

1 **Supplementary Legends**

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3 **Supplementary Figure 1 Fibronectin and interstitial fluid localization at the**
4 **neurectoderm-to-prechordal plate interface during zebrafish gastrulation.**

5 (a, b, c) Immunofluorescence confocal images of the neurectoderm (ecto)-to-
6 prechordal plate (ppl) interface (white dashed line) in a wild type (wt) embryos at 6
7 (a), 8 (b), and 9 (c) hpf showing Fibronectin staining (pseudo-colored with Fire LUT)
8 in maximum intensity projections of dorsal views (top panels) and sagittal sections
9 (middle panels); red dashed line outlines position of ppl leading edge cells; blue
10 dashed line indicates ecto-to-EVL interface, and yellow dashed line shows YSL
11 interface to ppl and ecto; bottom panels are sagittal sections of the ecto-to-ppl
12 interface stained for F-actin (phalloidin) to mark this interface; double-sided arrows
13 indicate animal (A) to vegetal (V) and dorsal (D) to ventral (V) embryo axes; asterisk
14 labels ppl leading edge cell; scale bar, 20 μm .

15 (d) Multiphoton live cell images showing interstitial fluid (IF) accumulation (dextran-
16 Alexa Fluor 647, left panel), F-actin localization (*Tg(actb1:lifect-GFP)*, middle
17 panel) and a combination of those different labels (right panel) at the ecto-to-ppl
18 interface (white dashed line) at 7 hpf; red arrows indicate extracellular cavities filled
19 with IF at the ecto-to-ppl and ecto-to-YSL interfaces; white arrows indicate ecto-to-
20 ppl cell-cell contacts devoid of IF accumulations; blue dashed line indicates ecto-to-
21 EVL interface, and yellow dashed line shows YSL interface to ppl and ecto; double-
22 sided arrows indicate AV and dorsal DV embryo axes; asterisk labels ppl leading
23 edge cell; scale bar, 20 μm .

24 (e) Multiphoton live cell image of *Tg(gsc:GFP)* embryo ($t = 120$ min, 8 hpf) with
25 pseudo-colored spots marking positions of nuclei within the axial mesendoderm
26 (green); dorsal view with double-sided arrows indicating AP to VP and left (L) to
27 right (R) embryo axes; color-code indicates mean total cell speeds of axial
28 mesendoderm cells moving to the animal pole after internalization (cyan, 0-2 and
29 yellow/magenta >2 $\mu\text{m}/\text{min}$); position of anterior (ppl) and posterior mesendoderm
30 marked; scale bar, 50 μm .

31 (f) Average instantaneous cell speeds in $\mu\text{m}/\text{min}$ of internalized axial mesendoderm
32 cells in wt embryos ($n=6$ embryos) plotted along the normalized distance along the
33 AV axis from anterior (0) to posterior (1); green dashed line marks position of
34 transition from anterior (ppl) to posterior axial mesendoderm, error bars, s.e.m.

35 **Supplementary Figure 2 Prechordal plate and neurectoderm cell movements and**
36 **neural plate positioning in wild type and MZ*oep* mutant embryos.**

37 (a) Fluorescent images of a wild type (wt) *Tg(gsc:GFP)* embryo showing
38 neurectoderm nuclei (H2A-BFP, cyan) and *gsc*-expressing GFP-labeled prechordal
39 plate (ppl) cells at a representative time point during gastrulation ($t = 65$ min, 7.1 hpf);
40 dorsal and sagittal (dorsal up) sections through the embryo (yellow tags in upper
41 panel mark sagittal section plane in lower panel); animal (AP) and vegetal pole (VP)
42 indicated by arrows; scale bar, 100 μm .

43 (b) Correlation of ppl cell movements in a wt embryo at a representative time point
44 during gastrulation ($t = 111.7$ min, 7.9 hpf); ppl cells are visualized as arrows in a 2D
45 plot and color-coded corresponding to their 3D correlation values between 1 (red,
46 maximum correlation) and -1 (blue, minimum correlation); every 3rd cell is plotted;
47 AP, animal pole; VP, vegetal pole; scale bar, 50 μm .

48 (c) Average degree of alignment of ppl cell movements in wt embryos ($n=5$ embryos)
49 plotted from 6 to 8 hpf (120 min); the order parameter corresponds to the degree of
50 alignment ranging from 0 (disordered movement) to 1 (highly ordered movement);
51 error bars, s.e.m.

52 (d) Mean instantaneous cell speed and directionality of ppl cells in a wt embryo ($n=5$
53 embryos) calculated from 6 to 8 hpf are plotted as bar graphs; error bars, s.e.m.

54 (e) Schematic illustration of global neurectoderm velocity measurements at the dorsal
55 side of the embryo; the neurectoderm was segmented into 100 x 200 μm sectors along
56 the AV axis (V_{AV}); sectors were positioned and color-coded relative to the ppl leading
57 edge (yellow dot), or fixed for cases without ppl cells; A1-3 and P1-3, sector anterior
58 and posterior of the ppl leading edge, respectively; mean V_{AV} velocities in the
59 different sectors were calculated for each time frame.

60 (f) Mean movement velocities ($\mu\text{m}/\text{min}$) along the AV axis (V_{AV}) of neurectoderm
61 cells in wt embryos ($n=6$ embryos) plotted from 6 to 8 hpf (120 min); colors of curves
62 correspond to respective sectors in (e); error bars, s.e.m.

63 (g) Schematic illustration of global 3D movement correlation analysis between
64 neurectoderm and ppl cells in defined sectors along the AV axis of the embryo. For
65 3D correlation calculations, neurectoderm cell velocities along the AV (V_{AV}), left-
66 right (LR) (V_{LR} ; see (e)) and dorsal-ventral (DV) axis (V_{DV} in sectors of 130x100 μm)
67 were measured; sectors were positioned and color-coded relative to the ppl leading

68 edge (yellow dot); A1-3 and P1-3, sector anterior and posterior of the ppl leading
69 edge (yellow dot), respectively.

70 (h) 3D movement correlation between leading edge ppl and adjacent neurectoderm
71 cells in defined sectors along the AV axis of wt embryos (n=6 embryos) plotted from
72 6 to 8 hpf (120 min); colors of curves correspond to respective sectors in (e) and (g);
73 error bars, s.e.m.

74 (i) Fluorescent images of a *MZoep;Tg(dharma:EGFP)* mutant embryo showing
75 neurectoderm nuclei (H2A-BFP, cyan) and Dharma (*dharma:EGFP*, green, marked
76 with asterisk) expression at the dorsal blastoderm margin at a representative time
77 point during gastrulation (t = 74.22 min, 7.2 hpf); dorsal and sagittal (dorsal up)
78 sections through the embryo (yellow tags in upper panel mark sagittal section plane in
79 lower panel); animal (AP) and vegetal pole (VP) indicated by arrows; scale bar, 100
80 μm .

81 (j) Mean movement velocities ($\mu\text{m}/\text{min}$) of neurectoderm cells along the AV axis
82 (V_{AV}) in *MZoep* mutant embryos (n=4 embryos) plotted over from 6 to 8 hpf (120
83 min); colors of curves correspond to sectors outlined in (e); error bars, s.e.m.

84 (k, l) Anterior neural anlage in wt (k) and *MZoep* mutant (l) embryos marked by
85 whole-mount *in situ* hybridization of *otx2* mRNA expression at consecutive stages of
86 gastrulation from 70% epiboly to bud stage (7 - 10hpf); posterior axial mesoderm was
87 detected by *no tail (ntl)* mRNA expression (arrows); animal pole (dorsal down),
88 dorsal (animal pole up) and lateral (dorsal right) views are shown; arrowheads mark
89 the anterior most edge of the neural plate; scale bars 200 μm .

90 (m) Quantitative analysis of neural plate position during gastrulation in *MZoep* versus
91 wt embryos. The angle ($^{\circ}$) between the vegetal pole and the anterior border of the *otx2*
92 expression domain was measured for embryos at different stages during gastrulation
93 (k, l) and plotted as box-whisker graphs; n, embryos analyzed from 4 independent
94 experiments; student's t-test (*P* value indicated) for all graphs comparing same stages;
95 ***, *P* <0.001, (ns) non significant, *P* >0.05; n (wt, bud) = 36, n (wt, 90%) = 36, n
96 (wt, 80%) = 34, n (wt, 70%) = 29, n (*MZoep*, bud; *P* <0.0001) = 24, n (*MZoep*, 90%;
97 *P* <0.0001) = 36, n (*MZoep*, 80%; *P* <0.0001) = 20, n (*MZoep*, 70%; *P* <0.358) = 18;
98 box plot centre, median; red dot, mean; upper whisker, maximum; lower whisker,
99 minimum.

