

Figure S2: ΔNter-GFP is still targeted to the ER membrane but does not translocate to the tight junctions as polarity develops. A: representative x-y confocal sections showing overlapping distribution of IP<sub>3</sub>R1-GFP or  $\Delta$ Nter-GFP and the ER marker PDI in subconfluent MDCK cells. **B-D**: Representative x-y confocal sections of polarized MDCK cells expressing either IP<sub>3</sub>R1-GFP or  $\Delta$ Nter-GFP and stained for PDI (B), IP<sub>3</sub>R3 (C) or ZO-1 (D) demonstrating that, while IP<sub>3</sub>R1-GFP and endogenous IP<sub>3</sub>R3 translocate to the cell periphery upon MDCK epithelial cell differentiation,  $\Delta$ Nter-GFP remains evenly distributed in the ER and the nucleoplasmic reticulum. Scale bar, 10 μm. E: x-z reconstructions showing that, in contrast to IP<sub>3</sub>R1-GFP,  $\Delta$ Nter-GFP does not accumulate at the tight junction level as defined by ZO-1 staining in polarized cells. This is likely not due to the saturation of the translocation mechanisms, as endogenous IP<sub>3</sub>Rs are normally recruited to the apex of the lateral membrane in  $\Delta$ Nter-GFP expressing cells. (x-z), 7,5 μm.