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# Scribble controls social behaviors through the regulation of the ERK/Mnk1 pathway

Maïté M. Moreau<sup>1</sup>, Susanna Pietropaolo<sup>2</sup>, Jérôme Ezan<sup>1</sup>, Benjamin J.A. Robert<sup>1</sup>, Sylvain Miraux<sup>3</sup>, Marlène Maître<sup>1</sup>, Yoon Cho<sup>2</sup>, Wim E. Crusio<sup>2</sup>, Mireille Montcouquiol<sup>1\*</sup> & Nathalie Sans<sup>1\*</sup>

1. Univ. Bordeaux, INSERM, Neurocentre Magendie, U1215, F-33077 Bordeaux, France

2. Univ. Bordeaux, CNRS, Aquitaine Institute for Cognitive and Integrative Neurosciences, UMR5287, F-33405 Bordeaux, France

3. Univ. Bordeaux, Centre de Résonance Magnétique des Systèmes Biologiques UMR5536, F-33077, France

4. Correspondence : Dr N. Sans or Dr. M.M. Moreau, Neurocentre Magendie U1215, Planar Polarity and Plasticity team, 146 rue Léo-Saignat, 33077 Bordeaux Cedex, France. E-mail: [nathalie.sans@inserm.fr](mailto:nathalie.sans@inserm.fr) & [maite.moreau@inserm.fr](mailto:maite.moreau@inserm.fr)

\* These senior authors contributed equally to this work

## Abstract

Social behavior is a basic domain affected in several neurodevelopmental disorders. Indeed, deficits in social interest, interactions and recognition represent core symptoms of Autism Spectrum Disorder but are also found associated with a heterogeneous set of neuropsychiatric and rare disorders. The *SCRIB* gene that codes for the polarity protein SCRIBBLE has been identified as a risk gene for spina bifida, the most common type of open neural tube defect, found at high frequencies in autistic patients, as other congenital anomalies, while the deletions/mutations of the 8q24.3 region encompassing *SCRIB* genes is associated with multisyndromic and rare disorders. Nonetheless, the potential link between *SCRIB* and ASD-relevant social phenotypes has not been investigated yet. Hence, we performed an extensive behavioral characterization of the *circletail* line that carries a mutated version of *Scrib*. *Scrib<sup>crc/+</sup>* mice displayed reduced social interest, lack of preference for social novelty and social reward, and reduced social habituation while other behavioral domains were unaltered. Social deficits were associated with reduced hippocampal volume, upregulation of ERK phosphorylation in specific hippocampal regions, together with increased c-Fos activity in the same brain areas. Importantly, the social alterations were rescued by both direct and indirect pERK inhibition. These results support a specific link between polarity genes, social behaviors and hippocampal functionality, thus suggesting a role for *SCRIB* in the etiopathology of neurodevelopmental disorders. Furthermore, our data demonstrate the crucial role of the MAPK/ERK signaling pathway, in underlying the social deficits induced by *SCRIB* mutation, thus supporting its relevance as a therapeutic target.

## 38 Introduction

39  
40 Social approaches, interactions and relationships are the basis of our normal and modern life (Adolphs,  
41 2009). These social relationships rely on social memory and are built on individual abilities to differentiate  
42 other person, to remember them in order to have the appropriate behavioral responses based upon  
43 previous encounters. Dealing with these social situations can be considered as problematic for some  
44 individuals. Given the importance of social interactions in our everyday life, it is crucial to understand their  
45 bases at molecular, neural and network levels to develop new pharmacotherapeutics that can ease  
46 dysfunctional social interactions. To some extent, these social dysfunctions become characteristic  
47 symptoms of various psychiatric illnesses, including several neurodevelopmental disorders (NDDs) and  
48 genetic disorders such as autism, but are also found in mood and anxiety disorders that probably stem  
49 their origin in prenatal life during fetal development (Courchesne et al., 2019).

50 *SCRIB* gene (*SCRIBBLE*, *SCRIB1*; OMIM# 607733), located on chromosome 8, encodes for a protein named  
51 Scribble (Scrib or Scrib1) which is key in many developmental process including cell proliferation,  
52 migration, and polarity (Bonello and Peifer, 2019). Scrib is also known for its involvement in planar cell  
53 polarity signaling that ultimately regulates embryonic and postnatal development (Montcouquiol et al.,  
54 2003, 2006a; Moreau et al., 2010; Murdoch et al., 2003). Mutations in *SCRIB* are described in patients  
55 with spina bifida (Lei et al., 2013; Robinson et al., 2012; Wang et al., 2019), one of the most common  
56 forms of neural tube defect, found at high frequency in patients of Autism Spectrum disorder (ASD)  
57 (Dawson et al., 2009; Lauritsen et al., 2002; Timonen-Soivio et al., 2015). Indeed, accumulating evidence  
58 suggests a role of *SCRIB*, together with other polarity genes, in neurodevelopmental and autistic disorders  
59 (for review, see (Sans et al., 2016)). Mutations 8q24.3 in the locus encompassing the *SCRIB* gene have  
60 been linked to the rare Verheij syndrome (VRJS, OMIM #615583) characterized by intellectual disability  
61 (Dauber et al., 2013) and to ASD as well (Hu et al., 2015; Iossifov et al., 2012; Neale et al., 2012). Moreover,  
62 Scrib is also known to play a role in the modulation of the MAPK signaling and to interact directly with ERK  
63 (Nagasaka et al., 2010). Chromosomal deletions encompassing ERK-encoding *MAPK* genes are commonly  
64 associated with neurodevelopmental disorders (NDDs) and ASDs (Campbell et al., 2008; Fernandez et al.,  
65 2010; Saitta et al., 2004). Furthermore, abnormalities in ERK functionality have been described in several  
66 mouse models of NDDs and ASD (Bagni and Zukin, 2019; Courchesne et al., 2019; Faridar et al., 2014;  
67 Vithayathil et al., 2018). Mouse studies have also shown a major role of Scrib in modulating dendritic  
68 arborization and connectivity, post-endocytic NMDA receptor trafficking and hippocampal synaptic  
69 plasticity (Moreau et al., 2010; Hilal et al., 2017), i.e. processes that are known to play a key role in the  
70 etiopathology of in NDDs and ASD (Bagni and Zukin, 2019; Cheng et al., 2017; Lin et al., 2016). The  
71 potential link between *SCRIB*, brain development and function and underlying ASD-like behaviors are

72 poorly understood. Also, understanding of the molecular systems involved in the molecular pathology of  
73 *SCRIB* -associated neurodevelopmental disorders including autism form the bases of ongoing work to  
74 build autism biomarkers (Diaz-Beltran et al., 2016).

75 To fully understand the physiological and pathophysiological mechanisms regulated by *Scrib*, we  
76 conducted an extensive neurobehavioral characterization of heterozygous *circletail* mutant mice  
77 (*Scrib*<sup>*crc/+*</sup>), carrying a mutation in *Scrib* to investigate a potential link between this polarity gene and ASD-  
78 like behaviors. Our focus was on social behaviors (social interest, preference for social novelty and social  
79 reward in the three-compartment test, direct social interaction with assessment of ultrasonic  
80 communication, social habituation), as they represent core symptoms of ASD that are also common to  
81 multiple NDDs. Non-social behaviors were assessed using tests for locomotion (open field), emotionality  
82 (Elevated plus-maze, dark-light box and neophobia), repetitive behaviors (marble, grooming), olfactory  
83 discrimination/habituation, sensory-motor responsiveness (acoustic startle and its prepulse inhibition)  
84 cognitive abilities (based on novelty preference, i.e., object recognition and spontaneous alternation in  
85 the Y maze). This variety of tests allowed us to evaluate whether the potential impact of *Scrib* mutation  
86 on social behaviors could (i) be confounded by the interference of other behavioral alterations (e.g.,  
87 changes in general activity, emotionality, olfaction), (ii) be specific for this domain or instead affects  
88 general novelty preference and detection (e.g., object recognition, spontaneous alternation, olfactory  
89 habituation). We then investigated the potential brain mechanisms underlying the behavioral effects of  
90 *Scrib* mutation using MRI and c-Fos expression approaches, as well as assessing the functionality of the  
91 MAPK/ERK pathways. Finally, we investigated whether the behavioral deficits of *Scrib*<sup>*crc/+*</sup> mice could be  
92 specifically rescued by inhibiting the MAPK/ERK pathway through systemic administration of MEK and  
93 Mnk1 inhibitors. Mnk1 kinase is a known downstream target of ERK signaling and a recent study suggested  
94 it could be a part of a molecular signature for ASD (Lim et al., 2013; Rosina et al., 2019). Importantly, we  
95 were able to specifically rescue these behavioral deficits by systemic administration of MEK and Mnk1  
96 inhibitors of the MAPK/ERK pathway in adult mice. This supports the hypothesis that the MAPK/ERK  
97 pathway is hyperactive in the *Scrib*<sup>*crc/+*</sup> mice, and this underlies the sociability deficits. Altogether, our  
98 results suggest that social behaviors are controlled by a *Scrib* regulation of the ERK-Mnk1 overlapping  
99 mechanisms of phosphorylation.

