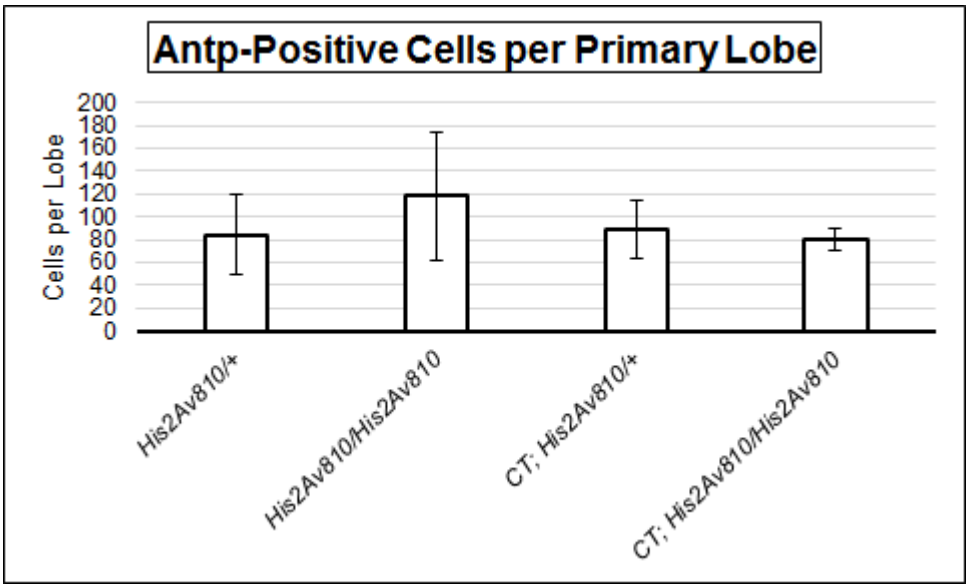
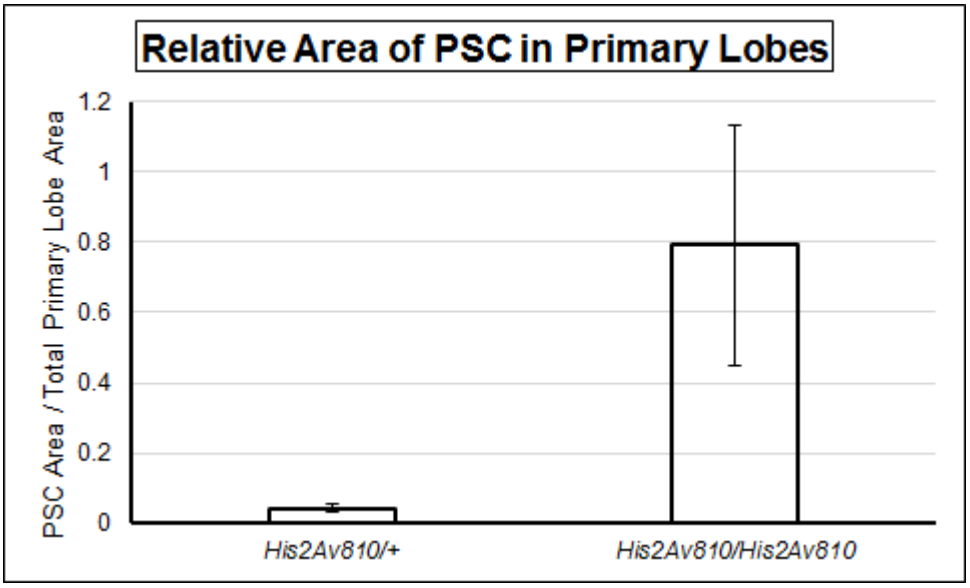
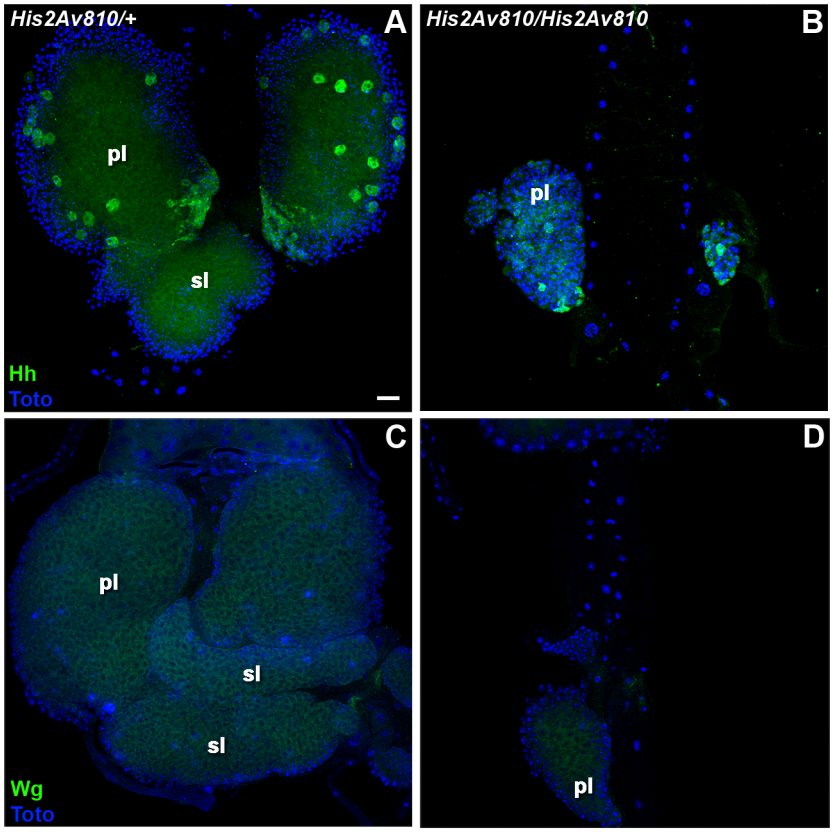




