Supplemental Materials

Molecular Biology of the Cell

Ruch et al.

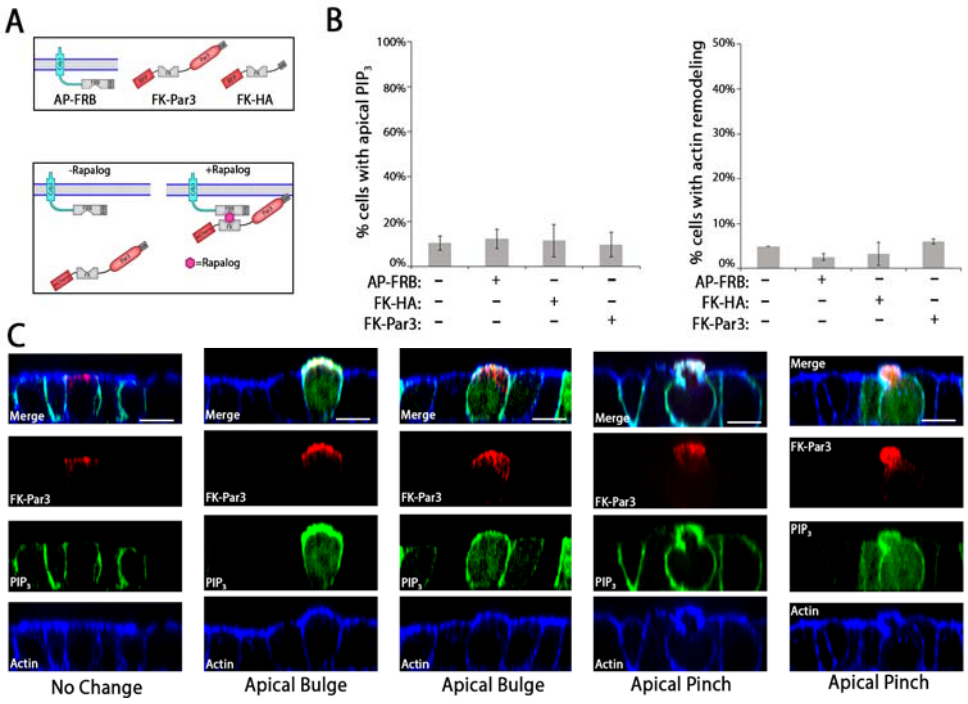

Figure S1. Expression of CID constructs and images of apical membrane remodeling. (A) Diagram depicting the constructs and CID strategy. Rapalog addition induces apical localization of FK-Par3 and FK-HA. **(B)** Quantitation of apical PIP_3 and actin rearrangement in cells expressing the indicated constructs. Error bars=SEM. P-value determined by one-way ANOVA followed by post-hoc Tukey's test. N=3. Scale bar=10 µm. **(C)** Representative single XZ slice from confocal micrograph of MDCK cells expressing PH-Akt-GFP (PIP_3, green) transfected with AP-FRB and AP-Par3 (red) and then treated with 200 nM Rapalog (+Rapa) for 60 mins. Shown are examples of the various actin (blue) rearrangement morphologies that were scored as "positive", including apical bulge and apical pinch.

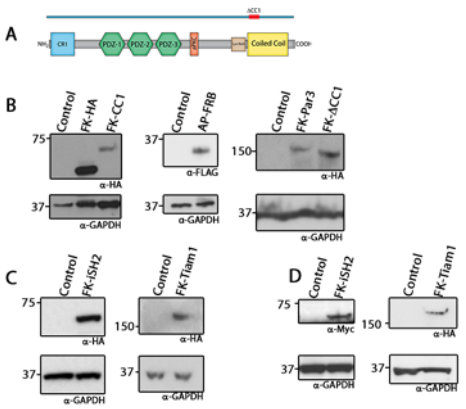

Figure S2. Par3 diagram and Western blots of CID constructs. (A) Diagram of the major domains of Par3. The ΔCC1 deletion is denoted in red. **(B-E)** Western blot of untransfected MDCK cells (control) or cells individually transfected with the indicated constructs. GAPDH serves as a loading control. Molecular weight markers are indicated. FK constructs were detected using an anti-HA antibody. For **(E)**, FK-iSH2 was detected using a Myc antibody and FK-Tiam1 was detected using an HA antibody.

