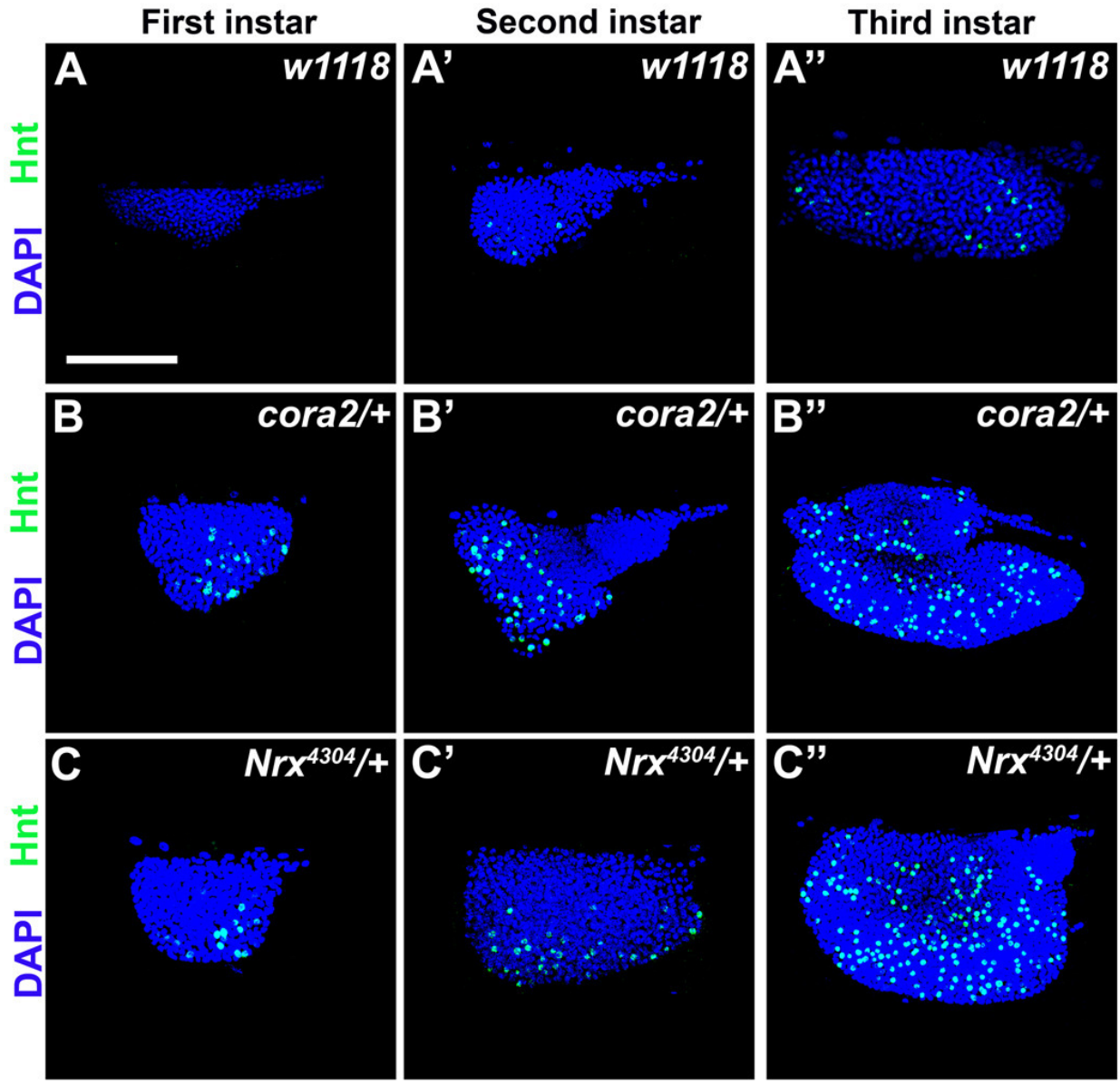
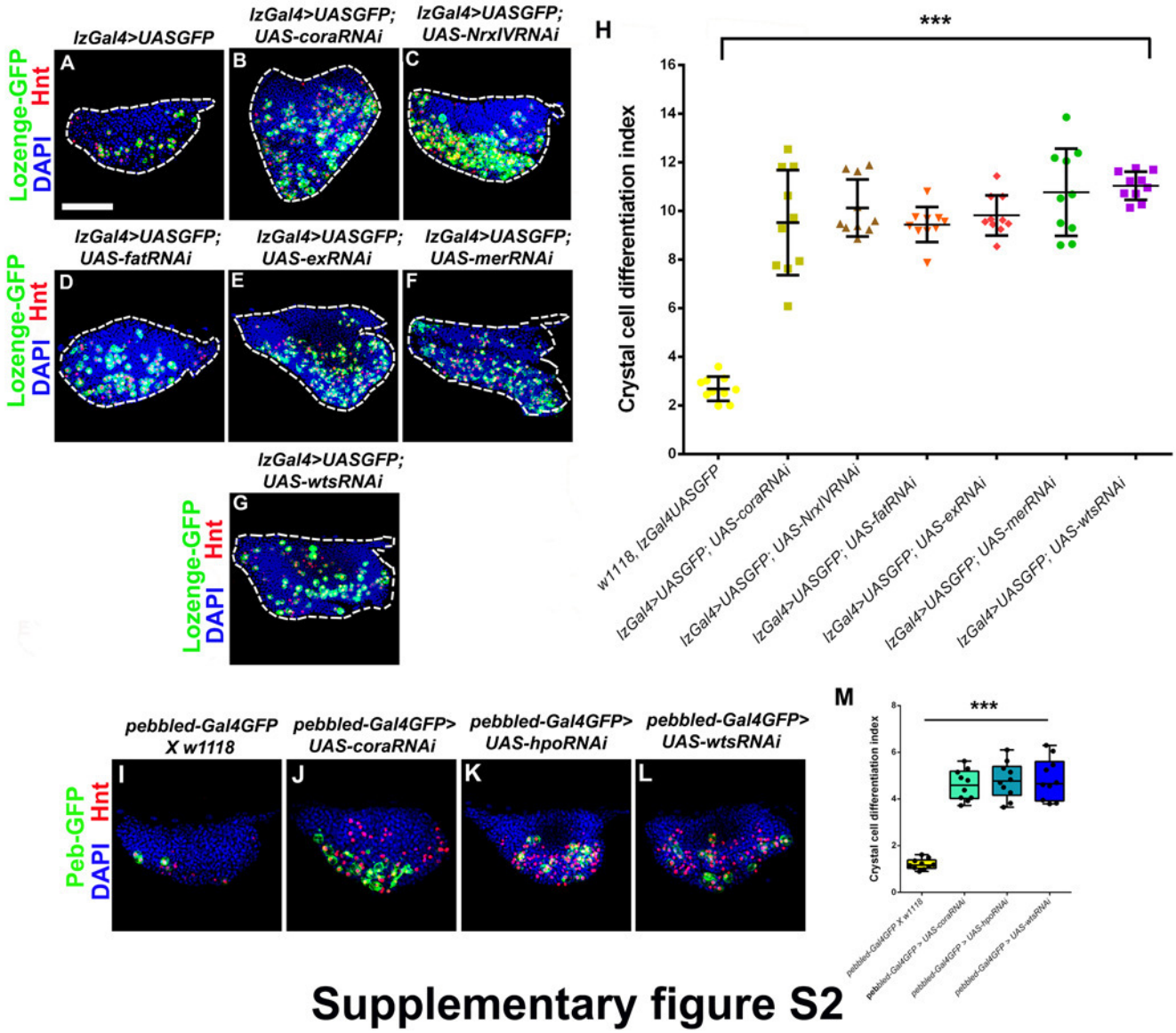


Figure S1: Septate junction component mutants show increased crystal cell differentiation from early stages of larval development. (Related to Figure 1)

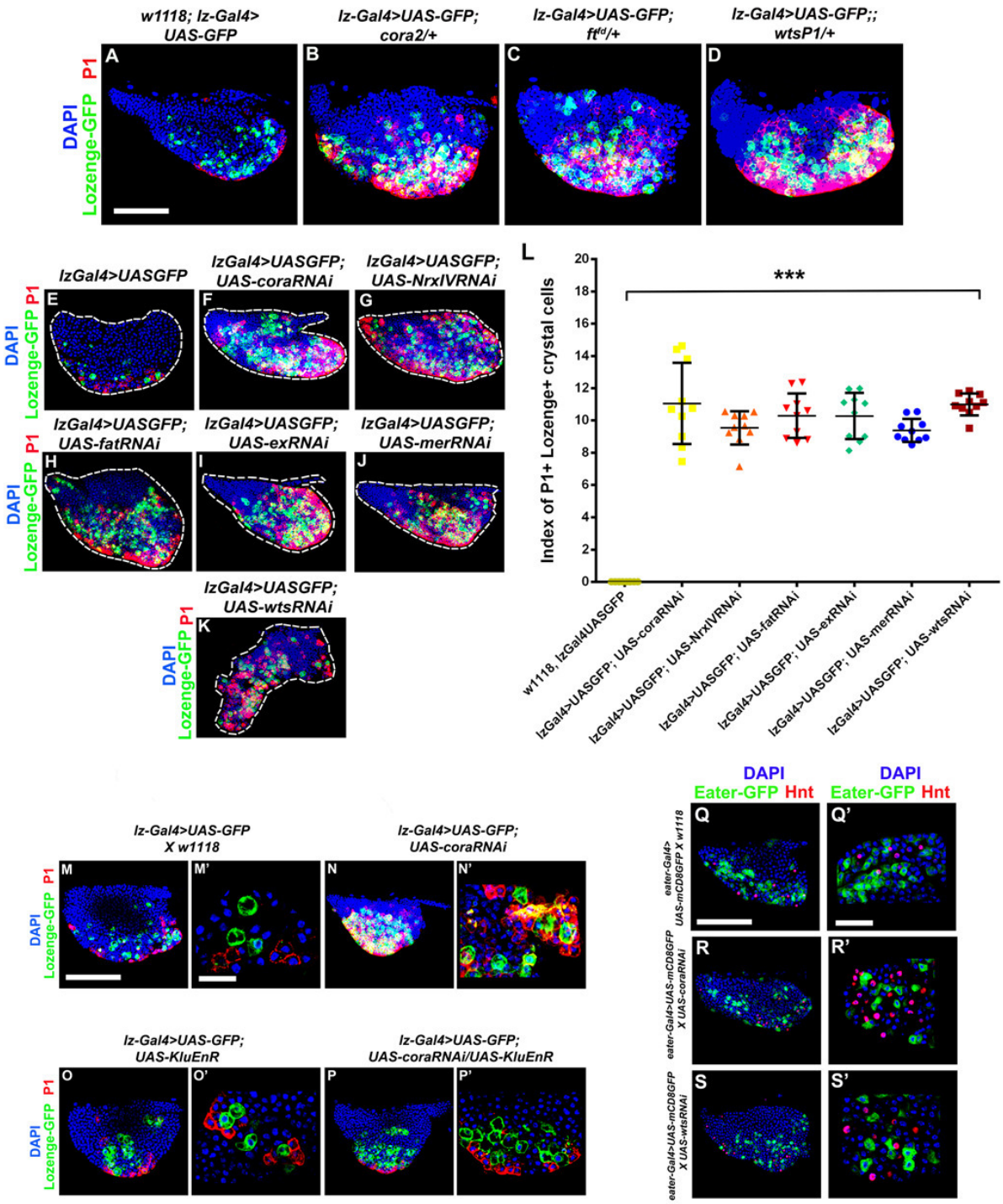
(A-C) Representative primary lymph glands from first, second and third larval instar wild type control larva (*w1118*; A) and larva heterozygous for mutant null alleles of *coracle* (*cora2/+;* B) or *NrxIV* (Nrx^{4304}/+; C). Crystal cells labelled with Hnt (green; Nuclei are marked with DAPI (Blue). Scale bar: 40µm (A-C'').



