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## Sensory neurons contacting the cerebrospinal fluid require the Reissner fiber to detect spinal curvature in vivo

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1 **Title**

2 Sensory neurons contacting the cerebrospinal  
3 fluid require the Reissner fiber to detect  
4 spinal curvature *in vivo*

5

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20

21 **Summary**

22 Recent evidence indicate active roles for the cerebrospinal fluid (CSF) on body axis  
23 development and morphogenesis of the spine implying CSF-contacting neurons (CSF-cNs) in  
24 the spinal cord. CSF-cNs project a ciliated apical extension into the central canal that is  
25 enriched in the channel PKD2L1 and enables the detection of spinal curvature in a directional  
26 manner. Dorsolateral CSF-cNs ipsilaterally respond to lateral bending while ventral CSF-cNs  
27 respond to longitudinal bending. Historically, the implication of the Reissner fiber (RF), a long  
28 extracellular thread in the CSF, to CSF-cN sensory functions has remained a subject of debate.  
29 Here, we reveal using electron microscopy in zebrafish larvae that the RF is in close vicinity with  
30 cilia and microvilli of ventral and dorsolateral CSF-cNs. We investigate *in vivo* the role of cilia  
31 and the Reissner fiber in the mechanosensory functions of CSF-cNs by combining calcium  
32 imaging with patch-clamp recordings. We show that disruption of cilia motility affects CSF-cN  
33 sensory responses to passive and active curvature of the spinal cord without affecting the  
34 Pkd2l1 channel activity. Since ciliary defects alter the formation of the Reissner fiber, we  
35 investigated whether the Reissner fiber contributes to CSF-cN mechanosensitivity *in vivo*. Using  
36 a hypomorphic mutation in the *scospondin* gene that forbids the aggregation of SCO-spondin  
37 into a fiber, we demonstrate *in vivo* that the Reissner fiber *per se* is critical for CSF-cN  
38 mechanosensory function. Our study uncovers that neurons contacting the cerebrospinal fluid  
39 functionally interact with the Reissner fiber to detect spinal curvature in the vertebrate spinal  
40 cord.

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**Keywords**

Cerebrospinal fluid (CSF), CSF-contacting neurons (CSF-cNs), mechanoreception, the Reissner fiber (RF), Polycystin Kidney Disease 2 Like 1 (PKD2L1), SCO-spondin, motile cilia, Kolmer Agduhr cells (KAs), spinal cord, central canal.

**Introduction**

Cerebrospinal fluid (CSF) is secreted by the choroid plexuses and fills the ventricular cavities of the brain and the central canal of the spinal cord [1]. The CSF has been long assumed to form a passive fluid acting as a cushion, supporting the clearance of toxic products in the brain, thereby ensuring its mechanical protection and chemical homeostasis. However, multiple studies have shown that secretion and circulation of signaling molecules in the CSF contribute in the brain to neurogenesis in an age-dependent manner [2–7]. Furthermore, physico-chemical properties of the CSF also contribute to organogenesis outside of the nervous system. CSF content and cilia-driven flow control the geometry of the body axis during embryogenesis [8,9] as well as spine curvature in juvenile zebrafish [9,10]. Both the formation of the body axis and the spine organogenesis appear linked to urotensin-related peptides expressed in the spinal cord by cerebrospinal fluid-contacting neurons (CSF-cNs) [9,11].

CSF-cNs are found in the spinal cord in many vertebrate species [12–14]. Spinal CSF-cNs are GABAergic sensory neurons that extend an apical extension into the lumen of the central canal. This apical extension is composed of one motile cilium and numerous microvilli that bath in the CSF [14–20]. Dorsolateral and ventral CSF-cNs originate from two distinct progenitor domains in zebrafish [18,19,21–23] as in mouse [24], and are characterized by different axonal targets [18], and morphology of the apical extension [17]. Both CSF-cN types respond to spinal curvature in a directional manner: while dorsolateral CSF-cNs respond ipsilaterally to lateral bending [16] ventral CSF-cNs are recruited during longitudinal contractions of the spinal cord [25].

Due to the morphological resemblance between CSF-cNs and hair cells, Kolmer had proposed that these cells could constitute a novel sensory organ, referred to as the “SagittalOrgan”, acting as a third ear in the vertebrate spinal cord [13]. This hypothesis has been discussed several times since [12,26–28], but data were based on sparse electron

76 microscopy and not functional evidence. Identifying genetic markers of CSF-cNs over the last  
77 decade [29–31] has allowed novel investigation of their function. Based on these recent studies,  
78 we know that spinal CSF-cNs sense changes in pH and osmolarity [32–35] as well as  
79 mechanical stretch of the spinal cord [16,25,33,36]. Mechanotransduction in CSF-cNs relies on  
80 the Polycystic Kidney Disease 2-Like 1 (PKD2L1) channel [16,36], a member of the Transient  
81 Receptor Potential (TRP) channel family, which is also a specific marker of these cells in the  
82 vertebrate spinal cord [19,30,34,35]. The PKD2L1 channel is enriched in the CSF-cN apical  
83 extension [19,36], which differentiates in the larva and likely constitutes the sensory apparatus  
84 of CSF-cNs. Concordantly, the length of microvilli in the apical extension tunes the  
85 mechanosensory response of CSF-cNs [17].

86  
87 In the lumen of the central canal, the Reissner fiber (RF) is a long extracellular thread  
88 extending caudally from the diencephalic third ventricle to the central canal of the spinal cord  
89 and is mainly composed of the aggregation of the SCO-spondin glycoprotein [26,37,38]. A  
90 century ago, Kolmer and Agduhr noticed the presence of the RF in close vicinity with the CSF-  
91 cN apical extension bathing in the CSF [39]. This proximity led Kolmer to hypothesize a possible  
92 functional interaction between the RF and CSF-cNs [13], a hypothesis not favored by Agduhr  
93 [12]. Here, we revisited this question by investigating in zebrafish larvae the role of motile cilia  
94 and the RF in CSF-cN mechanotransduction.

95  
96 We show that disruption of cilia motility affects mechanosensory responses of both  
97 ventral and dorsolateral CSF-cNs without affecting Pkd2l1 channel activity. We found using  
98 electron microscopy that the RF and CSF-cN apical extension come together in close proximity  
99 in the lumen of the central canal. We show that in *scospondin* hypomorph lacking the fiber in the  
100 central canal, CSF-cNs lose their mechanoreception without disrupting Pkd2l1 channel  
101 spontaneous opening. Our results demonstrate the need of the RF for transmitting mechanical  
102 deformations associated with spinal curvature to sensory neurons lining the central canal.  
103 Altogether, our results validate Kolmer’s hypothesis who suggested that CSF-cNs may  
104 functionally interact with the Reissner fiber *in vivo* to form a sensory organ in the vertebrate  
105 spinal cord.

106

## 107 **Results**

108

### 109 **Motile cilia are required for CSF-cN response to muscle contraction and spinal curvature** 110 ***in vivo***

111

112 We previously showed that cilia functions are necessary for spontaneous activity of CSF-  
113 cNs in the embryos [36]. Now, in order to test the role of motile cilia in CSF-cN response to  
114 spinal curvature in the larval stage when their apical extension is differentiated, we induced  
115 escape responses in 3 days post fertilization (dpf) larvae by puffing artificial cerebrospinal fluid  
116 (aCSF) in the otic vesicle (Figure 1). We monitored in the sagittal plane CSF-cN activity with the  
117 genetically-encoded calcium sensor GCaMP5G expressed under the CSF-cN specific promoter  
118 *pkd2l1* in *Tg(pkd2l1:GCaMP5G)* transgenic larvae [18,29,36] (Figure 1A1, 1A2, 1B2, see also  
119 Video S1). In this paradigm, larvae were pinned on the side and muscle contractions  
120 subsequent to the otic vesicle stimulation occurred in lateral and horizontal directions, which  
121 lead to calcium transients in dorsolateral and ventral CSF-cNs (Figure 1A2 and 1A3). The  
122 amplitude of calcium transient in dorsolateral CSF-cNs was larger than in ventral CSF-cNs  
123 (mean  $\Delta F / F = 87.2 \pm 5 \%$  from 211 dorsolateral CSF-cNs, vs.  $57.4 \pm 3.3 \%$  from 168 ventral  
124 CSF-cNs in 15 control sibling larvae; linear mixed model (type II Wald chi-square test):  $p < 1 \times$   
125  $10^{-4}$ , see Methods S1), likely due to the muscle contractions being mainly lateral during the  
126 escape. We used the *cfap298<sup>tm304</sup>* mutation affecting the Cfap298 protein enriched at the base  
127 of cilia, which leads to defects in dynein arms, defective motility and polarity of cilia [40–42]. In  
128 the *cfap298<sup>tm304/tm304</sup>* mutant larvae, the response of CSF-cNs was overall massively reduced  
129 (mean  $\Delta F / F = 19.0 \pm 1.9 \%$  from 153 dorsolateral CSF-cNs and  $15.5 \pm 1.4 \%$  from 104 ventral  
130 CSF-cNs in 11 mutant larvae;  $p < 1 \times 10^{-4}$  for dorsolateral CSF-cNs and  $p < 1 \times 10^{-3}$  for ventral  
131 CSF-cNs between mutant and control siblings; Figure 1A2 and 1A3; Video S1 and S2, Table  
132 S1). The proportion of CSF-cNs recruited decreased from 68.2 % to 29.4 % for dorsolateral  
133 CSF-cNs and from 64.3 % to 31.7 % for ventral CSF-cNs (Figure 1A3).

