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A mechanosensitive vascular niche for *Drosophila* hematopoiesis

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1 **A mechanosensitive vascular niche for *Drosophila* hematopoiesis**

2

3 **Short title:** A mechanosensitive hematopoietic niche

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6 **One sentence summary:** Mechano-sensing of blood flow by vascular niche cells regulates
7 hematopoietic progenitor maintenance

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16

17

18 **Abstract**

19 **Hematopoietic stem and progenitor cells maintain blood cell homeostasis by integrating**
20 **various cues provided by specialized microenvironments or niches. Biomechanical forces**
21 **are emerging as key regulators of hematopoiesis. Here we report that mechanical stimuli**
22 **provided by blood flow in the vascular niche control *Drosophila* hematopoiesis. In**
23 **vascular niche cells, the mechanosensitive channel Piezo transduces mechanical forces**
24 **through intracellular calcium upregulation, leading to Notch activation and repression**
25 **of FGF ligand transcription, known to regulate hematopoietic progenitor maintenance.**
26 **Our results provide insight into how the vascular niche integrates mechanical stimuli to**
27 **regulate hematopoiesis.**

28

29 **Main text**

30 In adult Mammals, hematopoiesis takes place in bone marrow where hematopoietic stem and
31 progenitor cells (HSPCs) are maintained by specialized microenvironments, or niches. HSPCs
32 localize around arterioles and sinusoid blood vessels. Endothelium cells, which line the lumen
33 of blood vessels and perivascular cells, constitute the vascular niche¹⁻⁸. Endothelium cells are
34 exposed to mechanical forces linked to blood flow^{9,10}. Whether mechanosensing by
35 endothelium/vascular niche cells regulates HSPC maintenance is unknown. We use
36 *Drosophila* as a model system to ask whether mechanical stimulation resulting from blood
37 flow affects the transcription of signals secreted by cardiac cells, which correspond to
38 vascular niche cells in *Drosophila*, and thereby regulates hematopoietic progenitor
39 maintenance.

40 The *Drosophila* larval hematopoietic organ, called the lymph gland, has emerged as an
41 attractive *in vivo* model to study how hematopoietic progenitor maintenance and
42 differentiation are regulated by their microenvironment¹¹⁻¹³. The lymph gland is aligned along
43 the cardiac tube which corresponds to the vascular system, and is composed of hematopoietic

44 progenitors, differentiated blood cells and the Posterior Signaling Center (PSC). The latter
45 corresponds to the niche (Fig. 1a) since the PSC produces various signals that regulate lymph
46 gland homeostasis, i.e., the balance between progenitors and differentiated blood cells^{12,13}.
47 Recently we established that cardiac cells produce the Fibroblast Growth Factor (FGF) ligand
48 Branchless (Bnl). Though the activation of its FGF receptor in progenitors, Bnl controls
49 progenitor maintenance and prevents their differentiation into crystal cells and plasmacytes,
50 two mature blood cell types that differentiate under physiological conditions. Thus the cardiac
51 tube corresponds to a second niche which directly regulates the extent of progenitor
52 differentiation in the lymph gland¹⁴. The cardiac tube is composed of one anterior part (aorta)
53 in contact with the lymph gland containing non-contractile cardiomyocytes, and a posterior
54 contractile heart chamber where cardiomyocytes by contracting rhythmically, provide the
55 propelling force for hemolymph/blood flow circulation within the cardiac lumen¹⁵
56 (Supplementary Fig. 2a). In insects, the hemolymph provides nutriment and conveys signals,
57 but does not supply oxygen which is achieved via the tracheal system. Analyses of
58 *Drosophila* cardiac physiology further established that, as in Mammals, the blood flow
59 generates intra-cardiac mechanical forces¹⁶. Here our data strongly support the conclusion that
60 mechanical constraints exerted by blood flow on vascular niche cells modify their secreted
61 signals and subsequently impact hematopoietic progenitor homeostasis.

62 **Results**

63 **Heartbeat regulates lymph gland homeostasis**

64 A primary function of *Drosophila* heart is to pump hemolymph throughout the body in an
65 open circulatory system¹⁵. We asked whether mechanical constraints exercised by blood flow,
66 resulting from cardioblast contraction, could modify lymph gland hematopoiesis. To stop
67 blood flow, we expressed either a constitutively active form of Ork1 (Ork1-deltaC (Ork1 Δ C)),
68 a potassium channel subunit required to set heartbeat frequency¹⁷ or a knockdown of Myosin

69 heavy chain (Mhc, necessary for muscle contraction) specifically in cardiac cells using two
70 independent Gal4 drivers NP1029 and Hand Δ C^{14,18}. Both Mhc knockdown and Ork1 Δ C
71 expression in cardiac cells from the second larval stage (L2) on, resulted in heartbeat arrest
72 (Fig. 1b-c and movies 1-3, Supplementary Fig. 1b) without affecting heart morphology
73 (Supplementary Fig. 1a). In all these contexts, heart failure resulted in decreased cells
74 differentiation in the lymph gland, for both crystal cells (labelled by Hindsight (Hnt)
75 antibody) (Fig. 1e-g, i, Supplementary Fig. 1f-k) and plasmatocytes (P1 antibody labelling)
76 (Supplementary Fig. 1c-e). In agreement with published data¹⁷ an increased heart rate was
77 observed when Ork1-RNAi was expressed with the Hand Δ cardiac driver (Fig. 1d, movie 4-
78 5), and this resulted in increased crystal cell differentiation compared to control (Fig. 1h-i).
79 We then analyzed the effect of heart arrest on hematopoietic progenitors. The transcription
80 factor Collier (Col) (also known as Knot) is expressed at high levels in the PSC niche and at
81 lower levels in core progenitors, a subset of hematopoietic progenitors maintained by cardiac
82 cell signals^{14,19,20} (Fig. 1a). Compared to wild type, higher numbers of Col⁺ progenitors were
83 recorded, indicating that heartbeat controls core progenitor maintenance (Fig. 1j-m,o and
84 Supplementary Fig. 1l-n). Since the PSC is also known to control lymph gland homeostasis²⁰⁻
85 ²⁶ we looked at PSC cell numbers by Antennapedia (Antp) immunostaining. No difference in
86 PSC cell numbers was observed (Supplementary Fig. 1o-q). Altogether, these data establish
87 that in third instar (L3) larvae the heartbeat, and thereby blood flow, trigger progenitor
88 differentiation into mature blood cells. These results indicate that mechanical stimuli resulting
89 from blood flow may be critical regulators of hematopoiesis.

90 **Mechanosensitive ion channel Piezo controls lymph gland homeostasis**

91 Heartbeat imposes mechanical constraints on cardiac cells¹⁶. Piezo encodes a
92 mechanosensitive cation channel that directly senses mechanical tension in lipid bilayers²⁷
93 and is responsible for mechano-reception in many cell types^{28,29}. A single *piezo* gene is

94 present in the *Drosophila* genome²⁹⁻³³. Piezo is expressed in larval cardiomyocytes, but no
95 function in these cells has been reported so far²⁹. To document its expression, we used *piezo-*
96 *Gal4*, where Gal4 is under the control of a *piezo* enhancer²⁹ and the Piezo knock-in Gal4
97 allele *piezo-Gal4 (KI)*³⁰ to express UAS-mcd8GFP. In both cases GFP was expressed in
98 cardiac cells and at low levels in a subset of crystal cells (Fig. 1n and Supplementary Fig. 2a-
99 c). We investigated whether in cardiac cells Piezo regulates lymph gland hematopoiesis. To
100 monitor blood cell differentiation and progenitor maintenance, we looked at crystal cell
101 differentiation and Col⁺ expression, respectively. In two null mutant contexts: *piezo-KO* and
102 *piezo-Gal4(KI)*³⁰, crystal cell indexes were lower than in the control (Fig. 1p, r and
103 Supplementary Fig. 2d-e). Similar results were obtained by knocking down *piezo* in cardiac
104 cells by RNAi (Fig. 1q, s; Supplementary Fig. 2i-l) or by over-expressing mPiezo1-2336-
105 Myc, which encodes a nonfunctional channel³¹ (Supplementary Fig. 2m-o). Higher numbers
106 of Col⁺ progenitors were observed when *piezo* was knocked down in cardiac cells
107 (Supplementary Fig. 2f-h). Altogether these data establish that *piezo* expression in cardiac
108 cells regulates lymph gland homeostasis. To determine whether its function is specifically
109 required in anterior aorta cells which are in contact with the lymph gland, we used the 76E11-
110 Gal4 cardiac cell driver expressed only in the anterior aorta cells of late L2/early L3 larvae
111 (Supplementary Fig. 2p). Applying *piezo* knockdown only after the L2 stage using the Gal80^{ts}
112 system³⁴, led to a decrease in crystal cell index (Supplementary Fig. 2q-s), which means that
113 *piezo* is required in anterior aorta cells to control blood cell differentiation. Finally, we
114 showed that *piezo* knockdown in cardiac cells does not affect heartbeat (Supplementary Fig.
115 2t-u and movie 6). In summary, our data indicate that the function of *piezo* is required in L3
116 larval anterior aorta cells to control lymph gland homeostasis non-cell-autonomously. Since
117 *piezo* knockdown and heart failure led to similar lymph gland defects, we asked whether both
118 were functionally linked. Expression of a constitutively active form of Piezo, mPiezo1-TriM³¹