Supplementary figure S2

Figure S2: Cora and NrxIV function in the crystal cell lineage to regulate differentiation (Related to Figure 2)

(A-H) Crystal cell differentiation in representative control (UAS-GFP; A) primary lymph glands and following knockdown of *cora* (B), *NrxIV* (C), *fat* (D), *ex* (E), *mer* (F), *wts* (G) driven by the crystal cell lineage specific driver *lz-Gal4*. Crystal cells labelled with Hnt (Red), all hemocytes labelled by expression of UAS-GFP (green). (H) Quantification of crystal cell differentiation index (in arbitrary units) for genotypes shown in A-G. (I-L) Crystal cell differentiation in representative control (UAS-GFP; I) primary lymph glands and following knockdown of *cora* (J), *hippo* (K) and *warts* (L) driven by an alternate crystal cell lineage specific driver *pebbled-Gal4*. (M) Quantification of crystal cell differentiation index (in arbitrary units) for genotypes shown in I-L. Nuclei are marked with DAPI (Blue). Statistical significance was estimated using unpaired t-test with Welch's correction. Error bars indicate SD of the mean. *** indicates $P<0.001$. Scale bar: 40µm (A-G, I-L).

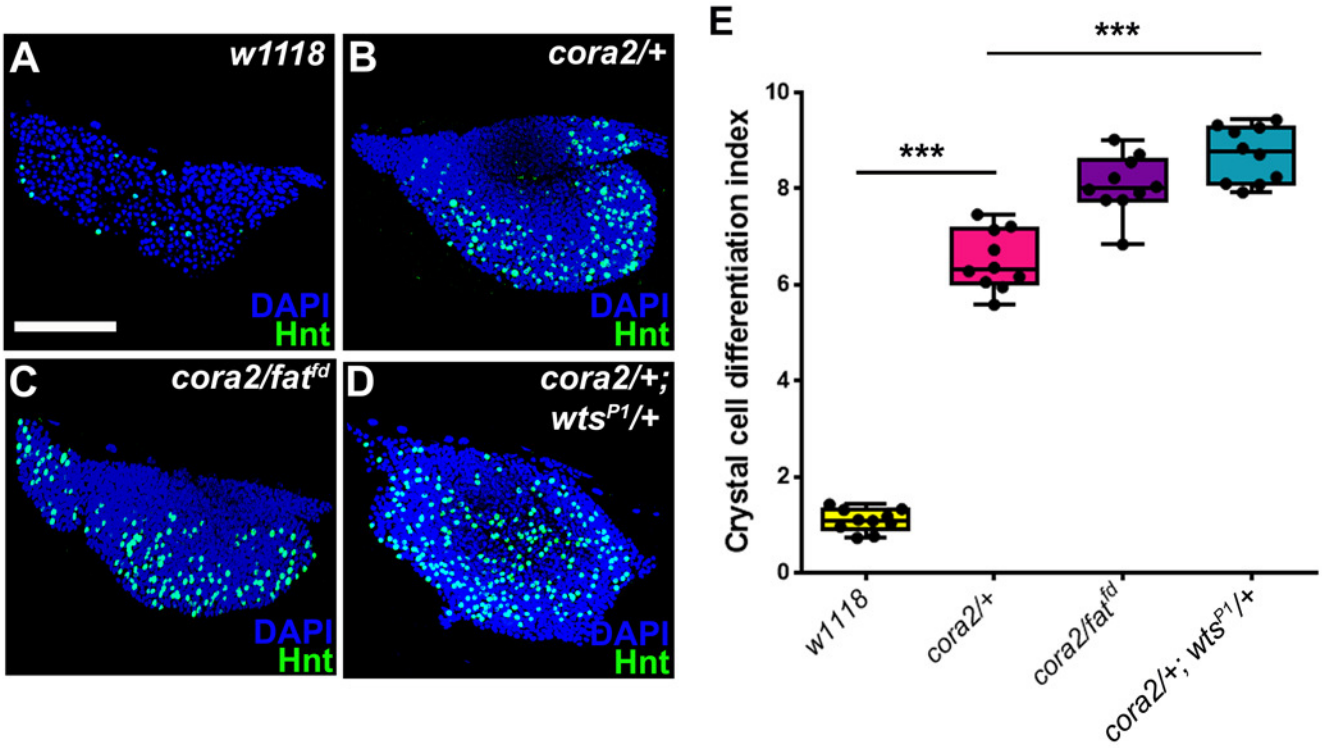


Supplementary figure S3

Figure S3: Loss of Cora, NrxIV or Hippo pathway activity results in hemocytes adopting mixed cell fate. (Related to Figure 2)

(A-D) Hemocyte differentiation in representative wild type control (*w1118*; A), heterozygous mutants for *cora* (*cora2*/+; B), *fat* (*fat*^{fd}/+; C), *wts* (*wtsP1/+;* D). Staining shown is for the crystal cell marker Lz (labelled using *lz-Gal4* to drive GFP (Green)), the plasmatocyte marker P1

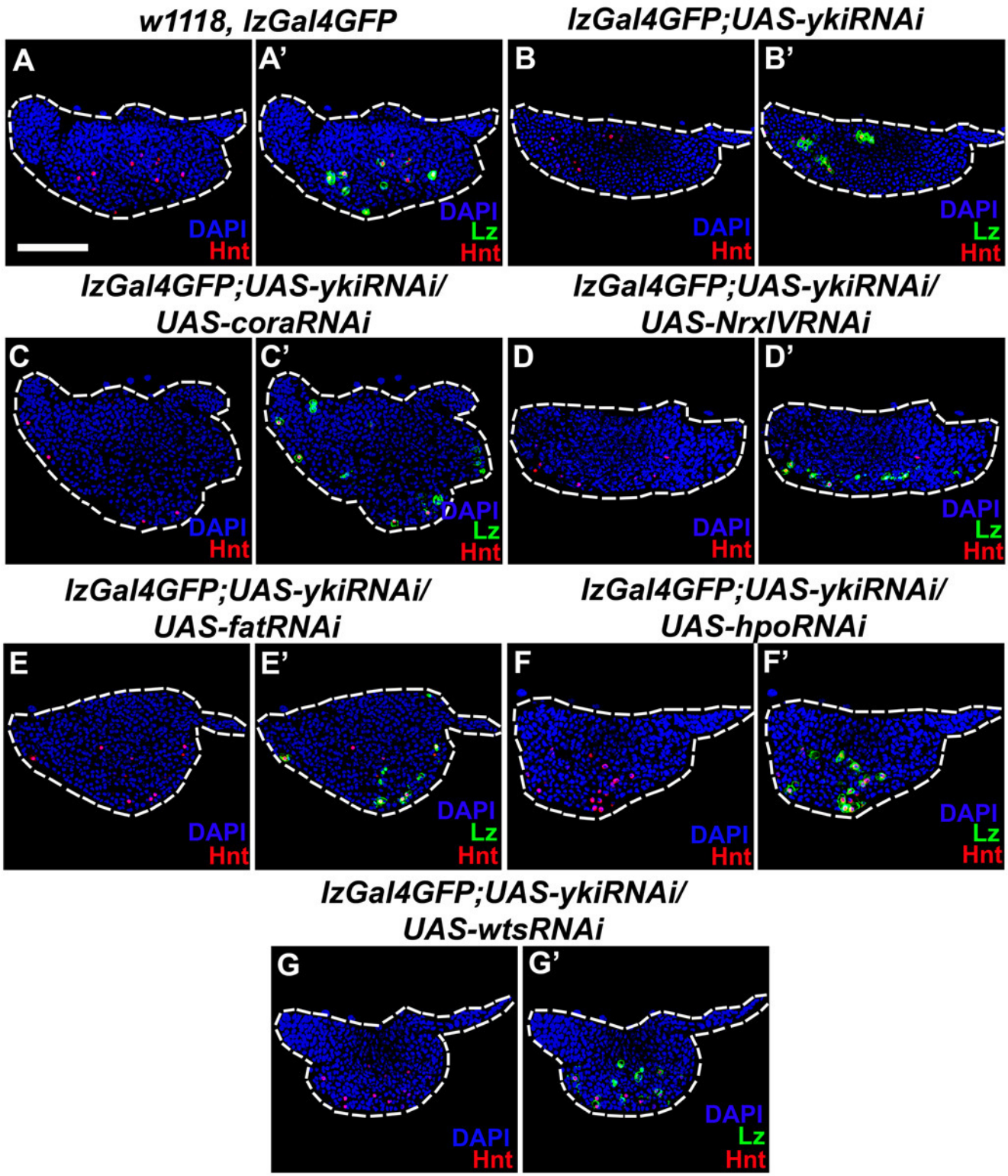
(Red), and nuclei (marked with DAPI (Blue). (E-K) Hemocyte differentiation in representative primary lymph glands in control (UAS-GFP; E) or following crystal specific knockdown (using *lz-Gal4*) mediated by RNAi of *cora* (F) and *NrxIV* (G), *fat* (H), *expanded* (I), *merlin* (J), *warts* (K). Staining shown is for the crystal cell marker Lz (labelled using *lz-Gal4* to drive GFP (Green), the plasmatocyte marker P1 (Red), and nuclei (marked with DAPI (Blue). (L) Quantification of the index of dual P1/*lz-Gal4* positive (in arbitrary units, see materials and methods) for genotypes shown in E-K. (M-P) Hemocyte differentiation in representative primary lymph glands in control (*lz-Gal4>UAS-GFP X w1118*; M-M') or following crystal specific knockdown (using *lz-Gal4*) mediated by RNAi of *cora* (*lz-Gal4>UAS-GFP; UAS-coraRNAi*, N-N') or upon expression of KluEnR alone (*lz-Gal4>UAS-GFP; UAS-KluEnR*, O-O') or in the genetic background of *cora* knockdown (*lz-Gal4>UAS-GFP; UAS-coraRNAi/UAS-KluEnR*, P-P'). (M'-P') are high magnification images of hemocytes showing co-staining for crystal cells and plasmatocytes from corresponding genotypes in M-P. Staining shown is for the crystal cell marker Lz (labelled using *lz-Gal4* to drive GFP (Green), the plasmatocyte marker P1 (Red), and nuclei (marked with DAPI (Blue). (Q-S) Hemocyte differentiation in representative primary lymph glands in control (*eater-Gal4>UAS-GFP X w1118*; Q-Q') or following plasmatocyte specific knockdown (using *eater-Gal4*) mediated by RNAi of *cora* (*eater-Gal4>UAS-GFP; UAS-coraRNAi*, R-R') or *wts* (*eater-Gal4>UAS-GFP; UAS-wtsRNAi*, S-S'). (Q'-S') are high magnification images of hemocytes showing co-staining for crystal cells and plasmatocytes from corresponding genotypes in Q-S. Staining shown is for the crystal cell marker Hnt (Red), the plasmatocytes are marked by eater-Gal4 driving GFP expression (Green), and nuclei (marked with DAPI (Blue). Statistical significance was estimated using unpaired t-test with Welch's correction. Error bars indicate SD of the mean. *** indicates $P<0.001$. Scale bar: 40µm (A-K, M-S), 20µm (M'-S').