134 As the *cfap298* mutation may alter cilia in the otic vesicle, defects in inner ear hair cells  
135 could be responsible of the decreased response of CSF-cNs. We therefore examined CSF-cN  
136 responses to passive curvature of the spinal cord from paralyzed 3 dpf *Tg(pkd2l1:GCaMP5G)*  
137 larvae mounted to record from a coronal view dorsolateral CSF-cNs (Figure 1B1) that are  
138 selectively activated by lateral bending of the spinal cord [16,17]. Lateral bending of the tail in  
139 control larvae induced as previously reported [16] calcium transients in dorsolateral CSF-cNs  
140 (Figure 1B1). In *cfap298<sup>tm304/tm304</sup>* larvae, the response of CSF-cNs was overall massively  
141 reduced (mean  $\Delta F / F = 32.30 \pm 2.7 \%$  from 490 dorsolateral CSF-cNs in 15 mutant larvae vs.  
142  $100.7 \pm 4.3 \%$  from 830 dorsolateral CSF-cNs in 26 control siblings;  $p < 2 \times 10^{-9}$ ; Figure 1B1 and  
143 1B2; Video S2, Table S1, Methods S1). The proportion of dorsolateral CSF-cNs recruited  
144 decreased from 62.2 % to 28.4 % (Figure 1B3). Altogether, our results indicate that cilia motility  
145 and polarity are necessary for mechanosensory functions of CSF-cNs *in vivo*.

146

147 **Mutants with defective cilia maintain functional Pkd2l1 channels on CSF-cN apical**  
148 **extension**

149  
150 We previously showed that mechanical activation of CSF-cNs requires Pkd2l1 channels  
151 *in vivo* [16] and *in vitro* [36]. To confirm the presence and localization of Pkd2l1 at the apical  
152 extension of CSF-cNs in the *cfap298<sup>tm304/tm304</sup>* mutant at 3 dpf, we performed  
153 immunohistochemistry in *Tg(pkcd2l1:GCaMP5G); cfap298<sup>tm304/tm304</sup>* larvae. Pkd2l1 localized at  
154 the apical extension of CSF-cNs in *cfap298<sup>tm304/tm304</sup>* larvae similarly to control (Figure 2A).

155 In order to confirm the functionality of Pkd2l1 channels in CSF-cNs, we performed *in vivo*  
156 whole-cell voltage clamp recordings in 3 dpf *Tg(pkcd2l1:GAL4;UAS:mCherry); cfap298<sup>tm304/tm304</sup>*  
157 larvae. We observed spontaneous Pkd2l1 channel openings with similar properties in both  
158 *cfap298<sup>tm304/tm304</sup>* mutants and their control siblings (Figure 2B, 2C Two-sample Kolmogorov-  
159 Smirnov test:  $p > 0.5$ , Table S2). Furthermore, we did not notice any effect of cilia defects on  
160 CSF-cN passive properties (Figure 2D1, 2D2, 2D3,  $p > 0.2$ , Table S3) or firing patterns upon  
161 current injection (Figure 2E). Hence, cilia impairment decreases CSF-cN mechanosensory  
162 function without affecting their intrinsic excitability nor Pkd2l1 spontaneous channel openings.

163

164 **Mechanosensory function of CSF-cNs requires the Reissner fiber**

165

166 The *cfap298<sup>tm304</sup>* mutation affecting cilia polarity and motility [40,42] has been shown to *i)*  
167 reduce CSF flow [10,43] and CSF transport [36], *ii)* reduce the diameter of the central canal [43]  
168 and *iii)* forbids the formation of the RF [8]. Parameters such as CSF flow, diameter of the central  
169 canal and the presence of the RF could all contribute to the defect observed in CSF-cN sensory  
170 function.

171 Since the role of the RF in spinal mechanoreception is of peculiar interest, we took  
172 advantage of the hypomorphic mutation *scospondin<sup>icm15</sup>* in the gene *scospondin* in which a 5  
173 amino acid insertion in the EMI domain forbids the aggregation of SCO-spondin into the  
174 Reissner fiber [8]. Response of dorsolateral and ventral CSF-cN to active tail bending was  
175 largely abolished in *Tg(pkcd2l1:GCaMP5G); scospondin<sup>icm15/icm15</sup>* mutant larvae (Figure 3A1, A2,  
176 Video S3, Table S4 ; dorsolateral CSF-cNs: mean  $\Delta F / F = 57.3 \% \pm 3.0 \%$  from 180 cells in 12  
177 control sibling, vs.  $7.1 \% \pm 0.6 \%$  from 167 cells in 11 mutant larvae, linear mixed model (type II  
178 Wald chi-square test):  $p < 1 \times 10^{-4}$ ; ventral CSF-cNs: mean  $\Delta F / F = 31.3 \pm 2.1 \%$  from 128 cells  
179 in control sibling, vs.  $10.9 \pm 1.4 \%$  from 146 cells from mutant larvae, linear mixed model (type II  
180 Wald chi-square test):  $p < 5 \times 10^{-4}$ , see Methods S1). In response to passive tail bending (Figure  
181 3B1), the calcium transients recorded in dorsolateral CSF-cNs showed a threefold reduction in  
182 *scospondin<sup>icm15/icm15</sup>* mutant larvae (Figure 3B2, mean  $\Delta F / F = 194.0 \pm 17.8 \%$  from 276 cells in  
183 11 control siblings, vs.  $56.2 \pm 13.1 \%$  from 165 cells from in mutant larvae,  $p < 0.005$ , see

184 Methods S1). In the active assay, the proportion of dorsolateral CSF-cNs recruited was  
185 decreased by 90.8 % and by 77.8 % for ventral CSF-cNs (Figure 3A2). After passive  
186 stimulation, the proportion of responding dorsolateral CSF-cNs was decreased by 72.1 %  
187 (Figure 3B2, Table S4). Altogether, our results demonstrate that CSF-cNs require the RF to  
188 optimally respond to mechanical stimuli associated with spinal curvature *in vivo*.

189

### 190 **Pkd2l1 channels in CSF-cNs remain functional when the Reissner fiber is absent**

191

192 The RF could be required for CSF-cNs to express the Pkd2l1 channel at the membrane.  
193 To verify that CSF-cNs properly express Pkd2l1 in their apical extension, we first performed  
194 immunohistochemistry on *Tg(pkd2l1:GCaMP5G); scospondin<sup>icm15/icm15</sup>* and found that the Pkd2l1  
195 protein was still enriched in CSF-cN apical extension of larvae lacking the RF (Figure 4A).  
196 Accordingly, whole-cell patch-clamp recording of CSF-cNs in *Tg(pkd2l1:GAL4;UAS:mCherry);*  
197 *scospondin<sup>icm15/icm15</sup>* revealed spontaneous unitary current (Figure 4B-C, Two-sample  
198 Kolmogorov-Smirnov test:  $p > 0.8$ , Table S5), most likely reflecting functional Pkd2l1 channels  
199 [34,36]. We tested whether the absence of the RF could affect the intrinsic properties of CSF-  
200 cNs and found no difference in membrane resistance, membrane capacitance, or resting  
201 membrane potential (Figure 4D1-3;  $p > 0.07$ , Table S6). The firing pattern of CSF-cNs in  
202 *scospondin<sup>icm15/icm15</sup>* upon current injection was comparable to controls (Figure 4E). Altogether,  
203 our results show that the absence of the RF does not alter the localization of Pkd2l1 channel to  
204 the apical extension, the spontaneous Pkd2l1 channel properties nor the intrinsic excitability of  
205 CSF-cNs.

### 206 **In the central canal, the Reissner fiber is in close vicinity with CSF-cN apical extension**

207

208 Given that in the absence of the RF, CSF-cN sensitivity to spinal curvature is largely  
209 reduced despite the cells retaining their intrinsic properties and Pkd2l1 channel activity, we  
210 investigated where the RF is localized in the central canal relative to the apical extension of  
211 CSF-cNs. In live larvae, the lumen of the central canal is typically  $8.7 \pm 0.4 \mu\text{m}$  width and  $10.2 \pm$   
212  $0.7 \mu\text{m}$  height (measured from 4 and 9 larvae respectively, Figure 5A, 5C and 5D, Table S7)  
213 and CSF-cNs extend their dendritic apical extension by typically  $2.9 \pm 0.1 \mu\text{m}$  height (measured  
214 from 9 cells in 4 larvae, Table S7) towards the center of the central canal, suggesting that CSF-  
215 cNs cover a substantial portion of the central canal in living zebrafish larvae.

216 To assess the relative organization of the RF and CSF-cN apical extension, we immuno-  
217 stained for the Reissner fiber material as previously described [8] in 3 dpf  
218 *Tg(pkd2l1:GAL4;UAS:tagRFP-CAAX)* larvae. As the process of fixation can alter the shape of

219 cavities filled with CSF such as the central canal, we determined the impact of fixation on the  
220 width and height of the central canal measured in 3 dpf *Tg(cdh2:cdh2-GFP*;  
221 *pkd2l1:GAL4;UAS:tagRFP)* larvae imaged live and after fixation followed by either co-  
222 immunostaining for GFP and tag-RFP, or ZO-1 and tag-RFP (Figure 5A-D). PFA fixation  
223 induced a shrinking of the central canal along the midline (Figure 5A and 5B): in the transverse  
224 plane, the lumen of the central canal after fixation became narrower ( $3.5 \pm 0.3 \mu\text{m}$ , Two-sample  
225 Kolmogorov-Smirnov test:  $p < 0.05$ ) and more elongated along the dorsoventral axis ( $14.1 \pm 1.2$   
226  $\mu\text{m}$ ,  $p < 0.002$ ) (Figure 5C and 5D, Table S7). In these conditions, we investigated the  
227 approximate position of the RF relative to the apical extension of dorsolateral (Figure 5E1) and  
228 ventral (Figure 5E2) CSF-cNs in 3 dpf *Tg(pkd2l1:GAL4;UAS:tagRFP-CAAX)* larvae co-  
229 immunostained for RF and tag-RFP. The RF was in close vicinity of two third of dorsolateral  
230 CSF-cNs (Figure 5E1, top panel and 5F) and one third of ventral CSF-cNs (Figure 5E2, top  
231 panel and 5F; Two-sample Kolmogorov-Smirnov test:  $p < 5 \times 10^{-4}$ ).