Supplementary Figure S1

PSC cell number is unaffected, but non-PSC cells are greatly diminished in a *His2Av* null mutant. Top panel: Antp-positive cells were counted in primary lobes of lymph glands from larvae of the indicated genotypes. Bars indicate the mean values of four to six samples for each genotype with error bars indicating standard deviations. No statistically significant differences ($P < 0.05$) between pairs of genotypes were detected using a two-tailed Student t-test. Bottom panel: Image J was used to measure the areas of entire primary lymph gland lobes and the areas occupied by Antp-positive cells of the PSC. Bars indicate the mean values of the ratio of PSC area to total area for each genotype with error bars indicating standard deviations. A statistically significant difference ($P = 0.0005$) between the two genotypes was detected using a two-tailed Student t-test.

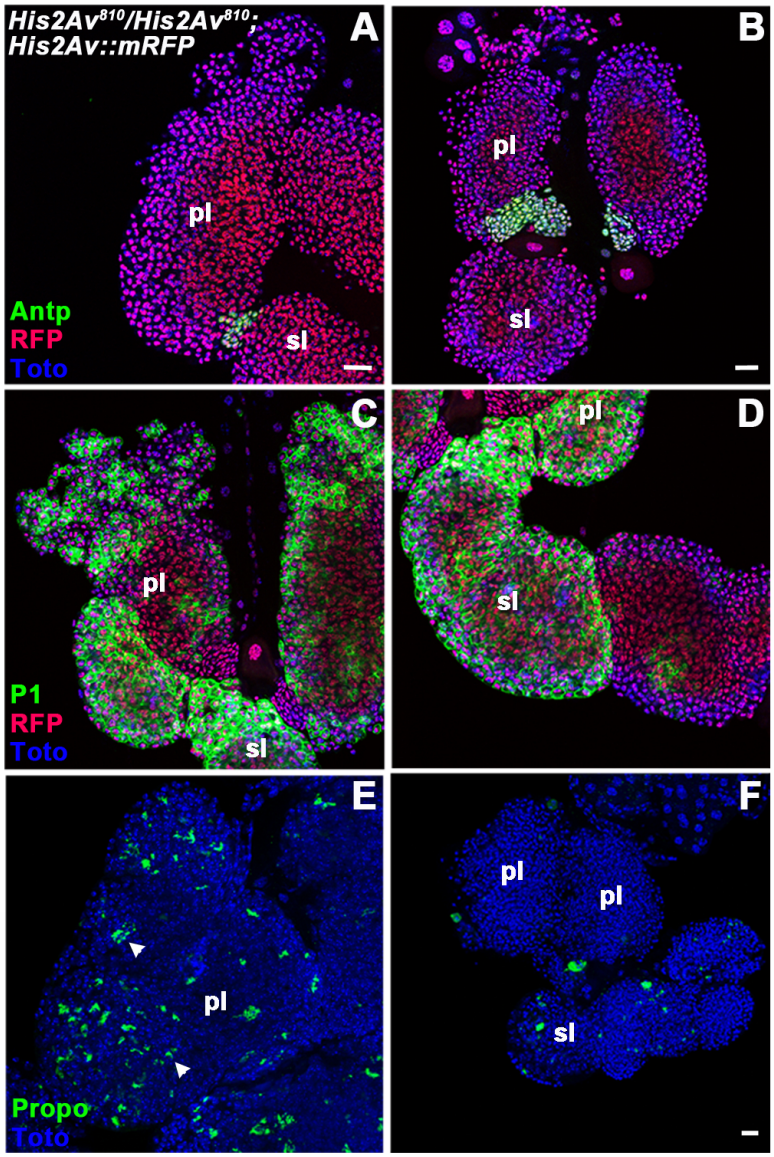


Grigorian, et al, Supplementary Figure S2

Supplementary Figure S2

