Schmidt et al., http://www.jcb.org/cgi/content/full/jcb.201202126/DC1

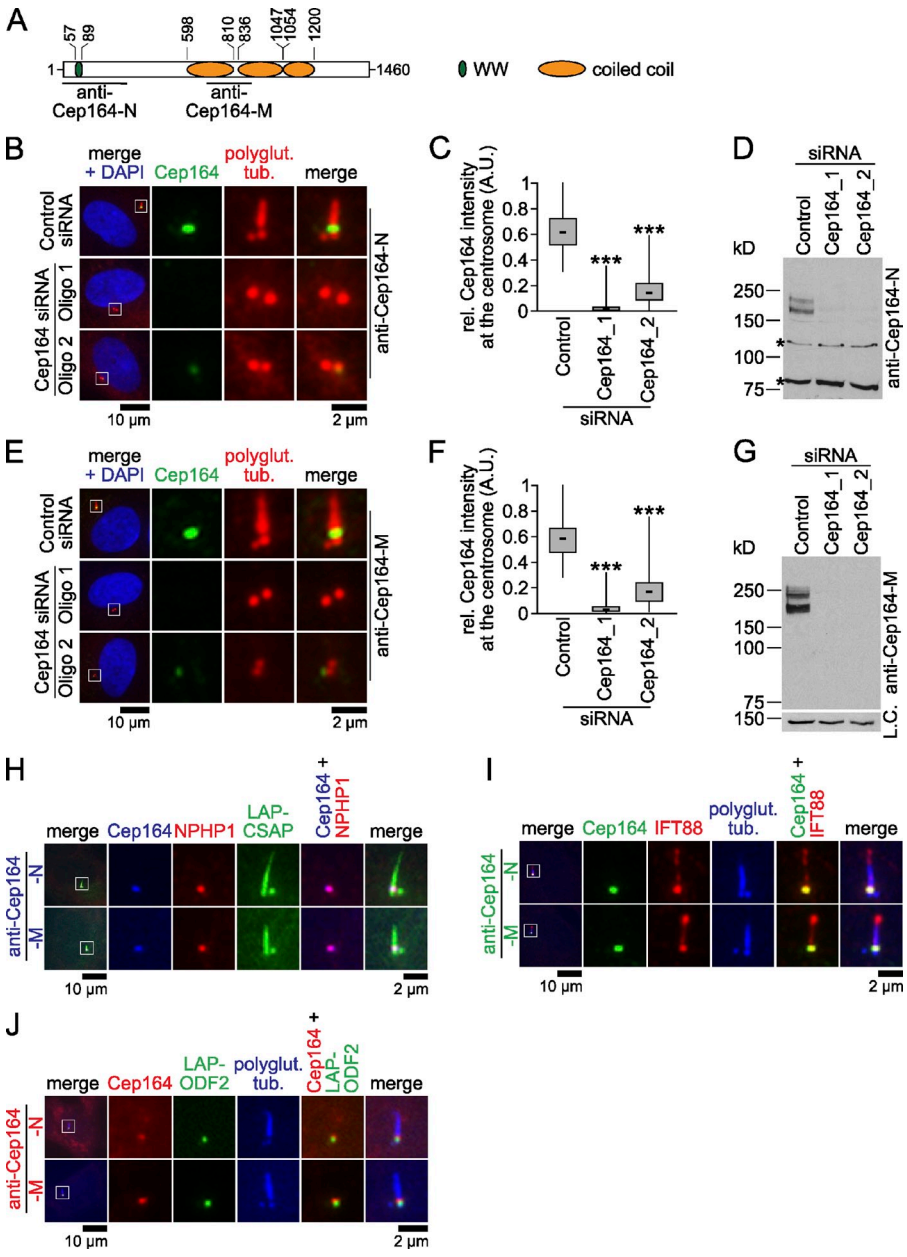

Figure S1. **Characterization of Cep164 antibodies.** (A) Scheme illustrating Cep164 domains. Numbers indicate amino acid positions. The horizontal lines below indicate regions used as antigens for antibody production. Anti–Cep164-N is directed against amino acids 1–298, anti–Cep164-M is directed against amino acids 665–873. (B) RPE1 cells were control depleted (top) or depleted for Cep164 using Cep164 siRNA1 (middle) or Cep164 siRNA2 (bottom) for 48 h with serum followed by 24 h without serum. Cells were fixed and stained with anti–Cep164-N (green) and anti-polyglutamylated tubulin antibodies (red) and for DNA (blue). Cep164 localizes at the distal part of the mother centriole, while Cep164 and ciliary staining is lost upon Cep164 depletion. (C) Box-and-whisker plots showing the relative Cep164 signal intensity at the centrosome quantified from cells treated as in B to confirm the efficient depletion of Cep164 using two independent siRNAs and the specificity of anti–Cep164-N. More than 100 cells were quantified from each sample. (D) Western blot analysis of cells treated as