Supplementary figure S4

Figure S4: Loss of Hippo pathway components enhances the crystal cell differentiation defects caused by removing one copy of *cora* (Related to Figure 3)

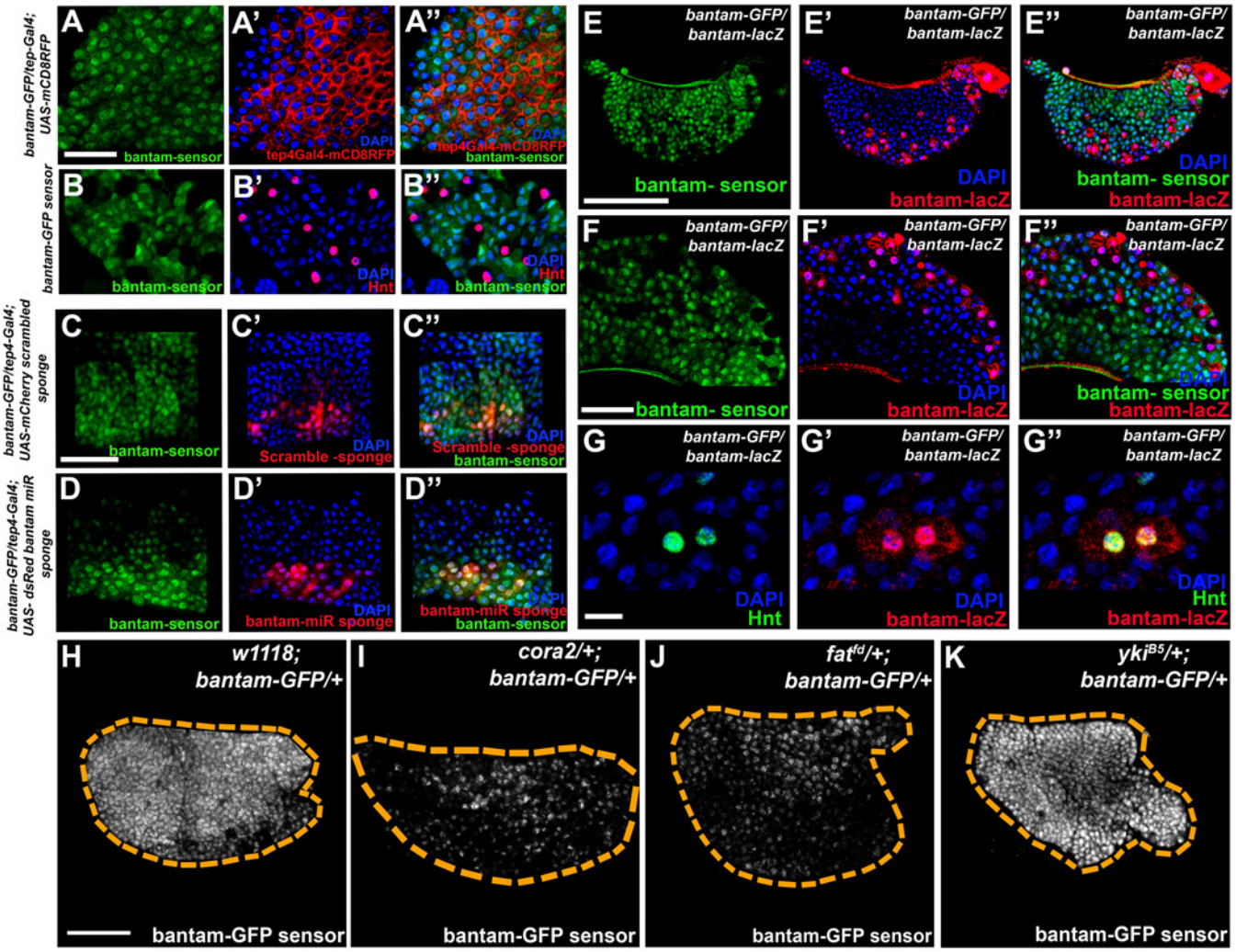
(A-D) Crystal cell differentiation in representative primary lymph glands from control (w^{1118}; A), heterozygous mutants for *cora* (*cora2/+*; B), double heterozygotes for *cora* and *fat* (*cora2/*fat^{fd}; C), and *cora* and *wts* (*cora2/+*; wts^{P1}*/+;* D). Crystal cells labelled with Hnt (green), nuclei are marked with DAPI (Blue). (E) Quantification of crystal cell differentiation index (in arbitrary units) for genotypes shown in A-D. Statistical significance was estimated using unpaired t-test with Welch's correction. Error bars indicate SD of the mean. *** indicates P<0.001. Scale bar: 40µm (A-D).



Supplementary figure S5

Figure S5: Rescue of crystal cell differentiation defects induced by knockdown of SJ or Hippo pathway components by simultaneous knockdown of *yorkie* (Related to Figure 3)

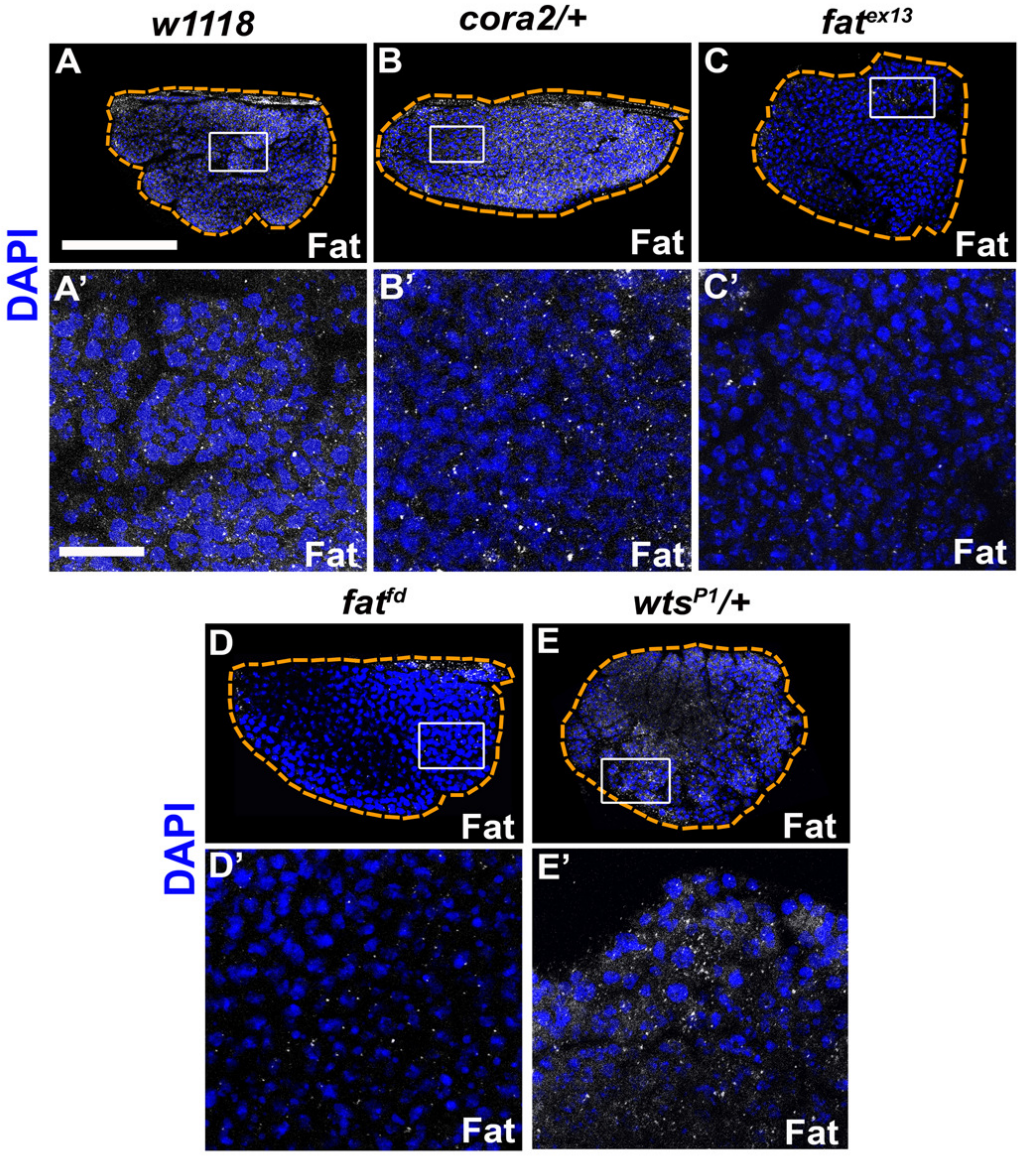
(A-G') Crystal cell differentiation in representative control primary lymph glands (w^{1118}; A-A') and following crystal cell specific (*lz-Gal4* driven) knockdown of *yorkie* by itself (B-B') or *yorkie* knockdown combined with UAS lines for the expression of RNAi lines targeting *cora* (C-C'), *NrxIV* (D-D'), *ft* (E-E'), *hippo* (F-F'), *wts* (G-G'). Crystal cells labelled with Hnt (Red), *lz-Gal4* expression marked using UAS-GFP (green), Nuclei are marked with DAPI (Blue). Scale bar: 40µm (A-G').

