Report

Yorkie and Scalloped Signaling Regulates Notch-Dependent Lineage Specification during *Drosophila* Hematopoiesis

Gabriel B. Ferguson[1,2] and Julian A. Martinez-Agosto[1,2,3,*]
[1]Molecular Biology Interdepartmental Ph.D. Program
[2]Department of Human Genetics
[3]Molecular Biology Institute, Jonsson Comprehensive Cancer Center, UCLA Broad Stem Cell Center, and Mattel Children's Hospital UCLA
David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA

Summary

Cellular microenvironments established by the spatial and temporal expression of specific signaling molecules are critical for both the maintenance and lineage-specific differentiation of progenitor cells. In *Drosophila*, a population of hematopoietic progenitors, or prohemocytes, within the larval lymph gland [1] gives rise to three mature cell types: plasmatocytes, lamellocytes, and crystal cells. Removal of the secreted signaling molecules Hedgehog [2] and PVF1 [3] from the posterior signaling center (PSC) [2, 4, 5], which acts as a niche, leads to a loss of progenitors and complete differentiation of the lymph gland. Here, we characterize a novel population of signaling cells within the lymph gland, distinct from the PSC, that are required for lineage-specific differentiation of crystal cells. We provide evidence that Yorkie [6] and Scalloped [7], the *Drosophila* homologs of YAP and TEAD, are required in lineage-specifying cells to regulate expression of *Serrate*, the Notch ligand responsible for the initiation of the crystal cell differentiation program [5, 8]. Genetic manipulation of *yorkie* and *scalloped* in the lymph gland specifically alters *Serrate* expression and crystal cell differentiation. Furthermore, *Serrate* expression in lineage-specifying cells is eliminated in the lymph gland upon the immune response induced by wasp parasitization to ensure the proper differentiation of lamellocytes at the expense of crystal cells. These findings expand the roles for Yorkie/Scalloped beyond growth to encompass specific cell-fate determination in the context of blood development. Similar regulatory functions may extend to their homologs in vertebrate progenitor cell niches that are required for specifying cell fate.

Results and Discussion

Yorkie and Scalloped Are Required for Crystal Cell Formation in the Lymph Gland

Differentiating hemocytes in the lymph gland (LG) are restricted to the periphery or cortical zone (CZ) of the organ (Figure 1A). These hemocytes originate from a population of progenitors termed prohemocytes (PHs) that are located in the medullary zone (MZ; Figure 1A) and are maintained by the posterior signaling center (PSC; Figure 1A). PHs transition through an intermediate progenitor (IP) [9] state (Figure 1A), where they express both progenitor (*dome*+) and early

differentiation (Pxn+) markers [10]. These IPs will eventually fully mature into plasmatocytes (PLs), crystal cells (CCs), which are specified by Notch signaling [5, 8], or lamellocytes. CCs are marked by crystalline inclusions that contain prophenoloxidase (ProPO), which is essential in the immune response [1]. These cells mature from newly specified CC progenitors (CCPs), which express Lozenge (Lz) [5], the *Drosophila* homolog of Runx1, into functional ProPO+ cells.

Scattered among differentiating cells, we observe a population of Yorkie (Yki)-expressing cells (Figures 1B–1D). Similarly, Yki's binding partner Scalloped (Sd) is expressed in clusters of cells found throughout the CZ (Figures 1E–1G), where it is coexpressed with Yki (Figure 1F, arrows). In addition, Yki+ and *sd*+ cells are observed adjacent to each other (Figure 1F, arrowhead). Yki is also observed in 77% of Lz+ CCPs [5] (Figure 1C) but only in 8% of *sd*+ cells. Similarly, only a small percentage of Lz+ cells express *sd* (Figure S1A, arrowheads, available online). Yki is also present in *Black cells-GFP*+ cells (Figure 1D), a marker of mature CCs (Figure S1B). A small number of *sd*+ cells are also ProPO+ (Figure 1G, arrow), while a subset of *sd*+ cells are observed adjacent to mature CCs but do not express CC markers (Figure 1G, arrowheads). Furthermore, lineage-tracing analysis with *sd-gal4, UAS-GFP* identified ProPO+-traced cells that do not express GFP (Figure 1G, inset), suggesting that *sd* is only transiently expressed in this population of CCs. Notch is also observed in a subset of *sd*+ cells (Figure S1C, arrow); however, the majority of Notch+ cells do not coexpress *sd* but are located adjacent to *sd*+ cells (Figure S1C, arrowhead). These observations demonstrate that Yki and Sd are present both in CCs and in neighboring populations.

We next generated *sd* and *yki* mutant clones to interrogate their function in the LG. While *yki* clones are extremely small or absent in the LG (data not shown), we do observe a very striking absence of mature ProPO+ CCs in *sd* loss-of-function mutant clones (Figures S1D and S1E), confirming a requirement for Sd in CC formation. To gain further insight into their role in CC differentiation, we manipulated *yki* and *sd* expression using the *hand lineage tracing* (*HLT*) driver, which clonally expresses *gal-4* throughout the LG (Figures S1F–S1J correspond to Figures 1H–1L, respectively). We observe an increase of Lz+ CCPs (Figures 1H, 1I, and 1Q) upon LG-specific overexpression of *yki*WT. Conversely, depletion of *yki* (Figures 1J and 1Q) or *sd* (Figures 1K and 1Q) causes a decrease in Lz+ cells. Importantly, depletion of *sd* blocks the increase in CCPs observed upon *yki*WT overexpression (Figures 1L and 1Q), demonstrating that Sd is required for Yki's function in CC differentiation. The extent of CC loss in this background is milder compared to *sd* depletion alone (Figure 1Q), which could be explained by low levels of remaining Sd interacting with an overabundance of Yki.