119 in cardiac cells (*HandΔ*) led to a significant increase in crystal cell differentiation (Fig. 1t, v).
120 In addition, activation of Piezo function in larvae with reduced heart contractility (*Ork1ΔC*)
121 rescued lymph gland defects as it restored a wild type number of crystal cells (Fig. 1u-v).
122 Overall, these data indicate that in aorta cells *piezo* acts downstream of heartbeat to control
123 lymph gland hematopoiesis.

124 **Piezo and heartbeat control expression of the FGF ligand Bnl**

125 *Drosophila* cardiac cells exert a function similar to the mammalian vascular niche. The FGF
126 ligand Bnl secreted by cardiac cells, activates in L3 larvae the FGF pathway in hematopoietic
127 progenitors, which in turn controls lymph gland homeostasis¹⁴. To investigate whether Bnl
128 levels in cardiac tube are dependent on *piezo* and/or on heartbeat, we analyzed endogenous
129 Bnl expression using the *bnl*:GFP^{endo} knock-in allele³⁵. When the heart was arrested or *piezo*
130 knocked down in cardiac cells, more *bnl*:GFP^{endo} was observed in cardiac cells (Fig. 2a-e),
131 indicating that *piezo* and heartbeat control Bnl protein levels in cardiac tube. We then
132 established that Piezo and heartbeat repressed *bnl* transcription in cardiac cells, since *bnl*
133 transcription, quantified by *in situ* hybridization, was higher when either *piezo* was knocked
134 down or the heart arrested (Fig. 2f-j).

135 To investigate the functional link between *bnl* and *piezo* in cardiac cells and lymph gland
136 hematopoiesis, we monitored blood cell differentiation using crystal cell differentiation as a
137 readout, and asked whether we could rescue the decreased crystal cell numbers due to *piezo*
138 knockdown by knocking down *bnl* in cardiac cells. While the expression of *bnl*-RNAi in
139 cardiac cells increased crystal cell numbers, in agreement with the previous report¹⁴, and
140 *piezo*-RNAi reduced crystal cell numbers, simultaneous expression of *bnl*-RNAi and *piezo*-
141 RNAi restored wild type crystal cell numbers (Fig. 2k-l, n). Altogether, these data indicate
142 that Piezo functions by repressing *bnl* expression. Furthermore, since heartbeat activates Piezo
143 which represses Bnl expression, we wondered whether Bnl reduction in cardiac cells can

144 compensate for heart arrest. Crystal cell differentiation returned to normal when *bnl-RNAi*
145 was expressed in a context where the heart was blocked (*HandΔ>bnl-RNAi>Ork1ΔC*, Fig.
146 2m, o). In conclusion, both heartbeat and Piezo control lymph gland hematopoiesis by
147 repressing *bnl* expression in cardiac cells. All these data lead us to propose that heartbeat
148 activates the mechanosensitive channel Piezo in aorta cells, which in turn represses *bnl*
149 transcription and therefore regulates lymph gland homeostasis.

150 **Notch acts downstream of Piezo to repress *bnl* expression**

151 To further decipher the molecular machinery downstream of Piezo, we first analyzed Ca^{2+}
152 levels, since Piezo triggers upregulation of cytosolic Ca^{2+} ³⁶⁻³⁹. Using the Ca^{2+} sensor
153 GCaMP3⁴⁰ we observed that knocking down *piezo* in cardiac cells (Fig. 3a-c) or blocking
154 heartbeat (Supplementary Fig. 3a-c) led to decreased fluorescence compared to control,
155 reflecting reduced Ca^{2+} levels. Thus heartbeat, via blood flow, activates Piezo which in turn
156 regulates cytosolic Ca^{2+} levels in cardiac cells.

157 We subsequently focused on Notch (N) signaling since Piezo, by modulating Ca^{2+} levels,
158 regulates N signaling in several cell types such as mouse endothelial cells ⁴¹, zebrafish cardiac
159 valve cells⁴² and *Drosophila* gut cells³⁰. In *Drosophila* *bnl* transcription is repressed by N
160 signaling in the embryonic tracheal system⁴³. We therefore hypothesized that in cardiac cells
161 Piezo could activate N signaling, which in turn would repress *bnl* expression. We therefore
162 investigated the role of N signaling in cardiac cells. The Notch Responsive Element
163 (Notch[NRE]-GFP) ⁴⁴ is a N signaling responsive enhancer. It is expressed in cardiac cells
164 (Fig. 3d-d') dependent on N signaling, since it is reduced when a dominant negative form of
165 N (N^{xho}) is expressed in cardiac cells (Supplementary Fig. 3e-e', g). These data indicate that
166 the N pathway is activated in cardiac cells. To strengthen this conclusion, we analyzed the
167 expression of NRE-GFP, a synthetic reporter for N pathway activation, and of E(spl)mbeta-
168 HLH-GFP, which reports on canonical N pathway activation⁴⁵. For both reporters, GFP was

169 expressed in cardiac cells, confirming that the canonical N pathway is activated in cardiac
170 cells (Supplementary Fig. 3i-j'). When *piezo* was knocked down in cardiac cells (Fig. 3e-f) or
171 when heartbeat was arrested (Supplementary Fig. 3f-f', h), Notch[NRE]-GFP expression was
172 reduced establishing that heartbeat and *piezo* activate N signaling. Furthermore we showed
173 that N signaling is dependent on Ca²⁺ levels, since Notch[NRE]-GFP expression was reduced
174 when Ca²⁺ levels were decreased in cardiac cells. This was achieved using either CamKII
175 knockdown (CamKII-RNAi) or with the inhibition of ER-mediated Ca²⁺ release in the cytosol
176 by knocking down IP3R (IP3R-RNAi)⁴⁶ (Fig. 3g-i). Collectively, these data indicate that
177 Piezo through the control of intracellular Ca²⁺ levels activates N signaling. We then
178 investigated the N/Bnl relationship. Bnl levels, as visualized with *bnl*:GFP^{endo}, were higher in
179 cardiac tube when the N pathway was inhibited (N^{Xho}, Fig. 3j-l) or when *N-RNAi* was
180 expressed in cardiac cells (Supplementary Fig. 3k-m). This indicates that N signaling
181 represses *bnl* expression in cardiac cells.

182 We asked whether N signaling in cardiac cells is required for lymph gland homeostasis. We
183 monitored blood cell differentiation using crystal cell differentiation as a readout. When N
184 signaling was inhibited by expressing (N^{Xho}) with either of the two heart drivers (Fig. 3m, o
185 and Supplementary Fig. 3n-o, q), crystal cell numbers were lower than in the control.
186 Reduced crystal cell differentiation was also seen upon expression of *N-RNAi* in cardiac cells
187 (Supplementary Fig. 3r-t) or of a dominant negative form of *mastermind* (*mam*^{DN}). Mam is
188 the coactivator of NICD to regulate N target gene expression⁴⁵ (Supplementary Fig. 3p-q). In
189 conclusion, the N signaling pathway is activated in cardiac cells and regulates lymph gland
190 hematopoiesis non-cell autonomously via Bnl. Piezo activation and N loss-of-function have
191 opposite effects on crystal cell numbers. Simultaneous expression of a constitutively active
192 form of Piezo (*mpiezoI-TriM*) and N^{Xho} (N inhibition) led to increased crystal cell numbers
193 compared to inactivation of N signaling alone (Fig. 3n-o), establishing that Piezo and N are

194 functionally linked and that Piezo in part functions through N signaling. Finally, knockdown
195 of *bnl* in cardiac cells compensates for the crystal cell differentiation defect due to N signaling
196 inhibition, indicating that N pathway regulates crystal cell differentiation via the repression of
197 *bnl* transcription in cardiac cells (Fig. 3p-r).