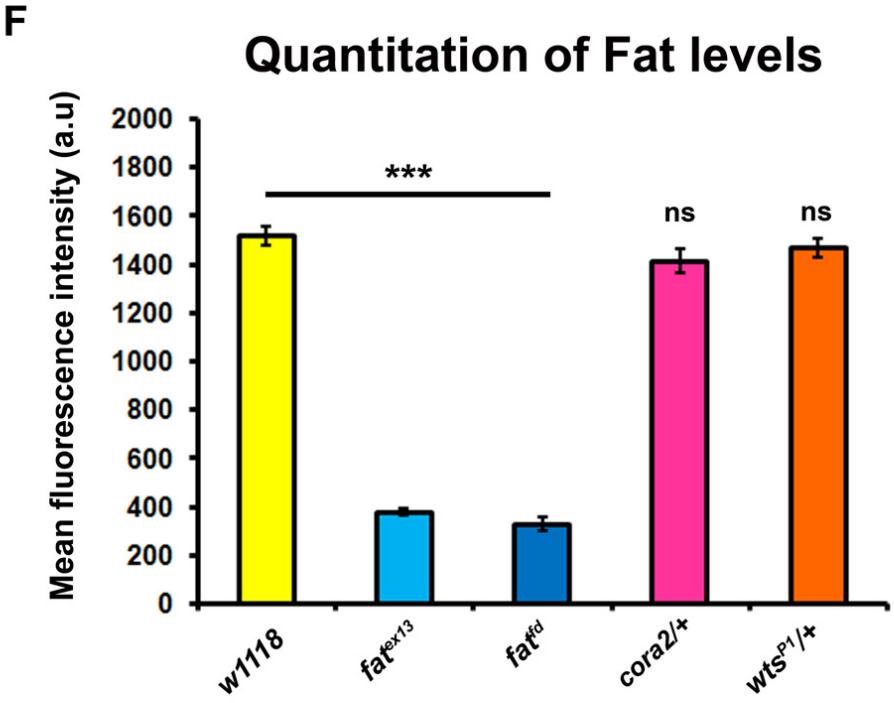


Supplementary figure S6

Figure S6: Expression of microRNA reporter *bantam-GFP* in the LG of larva with reduced levels of Cora or Hippo pathway components. (Related to Figure 3)

(A-D) Lymph gland hemocytes showing bantam-GFP sensor expression (Green) co-stained with prohemocyte marker tep-Gal4 driven UAS-mCD8RFP (Red, A-A'') or crystal cell marker Hnt (Red, B-B'') or upon *tep-Gal4* (prohemocyte specific) mediated over-expression of dsRed tagged bantam microRNA sponge (D-D'', Red) and mCherry tagged scrambled sponge (C-C'', Red). (E-F'') Lymph gland hemocytes showing bantam-GFP sensor expression (Green) and bantam-lacZ expression (Red). F-F'' are high magnification images showing bantam-GFP sensor (Green) and bantam-lacZ (Red) expression. (G-G'') Lymph gland cells marked with bantam-lacZ (Red) co-stained with crystal cell marker, Hnt (Red). (H-K) microRNA reporter, bantam-GFP sensor (white) expression in control larva (*w1118; bantam-GFP*/+; H) or larvae bearing heterozygous mutant alleles of *cora* (*cora2/+; bantam-GFP*/+; I) or components of the core Hippo pathway: *fat* (*fat^{fd}*/+; *bantam-GFP*/+; J) and *yorkie* (*yki^{B5}*/+; *bantam-GFP*/+; K). Dotted brown lines indicate the boundary of the primary lymph gland lobe. Scale bar: 40µm (E-E'', H-K), 20µm (A-D'', F-F'') and 15µm (G-G'').

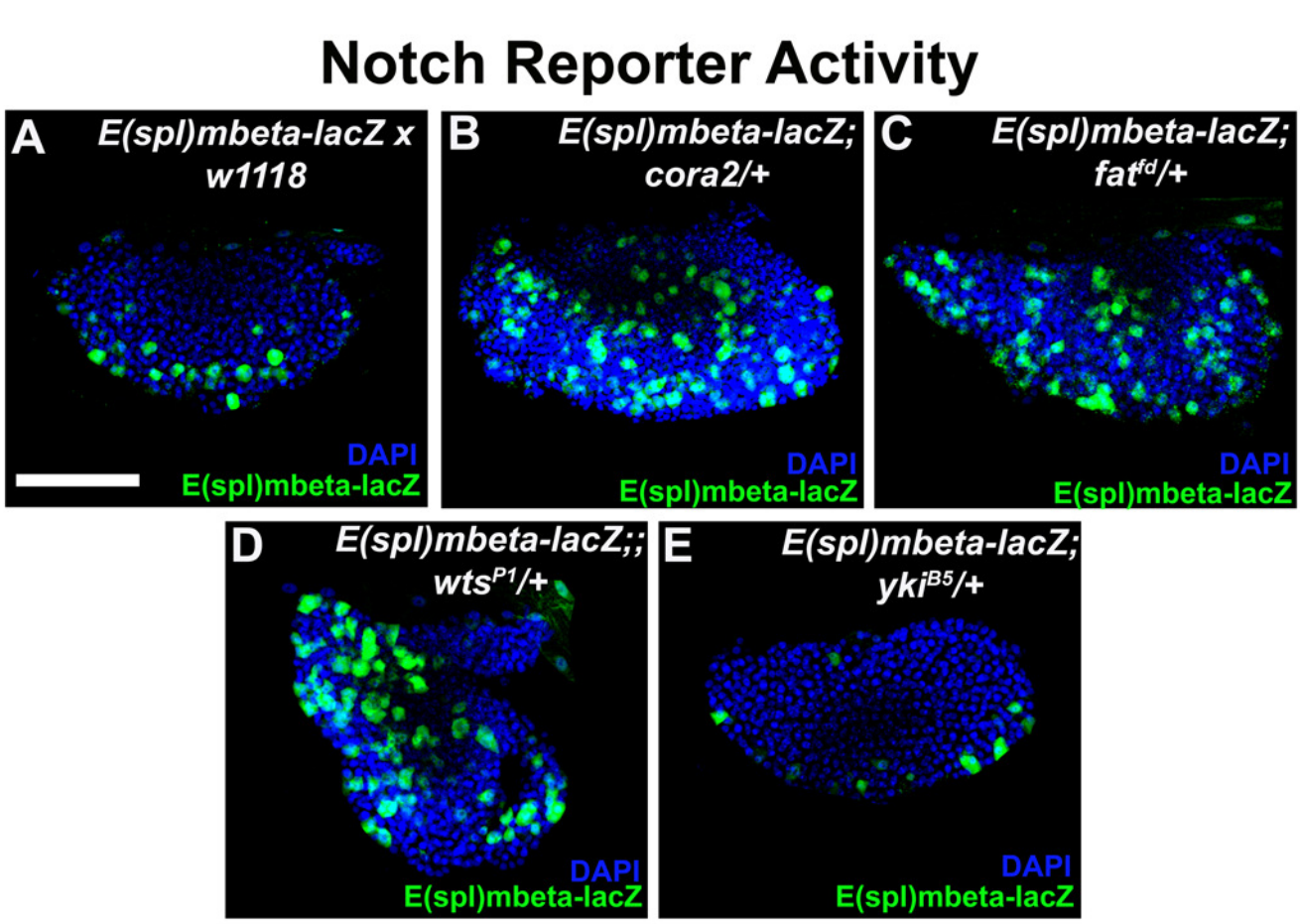


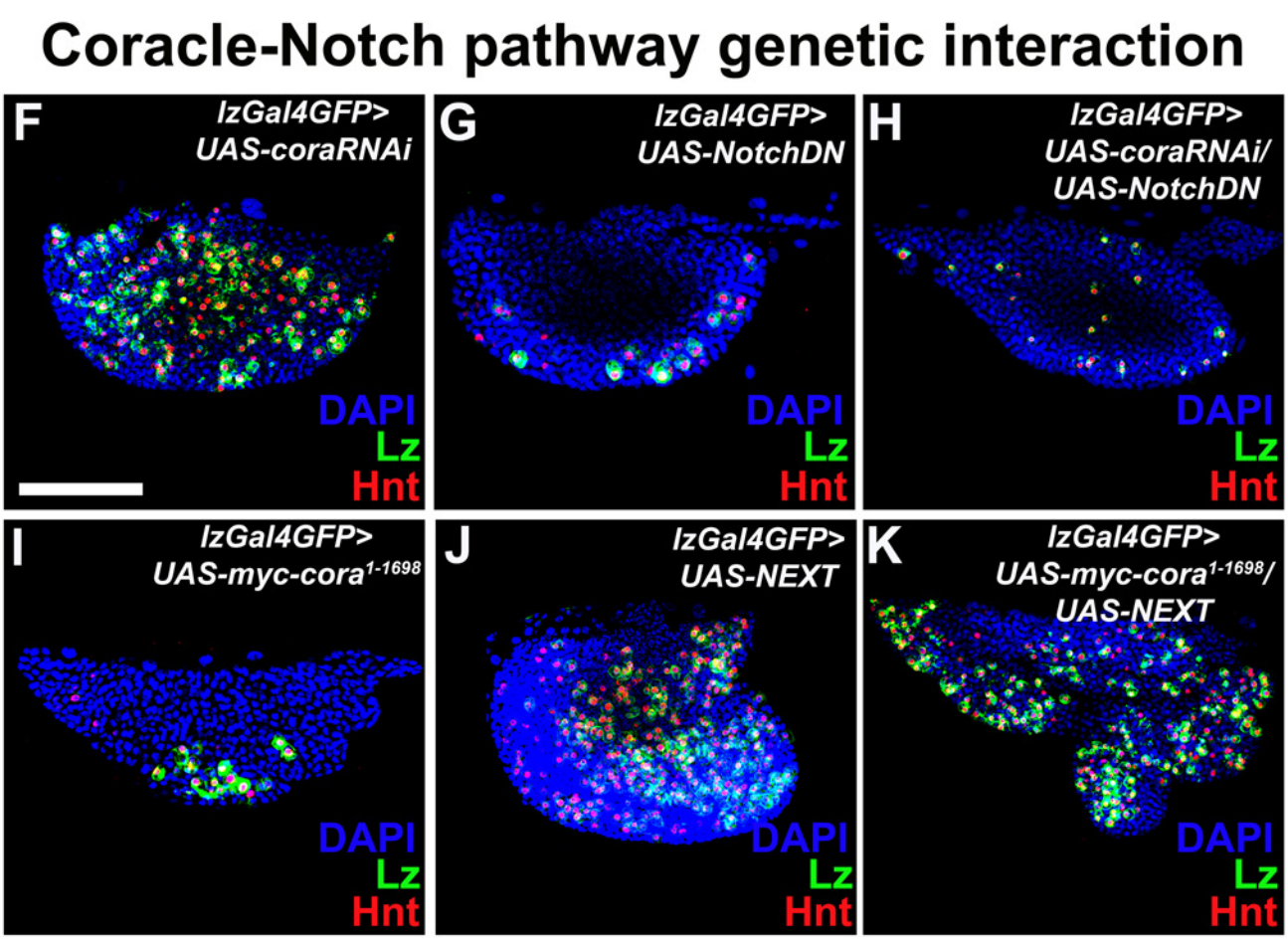


Supplementary figure S7

Figure S7: Fat localization in *cora*, *fat* and *wts* mutants. (Related to Figure 5)

(A-E) Representative high magnification confocal sections (A'-E') of the hemocytes indicated with the boxed region in the primary lymph glands showing Fat expression (white, nuclei labelled with DAPI in blue) in wild type control (*w1118*; A), heterozygous *cora* allele ($cora^{2}$/+; B), two *fat* mutants (fat^{ex13}, fat^{fd}; C & D respectively), or heterozygous *warts* mutant (wts^{P1}/+; E) respectively. (F) Quantification of Fat expression (in arbitrary units, see materials and methods) for genotypes shown in A-E. For (F) Statistical significance was estimated using unpaired t-test with Welch's correction, *** indicates P<0.001. Error bars indicate standard error of the mean. Scale bars: 40µm (A-E), 20µm (A'-E').

