Expanded View Figures

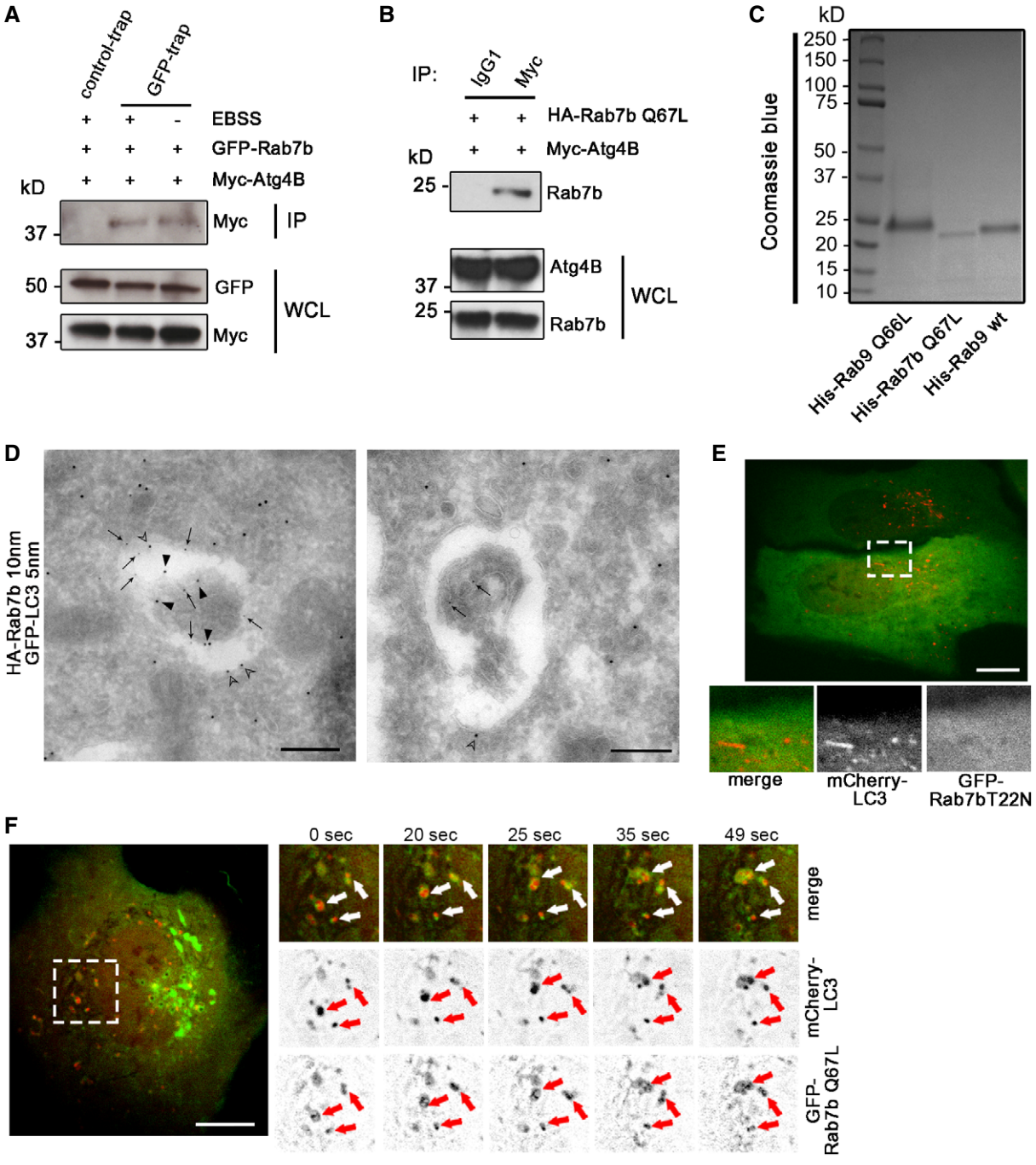

Figure EV1.

Figure EV1. Rab7b associates with Atg4B and autophagosomes.

A U2OS cells were transiently co-transfected with myc-Atg4B and GFP-Rab7b, incubated in CM or starved for 2 h, and lysed, followed by co-IP with GFP-Trap magnetic agarose beads. Whole-cell lysates (WCL) and immunoprecipitates (IP) were subjected to Western blot analysis using the indicated antibodies.

