# PDGF/VEGF homologues control blood cell numbers in *Drosophila*

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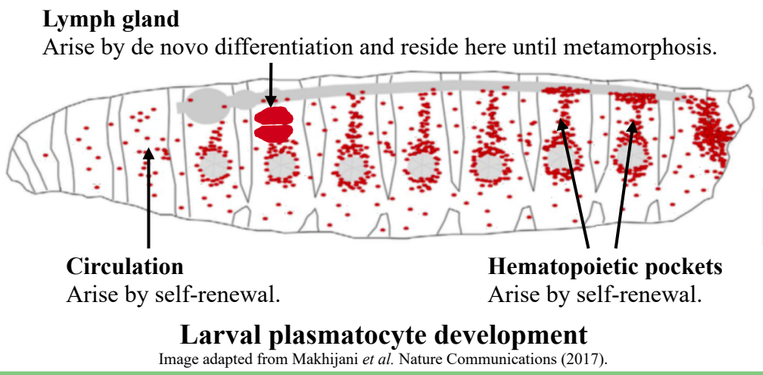
## Background

- Macrophages are a critical component of the mammalian immune system and were recently found to replenish their numbers via self-renew.[2]
- Macrophage self-renewal remains poorly understood.
- In *Drosophila melanogaster,* >90% of blood cells (called hemocytes) are macrophage-like plasmatocytes that play critical roles in immunity and tissue remodeling.[3]
- New plasmatocytes arise by de novo differentiation in the embryo and in three locations during the larval stages (right).




**Larval plasmatocyte development**
Image adapted from Makhijani *et al.* Nature Communications (2017).

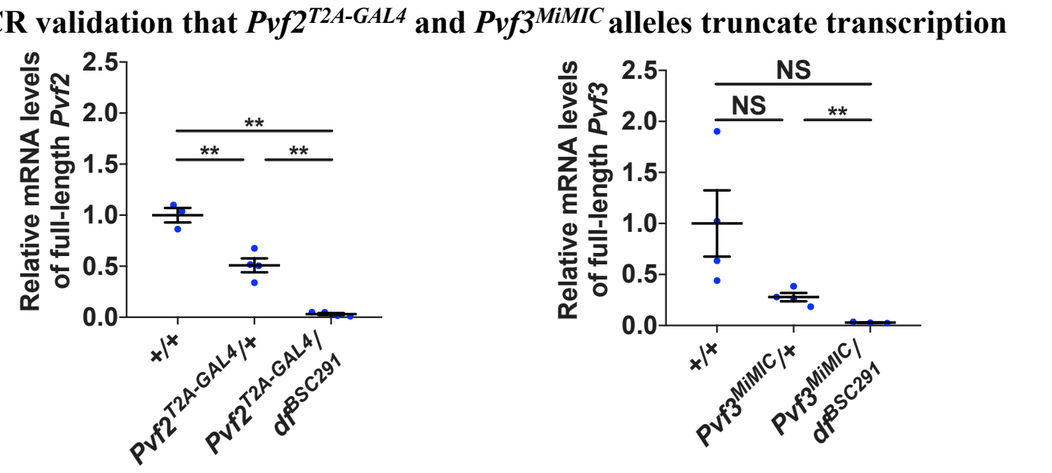
- Larval plasmatocyte development can be used as a model for the study of macrophage self-renewal.[4]
- The sole *Drosophila* homologue of the mammalian PDGF and VEGF receptors (Pvr) is one of few factors that has been shown to control plasmatocyte self-renewal.[5]
- Pvr is required in larval hemocytes in this role.[5]
- Pvr has 3 ligands (Pvf1-3).[6]
- To understand the mechanisms of plasmatocyte self-renewal, we sought to identify the ligands that activate Pvr in this role and explore how they are controlled.

