Esk et al., http://www.jcb.org/cgi/content/full/jcb.200908057/DC1

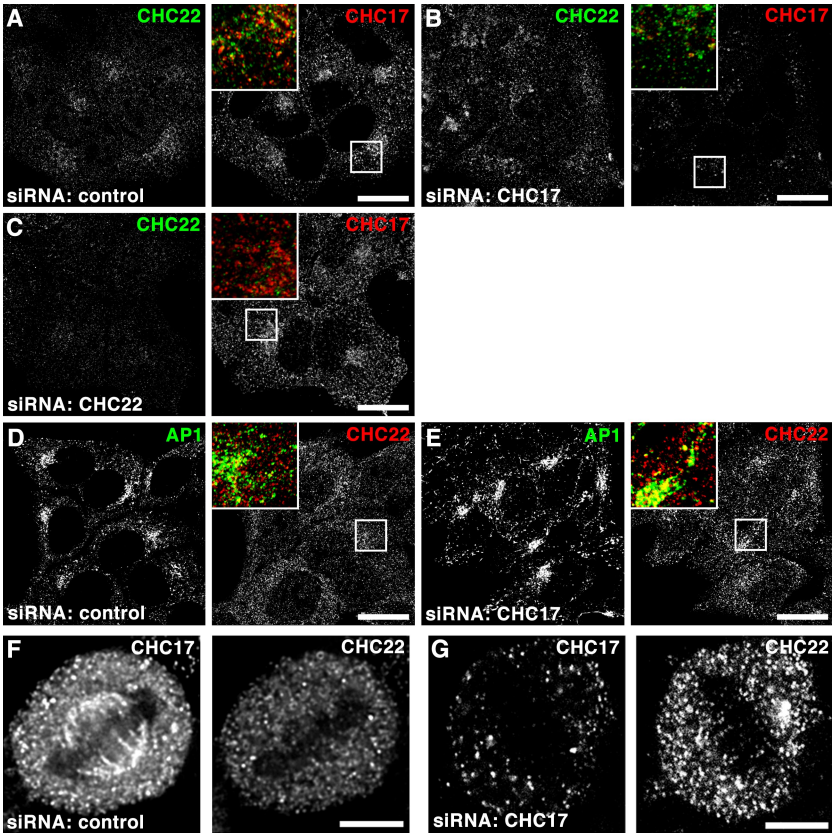

Figure S1. **CHC17 and CHC22 show only minor colocalization on membranes and the mitotic spindle.** (A–C) HeLa cells treated with control siRNA (A) or siRNA to deplete CHC17 (B) or CHC22 (C) were processed for immunofluorescence and labeled for CHC22 (green in merged insets) and CHC17 (red in merged insets) as indicated at the top. Each individual label is shown as a black and white image. Bars, 20 µm. (D and E) HeLa cells treated with control siRNA (D) or siRNA to deplete CHC22 (E) were processed for immunofluorescence and labeled for AP1 (green in merged insets) and CHC22 (red in merged insets) as indicated at the top. Bars, 20 µm. (F and G) HeLa cells were treated with control siRNA (F) and siRNA targeting CHC17 (G) and double labeled with antibodies against CHC17 and CHC22. Mitotic cells were identified by the presence of condensed chromosomes and typical images are shown. Bars, 5 µm.