100

101

102

## 103 **Results**

### 104 ***Scrib<sup>crc/+</sup> mice have deficits in social interest and lack of preference for social novelty and social reward***

105 To determine whether the *Scrib<sup>crc/+</sup>* mouse has social deficit, we first tested the mice in the social interest  
106 and preference for social novelty paradigm using the three-compartment test (Figure 1A). During  
107 habituation, the time spent in the right/left chamber during habituation was not different between the  
108 WT and *Scrib<sup>crc/+</sup>* (no genotype x chamber interaction:  $F_{1,34} < 1$ , n.s; Figure S1A). In the social interest test  
109 (Figure 1C), WT mice had a significantly higher preference for the stranger male mouse (S1, social  
110 stimulus) than *Scrib<sup>crc/+</sup>* animals (genotype x stimulus interaction;  $F_{1,34} = 21.34$ ,  $p < 0.0001$ ). Accordingly,  
111 WT spent more time spent around the small wire cage containing the stranger (S1) than the non-social  
112 novel stimulus (O) ( $t_{16} = 6.57$ ,  $p < 0.0001$ ; Figure 1C), whereas *Scrib<sup>crc/+</sup>* mice showed no preference for the  
113 stranger male mouse ( $t_{18} = 0.22$ ; ns; Figure 1C). At the end of the social interest test, each mouse was  
114 tested for social novelty preference when a novel social stimulus (S2) replacing the non-social one (O)  
115 (Figure 1D). WT mice showed a clear preference for the compartment containing the novel (S2) rather  
116 than the familiar social stimulus (S1), while this preference was not observed in *Scrib<sup>crc/+</sup>* mice, which spent  
117 approximately the same amount of time in the two compartments (genotype x stimulus interaction:  $F_{1,34}$   
118  $= 4.88$ ,  $p < 0.05$ ; S1 vs. S2, Bonferroni corrected  $t$ -test: WT:  $t_{16} = 3.84$ ,  $p < 0.001$ ; *Scrib<sup>crc/+</sup>*:  $t_{18} = 0.84$ , n.s;  
119 Figure 1D). For all trials, the number of entries in both stimulus chambers was similar for both genotypes  
120 (Figure S1B-D), suggesting no difference in general exploration levels. All together these results show that  
121 *Scrib<sup>crc/+</sup>* mice have a deficit in the natural preference for social interaction and in social novelty  
122 recognition.

123 We then assessed the preference for a more attractive, rewarding stimulus, i.e., an adult female (F), in  
124 the three-compartment apparatus compared to a stranger male mouse stimulus (M) (Figure 1B). During  
125 the test, WT mice showed a preference for the female stimulus (F), which was absent in mutants  
126 (genotype x stimulus interaction:  $F_{1,24} = 6.447$ ,  $p < 0.018$ ; Bonferroni corrected  $t$ -test: WT:  $t_{24} = 2.58$ ,  $p <$   
127  $0.05$ ; *Scrib<sup>crc/+</sup>*:  $t_{24} = 0.89$ , n.s ; Figure 1E). These results show that *Scrib<sup>crc/+</sup>* display lack of preference for  
128 social reward.

129

### 130 ***Scrib<sup>crc/+</sup> mice display deficits in social habituation with a juvenile but normal social interaction and*** 131 ***communication with adults***

132 Next, we used a direct social interaction test to assess ultrasonic vocalizations during the direct social  
133 behavior. The time spent in social investigation was similar between *Scrib<sup>crc/+</sup>* mice and their WT  
134 littermates indicating that *Scrib<sup>crc/+</sup>* mice have no major defect in direct social interaction with an adult  
135 female ( $t_{24} = 0.16$ , n.s; Figure S1E). No difference in the ultrasonic vocalizations emitted during the social

136 interaction session was observed, in terms of both number and mean duration (no genotype effect:  $F_{1,22}$   
137  $< 1$ , n.s.; Figure 1F), indicating that *Scrib<sup>crc/+</sup>* mice have no communication defects with an adult female.

138 Next, we tested the mutant mice in a social habituation paradigm (Figure 1G) that provides a measure  
139 of hippocampal- and amygdala-dependent social memory (Kogan et al., 2000). As expected, WT and  
140 *Scrib<sup>crc/+</sup>* mice showed a characteristic social habituation, i.e. decrease in duration of social investigation  
141 with a juvenile male during the two repeated presentations of the same stimulus mouse (Day effect:  $F_{1,48}$   
142  $= 5.63$ ,  $p < 0.05$ ). In contrast, *Scrib<sup>crc/+</sup>* mice presented a slower habituation compared to WT (genotype x  
143 day interaction:  $F_{1,48} = 5.97$ ,  $p < 0.05$ ; Bonferroni corrected *t*-test: Day 2 comparison Test, WT vs *Scrib<sup>crc/+</sup>*;  
144  $t_{24} = 3.40$ ,  $p < 0.01$ ; Figure 1G). These data indicate that *Scrib<sup>crc/+</sup>* mice have normal direct social interaction  
145 with a juvenile mouse, but have a deficit in social habituation.

146

#### 147 ***Scrib<sup>crc/+</sup> mice do not have repetitive behavior or sensorimotor gating defect***

148 To determine whether the social behavior deficits were accompanied by other type of difficulties, we first  
149 investigated potential repetitive behaviors. The *Scrib<sup>crc/+</sup>* mice showed normal rearing (WT vs. *Scrib<sup>crc/+</sup>*, *t*-  
150 test,  $t_{23} = 0.011$ , n.s; Table S1) and self-grooming in their home cages, similar to control mice (WT vs.  
151 *Scrib<sup>crc/+</sup>*, *t*-test,  $t_{23} = 0.68$ , n.s; Table S1). Also, no differences between groups were found when comparing  
152 the number of repetitive entries in the same arm of the Y-maze test (percentage correct alternations; WT  
153 vs. *Scrib<sup>crc/+</sup>*, *t*-test,  $t_{23} = 0.37$ , n.s; Table S1), and the number of repetitive digging behavior in the marble-  
154 burying test (WT vs. *Scrib<sup>crc/+</sup>*, *t*-test,  $t_{23} = 0.44$ , n.s; Table S1). Using the startle response and prepulse  
155 inhibition (PPI), we found that *Scrib<sup>crc/+</sup>* had normal sensory-motor gating (pulse effect for WT and  
156 *Scrib<sup>crc/+</sup>* respectively,  $F_{2,4} = 269.98$  and  $10.05$ , n.s; Table S2). Altogether, these results did not reveal  
157 problem of stereotyped behaviors and sensory-motor integration in *Scrib<sup>crc/+</sup>* mice.

158

#### 159 ***The social deficits in Scrib<sup>crc/+</sup> mice are not a consequence of anxiety, alterations in locomotion, or*** 160 ***neophobia***

161 The social behavior deficits found in the *Scrib<sup>crc/+</sup>* mice could be the result of increased anxiety, altered  
162 locomotion, neophobia, or impairment in general novelty recognition which are all crucial for social  
163 interest and novelty. We did not detect a deficit in any of these behaviors when we submitted the *Scrib<sup>crc/+</sup>*  
164 mice to a battery of test (Table S1). We also used a familiar open field environment to ascertain the intact  
165 abilities of WT and mutant mice to explore novel objects (Figure S2A-C) as described previously (Dulawa  
166 et al., 1999). In the open field, both WT and *Scrib<sup>crc/+</sup>* mice completed a high number of entries and spent  
167 more time in the center after introduction of the object (effect of object:  $F_{1,44} = 52.23$ ,  $p < 0.0001^{***}$  for  
168 entries;  $F_{1,44} = 46.96$ ,  $p < 0.0001^{***}$  for time; no genotype effect:  $F_{1,44} = .75$ , n.s for time;  $F_{1,44} = 0.06$ , n.s  
169 for entries; Figure S2A), confirming that the novel object elicited curiosity.

170  
171 These data suggest that *Scrib<sup>crc/+</sup>* mice have normal behavioral responses to novelty in the object  
172 exploration test. Next, we showed that both groups of mice spent a comparable amount of time exploring  
173 the environment with two identical objects (no object effect:  $F_{1,24} < 1$ , n.s; no genotype effect:  $F_{1,24} < 1$ ,  
174 n.s; Figure S2B). When one object was replaced by a new object, both groups exhibited a significant  
175 preference for the novel object (Object effect:  $F_{1,24} = 12.84$ ,  $p < 0.01^{**}$ ; no genotype effect:  $F_{1,24} < 1$ , n.s;  
176 no genotype x object interaction:  $F_{1,24} < 1$ , n.s; Figure S2C). These data indicate that *Scrib<sup>crc/+</sup>* and WT mice  
177 have a normal object-recognition memory and do not display neophobia.

178  
179 ***Scrib<sup>crc/+</sup> mice have normal olfactory discrimination***  
180 Since social behavior in mice is strongly dependent on olfaction (Farbman, 1994; Guillot and Chapouthier,  
181 1996), we evaluated whether *Scrib<sup>crc/+</sup>* mice have olfactory deficits. We tested both WT and *Scrib<sup>crc/+</sup>* mice  
182 in an olfactory-guided foraging task (Moy et al., 2004). The time required to locate the food buried under  
183 the sawdust was similar for both genotypes without food deprivation conditions (WT vs. *Scrib<sup>crc/+</sup>*, *t*-test,  
184  $t_{26} = 0.40$ , n.s; Figure S3A) or following food deprivation conditions (data not shown). Habituation and  
185 dishabituation (Figure S3B) were observed for social and non-social odor cues with no statistical difference  
186 between WT and *Scrib<sup>crc/+</sup>* mice (no genotype effect:  $F_{1,187} = 1.87$ , n.s). Both genotypes displayed similar  
187 levels of habituation indicated by a decrease in the time spent sniffing the odorant stimulus following its  
188 repeated presentation and comparable levels of dishabituation, indicated by increased time sniffing a  
189 novel odorant stimulus (odor effect:  $F_{11,187} = 22.54$ ,  $p < 0.0001$ ; no odor x genotype interaction:  $F_{11,187} =$   
190 1.30, n.s; Figure S3B). These data indicate that *Scrib<sup>crc/+</sup>* mice do not have olfactory deficits. Altogether,  
191 our data rule out abnormal motor function, general novelty recognition problems, or sensory alterations  
192 as the cause of the reported social deficits in the *Scrib<sup>crc/+</sup>* mutant mice.