Hedgehog and Wingless proteins are present in the cells of *His2Av* null mutant lymph glands. Larval lymph glands of the indicated genotypes were dissected, fixed, and stained with either anti-Hedgehog antibody (Hh, green, A-B) or with anti-Wingless antibody (Wg, green, C-D), as well as with the DNA dye TOTO-3 (Toto, blue, A-D). A tight cluster of Hh-positive cells is present in the PSC regions of each primary lymph gland lobe in *His2Av*^{810} / + heterozygous larvae as well as scattered Hh-positive hemocytes in the cortical zone (A). A tight cluster of Hh-positive cells is also present in the PSC regions of each primary lymph gland lobe in *His2Av*^{810} / *His2Av*^{810} homozygous larvae (B). Few if any prohemocytes are present in the homozygous mutant lymph glands. The differences in relative areas of the primary lobe occupied by Hh-positive cells were similar to those observed for Antp-positive cells in Figure S1 (0.25 for

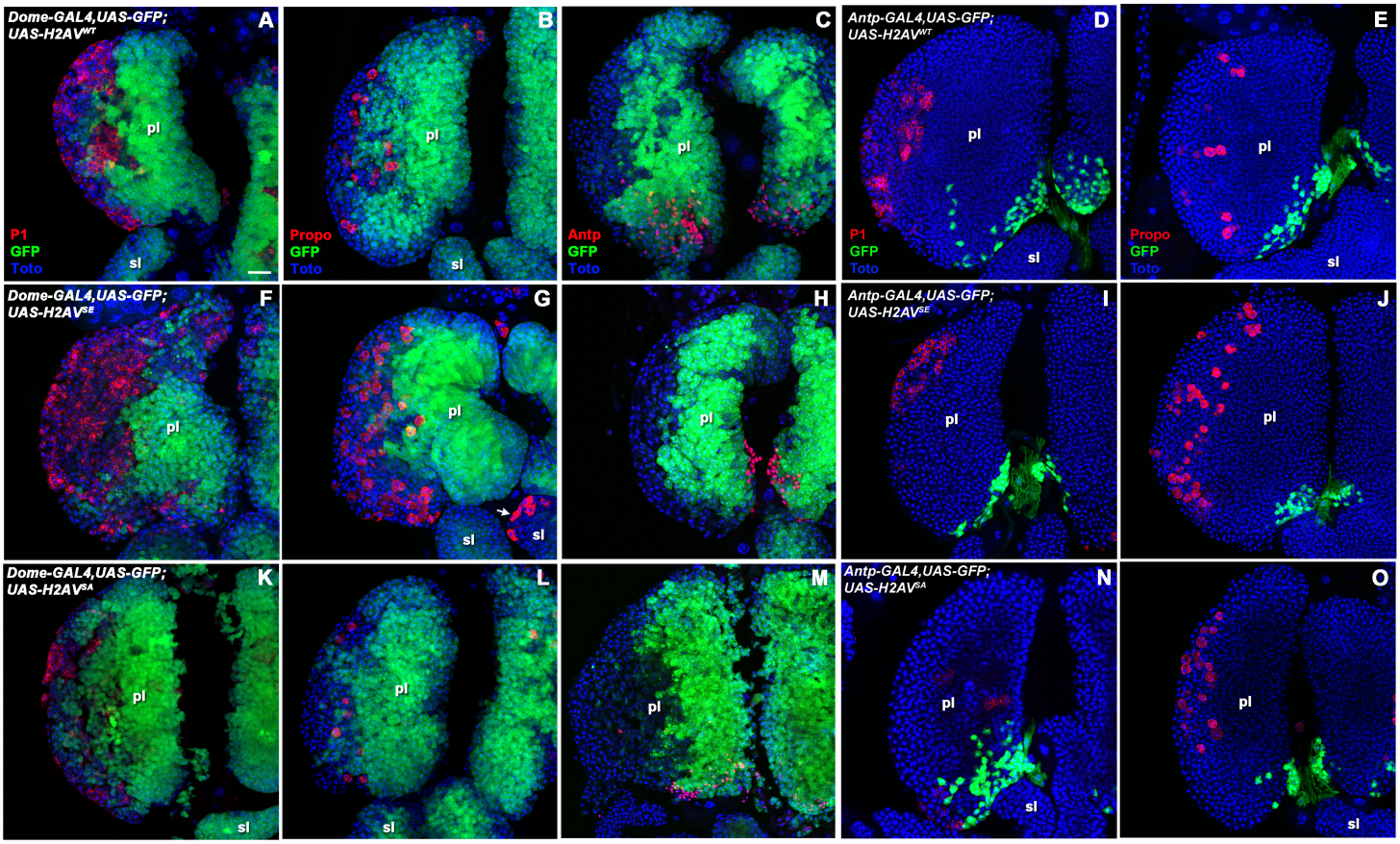
homozygotes; 0.08 for heterozygotes). Wg expression can be seen in the primary and secondary lobes of the *His2Av*^{810} / + heterozygous larval lymph gland (C). Wg expression is also seen in what remains of the primary lymph gland lobe of *His2Av*^{810} / *His2Av*^{810} homozygous larvae (D). Abbreviations: pl: primary lobe; sl: secondary lobe. Scale Bars: A-D, 20μm.



