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Supplemental information

Consecutive functions of small GTPases guide HOPS-mediated tethering of late endosomes and lysosomes

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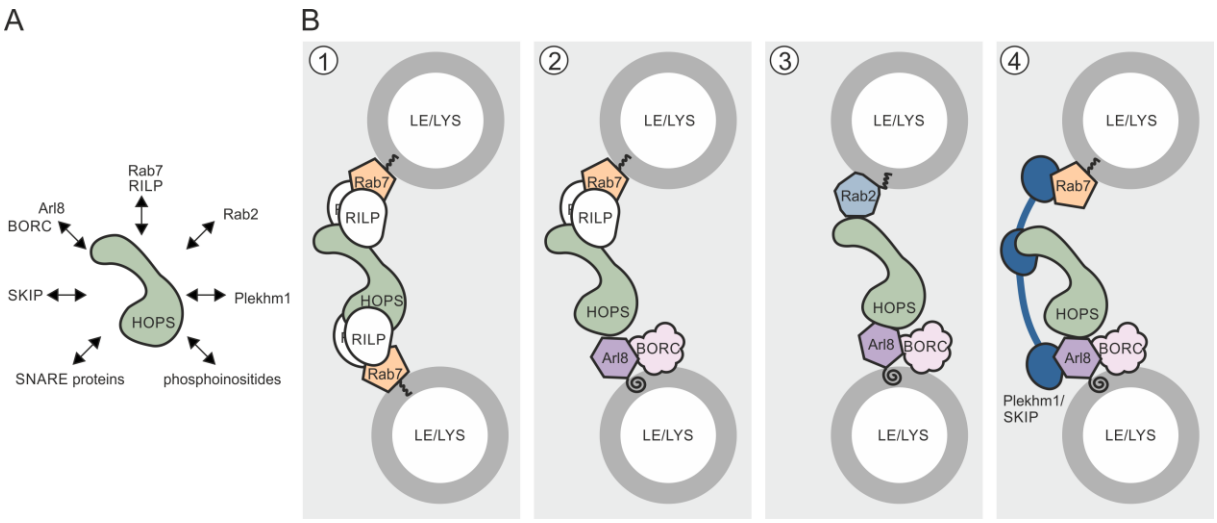

Figure S1 (related to Introduction and Figure 7 N). The HOPS tethering bridge between LEs and LYSs. (A) Summary of the various interactors of HOPS. (B) Schematic representation of scenarios of how HOPS may bridge late endocytic compartments during tethering. Note that neither of the depicted HOPS interactors had been assigned to LEs or LYSs. See text for details.

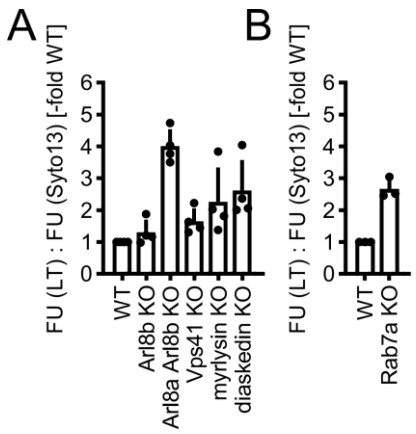

Figure S2 (related to Figure 2). Rab7, Arl8, HOPS or BORC KO cells are not defective in endosome acidification. (A, B) Cells were seeded in 96-well plates and cultured for 16 h at 37°C. Cells were incubated with DMEM/FBS containing 100 nM LT and 1 µM Syto13 at 37°C for 30 min. Using a spectrofluorometer, the LT and Syto13 fluorescence intensities per well were measured. To normalize the LT fluorescence per well to the number of cells analyzed, the ratios between the fluorescence intensities of LT and Syto13 were calculated. The LT:Syto13 ratio for WT HeLa cells was set as 1. Data are means and SDs from 4 (A) or 3 (B) independent experiments.

