

1 **Supplementary Figures and *Drosophila* genetics**

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

3 (a) Cardiac cells express a membrane-bound GFP (*NP1029>mcD8-GFP*, green). Transversal sections
4 of the anterior aorta are shown. (b) No difference is observed in the lumen upon heartbeat arrest (*Ork1ΔC*
5 or *Mhc-RNAi*). (c) Heart contraction number per 20 seconds when the heart is arrested
6 (*HandΔ>Ork1ΔC*). (d-e, g-h) Compared to the control (d, g) fewer crystal cells (Hnt, green) are
7 observed when the heart is arrested (*HandΔ>Ork1ΔC*, e and *NP1029>Mhc-RNAi*, h). (f, i) Crystal cell
8 index. (j-k) Col (red) is expressed at high levels in the PSC (arrow) and at lower levels in core
9 progenitors (*). Compared to the control (j) higher levels of Col are observed in core progenitors when
10 the heart is arrested (*Hand>Ork1ΔC*, k). (l) Core progenitor index. (m-n) Higher magnification view of
11 PSC cells labelled by Antp (red) antibody. No difference in PSC cell numbers is observed when heart
12 arrested (n) compared to the control (m). (o) Quantification of PSC cell numbers.

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

14 (a-b) *piezo(enhancer)>mcD8-GFP* in L3 larvae. Col (red) labels PSC (white arrow) and a subset of
15 lymph gland cells in anterior and posterior lobes (red arrows). PC for pericardial cells. In L3 larvae,
16 *piezo* (green) is expressed in cardiac cells (white arrowhead). (b) Higher magnification view of lymph
17 gland cortical zone expressing *piezo-Gal4>mcd8-GFP* (green), *piezo* is expressed in crystal cells (Hnt,
18 red). (c) Decreasing *piezo* in crystal cells by using the *lozenge (lz)-Gal4* driver leads to increased crystal
19 cell numbers (d-e) *piezo(enhancer)>mcD8-GFP* (d) and *piezo-Gal4(KI)>mcD8GFP* (e) in L2 larvae.
20 *piezo* (green) is expressed in aorta cells (white arrowhead). (f-g) In *piezo* null mutant, crystal cell (Hnt,
21 green) differentiation is decreased (g). (i-j and m-n) Compared to the controls (i, m), fewer crystal cells
22 (Hnt, green) are observed when *piezo* knocked down in cardiac cells (j) or when a nonfunctional channel
23 encoded by *mPiezo1-2336-Myc* expressed in cardiac cells (n). (k-l, o) Crystal cell index. (p) *76E11-*
24 *Gal4>mcD8-GFP* is green; Col (red) labels the PSC (white arrow), lymph gland posterior lobes (red
25 arrows) and pericardial cells (PC). In L3 larvae, *76E11-Gal4* promotes expression of membrane-bound
26 GFP in anterior aorta cells (green). (q-r) Compared to the control (q), fewer crystal cells (Hnt, green)
27 are observed when *piezo* is knocked down in cardiac cells using the *76E11-Gal4* driver (r). (s) Crystal

28 cell index. (t) Kymograph of heartbeat in control (*HandΔ>*) and when *piezo* knocked down in cardiac
29 cells (*HandΔ>piezo-RNAi*). (u) Number of heart contractions per 20 seconds. No difference compared
30 to the control is observed when *piezo* knocked down in cardiac cells.

31 **Figure S3: Piezo in progenitors does not regulate crystal cell differentiation and Col+ progenitors**
32 **are rescued by activating Piezo when heartbeat is blocked.**

33 (a-b, d-e) Crystal cell differentiation (Hnt, green) is not affected when *piezo* is knocked down in
34 progenitors using *tep4* (core-progenitors, a-b) or *dome* (MZ progenitors, d-e) drivers. (c, f) Crystal cell
35 index. (g-h, i-j) Active form of *piezo* (*mPiezol-TriM*) with arrested heart (*Ork1ΔC*) restores wild type
36 levels of Col expression (red) in progenitors (j). (k) core progenitor index

37 **Figure S4: Heartbeat activates N signaling which regulates Bnl levels .**

38 (a-b) GCaMP3 Ca²⁺ sensor (green) expressed under the control of the *NP1029* driver. GCaMP3 intensity
39 decreases when heart arrested (b) compared to the control (a). (c-d) Quantification of GCaMP3 mean
40 intensity in anterior (c) and posterior aorta (d). (e-g') Close-up view of cardiac tube of larvae expressing
41 *Notch[NRE]-GFP* (green in e-g and white in e'-g') and Col (red) that labels core progenitors (e-g).
42 Dotted lines indicate cardiac tube outline. Compared to the control (e-e'), *Notch[NRE]-GFP* in cardiac
43 cells decreases when N signaling is inhibited in cardiac cells (f-f') or when the heart is arrested (g-g').
44 (h-j) Quantification of *Notch[NRE]-GFP* levels in the aorta. (k-l') Close-up view of cardiac tube of
45 larvae expressing *NRE-GFP* (green, k) or *E(spl)mbeta-GFP* (green, l) and Col (red) that labels core
46 progenitors. (m-n', p-q') Close-up view of cardiac tube of larvae expressing *bnl:GFP^{endo}* (green in m-n,
47 p-q and white in m'-n', p'-q') and Col (red) in core progenitors (m-n, p-q). *bnl:GFP^{endo}* expression
48 increases when N signaling is inhibited in cardiac cells (n-n') compared to the control (m-m').
49 *bnl:GFP^{endo}* expression increases when an active form of *E(spl)mbeta* is expressed in cardiac cells (q-
50 q') compared to the control (p-p'). (o, r) Quantification of *bnl:GFP^{endo}* cytoplasmic granules relative to
51 volume.