in B showing the efficient depletion of Cep164 using siRNA1 and siRNA2 and the specificity of anti–Cep164-N. Unspecific protein bands are marked with asterisks and serve as loading control. (E) RPE1 cells were treated and stained as in B except that anti–Cep164-M (green) was used. (F) Box-and-whisker plots showing the relative Cep164 signal intensity at the centrosome quantified from cells treated as in B to confirm the specificity of anti–Cep164-M. More than 100 cells were quantified from each sample. (G) Western blot analysis from cells treated as in B showing the efficient depletion of Cep164 using two independent siRNAs and the specificity of anti–Cep164-M. L.C., loading control. (H–J) Co-staining of Cep164 using anti–Cep164-N (top panels) or anti–Cep164-M (bottom panels) with antibodies against NPHP1 (H), IFT88 (I), and ODF2 (J). In H and I, RPE1 cells stably expressing LAP-CSAP (green) were used, whereas LAP-ODF2 (J) was stably expressed in NIH 3T3 cells. The CSAP protein localizes to both centrioles and the axoneme and served as a centriolar and ciliary marker (Backer et al., 2012). RPE1 cells were serum starved for 48 h, whereas NIH 3T3 cells were serum starved for 24 h. Cells were fixed and stained with the indicated antibodies. Merged images are shown in the left panels of B, E, and H–J. Regions within the white boxes are shown at higher magnification to the right. A. U., arbitrary units.

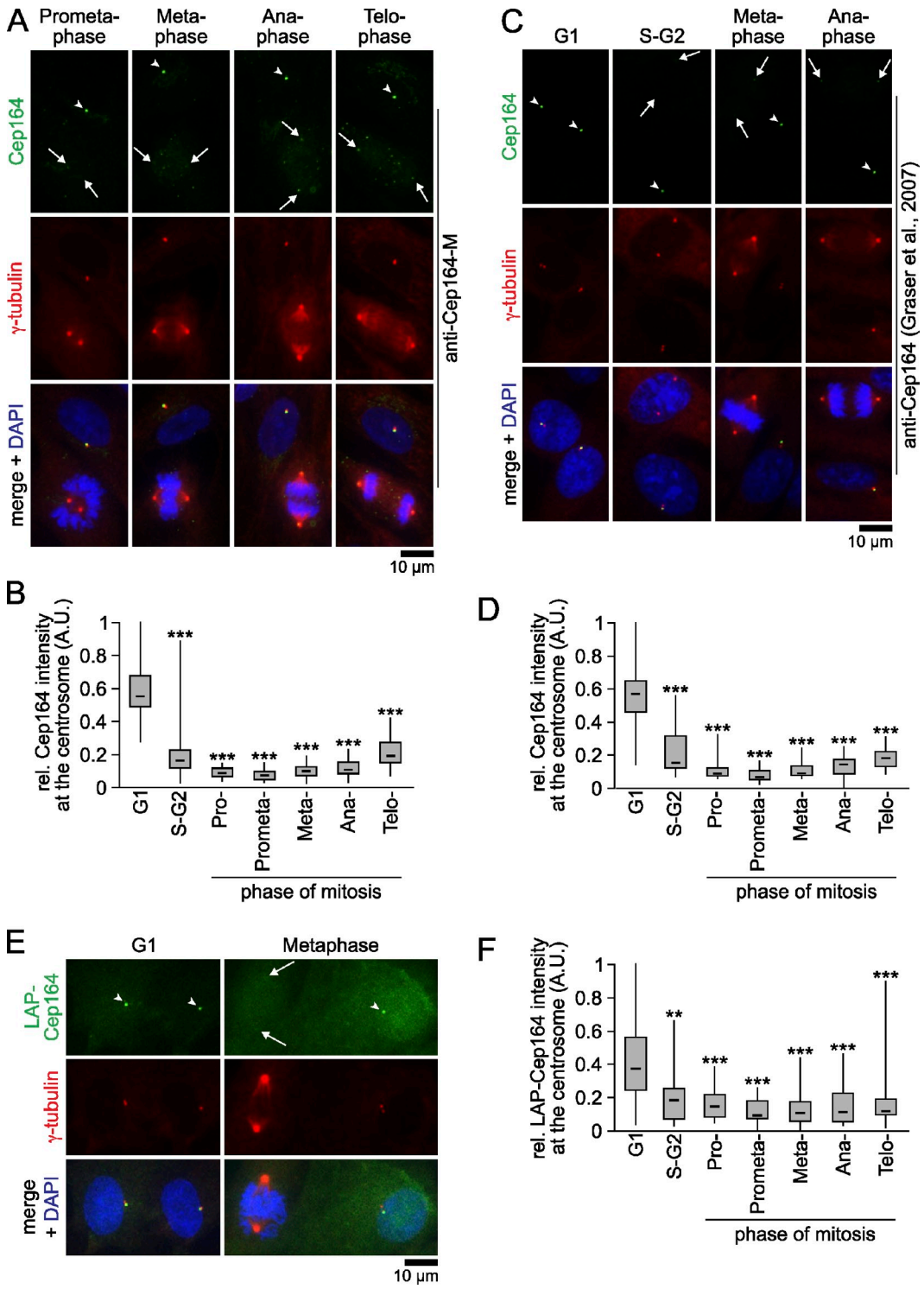

Figure S2. **Cep164 localization during the cell cycle.** (A) RPE1 cells were fixed and stained with anti-Cep164-M, anti-γ-tubulin (red), and for DNA (blue). Centrosomes of representative cells in G_1 (arrowheads) and mitosis (arrows) are indicated. (B) Quantification of A. Box-and-whisker plots show the relative Cep164 signal intensity at the centrosome (100 cells were scored per cell cycle phase). (C) RPE1 cells were fixed and stained for Cep164 (green; Cep164-antibody, Graser et al., 2007), γ-tubulin (red), and DNA (blue). Centrosomes of representative cells in G_1 (arrowheads), S-G_2 and mitosis (arrows) are indicated. (D) Quantification of C, as described in B. (E) Stably LAP-Cep164 (green) expressing RPE1 cells were fixed and stained for γ-tubulin (red) and DNA (blue). Centrosomes of representative cells in G1 (arrowheads) and mitosis (arrows) are indicated. (F) Quantification of E, as described in B. A.U., arbitrary units.

