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Adrenergic activation modulates the signal from the Reissner fiber to cerebrospinal fluid-contacting neurons during development

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1 **Title**

2 Adrenergic activation modulates the signal from the Reissner fiber to cerebrospinal fluid-
3 contacting neurons during development

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13

14 **Abstract**

15 The cerebrospinal fluid (CSF) contains an extracellular thread conserved in
16 vertebrates, the Reissner fiber, which controls body axis morphogenesis in the zebrafish
17 embryo. Yet, the signaling cascade originating from this fiber to ensure body axis
18 straightening is not understood. Here, we explore the functional link between the Reissner
19 fiber and undifferentiated spinal neurons contacting the CSF (CSF-cNs). First, we show that
20 the Reissner fiber is required in vivo for the expression of urp2, a neuropeptide expressed in
21 CSF-cNs. We show that the Reissner fiber is also required for embryonic calcium transients
22 in these spinal neurons. Finally, we study how local adrenergic activation can substitute for
23 the Reissner fiber-signaling pathway to CSF-cNs and rescue body axis morphogenesis. Our
24 results show that the Reissner fiber acts on CSF-cNs and thereby contributes to establish
25 body axis morphogenesis, and suggest it does so by controlling the availability of a chemical
26 signal in the CSF.

27 **Introduction**

28 One of the major questions in the study of multicellular organism development is to
29 understand how precise morphogenesis is ensured during embryonic and postembryonic
30 stages while the animal grows into an adult. In particular, this process requires coordination
31 between cell specification signals and the control of the tissue shape (Chan et al., 2017). It
32 has recently emerged that the cerebrospinal fluid (CSF) contains many signals important for
33 cell differentiation, and is an important route for the control of morphogenesis (Fame and
34 Lehtinen, 2020). The CSF is a complex liquid filling the central nervous system cavities
35 containing a set of diffusible signaling cues guiding neurogenesis (Lehtinen et al., 2011) and
36 brain morphology in a tissue autonomous manner (Kaiser et al., 2019; Langford et al., 2020).
37 The CSF circulation is in part generated by the coordinated movement of cilia projecting to
38 the lumen of brain ventricles and central canal of the spinal cord (Faubel et al., 2016;
39 Hagenlocher et al., 2013; Olstad et al., 2019; Sternberg et al., 2018; Thouvenin et al., 2020).

40 The role of cilia in the control of CSF composition and circulation has recently gained a
41 special attention in zebrafish. Indeed, disruption of cilia motility in this species has been long
42 known to induce a typical phenotype consisting in a downward curvature of the posterior axis
43 of the embryo (Brand et al., 1996; Jaffe et al., 2016; Kramer-Zucker et al., 2005; Sullivan-
44 Brown et al., 2008). This phenotype has recently been linked to a role of the CSF in body
45 axis curvature establishment and maintenance downstream of cilia function (Cantaut-Belarif
46 et al., 2018; Grimes et al., 2016). We previously showed that the Reissner fiber (RF), a
47 component of the CSF, is important for axis morphogenesis in the zebrafish embryo (Cantaut-
48 Belarif et al., 2018). The Reissner fiber (Reissner, 1860) is an acellular proteinous thread
49 bathing in the brain and spinal cord cavities early in development (Troutwine et al., 2019),
50 formed by the aggregation of the SCO-spondin glycoprotein, secreted into the CSF by the
51 subcommissural organ and the floor plate (Lehmann and Naumann, 2005; Meiniel et al.,
52 2008). This fiber fails to assemble in cilia-defective mutants, and *scospondin* mutant embryos
53 lacking the Reissner fiber exhibit a curled-down axis despite exhibiting normal cilia motility
54 and CSF flow (Cantaut-Belarif et al., 2018; Rose et al., 2020). These observations suggest
55 that the fiber itself plays a role in the control of body axis morphogenesis in the embryo, and
56 that its absence causes a curled-down phenotype, including in cilia-defective mutants.
57 However, the signal linking the presence of the Reissner fiber and its long-range effect on
58 body axis morphogenesis is not fully understood.

59 Recent studies suggest that bath applications of monoamines can restore a straight
60 axis in embryos with a curled-down phenotype (Lu et al., 2020; Zhang et al., 2018).
61 Monoamines are widespread neurotransmitters and neuromodulators that include

62 epinephrine and norepinephrine, and influence many neurophysiological processes in the
63 adult such as sleep control (Singh et al., 2015). Radiolabeled norepinephrine can bind the
64 Reissner fiber in the CSF of rats (Caprile et al., 2003) and frogs (Diederer et al., 1983). The
65 study of their role in development is emerging, with a special focus on neuronal precursors
66 proliferation and differentiation (Berg et al., 2013).

67 In addition, the signal controlling axis morphogenesis downstream of CSF flow has
68 been associated with changes in the expression of Urp1 and Urp2 peptides (Lu et al., 2020;
69 Zhang et al., 2018). These peptides belong to a family of neuropeptides similar to Urotensin-
70 II previously shown to mediate endocrine, cardiac and neurophysiological functions (Vaudry
71 et al., 2015). In the zebrafish embryo, *urp1* and *urp2* are expressed along the antero-
72 posterior axis of the neural tube in a subset of spinal sensory neurons (Quan et al., 2015)
73 called CSF-contacting neurons (CSF-cNs). CSF-cNs extend into the CSF a microvilliated
74 apical extension that starts differentiating in the embryo and becomes fully mature in the
75 larva to tune the mechanosensory responses of CSF-cNs upon tail bending (Desban et al.,
76 2019). These conserved interoceptive neurons form two populations of different
77 developmental origins that are located ventrally and dorso-laterally relative to the central
78 canal of the zebrafish spinal cord (Djenoune et al., 2017; Park et al., 2004). At the embryonic
79 stage, undifferentiated CSF-cNs that are located ventrally in the neural tube are the only
80 ones expressing Urp neuropeptides and exhibiting spontaneous calcium transients at rest
81 (Sternberg et al., 2018). Recently, the Reissner fiber was shown to be required in the larva
82 for the mechanosensory function leading to intracellular calcium increase in differentiated
83 CSF-cNs (Orts-Del'Immagine et al., 2020). However, the role of the Reissner fiber in CSF-cN
84 spontaneous activity at the embryonic stage when they express Urp neuropeptides is
85 unknown.

86 In this study, we aimed to better understand the signal linking the Reissner fiber to
87 body axis morphogenesis. To investigate whether the Reissner fiber is required for a signal
88 towards the immature CSF-cNs, we sought to decipher the nature of this signal and how it
89 affects at long distance body axis straightening. We show that the Reissner fiber is required
90 for a signal controlling *urp2* expression. Using *in vivo* calcium imaging, we report that the
91 Reissner fiber is also required for calcium signaling in *urp2*-expressing ventral CSF-cNs,
92 confirming the existence of a crosstalk between the Reissner fiber and undifferentiated CSF-
93 cNs at the embryonic stage. Using the *pkd2/1* mutant, we show that the loss of calcium
94 signaling in ventral CSF-cNs does not lead to a loss of *urp2* expression nor embryonic body
95 axis curvature. Finally, we show that epinephrine and norepinephrine can restore the
96 Reissner fiber-dependent signal when injected locally in the brain ventricles, and can restore
97 body axis defects of *scospondin* mutants. Our work demonstrates that the Reissner fiber-

98 dependent signal to ventral CSF-cNs contributes to body axis straightening and is modulated
99 by adrenergic ligands.

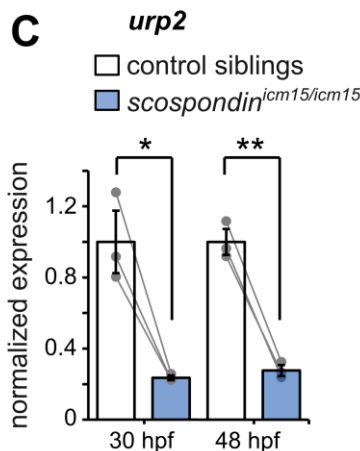
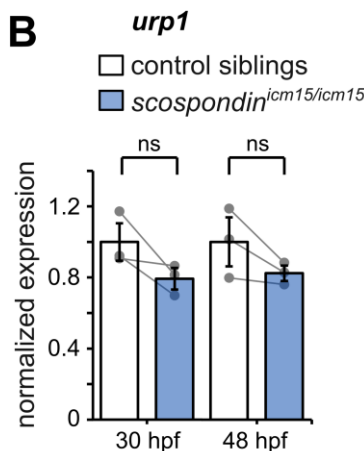
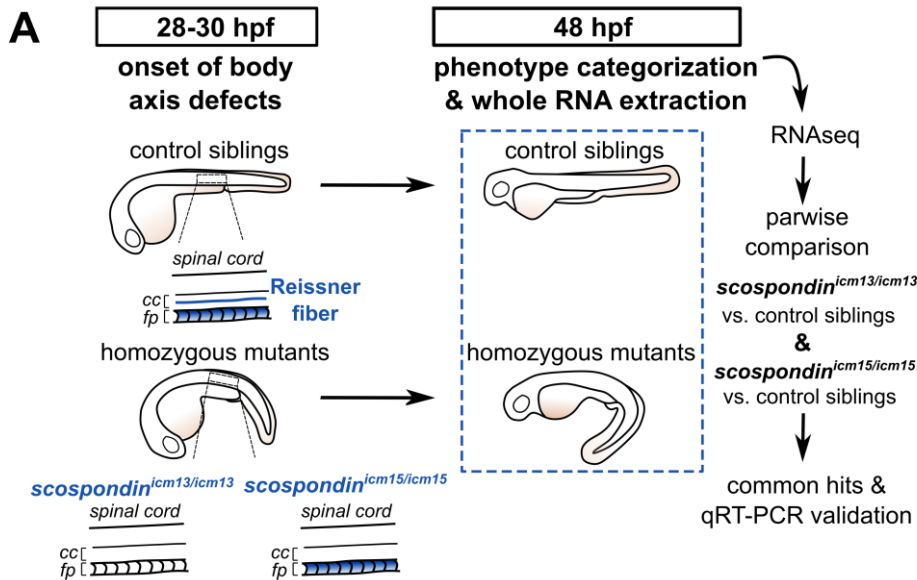
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101 **Results and Figures**

102

103 **The Reissner fiber controls *urp2* gene expression**

104 To explore the signals downstream the Reissner fiber, we performed a transcriptomic
 105 analysis of *scospondin* mutants (Figure 1).



106

107 **Figure 1. The Reissner fiber is required for *urp2* but not *urp1* gene expression.**

108 (A) Schematic of the experimental design. Embryos obtained from *scospondin*^{icm15/+} or
 109 *scospondin*^{icm13/+} incrosses were raised until 48 hpf and categorized according to their external
 110 phenotype: straight body axis (control siblings, top) or curled-down body axis (homozygous mutants,
 111 bottom) prior to RNA extraction. RNA sequencing was performed on 3 independent replicates for each
 112 allele and allowed pairwise comparisons of transcriptomes to identify commonly regulated genes.
 113 qRT-PCR experiments were performed at 30 hpf to validate transcriptomic data at the onset of body
 114 axis defects induced by the loss of the Reissner fiber and at 48 hpf when the phenotype is fully
 115 developed (48 hpf). Null *scospondin*^{icm13/icm13} and hypomorphic *scospondin*^{icm15/icm15} mutant embryos
 116 share the same peculiar curled-down phenotype induced by the loss of the Reissner fiber in the

117 central canal of spinal cord (cc). However, *scospondin*^{icm15/icm15} mutants retain SCO-spondin protein
118 expression in secretory structures such as the floor plate (fp). (B, C) qRT-PCR analysis of mRNA
119 levels of *urp1* (B) and *urp2* (C) in *scospondin*^{icm15/icm15} mutants (blue) compared to their control siblings
120 (white) at 30 and 48 hpf. Data are represented as mean \pm SEM. N = 3 independent biological
121 replicates for each condition. Each point represents a single experimental replicate. ns $p > 0.05$, *
122 $p < 0.05$, ** $p < 0.01$ (paired t-test).
123 See also Figure Supplement 1 and Source Data 1 & 2.

124

125 We took advantage of two previously-generated *scospondin* alleles (Cantaut-Belarif
126 et al., 2018) to evaluate transcriptional modifications associated with the curled-down
127 phenotype due to the loss of the Reissner fiber in the CSF. While the *scospondin*^{icm13} null
128 allele leads to a dual loss of the fiber in the central canal and of the SCO-spondin protein
129 detection in secretory structures, the *scospondin*^{icm15} hypomorphic allele retains protein
130 expression but solely precludes the Reissner fiber formation (Figure 1A). Both homozygous
131 mutants lack the Reissner fiber and exhibit a typical curled-down phenotype, which arises
132 from 28-30 hours post-fertilization (hpf) onwards and was undetectable beforehand (Cantaut-
133 Belarif et al., 2018), Figure 1A, Figure 1-figure supplement 1). We performed pairwise
134 comparisons of the transcriptomes of homozygous mutants versus control siblings at 48 hpf
135 when the body axis curvature defect is fully penetrant (Cantaut-Belarif et al., 2018). The
136 resulting lists of up- and down-regulated transcripts that were common to the two *scospondin*
137 alleles are presented in Figure 1-Source Data 2. Very few genes exhibited an important
138 change in expression, and only a handful changed more than two folds. Noticeably, we
139 observed a strong reduction of *urp2* gene expression in curled-down mutant embryos lacking
140 the Reissner fiber compared to their control siblings (mean \pm SEM fold decrease: 4.07 ± 1.2
141 in *scospondin*^{icm13/icm13} and 4.69 ± 1.77 in *scospondin*^{icm15/icm15}, n=3 replicates each, p-value <
142 0.00005, GLM test; see Material and Methods and Figure 1-Source Data 2 for details). There
143 was no significant decrease in *urp1* transcript levels (mean \pm SEM fold decrease: 0.97 ± 0.06
144 in *scospondin*^{icm13/icm13} and 1.22 ± 0.25 in *scospondin*^{icm15/icm15}, n=3 replicates each, p-value =
145 $p = 0.191$, GLM test).

146 The gene *urp2* encodes for a secreted neuropeptide belonging to the Urotensin-II-
147 related-peptide family (Tostivint et al., 2014). Together with *urp1*, these transcripts have
148 recently been identified as both strongly downregulated in curled-down mutants (Lu et al.,
149 2020; Zhang et al., 2018). To ascertain our RNAseq results and confirm the difference we
150 observed with previous results, we carried out qRT-PCR analysis of *urp1* and *urp2*
151 expression levels in the hypomorphic *scospondin*^{icm15} allele. Interestingly, we observed that
152 *urp1* expression level is not significantly decreased in *scospondin* homozygous mutants
153 compared to their control siblings, neither at 30 hpf nor at 48 hpf (Figure 1B). Consistently

154 with transcriptomic results, *urp2* expression level shows a strong decrease at 48 hpf in
155 mutant embryos compared to their control siblings (3.6 ± 0.2 fold decrease; mean \pm SEM;
156 Figure 1C). This is also true at the onset of the curled-down phenotype (30 hpf: 4.2 ± 0.5 fold
157 decrease; mean \pm SEM; Figure 1C) indicating that *urp2* gene expression level is affected
158 when embryos start to develop an abnormal morphogenesis of the posterior axis. Taken
159 together, these data show that in zebrafish embryo the presence of the Reissner fiber in the
160 CSF is required for the normal expression level of *urp2*, but not *urp1*.

