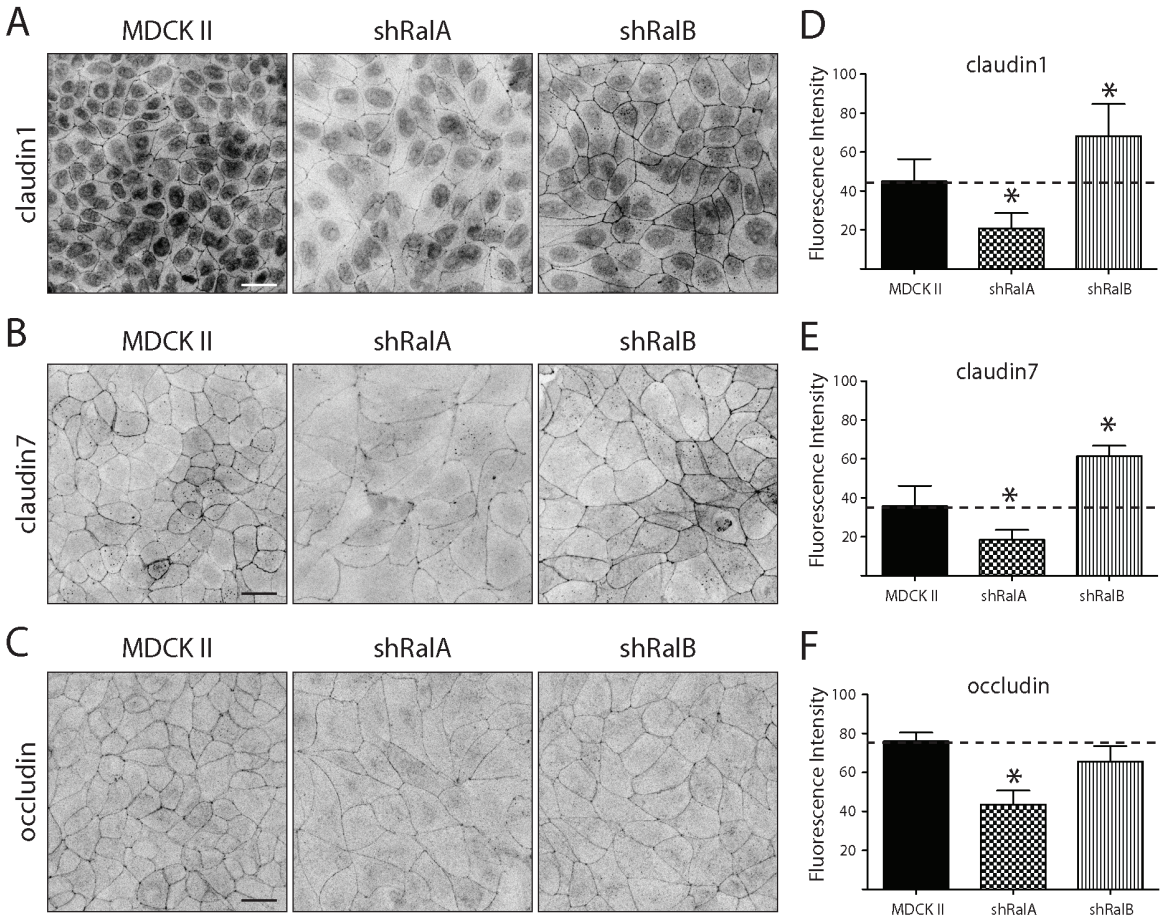
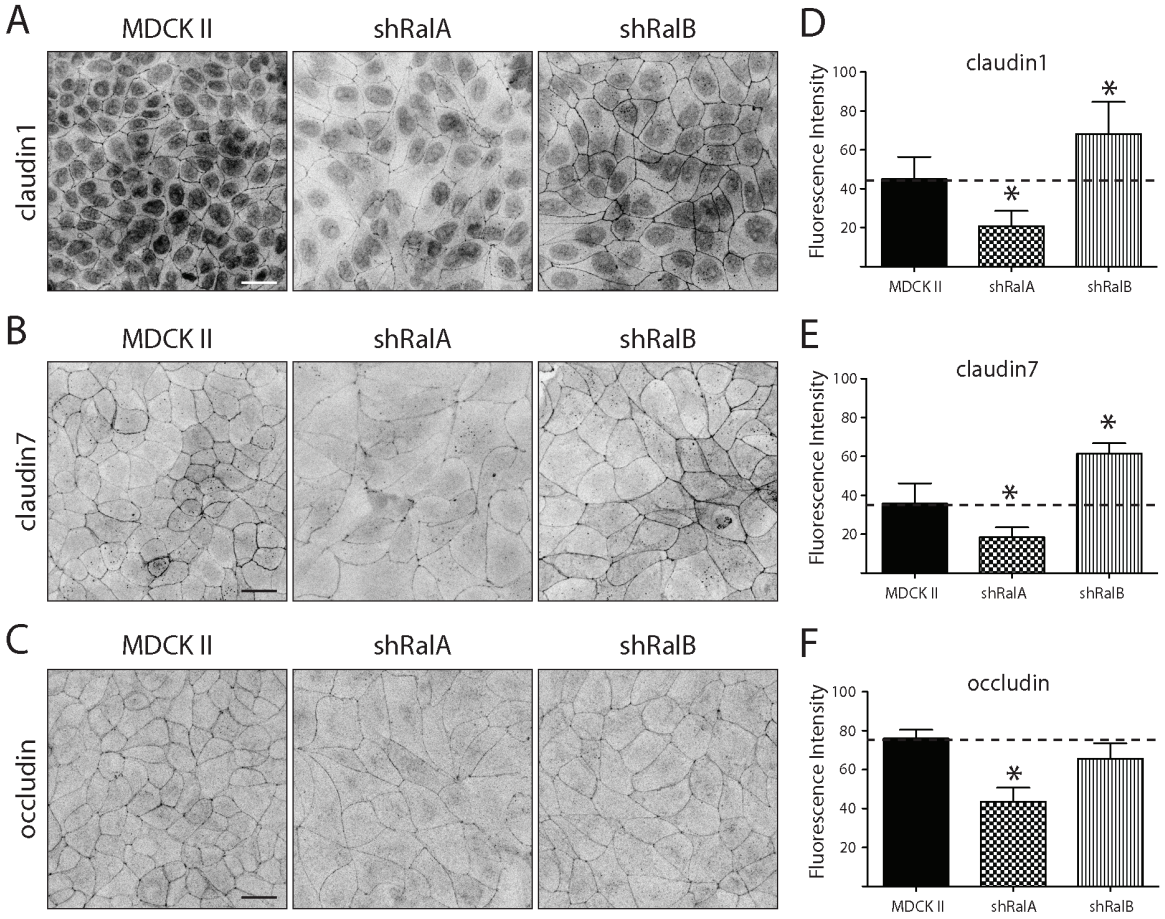
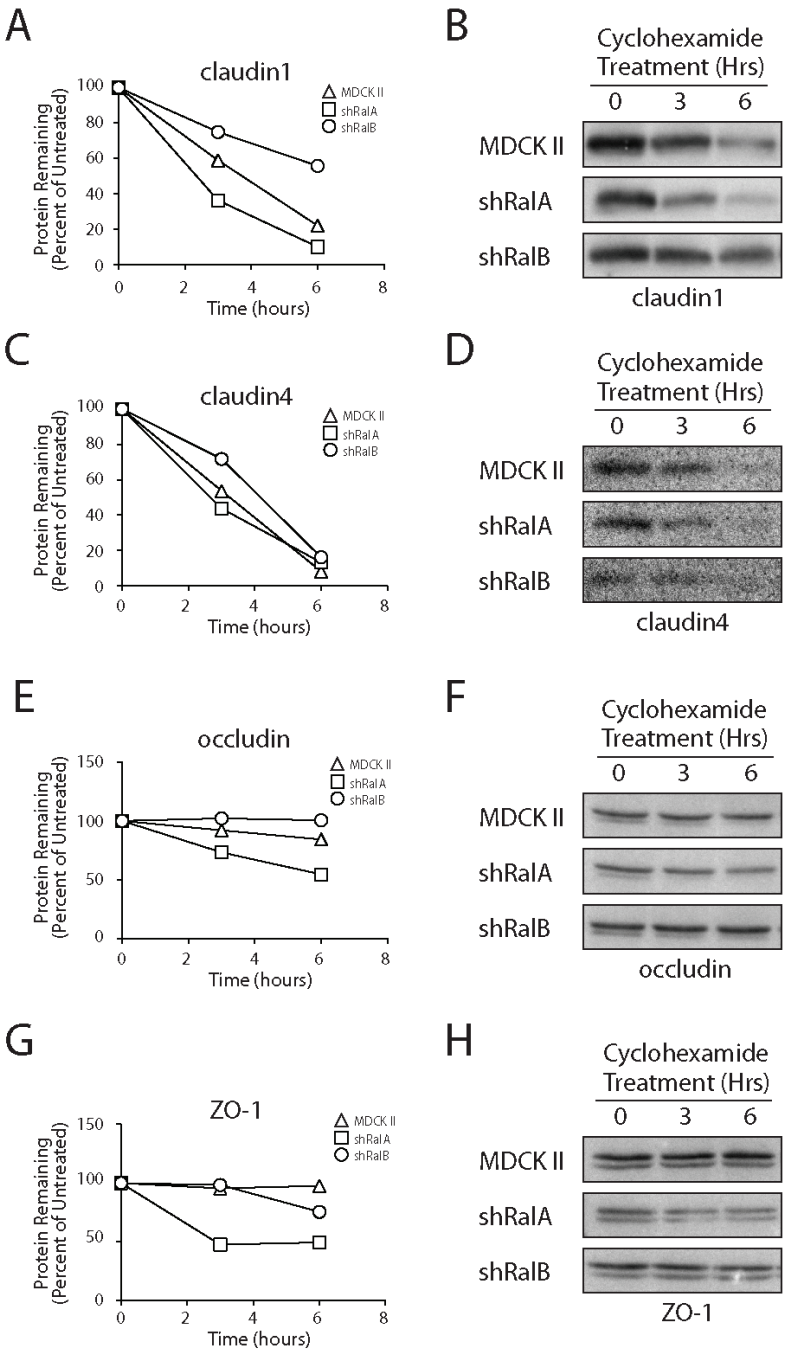


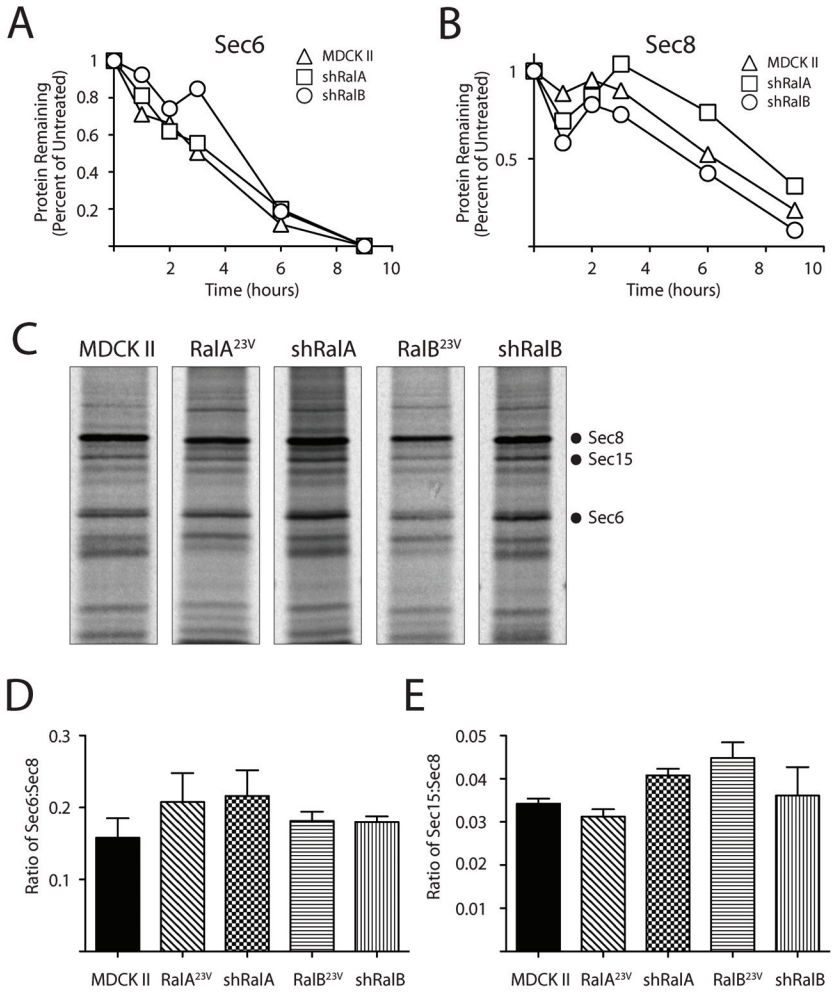
Supplemental Figure 1. TJ composition is affected by Ral knockdown. (A-C) RalA and RalB knockdown differentially affects the incorporation of components into the TJ. MDCK II, shRalA and shRalB cells were subjected to a Ca^{2+}-switch and processed for immunofluorescence 10 hours later. For each protein examined (claudin1, claudin7 and occludin), optical sections were merged into a single stacked image and inverted. Bars, 20 μm. (D-F) Fluorescence intensities of TJ labeling in each cell type were traced and quantified using ImageJ software. Images are representative of three independent experiments. Error bars represent SD of 5 lengths of TJ. Asterices, $p<.05$.



