Supplemental Materials

Molecular Biology of the Cell

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Legends for Supplementary Figures

Figure S1. The expression of exogenous MgcRacGAP in double-KD cells reduces Rac1 activation and TJ formation during the calcium-switch. **(A):** Rac1 and RhoA activation assays of WT, single-KD (either CGN-KD or CGNL-KD) and double-KD cells at different times after the calcium switch. Note that peaks of Rac1 activation occur at 10', 20', and 3 hr in all cell lines except for CGNL-KD cells, and RhoA activity is down-regulated at 8 hr only in WT cells. **(B):** Transepithelial electrical resistance (TER, ohm.cm^2) of three different stable clones of double-KD CGN(-)/CGNL1(-) cells, showing a peak of TER during the calcium-switch. **(C):** Histogram showing the relative mRNA levels for Asef, Vav22, Rich-1 and MgcRacGAP in WT, single (CGN(-), CGNL1(-)), and double-KD cells, taking WT levels as 100%, as determined by qRT-PCR. **(D):** Immunoblotting analysis of either total lysates (RIPA) or fractionated lysates of WT, CGN(-), CGNL1(-), and double-KD cells with anti-MgcRacGAP. **(E)**: Rac1 activation assays in either single KD (CGNL1(-)) or three independent double-KD cell clones (a, b and c) stably expressing or not (-) an exogenous human (h) FLAG-tagged MgcRacGAP protein, during the calcium-switch assay (0, 10' and 3h time points). **(F):** Pattern of development of TER in three independent double-KD clones expressing (a, b, and c) or not the exogenous human (h) FLAG-tagged MgcRacGAP protein, during the calcium switch.

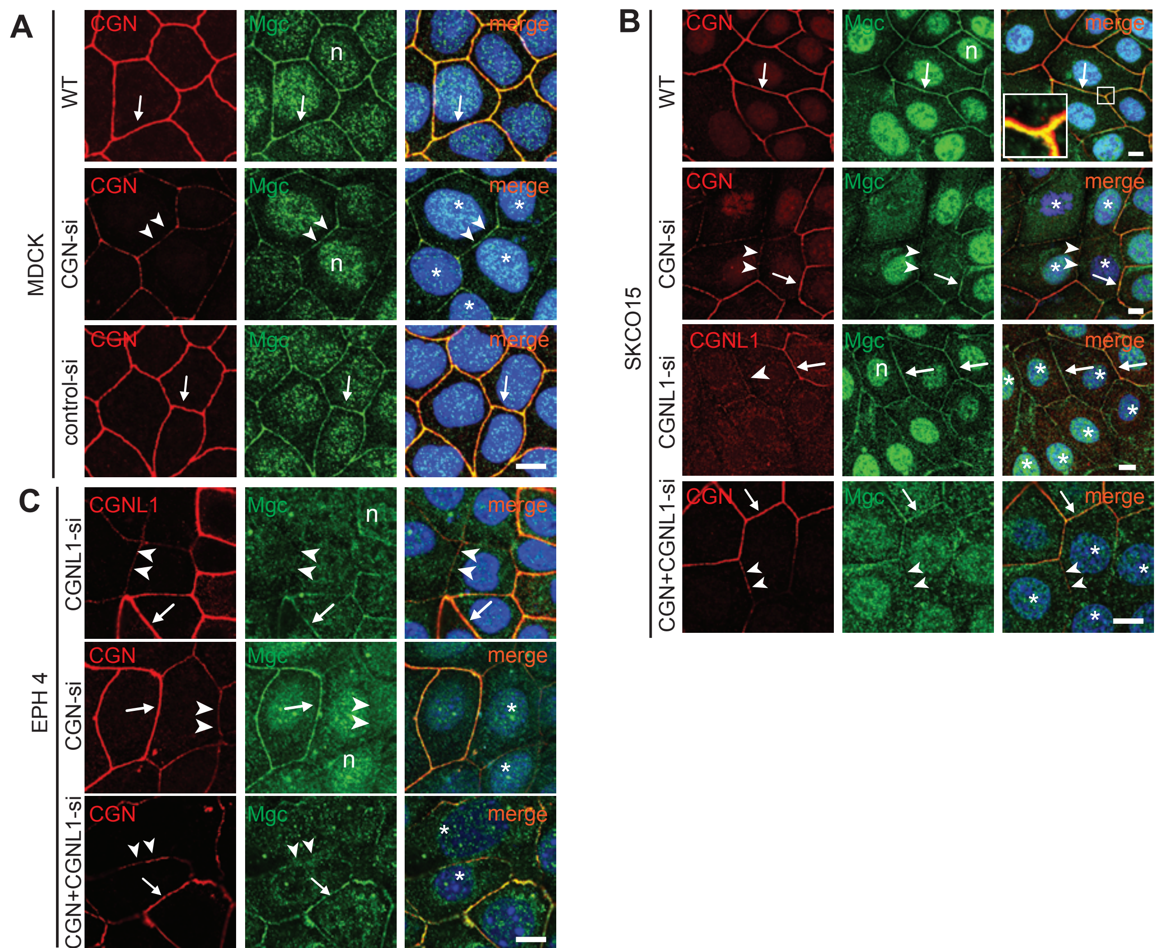
Figure S2. Effect of CGN and CGNL1 depletion on the localization of MgcRacGAP in different epithelial cell types. Immunofluorescence analysis (CGN/CGNL1-red, MgcRacGAP-green, ZO-1-gray, DAPI-blue) of **(A):** WT MDCK cells, and MDCK cells transfected with either CGN or control siRNA; **(B):** either WT SKCO-15 cells (WT), or a mix of WT cells and cells where either CGN (CGN-si) or both CGN and CGNL1 (CGN+CGNL1-si) were depleted through siRNA expression. The magnified square in the WT merge image shows the partial co-localization of CGN and MgcRacGAP labelling. Note that upon CGNL1 depletion MgcRacGAP is still detectable at junctions (arrow in CGNL1si, green). **(C):** mixes of WT Eph4 cells and Eph4 cells where either CGNL1 (CGNL1-si), CGN (CGN-si) or both CGN and CGNL1 (CGN+CGNL1-si) were depleted through siRNA expression. “n” = nuclear labelling for MgcRacGAP. Asterisks in merge images=siRNA-depleted cells. Double arrowheads= junctions with reduced CGN and MgcRacGAP labelling. Arrows= junctions between WT cells showing both CGN and MgcRacGAP labelling. Bar = 5 μm.