161

162 **The Reissner fiber is required for calcium signaling in *urp2* expressing CSF-cNs**

163 The expression of Urotensin-II-related peptides is restricted to the ventral population
164 of CSF-cNs (Quan et al., 2015), known to exhibit spontaneous intracellular calcium variations
165 around 30 hpf (Sternberg et al., 2018) when the curled-down phenotype becomes visible in
166 *scospondin* mutants (Cantaut-Belarif 2018 and Figure 1-figure supplement 1). Curled-down
167 cilia-defective embryos lack these early calcium transients (Sternberg et al., 2018) and do
168 not form a proper Reissner fiber (Cantaut-Belarif et al., 2018). We therefore hypothesized
169 that the Reissner fiber may functionally interact with ventral CSF-cNs that are expressing
170 *urp2*.

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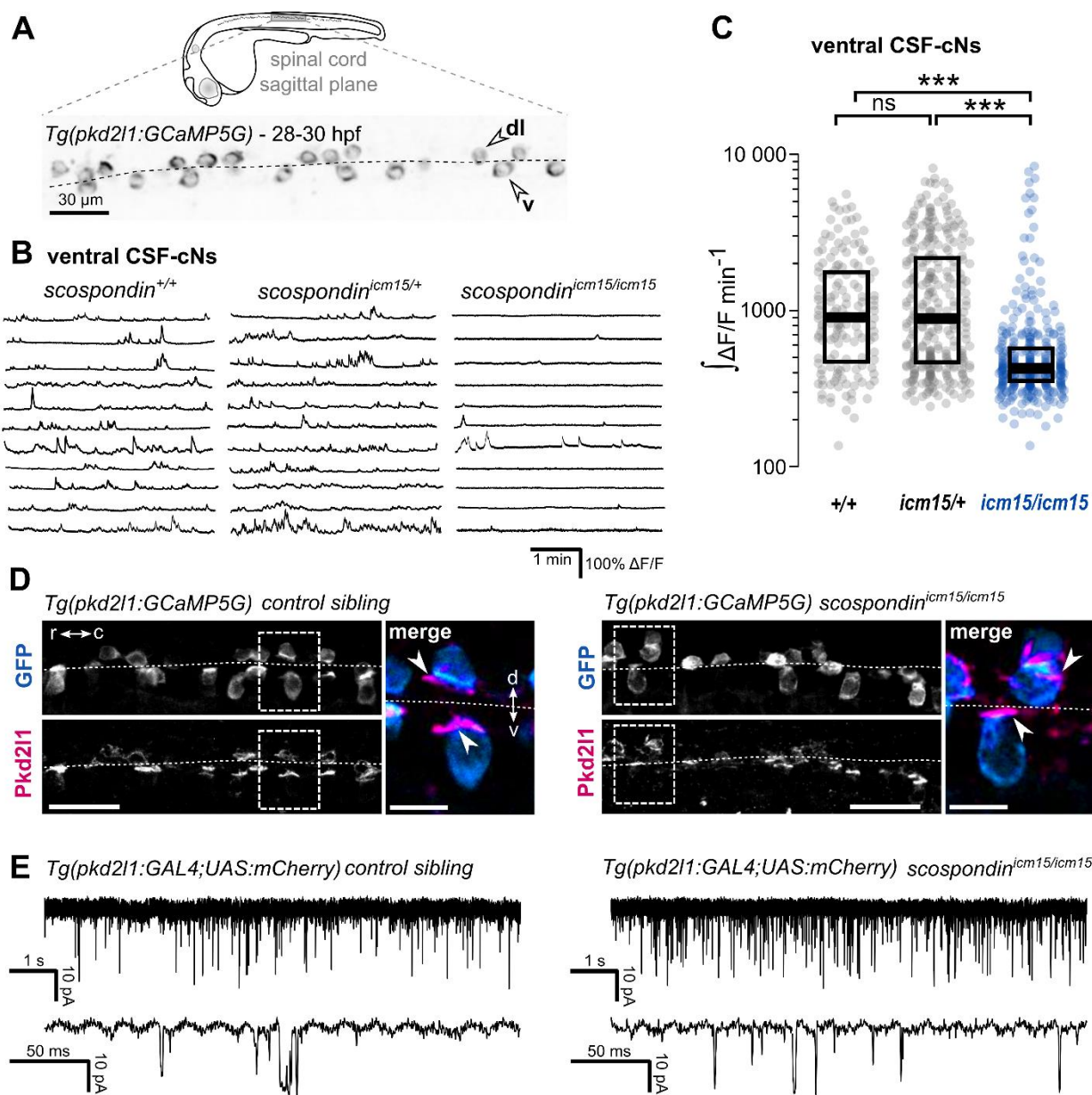
172 To address this question, we performed *in vivo* population calcium imaging at 28-30
173 hpf using the *Tg(pkcd2l1:GCaMP5G)* line labeling the dorso-lateral and ventral CSF-cNs in
174 the spinal cord (Figure 2A, Figure 2-video 1). As previously described, we observed that
175 ventral CSF-cNs exhibit spontaneous calcium transients in wild type embryos (*scospondin*^{+/+},
176 Figure 2B, Figure 2-video 1). Quantification of the integrated fluorescence variations over
177 time showed that in heterozygous *scospondin*^{icm15/+} embryos, which display a straight body
178 axis and form a proper Reissner fiber (Cantaut-Belarif et al., 2018), ventral CSF-cNs retained
179 the same level of activity than in wild type (Figure 2C). On the contrary, homozygous
180 *scospondin*^{icm15/icm15} embryos exhibited a 52.8% decrease of calcium activity compared to
181 wild type (Figure 2C). A similar reduction of 39% occurred in the null *scospondin*^{icm13/icm13}
182 mutant compared to wild type siblings (Figure 2-figure supplement 2A, 2B). These data show
183 that the loss of the Reissner fiber reduces spontaneous calcium variations of ventral CSF-
184 cNs during the critical time window for body axis straightening. On the contrary, the sparse
185 spontaneous calcium transients in dorsal CSF-cNs were not affected by the absence of the
186 Reissner fiber in both *scospondin* alleles (Figure 2-figure supplement 2C, 2D).

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Figure 2. The Reissner fiber is required for the spontaneous calcium variations of ventral CSF-contacting neurons.

(A) 28-30 hpf embryos expressing the GCaMP5G calcium reporter in CSF-contacting neurons were imaged on the lateral side. *Tg(pkcd211:GCaMP5G)* embryos label both dorso-lateral (dl, above the dotted line) and ventral (v, below the dotted line) CSF-contacting neurons (arrowheads). Scale bar: 30 μm . (B) Representative traces of calcium variations in individual ventral CSF-contacting neurons in wild type (*scospondin*^{+/+}), heterozygous (*scospondin*^{*icm15*/+}) and *scospondin*^{*icm15/icm15*} mutants. Sample traces from individual cells with integral $\Delta F/F$ values ranging around the median distribution of the imaged population are represented for each genotype (n = 11 cells). (C) Quantification of the normalized integrated calcium variation over time of ventral CSF-contacting neurons in wild type (+/+), heterozygous (*icm15*/+) and *scospondin*^{*icm15/icm15*} mutants (*icm15/icm15*, blue). Data were collected

204 from 5 independent experiments and include 10 wild type embryos (n=146 cells), 20 heterozygous
205 embryos (n=287 cells) and 21 *scospondin*^{icm15/icm15} mutants (n=307 cells). Each point represents a
206 single cell. Bottom and top edges of the boxes indicate the 1st and 3rd quartiles. Bold lines represent
207 the median value for each distribution. ns p>0.05, *** p<0.001 (Kolmogorov-Smirnov test). (D)
208 Immunohistochemistry for Pkd2l1 (magenta) and GFP (blue) in *Tg(pkd2l1:GCaMP5G)* embryos at 30
209 hpf in the spinal cord of a control sibling (left) and *scospondin*^{icm15/icm15} mutant (right). Scale bar: 30 μ m.
210 Magnification of the area delineated by dotted line boxes is represented for each condition (r: rostral,
211 c: caudal, d: dorsal, v: ventral). Scale bar: 10 μ m. *scospondin*^{icm15/icm15} embryos show a similar
212 localization of the Pkd2l1 protein at the developing apical extension (arrowheads) of the CSF-cNs
213 (labeled by the GFP antibody, blue) compared to control siblings. (E) *In vivo* voltage-clamp recordings
214 from CSF-contacting neurons in the *Tg(pkd2l1:GAL4;UAS:mCherry)* line at 30 hpf in control embryos
215 (left) and *scospondin*^{icm15/icm15} mutants (right). Note the extensive number of events in both conditions
216 (top traces). Bottom traces represent higher temporal magnifications and allow distinguishing single
217 channel openings.
218 See also Figure Supplement 1 and Video 1.

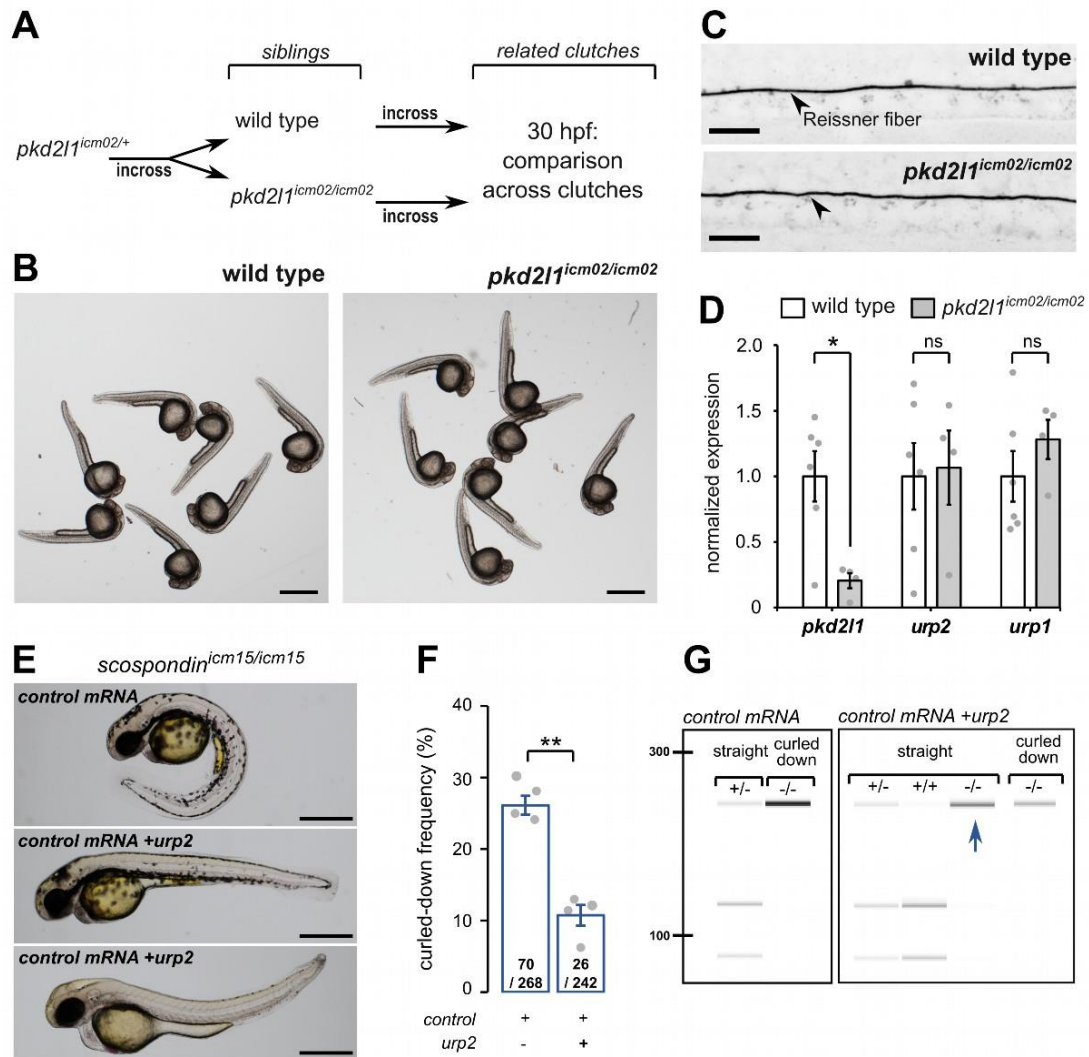
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220 Early calcium transients in CSF-cNs require the non-selective cationic channels
221 permeable Pkd2l1 channel *in vivo* (Sternberg et al., 2018) that is permeable to calcium ions.
222 The reduction in calcium activity when the Reissner fiber is lacking could therefore be due to
223 a defect in the Pkd2l1 channel localization or opening probability. *In toto*
224 immunohistochemistry for Pkd2l1 protein showed that this channel is enriched at the
225 differentiating apical extension of CSF-cNs in curled-down *scospondin*^{icm15/icm15} mutants as
226 well as in control embryos (Figure 2D). In order to assess Pkd2l1 channel properties, we
227 performed *in vivo* whole cell voltage clamp recordings in double transgenic
228 *Tg(pkd2l1:GAL4;UAS:mCherry)* embryos. In the absence of a Reissner fiber in
229 *scospondin*^{icm15/icm15} mutants, we observed spontaneous Pkd2l1 channel openings that were
230 similar to control embryos (Figure 2E). Thus, the loss of the Reissner fiber decreases CSF-
231 cNs intracellular calcium variations without preventing the opening nor the localization of
232 Pkd2l1 channels in the differentiating apical extension of the cells. Altogether, these data
233 suggest that the Reissner fiber is required for a signal acting on ventral CSF-cNs that
234 controls both the spontaneous calcium variations and the expression of *urp2* in the embryo.
235

236 **Loss of *urp2* expression is only observed in mutants devoid of the Reissner fiber,** 237 **independently of calcium variations in CSF-cNs**

238 As the Reissner fiber is required for both ventral CSF-cNs spontaneous calcium
239 transients and *urp2* gene expression, we tested whether intracellular calcium variations are
240 necessary for a normal *urp2* expression and axis straightness. We took advantage of the

241 *pkd211^{icm02/icm02}* mutant where the embryonic activity of CSF-cNs is abolished (Sternberg et
 242 al., 2018).