B U2OS cells were transiently co-transfected with HA-Rab7b Q67L and myc-Atg4B, starved for 2 h, and lysed, prior to co-IP with an antibody against myc or an isotype control (IgG1). Whole-cell lysates (WCL) and immunoprecipitates (IP) were subjected to Western blot analysis using the indicated antibodies.

C Coomassie blue staining of bacterially expressed His-Rab9 Q66L, His-Rab7b Q67L, and His-Rab9 wt purified using cobalt-coated Dynabeads.

D Double immunogold labeling of U2OS cells stably transfected with GFP-LC3 and transiently transfected with HA-Rab7b. HA-Rab7b (10 nm gold) localizes to outer (open arrowheads) and inner (filled arrowheads) membranes of autophagosomes, identified by LC3 labeling (5 nm gold, arrows) and characteristic ultrastructure (cytoplasmic mass surrounded with electron lucent area representing double membrane). Scale bars: 200 nm.

E, F Live-cell imaging of U2OS cells transiently transfected with mCherry-LC3 and GFP-Rab7b T22N (E) or mCherry-LC3 and GFP-Rab7b Q67L (F). Red and white arrows indicate vesicles positive for both mCherry-LC3 and GFP-Rab7b Q67L. Scale bar: 15 μm.

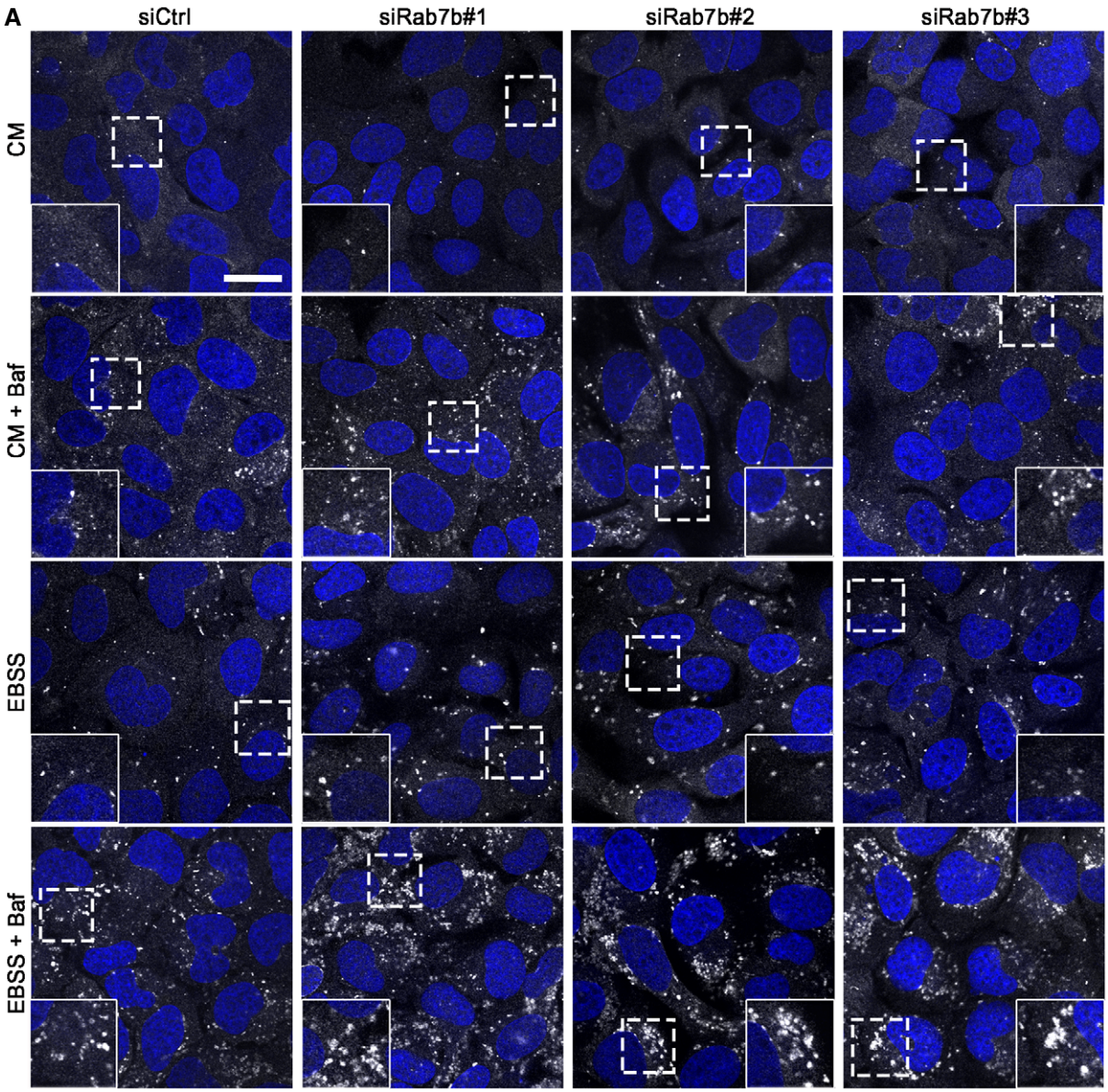

Figure EV2. Depletion of Rab7b increases the size of LC3-positive puncta.

U2OS cells transfected with control siRNA (siCtrl) or three different siRNAs against Rab7b (#1, #2, or #3) were incubated for 2 h in either CM, CM with BafA1, EBSS, or EBSS with BafA1, before fixation and staining with anti-LC3 and Hoechst. The insets show magnifications of the boxed areas. Scale bar: 20 μm.

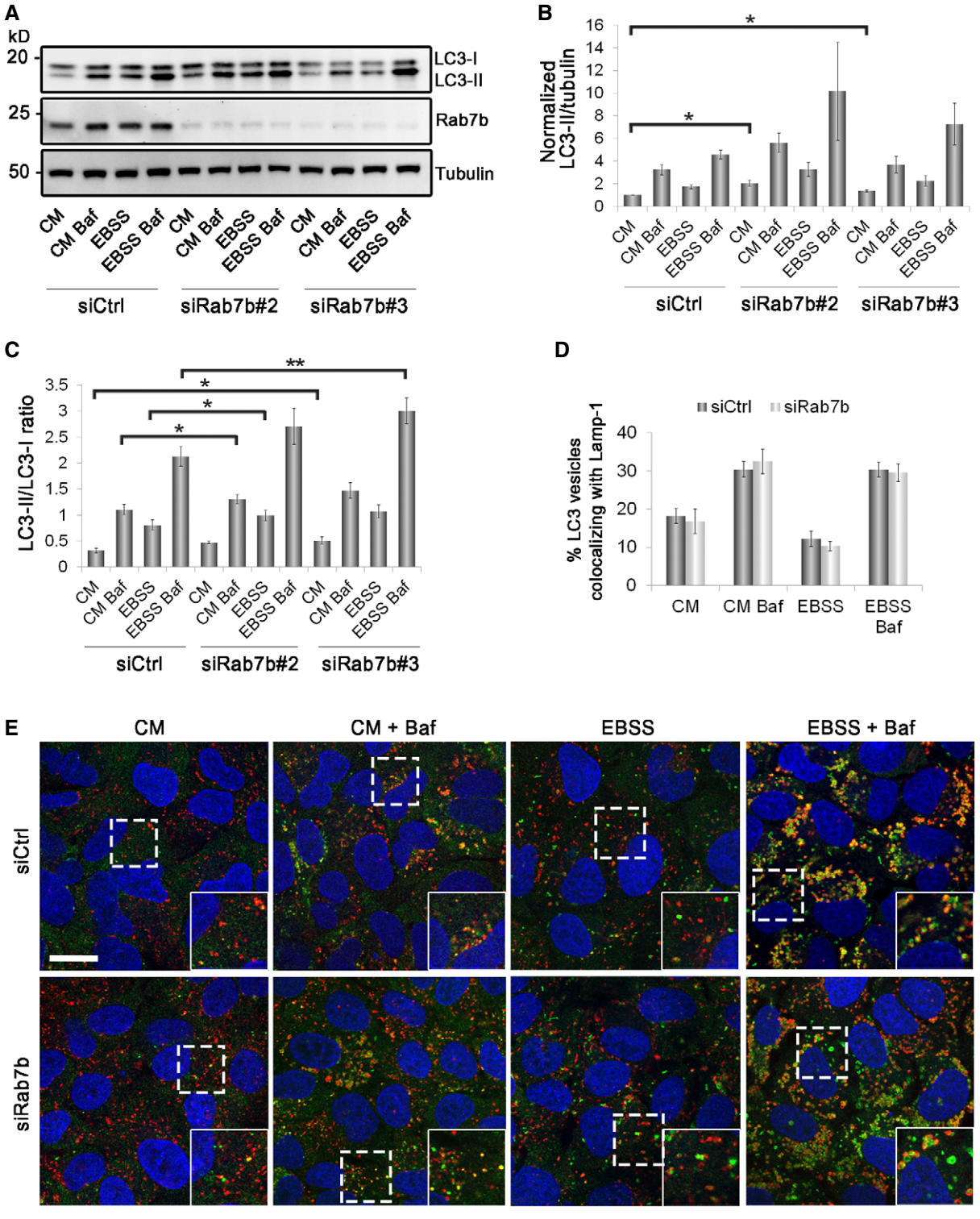

Figure EV3.

