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online data

## SUPPLEMENTARY ONLINE DATA

# Sorting nexin 27 (SNX27) associates with zonula occludens-2 (ZO-2) and modulates the epithelial tight junction

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Supplementary Figures S1–S4 can be found on the following pages.



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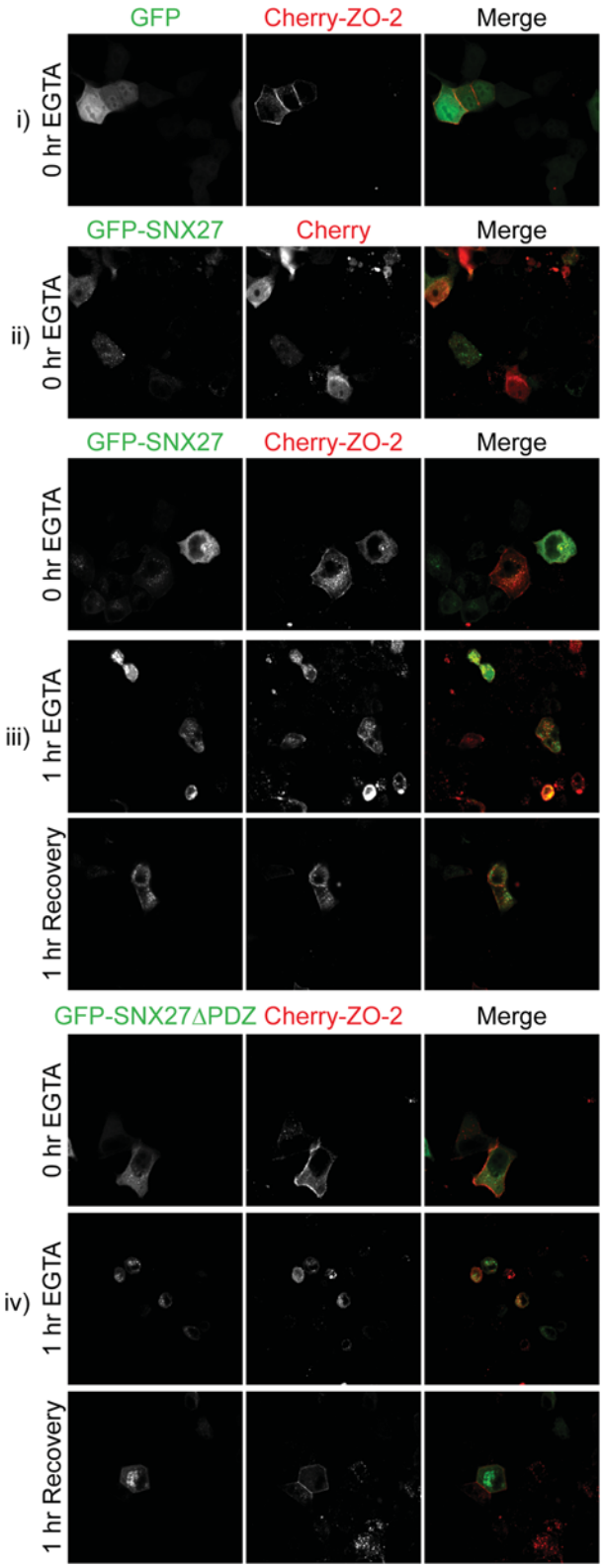


**Figure S1 Additional images for Figure 4 of the main text of mpkCCD cells transiently transfected with the indicated GFP and mCherry constructs**

The cells were grown to confluence and subjected to the calcium-switch procedure for the indicated times. Cells were fixed and visualized with confocal microscopy.

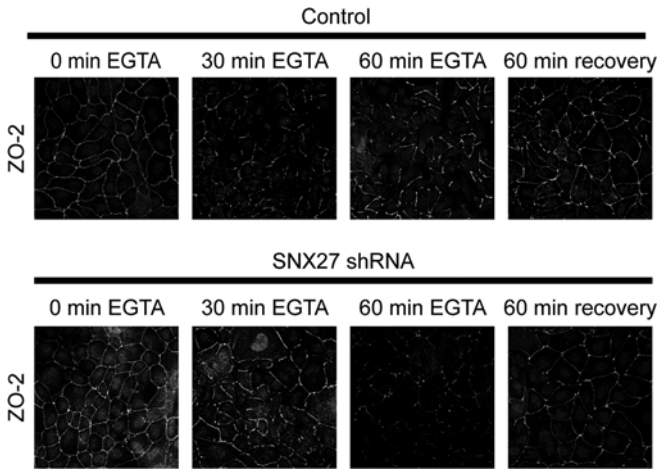


**Figure S2 SNX27 knockdown does not alter ZO-2 localization during calcium switch**

Control or SNX27-knockdown cells were subjected to calcium switch and fixed at specified time points. Cells were stained using a polyclonal antibody against ZO-2 and visualized by confocal microscopy.