Grigorian, et al, Supplementary Figure S3

Supplementary Figure S3

An H2AV-mRFP fusion protein rescues larval lymph gland structure and function in a *His2Av* null mutant. A transgene expressing an H2AV protein fused to mRFP *P{His2Av::mRFP}* was tested for lymph gland rescue in a $His2Av^{810}$ / $His2Av^{810}$ homozygous mutant background. Immunostaining was used to detect H2AV-mRFP (RFP, red; A-D), the PSC (Antp, green;A-B), plasmatocytes (P1,green; C-D), and crystal cells (Propo, green; E-F). Nuclei were stained with a DNA dye (Toto, blue; A-F). Arrowheads mark areas where crystal cells appear to have burst and left debris (E). Panels B and F are shown at lower magnification to facilitate visualization of the secondary lobes. Abbreviations: pl: primary lobe; sl: secondary lobe. Scale Bars: A,C,D,E, 20μm ; B, 20μm ; F, 20μm.



Grigorian, et al, Supplementary Figure S4

Supplementary Figure S4

Ectopic expression of H2AV proteins leads to changes in the lymph gland. *Domeless-GAL4* (*Dome-GAL4*) was used to drive the expression of *UAS-GFP* along with *UAS-H2AV^{WT}* (A-C), *UAS-H2AV^{SE}* phospho-mimic (F-H), or *UAS-H2AV^{SA}* phospho-mutant (K-M) in an otherwise wild-type *His2Av* background. Lymph glands were immunostained for the presence of plasmatocytes (P1, red; A,F,K), crystal cells (Propo,red; B,G,L), or the PSC (Antp, red; C,H,M). The arrow points to an area of abnormal crystal cell differentiation within the secondary lobe (G). *Antennapedia-GAL4* (*Antp-GAL4*) was used to drive *UAS-GFP* along with *UAS-H2AV^{WT}* (D,E), *UAS-H2AV^{SE}* phospho-mimic (I,J), or *UAS-H2AV^{SA}* phospho-mutant (N,O). Plasmatocytes (P1, red; D,I,N) and crystal cells (Propo, red; E,J,O) were detected by immunostaining. GFP indicates the areas in which the GAL4 drivers are active (green; A-O). Nuclei were labeled with a DNA dye (Toto, blue; A-O). Abbreviations: pl: primary lobe; sl: secondary lobe. Scale Bars: A-O, 20μm.

The arrow points to an area of abnormal crystal cell differentiation within the secondary lobe (G). *Antennapedia-GAL4* (*Antp-GAL4*) was used to drive *UAS-GFP* along with *UAS-H2AV*^{WT} (D,E), *UAS-H2AV*^{SE} phospho-mimic (I,J), or *UAS-H2AV*^{SA} phospho-mutant (N,O). Plasmatocytes (P1, red; D,I,N) and crystal cells (Propo, red; E,J,O) were detected by immunostaining. GFP indicates the areas in which the GAL4 drivers are active (green; A-O). Nuclei were labeled with a DNA dye (Toto, blue; A-O). Abbreviations: pl: primary lobe; sl: secondary lobe. Scale Bars: A-O, 20μm.