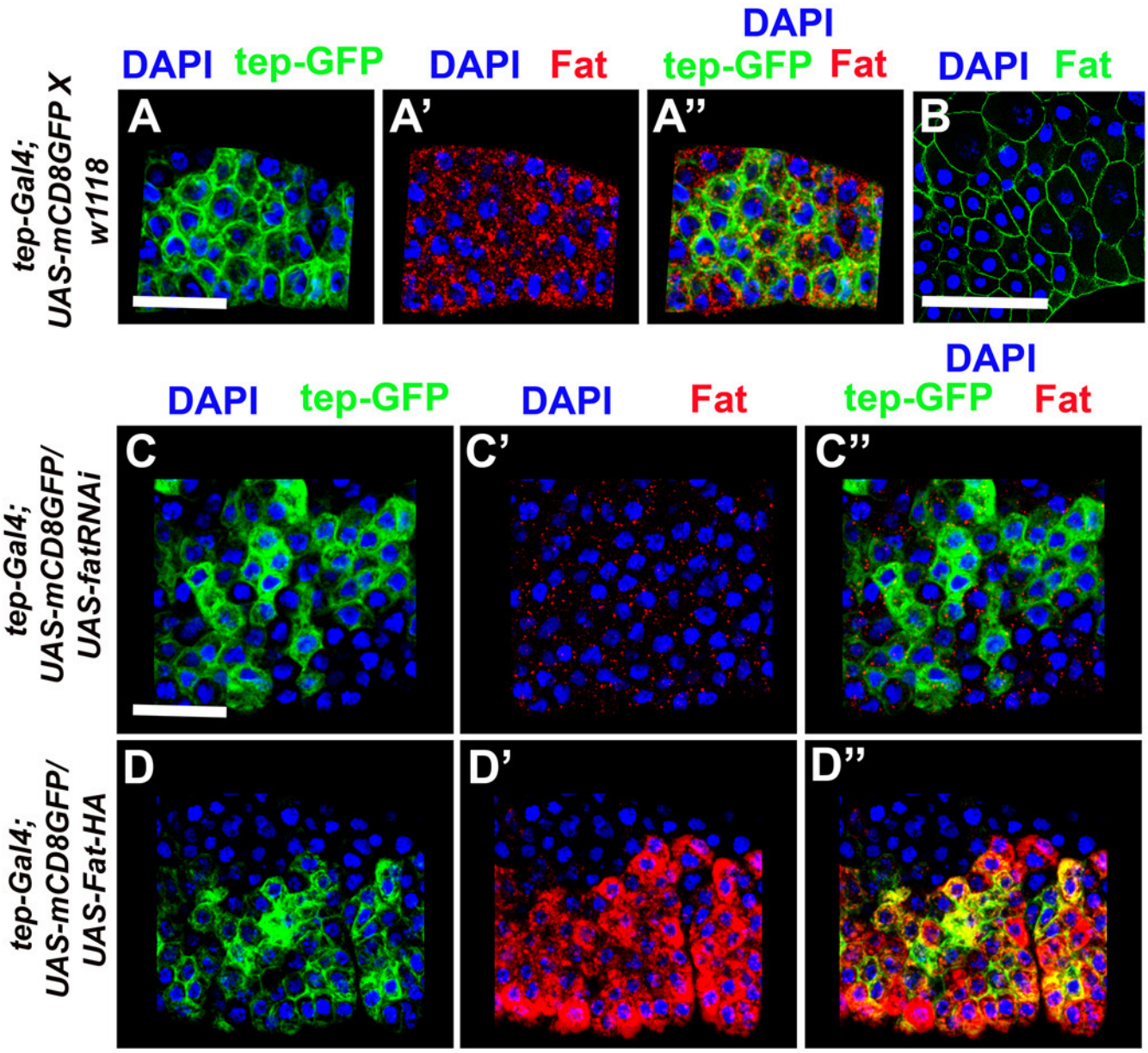




Supplementary figure S8

Figure S8: Crystal cell differentiation following Cora knockdown occurs via Hippo-mediated Notch activation.

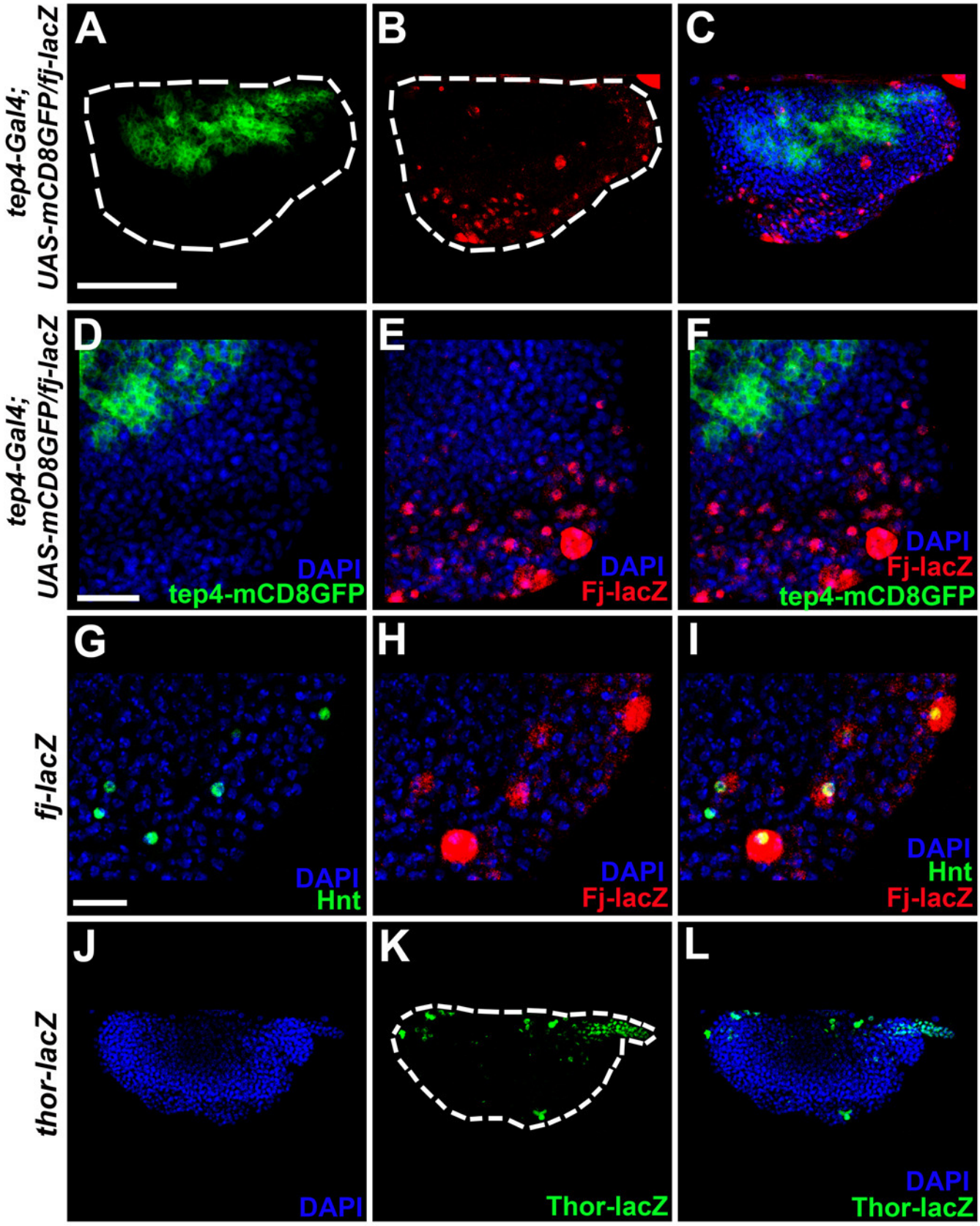
(A-E) Expression of the Notch reporter transgene *E(spl)-mbetalacZ* (in Green, nuclei labelled with DAPI in blue) in representative primary lymph glands from a wild type control (*w1118*; A) or larva heterozygous for mutant alleles of *cora* (*cora2*/+; B), *fat* (fat^{fd}/+; C), *warts* (wts^{P1}/+; D), and *yorkie* mutant (yki^{B5}/; E). (F-K) Crystal cell differentiation in representative primary lymph glands following crystal cell specific (*lz-Gal4* driven) RNAi-mediated knockdown of *cora* (F), expression of a dominant negative *notch* transgene (UAS-NotchDN) (G), or combined knockdown of *cora* and expression of dominant negative *notch* (H). (I-K) Crystal cell differentiation in representative lymph glands following crystal cell specific (*lz-Gal4* driven) over-expression of *cora* (UAS-*myc*-$cora^{1\text{-}1698}$; I), expression of a constitutively active *notch* transgene (UAS-NEXT) (J), or combined over-expression of *cora* with constitutively active *notch* (K). (F-K) Crystal cells labelled with Hnt (Red), *lz-Gal4* expression marked using UAS-GFP (green), Nuclei are marked with DAPI (Blue). Scale bar: 40µm (A-K).



Supplementary figure S9

Figure S9: Validation of Fat expression in lymph gland hemocytes. (Related to Figure 5).

(A-A'') Expression of Fat (Red) in lymph gland prohemocytes marked with membrane tagged UAS-mCD8GFP (Green) driven by *tep4-Gal4*. (B) Fat expression (Green) in salivary gland epithelial cells. Validation of Fat expression upon knockdown (*tep4-Gal4; UAS-mCD8GFP/UAS-fatRNAi*; C-C'') or over-expression (*tep4-Gal4; UAS-mCD8GFP/UAS-Fat-HA*; D-D'') of Fat in prohemocytes using *tep4-Gal4*. Prohemocytes are marked with GFP (Green) driven by *tep4-Gal4*. Nuclei are marked with DAPI (Blue). Scale Bars: 40µm (B) 20µm (A-A'', C-D'').



Supplementary figure S10

Figure S10: Validation of Fj-lacZ expression in lymph gland hemocytes.

(Related to Figure 3).

(A-F) Primary lymph gland lobe hemocytes showing expression of Fj-lacZ (Red) along with prohemocytes marked with GFP (Green) driven by *tep4-Gal4*. D-F are high magnification images of the corresponding primary lymph gland lobe shown in A-C. Fj-lacZ expression (Red) in lymph gland hemocytes co-stained with crystal cell marker Hnt (Green; G-I). Primary lymph gland lobe showing Thor-lacZ expression (Green) in the PSC as a lacZ control. Nuclei are marked with DAPI (Blue). Scale Bars: 40µm (A-C, J-L) 20µm (D-I).

