# Supplemental Materials

***Molecular Biology of the Cell***

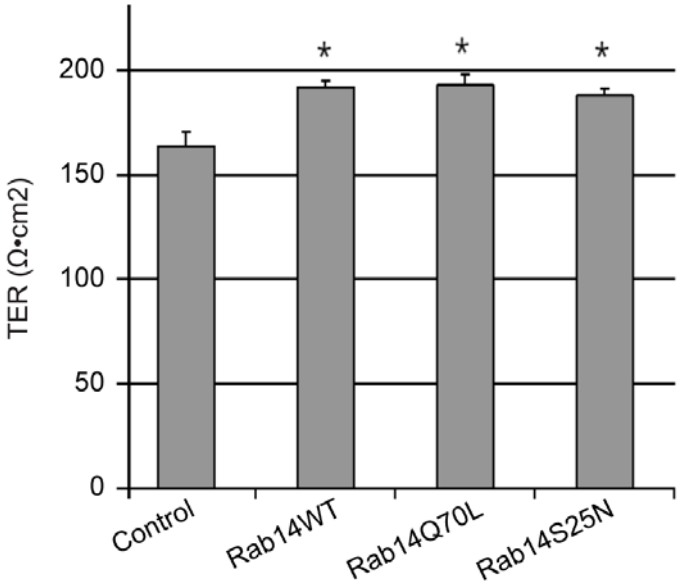
Lu et al.

**Supplemental Figure 1.** A. Expression of Rab14-WT, Rab14-Q70L or Rab14-S25N slightly but significantly increases the TER of MDCK monolayers. B. Claudin-1 does not colocalize with Rab11 or Rab13. Scale bar, 10μm. *$p < 0.01$.

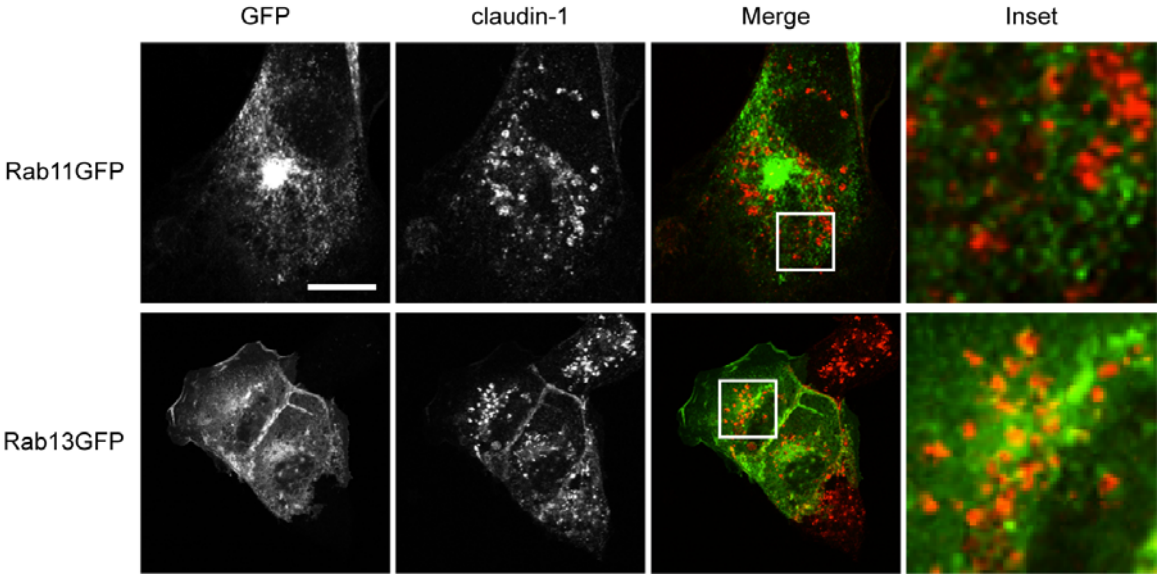
**Supplemental Figure 2.** Cells expressing Rab14-GFP were plated on Transwell filters and grown for 7 days, followed by fixation and labeling for the tight junction proteins indicated. Puncta of claudins -1, -2, and -4 colocalize with Rab14-GFP at the cell periphery (arrows). There is no colocalization with occludin. Scale bar, 10μm.

