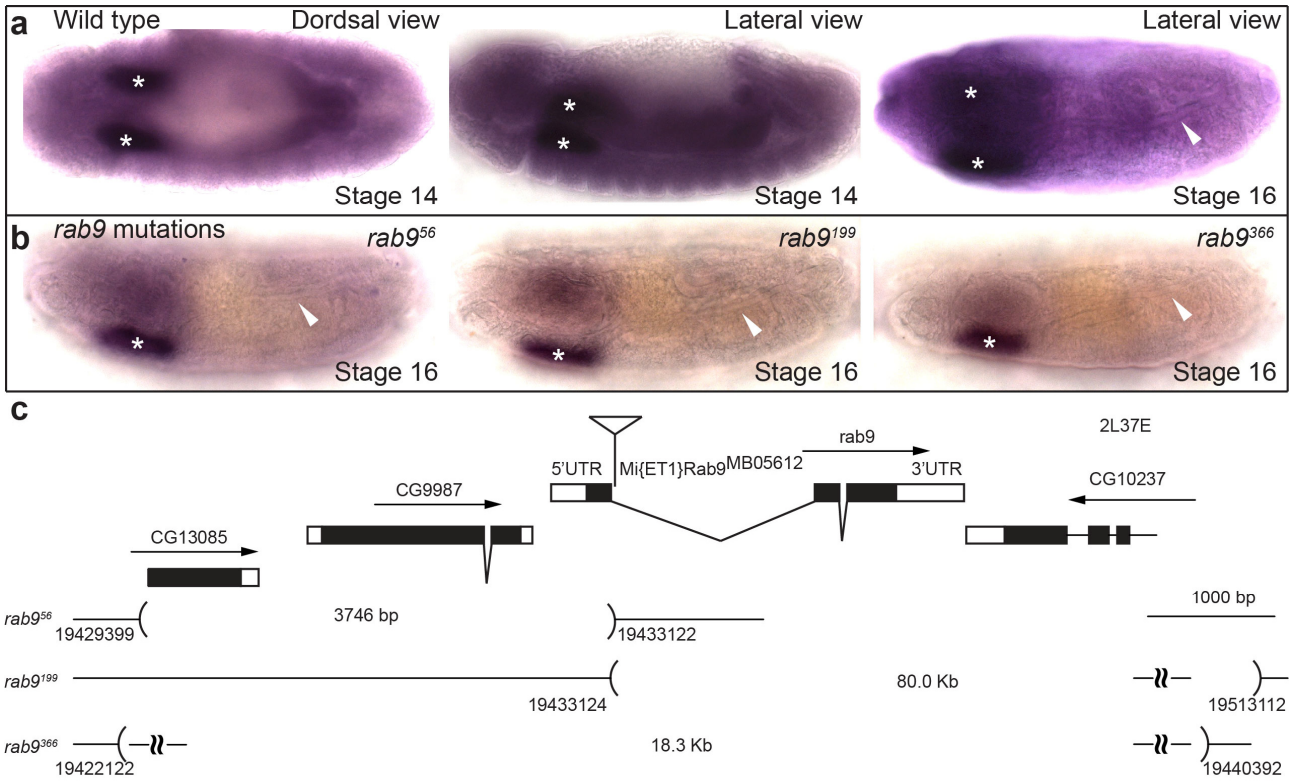
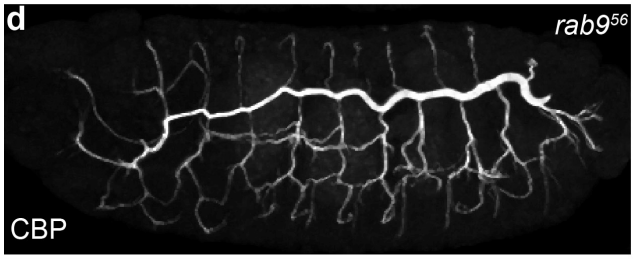
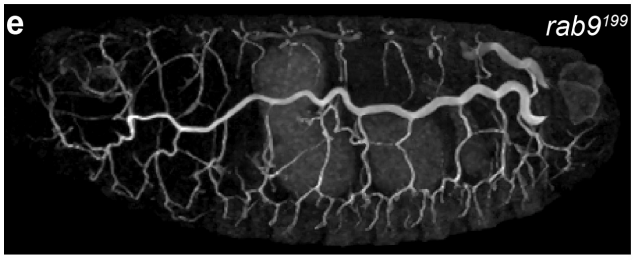
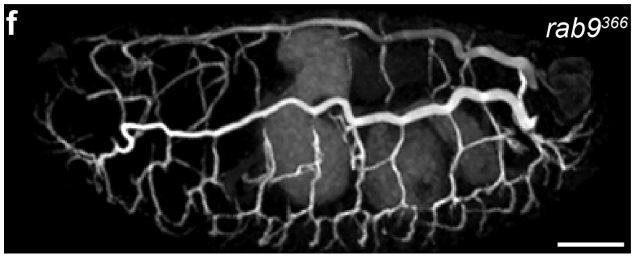
Supplementary Figures

