Figure S1  *Drosophila* immune cells move into the tail during development by penetrating an epithelial barrier. Schematic drawings show embryos from a lateral perspective with hemocytes (green) migrating into the tail during st 11-12. Hemocyte positions sketched from *in situ* of PVR. (a-c) Lateral confocal images of fixed wild type srpHemoGAL4 UAS-GFP embryos which express GFP in hemocytes; pictures correspond to the boxed area in the appropriate embryo schematic. Embryos are from successively later stages as indicated above and were stained with anti-Cadherin (red) and anti-GFP (green) antibodies as well as DAPI to visualize nuclei (blue). Hemocytes moving into the tail are indicated with white arrows; examining these locations in the ′ panels which show only the Cadherin channel reveals that a breach in the Cadherin barrier appears as hemocytes move into the tail. Scale bars, 25 μm. (d) Stills from 2-photon movie of *ubi:shg-GFP; srpHemoGAL4 UAS-GFP* embryos. Shg-GFP outlines all cells and hemocyte-expressed GFP is seen in cytoplasm. (d) Lateral view starting st 11. Movie (Supplementary Information, Movie S1) corresponds to the dashed boxed area in the late st 11 schematic embryos above. Scale bars, (a-c) 25 μm or (d) 20 μm. Anterior to left and dorsal up in all panels.
Figure S2 Identification of the gene, rhoL, and an allele rhoL^{10-161} that strongly reduces rhoL expression. (a) Schematic of genomic region. The white box represents rhoL mRNA and the black box within it corresponds to the ORF; transposon insert site indicated with triangle and transposon excision mutant indicated above. (b) A Clustal W alignment of all human and Drosophila Rho GTPases; RhoL falls in a branch with Rac1, Cdc42 and 5 other Human Rhos. A box surrounds Drosophila RhoL; arrows indicate other Drosophila Rhos. (c) In situ hybridizations with rhoL probe on a rhoL^{10-161} embryo. (d) RT-PCR after 27 cycles utilizing rhoL and rp49 loading control primers on RNA from wild type and rhoL^{10-161}. These results show severely reduced expression in the rhoL^{10-161} mutant.
Figure S3 RhoL is not required for migration along the anterior vnc or to alter Cadherin levels. Mmp1 and 2 are not required for invasive hemocyte migration into the tail. (a, b) Stills from 2-photon multiplane movies of (a) wild type and (b) rhoLXA12 embryos expressing Moesin-GFP in hemocytes under the control of the 8-163 GAL4 driver. Movies focus on the solid boxed area in the embryo schematic above, and thus show hemocytes migrating along the anterior ventral nerve cord. Blue arrow in schematic indicates direction of hemocyte movement. Arrows in (a, b) indicate a single hemocyte moving over time. The elapsed number of minutes is indicated. (c-e) Confocal images of fixed early st 12 embryos stained with anti-Cadherin (red) and (c, d) hemocyte-expressed anti-CD2 or (e) anti-GFP antibodies to visualize hemocytes (green). Hemocytes move normally into the tail in (c) mmp1 Q112 mmp2 W307 heterozygous and (d) homozygous embryos as well as (e) embryos expressing the protease inhibitors TIMP and RECK. (f) Wild type and (g) rhoLXA12 embryos expressing lacZ in hemocytes under the control of the 8-163GAL4 driver. Embryos were stained with anti-DE-Cadherin antibodies (red, single channel shown in f, g) and anti-β-Gal antibodies (green, single channel shown in f’, g’) to visualize the level of DE-Cadherin in hemocytes. Merge shown in (f”, g”). No strong Cadherin staining was seen in hemocytes in either genotype.
**Figure S4** *inflated* and *dizzy* mutants display normal dorsal vessel migration. Confocal images of the dorsal vessel of fixed (a) wild type, (b) *dizzyΔ1* and (c) *inflated* embryos stained with antibodies against (a, c) β-Gal or (b) CD2 to visualize hemocytes (green, white) and Cadherin (red) to visualize the surrounding tissue. (d) Quantitation of the % of embryos with at least one hemocyte in the central four segments of the dorsal vessel in the genotypes indicated.
**Supplementary Movie Legends**

**Movie S1** Lateral view of hemocytes migrating in a wild type embryo, all cell outlines labeled. Lateral movie of an embryo expressing Cadherin-GFP ubiquitously to mark cell outlines and Moe-GFP in hemocytes, showing movement of hemocytes into tail before germband retraction. Stills shown in Figure S1d.

**Movie S2** Single plane dorsal view of hemocytes invading the tail in a wild type embryo, all cell outlines labeled. Single plane dorsal movie of an embryo as in Movie S1 showing hemocytes moving into the tail, along the junction of the hindgut. Stills shown in Figure 1d.

**Movie S3** Multiplane dorsal movie of hemocytes invading the tail with chain migration in a wild type embryo, all cell outlines labeled. Multiplane dorsal movie of an embryo as in Movie S1 showing hemocytes engaging in chain migration as they move around the hindgut. Stills shown in Figure 1e.

**Movie S4** Lateral view of hemocytes migrating into the tail in a wild type embryo, hemocytes labeled. Lateral movie of the tail of a wild type embryo expressing Moe-GFP in hemocytes; hemocytes move into the tail. Stills shown in Figure 3c.

**Movie S5** Lateral view of hemocytes failing to migrate into the tail in a rhoLXA12 embryo, hemocytes labeled. Lateral movie of the tail of a rhoLXA12 embryo expressing Moe-GFP in hemocytes showing hemocytes moving up to the junction of the tail and following it during germband retraction, but failing to enter. Stills shown in Figure 3d.

**Movie S6** Lateral view of hemocytes migrating along the ventral nerve cord in a wild type embryo, hemocytes labeled. Lateral movie of a wild type embryo expressing Moe-GFP in hemocytes showing hemocytes moving towards the posterior along the anterior most section of the ventral nerve cord. Stills shown in Supplementary Information, Figure S3a.

**Movie S7** Lateral view of hemocytes migrating along the ventral nerve cord in a rhoLXA12 embryo, hemocytes labeled. Lateral movie of a rhoLXA12 embryo expressing Moe-GFP in hemocytes; hemocytes move normally towards the posterior along the anterior most section of the ventral nerve cord. Stills shown in Supplementary Information, Figure S3b.