Based on the pattern of expression (Figures 1E–1G) and the functional results upon *sd* depletion (Figures 1K and 1L), we further investigated the relationship between Yki and Sd in the context of CC differentiation by manipulating *yki* and *sd* levels with *sd-gal4.* We observe a significant increase in CCP numbers (Figures 1M, 1N, and 1R) when *yki*WT is overexpressed in *sd*+ cells. Similarly, depletion of *yki* in *sd*+ cells

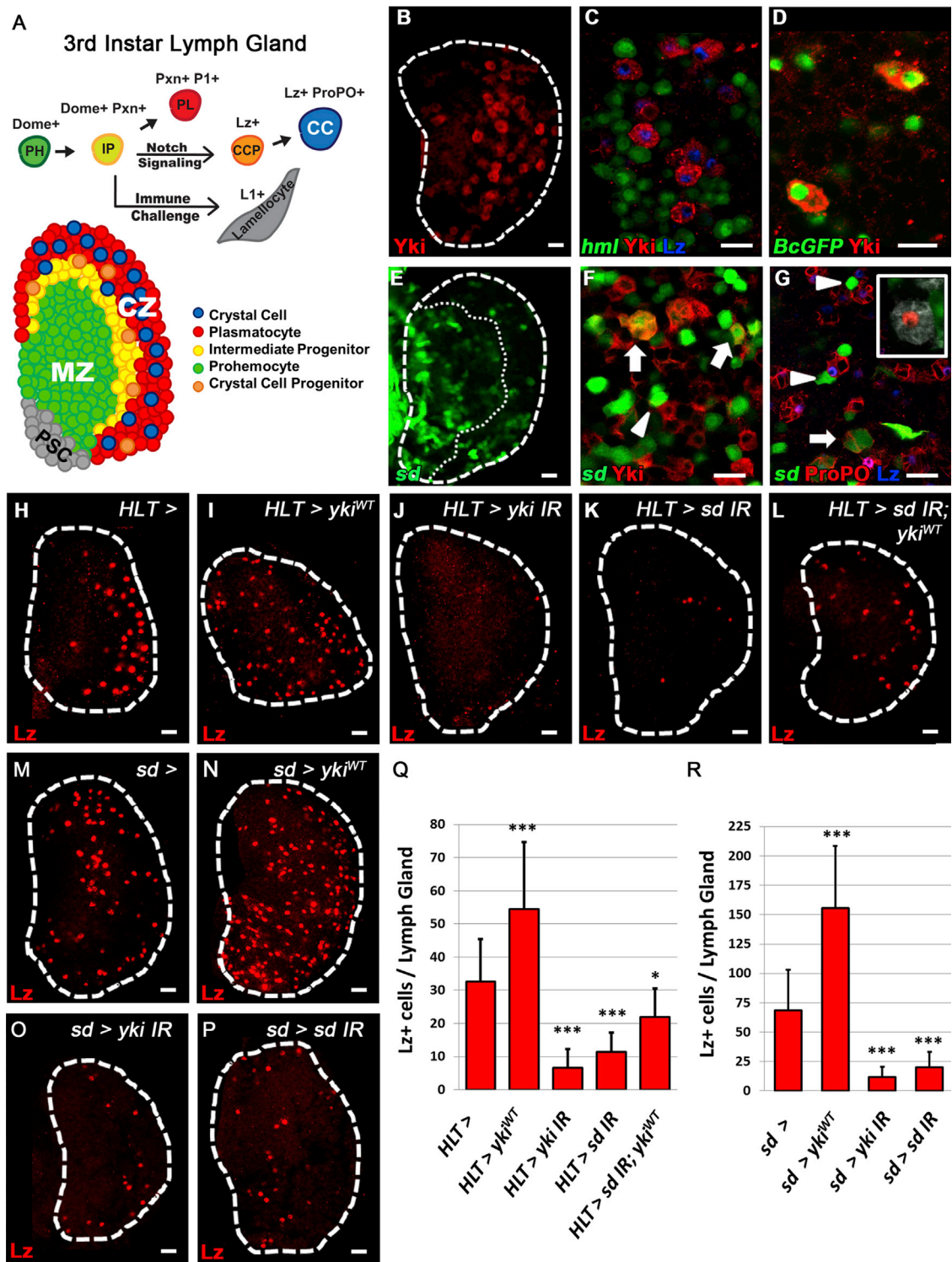*Correspondence: julianmartinez@mednet.ucla.edu

Figure 1. Scalloped and Yorkie Are Required for Proper Crystal Cell Differentiation

Crystal cell progenitors (CCPs) are labeled with Lz (H–P, red).

(A) Schematic of the third-instar lymph gland and hemocyte differentiation. Gray, PSC; green, prohemocytes (PH) of the MZ; yellow, intermediate progenitors (IP); red, plasmatocytes (PL); blue, crystal cells (CC) in the CZ.

(B) Yki (red) is expressed in scattered cells of the CZ in a third-instar lymph gland.

(C) Yki (red) is observed in CCPs (Lz; blue) among differentiating hemocytes (*hml*; green) of the CZ.

(D) Yki (red) is present in mature CCs labeled with *Black cells-GFP* (green).

(E) *sd* (*sd-gal4>UAS-2xEGFP*; green) is expressed in clusters of cells scattered throughout the lymph gland. CZ is demarcated by a dotted line.

(F and G) *sd* (green) is present in a subset of Yki+ cells (F, arrows) and mature CCs (G, arrows) and is also seen adjacent to Yki+ cells (F, arrowhead) and CCs

causes a dramatic loss of Lz$^+$ cells (Figures 1O and 1R), as does *sd* downregulation (Figures 1P and 1R). Importantly, manipulation of *yki* and *sd* levels with *sd-gal4* or *HLT* drivers does not significantly alter differentiation of plasmatocytes (Figures S1K and S1L). Taken together, these observations provide evidence of an integral role for both Yki and Sd specifically in CC differentiation.