243

244

245 **Figure 3. *urp2* expression is Pkd211-independent and is important for the Reissner fiber-**
 246 **dependent straightening of the embryonic posterior axis.**

247 (A) Adult wild type and *pkd211^{icm02/icm02}* siblings were incrossed to generate related clutches that were
 248 analyzed at 30 hpf. (B) Representative pictures of wild type (left) and *pkd211^{icm02/icm02}* embryos (right)

249 at 30 hpf. Note that mutant embryos develop a straight posterior axis. Scale bar: 0.5 mm. (C)

250 Representative immunohistochemistry for the Reissner fiber imaged from the spinal cord of a wild type

251 (top, one representative embryo out of 20) and a *pkd211^{icm02/icm02}* embryo (bottom, one representative

252 embryo out of 20). Note that the Reissner fiber forms properly in the mutant. Scale bars: 10 μ m. (D)

253 qRT-PCR analysis of mRNA levels of *pkd211*, *urp2* and *urp1* in wild type (white) and *pkd211^{icm02/icm02}*

254 embryos (grey). Data are represented as mean \pm SEM. N = 6 independent replicates for wild-type and

255 4 for *pkd211^{icm02/icm02}*. Each point represents a single experimental replicate. ns p > 0.05, * p < 0.05

256 (unpaired t-test). (E) Representative pictures of *scospondin^{icm15/icm15}* mutant embryos at 48 hpf after

257 one cell stage injections of a control mRNA alone or of a mix containing a control mRNA and *urp2*
258 mRNA (middle and bottom). Note that upon control injections, *scospondin*^{*icm15/icm15*} mutants display at
259 typical curled-down phenotype, while *urp2* overexpression can lead to straightened (middle) or slightly
260 curled-up posterior axis (bottom). Scale bar: 0.5 mm. (F) Quantification at 48 hpf of curled-down
261 frequency in embryos obtained from *scospondin*^{*icm15/+*} incrosses upon control mRNA injections (n=70
262 curled-down animals out of 268) or *urp2* mRNA overexpression (26 curled-down embryos out of 242).
263 Data were collected from 4 independent clutches and represented as mean ±SEM. ** p<0.01 (paired t-
264 test). (G) Injected embryos were genotyped at 48 hpf based on the loss of a restriction site in the
265 *scospondin* mutant allele leading to a band resistant to digestion (-/-). While mutant animals are
266 exclusively curled-down in control conditions, *urp2* mRNA overexpression leads to the detection of
267 mutant animals displaying a straight body axis (blue arrow).
268 See also Figure supplement 1.

269

270 Thanks to the viability of *pkd2l1*^{*icm02/icm02*} zygotic mutants, we generated genetically-
271 related clutches that were either fully wild type or fully maternal & zygotic (MZ) homozygous
272 mutant (Figure 3A). We observed that *pkd2l1*^{*icm02/icm02*} MZ mutant embryos did not display
273 any defect in body axis morphogenesis and were morphologically undistinguishable from wild
274 type embryos (Figure 3B). Using immunohistochemistry, we observed that *pkd2l1*^{*icm02/icm02*}
275 mutants form a normal Reissner fiber in the central canal of the spinal cord at 30 hpf (Figure
276 3C). Next, using qRT-PCR, we tested whether *urp1* and *urp2* gene expression levels were
277 diminished by the absence of calcium transients in CSF-cNs (Figure 3D). As the mutation in
278 the *icm02* allele generates a premature stop codon in the *pkd2l1* gene (Böhm et al., 2016),
279 one can predict that the *pkd2l1* mRNA would be degraded by nonsense-mediated decay.
280 Indeed, mutant clutches displayed a 4.9 fold decrease in *pkd2l1* transcripts level compared
281 to wild type counterparts (Figure 3D). Interestingly, we observed that *urp2* and *urp1* gene
282 expression were not decreased in *pkd2l1*^{*icm02/icm02*} embryos compared to wild types (Figure
283 3D). Altogether, these data show that the loss of Pkd2l1-driven calcium transients in CSF-
284 cNs does not lead to a decreased expression of *urp1* and *urp2* transcripts, rejecting the
285 hypothesis of a direct requirement for *urp2* expression of Pkd2l1-dependent intracellular
286 calcium variations. Instead, our results show a strict correlation between the presence of the
287 Reissner fiber, a normal *urp2* expression level and the proper morphogenesis of the
288 embryonic body axis. Altogether, our results prompted us to hypothesize that the aberrant
289 posterior axis curvature in absence of the Reissner fiber is a consequence of the decreased
290 *urp2* expression.

291

292 ***urp2* expression in the absence of the Reissner Fiber can restore posterior axis**
293 **defects**

294 Given the strict correlation between *urp2* expression and body axis straightening, we
295 hypothesized that restoring *urp2* levels would rescue the posterior axis curvature developed
296 by *scospondin*^{icm15/icm15} mutants. To test this hypothesis, we performed one cell stage *urp2*
297 mRNA injections on clutches obtained from *scospondin*^{icm15/+} parents. We sorted the injected
298 embryos at 48 hpf into two morphological categories: curled-down and non-curled-down
299 (Figure 3E, 3F). In the four independent experiments conducted, injections with a control
300 mRNA led to a proportion of curled-down embryos close to 25%, as expected (Figure 3F).
301 On the contrary, embryos overexpressing *urp2* showed much lower proportions of curled-
302 down embryos ($10.7 \pm 1.5\%$; mean \pm SEM; Figure 3F), suggesting that some homozygous
303 mutants are rescued in this condition. To confirm this rescue, we genotyped curled-down and
304 non-curled-down 48 hpf embryos injected with a control mRNA or *urp2* mRNA (Figure 3E,
305 3G, Figure3-figure supplement 1). As expected, wild type and heterozygous embryos
306 injected with the control mRNA displayed a straight body axis, while *scospondin*^{icm15/icm15}
307 showed a downward curvature of the posterior axis. Instead, *urp2* overexpression lead to
308 detect straight or slightly curled-up *scospondin*^{icm15/icm15} embryos at several instances (Figure
309 3E, 3G, Figure3-figure supplement 1). Thus, restoring higher *urp2* levels is sufficient to
310 prevent the embryonic posterior axis defects in *scospondin* mutants. These observations
311 confirm that *urp2* neuropeptide expressed in CSF-cNs may signal at long range, as
312 suggested from the expression of the receptor in dorsal somites (Zhang 2018), to ensure a
313 proper axis morphogenesis.

314

315 **Epinephrine and norepinephrine restore morphogenesis of the posterior axis and *urp*** 316 **expression in *scospondin* mutants**

317 Based on our observations, we assumed that the Reissner fiber is necessary for the
318 activity of at least one signaling pathway regulating *urp2* gene expression together with body
319 axis morphogenesis. Epinephrine and norepinephrine belong to the monoamine
320 neurotransmitter family and are known to bind the Reissner fiber in rats (Caprile et al., 2003)
321 and frogs (Diederer et al., 1983). Recently, systemic bath applications of monoamines have
322 been described to rescue body axis defects in curled-down cilia defective mutants (Lu et al.,
323 2020, (Zhang et al., 2018). We investigated the possible role of epinephrine and
324 norepinephrine in the regulation of the RF signaling pathways.

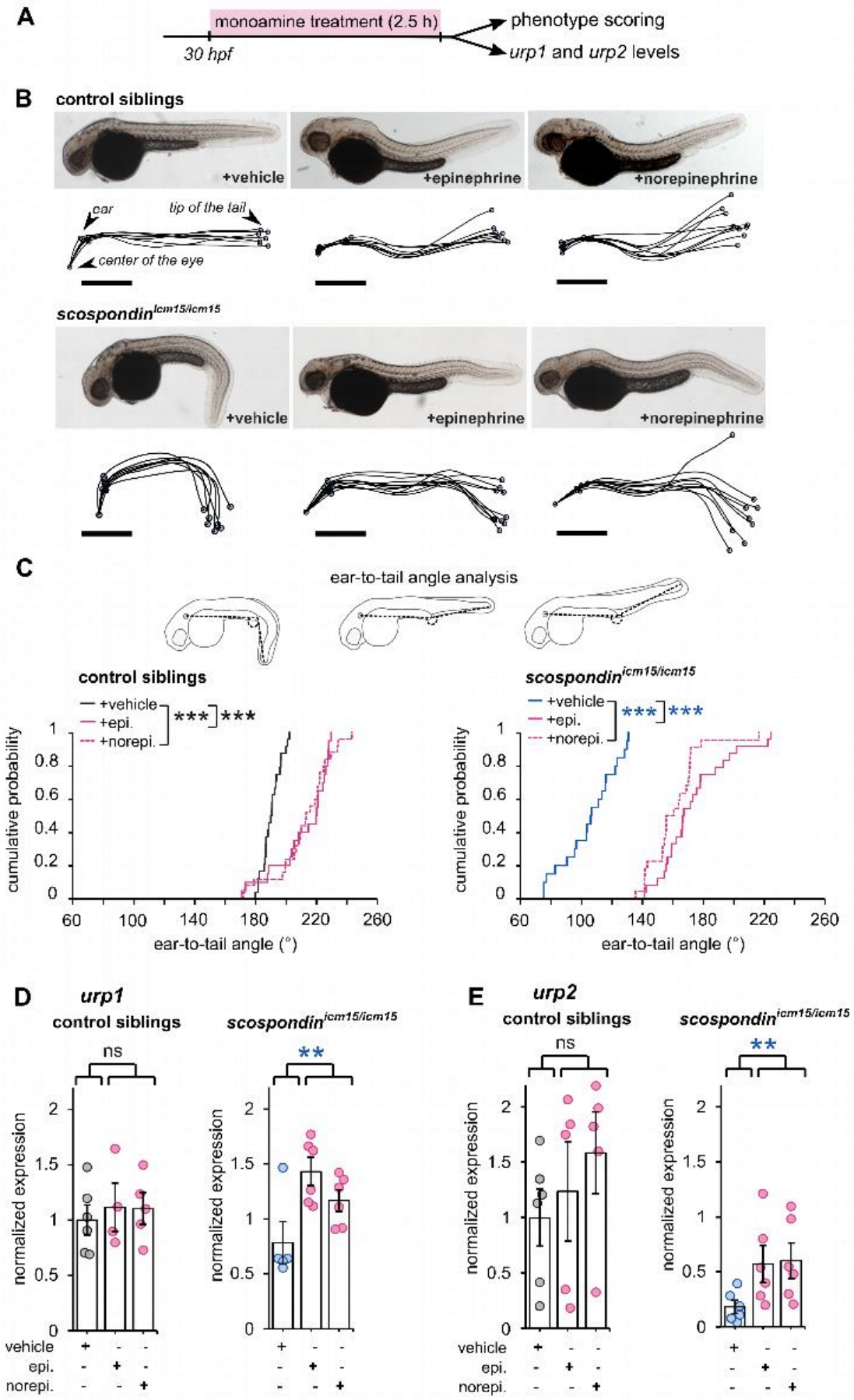
325 First, we tested if epinephrine and norepinephrine could influence the curled-down
326 phenotype developed by *scospondin*^{icm15/icm15} mutants. We compared sibling animals
327 generated from incrosses of *scospondin*^{icm15/+}. We first analyzed the effect of 2.5 hours bath
328 applications of epinephrine and norepinephrine on control embryos at 30 hpf (Figure 4A).
329 Epinephrine and norepinephrine have a moderate impact on the shape of the head-to-tail

330 axis of initially straight embryos, which display a slight curled-up phenotype after exposure to
331 monoamines (Figure 4B). To estimate the straightness of the posterior axis, we quantified
332 the angle formed between the ear, the caudal limit of the yolk extension and the tip of the tail
333 (Figure 4C, top panel). This angle is distributed around 190.6° in control embryos exposed to
334 a vehicle solution (median value; Figure 4C, bottom left graph). Comparatively, control
335 embryos exposed to monoamines exhibit a distribution of ear-to-tail angles shifted towards
336 slightly higher values (median values: 220.4° and 213.6° for epinephrine and norepinephrine,
337 respectively; Figure 4C, bottom right graph). Next, we analyzed the effect of epinephrine and
338 norepinephrine on *scospondin*^{icm15/icm15} embryos. While mutant animals exposed to a vehicle
339 solution display a typical curled-down body axis, *scospondin*^{icm15/icm15} mutants treated with
340 monoamines exhibit a reduction in the downward curvature of the posterior axis (Figure 4B).
341 Quantifications of ear-to-tail angles in *scospondin*^{icm15/icm15} mutants show a large increase of
342 the median angle value after exposure to monoamines, compared to embryos treated with a
343 vehicle solution (106.5° for vehicle, 167.1° for epinephrine and 158.5° for norepinephrine;
344 median values; Figure 4C). Altogether, these data show that epinephrine and norepinephrine
345 can partially restore the posterior axis geometry of *scospondin*^{icm15/icm15} mutants, suggesting
346 that monoamines can rescue the Reissner fiber-dependent signal required for a straight
347 embryonic body axis.

348 Next, we analyzed the effect of epinephrine and norepinephrine on *urp1* and *urp2*
349 gene expression using qRT-PCR. In control siblings, *urp1* expression remains comparable
350 after vehicle, epinephrine or norepinephrine exposure (Figure 4D). Consistently with our
351 previous results, *urp1* expression is not significantly modified in curled-down
352 *scospondin*^{icm15/icm15} embryos compared to their control siblings receiving the same treatment
353 (1.01 +/- 0.17 fold change; mean ± SEM; Figure 4D). However, we observed a slight
354 increase of *urp1* expression in curled-down mutant embryos treated with epinephrine and
355 norepinephrine compared to vehicle treatment (1.58 ± 0.43 and 1.28 ± 0.23 fold changes for
356 epinephrine and norepinephrine respectively; mean ± SEM). As expected, *urp2* expression
357 level is significantly decreased in curled-down *scospondin*^{icm15/icm15} mutants compared to
358 straight siblings (0.22 ± 0.06 fold change; mean ± SEM; Figure 4E). Epinephrine and
359 norepinephrine treatments also do not change *urp2* expression in control siblings, but
360 significantly increase it in curled-down homozygous mutant embryos (7.42 ± 4.23 and 4.67 ±
361 1.15 fold changes for epinephrine and norepinephrine respectively; mean ± SEM; Figure 4E).

362 These observations show that the rescue of the posterior axis curvature of
363 *scospondin* homozygous mutant embryos by monoamines is associated with an increase of
364 the expression of *urp1* and *urp2* neuropeptides. Epinephrine and norepinephrine
365 compensate the loss of the Reissner fiber both on Urotensin-II-related neuropeptides

366 expression and on posterior axis curvature, suggesting that these compounds act on the
367 Reissner fiber-dependent signaling pathway in the embryonic CSF.



