Supporting Information

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SI Materials and Methods

siRNAs. The following siRNAs were used: coiled-coil domain containing 41 (CCDC41)-1, AGGUGAAGUUGGUGACUCA; CCDC41-2, GGAACUAGUUAGAGUCAAG; CCDC41-3, GGAACAACUUGCUCGAGAA; Cep164, GAGUGAAGGUGUAUCGCUU (1); intraflagellar transport protein 20 (IFT20), CAGCAACUUCAAGCCCUAAUA (2); intraflagellar transport protein 88 (IFT88)-1, CGACUAAGUGCCAGACUCAUU (3); IFT88-2, CCGAAGCACUUAACACUUA (3); and centriolar coiled-coil protein 110 (CP110), AAGCAGCAUGAGUAUGCCAGU (4).

Individual CCDC41 siRNAs were used for the experiments shown in Fig. 2 *A* and *B*. For the other experiments, CCDC41-1 and CCDC41-2 siRNAs were used as a pool, and the total siRNA concentration was 10 nM. IFT88-1 and IFT88-2 siRNAs were also used as a pool.

Antibodies. The following antibodies were used: rabbit anti-CCDC41 (Sigma), mouse anti–γ-tubulin (Abcam), mouse anti–glutamylated-tubulin (Adipogen), rabbit anti-centrosomal protein 164 (Cep164) (gift from Erich Nigg, University of Basel, Basel, Switzerland), mouse anti-outer dense fiber of sperm tails 2 (Odf2) (Abnova), mouse anti-Ninein (Millipore), rabbit anti-centrosomal protein 97 (Cep97) (Novus), rabbit anti–ADP-ribosylation factor-like 13b (Arl13b) (Proteintech), rabbit anti-IFT20 (Proteintech), rabbit anti-CP110 (Proteintech), mouse anti–acetylated-tubulin (Sigma), mouse anti–α-tubulin (Sigma), rabbit anti-green fluorescent protein (GFP) (Abcam), rabbit anti-tectonic family member 1 (TCTN1) (Proteintech), rabbit anti-IFT88 (Proteintech), mouse anti-Giantin (Abcam), and mouse and rabbit anti-FLAG (Sigma).

Imaging. Images were obtained using a DeltaVision Deconvolution System equipped with a cooled CCD camera (Applied Precision). Image stacks were collected with a *z*-step size of 0.2 μm and deconvolved with DeltaVision software. One deconvolved image from each image stack was presented in the figures without *z*-plane projection.

1. Graser S, et al. (2007) Cep164, a novel centriole appendage protein required for primary cilium formation. *J Cell Biol* 179(2):321–330.
2. Inoko A, et al. (2012) Trichoplein and Aurora A block aberrant primary cilia assembly in proliferating cells. *J Cell Biol* 197(3):391–405.
3. Robert A, et al. (2007) The intraflagellar transport component IFT88/Polaris is a centrosomal protein regulating G1-S transition in non-ciliated cells. *J Cell Sci* 120:628–637.
4. Spektor A, Tsang WY, Khoo D, Dynlacht BD (2007) Cep97 and CP110 suppress a cilia assembly program. *Cell* 130(4):678–690.