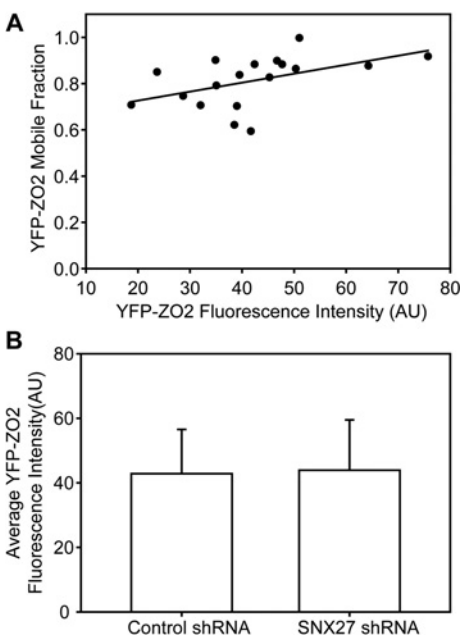


**Figure S3 YFP–ZO-2 expression levels does not significantly alter the mobile fraction**

(**A**) Average fluorescence intensity of YFP–ZO-2 compared with the mobile fraction (linear regression analysis, $R = 0.53$ and slope $= 0.0048$). (**B**) Average initial YFP–ZO-2 fluorescence intensity of control and SNX27-knockdown mpkCCD cells analysed by FRAP (error bars represent the S.D.).

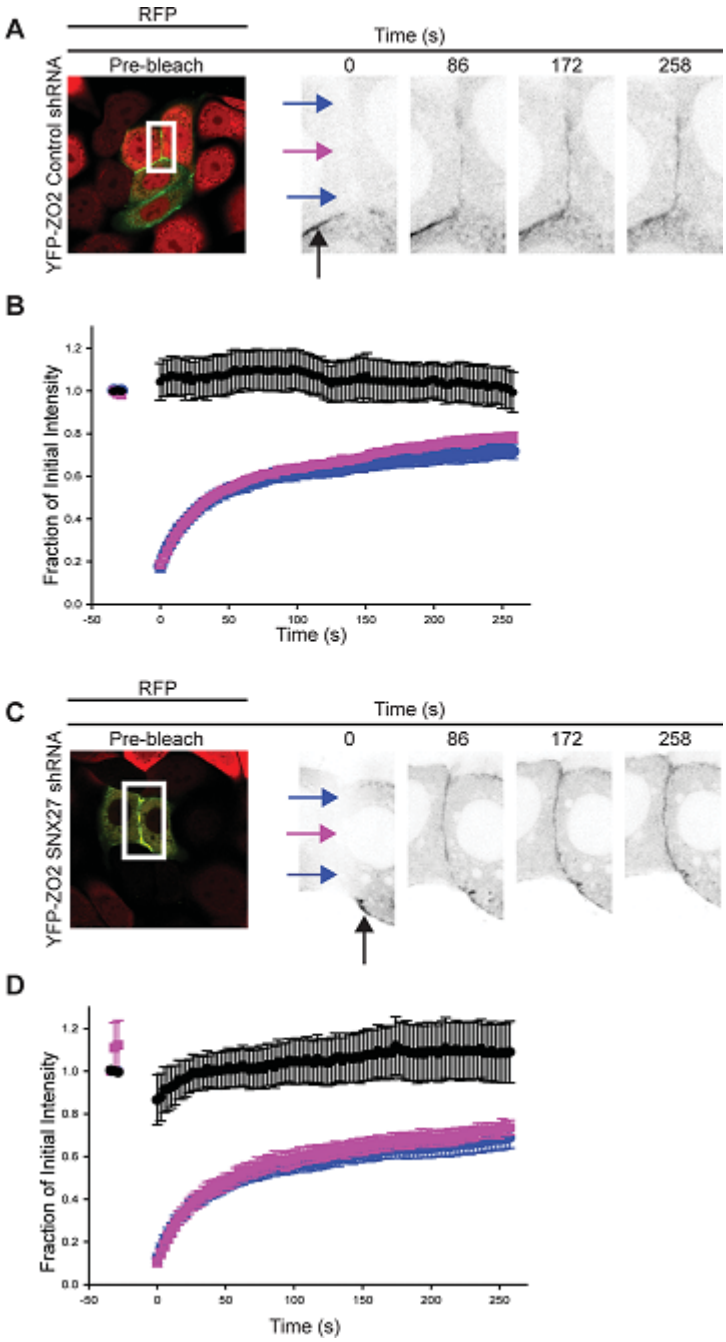


**Figure S4 Pertubation of ZO-2 kinetics in SNX27-knockdown cells is due to hindrance of cytoplasmic/TJ exchange**

Control or SNX27-knockdown cell monolayers were transiently transfected with YFP–ZO-2 (**A** and **C**). Points of cell–cell contact in both YFP- and RFP-expressing cells were photobleached and the rate of fluorescence recovery of ZO-2 at the TJ for the indicated times and regions of interest (arrows) was monitored by YFP and quantified (**B** and **D**). The colour of lines in (**B**) and (**C**) represent the co-ordinated region of interest monitored in (**A**) and (**C**). Cells expressing shRNA and YFP–ZO-2 were identified through RFP and YFP fluorescence. High magnification images of TJ sections at the indicated times are shown (error bars represent the S.D.).

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