193  
194 ***c-Fos levels are increased in the DG and CA3 region of the hippocampus of Scrib<sup>crc/+</sup> mice after social***  
195 ***exposure***

196 To specifically map brain regions activated or not during the social interest test and potentially affected  
197 by *Scrib* loss, we first performed *c-Fos* and *Zif268* neuroimaging (Figure 2). Both WT and *Scrib<sup>crc/+</sup>* mice  
198 were subjected to the social interest test in the three-compartment chamber and left undisturbed in their  
199 home cages for 1 h before perfusion (Social condition). In our control control group, both WT and *Scrib<sup>crc/+</sup>*  
200 mice were placed in the empty three-compartment chamber for the same time and then returned to their  
201 home cages until tissue collection (Control condition). In WT mice, after the social interest test, we  
202 observed an increase in the density of *c-Fos*-positive cells compared to the control condition in restricted  
203 areas of the basal forebrain including hippocampus areas (DG, CA1, CA3), enthorinal cortex (EntCx),

204 medial nucleus of the amygdala (MeA), motor (MCx) and piriform cortex (PirCx), striatum (ST) as well as  
205 in the granular cells of the olfactory bulb (MOB Gcells) (Figure S4A-B). In *Scrib<sup>crc/+</sup>* mice, this pattern was  
206 modified. After the social interest test, we found that the same regions were activated as in the WT group  
207 but also the density of c-Fos-positive cells was significantly increased in the Sensorimotor cortex (S1Cx)  
208 and in the cortical nucleus of the amygdala (CoA). On the other hand, the dorso lateral part of the striatum  
209 (STDL) was not activated in the *Scrib<sup>crc/+</sup>* mutant after social interest test compared to the *Scrib<sup>crc/+</sup>* mice  
210 control group (Fig 2B and Figure S4B). Furthermore, in both control and social interest condition this  
211 pattern was modified specifically by the mutation. We found that the number of c-Fos -positive cells  
212 measured were significantly increased in the olfactory bulb (AOB/MOB) and decreased in the piriform  
213 cortex (PirCx) in the *Scrib<sup>crc/+</sup>* mutant compared to WT (Fig 2B and Figure S4C-D). These observations show  
214 that c-Fos activity in these structures is modulated by Scrib expression levels but are independent of the  
215 social behavior task. However, after social interest test a more robust increase of number of c-Fos -  
216 positive cells expression was observed in the amygdala (CoA,  $\approx +56\%$ ), the DG ( $\approx +12\%$ ) and CA3 ( $\approx +55\%$ )  
217 region of the hippocampus and the Cortex (S1,  $\approx +48,7\%$ ) in *Scrib<sup>crc/+</sup>* compared to WT mice but not in the  
218 CA1 or the medial nucleus of the Amygdala (MeA) (Fig 2D and Figure S4D). This up-regulation of c-Fos  
219 levels in the CA3 and DG of the *Scrib<sup>crc/+</sup>* mice was the direct consequence of the social interest exposure,  
220 because the expression levels of c-Fos in the control animals were comparable between the WT and the  
221 *Scrib<sup>crc/+</sup>* mice in these two regions (Figure 2D). In the CA3 and DG of the hippocampus, we found a similar  
222 up-regulation of *Zif268*-positive cells (Table S3). These results show that the DG and CA3 regions of the  
223 hippocampus are the main activated ones during the social interest test in the *Scrib<sup>crc/+</sup>* mutant mice.

224

### 225 **Reduced structural integrity of the hippocampus *hippocampus of Scrib<sup>crc/+</sup> mice***

226 Next, we investigated whether or not the behavioral alteration associated with c-Fox expression was  
227 accompanied by a change in size/volume of brain regions. Magnetic Resonance Imaging (MRI) analysis  
228 showed no major changes in the global volume of the brain (Figure 3), confirming a previous study  
229 (Moreau et al., 2010). However, MRI revealed an increase in the volume of the olfactory bulbs and a  
230 decrease in the volume of the hippocampus (Figure 3A,B) that was confirmed using 3D reconstruction  
231 analysis on brain sections of *Scrib<sup>crc/+</sup>* mutant mice (Figure 3C,D). Together, these findings point to an  
232 alteration in the structure and /or connectivity between the OB maybe to compensate the defects the  
233 hippocampus in the mutant mice.

234

### 235 ***Reduction of Scrib levels leads to significant increase in ERK phosphorylation in the hippocampus of*** 236 ***Scrib<sup>crc/+</sup> mice after social exposure***

237 Scrib can inhibit the activation of extracellular signal regulated kinase (ERK) pathways in various systems  
238 (Bonello and Peifer, 2019; Jarjour et al., 2015; Nagasaka et al., 2010) and ERK and the mitogen activated

239 protein kinase (MAPK) signaling pathway are linked to neurodevelopmental disorders and more  
240 specifically ASD (Bagni and Zukin, 2019; Courchesne et al., 2019; Faridar et al., 2014; Vithayathil et al.,  
241 2018). Thus, we assessed the level of activated phosphorylated ERK in the three subfields of the  
242 hippocampus of *Scrib<sup>crc/+</sup>* mice in response to the social interest test. Our data show that ERK1 and ERK2  
243 were activated in the three regions of the hippocampus of both mice the mice which performed the test  
244 (both *Scrib<sup>crc/+</sup>* and WT) compared to the unexposed group (Figure 4A-C). Two-way ANOVA indicated a  
245 main effect of the social test exposure on the p-ERK1 levels on both genotype of each subfield (Social test  
246 effect, CA1:  $F_{1,12} = 16.24$ ,  $p < 0.01$ ; CA3:  $F_{1,12} = 12.47$ ,  $p < 0.01$ ; DG:  $F_{1,12} = 6.09$ ,  $p < 0.05$ ; Figure 4B). Exposure  
247 to the test also significantly increased ERK2 phosphorylation levels in both genotype in CA3 (Social test  
248 effect, CA1:  $F_{1,16} = 0.11$ , *n.s.*; CA3:  $F_{1,15} = 10.65$ ,  $p < 0.01$ ; DG:  $F_{1,16} = 4.131$ , *n.s.*; Figure 4C). On the other  
249 hand, the reduction of Scrib levels in the *Scrib<sup>crc/+</sup>* mice leads to a hyper-activation of ERK during social  
250 exposure in the DG and the CA3, but not in the CA1 region, compared to WT mice performing the same  
251 test (genotype x exposure interaction: pERK1-CA3:  $F_{1,12} = 6.41$ ,  $p < 0.05$ ; pERK2-DG:  $F_{1,16} = 8.96$ ,  $p < 0.01$ ;  
252 pERK2-CA3:  $F_{1,15} = 9.88$ ,  $p < 0.01$ ). In conclusion, ERK phosphorylation was significantly higher in the  
253 *Scrib<sup>crc/+</sup>* mice hippocampus compared to WT after the social interest test (Figure 4B and Figure 4C).

254  
255 ***Scrib<sup>crc</sup> form is crucial for ERK pathway activation***  
256 To confirm the direct link between Scrib levels and ERK activation, we used a classical ERK signaling  
257 pathway *in vitro* reporter assay using SRE-LUC as a reporter gene. As expected and illustrated Figure 4D,  
258 increased levels of full length Scrib reduced the serum-induced SRE-LUC reporter gene expression in a  
259 dose-dependent manner, supporting the inhibitory role of Scrib on ERK signaling (ANOVA,  $F_{11,132} = 217.4$ ;  
260  $p < 0.0001$ ). The C-ter truncated form of Scrib (the one expected from *Scrib<sup>crc</sup>* mutation) exhibited a  
261 biphasic response. Lower doses significantly increased the luciferase expression when compared to the  
262 same concentration of full length Scrib (Scrib vs. *Scrib1<sup>crc</sup>*; Bonferroni comparison *t*-test: 25 ng:  $t_{16} = 13.68$ ,  
263  $p < 0.001$ ; 50 ng:  $t_{16} = 9.248$ ,  $p < 0.001$ ; 100 ng:  $t_{34} = 8.57$ ,  $p < 0.001$ ; 250 ng:  $t_{16} = 3.31$ ,  $p < .05$ ), while higher  
264 doses were inhibitory, similar to full length Scrib, although not to the same extent (for illustration,  
265 compare Scrib and *Scrib<sup>crc</sup>* at 500 ng/ml;  $t_{16} = 3.31$ , *n.s.*). These results are consistent with an inhibitory role  
266 of Scrib on the ERK pathway, an inhibition that is reduced in *Scrib<sup>crc</sup>* mutants due most probably to the  
267 absence of one ERK binding site in the truncated protein. The mechanisms leading to the potentiating  
268 effect observed for lower levels of *Scrib<sup>crc</sup>* are unclear at the moment, but it could be the result of a  
269 dominant-negative effect of *Scrib<sup>crc</sup>* on full length Scrib. This dual effect is consistent with ERK  
270 hyperphosphorylation in the *Scrib<sup>crc/+</sup>* mice, and could explain part of the complex phenotype in these  
271 mice.

272

273 ***Specific inhibition of ERK rescues social deficits in *Scrib*<sup>crc/+</sup> mice***

274 The lack of social interest in *Scrib*<sup>crc/+</sup> mutant mice is associated with abnormally high levels of activated  
275 ERK levels in the hippocampus. If true, we should be able to rescue that phenotype by inhibiting ERK over-  
276 activation in the hippocampus via the administration of an inhibitor of the mitogen-activated protein  
277 kinase (MEK) SL327, as done previously (Fernandez et al., 2008).

