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Supporting Material

Two-path impedance spectroscopy for measuring paracellular and transcellular epithelial resistance

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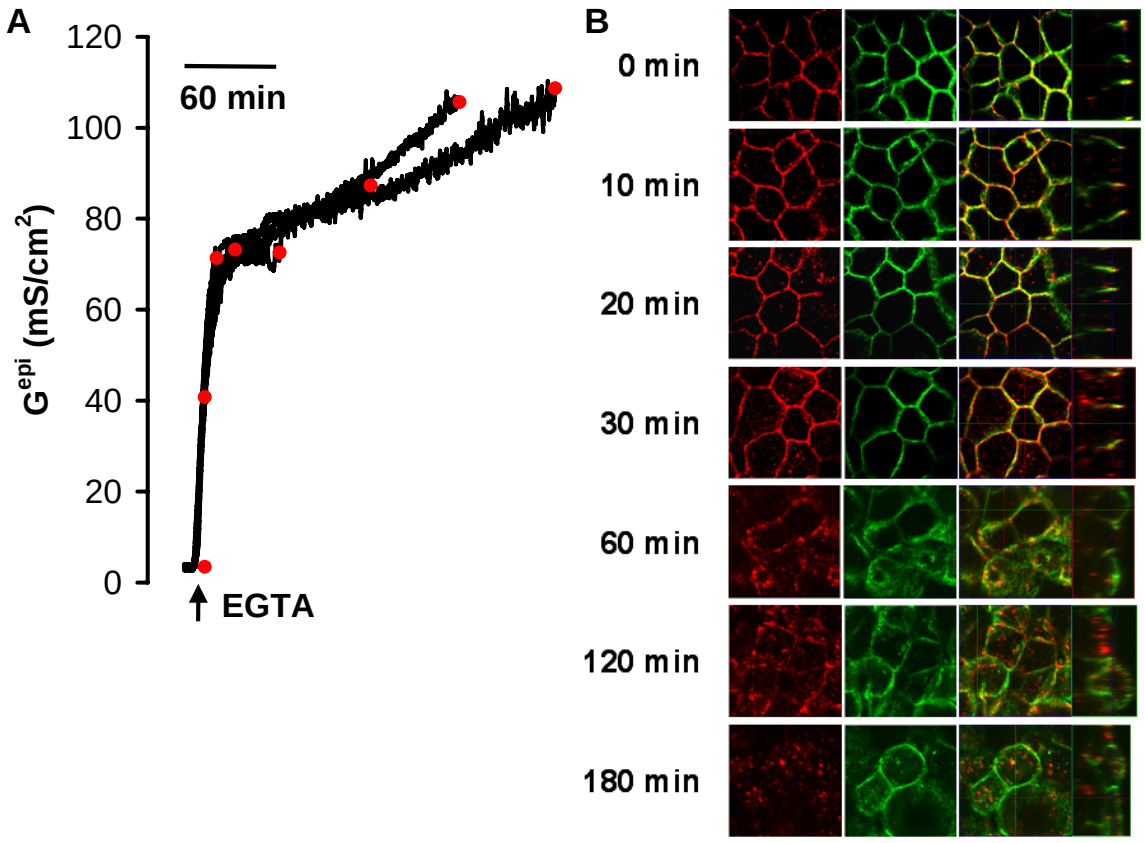

Fig. S1:
A) Chelating extracellular Ca^{2+} by the application of EGTA (arrow) caused an instantaneous increase in transepithelial conductance (each trace is a mean of 2- 5 experiments). Red dots indicate time of fixation.

B) After fixation, filters were stained for claudin-5 (red) and E-cadherin (green). Z-stacks show claudin-5 localization apical (left) to E-cadherin. During the first 30 min, the interval used for evaluation of flux and impedance data, the tight junction pattern appears to remain intact. The tight junction pattern is lost one hour after EGTA application. Claudin-5 is internalized, E-cadherin is distributed in the entire plasma membrane. The loss in tight junction integrity is associated with a further, slow increase in transepithelial conductance (A).

