JCB

# Supplemental material

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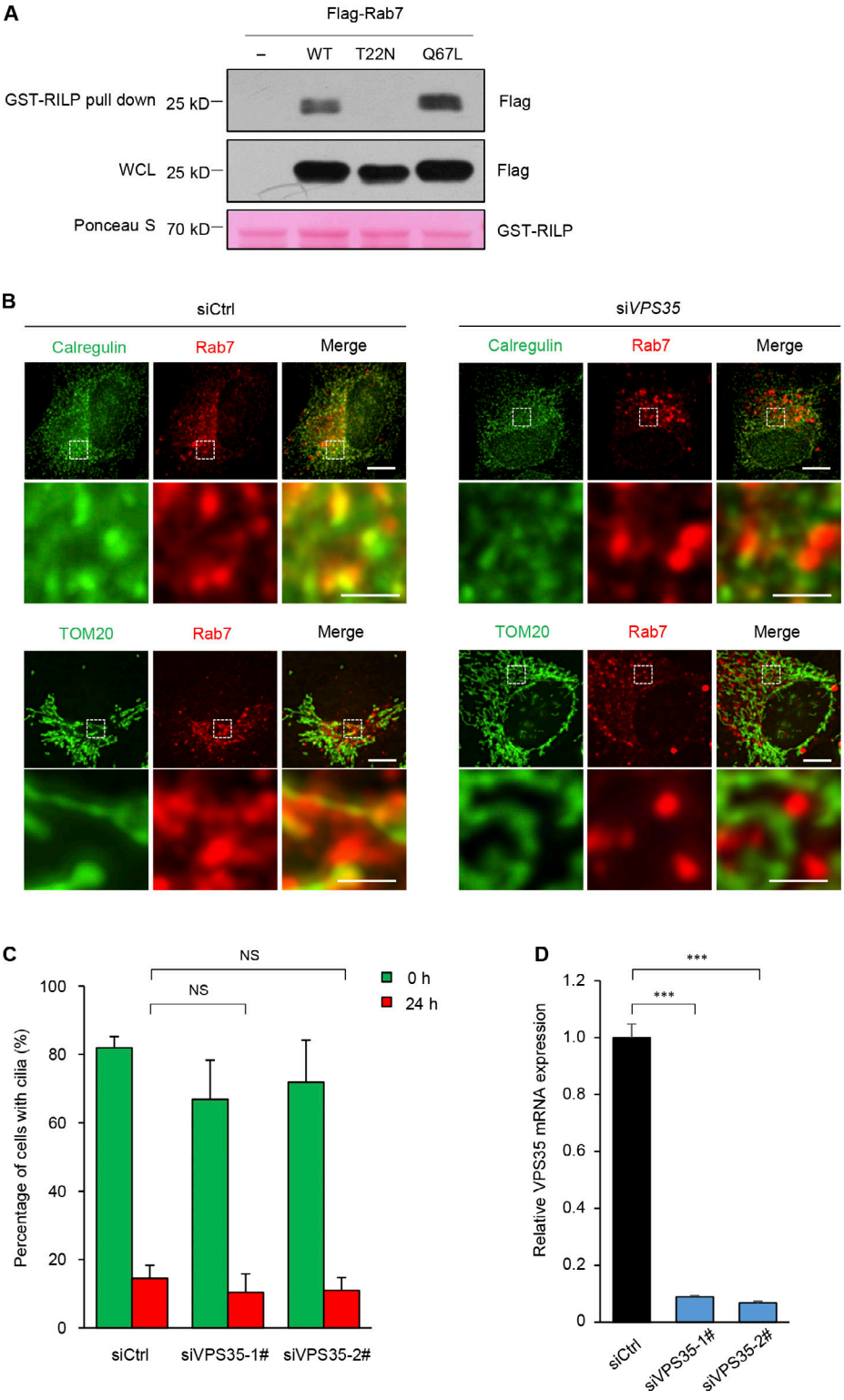


