RESEARCH ARTICLE

Figures and figure supplements

The vascular niche controls Drosophila hematopoiesis via fibroblast growth factor signaling

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Figure 1. Lymph gland organization and RNAi screen results. (a) Representation of lymph gland anterior and posterior lobes from third instar larvae. The anterior lobe is composed of progenitors (red) and core progenitors (hatched red), and the cortical zone (CZ, green). The PSC is blue and

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the cardiac tube (CT)/vascular system, is orange. PC corresponds to pericardial cell. (**b**) Summary of the screen performed by expressing RNAi in cardiac cells using the *hand*Δ*-gal4* driver. The number of genes corresponding to the different classes of phenotype is given. Subsequent panels illustrate the control and observed lymph gland defects (**c, d, g, j**). Anterior lobe and PSC are delimited by white and yellow dashed lines, respectively. Black-cell-GFP (BcGFP, white) labels crystal cells and Antp (black) the PSC. (**c', d', g', j'**) BcGFP is in green; (**e, h, k**) PSC cell numbers; (**f, i, l**) Crystal cell index. (**c–f**) Reducing *ilp6* in cardiac cells (**d, d'**) augments PSC cell number (**e**) without affecting crystal cell differentiation (**f**); this defines class 1. (**g–i**) Knocking down *dachsous (ds)* in cardiac cells (**g, g'**) decreases PSC cell number (**h**) and increases crystal cell index (**i**); this defines class 2. (**j–l**) Reducing *pvf3* in cardiac cells (**j, j'**) does not modify PSC cell number (**k**) but increases crystal cell differentiation (**l**); this defines class 3. (**m, n**) *tep4* (red) labels core progenitors. Decrease in *tep4* expression is observed when *pvf3* is knocked down in cardiac cells. (**o**) *tep4* index. For all quantifications and figures, statistical analysis *t*-test (Mann-Whitney nonparametric test) was performed using GraphPad Prism five software. Error bars represent SEM and *p<0,1; **p<0,01; ***p<0,001; ****p<0,0001 and ns (not significant). In all confocal pictures nuclei are labeled with Topro (blue) and scale bars = 20 µm.

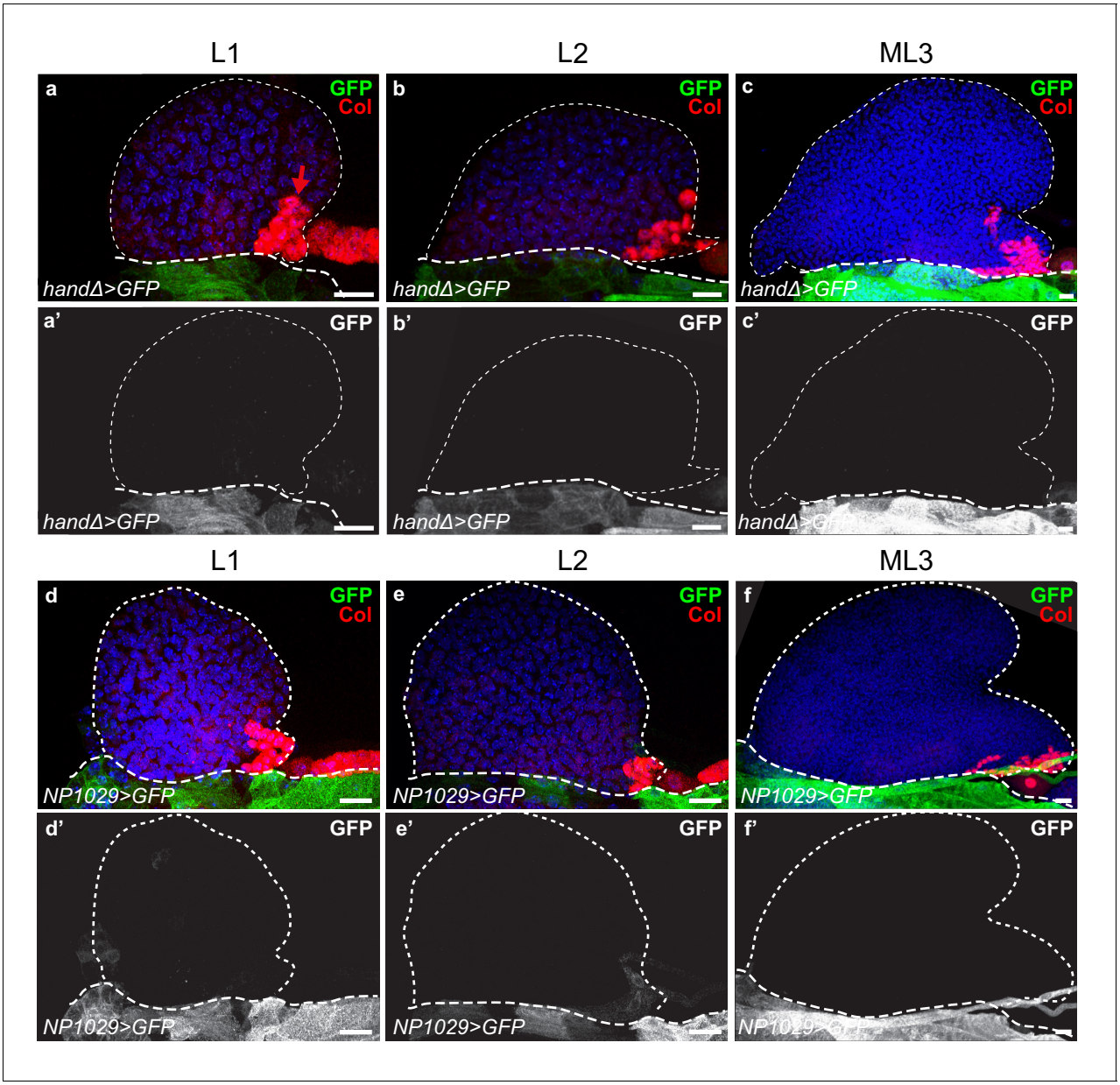

Figure 1—figure supplement 1. Expression pattern of *handΔ-gal4* and *NP1029-gal4* driver in lymph glands during larval development. (**a–c'**) *HandΔ-gal4 > mcd8* GFP is green in (**a–c**) and white in (**a'–c'**). (**d–f'**) *NP1029-gal4 > mcd8* GFP is green in (**d–f**) and white in (**d'–f'**). The PSC is labeled by Col (red and arrow in a); thin and thick dashed lines indicate the contours of the lymph gland and the cardiac tube, respectively.

