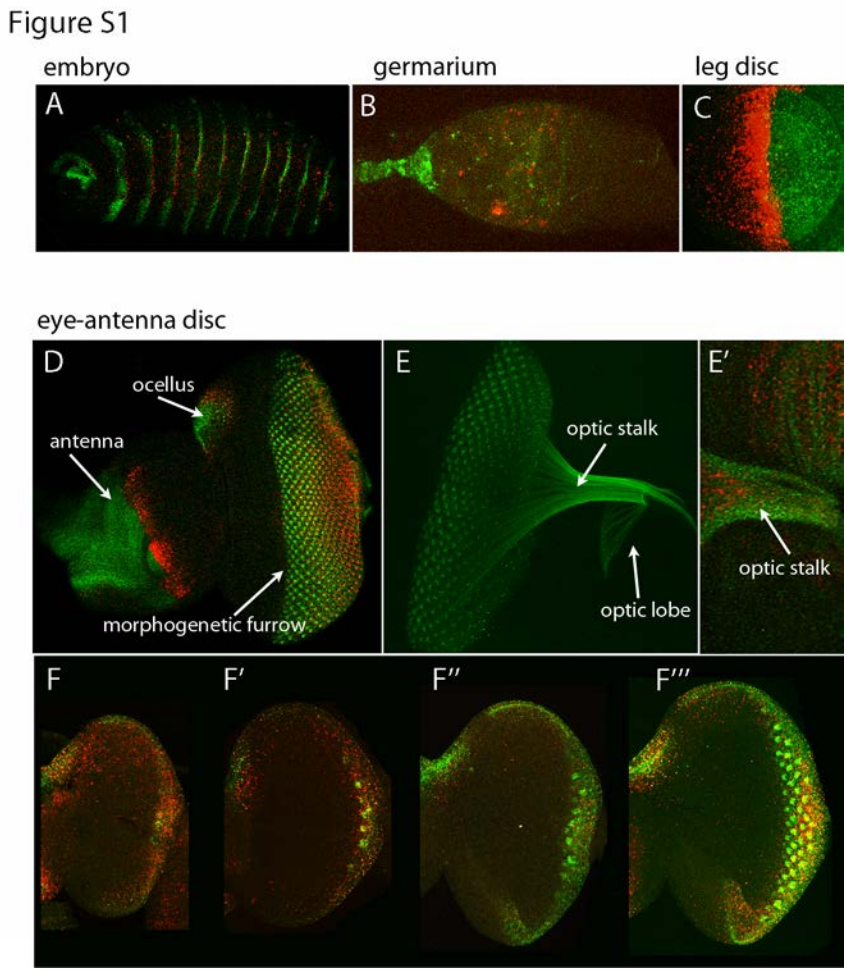


Figure S1. Distributions of Hh:GFP and Ptc:mCherry

Hh:GFP and Ptc:mCherry fluorescence in preparations of a late stage embryo (A), germarium (B), L3 leg disc (C), L3 eye-antennal disc (D-F"'). (F-F"') Successively later stages of early L3 eye disc showing increase in number of ommatidial clusters. Scale bars: (A) 100 μm; (B) 10 μm

