Supplemental Materials

Molecular Biology of the Cell

Farr et al.

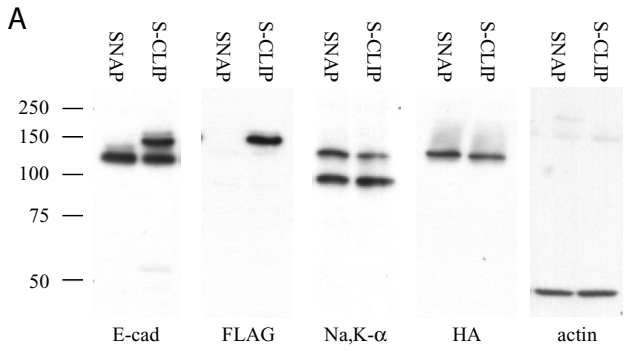
Supplemental Figure-1. Generation and validation of the SNAP-CLIP cell line (A) Samples were lysed in Triton X-100 buffer and analyzed by 8% SDS PAGE prior to western blotting with the indicated antibodies. (B) Filter grown cells were fixed and stained with the indicated antibodies as described in the legend for Figure 4. E-cadherin (red), gp135 (blue) and ZO-1 (green).

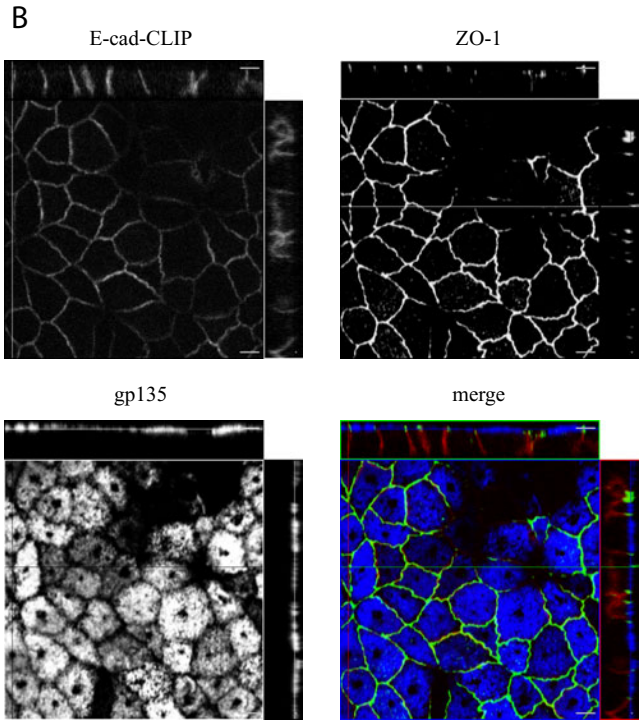
Supplemental Figure 2. E-cadherin exits the ER more rapidly than does the Na,K-ATPase. SNAP-CLIP cells were blocked and pulsed for the indicated times, and newly synthesized E-cadherin and Na,K-ATPase were recovered with BG-biotin and streptavidin as described in the Methods Section. Recovered proteins were mock treated or subjected to digestion with Endo H or PNGase F, after which they were separated by SDS-PAGE and analyzed by western blotting using antibodies directed against E-cadherin or the Na,K-ATPase β-subunit. At the earliest time point tested for E-cadherin the high molecular weight precursor form was detected, but the protein had already acquired resistance to Endo H digestion. In contrast, the 45 kDa Endo H-sensitive immature form of the Na,K-ATPase β-subunit was still detected 30 minutes after the completion of a 30 minute pulse incubation. After 60 minutes of chase essentially all of the newly synthesized Na,K-ATPase β-subunit was in the 55 kDa Endo H-resistant mature form.

Supplemental Figure-3. During the 14°C Block, sodium pump and E-cadherin signals predominantly co-localize in the Golgi complex. (A-B) SNAP-CLIP cells were blocked and pulsed as described in the legend for Figure 4 prior to incubation of cells at