52 **Figure S5: N signaling in cardiac cells regulates blood cell differentiation.**

53 (a-b, d-e) Compared to controls (a, d), crystal cell (Hnt, green) differentiation decreases when the N
54 pathway is inhibited in cardiac cells by expressing a dominant negative form of N (*N^{xho}*, b) or *N-RNAi*

55 (e). (c, f) Crystal cell index. (g-i) Compared to the control (g) fewer plasmatocytes (P1, red) are observed
56 when the N pathway is inhibited by expressing *N-RNAi* (h) or a dominant negative form of N (*N^{rho}*, i).
57 (j) Plasmatocyte index. (k-l) Col (red) labels core progenitors (*) and the PSC (arrow). Compared to the
58 control (k) core progenitors are more abundant when the N pathway is inhibited (l). (m) Core-progenitor
59 index.

60 **Figure S6: Several genes of N signaling are required in cardiac cells to regulate blood cell**
61 **differentiation.**

62 (a-b, d-e, g-h) Compared to control (a, d, g) crystal cell (Hnt, green) differentiation decreases when a
63 dominant negative form of *mam* (*mam^{DN}*, b), an activated form of *E(spl)mbeta* (c), a dominant negative
64 form of Kuz (*kuz^{DN}*, i), or *kuz-RNAi* (m) are expressed in cardiac cells. Compared to *kuz^{DN}* alone (i)
65 crystal cell (Hnt, green) differentiation is increased when *mPiezo1-TriM* and *kuz^{DN}* are simultaneously
66 expressed in cardiac cells (j). (c, f, k, n) Crystal cell index.

67 **Figure S7: N signaling is required in cardiac cells to regulate plasmatocyte differentiation and for**
68 **the maintenance of the Col+ progenitor pool.**

69 (a-b, d-e, f-g) Plasmatocyte differentiation (P1, red). *Kuz* knockdown in cardiac cells (b) leads to a
70 decrease in plasmatocyte differentiation compared to the control (a). (c) plasmatocyte index. Compared
71 to *kuz^{DN}* alone (f), plasmatocyte (P1, red) differentiation is increased when *mPiezo1-TriM* and *kuz^{DN}* are
72 simultaneously expressed in cardiac cells (g). (h) Plasmatocyte index. (i) Crystal cell index. Compared
73 to *kuz^{DN}* alone, crystal cell differentiation is increased when *bnl-RNAi* and *kuz^{DN}* are simultaneously
74 expressed in cardiac cells. (j) Plasmatocyte index. Plasmatocyte differentiation is increased when *bnl-*
75 *RNAi* and *kuz^{DN}* are simultaneously expressed in cardiac cells compared to *kuz^{DN}* alone. (k) core
76 progenitor index. Col+ core progenitor defect observed when *bnl* is knocked down (*bnl-RNAi*) is not
77 rescued by simultaneous *bnl* knockdown and N inhibition (k).

78 **Figure S8: Modulation of heartbeat rate during larval development regulates plasmatocyte**
79 **differentiation.**

80 (a-d) While plasmatocytes (P1, red) are seldom found in late L2 (a), they massively differentiate in mid
81 L3 larvae (b). Premature plasmatocyte differentiation is observed in late L2 larvae when heartbeat

82 accelerated (c), or when a constitutive active form of Piezo is expressed in cardiac cells (d). (e-f)
83 Plasmatocyte index.

84

85 **Movie 1:** heartbeat in control *NP1029, UAS-dicer; mcD8-GFP>w¹¹¹⁸*

86 **Movie 2:** heart is arrested in *NP1029, UAS-Dicer, mcD8-GFP>Ork1ΔC*

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

88 **Movie 4:** heartbeat in control *HandΔ, UAS-Dicer; HandC-GFP> w¹¹¹⁸*

89 **Movie 5:** heartbeat is increased in *HandΔ, UAS-Dicer ; HandC-GFP>Ork1-RNAI*

90 **Movie 6:** heartbeat in *HandΔ, UAS-Dicer ; HandC-GFP >piezo- RNAi*

91 **Movie 7:** heartbeat in L2 larvae in *HandΔ, UAS-Dicer ; HandC-GFP> w¹¹¹⁸*

92 **Movie 8:** heartbeat in L3 larvae in *HandΔ, UAS-Dicer ; HandC-GFP> w¹¹¹⁸*

93

94 **Drosophila genetics:** Fly crosses for each figure.

95 **Figure 1b:** *NP1029-gal4; UAS-dicer* crossed with *UAS-mcd8GFP*; *NP1029-gal4; UAS-dicer* crossed
96 with *UAS-mcd8GFP; UAS-Ork1ΔC*; *NP1029-gal4; UAS-dicer* crossed with *UAS-mcd8GFP; UAS-Mhc-*
97 *RNAi*; *HandΔ, UAS-dicer; HandC-GFP* crossed with *w 1118*; *HandΔ, UAS-dicer; HandC-GFP* crossed
98 with *UAS-Ork1-RNAi*; **1e, 1j, 1p:** *NP1029-gal4; UAS-dicer* crossed with *w 1118*; **1f, 1k, 1q:** *NP1029-*
99 *gal4; UAS-dicer* crossed with *UAS-Ork1ΔC*; **1g, 1m, 1s:** *NP1029-gal4; UAS-dicer* crossed with *UAS-*
100 *Mhc-RNAi*; **1h, 1n, 1t:** *NP1029-gal4; UAS-dicer* crossed with *UAS-Ork1-RNAi*.

101 **Figure 2a:** *piezo-gal4(KI)* crossed with *UAS-mcd8GFP*; **2b:** *piezo (enhancer)-gal4* crossed with *UAS-*
102 *mcd8GFP*; **2c:** *w 1118*; **2d:** *piezo (KO)*; **2e, 2n, 2p:** *NP1029-gal4; UAS-dicer* crossed with *w 1118*; **2f,**
103 **2o, 2q:** *NP1029-gal4; UAS-dicer* crossed with *UAS-piezo-RNAi*; **2i, 2t:** *HandΔ, UAS-dicer* crossed with
104 *w 1118*; **2j, 2u:** *HandΔ, UAS-dicer* crossed with *UAS-mPiezo1-TriM*; **2k, 2v:** *HandΔ, UAS-dicer* crossed
105 with *UAS-Ork1ΔC*; **2l, 2w:** *HandΔ, UAS-dicer; UAS-mPiezo1-TriM* crossed with *UAS-Ork1ΔC*.