Figure S2

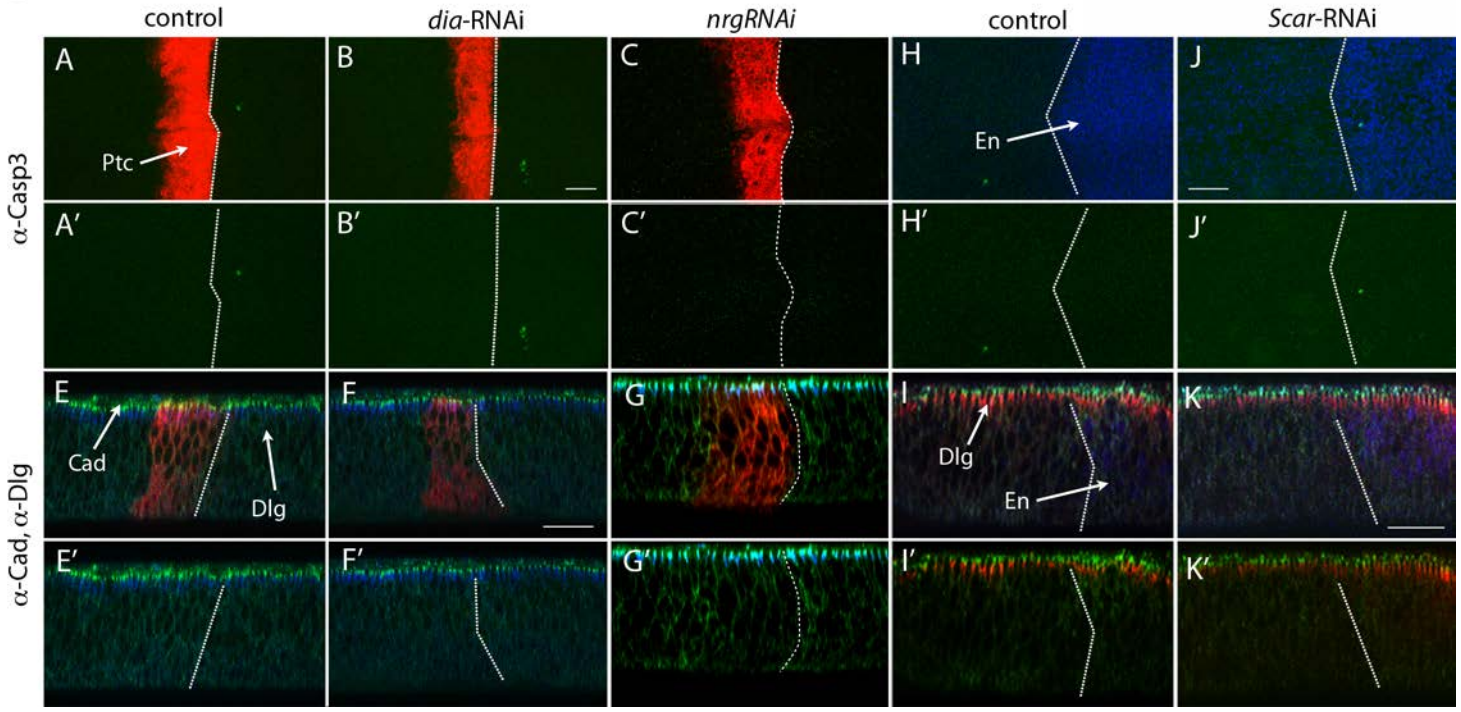


Figure S2. Neither cell death nor apicobasal polarity affected by expression of *dia*RNAi, *Scar*RNAi and *nrg*RNAi

(A,C,E,G,I) Frontal images of the pouch region of late L3 wing discs with dying cells marked with α-cleaved Caspase 3 staining (green); *ptc* domain (red); posterior compartment marked with α-Engrailed (blue). (B,D,F,H-J') Sagittal images of the pouch region of late L3 discs marked with α-Cad2 (green); α-Discs large (blue in B,D,F; red in H,J); *ptc* domain (red in B,D,F); posterior compartment marked with α-Engrailed (blue in H,J). Genotypes: (A-D) *ptc*-Gal4 *tub*-Gal80^{ts} *UAS*-CD8:mCherry *UAS-dia*RNAi; (E,F) *ptc*-Gal4 *tub*-Gal80^{ts} *UAS*-CD8:GFP *UAS-nrg*RNAi; (G-J) *ptc*-Gal4 *tub*-Gal80^{ts}*UAS-Scar*RNAi. (A,B,G,H) incubated at 18°C; (C-F,I,J) incubated at 18°C for approximately eight days followed by incubation at 29°C for 24-36 hrs prior to dissection. Dotted lines mark the A/P boundary. Scale bars: 20 μm.