While overexpression of *sd* with the CCP driver *lz-gal4* increases CC numbers (Figure S1M–S1O), overexpression of *yki*^{WT} does not affect CCs (Figures S1M and S1P). We do observe a remarkable decrease in mature CCs when both *sd* and *yki* are depleted in CCPs (Figure S1M, S1Q, and S1R). In addition, we observe striking ectopic expression of Yki and Lz in early-second-instar LGs upon overexpression of an activated form of Notch (Figures S1S and S1T). Furthermore, although *Notch* mutant LGs do not express Yki (Figures S1V and S1W), we do observe Yki expression in scattered cells of the CZ in *lz*^{R15} mutant LGs (Figure S1U). These findings indicate that Yki is specifically upregulated by Notch signaling independent of Lz early in the CC differentiation program and that Yki and Sd are required within CCPs to maintain normal CC numbers.

Yorkie and Scalloped Promote Serrate Expression in Lineage-Specifying Cells

While overexpression of *yki* throughout the LG (Figure 1I) or specifically in *sd*-expressing cells (Figure 1N) significantly increases CCP numbers, a similar increase in CCs is not observed when *yki* is overexpressed in CCPs that have already been specified (Figure S1P). This discrepancy suggests that Yki can promote CC formation independent of any effects within already committed CCPs, perhaps due to limited availability of Sd in these cells. This finding, along with the observation that *sd*$^+$ cells are frequently observed adjacent to CCs (Figure 1G), suggested that there may be a non-cell-autonomous role for Yki in CC differentiation, possibly through regulation of the Notch ligand Serrate (Ser).

Ser is highly expressed in the PSC (Figure 2A) [5, 11]; however, Ser function in this compartment is not required for CC differentiation (Figures S2A–S2C) [4]. Interestingly, both CCs [2, 11] and *Ser*$^+$ cells [11] are still observed in LGs that lack the PSC, and *Ser*$^+$ cells have also been observed outside of the PSC [5]. We confirmed the presence of *Ser*$^+$ cells within the CZ of third-instar LGs (Figure 2A, arrowhead). Inhibition of Ser in differentiating hemocytes of the CZ (Figures S2D–S2F) or MZ prohemocytes (Figures S2G–S2I) does not affect CC differentiation. However, LG-wide inhibition of Ser significantly decreases CC differentiation (Figures S2J–S2L) demonstrating that Ser function is required in a subset of cells that are distinct from the PSC, hematopoietic progenitors, or differentiating hemocytes.

Having demonstrated that Yki and Sd can regulate CC numbers within the LG, we asked whether they are specifically required in *Ser*$^+$ cells for CC formation. Indeed, we observe a significant decrease in CC numbers upon depletion of *yki* or *sd* in these *Ser*$^+$ cells (Figures 2B–2D and 2I), demonstrating a requirement for Yki and Sd in these signaling cells, which are also observed adjacent to CCs (Figure 2E). Depletion of *yki* or *sd* in the PSC using the *Antp-gal4* driver does not affect CC differentiation (Figures 2F–2H and 2J). Therefore, Yki and Sd function is required specifically in *Ser*$^+$ cells independent of the PSC for proper CC differentiation.

To gain further insight into the identity of *Ser*$^+$ cells in the LG, we performed a comprehensive analysis of hemocyte differentiation markers. Using a *LacZ* reporter of *Ser* expression, we confirmed that the population of *Ser*$^+$ cells is located in the CZ (Figure 3A). These cells do not express markers of differentiating hemocytes (Figure 3B), but they are observed in close proximity to both CCPs (Figure 3C) and mature CCs (Figure 3D). Furthermore, *Ser*$^+$ cells in the CZ coexpress *sd* (Figure 3E) and Yki (Figures S3A–S3A$''$). It is important to reiterate that these Yki$^+$ *sd*$^+$ *Ser*$^+$ cells do not express any other hemocyte markers (Figures 3B–3D) and are lineage traced from a *sd*$^+$ cell (Figures S3B–S3B$''$). We also observe a subset of *Ser*$^+$ cells that arise from a *dome*$^+$ precursor (Figures S3C–S3C$''$, arrowhead), but not all *Ser*$^+$ cells originate from this population (Figures S3C–S3C$''$, arrow). Importantly, *Ser*-expressing cells do not contribute to the CC lineage (Figures S3D–S3D$''$). These data demonstrate that this unique population of *Ser*$^+$ cells expresses both *sd* and Yki and represents a dedicated signaling cell that is distinct from other cell types in the LG.

Similar to the requirement of Yki and Sd in *Ser*$^+$ cells, depletion or inhibition of Ser in *sd*$^+$ cells is sufficient to block CC differentiation (Figures 3F–3H and 3P). This demonstrates that Ser is uniquely required in *sd*$^+$ cells and in no other LG cell populations (compare to Figures S2A–S2I) for CC differentiation. The Yki-mediated increase in CC numbers previously observed (Figure 1N) is blocked by overexpression of *Ser*^{DN} (Figures 3I and 3P), whereas overexpression of *Ser* rescues (Figures 3J and 3P) the loss of CCs observed upon *yki* knockdown (Figure 1O). In addition, overexpression of *yki*^{WT} in the LG increases *Ser* expression in the CZ (Figures 3K and 3L). Similarly, downregulation of *yki* or *sd* specifically in *sd*$^+$ cells causes a significant decrease in the number of *Ser*$^+$ signaling cells (Figures 3M–3O and 3Q) and a corresponding decrease in CCP numbers (Figures 1O, 1P, and 1R). However, overexpression of either *yki*^{WT} (Figures S3E and S3G) or *Ser* (Figures S3F and S3G) specifically in *Ser*$^+$ cells does not affect CC differentiation (compare to Figure 2B), suggesting that changes in CC number upon *yki*^{WT} overexpression are due to an increase in the number of *Ser*$^+$ cells (Figure 3L). These results demonstrate that Yki and Sd have definitive roles in CC specification by regulating *Ser* expression in a distinct population of cells within the LG that we have termed lineage-specifying cells (LSCs).


