

Figure S1. ARNO is required for the glucose-induced translocation of EPI64.

(A) ARNO-silenced MIN6 cells were incubated with 3 or 20 mM glucose for 2 min. The cells were immunostained with anti-EPI64 antibody. Scale bars, 10 μ m. (B) TIRF images were sampled in living MIN6 cells expressing GFP-EPI64 and mCherry-ARNO. Scale bars, 10 μ m.

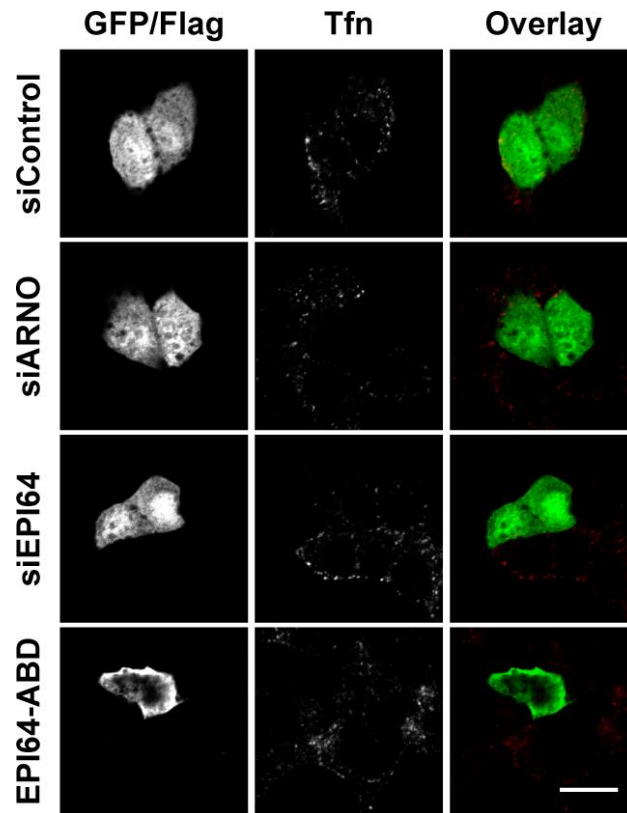


Figure S2. The crosstalk between EPI64 and ARNO signaling regulates clathrin-dependent endocytosis.

ARNO- or EPI64-silenced MIN6 cells expressing GFP were incubated with Alexa-568-labeled transferrin (red) in the presence of 3 mM glucose for 5 min followed by immunofluorescence analysis. Scale bars, 10 μ m.

