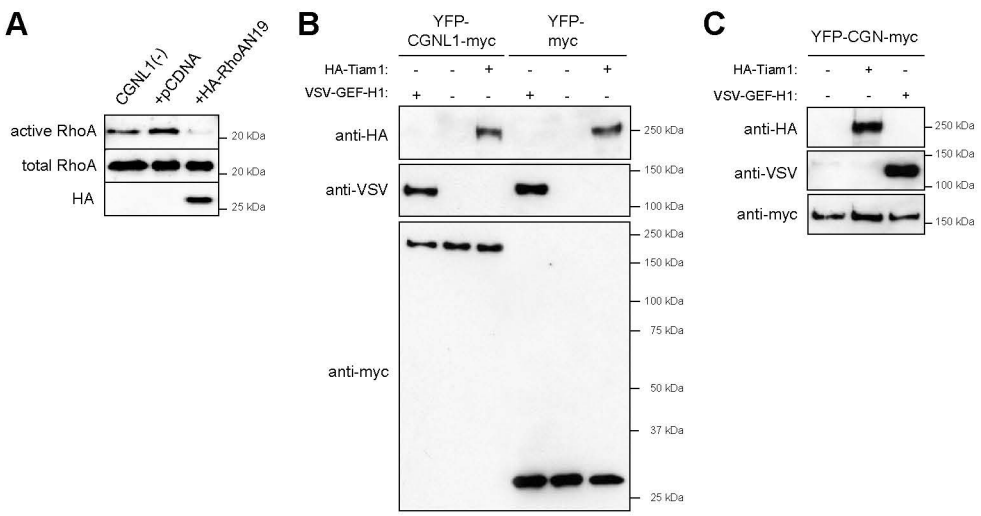

Figure S1 Immunoblot analysis of RhoA activity and exogenous protein expression in our experimental cell lines. (A) Expression of dominant-negative RhoA reduces RhoA activity in CGNL1(-) cells. Active RhoA, isolated by affinity purification on GST-RBD beads, is substantially decreased following expression of RhoAN19, but not empty vector. (B, C) YFP- and myc-tagged paracingulin (B), YFP- and myc-tagged cingulin (C), HA-tagged Tiam1 (B, C), and VSV-tagged GEF-H1 (B, C) are expressed after transfection of the respective constructs into WT MDCK cells, and show the appropriate molecular sizes, as detected by immunoblotting with antibodies against HA, VSV and myc. Control cells were transfected with YFP-myc.

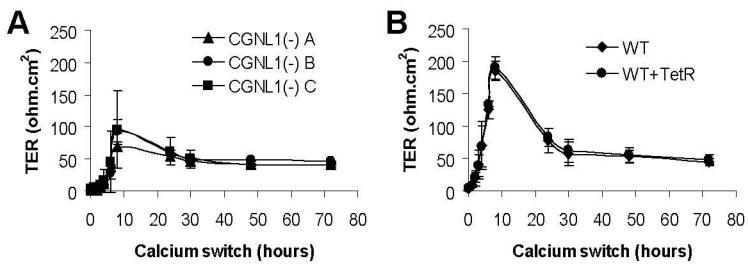

Figure S2 Depletion of paracingulin abolishes the peak of TER in three independent CGNL1(-) cell clones. (A) Three independent clones of CGNL1(-) cells (A, B, and C) show a decreased peak of TER during the calcium-switch. (B) WT cells expressing the TetR show the same pattern of TER development as WT cells, and display a peak of about 180 ohm.cm^2 8 h after the calcium-switch.