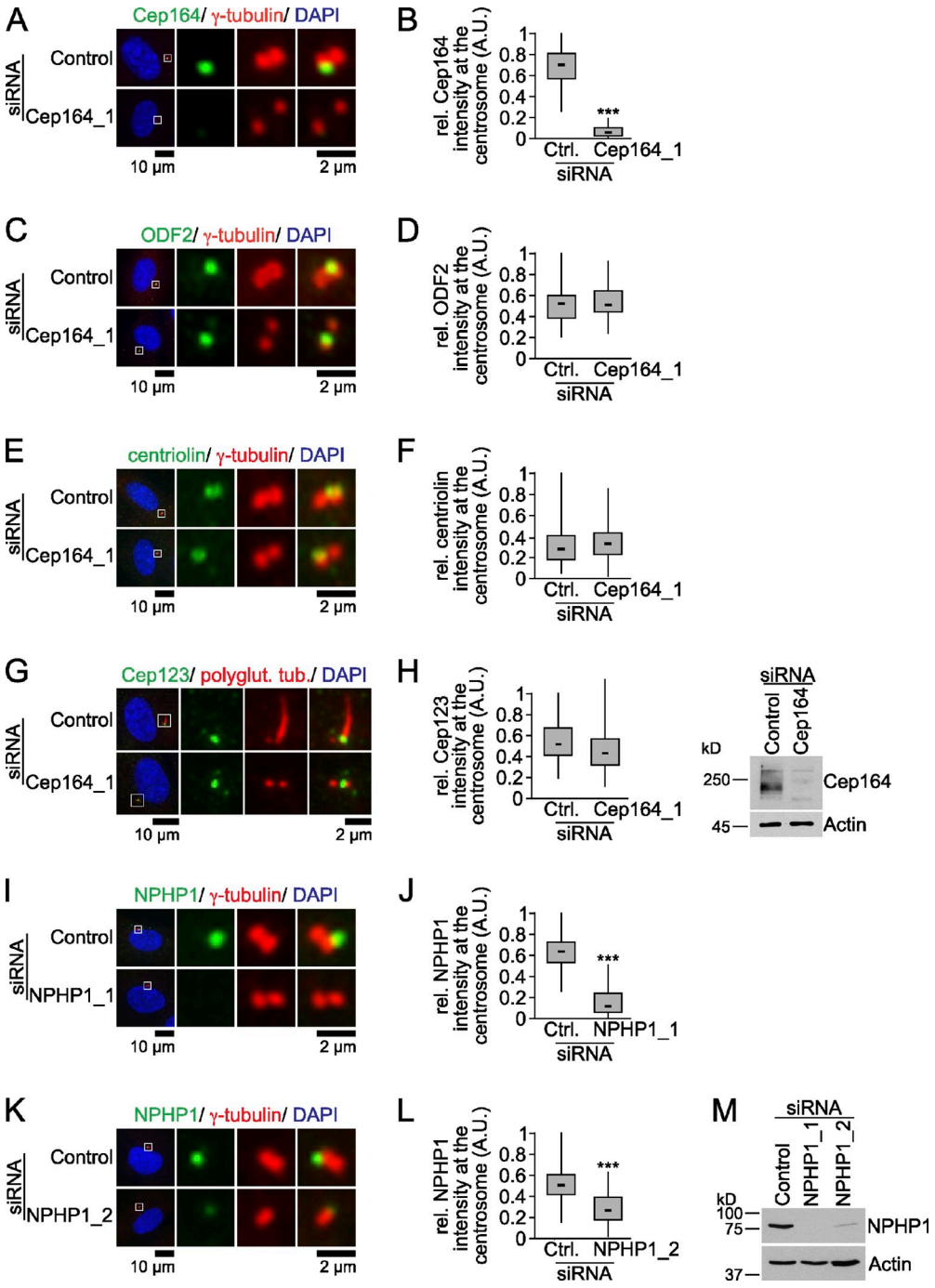

Figure S3. **The subdistal appendage proteins ODF2 and centriolin and the distal appendage protein Cep123 localize to the M-centriole independently of Cep164.** (A) RPE1 cells were control depleted or depleted for Cep164 for 24 h with serum followed by 48 h without serum. Cells were fixed and stained for Cep164 (green), γ-tubulin (red), and DNA (blue). (B) Box-and-whisker plots show the relative Cep164 signal intensity at the centrosome from cells treated as in A. 150 cells were quantified for each sample. One representative experiment out of three is shown. (C) RPE1 cells were treated as in A and stained for ODF2 (green), γ-tubulin (red), and DNA (blue). (D) Quantification of C, as described in B. 110 cells were quantified for each sample. One representative experiment out of two is shown. (E) RPE1 cells were treated as in A and stained for centriolin (green), γ-tubulin (red), and DNA (blue). (F) Quantification of E, as described in B. 170 cells were quantified for each sample. One representative experiment out of two is shown. (G) RPE1 cells were treated as in A and stained for Cep123 (green), polyglutamylated tubulin (red), and DNA (blue). (H) Quantification of G. Box-and-whisker plots show the relative Cep123 signal intensity at the centrosome. 