Co-immunoprecipitated proteins were determined by
186 Western blot analysis.

187

188 **Immunocytochemistry**

189 Surface staining was performed on low-density hippocampal cultures (20,000 cells/2 cm²) at 15
190 DIV. Cells were washed with artificial cerebrospinal fluid (aCSF) containing 124 mM NaCl, 5
191 mM KCl, 1.23 mM NaH₂PO₄, 26 mM NaHCO₃, 10 mM Dextrose, 1 mM MgCl₂, 1 mM CaCl₂,
192 and supplemented with 10% BSA. Cells were incubated with aCSF containing primary antibody
193 for one hour at 20°C. After washing with aCSF/10% BSA, cells were incubated with secondary
194 antibody for one hour at 20°C before fixation with 4% paraformaldehyde for 15 minutes (Gu &
195 Haganir, 2016; Noel et al., 1999). For total staining, N2a and primary cells were fixed with 4%
196 paraformaldehyde, permeabilized with 0.1% Triton-X100 and incubated with primary antibodies
197 overnight and secondary antibodies for one hour at room temperature. Cells were labeled with
198 DAPI and coverslips were mounted on glass slides with ProLong Diamond antifade mounting
199 solution (Thermo Fisher Scientific Cat#P36934) prior to imaging. Images were taken using a Zeiss
200 LSM-780 scanning confocal microscope with a 40x objective/1.30 EC-plan-neofluar oil or a 63x
201 objective/1.40 plan-apochromat oil for N2a cells, and a 63x objective/1.40 plan-apochromat oil
202 and 4x zoom for primary cells. For co-localization analysis, cadherin-8-positive puncta were
203 manually counted and classified as either co-localizing with PSD-95 or GAT-1 (partially or totally
204 overlapping puncta), being adjacent to PSD-95 or GAT-1 (puncta that were in close proximity and
205 touching each other) or cadherin-8 puncta that were PSD-95- or GAT-1-negative.

206

207 **Direct stochastic optical reconstruction microscopy (dSTORM) imaging**

208 67,000 cells prepared from P0 C57BL/6 mouse hippocampus were plated onto Fluorodish 35 mm
209 dishes with 23 mm #1.5-glass bottoms (Cat# FD35-100). At 15 DIV, cells were surface stained
210 with either cadherin-8 or neuroligin-1 primary antibody and Alexa647-conjugated secondary
211 antibody as described above. After surface staining, cells were fixed, permeabilized and

212 immunostained with PSD-95 primary antibody and Alexa488-conjugated secondary antibody and
213 maintained in PBS prior to imaging. Imaging was performed using a Nikon N-STORM
214 microscope, using the following buffer system: 150 mM tris-HCl pH 8.0, 100 mM MEA-HCl
215 (Millipore Sigma Cat#M6500), 3% Oxyfluor (Millipore Sigma Cat#SAE0059), and 2% DL-
216 Lactate (Millipore Sigma Cat#L1375) (Nahidiazar, Agronskaia, Broertjes, van den Broek, &
217 Jalink, 2016). A minimum of 20,000 frames were obtained using 100% laser power of 488 and
218 647 nm channels with 16 ms exposure time using elliptical lens for 3D analysis. STORM
219 processing was performed using the Fiji (NIH) plug-in ThunderSTORM (Ovesný, Křížek,
220 Borkovec, Svindrych, & Hagen, 2014). For nearest neighbor analysis, the ThunderSTORM CBC
221 function was used to compare the cadherin-8 or neuroligin-1 localization tables to the
222 corresponding PSD-95 table. Individual molecules of either cadherin-8 or neuroligin-1 were
223 manually identified, and the closest molecule of PSD-95 was quantified. 50 fluorescence particles
224 per image were examined, unless fewer than that were available for analysis. For a negative
225 control, numbers between 0-400 were randomly generated using Microsoft Excel. For comparison
226 between random, cadherin-8, and neuroligin-1, values were binned at 10 nm intervals and plotted
227 using Graph Pad Prism 8 software (GraphPad Prism Software, RRID: SCR_002798).

228

229 **Morphometric analysis**

230 Cultured *Cdh11* wild-type and knockout hippocampal neurons (300,000 cells/2 cm²) were
231 transfected at 9 DIV with the pLL3.7-GFP vector (Addgene Cat#11795) using Lipofectamine 2000
232 (Invitrogen Cat#11668-019) and fixed at 15 DIV with 4% paraformaldehyde. Z-stack images were
233 taken using Zeiss LSM-780 scanning confocal microscope with a 63x objective/1.40 plan-
234 apochromat oil at 1500x1500 resolution and stitched with 2x2 frames. For dendritic spine analysis,
235 two to three dendrite segments per neuron were randomly selected from primary, secondary and
236 tertiary branches on the apical dendrite. The number of dendritic spines ($\leq 2 \mu\text{m}$ in length) was
237 quantified using Fiji (NIH). For analysis of dendritic morphology, neurons were traced and the
238 total dendrite length and branch tip number were quantified using Fiji (NIH) with NeuronJ plugin
239 (Meijering et al., 2004). Neurons were further analyzed using Fiji (NIH) with Sholl analysis plugin
240 (Ferreira et al., 2014). From the center of the cell body, concentric circles having 10 μm increments
241 in radius were defined and the number of traced dendrites crossing each circle was quantified. The
242 complexity of dendritic arbors was analyzed by the area under the curve (AUC) using Graph Pad
243 Prism 8 software (GraphPad Prism Software, RRID: SCR_002798).

244

245 **Calcium imaging**

246 *Cdh11* wild-type and knockout hippocampal neurons were seeded in triplicates at a density of
247 30,000 cells per well on a 96-well plate coated with 20 $\mu\text{g/ml}$ poly-D-lysine (Millipore Sigma
248 Cat#P6407). At 7 DIV neurons were infected with the IncuCyte NeuroBurst Orange lentivirus
249 under a synapsin promotor (Essen Bioscience Sartorius Cat# 4736). After 24 hours, virus was
250 removed by changing the media. Cells were imaged at 15 DIV for 24 hours using the IncuCyte S3
251 system (Essen Bioscience Sartorius Cat#4763). Using the IncuCyte S3 2019A software (Essen
252 Bioscience Sartorius) the following parameters were calculated: number of active neurons, mean
253 correlation of activity, mean burst strength, and mean burst rate. The total number of active neurons
254 was identified by the analysis definition. For the mean correlation of activity the temporal pattern

255 of the change in fluorescent intensity for each active neuron was compared to every other active
256 neuron in the image. A value between -1 and 1 was generated, with 0 being completely random
257 (no correlation) and 1 being identical patterns of change in fluorescent intensity (highly
258 correlated). Fisher r-to-z transformation was applied to assess the significance between correlation
259 coefficients. The mean burst strength was analyzed by integrating the area under the curve divided
260 by its duration. This value was calculated for each burst individually and then averaged for each
261 active neuron, followed by averaging across the entire image. To calculate the mean burst rate the
262 total number of bursts for each active neuron was divided by minutes of scan time, followed by
263 averaging the values for all active neurons across the entire image. The total number of cells was
264 obtained by counting DAPI-positive nuclei. InCuCyte NeuroLight Orange lentivirus (Essen
265 Bioscience Sartorius Cat# 4758) was used to measure the infection rate of *Cdh11* wild-type and
266 knockout hippocampal neurons. Neurons were infected at 7 DIV and the virus was removed after
267 24 hours by changing the media. Neurons were fixed with 4% paraformaldehyde at 16 DIV and
268 imaged using Evos Auto 2.0 system (Invitrogen) with a 10x objective. The number of infected
269 cells was counted and subsequently normalized to the total cell number determined by DAPI.

270

271 **Electrophysiology**

272 Electrophysiological recordings were conducted using *ex vivo* brain slices of *Cdh11* wild-type and
273 knockout littermate mice between P21 and P24. Mice were anesthetized with isoflurane,
274 decapitated, and brains removed into ice-cold sucrose cutting solution containing (in mM): 215
275 Sucrose, 2.5 KCl, 1.25 NaH₂PO₄, 2.8 NaHCO₃, 7 Dextrose, 3 Na-Pyruvate, 1 Na-Ascorbate, 0.5
276 CaCl₂, 7 MgCl₂ (pH 7.4, bubbled with 95% CO₂/5% O₂). Near-horizontal slices containing
277 hippocampus, 300 μm thick, were sectioned with a vibrating microtome (VT1200S, Leica
278 Biosystems). After cutting, slices were transferred into a warmed recovery chamber filled with
279 bubbled artificial cerebrospinal fluid (aCSF) containing the following (in mM): 125 NaCl, 2.5
280 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 25 Dextrose, 2 CaCl₂, 1 MgCl₂, 3 Na-Pyruvate, and 1 Na-
281 Ascorbate. After recovering for 30 minutes at a temperature of 34°C, the slices recovered for at
282 least another 30 minutes at room temperature prior to recording. For recording, slices were
283 transferred to a recording chamber superfused with the same aCSF used in the recovery chamber,
284 maintained at 32°C. Miniature excitatory postsynaptic currents (mEPSCs) were recorded in
285 voltage-clamp mode ($V_h = -76$ mV) in the presence of 100 μM DL-AP5, 10 μM tetrodotoxin, and
286 20 μM gabazine (channel blockers acquired from Tocris). Glass pipettes pulled to a resistance of
287 2-6 MΩ were filled with internal solution containing the following (in mM): 130 K-gluconate, 10
288 KCl, 10 HEPES, 0.2 EGTA, 4 MgATP, 0.5 Na₂GTP, 10 Na₂-phosphocreatine, and 5 QX-314
289 chloride, pH adjusted to 7.3 with KOH, 280–290 mOsm. Pyramidal neurons in stratum pyramidale
290 of CA1 in dorsal hippocampal slices were visually identified for recording. Only cells with a series
291 resistance ≤ 30 MΩ, input resistance > 80 MΩ, and a resting membrane potential ≤ -50 mV were
292 accepted for final analysis. Whole-cell parameters were monitored throughout the recording with
293 a 100 ms, -10 mV step delivered every 30 sec. Recordings were made using an Axon MultiClamp
294 700B amplifier (Molecular Devices). Data were filtered at 2 kHz and digitized at 10 kHz with a
295 National Instruments digital-analog converter under the control of Igor Pro software
296 (WaveMetrics, RRID: SCR_000325). The frequency, amplitude, rise and decay times, and charge
297 of mEPSCs were analyzed with Mini Analysis software (Synaptosoft, RRID: SCR_002184), with
298 a threshold of 3× RMS noise for event discrimination. 100-200 well-isolated events were used for

299 these analyses in wild-type neurons; because of extremely low event frequency, a minimum of 50
300 events were used in knockout neurons.

