Willenborg et al., http://www.jcb.org/cgi/content/full/jcb.201011112/DC1

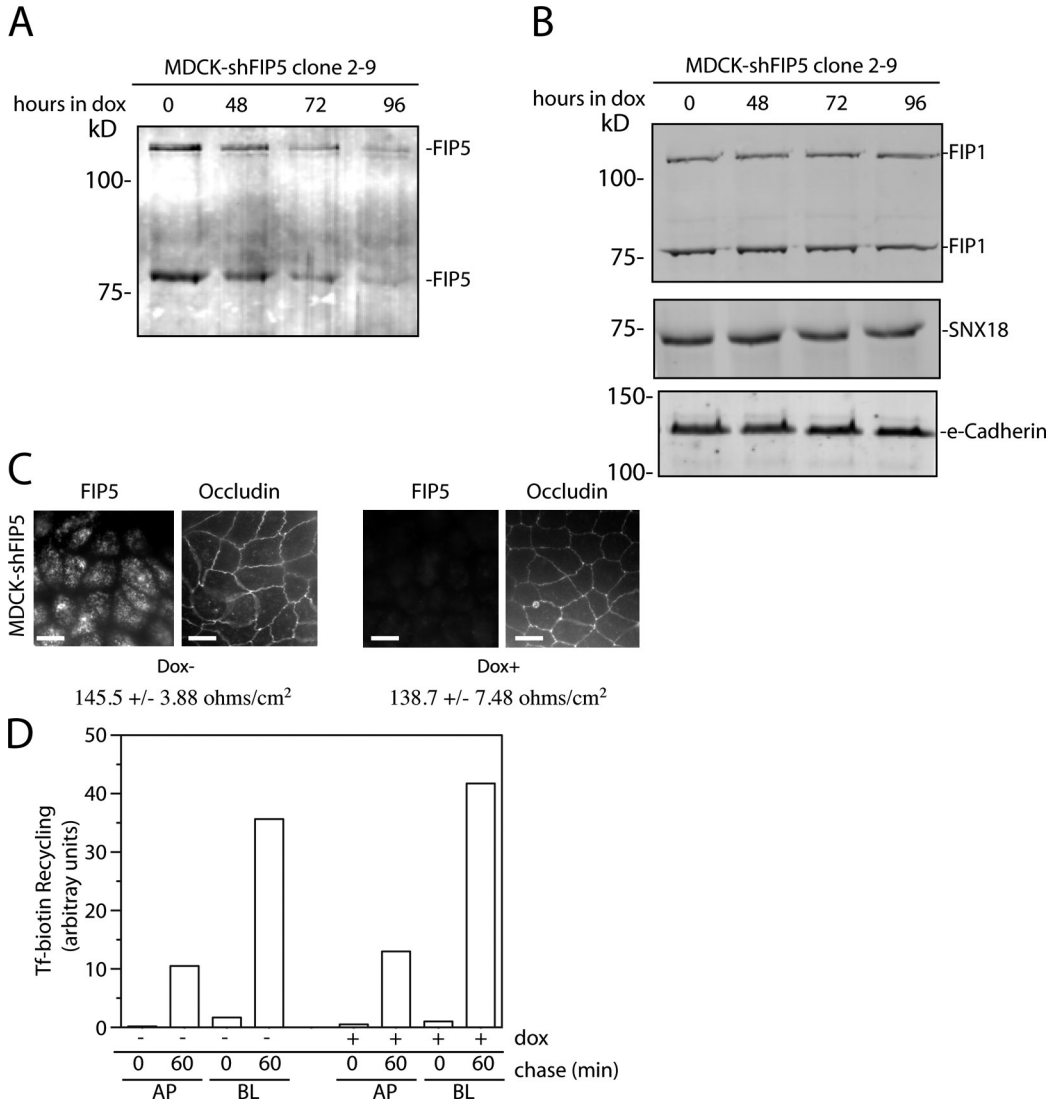

Figure S1. **Characterization of MDCK cell lines expressing tet-inducible FIP5 shRNA.** (A and B) MDCK-shFIP5 cells were grown in the presence of 1 µg/ml dox for varying amounts of time. Cells were then lysed and immunoblotted with anti-FIP5, anti-FIP1, anti-SNX18, and anti–e-Cadherin antibodies. Note that FIP5 and FIP1 exist as two alternatively spliced isoforms. FIP5 shRNA knocks down both FIP5 isoforms. (C) Filter-grown polarized MDCK-shRNA cells were incubated in the presence or absence of 1 µg/ml dox. Cells were then fixed and stained with anti-FIP5 and anti-Occludin antibodies. The numbers below the images are the measured trans-epithelial resistances of the monolayers just before cells were fixed for microscopy. The data shown are the mean and standard deviation from four separate experiments. Bars, 3 µm. (D) Quantitation of Tf-biotin recycling to the apical or basolateral PM domains in cells grown in the presence or absence of 1 µg/ml dox. AP, apical media; BL, basolateral media. Data shown are the average of two independent experiments.

