Zanet et al., http://www.jcb.org/cgi/content/full/jcb.201110135/DC1

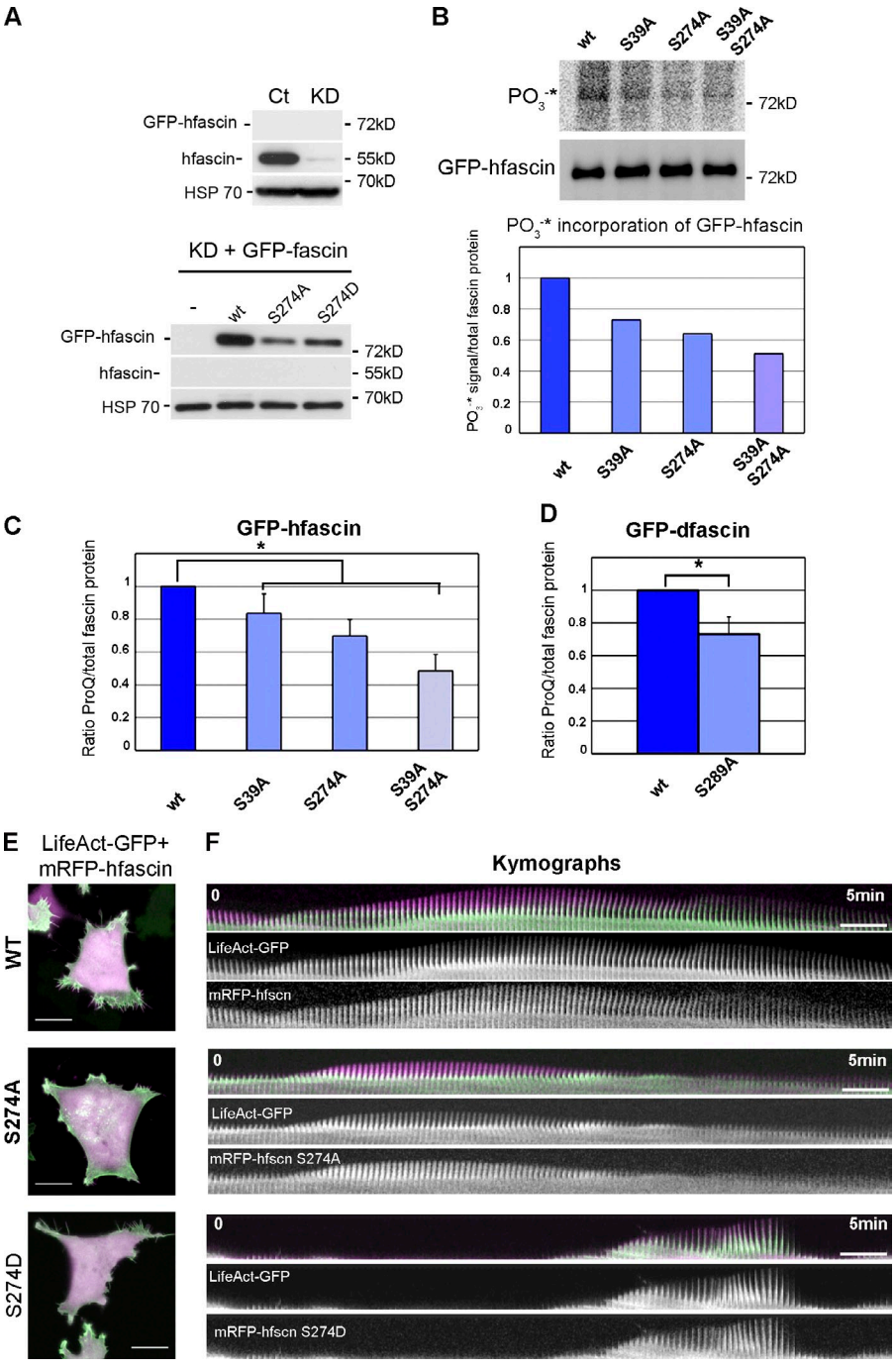


Figure S1. **Dynamics of filopodia are regulated by residue S274 in hfascin.** (A) Stable knockdown (KD) of endogenous fascin and hfascin transgene overexpression in MDA-MB-231 cells shown by Western blotting. Endogenous fascin was significantly reduced in knockdown cells compared with control (Ct), and GFP-fascin transgenes resistant to the shRNA could be expressed in fascin knockdown cells. Both endogenous and GFP-fascin were detected with an antifascin antibody. (B) MDA-MB-231 cells were transfected with hfascin transgenes, and phosphorylation was examined by metabolic incorporation of radioactive orthophosphate (PO_3^{-*}). Blots and bar graph reveal the levels of phosphate incorporation versus total protein. Because of the limited institutional allowance of radioactivity, this experiment was completed once. (C) Different mutant forms of hfascin were immunoprecipitated, and their phosphorylation levels were assessed by Pro-Q