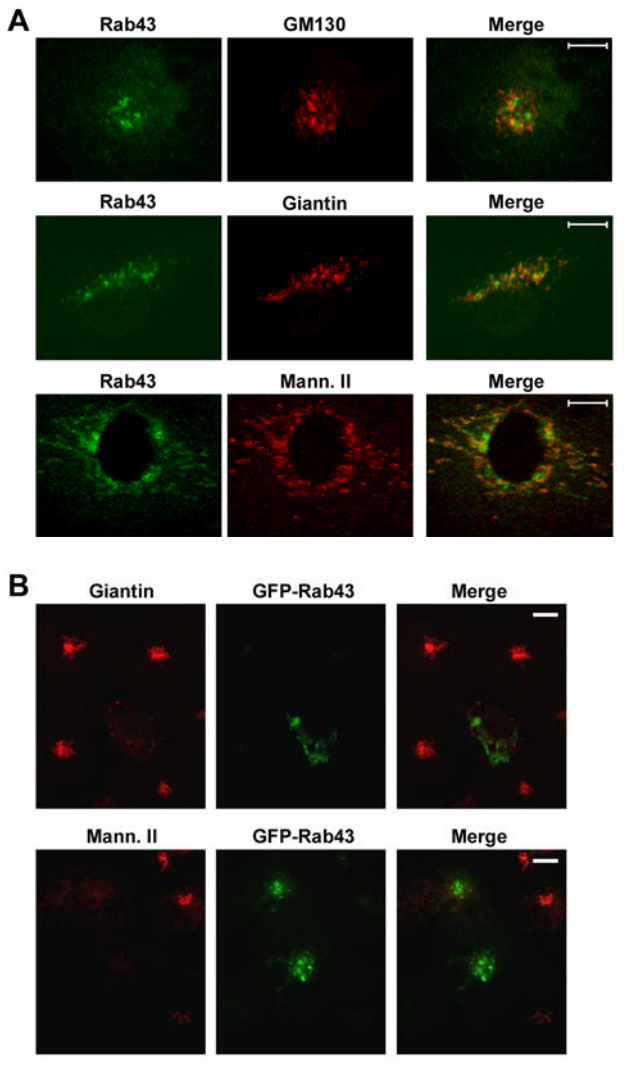
Supplemental Materials

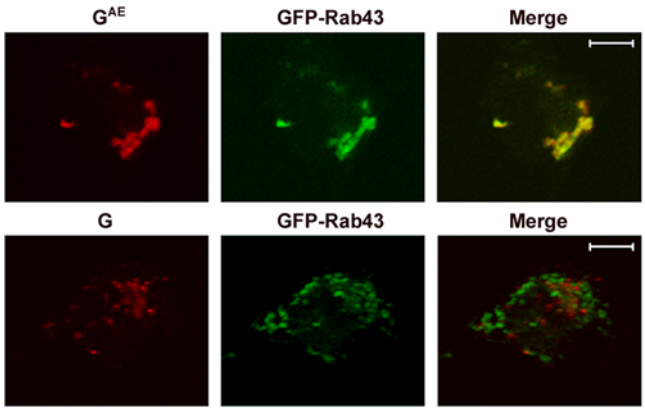
Molecular Biology of the Cell

Cox et al.