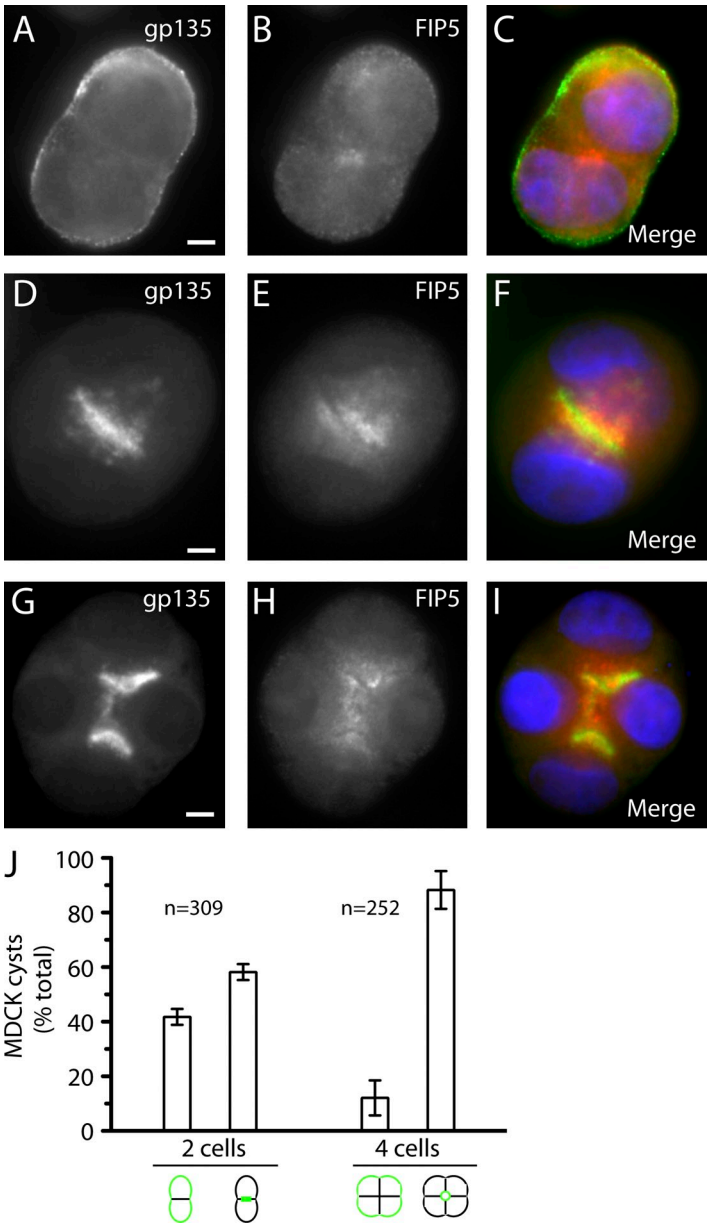

Figure S2. **Early stages of apical lumen formation in MDCK cells.** (A–I) MDCK cells were plated in 3D cultures and incubated for 24 h. Cells were then fixed and stained with anti-gp135 (A, D, and G) and anti-FIP5 (B, E, and H) antibodies. (A–F) Cysts at the two-cell stage. (G–I) Cysts at the four-cell stage. Yellow staining in C, F, and I shows the extent of gp135 and FIP5 colocalization. Bars, 3 µm. (J) Quantitation of cells with and without a formed lumen at the two and four cell stages. Data shown are the means and standard deviations derived from three independent experiments (error bars). *n* is the number of cysts analyzed.