(G, arrowheads). As shown in the inset in (G), lineage-traced (*sd-gal4, UAS-GFP>UAS-FLP, A5C-FRT-STOP-FRT-LacZ*; red) mature CCs (ProPO; white) do not express *sd* (green).

(H–L) For each panel, the corresponding pattern of *HLT>GFP* expression is demonstrated in Figures S1F–S1J. A wild-type (WT) lymph gland is shown in (H). Widespread overexpression of *yki*^{WT} in the lymph gland increases CCP numbers (I), whereas depletion of *yki* (J) or *sd* (K) blocks CC formation. *sd* knockdown blocks the increase of CCPs observed upon overexpression of *yki*^{WT} (L).

(M–P) A WT lymph gland is shown in (M). Overexpression of *yki*^{WT} in *sd*-expressing cells (*sd-gal4>*) increases CCP numbers (N), while depletion of *yki* (O) or *sd* (P) strongly inhibits CC differentiation.
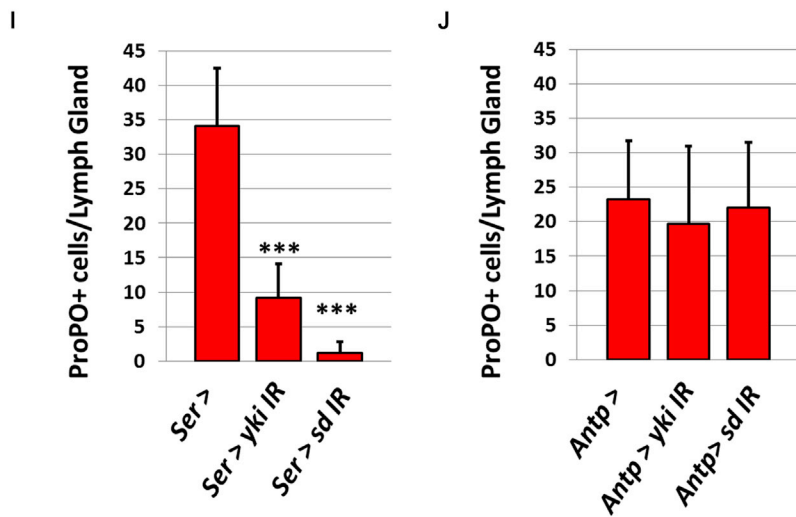
(Q and R) Quantification of (H)–(P) (n = 10). Statistical analysis was performed using a two-tailed Student's t test, and error bars represent 1 SD. *p < 0.05, ***p < 0.001.

Scale bar, 10 μm. See also Figure S1.
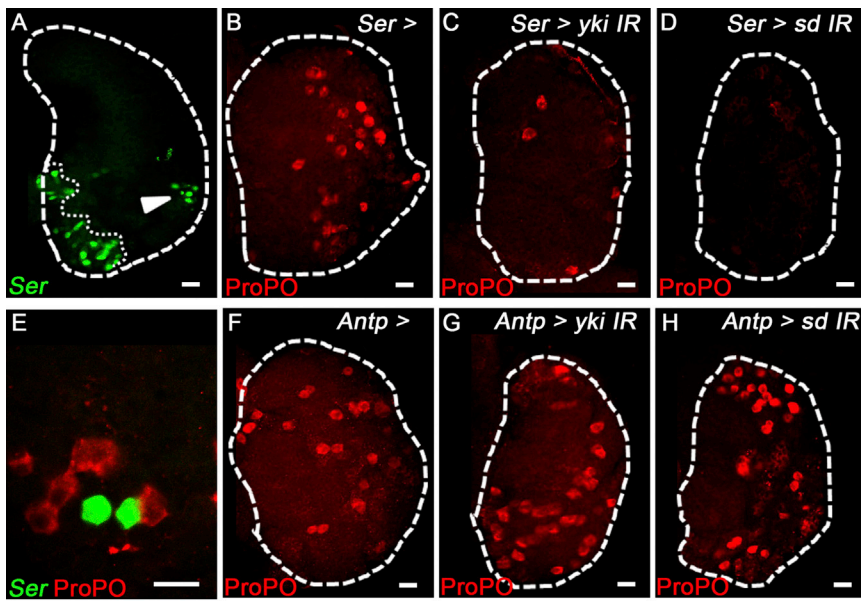
Figure 2. Yorkie and Scalloped Are Required Specifically in *Serrate*-Expressing Cells for Proper Crystal Cell Differentiation

Green labels *Ser*⁺ cells (Ser-gal4, UAS-GFP) (A and E), and red labels ProPO⁺ CCs.

(A) Ser-expressing cells (arrowhead) observed in the periphery of the CZ, distinct from the PSC (outlined by a dotted line).

(B) The WT.

(C and D) Knockdown of *yki* (C) or *sd* (D) in *Ser*⁺ cells blocks CC formation.

(E) *Ser*⁺ cells observed in direct contact with CCs in the CZ.

(F) The WT.

(G and H) Knockdown of *yki* (G) or *sd* (H) in the PSC (*Antp-gal4>*) has no effect on CC formation.

(I) Quantification of *yki* and *sd* knockdown in *Ser*⁺ cells.

(J) Quantification of *yki* and *sd* knockdown in the PSC (n = 10).