278 WT and mutants were assessed in the three-compartment test one hour after vehicle or 30 mg/kg SL327  
279 treatment (Figure 5A). Each treatment group was compared with its own saline control. *Scrib*<sup>crc/+</sup> treated  
280 with SL327 showed normal levels of social interest comparable to their treated WT littermates (genotype  
281 x stimulus x SL327;  $F_{1,60} = 10,36$ ;  $p < 0.01^{**}$ ; O vs S1; Bonferonni's multiple comparisons test: WT-SL327,  
282  $t_7 = 7.87$ ,  $p < 0.0001^{***}$ ; *Scrib*<sup>crc/+</sup>-SL327,  $t_7 = 6.35$ ,  $p < 0.0001^{***}$ ; Figure 5D). The effect is comparable to  
283 the WT in saline condition (WT-Saline,  $t_{11} = 8.80$ ,  $p < 0.0001^{***}$ ; Figure 5D) and different from the *Scrib*<sup>crc/+</sup>  
284 in the saline condition (*Scrib*<sup>crc/+</sup>-saline,  $t_9 = 1.17$ , *n.s.*; Figure 5D). The same rescued effects were observed  
285 on the preference for social novelty (genotype x stimulus x SL327 effect;  $F_{1,64} = 25.81$ ;  $p < 0.0001^{***}$ ; S1  
286 vs S2; Bonferonni's multiple comparisons test: WT-SL327,  $t_8 = 4.20$ ,  $p < 0.001^{***}$ ; *Scrib*<sup>crc/+</sup>-SL327,  $t_8 =$   
287  $6.90$ ,  $p < 0.001^{***}$ ; Figure 5E).

288  
289 ***Downstream of ERK signaling, the specific inhibition of Mnk1 in *Scrib*<sup>crc/+</sup> mice also rescues social deficits***

290 Mnk1 kinase is a known downstream target of ERK signaling and a recent study suggested it could be a  
291 part of a molecular signature for ASD (Bagni and Zukin, 2019; Lim et al., 2013). To determine whether the  
292 inactivation of Mnk1 could rescue the social deficits in the *Scrib*<sup>crc/+</sup> mice, we used CGP57380, a cell-  
293 permeable pyrazolo-pyrimidine compound that acts as a selective inhibitor of Mnk1, but has no inhibitory  
294 activity against ERK1/2 (Figure 5A). We found that Mnk1 inhibition improved social behavior in the  
295 *Scrib*<sup>crc/+</sup> mice (genotype x stimulus x CGP57380 effect;  $F_{1,68} = 13,69$ ;  $p < 0.001^{***}$ ; O vs S1; Bonferonni's  
296 multiple comparisons test: *Scrib*<sup>crc/+</sup>-CGP57380,  $t_9 = 8.04$ ,  $p < 0.0001^{***}$ ; Figure 5E). The same effects were  
297 observed on the preference for social novelty in treated conditions (no genotype x stimulus x CGP57380  
298 effect; genotype x stimulus effect:  $F_{1,62} = 6.18$ ,  $p < 0.05^*$ ; S1 vs S2; Bonferonni's multiple comparisons  
299 test: *Scrib*<sup>crc/+</sup>-CGP57380,  $t_8 = 4.46$ ,  $p < 0.0001^{***}$ ; Figure 5E). These data demonstrate that the  
300 impairments in social recognition observed in *Scrib*<sup>crc/+</sup> mutant is in part due to an increase in ERK/Mnk1  
301 pathway activity, consecutive to a reduction of Scrib levels in the hippocampus. Further, we demonstrate  
302 in our mouse model that social interest deficit can be rescued in adult animal by modulating two biological  
303 targets which are part of a major signaling pathway.

304

## 305 Discussion

306 This study provides extensive evidence for the role of Scrib in the regulation of social behaviors, mediated  
307 by hippocampal functionality and -more specifically- by the ERK/Mnk signaling pathway. Three major  
308 findings were obtained from this study. First, we report that *Scrib<sup>crc/+</sup>* mice have behavioral alterations that  
309 are specific to the social domain, in the absence of changes in other abilities. Second, these social deficits  
310 are associated with an increased c-Fos activity in the hippocampus and an over-activation of  
311 phosphorylated ERK. Third, the social abnormalities of *Scrib<sup>crc/+</sup>* mice could be rescued by the  
312 administration of MEK/ERK/Mnk1 inhibitors. Our study identifies that the hyperactivation of ERK and Mnk  
313 and the dysregulation of the ERK/Mnk1 signaling pathways in the absence of a fully functional Scrib  
314 protein is the key molecular mechanism that triggers social behavior defects in the *Scrib<sup>crc/+</sup>* mutant mice.  
315 Taken together, our findings suggest that Scribble controls ERK and Mnk1 signaling molecule levels of  
316 phosphorylation during social behavior.

### 317 Scrib mutation induces behavioral alterations that are specifically social

318 *Scrib<sup>crc/+</sup>* mice displayed lack of social interest and of preference for social novelty and social reward in the  
319 three-compartment test, as well as reduced social habituation. The qualitative patterns of social  
320 interaction, as assessed in the direct social interaction test, were unaltered, as well as ultrasonic  
321 communication. As we performed mainly quantitative measures of ultrasonic frequency and duration, it  
322 is still possible that subtle differences in communication may be revealed, for instance through qualitative  
323 spectrographic analyses as suggested by studies on mouse models of ASD (Scattoni et al., 2013).  
324 Nonetheless, our findings undoubtedly indicate that social recognition and detection of social novelty  
325 represent the major domains that are robustly affected by *Scrib<sup>crc</sup>* mutation. Based on these results, we  
326 have therefore focused on these social domains to identify the underlying neuromolecular mechanisms.  
327 The use of the three-compartment test further simplifies the procedures to be applied for the future and  
328 it enhances reproducibility across studies, by applying automatic measurements of compartment  
329 preference, thus avoiding the potential confounding impact of inter-observer variations of direct  
330 interaction analysis.

331 Importantly, the social deficits of *Scrib<sup>crc/+</sup>* mouse mutant were not accompanied by other behavioral  
332 alterations. This allowed us to rule out the confounding role of olfactory, emotional and sensory motor  
333 alterations, but also to exclude a general novelty discrimination deficit, as supported by the lack of  
334 abnormalities in the Y maze and object recognition tests. Our data therefore suggest that the role of *SCRIB*  
335 in the etiopathology of ASD would be specific for social alterations, without other additional sensori-  
336 motor and emotional symptoms or cognitive deficits. This does not undermine the importance of the Scrib  
337 *crc* mouse line to investigate ASD and NDDs on the contrary. First, the social phenotype represents the

338 most relevant core symptom of ASD-related pathologies and it is common to a variety of NDDs.  
339 Interestingly, other polarity genes have been linked with NDDs or more specifically ASD-like phenotypes  
340 (Sans et al., 2016). Notably, mutations in core PCP genes of the Dishevelled (*DVL*) or Prickle (*PK*) families  
341 are found in autistic patients (Dong et al., 2014; Gilman et al., 2011; Sowers et al., 2013), while the  
342 transgenic mouse model with mutations in two Disheveled genes, *Dvl1* and *Dvl3*, display social behavioral  
343 abnormalities (Belinson et al., 2016; Gilman et al., 2011). Thus, our findings underscore the interest in  
344 studying the specific link between other polarity genes and social behaviors. Second, it is possible that  
345 *Scrib* may be linked to specific types of ASD or NDDs, for instance the high functioning or Asperger's  
346 syndromes: this hypothesis could be supported by the higher spatial memory abilities previously  
347 described in the water maze task in *Scrib* mutant mice (Moreau et al., 2010), and would deserve further  
348 investigation.

#### 349 **A central role of the hippocampus in *Scrib* dependent social dysfunctions**

350 Using MRI analysis, we found no major structural alterations in the brain of our mutants except larger  
351 olfactory bulbs and a smaller hippocampus. Our data are in agreement with studies showing that the  
352 hippocampus plays a critical role in social memory and recognition (Kogan et al., 2000; Maaswinkel et al.,  
353 1996; Okuyama, 2018; Raam et al., 2017; Tzakis and Holahan, 2019). It is possible that social memories  
354 also rely on different subregions of the hippocampus, different networks and connections and/or different  
355 molecular pathways. More than one of them could be affected in the *circletail* mutant. It has recently  
356 been shown that a brain network composed of the hippocampus/mPFC/ACC/amygdala was required for  
357 the consolidation of social recognition memory (Tanimizu et al., 2017). In the *Scrib<sup>crc/+</sup>* mice, both the  
358 hippocampus and amygdala showed an excessive c-Fos induction, a result that might highlight an  
359 abnormal regulation of neuronal activity resulting in deficient social memory to form properly. Moreover,  
360 MRI studies have highlighted reduced volume of amygdala or hippocampus, in children and adult ASD  
361 patients compared to typical subjects (Stanfield et al., 2008; Via et al., 2011) as well as in adult patients  
362 with spina bifida (Treble-Barna et al., 2015). It will be interesting to further explore the role of the  
363 hippocampus in these different forms of memory.

#### 364 **The ERK/Mnk1 pathway mediates the impact of *Scrib* on social behavior: a novel therapeutic target?**

365 Our data show that social exposure leads to an increased neuronal and MAPK signaling activity of the  
366 hippocampus in *Scrib<sup>crc/+</sup>* mice compared to WT mice. Though various signaling pathway have been  
367 associated with ASD, this major signaling pathway is linked to neurodevelopmental disorders and more  
368 specifically ASD (Courchesne et al., 2019; Faridar et al., 2014; Vithayathil et al., 2018) and the ERK  
369 MAPK/ERK pathway is hyperactive in at least a subpopulation of autism spectrum disorder (Kalkman,  
370 2012). Because *Scrib* levels are reduced in *Scrib<sup>crc/+</sup>* mice, it is reasonable to think that this hyper-

371 phosphorylation of ERK1/2 is the results of a release of inhibition of the MAPK cascade. This hypothesis is  
372 largely supported by studies is different tissue and cell types, demonstrating that full length Scrib inhibits  
373 ERK phosphorylation by interacting directly with ERK and inhibiting its subsequent translocation to the  
374 nucleus (Dow and Humbert, 2007; Nagasaka et al., 2013). Our luciferase assay demonstrated that MAPK  
375 activity reduction was dose-dependent, as more Scrib lead to more inhibition. We further found a biphasic  
376 regulation of the mutated *Scrib<sup>crc</sup>* form of Scrib, suggesting a tight and dynamic regulation of MAPK activity  
377 depending on the levels of Scrib but also on the domains of the proteins available in neurons. Similar  
378 complex effect of a mutation is observed in a neuroligin-3 knock-in mouse, with a R451C-substitution  
379 likely acting as a gain-of-function mutation (Etherton et al., 2011). In this study, the authors observed that  
380 although only 10% of the neuroligin-3 protein remains in the knock-in mice, this remaining neuroligin-3  
381 protein lead to an inhibition of synaptic transmission whereas the full neuroligin-3 KO exerts no such  
382 effect. Consistent with this, chromosomal duplications/deletions of 16p11.2, which includes the MAPK3  
383 gene encoding for ERK1, or a microdeletion on chromosome 22, which contain the MAPK1 gene coding  
384 for ERK2 are commonly found associated with neurodevelopmental disorders and ASD (Campbell et al.,  
385 2008; Fernandez et al., 2010; Saitta et al., 2004).