369 **Figure 4. Epinephrine and norepinephrine compensate the loss of the Reissner fiber for body**
370 **axis straightening and increase *urp* expression.**

371 (A) Curled-down *scospondin*^{icm15/icm15} mutants and their control siblings were sorted at 30 hpf
372 according to the geometry of their posterior axis and then exposed to a E3 solution (vehicle),
373 epinephrine or norepinephrine for 2.5 hours prior to phenotype scoring and RNA extraction. (B)
374 Representative pictures of control siblings (top) and *scospondin*^{icm15/icm15} mutants (bottom) after vehicle
375 (left), epinephrine (middle) or norepinephrine (right) treatments. For each condition, the global
376 morphologies of treated embryos are represented by superimposed traces linking the center of the
377 eye, the ear and the tip of the tail in one representative clutch. Scale bar: 0.5 mm. (C) Quantification of
378 the angle formed between the ear, the caudal limit of the yolk extension and the tip of the tail (as
379 shown on the schematics, top) in control siblings (bottom left) and *scospondin*^{icm15/icm15} mutants
380 (bottom right). Data were collected from 3 independent experiments and include 24, 20, 25 control
381 siblings treated with a vehicle solution, epinephrine, norepinephrine respectively (black, solid pink and
382 dotted pink line respectively) and 20, 24, 22 *scospondin*^{icm15/icm15} embryos treated with a vehicle
383 solution, epinephrine, norepinephrine respectively (blue, solid pink and dotted pink line respectively)
384 *** p<0.001 (Kolmogorov-Smirnov test). (D, E) qRT-PCR analysis of the mRNA level of *urp1* (D) and
385 *urp2* (E) in control siblings (left) and *scospondin*^{icm15/icm15} embryos (right). Data are represented as
386 mean ± SEM. n=4 to 6 independent replicates for each condition. Each point represents a single
387 experimental replicate. ns p>0.05, ** p<0.01 (GLM test).

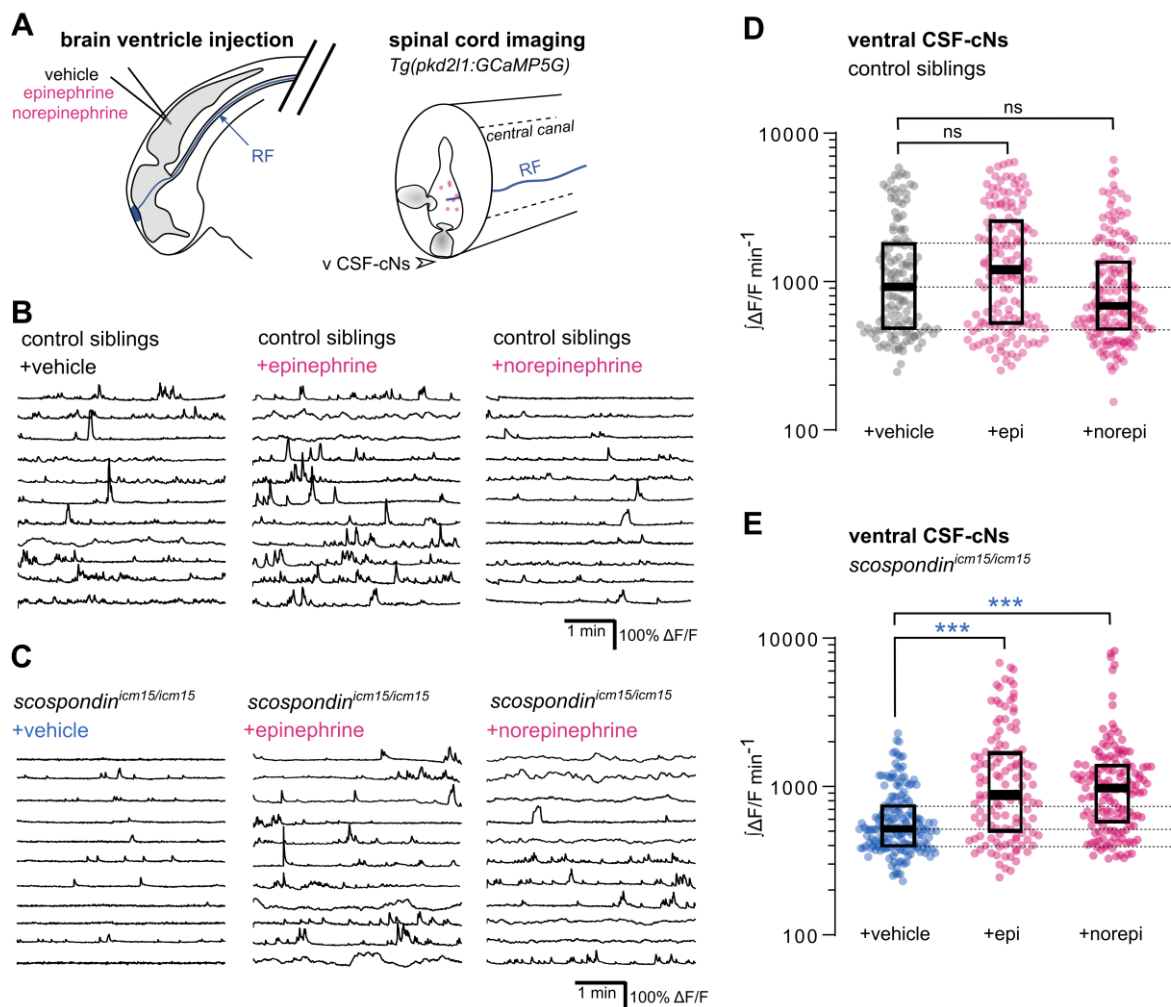
388

389 **Epinephrine and norepinephrine restore the Reissner fiber-dependent calcium**
390 **signaling in ventral CSF-cNs of *scospondin* mutants**

391 The Reissner fiber is required for three concomitant events: intracellular calcium
392 variations and *urp2* expression in ventral CSF-cNs, and the straightening of the posterior
393 axis. We therefore asked whether the delivery of monoamines in CSF could also restore
394 calcium variations in ventral CSF-cNs. To address this question, we performed hindbrain
395 ventricle injections of epinephrine or norepinephrine in the *Tg(pkd211:GCaMP5G)*;
396 *scospondin*^{icm15} embryos at 30 hpf, and recorded calcium variations in ventral CSF-cNs within
397 the spinal cord 20 to 60 minutes after the injections (Figure 5A). In order to assess how the
398 monoamines used diffuse in the central canal of the spinal cord and whether they are stable
399 enough in the CSF, we performed immunostainings against norepinephrine 30 and 60
400 minutes after hindbrain ventricle injections (Figure 5- figure supplement 1). We observed an
401 abundant norepinephrine-positive signal in the central canal of the rostral most region of the
402 spinal cord 30 minutes post-injection, and the median and caudal most-regions 60 minutes
403 post injection, which was absent in control injections (Figure 5- figure supplement 1). This
404 observation indicates that norepinephrine diffuses down the central canal when injected in
405 the same conditions used for *in vivo* calcium imaging and that it is stable in the CSF after an

406 hour, in agreement with former results in the mammalian ventricular system of dogs (Maas
 407 and Landis, 1965), sheeps (Forbes and Baile, 1974) and rats (Fuxe and Ungerstedt, 1966;
 408 Levitt et al., 1983).

409 We first analyzed the effect of epinephrine and norepinephrine injections on control
 410 embryos displaying a straight body axis at 30 hpf and observed that an exogenous delivery
 411 of monoamines did not influence the basal calcium variations of ventral CSF-cNs (Figure 5B,
 412 5D). Next, we analyzed the effect of epinephrine and norepinephrine in *scospondin*^{icm15/icm15}
 413 mutants (Figure 5C, 5E, Figure5-video 1). As described previously, ventral CSF-cNs
 414 displayed a 44.1% decrease of calcium variations in *scospondin*^{icm15/icm15} embryos compared
 415 to control siblings in control condition with vehicle injections (median value; Figure 5D, 5E),
 416 confirming



417
 418 **Figure 5. Local monoamine delivery restores calcium variations of ventral CSF-contacting**
 419 **neurons in *scospondin* mutants.**

420 (A) *Tg(pkcd211:GCaMP5G)* embryos we used to perform hindbrain ventricle injections at 30 hpf of
 421 artificial CSF (vehicle), epinephrine or norepinephrine (left). Intracellular calcium variations in ventral
 422 CSF-contacting neurons (v CSF-cNs, arrowhead) were recorded in the spinal cord 30 minutes after
 423 the injection to allow monoamines (pink dots) diffusing down the central canal where bathes the

424 Reissner fiber (RF) in control embryos. **(B, C)** Representative traces of calcium variations of individual
425 ventral CSF-contacting neurons in control siblings **(B)** and *scospondin*^{icm15/icm15} mutants **(C)** after
426 vehicle (left), epinephrine (middle) and norepinephrine injections (right). Sample traces from individual
427 cells with integral $\Delta F/F$ values ranging around the median distribution of the imaged population are
428 represented for each condition (n=11). **(D, E)** Quantification of the normalized integrated intracellular
429 calcium variation over time of ventral CSF-contacting neurons in control siblings **(D)** and
430 *scospondin*^{icm15/icm15} mutants **(E)**. Data were collected from 3 independent experiments and include 9,
431 11 and 12 control embryos recorded after vehicle, epinephrine and norepinephrine injections
432 respectively (n=131, 150 and 164 cells respectively) and 11, 10 and 10 *scospondin*^{icm15/icm15} mutants
433 after vehicle, epinephrine and norepinephrine injections respectively (n=168, 124 and 150 cells
434 respectively). Each point represents a single cell. Bottom and top edges of the boxes indicate the 1st
435 and 3rd quartiles. Dotted lines represent the distribution range around the 1st and 3rd quartiles of control
436 embryos injected with a vehicle solution. Bold lines represent the median value for each distribution.
437 ns p>0.05, *** p<0.001 (Kolmogorov-Smirnov test).

438 See also Figure supplement 1 and Video 1.

439

440 the observations reported in Figure 3. Interestingly, injections of epinephrine or
441 norepinephrine in *scospondin*^{icm15/icm15} mutants increased the median $\int \Delta F/F \text{ min}^{-1}$ by 70.02%
442 and 88.6% respectively compared to vehicle injections (Figure 5E). Moreover, the rescue of
443 the spontaneous activity of CSF-cNs by monoamines in mutant embryos reached
444 comparable levels to those observed in control siblings (Figure 5D-E). These data show that
445 epinephrine and norepinephrine restore the Reissner fiber-dependent calcium transients in
446 ventral CSF-cNs in *scospondin* mutants.

447 Altogether, our results are compatible with the existence of a signal that links the
448 Reissner fiber to both *urp2* expression and calcium variations in ventral CSF-cNs that can be
449 modulated by monoaminergic activation. These observations suggest that endogenous
450 epinephrine and norepinephrine may act locally to tune calcium signaling in CSF-cNs.

451

452 **Norepinephrine can be detected in the embryonic CSF and adrenergic receptors are** 453 **expressed by spinal cells contacting the CSF**

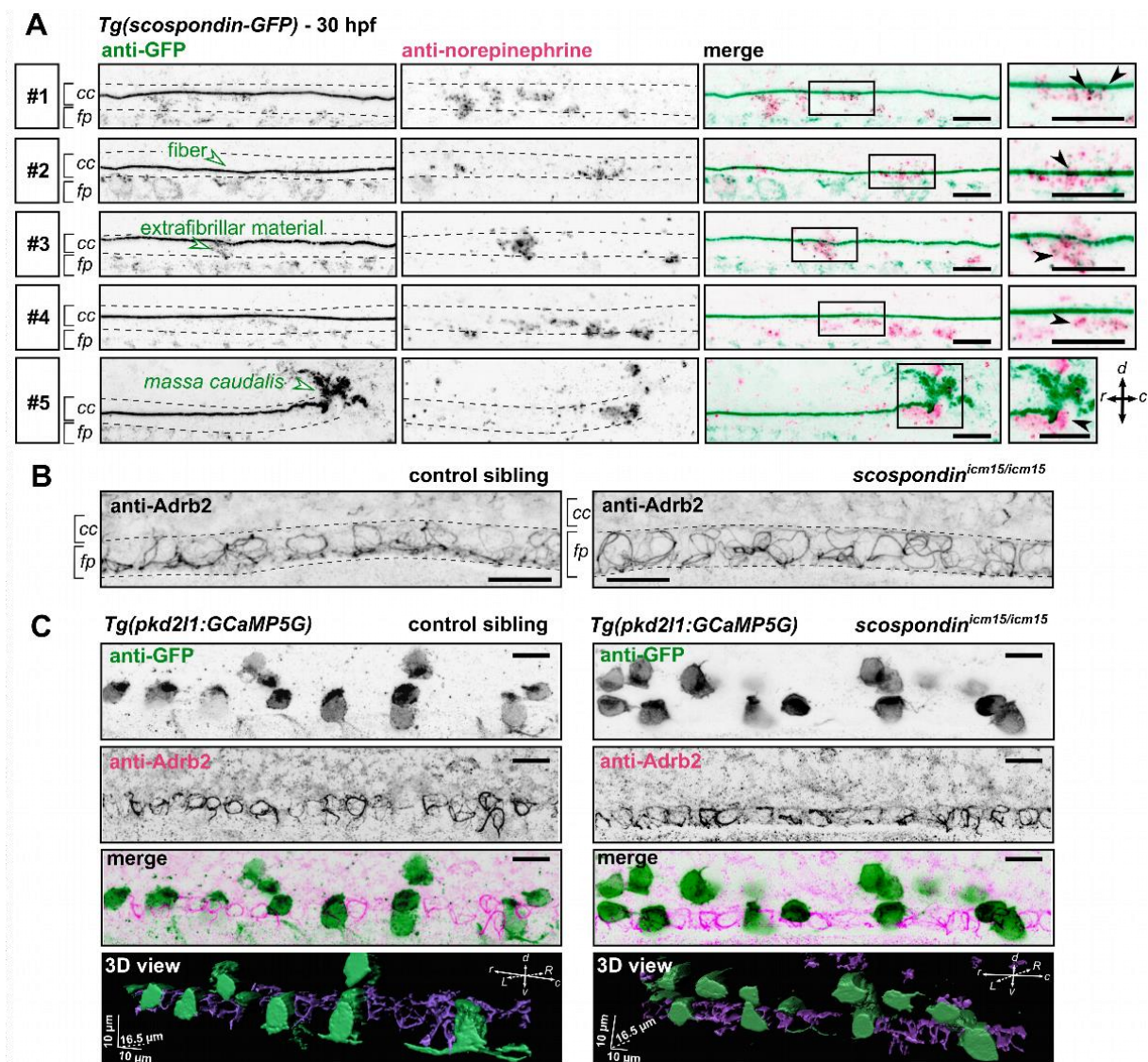
454 To assess that this modulation takes place at the interface between CSF and the cells
455 lining the central canal, we performed immunostainings against endogenous norepinephrine
456 at 30 hpf in *Tg(scospodin-GFP)* embryos labeling *in vivo* the Reissner fiber (Troutwine et
457 al., 2019). As previously described, SCO-spondin positive material in the CSF contributes
458 mainly to form the fiber, but is also present as punctated material in close vicinity the fiber,
459 referred to here as extrafibrillar material, (Troutwine et al., 2019, Figure 6A). We observed
460 norepinephrine positive puncta colocalized with the Reissner fiber as well as endogenous
461 norepinephrine positive signals following patterns similar to extrafibrillar material labelled by

462 the *Tg(scospondin-GFP)* transgene. This can also be observed closely at the level of the
463 *massa caudalis*, formed by the accumulation of SCO-spondin at the caudal end of the central
464 canal (Figure 6A). These observations suggest that norepinephrine is endogenously present
465 in the embryonic CSF where it is associated with the Reissner-positive material in the central
466 canal.

