SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Pvf1 and Pvr function in non-overlapping zones and loss of Pvr does not cause cell death

All lymph glands shown are from wandering third instar larvae, except panel **A-F** where they are second instar.

(**A-F**) Cell proliferation profile in lymph glands from mid-second instar larvae analyzed by BrdU (red) incorporation during S phase. These panels are identical to those shown in **Figure 1B-G** except for the fact that the green channel is shown here to emphasize that the BrdU incorporating cells do not express the differentiation marker Hml (green). (**A**) Control lymph glands (genotype: *Hml-gal4 UAS-2xEGFP*) at this stage have only a few proliferating cells (9 +/- 6 cells, n=6). (**B**) Induction of cell death in the differentiating blood cells by expression of Hid and Reaper (*Hml-gal4 UAS-2xEGFP UAS-hid UAS-rpr*) causes an increase in the number of BrdU positive cells (red), indicative of the loss of quiescence among the progenitors (compare with **A**). A similar loss of quiescence occurs upon either (**C**) down-regulation of Pvf1 in the PSC using $Pvf1^{RNAi}$ (*Antp-gal4 UAS-2xEGFP UAS-*$Pvf1^{RNAi}$), (**D**) down-regulation of Pvr in the differentiating cells using Pvr^{RNAi} (*Hml-gal4 UAS-2xEGFP UAS-*Pvr^{RNAi}; 39 +/- 7 cells, n=5, $p<0.004$ when compared with **A**), or (**E**) down-regulation of Adgf-A in the differentiating cells using $Adgf\text{-}A^{RNAi}$ (*Hml-gal4 UAS-2xEGFP UAS-*$Adgf\text{-}A^{RNAi}$). (**F**) Over-expression of *Adgf-A*

can suppress the proliferation phenotype due to loss of Pvr^{RNAi} (*Hml-gal4 UAS-2xEGFP UAS-*Pvr^{RNAi} *UAS-Adgf-A*; 17 +/- 7 cells, n=8, $p<0.03$ when compared with **D**).

(**G-J**) Down-regulation of *Pvf1* in (**H**) the MZ (*dome-gal4 UAS-2xEGFP UAS-*$Pvf1^{RNAi}$) or (**I**) the CZ (*Hml-gal4 UAS-2xEGFP UAS-*$Pvf1^{RANi}$) or (**J**) down-regulation of *Pvr* in the MZ (*dome-gal4 UAS-2xEGFP UAS-*Pvr^{RNAi}) does not cause loss of MZ progenitors.

(**K-N'**) Cell death assay by TUNEL staining (red in **K-N)** and differentiation marker Hml (green in **K'-N'**). The lymph glands shown in **K-N** are the same as the corresponding ones in **K'-N'**. (**K-K'**) Control (*Hml-gal4 UAS-2xEGFP*). (**L-L'**) As a positive control, over-expression of Hid and Reaper (*Hml-gal4 UAS-2xEGFP UAS-hid, UAS-rpr*) promotes cell death (red). (**M-M'**) Down-regulation of *Pvr* in the CZ (*Hml-gal4 UAS-2xEGFP UAS-*Pvr^{RNAi}) does not promote cell death. (**N-N'**) Down-regulation of *Adgf-A* in the CZ (*Hml-gal4 UAS-2xEGFP UAS-ADGF-*A^{RNAi}) does not promote cell death.

(**O-P)** Over-expression of the anti-apoptotic protein *p35* does not rescue the loss of MZ from down-regulation of *Pvr* in the CZ (*Hml-gal4 UAS-2xEGFP UAS-*Pvr^{RNAi} *UAS-p35*). The lymph gland shows expansion of differentiated cells expressing the Hml marker (green).

Figure S2. Expression and function of Pvf2

In **C-D**, differentiating cells are shown in red. In **E-J**, progenitors are marked with *dome-gal4 UAS-2xEGFP* (green). This figure shows that unlike the Pvf1/Pvr interaction in the CZ, which leads to the CZ signal, Pvf2 interacts with Pvr in the MZ to control E-cadherin expression necessary for maintaining the integrity and compactness of the MZ.

