Supplementary material

Gagnoux-Palacios et al., https://doi.org/10.1083/jcb.201805071

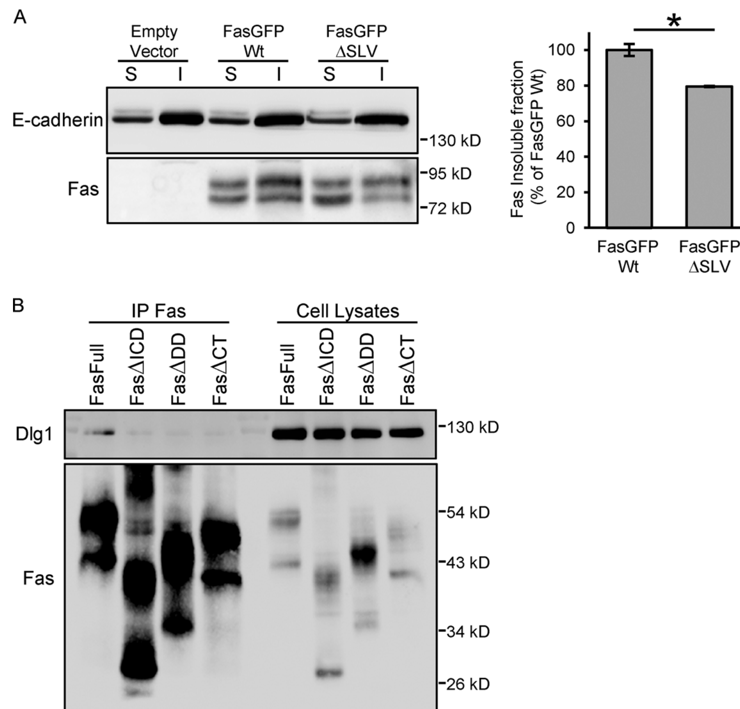


Figure S1. **Role of Fas PDZ binding domain in the regulation of Fas solubility and Dlg1 association. (A)** Detergent solubility of Fas-GFP (WT and ΔSLV mutant) stably expressed in HCT15 cells was analyzed by IB. S, soluble fraction; I, insoluble cytoskeletal fraction. E-cadherin was used as a control for membrane protein. The percentage of insolubility of WT and ΔSLV mutant Fas-GFP was quantified by densitometry and is shown on the right (n = 3). Graphs represent means ± SEM. *, $P < 0.05$; Student's t test. **(B)** IP experiments on 293T cell extracts expressing Dlg1 and either WT or Fas deletion mutant proteins. The presence of Dlg1 in the IP was detected by IB.

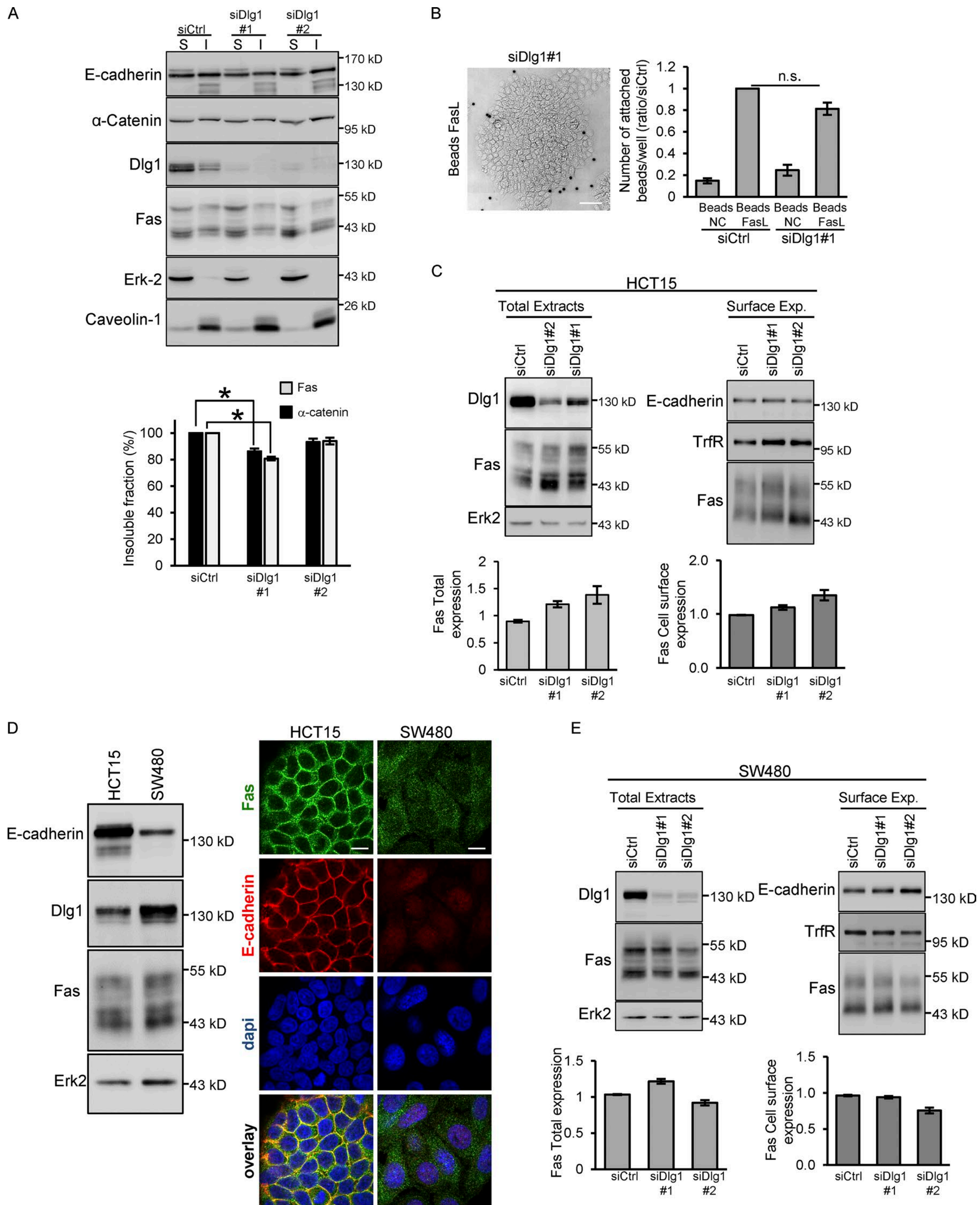
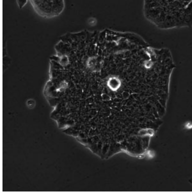
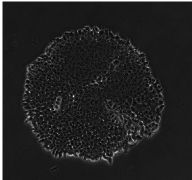


