Supplemental Data:

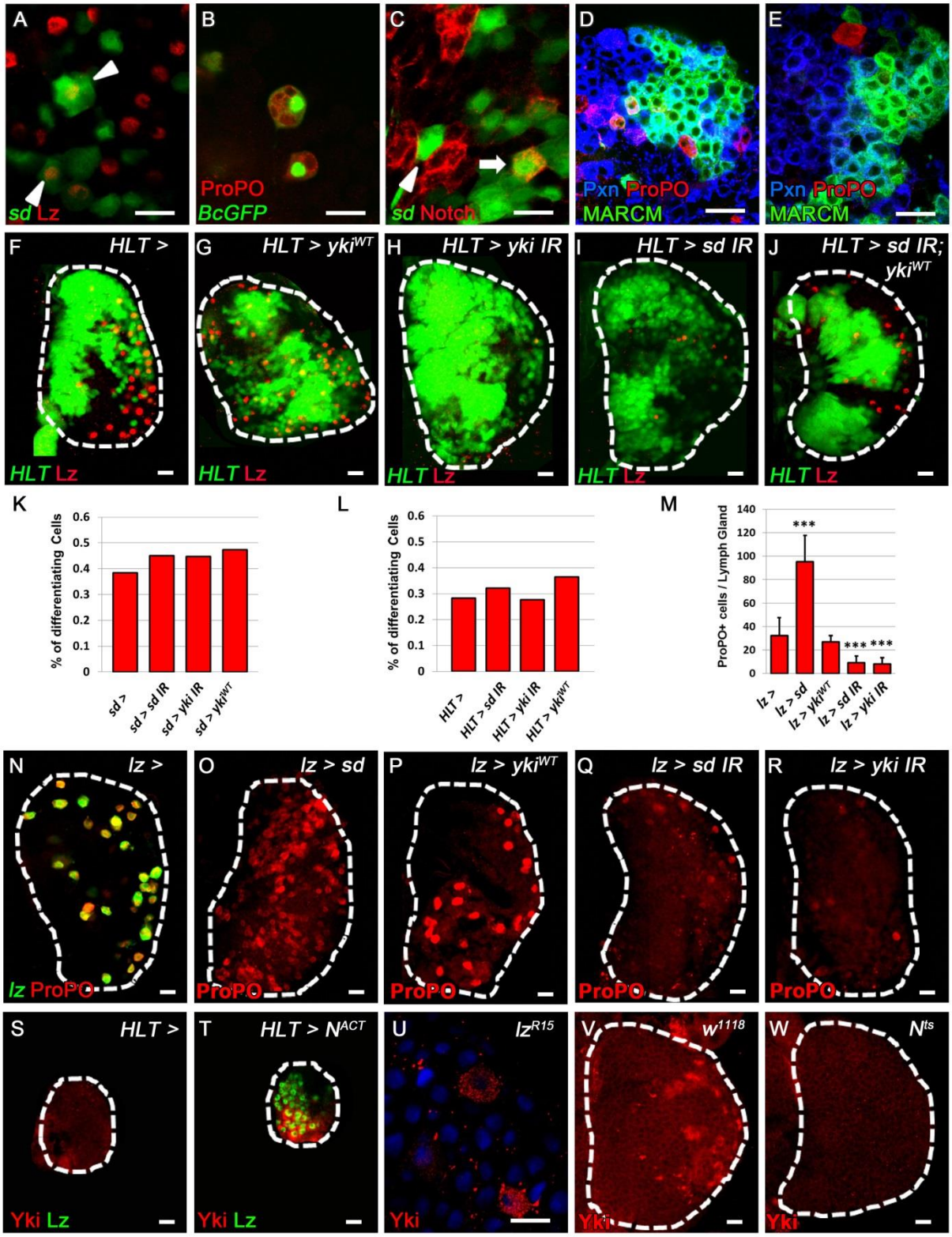

Figure S1, related to Fig. 1: Yorkie and Scalloped promote crystal cell formation. (A) *sd* (green) and Lz (red) expression in the CZ of a 3rd instar lymph gland. (B) Mature CC marker ProPO (red) is co-expressed in CCs labeled with *Black-cells (Bc)GFP* (green) (C) *sd* (green) co-localizes with Notch (red, arrow), and in adjacent cells (arrowheads). (D) CCs (ProPO, red) are observed within control MARCM clones (green, overlap yellow) in the CZ (Peroxidasin, Pxn, blue) of 3rd instar lymph glands, but are not observed in (E) *sd* mutant clones. (F-J) *HLT* clonal expression in the lymph gland (green). CCPs are labeled with Lz (red) (K-L) Quantification of changes to differentiation upon *yki* and *sd* manipulation in the lymph gland with *sd-gal4* (K) and *HLT* (L) (M) Quantification of Fig. S1N-R (n=10) (N) WT lymph gland. *Lz-gal4 >UAS-GFP* (green) labels CCPs and ProPO (red) labels mature CCs. (O) Over-expression of *sd* in CCPs (*lz-gal4 >*) increases CC numbers while (P) over-expression of yki^{WT} has no effect. (Q) Depletion of *sd* and (R) *yki* leads to significant loss of mature CCs. (S) WT 2nd instar LG does not express crystal cell markers Yki (red) and Lz (green). (T) Over-expression of Notch Activated (*HLT > UAS-*N^{Act}) drives ectopic expression of Yki (red) and Lz (green). (U) Yki (red) is expressed in lz^{r15} mutant lymph glands, nuclear marker (blue). (V) Yki (red) expression in 3rd instar lymph gland. (W) Yki expression is not observed in N^{ts} mutant lymph glands raised at non-permissive temperature. Scale bar 10 µm.