Figure S1. **The activity of Rab7 is required for the proper maintenance of ciliogenesis. (A)** Determination of the activity of Rab7 constructs. Different Rab7 proteins were expressed in HEK293T cells, and the corresponding cell lysates were incubated with GST-RILP beads for 1 h, followed by detection of proteins attached to the beads by Western blotting with the indicated antibodies and Ponceau S staining. WCL, whole cell lysis. **(B)** VPS35 knockdown inhibits Rab7 localization on ER and mitochondria membranes. Control cells and VPS35 knocked-down cells were co-stained for endogenous Rab7 (red) and endogenous ER marker (Calregulin, green, upper panels) or for endogenous Rab7 (red) and endogenous mitochondria marker (TOM20, green, lower panels). Scale bars, 10 µm (main image) and 2 µm (magnified regions). **(C)** RPE-1 cells transfected with the indicated siRNAs were subjected to cilia disassembly assay. The percentage of cells with primary cilia was quantified. Data are means ± SD of three independent experiments. Student's *t* test was performed. From left to right, cell *n* = 185, 182; 174, 182; and 164, 180. **(D)** Quantitative real-time PCR assay for VPS35 gene expression performed on RPE-1 cells transfected with the indicated siRNAs. Data are means ± SD of three independent experiments. Student's *t* test was performed. ***P < 0.001.