301

302 **Human induced pluripotent stem cell (iPSC)-derived cortical neural precursor cell cultures**

303 iPSC lines were derived from peripheral blood obtained from eight individuals with autism and
304 from four typically developing controls (DeRosa et al., 2018; Table 2). Peripheral blood
305 mononuclear cells (PBMCs) were isolated and cultured in suspension until transduction using
306 Oct4, Sox2, Klf4, and c-Myc Cytotune Sendai viruses (Life Technologies Cat# A16517) (DeRosa
307 et al., 2012). After PBMC transduction into iPSCs the media was supplemented with 10 μ M
308 CHIR99021 (Stemgent Cat#04-0004-10), 1 μ M PD325901 (Stemgent Cat#04-0006), 1 μ M
309 thiazovivin (Stemgent Cat#04-0017), and 10 μ M Y27632 (Stemgent Cat#04-0012-10) for 7 days.
310 At day 7, the media with small molecules was transitioned to mTeSR1 full stem cell maintenance
311 media (Stemcell Technologies Cat#85850) with the media being changed daily. iPSC colonies
312 were plated onto mouse embryonic feeders (MEFs) and grown for 7 days. Colonies were selected
313 showing proliferating cell clusters, indicative of reprogrammed cells. To derive cortical progenitor
314 neurons, selected iPSC colonies were dissociated via a 7-minute treatment with Accutase in the
315 presence of 20 μ M Y27632 and MEF feeders were removed using 0.1% gelatin as previously
316 described (Nestor et al., 2015; Phillips, Nestor, & Nestor, 2017). Dissociated iPSCs were exposed
317 to media containing small molecules (e.g. 10 μ M Y27632, 10 μ M SB431542 (Stemgent Cat#04-
318 0010-10), 1 μ M dorsomorphin (Stemgent Cat#04-0024), and 1 μ M thiazovivin) within growth
319 media as described previously (DeRosa et al., 2012). After patterning and neural induction, iPSC-
320 derived neuron progenitors were expanded using 6-well plates coated with 15 μ g/ml Poly-L-
321 Ornithine (Millipore Sigma Cat# P4957) and 10 μ g/ml laminin (Invitrogen Cat# 230171015)
322 within an enriched medium containing 1:1 mixture of DMEM/F12 (with L-Glutamine; Thermo
323 Fisher Cat# 11320-033) and Neurobasal medium (minus phenol red; Invitrogen Cat#12348017),
324 5 μ M forskolin (Millipore Sigma Cat# F6886), 60 ng/ml progesterone (Millipore Sigma
325 Cat#P8783), 16 μ g/ml putrescine (Millipore Sigma Cat#P7505), 5 μ g/ml N-acetyl-L-cysteine
326 (Millipore Sigma Cat#8199), 1% Insulin-Transferrin-Selenium-A (Gibco Cat#41400045), 1% B-
327 27 supplement (Gibco Cat#12587010), 0.5% N2 supplement (Gibco Cat#17502048), 1%
328 Antibiotic-Antimycotic, 30 ng/ml tri-iodothyronine (Millipore Sigma Cat#T6397), 40 ng/ml
329 thyroxine (Millipore Sigma Cat#T1775), 0.5% non-essential amino acids, 100 μ g/ml bovine-
330 serum albumen (Millipore Sigma Cat#4161) and 0.5% GlutaMAX (Invitrogen Cat#35050061).
331 Cells were harvested at 19 DIV in RIPA buffer (Cell Signaling Technologies Cat# 9806S)
332 supplemented with PMSF (Cell Signaling Technologies Cat#8553S) and protease and phosphatase
333 inhibitor cocktail (Thermo Fisher Scientific Cat#78442) and triplicates of each control and autism
334 line were analyzed by Western blot.

335

336 **Organoid culture and mRNA quantitation via qPCR**

337 Cortical organoid cultures were generated from four control and six autism lines (Table 2) as
338 previously described in Durens et al. (Durens et al., 2020). Organoids were cultured for 60 days,
339 and then transferred to RNAprotect Cell Reagent (Qiagen Cat#76526) for RNA stabilization. Two
340 organoids were pooled per biological replicate and triplicates of each control and autism line were
341 analyzed. Samples were stored at -80°C until processed. Samples in RNAprotect were thawed on

Cadherins in Development and Autism

342 ice and centrifuged at 5000 x g for 5 minutes after which supernatant was removed. Total RNA
343 was extracted using the RiboPure Kit (Thermo Fisher Scientific Cat#AM1924) following
344 manufacturer's specifications. RNA concentration was determined using NanoDrop 8000
345 Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific). DNA-free DNA removal kit
346 (Thermo Fisher Scientific Cat#AM1906) was used to remove residual DNA. Reverse transcription
347 (RT) was performed using 250 ng of DNase-treated RNA using the iScript cDNA synthesis kit
348 (Bio-Rad Cat#1708890) and the resulting RT reactions were diluted 1:5 with nuclease-free water.
349 qPCR was performed for the following genes: CDH8 (Forward – 5'-ACA GCG AAT TTT GAA
350 CCG CTC-3'; Reverse – 5'- TCC TCC CGG TCA AGT CTT TTT -3') and CDH11 (Forward –
351 5'-AGA GGT CCA ATG TGG GAA CG -3', Reverse: 5'- GGT TGT CCT TCG AGG ATA CTG
352 -3'). GAPDH was used as a reference gene (Genecopoeia Catalog#: HQP006940). qPCR was
353 performed with 2X All-in-One qPCR Mix (Genecopoeia Catalog#: QP001) using the following
354 reaction mix: 10µl All-in-One qPCR Mix (2x), 2 µl of 20µM primer, 5µl nuclease-free water, and
355 5 µl of cDNA at 1:5 dilution. Reactions were incubated at 95° for 10 minutes, followed by 40
356 cycles of 95° for 10 seconds, 60° for 20 seconds, and 72° for 30 seconds using the CFX96 Real-
357 time System (Bio Rad). Melt curves were generated after amplification by increasing temperature
358 from 72° to 95° at 0.5° increments. All reactions were performed in duplicates. Relative quantities
359 for each gene were analyzed using the comparative Ct method. Fold changes were calculated
360 relative to the average Ct values for control samples.

361

362 **Statistical analysis**

363 Statistical analysis was performed using Graph Pad Prism 8 software (GraphPad Prism Software,
364 RRID: SCR_002798). Unpaired two-tailed *t*-test was performed when comparing two groups and
365 one-way ANOVA with Tukey's or Dunnett's multiple comparison test was used to compare
366 differences between three or four groups. *P*-values were considered significant if ≤ 0.05 . Bar
367 graphs are displayed as mean \pm standard error of the mean (SEM).

368

369 **Results**

370 **Cadherin-8 and cadherin-11 show similar temporal and spatial expression patterns**

371 We compared the overall protein levels of classical type II cadherins cadherin-8 and cadherin-11
372 in C57BL/6 wild-type mouse whole brain samples taken at different ages of development from
373 embryonic day (E) 14 to postnatal day (P) 21, as well as in adulthood by Western blot (Figure 1A).
374 Cadherin-8 and cadherin-11 both exhibited relatively low expression at E14 but their levels
375 increased postnatally. Cadherin-8 levels readily increased at P1 and reached more than a 10-fold
376 increase by P7 and P14. The expression of Cadherin-8 dropped after P14 and remained at low
377 levels in adulthood (Figure 1B). Cadherin-11 levels reached an approximately 2-fold increase by
378 P7 and P14 compared to E14, but were reduced to P1 level by P21 and to E14 level by adulthood
379 (Figure 1B). Comparison to the developmental timeline showed that cadherin-8 and cadherin-11
380 expression correlated to time points during which processes of dendrite development and
381 synaptogenesis are prevalent, suggesting that they may play a roles in these processes. We then
382 analyzed the protein levels in specific brain regions at P14 (Figure 1C, D). Cadherin-8 expression
383 was significantly higher in the cortex, hippocampus and thalamus/striatum compared to the
384 cerebellum. Cadherin-11 showed a similar expression pattern, although differences between
385 regions were not statistically significant.

386 To further interrogate which brain cell type(s) express cadherins, protein levels of cadherin-
387 8 and cadherin-11 were measured in primary cortical neurons and glial cells cultured from mouse
388 cortices. The temporal expression patterns of cadherin-8 and cadherin-11 in primary cortical
389 neurons showed a gradual increase of both cadherins from 1 DIV to 14 DIV, recapitulating the
390 temporal expression profile observed in the mouse whole brain tissue (Supplementary Figure 3A,
391 B). In contrast to neurons, cadherin-8 was virtually undetectable in primary glial cells
392 (Supplementary Figure 3C). Cadherin-11 was expressed in both cortical neurons and glial cells,
393 but the protein levels in neurons were two-fold greater than in glial cells (Supplementary Figure
394 3D). These results demonstrate that both cadherins are preferentially expressed in neurons.

395

396 **Cadherin-8 is preferentially expressed in excitatory neurons and binds to neuroligin-1**

397 We next performed subcellular fractionation of forebrain tissue to isolate synaptic plasma
398 membrane (SPM) and postsynaptic density (PSD) to determine whether cadherins are enriched in
399 synaptic compartments (Figure 2A, B). The distribution of PSD-95, syntaxin-1, and β -actin were
400 evaluated to confirm successful separation and purity of different subcellular fractions. PSD-95
401 was used as a positive control for protein enrichment in SPM and PSD fractions - it was
402 significantly enriched in SPM and PSD fractions compared to total protein input (Figure 2B).
403 Syntaxin-1 was enriched in synaptosome (P3), SPM, and synaptic vesicles (S3), consistent with
404 its localization in the presynaptic membrane and its function in vesicle release. Cadherin-8 was
405 enriched 3.7-fold in the PSD fraction and cadherin-11 was enriched 2.6- and 3.4-fold in the SPM
406 and PSD fractions, respectively, compared to unfractionated lysate (Figure 2B).

407 Since cadherin-8 and cadherin-11 expression were both enriched in the PSD, we performed
408 co-immunoprecipitation (co-IP) experiments using anti-Cdh8 (Figure 2C) and anti-Cdh11
409 antibodies (Figure 2D) to determine the association of these two cadherins with specific
410 postsynaptic proteins. Both cadherin-8 and cadherin-11 immunoprecipitated β -catenin, the

411 conserved intracellular binding partner of classical cadherins (Seong et al., 2015). Neither
412 cadherin-8 nor cadherin-11 co-immunoprecipitated with PSD-95 or N-cadherin. Previous studies
413 found cooperative functions of classical cadherins with the excitatory synaptic adhesion molecule
414 neuroligin-1 (*Nlgn1*) during neural circuit development (Aiga, Levinson, & Bamji, 2011; Stan et
415 al., 2010; Yamagata, Duan, & Sanes, 2018). This prompted us to investigate whether cadherin-8
416 and cadherin-11 physically interact with neuroligin-1. Interestingly, neuroligin-1 was detected in
417 the immunoprecipitate of cadherin-8, but not cadherin-11. We confirmed this interaction *in vitro*
418 by co-expressing myc-tagged *Cdh8* together with HA-tagged *Nlgn1* in N2a cells that do not
419 express either protein (Supplementary Figure 4). Using either anti-HA or anti-myc antibodies to
420 selectively pull down neuroligin-1 and cadherin-8, respectively, we found that neuroligin-1 co-
421 immunoprecipitated cadherin-8 and vice versa. Consistent with these findings, immunostaining of
422 N2a cells overexpressing myc-tagged *Cdh8* and HA-tagged *Nlgn1* showed a partial co-localization
423 of these two proteins at the plasma membrane and at cell-cell contacts (Figure 2E).

