Developmental Cell

Active Hematopoietic Hubs in Drosophila Adults Generate Hemocytes and Contribute to Immune Response

Graphical Abstract

Highlights

- An active hematopoietic hub exists in the abdomen of adult Drosophila
- Progenitors within the hub can give rise to plasmatocytes and crystal cells
- Resident plasmatocytes show immune responses and proliferate upon infection
- Progenitors residing in the hub originate from the posterior lobes of lymph gland

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In Brief

Definitive hematopoiesis in Drosophila was thought to occur only in the larval hematopoietic organ, which ruptures upon pupation. Ghosh et al. now demonstrate the presence of active hematopoietic sites in the abdomen of adult flies, which can give rise to new blood cells and can respond to immune challenges.
Active Hematopoietic Hubs in Drosophila Adults Generate Hemocytes and Contribute to Immune Response

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SUMMARY

Blood cell development in Drosophila shares significant similarities with vertebrate. The conservation ranges from biphasic mode of hematopoiesis to signaling molecules crucial for progenitor cell formation, maintenance, and differentiation. Primitive hematopoiesis in Drosophila ensues in embryonic head mesoderm, whereas definitive hematopoiesis happens in larval hematopoietic organ, the lymph gland. This organ, with the onset of pupation, ruptures to release hemocytes into circulation. It is believed that the adult lacks a hematopoietic organ and survives on the contribution of both embryonic and larval hematopoiesis. However, our studies revealed a surge of blood cell development in the dorsal abdominal hemocyte clusters of adult fly. These active hematopoietic hubs are capable of blood cell specification and can respond to bacterial challenges. The presence of progenitors and differentiated hemocytes embedded in a functional network of Laminin A and Pericardin within this hematopoietic hub projects it as a simple version of the vertebrate bone marrow.

RESULTS AND DISCUSSION

Identification of Hematopoietic Clusters in Adult Drosophila

Employing hemolectin-Gal4, UAS-GFP, we have identified four hematopoietic blood cell clusters along the dorsal midline in the abdominal segments A1–A4 of adult flies (Figures 1A–1C* and S1A–S1E; Movies S1 and S2). Of the four clusters, the one in the abdominal segment A1 has the maximum aggregation of cells (Figure S1F) that occupies the area that spans the lateral segments A1–A4. These clusters harbor progenitor cells that trace their origin to the tertiary and quaternary lobes of larval lymph gland. Importantly, the precursors are capable of de novo generation of both plasmocytes and crystal cells. The surge of post-larval hematopoiesis partly relies on Notch signaling, and the resident plasmocytes are capable of responding to bacterial challenges. All these observations, put together, establish these active hematopoietic hubs as a simpler version of vertebrate bone marrow.
these hemocytes are located dorsally (Figure 1G). Thus, these clusters remain secluded from the abdominal cavity by the dorsal and the pericardial diaphragm (Wasserthal, 2007) (Figures 1H and S1N).

The hemocytes within the clusters are embedded in an extensive network of extracellular matrix proteins surrounding the heart and the pericardial cells. One of the important components of this network is the type IV collagen-like protein, Pericardin (Figure 1I; Movie S3). In homozygous mutant for lonely heart (loh), a gene encoding a secreted receptor of Pericardin (Drechsler et al., 2013), the hemocytes fail to form the cluster (Figures 1J–1M, S1M, and S1M*), as this network gets significantly affected. Similar result is observed upon knocking down the expression of Laminin A (Figure 1N), another important component of the network, by driving UAS-laminin A RNAi in the cardiac tube by mef2-Gal4 (Figure 1O). Based on expression and functional analyses, we conclude that both Pericardin and Laminin A function in maintaining adhesive interaction with the hemocytes aiding in formation of the clusters. Interestingly, Laminin A polypeptides (Gu et al., 2003; Siler et al., 2000) and collagen IV (Nilsson et al., 1998) are also prevalent in vertebrate bone marrow. Our finding that the blood cells are fenestrated in a functional network of Laminin A and Pericardin raised the speculation that these sites might function as bone marrow-like tissues in adults (Binggeli et al., 2005) (Figure 1S), and are Serpent (Srp) and lozenge-GFP further ascertains the above observations (arrowhead; Figure 2E). Interestingly, some of these Iz-GFP-positive cells still have low levels of Su(H)lacZ expression (arrowhead; Figure 2E). By 5 dpe, we observe an increase in the number of cells that are either expressing Iz-GFP (double arrowhead; Figure 2F) or have low levels of Su(H)lacZ expression along with Iz-GFP expression (single arrowhead in Figure 2F).

