Supporting Information

Zhou et al. 10.1073/pnas.1308419110

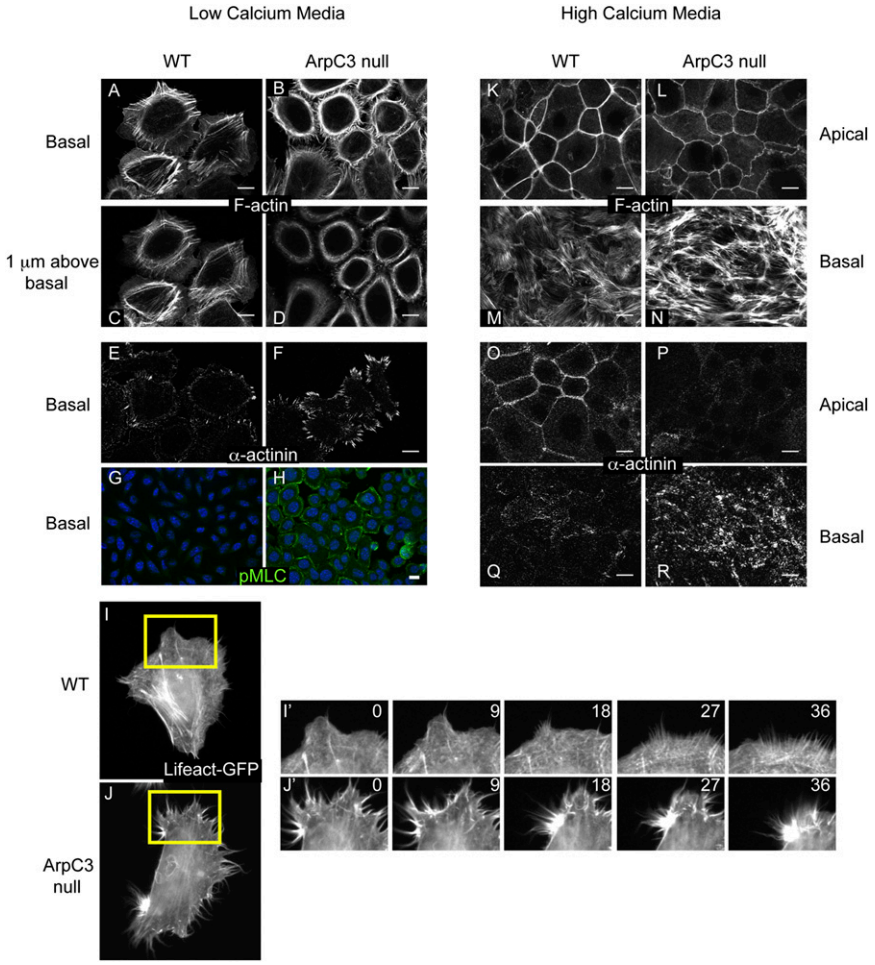

Fig. S1. Changes in F-actin organization in ArpC3-null keratinocytes. (*A–D*) Rhodamine red-X–phalloidin staining of F-actin in WT and ArpC3-null keratinocytes grown in low-calcium conditions. (*E* and *F*) α-Actinin localization at the base of WT and ArpC3-null keratinocytes. (*G* and *H*) Localization of phosphomyosin light chain (Thr18/Ser19) in WT and ArpC3-null keratinocytes. (*I* and *J*) Images of LifeAct-GFP transfected WT and ArpC3-null cells from which stills of time-lapse movies are drawn. (*K–N*) Rhodamine red-X–phalloidin staining of F-actin in WT and ArpC3-null keratinocytes grown in high-calcium conditions. Images from the apical and basal sides of cells are shown. (*O–R*) α-Actinin localization at the base of WT and ArpC3-null keratinocytes. Images from the apical and basal sides of cells are shown. (Scale bars: 10 μm.)