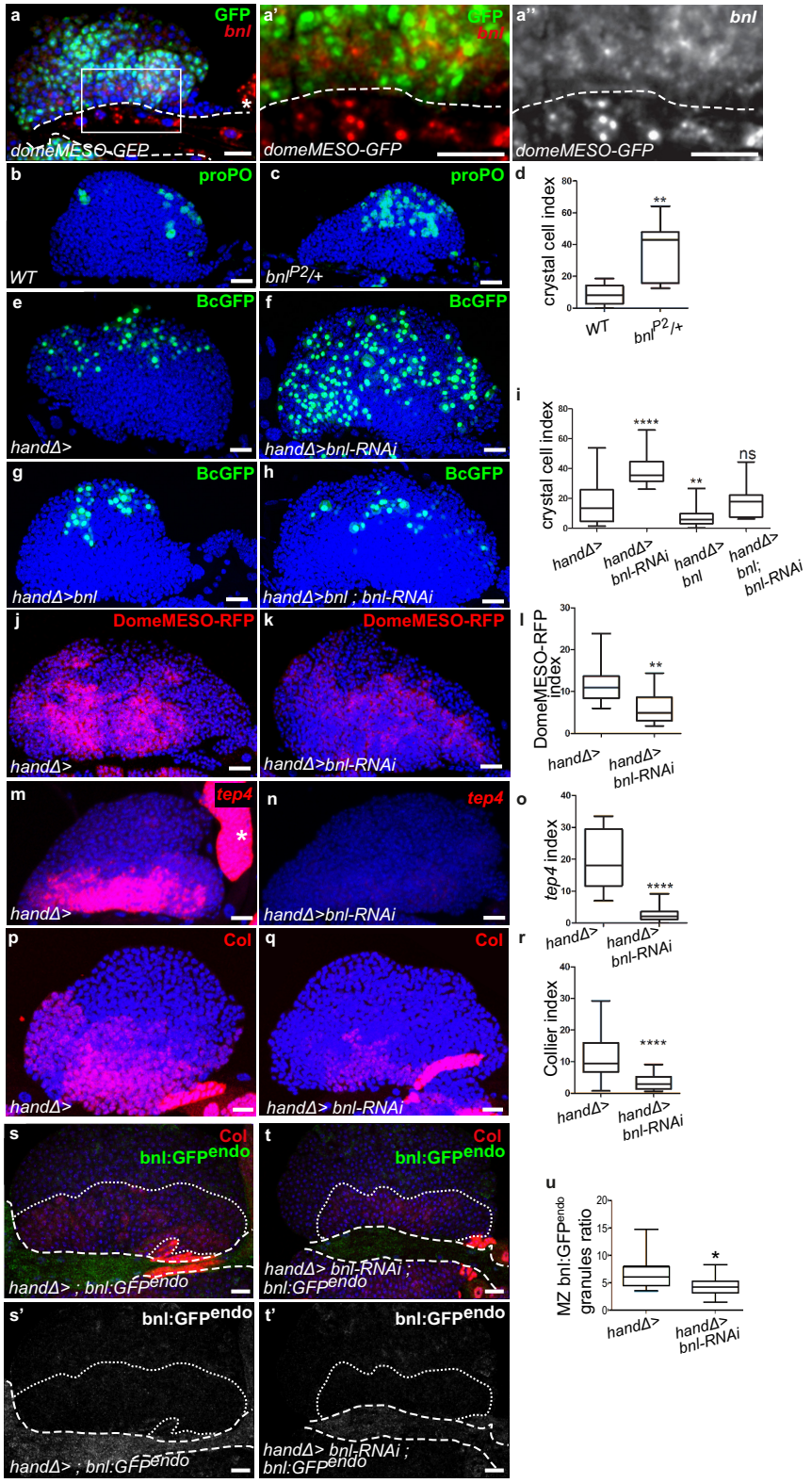

Figure 2. Ligand Bnl is expressed in cardiac cells and controls lymph gland homeostasis. (**a**) A maximum projection of 5 confocal lymph gland sections, *bnl* (red) is expressed in cardiac cells and MZ progenitors that express domeMESO-GFP (green). (**a', a''**) An enlarged view, *bnl* is red (**a'**) or white (**a''**). A

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white dashed line indicates the cardiac tube. * indicates a pericardiac cell. (**b, c**) proPO (green) labels crystal cells. *bnl*^{P2}/+ heterozygous mutant lymph glands have an increased number of crystal cells (**c**) compared to the control (**b**). (**e–f, g–h**) Black-cell GFP (BcGFP, green) labels crystal cells. (**d, i**) Crystal cell index. Co-expression of *bnl* and *bnl-RNAi* in cardiac cells restores the wildtype number of crystal cells (**i**). (**j, k**) DomeMESO-RFP (red) labels MZ progenitors. Compared to the control (**j**) barely detectable DomeMESO-RFP levels are observed when *bnl* is knocked down in cardiac cells (**k**). (**l**) DomeMESO-RFP index. (**m, n**) *tep4* labels core progenitors. Compared to the control (**m**) lower levels of *tep4* (red) are observed when *bnl* is knocked down in cardiac cells (**n**). (**o**) *tep4* index. (**p–q**) Col labels core progenitors. Compared to the control (**p**) lower levels of Col are observed in the core progenitors when *bnl* is knocked down in cardiac cells (**q**). (**r**) Col index. (**s–t'**) Maximum projection of 5 confocal sections of the lymph gland expressing *bnl:GFP* ^{endo} (green) and Col immunostaining that labels MZ progenitors (red). Compared to the control (**s, s'**) a decrease in bnl:GFP ^{endo} in green (**t**) and white (**t'**) is observed when *bnl* is knocked down in cardiac cells. Fine and thick dashed lines indicate the MZ and CT contours, respectively. (**u**) Bnl: GFP^{endo} granules ratio in the MZ.