The Cluster Houses Hemocyte Precursors

GATA factor Serpent (Srp) is expressed in low levels in all hemoocytes, including plasmatocytes and crystal cells (Figure 2A). However, the hemocyte precursor cell can be identified by the presence of high levels of Srp expression (Rehorn et al., 1996). Analysis of developing cluster at 2 dpe reveals the presence of cells positive for both Srp and hml (plasmatocytes) (Figures 2B–2B”, and a small subset of cells exclusively expressing Srp. No crystal cells (Hnt) are present in the cluster. In contrast, at 5 dpe, along with the two cell types mentioned above, some Srp- and Hnt-positive crystal cells are seen (Figures 2C–2C”). Quantitative analysis of the above observations clearly demonstrate an increase in the number of differentiated cells (plasmatocytes and crystal cells) with a concomitant decline in the number of cells exclusively expressing Srp at 5 versus 2 dpe (Figure S2A). These results also indicate that the Srp-positive cells within the cluster that do not express either hml or Hnt might be the precursor cells, yet to turn on differentiation.

We then followed the crystal cell development in the cluster. Since activation of Notch (N) pathway precedes Lz expression in crystal cells (Duvic et al., 2002), we generated a recombinant fly line with 12XSu(H)lacZ in the background of Iz-GFP, Su(H)lacZ-positive cells are first seen in the cluster 2 dpe (Figure 2D), whereas the expression of Iz-GFP is observed only on 3 dpe (double arrowhead; Figure 2E). Interestingly, some of these Iz-GFP-positive cells still have low levels of Su(H)lacZ expression (arrowhead; Figure 2E). By 5 dpe, we observe an increase in the number of cells that are either expressing Iz-GFP (double arrowhead; Figure 2F) or have low levels of Su(H)lacZ expression along with Iz-GFP expression (single arrowhead in Figure 2F).

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Figure 1. Adult Hematopoietic Hubs *Drosophila*

(A and B) Hemocyte clusters (A) in the abdominal segments on either side of dorsal midline (Dm) are closely associated with dorsal vessel (DV) (B). (C–C’’) 3D reconstruction of a fillet showing the position of four abdominal hemocyte clusters (green) above DV (red). Horizontal rotation angles are as mentioned.

(C’) Lateral view of (C), whereas (C’’) and (C’’’) are rotation of (C’) toward dorsal side.
observed (Figures 2I, 2I', and S2C–S2G'). This result in conjunction with our earlier observation establishes that crystal cells develop in adult cluster from high Srp-positive precursor cells, and this process requires N signaling. As a functional correlate to establish the presence of precursor cells in the cluster, we twitched N signaling to determine its effect on differentiation of crystal cells. Since the onset of Su(H)lacZ and Iz-GFP expression in the cluster is observed at 2 dpe and 3 dpe, respectively, we impaired N signaling in the precursors by driving UAS-N RNAi using hemese-Gal4 from 2 dpe. As shown in Figure 2K, this result in complete loss of crystal cells compared with that observed in WT clusters (Figure 2J). Interestingly, the marginal increase in the number of plasmatocytes observed by counting down N correlates with the number of crystal cells missing in this genetic background when compared to control (Figure 2M). Likewise, overexpressing N in these cells results in almost 7-fold increase in the number of crystal cells with a significant drop in the number of plasmatocytes (Figures 2L and 2M).

It is therefore quite evident from our results that the clusters of blood cells on dorsal side of adult fly are not a mere aggregation of hemocytes of embryonic and larval origin but also houses true progenitors. The very fact that they house blood cell precursors and exhibit dynamicity as de novo crystal cells get differentiated in them qualifies them to be considered as active hubs of hematopoiesis in adult.

