Lázaro-Diéguez et al., https://doi.org/10.1083/jcb.201608065

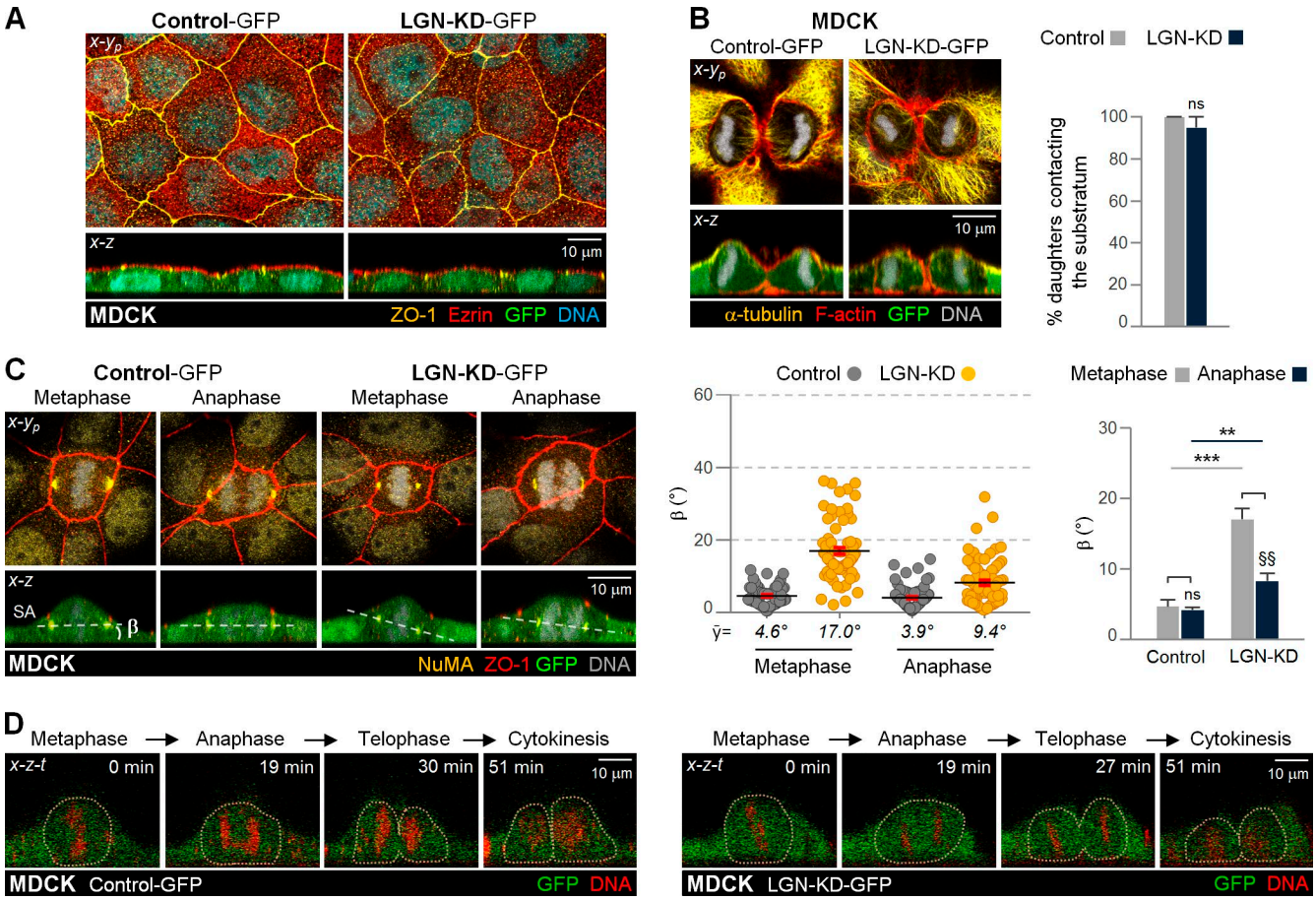


Figure S1. **Tilted metaphase spindles in LGN-depleted MDCK cells realign with the substratum during anaphase.** (A–C) Immunofluorescence analysis of control and LGN-KD MDCK monolayers (A), cytokinesis profiles with quantification of the percentage of daughter cells contacting the substratum (B), and metaphase or anaphase profiles with β angle quantification (C). n = 20–25 cells/experiment were analyzed for N = 3 experiments. Error bars indicate ±SEM (dot graph) or +SD (bar graphs). **, P ≤ 0.01; ***, P ≤ 0.001; §§, P ≤ 0.01; ns, not significant, analyzed by t test. Note that parental control and LGN-KD cells express GFP. (D) x-z-t confocal time-lapse sequence of control and LGN-depleted MDCK dividing cells. Dotted lines show cell contours.

