Gu et al., http://www.jcb.org/cgi/content/full/jcb.201010075/DC1

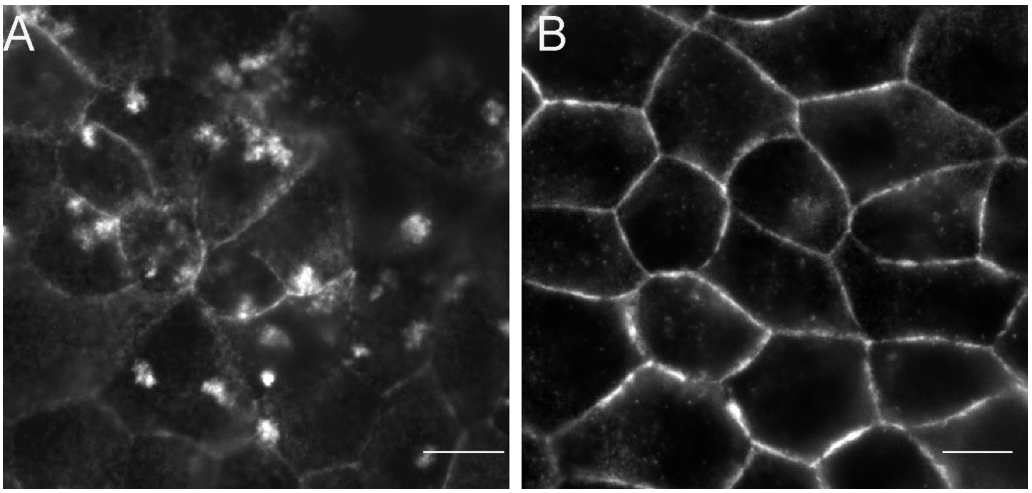


Figure S1. **S1P directly induces actin assembly.** Actin assembly at the apex of an MDCK monolayer to which 20 µg/mlS1P (A) or equal amount of total lipid extraction from *E. coli* (B) have been added.