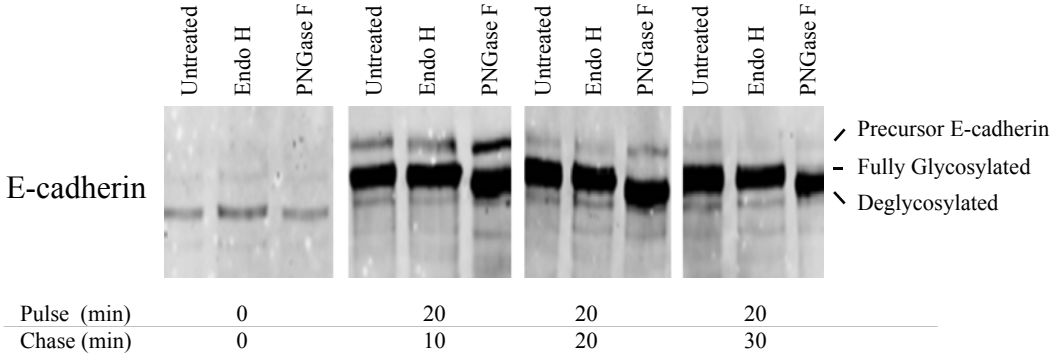
14°C. Samples were fixed and imaged as described above. CT-TMR is depicted in red, Alexa488-SNAP is shown in green and the indicated antibodies shown in blue. In panel A staining of the HA epitope tag (corresponding to the steady-state pool of Na,K-ATPase at the basolateral plasma membrane) is depicted in purple. (Bar = 5μ.)

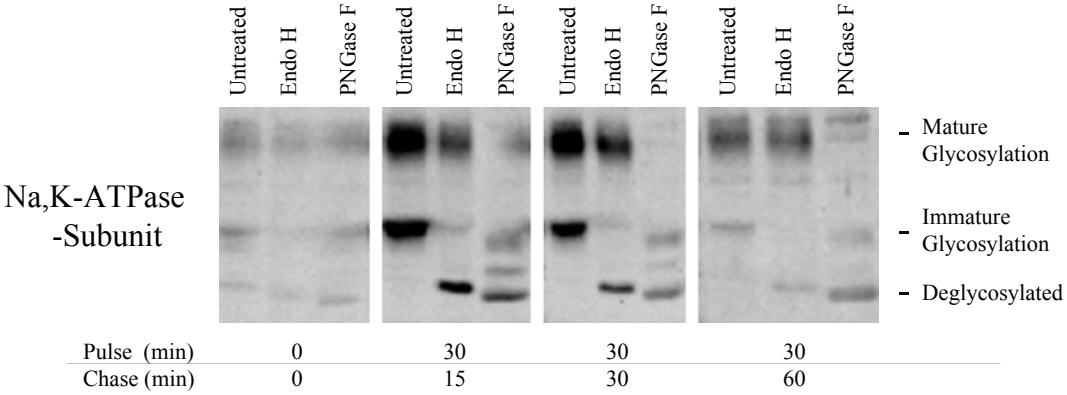
Supplemental Figure 4. Following incubation at 19^{o} C in the presence of BFA newly synthesized E-cadherin and Na,K-ATPase localize to different compartments. SNAP-CLIP cells were blocked and pulsed as described in the legend for Figure 4. Samples were incubated in the presence of 5 μg/ml BFA for 2h at 19^{o}C after which they were fixed, labeled and imaged. E-cadherin labeled with CT-TMR is depicted in red and Na,K-ATPase labeled with Alexa488-SNAP is shown in green. Staining for the Golgi marker Vti1a is depicted in blue. (Bar = 10 μ)