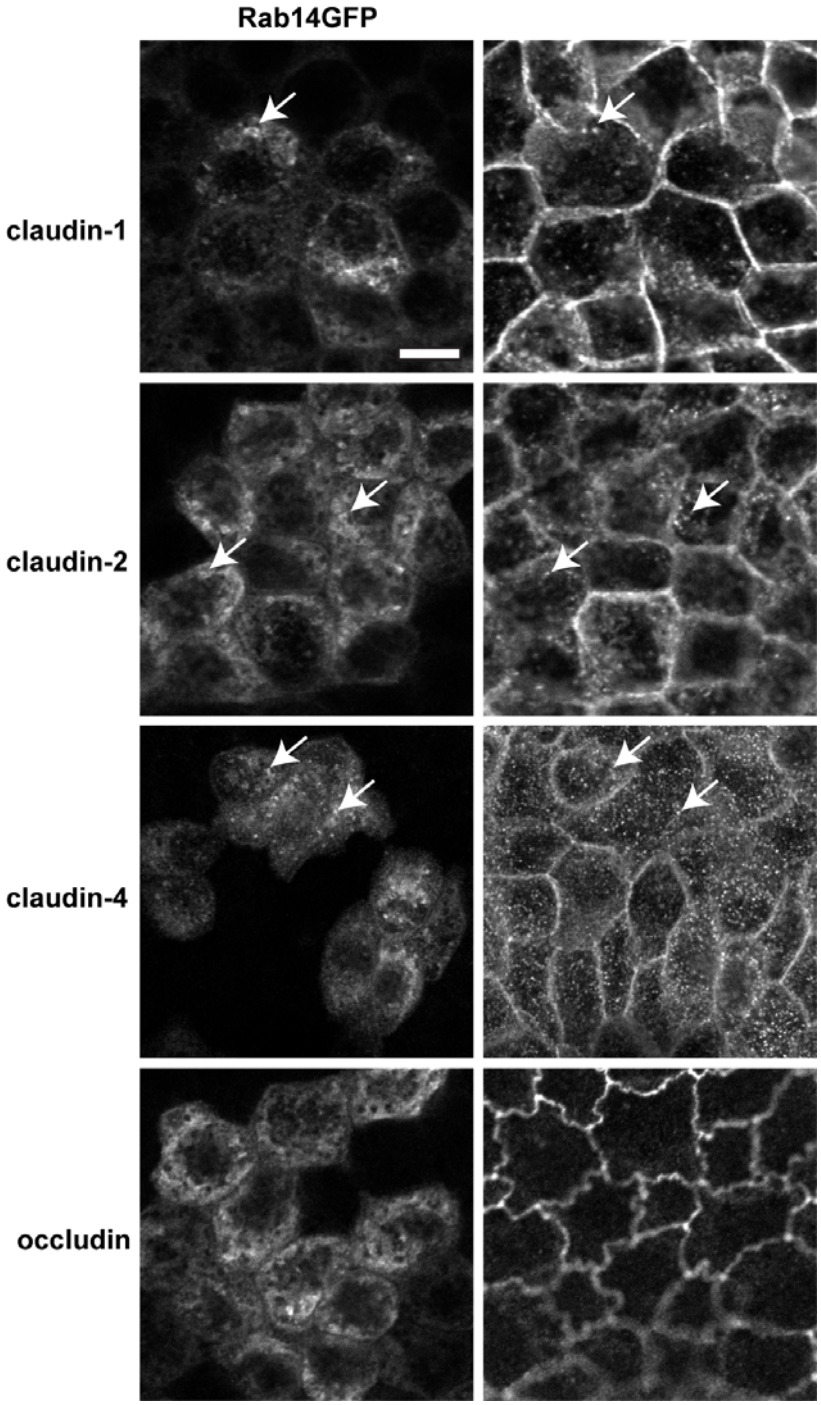
**Supplemental Figure 3.** A. Western blot analysis of expression levels of Rab14-GFP compared to endogenous Rab14. Over-expressed Rab14-GFP or mutant forms constitutes less than thirty percent of the total Rab14. B. Expression of Rab14-GFP, Rab14Q70L-GFP results in decreased claudin-2. There is no change observed in claudin-2 levels with Rab14S25N-GFP overexpression. These small changes are likely due to the presence of substantial functional endogenous protein.

