Kobayashi et al., http://www.jcb.org/cgi/content/full/jcb.201304153/DC1

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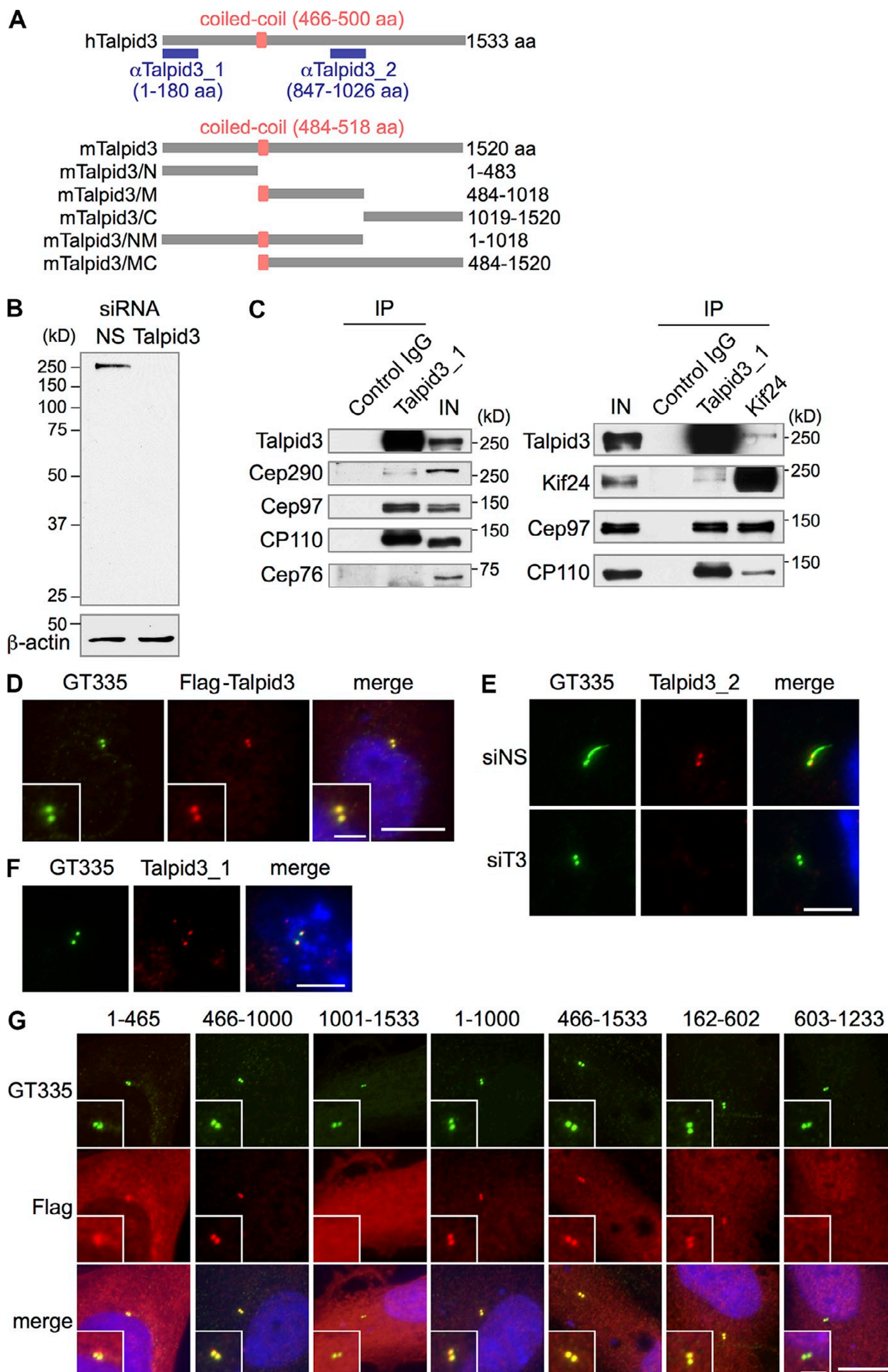