467 To address the question of the receptor associated to this signaling, we performed
468 immunostainings against the adrenergic receptor *Adrb2*, binding both epinephrine and
469 norepinephrine, which is described to be transiently expressed in the zebrafish nervous
470 system at early stages of embryonic life (Wang et al., 2009). We observed that *Adrb2* is
471 distributed ventrally in the neural tube, at the interface with the central canal, in a pattern that
472 suggests a membrane localization in both control siblings and *scospondin^{icm15/icm15}* mutants
473 (Figure 6B). Thus, *Adrb2* localization is suitable for binding endogenous ligands present in
474 the CSF.

475 Next, we addressed the question of whether *Adrb2* is expressed in CSF-contacting
476 neurons, which could explain the rescue of calcium transients observed in
477 *scospondin^{icm15/icm15}* mutants after epinephrine and norepinephrine injections. Double
478 immunostainings against *Adrb2* and GFP in *Tg(pkd2l1:GCaMP5G)* control siblings show that
479 both signals cover different domains in the ventral most region of the spinal cord (Figure 6C),
480 where the *Adrb2* positive domain remains in the ventral midline, inserted between two rows
481 of ventral CSF-contacting neurons (Figure 6-video 1). As ventral CSF-contacting neurons are
482 known to derive from lateral floor plate progenitors (Park et al., 2004; Yang et al., 2010), the
483 medial cells expressing *Adrb2* most probably correspond to the medial floor plate.
484 Importantly, the same distribution is observed in *scospondin^{icm15/icm15}* mutants (Figure 6C and
485 Figure 6-video 1). This observation suggests that the loss of calcium signaling in
486 *scospondin^{icm15/icm15}* mutants is unlikely due to a defect in the *Adrb2* distribution in the spinal
487 cord.

488 Altogether, our results indicate that endogenous adrenergic signals could modulate
489 the Reissner fiber-dependent signaling pathway that instructs body axis straightening during
490 embryonic development, and suggests that monoamines act on CSF-contacting neurons
491 through an indirect mechanism.



492

493 **Figure 6. The adrenergic receptor Adrb2 is expressed in cells ventral to the central canal in**
 494 **which norepinephrine can be detected.**

495 (A) Double immunodetection of GFP (left) and endogenous norepinephrine (middle) in the spinal cord
 496 of *Tg(scospodin-GFP)* embryos at 30 hpf imaged laterally. Merged signals and close up of boxed
 497 regions are represented on the right. 5 representative examples (#1 to #5) out of 26 embryos are
 498 shown (no signal was detected in 6 embryos out of 26 in total, n=2 independent experiments). In the
 499 central canal, norepinephrine positive signals can be detected as colocalized with the Reissner fiber
 500 itself (black arrowheads; embryos #1, 2, 3), with extrafibrillar material in the central canal (black
 501 arrowheads; embryos #4 and #5), and closely apposed to the *massa caudalis* located after the caudal
 502 limit of the central canal (black arrowhead; embryo #6). Scale bars: 10 μ m. r: rostral; c: caudal; d:
 503 dorsal; v: ventral; cc: central canal; fp: floor plate. (B) Immunohistochemistry for the adrenergic
 504 receptor Adrb2 in a 30 hpf control sibling (left, representative example out of 8 embryos) and a
 505 *scospodin^{icm15/icm15}* embryo (right, representative example out of 9 embryos). Adrb2 is distributed
 506 along the midline of the ventral most region of the neural tube (corresponding to the floor plate, fp)
 507 at the interface with the central canal (cc). Embryos are oriented rostral to the left and dorsal to the top.
 508 Scale bars: 10 μ m. (C) Sagittal views of double immunostainings against GFP and Adrb2 in the spinal

509 cord of *Tg(pkd2l1:GCaMP5G)* embryos at 30 hpf. Maximal z-projections are shown for a control sibling
510 (left; one embryo out of 12) and a *scospondin*^{*icm15/icm15*} mutant (right; one embryo out of 8). Merged
511 signals show that GFP positive and Adrb2 positive signals cover different domains in the ventral most
512 region of the neural tube. 3D reconstructions of the same field of views (bottom panel: GFP: green;
513 Adrb2: purple) further illustrate that Adrb2 is enriched in a distinct cell population that is medial to
514 ventral CSF-contacting neurons. Scale bars: 10 μ m. L: left; R: right;
515 See also Video 1.

516 **Discussion**

517 Using a combination of transcriptomic analyses together with *in vivo* calcium imaging
518 and pharmacology, we show here that the Reissner fiber is essential for signaling to the
519 developing CSF-contacting neurons (CSF-cNs). In these neurons, the Reissner fiber is
520 required for both *urp2* expression and spontaneous intracellular calcium variations. This
521 functional interaction between the Reissner fiber and ventral CSF-cNs is required for a
522 normal curvature of the developing posterior axis of zebrafish. Using monoamine injections
523 into the CSF, we show that the CSF-cNs response to the signal from the Reissner fiber can
524 be modulated by local adrenergic activation *in vivo*, suggesting that the Reissner fiber acts
525 by controlling the availability of a chemical signal in the CSF.

526

527 ***urp2* expression in ventral CSF-cNs depends on the presence of the Reissner fiber and**
528 **impacts on the curvature of embryonic axis**

529 Previous results in both cilia-defective and *scospondin* mutants suggested that mutants
530 that fail to form a Reissner fiber display a strong downregulation of both Urotensin-II-related
531 neuropeptides 1 and 2 in the embryo (Lu et al., 2020; Rose et al., 2020; Zhang et al., 2018).
532 Our new results contradict the observation that *urp1* is downregulated in *scospondin* mutants
533 (Lu et al., 2020). In the present study, our transcriptomic analysis of *scospondin* mutants
534 yielded surprisingly few candidates commonly misregulated in the two alleles, and *urp2* was
535 the only gene with a strong downregulation, while *urp1* showed no significant change. We
536 confirmed this result by qRT-PCR and showed again that only *urp2* is strongly
537 downregulated, while *urp1* transcripts levels were not significantly affected. In line with our
538 results, the investigation of a newly generated *scospondin* hypomorphic allele (Rose et al.,
539 2020) also revealed *urp2* as the major downregulated gene in homozygous mutant and did
540 not report a *urp1* downregulation.

541 We further show that *urp2* overexpression in *scospondin* mutants decreases the
542 frequency of curled-down phenotypes, confirming that *urp2* expression level is involved in the
543 control of embryonic axis curvature (Lu et al., 2020; Zhang et al., 2018). However, *urp2* may
544 not be the only determinant of embryonic axis morphogenesis downstream of the Reissner
545 fiber, as single *urp2* morpholino knockdown do not show a defective axis curvature (Zhang et
546 al., 2018). Parallel signaling pathways that would likely act post-transcriptionally and would
547 therefore not be detected using our transcriptomic strategy may be involved.

548 The discovery of a new hypomorphic allele for *scospondin* recently revealed an
549 inflammatory signature induced by the loss of the Reissner fiber at the embryonic stage
550 (Rose et al., 2020). In our work, we did not detect such a signature in our transcriptomic
551 analysis (see Figure 1-table supplement 1). We can speculate that these differences are due

552 to difference in the fish genetic background or husbandry conditions. Our work therefore
553 begs for further studies in order to decipher the molecular pathways downstream and/or in
554 parallel to *Urp2* that regulate the morphogenesis of the embryonic axis.

555

556 **Intracellular calcium variations in developing ventral CSF-cNs require the Reissner** 557 **fiber**

558 In the embryo, ventral CSF-cNs are spontaneously active via the opening of the *Pkd2l1*
559 calcium channel enriched at the level of the developing apical extension of the cells
560 (Sternberg et al., 2018). In addition to controlling *urp2* gene expression in CSF-cNs, we
561 report here that the Reissner fiber is also required for calcium signaling in *urp2*-expressing
562 cells. By investigating the *pkd2l1* mutant deprived of calcium signaling in embryonic CSF-
563 cNs, our work rejects the simple explanation that the Reissner fiber controls *urp2* mRNA
564 level and axis straightness by increasing calcium intracellular concentrations in CSF-cNs.
565 Indeed, the absence of *urp* genes downregulation observed in the *pkd2l1* mutant compared
566 to wild-type goes against this hypothesis.

567 One question remaining is how the Reissner Fiber controls calcium variations in ventral
568 CSF-cNs. We recently showed that the Reissner fiber is functionally coupled to the
569 mechano-sensory function of these interoceptive neurons in the larva (Orts-Del'Immagine et
570 al., 2020). However, embryonic CSF-cNs are not fully differentiated, as they do not harbor a
571 fully developed apical extension known to tune their mechanosensory function at larval stage
572 (Desban et al., 2019). We therefore favor the hypothesis that the Reissner fiber acts via the
573 modulation of the CSF content, which is supported by the restoration of calcium signaling in
574 CSF-cNs upon monoamine injections in the brain ventricles.

575

576 **Adrenergic activation restores the Reissner fiber-dependent signaling and axis** 577 **straightening**

578 Earlier reports suggest that the Reissner fiber can interact with different neuro-
579 modulators, including monoamines (Caprile et al., 2003; Diederer et al., 1983). Monoamines
580 globally supplied in the fish water were also found to rescue body curvature in Reissner fiber-
581 defective mutants (Lu et al., 2020; Zhang et al., 2018). They are therefore good candidates
582 to influence the Reissner fiber signal towards ventral CSF-cNs. We added to previous report
583 (Lu et al., 2020) that the action of both epinephrine and norepinephrine can compensate
584 locally, directly in the ventricular cavities, for the loss of the Reissner fiber on calcium
585 signaling. We also used quantitative PCR to confirm the regulation of *urp* neuropeptides
586 expression by both epinephrine and norepinephrine in *scospondin* mutants, extending the
587 previous result obtained using *in situ* hybridization after epinephrine treatments (Lu et al,

588 2020). These two monoamines rescue the *scospondin* phenotype for three features:
589 spontaneous calcium variations and Urotensin-II-related peptides expression in CSF-cNs,
590 and body axis curvature.

591 One possible interpretation of our results is that the Reissner fiber is essential for the
592 control of endogenous epinephrine and norepinephrine distribution in the embryonic CSF.
593 This is consistent with our immunodetection of norepinephrine in close vicinity with the
594 Reissner fiber in the embryonic central canal. This hypothesis is supported by the presence
595 of noradrenergic neurons in the embryonic hindbrain as early as 24 hpf (Holzschuh et al.,
596 2003), providing a potential source of monoamines that would need to be transported
597 caudally to the central canal of the spinal cord. This hypothesis is reinforced by our original
598 result showing the localization of the Adrb2 receptor at the interface with CSF in the ventral
599 part of the neural tube, ideally located to bind norepinephrine ligands that we found
600 distributed close to Reissner-positive material. Interestingly Adrb2 belongs to the G protein-
601 coupled receptors family and was identified to trigger cytoplasmic calcium raise *in vitro*
602 (Galaz-Montoya et al., 2017). In zebrafish, the adrenergic system plays important roles in the
603 control of wakefulness (Singh et al., 2015) and of cardiac contractions (Steele et al., 2011).
604 However, no morphological defects have been reported in animal missing the rate-limiting
605 enzyme for the synthesis of epinephrine and norepinephrine (Singh et al., 2015). This might
606 reflect the masking of the phenotype due to transcriptional adaptation to the genetic mutation
607 (Rossi et al., 2015). Alternatively, redundant signaling pathways might mask the role of
608 monoamines in axis curvature control. Nonetheless, our results support the idea that the
609 cross-talk between the Reissner fiber and undifferentiated CSF-cNs is likely to be of
610 chemical nature, possibly through monoamines themselves. They further support the idea
611 that monoamines act on CSF-cNs in an indirect manner. Future investigations will allow to
612 fully delineate the contribution and mechanism of action of endogenous monoamines on
613 ventral CSF-cNs signaling and body axis curvature.

614
615 Altogether, our study unravels a signal from the Reissner fiber to the developing CSF-
616 contacting neurons. We also show that adrenergic activation can modulate this signal during
617 embryonic body axis morphogenesis. Interestingly, a temporally controlled inactivation of cilia
618 motility leads to spine curves reminiscent of adolescent idiopathic scoliosis (Grimes et al.,
619 2016). Recent results suggest that, as in the embryo, the Reissner fiber and Urp reception in
620 slow muscles are also implicated in the maintenance of a straight spine during
621 postembryonic development (Lu et al., 2020; Rose et al., 2020; Troutwine et al., 2019; Zhang
622 et al., 2018). Our work will pave the way for future investigations to identify the potential of

623 the interplay between CSF-cNs and the adrenergic system to modulate the Reissner fiber-
 624 dependent morphogenesis of the spine in the juvenile.

