JCB

Supplemental material

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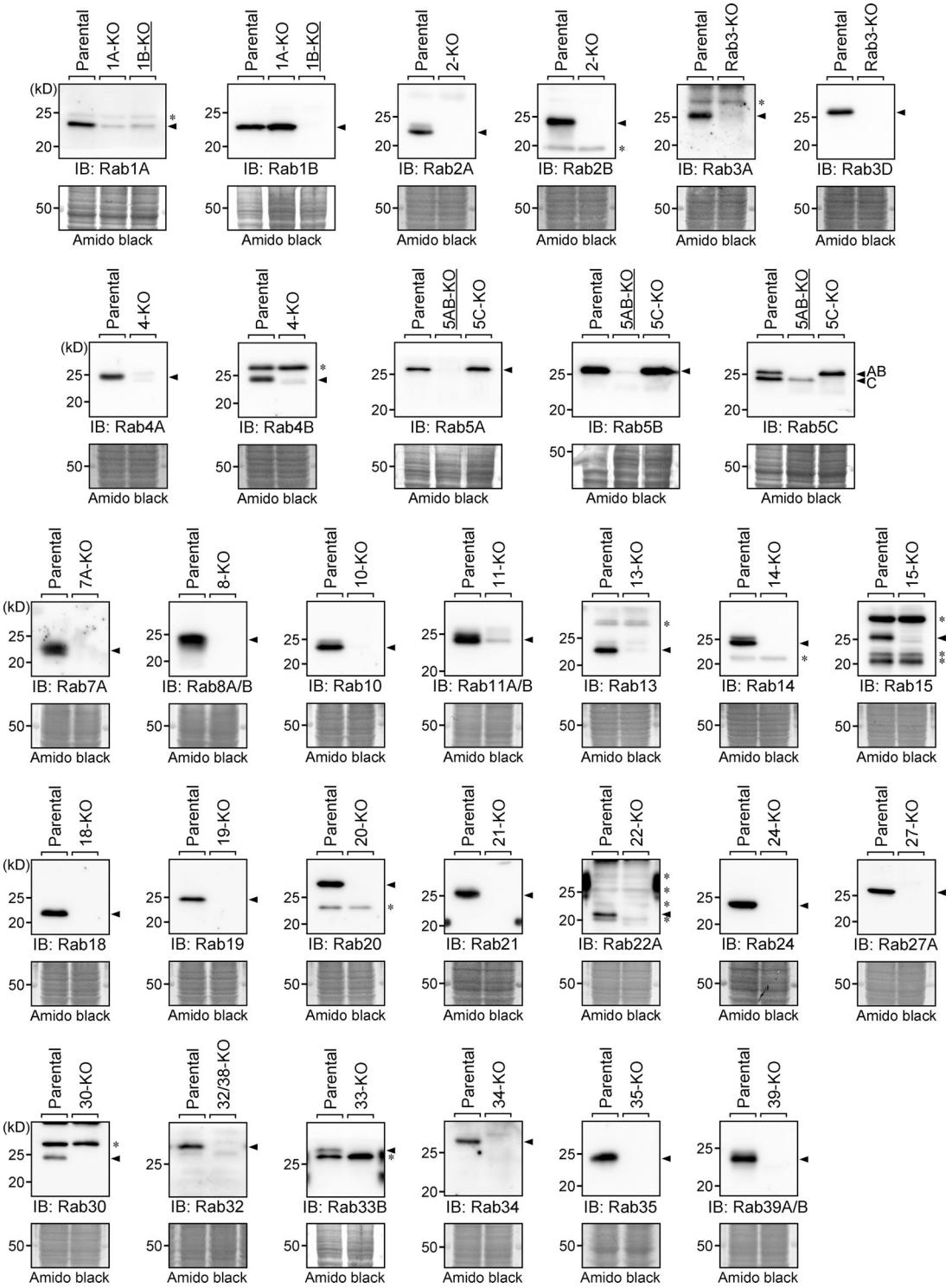

Figure S1. **Endogenous Rab expression in MDCK cells and its loss in Rab-KO cells.** Related to Table S1 and Fig. 1. Lysates of parental and Rab-KO cells were analyzed by immunoblotting (IB) with antibodies against the corresponding Rabs (indicated by arrowheads). Amido black staining was performed as a loading control. The asterisks indicate nonspecific bands of primary antibodies. Since these antibodies were raised against full-length Rab proteins, they may slightly recognize other related Rabs. Note that Rab1B-KO and Rab5A/B-KO cells (underlined) have mutated alleles of *Rab1A* and *Rab5C* (more specifically, they are Rab1A$^{+/-}$B$^{-/-}$ and Rab5A$^{-/-}$B$^{-/-}$C$^{+/-}$, respectively), thereby resulting in reduction of their protein levels.