106 **Figure 3a:** *HandΔ, UAS-dicer; bnl:GFPendo* crossed with *w 1118*; **3b:** *HandΔ, UAS-dicer;*
107 *bnl:GFPendo* crossed with *UAS-piezo-RNAi*; **3c:** *NP1029-gal4; bnl:GFPendo* crossed with *UAS-Mhc-*
108 *RNAi*; **3h:** *HandΔ; UAS-mcd8GFP* crossed with *w 1118*; **3i:** *HandΔ; UAS-mcd8GFP* crossed with *UAS-*

109 *dicer*; *UAS-piezo-RNAi*; **3j**: *NP1029-gal4* crossed with *UAS-Ork1ΔC*; **3m**: *NP1029-gal4*; *UAS-dicer*
110 crossed with *w 1118*; **3n**: *NP1029-gal4*; *UAS-dicer* crossed with *UAS-piezo-RNAi*; **3o**: *NP1029-gal4*;
111 *UAS-dicer* crossed with *UAS-bnl-RNAi*; **3p**: *NP1029-gal4*; *UAS-bnl-RNAi* crossed with *UAS-dicer*;
112 *UAS-piezo-RNAi*; **3r, 3w**: *HandΔ*, *UAS-dicer* crossed with *w 1118*; **3s, 3x**: *HandΔ*, *UAS-dicer* crossed
113 with *UAS-Ork1ΔC*; **3t, 3y**: *HandΔ*, *UAS-dicer* crossed with *UAS-bnl-RNAi*; **3u, 3z**: *HandΔ*, *UAS-dicer*;
114 *UAS-bnl-RNAi* crossed with *UAS-Ork1ΔC*.

115 **Figure 4a**: *NP1029-gal4*; *UAS-GCaMP3* crossed with *w 1118*; **4b**: *NP1029-gal4*; *UAS-GCaMP3*
116 crossed with *UAS-dicer*; *UAS-piezo-RNAi*; **4e**: *NP1029-gal4*; *Notch[NRE]-GFP* crossed with *w 1118*;
117 **4f**: *NP1029-gal4*; *Notch[NRE]-GFP* crossed with *UAS-dicer*; *UAS-piezo-RNAi*; **4i**: *NP1029-gal4*;
118 *Notch[NRE]-GFP* crossed with *UAS-CaMKII-RNAi*; **4j**: *NP1029-gal4*; *Notch[NRE]-GFP* crossed with
119 *UAS-IP3R-RNAi*; **4k**: *NP1029-gal4*; *bnl:GFPendo* crossed with *w 1118*; **4l**: *NP1029-gal4*;
120 *bnl:GFPendo* crossed with *UAS-N^{xho}*; **4o**: *HandΔ*, *UAS-dicer* crossed with *w 1118*; **4p**: *HandΔ*, *UAS-*
121 *dicer* crossed with *UAS-mPiezo1-TriM*; **4q**: *HandΔ*, *UAS-dicer* crossed with *UAS-N^{xho}*; **4r**: *HandΔ*, *UAS-*
122 *dicer*; *UAS-mPiezo1-TriM* crossed with *UAS-N^{xho}*; **4s**: *HandΔ*, *UAS-dicer* crossed with *UAS-bnl-RNAi*;
123 **4t**: *HandΔ*, *UAS-dicer*; *UAS-bnl-RNAi* crossed with *UAS-N^{xho}*.

124 **Figure 5a, 5b**: *bnl:GFPendo*; **5e, 5f**: *Notch[NRE]-GFP*; **5h, 5i**: *w 1118*; **5k**: *NP1029-gal4*; *UAS-dicer*
125 crossed with *UAS-Ork1-RNAi*; **5l**: *NP1029-gal4*; *UAS-dicer* crossed with *UAS-mPiezo1-TriM*; **5n**:
126 *HandΔ*, *UAS-dicer*; *bnl:GFPendo* crossed with *w 1118*; **5o**: *HandΔ*, *UAS-dicer*; *bnl:GFPendo* crossed
127 with *UAS-mPiezo1-TriM*; **5q**: *NP1029-gal4*; *Notch[NRE]-GFP* crossed with *w 1118*; **5r**: *NP1029-gal4*;
128 *Notch[NRE]-GFP* crossed with *UAS-mPiezo1-TriM*.

129 **Figure S1a**: *NP1029-gal4*; *UAS-dicer* crossed with *UAS-mcd8GFP*; *NP1029-gal4*; *UAS-dicer* crossed
130 with *UAS-mcd8GFP*; *UAS-Ork1ΔC*; and *NP1029-gal4*; *UAS-dicer* crossed with *UAS-mcd8GFP*; *UAS-*
131 *Mhc-RNAi*; **S1c**: *HandΔ*, *UAS-dicer* crossed with *w 1118*; *HandΔ*, *UAS-dicer* crossed with *UAS-*
132 *Ork1ΔC*; **S1d, S1j**: *HandΔ*, *UAS-dicer* crossed with *w 1118*; **S1e, S1k**: *HandΔ*, *UAS-dicer* crossed with
133 *UAS-Ork1ΔC*; **S1g, S1m**: *NP1029-gal4*; *UAS-dicer* crossed with *w 1118*; **S1h**: *NP1029-gal4*; *UAS-dicer*
134 crossed with *UAS-Mhc-RNAi2*; **S1n**: *NP1029-gal4*; *UAS-dicer* crossed with *UAS-Ork1ΔC*.