100 cells were quantified for each sample. The Western blot shows the efficiency of Cep164 depletion in G. One representative experiment out of three is shown. (I) RPE1 cells were control depleted or depleted for NPHP1 with siRNA1 for 24 h with serum followed by 48 h without serum. Cells were stained for NPHP1 (green), γ-tubulin (red), and DNA (blue). (J) Quantification of I. Box-and-whisker plots show the relative NPHP1 signal intensity at the centrosome. 150–200 cells were quantified for each sample. One representative experiment out of three is shown. (K) RPE1 cells were control depleted or depleted for NPHP1 with siRNA2 for 24 h with serum followed by 48 h without serum. Cells were stained for NPHP1 (green), γ-tubulin (red), and DNA (blue). (L) Quantification of K. Box-and-whisker plots show the relative NPHP1 signal intensity at the centrosome. More than 100 cells were quantified for each sample. (M) Western blot showing the specificity of anti-NPHP1 antibody. RPE1 cells were treated with the indicated siRNAs for 48 h and serum starved for the following 24 h. Proteins were detected with the indicated antibodies. Merged images are shown in the left panels of A, C, E, G, I, and K. Regions within the white boxes are shown at higher magnification to the right. A.U., arbitrary units.

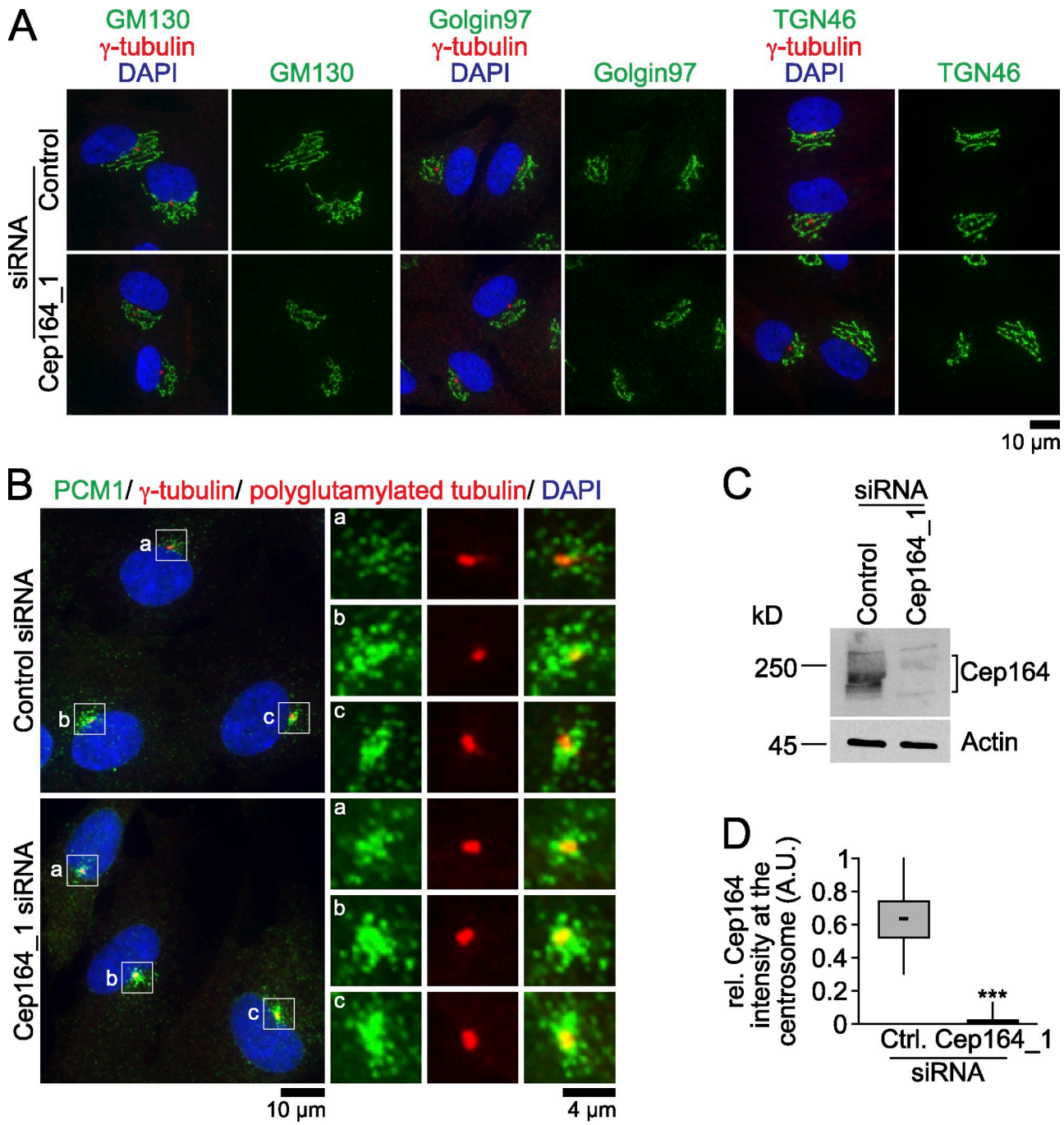