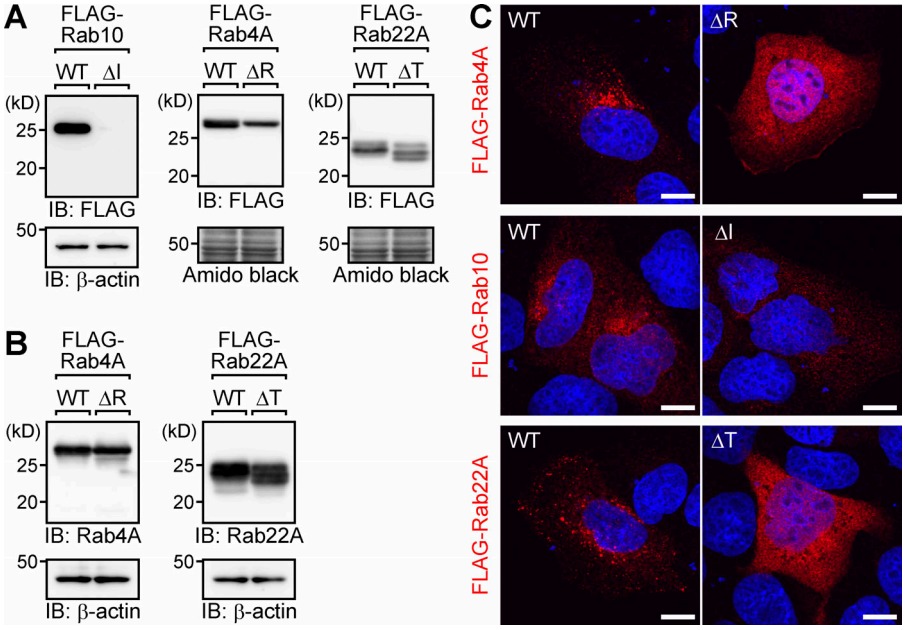

Figure S2. **Expression and localization of mutant Rab4A, Rab10, and Rab22A proteins that have one amino acid deletion.** Related to Table S1 and Fig. 1. **(A and B)** Expression of mutant Rab4A, Rab10, and Rab22A proteins in MDCK cells. FLAG-tagged Rab4A(ΔR), Rab10(ΔI), and Rab22A(ΔT) mutant (see Extended Fig. S1 in BioStudies for details) were overexpressed in MDCK cells, and their lysates were analyzed by immunoblotting (IB) with anti-FLAG, anti-Rab4A, and anti-Rab22A antibodies. **(C)** Localization of mutant Rab4A, Rab10, and Rab22A proteins in MDCK cells. FLAG-tagged Rab4A(ΔR), Rab10(ΔI), and Rab22A(ΔT) mutant were overexpressed in MDCK cells. The cells were then fixed with PFA and immunostained with an anti-FLAG (red) antibody (DAPI in blue). Scale bars, 10 µm.