## 1. *Pvf2* and *Pvf3* control larval hemocyte number

### Pvf mutants:

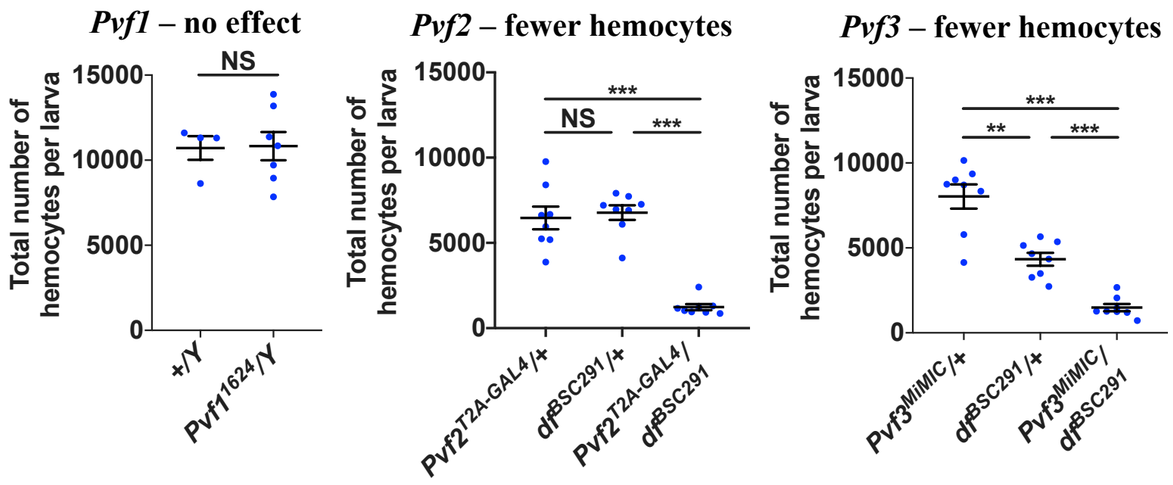
Pvf1: *Pvf1*$^{1624}$ allele does not produce detectable levels of *Pvf1* transcript.[7]
Pvf2: Generated *Pvf2*$^{T2A\text{-}GAL4}$ allele, which is predicted to truncate *Pvf2* transcription.
Pvf3: *Pvf3*$^{MiMIC}$ allele is predicted to truncate *Pvf3* transcription.
Note: *df*$^{BSC291}$ is a deficiency allele that removes *Pvf2*, *Pvf3* and several other genes.

**qPCR validation that *Pvf2*$^{T2A\text{-}GAL4}$ and *Pvf3*$^{MiMIC}$ alleles truncate transcription**




### Hemocyte numbers in *Pvf* mutants:

Hemocytes were identified and quantified using fluorescent markers that express in larval hemocytes (*hml*Δ*dsRed* for *Pvf1* and *Pvf2*, *hml*Δ*-GAL4>UAS-GFP* for *Pvf3*).[8-10]

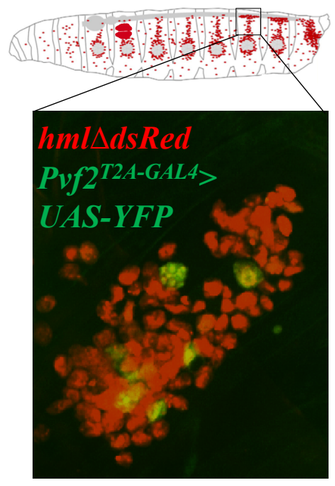


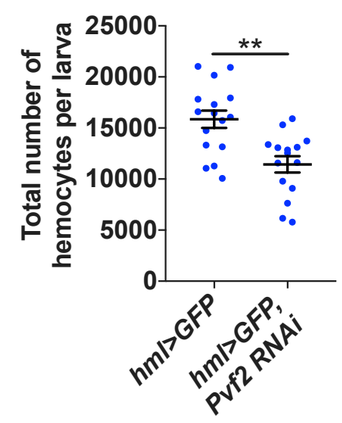

## 2. Larval hemocyte numbers depend on *Pvf2* expression in a novel hemocyte subpopulation

*Pvf2* is expressed in ~1% of larval hemocytes (right).

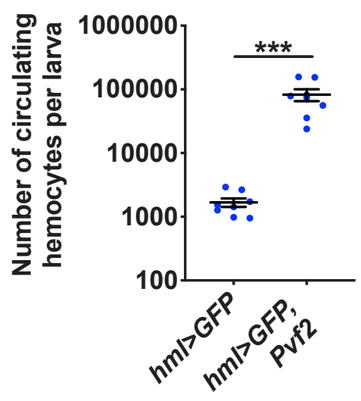
Knockdown of *Pvf2* in all larval hemocytes (far right) reduces total hemocyte number.