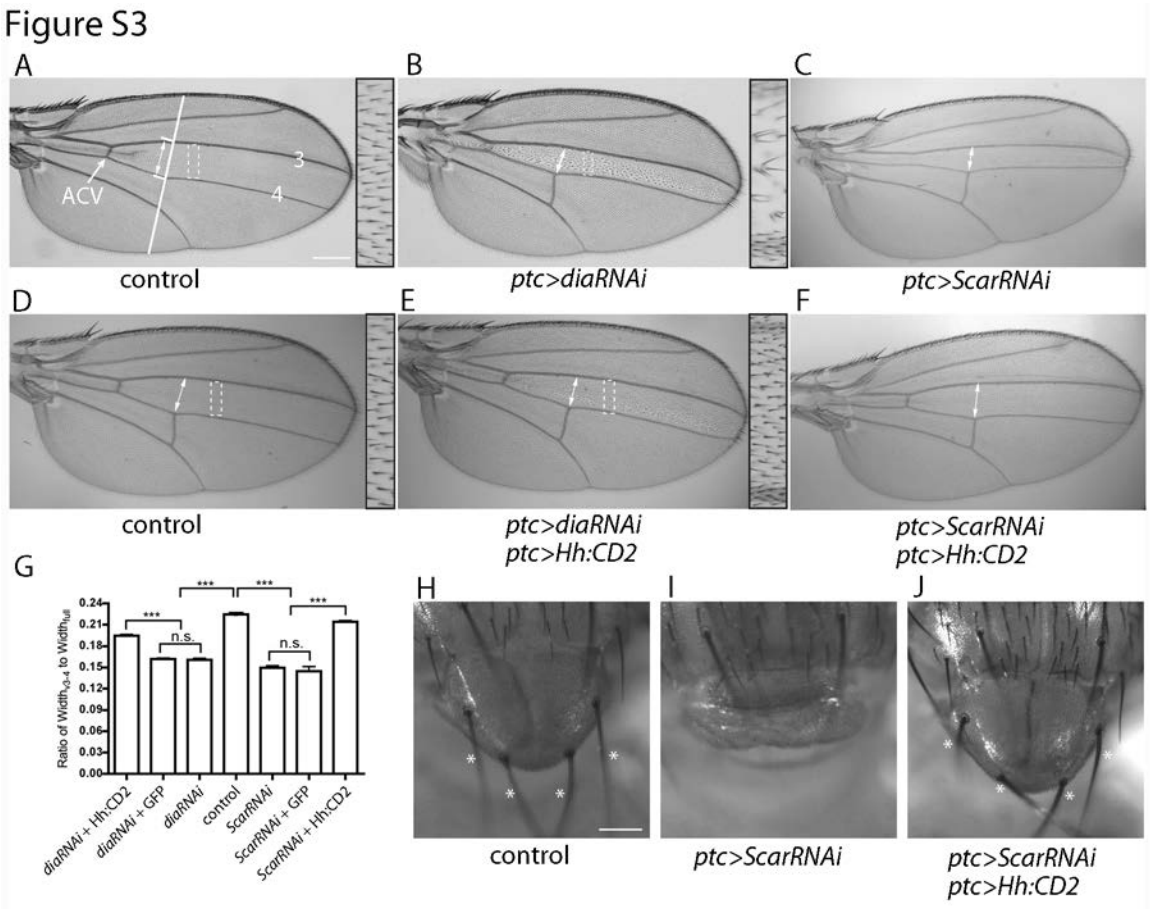
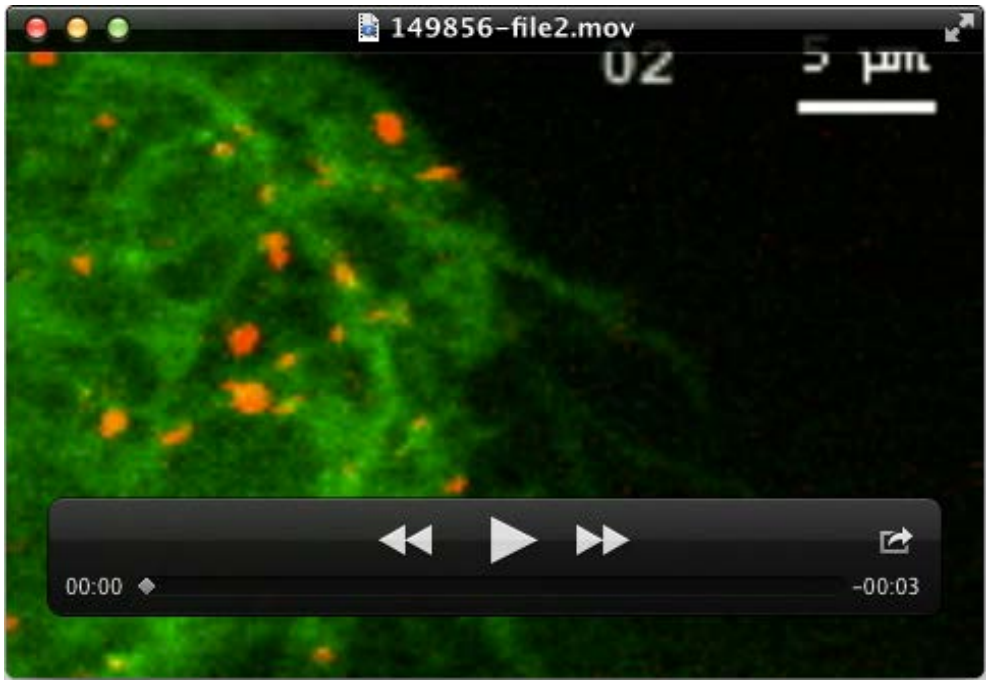


Figure S3. Wing blade and scutellum morphology of *dia*- and *Scar*-depleted flies