Figure S4. **Golgi structure and microtubule-dependent transport is intact in Cep164-depleted cells.** (A) RPE1 cells were control depleted or depleted for Cep164 for 24 h with serum followed by 48 h without serum. Cells were fixed and stained for GM130 (cis-Golgi), Golgin97 or TGN46 (trans-Golgi; green), and γ-tubulin (red). DNA (blue) was stained with DAPI. Staining of the Golgi structure (green) is shown to the right of each merged image. (B) RPE1 cells were treated as in A. Cells were fixed and stained for PCM1 (green), γ- and polyglutamylated tubulin (red), and DNA (blue). Merged images are shown in the left panels. Regions within the white boxes are shown at higher magnification to the right. (C) Western blot analysis of cells treated as in A and B showing an efficient depletion of Cep164. (D) Box-and-whisker plots of cells treated as in A and B and stained for Cep164. More than 100 cells were scored for each sample. A.U., arbitrary units.

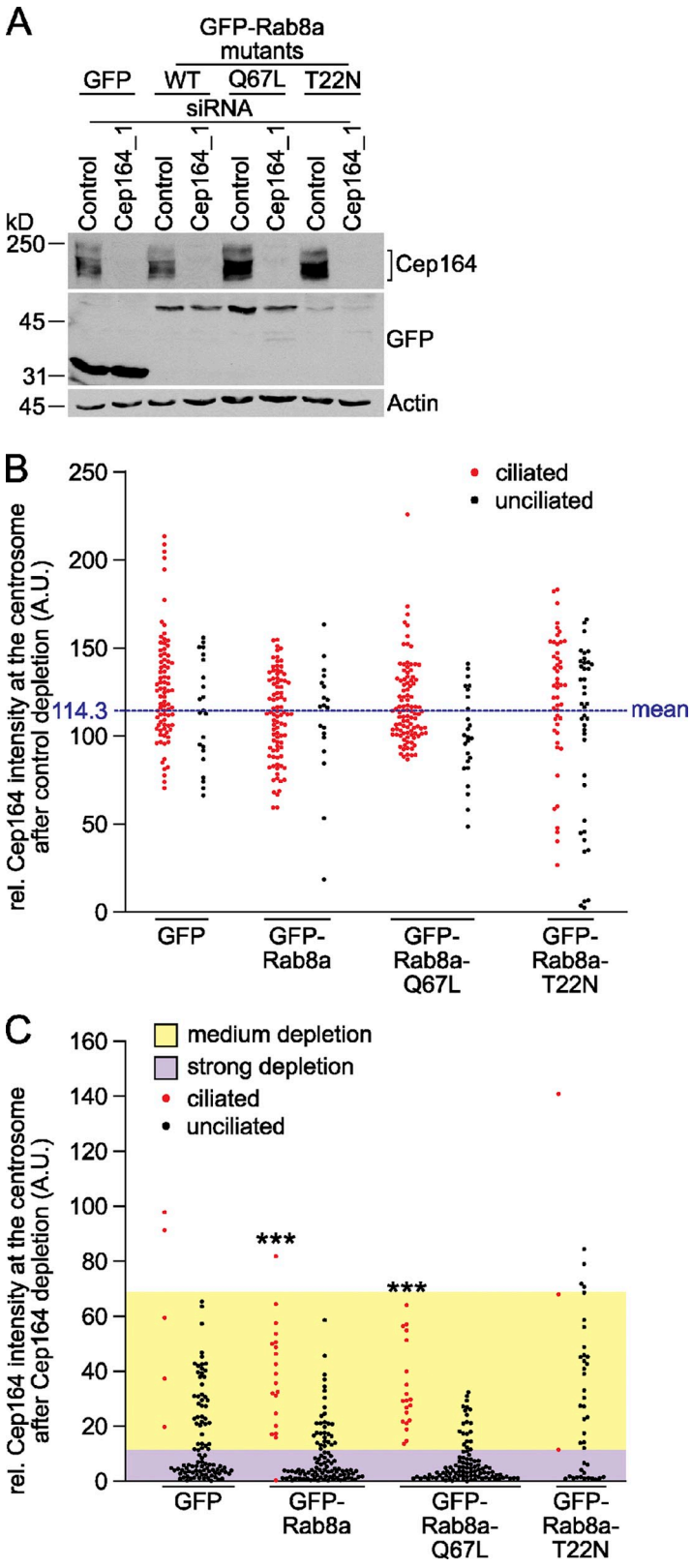

Figure S5. **Overexpression of Rab8a or constitutively active Rab8a can compensate for cilia loss due to a decreased Cep164 protein level.** (A) Western blot analysis of cells treated as in Fig. 9 A. Proteins were detected with the indicated antibodies. GDP-locked GFP-Rab8a-T22N was expressed at lower levels compared with wild-type or GTP-locked GFP-Rab8a most likely due to toxicity. (B and C) Graphs show raw data of the measurements of Cep164 signal intensities for control (B) or Cep164-depleted (C) RPE1 cells treated as in Fig. 9 A. Each dot represents the Cep164 signal intensity per centrosome for one cell overexpressing the indicated GFP construct. Red dots represent ciliated cells, and black dots represent unciliated cells. The mean signal intensity in B was calculated from all signal intensities of control depleted cells. The yellow area in C represents Cep164 signal intensities from Cep164-depleted cells that are reduced to 10–60% of the mean signal intensity measured for control depleted cells in B. This level of reduction is called "medium depletion". The purple area in C contains Cep164 signal intensities from Cep164-depleted cells reduced to 0–10% of the mean signal intensity in control depleted cells and is called "strong depletion". No significant difference in the Cep164 knockdown efficiencies between the cell lines (C) was detected by Student's *t* test, confirming the populations to be equal regarding Cep164 protein levels left at the centrosome. χ^2 test confirmed the increase in the percentage of ciliated cells after Cep164 depletion and overexpression of GFP-Rab8a or GFP-Rab8a-Q67L to be significant as indicated. Data shown in B and C are from a single representative experiment out of three repeats. For the experiment shown in B, 88–140 cells were analyzed per cell line. For the experiment shown in C, 43–120 cells were analyzed per cell line. A.U., arbitrary units.

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