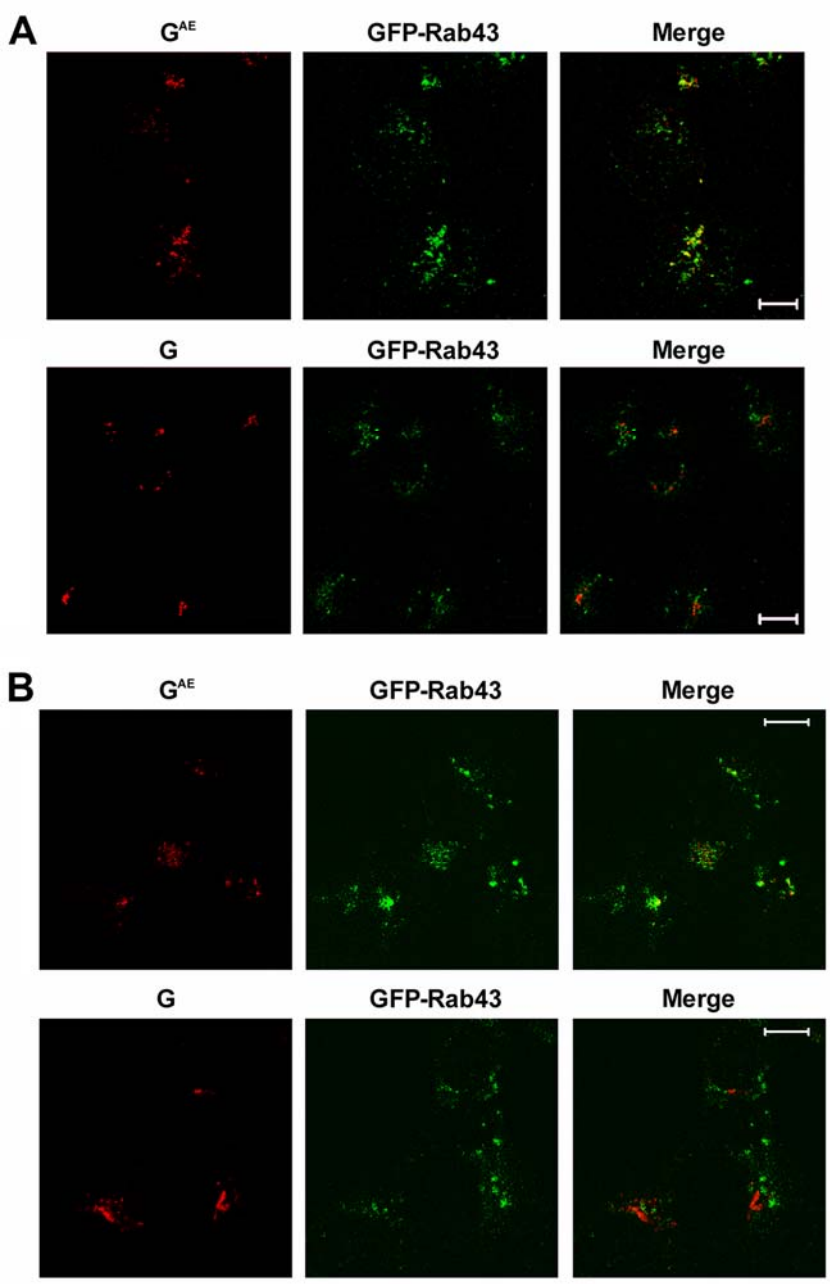


Supp. Fig. S1. GFP-Rab43 co-localization with various markers of the early secretory pathway. (A) PH5CH8cells were fixed and stained with rabbit antibodies specific GM130, giantin, or mannosidase II(red) and with a mouse monoclonal antibody specific for Rab43 (green). The cells were analyzed by confocal microscopy and the images shown are projections of confocal z-stacks.(B) COS7 cells expressing high levels of GFP-Rab43 were fixed and stained with antibodies specific for giantin or mannosidase II (Mann. II) and imaged by epifluorescence microscopy.Note the extensive vesiculation of the giantin-containing compartment and the complete

disruption of the mannosidase II-containing compartment in GFP-Rab43 expressing cells relative to the cells not expressing GFP-Rab43. White bars in A and B are 10μm.