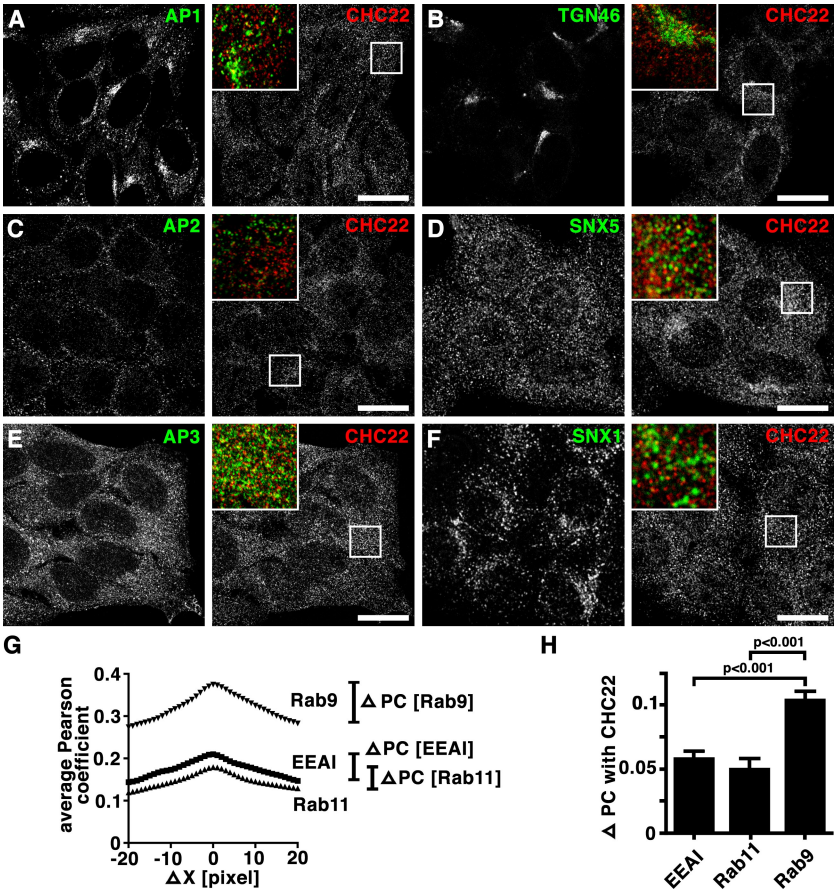

Figure S2. **Comparison of CHC22 distribution with membrane traffic markers.** (A–F) HeLa cells were grown on coverslips, processed for immunofluorescence, and labeled using a rabbit polyclonal antibody against CHC22 (right panels, red in merge). Simultaneous labeling was performed (left panels, green in merge) using mouse monoclonal antibodies against (A) AP1, (C) AP2, (E) AP3, and (F) SNX1, (B) a sheep polyclonal antibody against TGN46, or (D) a goat polyclonal antibody against SNX5. Bars, 20 µm. (G) HeLa cells were grown and processed as in Fig. 3. Pearson's correlation coefficients (PC) of individual cells were averaged for colocalization of CHC22 with EEA1, Rab11, and Rab9 (n = 12 for each double labeling from two different experiments). Plotted are PCs with the CHC22-containing channels moved by up to 20 pixels from the original image (Δ X pixels) to assess the probability of colocalization occurring by chance. On the right side Δ PC of EEA1, Rab11, and Rab9 indicates the difference of the highest to lowest PC for each dataset. (H) Averages ±SEM of the differences (Δ PC with CHC22) of the highest to lowest PC for each dataset were plotted for each marker indicated at the bottom. The higher Δ PC for Rab9 indicates greater nonrandom colocalization with CHC22 than for the other markers. P values for selected samples are indicated.