625 **Material and Methods**

626 **Key resources table**

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Genetic reagent (<i>D. rerio</i>)	<i>scospondin</i> ^{icm13}	(Cantaut-Belarif et al., 2018)	ZFIN : ZDB-ALT-181113-3	
Genetic reagent (<i>D. rerio</i>)	<i>scospondin</i> ^{icm15}	(Cantaut-Belarif et al., 2018)	ZFIN: ZDB-ALT-181113-4	
Genetic reagent (<i>D. rerio</i>)	<i>pkd211</i> ^{icm02}	(Böhm et al., 2016)	ZFIN : ZDB-ALT-160119-6	
Genetic reagent (<i>D. rerio</i>)	<i>Tg(pkd211:GCaMP5G)</i> ^{icm07Tg}	(Böhm et al., 2016)	ZFIN: ZDB-ALT-160119-4	
Genetic reagent (<i>D. rerio</i>)	<i>Tg(pkd211:GAL4)</i> ^{icm10Tg}	(Fidelin et al., 2015)	ZFIN: ZDB-ALT-150324-1	
Genetic reagent (<i>D. rerio</i>)	<i>Tg(UAS:mCherry)</i>	(Robles et al., 2014)	ZFIN: ZDB-ALT-130702-1	
Genetic reagent (<i>D. rerio</i>)	<i>Tg(scospodindin-GFP)</i>	(Troutwine et al., 2019)		R. S. Gray lab
Recombinant DNA reagent	<i>pCS2-urp2</i> plasmid	G. Pézeron, this work		Used for RNA synthesis
Recombinant DNA reagent	<i>pCS2-Ras-eGFP</i> plasmid	(Ségalen et al., 2010)		Used for RNA synthesis

Sequence-based reagent	URP2_BamHI_F gcgcgcGGATCCgtatct gtagaatctgctttgctgc	This work		forward oligonucleotide used for <i>urp2</i> cloning
Sequence-based reagent	URP2_XbaI_R gcgcgcTCTAGAggcag agggtcagtcgtgttat	This work		reverse oligonucleotide used for <i>urp2</i> cloning
Sequence-based reagent	<i>urp2</i> forward: CCACCGGATCACCA TCATTACC	This work		qPCR oligonucleotide
Sequence-based reagent	<i>urp2</i> reverse: GATGCCACCGCTGT CTATAGTG	This work		qPCR oligonucleotide
Sequence-based reagent	<i>urp1</i> forward: TGCGCTGCCTCTGT ATTCAG	This work		qPCR oligonucleotide
Sequence-based reagent	<i>urp1</i> reverse: CTTTGTCCGTCTTCA ACCTCTG	This work		qPCR oligonucleotide
Sequence-based reagent	<i>pkd2l1</i> forward: GCGAACTATGCCCA ATGAGG	This work		qPCR oligonucleotide
Sequence-based reagent	<i>pkd2l1</i> reverse: TCTCAAAGCTGTTCC CCACA	This work		qPCR oligonucleotide
Sequence-based reagent	<i>lsm12b</i> forward: GAGACTCCTCCTCC TCTAGCAT	This work		qPCR oligonucleotide
Sequence-based reagent	<i>lsm12b</i> reverse: GATTGCATAGGCTT GGGACAAC	This work		qPCR oligonucleotide
Antibody	Anti-Reissner fiber, rabbit , polyclonal	(Didier et al., 1995)	Courtesy of S. Gobron	Dilution 1:200

Antibody	Anti-GFP, chicken, polyclonal	Abcam, Cat# ab13970	RRID:AB_300798	Dilution 1:500
Antibody	Anti-Pkd2l1, rabbit, polyclonal	(Sternberg et al., 2018)		Dilution 1:200
Antibody	Anti-Norepinephrine, rabbit, polyclonal	Millipore, Cat# AB120	RRID:AB_90481	Dilution 1:100
Antibody	Anti-Adrb2, rabbit, polyclonal	ThermoFischer Scientific, Cat# PA5-80323	RRID:AB_2787652	Dilution 1:200
Antibody	Alexa Fluor-488 goat anti chicken IgG (H+L)	Molecular Probes, Cat# A-11039	RRID:AB_142924	Dilution 1:500
Antibody	Alexa Fluor-568 goat anti-rabbit IgG (H+L)	Molecular Probes, Cat# A-11036	RRID:AB_10563566	Dilution 1:500
Antibody	Alexa Fluor-488 donkey anti rabbit IgG (H+L)	Molecular Probes, Cat# A-21206	RRID:AB_2535792	Dilution 1:500
Chemical compound, drug	DL-Norepinephrine hydrochloride	Sigma	Cat# A7256	Dilution to 3mM
Chemical compound, drug	+/- Epinephrine hydrochloride	Sigma	Cat# E4642	Dilution to 3mM
Chemical compound, drug	MS 222	Sigma	Cat# E10521	Dilution to 0.2% w/v
Chemical compound, drug	alpha-bungarotoxin	Tocris	Cat# 2133	Dilution to 500 µM
Software, algorithm	MATLAB	MathWorks	RRID:SCR_001622	
Software, algorithm	Prism	GraphPad	RRID:SCR_002798	

Software, algorithm	Fiji	(Schindelin et al., 2012)	RRID:SCR_002285	
Software, algorithm	Imaris	Oxford Instruments	RRID:SCR_007370	

627

628

629 **Animal husbandry and genotyping**

630 All procedures were performed on zebrafish embryos between 30 and 48 hpf in accordance
631 with the European Communities Council Directive (2010/63/EU) and French law (87/848)
632 and approved by the Paris Brain Institute (Institut du Cerveau) and the French Ministry for
633 research (APAFIS agreement 2018071217081175). All experiments were performed on
634 *Danio rerio* embryos of AB, Tüpfel long fin (TL) and nacre background. Animals were raised
635 at 28.5°C under a 14/10 light/dark cycle until the start of the experiment. Genotyping was
636 performed as previously described for *scospondin*^{icm13} and *scospondin*^{icm15} (Cantaut-Belarif et
637 al., 2018) and *pkd211*^{icm02} (Böhm et al., 2016).

638

639 **RNA sample preparation**

640 For each experiment, condition (developmental time point and pharmacological treatment)
641 and phenotype (either straight or curled-down), 30 sibling embryos were sorted in
642 independent vials. After euthanasia in 0.2% MS 222 (Sigma, E10521), embryos were
643 resuspended in 1 mL of TrizolTM (ThermoFischer Scientific, 15596026) and dissociated by
644 multiple aspirations through the needle of a syringe. 200 µL chloroform were added prior to
645 centrifugation and extraction of the aqueous phase. Nucleic acids were precipitated using
646 700 µL isopropanol, and the pellet was resuspended in 200 µL water. RNAs were purified
647 using the RNeasy Micro Kit (Qiagen, 74004), following the provider's instruction. We
648 performed the optional on-column DNase treatment to improve RNAs purity. RNAs were
649 eluted in 30 µL water to ensure a high concentration, and the quality and quantity of the
650 extract was evaluated on a TapeStation System® (Agilent).

651

652 **RNA sequencing analysis**

653 mRNA library preparation was realized following manufacturer's recommendations (KAPA
654 mRNA HyperPrep Kit, Roche). Final samples pooled library preparations were sequenced on
655 Nextseq 500 ILLUMINA, corresponding to 2x30 millions of 75 base pair reads per sample
656 after demultiplexing. Quality of raw data was evaluated with FastQC (Andrews et al., 2010).

657 Poor quality sequences was trimmed or removed using Trimmomatic software (Bolger et al.,
658 2014) to retain only good quality paired reads. Star v2.5.3a (Dobin et al., 2013) was used to
659 align reads on GRCz11 reference genome using standard options. Quantification of gene
660 and isoform abundances was achieved using with Rsem 1.2.28, prior to normalization with the
661 edgeR bioconductor package (Robinson et al., 2009). Finally, differential analysis was
662 conducted with the GLM framework likelihood ratio test from edgeR.

663 The quality control and PCA analysis (not shown) indicated that one sample was not
664 reaching the same reproducibility as the two others, even after trimming and normalization.
665 We therefore used the average value of the two most reliable replicates to calculate the
666 average expression level (count per million, cpm) of all the 28 214 genes either in straight
667 controls or in curled-down embryos (average of the expression in the two alleles *icm13* and
668 *icm15*). We then filtered out low-expression genes that had an expression level below 1
669 c.p.m. in the average of the straight control replicates. We then kept genes that had an
670 average fold change between curled embryos and controls of 0.75 for down-regulated genes
671 (120 genes), and 1.45 for up-regulated genes (94 genes). We turned back to the original raw
672 cpm values of the three replicates in the two alleles for these two short-lists. We tested the
673 consistency of the fold change across replicates and across alleles with a general linear
674 model (GLM) (Nelder et al., 1972). The design matrix included 2 regressors of interest
675 (encoding whether the sample is a straight control or curled embryos) and 2 confounding
676 variables (the unwanted variability that might be associated with the two different genetic
677 environments of the two families of fish carrying the *icm13* and the *icm15* allele). Statistical
678 significance of the effect of interest (above and beyond confounding factors) was tested
679 using a t-test. Because of the potential false discovery associated with multiple testing, we
680 then used the Benjamini–Hochberg procedure (Benjamini, et al., 1995).

681 Potential genes of interest were sorted according to their increasing p-values. To shorten the
682 list of potential genes of interest, we determined at which rank i the p-value became higher
683 than the Benjamini–Hochberg criterion using $(i / \text{total nb}) * 0.2$. This method allows for up to
684 20% of false-positives, but avoids the rejection of true-positives that was manifest with a
685 more stringent correction as the Bonferroni correction. For example, the *scospondin* mRNA
686 level in the *icm13* allele is highly decreased, presumably through non-sense mediated decay:
687 the Bonferroni correction would have rejected this result as not significant, whereas the
688 Benjamini–Hochberg procedure keeps it in the list of potentially interesting genes (see Figure
689 1-Source Data 1 for the complete lists). However, this procedure requires a post-hoc
690 validation with an independent technique for each potential gene of interest, as we did by
691 qRT-PCR (see below).

692

693 **Quantitative RT-PCR**

694 1.2 µg of each RNA sample was retro-transcribed using the Verso cDNA Synthesis Kit
695 (ThermoFischer Scientific, AB1453B) following the provider's instructions. A 1:3 ratio of
696 random hexamer:polydT primers was used to favor mRNA amplification without a strong bias
697 for the 3' end of messengers. qPCR experiments were performed using the LightCycler® 480
698 SYBR Green I Master kit (Roche, 04707516001) on a LightCycler® 96 machine (Roche).
699 Each pair of primers (see Key Resource Table) was tested beforehand on a given cDNA
700 stock that was diluted in a series of 4 points. Linearity of CT variation with the cDNA dilution
701 as well as single peaks in the melting curves corresponding to single amplicons were
702 assessed. For each new RNA extraction, we tested that no amplification was detectable
703 when the PCR was performed directly on the RNA stock (-RT). We used the housekeeping
704 gene *lsm12b* as an internal reference in each experiment. qPCRs were repeated two to three
705 times for each cDNA stock and the average CT was used for further calculations. The
706 relative abundance of the gene of interest was evaluated using the CT comparison formula:
707 $2e^{-\Delta CT}$. All results were obtained on at least three biological replicates (except for *pkd2l1*
708 mutant extracts), each originating from a single mating.

709

710 **Monitoring of body axis curvature in developing embryos**

711 To characterize the exact evolution of the geometry of the posterior axis in
712 *scospondin*^{*icm15/icm15*} mutants during the early stages on embryogenesis (Figure supplement
713 1), we performed incrosses of *scospondin*^{*icm15/+*} parents. Eggs were collected 30 minutes
714 after mating and maintained at 28.5°C until 19.5 hpf. Embryos were staged according to
715 (Kimmel et al., 1995) at 19.5 hpf. Individual embryos were mounted laterally at 20 hpf in
716 1.5% low-melting point agarose, imaged using a AZ100M microscope (Nikon), unmounted
717 and kept at 28.5°C until they were remounted for the next stages (22, 24, 28 and 30 hpf).
718 The time window necessary for mounting-imaging-unmounting at each step was short
719 enough (10-15 min) to be neglected.

720

721 **Pharmacology and quantification of body axis curvature**

722 30 hpf embryos from *scospondin*^{*icm15/+*} incrosses were treated during 2.5 hours at 28.5°C with
723 E3 medium alone (vehicle), epinephrine hydrochloride (Sigma, E4642) or norepinephrine
724 hydrochloride (Sigma, A7256) both diluted to 3 mM in E3 medium (Figure 4). To avoid light-
725 induced oxidation of epinephrine and norepinephrine, dishes were covered with foil paper
726 during the incubation time. To ensure a proper quantification of ear-to-tail angles, embryos
727 were then fixed over-night in 4% PFA 6-well plates, rinsed 3 times during 45 minutes in 1X
728 PBS, mounted laterally in 1.5% low-melting point agarose and imaged using a AZ100M

729 microscope (Nikon). For each embryo, the angle between the ear, the caudal limit of the
730 yolk extension and the tip of the tail (Figure 4C) was quantified using Fiji (Schindelin et al.,
731 2012). Representative traces of the global morphology of the embryos after treatment were
732 drawn for all experimental conditions on one representative clutch (Figure 4B) by linking the
733 center of the eye to the ear and following the dorsal line linking somite boundaries until the
734 tip of the tail.

735

736 ***In vivo* calcium imaging**

737 *Tg(pkcd211:GCaMP5G)* embryos were manually dechorionated at 30 hpf, mounted laterally in
738 1.5% low-melting point agarose, and paralyzed by injecting 1-2 nL of 500 μ M alpha-
739 bungarotoxin (Tocris, 2133) in the caudal muscles of the trunk. When required (Figure 5),
740 hindbrain ventricle injections were performed using artificial cerebrospinal fluid (aCSF,
741 containing in mM: 134 NaCl, 2.9 KCl, 1.2 MgCl₂, 10 HEPES, 10 glucose and 2.1 CaCl₂; 290
742 mOsm.kg⁻¹, adjusted to pH 7.7–7.8 with NaOH) as a vehicle solution. Epinephrine
743 hydrochloride (Sigma, E4642) and norepinephrine hydrochloride (Sigma, A7256) were
744 diluted to 3 mM in aCSF before hindbrain ventricle injections, using a sharp funnel shape
745 needle with an approximate tip diameter of 1-3 μ m. Embryos were imaged 20 to 60 minutes
746 after hindbrain ventricle injections. Calcium imaging was performed at 4 Hz using a spinning
747 disk confocal microscope (Intelligent Imaging Systems, Denver) for 4 minutes. Imaging was
748 restricted to the region of the spinal cord located above the yolk extension. Regions of
749 interest were manually selected based on an average projection of the time-lapse and
750 according to the dorso-ventral position of the cells. The integrals of normalized $\Delta F/F$ signals
751 were calculated using a custom script on MATLAB (see full description in (Sternberg et al.,
752 2018)). The MATLAB codes used for data analysis are available at Github
753 <https://github.com/wyartlab/Cantaut-Belarif-et-al.-2020>.

754

755 ***In vivo* patch clamp recording**

756 Whole-cell recordings were performed in aCSF on 30 hpf double transgenic
757 *Tg(pkcd211:GAL4;UAS:mCherry)* embryos carrying the *scospondin*^{icm15} mutation and their
758 respective control siblings. Embryos were pinned through the notochord with 0.025 mm
759 diameter tungsten pins. Skin and muscle from two to three segments around segment 10 were
760 dissected out using a glass suction pipette. A MultiClamp 700B amplifier, a Digidata series 1440
761 A Digitizer, and pClamp 10.3 software (Axon Instruments, Molecular Devices, San Jose,
762 California, USA) were used for acquisition. Raw signals were acquired at 50 kHz and low-pass
763 filtered at 10 kHz. Patch pipettes (1B150F-4, WPI) with a tip resistance of 6–8M Ω were filled
764 with internal solution containing in mM: K-gluconate 115, KCl 15, MgCl₂ 2, Mg-ATP 4, HEPES-

765 free acid 10, EGTA 5 or 10, 290 mOsm/L, pH adjusted to 7.2 with KOH with Alexa 488 at 40 μ M
766 final concentration). Holding potential was – 85 mV, away from the calculated chloride reversal
767 potential ($E_{Cl} = - 51$ mV).

768

769 **Immunohistochemistry and confocal imaging**

770 Embryos were manually dechorionated and euthanized using 0.2% MS 222 (Sigma,
771 E10521) prior to fixation.

772 To detect the Pkd2l1 channel together with GFP (Figure 2D), 30 hpf embryos were
773 fixed 4 hours at 4°C in 4% PFA and then washed 3 times during 30 minutes in 1X PBS.
774 Embryos were blocked overnight at 4°C in a solution containing 0.7% Triton, 1% DMSO and
775 10% NGS. The primary antibodies were incubated overnight at 4°C in a solution containing
776 0.5% Triton, 1% DMSO and 1% NGS and subsequently washed 4 times during 1 hour in a
777 solution containing 0.5% Triton and 1% DMSO (washing solution). Secondary antibodies
778 were incubated 3 hours at room temperature in a solution containing 0.5% Triton, 1% DMSO
779 and 1% NGS. The mix of secondary antibodies was centrifuged 10 minutes at 10 000 rpm
780 prior to the incubation to pellet unconjugated dyes (supernatant only was used for the
781 incubation). Embryos were then washed 4 times during 2 hours using the washing solution
782 and thereafter overnight at 4°C in 1X PBS.