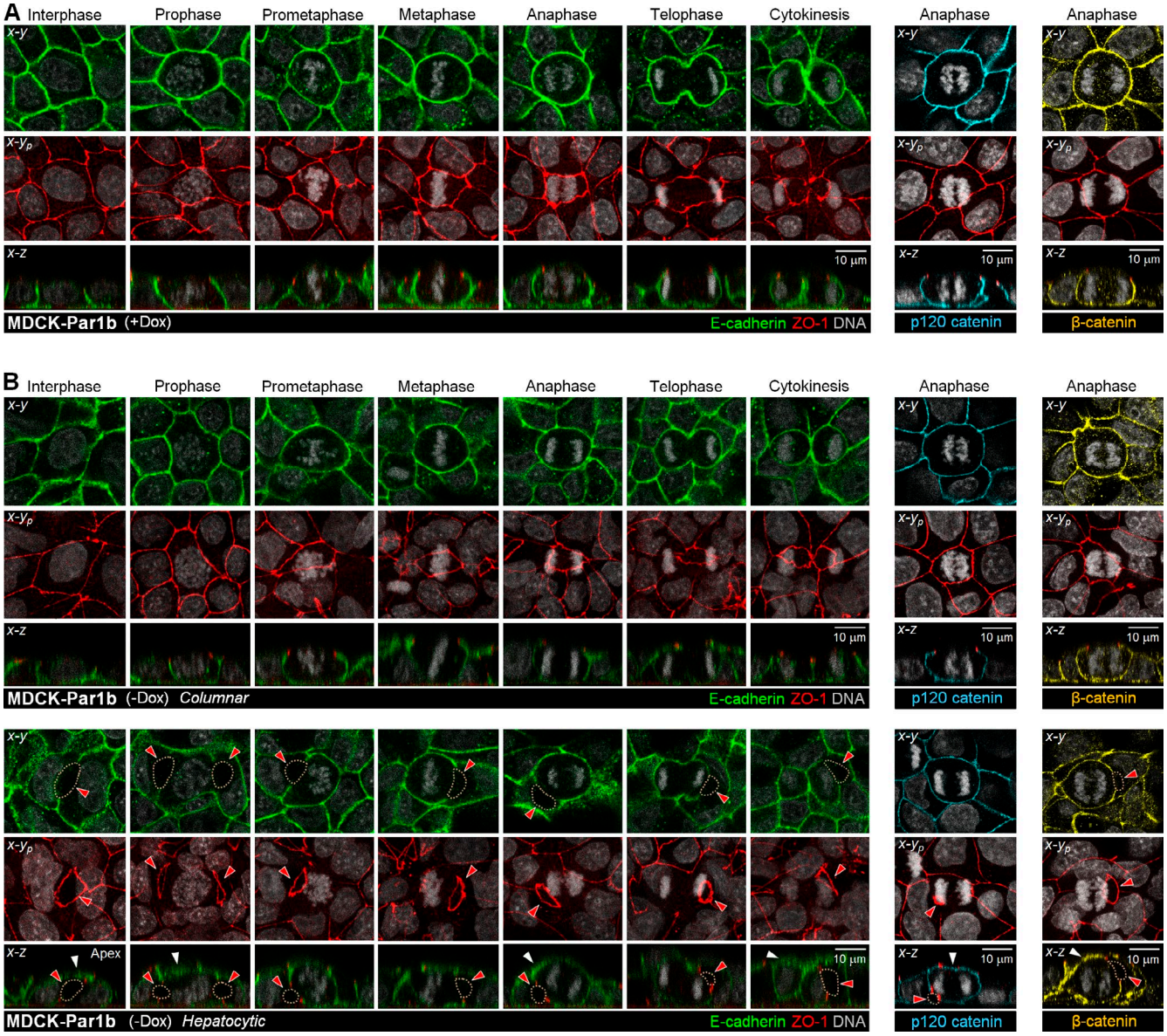


Figure S2. **Adherens junctions and TJs during mitosis in MDCK-Par1b cells.** (A and B) Confocal *x-y* and *x-z* sections and *x-y* projections (*x-y*$_p$) of MDCK-Par1b cells either uninduced (+Dox) or overexpressing Par1b (–Dox) fixed and stained as indicated. (B) Arrowheads indicate E-cadherin, p120 catenin, or β-catenin at the cell apex (white) or the hepatocytic-like lateral lumina (red).