(**A**) Pvf2 (red) is strongly expressed in the PSC (yellow) and the medullary zone (within the white dotted area) of the mid 2nd instar larvae (*Antp-gal4 UAS-2xEGFP Pvf2-lacZ*). The expression pattern is weaker in the mid third instar (**B**). TOPRO3 (blue) stains nuclei.

(**C**) Control (*Antp-gal4 UAS-2xEGFP*).

(**D**) *Pvf2* down-regulation in the PSC (*Antp-gal4 UAS-2xEGFP UAS-Pvf2^{RNAi}*) does not cause loss of cells in MZ.

(**E**) Control (*dome-gal4 UAS-2xEGFP*). The progenitor population is confined to the medullary zone.

(**F-H**) Down-regulation of *Pvf2* (**F**) (*dome-gal4 UAS-2xEGFP UAS-Pvf2^{RNAi}*), *Pvr* (**G**) (*dome-gal4 UAS-2xEGFP UAS-Pvr^{RNAi}*), and *shg* (**H**) (*dome-gal4 UAS-2xEGFP UAS-shg^{RNAi}*) in the MZ has no effect on the differentiation status of hematopoietic progenitors (green), but causes spillage of these progenitors throughout the lymph gland lobe including the CZ.

(**I**) Control (*dome-gal4 UAS-2xEGFP*). Shg *(Drosophila* E-cadherin) expression is confined to the MZ.

(**J**) Down-regulation of Pvr in the MZ (*dome-gal4 UAS-2xEGFP UAS-Pvr^{RNAi}*) reduces Shg expression (red).

Figure S3. Pvr/STAT/Adgf-A function in the CZ

In panels **C** and **D**, the GFP channel (*Hml-gal4 UAS-2xEGFP*) is represented in red for the sake of consistency in marking differentiating hemocytes in all panels in red.

(**A-B**) Activated Pvr (Pvr^{act}: red) immunostaining. (**A**) Control lymph gland (*Antp-gal4 UAS-2xEGFP*) (**B**) Down-regulation of Pvf1 in the PSC (*Antp-gal4 UAS-2xEGFP UAS-Pvf1^{RNAi}*) causes a decrease in Pvr^{act} (red) staining in the CZ (compare to **A**).

(**C-D**) Lack of Domeless function in the CZ. (**C**) Control lymph gland (*Hml-gal4 UAS-2xEGFP*). (**D**) Over-expression of *domeless* dominant negative in the CZ (*Hml-gal4 UAS-dome^{DN}*) does not cause loss of progenitors.

(**E-H"**) STAT-GFP reporter expression in third instar lymph glands from different trans-heterozygous genetic backgrounds. (**E-E"**) Control lymph gland (*10x Stat92E-GFP*/+). (**F-H"**) STAT-GFP reporter (green) expression is reduced in (**F-F"**) *Pvr*-/+*; STAT*-/+

(*10x Stat92E-GFP Pvr*^{C2195}/+; *Stat92E*^{06346}/+), (**G-G"**) *STAT-/+ Adgf-A-/+* (*10x Stat92E-GFP; Stat92E*^{06346}/+ *Adgf-A*^{karel}/+), and (**H-H"**) *Pvr-/+; Adgf-A-/+* (*10x Stat92E-GFP Pvr*^{C2195}/+; *Adgf-A*^{karel}/+) trans-heterozygous backgrounds.

(**J**) Quantification of transheterozygote phenotypes. In all transheterozygous genetic backgrounds, the size of the CZ as a proportion of the total size of the lymph gland is significantly increased from control [(cont=*Hml-gal4*, n=15), *Pvr*^{C2195}/+; *Stat92E*^{06346}/+ (n=17), *Stat92E*^{06346}/+ *Adgf-A*^{karel}/+ (n=13) and *Pvr*^{C2195}/+; *Adgf-A*^{karel}/+ (n=22).]

Figure S4. Adgf-A and AdoR function in the LG

In all panels, except **C** and **M'**, green represents differentiating hemocytes. All lymph glands are from third instar, except panels **H-J'**, **N-O** which are all second instar.