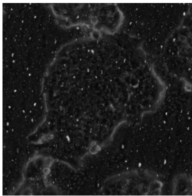
Figure S2. **Impact of Dlg1 polarity molecule on Fas expression. (A)** Detergent solubility of Fas, AJ components, and Dlg1 in HCT15 cells transfected by Ctrl or Dlg1 siRNAs was analyzed by IB as described above (Fig. 2 H). *, P < 0.05; Student's *t* test. **(B)** Binding assay of latex beads coated with FasL (Beads FasL) on HCT15 transfected with Dlg1 siRNA and followed by phase-contrast microscopy. FasL-coated or -noncoated (NC) beads attached to cells were counted using flow cytometry after cell lysis. Graphs represent means ± SEM (*n* = 3). **(C)** Cell surface expression of Fas was compared in Dlg1-knockdown and control HCT15 cells using biotinylation assays. Relative cell surface expression of Fas in Dlg1-knockdown and control cells was quantified by densitometry. Bars in graphs represent means ± SEM (*n* = 3). **(D)** Comparison of AJ components, Fas, and Dlg1 expression levels in HCT15 and SW480 cells by IB. Detection of E-cadherin and Fas localization by IF in HCT15 and SW480 cells. **(E)** Cell surface expression of Fas in Dlg1-knockdown and control SW480 cells was compared using biotinylation assays. Relative cell surface expression of Fas in Dlg1-knockdown and control cells was quantified by densitometry. Bars in graphs represent means ± SEM (*n* = 3). Bars: 50 µm (B); 10 µm (D).



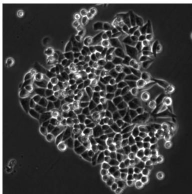
Video 1. **HCT15 cells forming cohesive islets were treated with 20 ng/ml of FasL in the presence of cross M2 antibody and immediately visualized by phase contrast using a video microscope for 12 h.** Frames were taken every 15 min for 12 h. Cell death is observed mainly in the periphery of the cell islet, suggesting that cells in the center of the islet are protected from Fas cell death signaling.



Video 2. **HCT15 cells were treated with staurosporine, a chemical inducer of apoptosis, and cells were filmed as described in Video 1.** Contrary to what happens with the FasL treatment, cells treated with staurosporine died with the same kinetic independently of their position within the cell islet. Frames were taken every 15 min for 12 h.



Video 3. **HCT15 were grown on a semipermeable cell culture insert and treated with FasL added apically or basolaterally.** Cell death is observed mainly in the periphery of the cell islet, demonstrating that the protective effect observed in cells located at the center of the islet cannot be explained by a restriction of FasL diffusion at the basal side of the cells. Frames were taken every 15 min for 12 h.



Video 4. **HCT15 were grown in culture media depleted of calcium before being treated with FasL and filmed in similar conditions to Video 1.** Absence of mature cell–cell contacts resulting from calcium depletion prevents the cell death–protective effect observed in control cells forming extensive cell–cell junctions. Indeed, FasL treatment induced cell death in all the cells independently of their position within the cell islet. Frames were taken every 15 min for 12 h.

Table S1 is a separate Excel document showing identification of Fas interactors by pulldown assay and LC-MS/MS analysis.

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