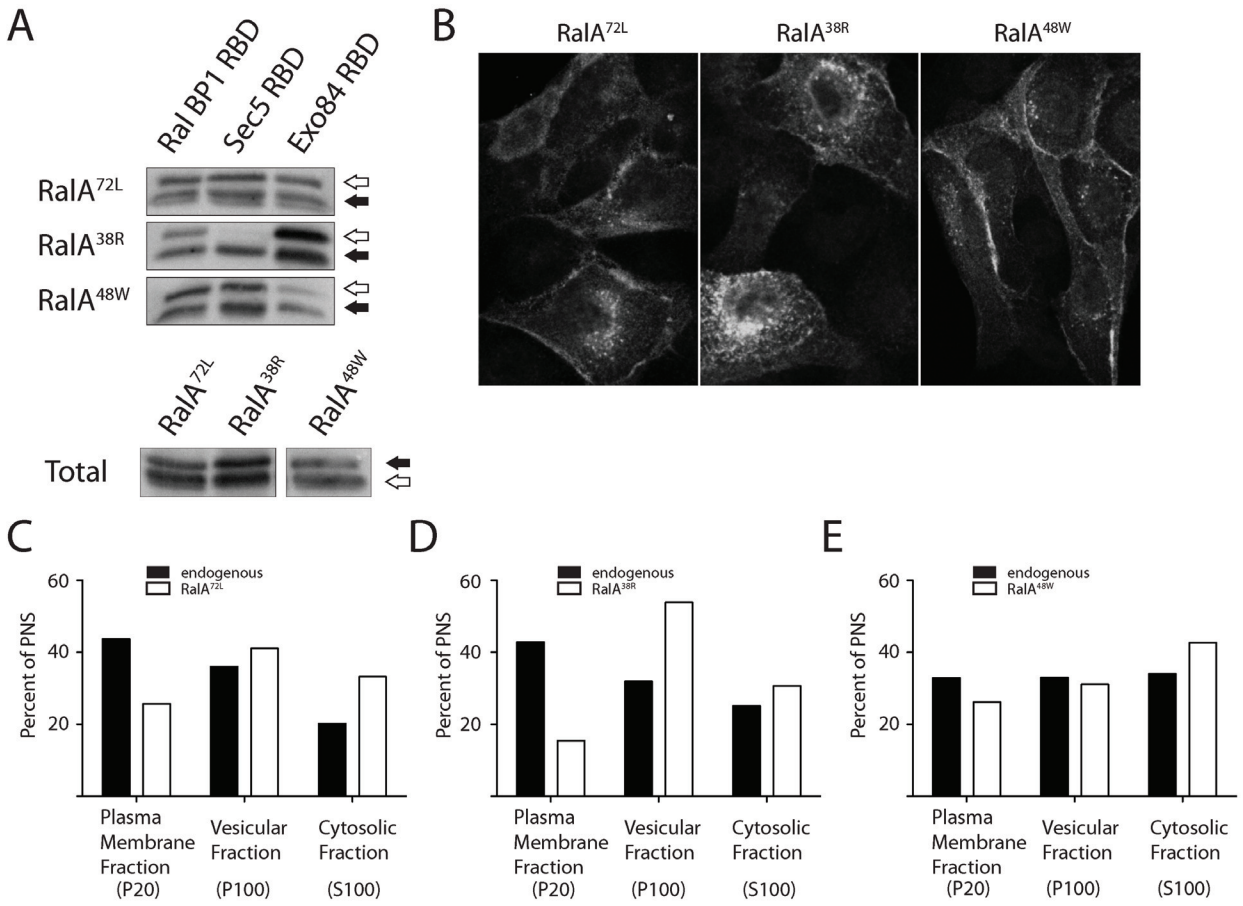
Supplemental Figure 2. Ral knockdown differentially affects claudin integration into TJs. (A-J) A Ca^{2+} switch was performed with MDCK II, shRalA and shRalB cells and samples were processed for Triton-X 100 solubility analysis and immunoblotting with indicated antibodies 0, 10, and 65 hours later. 10 hour immunoblot signals were quantified using phosphorimager screens and ImageQuant software. Shown are representative data of three independent experiments.