(A-F) Images of wings show that depletion of Dia and Scar in the *ptc* domain reduces Hh signaling. The intervein region between veins 3 and 4 (marked by double sided arrows) was reduced and the anterior cross vein (ACV) was absent in wings depleted for Dia (*ptc*-Gal4 *UAS*-Gal80^{ts} *UAS-dia*RNAi (B) or Scar (*ptc*-Gal4 *UAS*-Gal80^{ts} *UAS-Scar*RNAi (C) compared to control (A). The density of hairs in Dia-depleted wings was less that control (see high magnification insets of region boxed by dashed white line). Expression of CD2:Hh did not affect control wings (D) (*ptc*-Gal4 *UAS*-Gal80^{ts} *UAS*-Hh:CD2), but restored the 3-4 intervein region to normal size in Dia- and Scar-depleted wings (*ptc*-Gal4 *UAS*-Gal80^{ts} *UAS-dia*RNAi *UAS*-Hh:CD2 (E); *ptc*-Gal4 *UAS*-Gal80^{ts} *UAS-Scar*RNAi *UAS*-Hh:CD2 (F). Hair density in *dia*-depleted wings was also restored (E). (G) Bar graph showing normalized size of 3-4 intervein region in control and mutant wings. n=11 for all genotypes except n=10 for (D). The symbol (***) denotes P<0.001, two-tailed t-test; Bar value shown as mean ± s.d. (H-I) Depletion of *Scar* reduces the scutellum (I), but expression of Hh:CD2 (J) restores both scutellum morphology and the four scutellar macrochaetes (*) to normal (H). Genotypes are as in (A, C, F). Incubation was at 18°C for 8 days after egg laying, 29°C for 36 hrs, and 25°C until eclosion. Scale bars: (A) 200 μm; (H) 100 μm.

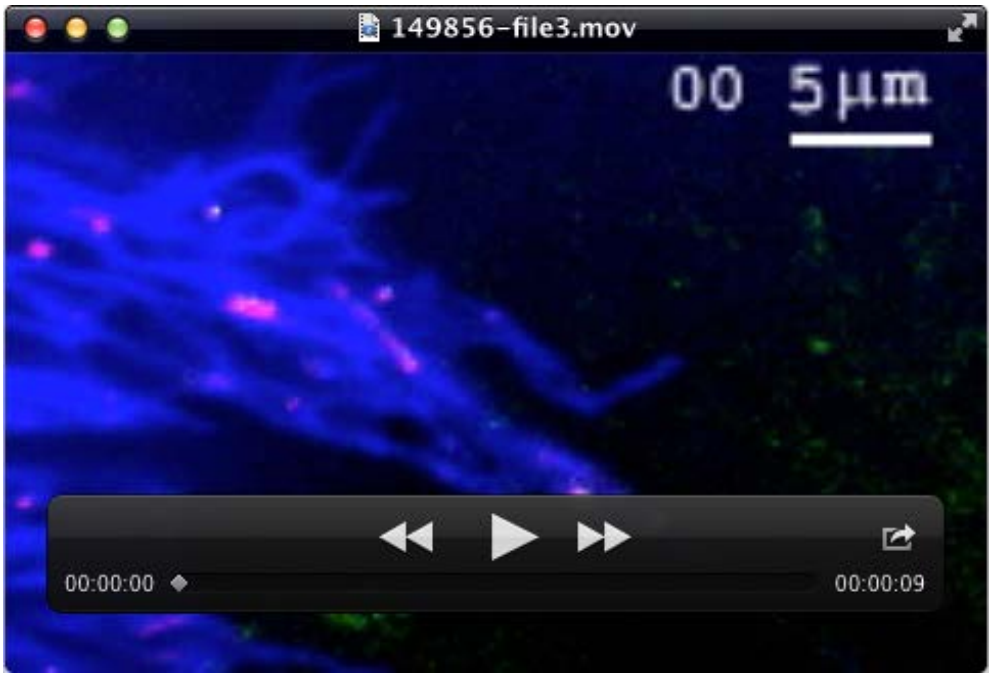
Movie 1. Cytoneme extension and retraction

Cytonemes from A compartment cells marked with membrane-tethered Cherry (*ptc*-Gal4 *UAS*-CD8:Cherry) orient towards the P compartment in the hinge region of this late 3rd instar wing disc. Their dynamic extension and retraction is recorded in this movie, which was taken at 12 second intervals in a 10 min period and is shown at 10 frames/second.



Movie 2. Ptc moves along cytonemes

BAC-encoded Ptc:mCherry marks motile puncta that travel in anterograde and retrograde directions along cytonemes that extend from A compartment cells marked with CD8:GFP (*ptc*-Gal4 *UAS*-CD8:GFP). The video was taken at the hinge region of a late 3rd instar wing disc; images were acquired every 15 seconds during an 8 min period and are shown at 10 frames/second.



Movie 3. Hh and Ptc colocalize in cytoneme-associated motile puncta

Puncta containing BAC-encoded Hh:GFP and BAC-encoded Ptc-mCherry move in cytonemes marked with CD4:IFP2.0-HO1 (*ptc*-Gal4 *UAS*-CD4:IFP2.0-HO1; blue) that extend from A compartment cells in the hinge region of a late 3rd instar wing disc. Movement is in a net retrograde direction towards the A compartment. Pictures were taken every 4 seconds during a 6.5 min period and are shown at 10 frames/second.