386  
387 We demonstrate in our mouse model that social interest deficit can be rescued in adult animal by  
388 modulating two biological targets which are part of a major signaling pathway. Mnk has emerged over the  
389 years as an important target in the ERK dependent signaling pathway that could be targeted to prevent  
390 ASD (Bramham et al., 2016). A recent study further suggests that MAPK activity might constitute a  
391 molecular signature of clinical severity in autism (Rosina et al., 2019). In that study, the authors suggest  
392 that the cascade ERK1-2/Mnk1/eIF4E may constitute a molecular signature predictive for early diagnosis  
393 of autism, notably severe ASD. Our results showed that inhibiting Mnk1 has similar effects than inhibiting  
394 directly ERK phosphorylation, both on sociability and on social novelty behavior, strongly suggesting it is  
395 a similar cascade that is deregulated in the *Scrib<sup>crc/+</sup>* mice.

396  
397 Alternatively, even if not mutually exclusive, Scrib could negatively regulate ERK activity indirectly,  
398 through the regulation of Rac1 activity. Many studies showed that Rac1 acts upstream of ERK1/2 through  
399 a p21-activated kinase (PAK) PAK/Raf/MEK signaling cascade, while *Scrib<sup>crc/+</sup>* mice display enhanced Rac1  
400 activity in the hippocampus (Moreau et al., 2010). Scrib could therefore be a negative regulator of Rac1  
401 signaling in the hippocampus. Since Rac1 can activate ERK1/2, decreased levels of Scrib would also lead  
402 to increased activation of pERK (Kennedy et al., 2005). Consistent with this hypothesis, Tonegawa and  
403 collaborators were able to rescue symptoms of Fragile X syndrome – another major developmental  
404 disorder often associated to autism - in mice using a dominant negative PAK transgene (Hayashi et al.,  
405 2007). PAK also acts downstream of Scrib (Montcouquiol et al., 2006b; Sans et al., 2016), so it is reasonable

406 to think that these proteins belong to the same molecular pathway, which appear as a good candidate for  
407 drugs that could improve social symptoms. Interestingly, PAK is also downstream of  $\beta$ PIX and SHANK  
408 proteins, which are main players in autistic behaviors with and without mental retardation (Park et al.,  
409 2003). Because of many similar phenotypes in *SHANK3* mouse models and in *Scrib*<sup>crc/+</sup> mice, it would be of  
410 interest to evaluate whether Scrib and Shank3 act in concert or in parallel upstream of the ERK pathway  
411 to regulate social behavior.

412

### 413 **Conclusion**

414 Altogether, this work identifies Scrib as an important regulator of social behavior in mice, through  
415 negative regulation of the ERK/Mnk signaling pathway, and that the *circletail* mutant mouse is a useful  
416 model on which the effects on social behavior of candidate therapeutical compounds could be tested. It  
417 is tempting to suggest that disruption of SCRIB-dependent signaling could underlie common genetic  
418 factors to NTD and ASD-like deficits, and may in some way predispose to development of these  
419 pathologies. In the future, it will be important to define the upstream and the other downstream effectors  
420 of Scrib implicated in the processing of the sensory inputs that play a pivotal role in social information  
421 integration.

422

## 423 **Materials and Methods**

### 424 ***Animals.***

425 Experiments were performed using heterozygous mice for the mutation (*Scrib<sup>cr/+</sup>*) and WT (*Scrib<sup>+/+</sup>*)  
426 littermates. All subjects were adult males of 10-11 weeks of age at the start of behavioral tests and housed  
427 in collective cages under standard laboratory conditions with a 12 h light/12 h dark cycle (light on: 07:00)  
428 with food and water supplied *ad libitum*. Juvenile male Swiss mice (21–28 days old, Janvier, France), or  
429 SV27 Female mice (10-12 weeks) were used as social stimulus for social interaction and communication  
430 analysis. Multiple cohorts of mice were used for the study, as described in the supplementary Materials  
431 and Methods section.

### 432 ***Ethics Statement.***

433 Experiments were approved by the local Animal Health and Care Committee and performed in strict  
434 compliance with the EEC recommendations for the care and use of laboratory animals.

### 435 ***Assessment of Social behaviours***

436 The multiple social tests used assessed multiple characteristics of social behaviors in different social  
437 contexts. All data from the three-compartment test were analyzed with Ethovision (Version 13, Noldus).

438 *Social interest and preference for social novelty in the three-compartment test.* The three-  
439 compartment test provided an evaluation of a social preference without mandatory direct social  
440 interaction; compared to direct social interaction tests, it allows to assess social behaviours in at context  
441 of a free choice, also avoiding inter-male aggression or sexual behaviours. The test was performed as  
442 previously described (Moreau et al., 2010). It consisted of three 10-min trials. During trial #1 (habituation),  
443 the tested mouse was allowed to explore the 3-chamber in which each end-chamber contained an empty  
444 small wire cage. In trial #2 (social interest), a stranger male mice (S1, social stimulus) was placed under a  
445 wire cage in one end-chambers while an object (O, non-social stimulus) was placed in the opposite end-  
446 chamber. For the trial #3 (social novelty), a second stranger male mice (S2) replaced the object. The tested  
447 mouse was free to choose between a caged novel stranger (S2, novel social stimulus) versus the same  
448 caged mouse in trial 2 (S1, familiar social stimulus).

449 *Preference for social reward in the three-compartment test.* The mouse was habituated to the 3-  
450 compartment apparatus for 10 min in the empty compartment. Then, we tested the social reward over 5  
451 min. We placed a novel adult male stranger mouse (M) under a cage in one end-chamber and an adult  
452 female stranger mice (F) was placed in the opposite end-chamber. The tested mouse was free to choose  
453 between a both stimuli (F or M). Exploration was assessed by automatically measuring the time spent in  
454 each contact area containing the stimulus cage during 5 min.

455 *Direct social interaction and communication.* The quality of the behavioural patterns of social  
456 behaviours were assessed using the direct social interaction test with an adult female in in the home-  
457 cage. This experimental setting is also typically used to analyze ultrasonic vocalizations in adult male mice  
458 (Portfors, 2007). Tested mice were isolated 24 hours before the test. An unfamiliar stimulus mouse (adult  
459 NMRI female) was introduced into the testing cage and left for 5 min. Testing sessions were recorded and  
460 videos were analyzed with Observer XT (Noldus), taking only the tested mice into account. An observer  
461 (blind to the animal genotype) scored the time spent performing each of the following social behavior:  
462 sniffing the head and the snout of the partner, its anogenital region, or any other part of the body. During  
463 the test an ultrasonic microphone (CM16/CMPA, Avisoft, Berlin, Germany) was suspended 2 cm above  
464 the cage lid. Vocalizations were recorded and afterwards analyzed as previously described (Pietropaolo  
465 et al., 2011).

466 *Social habituation.* Social habituation was analyzed through repeated encounters with a juvenile male  
467 to elicit social interest but minimize the risk of aggression. The test was performed on two consecutive  
468 days. On day 1, an unfamiliar stimulus mouse (a juvenile Swiss male, 3-4 weeks old) was introduced in the  
469 home cage of the tested subject and left for 2 min. This procedure was repeated on day 2 with the same  
470 stimulus mouse. Social investigation of the stimulus mouse by the tested subject was scored by a trained  
471 observer who timed the duration of the investigation with a hand-held stopwatch. Behaviors that were  
472 scored as social investigation included direct contact, sniffings and close following (< 1cm).

#### 473 ***Assessment of non-social behaviors***

474 Non-social tests for locomotion, emotionality, repetitive behaviors, olfactory discrimination/habituation,  
475 sensory-motor responsiveness and cognitive abilities including were evaluated as described in the  
476 supplementary section.

#### 477 ***Treatments.***

478 The MEK inhibitor  $\alpha$ -[amino[(4-aminophenyl)thio]methylene]-2-(trifluoromethyl)benzeneacetonitrile  
479 (SL327) (Sigma-Aldrich) was dissolved in a vehicle solution of 2.5% dimethyl sulfoxide (DMSO)/ 2.5%  
480 Cremophor EL saline solution at 30 mg/kg (Satoh et al., 2011). The Mink1 inhibitor CGP57380 (Sigma-  
481 Aldrich) was dissolved in a vehicle solution of 2.5% dimethyl sulfoxide (DMSO) at 10mg/Kg (Lim et al.,  
482 2013). SL327 and CGP57380 were injected intraperitoneally (IP) at a volume of 3.3 ml/kg one hour before  
483 social interest test. Control mice received the same volume of vehicle.

#### 484 ***c-Fos immunoreactivity and laser microdissection following the social interest test.***

485 For each genotype, we analyzed two groups of mice: one group exposed to the social interest test  
486 (habituation and social interest = social group) and one group unexposed to the social interest test  
487 (habituation alone = control group). The social group went through the 2 trials of the three-compartment

488 test described previously (trial 1 + trial 2). During the same period, the control group was exposed to the  
489 habituation test (trial1) during the same time in the same experimental room. At the end of the test, all  
490 mice were returned in their home cage and left undisturbed for 60 minutes (min).

