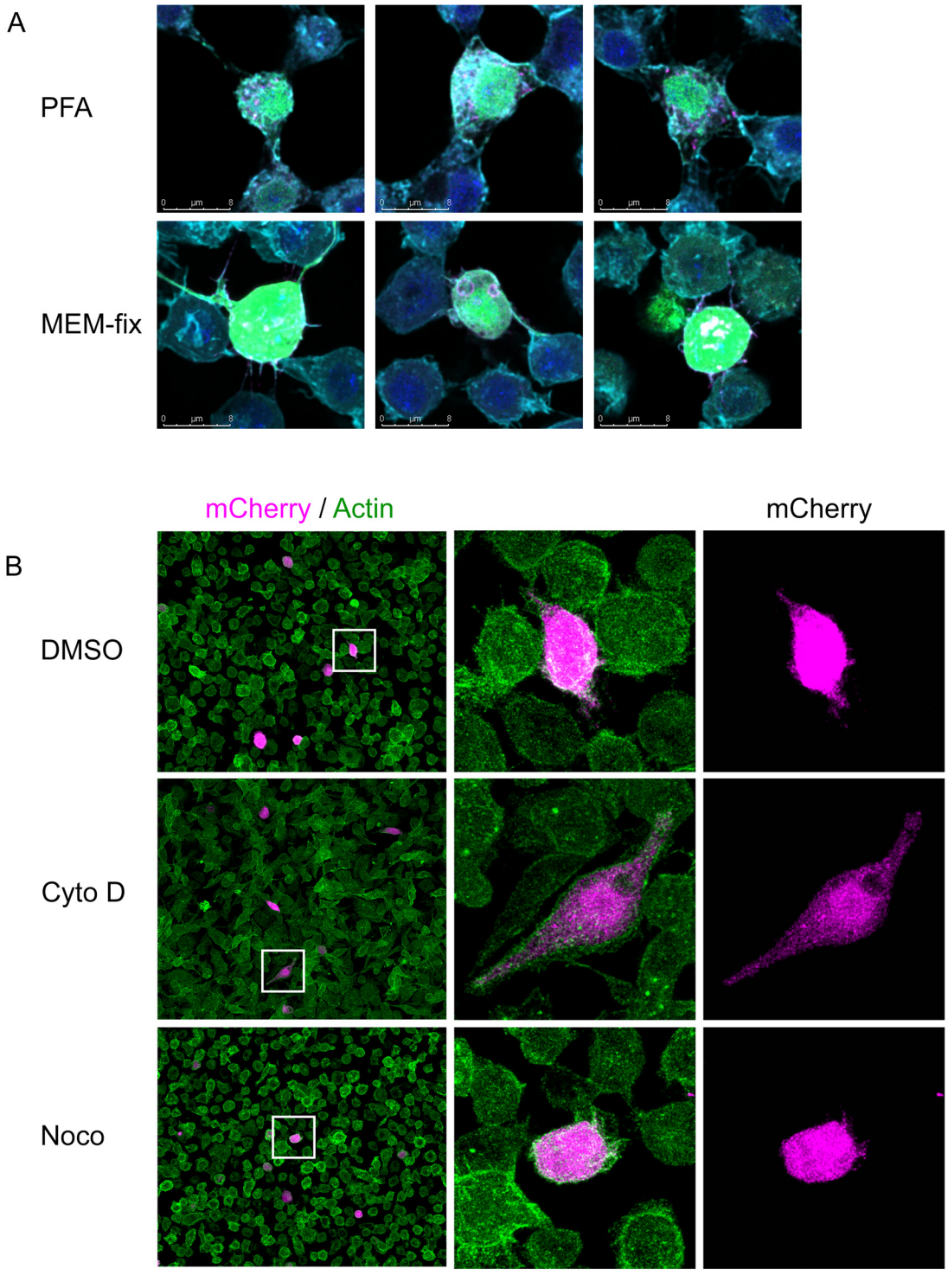
Supplementary Information

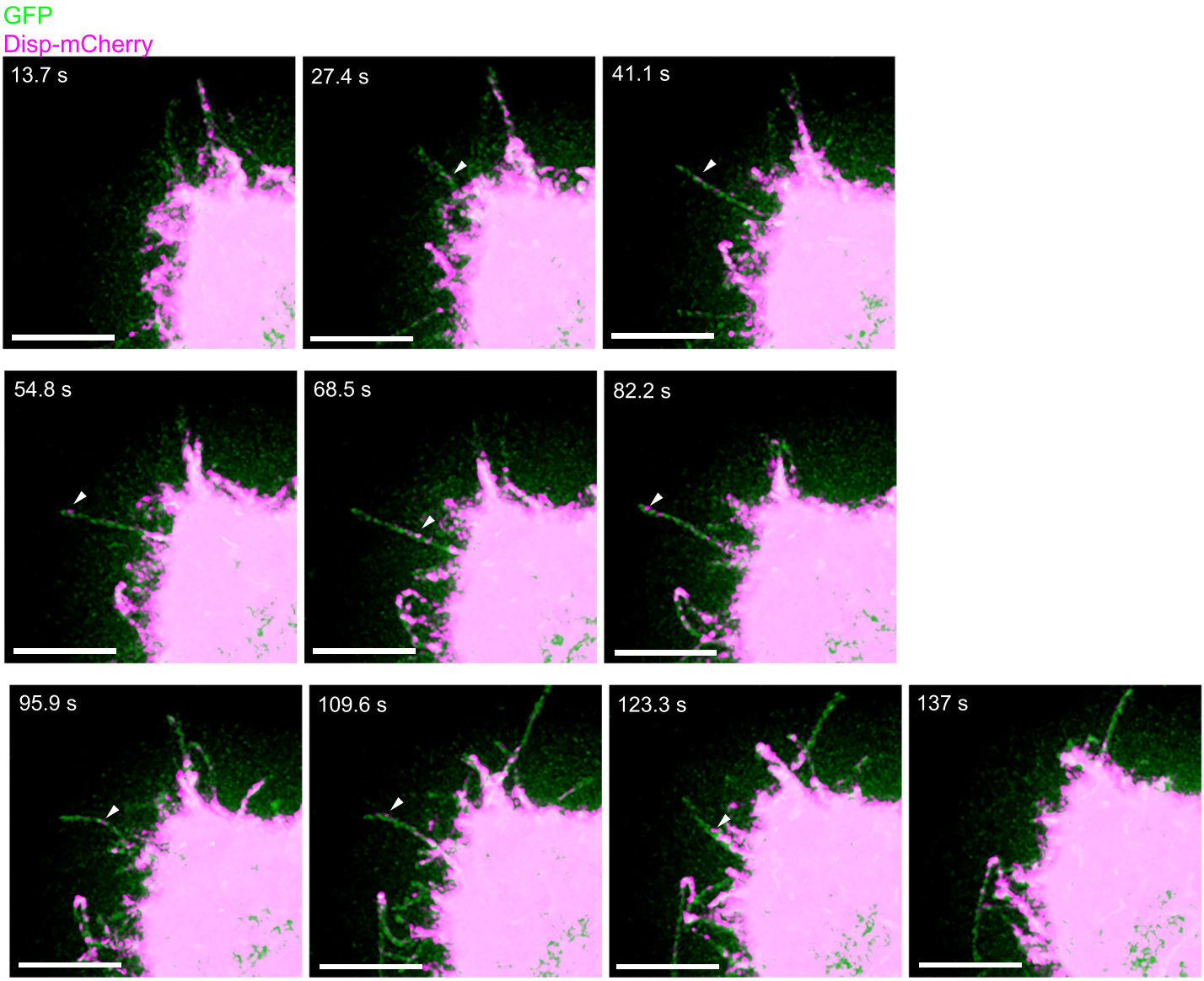
Supplementary Figures

Supplementary Figure 1



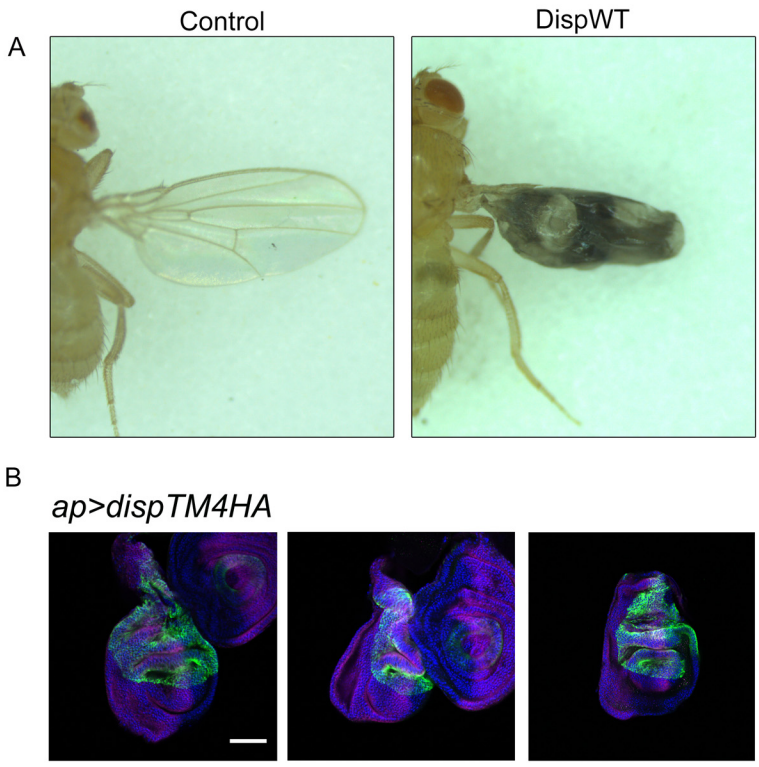
Supplementary Figure 1. A. S2 cells expressing GFP (green) and CD8-mCherry (magenta) were fixed with standard 4% PFA (top) or with MEM-fix (bottom). Actin (cyan) is stained with phalloidin. **B.