Supporting Information

Bras et al. 10.1073/pnas.1117317109

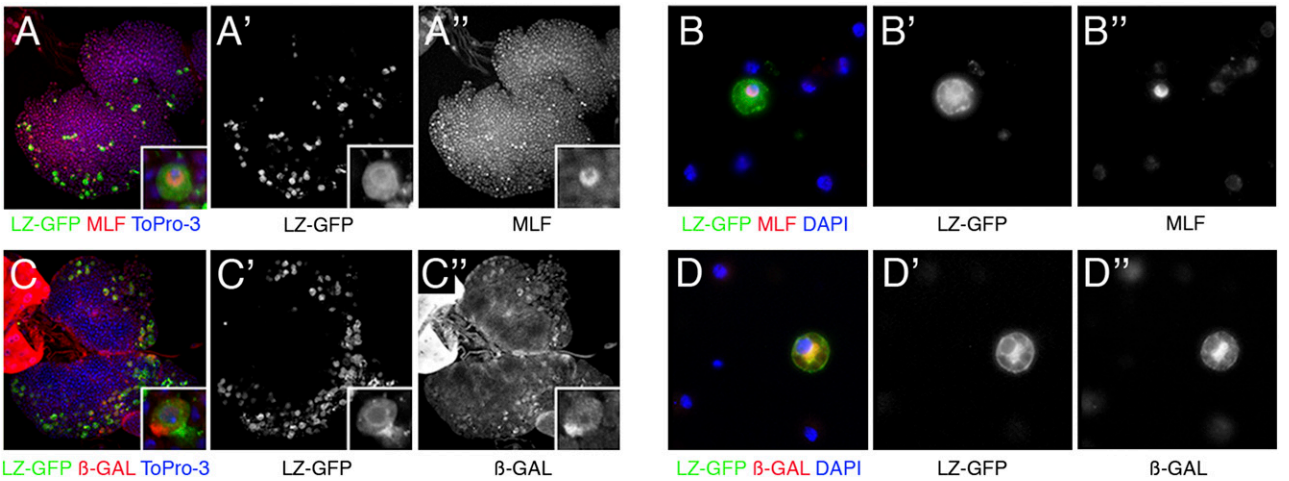

Fig. S1. *mlf* is expressed in larval crystal cells. Immunostaining against MLF (*A* and *B*) or β-galactosidase (*mlf-lacZ*) (*C* and *D*) on third instar larval lymph glands (*A* and *C*) or circulating blood cells (*B* and *D*). Crystal cells are labeled by GFP (*lz-Gal4*, *UAS-GFP*). (*Insets*, *A* and *C*) Higher-magnification views of LZ-GFP^{+} cells.

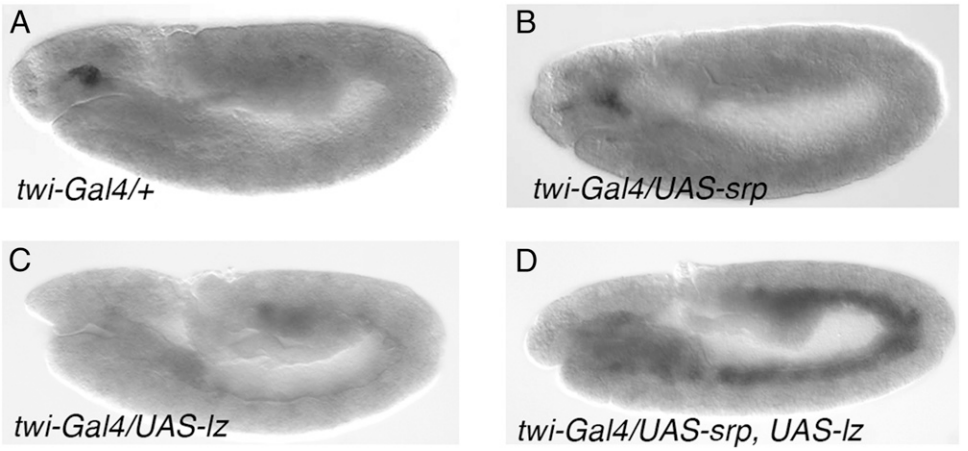

Fig. S2. *mlf* expression is activated by SRP/LZ in *Drosophila*. Lateral views of stage 11 embryos showing *mlf* expression as revealed by in situ hybridization. (*A*–*D*) Expression of the indicated *UAS* transgenes was driven in the mesoderm under the control of *twi-Gal4*. *twi-Gal4*–driven ectopic coexpression of SRP and LZ induces *mlf* transcription throughout the mesoderm.