232           At higher resolution, we characterized the ultrastructure of the central canal in sagittal  
233 sections of 3-4 dpf wild type larvae using transmission electron microscopy (Figure 6). In single  
234 sections (Figure 6A, 6B) and reconstruction of the RF and cilia in 3D using serial block face  
235 scanning electron microscope imaging (Figure 6C, 6D), the RF appeared as a long and thin  
236 thread (Figure 6A, 5B) with diameter of  $258.4 \text{ nm} \pm 6.8 \text{ nm}$  (26 measurements from 2 larvae)  
237 often in close contact with cilia (arrows) and microvilli (arrowheads) (Figure 6A). Cilia in contact  
238 with the RF had two central microtubules along the axoneme (Figure 6A1, 6A2, 6A3, 6A4),  
239 which typically characterize motile cilia found in ependymal radial glia [36,44–46] as well as  
240 CSF-cNs [16]. CSF-cNs have one motile cilium and many microvilli [16–18]. In these  
241 ultrastructure images, a subset of dorsolateral and ventral CSF-cNs extended toward the RF via  
242 both their microvilli (Figure 6A1, 6B, arrowheads) and their motile cilium (Figure 6B-D, see also  
243 Video S4).

244

## 245 **Discussion**

246 Our study demonstrates *in vivo* the functional coupling between the Reissner fiber and neurons  
247 contacting the cerebrospinal fluid in the spinal cord in order to detect spinal curvature. By  
248 analyzing the physiological and sensory properties of CSF-cNs in the *scospondin<sup>icm15</sup>* mutant  
249 lacking the Reissner fiber, we proved that Kolmer was correct when he speculated that neurons  
250 contacting the cerebrospinal fluid form a mechanosensory organ with the Reissner fiber in the  
251 vertebrate spinal cord [13].



252 **The Reissner fiber functionally interacts with CSF-cNs to sense spinal curvature *in vivo***

253  
254 We had formerly showed that CSF-cNs integrate *in vivo* mechanosensory inputs on the  
255 concave side during spinal curvature [16,17] in order to respond to lateral bending for  
256 dorsolateral CSF-cNs [16,17] and longitudinal bending for ventral CSF-cNs [25]. *In vivo*, this  
257 directional mechanosensory response of CSF-cNs requires the channel Pkd2l1 [16]. Recently,  
258 we showed that CSF-cNs when isolated *in vitro* keep their mechanosensory properties: the  
259 open probability of the channel Pkd2l1 is largely modulated by mechanical pressure on CSF-cN  
260 membrane [36]. Now, we made a new step in understanding how CSF-cNs detect curvature *in*  
261 *vivo*: we demonstrate that the RF enhance by at least threefold the response of CSF-cNs to  
262 spinal curvature *in vivo*.

263 Note that in *cfap298* mutants, the disruption of cilia motility leads to the absence of the  
264 Reissner fiber [8] together with a reduction in the dimensions of the central canal lumen [43]. In  
265 these mutants, we cannot exclude that the narrow central canal lumen could also contribute with  
266 the loss of Reissner fiber to the reduction of CSF-cN response. However, as RF loss is the only  
267 defect observed in the *scospondin<sup>icm15</sup>* mutant (the dimensions of the CC and cilia motility are  
268 not altered in this mutant), we formulate the parsimonious assumption that the Reissner fiber is  
269 the important factor in explaining the loss of CSF-cN mechanosensory response.

270

271 **How can the RF and CSF-cNs interact to sense spinal curvature?**

272

273 Recent evidence based on the labeling of SCO-spondin-GFP have revealed that the  
274 Reissner fiber *in vivo* is dynamic as well as straight as an arrow [47], which indicates that the  
275 fiber is under high tension *in vivo*. Given the dimensions of the central canal in living larvae  
276 (typically 10  $\mu\text{m}$  by 9  $\mu\text{m}$ ) compared to the height of CSF-cN apical extensions (typically 3-5  $\mu\text{m}$ )  
277 and the very thin diameter of the fiber (~200 nm), it is conceivable that at rest, the thin RF could  
278 sit in the center of the central canal away from CSF-cN apical extension. In contrast, when  
279 muscles contract on one side – left, right or ventrally– the Reissner fiber under tension could get  
280 closer to CSF-cNs during bending, which would enable their selective asymmetrical recruitment  
281 in the concave side ([16-17], see Graphical Abstract).

282 The nature of the functional interaction between the Reissner fiber and CSF-cN apical  
283 extension could rely on a transient or stable physical contact (see Graphical Abstract), which  
284 would amplify the mechanical force applied on the apical extension of CSF-cNs in an  
285 asymmetrical manner on the concave side during bending. Alternatively, from the fluid dynamics  
286 point of view, it is also highly conceivable that RF and CSF-cN apical extension *functionally*  
287 interact without the need for *physical* contacts. Indeed, the Reissner fiber could increase the

288 CSF flow gradient perceived by CSF-cN apical extension (see Graphical Abstract). We  
289 previously showed that CSF flow in the central canal is maximal close to the center and null  
290 along the central canal walls [43]. Remarkably, the most commonly used boundary condition in  
291 fluid dynamics is the non-slip condition, which claims that the velocity of the fluid at a solid wall  
292 is the same as that of the solid wall [48]. Therefore, CSF flow has to equalize the velocity of the  
293 fiber on the surface of the fiber itself (around 50-200 nm / s at most, [47]), which is negligible  
294 compared to the velocity of particles in the flow (around 10  $\mu\text{m}$  / s, [43]). Therefore, CSF-cN  
295 apical extension pointing towards the center of the lumen sits in a region of high CSF flow  
296 gradient precisely at the boundary between the high flow and the close-to-null flow point  
297 imposed by the Reissner fiber. This effect could be amplified by that CSF flow is largely  
298 increased by muscle contractions along the tail as reported in the brain ventricles [49] and in the  
299 central canal [43].

300 Due to the fixation artifact that we quantified here with classical immunostaining protocol,  
301 further investigations of the dynamic interactions of CSF-cN apical extensions together with  
302 labeled RF *in vivo* [47] occurring during spinal curvature will be necessary to distinguish  
303 between these hypotheses.

#### 304 **Comparison of CSF-cNs with inner ear hair cells**

305  
306 Kolmer and Agduhr originally compared CSF-cNs to hair cells due to the morphological  
307 similarity of the apical extension of both cell types. A century later, can we comment on the  
308 similarity of mechanisms underlying their mechanosensory functions? Of course, CSF-cNs with  
309 their coral-like shaped microvilli lack the regular staircase organization of stereocilia. However,  
310 CSF-cNs bear a kinocilium [15,16,50], similarly to inner ear hair cells from fish and amphibians  
311 [51]. We know from hair cells in amphibians that the active oscillations of the hair bundle amplify  
312 mechanical stimuli, which contributes to sound detection [52]. Similarly to vertebrate hair cells,  
313 mechanosensory transduction in the chordotonal sensory neurons that mediate hearing in  
314 *Drosophila* rely on the axonemal dynein of motile cilia [53,54]. Similarly, through active  
315 movements, CSF-cN kinocilium could contribute to the amplification of mechanosensory  
316 response. The specific role of CSF-cN kinocilium in mechanoreception will be the focus of future  
317 investigations based on tools for disrupting cilia only in CSF-cNs and not in other cell types.

#### 318 319 **Relevance for development of body axis and spine**

320  
321 Sensory systems are critical to guide symmetrical growth and balanced activation of  
322 motor circuits, but they can be also relevant for morphogenesis. We previously showed that

323 CSF-cNs modulate the spinal circuits controlling locomotion and active posture  
324 [16,25,29,31,36]. Recently, the RF and CSF-cNs have also been associated with the  
325 establishment of the body axis during embryogenesis [8] and of the spine morphogenesis in  
326 juvenile and adult zebrafish [9,36]. Multiple evidence suggest that CSF-cNs are relevant for  
327 spine morphogenesis: *pkd211* mutants deprived of CSF-cN sensory responses exhibit an  
328 increased curvature of the spine, reminiscent of kyphosis [36]. Furthermore, mutants for a  
329 receptor of the urotensin related peptides, which are solely produced by CSF-cNs in the spinal  
330 cord, exhibit a torsion of the spine, reminiscent of adolescent idiopathic scoliosis [9]. Finally, a  
331 recent report indicated that hypomorphic mutations in the *scospondin* gene induce 3D  
332 deformation of the spine [47]. Altogether, recent studies indicate that sensory neurons  
333 contacting the cerebrospinal fluid together with the Reissner fiber may contribute to the  
334 generation and maintenance of the shape of the spine. Future studies will investigate whether  
335 the functional coupling of CSF-cNs with the Reissner fiber that we have demonstrated here is  
336 relevant for sensing and adjusting morphogenesis of the spine.

337

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353

354

### 355 **Author Contributions**

356 A.O.D.I. performed all calcium imaging and electrophysiology experiments and analysis,  
357 A.O.D.I. and Y.C.-B. performed immunohistochemistry and confocal microscopy imaging, A.B.  
358 and D.L. performed E.M., O.T. provided guidance on automated analysis for calcium imaging,  
359 J.R. and F.K. helped with genotyping, P.B. provided help for 3D reconstruction, F.-X.L.

360 performed statistical analysis, P.-L.B. provided feedback on morphological analysis, C.W.  
361 conceived, funded and supervised the project. A.O.D.I. and C.W. conceived the project and  
362 wrote the article with inputs from Y.C.-B., P.-L.B. and all authors.