135 **Figure S2a**: *piezo (enhancer)-gal4* crossed with *UAS-mcd8GFP*; **S2b**: *piezo (KI)-gal4* crossed with
136 *UAS-mcd8GFP*; **S2d**: *piezo (enhancer)-gal4* crossed with *UAS-mcd8GFP*; **S2e**: *piezo-gal4(KI)* crossed

137 with *UAS-mcd8GFP*; **S2f**: *w 1118*; **S2g**: *piezo-gal4 (KI)*; **S2i**: *HandΔ*, *UAS-dicer* crossed with *w 1118*;
138 **S2j**: *HandΔ*, *UAS-dicer* crossed with *UAS-piezo-RNAi*; **S2m**: *HandΔ*, *UAS-dicer* crossed with *w 1118*;
139 **S2n**: *HandΔ*, *UAS-dicer* crossed with *UAS-mPiezo1-2336-Myc*; **S2p**: *76E11-gal4* crossed with *UAS-*
140 *mcd8GFP*; **S2q**: *tub-gal80ts*; *76E11-gal4* crossed with *w 1118*; **S2r**: *tub-gal80ts*; *76E11-gal4* crossed
141 with *UAS-dicer*; *UAS-piezo-RNAi*; **S2t**: *HandΔ*, *UAS-dicer*; *HandC-GFP* crossed with *w 1118*; *HandΔ*,
142 *UAS-dicer*; *HandC-GFP* crossed with *UAS-piezo-RNAi*.

143 **Figure S3a**: *tep4-gal4* crossed with *w 1118*; **S3b**: *tep4-gal4* crossed with *UAS-dicer*; *UAS-piezo-RNAi*;
144 **S3d**: *dome-gal4* crossed with *w 1118*; **S3e**: *dome-gal4* crossed with *UAS-dicer*; *UAS-piezo-RNAi*; **S3g**:
145 *HandΔ*, *UAS-dicer* crossed with *w 1118*; **S3h**: *HandΔ*, *UAS-dicer* crossed with *UAS-mPiezo1-TriM*; **S3i**:
146 *HandΔ*, *UAS-dicer* crossed with *UAS-Ork1ΔC*; **S3j**: *HandΔ*, *UAS-dicer*; *UAS-mPiezo1-TriM* crossed
147 with *UAS-Ork1ΔC*.

148 **Figure S4a**: *NP1029-gal4*; *UAS-GCaMP3* crossed with *w 1118*; **S4b**: *NP1029-gal4*; *UAS-GCaMP3*
149 crossed with *UAS-Mhc-RNAi*; **S4e**: *NP1029-gal4*; *Notch[NRE]-GFP* crossed with *w 1118*; **S4f**:
150 *NP1029-gal4*; *Notch[NRE]-GFP* crossed with *UAS-N^{aho}*; **S4g**: *NP1029-gal4*; *Notch[NRE]-GFP* crossed
151 with *UAS-Mhc-RNAi*; **S4k**: *NRE-GFP*; **S4l**: *E(spl)mbeta-GFP*; **S4m**, **S4p**: *HandΔ*, *UAS-dicer*;
152 *bnl:GFPendo* crossed with *w 1118*; **S4n**: *HandΔ*, *UAS-dicer*; *bnl:GFPendo* crossed with *UAS-Notch-*
153 *RNAi*; **S4q**: *HandΔ*, *UAS-dicer*; *bnl:GFPendo* crossed with *UAS-E(spl)mbeta-Act*.

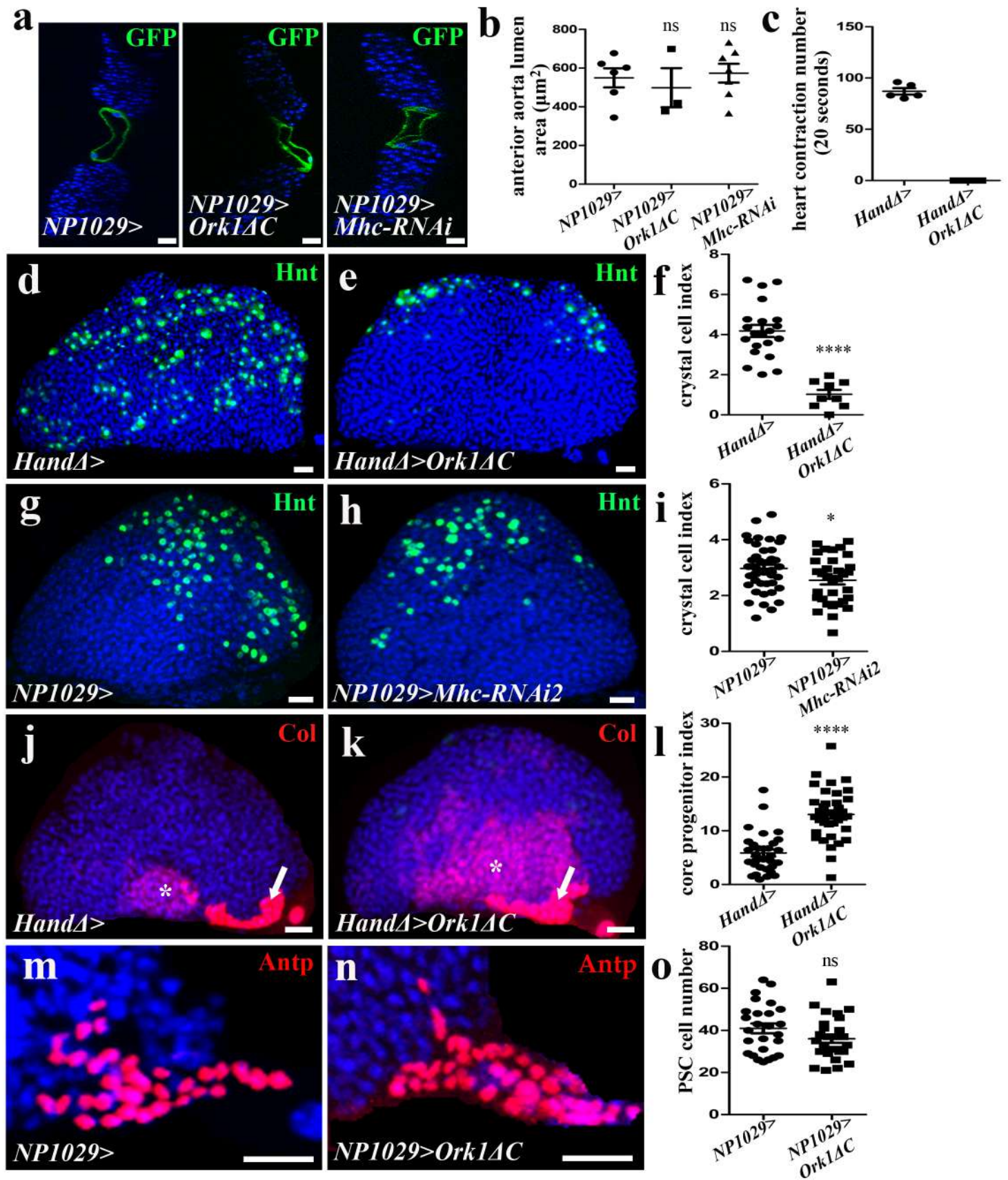
154 **Figure S5a**: *NP1029-gal4*, *UAS-dicer* crossed with *w 1118*; **S5b**: *NP1029-gal4*, *UAS-dicer* crossed with
155 *UAS-N^{aho}*; **S5d**, **S5g**, **S5k**: *HandΔ*, *UAS-dicer* crossed with *w 1118*; **S5e**, **S5h**: *HandΔ*, *UAS-dicer* crossed
156 with *UAS-Notch-RNAi*; **S5i**, **S5l**: *HandΔ*, *UAS-dicer* crossed with *UAS-N^{aho}*.