** S2 cells expressing mCherry were treated with 10 μm Cytochalasin D, 10 μM Nocodazole or DMSO vehicle control as in Fig. 1D-F. Actin (green) was stained with phalloidin. Wide field and zoom images are shown.

Supplemenatry Figure 2



Supplementary Figure 2. Still images from Movie 3 show tracking of a Disp-mCherry puncta along a cytoneme (arrowhead). The time stamp for each still is indicated. Disp-mCherry is magenta and GFP is green. Scale bar indicates 5 μm.

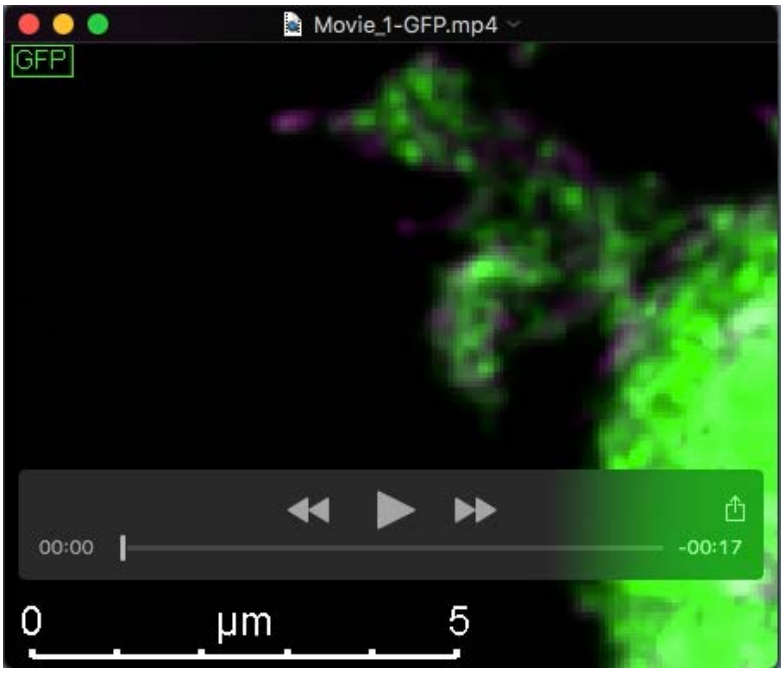
Supplementary Figure 3



