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Supplemental material

JCB

Sumigray et al., http://www.jcb.org/cgi/content/full/jcb.201206143/DC1

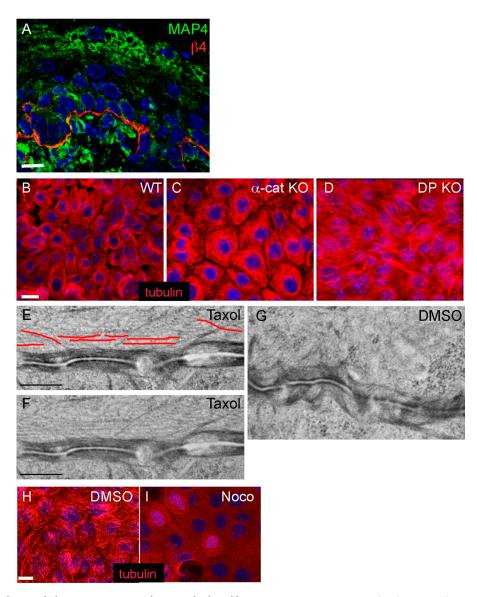
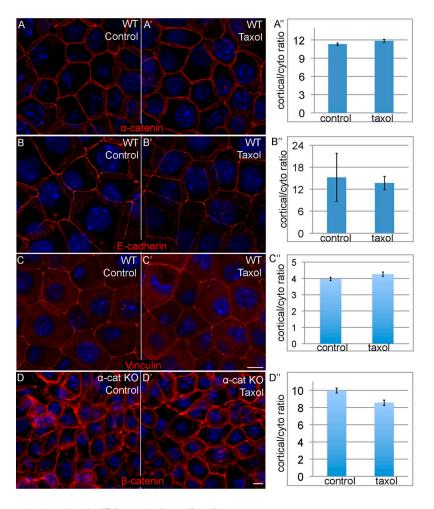


Figure S1. MAP4 and microtubule organization in epidermis and cultured keratinocytes. (A) MAP4 (green) localization in the upper differentiated layers of the epidermis. β 4-integrin (red) marks the epidermal–dermal boundary. Bar, 20 μ m. (B–D) β -Tubulin staining to highlight microtubules in WT (B), α -catenin–null (C), and desmoplakin-null (D) cells grown in low calcium containing media and treated with taxol. Bar, 10 μ m. (E–G) Ultrastructural analysis of cultured keratinocytes in taxol-treated (E and F) and control (G) cells. F is the same image as E with highlights for microtubules absent. Bar (E–G) 0.5 μ m. (H–I) Anti– β -tubulin immunofluorescence in control and nocodazole-treated keratinocytes.



E β -catenin IP/ α -catenin null cells

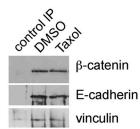


Figure S2. Localization of cell junction components after taxol treatment. Immunofluorescence analysis and quantitation of cortical/cytoplasmic ratio of α -catenin (A–A''), E-cadherin (B–B''), and vinculin (C–C'') in control and taxol-treated cells, as indicated. (D–D'') Localization of β -catenin in α -catenin–null, control, or taxol-treated cells. Bar, 10 μ m. (E) β -Catenin was immunoprecipitated from extracts of α -catenin–null cells (with or without pretreatment with taxol). Composition of complexes was analyzed by Western blotting with E-cadherin, β -catenin, and vinculin antibodies.

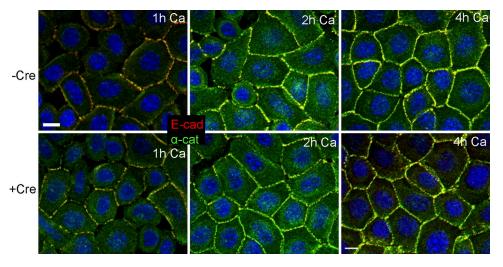


Figure S3. Normal recruitment of adherens junction proteins to cell junctions in myosin IIA-null keratinocytes. Myosin IIA fl/fl cells were treated with adenoviral-Cre or left untreated. After 48 h, calcium was added and the localization of E-cadherin (red) and α-catenin (green) was assessed. Bar, 10 μm.

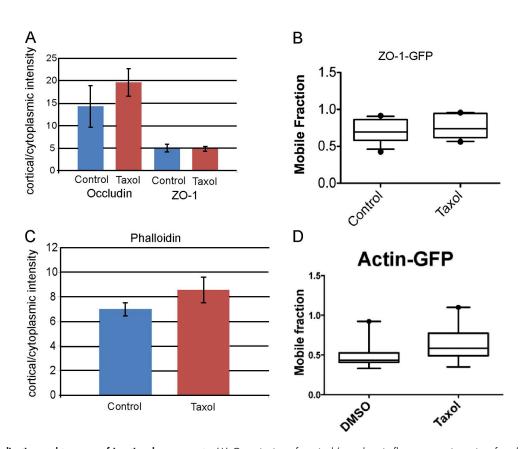


Figure S4. Localization and turnover of junctional components. (A) Quantitation of cortical/cytoplasmic fluorescence intensity of occludin and ZO-1 in control and taxol-treated cells. (B) Mobile fraction of ZO-1-GFP as determined by FRAP analysis in control and taxol-treated cells. (C) Quantitation of cortical/cytoplasmic fluorescence intensity of F-actin in control and taxol-treated cells. (D) Mobile fraction of actin-GFP as determined by FRAP analysis in control and taxol-treated cells.

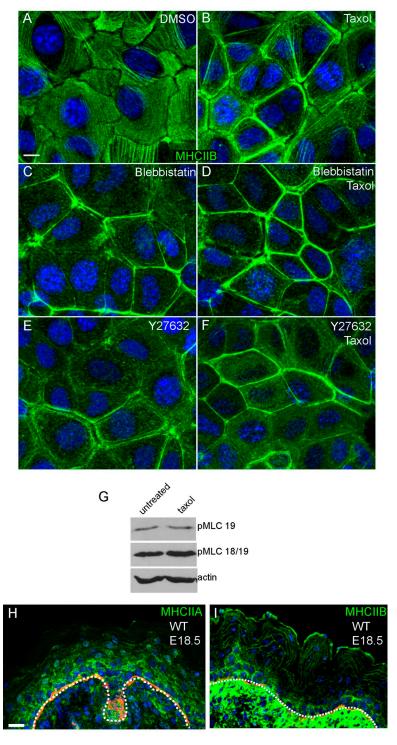


Figure S5. **Myosin II localization in vitro and in vivo.** (A–F) Myosin IIB (green) localization in cultured keratinocytes treated as indicated in the panels. Bar, 10 µm. (G) Western blots of myosin light chain phosphorylated at either residue 19, or 18 and 19. Lysates were prepared from control and taxol-treated keratinocytes. (H) Localization of myosin IIA (MHCIIA, green) in E18.5 mouse skin. Bar, 20 µm. (I) Localization of myosin IIB (MHCIIB, green) in E18.5 mouse skin. The dotted line marks the basement membrane in both H and I.