Figure S3. Exogenously expressed MgcRacGAP forms a complex with exogenously expressed CGN or CGNL1. **(A-B):** Immunoblotting analysis of immunoprecipitates of lysates of MDCK cells expressing (+) or not-expressing (-) exogenous myc-tagged MgcRacGAP, and either YFP-tagged CGNL1 ((A) or CGN (B), or YFP-myc (negative control). Immunoprecipitation was carried out using either anti-GFP antibodies, or anti-HA antibodies (negative control), and immunoblotting using anti-MgcRacGAP, to detect immunoprecipitated MgcRacGAP, or anti-myc, to detect exogenously expressed proteins (arrowheads) in IPs. The asterisk indicates a non-specific band, of size smaller than YFP-myc, recognized by the anti-myc antibodies. Note that MgcRacGAP is detected in both CGNL1 and CGN immunoprecipitates. **(C-D):** Immunoblotting analysis, with anti-myc antibodies, of the input of the lysates used for immunoprecipitation in panels A and B, respectively. Arrowheads indicate

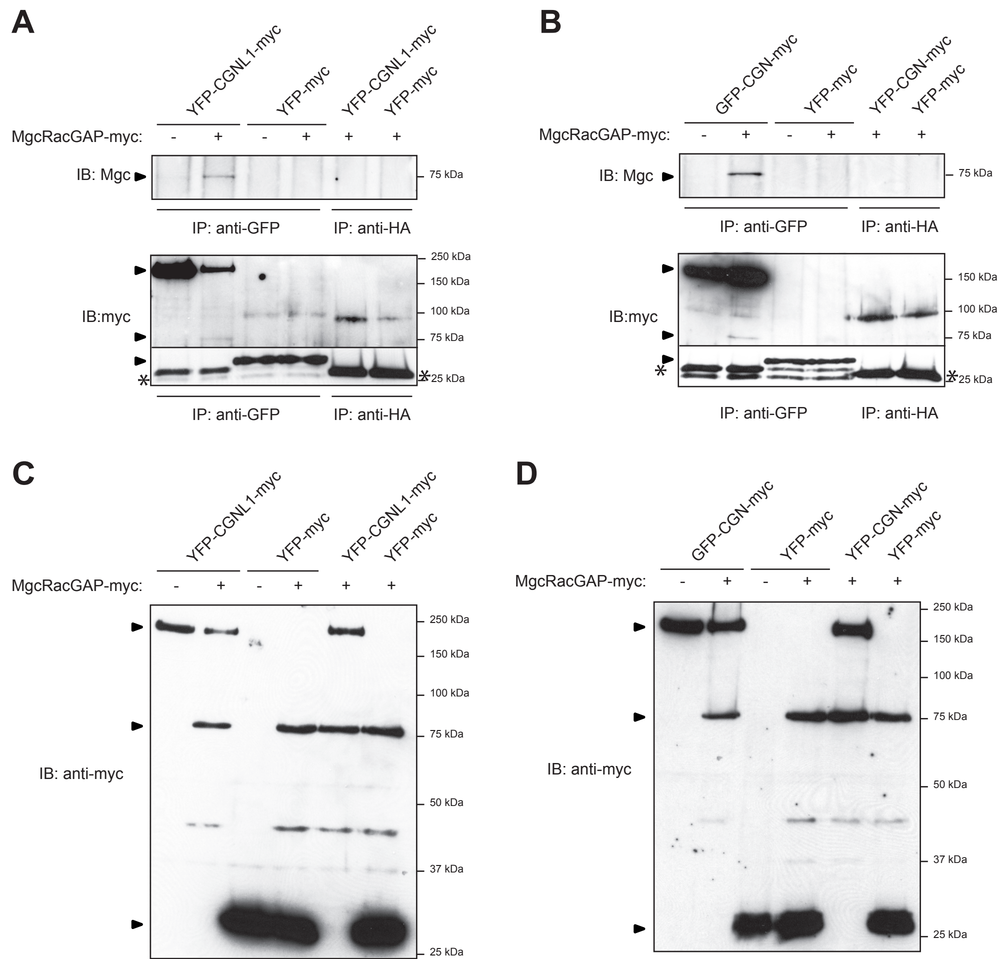
migration of YFP-CGNL1 or YFP-CGN, MgcRacGAP-myc, and YFP-myc, from top to bottom. Migration of molecular size markers is indicated on the right (kDa).