Figure S1. **Talpid3 interacts with CP110-containing protein complex and localizes to the centrosome in RPE1 cells.** (A) Schematic of human and mouse Talpid3, and mouse Talpid3 fragments. Red boxes show a coiled-coil domain. Blue bars show antigens for anti-Talpid3_1 and _2. (B) Western blotting of Talpid3 in cycling RPE1 cells treated with control (NS) or Talpid3 siRNA. β-Actin was used as a loading control. (C) Western blotting of endogenous Talpid3, Cep290, Cep97, CP110, Cep76, and Kif24 after immunoprecipitation from RPE1 cell extracts with antibodies indicated at the top of panel. IN, input. (D) RPE1 cells were transiently transfected with a plasmid expressing Flag-tagged Talpid3 and processed for immunofluorescence with anti-glutamylated tubulin (GT335, green) and anti-Flag (red) antibodies. (E) RPE1 cells transiently transfected with control (siNS) or Talpid3 siRNA were induced to quiescence for 48 h and processed for immunofluorescence with anti-glutamylated tubulin (GT335, green) and anti-Talpid3_2 (red) antibodies. (F) Cycling RPE1 cells were processed for immunofluorescence with anti-glutamylated tubulin (GT335, green) and anti-Talpid3_1 (red) antibodies. (G) RPE1 cells were transiently transfected with plasmids expressing Flag-tagged Talpid3 truncation mutants (end-points of each fragment are shown) and processed for immunofluorescence with anti-glutamylated tubulin (GT335, green) and anti-Flag (red) antibodies. Bars: (D and G) 10 µm; (D and G, insets) 2.5 µm; (E and F) 5 µm.