198 In conclusion, our data are consistent with the hypothesis that in cardiac cells Piezo
199 activates canonical N signaling, which subsequently represses *bnl* expression, which in turn
200 regulates lymph gland hematopoiesis (Fig. 3s).

201 **A physiological chronotropic effect controls lymph gland hematopoiesis**

202 We finally wondered what could be the physiological relevance of a control of lymph
203 gland hematopoiesis by heartbeat. When measuring heartbeat rate, we discovered that it
204 increases slightly during larval development (Fig. 4a and movies 7-8). We then looked at Bnl
205 expression and observed more abundant *bnl*:GFP^{endo} in the cardiac tube of late L2 larvae
206 compared to mid L3 larvae (Fig. 4b-d), indicating that Bnl levels in the cardiac tube decrease
207 between L2 and L3 larval stages. While there were no crystal cells in late L2 lymph glands,
208 many were found in mid L3 larvae (Fig. 4e-g), confirming published data indicating that
209 blood cell differentiation occurs in L3 larvae, mature blood cells being seldom found in L2
210 larvae¹³. Finally, we determined whether temporal differentiation of crystal cells is dependent
211 on heartbeat rate and/or Piezo activation. We showed that premature blood cell differentiation
212 occurs in late L2 larvae when heartbeat rate was increased (Ork1-RNAi) and when Piezo was
213 constitutively activated (mPiezo1-TriM) from L1 to L2 larval stages (Fig. 4h-j). All these data
214 strongly suggest that the temporal control, between L2 and L3 larval stages, of lymph gland
215 blood cell differentiation may be achieved through physiological modulation of the heartbeat
216 rate.

217 In conclusion, cardiac cells, previously established to act as vascular niche cells, may
218 directly sense mechanical forces provided by blood flow and adjust lymph gland

219 hematopoiesis accordingly. This mechanosensing is mediated by Piezo activation that
220 transduces the stimuli into cellular responses in vascular niche cells. Our findings show that
221 Piezo, activated by blood flow, promotes blood progenitor maintenance via N activation in
222 vascular niche cells, which in turn represses expression of the FGF ligand Bnl. Whether N
223 signaling represses *bnl* transcription in cardiac cells via *E(spl)* genes has to be confirmed. Bnl
224 secreted by vascular niche cells non-cell-autonomously activates the FGF pathway in
225 hematopoietic progenitors for their maintenance. These data demonstrate a direct link
226 between the blood flow rate and the maintenance of hematopoietic progenitors, and provide a
227 regulatory network involving mechanosensitive channels in hematopoietic vascular niche
228 cells to promote blood cell progenitor maintenance. Given the striking parallels between
229 *Drosophila* lymph gland and mammalian bone marrow hematopoiesis, there is promise that
230 our newly identified regulations are conserved and that mechanosensing of blood flow by
231 endothelial cells, a major cell type of the bone marrow vascular niche in mammals, may also
232 contribute to HSPC maintenance. Modification of blood pressure, such as during physical
233 activity, could therefore be a powerful way to regulate hematopoiesis.

234

235 **Materials and Methods.**

236 **Fly strains.** *w*¹¹¹⁸ (wild type, *WT*), *UAS-mcD8GFP*¹⁹, *handΔ-gal4*¹⁴, *NP1029-gal4*⁴⁷,
237 *BcGFP*⁴⁸, *bnl:GFP^{endo}*³⁵, *HandCGFP*⁴⁹, *UAS-Ork1ΔC* and *UAS-Ork1-RNAi*¹⁷, *UAS-Piezo-*
238 *RNAi1* (line 1), *UAS-mPiezo1-2336-Myc* and *UAS-mPiezo1-TriM*³¹, *Mhc-RNAi1* (line 1)
239 (F.Schnörrer), *Notch[NRE]-GFP* and *Notch-RNAi*⁴⁴, *NRE-GFP* and *UAS-Mam^{DN}*⁴⁵, and
240 *N^{xho}* (S.Bray). Other strains were provided by Bloomington (BL) and Vienna (VDRC)
241 *Drosophila* stock centers: *UAS-GCaMP3* (BL32116), *UAS-Dicer* (BL24650), *76E11-gal4*
242 (BL39933), *tub-gal80^{ts}* (BL7019), *UAS-piezo-RNAi2* (line 2) (VDRC v25780), *Mhc-RNAi2*
243 (line 2) (BL26299), *piezo-gal4* (KI) (BL78335), *piezo-gal4* (enhancer) (BL59266), *piezo*

244 (KO) (BL58770), UAS-bnl-RNAi (VDRC GD5730), UAS-CaMKII (BL29401), UAS-IP3R-
245 RNAi (BL25937) and E(spl)mbeta-HLH-GFP (BL65294). Crosses and subsequent raising of
246 larvae until late L1/early L2 stage were performed at 22°C, before shifting larvae to 25°C
247 until their dissection at L3 stage. For RNAi treatments the same procedure was followed
248 except that larvae were shifted to 29°C until their dissection. UAS-Dicer was introduced and
249 at least two independent RNAi lines per gene were tested. Controls correspond to Gal4 drivers
250 with UAS-Dicer crossed with w¹¹¹⁸. For gal80^{ts} experiments, crosses were initially maintained
251 at 18°C (permissive temperature) for 3 days after egg laying, and then shifted to 29°C until
252 dissection.

253 **Antibodies.** Primary antibodies were mouse anti-Hnt (1/100, Hybridoma Bank), mouse anti-
254 Col (1/40)¹⁹, mouse anti-P1 (1/30, I. Ando, Institute of Genetics, Biological Research Center
255 of the Hungarian Academy of Science, Szeged, Hungary), mouse anti-Antp (1/100,
256 Hybridoma Bank), chicken anti-GFP (1/500, Abcam). Secondary antibodies were Alexa
257 Fluor-488 and -555 conjugated antibodies (1/1000, Molecular Probes) and goat anti-Chicken
258 Alexa Fluor-488 (1/800, Molecular Probes). Nuclei were labeled with TOPRO3 (Thermo
259 Fisher Scientific). For detecting bnl:GFP^{endo} and Notch[NRE]-GFP, GFP immunostainings
260 were performed.

261 **Heart rate measurements and movies.** Larvae expressed HandC-GFP with GFP labelling of
262 cardioblasts and pericardial cells. Larvae were anaesthetized with FlyNap (Carolina
263 Biological, Burlington, NC, USA). 5µl of FlyNap put on a piece of cotton in a closed chamber
264 and larvae put in chamber for 160 seconds. Anaesthetized larvae deposited on a glass slide for
265 recording with a binocular microscope (SMZ18, Nikon). Anaesthetized larvae kept at 25°C
266 for 10 minutes before recording. For each individual, 20 second-recordings performed, and
267 this repeated twice with 10 minutes of rest in the dark. To measure heart contraction, movies
268 were converted into a kymograph using Fiji software. For each larva, number of heartbeat

269 contractions during 20 seconds corresponds to average of three measurements. At least 10
270 larvae per genotype scored and experiments reproduced at least three times. All recorded
271 larvae survived and gave rise to an adult. Statistical analyses *t*-test (Mann-Whitney
272 nonparametric test) performed using GraphPad Prism 5 software.

273 **Quantifications.** In all experiments, optimized confocal sections performed on Leica SP8
274 microscope for 3D reconstructions, and since number of lymph gland differentiated blood
275 cells fluctuates depending on the larval stage, and to limit discrepancies, all genotypes always
276 analyzed in parallel. For all quantifications, statistical analyses *t*-test (Mann-Whitney
277 nonparametric test) performed using GraphPad Prism 5 software.

278 **Blood cell and progenitor quantification.**

279 Crystal cells visualized by BcGFP or immunostaining with antibodies against Hnt.
280 Plasmatocytes and core progenitors labelled by P1 and Col immunostaining, respectively.
281 Nuclei labelled by TOPRO3. Number of crystal cells and volume (in μm^3) of each anterior
282 lymph gland lobe measured using Fiji software and 3DSuite plugin⁵⁰. Crystal cell index
283 corresponds to [(number of crystal cells)/(primary lobe volume)] x100. Plasmatocyte or
284 progenitor index corresponds to (plasmatocyte or progenitor volume/anterior lobe volume) x
285 10. At least 15 anterior lobes scored per genotype, and experiments reproduced at least three
286 times.

