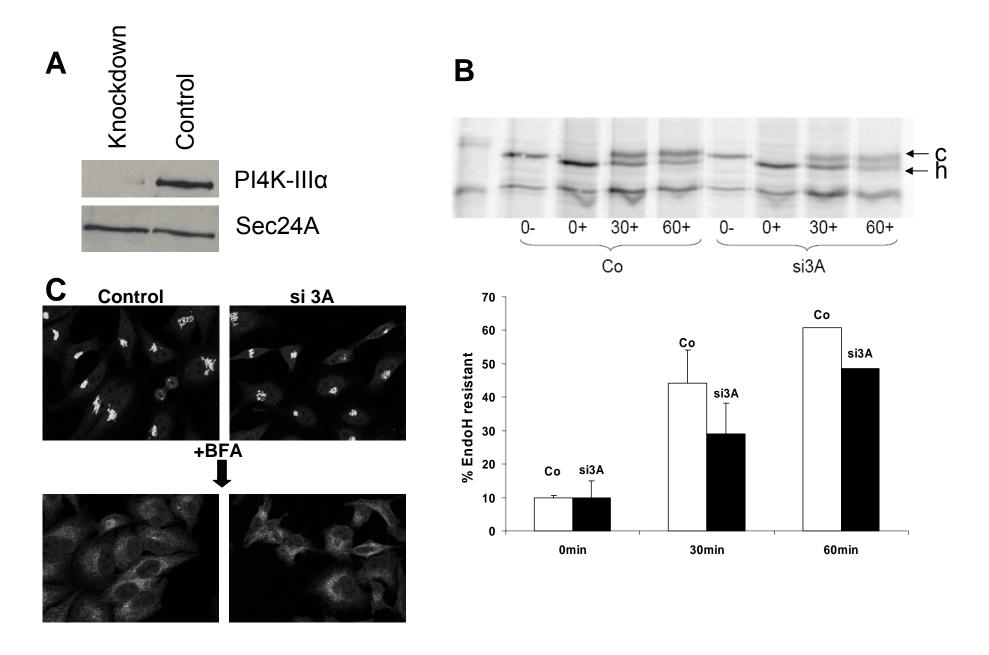
## **Supplementary figure legends**

Fig S1. Knockdown of PI4K-IIIa. A, HeLa cells were transfected with siRNA for PI4K-IIIa (Knockdown) or with a control siRNA (Control). After 72 h, the cells were lysed and subjected to SDES-PAGE. PI4K-III $\alpha$  and Sec24A were detected by immunoblotting. **B**, HeLa cells were transfected with siRNA for PI4K-IIIa (si3A) or with a control siRNA (co). After 48 h cells were transfected with GFP-tagged VSVG-tsO45. On the next day cells were incubated in methionin-free medium for 20 min and subsequently pulsed with 100  $\mu$ Ci for 10 minutes. Chase was performed in medium with non labelled methionin for 0, 30 and 60 minutes. The cells were lysed in VSVG was immunoprecipitated with an antibody to GFP. The immunoprecipitate was subjected to endoH digestion (+). Samples were separated by SDS-PAGE and detection was performed using a phosphor-imager. Mean  $\pm$  SD (n=3) for chase times 0 min and 30 min. c indicates the complex glycosylated, endoH-resistant form and h indicates the high mannose endoH-sensitive form. C, HeLa cells were transfected with siRNA for PI4K-IIIa (si3A) or with a control siRNA (control). After 72 h cells were treated with solvent (upper images) or with 5 µg/ml BFA for 40 min. Cells were then fixed and the Golgi was visulaized by immunofluorescence staining of giantin. D, HeLa cells were transfected with a control siRNA, siRNA to PI4K-IIIa, or with a siRNA to golgin 84. After 72h cells were fixed and co-stained for Sec31 (to visualize ERES) and to GM130 to (visualize the Golgi).

**Fig S2.** *Localization of basic residues on the Sec23-Sec24 dimer.* The model is based on the crystal structure of the dimer of Sec23A (pink)/Sec24A (blue) in complex with Sec22 (brown) reported by Mancias and Goldberg (2007). The white line indicates the curved membrane. The basic residues Arg and Lys were labeled in yellow. The model was generated using Cn3D software.

## Figure S1

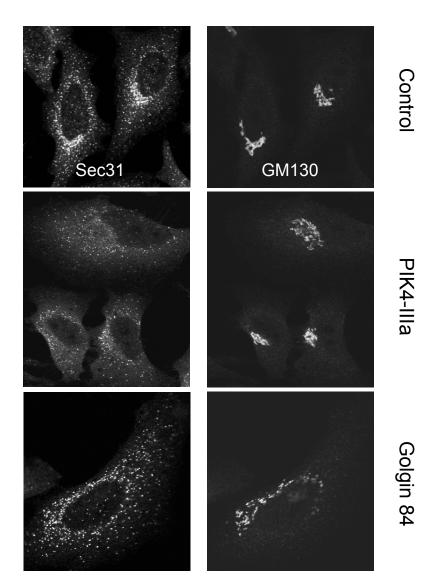


D

100% (± 8.6)

62.8% (± 14.5)

101.5% (± 4.3)



## Figure S2