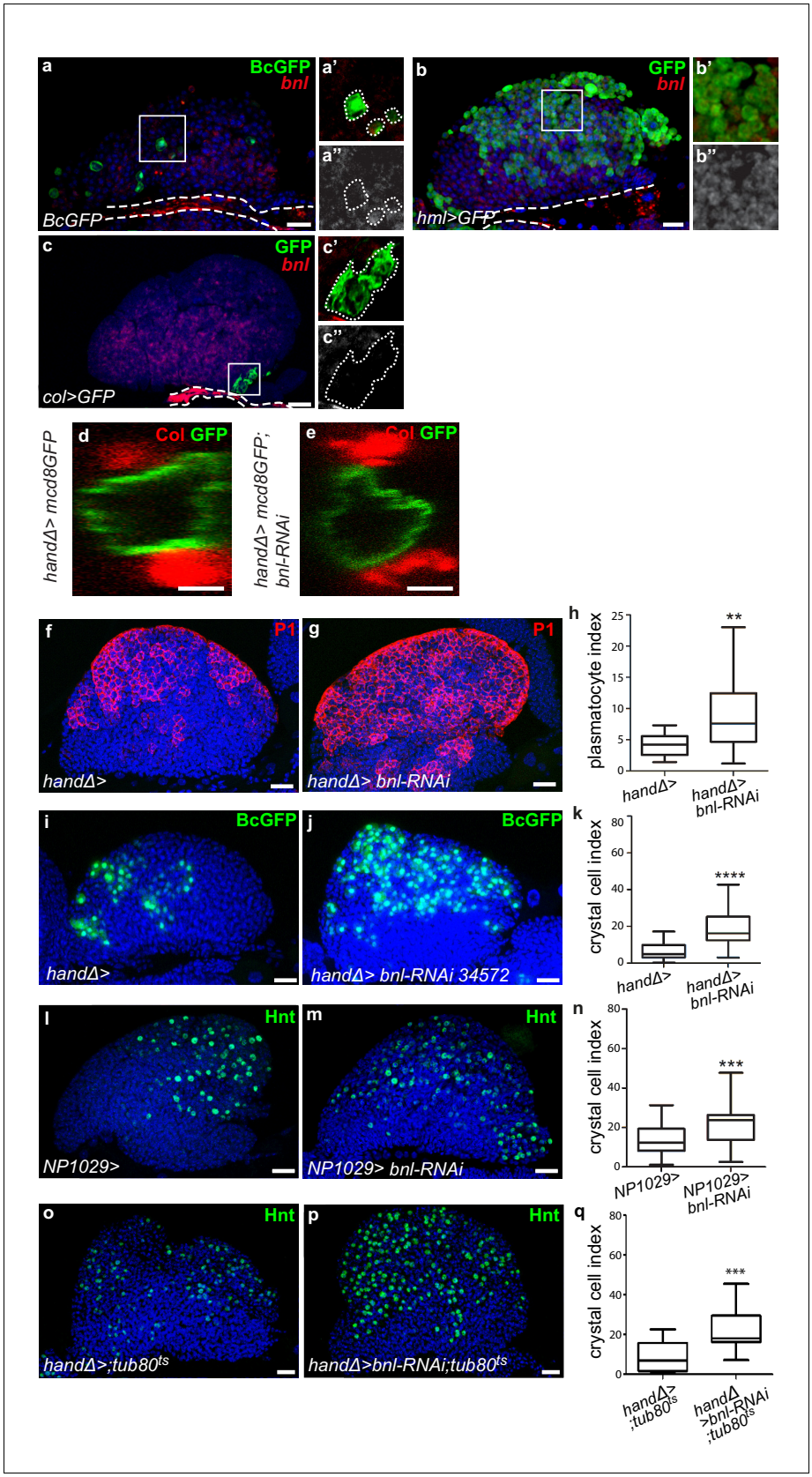

Figure 2—figure supplement 1. The ligand Bnl in cardiac cells controls lymph gland hemocyte differentiation homeostasis. (**a–c''**) A maximum projection of 5 confocal lymph gland sections, *bnl* is in red. (**a,b,c**) and white in

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(**a″**,**b″**,**c″**). BcGFP (green) labels crystal cells. (**a′–a″**) An enlarged view, crystal cells are green (**a′**) and *bnl* is white (**a″**). (**b–b″**) *hml >GFP* (green) labels differentiating hemocytes in the CZ. (**b′–b″**) An enlarged view, differentiating hemocytes are green (**b′**) and *bnl* is white (**b″**). (**c–c″**) *col >GFP* (green) labels PSC cells. (**c′–c″**) An enlarged view, the PSC is green (**c′**) and *bnl* is white (**c″**). White large dashed line indicates the cardiac tube contour. (**d, e**) Cardiac cells express mcd8-GFP (*hand*Δ*>mcd8* GFP, green). A transversal section is shown. PSC cells are labeled by Col (red). No difference in cardiac tube morphology is observed when *bnl* is knocked down in cardiac cells at the larval stage (**e**) compared to the control (**d**). (**f, g**) Plasmatocytes (red) are labeled by P1 antibody. An increase in plasmatocyte number is observed when *bnl* expression is decreased in cardiac cells (**g**) compared to the control (**f**). (**h**) Plasmatocyte index. (**i–j**) Crystal cells (GFP, green) express the Black-cell GFP (BcGFP) marker. An increase in crystal cell number (**j**) is observed when *bnl* expression is decreased in cardiac cells compared to the control (**i**), when another *bnl*-RNAi is used (**j**) or when another cardiac cell driver NP1029 is used (**l, m**). (**k, n**) Crystal cell index. (**o, p**) Hnt labels crystal cells. Compared to the control (**o**) crystal cell number is increased when *bnl* is knocked down in cardiac cells from the L2 stage by using the conditional Gal4/Gal80^{ts} system (**p**). (**q**) Crystal cell index.