287 **Quantification of UAS-GCaMP3 intensity.**

288 Volume of cardiac tube localized in between two lymph gland anterior lobes measured. For
289 GCaMP3, mean intensity of cytoplasmic GFP quantified using Fiji software and the 3DSuite
290 plugin⁵⁰. Mean intensity of GCaMP3 corresponds to sum intensity of GCaMP3 per cardiac
291 tube volume. At least 20 cardiac tubes scored per genotype, and experiments reproduced at
292 least three times.

293 **Quantification of *bnl*::GFP and Notch[NRE]-GFP dots, and *bnl* expression.**

294 A ROI including cardiac tube in between the anterior lymph gland lobes was selected. To
295 normalize with respect to background in tissue preparations, a ROI in the lymph gland cortical
296 zone was selected and used as background level. Volume of ROI and number of Bnl::GFP or
297 Notch[NRE]-GFP granules per ROI was quantified using Fiji software and DiAna plugin⁵¹.
298 The sum of GFP granules per volume was calculated. Notch[NRE]-GFP and bnl::GFP levels
299 correspond to GFP granules measured in the cardiac tube minus the sum of granules measured
300 in the cortical zone. At least 10 cardiac tubes scored per genotype. For *bnl* expression levels,
301 volume of cardiac tube and *bnl* fluorescence quantified using Fiji software and 3DSuite
302 plugin⁵⁰. *bnl* mean intensity corresponds to *bnl* fluorescence/volume. At least 6 cardiac tubes
303 scored per genotype. All experiments reproduced at least three times.

304

305 **Data availability.** The authors declare that the data supporting the findings of this study are
306 available within the article and its Extended Data Figs.

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425

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437 **Author contributions**

438 Conceived and designed the experiments: Y.T. and M.C. performed the experiments: Y.T.
439 and I.M.P.. Analyzed the data: Y.T., I.M.P., N.V. and M.C.. Contributed
440 reagents/materials/analysis tools: Y.T. and I.M.P.. Wrote the paper :Y.T. and M.C..

441 **Competing interests:** The authors declare no conflict of interest.

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443

444 **Figure 1: Heartbeat and Piezo expressed in cardiac cells control lymph gland** 445 **homeostasis.**

446 (a) The lymph gland is composed of progenitors (red) and core progenitors (hatched red) in a
447 medullary zone (MZ), and a cortical zone which contains differentiated hemocytes (CZ, dots).
448 The two niches, the PSC (pink) and the cardiac tube (orange) regulate different subsets of MZ
449 progenitors (black arrows). (b) Kymograph of heartbeat in control and when *Orkl Δ C*, *Mhc-*
450 *RNAi* and *Orkl-RNAi* are expressed with cardiac cell drivers. (c-d) Heart contraction numbers
451 per 20s. (e-h) Crystal cell differentiation (Hnt, green) when the heart is blocked (f-g) or
452 accelerated (h). (i) Crystal cell index. (j-k, m) Col (red,*) labels core progenitors and the PSC
453 (arrow). (l, o) Quantification. (n) *mcd8-GFP* (green) under *piezo-Gal4* is expressed in cardiac
454 cells (arrowhead). (p-q) Fewer crystal cells (Hnt, green) in a *piezo* null mutant (p) or when

455 *piezo* is knocked down in cardiac cells (q). (r-s) Crystal cell index. (t-u) Active form of *piezo*
456 (*mPiezo1-TriM*) with arrested heart (*Ork1ΔC*) restores wild type crystal cell numbers (u). (v)
457 Crystal cell index. Statistical analysis *t*-test (Mann-Whitney nonparametric test) performed
458 using GraphPad Prism 5 software. Error bars represent SEM and *P<0.1,** P< 0.01; ***P
459 <0.001; ****P <0.0001. ns (not significant). Nuclei labelled with Topro (blue) and scale bars
460 = 20μm.

461 **Figure 2: Heartbeat and Piezo control expression of FGF ligand Branchless (Bnl) in**
462 **cardiac cells and in turn Bnl regulates lymph gland hematopoiesis.**

463 (a-c') Enlarged view of larval cardiac tube expressing *bnl:GFP^{endo}* (green) and Col (red) in
464 core progenitors (a-c) and *bnl:GFP^{endo}* (white) in (a'-c'). Dotted lines indicate cardiac tube
465 contour. *bnl:GFP^{endo}* expression is higher when heart is blocked (b-c'). (d-e) Quantification.
466 (f-h') Enlarged view of cardiac tube when *piezo-RNAi* (g-g') or *Ork1ΔC* (h-h') expressed in
467 cardiac cells; *bnl* in red (f-h) and white (f'-h'). *bnl* expression in cardiac cells increases in
468 both conditions. (i-j) Quantification. (k-m) Crystal cell (Hnt, green) differentiation increases
469 when *bnl* knocked down in cardiac cells (k) and restored to wild type when *piezo* and *bnl*
470 simultaneously knocked down in cardiac cells (l). Crystal cell differentiation rescued when
471 *bnl* knocked down in cardiac cells simultaneously with heart arrest (m). (n-o) Crystal cell
472 index.

473 **Figure 3: Piezo regulates Ca²⁺ levels and activates N signaling, which controls blood cell**
474 **differentiation by repressing *bnl* transcription in cardiac cells.**

475 (a-b) GCaMP3 Ca²⁺ sensor (green) decreases when *piezo* knocked down in cardiac cells. (c)
476 Quantification. (d-e', g-h') Enlarged view of larval cardiac tube expressing *Notch[NRE]-GFP*
477 (green) and Col (red) in core progenitors (d-e, g-h) and *Notch[NRE]-GFP* (white) in (d'-e',
478 g'-h'). Dotted lines indicate cardiac tube contour. *Notch[NRE]-GFP* expression decreased
479 when *piezo* knocked down (e-e') and when Ca²⁺ levels decreased (g-h') in cardiac cells. (f, i)
480 Quantification. (j-k') *bnl:GFP^{endo}* expression increased when N signaling inhibited. (l)

481 Quantification. (m-n, p-q) Crystal cell (Hnt, green) differentiation reduced when N signaling
482 inhibited (m), and rescued when simultaneously N signaling is inhibited and Piezo is
483 constitutively activated (n). Crystal cell differentiation defect observed when N is inhibited
484 (N^{xho}), is rescued when simultaneously N signaling is inhibited and *bnl* is knocked down (q).
485 (o, r) Crystal cell index. (s) Representation of third instar larval lymph gland anterior lobe.
486 Blood flow resulting from heartbeat activates Piezo in cardiac/vascular cells leading to Ca^{2+}
487 increase which activates N signaling, which in turn represses *bnl* expression in vascular cells.
488 Bnl normally produced by vascular cells activates FGF pathway in progenitors, where it is
489 required for their maintenance at the expense of their differentiation.

490 **Figure 4: Physiological modulation of heartbeat rate during larval development**
491 **regulates hematopoiesis.**

492 (a) Heart contraction number per 20 seconds in late L2 and mid L3 larvae. (b-c') Enlarged
493 view of cardiac tube in larvae expressing *bnl:GFP^{endo}* (green) and Col (red) in core
494 progenitors (b, c) and *bnl:GFP^{endo}* (white) in (b', c') in late L2 (b, b') and mid L3 larvae (c,
495 c'). Dotted lines indicate cardiac tube contour. *bnl:GFP^{endo}* decreases in mid L3 compared to
496 late L2 larvae. (d) Quantification. (e-f, h-i) Hnt (green) labels crystal cells. While crystal cells
497 are seldom found in late L2 (e), they massively differentiate in mid L3 larvae (f). Premature
498 crystal cell differentiation is observed in late L2 larvae when heartbeat accelerated (h) or
499 when a constitutively activated form of Piezo expressed in cardiac cells (i). (g, j) Crystal cell
500 index.