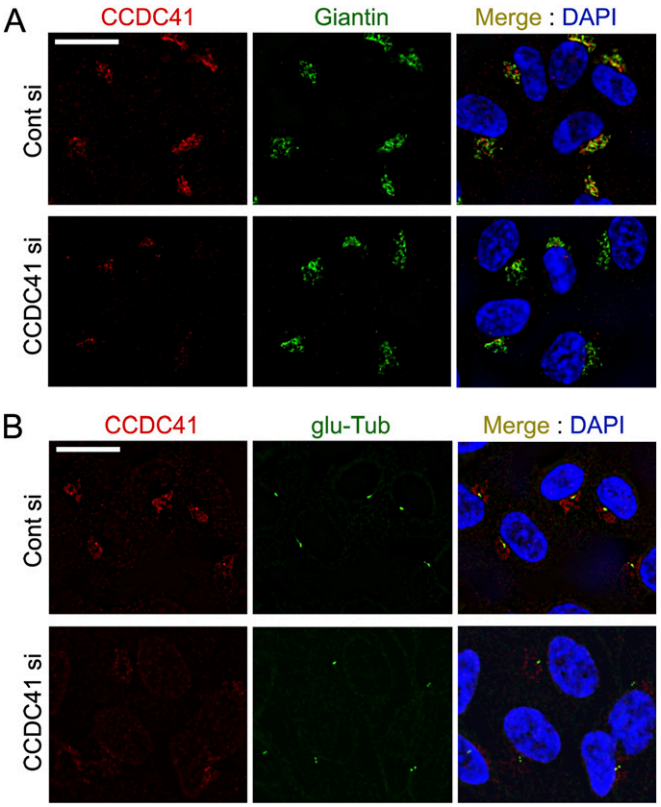

Fig. S1. Confirmation of Golgi and centriolar localization of CCDC41. (*A*) RPE1 cells were transfected with siRNAs for 2.5 d and double-stained with anti-CCDC41 and anti-Giantin antibodies. (*B*) RPE1 cells were transfected with siRNAs for 2.5 d (cells were serum-starved for the final 24 h), and double-stained with anti-CCDC41 and anti–glu-Tub antibodies. (Scale bars: 15 μm.)

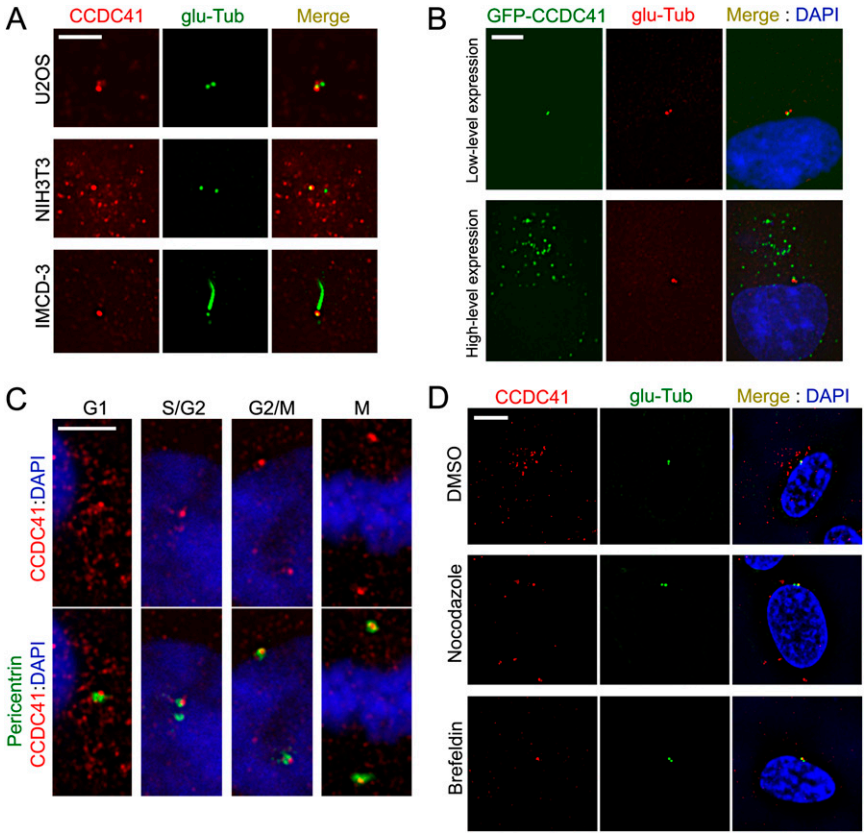

Fig. S2. Centriolar association of CCDC41. (*A*) U2OS, NIH 3T3, and IMCD-3 cells were double-stained with anti-CCDC41 and anti–glu-Tub antibodies. (*B*) RPE1 cells were transfected with GFP-CCDC41 plasmid for 8 h (*Upper*) or 16 h (*Lower*). Cells expressing high levels of GFP-CCDC41 display extracentrosomal GFP-CCDC41 accumulations. (*C*) CCDC41 localizes to centrioles in RPE1 cells at all stages of the cell cycle. Increasing levels of CCDC41 immunoreactivity were observed in a second parental centriole at the onset of centrosome separation. (*D*) RPE1 cells were treated with DMSO, nocodazole (3 μg/mL), or brefeldin A (1.4 μg/mL) for 2 h, and double-stained with anti-CCDC41 and anti–glu-Tub antibodies. (Scale bars: *A*–*C*, 5 μm; *D*, 10 μm.)