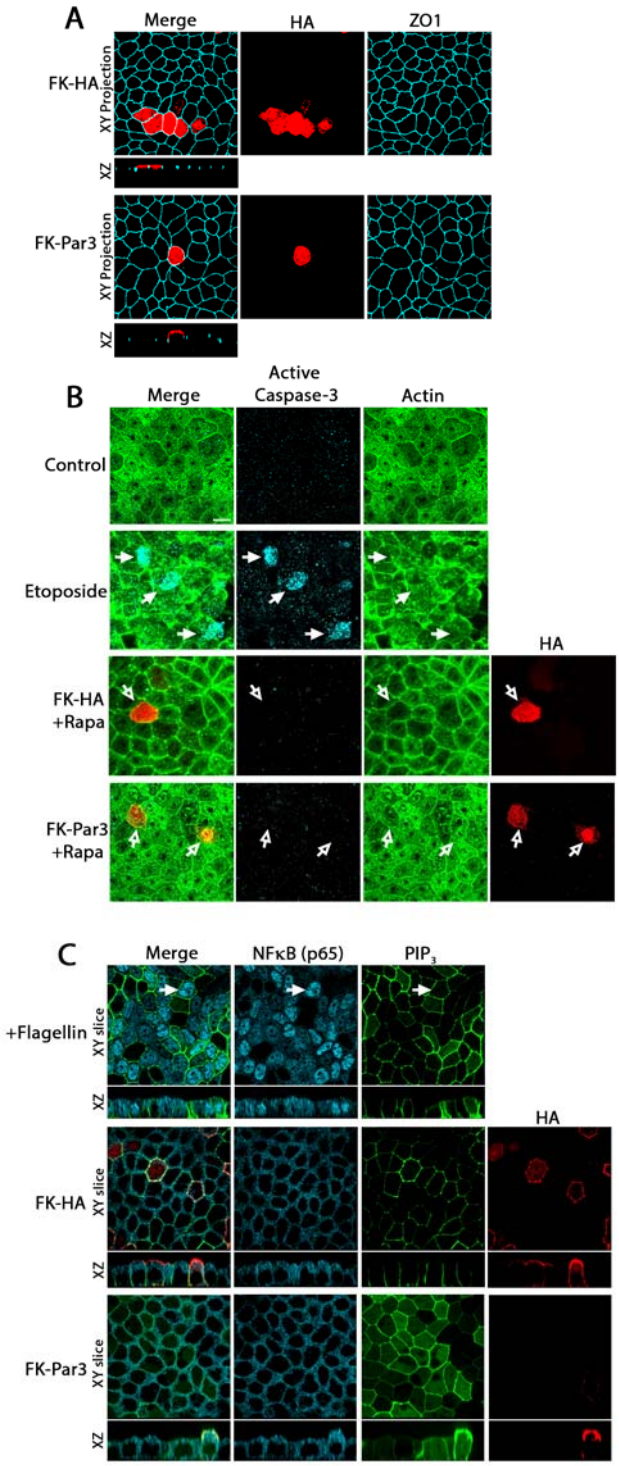

Figure S3. Apical membrane remodeling does not affect tight junctions, induce apoptosis, or activate NFκB. (A) Confocal XZ slice and max intensity XY projections of MDCK cells expressing AP-FRB and either FK-HA or FK-Par3 (red, HA) 60 mins after treatment with 200nM Rapalog. The tight junction protein ZO1 is shown in cyan. **(B)** Max intensity XY projections of MDCK cells that were untransfected

(control), treated with 500 μM etoposide for 3 hours, or that were transfected with AP-FRB and either FK-HA or FK-Par3 and then treated for 60 mins with 200nM Rapalog. Cells were stained for activated caspase-3 (cyan), actin (green, phalloidin), and HA (red). White arrows indicate cells with activated capase-3. Transparent arrows indicate transfected cells expressing FK-HA or FK-Par3. **(C)** MDCK cells expressing PH-Akt-GFP (green) were treated with purified 200 ng/ml *P. aeruginosa* flagellin for 60 mins or were transfected with AP-FRB and either FK-HA or FK-Par3 and treated with Rapalog for 60 mins. Arrows point to a cell with nuclear NFκB. Shown are XZ and XY slices stained for NFκB (cyan, p65), and HA (red). Scale bar=10 μm.