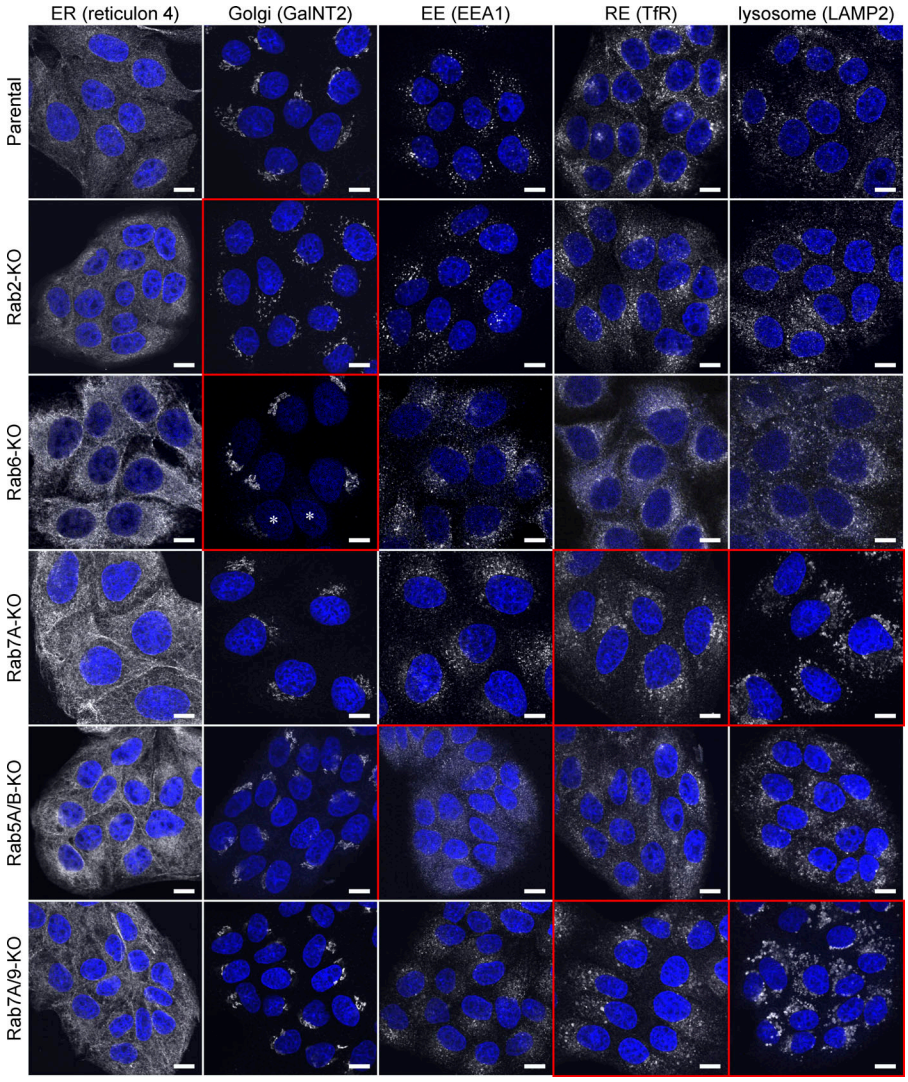

Figure S3. **Immunofluorescence analysis of the ER, the Golgi, early endosomes (EE), recycling endosomes (RE), and lysosomes in Rab-KO cells.** Related to Fig. 2. Rab-KO cells were fixed with PFA and immunostained for reticulon 4 (ER), GalNT2 (Golgi), EEA1 (EE), TfR (RE), and LAMP2 (lysosome; DAPI in blue). Scale bars, 10 µm. The red boxes indicate Rab-KO cells that showed abnormal organelle distribution and/or size (see Results for details).