157 **Figure S6a**, **S6l**: *NP1029-gal4*, *UAS-dicer* crossed with *w 1118*; **S6b**: *NP1029-gal4*, *UAS-dicer* crossed
158 with *UAS-mam^{DN}*; **S6d**, **S6g**: *HandΔ*, *UAS-dicer* crossed with *w 1118*; **S6e**: *HandΔ*, *UAS-dicer* crossed
159 with *UAS-E(spl)mbeta-Act*; **S6h**: *HandΔ*, *UAS-dicer* crossed with *UAS-mPiezo1-TriM*; **S6i**: *HandΔ*,
160 *UAS-dicer* crossed with *UAS-kuz^{DN}*; **S6j**: *HandΔ*, *UAS-dicer*; *UAS-mPiezo1-TriM* crossed with *UAS-*
161 *kuz^{DN}*; **S6m**: *NP1029-gal4*, *UAS-dicer* crossed with *UAS-kuz-RNAi*.

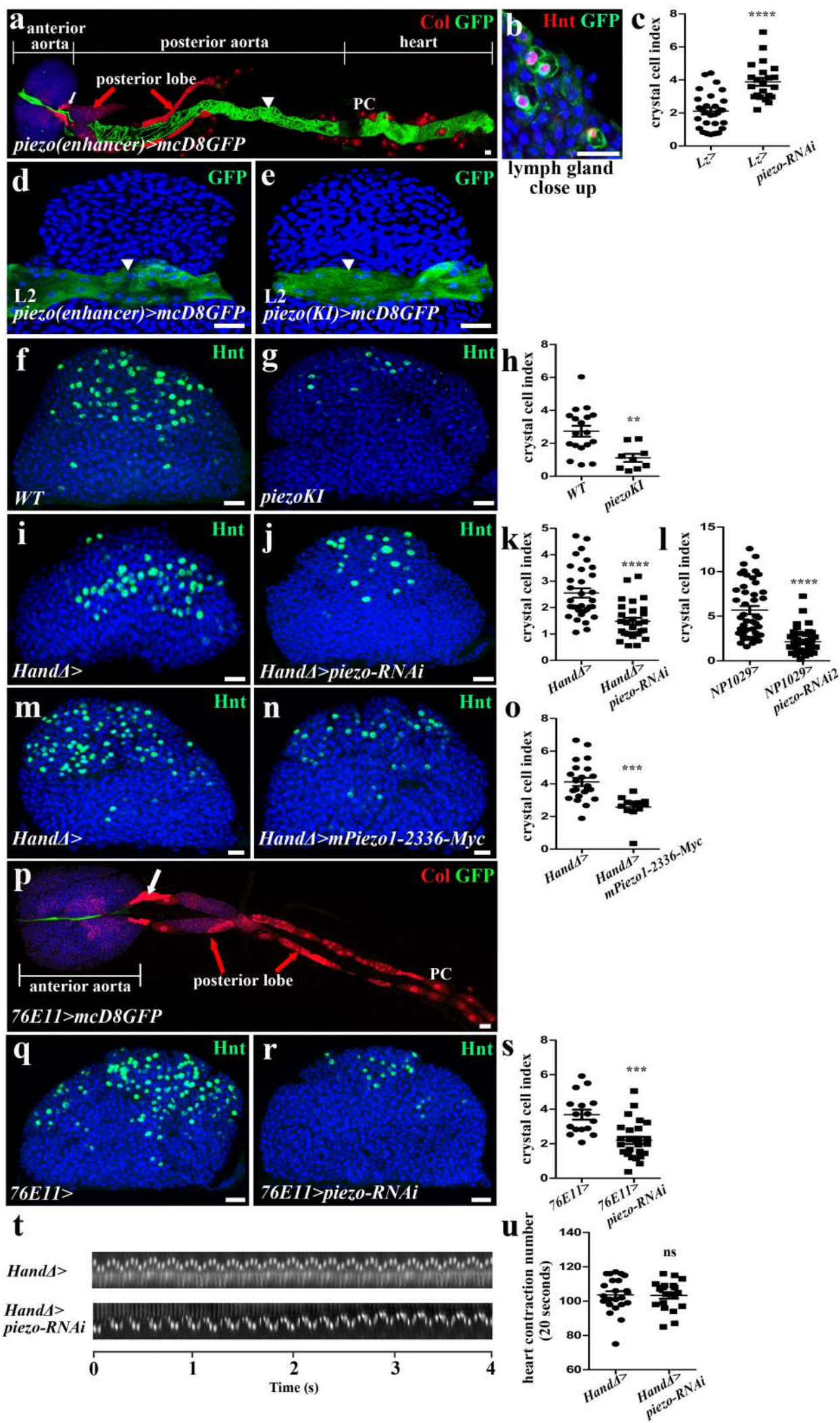
162 **Figure S7a**: *NP1029-gal4*, *UAS-dicer* crossed with *w 1118*; **S7b**: *NP1029-gal4*, *UAS-dicer* crossed with
163 *UAS-kuz-RNAi*; **S7d**: *HandΔ*, *UAS-dicer* crossed with *w 1118*; **S7e**: *HandΔ*, *UAS-dicer* crossed with
164 *UAS-mPiezo1-TriM*; **S7f**: *HandΔ*, *UAS-dicer* crossed with *UAS-kuz^{DN}*; **S7g**: *HandΔ*, *UAS-dicer*; *UAS-*

