Farr et al., http://www.jcb.org/cgi/content/full/jcb.200901021/DC1

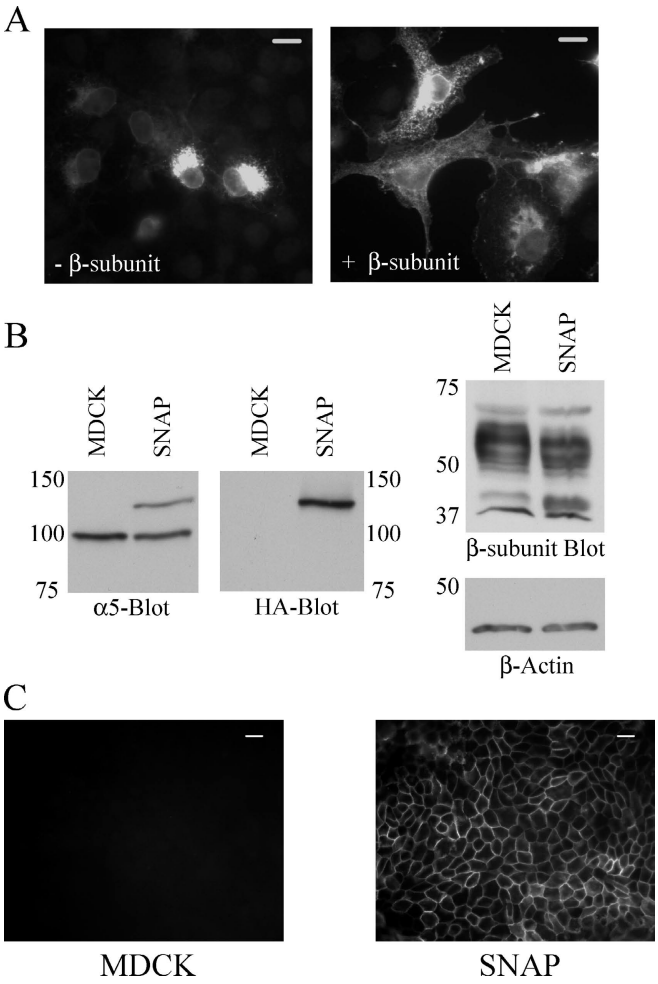

Figure S1. **Transient and stable expression of the SNAP-tagged Na pump.** (A) COS cells were transiently transfected with a plasmid encoding the SNAP-tagged Na,K-ATPase α1 subunit alone (−β subunit) or simultaneously with a plasmid encoding the Na,K-ATPase β1 subunit (+β subunit). 24 h after transfection, cells were fixed and processed for immunofluorescence with anti-HA antibodies. As expected, the SNAP-tagged α subunit is delivered to the cell surface only when coexpressed in mammalian cells in association with the Na,K-ATPase β subunit. (B) The rat α subunit used in the production of the SNAP-tagged construct is 1,000-fold less sensitive to the Na pump inhibitor ouabain than the canine pump expressed endogenously in MDCK cells. This was in fact observed and exploited to select a stably transfected MDCK cell line, indicating that addition of the SNAP tag does not affect pump activity. Western blots of wild-type MDCK cells and MDCK cells stably transfected with the SNAP-tagged Na pump are shown. Antibody α5 detects both endogenous Na pump and the SNAP tag fusion construct, whereas the SNAP construct was detected with anti-HA. gp58, an antibody which detects the Na,K-ATPase β subunit polypeptide, was used to measure total β subunit expression, and anti–β-actin was used as a loading control. Molecular mass is shown in kilodaltons. (C) Wild-type MDCK or SNAP cells were fixed and stained with TMR-STAR as described in Materials and methods. Bars, 20 μm.