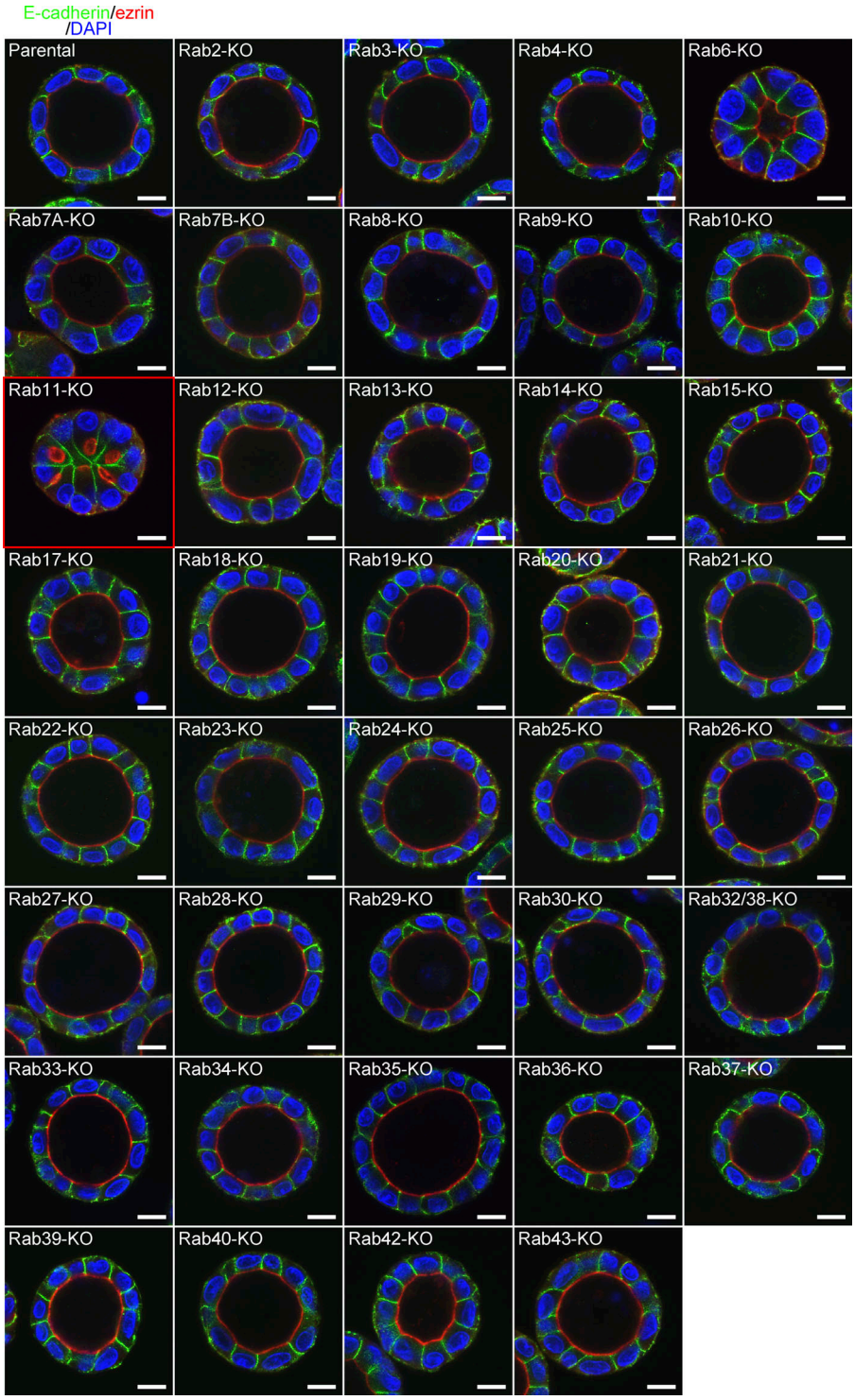

Figure S4. **Immunostaining of ezrin and E-cadherin in Rab-KO cysts.** Related to Fig. 4. Rab-KO cysts grown in collagen gel for 7 d were fixed with TCA and immunostained with anti-ezrin and anti–E-cadherin antibodies. Scale bars, 10 µm. The red box indicates Rab11-KO cysts that have multiple small lumens.