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102 **Supplementary Figure 3 Prechordal plate cell movements and neural plate**
103 **positioning in *cyc* and *slb* morphant embryos.**

104 (a, e) Fluorescence images of a Tg(*gsc*:GFP) *cyclops* (*cyc*) (a) and *silberblick* (*slb*)
105 morphant (e) embryo showing H2A-BFP expression (cyan) in all nuclei and GFP
106 (green, white outline) expression in *gsc*-expressing prechordal plate (ppl) cells at a
107 representative time point during gastrulation (a; t = 71.40 min, 7.2 hpf and e; t = 74.22
108 min, 7.2 hpf); dorsal and sagittal (dorsal up) sections through the embryo (yellow tags
109 in upper panel mark sagittal section plane in lower panel); animal (AP) and vegetal
110 pole (VP) indicated by arrows; red line in (e) indicates widened ppl internalization
111 zone; scale bar, 100 μ m.

112 (b, f) Number of internalized ppl cells in Tg(*gsc*:GFP) *cyc* (b; blue curve, n = 3
113 embryos) and *slb* (f; blue curve, n = 3 embryos) morphant embryos (blue curve, n = 3
114 embryos) versus wt (green curve, n = 6 embryos) embryos plotted between 6 and 8
115 hpf (120 min); error bars, s.e.m.

116 (c, g) Average degree of alignment of ppl cell movements in *cyc* (c) magenta
117 curve/squares, n = 3 embryos) and *slb* morphant (g; magenta curve/dots, n=3
118 embryos) versus wt (green curve/dots, see Supplementary Fig. 2c) embryos plotted
119 from 6 to 8/8.3 hpf (120/140 min); the order parameter corresponds to the degree of
120 alignment ranging from 0 (disordered movement) to 1 (highly ordered movement);
121 error bars, s.e.m.

122 (d, h) Mean instantaneous ppl cell speed and directionality of *cyc* [d; gray bar graph, n
123 = 4 embryos; $P(\text{speed}) = 0.0061$, $P(\text{dir}) = 0.033$] and *slb* morphant [gray bar graphs, n
124 = 3 embryos, $P(\text{speed}) = 0.0025$, $P(\text{dir}) < 0.0001$] versus wt (white bar graph, see
125 Supplementary Fig. 2d) embryos plotted as bar graphs; error bars, s.e.m.; student's t-
126 test for all graphs; ***, p < 0.001, **, p < 0.01; *, p < 0.05.

127 (i, j) Anterior neural plate anlage in *cyc* and *slb* morphant embryos marked by whole-
128 mount in situ hybridization of *otx2* mRNA expression at consecutive stages of
129 gastrulation from 70% epiboly to bud stage (7 - 10hpf); posterior axial mesoderm was
130 detected by *no tail* (*ntl*) mRNA expression (arrows); animal pole (dorsal down),
131 dorsal (animal pole up) and lateral (dorsal right) views are shown; arrowheads mark
132 the most anterior edge of the neural plate; scale bar 200 μ m.

133 (k) Quantitative analysis of neural plate position in *cyc* and *slb* morphant versus wt
134 embryos during gastrulation. The angle ($^{\circ}$) between the vegetal pole and the anterior
135 border of the *otx2* expression domain was measured for embryos at different stages

136 during gastrulation (i, j) and plotted as box-whisker graphs; n, embryos analyzed from
137 4 independent experiments; student's t-test (P value indicated) for all graphs
138 comparing same stages; ***, $P < 0.001$, ns (non significant), $P > 0.05$; n (wt, bud) =
139 36, n (wt, 90%) = 36, n (wt, 80%) = 34, n (wt, 70%) = 29, n (slb, bud; $P < 0.0001$) =
140 23, n (slb, 90%; $P < 0.0001$) = 17, n (slb, 80%; $P < 0.0001$) = 20, n (slb, 70%; $P =$
141 0.134) = 16, n (cyc, bud; $P < 0.0001$) = 40, n (cyc, 90%; $P < 0.0001$) = 39, n (cyc,
142 80%; $P < 0.0001$) = 32, n (cyc, 70%; $P = 0.851$) = 27; red dots mark mean values; box
143 plot centre, median; red dot, mean; upper whisker, maximum; lower whisker,
144 minimum.
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148 **Supplementary Figure 4 Prechordal plate cell movements and neural plate**
149 **positioning in wild type embryos overexpressing CA-Mypt within the yolk**
150 **syncytial layer.**

151 (a) Schematic illustration of *CA-Mypt* and *H2A-mCherry* mRNA injection into the
152 yolk syncytial layer (YSL) of an embryo at high stage (3.3 hpf).

153 (b) Confocal images of the enveloping layer (EVL)/YSL epiboly progression in F-
154 actin labeled *Tg(actb1:GFP-UtrCH)* wild type (wt) control (lower panel) and embryos
155 injected with constitutively active myosin II phosphatase mRNA into the YSL (*CA-*
156 *Mypt*, upper panel) at 8 hpf; both embryos were co-injected with *H2A-mCherry*
157 mRNA into the YSL to mark YSL nuclei.

158 (c) Quantification of the average advancement ($\mu\text{m}/\text{min}$) of the EVL margin of wt
159 control and *CA-Mypt* injected embryos between 7 and 9 hpf; student's t-test; ***, P
160 <0.001 ; $n = 4$ embryos; error bars, s.e.m.

161 (d) Fluorescence images of a *Tg(gsc:GFP)* embryo overexpressing *CA-Mypt* and
162 *H2A-mCherry* (magenta, arrows) within the YSL, also showing *H2A-BFP* expression
163 within all nuclei and *GFP*-expression in *gsc*-expressing prechordal plate (ppl)
164 progenitors (green, white outline) at a representative time point during gastrulation (t
165 $= 75$ min, 6.25 hpf); dorsal and sagittal (dorsal up) sections through the embryo
166 (yellow tags in upper panel mark sagittal section plane in lower panel); animal (AP)
167 and vegetal pole (VP) indicated by arrows; scale bar, $100\mu\text{m}$.

168 (e) Number of internalized ppl cells in *Tg(gsc:GFP)* embryos overexpressing *CA-*
169 *Mypt* within the YSL (blue curve, $n = 4$ embryos) versus wt embryos (green curve)
170 plotted from 6 to 8 hpf (120 min); error bars, s.e.m.

171 (f) Directional correlation of ppl cell movements in a wt embryo overexpressing *CA-*
172 *Mypt* within the YSL at a representative time point during gastrulation ($t = 77.40$ min,
173 6.8 hpf); ppl cells are visualized as arrows in a 2D plot and color-coded according to
174 their 3D correlation values between 1 (red, maximum correlation) and -1 (blue,
175 minimum correlation); every 3rd cell is plotted; AP, animal pole; VP, vegetal pole;
176 scale bar, $50\mu\text{m}$.

177 (g) Average degree of alignment of ppl movements in embryos overexpressing *CA-*
178 *Mypt* within the YSL (magenta curve/squares, $n = 3$ embryos) versus wt embryos
179 (green curve/dots, see Supplementary Fig. 1c) plotted from 6 to 8 hpf (120 min); the
180 order parameter corresponds to the degree of alignment ranging from 0 (disordered
181 movement) to 1 (highly ordered movement); error bars, s.e.m.

182 (h) Mean ppl cell instantaneous speed and directionality in CA-Mypt injected [gray
183 bar graphs, $n = 4$ embryos; $P(\text{speed}) = 0.323$, $P(\text{dir}) = 0.702$] versus wt (white bar
184 graphs, see Supplementary Fig. 2d) embryos plotted over 120min (6 to 8 hpf) as bar
185 graphs; error bars, s.e.m.; student's t-test for all graphs; ns (not-significant), $P > 0.05$.
186 (i) Anterior neural anlage in embryos overexpressing CA-Mypt within the YSL
187 marked by whole-mount *in situ* hybridization of *otx2* mRNA expression at
188 consecutive stages of gastrulation from 70% epiboly to bud stage (7 - 10hpf);
189 posterior axial mesoderm was detected by *no tail (ntl)* mRNA expression (yellow
190 arrows); animal pole (dorsal down), dorsal (animal pole up) and lateral (dorsal right)
191 views are shown; red arrowhead marks the most anterior edge of the neural plate; 200
192 μm .
193 (j) Quantitative analysis of neural plate position during gastrulation in embryos
194 overexpressing CA-Mypt in the YSL versus wt embryos. The angle ($^{\circ}$) between the
195 vegetal pole and the anterior border of the *otx2* expression domain was measured for
196 embryos at different stages during gastrulation (i) and plotted as box-whisker graphs;
197 n , embryos analyzed from 4 independent experiments; student's t-test (P value
198 indicated) for all graphs comparing same stages; **, $P < 0.01$; *, $P < 0.05$, (ns) non
199 significant, $P > 0.05$; n (wt, bud) = 36, n (wt, 90%) = 36, n (wt, 70%) = 29, n (CA-
200 Mypt, bud; $P = 0.49$) = 16, n (CA-Mypt, 90%; $P = 0.0259$) = 22, n (CA-Mypt, 80%;
201 $P = 0.0016$) = 34, n (CA-Mypt, 70%; $P = 0.0016$) = 12; red dots mark mean values;
202 box plot centre, median; red dot, mean; upper whisker, maximum; lower whisker,
203 minimum.
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206 **Supplementary Figure 5 Movement of transplanted prechordal plate cells in**
207 **MZoep mutant embryos.**

208 (a, h) Schematic illustration of a *MZoep;Tg(dharma:EGFP)* (a) and a
209 *MZoep;Tg(dharma:EGFP)* mutant embryo that was injected with *CA-Mypt* mRNA
210 into the YSL at high stage (h; 3.3 hpf) transplanted with prechordal plate (ppl) cells
211 (green) into the dorsal side at 60% epiboly (6 hpf); asterisk marks position of dorsal
212 marker Dharma; orange arrows indicate reduced vegetal-directed movement of EVL
213 margin (h); AP, animal pole; VP, vegetal pole; L, left; R, right.