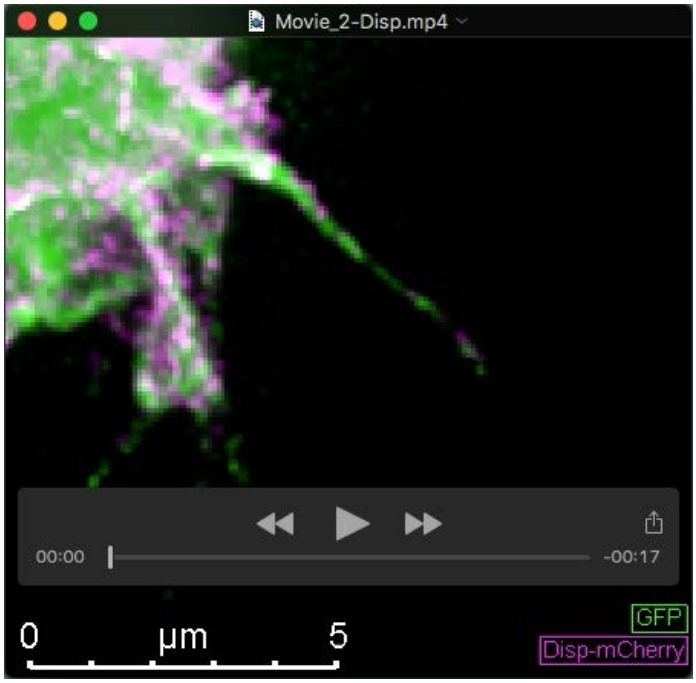
Supplementary Figure 3. A. Unmounted wings of control and *ap>dispHA* flies are shown to highlight the extent of wing blistering induced by DispHA overexpression. **B.** Wing imaginal discs from surviving *ap>dispTM4HA* 3rd instar larva are shown to highlight the severity of disc malformation resulting from DispTM4HA over-expression (green). Ci is shown in magenta and DAPI is blue. Scare bar indicates 50 μm.