491 *c-Fos and Zif-268 immunoreactivity.* Mice were anesthetized using a Phenobarbital i.p injection and  
492 transcardially perfused with physiological saline for 5 min followed by 4% paraformaldehyde diluted in  
493 0.1 M phosphate buffer pH 7.4 (PFA4%) for 15 min. The brain was removed, postfixed for 24 h in 4% PFA  
494 and cut with a vibratome. Free-floating coronal sections (40  $\mu$ m) were incubated in 0.3% Triton X-100,  
495 0.3% normal goat serum and c-fos or Zif-268 primary antibody (Santa Cruz Biotechnology). Next, the  
496 sections were incubated with biotinylated goat anti-rabbit IgG (Vector Laboratories) followed by ABC  
497 EliteKit (Vector Laboratories). Immunoreactive cells were visualized using a diaminobenzidine (DAB,  
498 Vector Laboratories) colorimetric reaction. Images were acquired with a Leica microscope and the number  
499 of c-Fos and Zif-268 positive cells was quantified automatically using Metamorph. Brain areas were  
500 identified using the stereotaxic atlas of Paxinos and Franklin (1997) (Paxinos and Franklin, 2019).

501 *Laser Capture Microdissection (LCM) analysis.* Mice were anesthetized by brief inhalation of isoflurane  
502 (5% in air), sacrificed by decapitation and the brain was rapidly dissected, snap frozen, and stored at -  
503 80°C. LCM was performed on coronal frozen sections as described (Maitre et al., 2011). The anterior  
504 hippocampal neuronal layers (B = -.94 à -2.7 mm) including the CA3, the CA1 and the DG were selectively  
505 captured on three different LCM caps. Following LCM, 150  $\mu$ l of extraction buffer was added to the caps  
506 and stored at -80°C until protein isolation.

507 *Western blot analysis.* Preparation for protein extracts was performed as previously described  
508 (Moreau et al., 2010). The concentration of protein was measured by a BCA assay kit and western blots  
509 were done on 10% SDS-PAGE using standard methods (Sans et al., 2005). Antibodies used were anti-Scrib  
510 antibody (AbMM468 (Montcouquiol et al., 2006a); anti-p44/42 MAPK (#9102) and anti-Phospho-p44/42  
511 MAPK (#9101) from Cell Signaling. Secondary antibodies used was anti-rabbit HRP-conjugated from GE  
512 Healthcare.

### 513 ***Cell Culture, Transfection, and the SRE-Luciferase Reporter Assay***

514 HEK293T cells were maintained in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10%  
515 fetal bovine serum (GIBCO) and were transfected using linear polyethylenimine (MW 25,000;  
516 Polysciences). SRE-Luciferase Reporter Assay was performed using the Dual-Luciferase assay system  
517 (Promega) as described previously (Ossipova et al., 2009). Each well in 12-well plates received 0.05  $\mu$ g of  
518 pTKRenilla-Luc, 1  $\mu$ g of pGL4.33[*luc2P/SRE/Hygro*] Vector DNA (Promega), and indicated amounts of  
519 human Scrib constructs (pCDNA3-eGFP-Scrib, pCDNA3-eGFP-Scrib<sup>Crc</sup>). Total DNA was adjusted to 2.05  $\mu$ g  
520 by supplementing pCDNA3 DNA. One day after transfection, cells were serum starved for 2 hr then treated

521 with 20% FBS containing medium for an additional 6 hr and harvested in 200 $\mu$ l of 1X passive lysis buffer  
522 for luciferase activity measurement. Results are expressed in terms of relative luciferase activity (ratio of  
523 firefly luciferase activity divided by Renilla luciferase activity), which were determined using a POLARstar  
524 Omega Multi-Mode Microplate Reader (BMG LABTECH).

### 525 ***Magnetic resonance imaging (MRI)***

526 Experiments were performed on a 4.7T Bruker system (Ettlingen, Germany) equipped with a 12-cm  
527 gradient system capable of 660 mT/m maximum strength. Measurements were performed with a  
528 birdcage resonator (25 mm diameter and 30 mm length) tuned to 200 MHz. Mice were anesthetized with  
529 isoflurane (1-1.5 % in air) and maintained at a constant respiration rate of  $75 \pm 15$  respirations/min. The  
530 animals were placed in a lying position within the magnet with the head at the centre of the NMR coil. A  
531 3D TrueFISP imaging sequence with alternating RF phase pulse method and sum of square reconstruction  
532 were used as already described (Miraux et al., 2008; Ribot et al., 2015). The following parameters were  
533 used: TE/TR: 2.5/5 ms; flip angle: 30°; bandwidth: 271 Hz/pixel; FOV: 20x20x16 mm; matrix: 128x128x102;  
534 spatial resolution: 156x156x156 mm<sup>3</sup>); number of averages: 4; 4 DeltaPhi values; total acquisition time:  
535 17 min 04 s.

### 536 ***3D volume reconstitution and surface rendering***

537 Consecutive z-series of coronal brain sections were stained with cresyl violet and scanned with  
538 Hamamatsu NANOZOOMER 2.0. All brain images were compiled into a stack file after being aligned using  
539 ImageJ software. 3D reconstruction and volume measurement were done with Imaris Scientific 3D/4D  
540 image processing and analysis software.

### 541 ***Statistical analysis.***

542 All animals were assigned randomly to the different experimental conditions. Normality and homogeneity  
543 of variances from the samples were tested with Shapiro-Wilk normality test and Bartlett test respectively.  
544 If data were parametric, Student's *t*-test (two comparisons), ANOVA 1-way (multiple comparisons),  
545 ANOVA 2-way or ANOVA 3-way were used. Otherwise non-parametric tests were used (Mann-Whitney or  
546 Kruskal-Wallis). When significant interaction effects of main factors were detected, post hoc analyses,  
547 recommended by GraphPad Prism 8 software (Bonferroni's for ANOVA and Dunn's for Kruskal-Wallis),  
548 were performed. Effects with  $p \leq .05$  was considered statistically significant.

549

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554

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567

## 568 **Author Contributions and information**

569 MMM, SP, WEC, MM and NS conceptualized the paper. MMM, SP, JE, BJAR, SM and MaM participated in  
570 the execution and analysis of experiments. MMM, SP, JE, NS designed and interpreted the results. MMM,  
571 SP, MM and NS wrote the paper; All authors edited and approved the paper. Equal senior authorship:  
572 Mireille Montcouquiol and Nathalie Sans