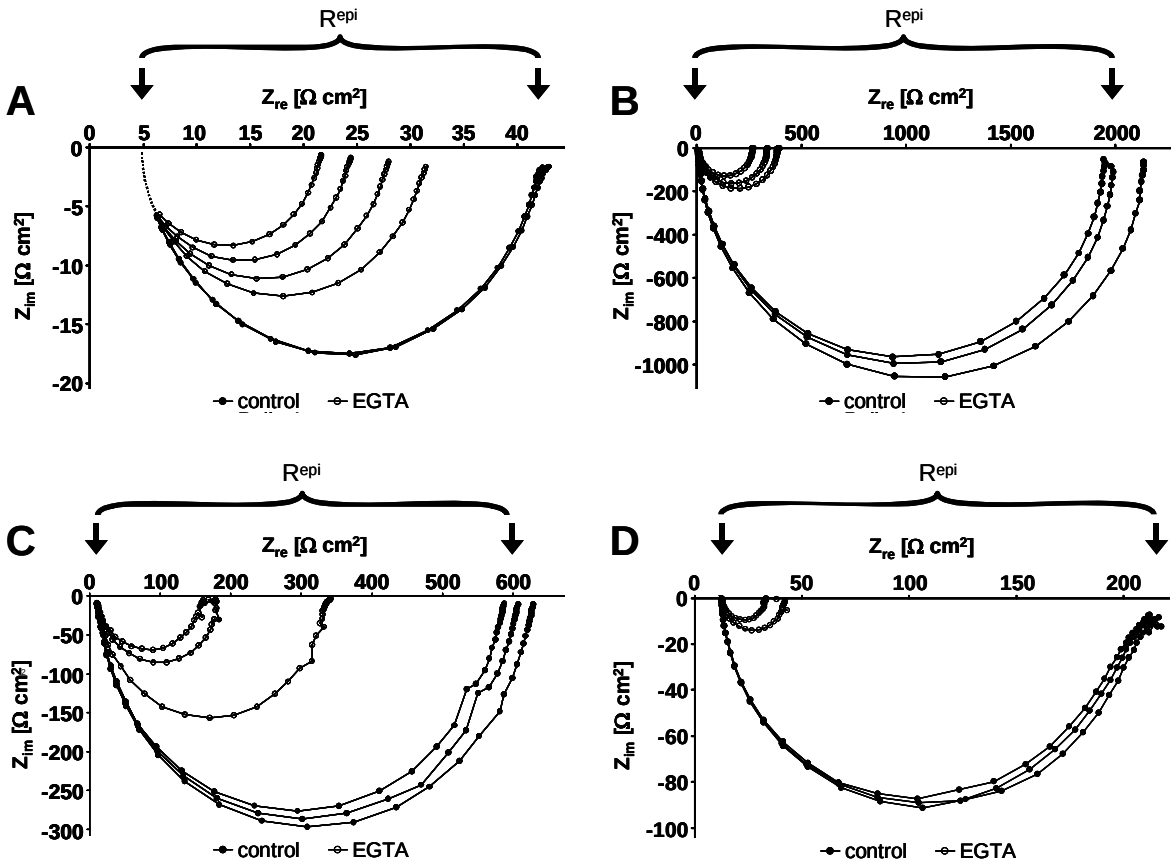

Fig. S2: Nyquist plot of impedance spectra recorded from MDCK-C11 (**A**), MDCK-C7 (**B**), MDCK-C7 transfected with Cldn10b (**C**), and Caco-2 (**D**) cell layers in the absence (●) and presence (O) of EGTA. Dotted line in **A**: extrapolation to obtain R^{sub}.

As previously descibed (5), transfection of MDCK-C7-cells with Cldn10b caused a reduction in transepithelial resistance (compare R^{epi} values in **B** and **C**).

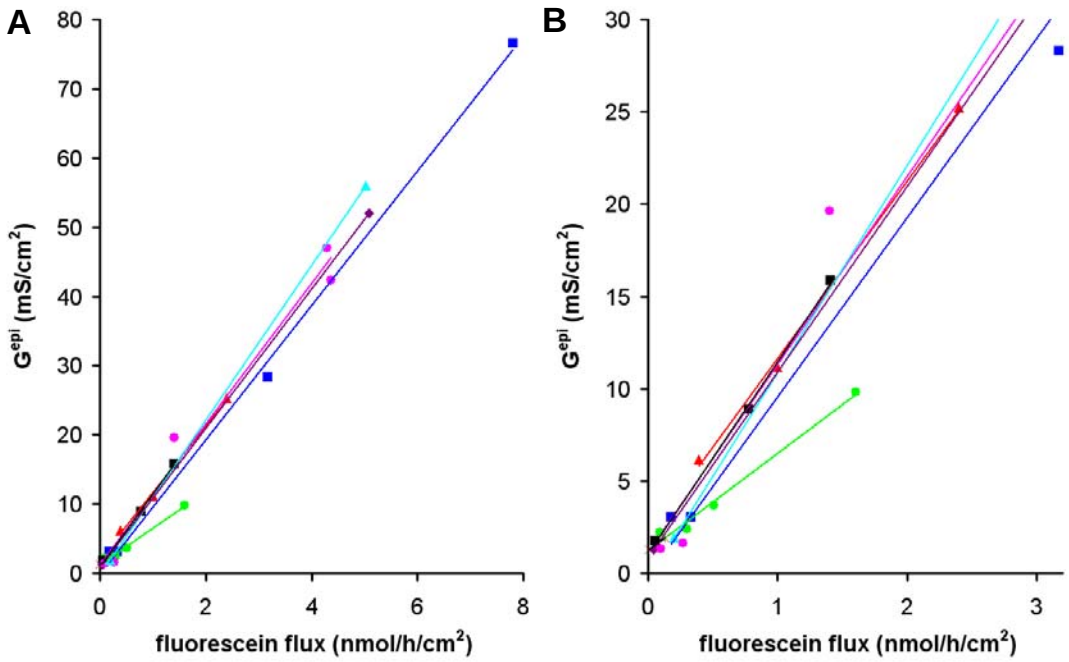

Fig. S3:
A) G^{epi} vs. fluorescein permeability plot from experiments on HT-29/B6 cell layers in the absence and presence of EGTA (same data as shown in Fig. 7a). G^{trans} and G^{para} from different experiments were calculated from the regression lines for each individual experiment.

B) Same as (A), but at an expanded scale to demonstrate the accuracy at the lowest values (obtained in the absence of EGTA).