Diamond staining. Bar graph showing the ratio of the Pro-Q signal to total fascin protein. Note that both S39 and S274 mutants show equivalent reductions. $n = 3$. (D) Wild-type (wt) *Drosophila* fascin and S289A were immunoprecipitated from embryos, and their phosphorylation levels were assessed by Pro-Q Diamond staining. Bar graph reveals the ratio of the Pro-Q signal to total fascin protein. $n = 6$. (E) LifeAct-GFP (green) and hfascin transgenes (purple) were expressed in MDA-MB-231 carcinoma cells depleted of endogenous fascin, and filopodia were imaged by time-lapse analysis. Bars, 10 µm. (F) Kymograph analysis of individual filopodia revealed that the S274A mutation prevented significant elongation of the filopodia and shortened its lifetime. In contrast, fascin S274D strongly reduced the stability of filopodia without significantly decreasing their length. Error bars show SEM. *, $P < 0.05$. Bars, 5 µm.

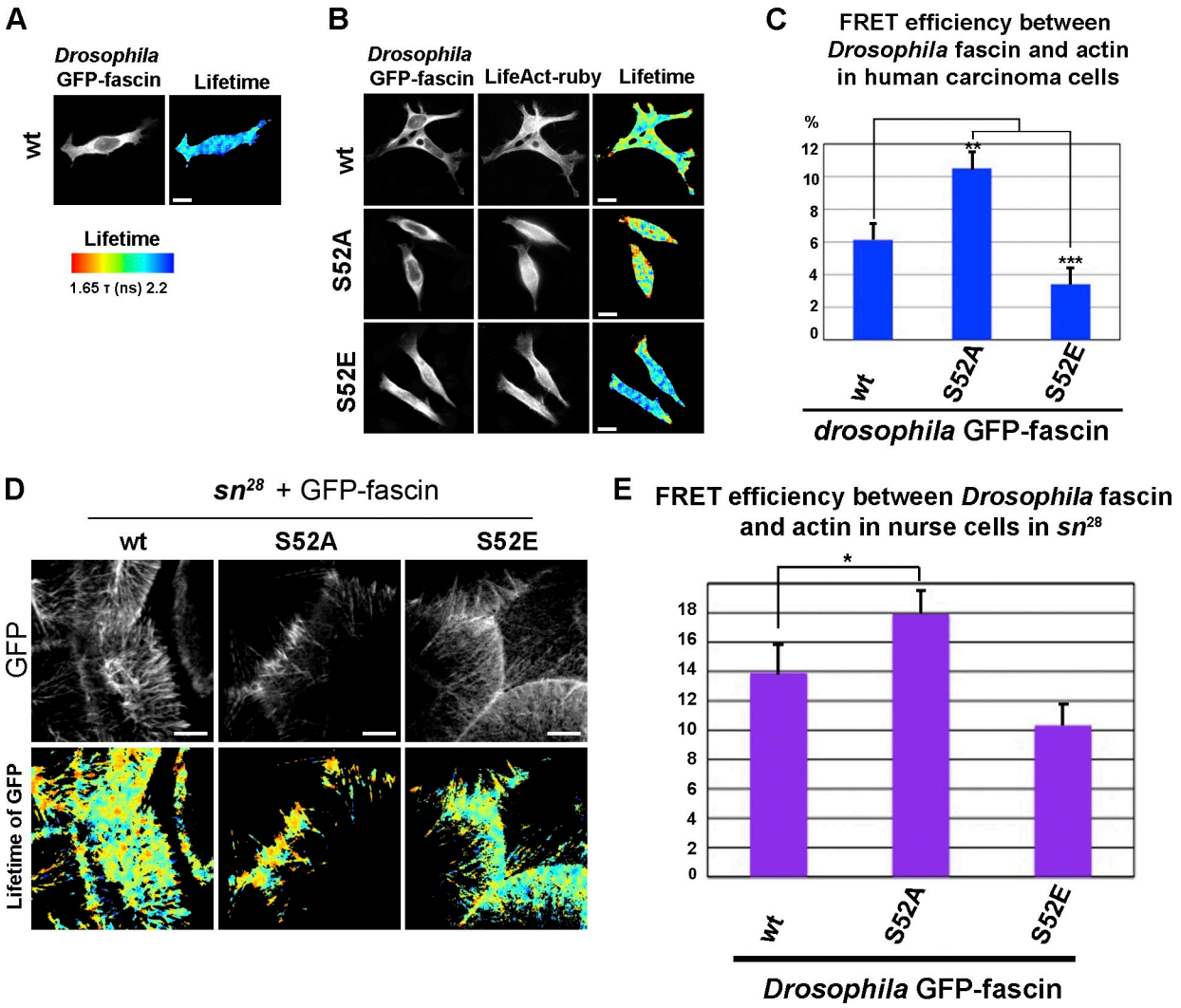
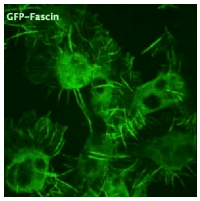
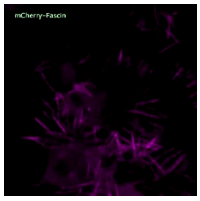