165 *mPiezo1-TriM* crossed with *UAS-kuz^{DN}*; **S7i, S7j**: *HandΔ*, *UAS-dicer* crossed with *w 1118*; *HandΔ*, *UAS-*
166 *dicer* crossed with *UAS-kuz^{DN}*; *HandΔ*, *UAS-dicer* crossed with *UAS-bnl-RNAi*; *HandΔ*, *UAS-dicer*;
167 *UAS-bnl-RNAi* crossed with *UAS-kuz^{DN}*; **S7k**: *HandΔ*, *UAS-dicer* crossed with *w 1118*; *HandΔ*, *UAS-*
168 *dicer* crossed with *UAS-bnl-RNAi*; *HandΔ*, *UAS-dicer*; *UAS-bnl-RNAi* crossed with *UAS-N^{xho}*.
169 **Figure S8a, S8b**: *NP1029-gal4*, *UAS-dicer* crossed with *w 1118*; **S8c**: *NP1029-gal4*, *UAS-dicer* crossed
170 with *UAS-Ork1-RNAi*; **S8d**: *HandΔ*, *UAS-dicer* crossed with *UAS-mPiezo1-TriM*.
171

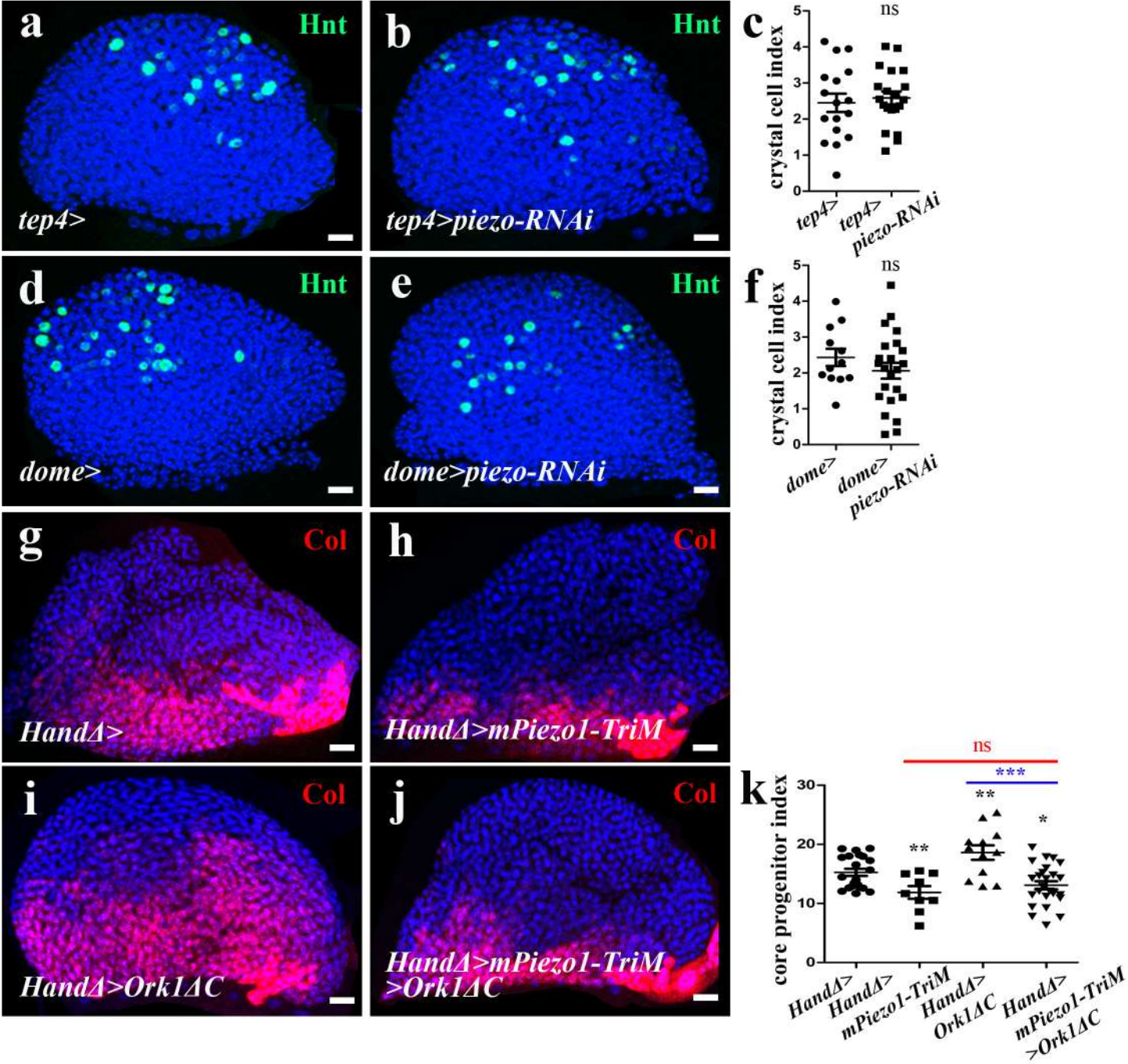
Sup Fig 1



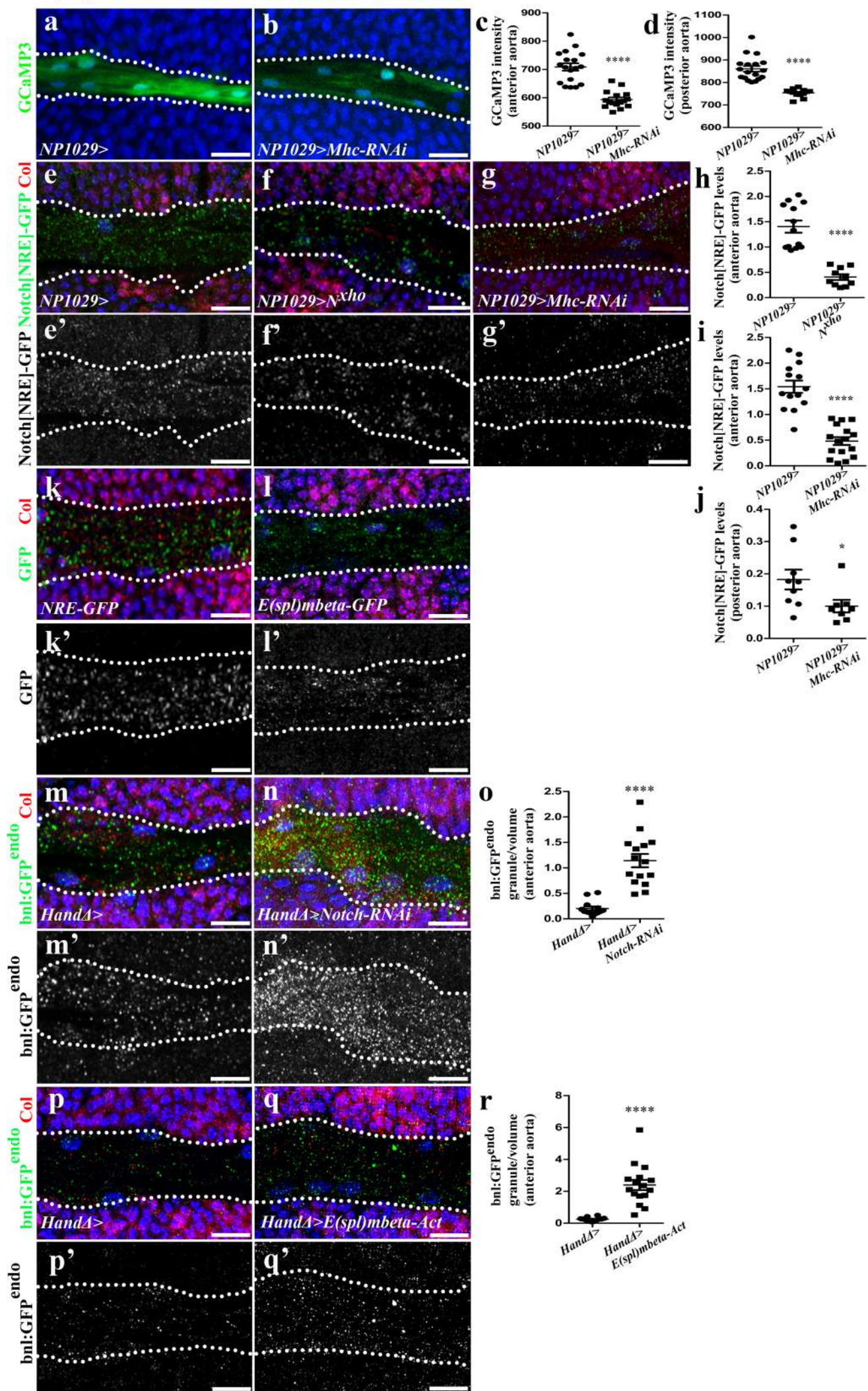
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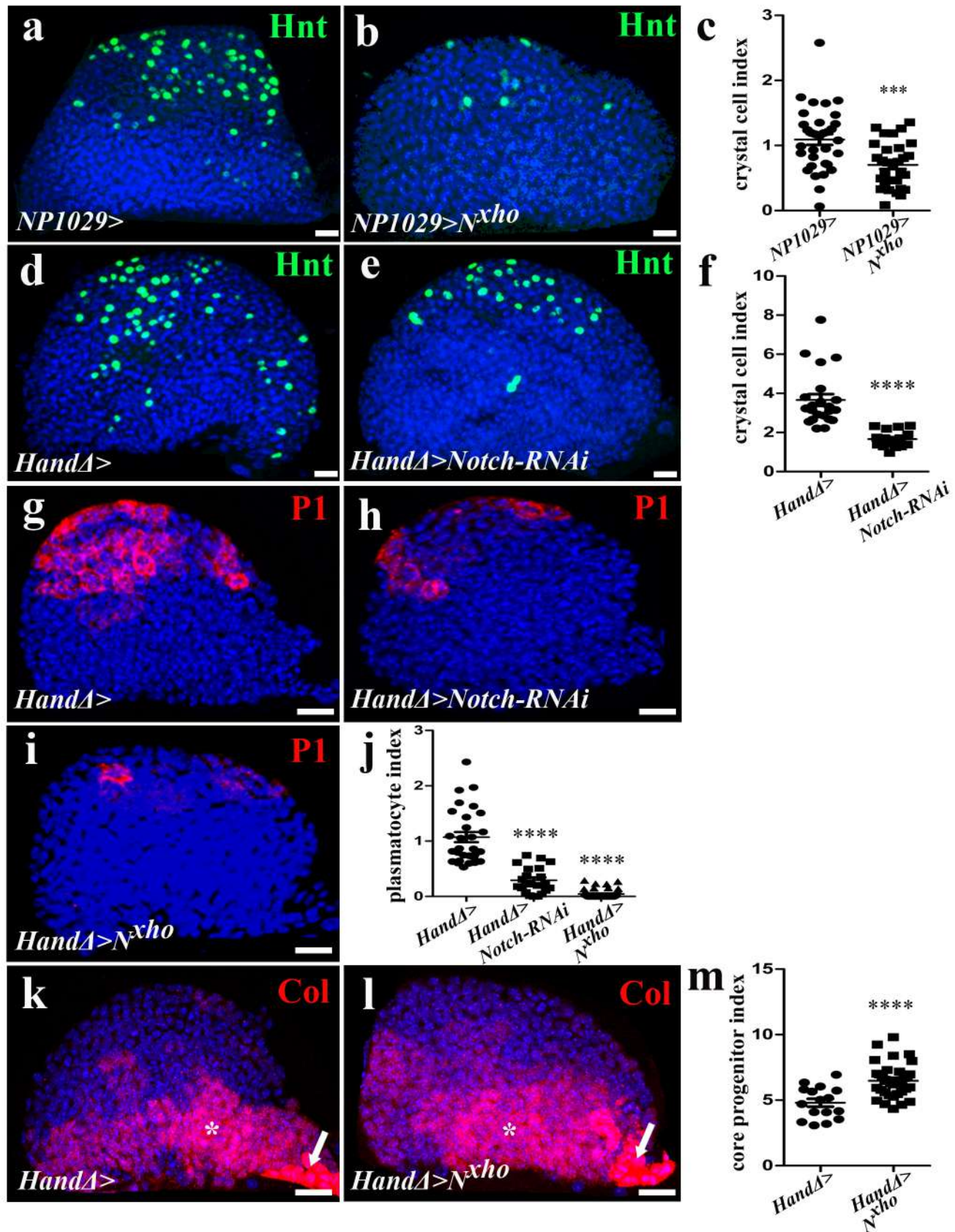
sup Figure 3