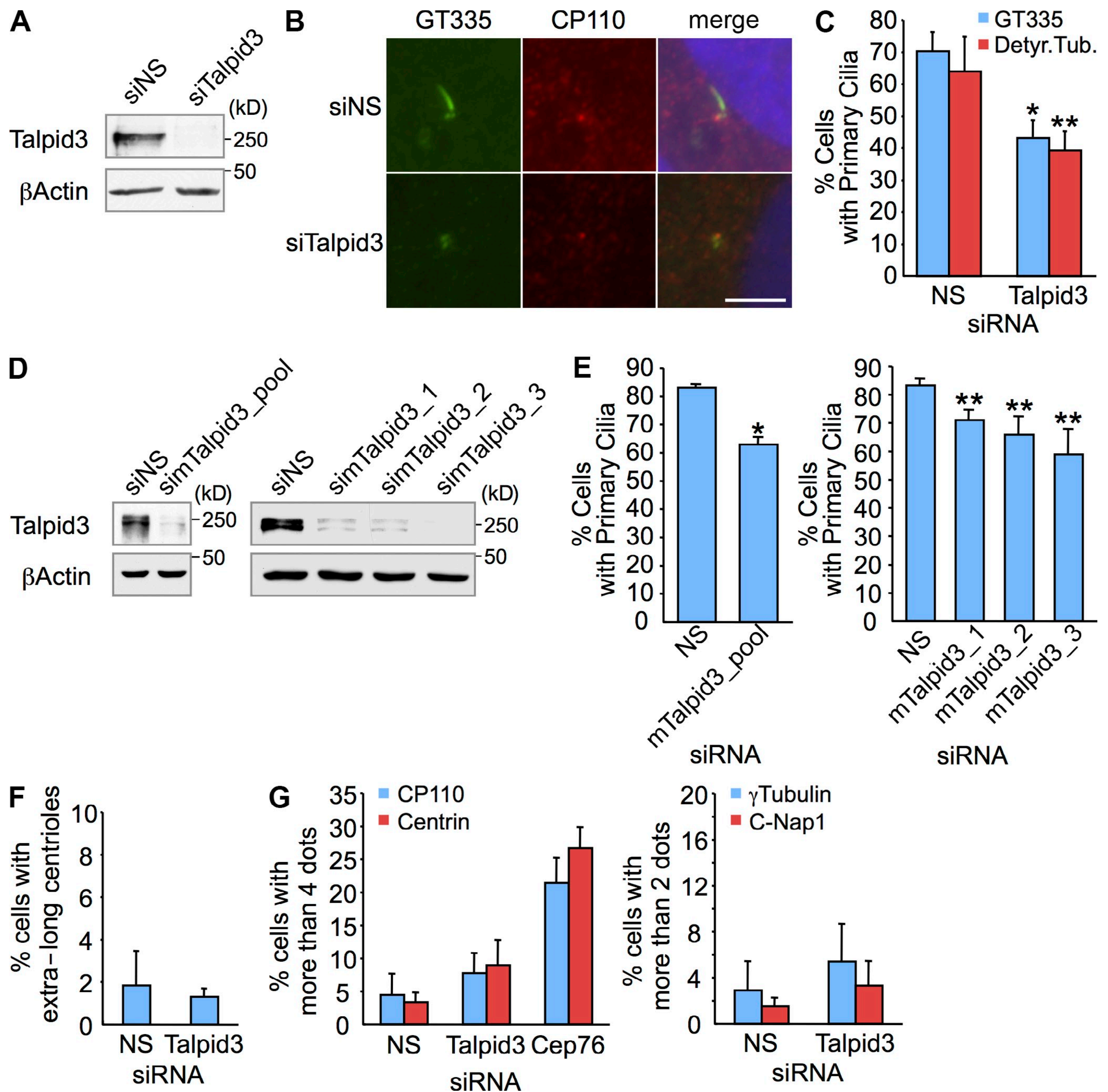
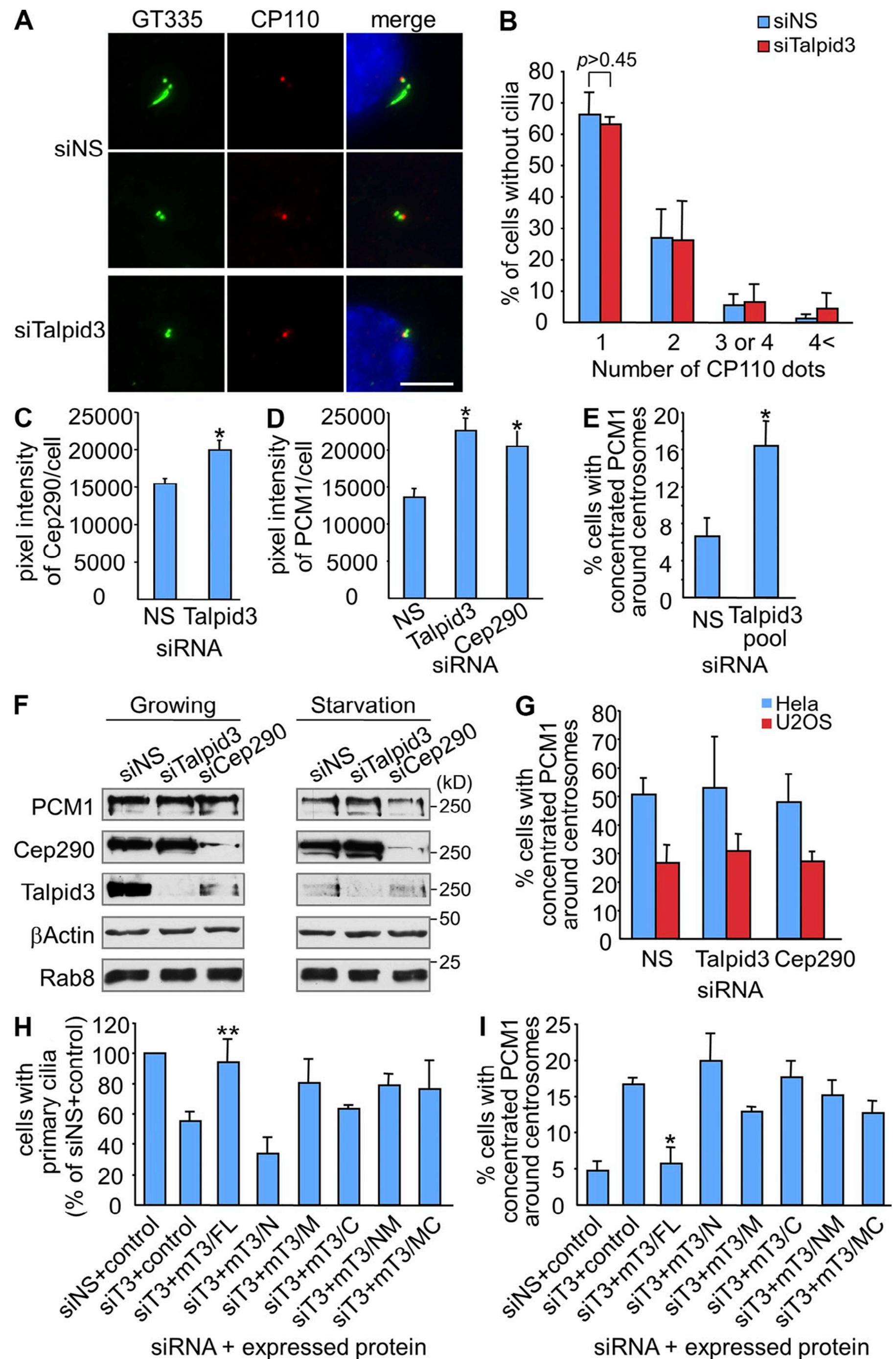