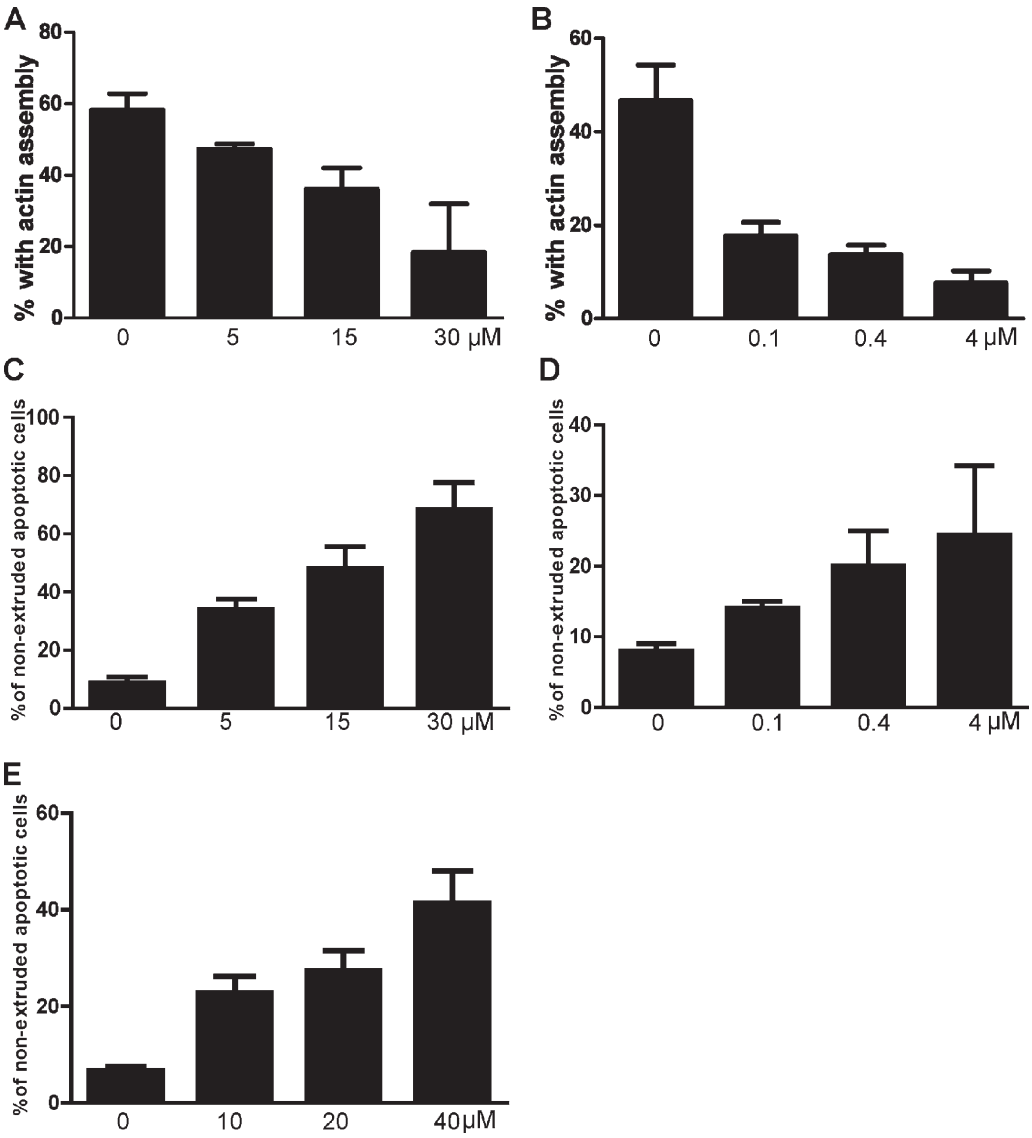


Figure S2. **Inhibition of actin assembly and apoptotic cell extrusion by inhibitors of SphKs is dose dependent.** (A and B) Quantification of actin assembly induced by cell fragments prepared from MDCK cells pretreated with different concentrations of SKI II (A) or SKI V (B). (C–E) Quantification of nonextruded apoptotic cells in increasing concentrations of SKI II (C), SKI V (D), or tDHS (E).

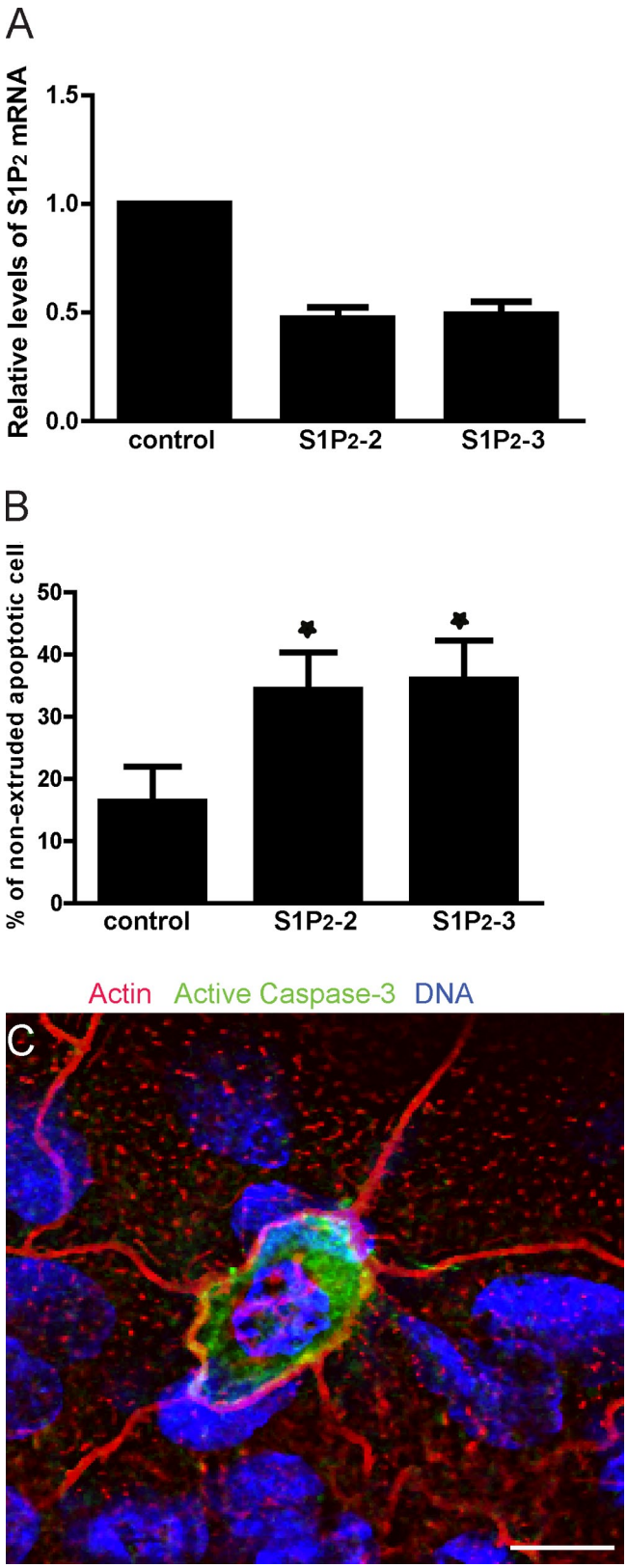
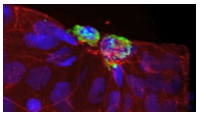
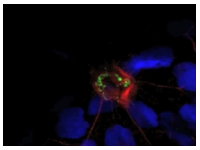