501 **Supplementary Figures**

502 **Figure S1: Heartbeat regulates lymph gland homeostasis.**

503 (a) Cardiac cells express *mcd8-GFP* (*NP1029>mcD8-GFP*, green). Transversal section
504 shown. No difference in cardiac tube morphology observed when heartbeat arrested
505 (*ORK1ΔC* or *Mhc-RNAi*). (b) Heart contraction number per 20 seconds when heart arrested

506 (*HandΔ>Ork1ΔC*). (c-d) P1 (red) labels plasmacytes (arrow). Compared to the control (c)
507 decreased P1 staining is observed when heart arrested (*NPI029>Ork1ΔC*, d). (e)
508 Plasmacyte index. (f, g) Hnt (green) labels crystal cells. Compared to the control (f) fewer
509 crystal cells are observed when heart arrested (*HandΔ>Ork1ΔC*, g). (h) Crystal cell index. (i-
510 j) Hnt (green) labels crystal cells. Compared to the control (i) fewer crystal cells are observed
511 when heart arrested (*NPI029>Mhc-RNAi*, j). (k) Crystal cell index. (l-m) Col (red) is
512 expressed at high levels in the PSC (arrow) and at lower levels in core progenitors (*).
513 Compared to the control (l) higher levels of Col are observed in core progenitors when heart
514 arrested (*Hand>Ork1ΔC*, m). (n) Core progenitor index. (o-p) Enlargement of PSC cells
515 labelled by Antp (red) antibody. No difference in PSC cell numbers is observed when heart
516 arrested (p) compared to the control (o). (q) Quantification of PSC cell number.

517 **Figure S2: Piezo expressed in anterior aorta cells controls lymph gland hematopoiesis.**

518 (a-b) *piezo(enhancer)>mcD8-GFP* and (c) *piezo-gal4(KI)>mcd8-GFP*. Col (red) labels PSC
519 (white arrow), lymph gland posterior lobes (red arrows) and pericardial cells (PC). In L3
520 larvae, *piezo* is expressed in cardiac cells (white arrowhead). (b) Enlargement of lymph gland
521 cortical zone expressing *piezo-gal4>mcd8-GFP* (green), *piezo* is expressed in crystal cells
522 labelled by Hnt (red). (d) In *piezo* null mutant, crystal cell (Hnt, green) differentiation is
523 decreased. (e) Crystal cell index. (f-g) Col (red) is expressed at high levels in the PSC (arrow)
524 and at lower levels in core progenitors (*). Compared to the control (f), number of core
525 progenitors increases when *piezo* knocked down in cardiac cells (g). (h) Core progenitor
526 index. (i-j, m-n) BcGFP (green, i-j) or Hnt (green, m-n) labels crystal cells. Compared to the
527 controls (i, m), fewer crystal cells are observed when *piezo* knocked down in cardiac cells (j)
528 or when a nonfunctional channel encoded by mPiezo1-2336-Myc expressed in cardiac cells
529 (n). (k-l, o) Crystal cell index. (p) *76E11-gal4>mcD8-GFP* is green; Col (red) labels the PSC
530 (white arrow), lymph gland posterior lobes (red arrows) and pericardial cells (PC). In L3

531 larvae, *76E11* is expressed in anterior aorta cells (green). (q-r) Hnt (green) labels crystal cells.
532 Compared to the control (q), fewer crystal cells are observed when *piezo* knocked down (r) in
533 cardiac cells using the *76E11-gal4* driver. (s) Crystal cell index. (t) Kymograph of heartbeat
534 in control (*handΔ>*) and when *piezo* knocked down in cardiac cells. (u) Number of heart
535 contractions per 20 seconds. No difference compared to the control is observed when *piezo*
536 knocked down in cardiac cells.

537 **Figure S3: Heartbeat activates N signaling which regulates blood cell differentiation.**

538 (a-b) GCaMP3 Ca^{2+} sensor (green) expressed under NP1029 driver. GCaMP3 intensity
539 decreases when heart arrested (b) compared to the control (a). (c) Quantification of GCaMP3
540 mean intensity. (d-f') Enlarged view of cardiac tube and a maximum projection of 10 confocal
541 lymph gland sections of larvae expressing *Notch[NRE]-GFP* (green) and Col (red) that labels
542 core progenitors (d-f) and *Notch[NRE]-GFP* (white) in (d'-f'). Dotted lines indicate cardiac
543 tube contour. Compared to the control (d-d'), *Notch[NRE]-GFP* in cardiac cells decreases
544 when N signaling inhibited in cardiac cells (e-e') or when and heart arrested (f-f'). (g-h)
545 Quantification of *Notch[NRE]-GFP* levels in cardiac cells. (i-j') Enlarged view of cardiac
546 tube of larvae expressing *NRE-GFP* (green, i) or *E(spl)mbeta-GFP* (green, j) and Col (red)
547 that labels core progenitors. For *NRE-GFP* and *E(spl)mbeta-GFP*, a maximum projection of 9
548 and 7 confocal lymph gland sections were used, respectively. (k-l') Enlarged view of cardiac
549 tube and a maximum projection of 10 confocal lymph gland sections of larvae expressing
550 *bnl:GFP^{endo}* (green) and Col (red) in core progenitors (k-l) and *bnl:GFP^{endo}* (white) in (k'-l').
551 *bnl:GFP^{endo}* expression increases when N signaling inhibited in cardiac cells (l-l') compared
552 to the control (k-k'). (m) Quantification in cardiac cells of *bnl:GFP^{endo}* cytoplasmic granules
553 relative to volume. (n-p, r-s) Hnt (green) labels crystal cells. Compared to controls (n, r),
554 crystal cell differentiation decreases when the N pathway inhibited by expressing a dominant

555 negative form of N (N^{xho} , o), a dominant negative form of *mam* (mam^{DN} , p), or *N-RNAi* (s) in
556 cardiac cells. (q, t) Crystal cell index.

557 **Movie 1:** heartbeat in control *NP1029; mcD8-GFP*>

558 **Movie 2:** heart is arrested in *NP1029, mcD8-GFP>Ork1ΔC*

559 **Movie 3:** heart is arrested in *NP1029, mcD8-GFP>Mhc-RNAi*

560 **Movie 4:** heartbeat in control *HandΔ; HandC-GFP*>

561 **Movie 5:** heartbeat is increased in *HandΔ; HandC-GFP>Ork1-RNAI*

562 **Movie 6:** heartbeat in *HandΔ; HandC-GFP >piezo- RNAi*

563 **Movie 7:** heartbeat in L2 larvae (*HandΔ; HandC-GFP*>)

564 **Movie 8:** heartbeat in L3 larvae (*HandΔ; HandC-GFP*>)