214 (b, i) Bright-field/fluorescence image of a *MZoep;Tg(dharma:EGFP)* mutant embryo
215 at 90% epiboly (9 hpf) and a *MZoep;Tg(dharma:EGFP)* mutant embryo at 80 %
216 epiboly (8 hpf) that overexpresses *CA-Mypt* and the nuclei marker H2A-mCherry
217 (red) within the YSL containing transplanted GFP-labeled ppl cells from *Tg(gsc:GFP)*
218 donor (b) and *Tg(gsc:GFP-CAAX)* donor (i) embryos; ppl cell nuclei are marked by
219 H2A-mCherry (i; red, co-localizes with green ppl cells); dashed white line indicates
220 position of transplanted ppl progenitors; arrowhead points at anterior edge of ppl
221 cells; asterisk marks *dharma:EGFP* signal at the dorsal side of the embryo; dorsal
222 (animal pole up, top panel) and lateral (dorsal right, bottom panel) views; scale bar,
223 200 μm .

224 (c, j) Fluorescence images of representative time points during gastrulation (c; t =
225 47.19 min, 6.8 hpf and j; t = 56.39 min, 6.9 hpf) showing a
226 *MZoep;Tg(dharma:EGFP)* (c) and a *MZoep;Tg(dharma:EGFP)* mutant embryo
227 which overexpresses *CA-Mypt* and the nuclei marker H2A-mCherry (magenta) within
228 the YSL (j) containing transplanted *gsc*-expressing GFP-labeled ppl cells (white
229 outline) from *Tg(gsc:GFP)* donor (c) and *Tg(gsc:GFP-CAAX)* donor (j) embryos; all
230 nuclei are marked by H2A-mCherry (c; magenta) and H2A-BFP (j; cyan) expression,
231 and the dorsal side of the embryos is marked by *dharma:EGFP* expression (green,
232 asterisk); dorsal and sagittal (dorsal up) sections through the embryo (yellow tags in
233 upper panel mark sagittal section plane in lower panel); animal pole (AP) and vegetal
234 pole (VP) indicated by arrows; scale bar, 100 μm .

235 (d) Protrusion orientation of ppl cells transplanted into *MZoep* mutants: top panel,
236 fluorescence image of ppl cells with cytoplasm in green (*gsc:GFP*) and nuclei in cyan
237 (H2A-BFP); animal pole up; scale bar, 20 μm . Bottom panel, polar plot or protrusion
238 orientation of transplanted ppl cells (n = 48 cells from 2 embryos) with 0 $^{\circ}$ = animal
239 pole, 180 $^{\circ}$ = vegetal pole.

240 (e) Number of ppl cells transplanted into *MZoep* mutant embryos (n=3 embryos)
241 plotted from 6 to 8 hpf (120 min); error bars, s.e.m.

242 (f) Directional correlation of transplanted ppl cell movements in a *MZoep* mutant
243 embryo at a representative time point during gastrulation ($t = 83.5$ min, 7.4 hpf); ppl
244 cells are visualized as arrows in a 2D plot and color-coded according to their 3D
245 correlation values between 1 (red, maximum correlation) and -1 (blue, minimum
246 correlation); every 5th cell is plotted; AP, animal pole; VP, vegetal pole; scale bar, 50
247 μm .

248 (g) Average degree of alignment of transplanted ppl cell movements in *MZoep*
249 mutant embryos (magenta curve/squares, n=3 embryos) versus endogenous ppl cell
250 movements in wt embryos (green curve/dots, see Supplementary Fig. 2c) from 6 to 8
251 hpf (120 min); the order parameter corresponds to the degree of alignment ranging
252 from 0 (disordered movement) to 1 (highly ordered movement); error bars, s.e.m.

253 (k) Mean neurectoderm cell velocities along the animal-vegetal (AV) axis (V_{AV})
254 (measurement area indicated by black box in Supplementary Fig. 2e) in *MZoep*
255 mutant embryos (red curve, n=3 embryos) and *MZoep* mutant embryos
256 overexpressing CA-Mypt in the YSL (black curve, n=3 embryos); error bars, s.e.m.
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259 **Supplementary Figure 6 Effect of external friction on one-dimensional**
260 **neurectoderm flow profile.**

261 (a) For capturing the flow profile induced solely by prechordal plate (ppl) cells,
262 *MZoep* mutants devoid of ppl cells were used to measure unperturbed epiboly
263 movements, and those movements were subtracted from the overall neurectoderm
264 flow field in wt embryos. This allowed decomposing the neurectoderm flow field and
265 obtaining the ppl-induced movement alterations only. In the 2D description,
266 neurectoderm flows exclusively within the experimental image plane (red square)
267 were taken into account.

268 (b) Theoretical 1D flow profile when the external friction coefficient ξ_0 between
269 neurectoderm and tissues other than the ppl, such as the yolk cell and/or EVL, is
270 varied. In case the external friction coefficient is increased, the range of flow
271 triggered by ppl cells is decreased (blue: $\xi_0/\bar{\eta} = 10^{-11}\mu\text{m}^{-2}$, orange: $\xi_0/\bar{\eta} =$
272 $10^{-5}\mu\text{m}^{-2}$, green: $\xi_0/\bar{\eta} = 10^{-4}\mu\text{m}^{-2}$, all curves: $f/\bar{\eta} = -4.2 \cdot 10^{-5}\mu\text{m}^{-1} \cdot \text{min}^{-1}$).

273 (c) Experimental velocities in wt embryos (blue dots) compared to theoretical flow
274 profiles for the parameter settings as used in Fig. 5 (red dotted line, $f/\bar{\eta} = -4.2 \cdot$
275 $10^{-5}\mu\text{m}^{-1} \cdot \text{min}^{-1}$, $\xi_0/\bar{\eta} = 1.6 \cdot 10^{-6}\mu\text{m}^{-2}$), and for zero external friction (green
276 line, $f/\bar{\eta} = -3.5 \cdot 10^{-5}\mu\text{m}^{-1} \cdot \text{min}^{-1}$, $\xi_0/\bar{\eta} = 0$). The experimental velocity profile
277 in wt embryos is well explained by either a small ($\xi_0 < \bar{\eta}/L_E^2$) or vanishing ($\xi_0 = 0$)
278 external friction coefficient.

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281 **Supplementary Figure 7 Prechordal plate cell movements and neural plate**
282 **positioning in *e-cadherin* morphant embryos.**

283 (a) Brightfield/fluorescence image of a wild type (wt) *Tg(gsc:GFP)* (top panel) and *e-*
284 *cadherin* (*e-cad*) morphant embryo (bottom panel) with *gsc*-expressing GFP-labeled
285 prechordal plate progenitor (ppl) cells (green, white outline) at 80% epiboly; dorsal
286 views, animal pole up; the increasing distance between the margins of the enveloping
287 layer (EVL; red dashed line) and deep cell/neurectoderm (blue dashed line) shows
288 (neur)ectoderm epiboly delay in *e-cad* morphant embryos; scale bar, 200 μm .

289 (b) Fluorescence images of a *Tg(gsc:GFP)* *e-cad* morphant embryo showing H2A-
290 mCherry (magenta) expression in all nuclei and GFP (green) expression in ppl cells
291 (white outline) at a representative time point during gastrulation ($t = 80.30$ min, 7.3
292 hpf); dorsal and sagittal (dorsal up) sections through the embryo (yellow tags in upper
293 panel mark sagittal section plane in lower panel); red and blue dashed lines as in (A);
294 animal pole (AP) and vegetal pole (VP) indicated by arrows; scale bar, 100 μm .