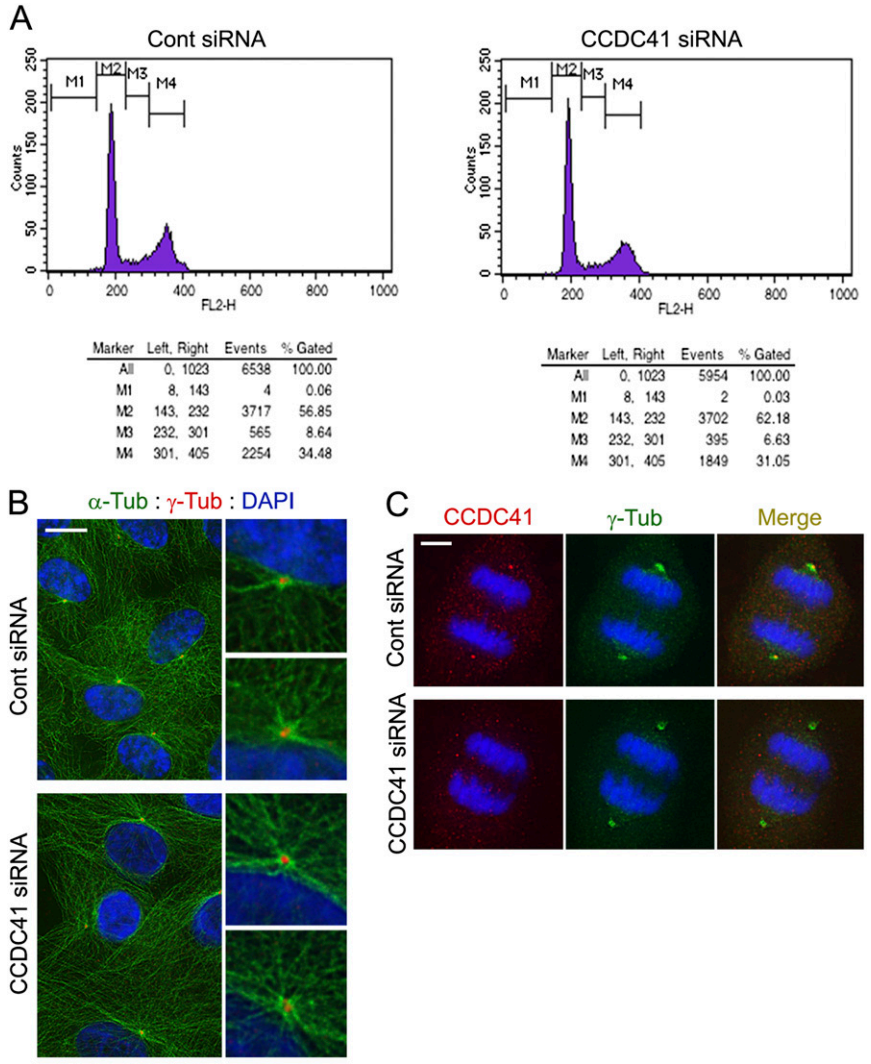

Fig. S3. CCDC41 is dispensable for cell cycle progression and microtubule organization. (*A*) RPE1 cells were transfected with the indicated siRNAs, and analyzed by flow cytometry. (*B*) RPE1 cells were transfected with siRNAs for 3 d and double-stained with anti–α-Tub and anti–γ-Tub antibodies. (*C*) RPE1 cells were transfected with siRNAs for 3 d and double-stained with anti-CCDC41 and anti–γ-Tub antibodies. (Scale bars: *B*, 10 μm; *C*, 5 μm.)