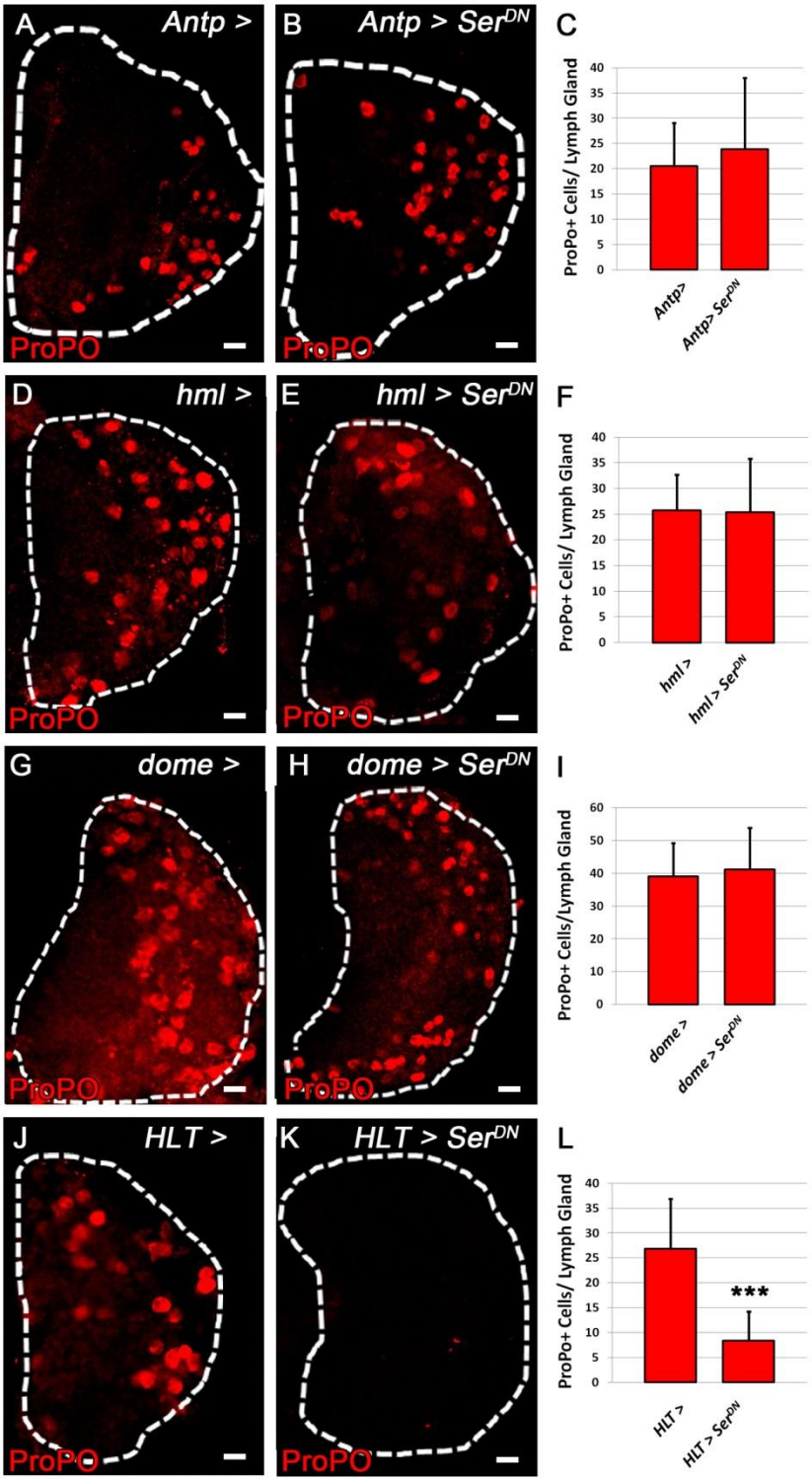

Figure S2, related to Fig. 2: Inhibition of Serrate in different populations of cells within the lymph gland. (A) WT lymph gland. (B) PSC specific inhibition of Serrate (*Antp-gal4 > Ser^{DN}*)

does not block crystal cell differentiation. (C) Quantification of Serrate inhibition in the PSC. (n=10) (D) WT lymph gland. (E) Inhibition of Serrate (*hml-gal4 > Ser^{DN}*) in differentiating hemocytes does not block crystal cell differentiation. (F) Quantification of Serrate inhibition in the CZ. (n=10) (G) WT lymph gland. (H) Inhibition of Serrate (*dome-gal4 > Ser^{DN}*) in prohemocytes does not block crystal cell differentiation. (I) Quantification of Serrate inhibition in the Medullary Zone. (n=10) (J) WT LG. (K) Inhibition of Serrate throughout the lymph gland (*HLT > Ser^{DN}*) significantly blocks crystal cell differentiation. (L) Quantification of Serrate Inhibition. (n=10) *** indicates pValue <.001. Scale bar 10 µm.