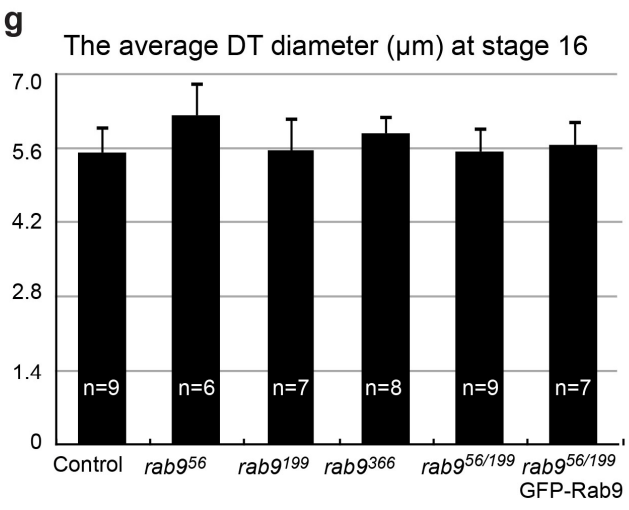
Supplementary Fig. S1









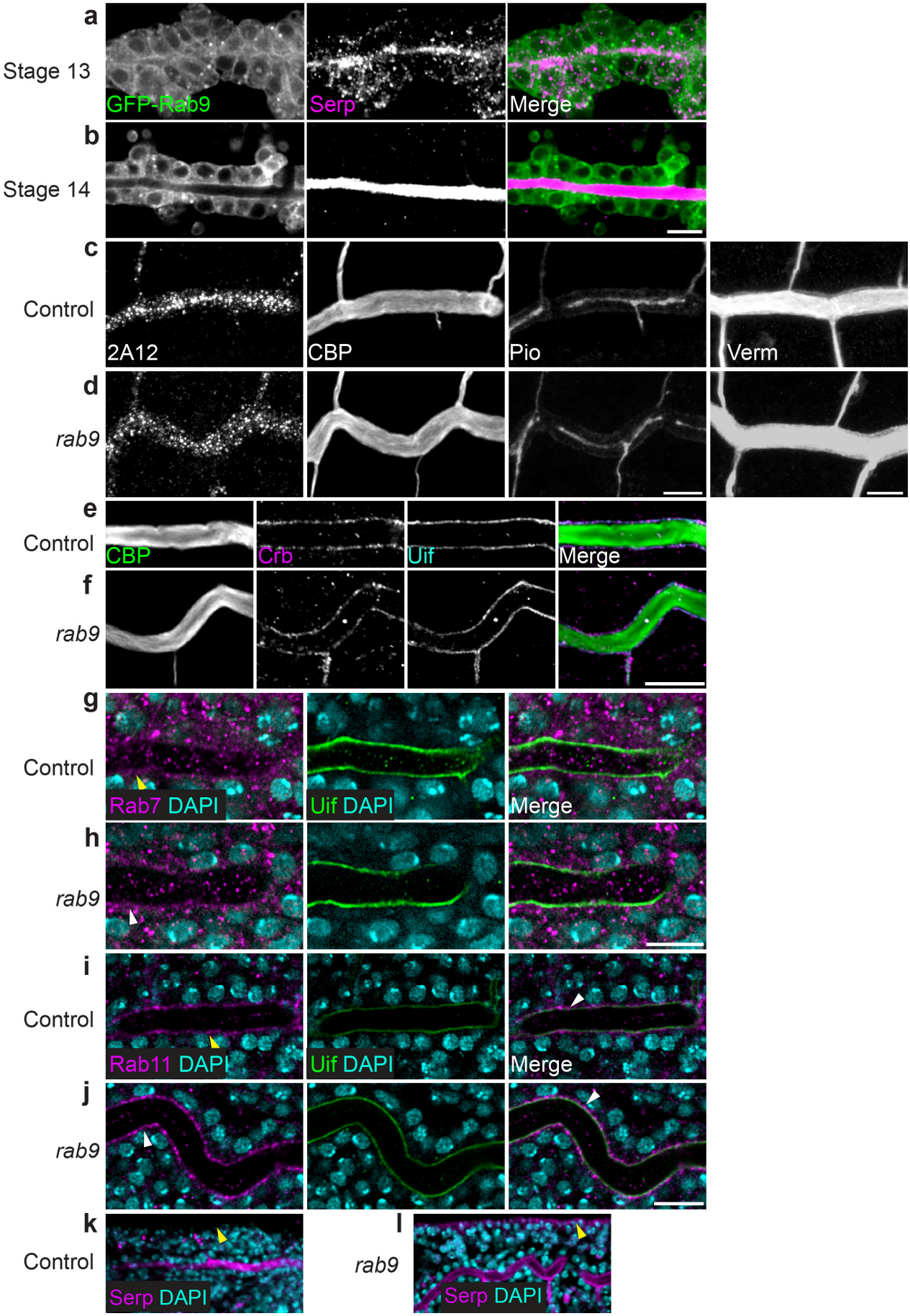


Supplementary Fig. S1

Rab9 regulates tracheal elongation rather than expansion.

(**a-b**) mRNA expression of *rab9* in wild-type (Oregon R) (**a**) and *rab9* mutant (**b**) embryos by *in situ* hybridization. White asterisks indicate non-specific staining of salivary glands. White arrowheads indicate the dorsal trunk (DT) of the tracheal tube in stage 16 embryos. (**c**) *rab9* gene organization and mutagenesis. Solid and empty boxes indicate exons and introns respectively. Brackets indicate the deleted region in the *rab9* locus. Triangle indicates the insertion position of the *Minos* transposon in the first intron of the *rab9* gene.(**d-f**) Stage16 *rab9* mutant embryos stained with CBP showing the DT. (**g**) Quantification of the DT diameter in control, *rab9* mutant, and rescued embryos at stage 16 (n=6-9 embryos). Scale bars represent 50 μm.