Statistical analysis was performed using a two-tailed Student's t test, and error bars represent 1 SD. ***p< 0.001. Scale bar, 10 μm. See also Figure S2.

lamellocytes (Figures 4E′ and 4G′) and a decrease in CCs (Figures 4E, 4F, and 4G) [9]. To further verify that this downregulation of *Ser* is required for a proper immune response, we ectopically expressed *Ser* in the LG and subjected these larvae to wasp parasitization. Unlike with the WT parasitized control (Figures 4G–4G″), we observe a significant increase in CCs when *Ser* is overexpressed either in prohemocytes and IPs (Figures 4H and 4I) or ubiquitously by *HLT* (Figures 4K, 4L, and 4N). Most strikingly, there is a significant inhibition of lamellocyte differentiation upon enforced expression of *Ser* (Figures 4G′, 4I′, and 4J). These findings demonstrate that downregulation of *Ser* is responsible for the decreased numbers of CCs observed in the LGs of wasp parasitized larvae and is essential for lamellocyte differentiation in the LG.

We next examined whether the alterations in cell fate and observed changes in *Ser* expression upon immune challenge were due to changes in Yki and Sd function. Interestingly, both Yki and *sd* are strongly expressed in lamellocytes, whereas expression of Yki and *sd* in other cells of the LG is severely diminished upon wasp parasitization (Figures S4A–S4B″). Given the expression of *sd* in lamellocytes, we used *sd-gal4* to interrogate the function of Yki and Sd in these cells. Downregulation of *yki* or *sd* levels has no effect on lamellocyte differentiation (Figures S4C–S4E), and overexpression of *yki*^WT^ is not sufficient to rescue loss of CCs in immune-challenged LGs (Figures 4M and 4N). These findings indicate that wasp parasitization regulates *Ser* expression in LSCs to allow for lamellocyte differentiation at the expense of CCs, while emphasizing the dynamic role for LSCs in maintaining LG homeostasis under normal and stress conditions.

Our findings demonstrate a novel role for Yki and Sd in the Notch-dependent lineage specification of CCs. Although expression of the Yki and Sd homologs, YAP1/TAZ and TEAD

Wasp Parasitization Triggers Cell-Fate Decisions Required for the Lymph Gland Immune Response by Altering Serrate Expression

Larval parasitization by the wasp *Leptopilina boulardi* elicits a strong cellular immune response in the *Drosophila* LG [12, 13] that is characterized by lamellocyte differentiation (Figure 4A), which is rarely observed in WT LGs. These large, flat cells defend the larva by engulfing invading pathogens or parasites, such as the *L. boulardi* eggs. Upon wasp parasitization, there is a robust increase in lamellocyte differentiation along with a corresponding decrease in CC differentiation [9]. However, the mechanism by which this change in lineage fate decisions is regulated has not been definitively determined, although it has been recently shown that Notch signaling blocks lamellocyte formation (Figure 4A) [14].

A possible explanation for the loss of CCs in the LG upon wasp parasitization could be that expression of *Ser* in LSCs is downregulated under these conditions as a requirement for lamellocyte differentiation. Indeed, we observe a significant decrease in the numbers of *Ser*⁺ LSCs in parasitized larvae (Figures 4B–4D) associated with an upregulation of
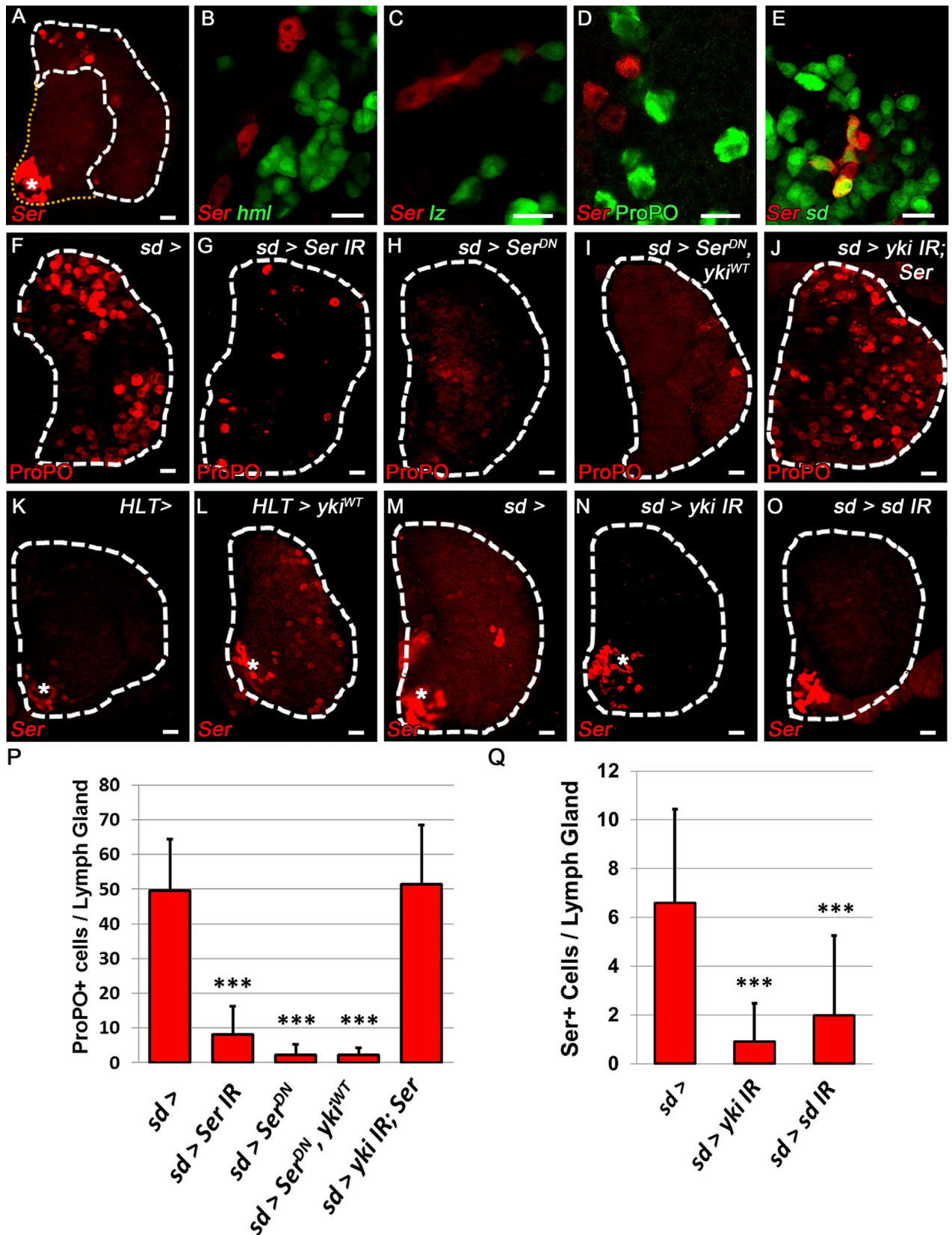
Figure 3. Yorkie and Scalloped Regulate *Serrate*-Expressing Lineage-Specifying Cell Numbers