424 The finding of an interaction of cadherin-8 with neuroligin-1 prompted us to further
425 examine the subcellular localization of cadherin-8 in neurons. The specificity of the anti-cadherin-
426 8 antibody was first validated in immunocytochemistry (Supplementary Figure 1C).
427 Immunofluorescence of surface-exposed cadherin-8 in 15 DIV hippocampal neurons revealed
428 partial co-localization with the excitatory synaptic markers PSD-95 and synapsin-1 (Figure 3A).
429 The majority of cadherin-8 localized to excitatory synaptic puncta, either co-localizing with PSD-
430 95 ($30.3\% \pm 2.0\%$) or located directly adjacent to PSD-95-positive puncta ($41.6\% \pm 2.5\%$), while
431 the remainder of cadherin-8 puncta ($28.1\% \pm 2.2\%$) did not localize to PSD-95-positive puncta
432 (Figure 3C). Hence, most, but not all, cadherin-8-positive puncta are associated with synapses.
433 Conversely, the majority of the cadherin-8-positive puncta did not associate with GAT-1-positive
434 puncta, markers of inhibitory synapses ($56.8\% \pm 3.4\%$; Figure 3B and D). However, a small
435 fraction of cadherin-8-positive puncta exhibited co-localization with ($10.0\% \pm 1.8\%$) or were
436 adjacent to GAT-1-positive puncta ($33.2\% \pm 2.6\%$). These data suggest that cadherin-8 is enriched
437 at/near excitatory synapses compared to inhibitory synapses, consistent with the potential role in
438 excitatory synaptic function. We performed direct stochastic reconstruction microscopy
439 (dSTORM) to further quantify cadherin-8 presence at excitatory synapses. We examined the
440 distance between PSD-95 and either cadherin-8 or neuroligin-1, a positive control for excitatory
441 synaptic localization (Figure 3E, F). For optimal two-color imaging, we used an Oxyfluor buffer
442 system (Nahidiazar et al., 2016). $33.3\% \pm 3.3\%$ of cadherin-8 puncta were located within 50 nm
443 of a PSD-95 puncta, significantly more than a randomized set of data points ($13.7\% \pm 1.0\%$; Figure
444 3G, I, J). This recapitulates the co-localization data obtained using standard confocal microscopy.
445 Interestingly, dSTORM imaging showed that significantly more neuroligin-1 puncta were within
446 50 nm of a PSD-95 punctum ($52\% \pm 3.8\%$) compared to cadherin-8 puncta and the randomized
447 data points (Figure 3H, I, J). Together, these data indicate that cadherin-8 localizes to excitatory
448 synapses but to a lesser extent than neuroligin-1. These findings are in line with the localization of
449 cadherin-8 and neuroligin-1 in N2a cells suggesting that these two proteins partially but not
450 completely localize to the same cellular compartment.

451

452 **Elevated cadherin-8 expression in *Cdh11*^{-/-} mice is accompanied by an increase of excitatory**
453 **synaptic proteins and dendrite complexity**

454 Our results suggest that cadherin-8 and cadherin-11 share partially overlapping expression
455 patterns. Alterations in excitatory synapse development is one of the hallmarks of autism (Forrest,
456 Parnell, & Penzes, 2018; Hutsler & Zhang, 2010). The *Cdh11* knockout mouse provided a means
457 to further examine the relationship between cadherin-8 and cadherin-11 and their potential
458 contributions to excitatory synaptic development (Horikawa et al., 1999). Western blot analysis
459 from P7 whole brain tissue confirmed that cadherin-11 was not detected in *Cdh11*^{-/-} brains (Figure
460 4A, B), while levels of cadherin-8 were significantly increased in *Cdh11*^{-/-} brains (Figure 4A, C).
461 This result indicates that cadherin-8 may exert a compensatory function in the absence of cadherin-
462 11.

463 Since we identified neuroligin-1 as a selective binding partner of cadherin-8, and
464 neuroligin-1 has been shown to be selectively involved in the development of excitatory synapses
465 (Song, Ichtchenko, Südhof, & Brose, 1999), we reasoned that neuroligin-1 levels may be altered
466 in *Cdh11*^{-/-} brains. Indeed, Western blot analysis from P7 *Cdh11*^{-/-} whole brain lysates showed
467 that neuroligin-1 was significantly increased compared to wild-type littermates (Figure 4D, E).
468 PSD-95 expression was also significantly increased in *Cdh11*^{-/-} brains (Figure 4D, F). In contrast,
469 there was no difference in levels of the inhibitory synaptic marker gephyrin (Figure 4D, G). We
470 then examined the density of dendritic spines, the major structures that harbor excitatory synapses,
471 in 15 DIV hippocampal cultures from *Cdh11* wild-type and knockout mice (Figure 4H).
472 Intriguingly, we did not observe changes in the dendritic spine density on primary, secondary and
473 tertiary dendrites of *Cdh11*^{-/-} neurons compared to wild-type neurons (Figure 4I; Primary – WT:
474 $0.7664 \pm 0.0301 \mu\text{m}^{-1}$, KO: $0.7086 \pm 0.0428 \mu\text{m}^{-1}$; Secondary – WT: $0.7070 \pm 0.0270 \mu\text{m}^{-1}$, KO:
475 $0.6527 \pm 0.0301 \mu\text{m}^{-1}$; Tertiary – WT: $0.6051 \pm 0.0265 \mu\text{m}^{-1}$, KO: $0.6440 \pm 0.0337 \mu\text{m}^{-1}$; Total –
476 WT: $0.6778 \pm 0.0194 \mu\text{m}^{-1}$, KO: $0.6607 \pm 0.0299 \mu\text{m}^{-1}$). We next analyzed the dendritic
477 morphology of *Cdh11* knockout and wild-type neurons from 15 DIV hippocampal cultures. Sholl
478 analysis revealed an increase in the dendritic arbor complexity in *Cdh11*^{-/-} neurons (area under
479 the curve (AUC): $3,051 \pm 156$) compared to wild-type neurons (Figure 4J, K; AUC: $2,551 \pm 153.1$).
480 The increase in dendritic complexity resulted from a significant increase in total dendrite length
481 (Figure 4L; WT: $2,509 \pm 174 \mu\text{m}$, KO: $3,034 \pm 162.7 \mu\text{m}$) as well as branches as revealed by
482 branch tip number (Figure 4M; WT: 29.17 ± 2.194 , KO: 35.4 ± 1.48). These data suggest that the
483 overall increased dendritic complexity of *Cdh11* knockout neurons may result in an increase of
484 excitatory postsynaptic sites without changing the spine density.

485

486 ***Cdh11*^{-/-} mice exhibit altered calcium activity and miniature excitatory postsynaptic currents**

487 The increase in excitatory synaptic protein expression in *Cdh11*^{-/-} brains and the increased
488 dendritic arborization in *Cdh11*^{-/-} neurons prompted us to investigate whether deletion of *Cdh11*
489 results in changes in neuronal and synaptic activity. We first transduced hippocampal neurons
490 prepared from *Cdh11* knockout and wild-type mice with the NeuroBurst Orange lentivirus
491 (EssenBioscience Sartorius) and imaged network calcium activity using the InCuCyte S3 Live-Cell
492 Analysis System for Neuroscience (EssenBioscience Sartorius) at 15 DIV (Figure 5A) for 24
493 hours. Interestingly, *Cdh11* knockout cultures showed significantly fewer active neurons
494 compared to wild-type cultures (Figure 5B; WT: $1,756 \pm 109.9$, KO: $1,238 \pm 159.1$), although
495 there was no difference in total cell number or infection rate between genotypes (Supplementary

496 Figure 5). In addition, the activity of *Cdh11*^{-/-} neurons was significantly less correlated when
497 compared to wild-type neurons (Figure 5C; WT: 1.405 ± 0.0681 , KO: 1.111 ± 0.0657). *Cdh11*^{-/-}
498 neurons further exhibited significantly reduced mean burst strengths (Figure 5D; WT: $0.6473 \pm$
499 0.0507 , KO: 0.4163 ± 0.0324), whereas mean burst rate was similar in *Cdh11* knockout and wild-
500 type cultures (Figure 5E; WT: $3.643 \pm 0.9519 \text{ min}^{-1}$, KO: $4.983 \pm 1.424 \text{ min}^{-1}$). Together, these
501 data show that *Cdh11* knockout neurons exhibit a significant reduction in neuronal activity
502 compared to wild-type neurons.

503 Because of the altered dendritic morphology and changes in calcium activity in *Cdh11*
504 knockout cultures, we next asked whether deletion of cadherin-11 affects the function of
505 hippocampal synapses in the whole organism. We used *ex vivo* hippocampal slices to examine the
506 effects of *Cdh11* knockout on AMPAR-mediated miniature excitatory postsynaptic currents
507 (mEPSCs) in CA1 pyramidal cells from wild-type and knockout mice aged P21-P24 (Figure 5F,
508 G). We found that miniature EPSC frequency was significantly lower in CA1 pyramidal cells from
509 *Cdh11* knockout mice compared to wild-type littermates (Figure 5H; WT: $1.16 \pm 0.1254 \text{ Hz}$, KO:
510 $0.5026 \pm 0.0445 \text{ Hz}$). Conversely, mEPSC amplitude was higher in *Cdh11* knockout neurons
511 (Figure 5I; WT: $11.28 \pm 0.4684 \text{ pA}$, KO: $14.12 \pm 1.078 \text{ pA}$). Analysis of the kinetics of CA1
512 mEPSCs found that there was no difference in the charge (Figure 5J; WT: $76.92 \pm 4.035 \text{ fC}$, KO:
513 $91.56 \pm 6.325 \text{ fC}$), rise time (Figure 5K; WT: $1.355 \pm 0.044 \text{ ms}$, KO: $1.355 \pm 0.064 \text{ ms}$) or decay
514 time constant between *Cdh11* knockout and wild-type mice (Figure 5L; WT: $4.940 \pm 0.194 \text{ ms}$,
515 KO: $5.091 \pm 0.228 \text{ ms}$). These data, demonstrating a reduction of mEPSC frequency and a
516 concurrent increase in mEPSC amplitude in *Cdh11* knockout hippocampus, supports the important
517 role of cadherin-11 in the formation and maintenance of synaptic networks. Combined with our
518 earlier data from cultured hippocampal neurons, the data suggest that loss of cadherin-11 results
519 in disturbed synaptic transmission potentially by reduced functional synaptic input to postsynaptic
520 cells.