S2 Cytoneme Live Imaging Methods

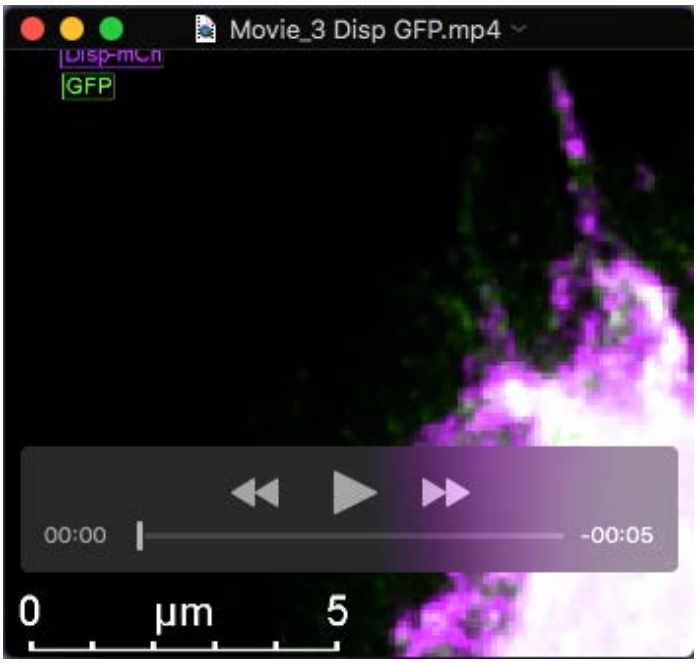
For live imaging, approximately 4 x 10^6 cells were transfected with *pAc-GFP, pAc-disp-mCherry*, or *pAc-CD8-mCherry* as described in the manuscript. After ~48 hours, cells were washed twice with serum-free S2 media, resuspended in fresh S2 media supplemented with 10% FBS, and then plated into glass bottom dishes (MatTek, P35G-0.170-14-C). Cells were allowed to settle for 1.5 hours before imaging using Leica TCS SP8 resonance scanner mode. Entire z-stacks were taken at ~12 second intervals using the 100X objective. Images were processed as detailed in the manuscript before constructing the maximum intensity projection. Movies 1 and 2 were encoded to match timeframes between GFP co-expressed with CD8-mCherry or Disp-mCherry captures. Movie 3 was captured using a Leica SP8 with resonance scanner using the 100X objective. Z-stacks were taken every ~13 seconds and the movie was encoded at 2 frames per second of video. Growth and retraction rates were calculated by measuring distance traveled by a cytoneme divided by time taken to reach lag phase. Total duration included projection and full retraction including lag phases between growth and retraction events.



Movie 1. Live imaging of cytonemes of a GFP and CD8-mCherry positive cell. Images of the full axial plane of the cell were taken at 11.245 second intervals**.** The video was encoded with a 2.4 frame/second rate. GFP (green) and CD8-mCherry (magenta) are located within extensions which originate from regions of the cell not in contact with the slide.



Movie 2. Live imaging of cytonemes of a GFP and Disp-mCherry positive cell. Images of the full axial plane of the cell were taken at 13.545 second intervals. The video was encoded with a 2 frame/second rate. In the time lapse, GFP (green) and Disp-mCherry (magenta) cytonemes exhibit slower retraction rates compared to control cells.



Movie 3. Live imaging of Disp-mCherry puncta in cytonemes. Images of the full axial plane of the cell were taken at 13 second intervals. The video was encoded with a 2 frame/second rate. Disp-mCherry (magenta) puncta travel along cytonemes. Cytoplasmic GFP is green.