Figure S2. **Ablation of Talpid3 results in cilia assembly defects in RPE1 and 3T3 cells.** (A) Western blotting of Talpid3 in cycling RPE1 cells treated with control (siNS) or Talpid3 siRNA for 48 h. β-Actin was used as a loading control. (B) RPE1 cells transiently transfected with control (siNS) or Talpid3 siRNA were serum starved into quiescence for 48 h and processed for immunofluorescence with anti-glutamylated tubulin (GT335, green) and anti-CP110 (red) antibodies. Bar, 5 µm. For each panel, DNA was stained with DAPI (blue). (C) The percentages of quiescent RPE1 cells with primary cilia were determined using either anti-glutamylated tubulin (GT335) or anti-detyrosinated tubulin (Detyr. Tub.) antibodies as markers. Average of three to six independent experiments is shown. *, P < 0.01; **, P < 0.05 compared with NS. (D) Western blotting of Talpid3 in cycling 3T3 cells treated with control (siNS) or mouse Talpid3 siRNA for 48 h. β-Actin was used as a loading control. (E) 3T3 cells transiently transfected with control (siNS) siRNA or siRNA targeting mouse Talpid3 were induced to quiescence for 48 h and processed for immunofluorescence with an anti-glutamylated tubulin (GT335) antibody. The percentages of quiescent 3T3 cells with primary cilia are shown. Average of three to four independent experiments is shown. *, P < 0.01; **, P < 0.05 compared with NS. (F and G) U2OS cells transiently transfected with control (siNS), Talpid3, or Cep76 siRNA were processed for immunofluorescence with (F) anti-centrin, or (G) anti-CP110 and anti-centrin, or anti–γ-tubulin and anti-C-Nap1 antibodies. The percentages of U2OS cells with (F) extra-long centrioles or (G) more than four CP110 or centrin dots and more than two γ-tubulin or C-Nap1 dots were determined.