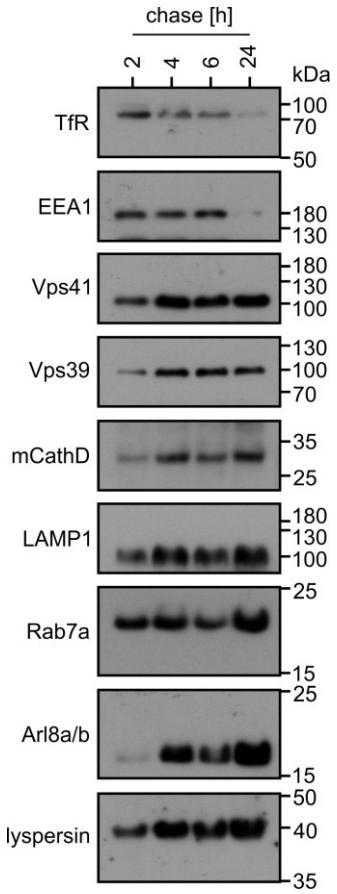

Figure S3 (related to Figure 3). FF endosomes lose early endocytic markers 24 h after endocytosis. FF compartments were purified from HeLa WT cells after 30 min of pulse and the chase periods indicated, adjusted to identical protein content and analyzed by immunoblotting.

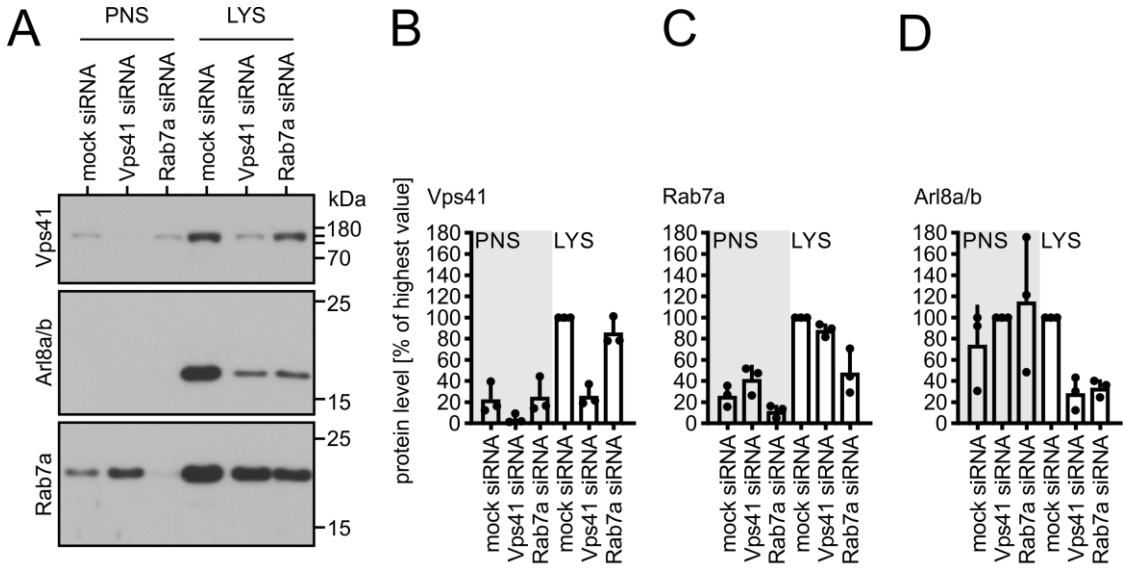

Figure S4 (related to Figure 4). Rab7 and Vps41 act upstream of Arl8 in J774E cells. (A) J774E cells were transfected with siRNAs targeting Vps41 or Rab7 or scrambled siRNA (mock). Twenty-four hours after transfection, cells were incubated with FF for 20 min/20 min (pulse/chase) and FF compartments were purified and analyzed by immunoblotting. (B-D) Quantification of immunoblots from 3 independent experiments as in A. Data are means and SDs.