Figure S4. Depletion of CGN and/or CGNL1 does not affect the localization of ECT2. Immunofluorescence analysis (CGN/CGNL1-red, ECT2-green, DAPI blue in merge images) of **(A):** mix of WT and CGN-KO keratinocytes, at different times (8 hr, 24 hr, 72 hr) after calcium-induced differentiation (the image WT+KO in Fig. 5 corresponds to the 48 hr time point, and the image WT in Fig. 5 is a magnification of a junction between WT cells in the 24 hr time point). **(B):** SKCO-15 cells: WT, or a mix of WT cells and cells where either CGN (CGN-si) or CGNL1 (CGNL1-si) are depleted through siRNA expression; Control-si was used as a negative control. Heterogeneous cellular labeling for ECT2 is indicated: CF= cleavage furrow; N = nuclear; Cyt = cytoplasmic; Neg= negative cell; Asterisks=KD/KO cells. Arrows= junctions between WT cells showing both CGN/CGNL1 and ECT2 labelling. Bar = 5 μm.

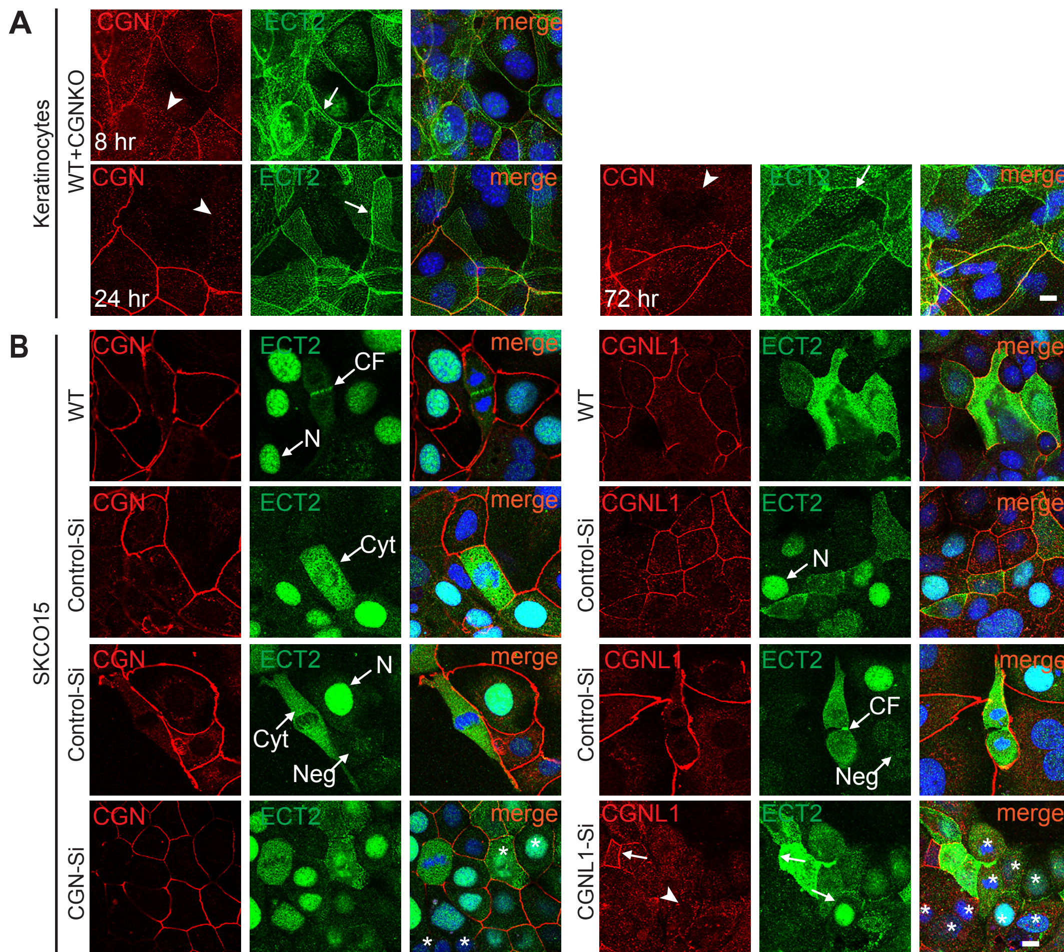
Supplementary Figure 1



Supplementary Fig. 2



Supplementary Fig. 3



Supplementary Fig. 4