521

522 **CDH8 and CDH11 levels are altered in autism-specific iPSC-derived cortical neuronal** 523 **progenitor cells and organoids**

524 As autism spectrum disorder is a genetically complex condition with broad heterogeneity across
525 individuals, we investigated the expression of CDH8 and CDH11 in human tissue using samples
526 from autistic and typically-developing control individuals to determine whether the levels of these
527 cadherins are commonly altered in autism. iPSC-derived cortical neural progenitor cells (NPCs)
528 from four neurotypical control and eight autistic individuals were cultured for 19 DIV and cell
529 lysates were harvested for protein analysis (Table 2; Figure 6A, B). Although none of these
530 individuals carried risk variants in either of these cadherins (DeRosa et al., 2018), CDH8 levels
531 were significantly increased (Figure 6A) whereas CDH11 levels were significantly decreased
532 (Figure 6B) in iPSC-derived cortical NPCs from autistic individuals compared to neurotypical
533 control cells. This expression pattern recapitulated the expression profile observed in the *Cdh11*
534 knockout mice and supported the use of this mouse to investigate potential mechanistic alterations
535 occurring in autism. We next performed quantitative PCR (qPCR) to analyze mRNA expression
536 of CDH8 and CDH11 in iPSC-derived cortical organoids generated from autistic and typically-
537 developing control individuals that were grown in culture for 60 DIV (Table 2; Figure 6C, D).
538 These cortical organoids mimic early cortical development with active excitatory and inhibitory
539 neurotransmission (Durens et al., 2020). In line with the expression profile in the NPCs, autism-

Cadherins in Development and Autism

540 specific iPSC-derived cortical organoids showed a significant increase of CDH8 (Figure 6C) and
541 a concomitant decrease of CDH11 (Figure 6D) compared to neurotypical control organoids.
542 Together, these results show that cadherin expression levels are altered in cells derived from
543 individuals with autism during early stages of neural circuit development. The differences in
544 expression between the autism-specific iPSC-derived neurons and neurotypical control neurons
545 suggest a potential role of CDH8 and CDH11 in autism pathophysiology.

546 Discussion

547 In this study, we used mouse tissues to systematically investigate the expression, localization and
548 function of the autism candidate risk genes cadherin-8 and cadherin-11. The comprehensive
549 expression analysis revealed that both of these type II classical cadherins are expressed in the
550 developing mouse brain at the right time and right place to regulate crucial steps in neural circuit
551 formation and to impact normal brain development. The analysis of *Cdh11*^{-/-} mouse brains further
552 suggests putative mechanisms to explain cellular and circuitry phenotypes observed in autism.
553 Expression analysis of cadherin-8 and cadherin-11 in NPCs and mature organoids generated from
554 autistic individuals demonstrate that levels of these two cadherins are altered throughout early
555 neural circuit development in autism. Together, our study not only reveals a detailed and novel
556 characterization of the autism-risk cadherins cadherin-8 and cadherin-11, but also provides
557 insights into the potential contributions of these cadherins in neural circuit formation and the
558 neurodevelopment of autism.

559 The cadherin superfamily consists of numerous subfamily members with similar protein
560 structures but distinct functions. Both, cadherin-8 and cadherin-11 belong to the same classical
561 type II cadherin subfamily. In addition to a similar structure, several studies have found similar
562 expression profiles and functions of cadherin-8 and cadherin-11 in the brain. Cadherin-8 is
563 important for synaptic targeting in the hippocampus (Bekirov, Nagy, Svoronos, Huntley, &
564 Benson, 2008), striatum (Friedman, Riemslagh, et al., 2015), and visual system (Duan,
565 Krishnaswamy, De la Huerta, & Sanes, 2014; Osterhout et al., 2011). Cadherin-11 is also strongly
566 expressed in the hippocampus and functions at glutamatergic synapses (Bartelt-Kirbach, Langer-
567 Fischer, & Golenhofen, 2010; Manabe et al., 2000; Paradis et al., 2007). In agreement with these
568 studies, our spatial expression analysis showed that cadherin-8 and cadherin-11 are enriched in
569 similar brain regions including cortex, hippocampus, and thalamus/striatum. The temporal
570 expression analysis showed that both cadherins exhibit peak expression in postnatal brains at the
571 time window that coincides with dendrite development and synaptogenesis, processes that have
572 been adversely implicated in the developing autism brain (Betancur et al., 2009; Bourgeron, 2009;
573 Hussman et al., 2011). In particular, cadherin-8 exhibited a dramatic but transient increase from
574 embryonic stage to the first postnatal week. Protein levels quickly declined later in development
575 and remained low in adulthood. This surge in expression strongly indicates a specific role for
576 cadherin-8 in regulating dendrite and synapse development. In contrast, cadherin-11 expression
577 levels were relatively steady before and after the peak increase at an early postnatal age. This
578 overlooked difference between cadherin-8 and cadherin-11 may indicate at least partially divergent
579 signaling pathways and functions mediated by these two closely related cadherins.

580 In addition, we found neuroligin-1 as a selective interaction partner of cadherin-8, but not
581 of cadherin-11. Although cadherin-8 levels were consistently increased in the absence of cadherin-
582 11, potentially due to compensation, the selective interaction between cadherin-8 and neuroligin-
583 1 may explain why the loss of cadherin-11 results in altered cellular and physiological phenotypes.
584 Morphometric analysis of *Cdh11* knockout neurons revealed an increased complexity of dendritic
585 arbors. This may be due to the increase of cadherin-8, as knockdown of cadherin-8 in rat cortical
586 neurons has been shown to impair dendritic arborization (Friedman, Riemslagh, et al., 2015).
587 Subsequently, the increase of dendrites may provide more available potential synaptic contacts
588 even though the density of dendritic spines is not changed. The increases of PSD-95 and
589 neuroligin-1 reflect an increase in potential excitatory postsynaptic sites, where cadherin-8 may
590 also regulate synaptic function by interacting with neuroligin-1.

Cadherins in Development and Autism

591 The increase of excitatory postsynaptic sites coupled with a reduction in calcium activity,
592 as indicated by the decreased correlation of activity and fewer active cells, as well as the reduction
593 in mEPSC frequency in *Cdh11* knockout mice suggests disruption of functional synaptic input
594 onto postsynaptic neurons. As a compensatory mechanism, neurons may increase postsynaptic
595 strength, as shown by the higher mEPSC amplitude, and search for more synaptic input, as shown
596 by the increased dendritic arborization and the increase in postsynaptic markers, in an effort to
597 reach homeostatic levels of input and activity. In line with this interpretation, calcium-imaging
598 further showed that *Cdh11* knockout neurons exhibited significantly reduced mean burst strengths,
599 reflecting an overall decrease in somatic calcium influx and potentially reduced neuronal activity.
600 Interestingly, *Cdh11* and *Nlgn1* knockout mice both exhibit behavioral deficits similar to
601 phenotypes observed in autism (Blundell et al., 2010; Wu et al., 2020), although *Nlgn1* knockout
602 animals exhibit impairment of excitatory synaptic function (Chanda, Hale, Zhang, Wernig, &
603 Südhof, 2017; Chubykin et al., 2007). This suggests that alterations in the number of excitatory
604 synapses in any direction is expected to disrupt the balance of excitation and inhibition in neuronal
605 circuitry, a common phenotype that has been observed in autism (Blatt et al., 2001; Gao & Penzes,
606 2015; Hussman, 2001). Further investigations are required to help elucidate a potential mechanism
607 underlying the altered synaptic activity in *Cdh11* knockout brains.

608 Members of the cadherin superfamily have emerged as candidate risk genes for autism in
609 multiple independent association studies (Camacho et al., 2012; Chapman et al., 2011; Crepel et
610 al., 2014; Cukier et al., 2014; Depienne et al., 2009; Girirajan et al., 2013; Hussman et al., 2011;
611 Kenny et al., 2014; Marshall et al., 2008; Morrow et al., 2008; Neale et al., 2012; O'Roak et al.,
612 2012; Pagnamenta et al., 2011; Sanders et al., 2011; van Harssel et al., 2013; K. Wang et al., 2009;
613 Willemsen et al., 2010). Despite these findings, no autism case reported to date has resulted from
614 monogenic alterations in cadherins. In fact, none of the individuals with autism tested in this study
615 harbor damaging mutations in the *CDH8* or *CDH11* genes (DeRosa et al., 2018). Yet, these
616 individuals consistently show altered expression patterns in *CDH8* and *CDH11* throughout neural
617 development from neural precursor cells to more mature organoids. This suggests that a pathway
618 involving *CDH8* and *CDH11* may be vulnerable in autism and that the balance of these two
619 cadherins may be important for normal brain function. In addition to specific autism-associated
620 mutations in cadherins, these molecules may be part of a broader pathway involving other genes
621 mutated in autism. Indeed, some of the autism lines examined in this study were also reported to
622 display misregulation of genes involved in cell-cell signaling and actin cytoskeleton signaling,
623 both processes that involve cadherins (DeRosa et al., 2018). The overall decrease in synaptic
624 activity that we observed in the *Cdh11* knockout neurons is consistent with the phenotypic
625 characterization of the iPSC-derived cortical neurons from autistic individuals performed by
626 DeRosa et al., as they reported decreased spontaneous spiking activity, as well as decreased
627 number of calcium transients in these neurons. The autism-specific iPSC-derived cortical neurons
628 also exhibited decreased migration of neuronal processes (DeRosa et al., 2018). Interestingly,
629 elevated expression of *CDH11* has been observed in glioblastoma and has been associated with
630 increased cell migration (Schulte et al., 2013). Thus, the reduced migration ability of the autism-
631 specific neurons may stem from the decreased *CDH11* expression that we observed in these autism
632 lines. These findings further emphasize the significance of our effort to investigate the involvement
633 of *CDH8* and *CDH11* in autism.

634 To our knowledge, our study is the first to show that cadherin levels are altered in cells
635 derived from individuals with autism. Based on the known functions of cadherins, both lower and

636 higher expression levels could directly affect neuronal connectivity by altering cell-cell adhesion,
637 downstream signaling and consequently synaptic function. The findings of this study strengthen
638 the hypothesis that cadherin signaling may represent an important pathway affected in autism. Our
639 current study suggests that excitatory neuronal development is a common function mediated by
640 cadherin-8 and cadherin-11. However, upstream factors affecting the expression of cadherin-8 and
641 cadherin-11 still require further investigation. Future studies should also aim to identify
642 mechanisms that connect different cadherins to other synaptic cell adhesion molecules. Such a
643 potential connection is described here between cadherin-8 and neuroligin-1, another autism risk
644 candidate adhesion molecule (Nakanishi et al., 2017). In conclusion, our study shows that
645 cadherin-8 and cadherin-11 play an important role in regulating excitatory neuronal development,
646 and that altered expression of these two cadherins likely contributes in part to the etiology of
647 autism.

648

649 **Funding**

650 This work is supported by Hussman Foundation grants HIAS#15007 and HIAS#18005 to JAF,
651 HIAS#18001 to SH, and HIAS#15003 and HIAS#18004 to YCL.

652

653 **Conflict of Interest**

654 The authors declare that the research was conducted in the absence of any commercial or financial
655 relationships that could be construed as a potential conflict of interest.