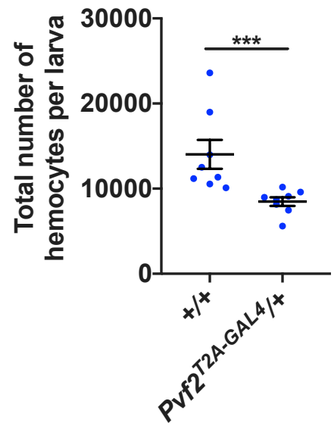
Larval hemocytes are thus a source of *Pvf2*.




Expression of *Pvf2* in all larval hemocytes results in hemocyte overproliferation (far left).




*Pvf2* is haploinsufficient in the control of larval hemocyte number (left).

Thus *Pvf2* expression levels in hemocytes is critical for controlling plasmatocyte self-renewal.

*Pvf2*-expressing hemocytes arise in the embryo (arrowed right, *srpHemo-H2A-mCherry* marks all embryonic hemocytes).[11]

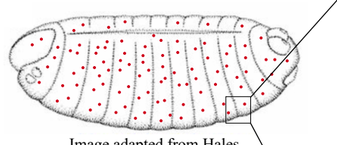
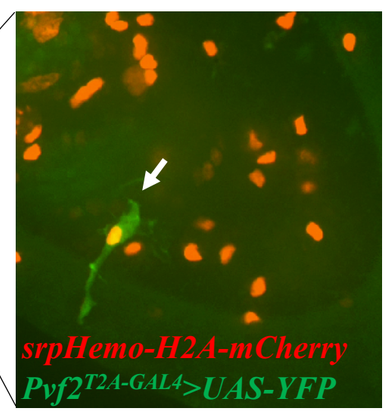
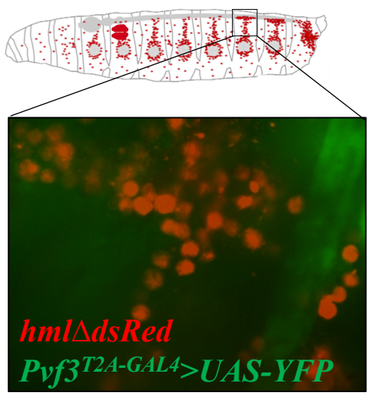


Image adapted from Hales *et al.* Genetics (2015).



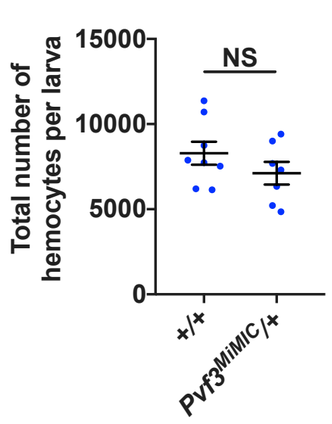

This may be a novel lineage of hemocytes specified during embryonic development.

## 3. *Pvf3* is not expressed in hemocytes




*Pvf3* is not expressed in larval hemocytes, thus this is not the source of Pvf3.

*Pvf3* is expressed in other organs that secrete proteins into circulation, such as the brain, prothoracic gland and fat body.

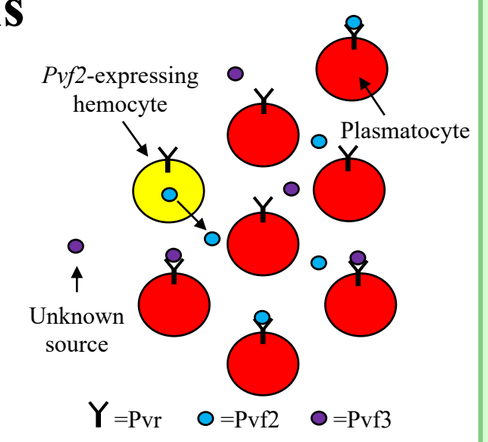



*Pvf3* is not haploinsufficient in this role.

Thus Pvf3 activity may be regulated post-transcriptionally.

## Conclusions

- A small number of hemocytes that express *Pvf2* are responsible for the global control of plasmatocyte self-renewal.
- Pvf3 controls larval hemocyte number via a different mechanism to Pvf2. Likely sources of Pvf3 are the brain, prothoracic gland and fat body.
- The findings presented here may provide insights into the control of mammalian macrophage self-renewal.




**Current model for Pvr-mediated control of plasmatocyte self-renewal**

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