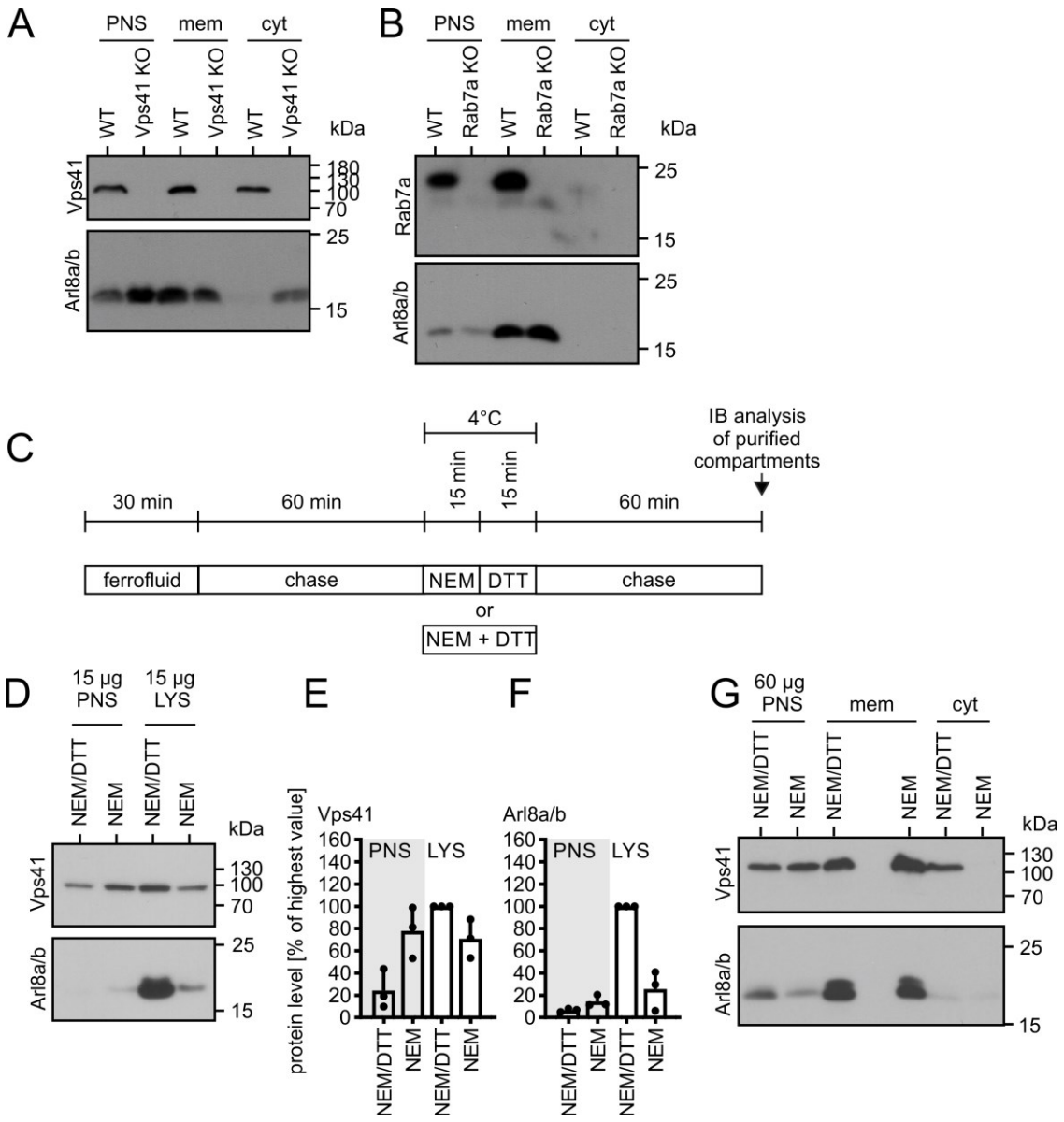

Figure S5 (related to Figure 4). Endosomes acquire Arl8 by fusion with Arl8-containing LYSs. (A) PNSs were prepared from HeLa Vps41 or Rab7 KO cells and from the corresponding WT cells and separated into membrane (mem) and soluble (cyt) protein fractions. The fractions were analyzed for presence of Arl8a/b (A, B), Vps41 (A) or Rab7 (B). One representative of 2 independent experiments. (C) Experimental setup. See text for details. PNSs and FF endosomes were prepared and analyzed by immunoblotting. Representative immunoblots (D) quantified in (E, F). Data are means and SDs from 3 independent experiments. (G) HeLa WT cells were incubated with NEM or DTT-pretreated NEM as in (C), PNSs were prepared and separated into a membrane (mem) and a soluble protein (cyt) fraction. Fractions were analyzed by immunoblotting for presence of Vps41 and Arl8a/b. Representative of 2 independent experiments.

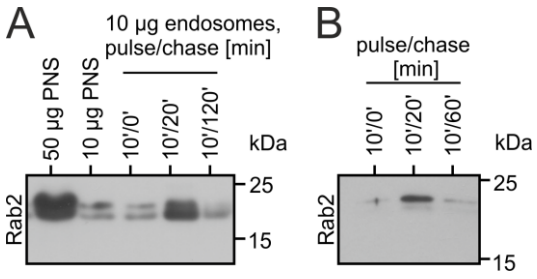

Figure S6 (related to Figure 6). Rab2a associates with maturing endosomes / phagosomes transiently. (A) FF compartments or (B) LBPs were purified from J774E cells after the pulse/chase periods indicated, adjusted to identical protein content or identical phagosome numbers (OD_{600}) and analyzed for Rab2 by immunoblotting. Samples are from the experiments described in Figure 3 H and J.