Supplementary Fig. S2

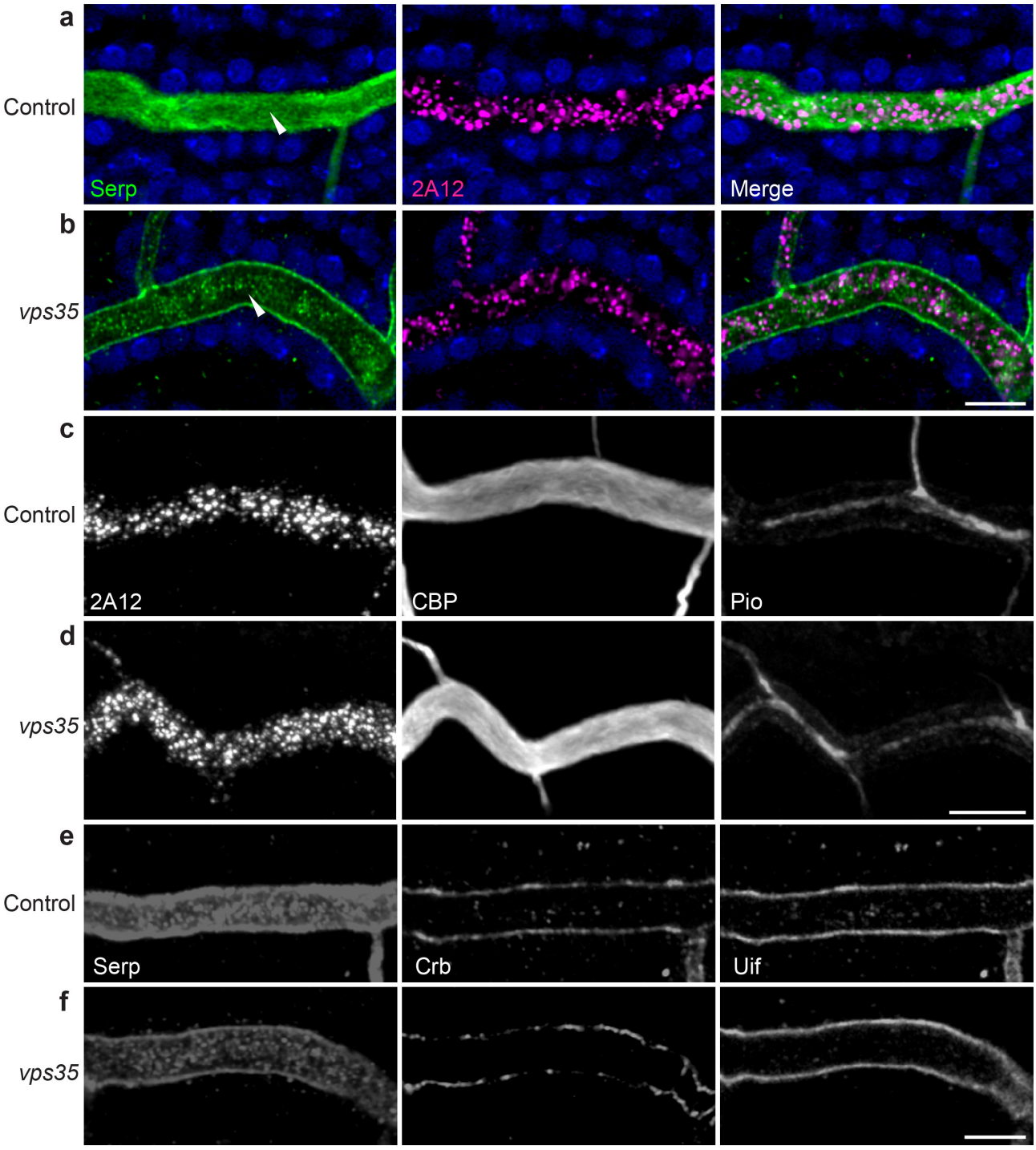


Supplementary Fig. S2

Rab9 specifically regulates Serp's localization and trafficking.

(**a,b**) GFP-Rab9 expressing embryos at stage13 and 14 were immunostained for Serp. (**c,d**) Stage 16 *rab9*^{56} heterozygous (**c**) and homozygous (**d**) embryos were immunostained for 2A12 antigen, CBP, Pio and Verm. 2A12, Chitin, Pio and Verm showed no discernible difference between the mutant and control embryos. (**e,f**) Stage 16 *rab9*^{56} mutant heterozygous (**e**) and homozygous (**f**) embryos immunostained for CBP, the membrane protein antigen Crb, and Uif. The apical membrane proteins Crb and Uif did not show any detectable differences between the control and *rab9* mutant embryos. (**g-j**) Stage 16 *rab9*^{56} mutant heterozygous (**g**,**i**) and homozygous (**h**,**j**) embryos immunostained for Rab7 and Uif (**g,h**), Rab11 and Uif (**i,j**). The localization of late endosomes and recycling endosomes did not change in *rab9* mutants (compare the white and yellow arrowheads in control **g** and **i** with *rab9* mutant **h** and **j** embryos, respectively). (**k,l**) Confocal sections of stage 16 control (**k**) and *rab9*^{56} mutant (**l**) embryos immunostained for Serp and DAPI. Yellow arrowheads point to the appearance of Serp at the surface of the epidermis in *rab9* mutant (**l**) compared with the control (**k**). Scale bars represent 10 µm.

