

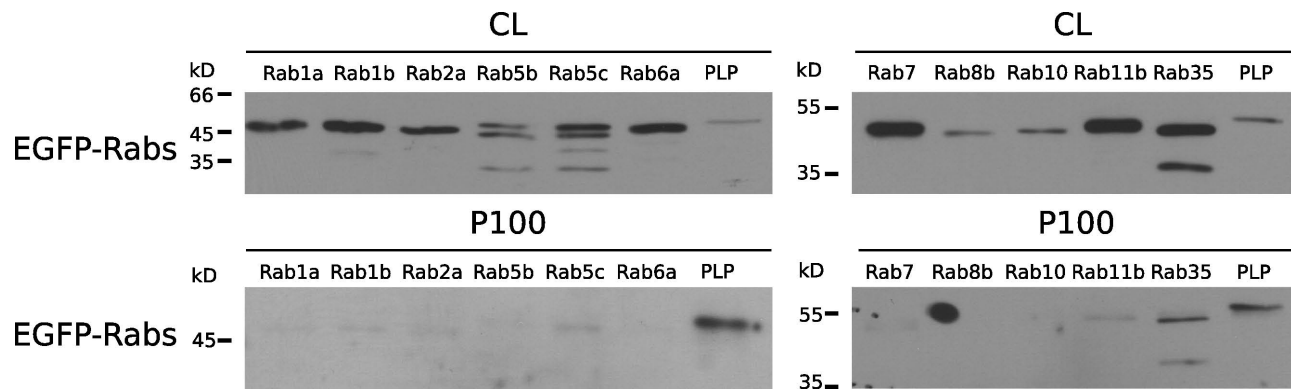
Hsu et al., <http://www.jcb.org/cgi/content/full/jcb.200911018/DC1>

Figure S1. **Analysis of Rabs in exosomes.** The Rabs identified in Oli-neu-derived exosomes by MS were expressed as EGFP fusion proteins in Oli-neu cells. 16 h after transfection, the cells were switched to serum-free medium, and the medium was collected after ~4 h of further incubation before submitting it to sequential centrifugation steps. The amount of the EGFP-Rab proteins was determined in the cell lysates (CL) and 100,000 g exosome pellets (P100) by Western blotting. PLP was used as reference. One representative experiment is shown.

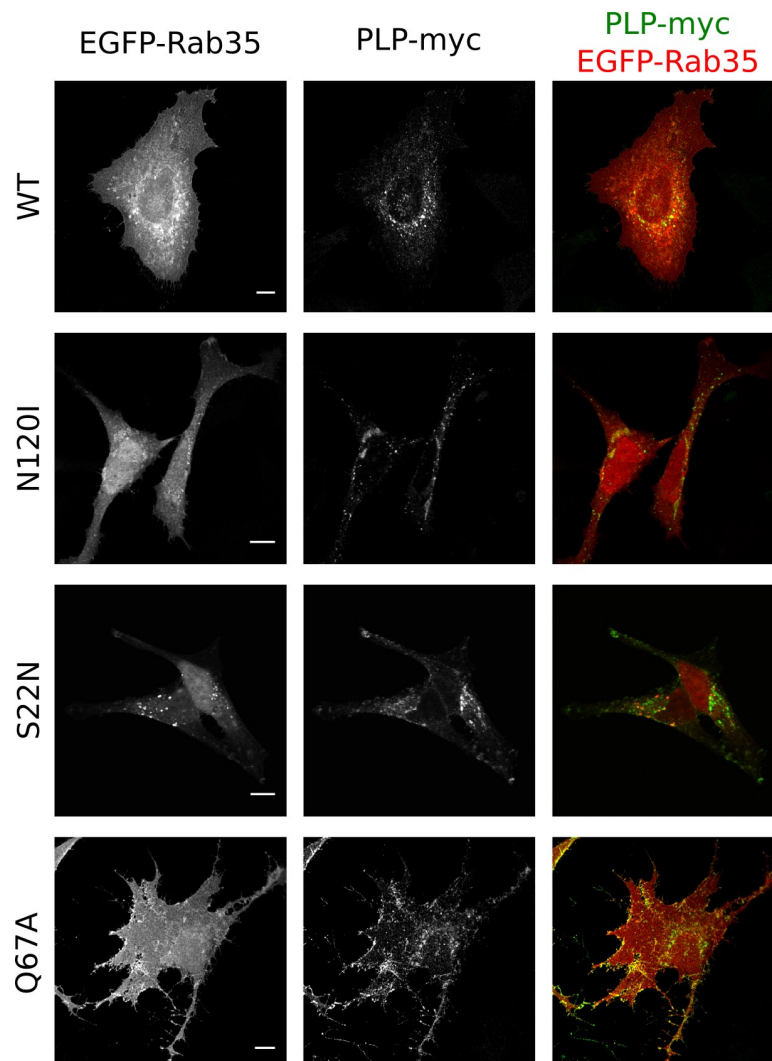


Figure S2. **Localization of Rab35 in HeLa cells.** HeLa cells were transfected with PLP-myc together with EGFP-Rab35^{S22N}, EGFP-Rab35^{N120I}, EGFP-Rab35^{Q67A}, or wild-type EGFP-Rab35 (WT) and analyzed by confocal microscopy. Bars, 10 μ m.