656

657 **Acknowledgements**

658 We want to thank Dr. Celine Plachez at the Hussman Institute for Autism for the technical support.
659 We also want to thank Drs. Louis DeTolla and Turhan Coksaygan at the University of Maryland
660 School of Medicine for providing veterinary services and consultation. We thank Dr. Olga
661 Latinovic from the imaging core at the Institute of Human Virology at the University of Maryland
662 School of Medicine for her help and assistance with the dSTORM imaging. Finally, we thank Drs.
663 John Hussman, Thomas Blanpied, and Anthony Koleske for the critical evaluation of this
664 manuscript.

665

666 **Author contribution statement**

667 JF, YL: Conceptualization. JF, RN, MB, MD, JN, XY, MN, SH, YL: Methodology. JF, RN, MB,
668 MD, YL: Investigation. JF, RN, MB, MD, YL: Formal Analysis. JF, YL: Writing – Original draft.
669 JF, RN, MB, MD, JN, XY, DD, MN, SH, GB, YL: Writing – Review and Editing. JF, YL:
670 Visualization. DD, MN, SH, GB, YL: Supervision. JF, YL: Project Administration. JF, SH, YL:
671 Funding Acquisition.

672

673 **Contribution to the field**

Cadherins in Development and Autism

674 Autism is a neurodevelopmental condition with high genetic and phenotypic heterogeneity.
675 Multiple genes have been implicated in autism. Among them, the cadherin superfamily of adhesion
676 molecules represent significant numbers of candidates. This study implicates roles of the classical
677 type II cadherins, cadherin-8 and cadherin-11, in the development of neural circuits and provides
678 novel insights into the association of these two cell adhesion molecules with autism. First, the
679 temporal and spatial expression pattern suggests that these two cadherins might act as regulators
680 of crucial steps during normal development of the nervous system, such as dendrite development
681 and synaptogenesis. Second, loss of cadherin-11 changes the expression level of not only cadherin-
682 8 and other proteins associated with excitatory synapses but also alters the morphology and activity
683 of excitatory neurons. Third, altered expression of cadherin-8 and cadherin-11 in human tissue
684 samples of autistic individuals suggests a direct effect of these molecules on neuronal function in
685 humans. Taken together, this study reveals novel contributions of two classical type II cadherins
686 in neural circuit formation and strengthens the hypothesis that they may contribute to aspects of
687 autism pathophysiology.

688

689 **Data availability statement**

690 The dataset supporting the conclusions of this study are available upon reasonable request by the
691 corresponding authors.

692

693 **References**

- 694 American Psychiatric Association (2013). Diagnostic and Statistical Manual of Mental Disorders,
695 5th Edn. Arlington, VA: American Psychiatric Publishing
- 696 Aiga, M., Levinson, J. N., & Bamji, S. X. (2011). N-cadherin and neuroligins cooperate to
697 regulate synapse formation in hippocampal cultures. *J Biol Chem*, *286*(1), 851-858.
698 doi:10.1074/jbc.M110.176305
- 699 Arikath, J., & Reichardt, L. F. (2008). Cadherins and catenins at synapses: roles in
700 synaptogenesis and synaptic plasticity. *Trends Neurosci*, *31*(9), 487-494.
701 doi:10.1016/j.tins.2008.07.001
- 702 Baio, J., Wiggins, L., Christensen, D. L., Maenner, M. J., Daniels, J., Warren, Z., . . . Dowling,
703 N. F. (2018). Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years -
704 Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States,
705 2014. *MMWR Surveill Summ*, *67*(6), 1-23. doi:10.15585/mmwr.ss6706a1
- 706 Bartelt-Kirbach, B., Langer-Fischer, K., & Golenhofen, N. (2010). Different regulation of N-
707 cadherin and cadherin-11 in rat hippocampus. *Cell Commun Adhes*, *17*(4-6), 75-82.
708 doi:10.3109/15419061.2010.549977
- 709 Basu, R., Taylor, M. R., & Williams, M. E. (2015). The classic cadherins in synaptic specificity.
710 *Cell Adh Migr*, *9*(3), 193-201. doi:10.1080/19336918.2014.1000072
- 711 Bekirov, I. H., Nagy, V., Svoronos, A., Huntley, G. W., & Benson, D. L. (2008). Cadherin-8 and
712 N-cadherin differentially regulate pre- and postsynaptic development of the hippocampal
713 mossy fiber pathway. *Hippocampus*, *18*(4), 349-363. doi:10.1002/hipo.20395
- 714 Bermejo, M. K., Milenkovic, M., Salahpour, A., & Ramsey, A. J. (2014). Preparation of synaptic
715 plasma membrane and postsynaptic density proteins using a discontinuous sucrose
716 gradient. *J Vis Exp*(91), e51896. doi:10.3791/51896
- 717 Betancur, C., Sakurai, T., & Buxbaum, J. D. (2009). The emerging role of synaptic cell-adhesion
718 pathways in the pathogenesis of autism spectrum disorders. *Trends Neurosci*, *32*(7), 402-
719 412. doi:10.1016/j.tins.2009.04.003
- 720 Blatt, G. J., Fitzgerald, C. M., Guptill, J. T., Booker, A. B., Kemper, T. L., & Bauman, M. L.
721 (2001). Density and distribution of hippocampal neurotransmitter receptors in autism: an
722 autoradiographic study. *J Autism Dev Disord*, *31*(6), 537-543.
- 723 Blundell, J., Blaiss, C. A., Etherton, M. R., Espinosa, F., Tabuchi, K., Walz, C., . . . Powell, C.
724 M. (2010). Neuroligin-1 deletion results in impaired spatial memory and increased
725 repetitive behavior. *J Neurosci*, *30*(6), 2115-2129. doi:10.1523/JNEUROSCI.4517-
726 09.2010
- 727 Bourgeron, T. (2009). A synaptic trek to autism. *Curr Opin Neurobiol*, *19*(2), 231-234.
728 doi:10.1016/j.conb.2009.06.003
- 729 Bourgeron, T. (2015). From the genetic architecture to synaptic plasticity in autism spectrum
730 disorder. *Nat Rev Neurosci*, *16*(9), 551-563. doi:10.1038/nrn3992
- 731 Camacho, A., Simón, R., Sanz, R., Viñuela, A., Martínez-Salio, A., & Mateos, F. (2012).
732 Cognitive and behavioral profile in females with epilepsy with PDCH19 mutation: two
733 novel mutations and review of the literature. *Epilepsy Behav*, *24*(1), 134-137.
734 doi:10.1016/j.yebeh.2012.02.023
- 735 Chanda, S., Hale, W. D., Zhang, B., Wernig, M., & Südhof, T. C. (2017). Unique versus
736 Redundant Functions of Neuroligin Genes in Shaping Excitatory and Inhibitory Synapse
737 Properties. *J Neurosci*, *37*(29), 6816-6836. doi:10.1523/JNEUROSCI.0125-17.2017

- 738 Chapman, N. H., Estes, A., Munson, J., Bernier, R., Webb, S. J., Rothstein, J. H., . . . Wijsman,
739 E. M. (2011). Genome-scan for IQ discrepancy in autism: evidence for loci on
740 chromosomes 10 and 16. *Hum Genet*, *129*(1), 59-70. doi:10.1007/s00439-010-0899-z
- 741 Chen, J., Yu, S., Fu, Y., & Li, X. (2014). Synaptic proteins and receptors defects in autism
742 spectrum disorders. *Front Cell Neurosci*, *8*, 276. doi:10.3389/fncel.2014.00276
- 743 Chih, B., Gollan, L., & Scheiffele, P. (2006). Alternative splicing controls selective trans-
744 synaptic interactions of the neuroligin-neurexin complex. *Neuron*, *51*(2), 171-178.
745 doi:10.1016/j.neuron.2006.06.005
- 746 Chubykin, A. A., Atasoy, D., Etherton, M. R., Brose, N., Kavalali, E. T., Gibson, J. R., &
747 Südhof, T. C. (2007). Activity-dependent validation of excitatory versus inhibitory
748 synapses by neuroligin-1 versus neuroligin-2. *Neuron*, *54*(6), 919-931.
749 doi:10.1016/j.neuron.2007.05.029
- 750 Crepel, A., De Wolf, V., Brison, N., Ceulemans, B., Wallegghem, D., Peuteman, G., . . . Peeters,
751 H. (2014). Association of CDH11 with non-syndromic ASD. *Am J Med Genet B*
752 *Neuropsychiatr Genet*, *165B*(5), 391-398. doi:10.1002/ajmg.b.32243
- 753 Cukier, H. N., Dueker, N. D., Slifer, S. H., Lee, J. M., Whitehead, P. L., Lalanne, E., . . . Pericak-
754 Vance, M. A. (2014). Exome sequencing of extended families with autism reveals genes
755 shared across neurodevelopmental and neuropsychiatric disorders. *Mol Autism*, *5*(1), 1.
756 doi:10.1186/2040-2392-5-1
- 757 Depienne, C., Bouteiller, D., Keren, B., Cheuret, E., Poirier, K., Trouillard, O., . . . Leguern, E.
758 (2009). Sporadic infantile epileptic encephalopathy caused by mutations in PCDH19
759 resembles Dravet syndrome but mainly affects females. *PLoS Genet*, *5*(2), e1000381.
760 doi:10.1371/journal.pgen.1000381
- 761 DeRosa, B. A., El Hokayem, J., Artimovich, E., Garcia-Serje, C., Phillips, A. W., Van Booven,
762 D., . . . Dykxhoorn, D. M. (2018). Convergent Pathways in Idiopathic Autism Revealed
763 by Time Course Transcriptomic Analysis of Patient-Derived Neurons. *Sci Rep*, *8*(1),
764 8423. doi:10.1038/s41598-018-26495-1
- 765 DeRosa, B. A., Van Baaren, J. M., Dubey, G. K., Lee, J. M., Cuccaro, M. L., Vance, J. M., . . .
766 Dykxhoorn, D. M. (2012). Derivation of autism spectrum disorder-specific induced
767 pluripotent stem cells from peripheral blood mononuclear cells. *Neurosci Lett*, *516*(1), 9-
768 14. doi:10.1016/j.neulet.2012.02.086
- 769 Duan, X., Krishnaswamy, A., De la Huerta, I., & Sanes, J. R. (2014). Type II cadherins guide
770 assembly of a direction-selective retinal circuit. *Cell*, *158*(4), 793-807.
771 doi:10.1016/j.cell.2014.06.047
- 772 Durens, M., Nestor, J., Williams, M., Herold, K., Niescier, R. F., Lunden, J. W., . . . Nestor, M.
773 W. (2020). High-throughput screening of human induced pluripotent stem cell-derived
774 brain organoids. *J Neurosci Methods*, *335*, 108627. doi:10.1016/j.jneumeth.2020.108627
- 775 Ferreira, T. A., Blackman, A. V., Oyrer, J., Jayabal, S., Chung, A. J., Watt, A. J., . . . van Meyel,
776 D. J. (2014). Neuronal morphometry directly from bitmap images. *Nat Methods*, *11*(10),
777 982-984. doi:10.1038/nmeth.3125
- 778 Forrest, M. P., Parnell, E., & Penzes, P. (2018). Dendritic structural plasticity and
779 neuropsychiatric disease. *Nat Rev Neurosci*, *19*(4), 215-234. doi:10.1038/nrn.2018.16
- 780 Friedman, L. G., Benson, D. L., & Huntley, G. W. (2015). Cadherin-based transsynaptic
781 networks in establishing and modifying neural connectivity. *Curr Top Dev Biol*, *112*,
782 415-465. doi:10.1016/bs.ctdb.2014.11.025