Supplemental Figure 3. Ral knockdown alters TJ protein stability. (A-H) RalA knockdown decreases stability of some TJ proteins while RalB knockdown increases stability of TJ proteins. Confluent MDCK II, shRalA and shRalB cells were left untreated or were treated with cyclohexamide for 3 or 6 hours. Following treatment, samples were processed and immunoblotting was performed with antibodies specific to the indicated proteins. Immunoblot signals were quantified using phosphorimager screens and ImageQuant software. Shown are representative data of three independent experiments.



Supplemental Figure 4. Ral knockdown does not affect stability or assembly of the Exocyst in MDCK II cells. (A, B) Sec6 and Sec8 stability is not affected by Ral knockdown. Confluent MDCK II, shRalA and shRalB cells were left untreated or were treated with cyclohexamide for 1, 2, 3, 6, or 9 hours. Following treatment, cell lysates were probed by immunoblot and quantified using phosphorimager screens and ImageQuant software. (C) MDCK II, RalA^{23V}, shRalA, RalB^{23V} and shRalB cells were pulse-labeled with ^{35}S methionine and Sec8 was immunoprecipitated. Positions of Sec8 and co-precipitating Sec6 and Sec15 were independently verified and are indicated by dots. (D-E) Ral does not affect assembly of newly synthesized Exocyst complex. Ratios of Sec6:Sec8 and Sec15:Sec8 in each cell type were quantified to determine amounts of newly assembled Exocyst in cells using phosphorimager screens and ImageQuant software. Error bars represent the SD of three independent experiments.



Supplemental Figure 5. Exocyst-uncoupled RalA mutants have different subcellular localizations. (A) Binding of RalA^{38R} and RalA^{48W} variants to Sec5 and Exo84 is specifically disrupted. Ectopic and endogenous active Ral (Ral^{GTP}) proteins were precipitated from lysates of MDCK II cells induced to express the indicated mutant GTPases, using glutathione beads coupled to either GST-Ral BP1, GST-Sec5 or GST-Exo84 Ral Binding Domain (RBD) fusion proteins. Precipitates and aliquots of total cell lysates were resolved by SDS-PAGE and immunoblotted with anti-RalA antibodies. Open arrows indicate positions of myc-tagged, ectopic RalA mutants and closed arrows indicate endogenous RalA. (B) RalA^{72L} was distributed between the plasma membrane and endomembranes, while RalA^{38R} localized primarily to intracellular structures and the majority of RalA^{48W} localized largely to the plasma membrane. MDCK II cells induced to express RalA^{72L}, RalA^{38R} and RalA^{48W} variants were seeded on coverslips at subconfluent densities and labeled with an anti-myc antibody. (D-F) Differential sedimentation analysis revealed a similar redistribution of mutant RalA as observed in (C). Non-

induced and cells induced to express RalA^{72L}, RalA^{38R} and RalA^{48W} variants were mechanically homogenized and organelles were separated by differential sedimentation. Aliquots of 20,000 and 100,000 x g pellets (P20 and P100) and 100,000 x g supernatent (S100) were processed by immunoblotting for RalA, and signals were quantified using a phosphorimager screen and ImageQuant software.