Red labels *Ser*⁺ LSCs (*SerLacZ*; A–E and K–O) and CCs (F–J).

(A) *Ser* expressing cells are located in the CZ, outlined by white hatch marks (the asterisk denotes the PSC).

has been previously described in mammalian hematopoietic stem cells (HSCs) [15, 16], no phenotypes have been observed upon manipulation of these factors in the HSC compartment [16]. Alternatively, we propose that a conserved role for YAP and TEAD signaling may reside in a non-cell-autonomous manner originating from lineage-specifying or niche cells, such as stromal cells of the bone marrow, thymic epithelium, and other sites of differentiation, such as the liver and spleen. Here, we have provided evidence for a novel regulatory role for Yki and Sd in promoting *Ser* expression in LSCs of the *Drosophila* LG while demonstrating LSC plasticity in immune-challenged larvae. Parasitization by the wasp *L. boulardi* necessitates a lineage switch from CCs to lamellocytes that is achieved by downregulation of *Ser*, allowing a common pool of hematopoietic progenitors to differentiate into lamellocytes.

Recently, it was shown that YAP regulates expression of Jagged1, the mammalian homolog of Ser, in hepatocytes [17, 18]. Hippo pathway signaling through YAP regulates liver cell-fate decisions [18], while misregulation of YAP leads to increased Jagged1 expression in a TEAD-dependent manner, causing irregular activation of the Notch pathway and hepatocellular carcinoma [17]. In addition, a biphasic lineage specification mechanism involving Notch signaling is required in the specification of megakaryocyte-erythroid progenitor cells into erythrocytes at the expense of megakaryocytes [19] under stress conditions. Given the presence of YAP and TEAD within mammalian hematopoietic compartments [15, 16, 20, 21], a similar requirement for these factors may be necessary for the regulation of Notch-dependent lineage specification.

We have described a similar role for Yki and Sd in regulating *Ser*⁺ LSCs in the *Drosophila* LG. Our results demonstrate a mechanism where a small number of Ser-expressing LSCs are tightly regulated by limiting availability of Yki and Sd, as perturbations to either of these factors alters CC differentiation. Furthermore, overexpression of *Ser* in the CZ increases CC numbers significantly (G.B.F. and J.A.M.-A., unpublished data), demonstrating the sensitivity to changes in Notch ligand availability in the LG [14]. Our finding that *Ser* expression in LSCs is specifically downregulated upon wasp parasitization further demonstrates that these signaling cells are in fact dynamically regulated within the LG. In total, these mechanisms further expand our understanding of hematopoietic niches and the regulation of signaling molecules that characterize hematopoietic microenvironments.

Experimental Procedures

Genetic Analysis

All crosses were reared at 29°C. Multiple *yki* (VDRC: 104253 and NIG: 4005R-1) and *sd* (VDRC: 101497 and NIG: 8544R-3) RNAi constructs were tested and yielded similar phenotypes. In the case of *HLT* experiments, the *Hand-gal, UAS-2xEGFP,UAS-FLP; A5C-FRT-STOP-FRT-GAL4* genetic background was used to generate lymph gland specific clones expressing the UAS construct of interest. For *sd* knockdown experiments, *UAS-dicer2* was used in the background to enhance phenotypes. Inhibition of Ser in hematopoietic progenitors of the MZ was achieved using the *dome-gal4, UAS-mCD8::GFP; gal80*ᵗˢ stock, with larvae reared at 18°C. After 48 hr, larvae were shifted to 29°C. *Notch*ᵗˢ larvae were raised at 29°C. All crosses involving *SerLacZ* reporter analysis were performed using two copies of *SerLacZ* in the background, except for the experiment involving overexpression of *UAS-yki*ᵂᵀ or its corresponding control.

Immunohistochemistry

LGs were dissected as previously described in 1× PBS and fixed in 3.7% paraformaldehyde for 20 min. LGs were then blocked for 30 min in 10% normal goat serum (NGS) in 0.4% Triton X/PBS (PBT). Antibodies were appropriately diluted in PBT and allowed to incubate with samples overnight at 4°C. LGs were washed four times for 15 min in PBT and were blocked again with 10% NGS in PBT for 30 min. Secondary antibodies were appropriately diluted in 10% NGS in PBT and allowed to incubate with samples overnight. LGs were then washed four times for 15 min in PBT and were mounted in Vectashield Mounting Medium. Samples were imaged using a Carl Zeiss LSM 310 laser scanning confocal microscope. A middle section of a z stack was used in every image.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.09.081.

References

1. Lebestky, T., Chang, T., Hartenstein, V., and Banerjee, U. (2000). Specification of Drosophila hematopoietic lineage by conserved transcription factors. Science *288*, 146–149.