- 783 Friedman, L. G., Riemsлагh, F. W., Sullivan, J. M., Mesias, R., Williams, F. M., Huntley, G. W.,
784 & Benson, D. L. (2015). Cadherin-8 expression, synaptic localization, and molecular
785 control of neuronal form in prefrontal corticostriatal circuits. *J Comp Neurol*, 523(1), 75-
786 92. doi:10.1002/cne.23666
- 787 Gao, R., & Penzes, P. (2015). Common mechanisms of excitatory and inhibitory imbalance in
788 schizophrenia and autism spectrum disorders. *Curr Mol Med*, 15(2), 146-167.
- 789 Girirajan, S., Dennis, M. Y., Baker, C., Malig, M., Coe, B. P., Campbell, C. D., . . . Eichler, E. E.
790 (2013). Refinement and discovery of new hotspots of copy-number variation associated
791 with autism spectrum disorder. *Am J Hum Genet*, 92(2), 221-237.
792 doi:10.1016/j.ajhg.2012.12.016
- 793 Gu, Y., & Haganir, R. L. (2016). Identification of the SNARE complex mediating the exocytosis
794 of NMDA receptors. *Proc Natl Acad Sci U S A*, 113(43), 12280-12285.
795 doi:10.1073/pnas.1614042113
- 796 Hirano, S., & Takeichi, M. (2012). Cadherins in brain morphogenesis and wiring. *Physiol Rev*,
797 92(2), 597-634. doi:10.1152/physrev.00014.2011
- 798 Horikawa, K., Radice, G., Takeichi, M., & Chisaka, O. (1999). Adhesive subdivisions intrinsic
799 to the epithelial somites. *Dev Biol*, 215(2), 182-189. doi:10.1006/dbio.1999.9463
- 800 Huguet, G., Ey, E., & Bourgeron, T. (2013). The genetic landscapes of autism spectrum
801 disorders. *Annu Rev Genomics Hum Genet*, 14, 191-213. doi:10.1146/annurev-genom-
802 091212-153431
- 803 Hulpiau, P., & van Roy, F. (2009). Molecular evolution of the cadherin superfamily. *Int J*
804 *Biochem Cell Biol*, 41(2), 349-369. doi:10.1016/j.biocel.2008.09.027
- 805 Hussman, J. P. (2001). Suppressed GABAergic inhibition as a common factor in suspected
806 etiologies of autism. *J Autism Dev Disord*, 31(2), 247-248.
- 807 Hussman, J. P., Chung, R. H., Griswold, A. J., Jaworski, J. M., Salyakina, D., Ma, D., . . .
808 Pericak-Vance, M. A. (2011). A noise-reduction GWAS analysis implicates altered
809 regulation of neurite outgrowth and guidance in autism. *Mol Autism*, 2(1), 1.
810 doi:10.1186/2040-2392-2-1
- 811 Hutsler, J. J., & Zhang, H. (2010). Increased dendritic spine densities on cortical projection
812 neurons in autism spectrum disorders. *Brain Res*, 1309, 83-94.
813 doi:10.1016/j.brainres.2009.09.120
- 814 Inoue, T., Tanaka, T., Suzuki, S. C., & Takeichi, M. (1998). Cadherin-6 in the developing mouse
815 brain: expression along restricted connection systems and synaptic localization suggest a
816 potential role in neuronal circuitry. *Dev Dyn*, 211(4), 338-351. doi:10.1002/(SICI)1097-
817 0177(199804)211:4<338::AID-AJA5>3.0.CO;2-I
- 818 Jeste, S. S., & Geschwind, D. H. (2014). Disentangling the heterogeneity of autism spectrum
819 disorder through genetic findings. *Nat Rev Neurol*, 10(2), 74-81.
820 doi:10.1038/nrneurol.2013.278
- 821 Joensuu, M., Lanoue, V., & Hotulainen, P. (2017). Dendritic spine actin cytoskeleton in autism
822 spectrum disorder. *Prog Neuropsychopharmacol Biol Psychiatry*.
823 doi:10.1016/j.pnpbp.2017.08.023
- 824 Kenny, E. M., Cormican, P., Furlong, S., Heron, E., Kenny, G., Fahey, C., . . . Morris, D. W.
825 (2014). Excess of rare novel loss-of-function variants in synaptic genes in schizophrenia
826 and autism spectrum disorders. *Mol Psychiatry*, 19(8), 872-879.
827 doi:10.1038/mp.2013.127

- 828 Korematsu, K., & Redies, C. (1997). Expression of cadherin-8 mRNA in the developing mouse
829 central nervous system. *J Comp Neurol*, *387*(2), 291-306.
- 830 Lin, Y. C., Frei, J. A., Kilander, M. B., Shen, W., & Blatt, G. J. (2016). A Subset of Autism-
831 Associated Genes Regulate the Structural Stability of Neurons. *Front Cell Neurosci*, *10*,
832 263. doi:10.3389/fncel.2016.00263
- 833 Manabe, T., Togashi, H., Uchida, N., Suzuki, S. C., Hayakawa, Y., Yamamoto, M., . . . Chisaka,
834 O. (2000). Loss of cadherin-11 adhesion receptor enhances plastic changes in
835 hippocampal synapses and modifies behavioral responses. *Mol Cell Neurosci*, *15*(6), 534-
836 546. doi:10.1006/mcne.2000.0849
- 837 Marshall, C. R., Noor, A., Vincent, J. B., Lionel, A. C., Feuk, L., Skaug, J., . . . Scherer, S. W.
838 (2008). Structural variation of chromosomes in autism spectrum disorder. *Am J Hum*
839 *Genet*, *82*(2), 477-488. doi:10.1016/j.ajhg.2007.12.009
- 840 Meijering, E., Jacob, M., Sarria, J. C., Steiner, P., Hirling, H., & Unser, M. (2004). Design and
841 validation of a tool for neurite tracing and analysis in fluorescence microscopy images.
842 *Cytometry A*, *58*(2), 167-176. doi:10.1002/cyto.a.20022
- 843 Morrow, E. M., Yoo, S. Y., Flavell, S. W., Kim, T. K., Lin, Y., Hill, R. S., . . . Walsh, C. A.
844 (2008). Identifying autism loci and genes by tracing recent shared ancestry. *Science*,
845 *321*(5886), 218-223. doi:10.1126/science.1157657
- 846 Nahidiazar, L., Agronskaia, A. V., Broertjes, J., van den Broek, B., & Jalink, K. (2016).
847 Optimizing Imaging Conditions for Demanding Multi-Color Super Resolution
848 Localization Microscopy. *PLoS One*, *11*(7), e0158884.
849 doi:10.1371/journal.pone.0158884
- 850 Nakanishi, M., Nomura, J., Ji, X., Tamada, K., Arai, T., Takahashi, E., . . . Takumi, T. (2017).
851 Correction: Functional significance of rare neuroligin 1 variants found in autism. *PLoS*
852 *Genet*, *13*(10), e1007035. doi:10.1371/journal.pgen.1007035
- 853 Neale, B. M., Kou, Y., Liu, L., Ma'ayan, A., Samocha, K. E., Sabo, A., . . . Daly, M. J. (2012).
854 Patterns and rates of exonic de novo mutations in autism spectrum disorders. *Nature*,
855 *485*(7397), 242-245. doi:10.1038/nature11011
- 856 Nestor, M. W., Jacob, S., Sun, B., Prè, D., Sproul, A. A., Hong, S. I., . . . Noggle, S. A. (2015).
857 Characterization of a subpopulation of developing cortical interneurons from human
858 iPSCs within serum-free embryoid bodies. *Am J Physiol Cell Physiol*, *308*(3), C209-219.
859 doi:10.1152/ajpcell.00263.2014
- 860 Noel, J., Ralph, G. S., Pickard, L., Williams, J., Molnar, E., Uney, J. B., . . . Henley, J. M.
861 (1999). Surface expression of AMPA receptors in hippocampal neurons is regulated by
862 an NSF-dependent mechanism. *Neuron*, *23*(2), 365-376.
- 863 O'Roak, B. J., Vives, L., Girirajan, S., Karakoc, E., Krumm, N., Coe, B. P., . . . Eichler, E. E.
864 (2012). Sporadic autism exomes reveal a highly interconnected protein network of de
865 novo mutations. *Nature*, *485*(7397), 246-250. doi:10.1038/nature10989
- 866 Osterhout, J. A., Josten, N., Yamada, J., Pan, F., Wu, S. W., Nguyen, P. L., . . . Huberman, A. D.
867 (2011). Cadherin-6 mediates axon-target matching in a non-image-forming visual circuit.
868 *Neuron*, *71*(4), 632-639. doi:10.1016/j.neuron.2011.07.006
- 869 Ovesný, M., Křížek, P., Borkovec, J., Svindrych, Z., & Hagen, G. M. (2014). ThunderSTORM: a
870 comprehensive ImageJ plug-in for PALM and STORM data analysis and super-resolution
871 imaging. *Bioinformatics*, *30*(16), 2389-2390. doi:10.1093/bioinformatics/btu202
- 872 Pagnamenta, A. T., Khan, H., Walker, S., Gerrelli, D., Wing, K., Bonaglia, M. C., . . . Monaco,
873 A. P. (2011). Rare familial 16q21 microdeletions under a linkage peak implicate cadherin