783 To detect the Reissner fiber (Figure 3C), 30 hpf embryos were fixed 4 hours at 4°C in
784 4% PFA and washed 3 times during 30 minutes in 1X PBS. Embryos were then blocked
785 overnight in a solution containing 0.5% Triton, 1% DMSO and 10% NGS. The primary
786 antibody was diluted in a solution containing 0.5% Triton, 1% DMSO and 1% NGS and
787 incubated overnight at 4°C. Embryos were subsequently washed 4 times during 1 hour in a
788 solution containing 0.5% Triton and 1% DMSO (washing solution). Secondary antibodies
789 were incubated 3 hours at room temperature in a solution containing 0.5% Triton, 1% DMSO
790 and 1% NGS. The mix of secondary antibodies was centrifuged 10 minutes at 10 000 rpm
791 prior to the incubation to pellet unconjugated dyes (supernatant only was used for the
792 incubation). Embryos were subsequently washed 4 times during 2 hours using the washing
793 solution and thereafter overnight at 4°C in 1X PBS.

794 To detect exogenous (Figure Supplement 4) and endogenous (Figure 5A)
795 norepinephrine alone (Figure Supplement 4) or together with GFP (Figure 5A), we used an
796 antibody designed against a Glutaraldehyde-conjugated form of norepinephrine (Millipore,
797 AB120). Thus, 30 hpf embryos were fixed 1.5 hour at 4°C under agitation in a 4% PFA-
798 0.125% Glutaraldehyde-3% sucrose solution to allow reactivity. Embryos were then washed
799 3 times in 1X PBS during 30 minutes and the yolks and skin from rostral parts of the trunk

800 were removed. Embryos were blocked overnight at 4°C in a solution containing 0.6% Triton,
801 1.2% DMSO and 10% normal goat serum (NGS). An additional blocking step was performed
802 during 3 hours at room temperature the day after. Primary antibodies were incubated in a
803 solution containing 1% Triton, 1% DMSO and 1% NGS during one day at room temperature
804 plus one night at 4°C. Embryos were then washed extensively 4 times during 2 hours in a
805 solution containing 0.5% Triton and 1% DMSO (washing solution). Secondary antibodies
806 were incubated 3 hours at room temperature in a solution containing 1% Triton, 1% DMSO
807 and 1% NGS. The mix of secondary antibodies was centrifuged 10 minutes at 10 000 rpm
808 prior to the incubation to pellet unconjugated dyes (supernatant only was used for the
809 incubation). Embryos were subsequently washed 4 times during 2 hours using the washing
810 solution, 4 times during 1 hour using a solution of 50% washing solution-50% 1X PBS and
811 thereafter overnight at 4°C in 1X PBS.

812 To detect *Adrb2* alone (Figure 6B) or together with GFP (Figure 6C), embryos were
813 fixed 2 hours at 4°C in a 4% PFA-3% sucrose solution and washed 3 times in 1X PBS during
814 30 minutes. The yolks and the skin from the rostral part of the trunk were removed and
815 embryos were blocked overnight in a solution containing 0.6% Triton, 1.2% DMSO and 10%
816 normal goat serum (NGS). Primary antibodies were incubated at 4°C during 2 days in a
817 solution containing 1% Triton, 1% DMSO and 1% NGS. Embryos were washed extensively 4
818 times during 2 hours in a solution containing 0.5% Triton and 1% DMSO (washing solution).
819 Secondary antibodies were incubated 3 hours at room temperature in a solution containing
820 1% Triton, 1% DMSO and 1% NGS. The mix of secondary antibodies was centrifuged 10
821 minutes at 10 000 rpm prior to the incubation to pellet unconjugated dyes (supernatant only
822 was used for the incubation). Embryos were subsequently washed using the washing
823 solution 4 times during 2 hours, 4 times during 1 hour using a solution of 50% washing
824 solution-50% 1X PBS and thereafter washed in 1X PBS overnight at 4°C.

825 The following dilutions of primary antibodies were used: rabbit anti-Reissner fiber
826 1:200 (Didier et al., 1995), rabbit anti-Pkd211 1:200 (Sternberg et al., 2018), chicken anti-GFP
827 1:500 (Abcam ab139170), rabbit anti-norepinephrine 1:100 (Millipore, AB120), rabbit anti-
828 *Adrb2* 1:200 (ThermoFischer Scientific, PA5-80323). All secondary antibodies were from
829 Molecular Probes© and used at 1:500. Systematic omission of the primary antibody
830 confirmed the specificity of the immunostaining results. Zebrafish embryos were mounted
831 laterally in Vectashield® Antifade Mounting Medium (Clinisciences, H1000) and imaged on
832 an inverted SP8 DLS confocal microscope (Leica). Images were then processed using Fiji
833 (Schindelin et al., 2012). Maximal Z-projections of 6-9 microns in depth are represented in
834 Figure 2D and Figure 3C, 9-12 microns in depth in Figure 6A, 6B and Figure Supplement 4B
835 and 18-22 microns in Figure 6C. 3D views shown in Figure 6C were obtained using Imaris.

836

837 **RNA microinjections**

838 To produce mRNA, *urp2* CDS was amplified from cDNA by PCR and cloned (BamHI - XbaI)
839 into pCS2+. Messenger RNA were produced with the mMESSAGE mMACHINE™ kit
840 (Ambion™). 1 nL RNA-containing solution was injected into 1- to 2-cell stage embryos
841 obtained from *scospondin*^{icm15/+} incrosses. Each clutch was separated into three groups:
842 uninjected and injected either with a control mRNA (100 ng / μL, *ras-eGFP* encoding for a
843 membrane tagged GFP, (Ségalen et al., 2010) or with a mix containing control mRNA and
844 *urp2* mRNA (100 ng / μL total, 1:1 ratio). To assess for injection quality, GFP-positive
845 embryos were first sorted out at 1 dpf and then scored at 48 hpf for body axis curvature
846 defects.

847

848 **Statistics**

849 All values are represented as boxplots (median ± interquartile range) or mean ± SEM (stated
850 for each in the figure legend). All statistics were performed using MATLAB and Excel. In the
851 figure panels, asterisks denote the statistical significance calculated using the appropriate
852 test (stated for each test in the legends): *, p<0.05; **, p<0.01; ***, p<0.001; ns, p>0.05.

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868

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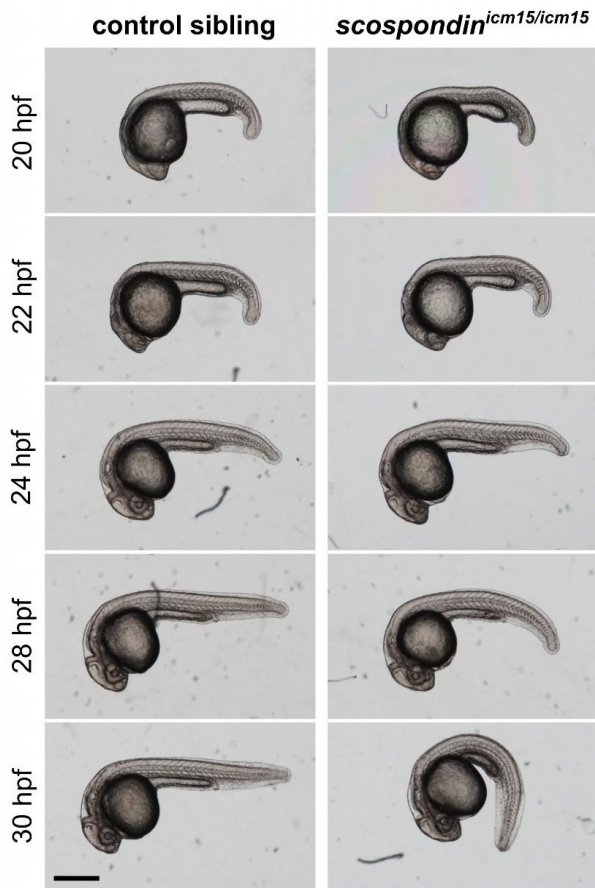
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1053 **Supplementary Figures**



1054

1055 **Figure 1 - Figure Supplement 1. Time course of the evolution of body axis geometry**
1056 **from 20 to 30 hpf in *scospondin*^{icm15/icm15} mutants.**

1057 Representative images of the morphology of a control sibling (left) and a *scospondin*^{icm15/icm15}
1058 embryo (right) followed over time at 20, 22, 24, 28 and 30 hpf. Embryos were obtained from
1059 a mating of *scospondin*^{icm15/+} parents, staged at 20 hpf and maintained at 28.5°C. Body axis
1060 curvature in *scospondin*^{icm15/icm15} mutants becomes visible as early as 28 hpf and fully
1061 recognizable at 30 hpf. Scale bar: 0.5 mm.

1062 **Figure 1 - source data 1. Data for figure 1B-C**

1063

1064 **Figure 1 - source data 2. Up- and down-regulated transcripts in curled-down**
1065 ***scospondin* mutants compared to their control siblings.**

1066 After filtering for low expression, we kept a list of genes that were differentially expressed in
1067 null *scospondin*^{icm13/icm13} and hypomorphic *scospondin*^{icm15/icm15} mutant embryos at 48 hpf. The
1068 table shows the average fold changes in the two alleles and their mean. Genes are ranked
1069 based on their p-value that compare the reproducibility of the change inside and between the
1070 two alleles using a GLM framework. We adjusted the false discovery rate due to multiple
1071 comparisons using the Benjamini-Hochberg procedure (see Material and Methods for
1072 details). The raw RNA-seq data have been deposited in the ArrayExpress database at
1073 EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-9615.

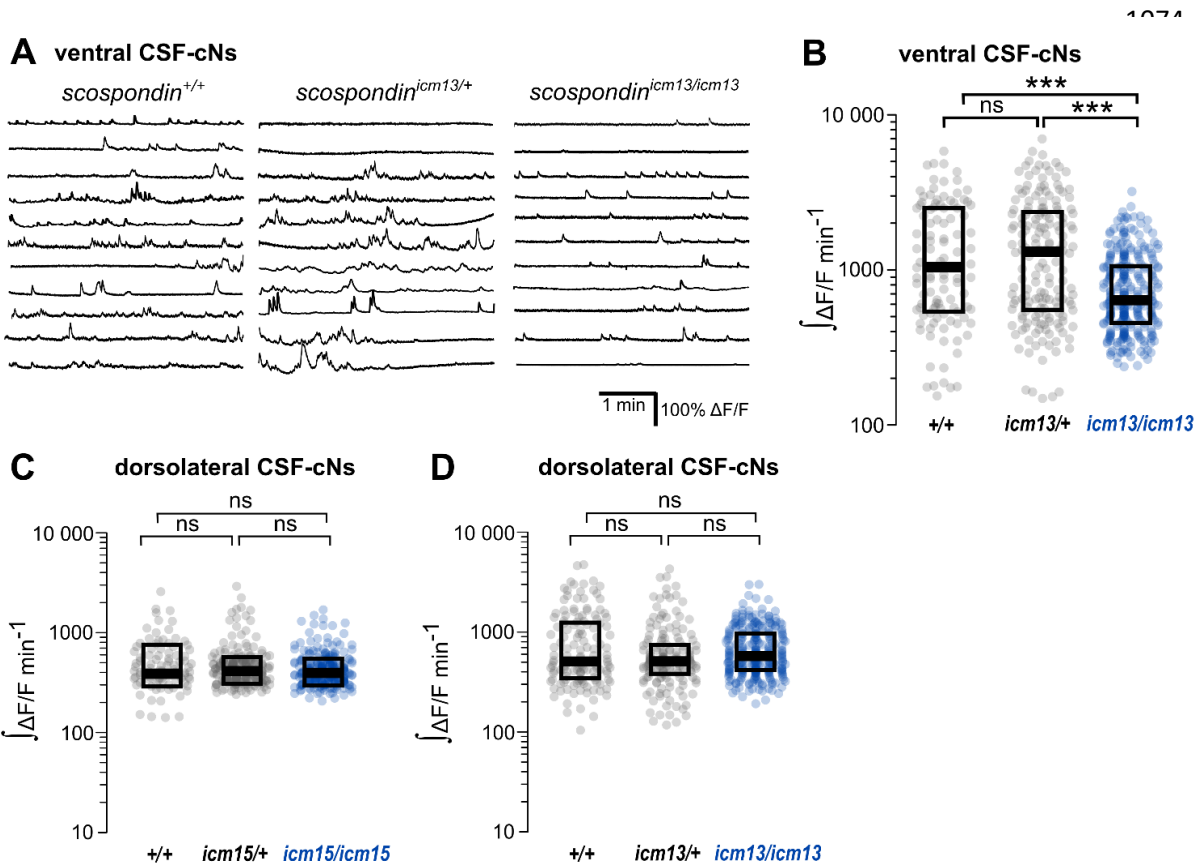
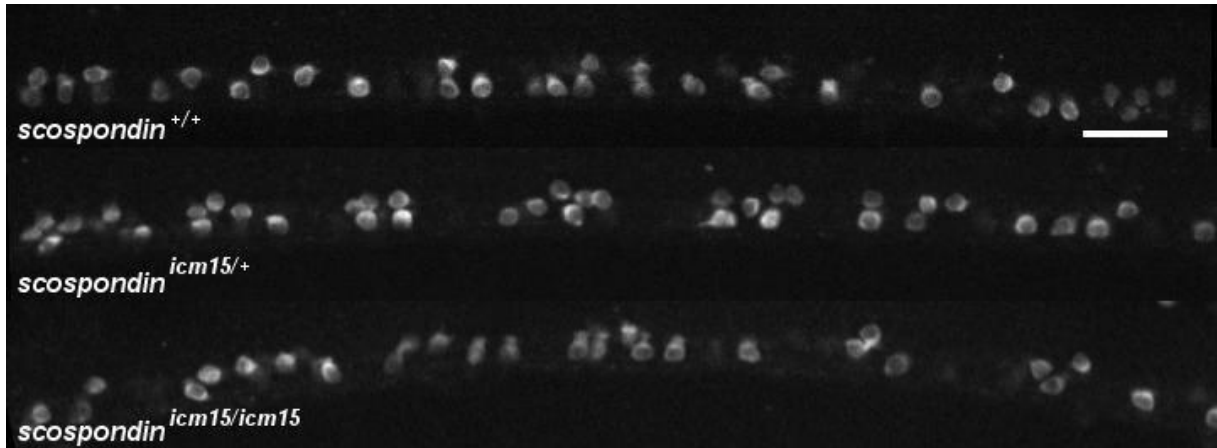


Figure 2 -
Figure Supplement 1. The spontaneous activity of dorso-

1091 lateral CSF-contacting neurons does not require the Reissner fiber in the embryo.