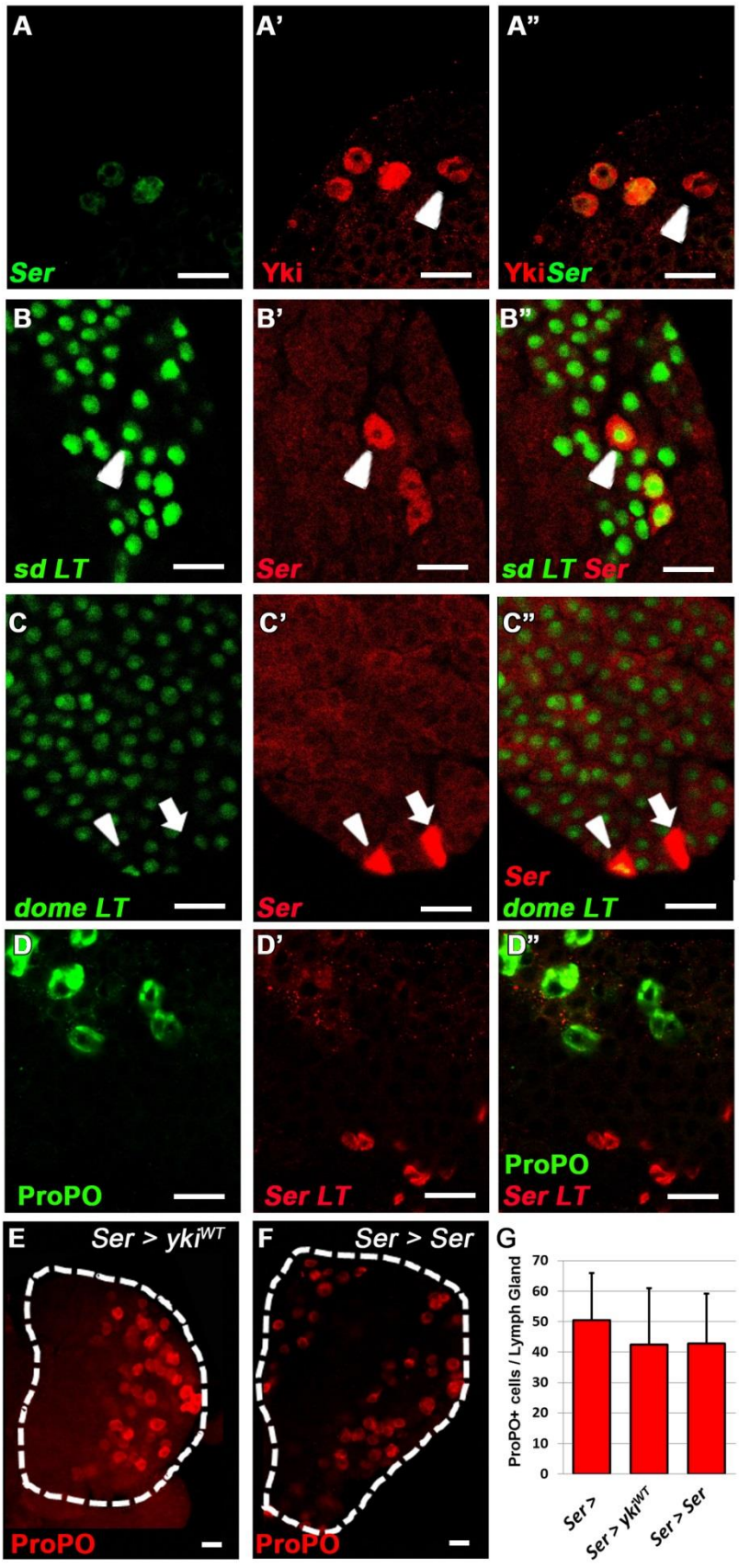
A
Ser
A'
Yki
A"
YkiSer
B
sd LT
B'
Ser
B"
sd LT Ser
C
dome LT
C'
Ser
C"
Ser
dome LT
D
ProPO
D'
Ser LT
D"
ProPO
Ser LT
E
Ser > yki^WT
ProPO
F
Ser > Ser
ProPO
G
ProPO+ cells / Lymph Gland
70
60
50
40
30
20
10
0
Ser>
Ser > yki^WT
Ser > Ser

Figure S3, related to Fig. 3: Characterization of Lineage Specifying Cells. (A-A") *Ser* (A, green) and Yki (A', red) co-localize (A", yellow), but not in all Yki^{+} cells (Arrowhead). (B-B") Lineage traced *sd*$^{+}$ cells (B, green) and *Ser* (B', red) co-localize (B", yellow, Arrowhead). (C-C") Lineage traced *dome*$^{+}$ cells (B, green) and *Ser* (B', red) co-localize (B", yellow, Arrowhead), but not all *Ser*$^{+}$ cells are traced from *dome* (Arrow). (D-D") ProPO^{+} CCs (D, green) do not co-localize with lineage traced *Ser*$^{+}$ cells (D', red). (E-G) Overexpression of (E) *yki*^{WT} or (F) *Ser* in *Ser*+ cells (*Ser-gal4* >) does not affect crystal cell (ProPO, red) differentiation. (G) Quantification of Fig. S3E-F (n=10). Scale bar 10 μm.