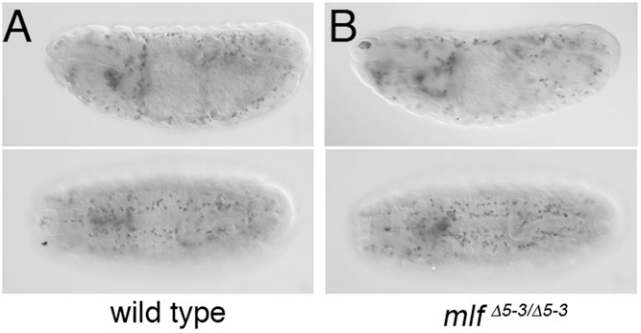

Fig. S3. *mlf* mutation does not impair embryonic plasmatocyte development. In situ hybridization against *pxn* was used to monitor plasmatocyte differentiation in WT (*A*) and *mlf* mutant (*B*) stage 16 embryos. (*Upper*) Lateral views. (*Lower*) Dorsal views.

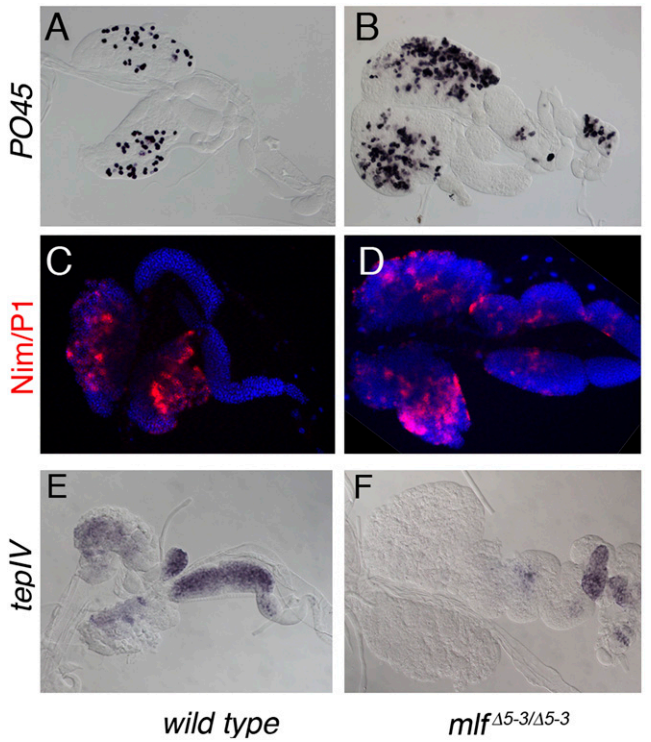

Fig. S4. *mlf* is required to maintain lymph gland homeostasis. Lymph glands from WT (*Left*) or $mlf^{-/-}$ (*Right*) third instar larvae are shown. In the absence of *mlf*, the lymph gland is hypertrophied, and both crystal cell differentiation and plasmatocyte differentiation are increased, notably in the secondary lobes (which normally contain only prohemocytes), whereas the prohemocyte population is decreased in the primary lobes. (*A* and *B*) In situ hybridization against *PO45* was used to monitor crystal cell differentiation. (*C* and *D*) Immunostaining against Nimrod/P1 was used to assess plasmatocyte differentiation. (*E* and *F*) In situ hybridization against *tepIV* was used to label the prohemocytes.