1092 (A) Traces of calcium variations in ventral CSF-contacting neurons at 30 hpf in wild type
 1093 (*scospondin*^{+/+}), heterozygous (*scospondin*^{icm13/+}) and *scospondin*^{icm13/icm13} null mutants.
 1094 Sample traces from individual cells with integral $\Delta F/F$ values ranging around the median
 1095 distribution of the imaged population are represented for each genotype (n=11). (B)
 1096 Quantification of the normalized integrated calcium variation over time of ventral CSF-
 1097 contacting neurons in wild type (+/+), heterozygous (*icm13/+*) and *scospondin*^{icm13/icm13}
 1098 mutants (*icm13/icm13*, blue). Data were collected from 3 independent experiments and
 1099 include 8 wild type embryos (n=107 cells), 13 heterozygous embryos (n=176 cells) and 22
 1100 *scospondin*^{icm15/icm15} mutants (n=277 cells). Each point represents a single cell. Bottom and
 1101 top edges of the boxes indicate the 1st and 3rd quartiles. Bold lines represent the median
 1102 value for each distribution. ns p>0.05, *** p<0.001 (Kolmogorov-Smirnov test). (C, D)
 1103 Quantification of the normalized integrated calcium variations over time of dorsolateral CSF-
 1104 contacting neurons in 30 hpf embryos obtained from *scospondin*^{icm15/+} incrosses (C) and
 1105 *scospondin*^{icm13/+} incrosses (D) n=95, 243 and 255 cells in wild type, heterozygous and
 1106 *scospondin*^{icm15/icm15} embryos respectively (C); n=141, 176 and 168 cells in wild type,
 1107 heterozygous and *scospondin*^{icm13/icm13} embryos respectively (D). ns p>0.05 (Kolmogorov-
 1108 Smirnov test).

1109



1110

1111

1112 **Figure 2- Video 1. Intracellular calcium transients of ventral CSF-contacting neurons**
1113 **are reduced in *scospondin* mutants.**

1114 Sagittal views of the spinal cord of *Tg(pkd2l1:GCaMP5G)* embryos at 30 hpf. *scospondin*^{+/+}
1115 (top), *scospondin*^{icm15/+} (middle) and *scospondin*^{icm15/icm15} siblings (bottom) are represented. In
1116 wild type and heterozygous embryos, ventral CSF-contacting neurons are more active than
1117 dorso-lateral ones. Note the sharp decrease in calcium variations of ventral CSF contacting
1118 neurons in the homozygous mutant embryo compared to wild type and heterozygous
1119 counterparts. Data were collected at 4 Hz and displayed at 80 Hz. Scale bar: 30 μm.

1120

1121 **Figure 2 - source data 1. Data for figure 2C and figure supplement 1B-C**

1122

A

control mRNA injection

	+/+	+/-	-/-
straight	3	8	0
curled up	0	0	0
curled down	0	0	12

Bcontrol mRNA +*urp2* injection

	+/+	+/-	-/-
straight	20	43	2
curled up	1	3	2
curled down	0	0	9

1123

1124 **Figure 3 -figure supplement 1. *urp2* overexpression rescues body axis curvature**1125 **defects in *scospondin* mutants.**

1126 Summary of straight, curled-up and curled-down phenotypes detected in wild type (+/+),

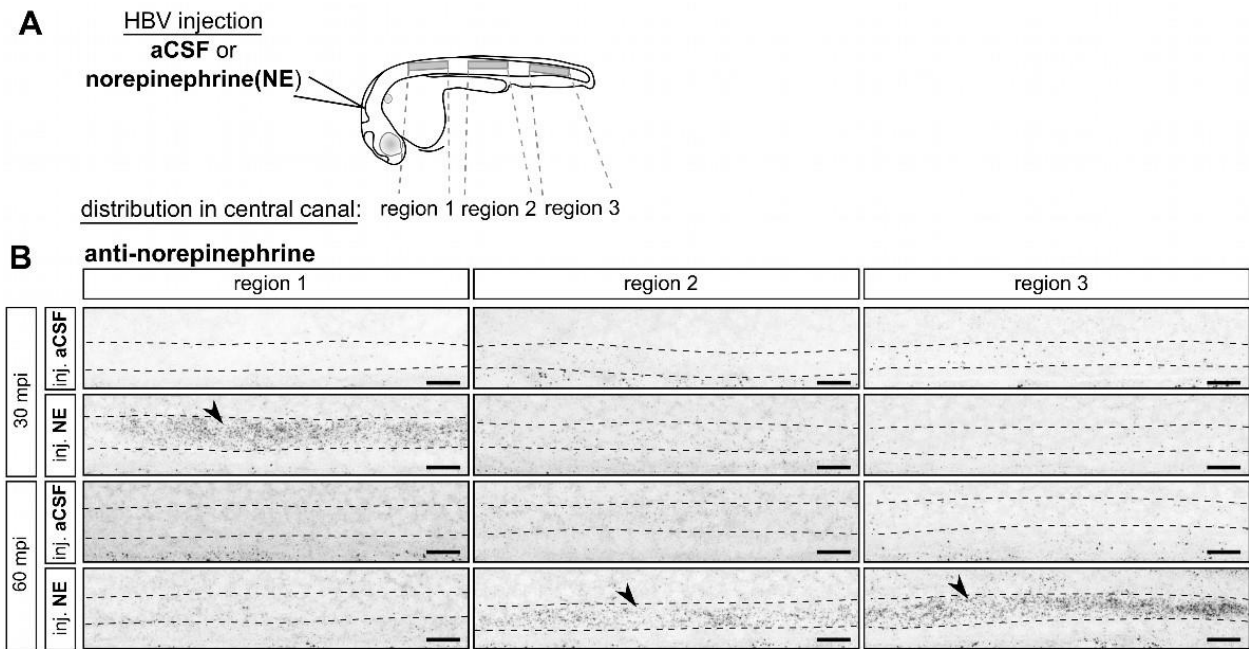
1127 *scospondin*^{*icm15/+*} (+/-) and *scospondin*^{*icm15/icm15*} mutant embryos (-/-) at 48 hpf after one cell1128 stage injection of control mRNA (**A**) or control +*urp2* mRNA (**B**). Note that 2 mutant (-/-)1129 embryos were detected as straight and 2 as curled-up upon *urp2* overexpression. Data were

1130 collected over 3 independent clutches.

1131

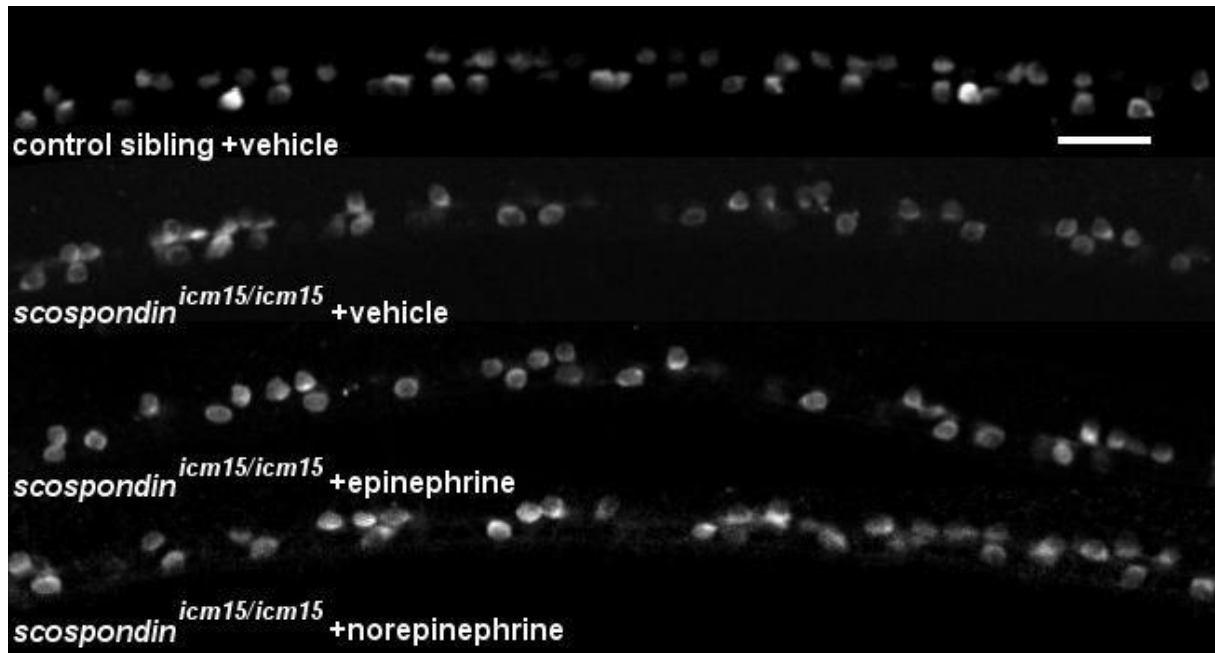
1132 **Figure 3 - source data 1. Data for figure 3D**

1133 **Figure 4 - source data 1. Data for figure 4C-E**



1134 **Figure 5-Figure Supplement 1. Exogenous norepinephrine injected in brain ventricles**
 1135 **is transported to the spinal cord and saturates the central canal 60 minutes post-**
 1136 **injection.**

1137 (A) 30 hpf embryos were mounted laterally and injected in the hindbrain ventricle (HBV) with
 1138 either aCSF or 3mM norepinephrine (NE). Embryos from each group were fixed 30 and 60
 1139 minutes post-injection (mpi) and processed for an immunostaining against norepinephrine.
 1140 Three regions in the spinal cord were imaged for each experimental condition (boxed
 1141 regions): above the yolk (rostral, region 1), above the yolk extension (middle, region 2) and
 1142 after the anal region (caudal, region 3). (B) Representative maximal z-projections of
 1143 norepinephrine-positive signals detected after either the injection of aCSF (inj. aCSF) or
 1144 exogenous norepinephrine (inj. NE), 30 and 60 minutes post-injection (mpi). The same
 1145 imaging parameters and image processing parameters were applied to all experimental
 1146 groups to avoid saturation for the most intense signals. Sagittal views of the three regions of
 1147 the spinal cord shown in (A) are represented for a single representative embryo (n=7
 1148 embryos 30 minutes post-aCSF injection; n=7 embryos 30 minutes post-norepinephrine
 1149 injection; n=6 embryos 60 minutes post-aCSF injection; n=8 embryos 60 minutes post-
 1150 norepinephrine injection). Note that norepinephrine injected in the HBV saturates the central
 1151 canal of the spinal cord (delineated by dotted lines) in the rostral most region 30 minutes
 1152 post-injection, and the caudal most region 60 minutes post-injection (arrowheads). Embryos
 1153 are oriented dorsal to the top and rostral to the left. Scale bars: 10 μ m.
 1154



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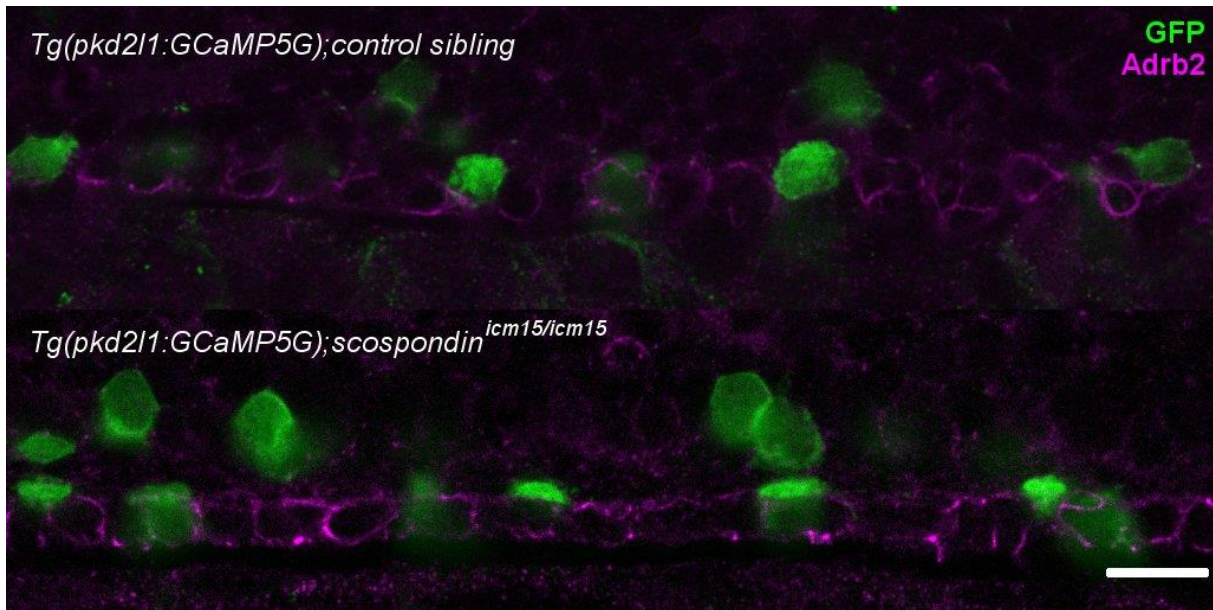
1157 **Figure 5- Video 1. Epinephrine and norepinephrine restore intracellular calcium**
1158 **transients of ventral CSF-contacting neurons in *scospondin* mutants.**

1159 Sagittal views of the spinal cord of *Tg(pkd211:GCaMP5G)* embryos at 30 hpf in a straight
1160 control sibling (top) and curled-down *scospondin*^{*icm15/icm15*} mutants after vehicle, epinephrine
1161 or norepinephrine injections in the hindbrain ventricle. Note that calcium transients are
1162 restored in a subset of ventral CSF-contacting neurons of *scospondin*^{*icm15/icm15*} embryos that
1163 received epinephrine and norepinephrine injections compared to mutants that received a
1164 vehicle injection. Data were collected at 4 Hz and displayed at 80 Hz. Scale bar: 30 μ m.

1165

1166 **Figure 5 - source data 1. Data for figure 5D-E**

1167



1168

1169

1170 **Figure 6 - Video 1. ADRB2 is expressed in the midline of the ventral most region of the**
1171 **neural tube that is different from that of CSF-contacting neurons.**

1172 Z-stack of a *Tg(pkd2l1:GCaMP5G)* control sibling (top) and a *Tg(pkd2l1:GCaMP5G);*
1173 *scospondin^{icm15/icm15}* mutant (bottom) at 30 hpf immunostained against GFP (green) and
1174 ADRB2 (magenta). In both cases, ADRB2-positive domains are inserted between two rows of
1175 GFP-positive ventral CSF-contacting neurons (both domains are not colocalized). The video
1176 represents a 20.5 μm deep slice (0.5 μm z-step) for both embryos. Scale bar: 10 μm .