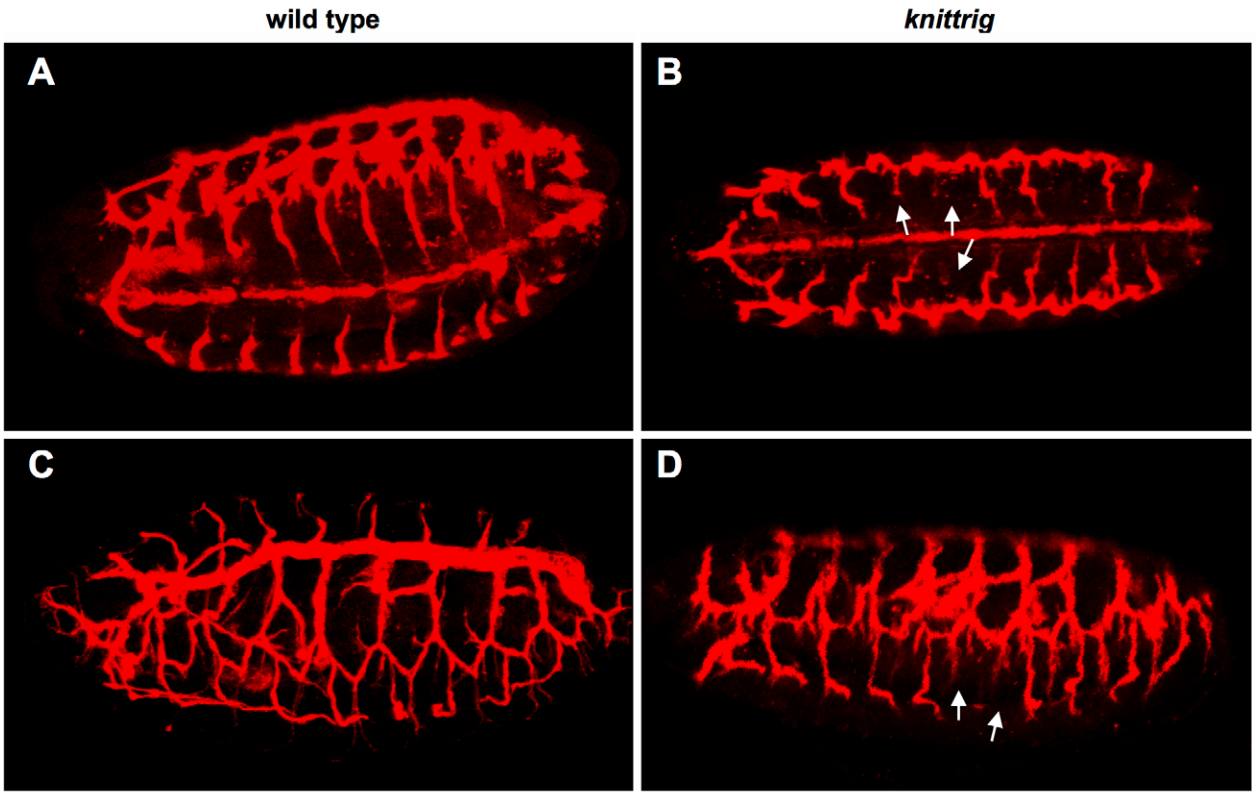
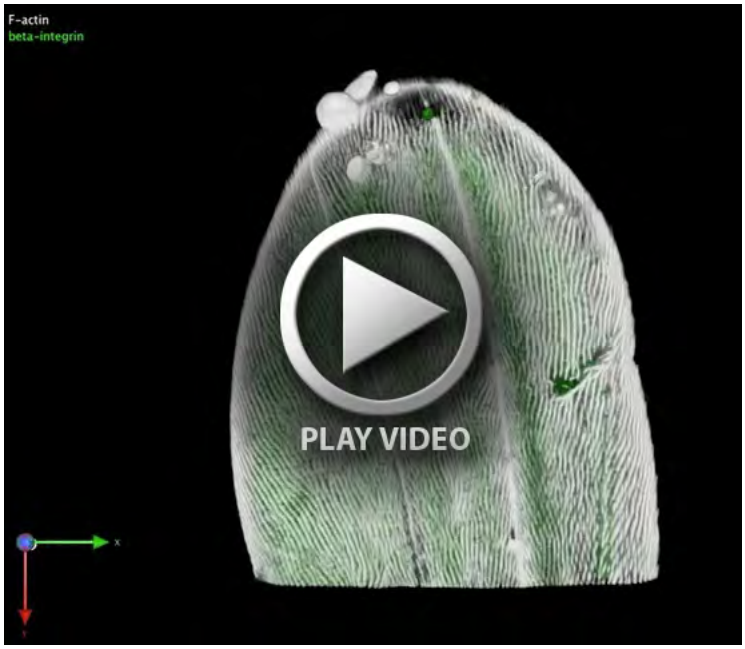
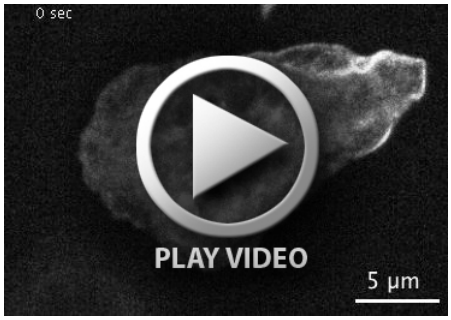