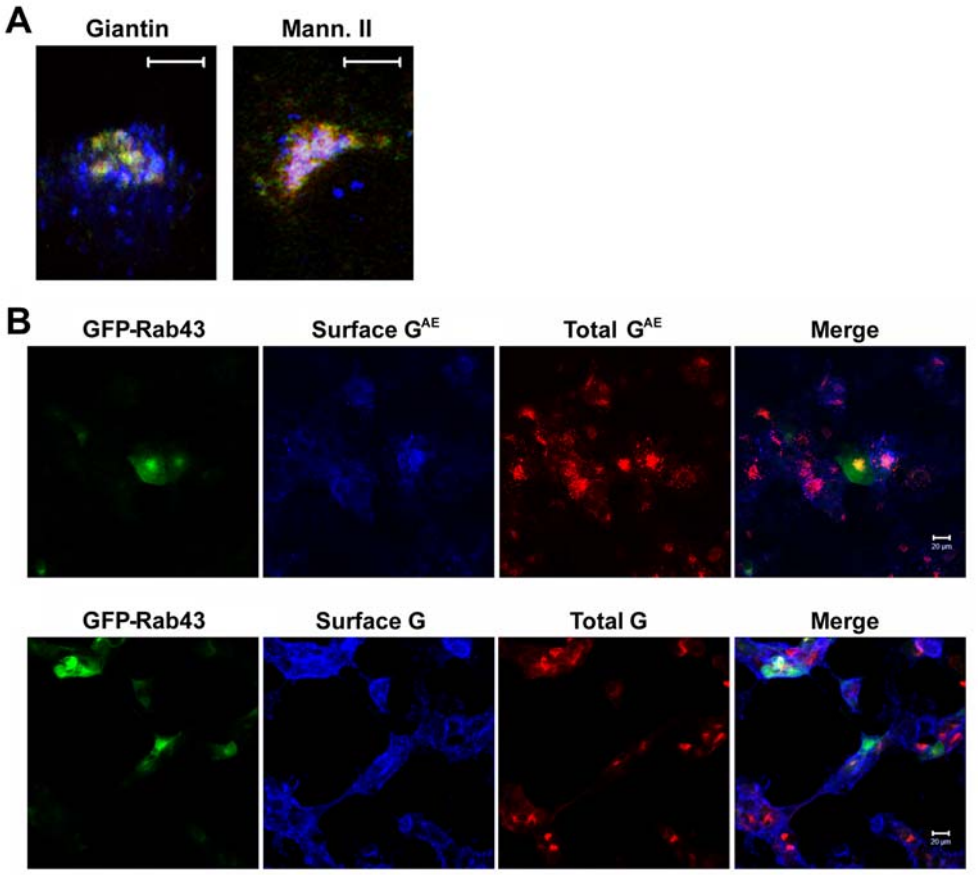


Supp. Fig. S2. 3-D projections illustrating the differential effect of GFP-Rab43 on G^{AE} and G sorting. COS7 cells transfected with GFP-Rab43were subsequently infected with replication-defective adenoviruses encodingG^{AE}-Ds or G-Ds and grown at the restrictive temperature for 24 hours. The cells were then shifted to permissive temperature for 30 minutes, fixed and imaged by confocal microscopy. The images shown are projections of confocal z-stacks that illustrate that G^{AE} accumulates in a GFP-Rab43-positive compartment, while G does not.



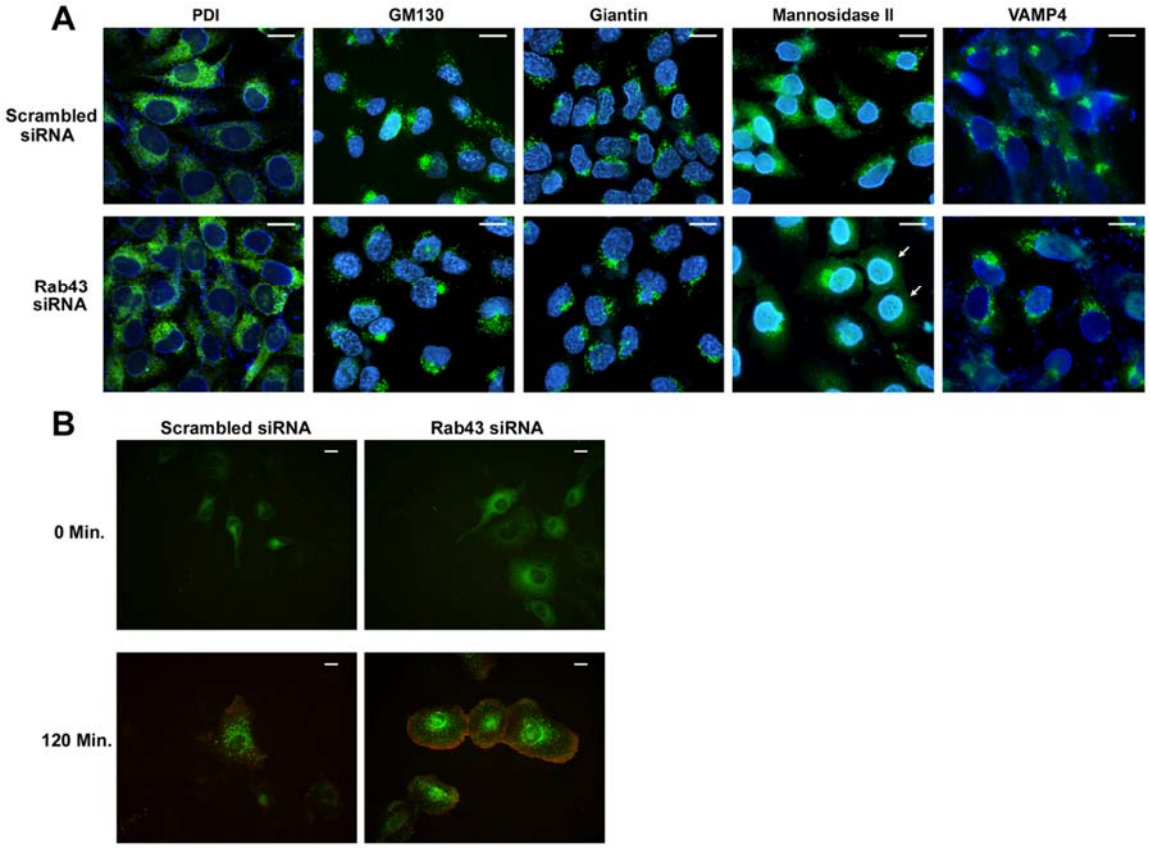
Supp. Fig. S3. Low power images illustrating the differential effect of GFP-Rab43on $G^{AE}$and G sorting. (A) COS7 cells or (B) PH5CH8 cells were transfected with GFP-Rab43and subsequently infected with adenoviruses encodingG^{AE}-Ds or G-Ds and grown at the restrictive temperature for 24 hours. The cells were then shifted to permissive temperature for (A) 30 or (B) 60 minutes, fixed and imaged by confocal microscopy. These lower magnification images show that the majority of G^{AE}