573

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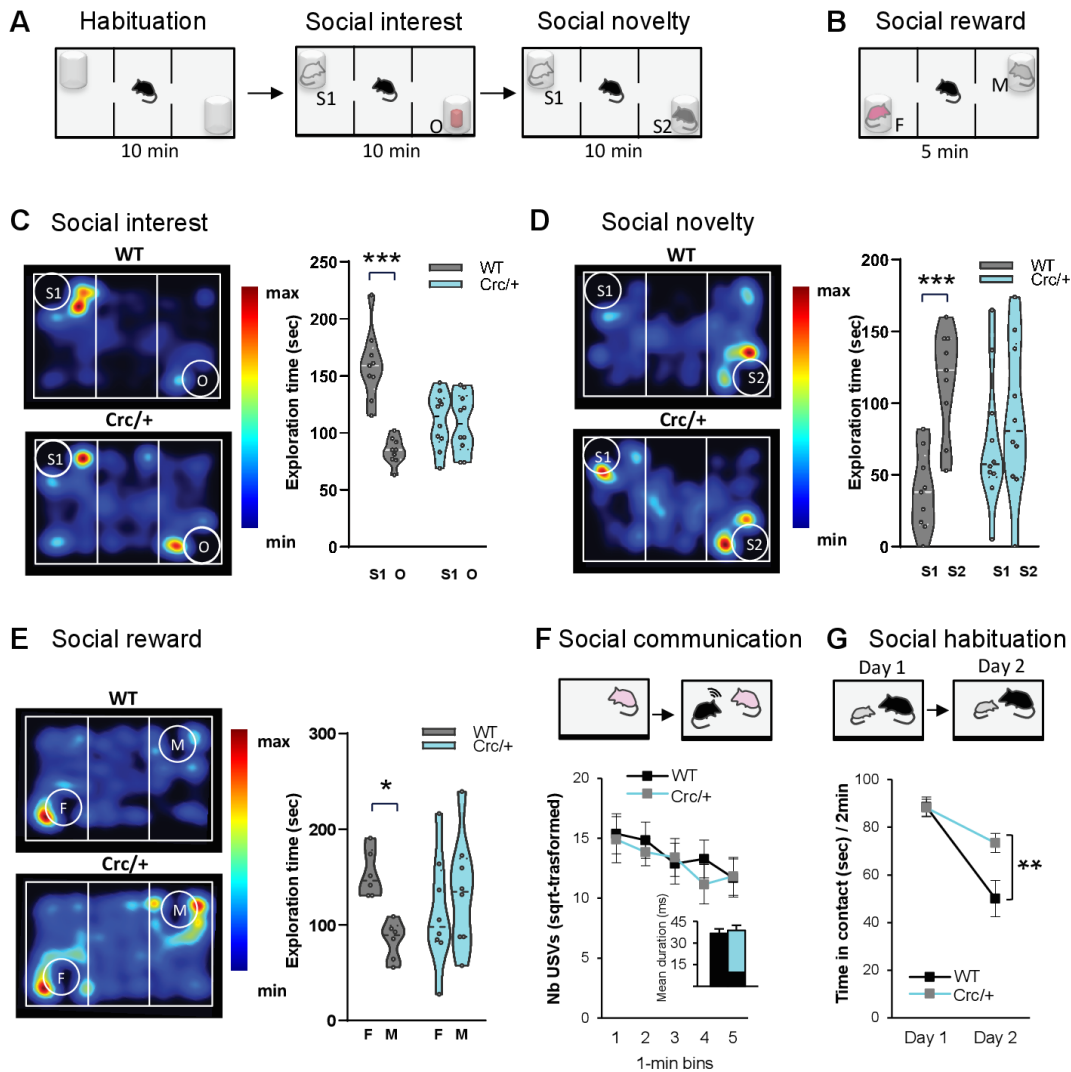
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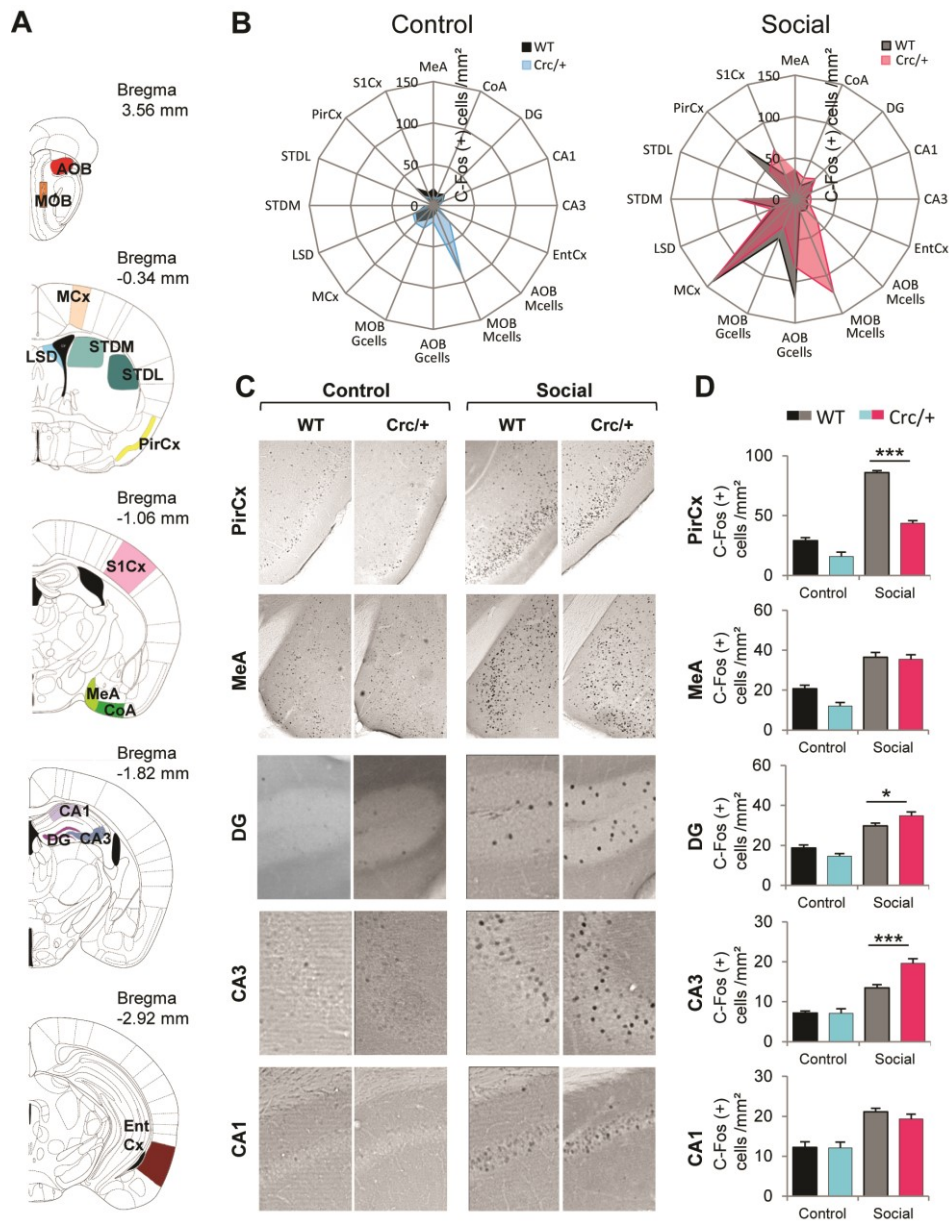
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755 **Figures**  
756

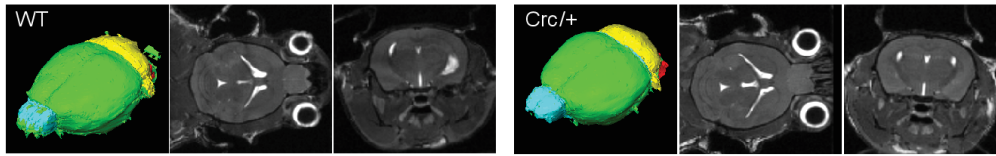


757 **Figure 1 *Scrib1<sup>crc/+</sup>* mutant mice display selective deficits of social interactions.** **A-B** Experimental design  
758 protocol of the three-chambered choice test. **A, C** Time spent sniffing with stranger male mice (S1, social  
759 stimulus) versus the object (O, non-social stimulus) during the social interest test. Here, control show a  
760 preference for the stranger mice while *Scrib1<sup>crc/+</sup>* mice show no preference between the stranger mice  
761 and the object. **A, D** Time spent sniffing with novel male mice (S2) versus familiar male (S1) mice during  
762 the social novelty test. Control group show a high preference for the novel stranger mice but *Scrib1<sup>crc/+</sup>*  
763 mice show no preference between the novel stranger mice and the familiar male mice. **B, E** Time spent  
764 sniffing a male (M) versus a female (F) mouse during the social reward test. Controls show a preference  
765 for the female mice during the first 5 min of the test while *Scrib1<sup>crc/+</sup>* mice did not. **F** Experimental design  
766 protocol of the direct social interaction with female during 5 min. Mean of duration and number of  
767 ultrasound vocalizations during a 5 min test. **G** Experimental design protocol of the direct social  
768 interaction/habituation with a juvenile. Mean investigation duration with a juvenile during initial social  
769 recognition (day 1) and social habituation (day 2). For c, d, and e data is presented as median with 25<sup>th</sup>  
770 and 75<sup>th</sup> percentile and single data points are shown as dot. For f and g data is presented as mean ±SEM.  
771 The shaded area represents the probability distribution of the variable. \* $p \leq 0.05$ ; \*\* $p \leq 0.01$  and \*\*\* $p \leq$   
772 0.001  
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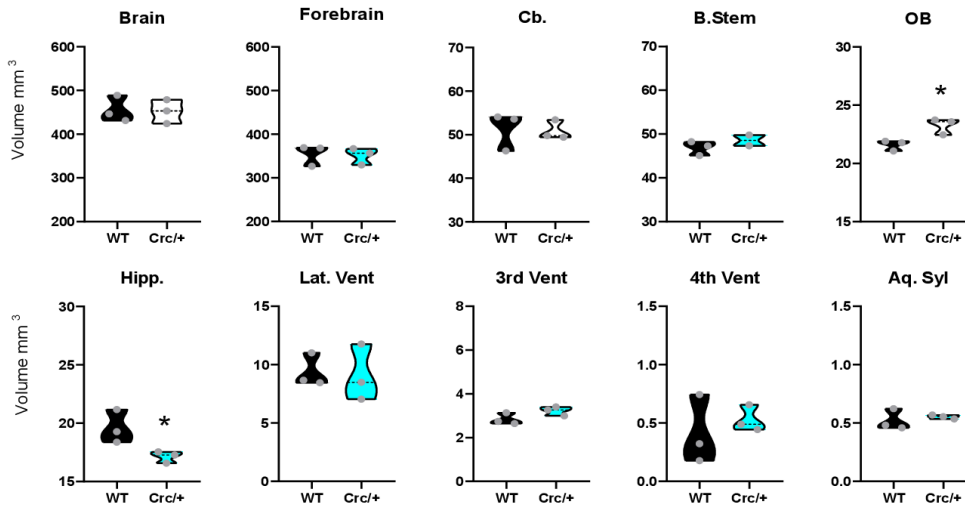


775  
 776 **Figure. 2 Specific patterns of neural activation after social interest test in *Scrib1<sup>crc/+</sup>* mice.** **A** Coronal  
 777 section of neuroanatomical areas analyzed for c-Fos immunoreactivity after a social interest test adapted  
 778 from Paxinos and Franklin (1997). Accessory olfactory bulb (AOB) and the main olfactory bulb (MOB)  
 779 granular (Gcells) and mitral cell subdivisions (Mcells); the motor cortex ( MCx); the dorsal portion of the  
 780 lateral septum (LSD); the dorso medial (STDM) and the dorso lateral (STDL) part of the striatum; the  
 781 piriform cortex (PirCx); the somatosensoriel cortex (S1Cx); the medial nucleus (MeA) and the cortical  
 782 nucleus of the amygdala (CoA); the CA1 (CA1) and the CA3 (CA3) subregion of the hippocampus; the  
 783 dentate gyrus (DG) and the lateral entorhinal cortex (EntCx). **B** Radar chart recapitulate the activate brain  
 784 region as measured by change in c-Fos immunoreactive cells in WT and *Scrib1<sup>crc/+</sup>* mice in the control and  
 785 social condition. **C** Representative microphotographs showing c-Fos-positive cells (dark dots) in the PirCx,  
 786 MeA, DG, CA3 and CA1 of WT mice or *Scrib1<sup>crc/+</sup>* mice that were killed 1 hour after the control/social test.  
 787 **D** Number of c-Fos-positive cells per mm<sup>2</sup> (mean ± SEM) was calculated for each genotype and exposure  
 788 condition. All data are presented as mean ± SEM. \**p* ≤ 0.05 and \*\*\**p* ≤ 0.001.  
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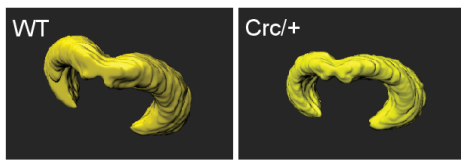
### A MRI