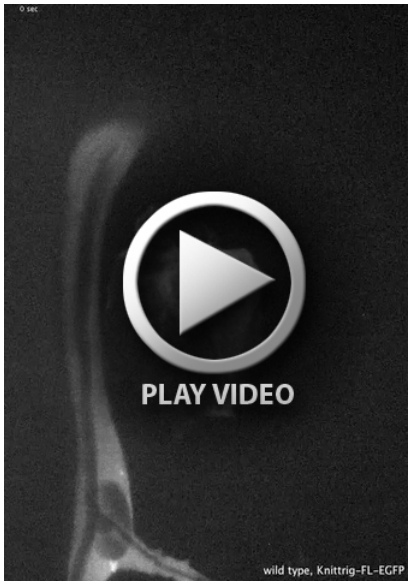
Fig. S1. *knittrig* function is required in trachea cells. Defects in the embryonic tracheal-system in *knittrig* mutant embryos are shown. To visualize the tracheae tau-lacZ transgene was driven under control of the *btl*-gal4 in mutant embryos. In the mutant embryos one or more ganglionic branches are not formed properly (arrows in B, D) in comparison to wild type embryos (A, C).



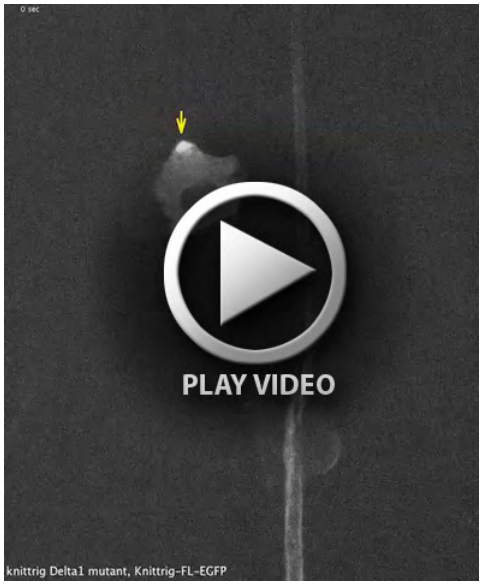
Movie 1. 3D reconstruction of a *knittrig* mutant wing of a late pupa (36h APF), reconstructed from confocal images, co-stained for F-actin (phalloidin, white) and β-integrin (green). No morphological defects are visible.



Movie 2. Spinning disc microscopy video of an isolated pupal macrophage expressing EGFP-tagged full-length Knittrig driven by the *hml*Δ-gal4 driver, immediately recorded after plating.



Movie 3. Random migration of a macrophage expressing EGFP-Knittrig-FL imaged from a wild type pupa (4 APF). The cell migrates along a trachea.



Movie 4. (a,b) Two examples of macrophages expressing EGFP-Knittrig-FL imaged from a *knittrig*$^{\Delta 1}$ mutant pupa (4 APF). The positions of the dynamic Knittrig localization is marked by yellow arrows.

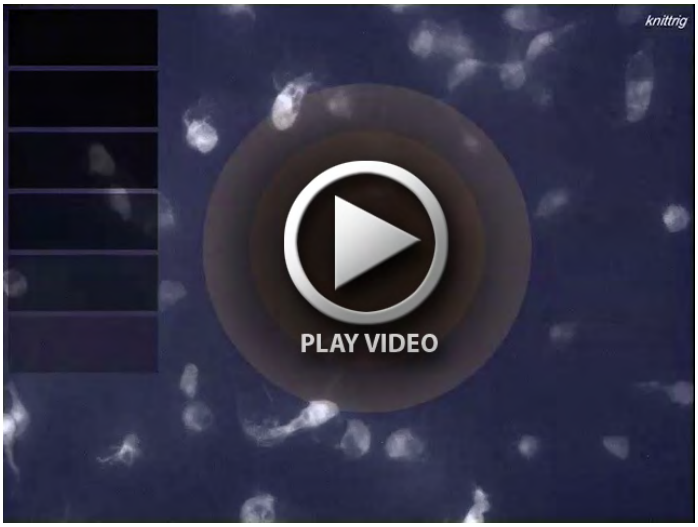
Movie 5. Directed cell migration of wild type macrophages expressing cytoplasmic EGFP imaged from a wing of a wild type pupa (20 APF) upon laser induced ablation of a single cell (position is marked by yellow circle).