565

Fig1

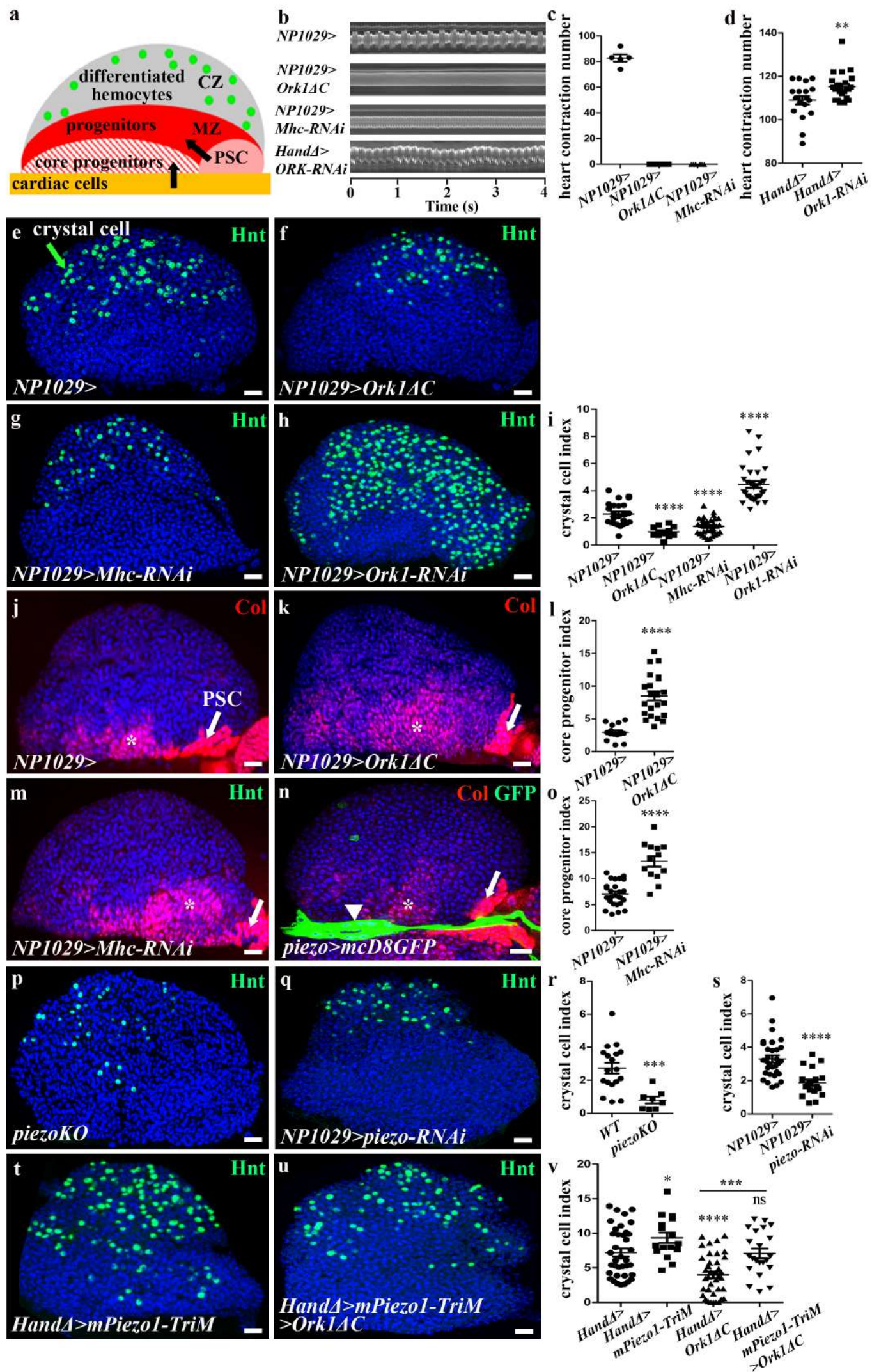


Figure 2: *piezo* controls *bnl* levels

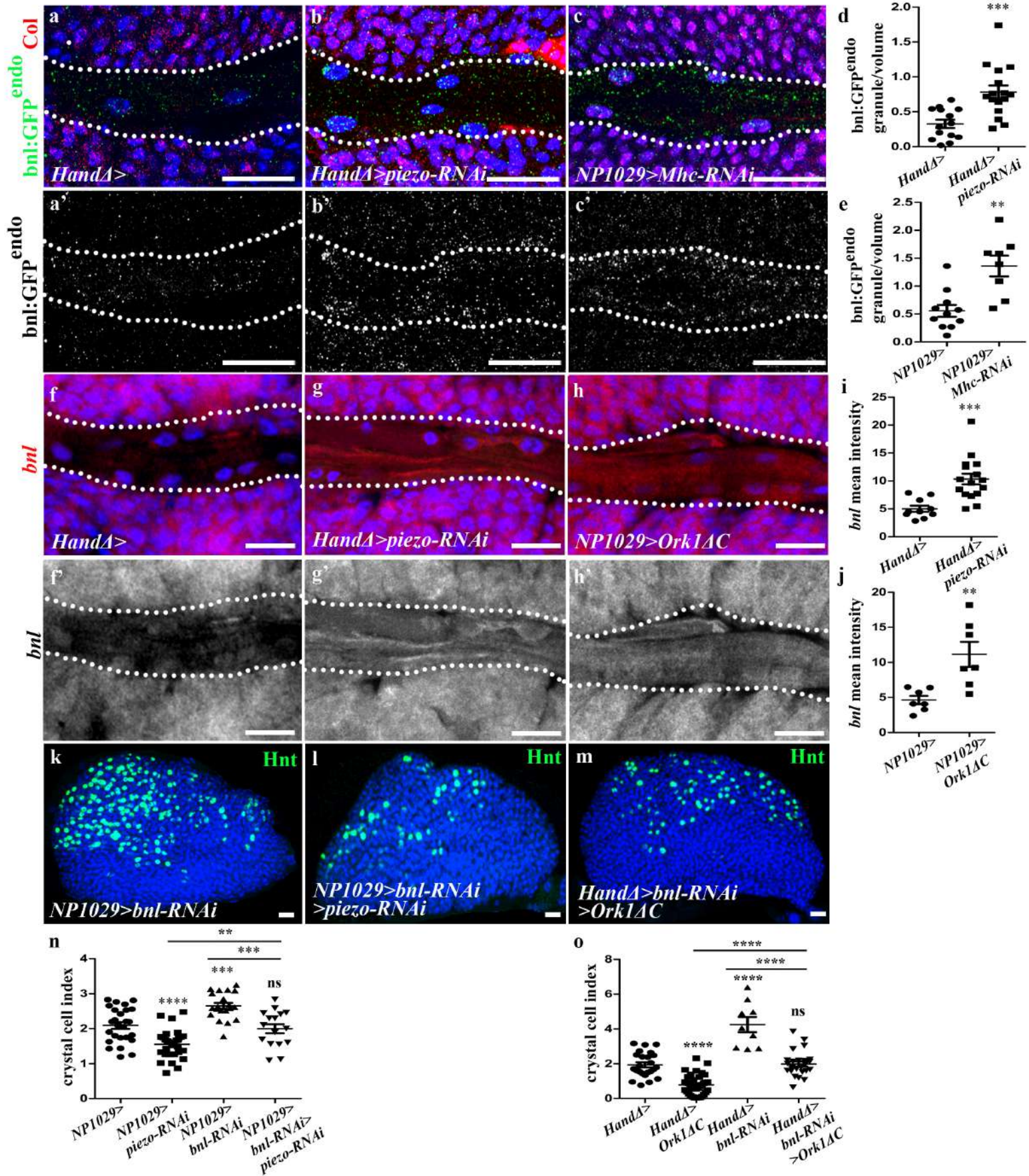