295 (c) Number of internalized ppl cells in *Tg(gsc:GFP)* *e-cad* morphant (blue curve, $n=4$
296 embryos) versus wt (green curve) embryos plotted from 6 to 8 hpf (120 min); error
297 bars, s.e.m.

298 (d) Correlation of ppl cell movements in a *e-cad* morphant embryo at a representative
299 time point during gastrulation ($t = 80$ min, 7.3 hpf); ppl cells are visualized as arrows
300 in a 2D plot and color-coded according to their 3D correlation values between 1 (red,
301 maximum correlation) and -1 (blue, minimum correlation); every 3rd cell is plotted;
302 AP, animal pole; VP, vegetal pole; scale bar, 50 μm .

303 (e) Average degree of alignment of ppl movements in *e-cad* morphant (magenta
304 curve/squares, $n=3$) versus wt (green curve/dots, see Supplementary Fig. 2c) embryos
305 from 6 to 8 hpf (120 min); the order parameter corresponds to the degree of
306 alignment, ranging from 0 (disordered movement) to 1 (highly ordered movement);
307 error bars, s.e.m.

308 (f) Mean ppl instantaneous speed and directionality in *e-cad* morphant [gray bar
309 graphs, $n=4$ embryos; $P(\text{speed}) = 0.0362$; $P(\text{dir}) = 0.222$] versus wt (white bar graphs,
310 see Supplementary Fig. 2d) embryos plotted as bar graphs; error bars, s.e.m; student's
311 t-test for all graphs; *, $P < 0.05$; (ns) non significant, $P > 0.05$.

312 (g) Model of friction generation under E-cadherin reduced conditions (compare with
313 wt in Fig. 6f) in *e-cadherin* morphant embryo leads to decreased friction at the ppl-to-
314 neurectoderm (ecto) interface and to non-graded velocities within the ppl (left panel;

315 F_f , friction force; orange dashes indicate remaining cadherin); reduced E-cadherin-
316 mediated adhesion between ppl and neurectoderm leads to loss of frictional drag and
317 vegetal-directed movements (red arrow) of neurectoderm cells (right panel; yellow
318 arrows indicate ppl movement); double-sided arrows indicate embryonic axes, animal
319 (A) to vegetal (V), dorsal (D) to ventral (V).

320 (h) 2D tissue flow map indicating velocities ($\mu\text{m}/\text{min}$) of neurectoderm (ectoderm)
321 cell movements along the AV (V_{AP}) and left-right (LR) (V_{LR}) axis at the dorsal side of
322 a *MZoep* embryo overexpressing CA-Mypt within the YSL and transplanted with *e-*
323 *cad* morphant ppl cells ($t = 41.40$ min, 6.7 hpf) at a representative time point; average
324 velocity vector for each defined area is indicated and color-coded ranging from 0
325 (blue) to 2 (red) $\mu\text{m}/\text{min}$; positions of all/leading edge ppl cells are marked by
326 black/green dots; black boxed area was used for mean velocity measurements in (i);
327 scale bar, 100 μm .

328 (i) Mean movement velocities ($\mu\text{m}/\text{min}$) along the AV axis (V_{AV}) of ppl leading edge
329 progenitor cells (green curve, left y-axis) and neurectoderm (ecto) cells positioned
330 above the ppl leading edge (black boxed area in h; red curve, right y-axis) in *MZoep*
331 embryos overexpressing CA-Mypt within the YSL and transplanted with *e-cad*
332 morphant ppl cells ($n=4$ embryos) plotted from 6 to 8 hpf; vertical dashed line
333 indicates start of vegetal-directed movements of ppl cells; error bars, s.e.m.

334 (j) 3D directional correlation values between leading edge ppl and adjacent
335 neurectoderm (ecto) cells in *MZoep* embryo overexpressing CA-Mypt within the YSL
336 and transplanted with *e-cad* morphant ppl cells ($t = 41.40$ min, 6.7 hpf) at a
337 representative time point during gastrulation; degree of correlation is color-coded
338 ranging from 1 (red, highest) to -1 (white, lowest); average neurectoderm velocities for
339 each defined area are marked; black boxed area was used for local correlation
340 measurements in (k); scale bar, 100 μm .

341 (k) 3D directional correlation values between leading edge ppl and adjacent
342 neurectoderm (ecto) cells (black boxed area in j) in *MZoep* embryos overexpressing
343 CA-Mypt within the YSL and transplanted with *e-cad* morphant ppl cells ($n=4$
344 embryos) plotted from 6 to 8 hpf; error bars, s.e.m.

345 (l) Anterior neural anlage in *e-cad* morphant embryos marked by whole-mount *in situ*
346 hybridization of *otx2* mRNA expression at consecutive stage during gastrulation from
347 70% to 90% epiboly (7 - 9hpf); posterior axial mesoderm was detected by *no tail* (*ntl*)
348 mRNA expression (yellow arrows), animal pole (dorsal down), dorsal (animal pole

349 up) and lateral (dorsal right) views are shown; red arrowheads mark the most anterior
350 edge of the neural plate; scale bar, 200 μm .

351 (m) Quantitative analysis of neural plate position during gastrulation in *e-cad*
352 morphant versus wt embryos; the angle ($^{\circ}$) between the vegetal pole and the anterior
353 border of the *otx2* expression domain was measured for embryos at different stages (l)
354 and plotted as box-whisker graphs; n, embryos analyzed from 4 independent
355 experiments; student's t-test (*P* value indicated) for all graphs comparing same stages;
356 ***, *P* <0.001, (ns) non significant, *P* >0.05; n (wt, 90%) = 36, n (wt, 80%) = 34, n
357 (wt, 70%) = 29, n (*e-cad*, 90%; *P* <0.0001) = 30, n (*e-cad*, 80%; *P* <0.0001) = 37, n
358 (*e-cad*, 70%; *P* = 0.00036) = 41; box plot centre, median; red dot, mean; upper
359 whisker, maximum; lower whisker, minimum.

360

361

362 **Supplementary Figure 8 Alterations in ectoderm movements upon application of**
363 **E-cadherin mediated friction *ex vivo* and shear-strain-induced neurectoderm**
364 **tissue deformation *in vivo*.**

365 (a) Bright-field/fluorescence image showing setup of magnetic polystyrene beads (20
366 μm diameter) and fluorescent reference beads (red, 4 μm diameter) attached to a glass
367 plate used to apply friction onto ectoderm cells; dashed line outlines shape of
368 polystyrene cluster; scale bars, 100 μm and 20 μm for magnified area.

369 (b) Western Blot analysis showing detection of E-cadherin ectodomain (80 kDa)
370 eluted from magnetic polystyrene beads coupled to E-cadherin-Fc Chimera (E-Fc) or
371 uncoated control beads; molecular weight markers, 100 and 200 kDa.

372 (c) Section of maximum projection confocal image (see Fig. 7a; $t = 19.33$ min)
373 showing top plate with fluorescent reference beads and selected beads are highlighted
374 (red arrows in xy and yz cross-section); cross-section (yz; red rectangle) shows the
375 position of E-Fc-coated beads (outlined in orange) at the ectoderm cell interface
376 (yellow dashed line); direction of beads movement (top plate; - y; velocity ~ 1.5
377 $\mu\text{m}/\text{min}$) is indicated; scale bars, 100 μm in xy and 20 μm in yz.

378 (d) Shear strain-induced neurectoderm tissue deformations of wild type (wt, upper
379 panels; $n=3$ embryos) and *MZoep* (lower panels; $n=3$ embryos) embryos plotted as
380 time-averaged strain values for each domain (50 x 50 μm); average shear strain rate is
381 color-coded according to amount of plane distortion [minimum green (0) to maximum
382 red ($5 \times 10^{-3} \text{s}^{-1}$)]; tissue flows of neurectoderm are indicated as time-averaged
383 velocities; dashed line indicates ppl and black dot marks ppl leading edge as reference
384 point in wt and *MZoep*; rectangle outlines area used for defining sectors along the
385 animal-vegetal (AV) axis in (e).

386 (e) Mean shear strain rates of neurectoderm tissue of wt (upper panels; $n=3$ embryos)
387 and *MZoep* (lower panels; $n=3$ embryos) in defined sectors (100 x 200 μm) are
388 plotted along the AV axis over time of gastrulation (plotted from 6.3 to 7.3 hpf in 10
389 min intervals); sectors were positioned and color-coded relative to the ppl leading
390 edge (anterior A1-2 and posterior P1-2 of the ppl leading edge; for detailed
391 description refer to Supplementary Fig. 1e); amount of plane distortion [minimum
392 green (0) to maximum red ($10 \times 10^{-3} \text{s}^{-1}$)] is plotted along the y-axis;

393 (f) Neurectoderm tissue strain rate maps derived by subtraction of time-averaged
394 shear strain values of wt from *MZoep* embryos ($n=3$ embryos); color-code as in (f);

395 tissue flows of neurectoderm are indicated as time-averaged velocities; dashed line
396 indicates ppl and black dot marks ppl leading edge as reference point.