363

364

### 365 **Declaration of interests**

366 The authors declare no conflict of interest.

367

368

### 369 **Figures and legends**

370

#### 371 **Figure 1. The sensory response of CSF-cNs to spinal curvature is altered in mutants** 372 **with defective motile cilia.**

373 **(A)** CSF-cN calcium transients calculated from the changes in fluorescence of the  
374 genetically-encoded calcium indicator GCaMP5G in 3 dpf *Tg(pkd2l1:GCaMP5G)* transgenic  
375 larvae are large for control siblings (top, green) and strongly reduced in *cfap298<sup>tm304/tm304</sup>*  
376 mutants (bottom, blue) during active bends where muscle contraction is induced by pressure-  
377 application of aCSF in the otic vesicle (see also **Video S1**; **Video S2**).

378 **(A1)** Diagram representing a 3 dpf larva view in sagittal plane.

379 **(A2)** Time projection stack of 3 optical sections imaged from the sagittal plane (left) showing  
380 for illustration purposes a subset of 10 spinal CSF-cNs expressing GCaMP5G in 3 dpf  
381 *Tg(pkd2l1:GCaMP5G)*. Green and blue lines around soma delimitate ROIs used to calculate  
382  $\Delta F / F$  traces on the right.

383 **(A3)** Quantification of corresponding  $\Delta F / F$  in dorsolateral and ventral CSF-cNs in control  
384 siblings (green) and *cfap298<sup>tm304/tm304</sup>* mutants (blue), (mean  $\Delta F / F = 87.2 \pm 5.0$  % from 211  
385 dorsolateral CSF-cNs and mean  $\Delta F / F = 57.4 \pm 3.3$  % from 168 ventral cells in 15 control  
386 siblings vs. mean  $\Delta F / F = 19.0 \pm 1.9$  % from 153 dorsolateral cells and mean  $\Delta F / F = 15.5 \pm$   
387  $1.4$  % from 104 ventral cells in 11 *cfap298<sup>tm304/tm304</sup>* larvae, linear mixed model (type II Wald  
388 chi-square test);  $p < 1 \times 10^{-4}$  between dorsolateral and ventral CSF-cNs in control siblings,  $Df = 613$ ;  
389  $p < 1 \times 10^{-4}$  between dorsolateral CSF-cNs in mutants vs control siblings,  $Df = 30.4$ ;  $p$   
390  $< 1 \times 10^{-3}$  between ventral CSF-cNs in mutants vs control siblings,  $Df = 32.9$ ;  $p > 0.5$  between  
391 dorsolateral and ventral CSF-cNs in *cfap<sup>298tm304/tm304</sup>* mutants,  $Df = 614$ ). Pie charts represent  
392 the percentage of responding cells (see also **Table S1**).

393 **(B)** CSF-cN calcium transient recorded in 3dpf *Tg(pkd2l1:GCaMP5G)* transgenic larvae after  
394 a passive bend of the tail (passive stimulation) in paralyzed control siblings (top, green) and  
395 *cfap298<sup>tm304/tm304</sup>* mutant fish (bottom, blue).

396 **(B1)** Diagram showing the coronal plane of a 3 dpf.  
397 **(B2)** Time projection stack of 3 optical sections imaged from the coronal plane (left) showing  
398 for illustration purposes a subset of 10 dorsolateral CSF-cNs expressing GCaMP5G in 3 dpf  
399 *Tg(pkd2l1:GCaMP5G)*. Green and blue lines around soma delimitate ROIs used to calculate  
400  $\Delta F / F$  traces upon passive mechanical stimulation represented on the right.  
401 **(B3)** Quantification of corresponding  $\Delta F / F$  in CSF-cNs represented (as in **(A2)**) during a  
402 passive bend (mean  $\Delta F / F = 100.7 \pm 4.3$  % from 830 cells in 26 control siblings vs.  $32.3 \pm$   
403  $2.7$  % from 490 cells in 15 *cfap298<sup>tm304/tm304</sup>* larvae, linear mixed model (type II Wald chi-  
404 square test);  $p < 2 \times 10^{-9}$ ; Df = 1; Chi2 = 36.41). Pie charts represent the percentage of  
405 responding cells (see also **Table S1**).

406 R, rostral; V, ventral dl: dorsolateral CSF-cNs, v: ventral CSF-cNs. Time projection stacks  
407 were constructed from 3 to 4 series of images (corresponding to 0.75 s - 1 s integration  
408 time). Each data plotted in **(A3)** and **(B3)** represents one recording from one cell, the central  
409 mark on the box plot indicates the median, the bottom and top edges of the box indicate the  
410 25th and 75th percentiles. The whiskers extend to the most extreme data points that are not  
411 considered outliers, outliers are identified with a "+" symbol. Scale bars are 500  $\mu$ m in **(A1)**  
412 and **(B1)** and 50  $\mu$ m in **(A2)** and **(B2)** (left panel).

413

414 **Figure 2. CSF-cNs in *cfap298* mutant with defective motile cilia exhibit functional**  
415 **Pkd2l1 channels.**

416 **(A)** Immunohistochemistry with anti-GFP and anti-Pkd2l1 antibodies in 3 dpf  
417 *Tg(pkd2l1:GCaMP5G)* control sibling (left) and *cfap298<sup>tm304/tm304</sup>* mutant (right) larvae shows  
418 in the spinal cord similar localization for Pkd2l1 protein in the apical extension of CSF-cNs  
419 (seen in 14 mutant larvae and 8 control siblings).

420 **(B)** *In vivo* whole cell patch-clamp recordings from CSF-cNs using voltage clamp (VC)-mode  
421 in 3 dpf *Tg(pkd2l1:GAL4;UAS:mCherry)* show single channel openings in control sibling (left)  
422 and in *cfap298<sup>tm304/tm304</sup>* (right). Bottom traces represent at higher temporal magnification the  
423 data from top traces.

424 **(C)** Data plot of unitary current properties show that mutant larvae with defective motile cilia  
425 show proper spontaneous channel opening properties (Mean  $NP_0 = 9 \times 10^{-4} \pm 2 \times 10^{-4}$  in  
426 control siblings vs.  $1.10^{-3} \pm 0.6 \times 10^{-3}$  in mutants, Two-sample Kolmogorov-Smirnov test,  $p >$   
427  $0.5$ , ks2stat = 0.39 ; Mean unitary current amplitude =  $-18.8 \pm 0.3$  pA for control siblings vs.  $-$   
428  $18.3 \pm 1.0$  pA in mutant larvae,  $p > 0.8$ , ks2stat = 0.28; Mean duration of single opening =  $3.0$   
429  $\pm 0.5$  ms in control sibling vs.  $2.8 \pm 0.3$  ms in mutant larvae,  $p > 0.8$ , ks2stat = 0.28; n = 9  
430 cells in control, n = 6 cells in *cfap298<sup>tm304/tm304</sup>* larvae) (see also **Table S2**).

431 **(D)** Quantification of CSF-cN basic intrinsic electrophysiological properties (see also **Table**  
432 **S3**).

433 **(D1)** CSF-cN resting membrane potential is not affected in *cfap298<sup>tm304</sup>* mutant larvae (Mean  
434 membrane potential = - 43.4 ± 1.1 mV in control sibling vs. - 44.0 ± 1.3 mV in mutant larvae,  
435 Two-sample Kolmogorov-Smirnov test,  $p > 0.8$ ,  $ks2stat = 0.30$ ,  $n = 8$  cells in control,  $n = 5$   
436 cells in *cfap298<sup>tm304/tm304</sup>* larvae).

437 **(D2)** Quantification of membrane resistance reveals no change in *cfap298<sup>tm304/tm304</sup>* mutant  
438 larvae (Mean membrane resistance = 3.9 ± 0.6 GΩ in control siblings vs. 5.5 ± 1.6 GΩ in  
439 mutant larvae, Two-sample Kolmogorov-Smirnov test,  $p > 0.8$ ,  $ks2stat = 0.29$ ;  $n = 11$  cells in  
440 controls,  $n = 7$  cells in *cfap298<sup>tm304/tm304</sup>* larvae)

441 **(D3)** CSF-cN membrane capacitance is not affected in *cfap298<sup>tm304</sup>* mutant larvae (Mean  
442 membrane capacitance = 2.2 ± 0.1 pF in control sibling vs. 2.1 ± 0.1 pF in mutant larvae,  
443 Two-sample Kolmogorov-Smirnov test,  $p > 0.2$ ;  $ks2stat = 0.44$ ,  $n = 11$  cells in control,  $n = 7$   
444 cells in *cfap298<sup>tm304/tm304</sup>* larvae).

445 **(E)** CSF-cN action potential discharge recorded in current clamp (CC)-mode in response to  
446 successive current steps (100 ms-long pulses from 2 pA to 10 pA in 2 pA increments).  
447 NP<sub>0</sub>, opening probability. Each data point represents one recording from one cell; plots use  
448 median as measure of central tendency (central mark on the box plot), bottom and top edges  
449 of the box indicate the 25th and 75th percentiles. The whiskers extend to the most extreme  
450 data points without considering outliers, which are identified with a “+” symbol. Scale bars is  
451 20 μm in **(A)**.

452

453 **Figure 3. The absence of the Reissner fiber reduces the sensory response of CSF-cNs**  
454 **to spinal curvature.**

455 **(A)** CSF-cN calcium transient recorded in 3dpf *Tg(pkd211:GCaMP5G)* after active bends due  
456 to muscle contraction induced by pressure-application of aCSF in the otic vesicle in  
457 *scospondin<sup>icm15/icm15</sup>* mutant larvae (right, blue) and control sibling (left, green) (see also  
458 **Video S3**).