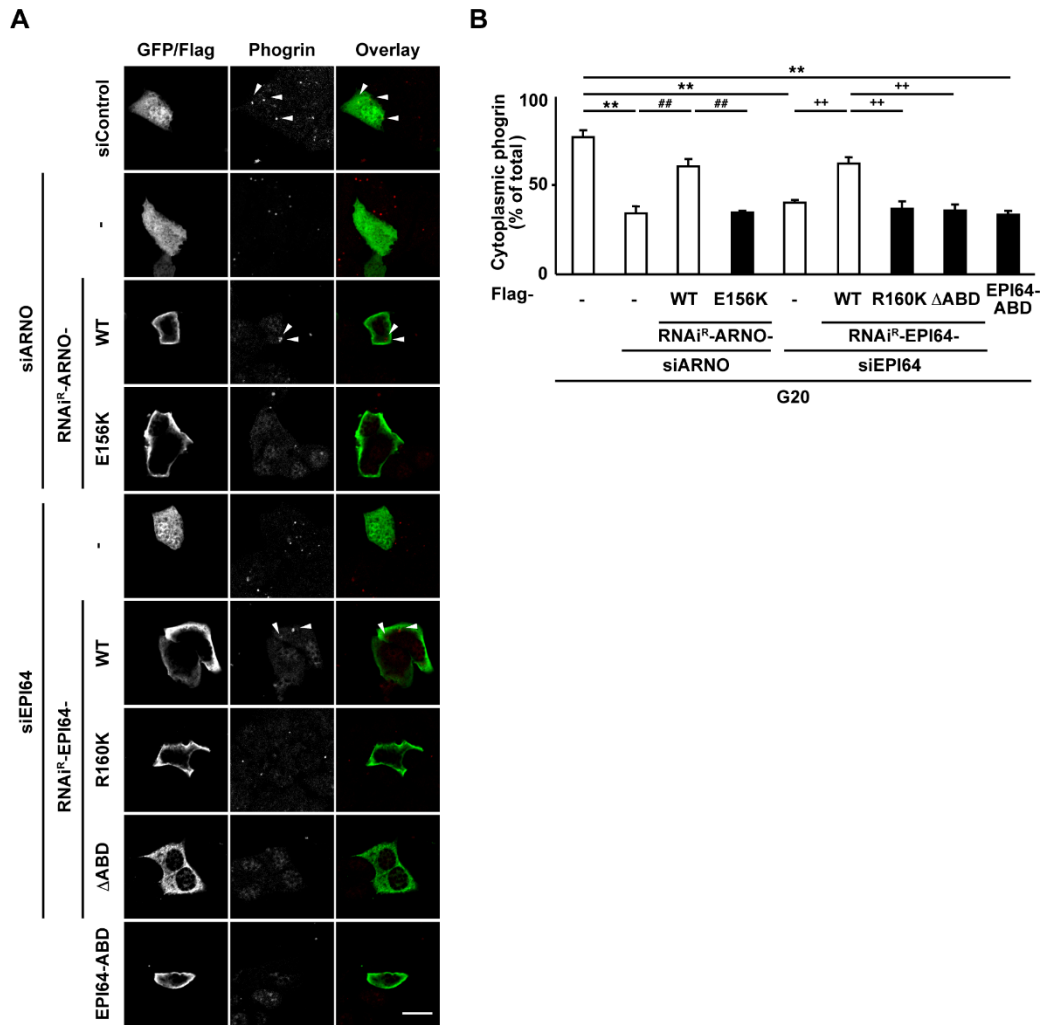
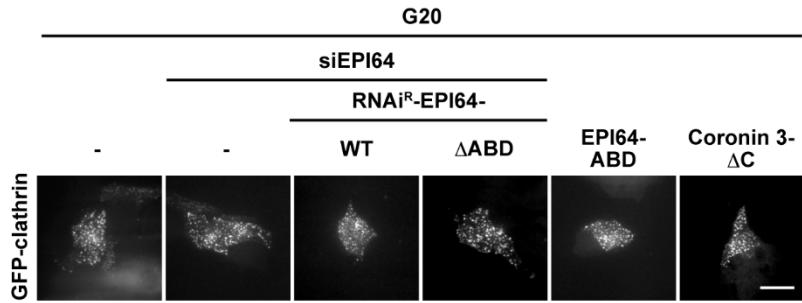


Figure S3. The crosstalk between EPI64 and ARNO signaling regulates endocytosis of insulin granules.

(A) ARNO- or EPI64-silenced MIN6 cells expressing GFP, Flag-ARNO, or Flag-EPI64 were incubated with Alexa-568-labeled anti-phogrin antibody (red) in the presence of 20 mM glucose for 15 min followed by immunofluorescence analysis. Arrowheads denote Alexa-568-labeled anti-phogrin antibody localized in MIN6 cells. (B) The percentage of transfected cells that showed a cytoplasmic distribution of Alexa-568-labeled anti-phogrin antibody was analyzed. More than 40 randomly selected cells (more than 8 cells/experiment) were examined. Data are expressed as means \pm s.d. from 4 independent experiments. The statistical significance of differences between means was assessed by ANOVA (Tukey-Kramer's method). ** $p < 0.01$ vs. control siRNA, ## $p < 0.01$ vs. RNAi^R-ARNO-WT, ++ $p < 0.01$ vs. RNAi^R-EPI64-WT. Scale bars, 10 μ m.

A



B

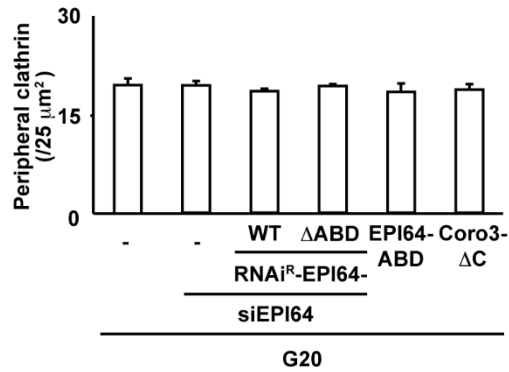


Figure S4. EPI64 regulates endocytosis at the late stage after scission from the plasma membrane.

(A) TIRF images were sampled in EPI64-silenced MIN6 cells expressing GFP-clathrin and Flag-EPI64 or cells expressing GFP-clathrin and Flag-EPI64 or Flag-coronin 3. (B) Individual vesicles were calculated in a $5 \times 5 \mu\text{m}$ square. More than 5 randomly selected cells (>5 squares/cell) were examined. Data are expressed as means \pm s.d. from 5 independent experiments. The statistical significance of differences between means was assessed by ANOVA (Tukey-Kramer's method). Scale bars, $10 \mu\text{m}$.