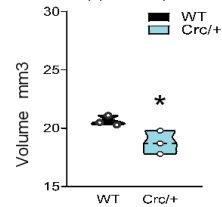
### B



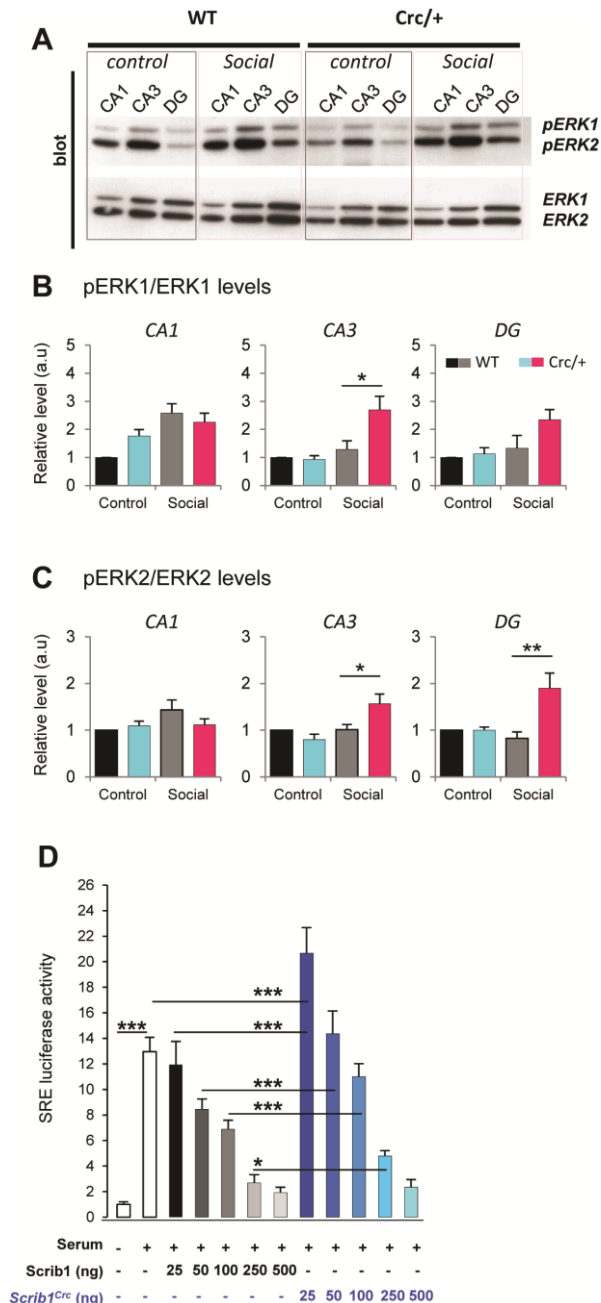
### C 3D reconstruction



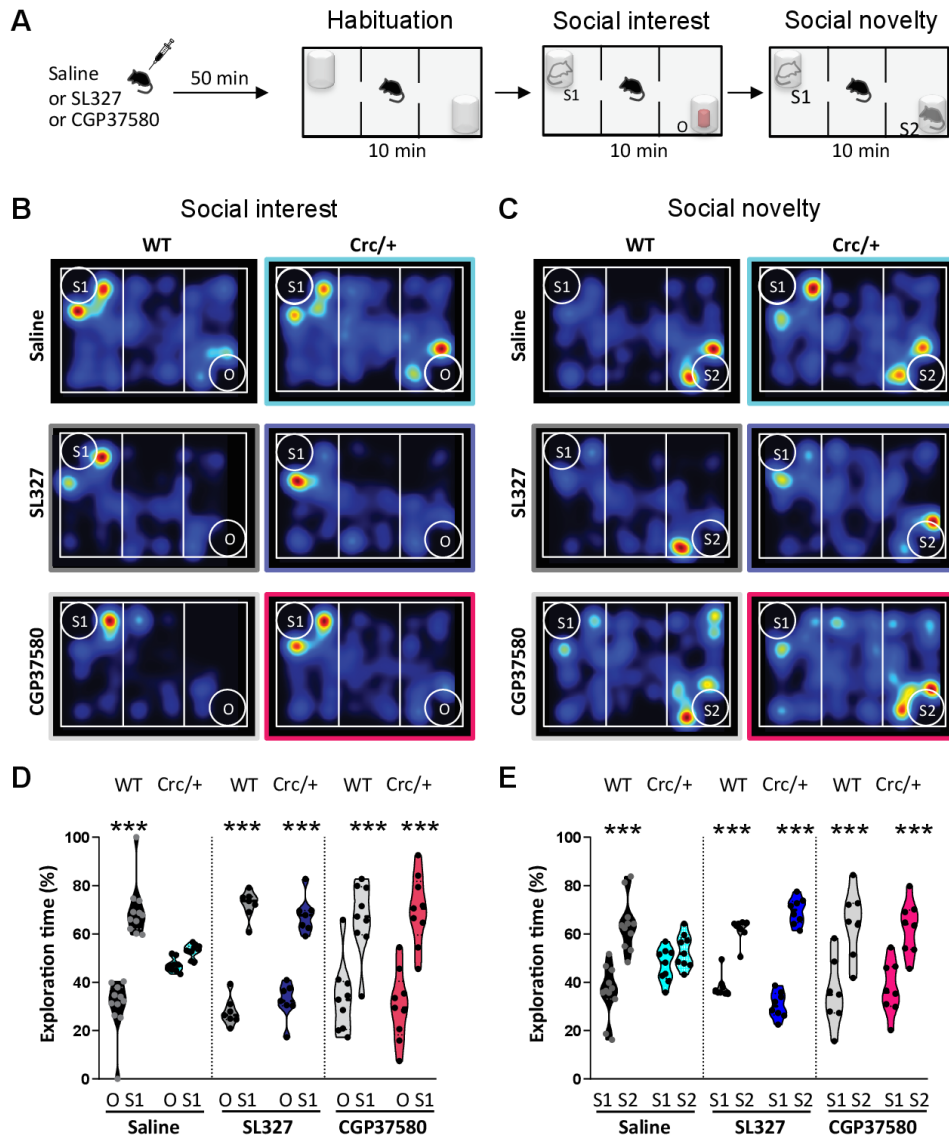
### D



790  
 791 **Figure 3. *Scrib1* mutation decreases hippocampus volume.** A MRI 3D reconstruction (left); sagittal  
 792 (middle) and coronal (right) maps of a WT and *Scrib1<sup>crc/+</sup>* mice brain in vivo. B MRI volume quantification  
 793 show a specific reduction of the hippocampus volume and increased olfactory bulb volume in *Scrib1<sup>crc/+</sup>*  
 794 mice brain. C Imaris 3D reconstruction of a WT and *Scrib1<sup>crc/+</sup>* mice hippocampus. D Volume  
 795 quantification show reduction of hippocampal formation in the *Scrib1<sup>crc/+</sup>* mice. All data are presented as  
 796 median with 25th and 75th percentile and single data points are shown as dot. The shaded area  
 797 represents the probability distribution of the variable. \* $p \leq 0.05$ .  
 798



799  
800 **Figure 4. Scrib1 mutation modulates ERK proteins levels in the hippocampus and has a biphasic effect**  
801 **on the ERK signaling pathway. A** CA1, CA3 and DG lazer capture homogenates from control and social  
802 WT and *Scrib1<sup>crc/+</sup>*. Mice are analyzed simultaneously for phospho-ERK1/2 (pERK1/2) and ERK1/2 by  
803 quantitative Western blotting. **B** Levels of phospho-ERK1 and **C** phospho-ERK2 in CA1, CA3 and DG. In  
804 control mice and *Scrib1<sup>crc/+</sup>* mice, ERK1 and ERK2 phosphorylation level is significantly more elevated  
805 after social interest in CA3 and DG of *Scrib1<sup>crc/+</sup>* mice. **D** Luciferase reporter assay data from HEK293 cells  
806 transfected with the SRE-LUC reporter vector along with increasing amount of the Scrib1 or *Scrib1<sup>crc</sup>*  
807 expression plasmid as indicated. Twenty-four hrs after transfection, cells were lysed after being serum-  
808 starved for 2hrs then activated for 6hrs with 20% FBS as indicated. Activation threshold of the serum-  
809 induced ERK pathway is indicated by the horizontal dashed line. Scrib1 represses the ERK-dependent  
810 transcriptional activation of the SRE-Luc promoter in a dose-dependent manner. *Scrib1<sup>crc</sup>* displayed a  
811 biphasic dose-response curve with an increased activation of the SRE-LUC activity effect at 25ng,  
812 whereas a significant inhibitory effect was observed at 100ng and higher doses. All data are presented  
813 as mean  $\pm$ SEM. \* $p \leq 0.05$  and \*\*\* $p \leq 0.001$ .



814  
 815 **Figure 5. The inhibition of ERK in the *Scrib1<sup>arc/+</sup>* mice reverses the autistic-like social deficits.**  
 816 **A** Experimental design protocol of the three-chambered choice test. **B, D** Time spent sniffing with  
 817 stranger male mice (S1) versus object (O) during the social interest test after vehicle, SL327 or CGP37580  
 818 treatment. **C, E** Time spent sniffing with novel male mice (S2) versus familiar male mice (S1) during the  
 819 social novelty test after vehicle, SL327 or CGP37580 treatment. Here, *Scrib1* mutation causes a deficit in  
 820 both social interest and social novelty test that is rescue by inhibition of ERK signaling. All data are  
 821 presented as median with 25<sup>th</sup> and 75<sup>th</sup> percentile and single data points are shown as dot. The shaded  
 822 area represents the probability distribution of the variable. \*\*\* $p \leq 0.001$ .