459 **(A1)** Time projection stack of 3 optical sections imaged from the sagittal plane showing  
460 dorsolateral and ventral CSF-cNs expressing GCaMP5 (top) in the spinal cord with ROIs on  
461 somas (green and blue line) used to calculate  $\Delta F / F$  traces below.

462 **(A2)** Quantification of calcium transients in dorsolateral and ventral CSF-cNs in control and  
463 *scospondin<sup>icm15/icm15</sup>* mutants in control sibling (green circle) and mutant larvae (blue circle),  
464 (mean  $\Delta F / F = 57.3 \pm 3.0$  % from 180 dorsolateral cells and mean  $\Delta F / F = 31.3 \pm 2.1$  from  
465 128 ventral cells in 12 control siblings vs. mean  $\Delta F / F = 7.1 \pm 0.6$  % from 167 dorsolateral  
466 cells and mean  $\Delta F / F = 10.9 \pm 1.4$  % from 146 ventral CSF-cNs in 11 *scospondin<sup>icm15/icm15</sup>*  
467 larvae, linear mixed model (type II Wald chi-square test);  $p < 1 \times 10^{-4}$  between dorsolateral

468 and ventral CSF-cNs in control sibling; Df = 602;  $p < 1 \times 10^{-4}$  between dorsolateral CSF-cNs  
469 in control siblings vs. mutant larvae; Df 21.4;  $p < 5 \times 10^{-4}$  between ventral CSF-cNs in control  
470 siblings vs. mutant larvae; Df 22.6;  $p > 0.05$  between dorsolateral and ventral CSF-cNs in  
471 *scospondin*<sup>icm15/icm15</sup> larvae, Df 601). Pie charts represent the percentage of responding cells  
472 (see also **Table S4**).

473 **(B)** CSF-cN calcium transient after a passive bend of the tail in paralyzed control siblings  
474 (left, green) and *scospondin*<sup>icm15/icm15</sup> mutant (right, blue) larvae.

475 **(B1)** Time projection stack of 3 optical sections imaged from the coronal plane shows CSF-  
476 cNs expressing GCaMP5 protein (top). ROIs (green and blue lines) used to calculate  $\Delta F / F$   
477 traces upon passive mechanical stimulation.

478 **(B2)** Same as **(A2)** during passive bending (mean  $\Delta F / F = 194.0 \pm 17.8$  % from 276 cells in  
479 11 control siblings vs. mean  $\Delta F / F = 56.2 \pm 13.1$  % from 165 cells in 8 *scospondin*<sup>icm15/icm15</sup>  
480 larvae, linear mixed model (type II Wald chi-square test);  $p < 5 \times 10^{-3}$ ; Df = 1; Chi2 = 8.18)  
481 (see also **Table S4**).

482 Scale bars are 50  $\mu\text{m}$  in **(A1)** and **(B1)**. dl: dorsolateral CSF-cNs, R, rostral; V, ventral, v:  
483 ventral CSF-cNs. Z-projection stacks were constructed from 3 series of images  
484 (corresponding to 0.75 s - 1 s integration time). For box plots, each data point in **(A2)** and  
485 **(B2)** represents one recording from one cell, the central mark indicates the median, the box  
486 bottom and top edges indicate the 25th and 75th percentiles.

487

488 **Figure 4. *scospondin* mutants deprived of the Reissner fiber exhibit functional Pkd211**  
489 **channels**

490 **(A)** Immunohistochemistry for GFP and Pkd211 in 3dpf *Tg(pkcd211:GCaMP5G)* control siblings  
491 (left) and *scospondin*<sup>icm15/icm15</sup> mutant larvae (right) shows that Pkd211 protein is localized in  
492 CSF-cN apical extension (sagittal view, n = 15 control sibling and 9 mutant larvae).

493 **(B)** *In vivo* whole cell patch-clamp recording of CSF-cNs in voltage clamp (VC)-mode  
494 targeted for mCherry in 3dpf *Tg(pkcd211:GAL4;UAS:mCherry)* exhibits single channel opening  
495 in control sibling (left) and in *scospondin*<sup>icm15/icm15</sup> mutant (right) larva. Bottom traces  
496 represent a higher magnification from the top trace.

497 **(C)** Unitary currents reflecting spontaneous channel opening properties are not affected in  
498 *scospondin*<sup>icm15/icm15</sup> mutants compared to control siblings  
499 (Mean  $\text{NP}_0 = 2.4 \times 10^{-3} \pm 1.3 \times 10^{-3}$  in control sibling vs.  $1.6 \times 10^{-3} \pm 0.5 \times 10^{-3}$  in mutant, Two-  
500 sample Kolmogorov-Smirnov test,  $p > 0.9$ , ks2stat = 0.23 ; Mean unitary current amplitude =  
501  $-18.6 \pm 0.3$  pA in control sibling vs.  $-18.7 \pm 0.9$  pA in mutant larvae,  $p > 0.8$ , ks2stat = 0.33 ;  
502 Mean duration of single opening =  $3.7 \pm 0.5$  ms in control sibling vs.  $5.1 \pm 1.5$  ms in mutant

503 larvae,  $p > 0.8$ ,  $ks2stat = 0.33$ ;  $n = 5$  cells in control siblings,  $n = 6$  cells in  
504 *scospondin*<sup>icm15/icm15</sup> larvae) (see also **Table S5**).

505 **(D)** Quantification of CSF-cN basic intrinsic electrophysiological properties (see also **Table**  
506 **S6**).

507 **(D1)** CSF-cN resting membrane potential remains unaffected in *scospondin*<sup>icm15/icm15</sup> mutants  
508 (Mean membrane potential =  $-45.5 \pm 2.0$  mV  $n = 4$  cells in control sibling vs.  $-44.5 \pm 1.8$   
509 mV,  $n = 4$  cells in mutant larvae, Two-sample Kolmogorov-Smirnov test,  $p > 0.9$ ,  $ks2stat =$   
510  $0.25$ ).

511 **(D2)** CSF-cN membrane resistance is not altered in *scospondin*<sup>icm15/icm15</sup> mutants (Mean  
512 membrane resistance =  $6.9 \pm 1.4$  G $\Omega$  in control sibling vs.  $10.4 \pm 2.1$  G $\Omega$  in mutant larvae,  
513 Two-sample Kolmogorov-Smirnov test,  $p > 0.08$ ,  $ks2stat = 0.67$ ;  $n = 6$  cells in control,  $n = 6$   
514 cells in *scospondin*<sup>icm15/icm15</sup> larvae).

515 **(D3)** CSF-cN membrane capacitance is comparable between *scospondin*<sup>icm15/icm15</sup> mutants  
516 and control larvae (Mean membrane capacitance =  $2.2 \pm 0.2$  pF  $n = 6$  cells in control sibling  
517 vs.  $2.1 \pm 0.2$  pF  $n = 6$  cells in *scospondin*<sup>icm15/icm15</sup> larvae, Two-sample Kolmogorov-Smirnov  
518 test,  $p > 0.8$ ,  $ks2stat = 0.33$ ).

519 **(E)** Discharge of action potential in CSF-cNs recorded in current clamp (CC)-mode in  
520 response to successive current steps (100 ms long-pulses from 2 pA to 10 pA in 2 pA  
521 increments).

522 Scale bars is 20  $\mu$ m in **(A)**. NP<sub>0</sub>, opening probability. Each data point represents one recording  
523 from one cell; plots use median as measure of central tendency (central mark on the box  
524 plot), the bottom and top edges of the box indicate the 25th and 75th percentiles. The  
525 whiskers extend to the most extreme data points without considering outliers, and the outlier  
526 is identified with a "+" symbol.

527

528 **Figure 5. The Reissner fiber is in close vicinity of the apical extension of CSF-cNs.**

529 **(A)** Z projection stack of a few optical sections imaged in the sagittal plane in living 3 dpf  
530 *Tg(cdh2:cdh2-GFP; pkd2l1:GAL4; UAS:tagRFP-CAAX)* larvae shows the height of the central  
531 canal *in vivo*.

532 **(B)** Z projection stack of a few optical sections imaged in the sagittal plane in 3 dpf  
533 *Tg(pkd2l1:GAL4; UAS:tagRFP-CAAX)* larvae after co-immunostaining for ZO-1 and tag-RFP  
534 used to measure the height of the central canal in fixed whole mount larva.

535 **(C)** Comparison of the size of the central canal between live and fixed whole mount 3 dpf  
536 larva reveals an artifact of fixation (see also **Table S7**).

537 **(C1)** Measurement of the height of the central canal shows that the central canal has a  
538 higher height after fixation (mean height =  $10.2 \pm 0.7$   $\mu$ m from 9 live larvae vs.  $14.1 \pm 1.2$   $\mu$ m



539 in 13 fixed whole mount larvae, Two-sample Kolmogorov-Smirnov test,  $p < 0.03$ ,  $ks2stat =$   
540  $0.62$ ).

541 **(C2)** Measurement of the width of the central canal reveals that the central canal is two times  
542 narrower after fixation (mean width =  $8.7 \pm 0.4 \mu\text{m}$  from 4 live larvae vs.  $3.5 \pm 0.3 \mu\text{m}$  in 10  
543 fixed whole mount larvae, Two-sample Kolmogorov-Smirnov test,  $p < 2 \times 10^{-3}$ ,  $ks2stat = 1$ ).

544 **(D)** Diagram representing the effect of 4 % PFA fixation on the shape of the central canal.

545 **(E)** Z projection stack of a few optical sections imaged after immunohistochemistry for tag-  
546 RFP and Reissner material in 3 dpf *Tg(pkd211:GAL4;UAS:tagRFP-CAAX)* larvae reveals that  
547 the apical extension of dorsolateral and ventral CSF-cNs are in close vicinity of the RF.