(**A-C**) Expression of Hh in the PSC. (**A**) Control lymph gland (*Hml-gal4 UAS-2xEGFP*). (**B**) Down-regulation of Adgf-A in the CZ does not alter Hh expression (red; *Hml-gal4 UAS-2xEGFP UAS-Adgf-A*^{RNAi}). (**C**) Adgf-A homozygous mutants (*Adgf-A*$^{karel/karel}$) show intact expression of Hh (red) and Antennapedia (green) in the PSC.

(**D-E**) Reactive oxygen species (ROS) in progenitors. (**D**) Control lymph gland (*Hml-gal4 UAS-2xEGFP*) shows high levels of ROS (red) in the MZ. (**E**) Down-regulation of Adgf-A in the CZ (*Hml-gal4 UAS-2xEGFP UAS-Adgf-A*^{RNAi}) shows no change in ROS levels in early lymph glands.

(**F-G**) Over-expression of Adgf-A. (**F**) Control lymph gland (*Hml-gal4 UAS-2xEGFP*) (**G**) Over-expression of Adgf-A in the CZ (*Hml-gal4 UAS-2xEGFP UAS-Adgf-A*) does not alter the progenitor population.

(**H-L**) Loss of AdoR rescues the Adgf-A differentiation phenotype. BrdU (red) incorporation assay marks proliferating cells in lymph glands from mid 2nd instar larvae. (**H** and **H'**) Control (*Hml-gal4 UAS-2xEGFP*, 19$\pm$5 BrdU positive cells, n=4). (**I** and **I'**) Down-regulation of Adgf-A in the CZ (*Hml-gal4 UAS-2xEGFP UAS-Adgf-A^{RNAi}*, 35$\pm$15 BrdU positive cells, n=8) is rescued by single copy loss *AdoR* (**J** and **J'**; *Hml-gal4 UAS-2xEGFP UAS-Adgf-A^{RNAi}; AdoR^{KGex}/+*, 20$\pm$7 BrdU positive cells, n= 8, p=0.005). (**K-L**) (**K**) Downreguation of Adgf-A in the CZ causes loss of progenitors (*Hml-gal4 UAS-2xEGFP UAS-Adgf-A^{RNAi}*). (**L**) Single copy loss of AdoR rescues the *Adgf-A^{RNAi}* differentiation phenotype (*Hml-gal4 UAS-2xEGFP UAS-Adgf-A^{RNAi};AdoR^{KGex}/+*).

(**M-M'**) PKA regulates Ptc levels in progenitors. A large FLP-out clone expressing a dominant negative form of PKA (green) shows increased Ptc (red) expression compared to wild-type levels (arrowhead). Note that the smaller region enclosed within the dotted white line is wild type for PKA.

(**N-O**) Down-regulation of PKA function in progenitors does not alter proliferation. (**N**) Control (*dome-gal4*) (**O**) Down-regulation of PKA function in progenitors in mid-second instar lymph glands (*dome-gal4 UAS-PKA^{EP2162}*) does not alter the number of cells incorporating BrdU.

(**P**) Homozygous *rutabaga* mutants (*rut*^{1}) have fewer differentiated hemocytes (green). Compare to **F**.

(**Q-T**) Activated Ci staining in lymph glands. (**Q**) Control (*WT*, *w*^{1118}) (**R**) *rutabaga* mutants (*rut*^{1}/*rut*^{1}) show an increase in levels of Ci^{act} expression. (**S**) *Adgf-A* mutants (*Adgf-A*^{karel}/*Adgf-A*^{karel}) show a reduction in Ci^{act} expression levels. (**T**) Single copy loss of PKA function in *Adgf-A* mutants rescues Ci^{act} expression (*PKA*^{EP}/*Cyo GFP; Adgf-A*^{karel}/*Adgf-A*^{karel}). Compare to **Q** and **S**.