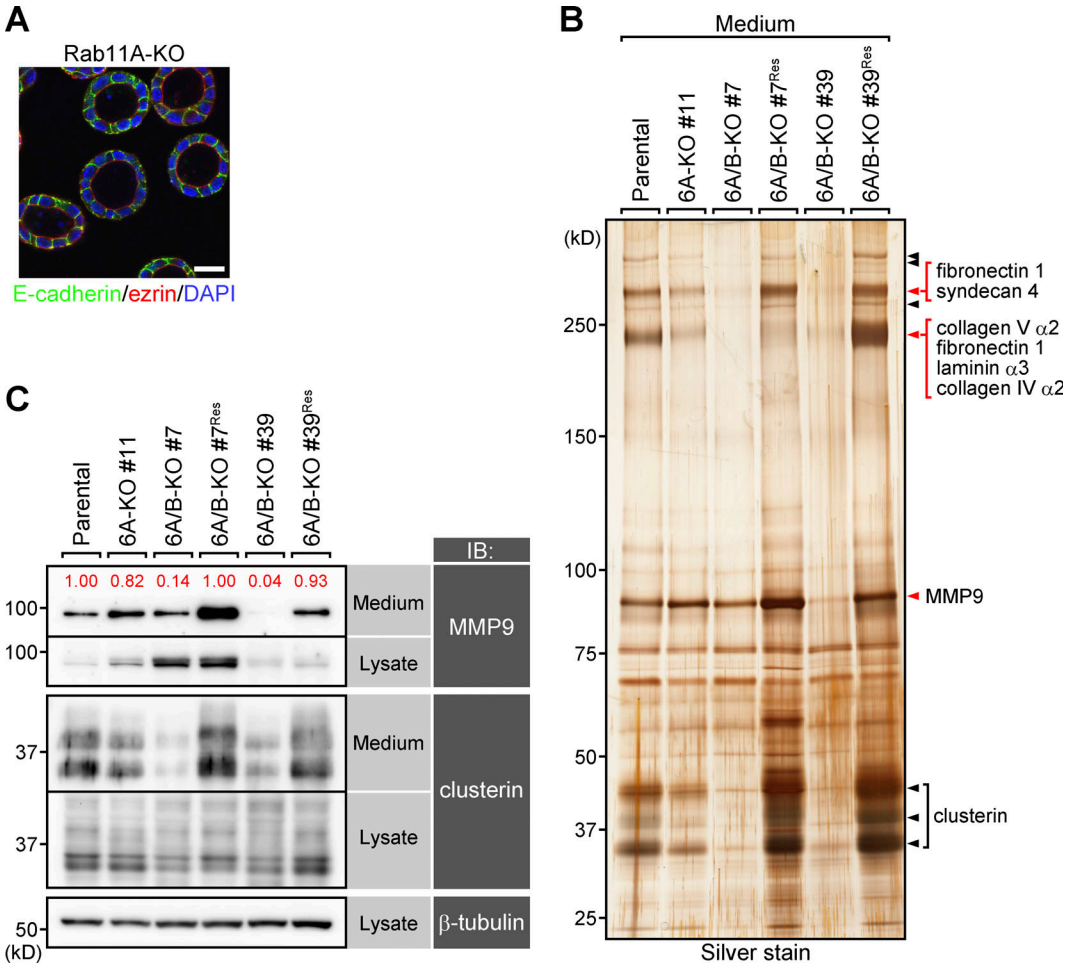

Figure S5. **Rab6 and Rab11 are required for normal epithelial morphogenesis.** Related to Fig. 4. **(A)** Immunostaining of ezrin and E-cadherin in Rab11A-KO cysts. Rab11A-KO cells were cultured in collagen gel for 7 d. The cells were fixed with TCA and immunostained with anti-ezrin (red) and anti–E-cadherin (green) antibodies (DAPI in blue). Scale bar, 20 µm. **(B)** Silver staining of the total secreted proteins shown in Fig. 4 D. Note that several bands (arrowheads) were greatly decreased in Rab6-KO cells, and the components of some of the bands (red arrowheads) were identified by mass spectrometry. The triplet bands around 37 kD were thought to be clusterin (Urban et al., 1987). MMP, matrix metalloproteinase. **(C)** Immunoblot (IB) analysis of MMP9 and clusterin in the total secreted proteins shown in Fig. 4 D. Note that although the levels of MMP9 expression varied among these cells, the medium/lysate ratios (indicated by red numbers) were markedly decreased in the Rab6-KO cells.