Origin of the Hematopoietic Precursors of the Hub

Upon identifying the hemocyte precursors, we sought to define their origin. In WT larvae, differentiation of plasmatocytes and crystal cells takes place in the primary lobe of the lymph gland, while secondary, tertiary, and quaternary lobes harbor the reserve blood cell precursors (Jung et al., 2005) (Figure 2N) that undergo differentiation only upon infection (Sorrentino et al., 2002). As reported earlier (Mondal et al., 2011), we found that driving PvrRNAi by hml-Gal4, UAS-GFP leads to differentiation of all cells present in the primary lobe of the lymph gland (Figure 2O). However, cells in the tertiary and quaternary lobes remain undifferentiated. Analyses of hematopoietic hub of UAS-PvrRNAi; hml-Gal4, UAS-GFP flies reveal a drastic reduction in the number of plasmatocytes when compared with control (Figures 2P and 2Q). We attribute this reduction in plasmatocyte number to the block in migration of differentiated hemocytes in circulation that results from knocking down Pvr (Brückner et al., 2004). Interestingly in these clusters we do find a large number of Srp-positive precursor cells (Figures 2Q and S2I–S2L). We presume that this relative increase in number of Srp positive cells when compared with control might be an outcome of preventing circulating plasmatocytes from migrating into the cluster. More importantly, the presence of Srp-positive precursors in the clusters together with the observation that the hemocyte precursors only reside in the tertiary and quaternary lobes of the lymph glands of larvae of this genotype strongly indicates that these precursors actually originate form tertiary and quaternary lobes of larval lymph gland.

Our analysis of collier expression in late third instar larval lymph gland reveals that apart from being expressed in the posterior signaling center (PSC) of the primary lobe (Crozatier et al., 2004), it is also expressed at a very high level in the hemocyte precursors of tertiary and quaternary lobes of the lymph gland (Figure 2R). On activation of G-TRACE construct by col Gal4 from late third instar larvae several col lineage traced cells (EGFP) are detected in the adult cluster (Figures S2–S2U and S2H–S2H'), thereby establishing that the hemocyte precursors present in the tertiary and quaternary lobes of larval lymph gland do migrate into these clusters. Some of these GFP positive collier lineage traced cells have high levels of Srp expression demonstrating their precursor state, while some have low levels of Srp expression (Figures S2–S2S'). Immunostaining of adult clusters having lineage traced cell (EGFP) with P1 and Hnt antibodies reveals that they are positive for either of this differentiation marker (Figures S2T–S2U'). In addition, there are also collier lineage traced cells that express neither of these markers (arrows in Figures S2T, S2U', and S2H–S2H') and therefore represent undifferentiated precursors. These results demonstrate that collier lineage traced progenitors in the hub originates from the hemocyte precursors present in the tertiary and quaternary lobes of the lymph gland.
of larval lymph gland and that they can give rise to both plasmacytes and crystal cells (Figure 2V).

Existence of a Post-larval Surge of Hematopoiesis in Drosophila

Next we wanted to investigate whether like crystal cells there is de novo differentiation of plasmacytes within the hub. For this purpose, we activated the G-TRACE cassette using hml-Gal4 with the aim to lineage trace (by EGFP expression) all plasmacytes that are formed during embryonic and larval stages, as well as to mark by RFP expression any plasmacyte that is formed de novo within the adult cluster (Figures 3B′ and S3D). Analysis of the cluster in adults at 5 dpe reveals the existence of plasmacytes that are either only lineage traced for hml expression (EGFP) or positive for both lineage traced and live expression (EGFP and RFP), or the ones that exhibits only RFP expression (arrow in Figures 3B–3D′). While RFP expression in the plasmacytoses confirms current expression of hml in these cells, the very existence of RFP-positive hemocytes that are not lineage traced definitely implies de novo origin of these plasmacytes in the hub. Analyses of the hub at 4 and 8 dpe give identical results (Figures S3A–S3G′).

Next, we wondered about the signal that triggers this surge of hematopoiesis in adult. For this purpose, we generated flies where the lineage-tracing cassette can be activated by notch-Gal4 in a genetic background that has a Gal80θ allele. Presence of Gal80θ allele would allow us to have a temporal control over the induction of the cassette by Gal4. Flies reared at 18°C for entire developmental period till 5 dpe (Figure 3E′) to keep the Gal80 active and in turn the Gal4 inactive served as control. In these flies none of the plasmacytoses (P1) are found to be N lineage traced, validating the tight regulation of Gal80 on the G-TRACE construct (Figures 3E–3F′). Interestingly, in the same genetic background upon activating G-TRACE system by notch-Gal4 10-hours post-pupation, we found that all the differentiating crystal cells are positive for both lineage traced and active N expression (Figures 3G–3H′′′). This observation is in tune with our finding that Su(H), a downstream target of N is active in differentiating crystal cells in the cluster.