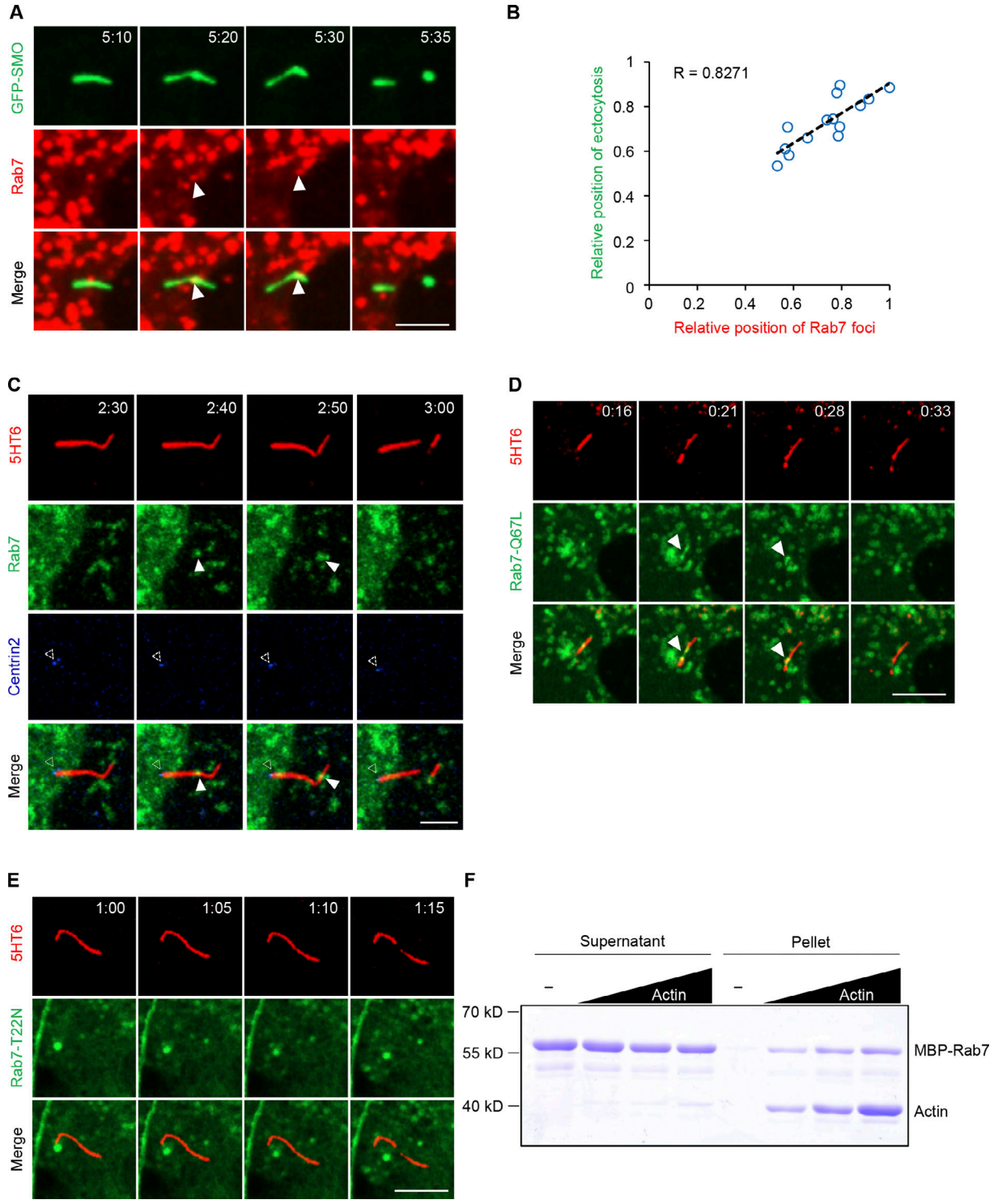


Figure S2. **Rab7 localization during cilia ectocytosis. (A)** Rab7 localization during cilia ectocytosis. Time-lapse images of RPE-1 cells expressing GFP-SMO and mCherry-Rab7 during serum restimulation. Arrowheads mark mCherry-Rab7 foci at the site of cilia excision. Time is in hour:minute. Scale bar, 5 µm. **(B)** Correlation plot of the intraciliary Rab7 position with position of excision, each provided as a relative ratio of cilia length. The position of Rab7 was measured at the estimated centroid position of mCherry-Rab7 foci at excision. Linear regression is indicated by the dashed line, with the Pearson correlation coefficient R value indicated. *n* = 14 cells from three independent experiments. **(C)** Rab7 localization during cilia ectocytosis. Time-lapse images of RPE-1 cells expressing 5HT6-mCherry, GFP-Rab7, and BFP-Centrin2 during serum restimulation. Solid arrowheads mark Rab7 foci at the site of cilia excision, and open arrowheads mark the base of cilia (centrosome). Time is in hour:minute. Scale bar, 5 µm. **(D and E)** Rab7-Q67L and Rab7-T22N localization during cilia ectocytosis. Time-lapse images of RPE-1 cells expressing 5HT6-mCherry and GFP-Rab7 mutants during serum restimulation. Arrowheads mark Rab7 mutant foci at the site of cilia excision. Time is in hour:minute. Scale bars, 10 µm. **(F)** Rab7 binds to F-actin in a dose-dependent fashion. Purified MBP-tagged Rab7 protein was incubated with 0, 2, 4, and 8 µM polymerized F-actin filaments and subjected to ultracentrifugation. Equal amounts of supernatant or pellet were subjected to SDS-PAGE, followed by Coomassie blue staining.