EXPERIMENTAL PROCEDURES

Drosophila stocks

All the *RNAi* stocks were obtained from VDRC (Vienna) and NIG (Kyoto), *UAS-Pvr*^{RNAi} (B. Shilo), *UAS-Pvr*^{DN} (P. Rorth and D. Montell), *10xSTAT-GFP* and *UAS-STAT*^{DN} (J. Darnell), JAK alleles (M. Zeidler), *Pvf2-lacZ* (M.A.Yoo), *UAS-PKA mC** and *mR** (J. Calderon), *UAS-*$G_{\alpha t}$ (J.E. Hooper), *UAS-Stat92E*^{act} and *UAS-dome*^{DN} (E. Bach), *Adgf-A*^{Karel}, *UAS-Adgf-A*, and *UAS-AdoR* (T. Dolezal), and *AdoR*^{KGex} (*AdoR*^{KG03964ex}; A. Sehgal). All Hop alleles, *hop*^{M38} and *hop*^{MSV1}, have been shown to block canonical JAK/STAT signaling as the *os*^{1} small eye phenotype is further enhanced by hypomorphic alleles of JAK (Tsai and Sun, 2004).

Immunohistochemistry

Antibodies: Rat αPvr and αPvf1 (B. Shilo), rabbit αHh (R. Holmgren), rabbit αRab11 (D. Ready), mouse αPvr^{act} (P. Rorth), mouse αBrdU (Sigma), rat αCi^{155} (DHSB), mouse αPxn (J. Fessler), and mouse αP1 (I. Ando).

For phagocytic assays, Fluorescent beads (FluoSpheres 0.1 μm carboxylate-modified microsphere, Invitrogen, Carlsbad, USA) were injected into the posterior lateral region of third instar larvae. One hour post-injection lymph glands were dissected and processed for imaging.

SUPPLEMENTARY REFERENCES

Tsai YC, Sun YH. Long-range effect of upd, a ligand for Jak/STAT pathway, on cell cycle in Drosophila eye development. Genesis 39(2):141-53

Supplemental Figure 1

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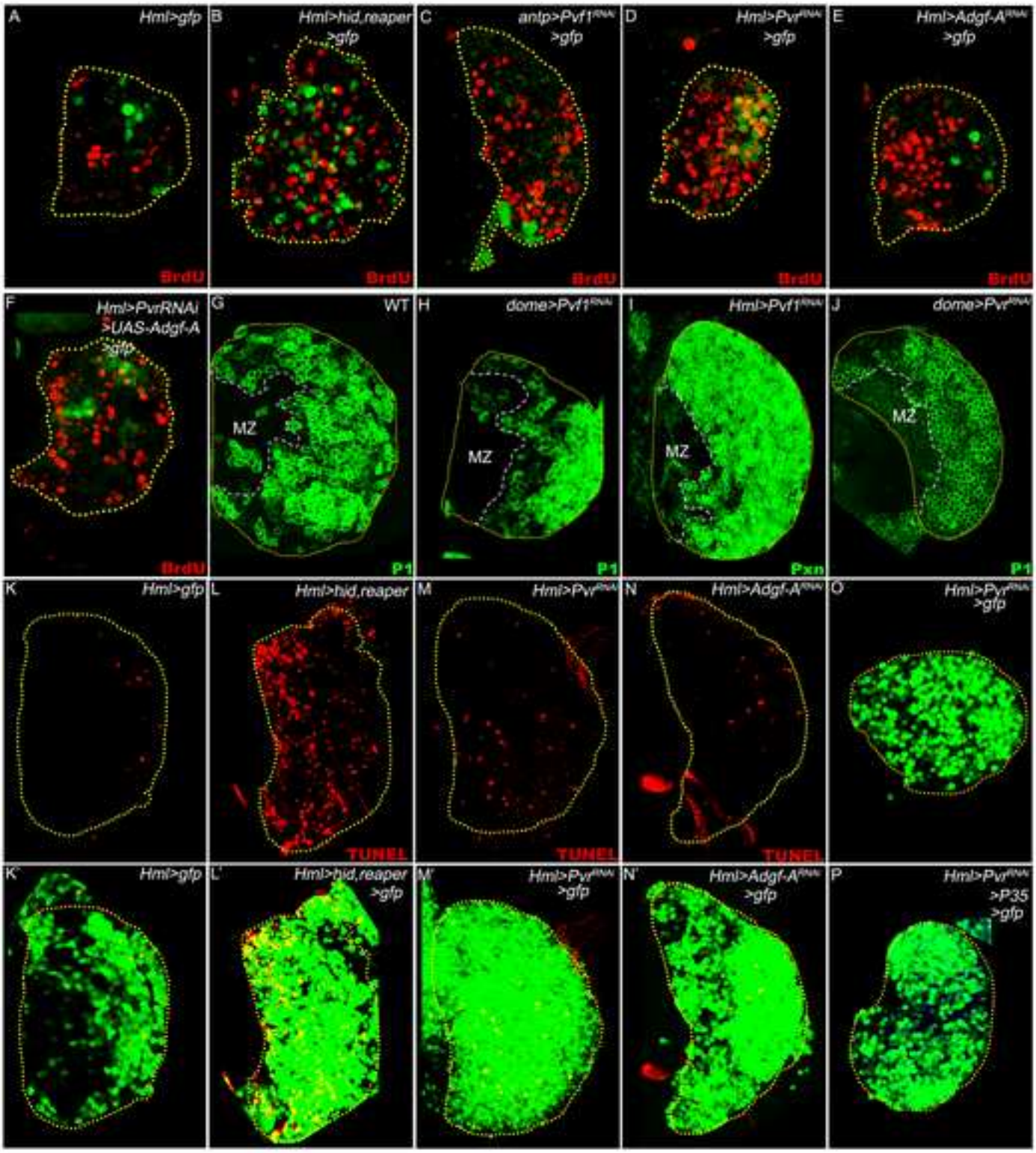