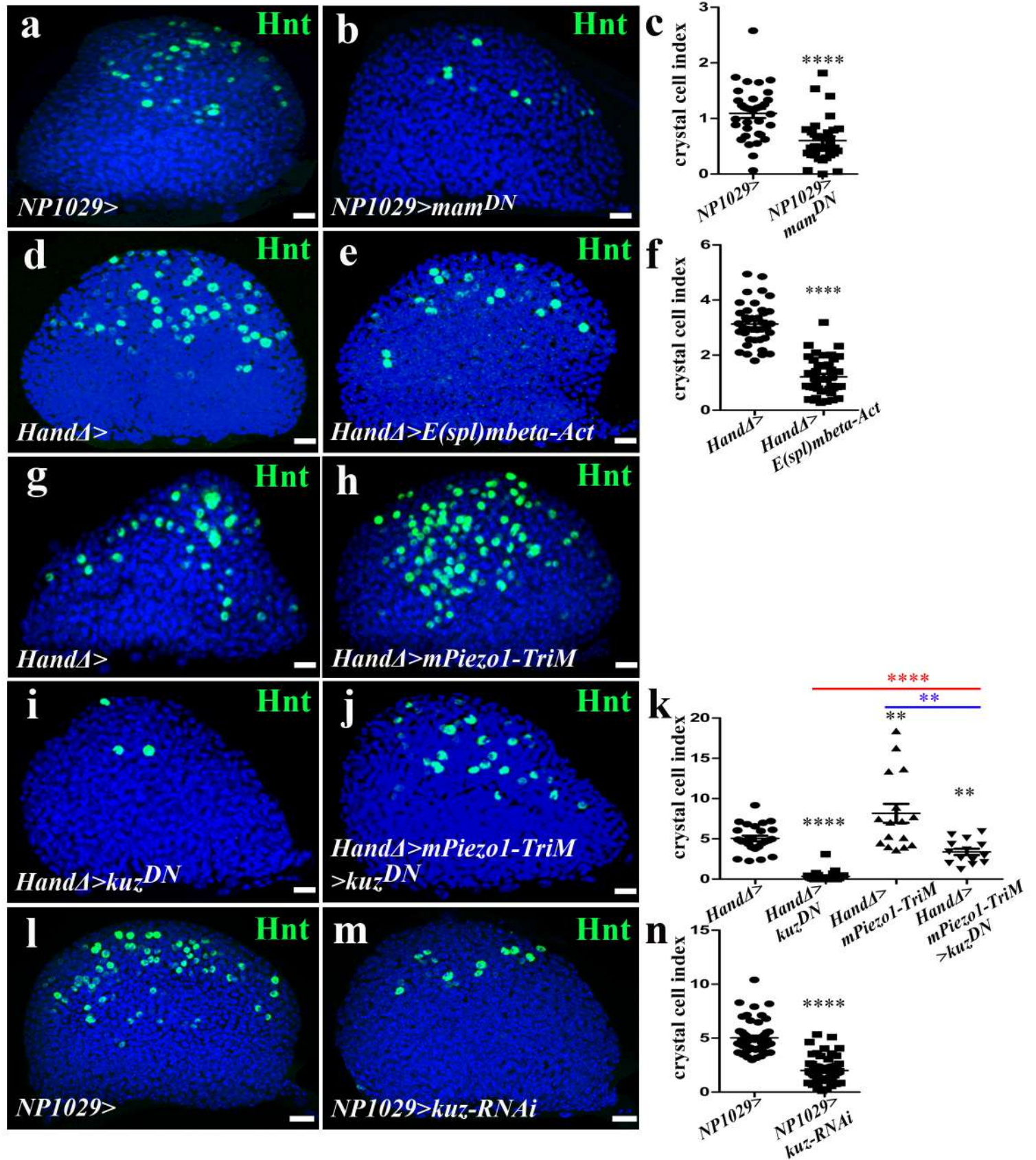
Sup Fig 4



Sup Fig 5



Sup Fig 6



Sup Fig 7