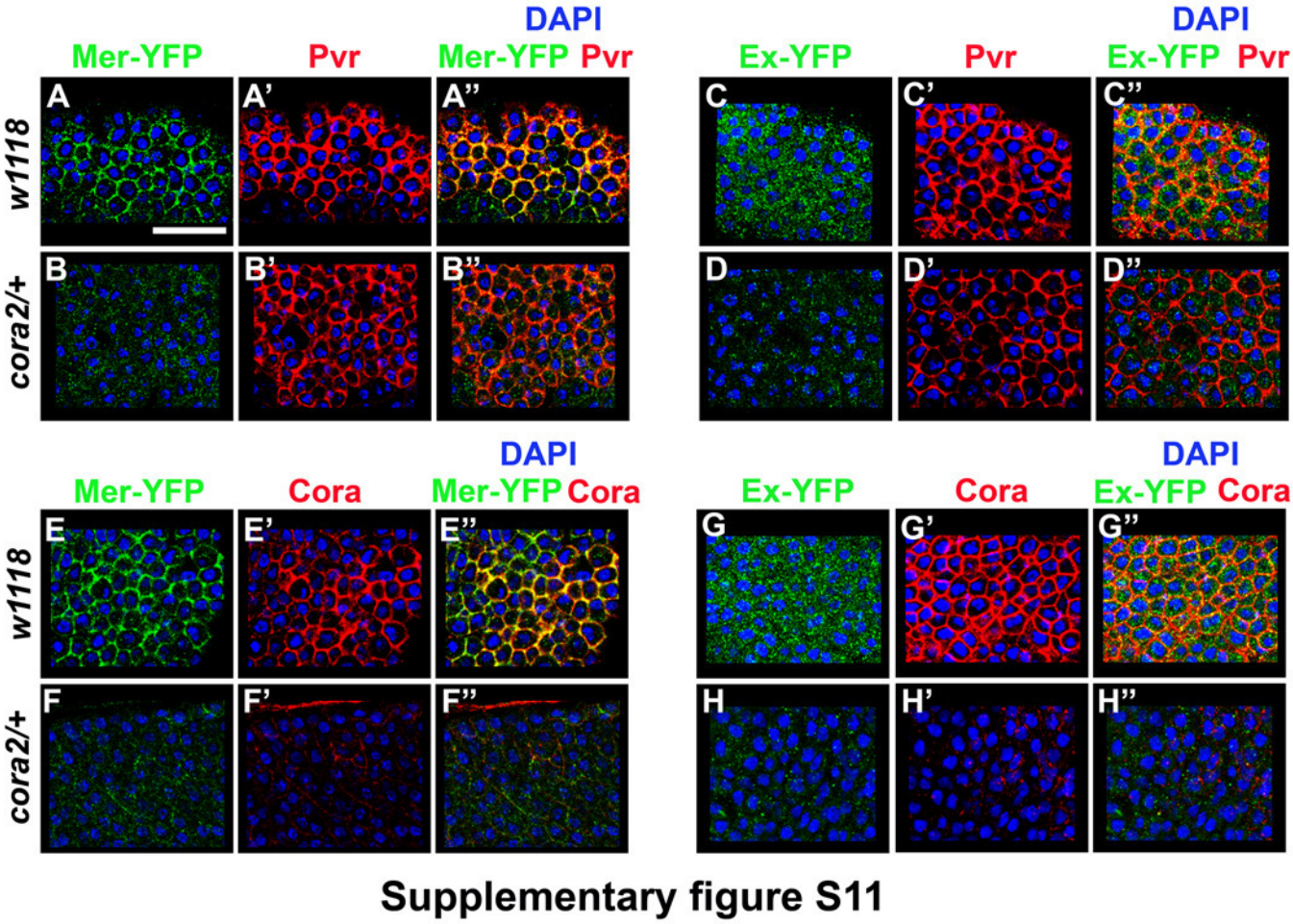
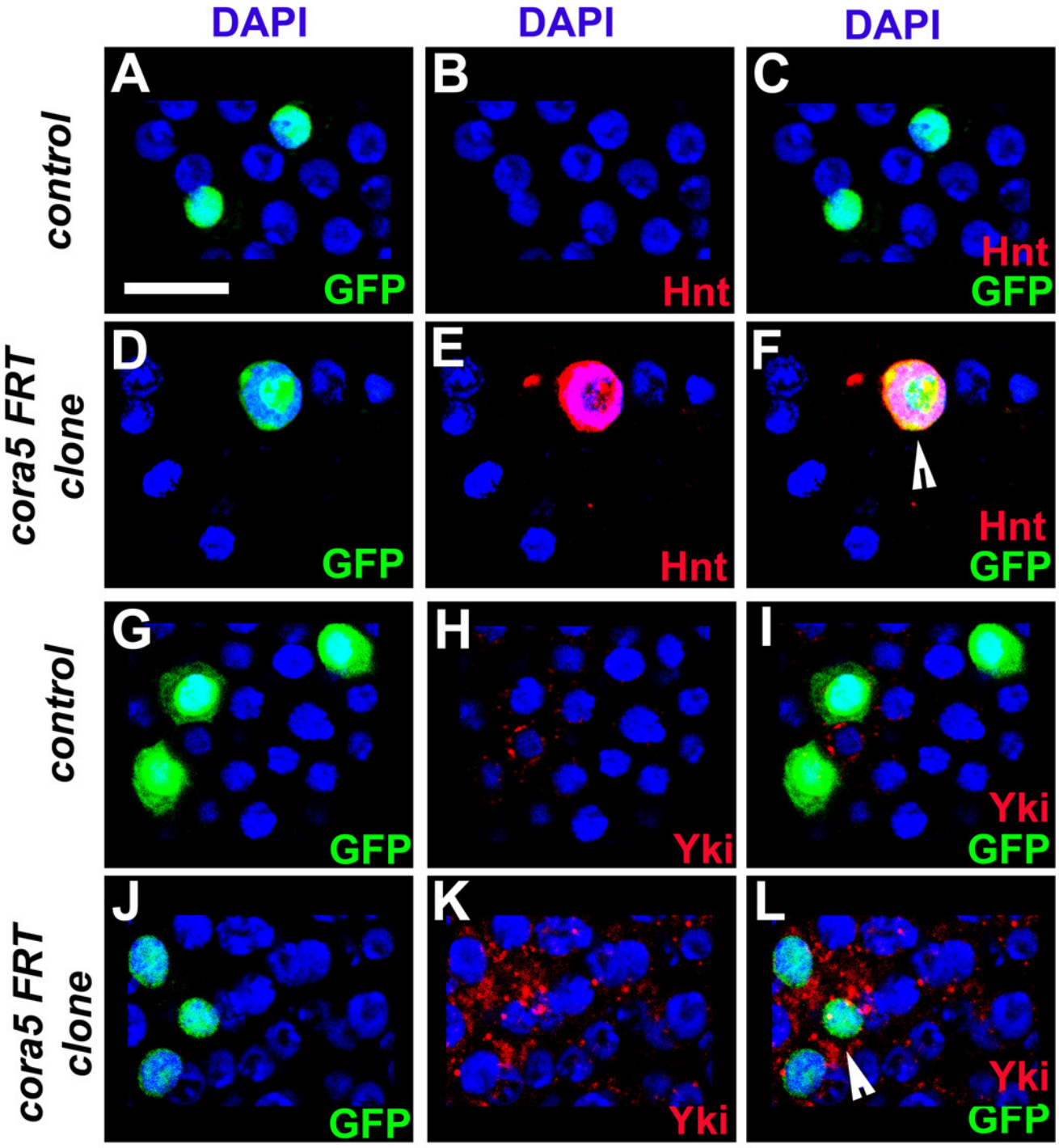


Figure S11: Expanded and Merlin localization is affected in *cora* mutant lymph gland hemocytes. (Related to Figure 5).

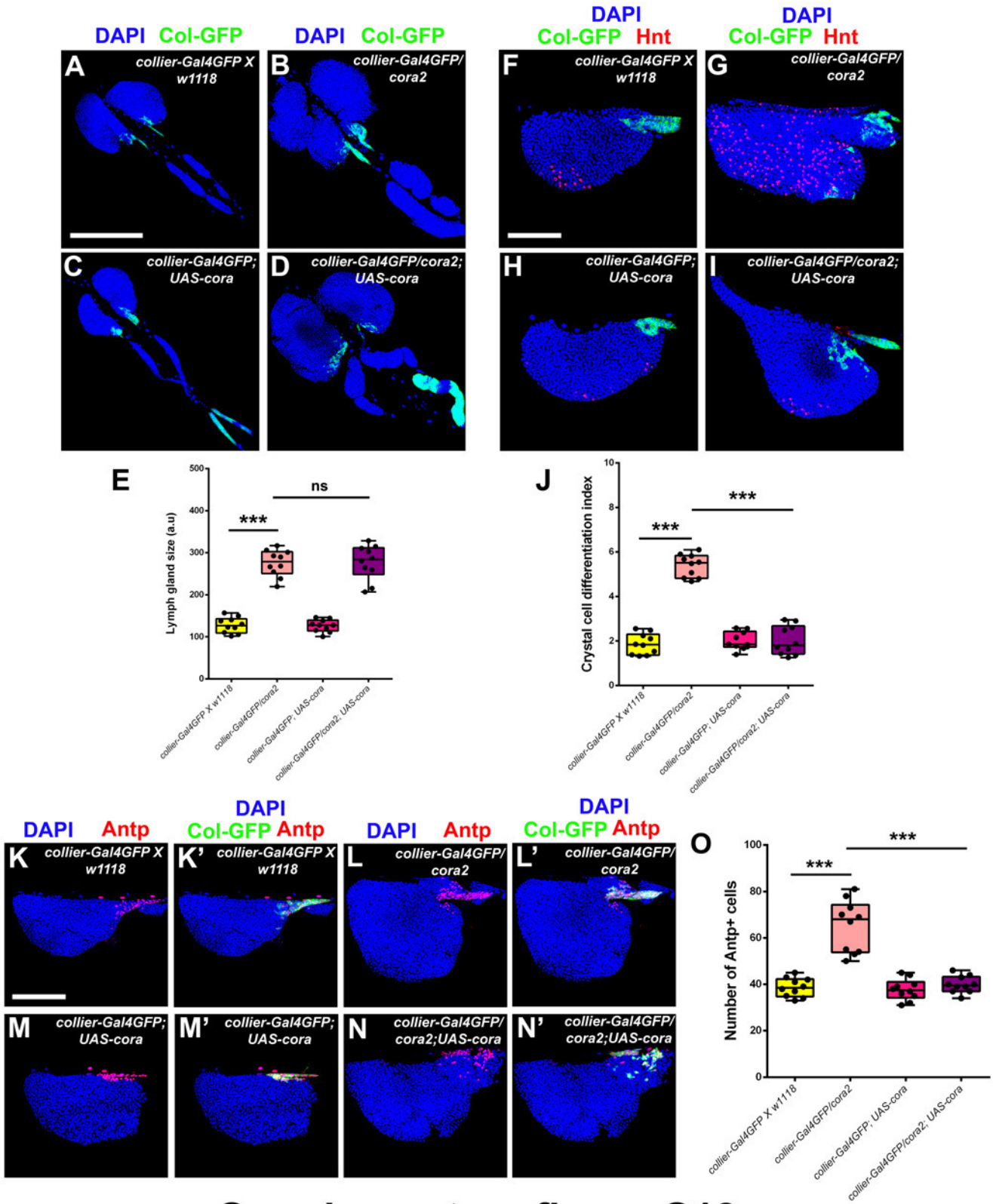
Lymph gland hemocytes co-stained with Merlin-YFP (Green) and membrane marker Pvr (PDF-VEGF Receptor, Red) or Coracle (Red) in wild type control (*w1118*; A-A'', E-E'') and larva heterozygous for mutant null alleles of *coracle* (*cora2/+* ; B-B'', F-F''). Lymph gland hemocytes co-stained with Expanded-YFP (Green) and membrane marker Pvr (PDF-VEGF Receptor, Red) or Coracle (Red) in wild type control (*w1118*; C-C'', G-G'') and larva heterozygous for mutant null alleles of *coracle* (*cora2/+* ; D-D'', H-H''). Nuclei are marked with DAPI (Blue). Scale Bars: 20µm (A-H'').



Supplementary figure S12

Figure S12: *cora* mutant clones differentiate into crystal cells and have high levels of Yorkie.