Supplementary Fig. S3

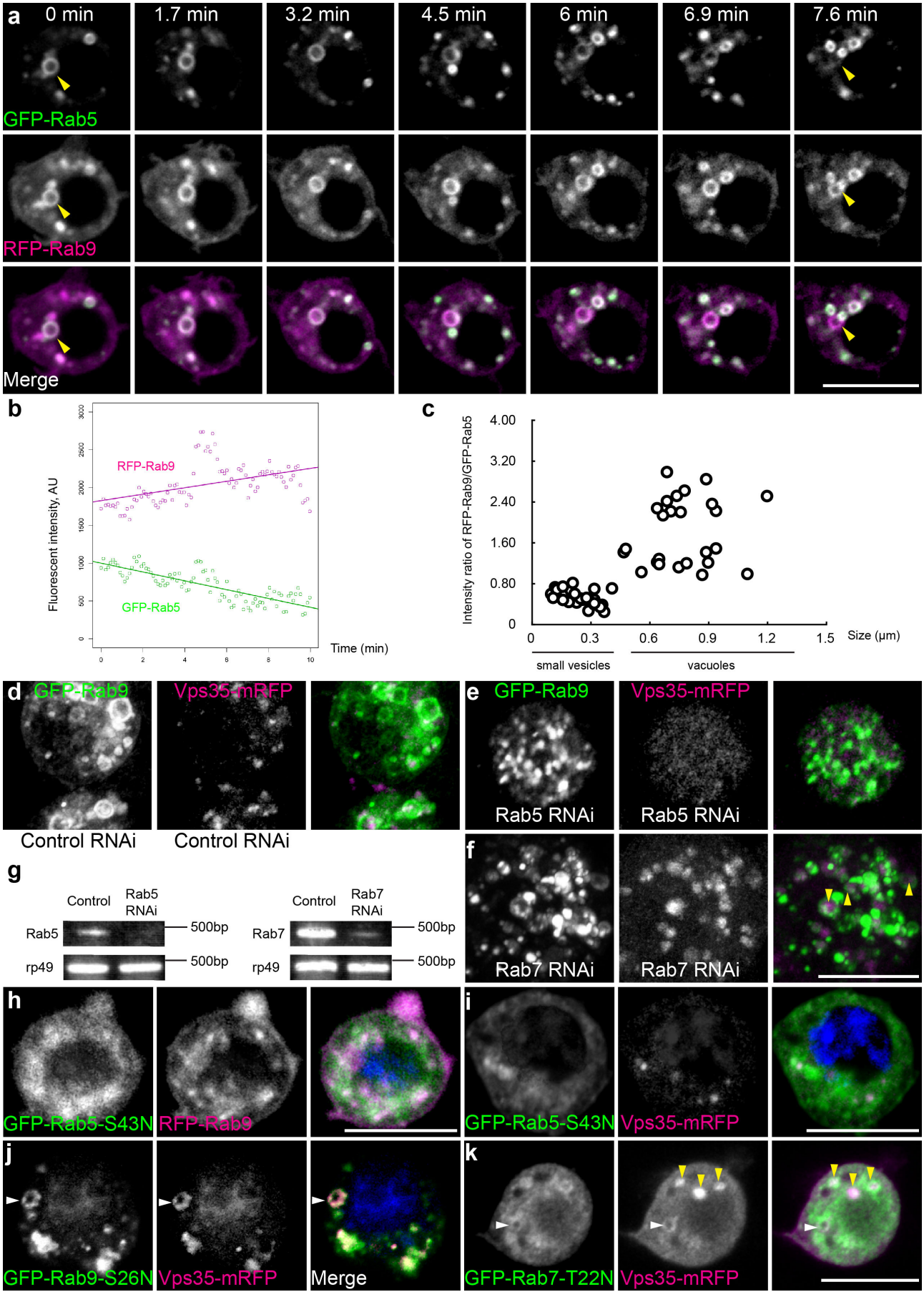


Supplementary Figure S3

Vps35 regulates the localization and trafficking of the specific luminal-protein.

(**a-f**) Stage 16 heterozygous (**a**,**c**,**e**) and homozygous (**b**,**d**,**f**) *vps35*^{1} embryos immunostained for Serp, 2A12 antigen (**a**,**b**), 2A12, CBP, and Pio (**c**,**d**), and Serp, Crb, and Uif (**e**,**f**), respectively. The white arrowheads in (**b**) shows the decrease of Serp in the lumen compared with that in control (**a**) embryo, whereas 2A12 (compare **b** with **a**, **d** with **c**), Chitin (compare **d** with **c**), and Pio (compare **d** with **c**) showed no difference between the mutant and control embryos. Scale bars represent 10 μm.

Supplementary Fig. S4



Supplementary Figure S4

Recruitment of Vps35 in endosomal membrane requires the function of Rab5, Rab9 and Rab7.

(**a**) Time-lapse images of S2 cells expressing GFP-Rab5 and RFP-Rab9. Yellow arrowheads show colocalization of GFP-Rab5 and RFP-Rab9 in a large vacuolar structure. Subsequently GFP-Rab5 was reduced, whereas RFP-Rab9 remained stable (see also supplementary Movie S2 