Movie 6. Directed cell migration of *knittrig*$^{\Delta 1}$ mutant macrophages imaged from a pupal wing (20 APF) upon laser induced ablation of a single cell.

Movie 7. Directed cell migration of rescued *knittrig*$^{\Delta 1}$ mutant macrophages imaged from a pupal wing (20 APF) upon laser induced ablation of a single cell.

Movie 8.

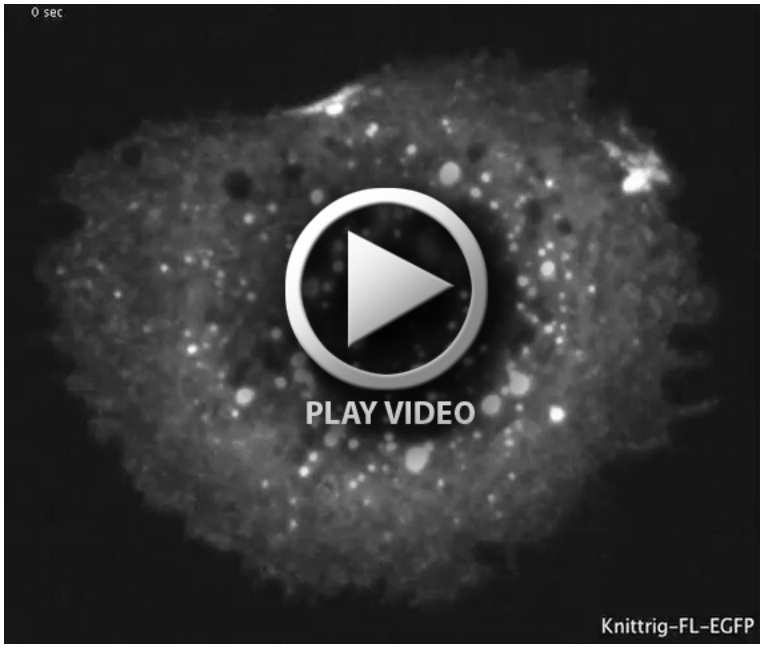
Movie 9.



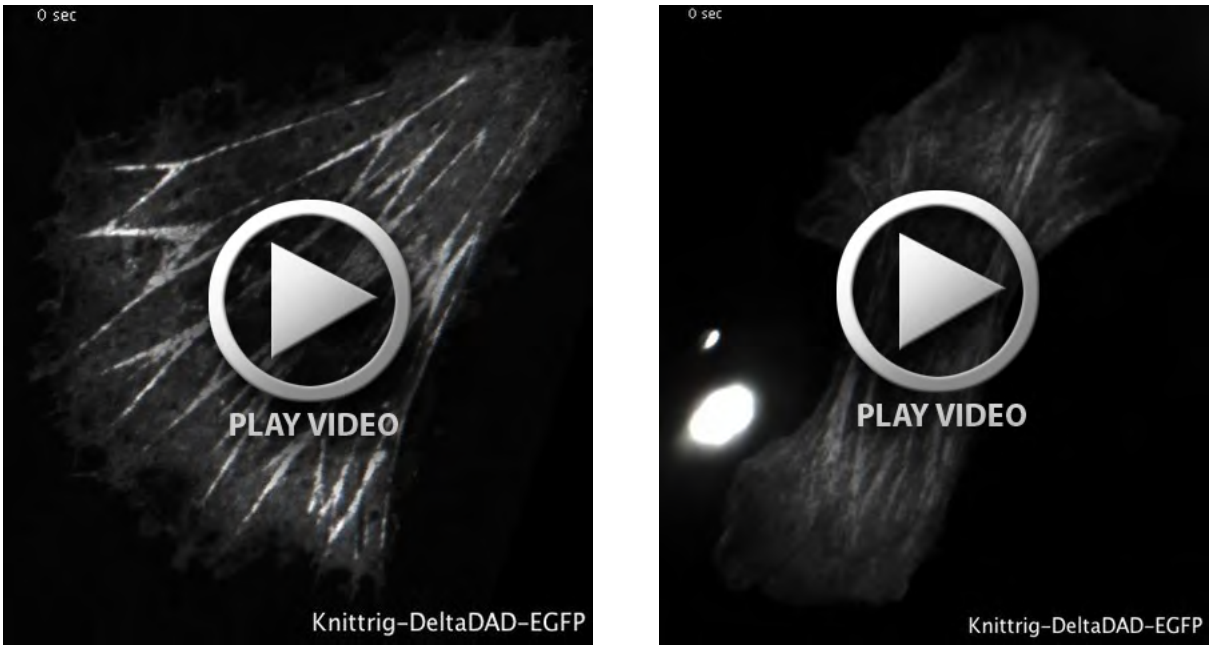
Movie 10.