- 874 8 (CDH8) in susceptibility to autism and learning disability. *J Med Genet*, 48(1), 48-54.
875 doi:10.1136/jmg.2010.079426
- 876 Paradis, S., Harrar, D. B., Lin, Y., Koon, A. C., Hauser, J. L., Griffith, E. C., . . . Greenberg, M.
877 E. (2007). An RNAi-based approach identifies molecules required for glutamatergic and
878 GABAergic synapse development. *Neuron*, 53(2), 217-232.
879 doi:10.1016/j.neuron.2006.12.012
- 880 Phillips, A. W., Nestor, J. E., & Nestor, M. W. (2017). Developing HiPSC Derived Serum Free
881 Embryoid Bodies for the Interrogation of 3-D Stem Cell Cultures Using Physiologically
882 Relevant Assays. *J Vis Exp*(125). doi:10.3791/55799
- 883 Redies, C., Hertel, N., & Hübner, C. A. (2012). Cadherins and neuropsychiatric disorders. *Brain*
884 *Res*, 1470, 130-144. doi:10.1016/j.brainres.2012.06.020
- 885 Rubinson, D. A., Dillon, C. P., Kwiatkowski, A. V., Sievers, C., Yang, L., Kopinja, J., . . . Van
886 Parijs, L. (2003). A lentivirus-based system to functionally silence genes in primary
887 mammalian cells, stem cells and transgenic mice by RNA interference. *Nat Genet*, 33(3),
888 401-406. doi:10.1038/ng1117
- 889 Sanders, S. J., Ercan-Sencicek, A. G., Hus, V., Luo, R., Murtha, M. T., Moreno-De-Luca, D., . . .
890 State, M. W. (2011). Multiple recurrent de novo CNVs, including duplications of the
891 7q11.23 Williams syndrome region, are strongly associated with autism. *Neuron*, 70(5),
892 863-885. doi:10.1016/j.neuron.2011.05.002
- 893 Schulte, J. D., Srikanth, M., Das, S., Zhang, J., Lathia, J. D., Yin, L., . . . Chen, A. (2013).
894 Cadherin-11 regulates motility in normal cortical neural precursors and glioblastoma.
895 *PLoS One*, 8(8), e70962. doi:10.1371/journal.pone.0070962
- 896 Seong, E., Yuan, L., & Arikath, J. (2015). Cadherins and catenins in dendrite and synapse
897 morphogenesis. *Cell Adh Migr*, 9(3), 202-213. doi:10.4161/19336918.2014.994919
- 898 Song, J. Y., Ichtchenko, K., Südhof, T. C., & Brose, N. (1999). Neuroligin 1 is a postsynaptic
899 cell-adhesion molecule of excitatory synapses. *Proc Natl Acad Sci U S A*, 96(3), 1100-
900 1105.
- 901 Stan, A., Pielarski, K. N., Brigadski, T., Wittenmayer, N., Fedorchenko, O., Gohla, A., . . .
902 Gottmann, K. (2010). Essential cooperation of N-cadherin and neuroligin-1 in the
903 transsynaptic control of vesicle accumulation. *Proc Natl Acad Sci U S A*, 107(24), 11116-
904 11121. doi:10.1073/pnas.0914233107
- 905 Suzuki, S. C., Inoue, T., Kimura, Y., Tanaka, T., & Takeichi, M. (1997). Neuronal circuits are
906 subdivided by differential expression of type-II classic cadherins in postnatal mouse
907 brains. *Mol Cell Neurosci*, 9(5-6), 433-447. doi:10.1006/mcne.1997.0626
- 908 Takeichi, M., & Abe, K. (2005). Synaptic contact dynamics controlled by cadherin and catenins.
909 *Trends Cell Biol*, 15(4), 216-221. doi:10.1016/j.tcb.2005.02.002
- 910 van Harssel, J. J., Weckhuysen, S., van Kempen, M. J., Hardies, K., Verbeek, N. E., de Kovel, C.
911 G., . . . Brilstra, E. H. (2013). Clinical and genetic aspects of PCDH19-related epilepsy
912 syndromes and the possible role of PCDH19 mutations in males with autism spectrum
913 disorders. *Neurogenetics*, 14(1), 23-34. doi:10.1007/s10048-013-0353-1
- 914 Wang, C., Pan, Y. H., Wang, Y., Blatt, G., & Yuan, X. B. (2019). Segregated expressions of
915 autism risk genes *Cdh11* and *Cdh9* in autism-relevant regions of developing cerebellum.
916 *Mol Brain*, 12(1), 40. doi:10.1186/s13041-019-0461-4
- 917 Wang, K., Zhang, H., Ma, D., Bucan, M., Glessner, J. T., Abrahams, B. S., . . . Hakonarson, H.
918 (2009). Common genetic variants on 5p14.1 associate with autism spectrum disorders.
919 *Nature*, 459(7246), 528-533. doi:10.1038/nature07999

Cadherins in Development and Autism

- 920 Willemsen, M. H., Fernandez, B. A., Bacino, C. A., Gerkes, E., de Brouwer, A. P., Pfundt, R., . .
921 . Kleefstra, T. (2010). Identification of ANKRD11 and ZNF778 as candidate genes for
922 autism and variable cognitive impairment in the novel 16q24.3 microdeletion syndrome.
923 *Eur J Hum Genet*, *18*(4), 429-435. doi:10.1038/ejhg.2009.192
- 924 Wu, N., Wang, Y., Pan, Y.-H., Yuan X. (2020). Identification of CDH11 as an ASD risk gene by
925 matched-gene co-expression analysis and mouse behavioral studies. *bioRxiv* doi:
926 10.1101/2020.02.04.931121
- 927 Yamagata, M., Duan, X., & Sanes, J. R. (2018). Cadherins Interact With Synaptic Organizers to
928 Promote Synaptic Differentiation. *Front Mol Neurosci*, *11*, 142.
929 doi:10.3389/fnmol.2018.00142
- 930

Cadherins in Development and Autism

931 **Table 1. Primary and secondary antibodies.** Abbreviations: PSD-95, postsynaptic density
932 protein-95; GAT-1, sodium- and chloride-dependent GABA transporter-1; GAPDH,
933 glyceraldehyde 3-phosphate dehydrogenase; HRP, horseradish peroxidase; aa, amino acid; WB,
934 Western blot; ICC, immunocytochemistry; IP: Immunoprecipitation.

Cadherins in Development and Autism

Primary						
Antibody	Host, isotype	Immunogen	Source	Cat #	Clone no./RRID	Dilution
Cadherin-8	Mouse	N-terminus, Mouse	DSHB	CAD8-1	RRID:AB_2078272	0.5 µg/mL (WB), 3-4 µg/mL (ICC), 2 µg (IP)
Cadherin-8	Goat, IgG	C-terminus, Human	Santa Cruz	sc-6461	C-18/RRID:AB_2078271	1:500
Cadherin-11	Mouse, IgG ₁	C-terminus, Human	Thermo Fisher Scientific	32-1700	5B2H5/RRID:AB_2533068	2 µg/mL (WB), 7 µg (IP)
PSD-95	Mouse, IgG _{2a}	aa 77-299, Human	Neuro Mab	75-028	K28/43/RRID:AB_2307331	1:100,000 (WB)
PSD-95	Guinea pig, IgG	aa 64-247, Mouse	Synaptic Systems	124014	RRID:AB_2619800	1:500 (ICC)
Syntaxin-1	Mouse, IgG _{2a}	N-terminus, Rat	Synaptic Systems	110111	78.3/RRID:AB_887848	1:10,000
Synapsin-1	Rabbit, IgG	Native protein purified from bovine brain	Abgent	P17599	N/A	1:1000
GAT-1	Rabbit, IgG	C-terminus, Rat	Phospho Solutions	880-GAT1	RRID:AB_2492119	1:200
Neuroigin-1	Mouse, IgG ₁	aa 718-843, Rat	Neuro Mab	75-160	N97A/31/RRID:AB_2235964	1:100
N-cadherin	Mouse, IgG ₁	aa 802-819, Mouse	BD Transduction Labs	610920	32/RRID:AB_2077527	1:1000
β-catenin	Mouse, IgG ₁	aa 571-781, Mouse	BD Transduction Labs	610153	14/RRID:AB_397554	1:700
Gephyrin	Mouse, IgG ₁	aa 326-550, Rat	Synaptic Systems	147111	3B11/RRID:AB_887719	1:1000
GAPDH	Rabbit, IgG	C-terminus, Human	Cell Signaling Technology	5174	D16H11/RRID:AB_10622025	1:1000
β-actin	Mouse, IgG _{2a}	N-terminus	Millipore Sigma	A5316	AC-74/RRID:AB_476743	1:5000

Cadherins in Development and Autism

HA	Rabbit, IgG	aa 98-106 of human influenza hemagglutinin	Millipore Sigma	H6908	RRID:AB_260070	1:1000 (WB), 1:25 (ICC), 4 µg (IP)
C-myc	Mouse, IgG ₁	aa 408-439 of human p62 ^{c-myc}	Millipore Sigma	M4439	9E10/RRID:AB_291324	1:3000 (WB), 1:500 (ICC)
Flag	Mouse, IgG _{2b}	DYKDDDDK tag	Thermo Fisher Scientific	MA191878	FG4R/RRID:AB_1957945	1:500 (ICC)
Secondary						
Antibody	Host, isotype	Immunogen	Source	Cat #	Clone no./RRID	Dilution
anti-rabbit IgG, HRP conjugate	Goat, IgG	IgG Rabbit	Cell Signaling Technology	7074P2	RRID:AB_2099233	1:7500
anti-mouse IgG, HRP conjugate	Goat, IgG	IgG Mouse	Thermo Fisher Scientific	A16072	RRID:AB_2534745	1:5000
anti-goat IgG, HRP conjugate	Donkey, IgG	IgG Goat	Thermo Fisher Scientific	A15999	RRID:AB_2534673	1:5000
anti-mouse Alexa488	Donkey, IgG	IgG (H+L) Mouse	Thermo Fisher Scientific	A21202	RRID:AB_141607	1:1000
Anti-mouse Alexa647	Donkey, IgG	IgG (H+L) Mouse	Thermo Fisher Scientific	A31571	RRID:AB_162542	1:1000
anti-rabbit Alexa488	Donkey, IgG	IgG (H+L) Rabbit	Thermo Fisher Scientific	A21206	RRID:AB_2535792	1:1000
anti-rabbit Alexa568	Donkey, IgG	IgG (H+L) Rabbit	Thermo Fisher Scientific	A10042	RRID:AB_2534017	1:1000
anti-rabbit Alexa647	Donkey, IgG	IgG (H+L) Rabbit	Thermo Fisher Scientific	A31573	RRID:AB_2536183	1:1000
anti-guinea pig Alexa488	Donkey, IgG	IgG (H+L) Guinea Pig	Jackson Immuno Research	706545148	RRID:AB_2340472	1:1000
anti-guinea pig Alexa568	Goat, IgG	IgG (H+L) Guinea Pig	Thermo Fisher Scientific	A11075	RRID:AB_2534119	1:1000

Cadherins in Development and Autism

936 **Table 2. Case information of control and autism-derived iPSC lines.** Sample identity number,
 937 gender, diagnosis and affected genes are listed for each iPSC line analyzed. Data was obtained
 938 from the Hussman Institute for Human Genomics at the University of Miami (Hussman et al.,
 939 2011; Cukier et al., 2014).
 940

	Sample ID	Gender	Diagnosis	Genes
Autism	110	M	Autism	<i>VPS13B, EFCAB5, TRIM55</i>
	700	M	Autism	<i>RBFOX1</i>
	725	M	Autism	<i>CEP290, NINL, SOS2, TRIM55, ZMYND17, BTN2A2, MDC1, FBXO40, KIAA1949, SLC8A3, TSPYL5</i>
	732	M	Autism	<i>CLCN2, F13A1, JARID2, STXBP5, C12orf73, C20orf118, FGD6</i>
	134	M	Autism	<i>CPZ, PRICKLE1, TOPOR5</i>
	691	M	Autism	<i>COL6A3, SLIT3, C2orf85, AB13BP, UIMC1</i>
	709	M	Autism (Twin)	<i>RBFOX1</i>
	710	M	Autism (Twin)	<i>RBFOX1</i>
Control	574	M	-	-
	321	M	-	-
	322	M	-	-
	324	M	-	-

941 **Figure Legends**

942 **Figure 1. Cadherin-8 and cadherin-11 show similar temporal and spatial expression**
943 **patterns.** (A) Temporal expression profile of cadherin-8 and cadherin-11 in mouse whole brain
944 harvested at different developmental ages. Adult mice were 5 months old. (B) Line graph of
945 temporal expression of the two cadherins. Values were normalized to P7. Cdh8: **** $p < 0.0001$,
946 * $p = 0.046$ (P1), * $p = 0.0123$ (P21) compared to P7; Cdh11: * $p = 0.017$ (E14), * $p = 0.0296$ (adult)
947 compared to P7; one-way ANOVA with Dunnett's multiple comparison test. N = 6 whole brains
948 per age from three independent litters. Expression profiles of (C) cadherin-8 and (D) cadherin-11
949 in different brain areas, including cortex (CX), hippocampus (HC), cerebellum (CB) and
950 thalamus/striatum (Th/St) at P14. ** $p = 0.0048$ between CX and CB, * $p = 0.0259$ between HC
951 and CB, * $p = 0.0326$ between CB and Th/St; one-way ANOVA with Tukey's multiple comparison
952 test. N = 4 samples per brain area with 2-3 pooled brain areas per sample. Cadherin signals were
953 normalized to GAPDH.

