**Fig S3. GSK-3 inhibitor blocks GSK-3 activity.**

(A) MDCK cells were transduced with adenoviral vectors expressing FIP5-wt-GFP (top panel), FIP5-T276D-GFP (upper middle panel), FIP5-T276A (bottom middle panel) or FIP5-S188A (bottom panel). Varying adenovirus concentrations were tested. Asterisks mark the used concentrations in all the remaining experiments. Please note that blots show three FIP5 isoforms, top and bottom bands are endogenous FIP5 splice variants. The middle band is GFP-tagged FIP5.

(B) Varying concentrations of GSK-3 inhibitor resulted in dephosphorylation of the GSK-3 substrate β-catenin. FIP1 and TfR were loading controls. (C-D) Treating MDCK cells with GSK-3 inhibitor but not DMSO for 24 h led to nuclear translocation of β-catenin. Scale bars: 10 μm.

(E) Filter-grown MDCK cells were treated for 3 h with varying concentrations of GSK-3 inhibitor. Cells were then fixed and stained with anti-pFIP5-T276 antibodies, rhodamine-phalloidin and DAPI. The levels of pFIP5-T276 signal in metaphase cells were then measured. At least 10 randomly picked cells were analyzed for each condition. Data shown are means and standard deviations from three independent experiments (p values as indicated). (F) MDCK-shFIP5 cells were incubated in the presence or absence of doxycycline. Doxycycline treated cells were then transduced with or without shRNA-resistant FIP5-T276A-GFP. The levels of the endogenous FIP5 and FIP5-T276A-GFP were analyzed using anti-FIP5 antibody.