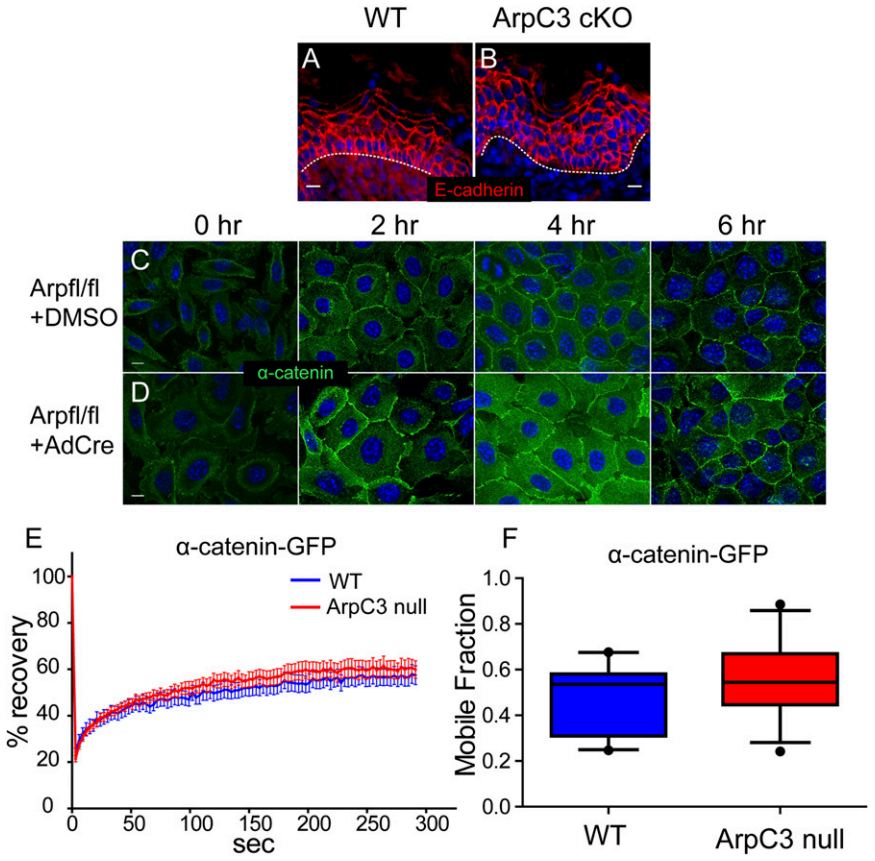

Fig. S2. Lack of notable adherens junction defects upon loss of ArpC3. (*A* and *B*) Localization of E-cadherin (red) in WT and ArpC3 conditional knockout (cKO) epidermis. The dashed line indicates the basement membrane. (*C* and *D*) Time course of adherens junction assembly upon addition of 1.2 mM calcium to the media. The cortical recruitment of α-catenin is shown over a 6-h experiment. (*E*) Fluorescence recovery after photobleaching analysis of α-catenin–GFP in WT and ArpC3-null keratinocytes. (*F*) Quantification of the mobile fraction of α-catenin–GFP in WT and ArpC3-null keratinocytes. Boxes represent 25% to 75%, whiskers represent 5% to 95%.