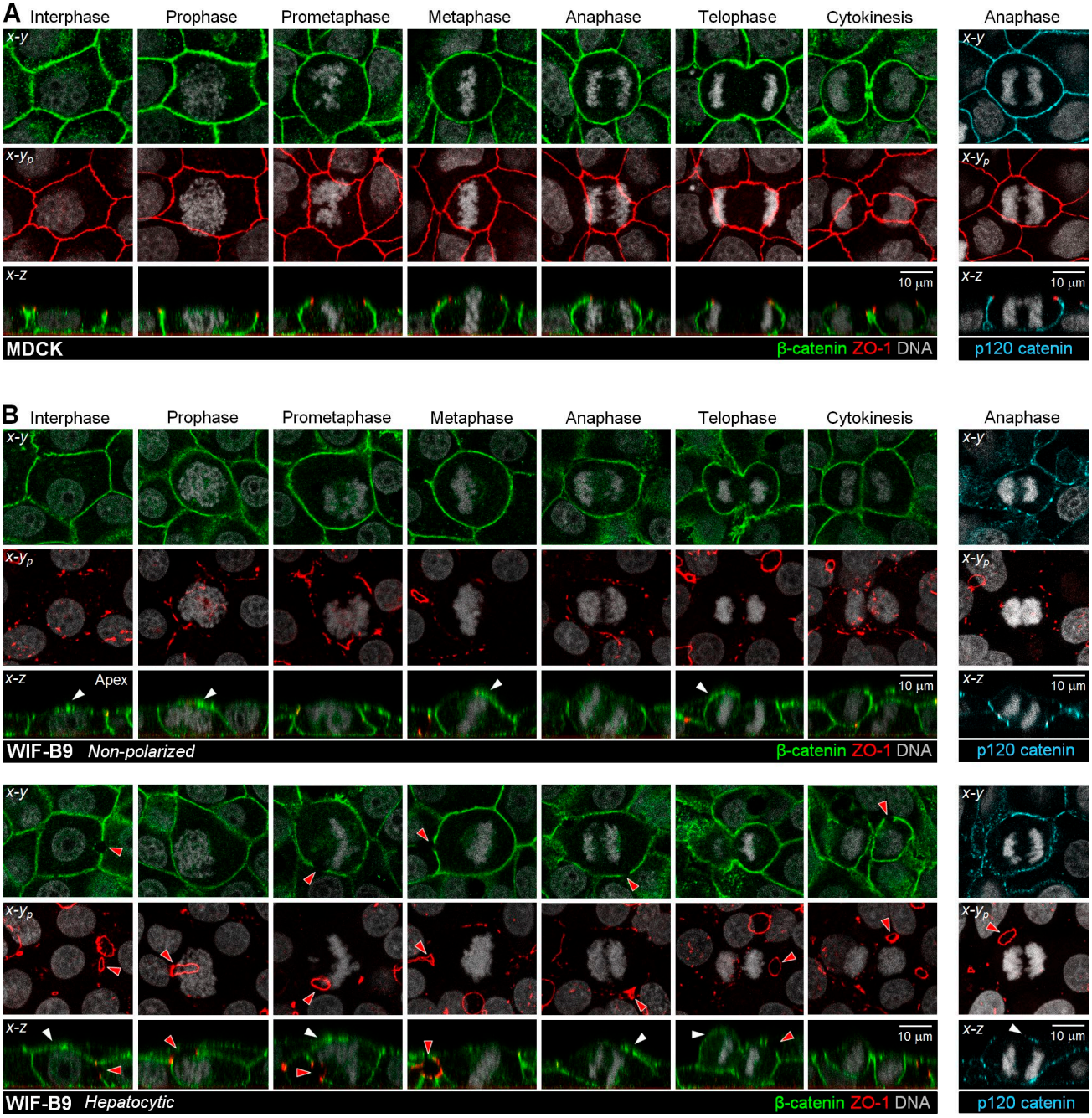


Figure S3. **Adherens junctions and TJs during mitosis in MDCK and WIF-B9 cells.** (A and B) Confocal *x-y* and *x-z* sections and *x-y* projections (*x-y*$_p$) of MDCK and WIF-B9 cells fixed and stained as indicated. (B) Arrowheads indicate β-catenin and p120 catenin localization at the cell apex (white) or the hepatocytic lateral lumina (red).