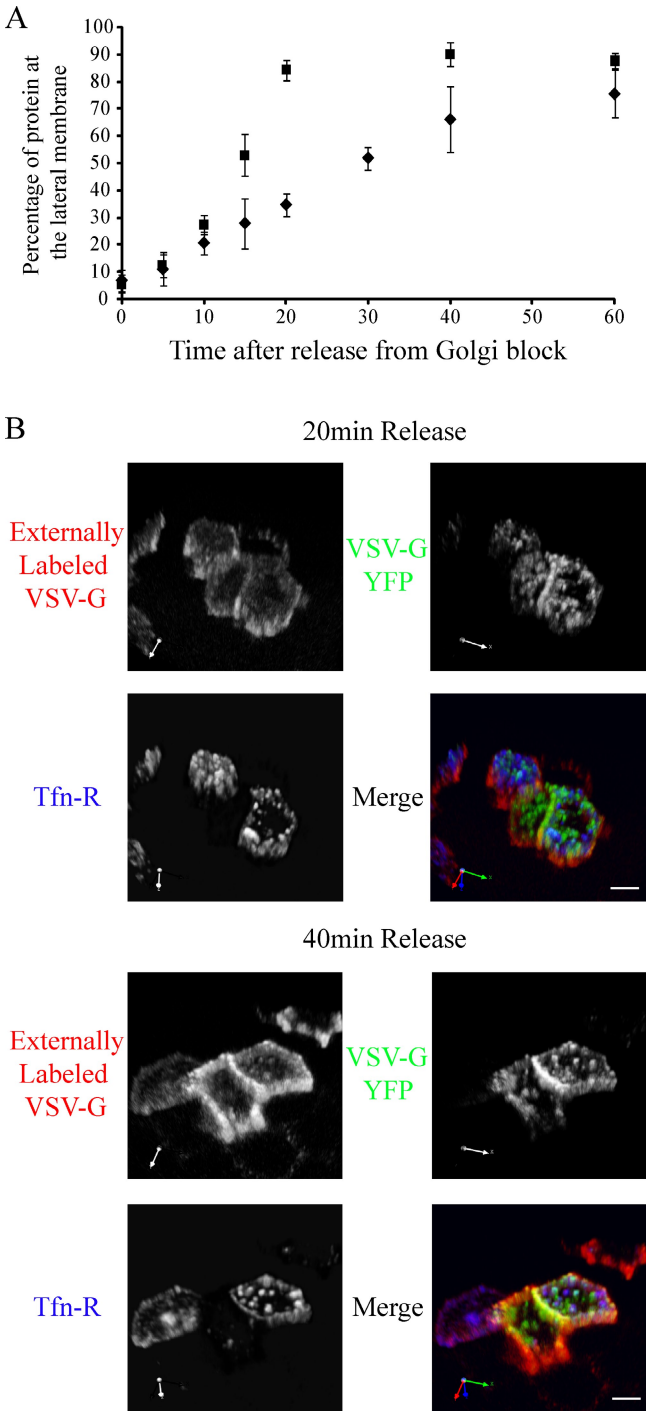

Figure S2. **Delivery of Golgi-accumulated Na pump to the lateral membrane.** (A) SNAP cells were infected with adenovirus expressing VSV-G–YFP Ts 045 as described in Fig. 5 B and Golgi blocked as in Fig. 3 B. Lateral membrane was detected with anti-HA antibody, whereas TGN-accumulated Na pump was labeled with TMR-STAR (■), and VSV-G (◆) was imaged by YFP fluorescence. Confocal z stacks were generated for each sample, and colocalization with the lateral membrane was determined as the percentage of total protein fluorescence throughout the entire stack that colocalized with HA staining at the membrane for the indicated time points after release from the Golgi block. Colocalization was quantified using the enhanced colocalization tool (LSM Image Examiner software). A minimum of 10 cells was analyzed for each time point, and the experiment was repeated in triplicate. Data are represented as mean ± SD. (B) SNAP cells were infected as in Fig. 5 B and Golgi blocked as in Fig. 3 B. However, during the Golgi block, an antibody directed against an extracellular epitope of VSV-G was added to the medium and maintained throughout the experiment. At the indicated time points after release from the Golgi block, cells were washed extensively at 4°C, fixed, and processed to reveal the localization of the surface-bound antibody (externally labeled VSV-G; red) and Tfn-R (blue). Very little of the VSV-G–YFP signal that remains intracellular after release from the Golgi block colocalizes with anti–VSV-G internalized from the surface or with Tfn-R, indicating that this intracellular pool does not represent VSV-G protein that has been delivered to the surface and internalized by endocytosis. Rather, this pool most probably represents VSV-G protein that has yet to complete its transit from the TGN to the PM. Bars, 5 µm.