Figure S3. **Ablation of Talpid3 causes accumulation of centriolar satellite markers without affecting protein levels.** (A) RPE1 cells transiently transfected with control (siNS) or Talpid3 siRNA were induced to quiescence for 48 h and processed for immunofluorescence with anti-glutamylated tubulin (GT335, green) and anti-CP110 (red) antibodies. Bar, 5 µm. For each panel, DNA was stained with DAPI (blue). (B) The percentages of nonciliated RPE1 cells with the indicated number of CP110 dots were determined. (C and D) RPE1 cells transiently transfected with control (siNS), Talpid3, or Cep290 siRNA were induced to quiescence for 48 h and processed for immunofluorescence with anti-glutamylated tubulin and (C) anti-Cep290 or (D) anti-PCM1 antibodies. The pixel intensities of (C) PCM1 or (D) Cep290 staining in the vicinity of centrosome are shown. (E) RPE1 cells transiently transfected with control (siNS) or Talpid3pool siRNAs were induced to quiescence for 48 h and processed for immunofluorescence with anti-glutamylated tubulin (GT335, green) and anti-PCM1 (red) antibodies. The percentage of quiescent RPE1 cells with accumulations of PCM1 granules around the centrosomes is shown. Average of three independent experiments is shown. *, P < 0.01 compared with NS. (F) Western blots of PCM1, Cep290, Talpid3, β-actin, and Rab8a in cycling (growing) or quiescent (starvation for 48 h) RPE1 cells treated with control (siNS), Talpid3, or Cep290 siRNA are shown. (G) Hela or U2OS cells transiently transfected with control (siNS), Talpid3, or Cep290 siRNAs were cultured in serum-free medium for 48 h and processed for immunofluorescence with anti-glutamylated tubulin (GT335) and anti-PCM1 antibodies. The percentage of cells with accumulations of PCM1 granules around the centrosomes is shown. Averaged data for three independent experiments are shown. (H) RPE1 cells treated with control (siNS) or Talpid3 were transiently transfected with plasmids expressing GFP and Flag or Flag-mTalpid3 truncations and were induced to ciliate through serum deprivation for 48 h. Cells were processed for immunofluorescence with an anti-glutamylated tubulin (GT335) antibody. The percentages of GFP-positive RPE1 cells with primary cilia were determined. Averages for three independent experiments are shown. (I) RPE1 cells treated with control (siNS) or Talpid3 siRNAs were transiently transfected with plasmids expressing GFP-Centrin2 and Flag or Flag-mTalpid3 truncations, and induced to quiescence for 48 h. Cells were processed for immunofluorescence with an anti-PCM1 antibody. The percentages of GFP-Centrin2–positive RPE1 cells with concentrated PCM1 granules around centrosomes were determined. Average of three independent experiments is shown. (H and I) T3 denotes Talpid3. mTalpid3 fragments used here are shown in Fig. S1 A. *, P < 0.01; **, P < 0.05 compared with siT3 + control.