(B) *Ser* does not colocalize with the PL marker *hml*.
(Cand D) *Ser*⁺ cells are observed adjacent to *lz*⁺ CCPs (C; green) and ProPO⁺ mature CCs (D; green) but do not colocalize.
(E) *sd* (green) is coexpressed with *Ser* (red).
(F) A WT lymph gland.
(G and H) Depletion (G) or inhibition (H) of *Ser* function in *sd*-expressing cells (*sd-gal4>*) blocks CC differentiation.
(I) CC differentiation is similarly blocked by inhibition of *Ser* after overexpression of *yki*ᵂᵀ.
(J) Loss of CCs observed upon *yki* depletion is rescued by overexpression of *Ser*.
(K) *Ser* expression in larvae containing a single copy of *SerLacZ* is only observed in the PSC (asterisk).
(L) Overexpression of *yki*ᵂᵀ (*HLT>*) greatly increases *Ser* expression in lymph glands containing a single copy of *SerLacZ*.
(M) A WT lymph gland.
(N and O) Depletion of *yki* (N) or *sd* (O) in *sd*-expressing cells (*sd-gal4>*) blocks *Ser* expression outside of the PSC (asterisk).
(P and Q) Quantification of (F)–(J) and (M)–(O) (n = 10). Statistical analysis was performed using a two-tailed Student's t test, and error bars represent 1 SD. *** p < 0.001.
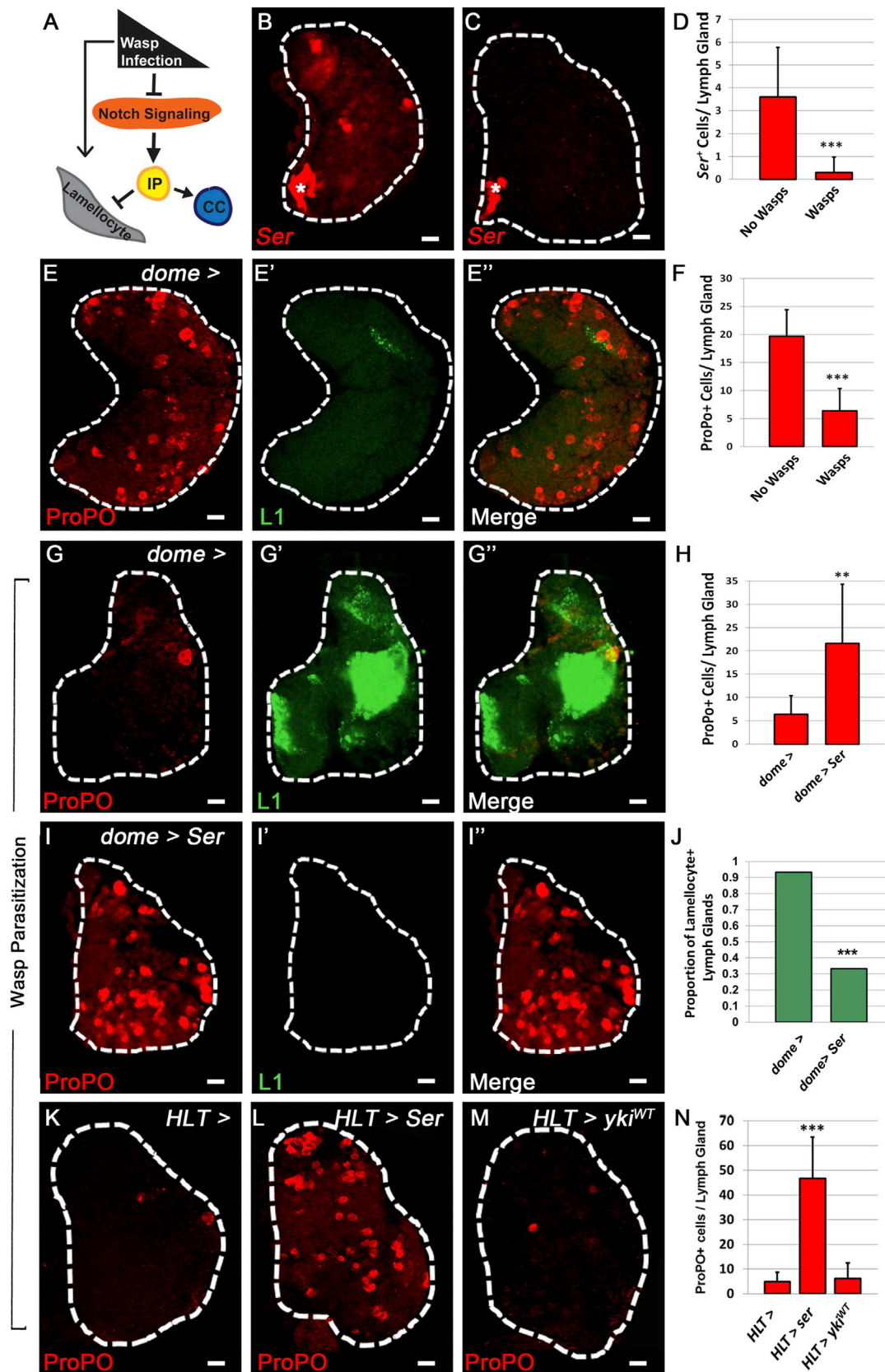Scale bar, 10 μm. See also Figure S3.

Figure 4. *Serrate* Expression in LSCs Is Downregulated in the Lymph Gland of Immune Challenged Larvae

(A) Schematic representation of the immune response generated upon wasp infection. Notch signaling, which promotes CC differentiation in the lymph gland, is blocked by wasp parasitization, allowing intermediate IPs to differentiate into lamellocytes [14]. The asterisk denotes the PSC.

2. Mandal, L., Martinez-Agosto, J.A., Evans, C.J., Hartenstein, V., and Banerjee, U. (2007). A Hedgehog- and Antennapedia-dependent niche maintains Drosophila haematopoietic precursors. Nature *446*, 320–324.

3. Mondal, B.C., Mukherjee, T., Mandal, L., Evans, C.J., Sinenko, S.A., Martinez-Agosto, J.A., and Banerjee, U. (2011). Interaction between differentiating cell- and niche-derived signals in hematopoietic progenitor maintenance. Cell *147*, 1589–1600.