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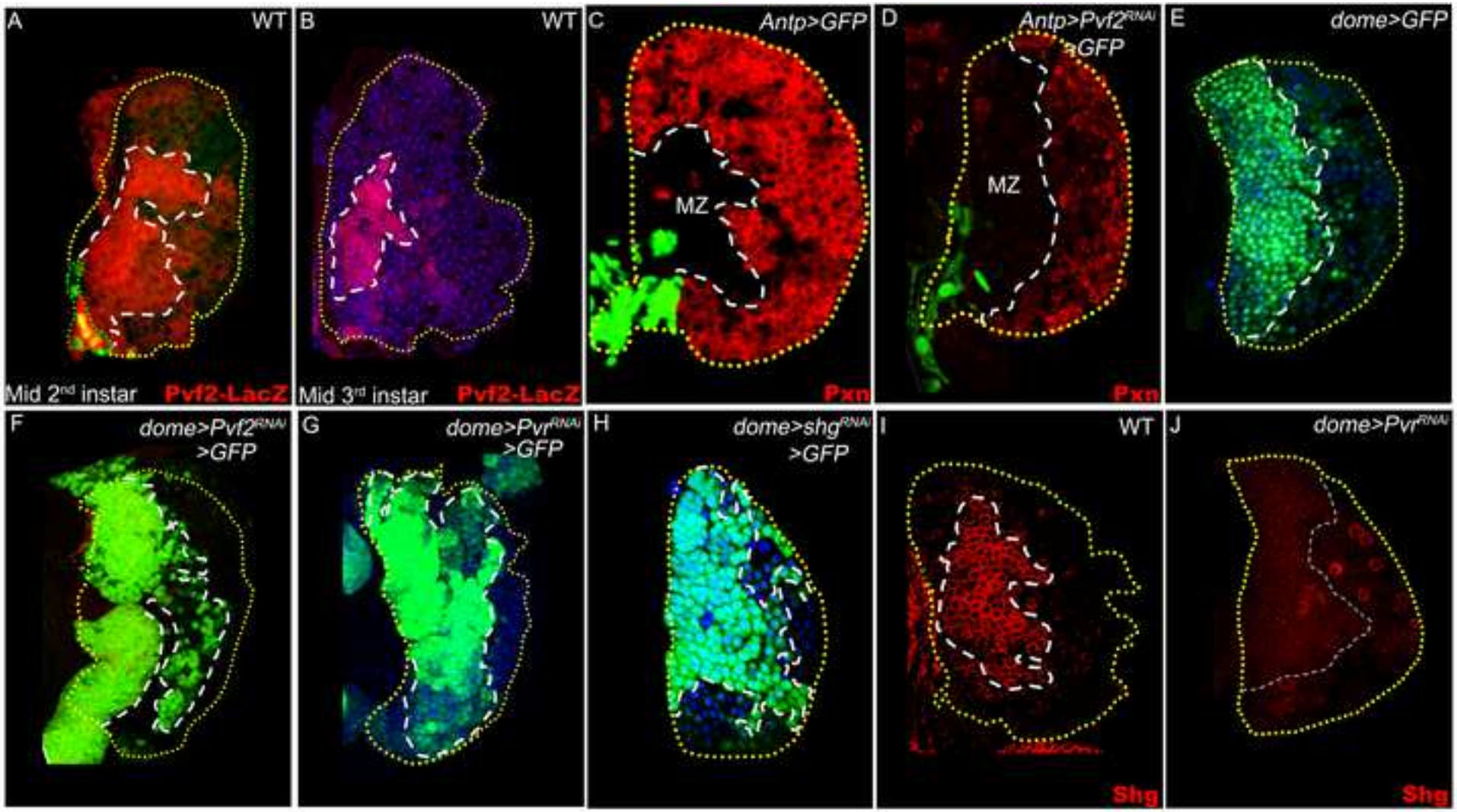

