Tzaban et al., http://www.jcb.org/cgi/content/full/jcb.200809122/DC1

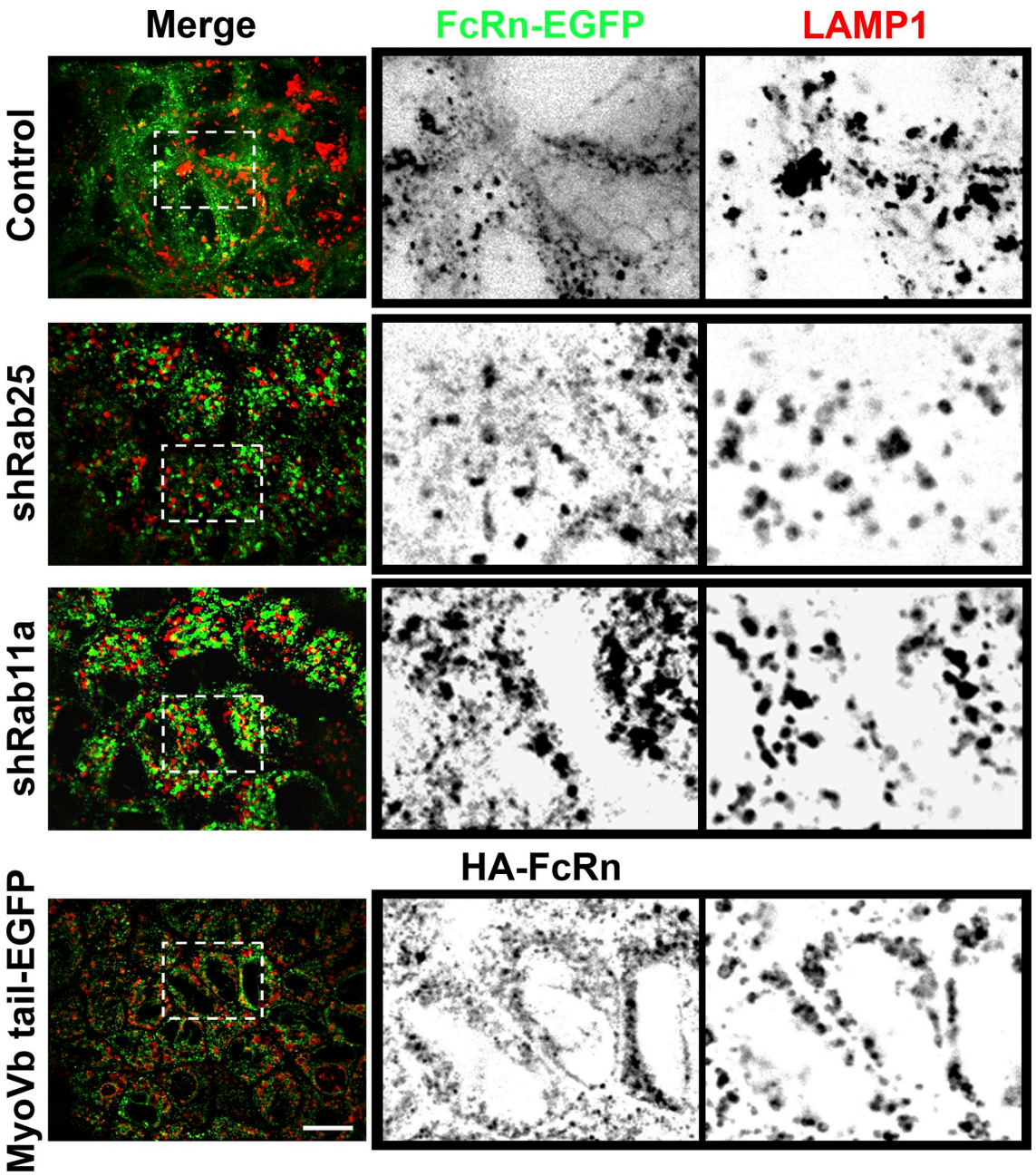


Figure S1. **Neither gene silencing of Rab25 or Rab11a nor overexpression of the tailless dominant-negative mutant of MyoVb diverts FcRn to the lysosomal compartment.** MDCK cells expressing FcRn-EGFP (top two rows) or HA-FcRn (bottom row) were incubated with doxycycline to induce the expression of shRNA targeting Rab25, Rab11a, or the MyoVb tail mutant. After 3 d, cells were fixed and stained for LAMP1 alone or together with HA staining (bottom row). 3D confocal stacks of images were acquired as described in Materials and methods, and selected middle sections are shown. Dashed boxes indicate the sections enlarged in the panels on the right.