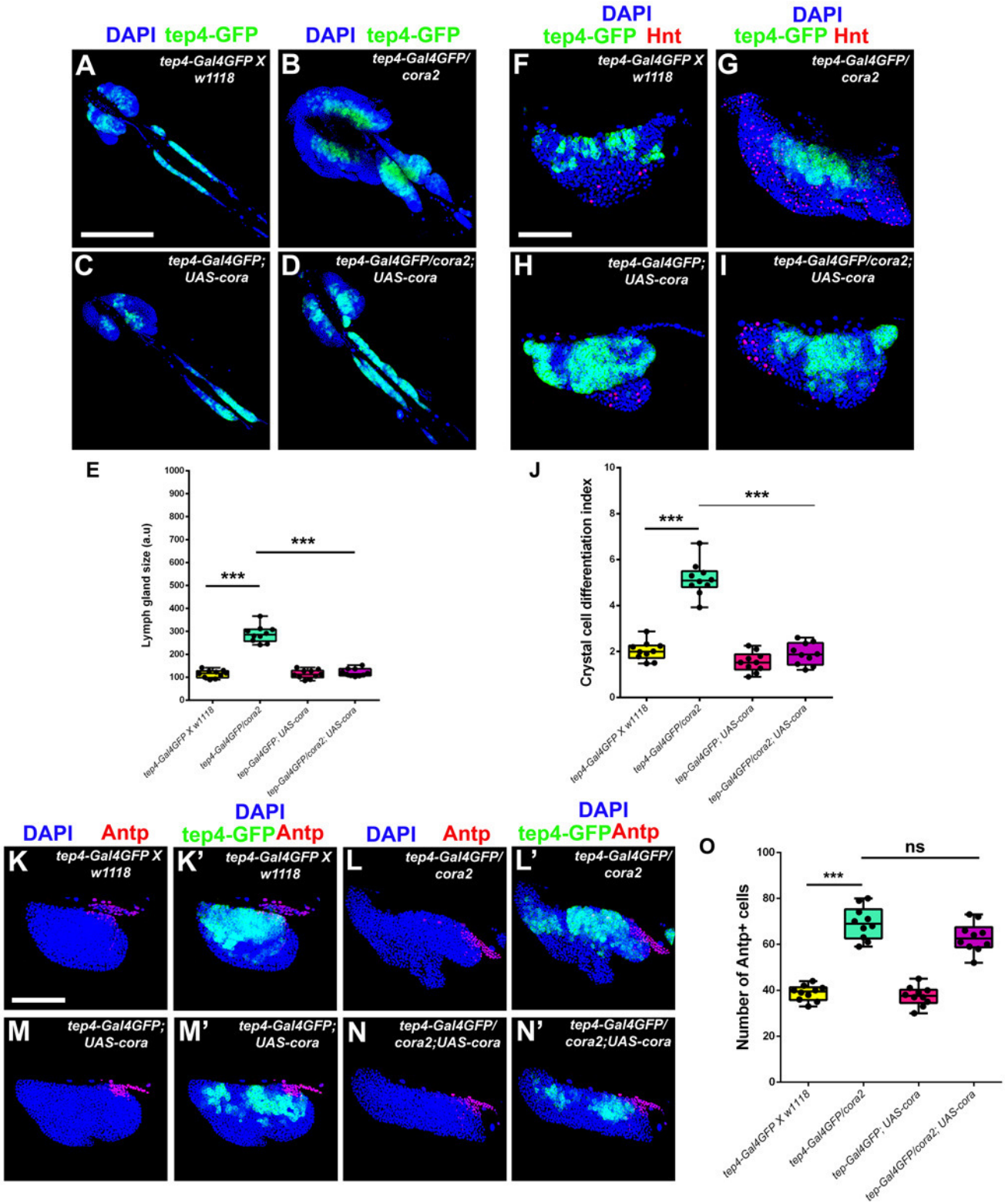
Lymph gland hemocytes consisting of GFP positive (Green) control clones (*Hemese-Gal4/ w[*]; P{w[+mC]=UAS-FLP.Exel}3, P{w[+mC]=Ubi-p63E(FRT.STOP)Stinger}15F2*; A-C, M-O) or *cora* mutant clones (*FRT43D/cora5FRT43D; Hemese-Gal4/w[*]; P{w[+mC]=UAS-FLP.Exel}3, P{w[+mC]=Ubi-p63E(FRT.STOP)Stinger}15F2*; D-F, P-R) co-stained with crystal cell marker Hnt (Red, A-F) or Yorkie (Red, M-R). Nuclei are labelled with DAPI (Blue). Scale Bars: 10µm (A-R).



Supplementary figure S13

Figure S13: Coracle expression in the PSC regulates niche cell numbers and crystal cell differentiation but not the overall lymph gland size.

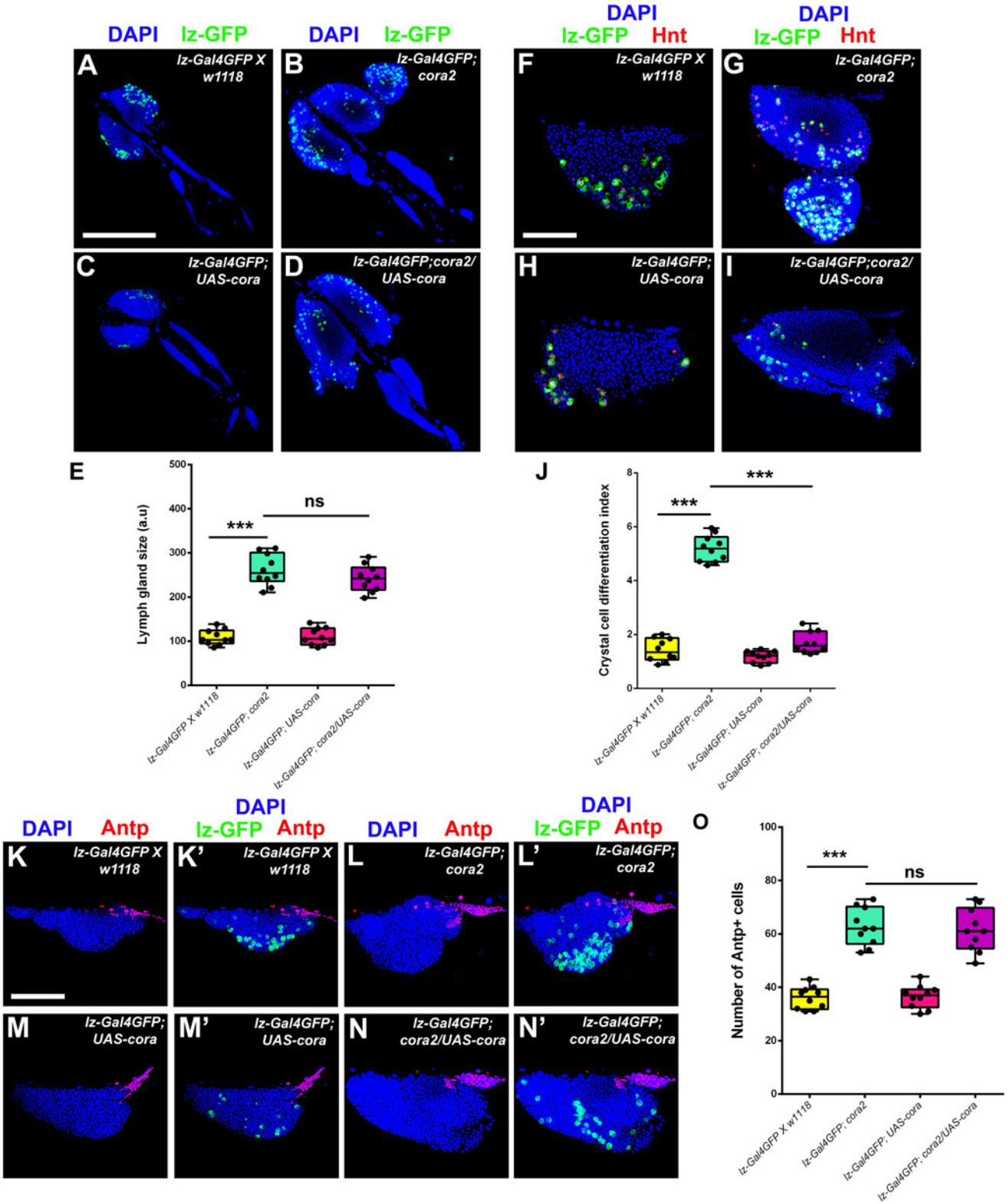
(A-D) Representative whole lymph glands in Gal4 control larva (*collier-Gal4GFP X w1118*; A), larva heterozygous for mutant null alleles of *coracle* (*collier-Gal4GFP/cora2;* B) or upon PSC specific over-expression of Cora alone (*collier-Gal4GFP; UAS-cora*, C) or in the *cora* mutant background (*collier-Gal4GFP/cora2; UAS-cora*, D). (E) Quantification of relative overall lymph gland organ size for genotypes shown in A-D. (F-I) Representative primary lymph gland lobes labelled with the crystal cell marker Hindsight (Hnt; Red) from Gal4 control larva (*collier-Gal4GFP X w1118*; F), larva heterozygous for mutant null alleles of *coracle* (*collier-Gal4GFP/cora2;* G), or upon PSC specific over-expression of Cora alone (*collier-Gal4GFP; UAS-cora*, H) or in the *cora* mutant background (*collier-Gal4GFP/cora2; UAS-cora,* I). (J) Quantification of crystal cell differentiation index (in arbitrary units) for genotypes shown in F-I. (K-N) Representative primary lymph gland lobes labelled with the niche cell marker Antennapedia (Antp; Red) from Gal4 control larva (*collier-Gal4GFP X w1118*; K-K'), larva heterozygous for mutant null alleles of *coracle* (*collier-Gal4GFP/cora2;* L-L'), or upon PSC specific over-expression of Cora alone (*collier-Gal4GFP; UAS-cora*, M-M') or in the *cora* mutant background (*collier-Gal4GFP/cora2; UAS-cora,* N-N'). (O) Quantification of niche cell numbers for genotypes shown in K-N'. Nuclei are marked with DAPI (Blue). PSC cells are marked with GFP (Green) driven by *collier-Gal4*. Statistical significance was estimated using unpaired t-test with Welch's correction, *** indicates $P<0.001$, ns indicates non-significant. Error bars indicate SD of the mean. Scale bars: 40µm (A-D, F-I and K-N').



Supplementary figure S14

Figure S14: Coracle expression in the medullary zone regulates crystal cell differentiation and overall lymph gland size but not niche cell numbers.