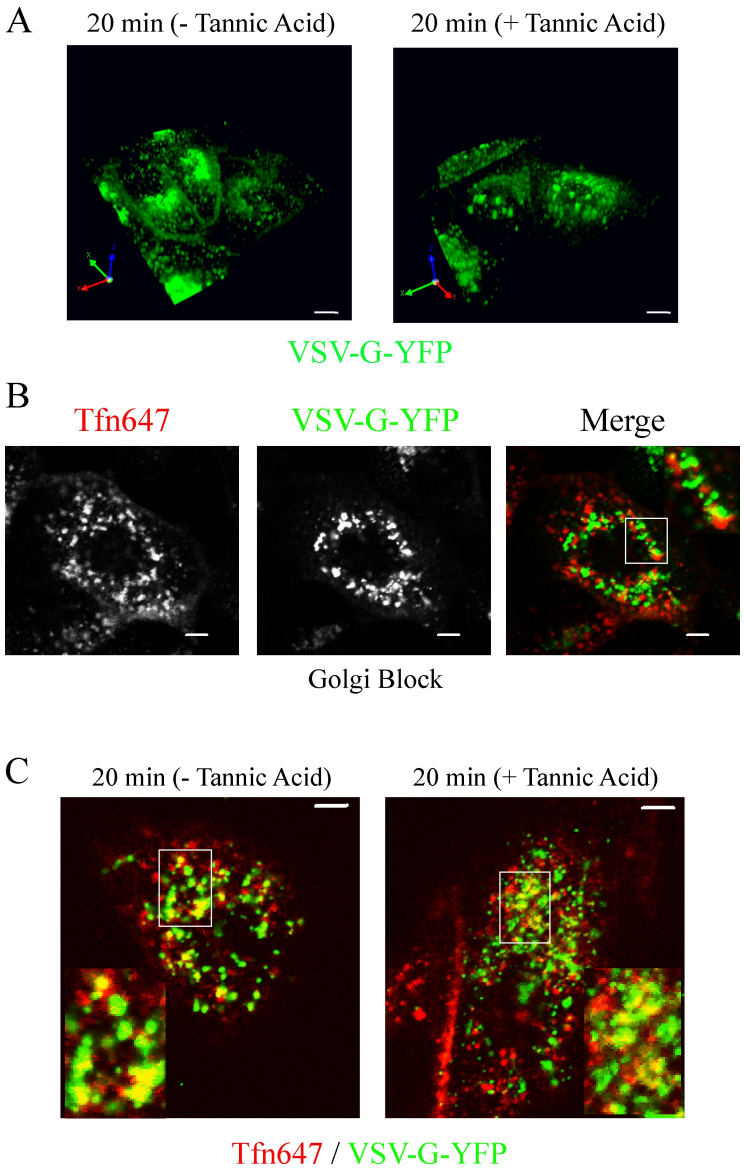

Figure S3. **VSV-G enters REs during biosynthetic delivery.** Subconfluent SNAP cells were infected with VSV-G/Tfn-R adenoviruses, and REs were labeled with Alexa Fluor 647–Tfn as described in Fig. 5 A. 10 min before the release of newly synthesized VSV-G protein from the 19°C Golgi block, cells were treated in the presence or absence of 0.5% tannic acid and either fixed immediately or warmed to 31°C to allow exit of VSV-G from the Golgi. (A) VSV-G traffics to the PM in the absence but not in the presence of tannic acid. Images are 3D reconstructions of confocal image stacks taken 20 min after release from the Golgi block. (B) Under Golgi block conditions, VSV-G and Tfn 647 reside within adjacent structures but do not significantly colocalize with one another. The inset shows that during the Golgi block, REs (red) and Golgi-localized VSV-G (green) reside in adjacent structures. (C) After release from the Golgi block, VSV-G enters Tfn 647–positive compartments in both control and tannic acid–treated cells. Insets show that upon leaving the Golgi, VSV-G colocalizes with REs, irrespective of tannic acid addition. Bars: (A and B) 5 µm; (C) 8 µm.