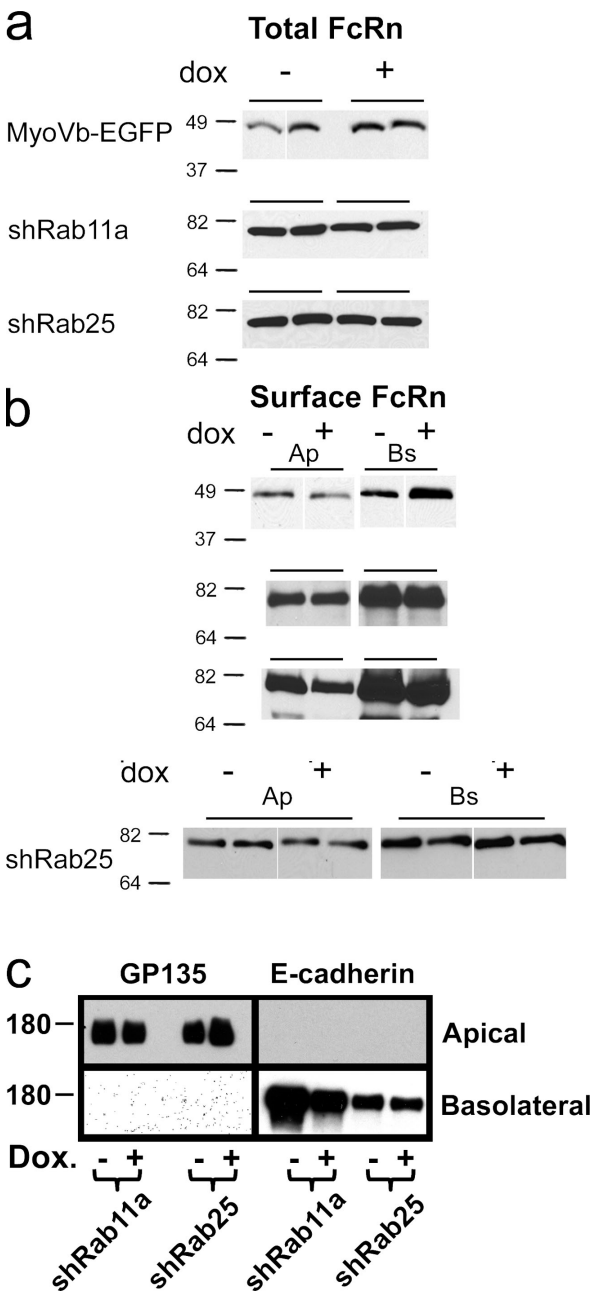


Figure S2. **Expression of MyoVb mutant or gene suppression of Rab11a or Rab25 has no detectable effect on total and cell surface levels of FcRn.** (a and b) Doxycycline-induced expression of MyoVb tail mutant and shRNA against Rab25 or Rab11a in MDCK cells. Cells with +/−dox were either directly lysed (a) or first subjected to selective cell surface biotinylation (b) on either the apical (Ap) or basolateral (Bs) cell surface, then lysed. Biotinylated proteins were pulled down using avidin-conjugated agarose beads. All samples were subjected to SDS-PAGE and immunoblotting against an HA epitope of FcRn. Two independent samples of each condition are shown in panel a. An additional example of shRNA against Rab25 is also shown (duplicates of independent samples). (c) Tight junctions remain intact and cells were polarized during these genetic perturbations. Cells with +/−dox were subjected to selective cell surface biotinylation on either the apical or basolateral cell surface, then lysed. Biotinylated proteins were pulled down using avidin-conjugated agarose beads. All samples were subjected to SDS-PAGE and immunoblotted against GP135 (apical marker) or e-cadherin (basolateral marker). Representative Immunoblots are shown. GP135 was only detected from apically biotinylated surface samples and not from basolaterally biotinylated samples. Opposite results were obtained for e-cadherin. Numbers to the left indicate kD.

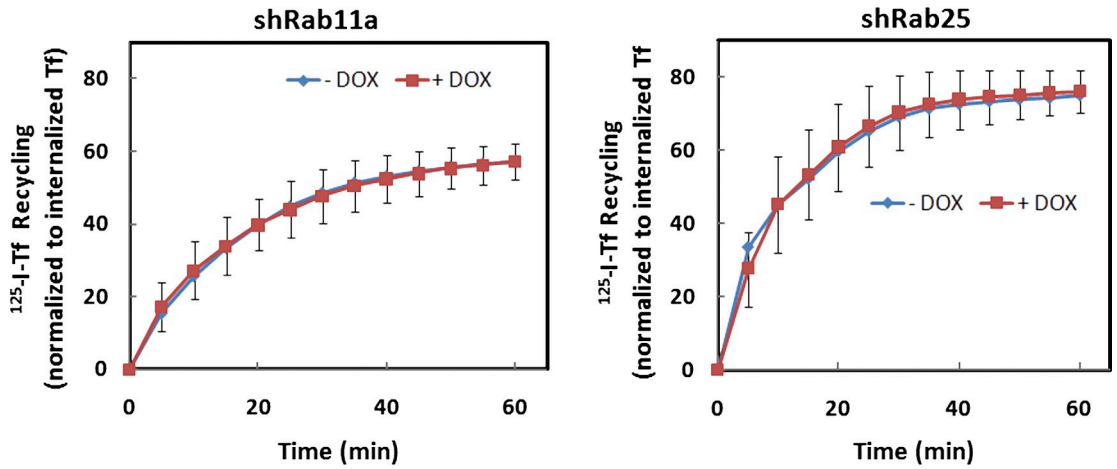
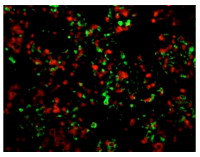


Figure S3. **Silencing Rab25 or Rab11a does not affect Tf recycling in polarized MDCK cells.** Induction of expression of shRNA targeting Rab25 and Rab11a in MDCK cells was performed as described previously. Recycling of ^{125}I-labeled human Tf was performed as described in Materials and methods. The line plot shows the lack of effect of the elimination of Rab25 and Rab11a in recycling of human Tf (each value is the mean of three independent samples). Standard errors are shown.



Video 1. **FcRn-EGFP is not present in acidic endosomes.** A 3D rendering of FcRn-EGFP cells stained for LAMP1. The cells were fixed and imaged by 3D confocal microscopy. Z series were collected from the bottom to the top of the cells every 0.25 µm. The video shows all optical sections and is played at 10 frames per second.