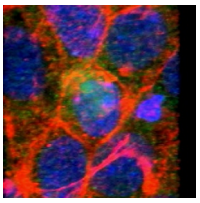
Figure S3. **Apoptotic cell extrusion requires the signaling mediated by $S1P_2$.** (A) qRT-PCR confirms knockdown of $S1P_2$ by $shS1P_2$-2 and $shS1P_2$-3 in HBE cells. (B) Quantification of nonextruded apoptotic cells in HBE monolayers expressing control shRNA or $shS1P_2$-2 or $shS1P_2$-3 shRNA after UV treatment. Asterisk indicates P values <0.05. (C) Projection of a nonextruded apoptotic cell on the epithelium of d 3 WT zebrafish larvae treated with JTE-013. Bar, 10 µm.



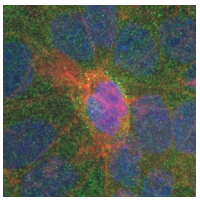
Video 1. **Tilting 3D reconstruction of two apoptotic cells that fail to extrude from the epidermis of a *mil* zebrafish embryo.** 3-d-old *mil* zebrafish larvae were treated with 1% DMSO on ice for 30 min, followed by recovery at 28°C for 10 min to induce apoptosis and stained with anti-active caspase-3 (green), Alexa Fluor 568–phalloidin for actin (red), and DAPI for DNA (blue). Confocal images were obtained using an inverted microscope (Eclipse TE300; Nikon) converted for spinning disc confocal microscopy (Andor) and were converted into Z series using MetaMorph.



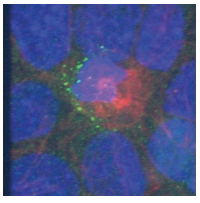
Video 2. **Z planes of an apoptotic cell that extrudes normally from the epidermis of a WT zebrafish embryo and an apoptotic cell fails to extrude from the epidermis of a *mil* zebrafish embryo.** 3-d-old WT and *mil* zebrafish larvae were treated with 1% DMSO on ice for 30 min, followed by recovery at 28°C for 10 min to induce apoptosis and stained with anti-active caspase-3 (green), Alexa Fluor 568–phalloidin for actin (red), and DAPI for DNA (blue). Confocal images were obtained using an inverted microscope (Eclipse TE300; Nikon) converted for spinning disc confocal microscopy (Andor) and were converted into Z series using MetaMorph.



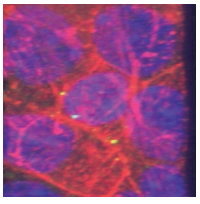
Video 3. **Localization of S1P during early stage of apoptotic cell extrusion.** An HBE monolayer was treated with short-wave UV to induce apoptosis and then stained with anti-S1P (green), Alexa Fluor 568–phalloidin for actin (red), and DRAQ5 (blue). Confocal micrographs were obtained using a microscope (TCS SP5; Leica) and Z series were reconstructed into 3D images using ImageJ and MetaMorph.



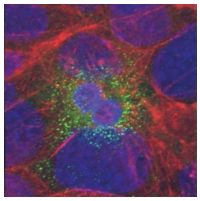
Video 4. **Localization of S1P during middle stage of apoptotic cell extrusion.** An HBE monolayer was treated with short-wave UV to induce apoptosis and then stained with anti-S1P (green), Alexa Fluor 568–phalloidin for actin (red), and DRAQ5 (blue). Confocal micrographs were obtained using a microscope (TCS SP5; Leica) and Z series were reconstructed into 3D images using ImageJ and MetaMorph.



Video 5. **Localization of S1P during late stage of apoptotic cell extrusion.** An HBE monolayer was treated with short-wave UV to induce apoptosis and then stained with anti-S1P (green), Alexa Fluor 568–phalloidin for actin (red), and DRAQ5 (blue). Confocal micrographs were obtained using a microscope (TCS SP5; Leica) and Z series were reconstructed into 3D images using ImageJ and MetaMorph.



Video 6. **SKI II blocks apoptotic cell extrusion by inhibiting S1P production.** An HBE monolayer was treated with short-wave UV to induce apoptosis in the presence of SKI II and then stained with anti-S1P (green), Alexa Fluor 568–phalloidin for actin (red), and DRAQ5 (blue). Confocal images were obtained using a microscope (TCS SP5; Leica) and Z series were reconstructed into 3D images using MetaMorph.



Video 7. **An apoptotic cell generates high levels of S1P, but does not extrude in the presence of the $S1P_2$ antagonist JTE-013.** An HBE monolayer was treated with short-wave UV to induce apoptosis in the presence of JTE-013 and then stained with anti-S1P (green), Alexa Fluor 568–phalloidin for actin (red), and DRAQ5 (blue). Confocal micrographs were obtained using a microscope (TCS SP5; Leica) and Z series were reconstructed into 3D images using ImageJ and MetaMorph.