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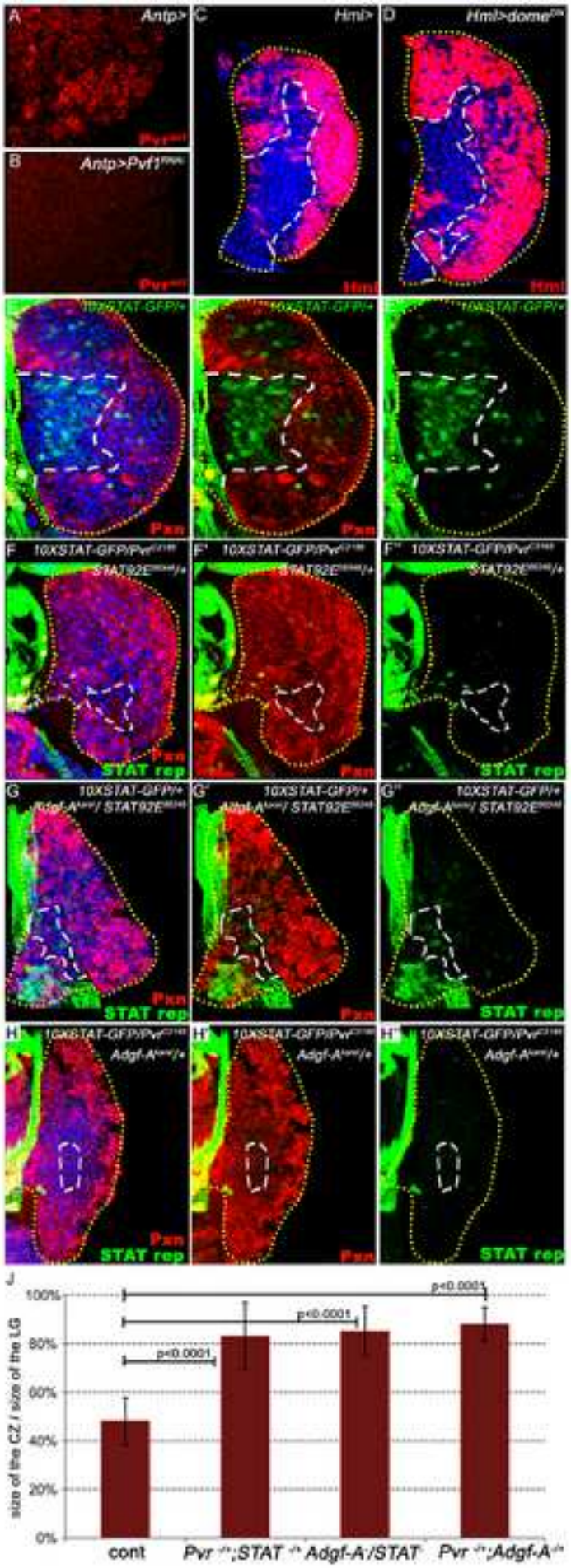

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