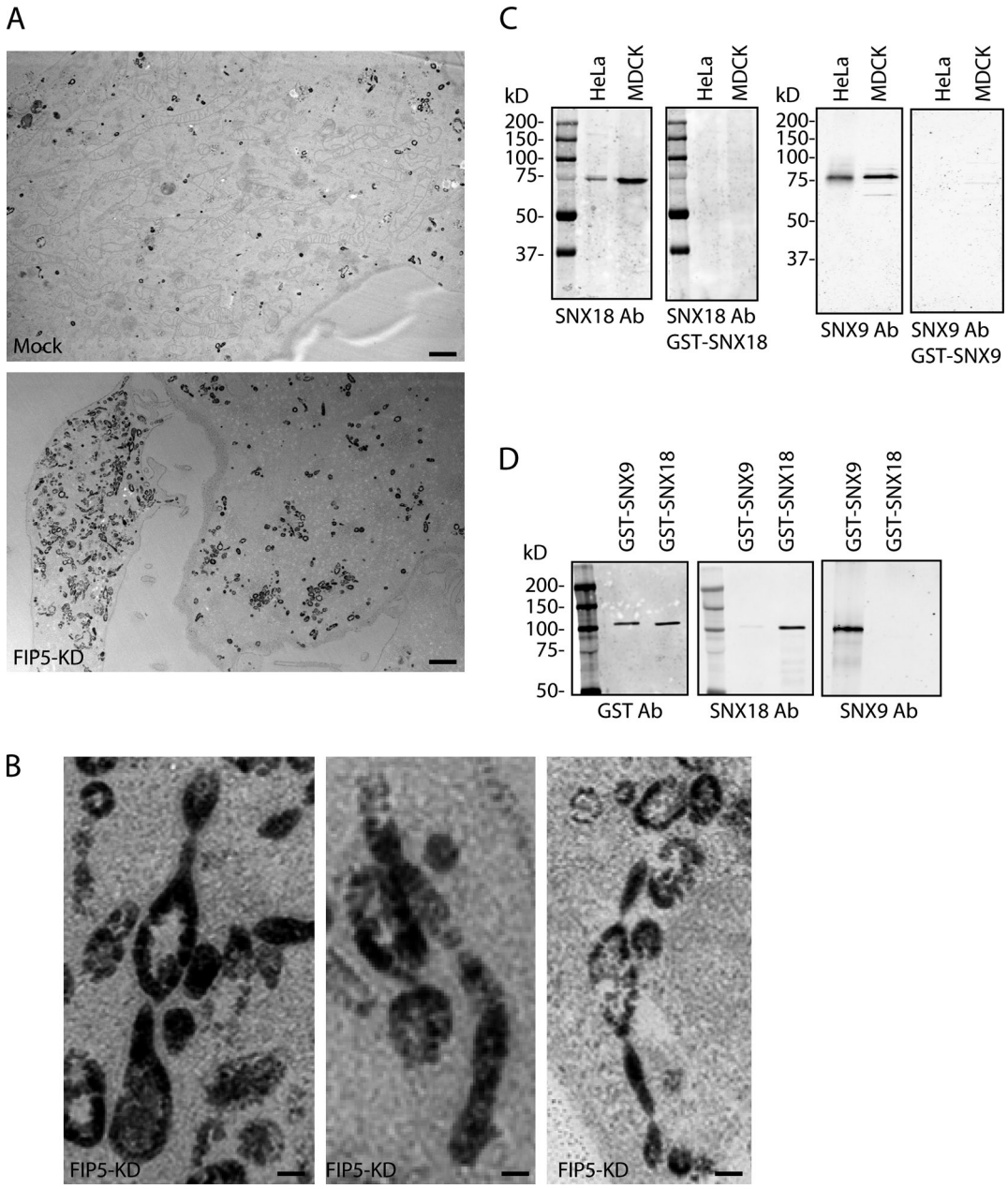

Figure S3. **The EM depicting beads-on-a-string endosomes in cells transfected with FIP5 siRNA.** (A) Mock (top) or FIP5 siRNA transfected (bottom) cells were incubated with Tf-HRP for 120 min at 37°C. The DAB reaction was performed before permeabilization, antibody incubation, and fixation. Electron-dense dark precipitate indicates DAB reaction product and highlights the localization of Tf-HRP. Bars, 5 µm. (B) Higher-magnification images from cells transfected with FIP5 siRNA and loaded with Tf-HRP. Bars, 50 nm. (C) HeLa and MDCK cell lysates were