397 (g) Illustration of shear strain tissue deformation in the neurectoderm; arrows indicate
398 direction of plane distortion of a tissue domain along the AV and left-right (LR) axis
399 dependent on the direction and magnitude of neurectoderm movements; shear strain-
400 induced domain angle of plane distortion can shrink (positive value) or enlarge
401 (negative value).

402

403

404 **Supplementary Video Legends**

405

406 **Supplementary Video 1 Live cell imaging of cell movements in wt embryo.**

407 Multiphoton time-lapse imaging of a wild type (wt) *Tg(gsc:GFP)* embryo with *gsc*-
408 expressing GFP-labeled prechordal plate progenitor (ppl) cells (green) and
409 neurectoderm cells at the dorsal side of the embryo from 6 to 8 hpf (123 min); all
410 nuclei were labeled with histone H2A-BFP; animal/vegetal pole, up/down.

411

412 **Supplementary Video 2 Live cell imaging of cell movements in MZ*oep* mutant**
413 **embryo.** Multiphoton time-lapse imaging of a *MZ*oep*;Tg(dharma:EGFP)* mutant

414 embryo (Dharma:EGFP signal green) showing neurectoderm cells at the dorsal side of
415 the embryo from 6 to 8.1 hpf (129 min); all nuclei were labeled with histone H2A-
416 BFP; animal/vegetal pole, up/down.

417

418 **Supplementary Video 3 2D velocities of neurectoderm cells in wt embryo.** Tissue

419 flow map indicating velocity vectors of neurectoderm cell movements along the
420 animal-vegetal (AV) (V_{AV}) and left-right (LR) (V_{LR}) axis at the dorsal side of a wild
421 type (wt) embryo between 6 to 8 hpf (117 min); average velocity vector for each
422 defined area is indicated and color-coded ranging from 0 (blue) to 2 (red) $\mu\text{m}/\text{min}$;
423 position of all/leading edge prechordal plate (ppl) cells are indicated as black/green
424 dots; xy-axes in μm ; time in mins; animal/vegetal pole, up/down.

425

426 **Supplementary Video 4 3D correlation of neurectoderm and prechordal plate**
427 **(pp) cell movements in wt embryo.** Movement correlation between neurectoderm

428 and underlying (ppl) cells at the dorsal side of a wild type (wt) embryo between 6 to 8
429 hpf (118 min); degree of correlation is color-coded ranging from 1 (red, highest
430 correlation) to -1 (white, lowest correlation); average neurectoderm movement
431 velocities and direction for each defined area are indicated by arrows; position of
432 all/leading edge ppl cells are indicated as white/green dots; blue arrow marks
433 movement direction of ppl leading edge cells; xy-axes in μm ; time in mins;
434 animal/vegetal pole, up/down.

435

436 **Supplementary Video 5 2D velocities of neurectoderm cells in MZ*oep* mutant**
437 **embryo.** Tissue flow map indicating velocity vectors of neurectoderm cell

438 movements along the animal-vegetal (AV) (V_{AV}) and left-right (LR) (V_{LR}) axis at the
439 dorsal side of a *MZoep* mutant embryo between 6 to 8 hpf (121 min); average
440 velocity vector for each defined area is indicated and color-coded ranging from 0
441 (blue) to 2 (red) $\mu\text{m}/\text{min}$; xy-axes in μm ; time in mins; animal/vegetal pole, up/down.

442

443 **Supplementary Video 6 3D correlation of neurectoderm and prechordal plate**
444 **(pp) cell movements in *CA-Mypt* injected embryo.** Movement correlation between
445 neurectoderm and underlying prechordal plate (ppl) cells at the dorsal side of a wt
446 embryo overexpressing *CA-Mypt* in the YSL between 6 to 8 hpf (118 min); degree of
447 correlation is color-coded ranging from 1 (red, highest correlation) to -1 (white,
448 lowest correlation); average neurectoderm movement velocities and direction for each
449 defined area are indicated by arrows; position of all/leading edge ppl cells are
450 indicated as white/green dots; blue arrow marks movement direction of ppl leading
451 edge cells; xy-axes in μm ; time in mins; animal/vegetal pole, up/down.

452

453 **Supplementary Video 7 2D velocities of neurectoderm cells in ppl-transplanted**
454 ***MZoep* mutant embryo.** Tissue flow map indicating velocity vectors of
455 neurectoderm cell movements along the animal-vegetal (AV) (V_{AV}) and left-right
456 (LR) (V_{LR}) axis at the dorsal side of a transplanted *MZoep* mutant embryo between 6
457 to 7.5 hpf (91 min); average velocity vector for each defined area is indicated and
458 color-coded ranging from 0 (blue) to 2 (red) $\mu\text{m}/\text{min}$; position of all/leading edge
459 transplanted prechordal plate (ppl) cells are indicated as black /green dots; xy-axis in
460 μm ; time in mins; animal/vegetal pole, up/down.

461

462 **Supplementary Video 8 2D velocities of neurectoderm cells in ppl-transplanted**
463 **and *CA-Mypt* injected *MZoep* mutant embryo.** Tissue flow map indicating velocity
464 vectors of neurectoderm cell movements along the animal-vegetal (AV) (V_{AV}) and
465 left-right (LR) (V_{LR}) axis at the dorsal side of a *MZoep* embryo overexpressing *CA-*
466 *Mypt* within the YSL between 6 to 8 hpf (120 min); average velocity vector for each
467 defined area is indicated and color-coded ranging from 0 (blue) to 2 (red) $\mu\text{m}/\text{min}$;
468 position of all/leading edge transplanted prechordal plate (ppl) cells are indicated as
469 black /green dots; xy-axis in μm ; time in mins; animal/vegetal pole, up/down.

470

471 **Supplementary Video 9 Arrangement of leading and trailing prechordal plate**
472 **(ppl) cells in wild type (wt) embryo.** Consecutive z-sections of a fluorescent imaging
473 stack showing lifeact-GFP (F-actin) expressing ppl cells transplanted into the ppl
474 leading edge of a wt embryo expressing Utrophin-mCherry (F-actin) and H2A-
475 mCherry (nuclei); section starts at the ppl-neurectoderm interface and progresses
476 through the leading edge ppl to the ppl-YSL interface; animal pole to the left; z-
477 section taken from movie 16 at $t = 12.36$ min; scale bar, $20 \mu\text{m}$.

478

479 **Supplementary Video 10 Life cell imaging of leading and trailing prechordal**
480 **plate (ppl) cells in wild type (wt) embryo.** Fluorescence time-lapse imaging of
481 lifeact-GFP (F-actin) expressing ppl cells transplanted into the ppl leading edge of a
482 wt embryo expressing Utrophin-mCherry (F-actin) and H2A-mCherry (nuclei)
483 starting at 70% epiboly (7 hpf); dorsal (top, animal pole left) and sagittal (bottom,
484 animal pole left) sections through the embryo with dual (left side) and single (right
485 side) color label; time in mins; scale bar, $20 \mu\text{m}$.