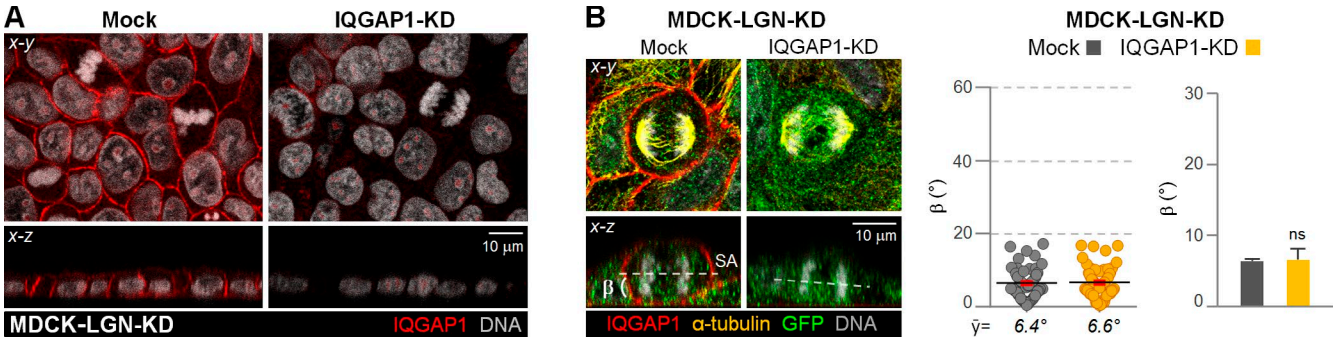


Figure S4. **E-cadherin binding partner IQGAP is not required for anaphase spindle realignment in LGN-KD cells.** Immunofluorescence analysis of LGN-KD (Mock) and LGN-IQGAP-KD double-depleted MDCK monolayers (A), and anaphase profiles with β angle quantification (B). Note that parental control and LGN-KD cells express GFP. *n* = 25 cells/experiment were analyzed for *N* = 3 independent experiments. Error bars indicate ±SEM (dot graph) or +SD (bar graphs). ns, not significant, analyzed by *t* test.

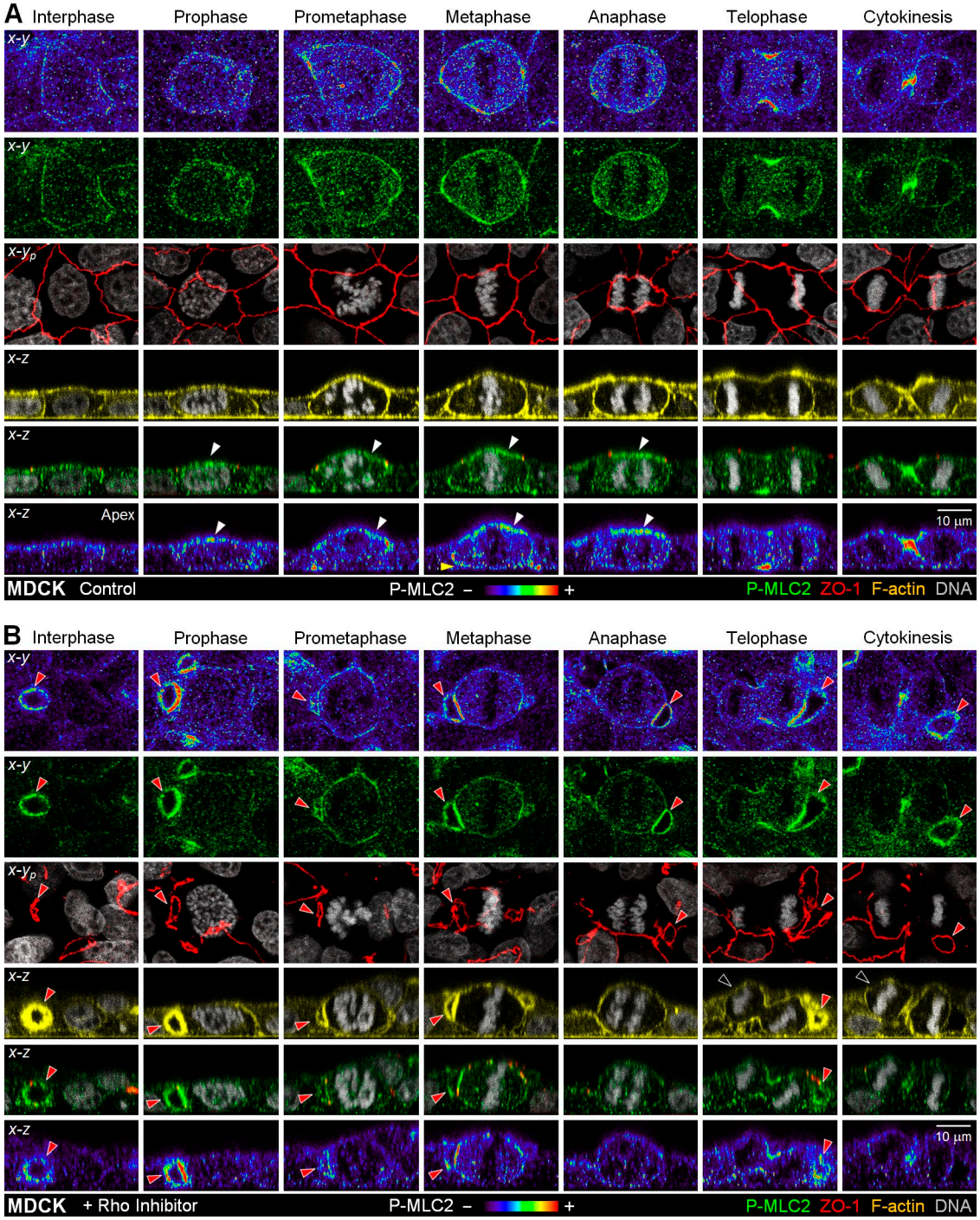
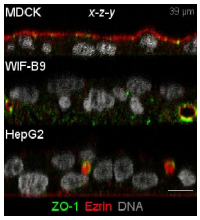
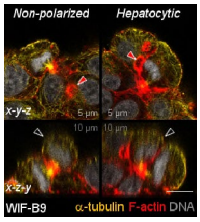