548 **(E1)** Dorsolateral CSF-cNs observed in coronal plane are found in the vicinity of the RF (top  
549 panel, In vicinity) or further away (bottom panel, Not in vicinity). Panel on the right represents  
550 a 3D reconstruction from the Z-stack on the left.

551 **(E2)** Same as **(E1)** imaged in the sagittal plane in order to visualize the proximity of ventral  
552 CSF-cNs with the RF.

553 **(F)** Dorsolateral CSF-cNs imaged in the coronal plane more often appear in close proximity  
554 to the RF than ventral CSF-cNs imaged in sagittal plane (mean fraction in vicinity =  $69.0 \pm$   
555  $2.9 \%$  from 281 dorsolateral CSF-cNs in 9 larvae vs.  $34.2 \pm 6.0 \%$  from 213 ventral CSF-cNs  
556 in 9 larvae; Two-sample Kolmogorov-Smirnov test,  $p < 5 \times 10^{-4}$ ;  $ks2stat = 0.89$ ).

557 Sale bar is  $20 \mu\text{m}$  in **(A)** and **(B)** and  $10 \mu\text{m}$  in **(E)** and **(F)**. cc: central canal; dl: dorsolateral  
558 CSF-cNs; H: Height; RF: the Reissner fiber; v: ventral CSF-cNs. Each data point represents  
559 one measure from one fish; plots use median as measure of central tendency (central mark  
560 on the box plot), the bottom and top edges of the box indicate the 25th and 75th percentiles.  
561 The whiskers extend to the most extreme data points without considering outliers, and the  
562 outlier is identified with a "+" symbol.

563

564 **Figure 6. The Reissner fiber can contact cilia and microvilli of CSF-cNs in the central**  
565 **canal.**

566 **(A, B)** Transmission electron microscopy (TEM) from spinal cord sectioned in the sagittal  
567 plane reveals motile cilium (black arrow) near or in contact with the Reissner fiber (RF) as  
568 well as microvilli from CSF-cNs (white arrowhead) near RF. Zoomed regions highlighted in  
569 different colors. **(A1)** The RF tends to be dorsally-located in the central canal and close to  
570 cilia (arrow), as well as microvilli (arrowhead) originating from identifiable dorsolateral CSF-  
571 cNs. **(A2), (A3), (A4)** Appositions onto the RF of motile cilia dotted of two central microtubules  
572 along the axoneme (arrow in **(A2), (A4)**) as well as microvilli from a ventral CSF-cN  
573 (arrowhead, **(A3)**).

574 **(B)** Zooms of lateral regions delineated in colored lines show that ventral CSF-cNs can  
575 apparently contact the RF via their microvilli (arrowheads), and their motile cilium (arrows).

576 **(C)** Z projection stack of 3 subsequent images acquired in the sagittal plane from serial block  
577 face scanning electron microscopy (SBF-SEM) highlighting apparent contacts between RF  
578 (blue ellipse) and dorsolateral CSF-cNs.

579 **(C1), (C2), (C3), (C4)** correspond to different position in the sagittal plane where multiple  
580 dorsolateral and ventral CSF-cNs can be observed along the rostro-caudal axis (see **Video**  
581 **S4**).

582 **(D)** 3D reconstruction after SBF-SEM imaging (60 sections of 40 nm Z steps and 7 nm  
583 thickness) shown in **(C)** and in **Video S4**.

584 Scale bars are 1  $\mu\text{m}$  in **(A1)** and **(A2)**, 200 nm in **(A3)** and **(A4)**, 2  $\mu\text{m}$  in **(B)**, 1  $\mu\text{m}$  in blue line  
585 delineated in **(B)**, 500 nm in orange line delineated in **(B)**, 200 nm in pink and green lines  
586 delineated in **(B)** and 5  $\mu\text{m}$  in **(C)**. C: caudal, cc: central canal, D: dorsal, dl: dorsolateral CSF-  
587 cNs, V: ventral: v: ventral CSF-cNs, R: rostral, RF: Reissner's fiber. Note that the background  
588 signal in the central canal obtained by TEM varies depending on the fixation methods: light  
589 background signal for PFA / TCA in **(A1), (A2)** and **(A3)** and dense background signal for  
590 PFA/Glutaraldehyde in **(A4), (B)** and **(C)**.

591

## 592 **STAR Methods**

593

### 594 **LEAD CONTACT AND MATERIALS AVAILABILITY**

595 Further information and requests for resources and reagents should be directed to and will be  
596 fulfilled by the Lead Contact, Claire Wyart ([claire.wyart@icm-institute.org](mailto:claire.wyart@icm-institute.org)).

597 This study did not generate new unique reagents.

598

599

### 600 **EXPERIMENTAL MODEL**

601 All procedures were performed on 3 days post fertilization (dpf) zebrafish larvae in accordance  
602 with the European Communities Council Directive (2010/63/EU) and French law (87/848) and  
603 approved by the Institut du Cerveau et de la Moelle épinière (ICM). All experiments were  
604 performed on *Danio rerio* embryos of AB, Tüpfel long fin (TL) and nacre background. Animals  
605 were raised at 28.5°C under a 14 / 10 light / dark cycle until the start of the experiment.

606

### 607 **METHOD DETAILS**

#### 608 **Immunohistochemistry**

609 3 dpf larva were euthanized in 0.2% Tricaine (MS-222, Sigma-Aldrich, Saint Louis, Memphis,  
610 USA) in system water and fixed 2 hours in phosphate-buffered saline (PBS) containing 4%  
611 paraformaldehyde (PFA) and 3% sucrose at 4°C. After three washes in PBS, skin from the

612 rostral trunk was partially removed and the yolk was removed. Samples were incubated  
613 overnight in a blocking buffer containing 0.5% Triton, 1% DMSO, 10% normal goat serum  
614 (NGS) in PBS. Primary antibodies were incubated 48 hours at 4°C in a blocking buffer  
615 containing 0.5% Triton, 1% DMSO and 1% NGS. All secondary antibodies were from  
616 Molecular Probes© (Invitrogen, Life Technologies, Carlsbad, California, USA) unless  
617 specified and used at 1:500 in blocking buffer, and incubated 2.5 hours at room temperature.  
618 The following primary antibodies were used for *in toto* immunohistochemistry: rabbit anti-  
619 Reissner fiber (1:200, polyclonal, custom-made) [8,57], rabbit anti-Pkd2l1 (1:200, custom-  
620 made, Sternberg et al., 2018), mouse anti-tagRFP (1:500, MA515257, Thermo Fischer  
621 Scientific, Waltham, Massachusetts, USA), mouse anti-ZO-1 (1:200, 339100, Invitrogen) and  
622 chicken anti-GFP (1:500, ab13970, Abcam, Cambridge, England). The following secondary  
623 antibodies were used at 1:500: Alexa Fluor-568 goat anti-rabbit IgG A11036, Alexa Fluor-488  
624 donkey anti-rabbit A21206, Alexa Fluor-647 goat anti-rabbit IgG A21244, Alexa Fluor-555  
625 goat anti-mouse IgG1 A21127, Alexa Fluor-568 goat anti-mouse A11004, Alexa Fluor-488  
626 goat anti chicken IgG A11039 (Thermo Fischer Scientific, Waltham, Massachusetts, USA).  
627 Zebrafish larvae were mounted dorsally (for imaging in coronal plane) or laterally (for imaging in  
628 sagittal plane) in Vectashield® Antifade Mounting Medium (Vector Laboratories, Inc.,  
629 Burlingame, California, USA) and imaged on an Olympus FV-1000 confocal microscope  
630 equipped with a 40X NA = 1.3 oil immersion objective (0.5 µm optical section and 0.35 µm  
631 optical section for images used to count contact with the RF (see Figure 5). Images were then  
632 processed using Fiji [58].

633

### 634 **Analysis of the proximity between CSF-cNs and the RF**

635 To image the Reissner fiber together with cerebrospinal fluid contacting neurons membranes  
636 in *Tg(pkcd2l1:GAL4;UAS:tagRFP-CAAX)*, zebrafish larvae were mounted dorsally or laterally  
637 in Vectashield Antifade Mounting Medium (Vector Laboratories, Inc., Burlingame, California,  
638 USA) and imaged on an SP8 X White Light Laser Leica inverted confocal microscope  
639 equipped with a 63X oil immersion objective (NA = 1.4). Dorsally mounted larvae allowed  
640 acquiring coronal planes ideally oriented to access the apical extensions of dorsolateral  
641 cerebrospinal fluid contacting neurons, while laterally mounted larvae allowed acquiring  
642 sagittal planes ideally oriented to access the apical extensions of ventral cerebrospinal fluid  
643 contacting neurons (see Figure 5). Z-stacks with a 250 nm step size (pixel size in the (x,y)  
644 plane: 144 nm) were acquired to estimate the proximity of the Reissner fiber with the apical  
645 extensions of cerebrospinal fluid contacting neurons in both cases. Four consecutive spinal  
646 cord regions were acquired along the rostro-caudal axis of the animals, respectively between  
647 segments 5 and 16. In order to correct for optical distortions taking place in the (x,y) and (z)  
648 planes, Z-stacks were first deconvolved for both the Reissner fiber fluorescence signals and

649 cerebrospinal fluid contacting neurons membrane signals, with the Huygens Professional  
650 version 19.04 (Scientific Volume Imaging, The Netherlands, <http://svi.nl>), using the CMLE  
651 algorithm, with a signal-to-noise ratio between 15 and 20, and up to 40 iterations. 3D stacks  
652 were then processed using Fiji [58]. Apposed or colocalized immunofluorescence signals  
653 from CSF-cNs and the Reissner fiber at least in a single plane after deconvolution were  
654 considered in close vicinity. The percentage of apical extensions in close vicinity with the  
655 Reissner fiber was then estimated for each fish. 3D views of the Reissner fiber and CSF-cNs  
656 from coronal and sagittal views (Figure 5E) were obtained using the 3D Viewer plugin on Fiji  
657 [59], after applying a median filter prior to 3D-segmentation.