In contrast to this, only a small subset of plasmacytoses (P1) is N lineage traced (Figures 3I–3K′ and S3H–S3M′). Since the lineage tracing system was activated 10-hr post-pupation, a time point by which disruption of the larval lymph gland takes place, none of the lineage traced GFP-positive cells represents the population of differentiated larval plasmacytoses that migrate to the hub. Rather, they represent the plasmacytoses that are born in the post-larval period. However, the number of N lineage traced plasmacytoses being very less compared with the total number of plasmacytoses that actually differentiate in the hub (compared with RFP expressing cells in Figures 3C–3D′′), we conclude that while a majority of the plasmacytoses generate de novo in the hub by a N independent mechanism, some of them depend on N for their genesis. In sum, our results demonstrate that N signal is involved in the genesis of crystal cells as well as of some plasmacytoses in the hematopoietic hubs of adult flies (Figure 3L).

Dynamic Hematopoietic Hub Generate Immune Response

Temporal studies using hml-GFP reveal that the number of plasmacytoses in the hub do not remain the same during the adult life. There is a gradual increase in the number till 5 dpe; thereafter, it remains more or less constant up to 8 dpe. However, with aging, a decline in the plasmacytose number in the hub is observed (Figures 4A–4F). Since we were able to detect several progenitors till 5 dpe, we speculate that the initial increment in number of plasmacytoses is partly due to the formation of new plasmacytoses from these precursors. However, upon analysis of the number of plasmacytoses present in adult circulation vis-a-vis to those present in the cluster at 2 and 5 dpe, we found that while there

**Figure 2. Hemocyte Progenitors Are Present within the Hematopoietic Hub in Adult**

(A) Plasmatocyte and crystal cell originate from precursors that express only Srp.

(B) Presence of plasmacytoses and few hemocytes that express only Srp (arrow) at 2 dpe in the hub.

(B′ and B″) Zoomed in regions of (B). Arrows denote cells that express only Srp.

(C) At 5 dpe, crystal cells, plasmacytoses, and several Srp positive (arrow) cells are seen in the cluster.

(C′ and C″) Zoomed in of (C) showing crystal cells (arrowhead) and Srp-positive hemocyte (arrow).

(D–G) Temporal kinetics of expressions of Su(H)LacZ (red) and Iz-GFP (green) in the cluster. At 2 dpe, only Su(H)LacZ-positive cells are seen (arrow, D). Expression of Iz-GFP is turned on in some Su(H)LacZ-positive cells by 3 dpe (arrowhead, E). At 5 (F) and 7 (G) dpe, while the number of Iz-GFP (double arrowhead) expressing cells increase, a concomitant decrease in Su(H)LacZ-positive cells (arrow) is observed.

(H–I′) Expressions of Srp (gray) and Su(H)LacZ (red) in the cluster. (H and H′) A cell (arrow) that has high levels of Srp expression also expresses Su(H)LacZ. (I and I′) With stabilization of Su(H)LacZ (red) expression, the Srp expression declines (I and I′).

(J–L) Crystal cell number compared with control (J) drastically reduces on knocking down Notch expression (K), while its overexpression causes huge increment.

(M) Quantitative analysis of (J)-(L).

(N) hml expression in differentiated cells of primary (1′) lobe of the lymph gland.

(O) Loss of Pvr in hml cells leads to complete differentiation of 1′ lobe and while tertiary (3′) and quaternary (4′) lobe cells (arrows) remain undifferentiated.

(p) hml-Gal4, UAS-GFP hematopoietic hub houses Srp (red) hml (green) and Hnt (cyan) expressing cells.

(Q) Only few hml (green) cells and Hnt (cyan) cells are seen in UAS-PvrRNAi; hml-Gal4, UAS-GFP hub, which has plenty of Srp (red, arrow) only cells.

(R) collar expressing (green) in PSC, 2′ lobe and the 3′ and 4′ lobes of late third instar larval lymph gland.

(S–S″) col-Gal4 lineage traced cells (green) and Srp (gray) expression in the cluster. (S′) and (S″) are zoomed in region of S showing collar lineage traced cells that are either high (arrowhead) or low (arrow) in Srp expression.