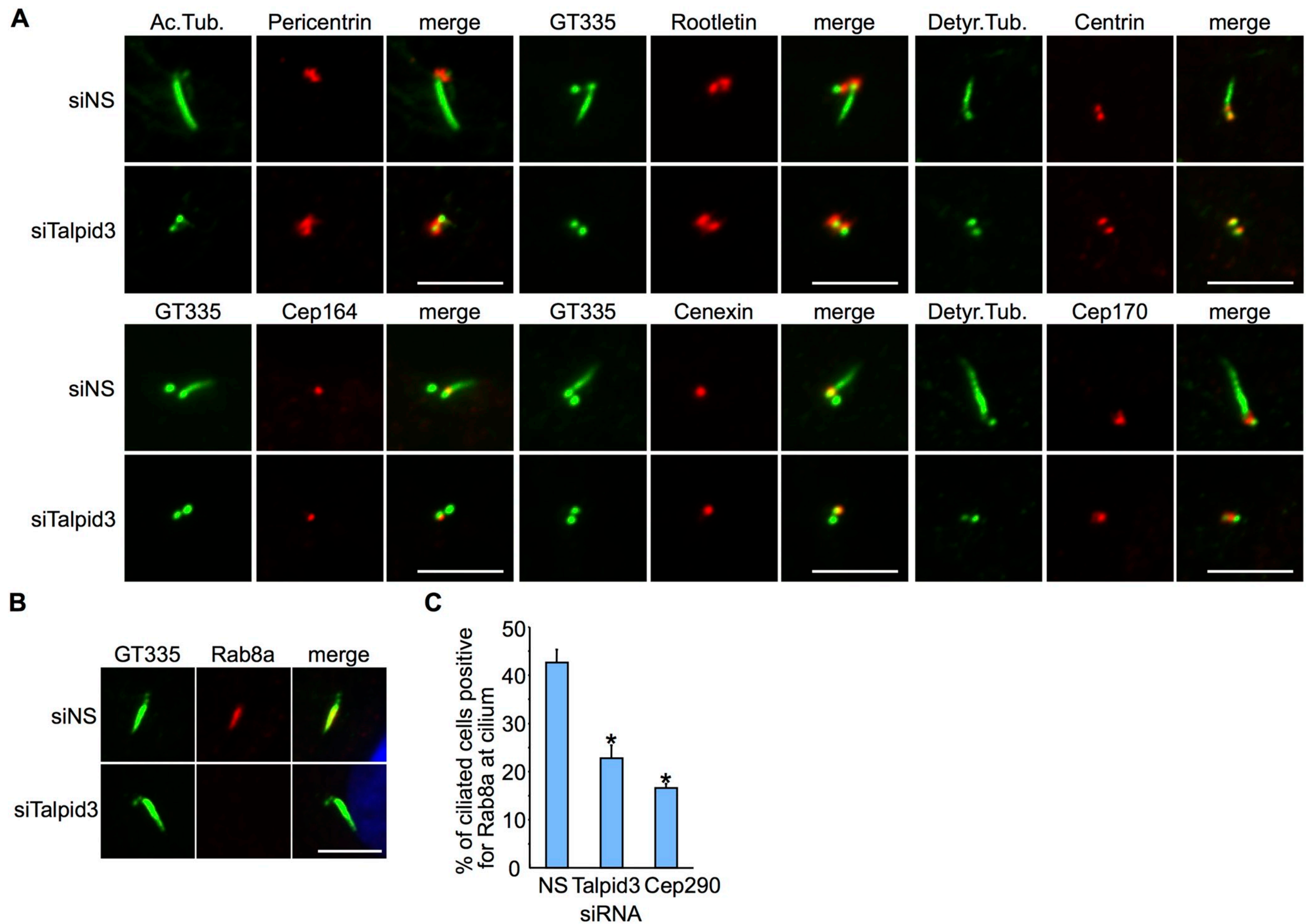
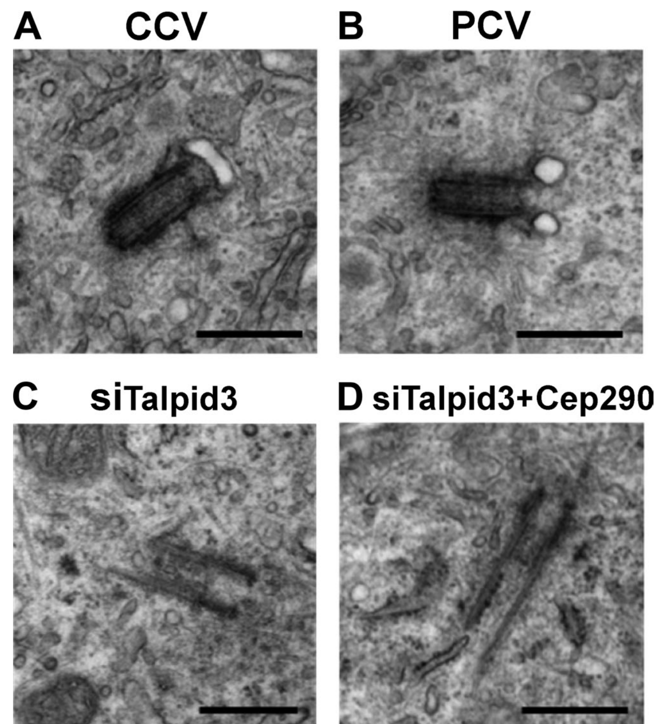