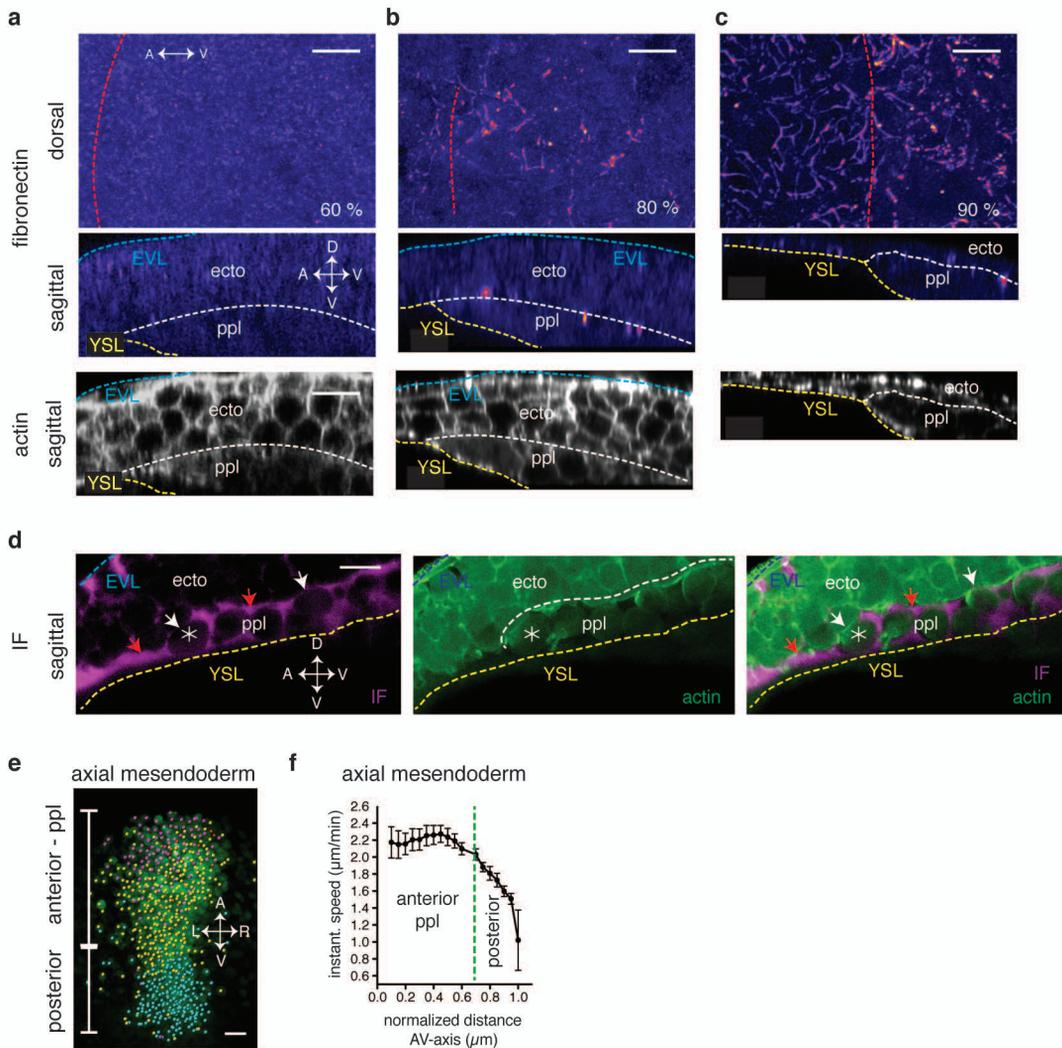
486

487 **Supplementary Video 11 2D velocities of neurectoderm cells in *e-cadherin***
488 **morphant embryo.** Tissue flow map indicating velocity vectors of neurectoderm cell
489 movements along the animal-vegetal (AV) (V_{AV}) and lefty-right (LR) (V_{LR}) axis at
490 the dorsal side of a *e-cadherin* morphant embryo between 6 to 8 hpf (120 min);
491 average velocity vector for each defined area is indicated and color-coded ranging
492 from 0 (blue) to 2 (red) $\mu\text{m}/\text{min}$; position of all/leading edge prechordal plate (ppl)
493 cells are indicated as black /green dots; xy-axis in μm ; time in mins; animal/vegetal
494 pole, up/down.

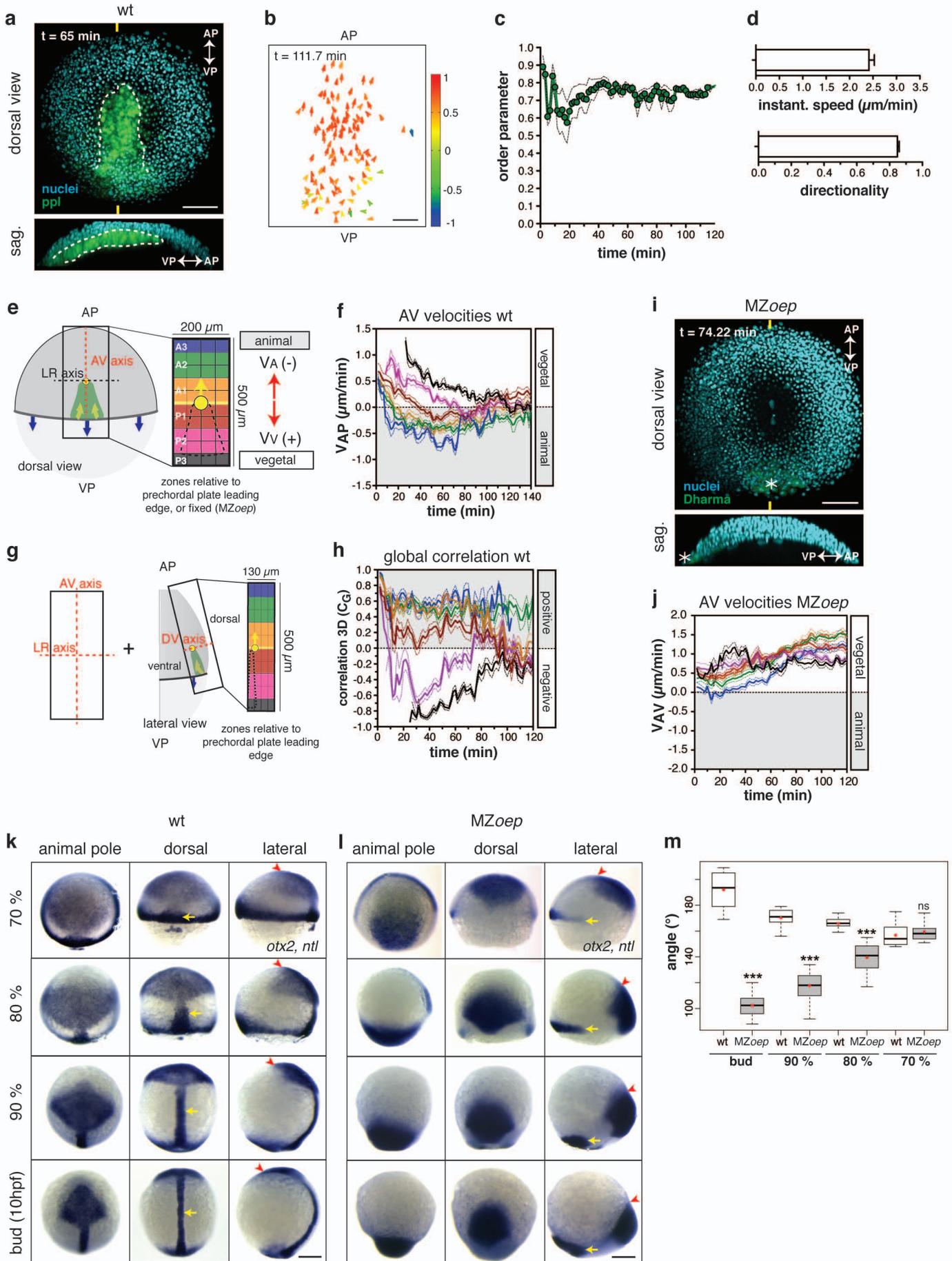
495

496 **Supplementary Video 12 3D correlation of neurectoderm and prechordal plate**
497 **(ppl) cell movements in *e-cadherin* morphant embryo.** 3D movement correlation
498 between neurectoderm and underlying prechordal plate (ppl) cells at the dorsal side of
499 *e-cadherin* morphant embryo 6 to 8 hpf (120 min); degree of correlation is color-
500 coded ranging from 1 (red, highest correlation) to -1 (white, lowest correlation);
501 average neurectoderm movement velocities and direction for each defined area are
502 indicated by arrows; position of all/leading edge ppl cells are indicated as white/green
503 dots; blue arrow marks movement direction of ppl leading edge cells; xy-axes in μm ;
504 time in mins; animal/vegetal pole, up/down.