658

## 659 **Morphological Analysis**

### 660 Analysis of the shape of the central canal

661 To measure the size of the central canal, we used 3 dpf zebrafish larvae where the apical  
662 junctions of the ependymal cells were visualized (with ZO-1 or Cadherin-2). For live imaging,  
663 we used the 3 dpf double transgenic *Tg(cdh2:cdh2-GFP; pkd2l1:GAL4;UAS:tagRFP-CAAX)*  
664 larva [56] in order to visualize the central canal and CSF-cNs *in vivo*. For images on fixed  
665 animals, we used antibodies against GFP (for *cdh2-GFP*), ZO-1 and tag-RFP. All images  
666 were acquired with an SP8 X White Light Laser Leica inverted confocal microscope equipped  
667 with a 40X water immersion objective (NA = 1). This image analysis was performed with Fiji.  
668 For the height of the canal, we acquired z-stacks of sagittal confocal sections spaced by 1  
669  $\mu\text{M}$ . A max z-projection of the slices encompassing the canal (typically 5 to 10 slices) was  
670 performed, and the height of the fluorescent signal was measured at 4 different fixed levels  
671 (spaced by 30  $\mu\text{M}$ ) and averaged. For the width of the canal, we acquired z-stacks of coronal  
672 confocal sections spaced by 1  $\mu\text{M}$ . We observed frequently that the canal was not straight in  
673 this axis, preventing us from quantifying the width on z-projection. Instead, we quantified at 4  
674 different fixed positions (spaced by 30  $\mu\text{M}$ ) the width of the fluorescent signal in the 5<sup>th</sup> slice  
675 above the floor plate signal (hence 4  $\mu\text{M}$  above the floor plate), and averaged it.

### 676 Measurement of the size of CSF-cN apical extensions

677 To measure the size of the apical extension *in vivo*, we used 3 dpf *Tg(cdh2:cdh2-GFP;*  
678 *pkd2l1:GAL4;UAS:tagRFP-CAAX)* zebrafish larvae where CSF-cNs were visualized through  
679 RFP signal. All images were acquired with an SP8 X White Light Laser Leica inverted  
680 confocal microscope equipped with a 40X water immersion objective (NA = 1). To measure  
681 the height of the apical extension, we acquired z-stacks of sagittal confocal sections spaced  
682 by 1  $\mu\text{M}$ . A max z-projection of the slices encompassing each CSF-cNs was performed and  
683 we drew polygons outlining their apical extension using the polygon tool in Fiji.sc as

684 described previously. An ellipse was fitted to the polygon and the height of the apical  
685 extension was measured as the height of the axis of the ellipse perpendicular to the floor  
686 plate as described previously [17,18].

687

### 688 **Calcium imaging**

689 All experiments were done on 3 dpf *Tg(pkd2l1:GCaMP5G); scospondin<sup>icm15/icm15</sup>* and  
690 *Tg(pkd2l1:GCaMP5G); cfap298<sup>tm304/tm304</sup>* and their respective siblings used as controls (*i.e.* wild  
691 type and heterozygous mutants from the same clutch).

### 692 **Active muscle contraction**

693 Unparalyzed 3 dpf larvae were pinned on their side through the notochord with 0.025 mm  
694 tungsten pins and bathed in artificial cerebrospinal fluid solution (aCSF, concentrations in mM:  
695 134 NaCl, 2.9 KCl, 1.2 MgCl<sub>2</sub>, 10 HEPES, 10 glucose and 2.1 CaCl<sub>2</sub>; 290 mOsm.kg<sup>-1</sup>, adjusted  
696 to pH 7.7–7.8 with NaOH). Active muscle contraction was induced by a 1 s-long pressure  
697 application of aCSF on the otic vesicle repeated 4-5 times with an inter trial interval of 1 minute.  
698 GCaMP5G fluorescence was excited by 490 nm illumination and monitored for 250 s at 4 Hz  
699 using an Examiner epifluorescence microscope (Zeiss, Göttingen, Germany) equipped with a 40  
700 X NA = 1.0 water-immersion objective and EMCCD camera Imagem X2 (Hamamatsu, Naka-ku,  
701 Japan). Images were acquired using Labview software (National Instruments, Austin, Texas,  
702 USA) for *cfap298<sup>tm304</sup>* mutants and using Hiris software (R&D Vision, Nogent-sur-Marne,  
703 France) for *scospondin<sup>icm15</sup>* larvae and reconstructed using Fiji.

### 704 **Passive spine bending**

705 3 dpf larvae were anesthetized in 0.02% Tricain (MS-222, SIGMA dorsally mounted in glass-  
706 bottom dishes (MatTek, Ashland, Massachusetts, USA) filled with 1.5% low-melting point  
707 agarose. Larvae were paralyzed by injecting 0.5 nl of 0.5 mM  $\alpha$ -Bungarotoxin in the musculature  
708 (Tocris Bioscience, Bristol, UK, [60] and placed in aCSF. After embedding, roughly half of the  
709 larval tail was freed unilaterally to provide access to a blunt 50  $\mu$ m diameter glass probe. Probe  
710 deflections were driven with a mechanotransducer device controlled through LabView software  
711 as done previously [16,17]. Calcium imaging was performed on a two-photon laser scanning  
712 microscope (2p-*vivo*, Intelligent Imaging Innovations, Inc., Denver, USA) using a 20X NA = 1.0  
713 objective. Lateral bending of the tail was induced by probe deflection and repeated 3 times  
714 every 14 to 17.5 s as done previously [16,17].

### 715 **Calcium imaging analysis**

716 Slow translational drifts of the image due to spine movement were corrected using image  
717 registration by taking as a reference image a max Z-projection of 3 consecutive images chosen  
718 when CSF-cNs are back to their position and bright after a muscle contraction. The regions of

719 interest (ROI) corresponding to each individual cell were drawn manually on the reference  
720 image. We identified CSF-cN calcium transients in response to spinal curvature by using either  
721 the motion artifact itself or a 200 ms-long flash of green light performed 16 s before the stimulus.  
722 The amplitude of the first CSF-cN calcium transients in response to passive and active spinal  
723 concave curvature were determined relative to baseline preceding the motion artifact with  
724 custom scripts written in MATLAB (MathWorks, Natick, Massachusetts, USA). For each  
725 contraction and each ROI,  $\Delta F / F$  was estimated as  $(F_{\text{GCaMP}} - F_{0\text{-GCaMP}}) / F_{0\text{-GCaMP}}$  with  $F_{\text{GCaMP}}$  is  
726 the fluorescence signal averaged over four time points (*i.e.* 1 s) around the peak after the first  
727 contraction and  $F_{0\text{-GCaMP}}$  is the baseline fluorescence average over 3 time points (*i.e.* 0.75 s)  
728 before each motion artifact. For each contraction, a new baseline was therefore estimated to  
729 prevent errors due to photobleaching or drifting during the recording. The percentage of  
730 responding cell was set as  $\Delta F / F > 1.96$  STD of the minimum value during the motion artefact.

731

### 732 ***In vivo* patch-clamp recording**

733 Whole-cell recordings were performed in aCSF on 3 dpf *Tg(pkd2l1:Gal4; UAS:mCherry)*  
734 carrying either the *scospondin*<sup>icm15</sup> or *cfap298*<sup>tm304</sup> mutation and their respective control siblings.  
735 Larva were pinned through the notochord with 0.025mm diameter tungsten pins. Skin and  
736 muscle from two to three segments around segment 10 were dissected out using a glass  
737 suction pipette. A MultiClamp 700B amplifier, a Digidata series 1440 A Digitizer, and pClamp  
738 10.3 software (Axon Instruments, Molecular Devices, San Jose, California, USA) were used for  
739 acquisition. Raw signals were acquired at 50 kHz and low-pass filtered at 10 kHz. Patch  
740 pipettes (1B150F-4, WPI) with a tip resistance of 5–8M $\Omega$  were filled with internal solution  
741 (concentrations in mM: K-gluconate 115, KCl 15, MgCl<sub>2</sub> 2, Mg-ATP 4, HEPES-free acid 10,  
742 EGTA 5 or 10, 290 mOsm/L, pH adjusted to 7.2 with KOH with Alexa 488 at 40  $\mu$ M final  
743 concentration). Holding potential was –85 mV, away from the calculated chloride reversal  
744 potential ( $E_{\text{Cl}} = -51$  mV). Analysis of electrophysiological data was performed offline using  
745 Clampex 10 software (Molecular Devices, San Jose, California, USA). Single channel events  
746 were identified using single-channel search in Clampfit (Molecular Devices, San Jose,  
747 California, USA), with a first level set at –15 pA from the baseline (level 0). Only events lasting  
748 longer than 1.2 ms were included for analysis. A 20 s window was used to identify channel  
749 events from a gap-free voltage-clamp recording from the first 1 to 3 min of recording. Passive  
750 properties were determined, in voltage-clamp mode at -85 mV, from the cell current response to  
751 a 10 mV hyperpolarization step (V step). Membrane resistance ( $R_m$ ) was estimated from the  
752 amplitude of the sustained current at the end of the 100 ms voltage step ( $R_m = V \text{ step} / I_m$ ).  
753 Membrane capacitance ( $C_m$ ) was estimated as the ratio between the cell decay time constant  
754 ( $t$ ), obtained from the exponential fit of the current decay and  $R_s$  ( $C_m \sim t / R_s$ ). Action potential

755 discharge was monitored in current-clamp mode in response to successive depolarizing current  
756 steps of 100 ms from – 2 pA to + 28 pA steps with a 2 pA increment after a fixed prepulse with -  
757 10 pA for 20 ms while holding the cell membrane potential at - 50 mV.