954 **Figure 2. Cadherin-8 and cadherin-11 are enriched in postsynaptic densities and cadherin-8**
955 **interacts with neuroligin-1.** (A) Forebrain tissues were subjected to synaptic fractionation
956 analysis to determine the subcellular localization of cadherin-8 and cadherin-11. Markers (PSD-
957 95 and syntaxin-1) were probed as control for purity of the fractionation. Total: total protein input,
958 P1: nuclear, S1: cytosol/membranes, P2: crude synaptosome, S2: cytosol/light membranes, P3:
959 synaptosome, SPM: synaptic plasma membrane, PSD: postsynaptic density, S3: synaptic vesicles.
960 (B) Quantification of protein enrichment in SPM and PSD fractions compared to total protein
961 input. PSD-95: ** $p = 0.0012$ between SPM and total, **** $p < 0.0001$ between PSD and total, * p
962 = 0.0444 between SPM and PSD; Cdh8: * $p = 0.0332$ between PSD and total; Cdh11: ** $p = 0.0039$
963 between SPM and total, ** $p = 0.0002$ between PSD and total; one-way ANOVA with Tukey's
964 multiple comparison test. N = 8 P21 mice, two pooled forebrains per sample. Immunoprecipitation
965 (IP) of (C) cadherin-8 and (D) cadherin-11 from P14 forebrain tissues. β -catenin signal is
966 detectable in the IP of cadherin-8 and cadherin-11 and neuroligin-1 signal is detectable in the IP
967 of cadherin-8. (E) Immunocytochemistry of N2a cells over-expressing cadherin-8-myc (magenta)
968 and neuroligin-1-HA (cyan), counterstained for DAPI (blue). Cadherin-8-myc and neuroligin-1-
969 HA show partial co-localization at cell-cell contacts (arrowheads) and at the cell membrane (open
970 arrowheads). Boxed areas are magnified. Scale bar = 20 μ m.

971 **Figure 3. Cadherin-8 localizes to excitatory synapses.** Representative confocal images showing
972 surface expressed cadherin-8 (cyan) on dendritic segments of cultured hippocampal neurons at 15
973 DIV co-immunostained with either the excitatory synaptic markers (A) PSD-95 (magenta) and
974 synapsin-1 (yellow), or the inhibitory synaptic marker (B) GAT-1 (magenta). Cadherin-8 puncta
975 co-localizing with PSD-95- or GAT-1-positive puncta (open arrowheads), being adjacent to PSD-
976 95- or GAT-1-positive puncta (arrowheads) and PSD-95- or GAT-1-negative cadherin-8 puncta
977 (arrows) were analyzed. Scale bar = 2 μ m. Representative synaptic puncta are magnified (boxed
978 areas, scale bar = 1 μ m). The synaptic profile of cadherin-8-positive puncta were quantified and
979 analyzed as fractions of co-localization with (C) PSD-95 or (D) GAT-1. PSD-95: ** $p = 0.0019$,
980 co-localization with PSD-95 versus adjacent to PSD-95; *** $p = 0.002$, adjacent to PSD-95 versus
981 PSD-95-negative puncta; GAT-1: **** $p < 0.0001$, comparison between all fractions; one-way
982 ANOVA with Tukey's multiple comparison test. N = 11-14 neurons, three independent cultures.
983 dSTORM imaging was performed on 15 DIV hippocampal neurons immunostained for PSD-95
984 (magenta) and either surface-expressed (E) cadherin-8 (cyan) or (F) neuroligin-1 (cyan). Scale
985 bar: 5 μ m. Insets (800 nm) show 3D proximity of PSD-95 to the surface stained proteins. Crosshair

986 shows YZ and XZ planes. **(G-J)** Nearest neighbor analysis of dSTORM data. Individual surface
987 stained **(G)** cadherin-8 and **(H)** neuroligin-1 puncta were isolated, and the nearest PSD-95 puncta
988 was determined. **(I)** Quantification and comparison of nearest neighbor analysis for cadherin-8,
989 neuroligin-1 and a randomized set of numbers generated between 0-400 nm. **(J)** Quantification
990 and comparison of the frequency distribution of cadherin-8 and neuroligin-1 puncta and a
991 randomized data set between 0 and 50 nm from the nearest PSD-95 puncta. $***p = 0.0001$, $****p$
992 < 0.0001 ; one-way ANOVA with Tukey's multiple comparison test. N = 23 neurons (Cdh8) and
993 19 neurons (Nlgn1), 18 random data points, three independent cultures. 50 particles per cell were
994 used, unless fewer particles were available.

995 **Figure 4. Elevated cadherin-8 expression in *Cdh11*^{-/-} mice is accompanied by an increase of**
996 **excitatory synaptic proteins and dendrite complexity.** **(A)** Western blot analysis of the
997 expression levels of cadherin-11 and cadherin-8 in P7 *Cdh11* wild-type (WT), heterozygous (HZ)
998 and knockout (KO) mouse brains. Cadherin-11 expression is not detectable in *Cdh11* KO brains.
999 Cadherin signals were normalized to GAPDH. Quantification of the expression of **(B)** cadherin-
1000 11 and **(C)** cadherin-8 in *Cdh11* WT, HZ and KO brains. Cdh11: $****p < 0.0001$, KO vs WT and
1001 KO vs HZ; $***p = 0.0002$, HZ vs WT; Cdh8: $*p = 0.0399$, KO vs WT; $*p = 0.0143$, KO vs HZ;
1002 one-way ANOVA with Tukey's multiple comparison test. N = 7 animals per genotype. **(D)**
1003 Western blot analysis of the expression profiles of neuroligin-1, PSD-95 and gephyrin in P7 *Cdh11*
1004 WT and KO mouse brains. Signals were normalized to β -actin. Quantification of the expression
1005 of **(E)** neuroligin-1, **(F)** PSD-95 and **(G)** gephyrin in *Cdh11* WT and KO brains. Nlgn1: $**p =$
1006 0.027 ; PSD95: $**p = 0.0022$; unpaired two-tailed *t*-test. N = 12 WT and 11 KO animals. **(H)**
1007 Confocal fluorochrome images of dendritic spines from primary, secondary and tertiary dendrites
1008 from 15 DIV *Cdh11* WT and KO hippocampal neurons transfected with pLL3.7-GFP. Scale bar =
1009 2 μ m. **(I)** Quantification of the mean spine density of 15 DIV *Cdh11* WT and KO hippocampal
1010 neurons. N = 33 WT and 28 KO neurons. **(J)** Representative images of reconstructed dendritic
1011 trees from 15 DIV *Cdh11* WT and KO hippocampal neurons. Scale bar = 100 μ m. **(K)** Sholl
1012 analysis of reconstructed neurons. Significant difference was determined by quantifying the area
1013 under the Sholl curve (AUC) between WT and KO neurons; $*p = 0.0269$, unpaired two-tailed *t*-
1014 test. N = 23 WT and 25 KO neurons. Quantification of **(L)** total dendrite length and **(M)** branch
1015 tip number of *Cdh11* WT and KO neurons. **(L)** $*p = 0.0323$; **(M)** $*p = 0.0212$; unpaired two-tailed
1016 *t*-test. N = 23 WT and 25 KO neurons.

1017 **Figure 5. *Cdh11*^{-/-} mice show altered calcium activity and miniature excitatory postsynaptic**
1018 **currents.** **(A)** Example calcium current traces from *Cdh11* wild-type (WT) and *Cdh11* knockout
1019 (KO) hippocampal cultures at 15 DIV recorded for 3 minutes using NeuroBurst Orange lentivirus
1020 calcium indicator. Averaged fluorescence intensity per cell of 1940 active neurons (WT) and 1009
1021 active neurons (KO) was plotted against time (sec). Measurement of **(B)** number of active neurons,
1022 **(C)** correlation of activity **(D)** mean burst strength and **(E)** mean burst rate of *Cdh11* WT and KO
1023 hippocampal neurons recorded at 15 DIV and 16 DIV. $*p = 0.0160$, $**p = 0.0049$, $***p = 0.0006$;
1024 unpaired two-tailed *t*-test. N = 6 WT and 7 KO cultures from two different time points. **(F)**
1025 Example current traces recorded from whole-cell voltage-clamp of hippocampal CA1 pyramidal
1026 neurons. **(G)** Examples of average (left) and scaled (right) mEPSC traces from *Cdh11* WT and KO
1027 cells. Measurement of **(H)** frequency, **(I)** amplitude, **(J)** charge, **(K)** rise time and **(L)** decay time
1028 constant of recorded mEPSCs. $****p > 0.0001$, $*p = 0.0166$; unpaired two-tailed *t*-test. N = 25
1029 WT and 23 KO neurons.

Cadherins in Development and Autism

1030 **Figure 6. iPSC-derived cortical NPCs and organoids from autistic individuals show altered**
1031 **expression of CDH8 and CDH11.** Western blot analysis and quantification of (A) CDH8 and (B)
1032 CDH11 expression in iPSC-derived cortical NPCs from typically-developing control and autistic
1033 individuals at 19 DIV. Quantification of the expression of each cadherin is represented as bar graph
1034 and as box and whisker plot. $**p = 0.0027$, $*p = 0.0323$; unpaired two-tailed t -test. N = 4 control
1035 and 8 autistic individuals, triplicates of each individual. Cadherin signals were normalized to
1036 GAPDH. Quantification of mRNA expression of (C) CDH8 and (D) CDH11 in cortical organoids
1037 derived from iPSCs of control and autistic individuals at 60 DIV via qPCR. $*p = 0.0473$, $***p =$
1038 0.0001 ; unpaired two-tailed t -test. N = 4 control and 6 autism individuals, triplicates of each
1039 individual.