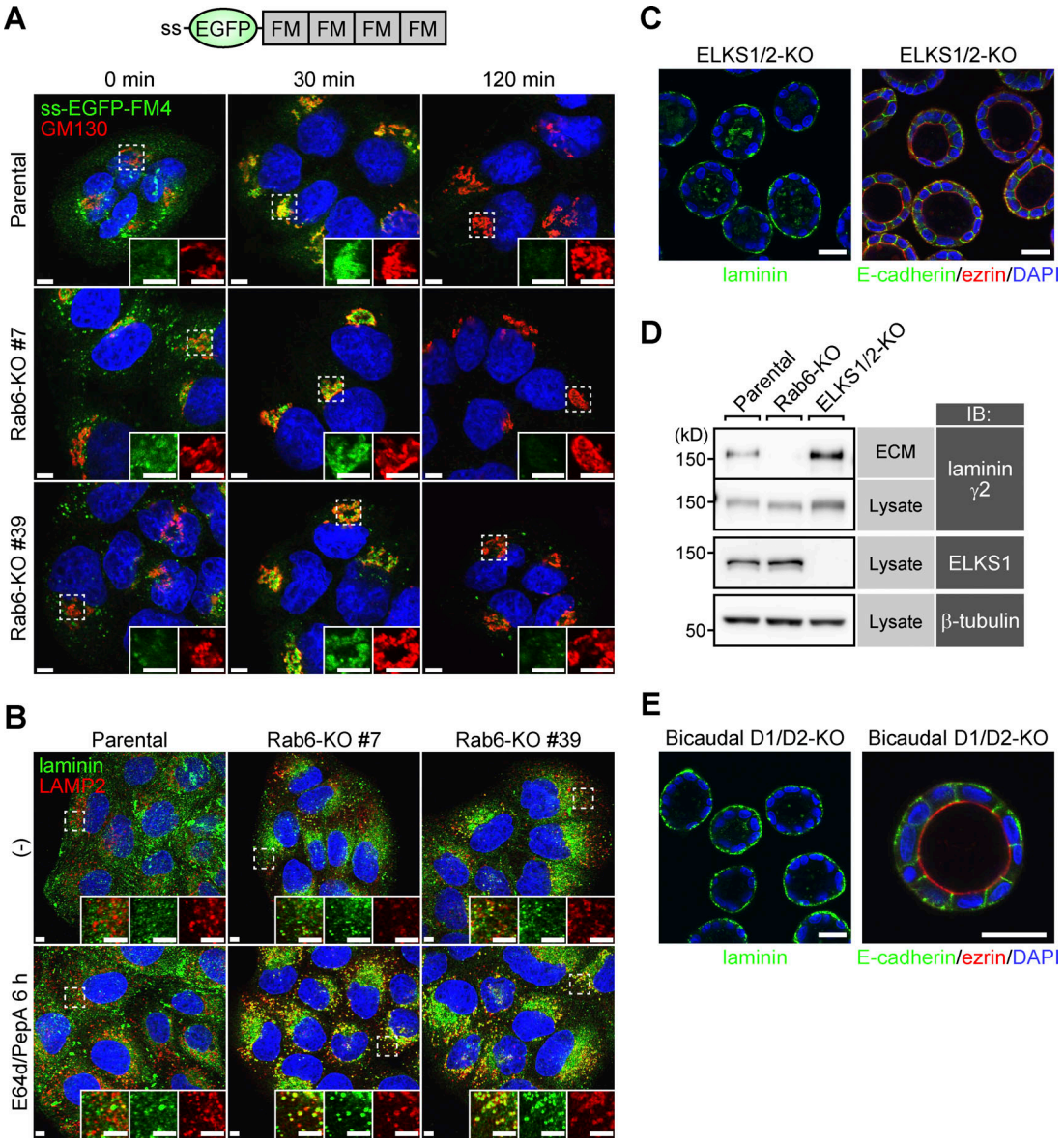

Figure S6. **Analysis of the secretory defect in Rab6-KO cells.** Related to Fig. 6. **(A)** Transport of ss-EGFP-FM4 in parental and Rab6-KO cells. Parental and Rab6-KO cells that stably express ss-EGFP-FM4 were treated with 100 ng/ml CHX and 250 µM D/D solubilizer to trigger synchronized transport of the cargo. After 0, 30, and 120 min, the cells were fixed with PFA and immunostained with an anti-GM130 antibody (red; DAPI in blue). Scale bars, 5 µm. **(B)** Endogenous laminin was also mistargeted to lysosomes in Rab6-KO cells. Parental and Rab6-KO cells were treated with 10 µM E64d and 20 µM pepstatin A for 0 and 6 h. The cells were then fixed with PFA and immunostained with anti-laminin (green) and anti-LAMP2 (red) antibodies (DAPI in blue). Scale bars, 5 µm. **(C)** Immunostaining of ELKS1/2-KO cysts. ELKS1/2-KO cells were cultured in collagen gel for 7 d. The cells were fixed with TCA and immunostained with anti-laminin (left) or anti-ezrin and anti–E-cadherin (right) antibodies. Scale bars, 20 µm. **(D)** Immunoblot (IB) analysis of laminin in ECM from ELKS1/2-KO cells. ECM and lysates from parental, Rab6-KO, and ELKS1/2-KO cells were analyzed by immunoblotting. **(E)** Immunostaining of Bicaudal D1/D2-KO cysts. Bicaudal D1/D2-KO cells were cultured in collagen gel for 7 d. The cells were fixed with TCA and immunostained with anti-laminin (left) or anti-ezrin and anti–E-cadherin (right) antibodies. Scale bars, 20 µm.

Reference

Urban, J., K. Parczyk, A. Leutz, M. Kayne, and C. Kondor-Koch. 1987. Constitutive apical secretion of an 80-kD sulfated glycoprotein complex in the polarized epithelial Madin-Darby canine kidney cell line. *J. Cell Biol.* 105:2735–2743. https://doi.org/10.1083/jcb.105.6.2735

Provided online are three tables in Excel. Table S1 is a list of the KO cells. Table S2 is a list of the proteins identified by quantitative mass spectrometry. Table S3 is a list of the materials used in this article.