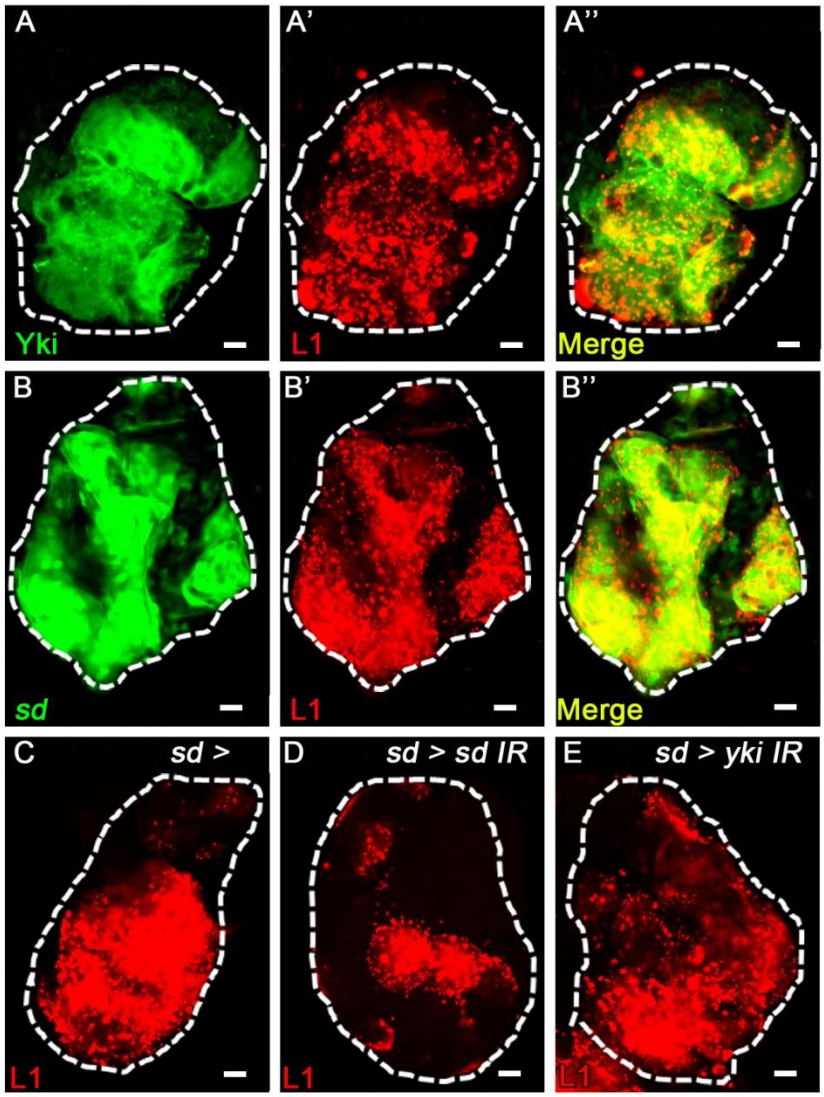

Figure S4, related to Fig. 4: Scalloped and Yorkie in lamellocytes. (A-A") Yki (A, green) is strongly expressed in lamellocytes labelled with L1 (A', red). (B-B") *sd* (B, green) is strongly expressed in lamellocytes labelled with L1 (B', red). (C-E) Lamellocytes labelled with L1 in wasp parasitized lymph gland. (C) WT lymph gland. (D) Depletion of *sd* or (E) *yki* with *sd-gal4* does not affect lamellocyte formation.