separated by SDS-PAGE and immunoblotted with anti-SNX18 or anti-SNX9 antibodies in the absence or presence of a 20-fold excess of recombinant purified GST-SNX18 or GST-SNX9. (D) 100 ng of GST-SNX9 and GST-SNX18 were immunoblotted with anti-GST (left), anti-SNX18 (middle), or anti-SNX9 (right) antibodies.

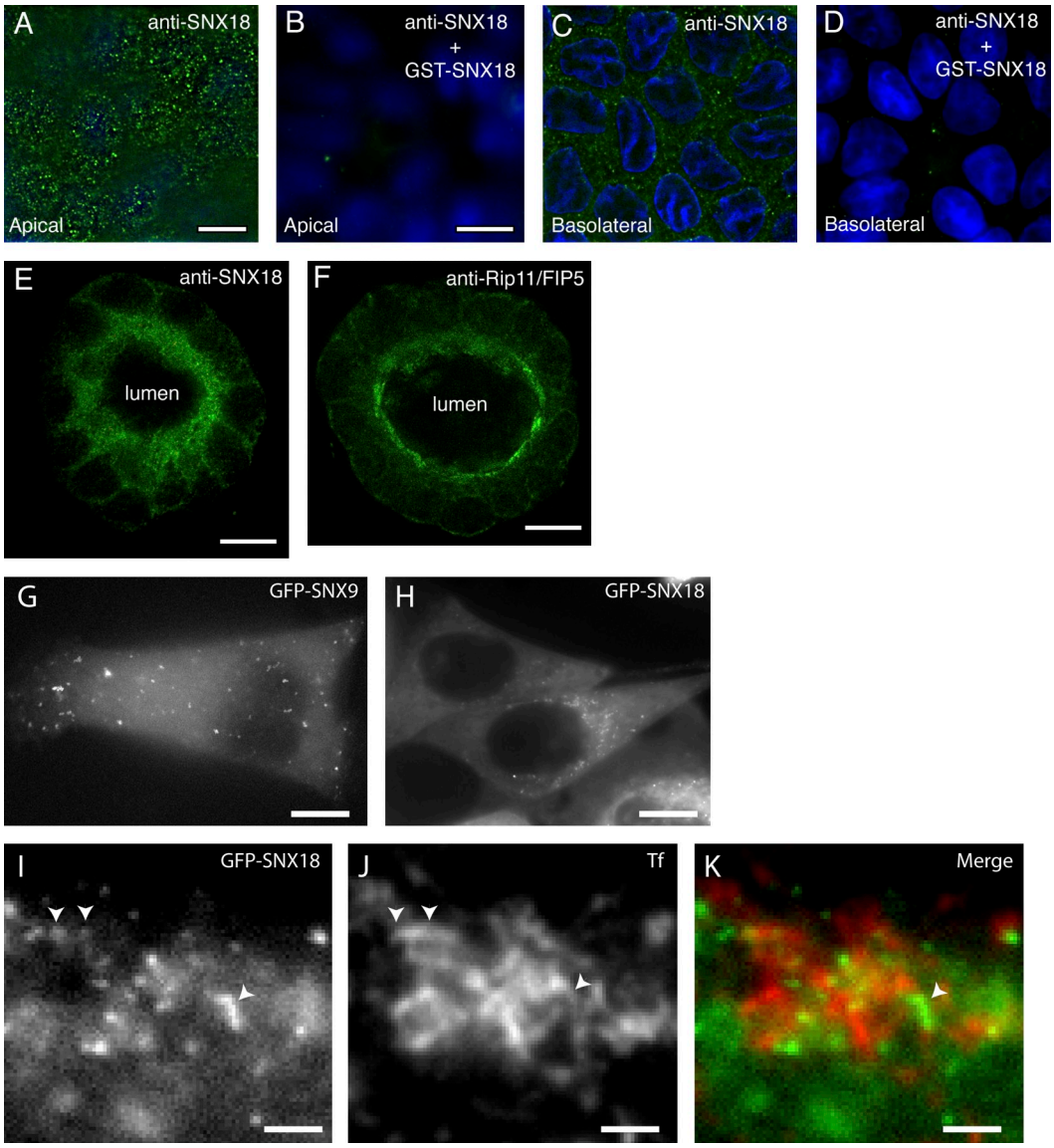

Figure S4. **SNX18 is enriched at endocytic organelles, but not at the PM in HeLa and polarized MDCK cells.** (A–D) Filter-grown, polarized MDCK cells were fixed and stained with anti-SNX18 antibody in the absence (A and C) or presence (B and D) of a 20-fold excess of GST-SNX18. Images in A and B were taken at the apical level. Images in C and D were taken at the basolateral level. (E and F) MDCK cells were grown in 3D cultures for 9 d. Cells were then fixed and stained with anti-SNX18 (E) or anti-FIP5 (F) antibodies. (G–K) HeLa cells were transfected with either GFP-SNX9 (G) or GFP-SNX18 (H–K). In I–K, cells were loaded with Tf-Alexa594 for 30 min before imaging. Arrowheads in I–K point to endocytic subdomains enriched in GFP-SNX18 and de-enriched in Tf-Alexa594. Bars: (A and B) 8 µm; (E and F) 12 µm; (G and H) 4 µm; (I–K) 1 µm.