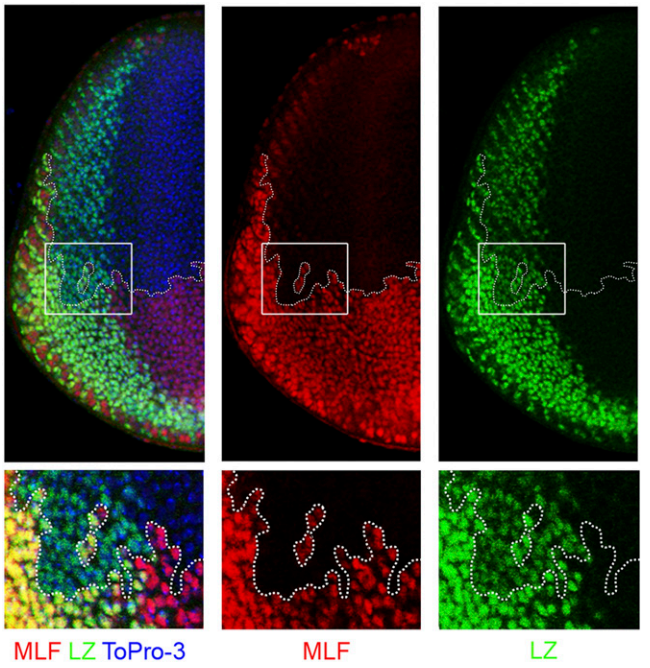

Fig. S5. MLF weakly stabilizes LZ expression in the eye. Immunostaining against MLF (red) and LZ (green) in third instar larvae eye disk carrying $mlf^{\Delta 5-3/\Delta 5-3}$ clones. High-magnification views of the boxed areas are shown in the lower panels. Dotted lines represent the boundaries of the clones. Mutant clones were induced 48 h after egg-laying by a 1-h heat shock at 37 °C in *yw, hs-flp; FRT42D* $mlf^{\Delta 5-3}$*/FRT42D arm-lacZ M(2)58F* larvae.

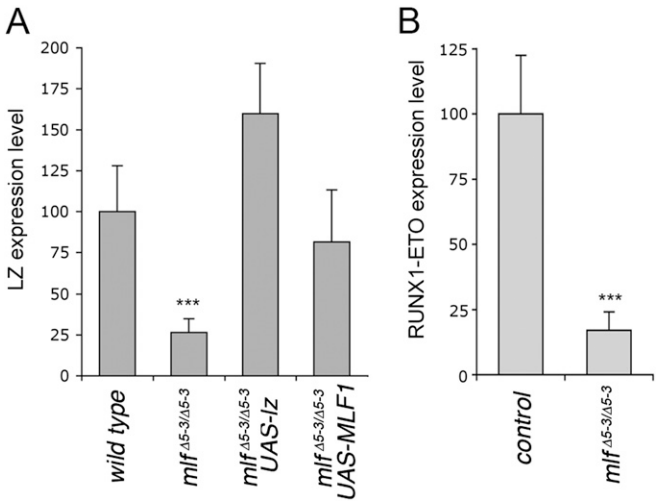

Fig. S6. MLF stabilizes LZ and RUNX1-ETO protein levels in vivo. (*A*) Fluorescent immunostaining against LZ was used to monitor LZ expression levels in circulating larval blood cells from *lz-Gal4, UAS-GFP* third instar larvae of the indicated genotype. (*B*) Fluorescent immunostaining against RUNX1-ETO was used to assess RUNX1-ETO expression levels in circulating larval blood cells from *lz-Gal4, UAS-GFP;UAS-RUNX1-ETO* (control) or *lz-Gal4, UAS-GFP;ml$^{\Delta 5-3}$/mlf$^{\Delta 5-3}$;UAS-RUNX1-ETO* (*ml$^{\Delta 5-3}$/mlf$^{\Delta 5-3}$*) third instar larvae. Fluorescent intensities were measured using ImageJ software on a minimum of 15 LZ-GFP^{+} cells of each genotype. ***$P < 0.0001$, Student *t* test.