◀ **Figure EV3. Rab7b depletion causes LC3-II accumulation but does not affect the fusion of autophagosomes with lysosomes.**

A Cell lysates from cells transfected with control siRNA or siRNAs against Rab7b were subjected to Western blot analysis with the indicated antibodies.

B, C Quantification of LC3-II levels normalized against tubulin and plotted relative to the intensities obtained in cells transfected with control siRNA in CM (B) and of LC3-II/LC3-I ratio (C). Data represent the mean ± s.e.m. of four independent experiments.

D U2OS cells transfected with control siRNA (siCtrl) or siRNA against Rab7b #1 (siRab7b) were incubated for 2 h in either CM, CM with BafA1, EBSS, or EBSS with BafA1, fixed, and stained with antibodies against LC3 and Lamp-1. The percentage of LC3 vesicles also positive for Lamp-1 per cell is represented in the graph. Data represent the mean ± s.e.m. of four independent experiments ($n > 60$ cells).

E Representative confocal fluorescence images showing LC3 (green), Lamp-1 (red), and Hoechst (blue) under normal or starvation conditions (with or without BafA1) in control cells transfected with a non-targeting siRNA or in cells depleted of Rab7b. The insets show magnifications of the boxed areas. Scale bar: 20 μm.

Data information: Statistical significance was evaluated in (B–D) using paired Student's *t*-test. *$P < 0.05$; **$P < 0.01$.