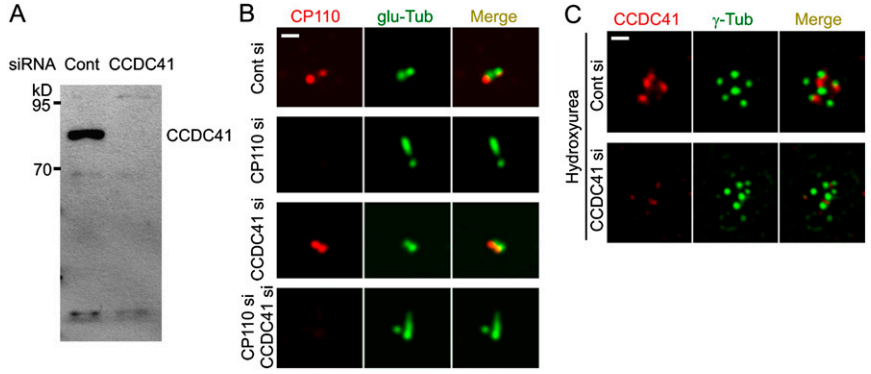

Fig. S4. CCDC41 is dispensable for centriole biogenesis and elongation in U2OS cells. (*A*) Western blot illustrating an efficient depletion of CCDC41 in U2OS cells. (*B*) U2OS cells were transfected with the indicated siRNAs for 3 d and double-stained with anti-CP110 and anti–glu-Tub antibodies. Note that CP110 depletion can induce abnormal centriole elongation in the absence of CCDC41. (*C*) U2OS cells were transfected with siRNAs, treated with hydroxyurea (4 mM) for 48 h, and double-stained with anti-CCDC41 and anti–γ-Tub antibodies. (Scale bars: *B* and *C*, 1 μm.)