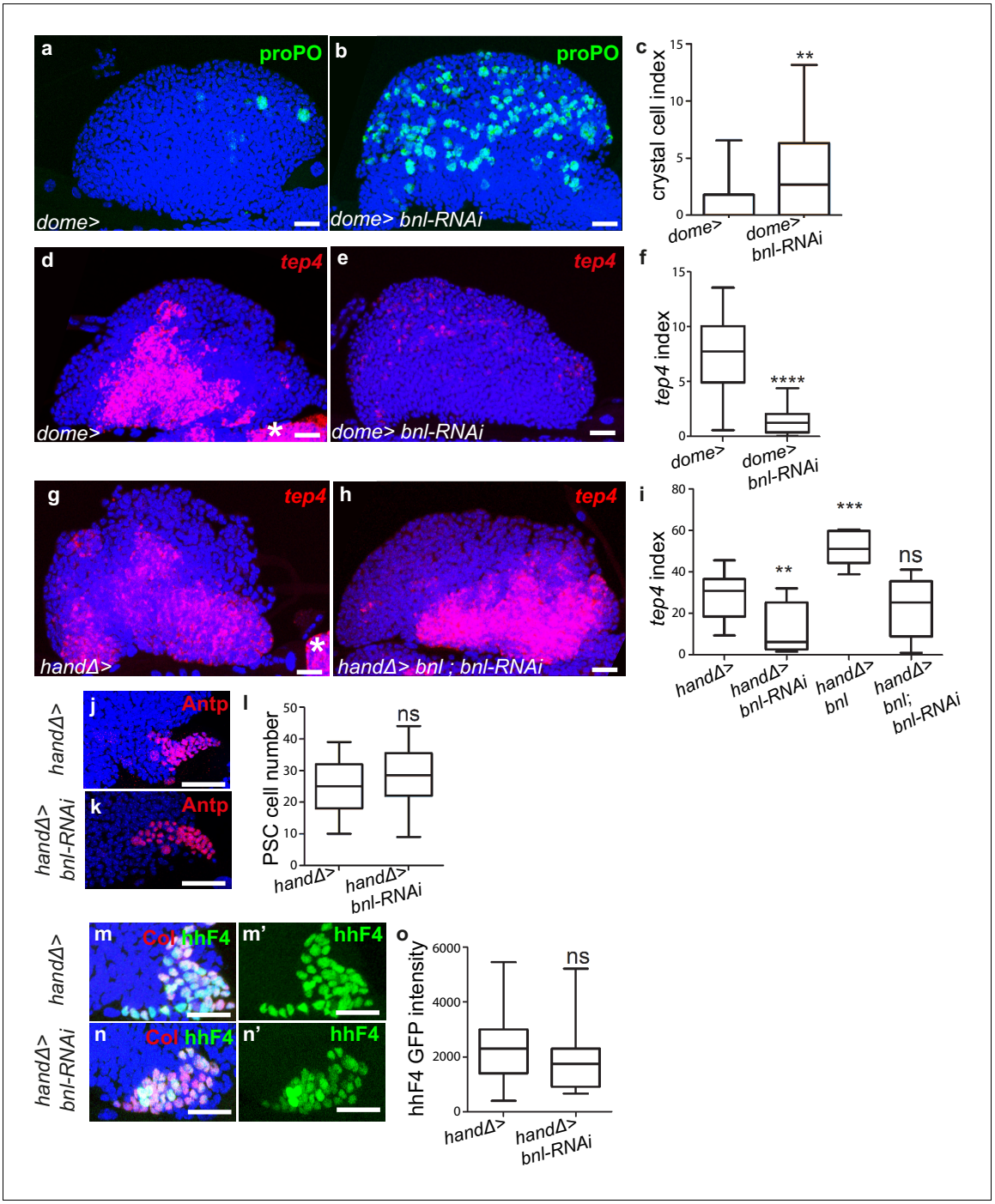

Figure 2—figure supplement 2. Whereas the ligand Bnl in cardiac cells does not control PSC cells, it is required in MZ progenitors to regulate lymph gland homeostasis. (**a, b**) Crystal cells are labeled by proPO antibody (green). An increase in crystal cell number is observed when *bnl* expression is decreased in MZ progenitors (**b**) compared to the control (**a**). (**c**) Crystal cell index. (**d–e, g–h**) *tep4* (red) is expressed in MZ progenitors. Compared to the control (**d**) barely detectable levels of *tep4* are observed when *bnl* is knocked down in MZ progenitors (**e**). Co-expression of *bnl* and *bnl*-RNAi in cardiac cells restores WT levels of *tep4* expression (**h and i**) compared to the control (**g**). (**f, i**) *tep4* index. (**j, k**) PSC cells are labeled by Antp (red) antibody. No difference in PSC cell numbers is observed when *bnl* is knocked down in cardiac cells (**k**) compared to the control (**j**). (**l**) Quantification of PSC cell number. (**m– n'**) hhF4-GFP labels PSC cells (GFP, green). No difference in hhF4-GFP expression is observed when *bnl* is knocked down in cardiac cells (**n**) compared to the control (**m**). (**o**) Quantification of hhF4-GFP intensity.

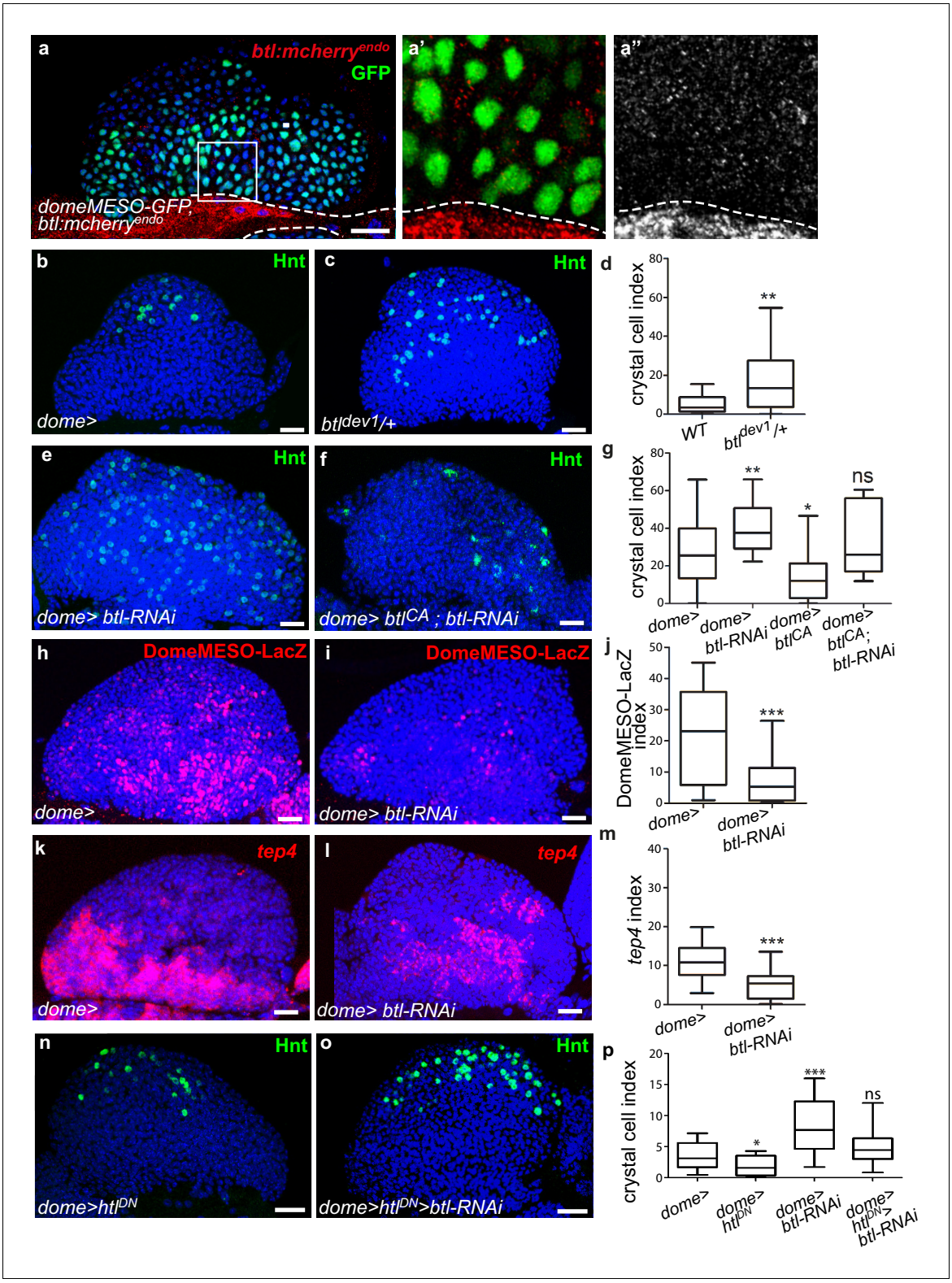

Figure 3. Receptor Btl is expressed in hematopoietic progenitors and required to control lymph gland homeostasis. (**a**) A maximum projection of 5 confocal lymph gland sections of larvae expressing *btl:cherry*^{endo} (red) and *domeMESO-GFP* that labels MZ progenitors (green). (**a', a''**) An enlarged view, *btl:cherry*^{endo} red (**a'**) or white (**a''**). Dashed lines indicate the cardiac tube contour. *btl:cherry*^{endo} is expressed in cardiac cells and MZ progenitors. (**b–c, e–f**) Hindsight (Hnt, green) labels crystal cells. Crystal cell differentiation is increased in *btl*^{dev1}/+ heterozygous mutant larvae (**c**) compared to the control (**b**). (**e, f**) Crystal cell numbers increase when *btl* is knocked down in progenitors (**e**) and crystal cell differentiation is rescued when a constitutive

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activated *btl* receptor (btl^{CA}) is expressed in the *btl-RNAi* context (**f**). (**d, g**) Crystal cell index. (**h, i**) DomeMESO-LacZ (red) labels MZ progenitors. Compared to the control (**h**) barely detectable domeMESO-LacZ levels are observed when *btl* is knocked down in progenitors (**i**). (**k, l**) Lower levels of *tep4* (red) are observed when *btl* is knocked down in progenitors (**l**) compared to the control (**k**). (**j, m**) DomeMESO-LacZ and tep4 index, respectively. (**n, p**) Crystal cell numbers decrease when a dominant negative *htl* receptor (htl^{DN}) is knocked down in progenitors (**n**) and crystal cell differentiation is increased when htl^{DN} is co-expressed with *btl-RNAi* (**o**). (**p**) Crystal cell index.