(T and T′) collar lineage traced cells (EGFP) in w; UAS-GTRACE/+;xn-Gal4/+ hub are either P1 (gray, membrane) or Hnt (gray, nucleus) or lack both Hnt and P1 expression (arrows in T–T′).

(U–U″) A crystal cell (Hnt) or plasmacytose (P1) can arise from collar lineage traced cells (EGFP). (U′) and (U″) are zoomed in image of U.

(V) Potential of Srp positive col lineage traced cells in the hematopoietic hub.

Gonocyte (Oe) Fb, fat body, PC, pericardial cell. Scale bar represents 20 μm. Error bar denotes SE. See also Figure S2.
is a decrease in the number of plasmatocytes in circulation from 2 to 5 dpe (Figure 4G), there is almost comparable increase in their number in the hub. We, therefore, presume that homing of circulating hemocytes also contributes in the initial increase of plasmatocytes in the hub. While the gradual loss in resident plasmatocytes suggests that the adult is regularly using the hemocytes from the hub as it ages.

Next, we addressed whether these plasmatocytes has the potential to mount response when immune challenged, thereby aiding the fly in combating infections. We observed that when challenged with RFP labeled E. coli, the plasmatocyte in the hub are able to phagocytose the bacteria (Figures 4H–4H’), confirming the fact that they are primed and ready for challenges imposed during adulthood. This observation of ours is in tune with the results of a previous study that demonstrated the existence of heart associated macrophages that can phagocytose (Horn et al., 2014).

Drosophila larval plasmatocytes are endowed with the capacity to divide in order to increase their population (Makhijani et al., 2011). However, it is believed that adult plasmatocytes lack the ability to proliferate and therefore considered to be in a state of senescence (Honti et al., 2014). Our data also endorse this finding, as no proliferating plasmatocytes are found in hematopoietic hub even after continuous 5 days of 5-bromo-2-deoxyuridine (BrdU) feeding (Figures 4I–4I’ and S4B1–S4B5). However, analysis of the hub in flies infected with E. coli reveals BrdU incorporation in several study that demonstrated the existence of N lineage traced (Figures S4C–S4F) highlights the contribution of the hub to fight out infection.

Summing up, this study unravels the presence of active hematopoietic hubs in Drosophila adults (Figure 4L). Refuting the existing notion that adults rely on long-lived hemocytes originating from embryonic and larval stages, we are successful in establishing that a surge of hematopoiesis happens in these hubs as the precursors present within differentiate into both crystal cells and plasmatocytes. The functionality of the hub gets further validated as we observed that besides exhibiting phagocytic activity the otherwise quiescent cells re-enter into proliferative mode in response to bacterial infection. These findings bring about a paradigm shift in our understanding of the process of hematopoiesis in Drosophila. With its well-characterized embryonic and larval hematopoietic activities, Drosophila has been serving as a powerful model for hematopoietic studies. In spite of that, the system seemed to be incomplete due to lack of detailed developmental analysis of hematopoiesis in adults. Our effort in establishing that the process of definitive hematopoiesis extends to adults expands the scope of exploiting this model system.

In vertebrates, the hematopoietic stem cells (HSCs) originate from hemangioblast and undergo maturation and expansion by a complex developmental process that requires the involvement of the yolk sac, the aorta-gonad-mesonephros (AGM) region, the placenta and the fetal liver before finally colonizing into bone marrow (Mikkola and Orkin, 2006). HSCs present within the bone marrow are however, not identical. Primarily based on their potential they are classified into three types: (1) myeloid biased, (2) lymphoid biased, and (3) balanced HSCs that are bipotent in nature and thus can give rise to both myeloid and lymphoid lineages (Müller-Sieburg et al., 2002). In a strikingly similar manner, the larval lymph gland houses blood cell precursors originating from hemangioblasts that arise from the embryonic cardiogenic mesoderm, a region analogous to the developing AGM of vertebrates (Mandal et al., 2004). Upon undergoing expansion within the lymph gland, some of these precursors actually home into the adult hematopoietic hub. Importantly, we found that the precursor cells within the hub are not homogeneous. While some of them are capable of differentiating into plasmatocytes independent of N signaling, there exist few bipotent precursors. Based on the results of N lineage trace experiments we speculate that these precursors initially turn on N and those that subsequently maintain N signaling adopts crystal cell fate. In contrast, failure to maintain N signaling triggers them to become plasmatocytes. This mirrors a striking resemblance to the different types of HSCs present in the vertebrate bone marrow. However, there being only myeloid lineage specification in flies, it seems that in this case the precursors are of two types: (1) biased for plasmatocyte lineage and (2) balanced bipotent precursors that can give birth to both plasmatocytes and crystal cells.