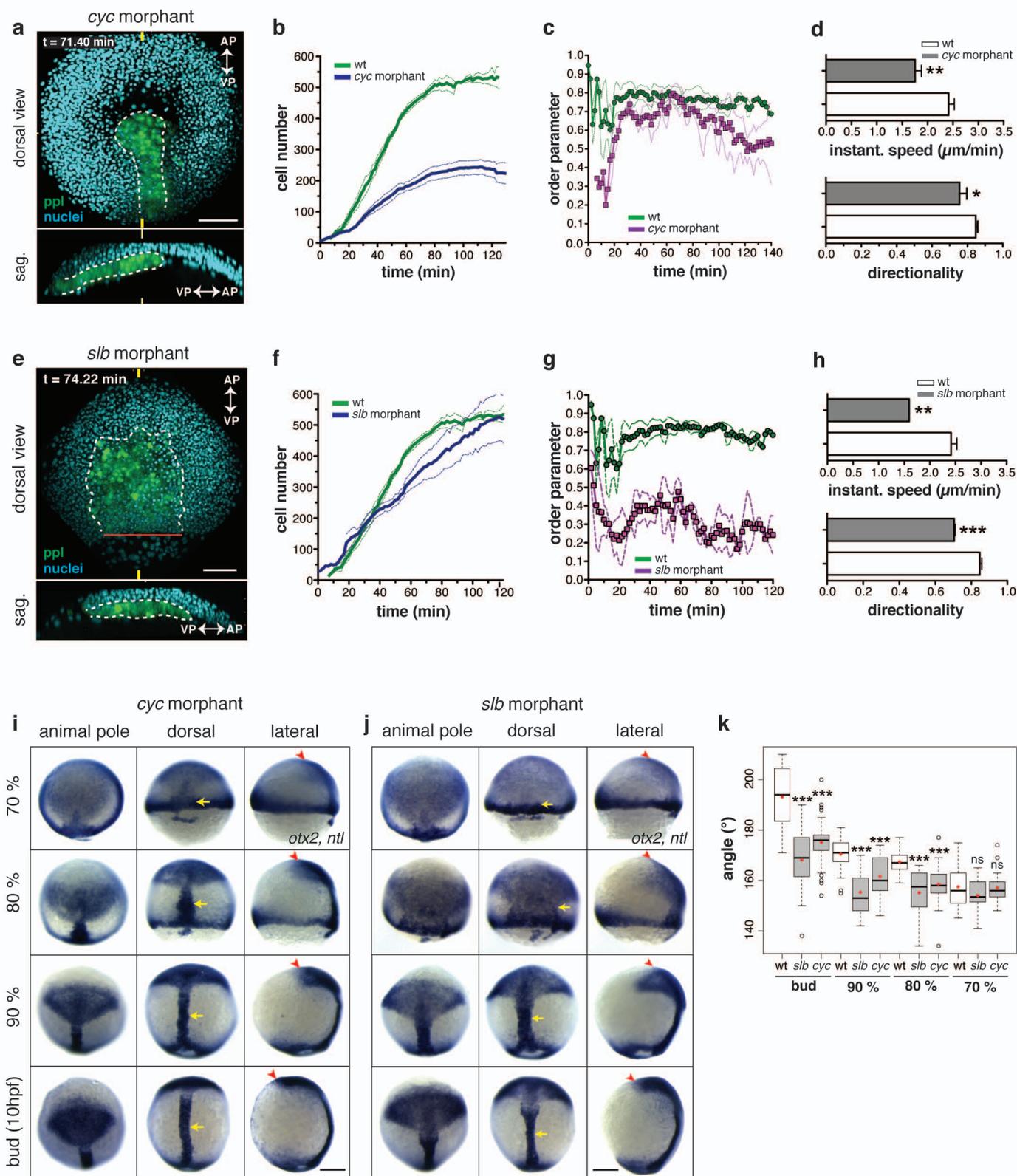
Supplementary Figure-1, Heisenberg



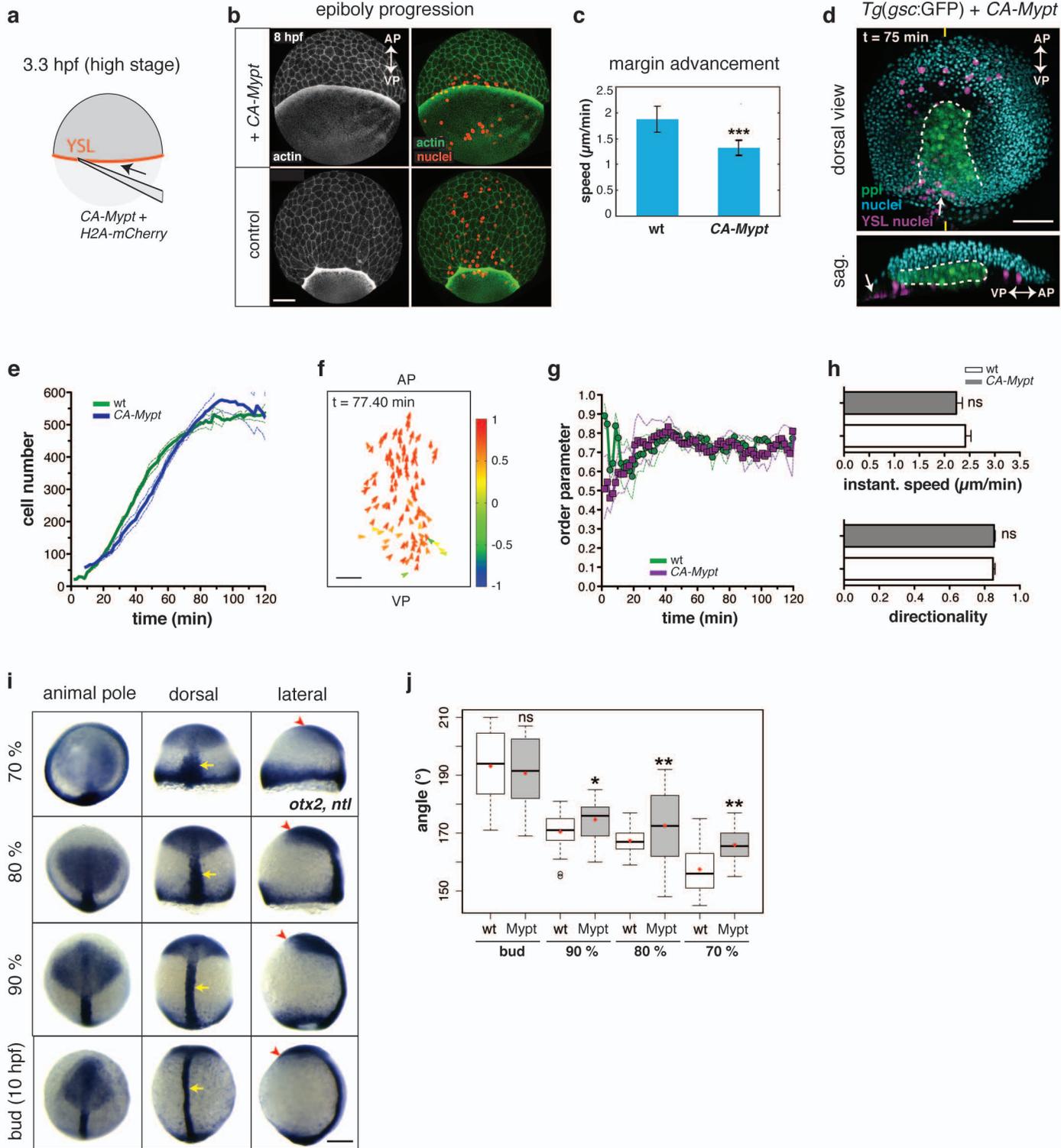
Supplementary Figure-2, Heisenberg



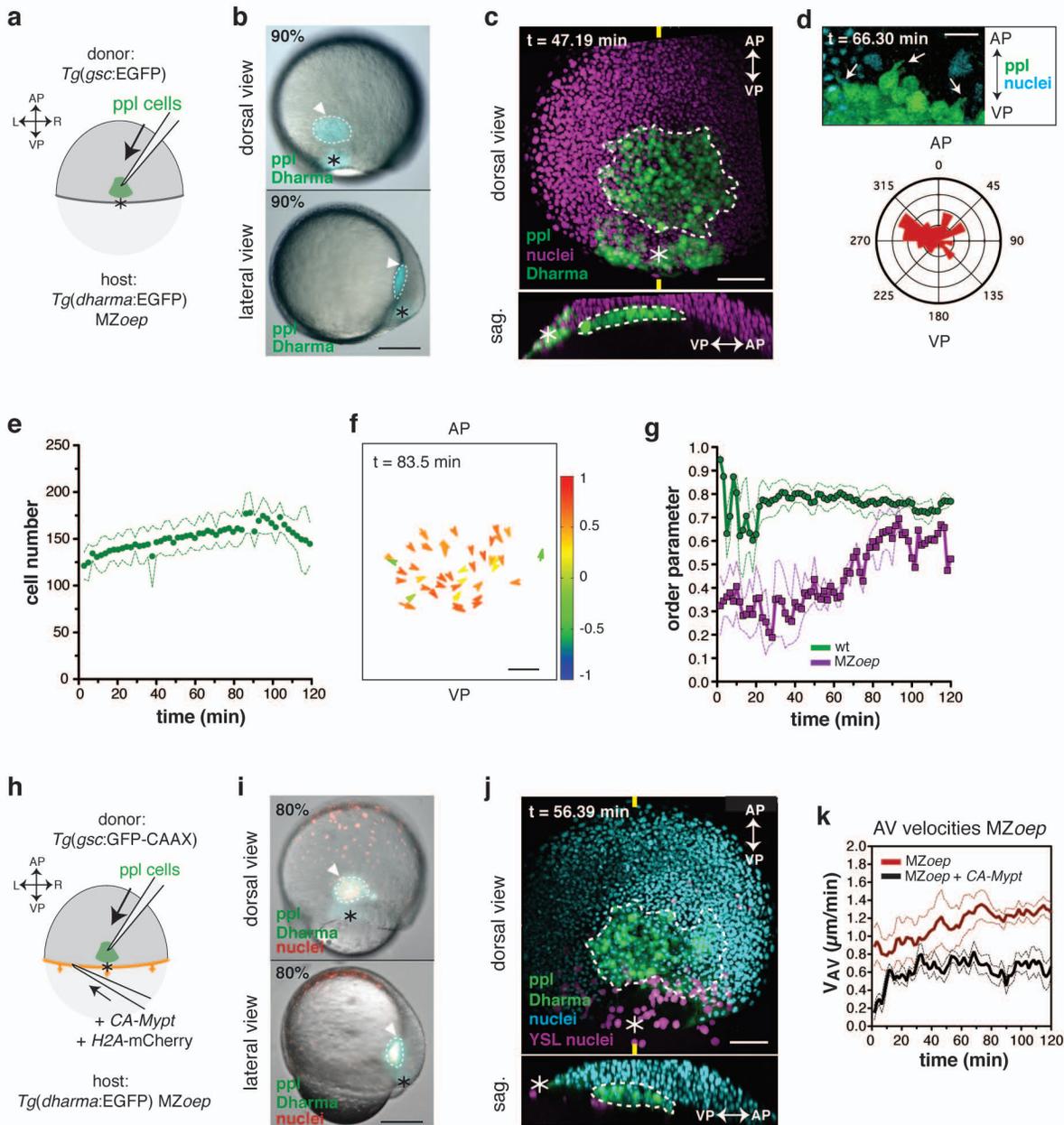
Supplementary Figure-3, Heisenberg



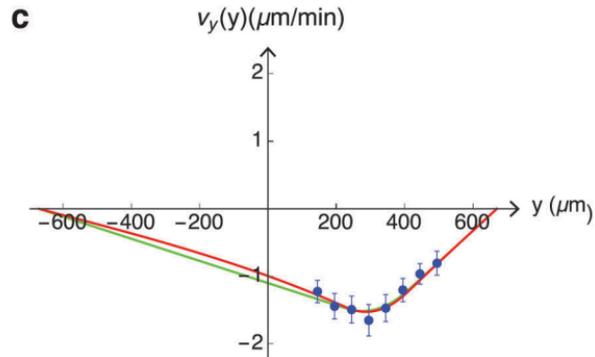