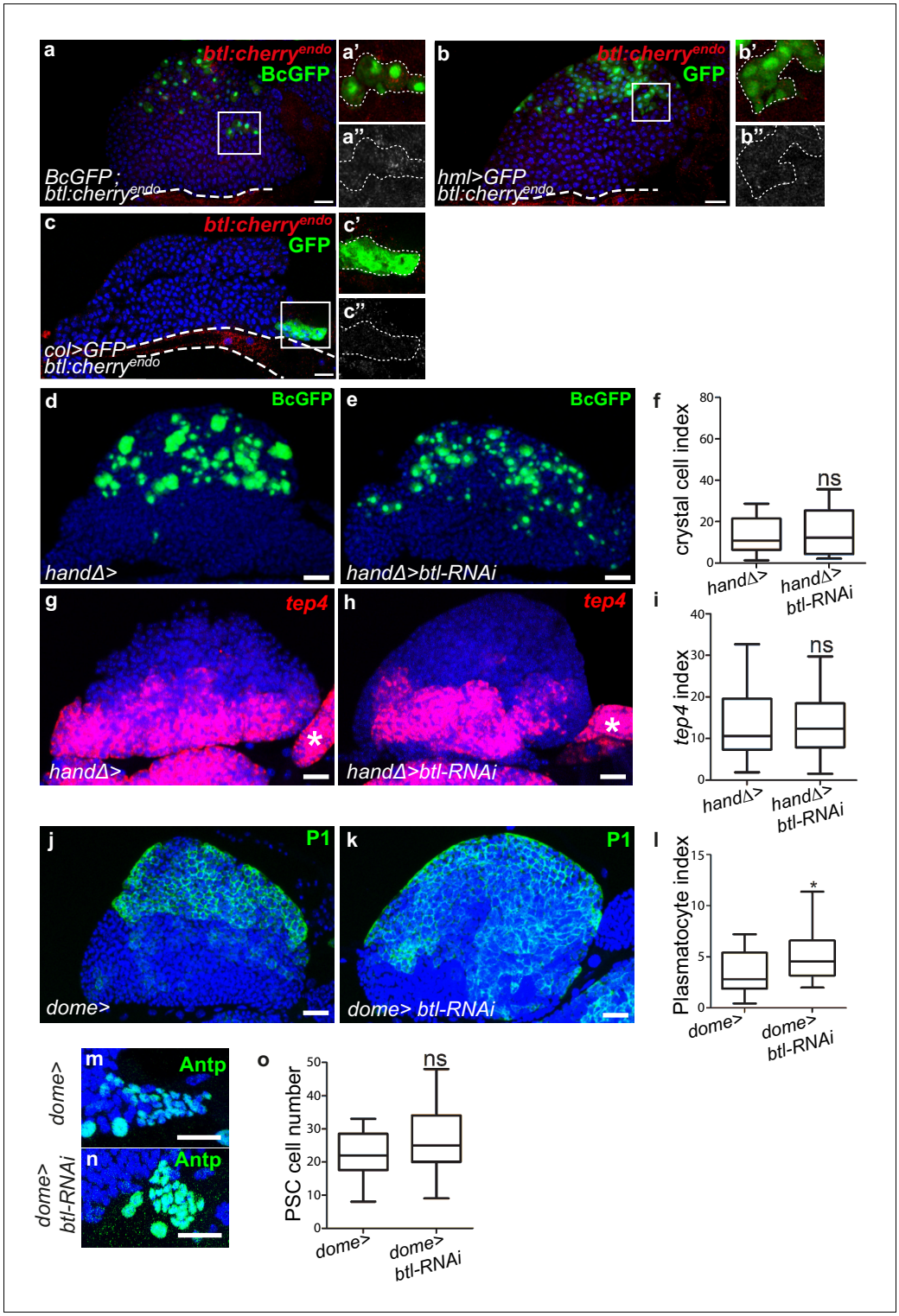

Figure 3—figure supplement 1. The Btl receptor in progenitors controls lymph gland hemocyte differentiation without affecting PSC size. (**a–c''**) A maximum projection of 5 confocal lymph gland sections of larvae expressing *btl:cherry^endo* (red). (**a–a'**) BcGFP (green) labels crystal cells. (**a'–a''**) An enlarged view, crystal cells are green (**a'**) and *btl:cherry^endo* is white (**a''**). (**b–b''**) *hml >GFP* (green) labels differentiating hemocytes in the CZ. (**b'–b''**) An enlarged view, differentiating hemocytes are green (**b'**) and *btl:cherry^endo* is white (**b''**). (**c–c''**) *Col >GFP* (green) labels PSC cells. (**c'–c''**) An enlarged view, the PSC is green (**c'**) and *btl:cherry^endo* is white (**c''**). (**a, b, c**) White large dashed line indicates the cardiac tube contour. (**d, e**) BcGFP (green) labeled crystal cells. No significant difference

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in crystal cell number is observed when *btl* expression is decreased in cardiac cells (**e**) compared to the control (**d**). (**f**) Crystal cell index. (**g, h**) Decreasing *btl* expression in cardiac cells (**h**) does not change *tep4* expression (red) compared to the control (**g**). (**i**) *tep4* index. (**j, k**) An increase in plasmatocyte number (labeled by P1 antibodies, green) is observed when *btl* expression is decreased in progenitors (**k**) compared to the control (**j**). (**l**) Plasmatocyte index. (**m, n**) PSC cells are labeled by Antp (green) antibody. No difference in PSC cell number is observed when *btl* is knocked down in progenitors (**n**) compared to control (**m**). (**o**) Quantification of PSC cell number.