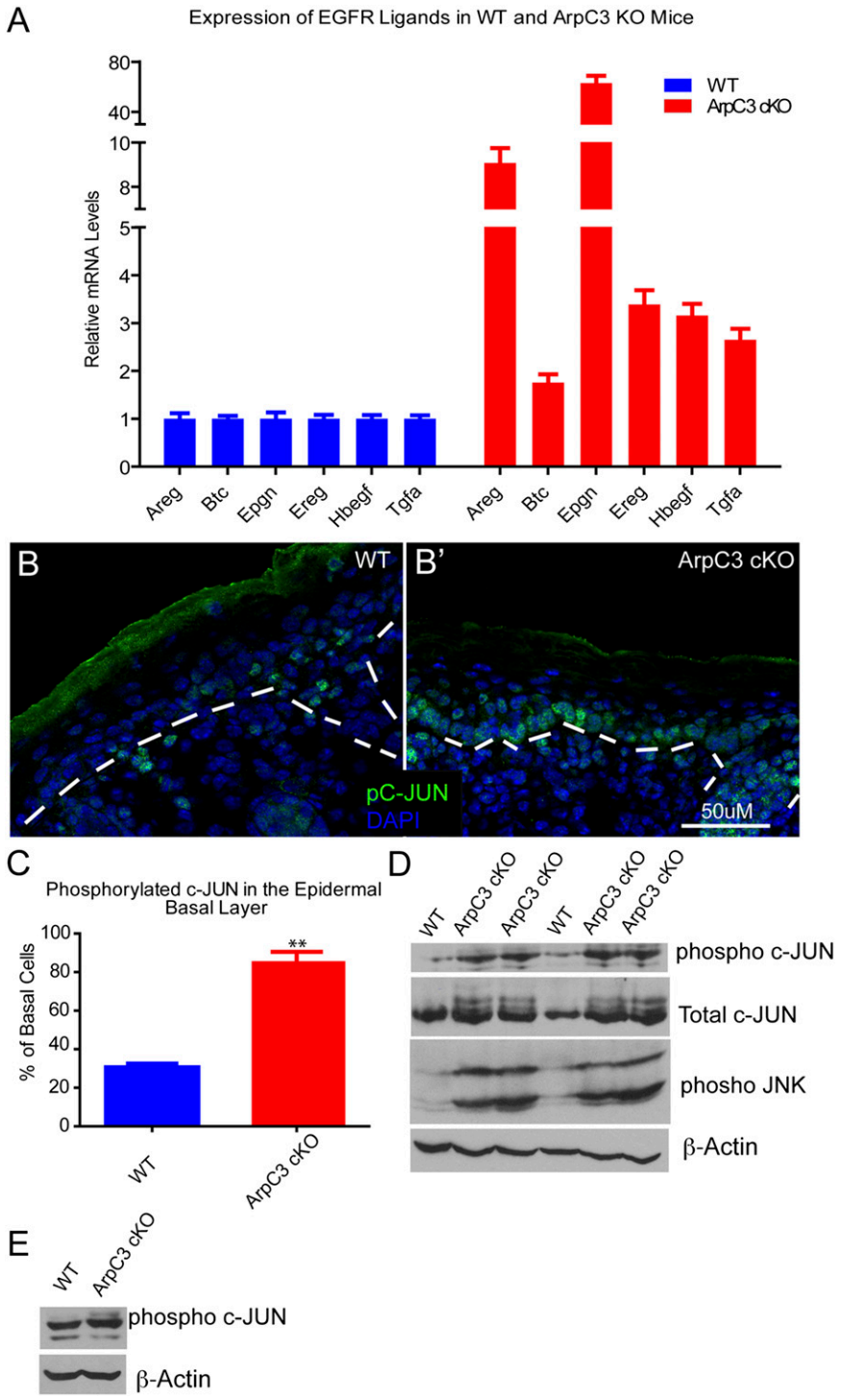

Fig. S3. Changes in EGFR ligand expression and Jun activation in ArpC3 cKO epidermis. (*A*) quantitative PCR analysis of the expression of six EGFR ligands in two WT and two ArpC3 cKO epidermal preparations (Areg, $P < 0.0001$; Btc, $P = 0.001$; Epgn, $P < 0.0001$; Ered, $P < 0.0001$; Hbegf, $P < 0.0001$; Tgfa, $P < 0.0001$). (*B* and *B′*) Immunofluorescence of active phospho c-Jun (green) in WT and ArpC3 cKO epidermis. The dashed line indicates the basement membrane. (C) Quantification of the number of basal cells that are positive for phospho–c-Jun in WT and ArpC3 cKO epidermis ($P = 0.0035$; $n > 300$ cells from each of two independent experiments). (*D*) Western blot analysis of phospho–c-Jun, total c-Jun, phospho-JNK, and total JNK in WT and ArpC3 cKO epidermal lysates. (*E*) Western blot analysis of phospho–c-Jun levels in WT and ArpC3-null keratinocytes.