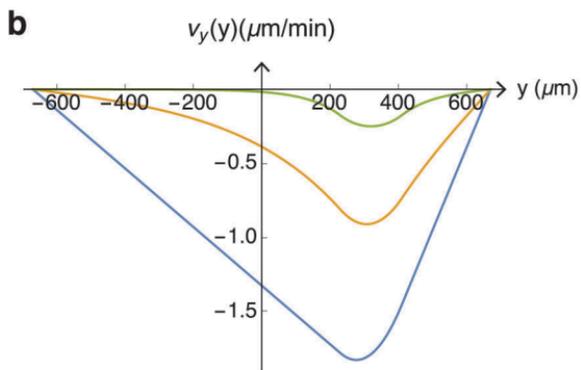
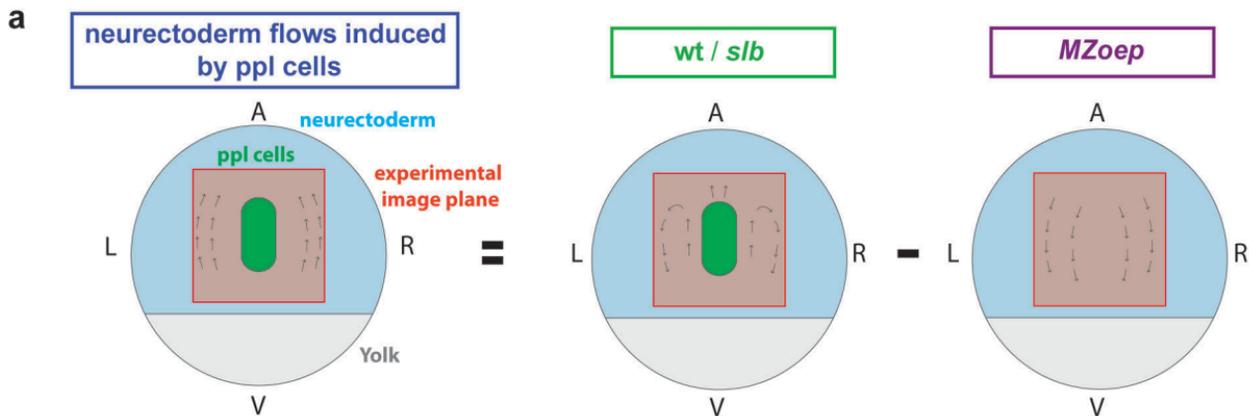
Supplementary Figure-4, Heisenberg



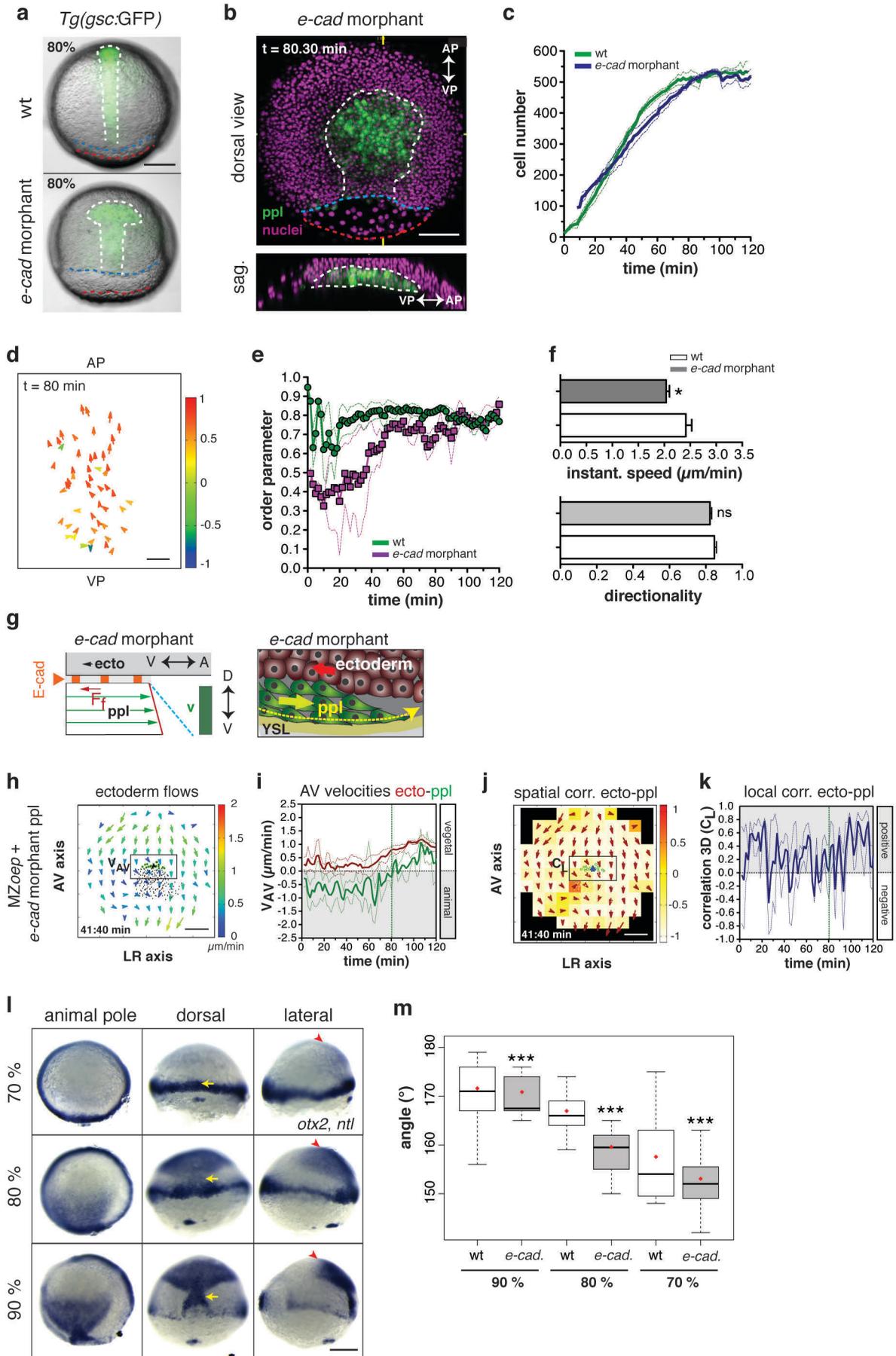
Supplementary Figure-5, Heisenberg



Supplementary Figure-6, Heisenberg



Supplementary Figure-7, Heisenberg



Supplementary Figure-8, Heisenberg