Supplemental Experimental Procedures:

Immunohistochemistry (continued):

The following primary antibodies were used: mouse anti- $Notch^{ECD}$ (Developmental Studies Hybridoma Bank, DSHB) (1:5), mouse anti-Lozenge (concentrated, DSHB) (1:40), rabbit anti-Prophenoloxidase (gift from Mike Kanost) (1:200), mouse anti-Peroxidasin (gift from J. Fessler) (1:400), mouse anti-GFP (Abcam) (1:100), mouse anti-L1(gift from Istvan Ando) (1:20), mouse anti-β Galactosidase (Millipore) (1:20), rabbit anti-Yorkie (gift from Kenneth Irvine) (pre-absorbed in cuticle at 1:200). The following secondary antibodies were used: anti-Mouse Cy3 (Jackson Immunoresearch) (1:400), anti-Mouse Alexa Fluor648 (Jackson Immunoresearch) (1:400), anti-Mouse FITC (Jackson Immunoresearch) (1:100), anti-Rabbit Cy3 (Jackson Immunoresearch) (1:400), anti-Rabbit Alexa Fluor648 (Jackson Immunoresearch) (1:400).

TUNEL staining: We modified the *In Situ cell death detection kit, TMR red* (Roche) protocol as follows: Larvae were dissected in PBS and fixed in 3.7% formaldehyde for 20 minutes at room temperature. LGs were then permeabilized in 0.4% PBT with 4 X 15 minute washes at RT. LGs were then further permeabilized in 100mM NaCitrate + 0.1% PBT (pH 6.0) on ice for 3 minutes and rinsed in PBS for 4X10 minutes. The TUNEL solution was made by combining Label Solution with Enzyme Solution at a 10:1 ratio. The plate containing tissues in TUNEL solution was incubated at 37°C for 1 hour and 15 minutes. At the end of incubation lymph glands were rinsed in 0.1% PBT for 6 X 5 minutes in glass wells, and mounted in Vectashield Mounting Medium.

MARCM clones: Female flies carrying the sd^{47M} mutation were mated to MARCM 19A males and allowed to lay eggs. 24 hours after egg-laying, the embryos were heat shocked at 29 degrees Celsius in a water bath for 2 hours. Larvae were then raised at RT and dissected at the wandering stage of the 3rd instar. Lymph Glands with control or *sd* clones in the Cortical Zone (Peroxidasin+) were scored based upon the presence or absence of crystal cells (ProPO+) within the clone. ProPO+ cells were observed in 9/20 control clones compared to 1/12 *sd* clones (pValue < .05).

All statistical analyses of crystal cell numbers were achieved using a Two-tailed Student's *t*-test. Lamellocyte quantification was determined by the percentage of Lymph Glands containing lamellocytes in WT (*dome* >) or enforced *Ser* expression (*dome* > *Ser*).

Wasp Parasitization: 8-10 female *L. boulardi* wasps and 5-6 male wasps were added to vials containing second instar *Drosophila* larvae, and were allowed to develop at 25°C. Wandering third instar larvae parasitized by wasp larvae were then dissected.

***Drosophila* stocks**:

The following stocks were used: *hml-gal4, UAS-2xEGFP; lz-gal4, UAS-mCD8::GFP ; Antp-gal4,UAS-2xEGFP* ; *Ser-gal4, UAS-GFP; G-TRACE; dome-gal4, UAS-2xEGFP; dome-gal4;gal80^{ts}; Hand-gal4,UAS-2xEGFP,UAS-FLP; A5C-FRT-STOP-FRT-GAL4* (*HLT) ; Notch^{ts}* (U. Banerjee); *UAS-FLP; A5C-FRT-STOP-FRT-LacZ; sd-gal4* (DGRC#113-596)*; UAS-yki IR(4005R-1); UAS-yki^{WT}; UAS-sd;UAS-2xEYFP; UAS-dicer2;UAS-Ser IR(#28713)* (Bloomington Stock Center); *UAS-yki IR; UAS-sd IR; diap1-GFP(3.5)*(J.Jiang)*; UAS-N^{Act}* (S.

Artavanis-Tsakonas); *UAS-Ser; UAS-Ser^{DN}* ; *SerLacZ* (E. Knust) ; *sd^{47M} FRT19A* (D. Pan)*;* and *hsflp, tubgal80 FRT19A/FM7; tub > mCD8::GFP/TM3, Ser* (B. Ohlstein), *Black cells-GFP* (Robert Schulz).