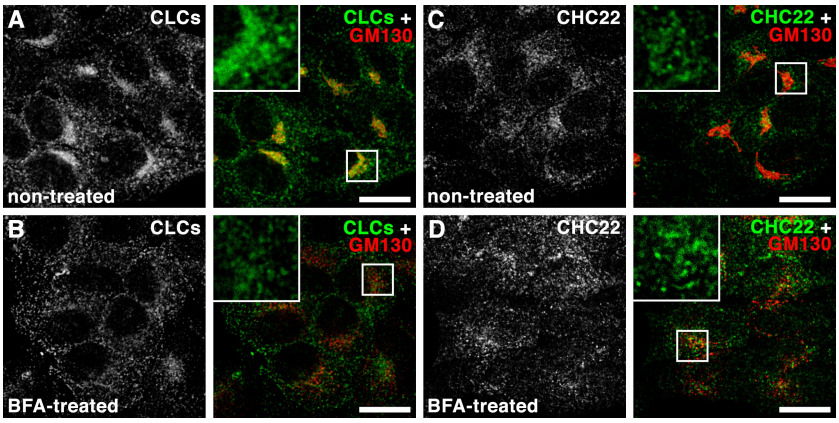

Figure S3. **Brefeldin A treatment does not alter CHC22 membrane association.** (A–D) HeLa cells were incubated in the presence of Brefeldin A (BFA-treated; B and D) for 60 min or not (nontreated; A and C) as indicated in each panel. Cultures were then processed for immunofluorescence and labeled using antibodies against CLCs (green; A and B), CHC22 (green; C and D), and GM130 (red; A–D) as indicated in each panel. GM130 served as a positive control for Golgi dissolution. The pairs of panels are images of the same samples with the left-hand panel showing individual antibody labeling in black and white and the right-hand panel showing the double-labeled image in color. Insets show magnification of boxed area for CLC (A and B) and CHC22 (C and D), green labeling only. Bars, 20 μm. Antibodies against CLC identify the bound CHC17 subunits of the clathrin triskelion.

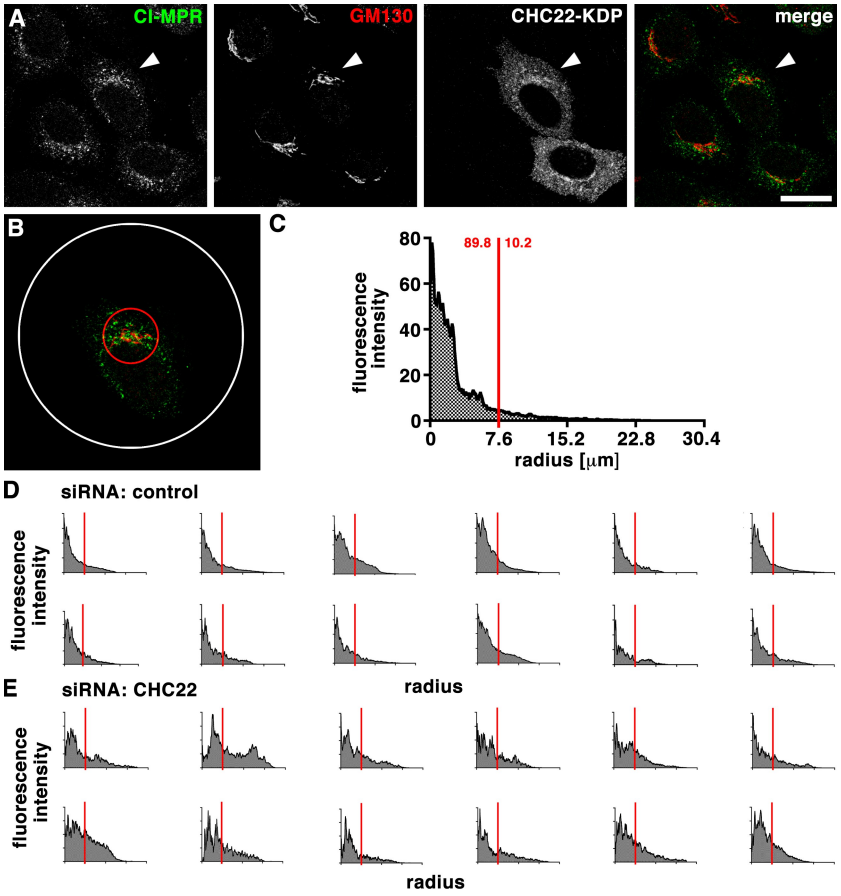

Figure S4. **Illustration of the method to measure fluorescent vesicles in the cell periphery.** (A) The panels shown in Fig. 4 C are reproduced here with an added merged image of CI-MPR (green) and GM130 (red) at the far right. All four panels are the same image of cells treated with control siRNA and transfected with an siRNA-resistant, FLAG-tagged CHC22 construct (CHC22-KDP, knock-down proof). Arrowhead marks the cell selected for analysis in B. Bar, 20 μm. (B) The cell marked in A is displayed with a red circle that surrounds the GM130 labeling of the Golgi representing the boundary between inner perinuclear and outer peripheral signals, and a white circle that marks the edge of the measurements. The center of both circles is the same and represents the center of the GM130 labeling (red), set manually. (C) Radial profile of CI-MPR fluorescence (green) in the cell shown in B. Red bar indicates threshold applied for perinuclear vs. peripheral classification, such that signals to the right of the red bar, corresponding to the area between the red and white circles in B, were classified as peripheral. Numbers indicate the percentage of total signal that is perinuclear (89.8) or peripheral (10.2). (D) Radial profiles of 12 individual cells transfected with control siRNA. Red mark indicates threshold applied for perinuclear vs. peripheral classification. (E) Radial profiles of 12 individual cells transfected with siRNA targeting CHC22. Red mark indicates threshold applied for perinuclear vs. peripheral classification. Note the wider distribution of CI-MPR fluorescence signal and larger percentage of peripheral signal for the CHC22-depleted cells compared with control cells in D. The percentages of total signals classified as peripheral were averaged for all cells for each experimental condition and plotted as bar diagrams (as seen in Figs. 4 and 7).