(A-D) Representative whole lymph glands in Gal4 control larva (*tep4-Gal4GFP X w1118*; A), larva heterozygous for mutant null alleles of *coracle* (*tep4-Gal4GFP/cora2;* B) or upon medullary zone specific over-expression of Cora alone (*tep4-Gal4GFP; UAS-cora*, C) or in the *cora* mutant background (*tep4-Gal4GFP/cora2; UAS-cora*, D). (E) Quantification of relative overall lymph gland organ size for genotypes shown in A-D. (F-I) Representative primary lymph gland lobes labelled with the crystal cell marker Hindsight (Hnt; Red) from Gal4 control larva (*tep4-Gal4GFP X w1118*; F), larva heterozygous for mutant null alleles of *coracle* (*tep4-Gal4GFP/cora2;* G), or upon medullary zone specific over-expression of Cora alone (*tep4-Gal4GFP; UAS-cora*, H) or in the *cora* mutant background (*tep4-Gal4GFP/cora2; UAS-cora*, I). (J) Quantification of crystal cell differentiation index (in arbitrary units) for genotypes shown in F-I. (K-N) Representative primary lymph gland lobes labelled with the niche cell marker Antennapedia (Antp; Red) from Gal4 control larva (*tep4-Gal4GFP X w1118*; K-K'), larva heterozygous for mutant null alleles of *coracle* (*tep4-Gal4GFP/cora2;* L-L'), or upon medullary zone specific over-expression of Cora alone (*tep4-Gal4GFP; UAS-cora*, M-M') or in the *cora* mutant background (*tep4-Gal4GFP/cora2; UAS-cora*, N-N'). (O) Quantification of niche cell numbers for genotypes shown in K-N'. Nuclei are marked with DAPI (Blue). Medullary zone prohemocytes are marked with GFP (Green) driven by *tep4-Gal4*. Statistical significance was estimated using unpaired t-test with Welch's correction, *** indicates $P<0.001$, ns indicates non-significant. Error bars indicate SD of the mean. Scale bars: 40µm (A-D, F-I and K-N').



Supplementary figure S15

Figure S15: Coracle expression in the crystal cells cell-autonomously regulates crystal cell differentiation but does not control overall lymph gland size and niche cell numbers.

(A-D) Representative whole lymph glands in Gal4 control larva (*lz-Gal4GFP X w1118*; A), larva heterozygous for mutant null alleles of *coracle* (*lz-Gal4GFP; cora2;* B) or upon crystal cell specific over-expression of Cora alone (*lz-Gal4GFP; UAS-cora*, C) or in the *cora* mutant background (*lz-Gal4GFP; cora2/UAS-cora,* D). (E) Quantification of relative overall lymph gland organ size for genotypes shown in A-D. (F-I) Representative primary lymph gland lobes labelled with the crystal cell marker Hindsight (Hnt; Red) from Gal4 control larva (*lz-Gal4GFP X w1118*; F), larva heterozygous for mutant null alleles of *coracle* (*lz-Gal4GFP; cora2;* G), or upon crystal cell specific over-expression of Cora alone (*lz-Gal4GFP; UAS-cora*, H) or in the *cora* mutant background (*lz-Gal4GFP; cora2/UAS-cora,* I). (J) Quantification of crystal cell differentiation index (in arbitrary units) for genotypes shown in F-I. (K-N) Representative primary lymph gland lobes labelled with the niche cell marker Antennapedia (Antp; Red) from Gal4 control larva (*lz-Gal4GFP X w1118*; K-K'), larva heterozygous for mutant null alleles of *coracle* (*lz-Gal4GFP; cora2;* L-L'), or upon crystal cell specific over-expression of Cora alone (*lz-Gal4GFP; UAS-cora*, M-M') or in the *cora* mutant background (*lz-Gal4GFP; cora2/UAS-cora,* N-N'). (O) Quantification of niche cell numbers for genotypes shown in K-N'. Nuclei are marked with DAPI (Blue). Crystal cells are also marked with GFP (Green) driven by *lz-Gal4* along with Hnt (Red). Statistical significance was estimated using unpaired t-test with Welch's correction, *** indicates $P<0.001$, ns indicates non-significant. Error bars indicate SD of the mean. Scale bars: 40µm (A-D, F-I and K-N').

Supplemental materials and methods:

***Drosophila* genetics:**

Prohemocyte specific or Intermediate/differentiating hemocyte specific RNAi or over-expression experiments were done using *tep4-Gal4* or *Pxn-Gal4* respectively. Crystal cell /maturing hemocyte specific knockdown or over-expression experiments were carried out using *lozenge-Gal4 (lz-Gal4)*. To validate the crystal cell differentiation phenotypes upon knockdown of *cora* or Hippo pathway components, an alternate crystal cell specific *pebbled-Gal4* driver was used for the crystal cell differentiation analysis. PSC specific over-expression experiments were performed using *collier-Gal4*. *eater-Gal4* was used for plasmatocyte lineage specific knockdown analysis. Heterozygous and homozygous mutants of the Hippo pathway components were used for the analysis of lymph gland tissue growth (by counting total number of cells) and crystal cell differentiation phenotypes. In addition to these, transheterozygotes of *warts* alleles were made namely: wts^{X1}/wts^{P1} and wts^{MGH1}/wts^{P1}. These alleles were originally balanced over *TM6, Tb* and upon genetic crossing; non-tubby larval progeny was selected for analysis. Total number of cells in the LG and crystal cell differentiation was analyzed in the transheterozygotes. SJ component mutant alleles or mutant alleles of the lateral polarity components Scrib or Dlg or hippo pathway mutant alleles were crossed to *w1118* (as wild type) for all the analysis using the heterozygotes. For analysis of Four-jointed expression using the Four-jointed LacZ reporter, the flies bearing the *fj-lacZ* were placed in the genetic background of either the heterozygous mutant allele of *cora* or the core hippo pathway components namely fat, *warts* and *yorkie*. To analyse whether Fj-lacZ is expressed in the lymph gland prohemocytes; *fj-lacZ* was genetically crossed to flies expressing *UAS-mCD8GFP* in the prohemocytes using *tep4-Gal4*. *thor-lacZ* was used as an unrelated lacZ control for the Fj-lacZ reporter analysis. *bantam-GFP* microRNA sensor was genetically placed in the genetic background of either the heterozygous mutant allele of *cora* or

the core hippo pathway components namely fat, *warts* and *yorkie* to analyze the transcriptional activation of Yorkie. bantam-GFP sensor was crossed to flies expressing *tep4-Gal4* driven mCD8RFP in the prohemocytes to analyze its expression in the prohemocytes. bantam-GFP sensor levels were analyzed upon expression of bantam microRNA sponge in the prohemocytes using *tep4-Gal4* as compared to the microRNA scramble sponge control. bantam-GFP sensor was also crossed to *bantam-lacZ* which reproduces the expression of the bantam microRNA to study the expression profile and validate the sensor. Double trans-heterozygotes of the mutant allele of *cora*, *NrxIV* or the core hippo pathway components with yorkie were generated to test if Yorkie genetically interacts with SJ components. To analyze Expanded and Merlin localization and recruitment in *cora* mutant genetic background, trans-heterozygotes of *expanded-YFP* or *merlin-YFP* were generated with the heterozygous mutant allele of *cora* (*cora2/CyO*). To analyze the activation of Notch pathway, Notch reporter *E(spl)mbeta-lacZ* was combined in a similar manner as *fj-lacZ* and then analyzed by staining with β-galactosidase antibody to detect β-gal positive cells. For all the *lz-Gal4, UAS-mCD8GFP* analysis; all the females used for the final genetic cross to obtain final progeny of interest had the following transgenes on their X-chromosome (*lz-Gal4, UAS-mCD8GFP/Fm7*). These females were crossed to males bearing *lz-Gal4, UAS-mCD8GFP*. These flies also contained corresponding *UAS-RNAi* or over-expression constructs on the 3rd chromosome. To study whether the crystal cells in the *cora* mutant genetic background or in the mutant background of the core hippo pathway components are dual positive for plasmatocyte antigen NimRodC1 (P1), *lz-Gal4* driving *UAS-GFP* was combined with heterozygous mutant allele of *cora* or the core hippo pathway components namely *fat* and *warts*. To study whether the dual positive differentiated hemocytes that are positive for both crystal cell and plasmatocyte markers are due to the mis-regulation of Klumpfuss; *UAS-KluEnR* (which acts as a gain of function construct upon over-expression) was over-expressed in *cora* knockdown

genetic background using crystal cell specific driver (*lz-Gal4*) to study whether the dual positive differentiated hemocyte phenotype is rescued. To check whether plasmatocyte lineage specific knockdown of *cora* or *warts* leads to the formation of dual positive differentiated hemocytes; plasmatocyte specific *eater-Gal4* was used for knockdown of *cora* or *warts* and then analyzed. Double trans-heterozygotes of the *cora* mutant allele (*cora2/CyO*) with the mutant alleles of core hippo pathway components namely *fat (fat^{fd}/CyO)* and *warts (wtsP1/Tm6Tb)* were generated to study whether they genetically act together in regulating crystal cell differentiation. To validate the Fat antibody and the expression of Fat in the lymph gland cells, Fat was depleted or over-expressed using *UAS-fatRNAi* or *UAS-fat-HA* construct. These constructs were driven in the prohemocytes using *tep-Gal4* to study Fat expression. Using FLP-FRT recombination, *cora* mutant clones were generated in the lymph gland. The construct used expresses Stinger (a nuclear-localized form of EGFP) under the control of a ubiquitin promoter upon FLP-mediated removal of an FRT cassette. *UAS-FLP* was driven by *Hemese-Gal4*. The control clones used for this analysis lacked the *cora* mutant (*cora5*) FRT allele cassette. PSC specific, MZ specific and crystal cell specific rescue analysis of the heterozygous *cora* mutant using the *UAS-cora* construct was performed using *collier-Gal4*, *tep4-Gal4* and *lz-Gal4* respectively.

Estimation of differentiation indices using MatLab:

To determine the number of differentiated crystal cells or plasmatocytes in the lymph gland, we developed custom cell counting scripts in MatLab. This script has been described and deposited in our previous study as Source code 1 and 2 (Khadilkar et al., 2017). We first filtered every image in the z-stack in the DAPI channel using a difference of Gaussians approach. A wide filter is used to remove background intensity, and a smaller filter is used to remove small objects. We applied each filter to the image and subtracted the result of the smaller filter from that of the wide filter, then thresholded the final image to generate a binary mask which effectively

identified cell nuclei. The script then automatically identified the bright spots within the 3 dimensional image corresponding to nuclei in order to determine their numbers and centroid coordinates. To calculate differentiation index, we determined what proportion of these cells also expressed markers for differentiation. The differentiation index was then calculated as follows: Differentiation index = Number of cells positive for differentiation marker identified by the script/total number of DAPI positive cells identified by the script. The index has been represented as a percentile. For each cell nucleus identified, we automatically measured intensity in the red channel within a search radius around the nucleus centroid. The search radius was defined as 1.5 times the average radius of nuclei. Nuclei surrounded by above threshold intensity in the red channel were considered differentiated. These numbers were then used to calculate the crystal cell differentiation indices or the total number of plasmatocytes. Index of dual positive crystal cells (for Lz-GFP and plasmatocyte marker, P1) was estimated by quantifying total GFP positive crystal cells overlapping with the P1 marker (Red) and then calculating the percentile of these dual positive cells with the total number of cells in the primary lymph gland lobe. In addition to this, Fj- LacZ reporter activity was analyzed by estimating the Fj-LacZ positive cells in the genetic background of heterozygous mutant allele of *cora* or core hippo pathway components was analyzed using this MatLab script and then plotted as an index of Fj-LacZ positive cells with respect to total number of cells in the primary lymph gland lobe. Number of larvae from which lymph glands were analyzed for all of the above analysis is 10 (n=10).