Movie 11.

Movies 8-11. Exemplary movies are given for wild type (M8), *knittrig* RNAi (M9), *knittrig*$^{\Delta 1}$ (M10) and rescued *knittrig*$^{\Delta 1}$ macrophages (M11). Calculated macrophage occurrences $\omega(R_i, t)$ are given for region R_0, R_1, R_2, R_3 and R_4 in red, dark orange, bright orange, yellow and blue respectively. The purple plot illustrates the $HMMS(t)$. Note that the y-axes scaling is adjusted during calculation. The values in the plots for regions R_0-R_4 increase the more macrophages are located in these regions. The $HMMS(t)$ increases with dense macrophage concentrations in R_0-R_3 and decreases with dense concentrations in R_4.

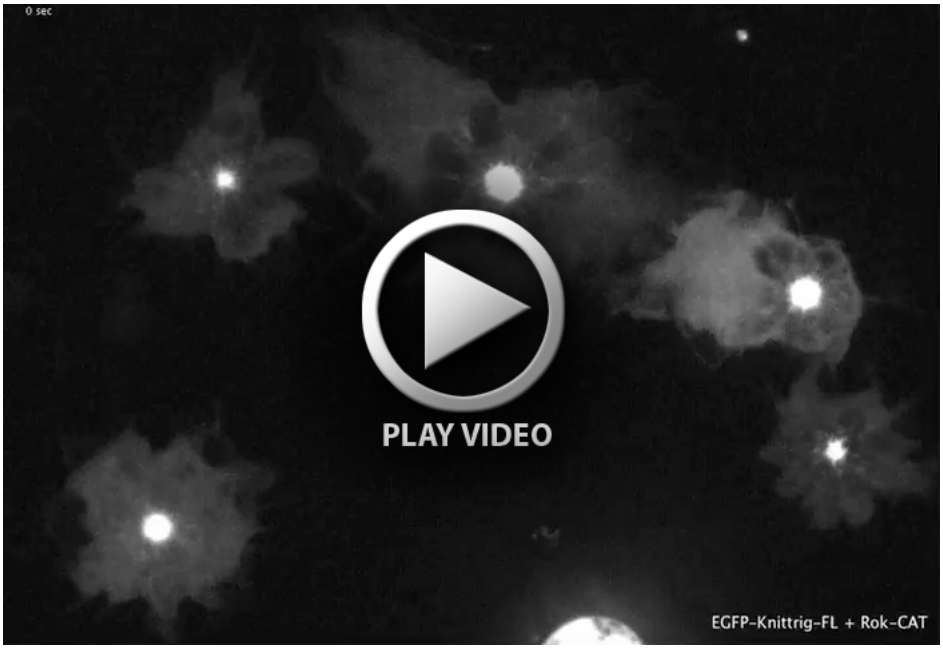


Movie 12. Video of a S2R+ cells expressing EGFP-Knittrig-FL, plated on glass.



Movie 13. (a,b) Two videos of S2R+ cells expressing EGFP-Knittrig-ΔDAD, plated on glass.

Movie 14. Video of a S2R+ cell expressing EGFP-Knittrig-ΔDAD, bleached at indicated regions at the tip and in the center of Knittrig marked stress fibers.



Movie 15. Video of S2R+ cells expressing EGFP-Knittrig-FL (white) together with activated Rho kinase Rok-CAT.

Movie 16. Video of S2R+ cells expressing EGFP-Knittrig-FL (white) together with activated Rho kinase Rok-CAT and Tubulin-Cherry (red).

Table S1. Primers

Primer name	Sequence	Destination vector
5-Knittrig-PA-FL-TOPO	CACCATGATTGTGAAAATGGAGCCGG	pENTR/pUASt-attB-rfa-EGFP
3-Knittrig-PA-FL-TOPO	CTAATAGGTTTGTATCAAGGCAGCC	pENTR/pUASt-attB-rfa-EGFP
3-Knittrig-PA-ΔDAD-TOPO	CTAGTCGCCATCGGTGAACTGC	pENTR/pUASt-attB-rfa-EGFP
3-Knittrig-PA-ΔB-TOPO	CTACGGCGTTGTCCGTGTTCCTG	pENTR/pUASt-attB-rfa-EGFP
5-Knittrig-PA-FH2-TOPO	CACCATGAGCTTGGCACCACCACCCATG	pENTR/pUASt-attB-rfa-EGFP
3-Knittrig-PA-FH2-TOPO	CTAGAACTGCTCCTCGCTTTGGC	pENTR/pUASt-attB-rfa-EGFP