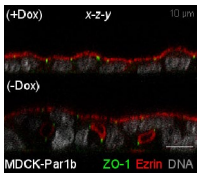
Figure S5. **Activated myosin II localization during mitosis in MDCK cells.** (A and B) Confocal *x-y* and *x-z* sections and *x-y* projections (*x-y*$_p$) of untreated (A) or Rho inhibitor–treated (B) MDCK cells fixed and stained as indicated. The P-MLC2 intensity spectrum map for the *x-y* and *x-z* views is shown. Arrowheads indicate P-MLC2 at the cell apex in prophase to anaphase cells (white) and at the base in metaphase (yellow), the hepatocytic-like lateral lumina (red), or daughter that lost substrate contact (black).



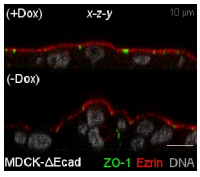
Video 1. **Hepatocyte-derived WIF-B9 and HepG2 cells form bilayers in 2D cell cultures.** Confocal *x-z-y* stacks (50 µm) of MDCK, WIF-B9, and HepG2 cells fixed and stained as indicated. Bar, 10 µm.



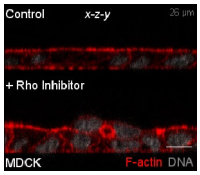
Video 2. **Cell divisions out of the monolayer in WIF-B9 cells.** Confocal *x-y-z* and *x-z-y* stacks of nonpolarized and hepatocytic WIF-B9 cells fixed and stained as indicated. Red arrowheads show hepatocytic lateral lumina. Black arrowheads show daughter that lost substrate contact. Bar, 10 µm.



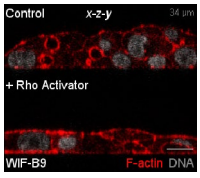
Video 3. **Par1b overexpression generates bilayers in MDCK cell cultures.** Confocal *x-z-y* stacks (50 µm) of uninduced (+Dox) or overexpressing Par1b (–Dox) MDCK-Par1b cells fixed and stained as indicated. Bar, 10 µm.



Video 4. **ΔEcad generates bilayers in MDCK cell cultures.** Confocal *x-z-y* stacks (50 µm) of uninduced (+Dox) or expressing ΔEcad (–Dox) MDCK-ΔEcad cells fixed and stained as indicated. Bar, 10 µm.



Video 5. **Rho inhibition promotes hepatocytic-type architecture in MDCK cell cultures.** Confocal *x-z-y* stacks (38 µm) of untreated cells (Control) or cells exposed to Rho inhibitor MDCK fixed and stained as indicated. Bar, 10 µm.



Video 6. **Rho activation promotes columnar-type architecture in WIF-B9 cell cultures.** Confocal *x-z-y* stacks (38 µm) of untreated cells (Control) or cells exposed to Rho activator WIF-B9 fixed and stained as indicated. Bar, 10 µm.