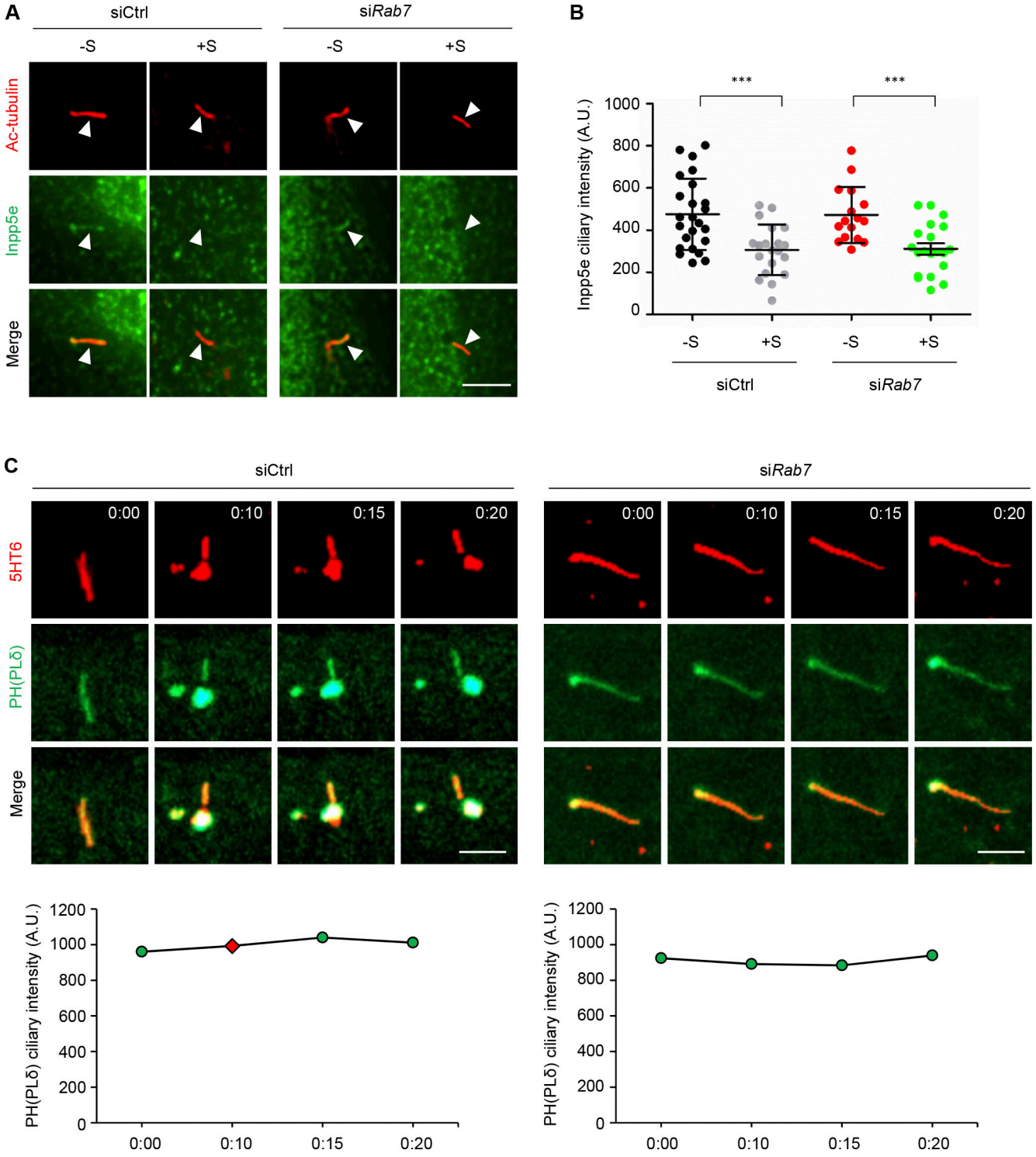


Figure S3. **Depletion of Rab7 does not affect ciliary Inpp5e and PI(4,5)P2 distribution during serum stimulation. (A)** Acetylated α-tubulin/Inpp5e immunofluorescence of cells treated with 0% FBS (–S) or 10% FBS (+S) for 4 h. Images of each wavelength are scaled to the same intensity range. Arrowheads mark cilia positions. Scale bar, 5 µm. **(B)** Inpp5e immunofluorescence signal intensity measurements in primary cilia (cilia background), as in A. Data are means ± SD. Student's *t* test was performed. ***P < 0.001. *n* = 24, 20, 16, and 20 cells, respectively. **(C)** Time-lapse images of the siCtrl or siRab7 cells expressing 5HT6-mCherry and GFP-PH(PLCδ) (a PI(4,5)P2 sensor) after serum stimulation for 6 h. Time is in hour:minute. Scale bars, 5 µm. PH(PLCδ) immunofluorescence signal intensity in the representative images was measured in primary cilia (cilia background [excluding released vesicles in control cells]) in the bottom panels. Red diamond in the control cell marks cilia ectocytosis time point. Data is shown as the representative result from three independent experiments.