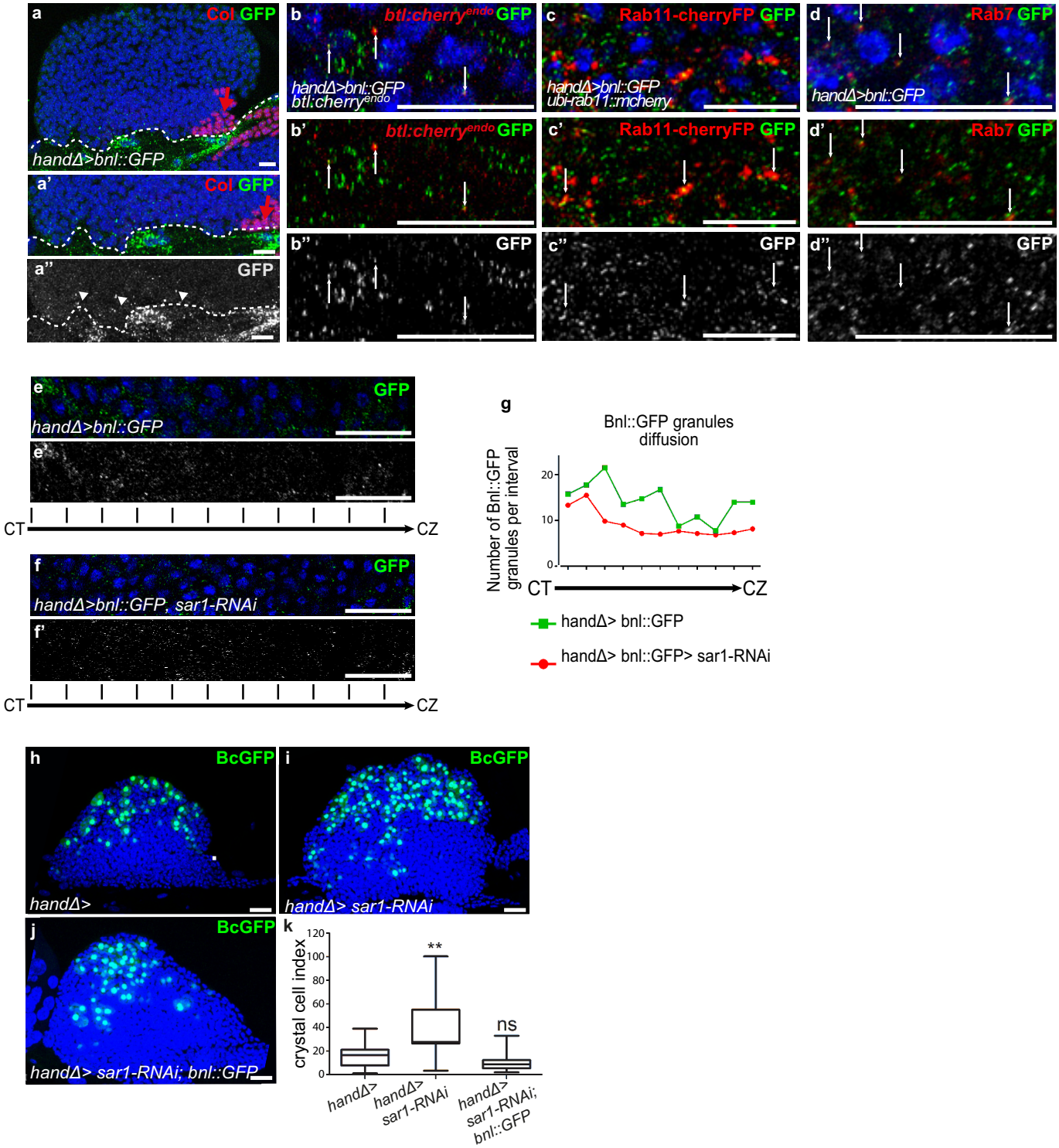

Figure 4. Ligand Bnl secreted by cardiac cells controls lymph gland crystal cell differentiation. (**a**) Active bnl::GFP fusion protein is expressed in cardiac cells using *handΔ-gal4* driver. Dashed lines indicate cardiac tube and the PSC is labeled by Collier (Col, red and red arrow). (**a', a''**) An enlarged view; Bnl::GFP is green (**a'**) or white (**a"**). Bnl::GFP positive granules are detected in cardiac and lymph gland cells (arrowheads). (**b–b"**) Enlargement of MZ area close to the cardiac tube in larvae expressing Bnl::GFP fusion protein (green) in cardiac cells (*handΔ-gal4 > Bnl::GFP*) and Btl:mcherry^{endo} (red). Bnl::GFP cytoplasmic punctate dots (green in b-b' and white in b') co-localize with Btl:mcherry^{endo} (yellow and arrows in b'). (**c,c"**) Enlargement of MZ

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area close to the cardiac tube in larvae expressing *ubi-Rab11cherryFP* (red), a marker for recycling endocytic vesicles; Bnl::GFP fusion protein (green) is expressed in cardiac cells (*handΔ-gal4 > Bnl::GFP*). Bnl::GFP cytoplasmic punctate dots (green in c-c' and white in c') co-localize with ubi-Rab11cherryFP (yellow and arrows in c'). (**d–d''**) Enlargement of MZ area close to the cardiac tube in larvae expressing Bnl::GFP fusion protein (green) in cardiac cells (*handΔ-gal4 > Bnl::GFP*) and Rab7 immunostainings (red in d, d' and white in d'). (**d–d'**) Bnl::GFP cytoplasmic punctuate dots co-localize with Rab7 positive dots (yellow and arrows in d'). (**e–f'**) Enlargement of lymph gland cross sections extending from the cardiac tube (CT) to the cortical zone (CZ). Bnl::GFP fusion protein, expressed in cardiac cells (*handΔ-gal4 > Bnl::GFP*) is green (**e, f**) and white (**e', f'**). Knocking down *sar1* in cardiac cells (**f, f'**) leads to a decrease in Bnl::GFP cytoplasmic punctate dots compared to the control (**e, e'**). (**g**) Quantification of Bnl::GFP cytoplasmic punctate dots/granules. (**h, j**) BcGFP (green) labels crystal cells. Knocking down *sar1* in cardiac cells (**i**) increases crystal cell numbers compared to the control (**h**). Crystal cell differentiation rescue is observed when bnl::GFP is co-expressed with *sar1-RNAi* (**j, k**). (**k**) Crystal cell index.