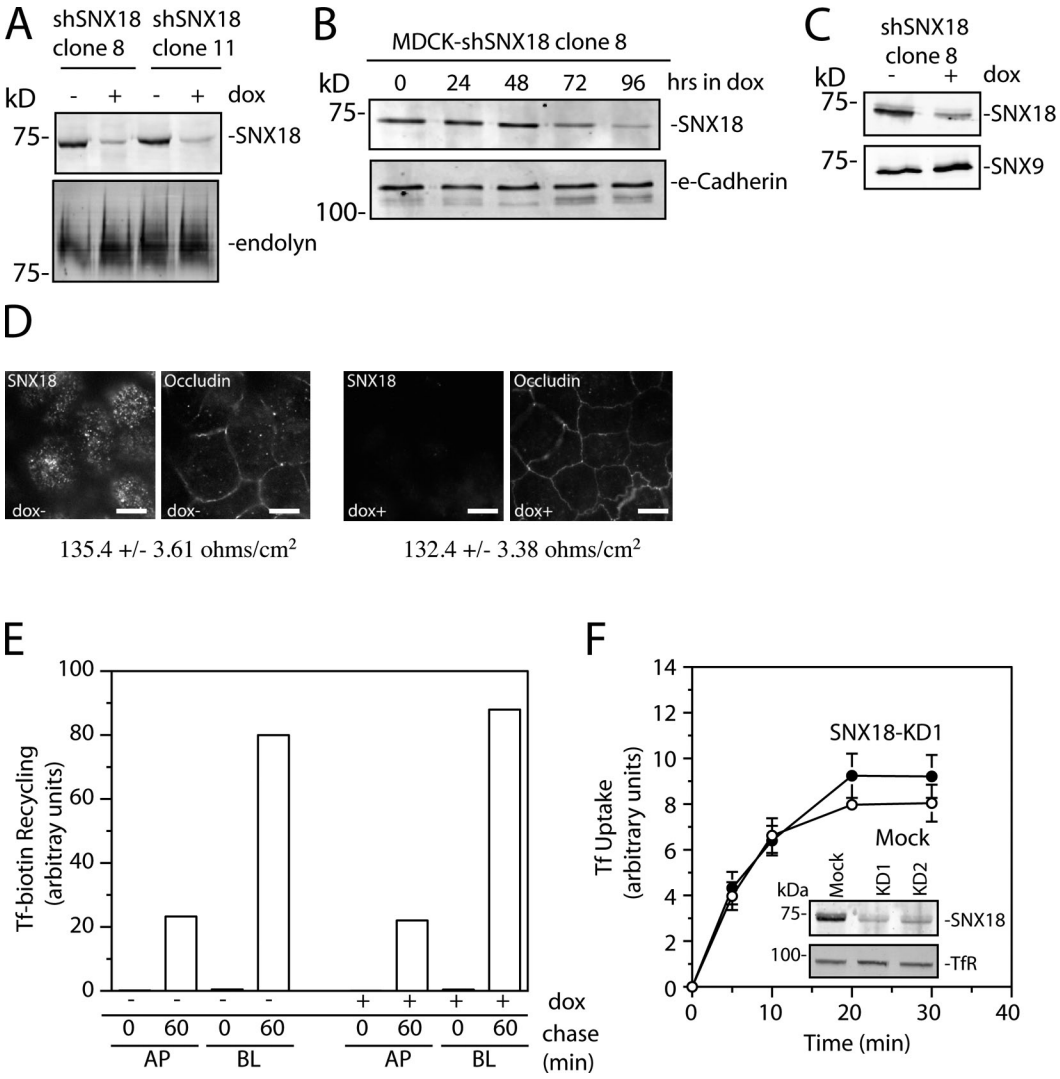

Figure S5. **Characterization of MDCK cell lines expressing tet-inducible SNX18 shRNA.** (A and B) MDCK-shSNX18 cells were grown in the presence of 1 µg/ml dox for varying amounts of time. Cells were then lysed and immunoblotted with anti-endolyn, anti-SNX18, and anti–e-Cadherin antibodies. (C) Lysates of mock or SNX18 siRNA-transfected HeLa cells immunoblotted with anti-SNX18 and anti-SNX9 antibodies. (D) Filter-grown, polarized MDCK-shSNX18 cells were incubated in the presence or absence of 1 µg/ml dox. Cells were then fixed and stained with anti-SNX18 and anti-Occludin antibodies. Numbers below images are the trans-epithelial resistances measured just before fixing cells for microscopy. The data shown are the mean and standard deviation from four separate experiments. Bars, 3 µm. (E) The quantitation of Tf-biotin recycling to the apical or basolateral PM in cells grown in the presence or absence of 1 µg/ml dox. Data shown are the average of two independent experiments. (F) Mock- or SNX18 siRNA-transfected HeLa cells were incubated with 20 µg/ml Tf-Alexa647. At different time points, cells were washed and fixed, and the amount of internalized Tf-Alexa647 was analyzed by flow cytometry. Data shown are the mean and standard deviations of three independent experiments (error bars).