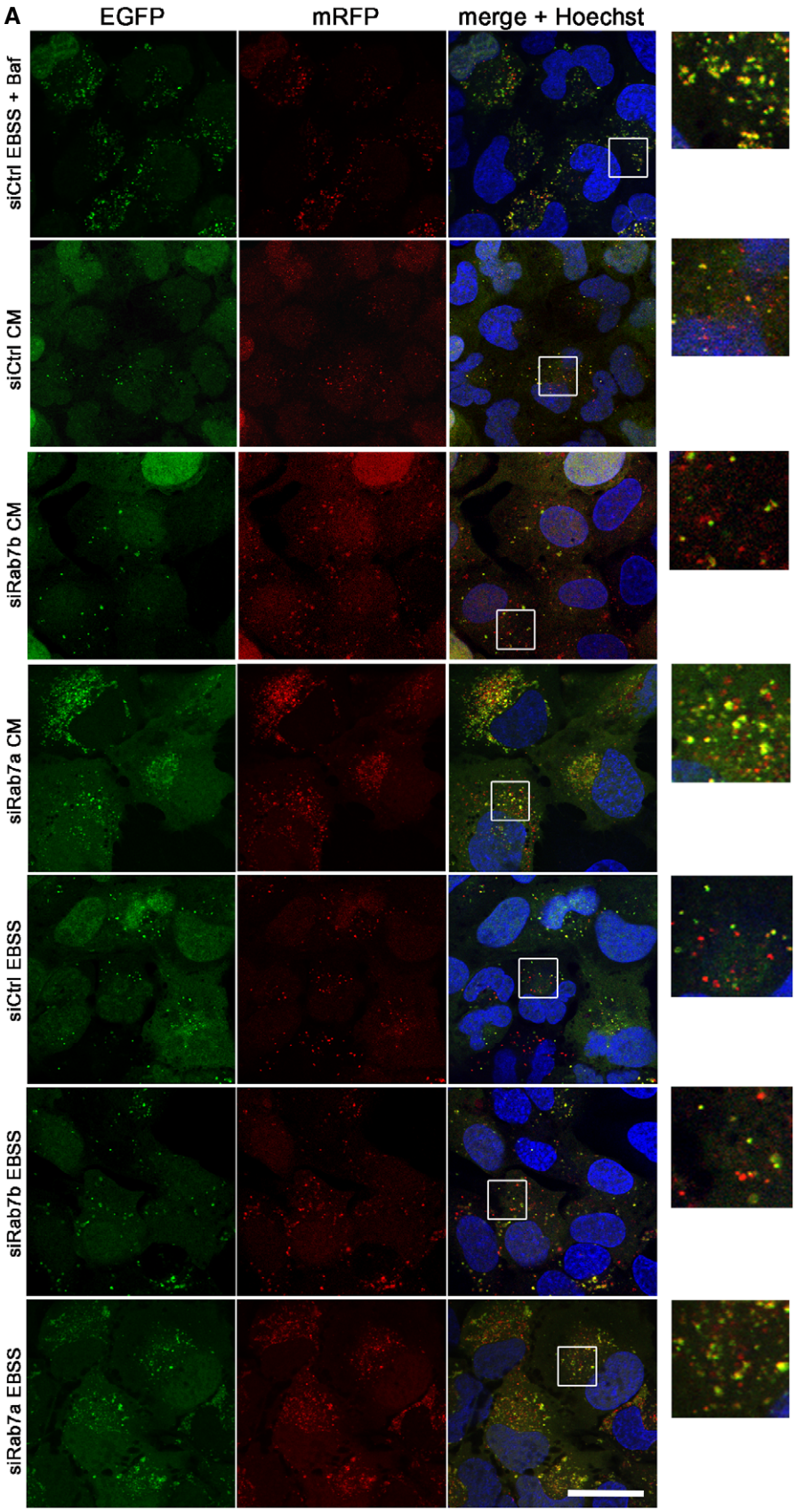

Figure EV4. Rab7b does not influence acidification of autophagosomes.

U2OS cells stably expressing mRFP-GFP-LC3 were transfected with either control siRNA (siCtrl) or siRNA against Rab7b or Rab7a and incubated for 2 h in CM, EBSS, or EBSS with BafA1. Subsequently, cells were fixed and stained with Hoechst (blue). The insets on the right side show magnifications of the boxed areas. Scale bar: 30 μm.

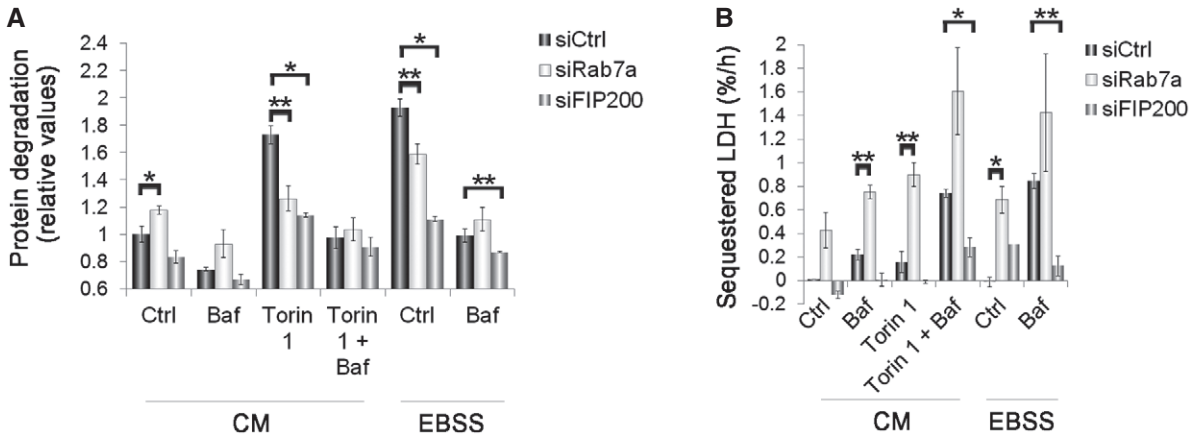

Figure EV5. Effect of Rab7a or FIP200 depletion on LLPD and LDH sequestration.

A U2OS cells transfected with control siRNA or siRNA against Rab7a or FIP200 were subjected to a LLPD assay. The quantification of the degradation rate for long-lived proteins is presented as mean ± s.e.m. of at least three independent experiments.

B U2OS cells transfected with control siRNA or siRNA against Rab7a or FIP200 were subjected to a LDH sequestration assay. In the graph, the net sequestered LDH per hour (%/h) is represented as mean ± s.e.m. of at least three independent experiments.

Data information: Statistical significance was evaluated in (A and B) using paired Student's *t*-test. *$P < 0.05$; **$P < 0.01$.