4. Krzemień, J., Dubois, L., Makki, R., Meister, M., Vincent, A., and Crozatier, M. (2007). Control of blood cell homeostasis in Drosophila larvae by the posterior signalling centre. Nature *446*, 325–328.

5. Lebestky, T., Jung, S.-H., and Banerjee, U. (2003). A Serrate-expressing signaling center controls Drosophila hematopoiesis. Genes Dev. *17*, 348–353.

6. Huang, J., Wu, S., Barrera, J., Matthews, K., and Pan, D. (2005). The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. Cell *122*, 421–434.

7. Wu, S., Liu, Y., Zheng, Y., Dong, J., and Pan, D. (2008). The TEAD/TEF family protein Scalloped mediates transcriptional output of the Hippo growth-regulatory pathway. Dev. Cell *14*, 388–398.

8. Duvic, B., Hoffmann, J.A., Meister, M., and Royet, J. (2002). Notch signaling controls lineage specification during Drosophila larval hematopoiesis. Curr. Biol. *12*, 1923–1927.

9. Krzemien, J., Oyallon, J., Crozatier, M., and Vincent, A. (2010). Hematopoietic progenitors and hemocyte lineages in the Drosophila lymph gland. Dev. Biol. *346*, 310–319.

10. Dragojlovic-Munther, M., and Martinez-Agosto, J.A. (2012). Multifaceted roles of PTEN and TSC orchestrate growth and differentiation of Drosophila blood progenitors. Development *139*, 3752–3763.

11. Crozatier, M., Ubeda, J.-M., Vincent, A., and Meister, M. (2004). Cellular immune response to parasitization in *Drosophila* requires the EBF orthologue collier. PLoS Biol. *2*, E196.

12. Rizki, T.M., and Rizki, R.M. (1992). Lamellocyte differentiation in Drosophila larvae parasitized by Leptopilina. Dev. Comp. Immunol. *16*, 103–110.

13. Sorrentino, R.P., Carton, Y., and Govind, S. (2002). Cellular immune response to parasite infection in the Drosophila lymph gland is developmentally regulated. Dev. Biol. *243*, 65–80.

14. Small, C., Ramroop, J., Otazo, M., Huang, L.H., Saleque, S., and Govind, S. (2013). An unexpected link between Notch signaling and ROS in restricting the differentiation of hematopoietic progenitors in Drosophila. Genetics *197*, 471–483.

15. Ramalho-Santos, M., Yoon, S., Matsuzaki, Y., Mulligan, R.C., and Melton, D.A. (2002). "Stemness": transcriptional profiling of embryonic and adult stem cells. Science *298*, 597–600.

16. Jansson, L., and Larsson, J. (2012). Normal hematopoietic stem cell function in mice with enforced expression of the Hippo signaling effector YAP1. PLoS ONE *7*, e32013.

17. Tschaharganeh, D.F., Chen, X., Latzko, P., Malz, M., Gaida, M.M., Felix, K., Ladu, S., Singer, S., Pinna, F., Gretz, N., et al. (2013). Yes-associated protein up-regulates Jagged-1 and activates the Notch pathway in human hepatocellular carcinoma. Gastroenterology *144*, 1530–1542.e12.

18. Yimlamai, D., Christodoulou, C., Galli, G.G., Yanger, K., Pepe-Mooney, B., Gurung, B., Shrestha, K., Cahan, P., Stanger, B.Z., and Camargo, F.D. (2014). Hippo pathway activity influences liver cell fate. Cell *157*, 1324–1338.

19. Oh, P., Lobry, C., Gao, J., Tikhonova, A., Loizou, E., Manent, J., van Handel, B., Ibrahim, S., Greve, J., Mikkola, H., et al. (2013). In vivo mapping of notch pathway activity in normal and stress hematopoiesis. Cell Stem Cell *13*, 190–204.

20. Xing, W., Kim, J., Wergedal, J., Chen, S.-T., and Mohan, S. (2010). Ephrin B1 regulates bone marrow stromal cell differentiation and bone formation by influencing TAZ transactivation via complex formation with NHERF1. Mol. Cell. Biol. *30*, 711–721.

21. Seo, E., Basu-Roy, U., Gunaratne, P.H., Coarfa, C., Lim, D.S., Basilico, C., and Mansukhani, A. (2013). SOX2 regulates YAP1 to maintain stemness and determine cell fate in the osteo-adipo lineage. Cell Rep. *3*, 2075–2087.

LSCs (*Ser*) are red in (B) and (C), and CCs (ProPO) are red in (E), (E″), (G), (G″), (I), (I″), and (K)–(M). Lamellocytes are labeled by green (L1).

(B) *Ser* expression in the WT.

(C) Loss of LSC *Ser* expression upon wasp parasitization.

(D) Quantification of (B) and (C) (n = 10).

(E–E″) WT lymph gland contains CCs (E) and lacks lamellocytes (E′).

(F) Quantification of (E) and (G) (n = 10).

(G–G″) Wasp parasitization eliminates CCs (G) and promotes lamellocytes (G′).

(H) Quantification of (G) and (I) (n = 10).

(I–I″) Enforced expression of *Ser* upon wasp parasitization rescues CC loss (I) and inhibits lamellocyte formation (I′).

(J) Quantification of (G′) and (I′) (n = 10).

(K) WT lymph gland.

(L and M) Overexpression of *Ser* (*HLT*>) rescues CC numbers upon wasp parasitization (L), but overexpression of *yki*^{WT} has no affect compared to the WT (M).

(N) Quantification of (K)–(M) (n = 10).

Statistical analysis was performed using a two-tailed Student's t test, and error bars represent 1 SD. **p < 0.01, ***p < 0.001. Scale bar, 10 μm. See also Figure S4.