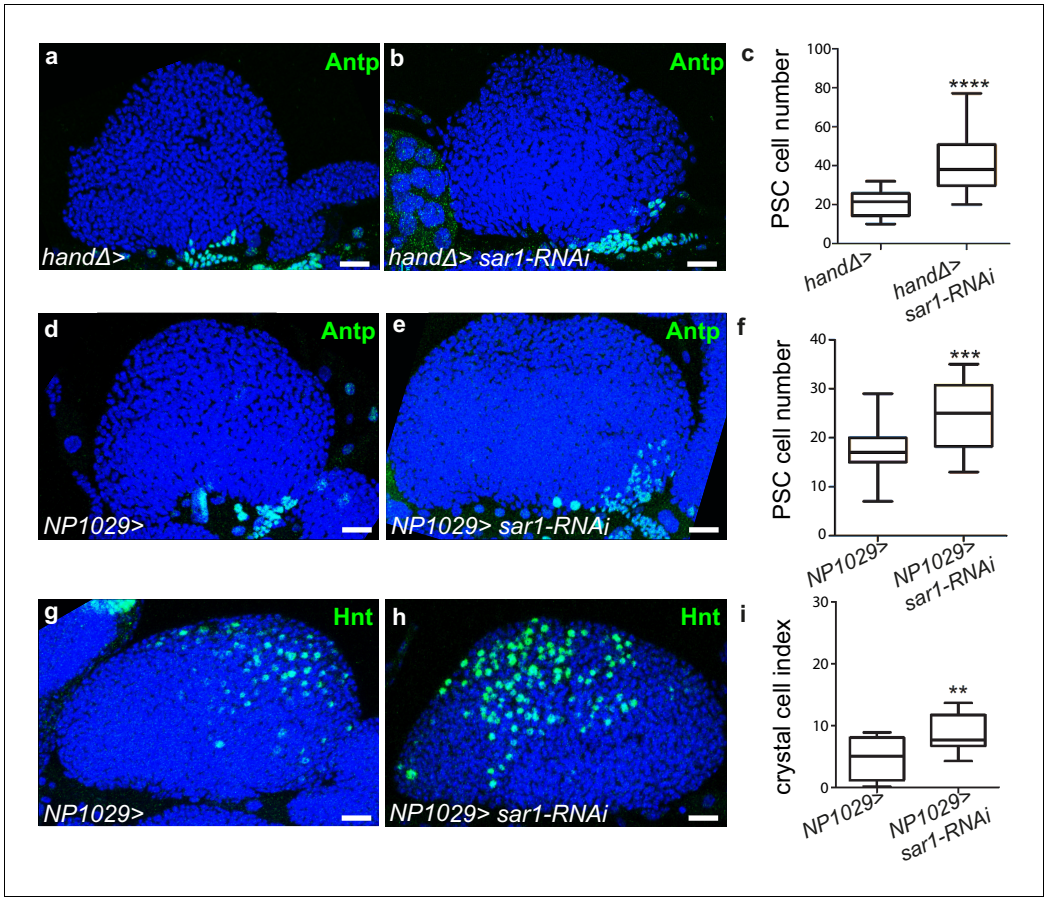

Figure 4—figure supplement 1. Knocking down *sar1* in cardiac cells impairs crystal cell differentiation and increases PSC size. (**a–b, d–e**) PSC cells are labeled with Antp (green) antibody. An increase in PSC cell numbers is observed when *sar1* is knocked down in cardiac cells using the *hand*Δ driver (**b**) compared to the control (**a**). (**c**) Quantification of PSC cell numbers. (**d–f**) An increase in PSC cell number is observed when *sar1* is decreased in cardiac cells using the *NP1029* driver (**e**) compared to the control (**d**). (**f**) PSC cell numbers. (**g, h**) Crystal cells are labeled with Hnt (green) antibody. An increase in crystal cell number is observed when *sar1* is knocked down in cardiac cells using *NP1029*, another cardiac cell driver (**h**) compared to the control (**g**). (**i**) Crystal cell index.

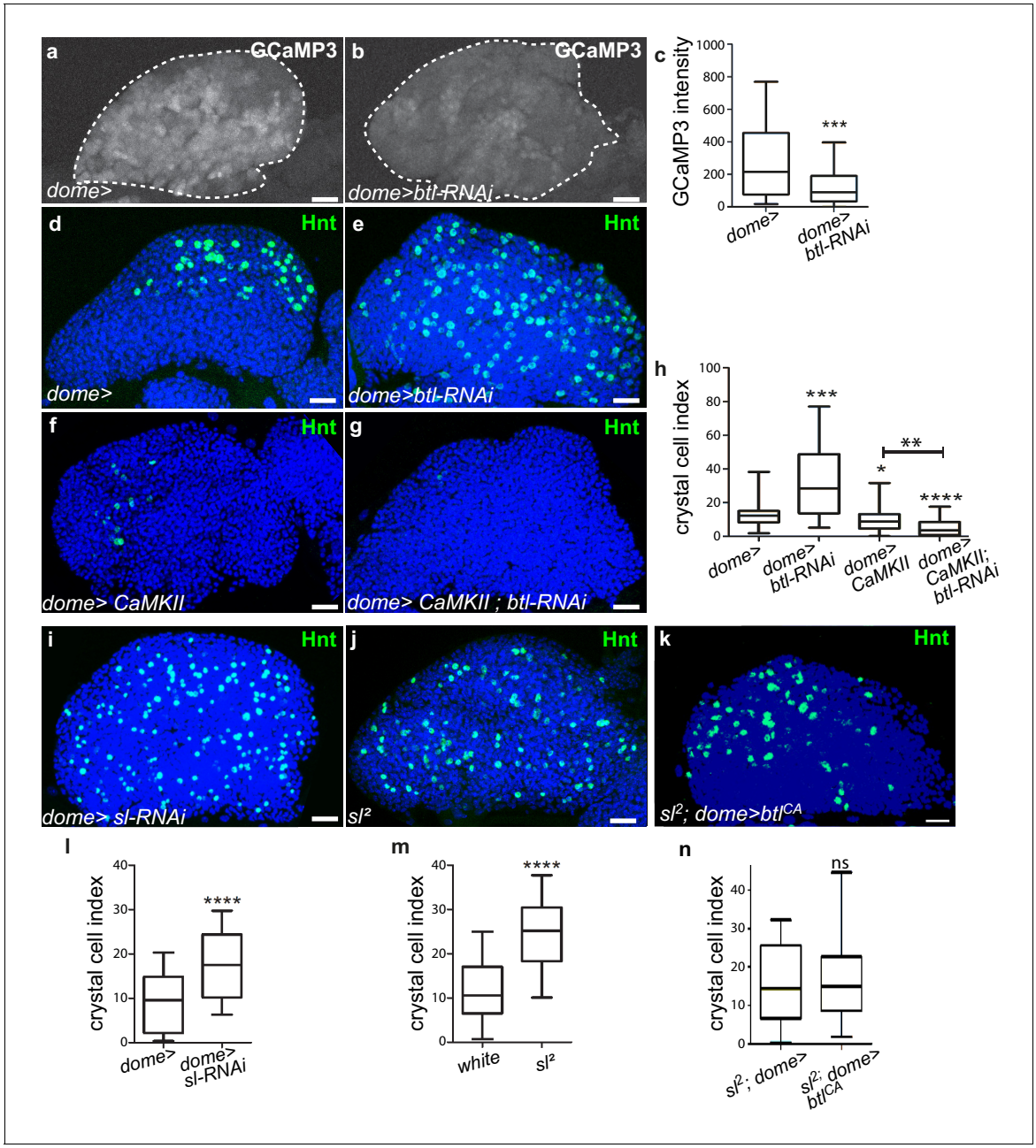

Figure 5. Btl receptor interacts genetically with CamKII to control blood cell differentiation by preventing high Ca^{2+} levels in progenitors. (**a, b**) GCaMP3 Ca^{2+} sensor (*dome >UAS-GCaMP3*) is white. GCaMP3 intensity decreases when *btl is* knocked down in MZ progenitors (**b**) compared to the control (**a**). (**c**) Quantification of GCaMP3 intensity. (**d–g, i–k**) Hnt (green) labels crystal cells. Crystal cell differentiation decrease is observed when Ca^{2+} levels increased due to CaMKII expression in progenitors (*dome >CaMKII,* **f**) compared to the control (**d**). Co-expression of CaMKII and *btl*-RNAi in progenitors (*dome >CaMKII; btl-RNAi*, **g**) leads to a decrease in crystal cell number compared to the *btl* knock-down alone (**e**). (**h, l–n**) Crystal cell index. Crystal cell differentiation increase is observed in sl^2 homozygous mutant larvae (**j, m**) and when *sl* is knocked down in progenitors (**i, l**) compared to the control (**d**). (**k, n**) No difference in crystal cell index is observed in sl^2 homozygous mutant larvae and in a sl^2 homozygous mutant where btl^{CA} is expressed in MZ progenitors (sl^2; *dome* >btl^{CA}).