accumulated in a GFP-Rab43-positive compartment in both cell types, while G did not. White bars in A and B are 20μm.



Supp. Fig. S4. $G^{AE}$and G trafficking in GFP-Rab43 expressing cells.(A) COS7 cells transfected with GFP-Rab43 were subsequently infected with adenovirus expressing G^{AE}-Ds, and grown at the restrictive temperature for 24 hours. The cells were then shifted to permissive temperature for 30 minutes and fixed. The cells were then permeabilized and stained with giantin or mannosidase II (Mann. II) antibodies followed by incubation with Alexafluor647 conjugated secondary antibodies. The cells were then imaged by confocal microscopy and 3-D projections of confocal Z-stacks of the 3-colored images are shown. The mannosidase II projection is the same cell shown in Fig. 4C. (B) Alternatively, COS7 cells transfected with GFP-Rab43 were subsequently infected with adenovirus encodingG^{AE}-Ds or G-Ds. The cells were shifted to permissive

temperature for 120 minutes and fixed with 4% paraformaldehyde in PBS. Non-permeabilized cells were then incubated with the I1 anti-G monoclonal antibody and surface bound antibodies were detected using an Alexafluor conjugated secondary antibody. The cells were imaged on a Zeiss LSM510 confocal microscope using a Plan-Neo 10X/0.3 objective with 3X digital magnification. Bars in A are 10μm. Bars in B are 20μm.



Supp. Fig. S5. Effect ofRab43 knockdown on the distribution of early secretory pathway markers and the surface delivery of G^{AE}.(A) PH5CH8 cells transfected with control scrambled or Rab43-specific SMART pool siRNAs were fixed four days post-transfection and stained with antibodies directed against protein disulfide isomerase (PDI), GM130, giantin, mannosidase II, or VAMP4. Nuclei were visualized by staining with Hoechst. Arrows indicate Rab43 knockdown cells with diffuse mannosidase II

staining. Analysis of >100 cells from 2 independent experiments revealed that 33% of cells transfected with Rab43 siRNAexhibited diffuse mannosidase II staining while 6% of cells transfected with the scrambled siRNA exhibited diffuse mannosidase II staining. (B) PH5CH8 cells transfected with the Rab43-specific SMART pool siRNAs or with the scrambled control siRNA were infected with adenovirus encoding G^{AE}-EGFPat 72 hours post-transfection. Following growth overnight at restrictive temperature, the cells were shifted to permissive temperature for 0 or 2 hours and fixed. Surface levels of G^{AE} in non-permeabilized cells were monitored by staining with the I1 monoclonal antibody and surface bound antibodies were detected using donkey anti-mouse IgG conjugated to Alexafluor 594. Bars in A are 50μm. Bars in B are 20 μm.