Conservation of hematopoietic events across the two taxa mentioned above along with the fact that the cells of the hub are nested in a functional network Laminin A and Pericardin projects this hematopoietic hub as a simple rendering of the vertebrate bone marrow. Given the fact that the vertebrate bone marrow is not easily accessible, we believe that our finding will...
Figure 4. The Hematopoietic Hub Is Functionally Active

(A–E) Plasmatocytes number in the hub at 2–35 dpe.

(F) Quantitative analysis of the data in (A)–(E).

(G) Comparative account of plasmatocytes number present in adult circulation at 2 and 5 dpe.

(H–H'') Plasmatocytes in the hub can phagocytose E. coli (H). (H') and (H'') are further zoomed in images of (H).

(I–I'') While fat body (Fb) cells incorporate BrdU (red, I') in adult fly, hemocytes of hub do not.

(J) Only on infection BrdU incorporation is seen.

(legend continued on next page)
establish *Drosophila* adult hematopoiesis as a simpler yet genetically amenable model to tease out normal and aberrant hematopoiesis and questions related to development, immunity, wound healing, and aging.

**EXPERIMENTAL PROCEDURES**

**Genetics**

hml-Gal4,UAS-2xEGFP, dot-Gal4, crq-Gal4, lz-Gal4, kn-Gal4, notch Gal4, met2-Gal4, UAS-N full, UAS-N RNAi, UAS-IanA RNAi, UAS-2xEGFP, UAS-nlsGFP, and ioh^M605750^ flies were obtained from Bloomington Stock Center. pxn-Gal4, gcm-Gal4, ZCL2897, 12xSuhIlacZ, G-TRACE, HHLT Gal4, and UAS-PvrRNAi and he-Gal4UAS-nlsGFP were provided by U. Banerjee, B Shilo, and I. Ando. For temporal control on Gal4 activity, temperature-sensitive allele of gal80 was used.

**Immunohistochemistry**

Antibodies P1, Srp, anti-proPO, and Laminin A were kind gifts from I. Ando, D. Hoshizaki, M. Kanost and S. Baumgartner. Hnt(1G9), Pro(EC11), anti-Brdu, and f-galactosidase were from Developmental Studies Hybridoma Bank, Abcam, and Promega, respectively. Secondary antibodies were from Jackson ImmunoResearch Laboratories. For all of the experiments, 5–8 dpe (unless mentioned) female flies were used. Tissues were fixed in 4% formaldehyde, blocked in 10% bovine serum albumin (BSA) before primary antibody incubation at 4 °C. Secondary antibody incubation was done at 4 °C. Imaging was done in Zeiss LSM 780 confocal microscope. Fly cuticle was imaged by autofluorescence at 633 nm. Images were processed by Image J and Photoshop CS3.

**Infection Studies**

Glass capillary was used to pierce the thorax of fly with E. coli expressing RFP. Infected flies were reared in normal food until dissection.

**BrdU Assay**

Flies 3 dpe were grown in corn meal yeast fly food supplemented with 200 μl of 6-mg/ml BrdU in PBS for 2 days before infecting with E. coli. They were then reared in BrdU food for 5 days, with two intermittent flips until dissection.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, four figures, and three movies and can be found with this article online at http://dx.doi.org/10.1016/j.devcel.2015.03.014.

**AUTHOR CONTRIBUTIONS**

S.G. did bulk of the experiments, imaging, and data analysis. A.S. helped in initial characterization. S.M. helped in interpretation and manuscript writing. Conception, designing, analysis, manuscript writing, and research supervision were by L.M.

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(J' and J") Zoomed region of (J).

(K) Quantification of BrdU incorporation in the hub of control (blue) and infected flies (red).

(L) Scheme depicting potential of the hematopoietic hub.

Scale bar represents 20 μm; 5 μm (H’). Error bar denotes SE. See also Figure S4.

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