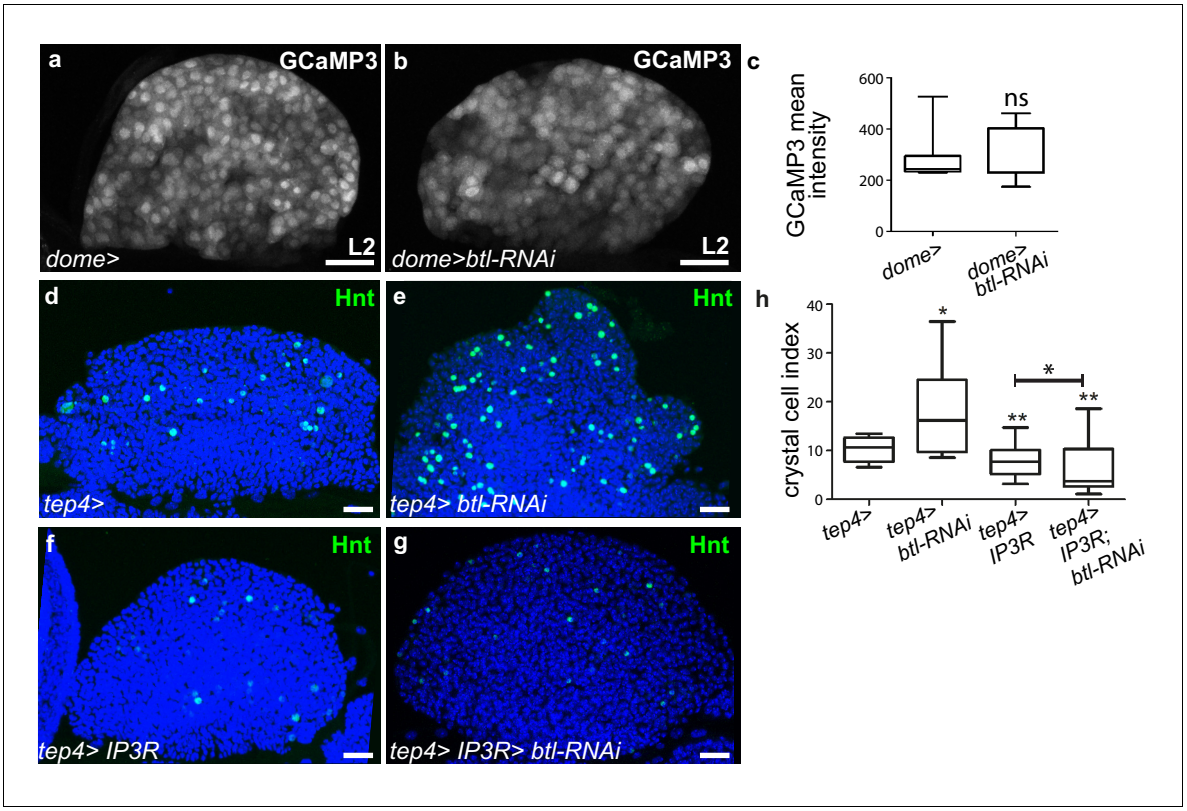

Figure 5—figure supplement 1. Ca^{2+} levels in progenitors regulate crystal cell differentiation. (**a, b**) L2 larval lymph glands and the GCaMP3 Ca^{2+} sensor (*dome >UAS-GCaMP3*) is white. No difference observed in GCaMP3 intensity between the control (**a**) and when *btl is* knocked down in MZ progenitors (**b**). (**c**) GCaMP3 intensity. (**d–e, f–g**) Crystal cells are labeled with Hnt (green) antibody. A decrease in crystal cell numbers is observed when *IP3R* is knocked down in progenitors using the *tep4* driver (**f**) compared to the control (**d**). Co-expression of *IP3R* and *btl*-RNAi in progenitors prevents crystal cell differentiation (**g**) compared to the control (**f**). (**h**) Crystal cell index.

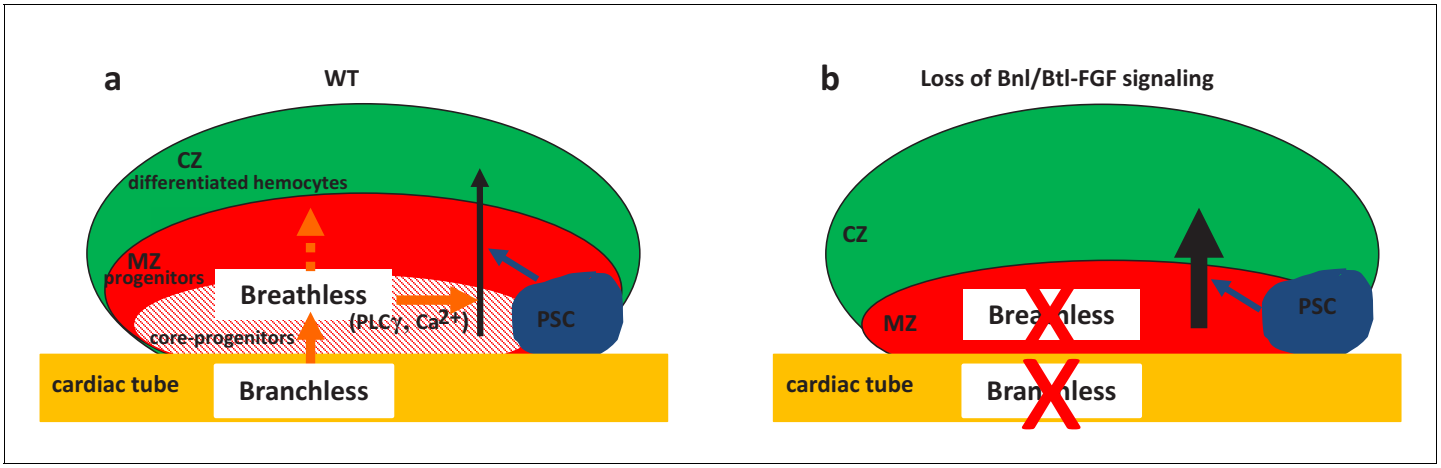

Figure 6. Two niches control lymph gland homeostasis. (**a–b**) Schematic representation of third instar larvae lymph gland anterior lobes. Progenitors and core progenitors are in red and hatched red, respectively. The cortical zone (CZ) is in green, the PSC and the cardiac tube (CT)/vascular system are in blue and orange, respectively. (**a**) In a wildtype (WT) lymph gland, under normal conditions the PSC, the first niche identified, regulates the maintenance of the progenitor pool except for core progenitors (blue arrow). Here, we show that by directly acting on core progenitors (orange arrow) the cardiac tube corresponds to a second niche present in the lymph gland. Bnl produced by cardiac cells activates its receptor Btl in progenitors. Btl-FGF activation regulates intracellular Ca^{2+} levels via PLCγ, and controls the maintenance of core progenitors and in turn the whole progenitor pool. (**b**) When *bnl* or *btl* are knocked down in cardiac cells and progenitors, respectively, an increase in blood cell differentiation in the CZ is observed at the expense of the progenitor pool.