Fig 3

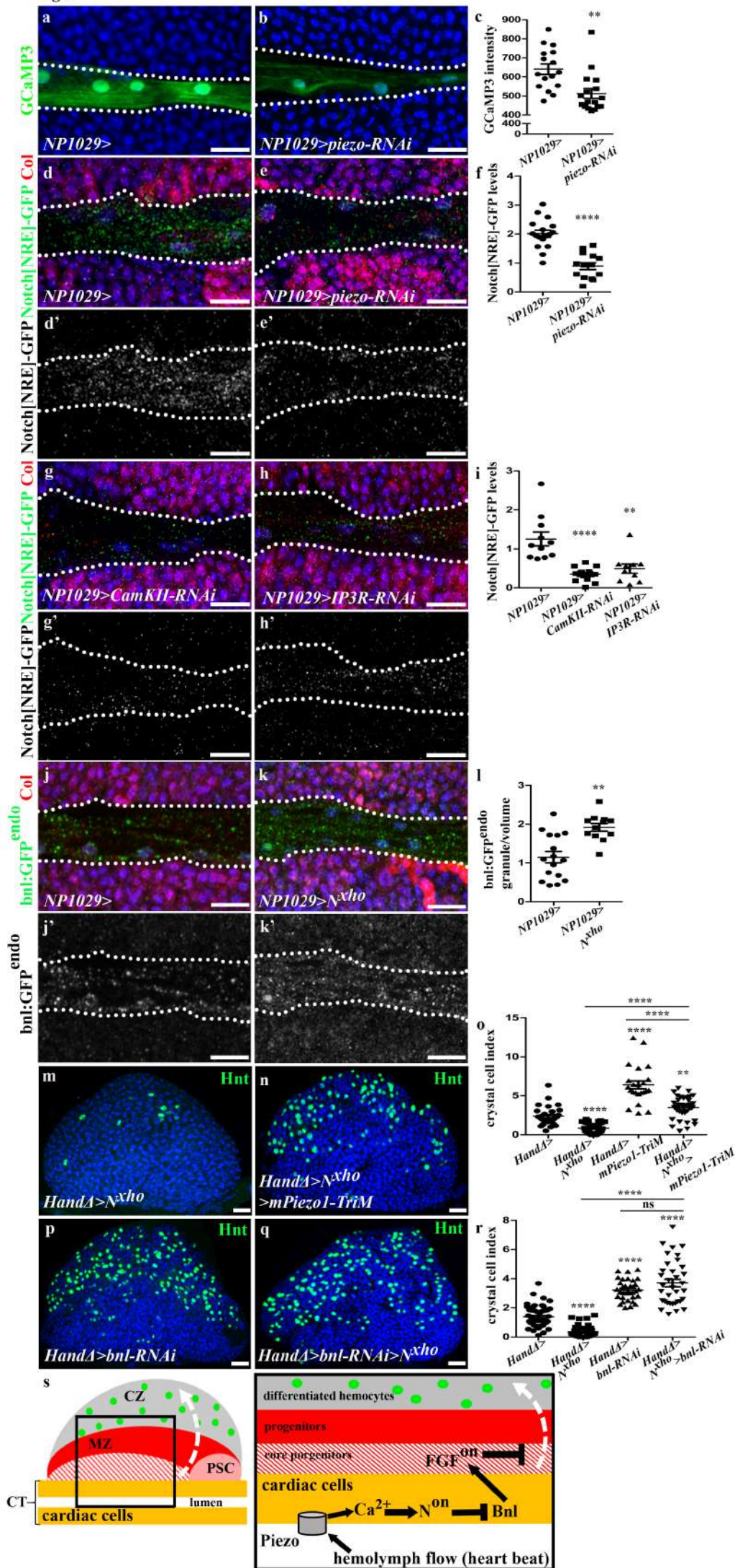
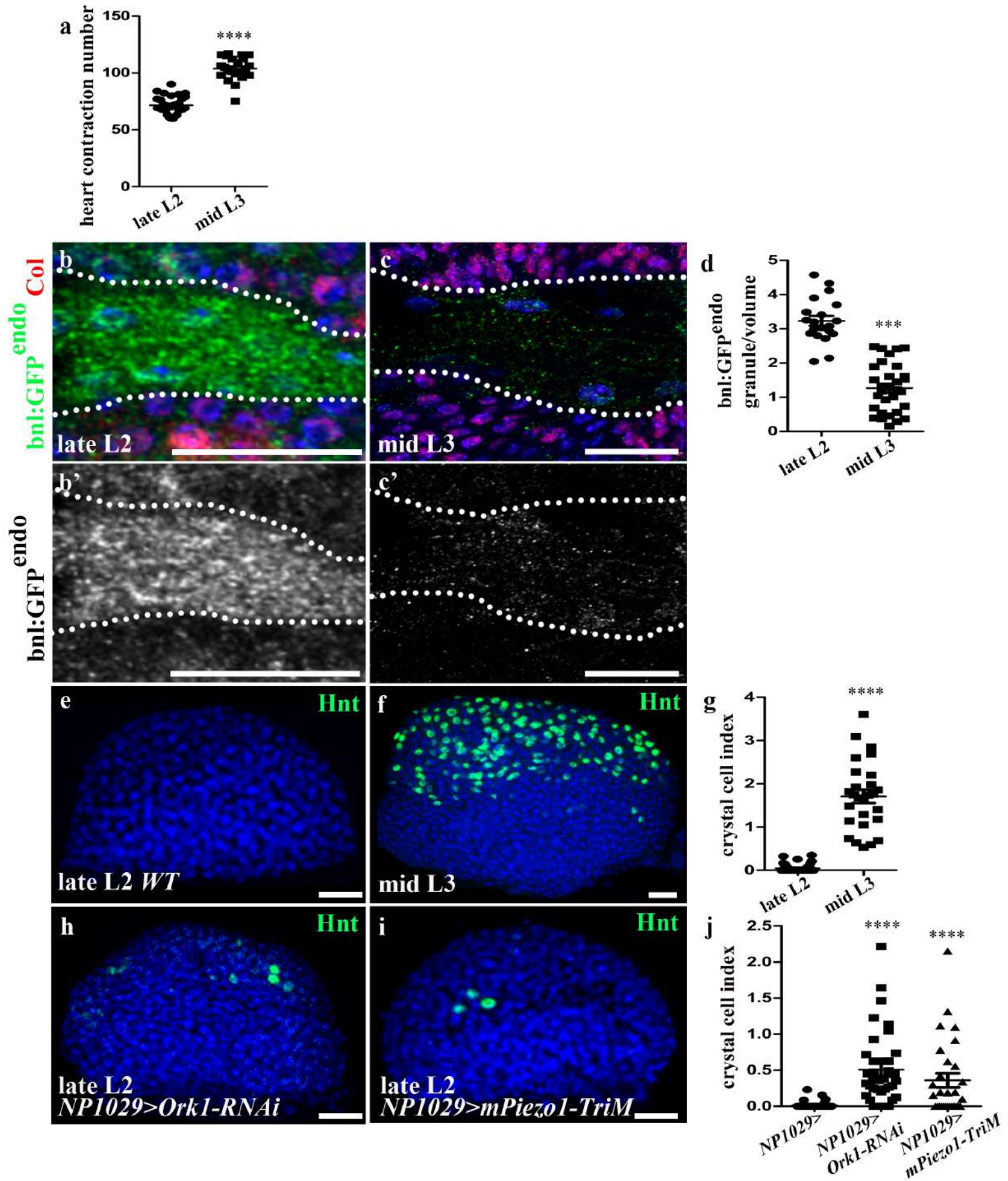
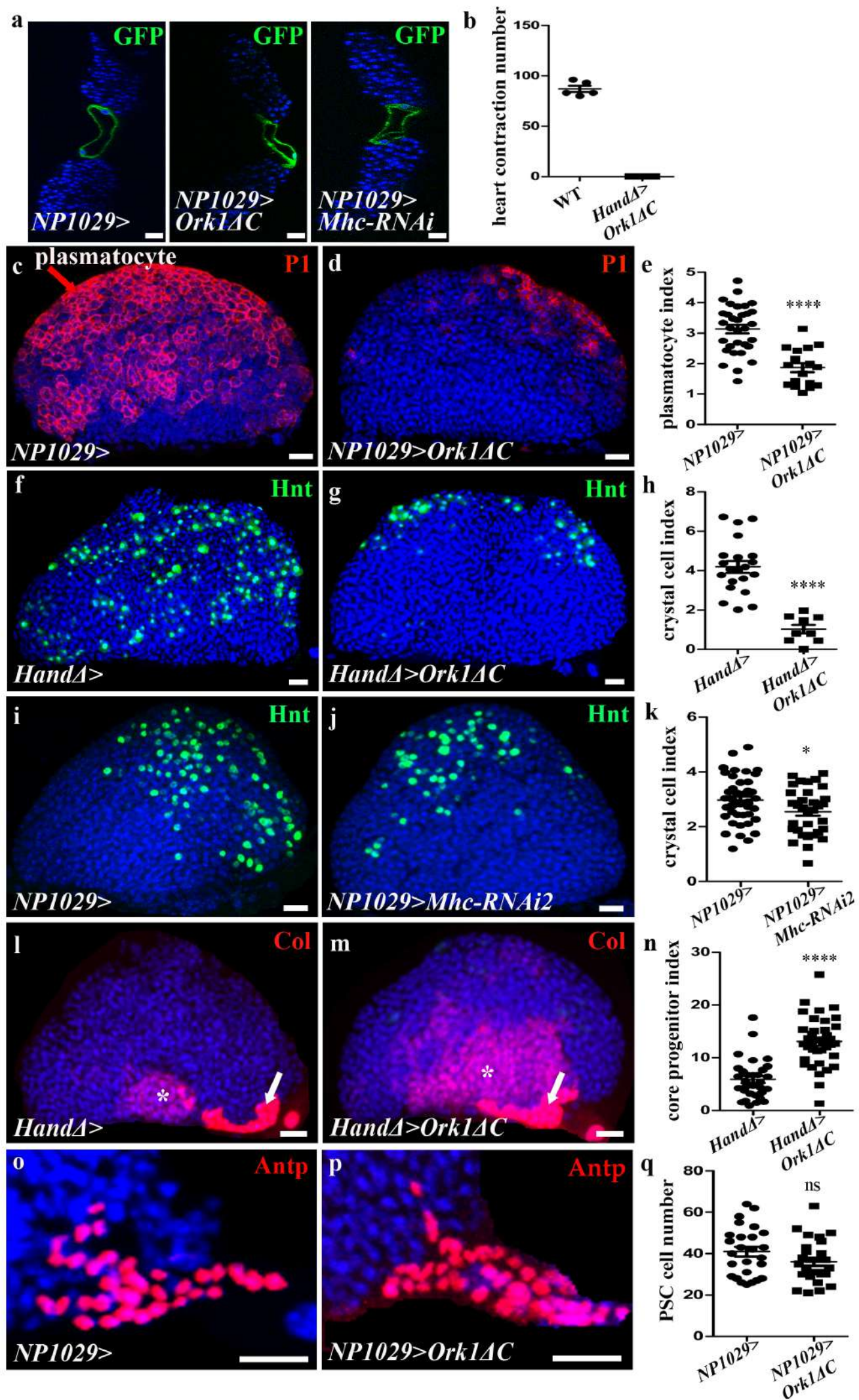