online). (**b**) Change in GFP-Rab5 and RFP-Rab9 fluorescence intensity in the vacuole (yellow arrowhead in Fig.S4a). The bold line represents mean values of three different locations in the vacuolar membrane. (**c**) The intensity ratio of RFP-Rab9 to GFP-Rab5 in small vesicles (diameter below 0.4 μm) and vacuoles (diameter between 0.4-1.3 μm). (**d**-**f**) S2 cells were co-transfected with GFP-Rab9 and Vps35-mRFP, then treated with control (**d**), Rab5 (**e**) and Rab7 (**f**) dsRNAs. (**g**) RT-PCR of Rab5, and Rab7 fragment from control and dsRNA treated cells. rp49 was amplified for the internal control. (**h**) S2 cells were co-transfected with dominant-negative GFP-Rab5-S43N, and RFP-Rab9. (**i**) S2 cells were co-transfected with dominant-negative GFP-Rab5-S43N, and Vps35-mRFP. (**j**) S2 cells were co-transfected with dominant-negative GFP-Rab9-S26N and Vps35-mRFP. White arrowhead shows mislocalization of Vps35-mRFP throughout the GFP-Rab9-S26N-labeled endosomal membrane. (**k**) S2 cells were co-transfected with dominant-negative GFP-Rab7-T22N, and Vps35-mRFP. Yellow arrowheads show Vps35-mRFP accumulated inside of the endosomes. Scale bars represent 10 μm.