Figure S4. **Talpid3 and Cep290 regulate targeting of Rab8a to the ciliary axoneme.** (A and B) RPE1 cells transiently transfected with control (siNS) or Talpid3 siRNA were induced to quiescence for 48 h and processed for immunofluorescence with indicated antibodies. Bars, 5 µm. For each panel, DNA was stained with DAPI (blue). (C) RPE1 cells transiently transfected with control (siNS), Talpid3, or Cep290 siRNA were induced to quiescence for 48 h and processed for immunofluorescence with anti-glutamylated tubulin (GT335) and anti-Rab8a antibodies. The percentages of ciliated RPE1 cells positive for Rab8a at cilia were determined. *, $P < 0.01$ compared with NS. Average of three independent experiments is shown.



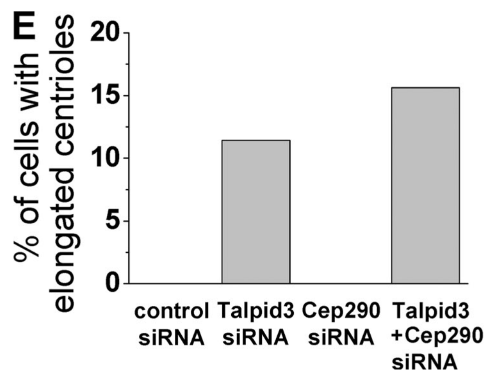
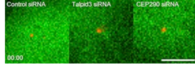


Figure S5. **Ablation of Talpid3 leads to elongated centrioles without ciliary vesicles.** (A and B) Representative images of capped ciliary vesicle (CCV, A) or primary ciliary vesicle (PCV, B) attached to the distal appendages of centrioles. (C and D) Representative images of elongated centrioles without ciliary vesicles of RPE1 cells treated with siRNA against Talpid3 (siTalpid3) or Talpid3 and Cep290 (siTalpid3 + Cep290). (E) Percentage of cells with elongated centrioles without ciliary vesicles at 24 h of serum starvation. n = 30 cells for control, n = 35 cells for Talpid3, n = 36 cells for Cep290, and n = 31 cells for Talpid3 + Cep290. siRNA-treated cells were observed from a single experiment. Bars, 500 nm.



Video 1. **The recruitment and elongation of GFP-Rab8a in control (NS)-, Talpid3-, or Cep290 siRNA-treated RPE1 cells.** GFP-Rab8a stably expressing RPE1 cells that had been treated with siRNAs against either control, Talpid3, or Cep290 were transiently transfected with a plasmid encoding tagRFP-Centrin2. After inducing the cells into a quiescent state, time-lapse fluorescence imaging was performed by taking cell images every 10 min for 4 h. Bar, 2.5 µm.

Table S1. **Data of tandem mass spectrometry after immuno-affinity chromatography**

Protein	Control IP	Talpid3 IP	Kif24 IP
CP110	0	1	77
Cep97	0	7	169
PCM1	0	6	0

Flag-Talpid3 or Flag-Kif24 was expressed in HEK293 cells and immuno-affinity purified with anti-Flag agarose beads. Bound proteins were eluted with Flag peptide, and the resultant eluates were subjected to mass spectrometric analysis. Numbers of peptides identified in the mass spectrometric analysis are shown.