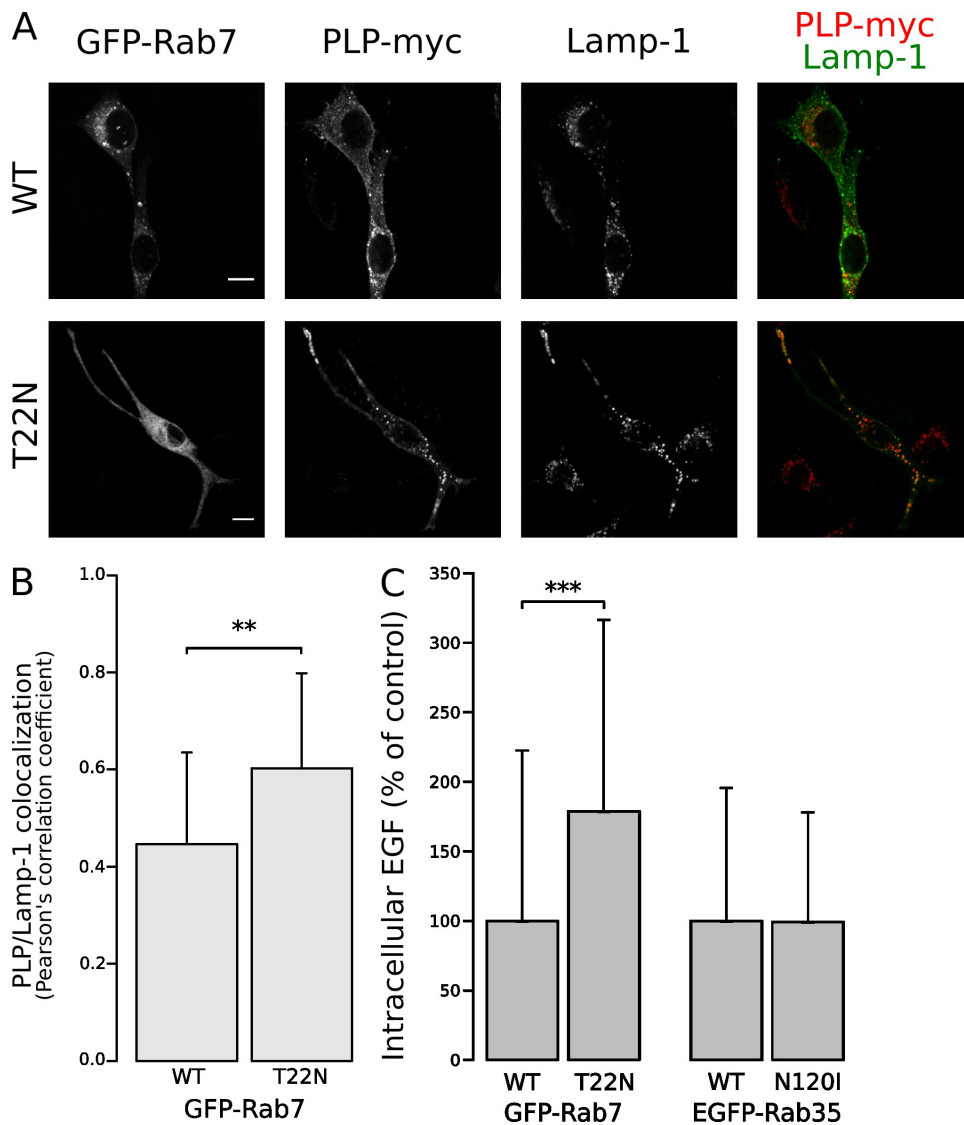


Figure S3. **Inhibition of Rab7 but not Rab35 inhibits EGF degradation.** (A) Oli-neu cells were cotransfected with EGFP-Rab7^{T22N} or wild-type GFP-Rab7 (WT) and PLP-myc and analyzed by confocal microscopy. (B) Quantification of colocalization of PLP and Lamp-1 is shown ($n = 25$ and 22). The values represent the mean \pm SD (**, $0.001 < P < 0.05$; Welch's two-sample t test). (C) Cells were transfected with EGFP-Rab7^{T22N} or EGFP-Rab35^{N120I} or the respective wild-type constructs together with EGF receptor-EGFP, incubated with rhodamine-labeled EGF for 15 min, washed, and incubated for 4 h in conditioned culture medium to allow EGF degradation. For quantification, images of randomly selected transfected cells were recorded, and fluorescence intensities were quantified. Values represent the mean \pm SD ($n = 54$ – 63 cells; ***, $P < 0.001$; Welch's two-sample t test). Bars, 10 μ m.

Table S1, included as an Excel file, shows the MS analysis of Oli-neu-derived exosomes.