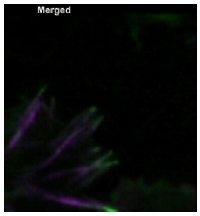
Figure S2. **FRET/FLIM analysis of *Drosophila* fascin and actin in nurse cells.** (A) Control experiment of GFP-fascin (donor) expressed in the absence of the LifeAct-Ruby (acceptor). Lifetime of the GFP is represented by a color code from no interaction (blue) to high interaction (red). (B) FRET/FLIM analysis of *Drosophila* GFP-fascin S52A/E and F-actin in SW480 cells. (C) Quantification of the FRET efficiency between *Drosophila* GFP-fascin with actin revealed that the S52A mutation enhanced and S52E decreased the interaction. $n = 6$; **, $P < 0.01$; ***, $P < 0.001$. (D) Image of the FRET/FLIM analysis of GFP-fascin S52A/E with actin in nurse cells in sn^{28} mutant flies. (E) Quantification of the FRET efficiency between *Drosophila* GFP-fascin S52A/E and F-actin. Note that the S52A mutation increased and S52E decreased actin-binding activity. $n = 6$; *, $P < 0.05$. wt, wild type. Error bars show means ± SD. Bars: (A and B) 10 µm; (D) 5 µm.



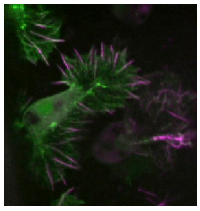
Video 1. **Expression of wild-type GFP-fascin and GFP-fascin S289A/D in macrophages in *sn*^{28} mutant embryos.** Images were analyzed by time-lapse confocal microscopy using a spinning-disk microscope (UltraVIEW VoX). Each frame was acquired every 10 s. Video is displayed at five frames/s.



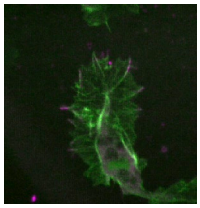
Video 2. **Coexpression of wild-type mCherry-fascin and GFP-fascinS289A in macrophages in *sn*^{28} mutant embryos.** mCherry-fascin (magenta); GFP-fascinS289A (green). Images were analyzed by time-lapse confocal microscopy using a spinning-disk microscope (UltraVIEW VoX). Each frame was acquired every 10 s. Video is displayed at five frames/s.



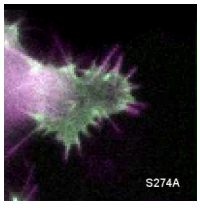
Video 3. **High magnification time-lapse sequence of a macrophage coexpressing wild-type mCherry-fascin and GFP-fascin S289A in *sn*^{28} mutant embryos.** mCherry-fascin (magenta); GFP-fascin S289A (green). Images were analyzed by time-lapse confocal microscopy using a spinning-disk microscope (UltraVIEW VoX). Note that GFP-fascin S289A is not incorporated into lamellae compared with wild-type mCherry-fascin. Each frame was acquired every 10 s. Video is displayed at five frames/s.



Video 4. **Coexpression of wild-type mCherry-fascin and LifeAct-GFP in macrophages in *sn*^{28} mutant embryos.** mCherry-fascin (magenta); LifeAct-GFP (green). Images were analyzed by time-lapse confocal microscopy using a spinning-disk microscope (UltraVIEW VoX). Each frame was acquired every 10 s. Video is displayed at five frames/s.



Video 5. **Coexpression of mCherry-fascin S289A and LifeAct-GFP in macrophages in *sn*^{28} mutant embryos.** mCherry-fascin S289A (magenta); LifeAct-GFP (green). Images were analyzed by time-lapse confocal microscopy using a spinning-disk microscope (UltraVIEW VoX). Each frame was acquired every 10 s. Video is displayed at five frames/s.



Video 6. **Coexpression of LifeAct-GFP and human mRFP-fascin transgenes in MDA-MB-231 cells.** Images were analyzed by time-lapse confocal microscopy using a confocal microscope (A1R). Each frame was taken every 2 s for 5 min. Video is displayed at five frames/s.