**A**

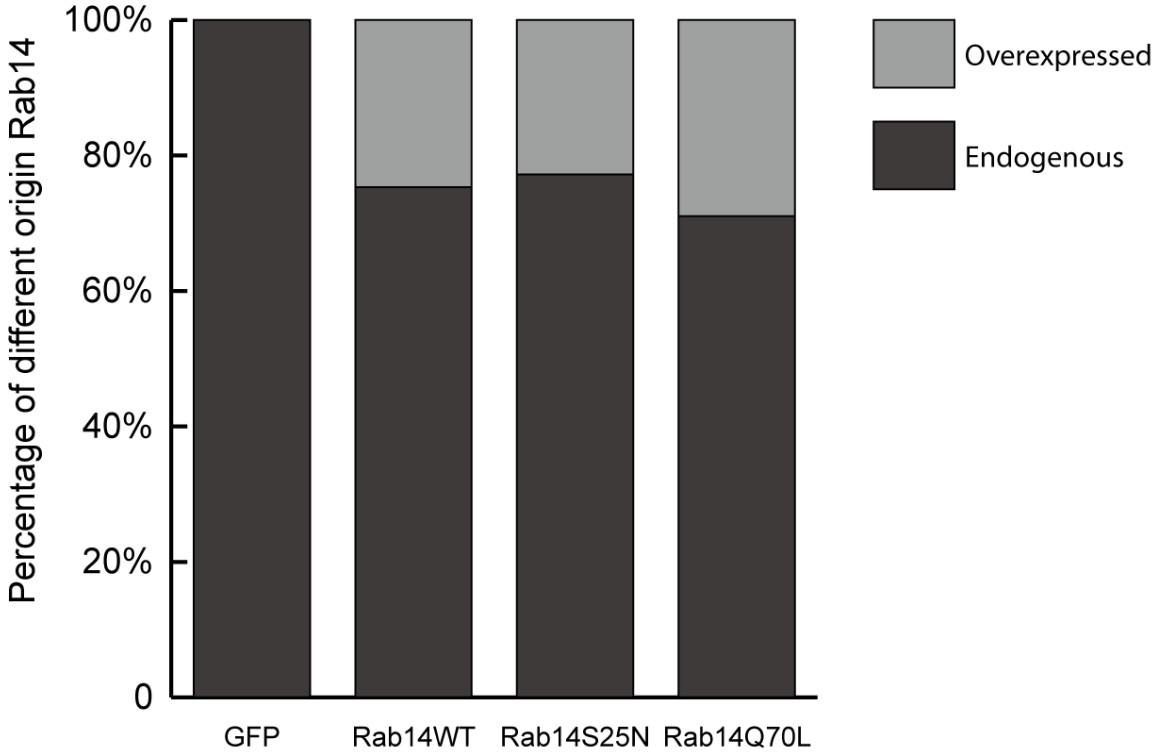

TER (Ω•cm2)
200
150
100
50
0
*
*
*
Control
Rab14WT
Rab14Q70L
Rab14S25N

**B**

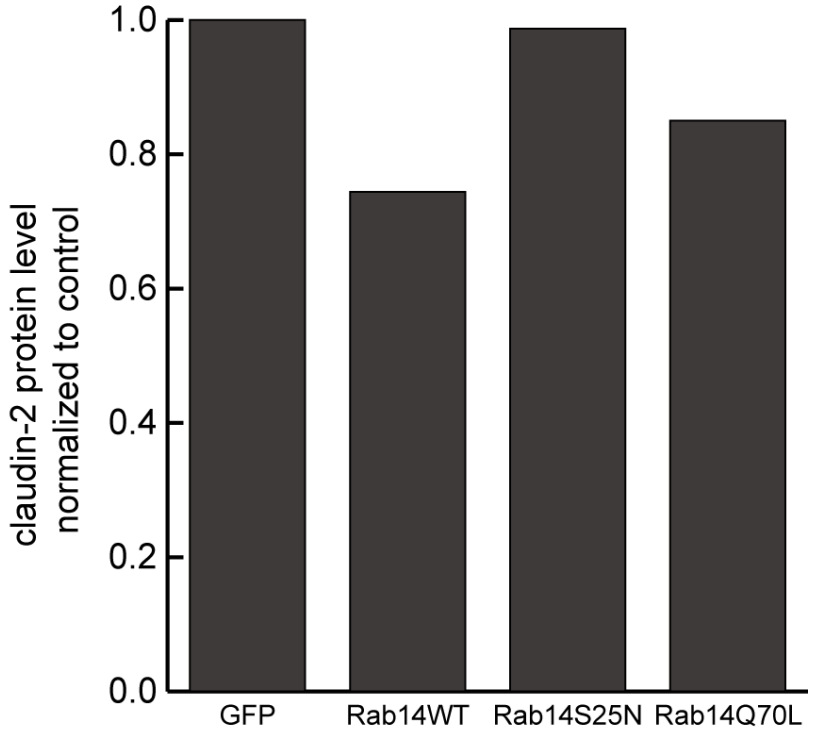

GFP
claudin-1
Merge
Inset
Rab11GFP
Rab13GFP


Rab14GFP
claudin-1
claudin-2
claudin-4
occludin

**A**


Percentage of different origin Rab14
100%
80%
60%
40%
20%
0
GFP
Rab14WT
Rab14S25N
Rab14Q70L
Overexpressed
Endogenous

**B**


claudin-2 protein level
normalized to control
1.0
0.8
0.6
0.4
0.2
0.0
GFP
Rab14WT
Rab14S25N
Rab14Q70L