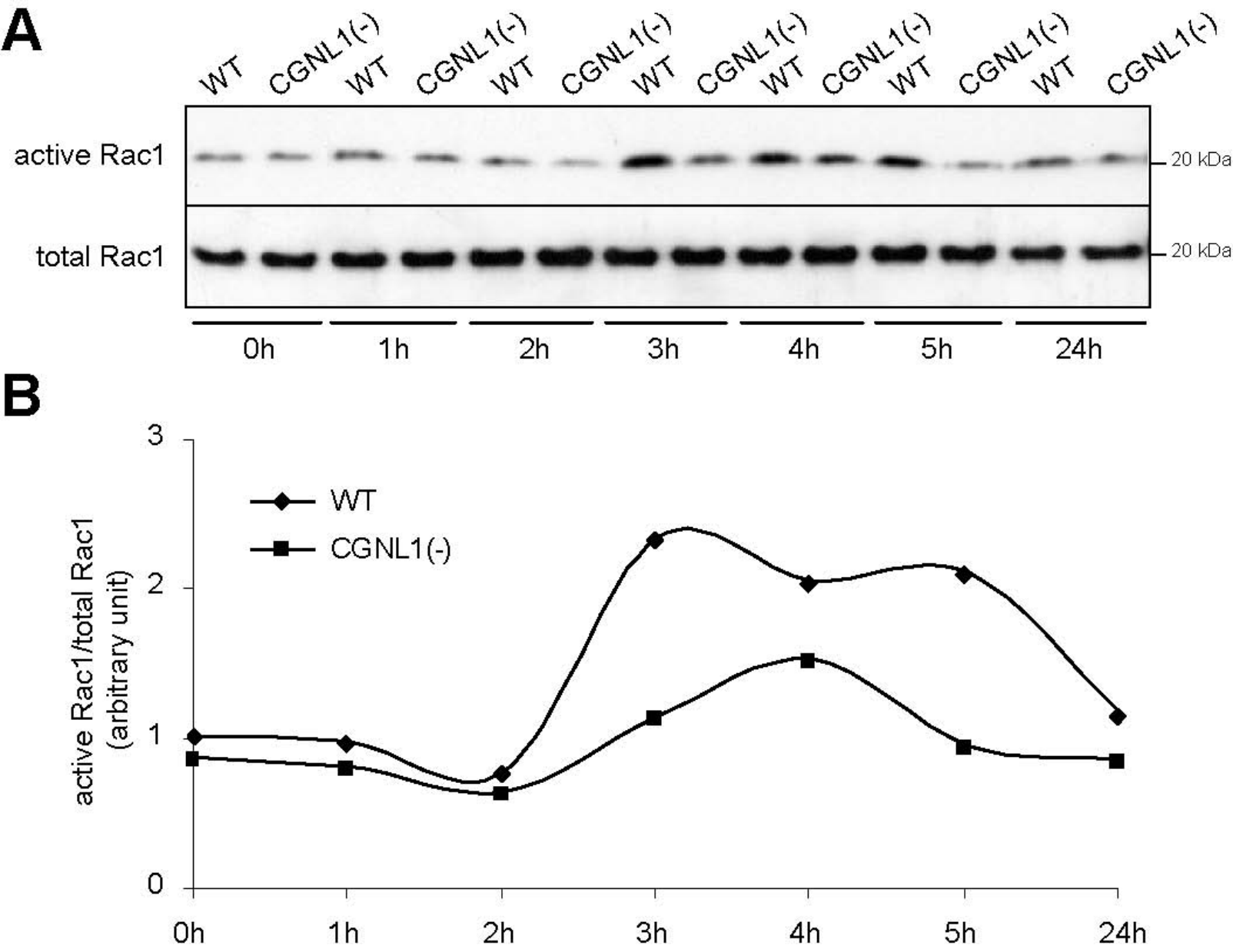

Figure S3 The second wave of Rac1 activation during the calcium-switch is decreased in CGNL1(-) MDCK cells. (A) Immunoblotting analysis and (B) densitometry of active Rac1 (isolated by affinity purification on GST-PBD beads) versus total Rac1 in cell lysates prepared at different times (indicated below each lane) show that CGNL1 depletion causes a decrease of the second peak of Rac1 activation during the calcium-switch.

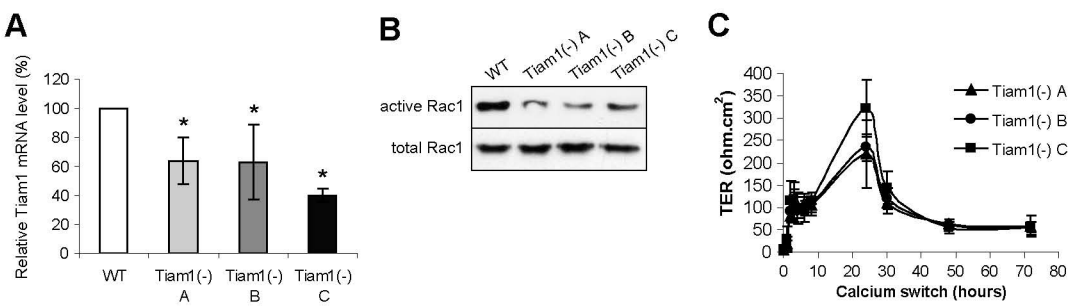

Figure S4 Tiam1 depletion in MDCK cells induces a delay of the TJ formation. (**A**) Tiam1 depletion in three independent Tiam1(-) cell clones. qRT-PCR analysis of Tiam1 mRNA levels in MDCK clones (A, B and C) expressing a shRNA against Tiam1. Relative mRNA levels are calculated as the ratio of the Tiam1 mRNA level in Tiam1(-) clones normalized to that of WT cells (100%). Values represent the means±S.D. of two independent RNA preparations. *$p<0.05$, compared with the Tiam1 mRNA level of WT cells. (**B**) Tiam1 depletion reduces Rac1 activity. Rac1 pull-down assay showing active versus total Rac1 activity in WT and Tiam1(-) cells. Data are representative of two independent experiments. (**C**) Tiam1 knockdown induces a delay in the apparition of the peak of TER in three independent Tiam1(-) cell clones during a calcium-switch assay. Formation of TJ is evaluated by measuring the TER of various Tiam1(-) clones (A, B and C). Data represent the means±S.D. of two independent experiments.