## 758 **Electron microscopy**

759 All the products used for electron microscopy were obtained from Electron Microscopy Science  
760 (EMS, distributor Euromedex, Souffleweyeshim, France).

### 761 Transmission electron microscopy

762 Samples were fixed in 0.5% glutaraldehyde 4% PFA in PBS, pH 7.4 for 2 hours at 4 °C. Some  
763 samples were treated with 1% trichloroacetic acid (TCA) within the fixative solution in order to  
764 better visualize preserve the Reissner fiber (as shown in Figure 6A1, 6A2, 6A3). Following three  
765 rinses with Na-cacodylate buffer 0.1 M pH = 7.4, sections were post-fixed with 1 % osmium  
766 tetroxide in the same buffer for 1 hour. Samples were dehydrated in a graded series of ethanol  
767 solutions (75, 80, 90 and 100 %, 5min each). Final dehydration was performed twice in 100 %  
768 acetone for 20 min. Infiltration with epoxy resin (Epon 812) was performed in 2 steps: overnight  
769 at + 4°C in a 1:1 mixture of resin and acetone in an airtight container and then, 2 hours at room  
770 temperature (RT) in pure resin. Finally, samples were placed in molds with fresh resin and  
771 cured at 56°C for 48 hours in a dry oven. Samples were sagittally cut in 0.5 µm semi-thin  
772 sections with an ultramicrotome EM UC7 (Leica, Wetzlar, Germany). Sections were stained with  
773 1% toluidine in borax buffer 0.1 M. Then ultra-thin sections (~ 70nm thick) were cut and  
774 collected on copper grid. Sections were then contrasted with Reynolds lead citrate for 7min[61].  
775 Observations were made with a HT 7700 electron microscope operating at 70kV (Hitachi, Ltd,  
776 Tokyo, Japan). Electron micrographs were taken with an integrated AMT XR41-B camera (2048  
777 x 2048 pixels, Advanced Microscopy Techniques Corp., Woburn, Massachusetts, USA).

### 778 Serial block face scanning electron microscopy

779 Samples (as shown in **Figure 6C** and **Video S3**) were fixed in 0.5% glutaraldehyde 4% PFA in  
780 PBS pH 7.4 for 2 hours at 4 °C. Following three rinses with Na-cacodylate buffer 0.1M pH = 7.4,  
781 sections were post-fixed for 30 minutes with 0.1% tannic acid in caco buffer as a mordant. After  
782 three rinses in caco buffer, samples were stained in freshly prepared (1% OsO<sub>4</sub>; 0,15%  
783 K<sub>4</sub>Fe(CN)<sub>6</sub>) solution for 1 hour. Samples underwent multiple incubation steps to increase the  
784 contrast: 20 min in 0.01% thiocarbohydrazide (TCH) at 60°C, 30 minutes in 1% OsO<sub>4</sub>, 60 min at  
785 4°C in 1% aqueous uranyl acetate and 30 min in a 0.66% lead nitrate in 30mM aspartic acid  
786 solution, pH = 5.5 at 60°C (Walton, 1979). Samples were dehydrated in graded ethanol at room  
787 temperature with a final dehydration in 100% acetone. Samples were then embedded in 50%  
788 resin / 50% acetone overnight at +4°C and dry at 60°C for 48 hours. Samples were sagittally  
789 sectioned in 7 nm-thick sections every 40 nm and imaged with a SBF-SEM. Sectioning and

790 scanning were performed with a TeneoVS electron microscope (FEI Company, Hillsboro,  
791 Oregon, USA) operating at 2kV-100pA- low vacuum (40Pa)-dwell time 1 $\mu$ s. Subsequently, 3D  
792 reconstruction was made using the Imaris software (Oxford instruments, Zurich, Switzerland).  
793

#### 794 **QUANTIFICATION AND STATISTICAL ANALYSIS**

795 All values are mean  $\pm$  standard error of the mean (S.E.M.) and represented as a box plot where  
796 the central mark on indicates the median, the bottom and top edges of the box indicate the 25th  
797 and 75th percentiles. The whiskers extend to the most extreme data points that are not  
798 considered outliers, outliers are identified with a “+” symbol.

#### 799 **Patch-clamp recording and Morphological study**

800 Statistical significance was determined using Two-sample Kolmogorov-Smirnov test (kstest2,  
801 MATLAB, MathWorks, Natick, Massachusetts, USA). A value of  $p \leq 0.05$  was considered  
802 significant.

#### 803 **Calcium imaging**

804 Stimulus artifact have been digitally removed in all figures. The data were analyzed with the  
805 repeated measure design. Values obtained from the response to active stimulation were  
806 analyzed using linear mixed-effects models (LMMs) with condition (control vs. mutant) and  
807 domain (dorsolateral vs. ventral CSF-cNs) as fixed effects and each independent fish (nested  
808 within clutch) as a random effect to account for the repeated measurements. Significance for  
809 the main effects of condition, domain and their interaction were then evaluated using ANOVA  
810 Type II Wald chi-square tests. The same analysis was conducted with the values obtained  
811 from response to passive stimulation, but for the condition factor only. All statistical analyses  
812 were conducted using R version 3.5.2 [62] and plots were generated with the ggplot2  
813 package. All LMMs were fitted using the function lmer in the lme4 package. ANOVA Type II  
814 Wald chi-square tests were performed using the function anova in the car package. Post hoc  
815 Tukey’s comparisons of the conditions within domains were made and plotted using the  
816 estimated marginal means from the emmeans package. To improve normality and  
817 homoscedasticity of residuals in the LMMs, response data were square root transformed on  
818 absolute values and then returned to their original sign prior to analysis. The level of  
819 statistical significance was set at  $p < 0.05$  for all tests (see Methods S1).  
820

#### 821 **DATA AND CODE AVAILABILITY**

822 The codes generated during this study are available at Github.  
823 [https://github.com/wyartlab/Orts-Del-Immagine\\_CurrentBio\\_2020](https://github.com/wyartlab/Orts-Del-Immagine_CurrentBio_2020)



824 The published article includes all statistical datasets generated during this study (see Methods  
825 S1).

826

## 827 **Supplemental Video**

828

829 **Video S1. Large calcium transients occur in CSF-cNs upon spinal curvature. Related**  
830 **to Figure 1.** Large calcium transients in dorsolateral and ventral CSF-cNs were induced by  
831 active muscle contractions after pressure-application of aCSF on the otic vesicle. Lateral  
832 view of the spinal cord in a 3 dpf *Tg(pkd211:GCaMP5G)* larva in which calcium imaging (raw  
833 data) was acquired at 4 Hz and is displayed at 30 Hz. Motion artifacts where cells are moving  
834 during contractions are excluded for subsequent analysis of  $\Delta F/F$ .

835

836 **Video S2. CSF-cN mechanosensitivity is disrupted in the *cfap* mutant with**  
837 **defective ciliary motility. Related to Figure 1.** Calcium transients in CSF-cNs  
838 induced by active muscle contractions after pressure-application of aCSF on the otic  
839 vesicle are massively reduced in *Tg(pkd211:GCaMP5G); cfap<sup>tm304/tm304</sup>* larvae. Lateral  
840 view of the spinal cord in which calcium imaging (raw data) was acquired at 4 Hz and is  
841 displayed here at 30 Hz. Motion artifacts where cells are moving during contractions  
842 are excluded for subsequent analysis of  $\Delta F/F$ .

843

844 **Video S3. The absence of the RF disrupts CSF-cN responses to spinal curvature.**  
845 **Related to Figure 3.** Calcium transients in CSF-cNs upon active muscle contractions after  
846 pressure-application of aCSF on the otic vesicle are massively reduced in  
847 *Tg(pkd211:GCaMP5G); scospondin<sup>icm15/icm15</sup>* larvae. Lateral view of the spinal cord in which  
848 calcium imaging (raw data) was acquired at 4 Hz and is displayed here at 30 Hz. Motion  
849 artifacts where cells are moving during contractions are excluded for subsequent analysis of  
850  $\Delta F/F$ .

851

852 **Video S4. The RF is in close proximity with cilia and CSF-cN apical extension in the**  
853 **central canal. Related to Figure 6.** SBF-SEM imaging (60 sections of 7nm-thickness and  
854 with 40 nm-Z step) is displayed at 15 Hz and show the organization of RF in the lumen of the  
855 central canal.

856

857

## 858 **Supplemental Tables**

859 **Table S1. Response of CSF-cNs to spine curvature in control sibling and in**  
860 ***cfap298<sup>tm304/tm304</sup>*. Related to Figure 1.**

861 **Table S2. Single channel opening properties of CSF-cNs measured in control and**  
862 ***cfap298<sup>tm304/tm304</sup>*. Related to Figure 2B and 2C.**

863 **Table S3. Intrinsic properties of CSF-cNs measured in control and *cfap298<sup>tm304/tm304</sup>*.**  
864 **Related to Figure 2D.**

865 **Table S4. Response of CSF-cNs to spine curvature in control sibling and in**  
866 ***scospondin<sup>icm15/icm15</sup>*. Related to Figure 3.**

867 **Table S5. Single channel opening properties of CSF-cNs measured in control and**  
868 ***scospondin<sup>icm15/icm15</sup>*. Related to Figure 4B and 4C.**

869 **Table S6. Intrinsic properties of CSF-cNs measured in control and**  
870 ***scospondin<sup>icm15/icm15</sup>*. Related to Figure 4D.**

871 **Table S7. Comparison of the size of the central canal and the CSF-cN apical extension**  
872 **in live and fixed tissue. Related to Figure 5.**

873

874

875 **Methods S1. Statistical analysis performed on the results from calcium imaging.**  
876 **Related to STAR Methods.** Description of statistical analysis performed on calcium imaging  
877 data using linear mixed models.

878

879

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881

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