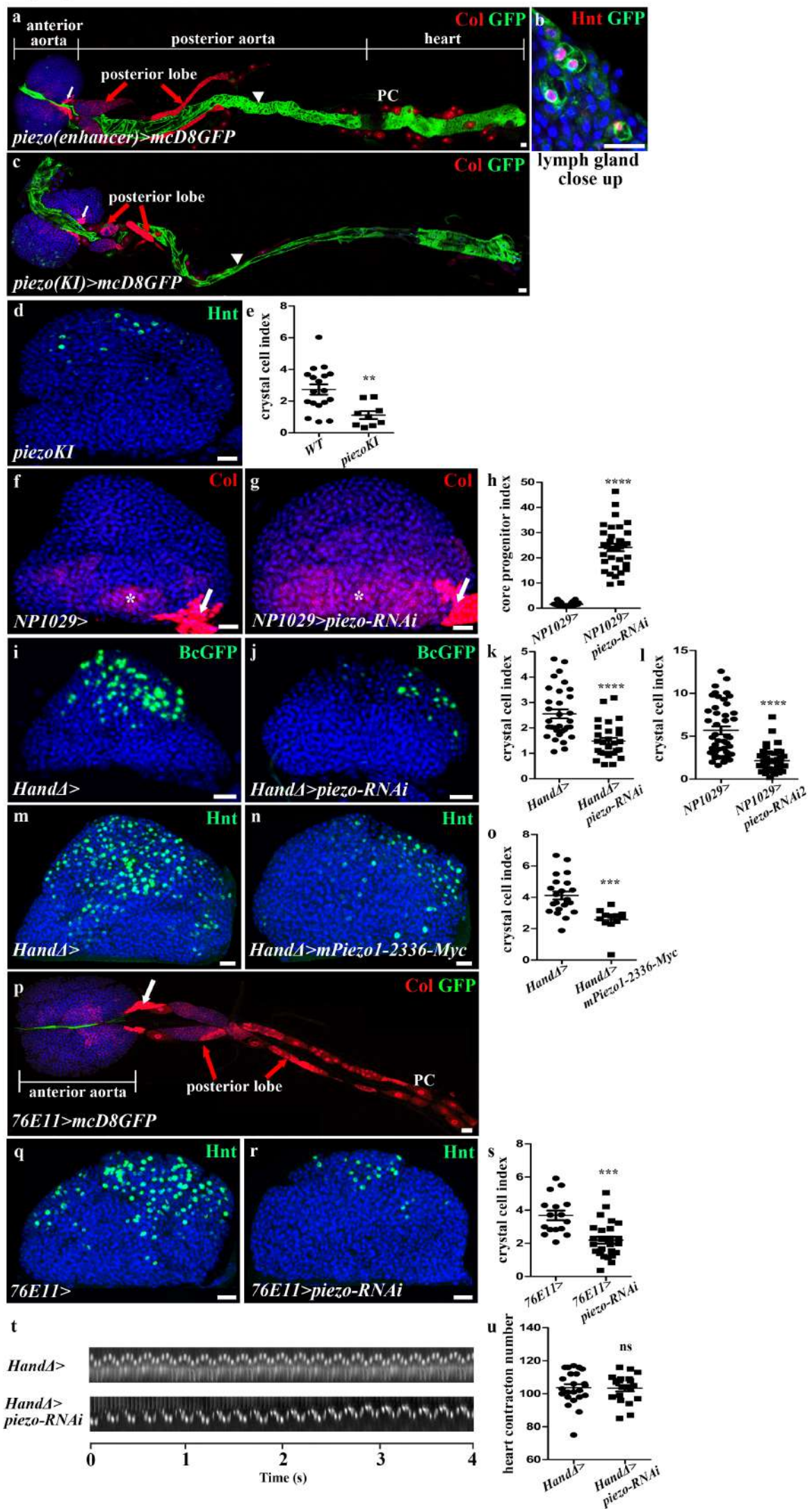
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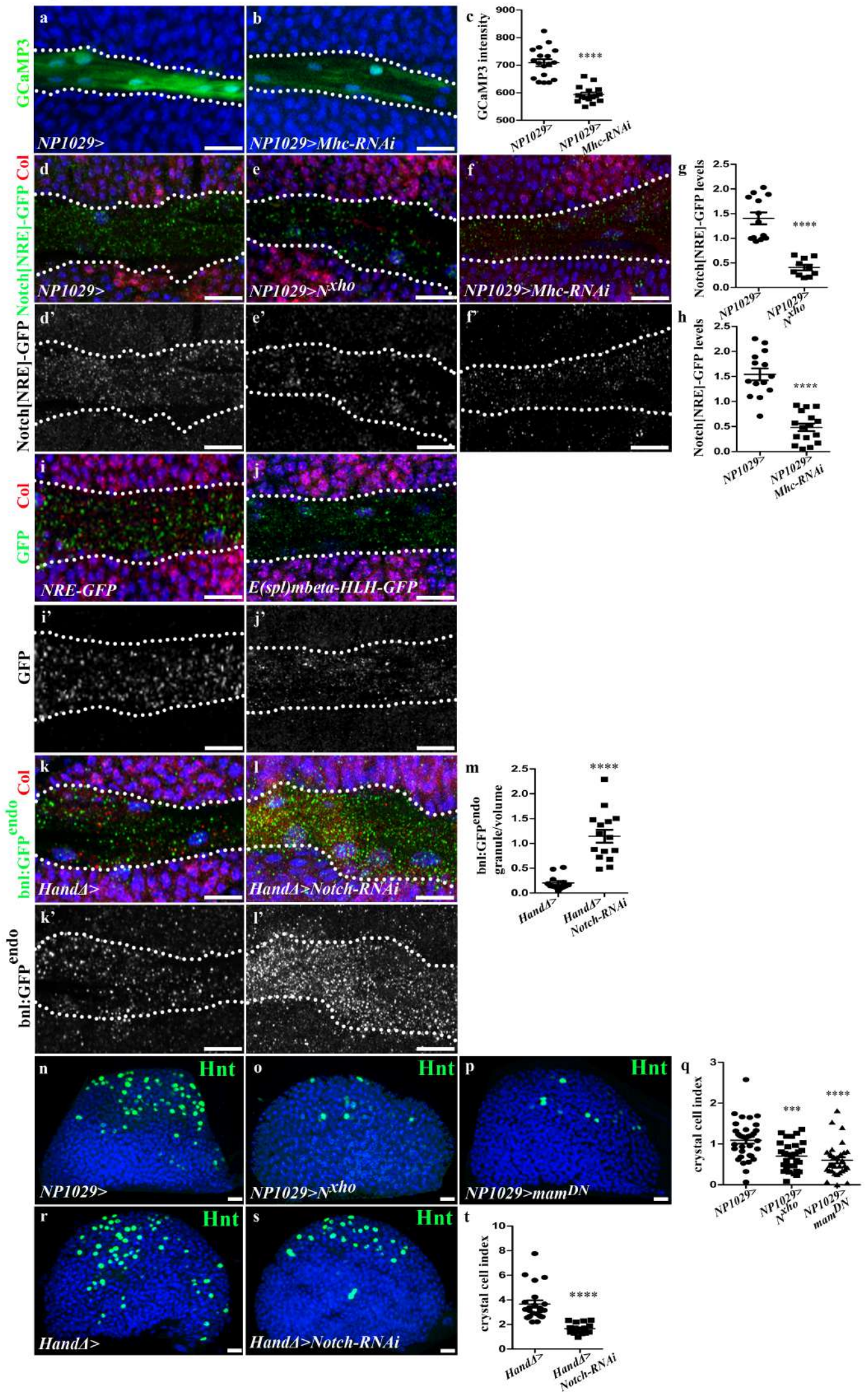
Sup Fig 1



Sup Fig 2



Sup Fig 3



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