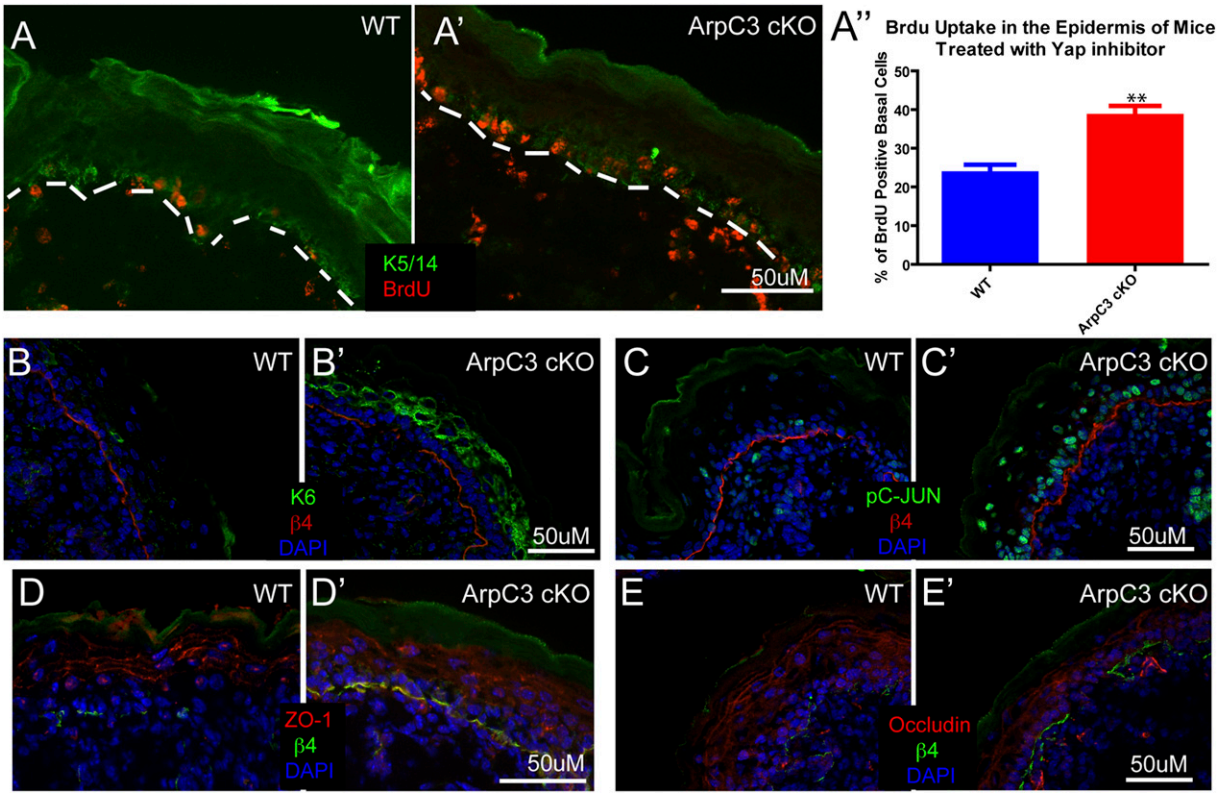

Fig. S4. YAP inhibition does not rescue proliferation, stress, or tight junction defects in ArpC3 cKO epidermis. (*A* and *A″*) Immunofluorescence and quantification of BrdU incorporation into basal cells of the epidermis in WT and ArpC3 cKO epidermis after treatment with verteporfin ($P < 0.0001$). (*B*–*E*) Localization of keratin 6, phospho–c-Jun, and the tight junction proteins ZO-1 and occludin in WT and ArpC3 cKO epidermis treated with verteporfin as indicated.