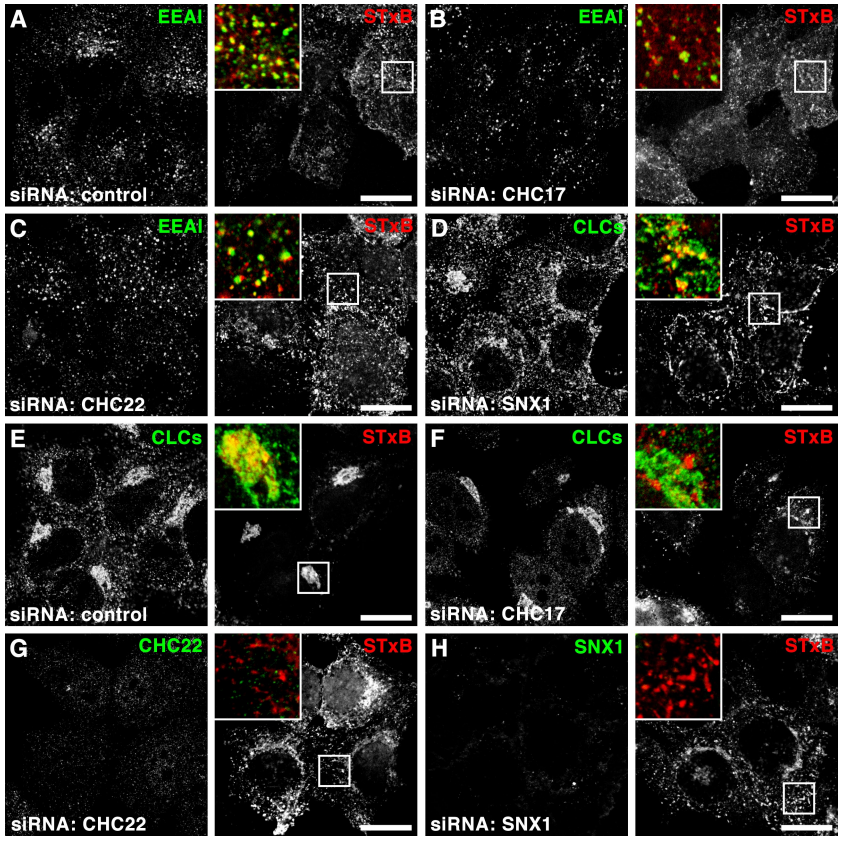

Figure S5. **Retrograde endosome-to-Golgi but not endocytosis of STxB is impaired in cells depleted of CHC17, CHC22, or SNX1.** (A–D) HeLa cells were treated with control siRNA (A) or siRNA targeting CHC17 (B), CHC22 (C), or SNX1 (D). Fluorescent STxB (red in merged insets) in fresh medium was bound to cells for 30 min on ice, washed in PBS, and chased for 15 min in fresh medium at 37°C. Cells were fixed, processed for immunofluorescence, and labeled using an antibody against EEA1 (green in merged insets) or CLC (green in merged insets). Bars, 20 µm. (E–H) HeLa cells were treated as in A–D but STxB (red in merged insets) internalization was allowed for 60 min. Cells were then fixed, processed for immunofluorescence, and labeled using antibodies against CLCs, CHC22, or SNX1 (green in merged insets). Labeling for CLCs was used to detect CHC17, as CLCs do not bind CHC22. CLC (F), CHC22 (G), and SNX1 (H) labeling indicates the degree of depletion of these proteins by siRNA. Note that CLC labeling reveals incomplete depletion of CHC17, with some residual stable CHC17 in the TGN region. Bars, 20 µm.