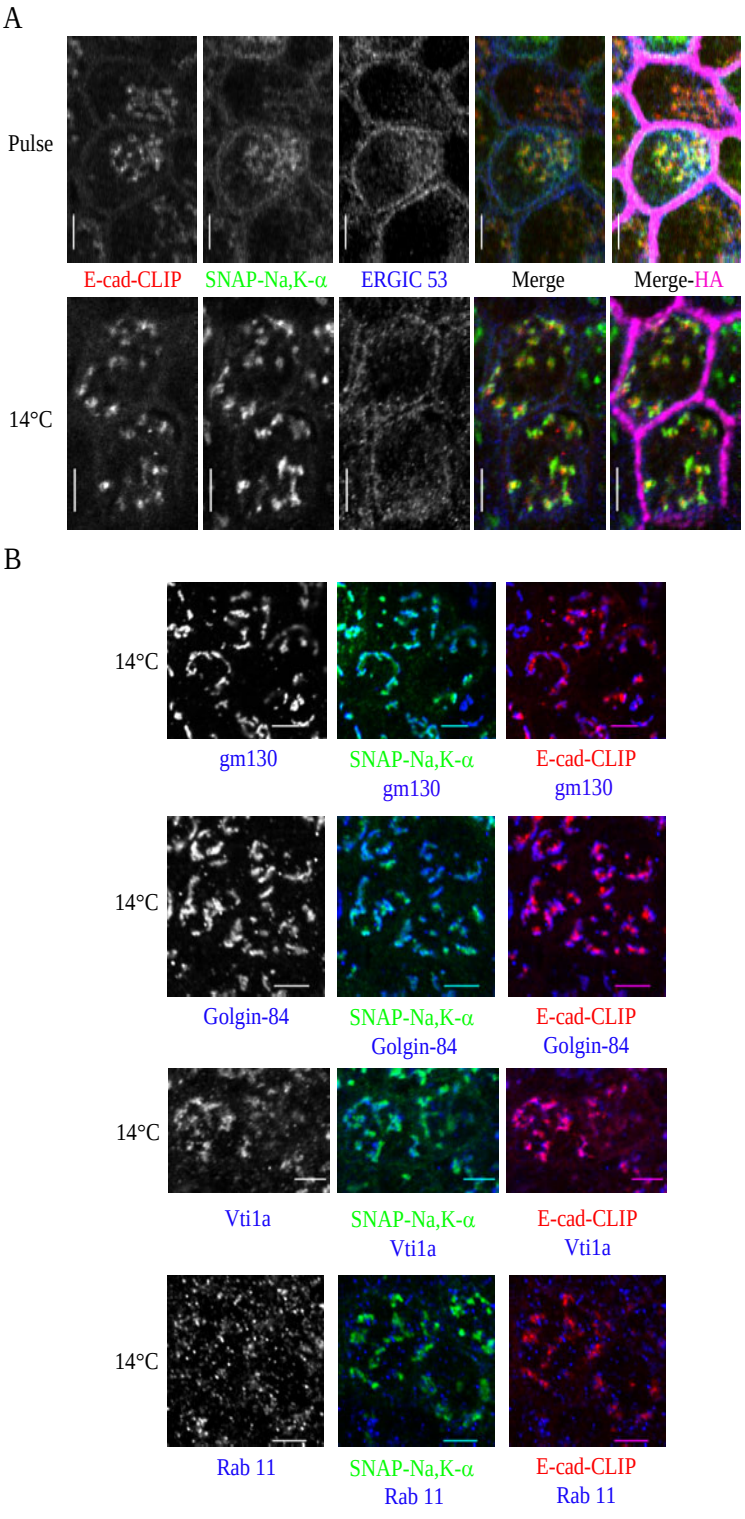


Supplemental Figure 1

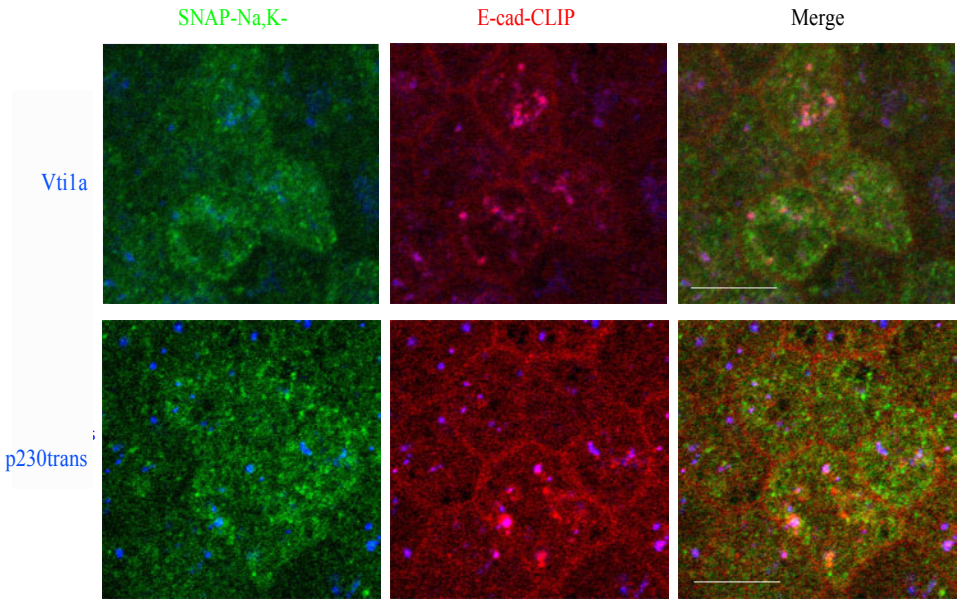




Supplemental Figure 2



Supplemental Figure - 3



Supplemental Figure - 4