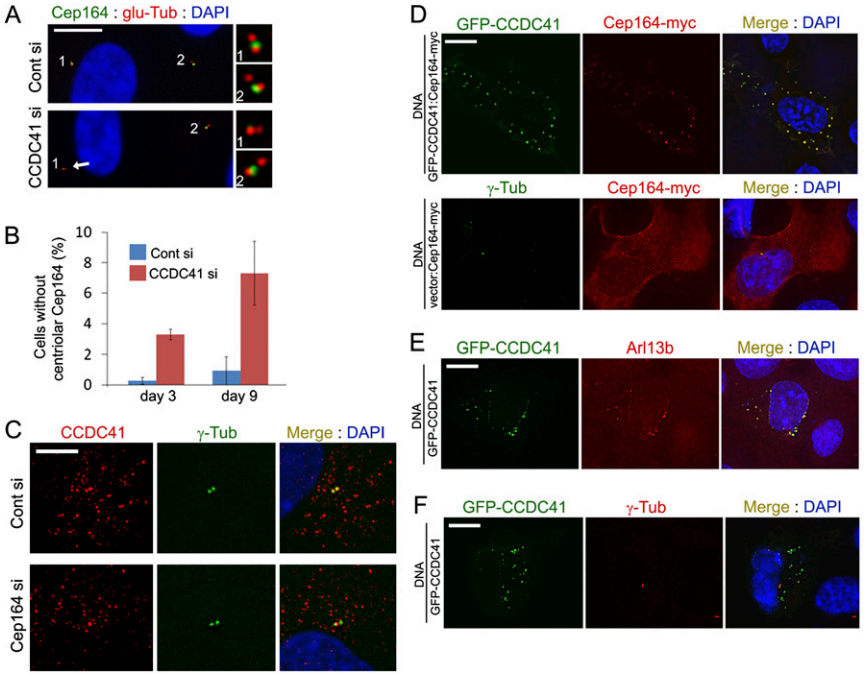

Fig. S5. Potential interaction between CCDC41 and Cep164. (*A*) Centriolar Cep164 staining was not detected in a small fraction of RPE1 cells transfected with CCDC41 siRNAs for 3 d without serum starvation. Arrow indicates a centrosome without Cep164 staining (*B*) Quantification of the percentage of cells that do not exhibit centriolar Cep164 staining. For 9-d experiments, siRNAs were repeatedly transfected at days 0, 3, and 6. (*C*) RPE1 cells were transfected with Cep164 siRNA for 3 d and double-stained with anti-CCDC41 and anti–γ-Tub antibodies. (*D*) RPE1 cells were cotransfected with GFP-CCDC41 and Cep164-myc for 8 h and stained with anti-myc antibody. (*E*) RPE1 cells were transfected with GFP-CCDC41 plasmid for 16 h and stained with anti-Arl13b antibody. (*F*) RPE1 cells were transfected with GFP-CCDC41 for 8 h and stained with anti–γ-Tub antibody. (Scale bars: *A* and *D*–*F*, 10 μm; *C*, 5 μm.)

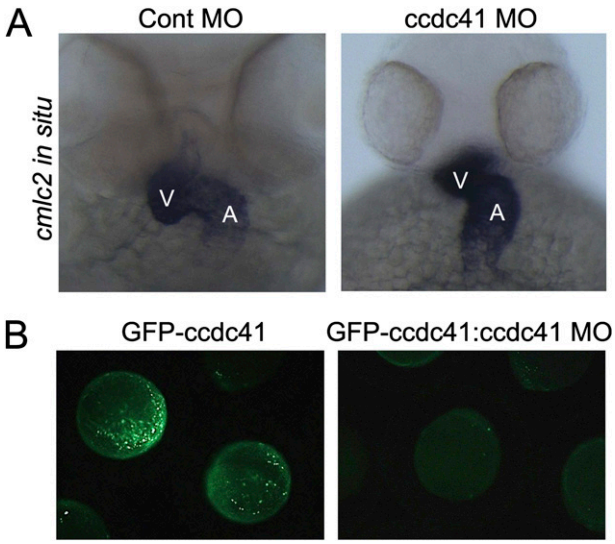

Fig. S6. Efficiency test of ccdc41 knockdown in zebrafish. (*A*) Morpholino-mediated knockdown of ccdc41 does not affect left/right body patterning. A, atrium; *cmlc2*, heart marker; V, ventricle. (*B*) Fluorescence image of zebrafish embryos coinjected with GFP-ccdc41 reporter vector and ccdc41 morpholino (MO). Note the absence of detectable GFP fluorescence in ccdc41 morpholino-injected embryos.

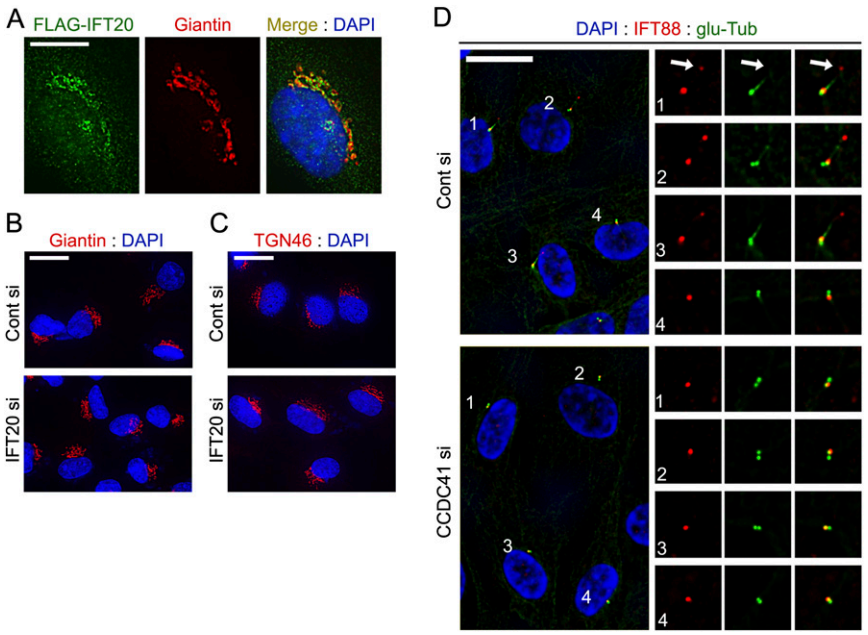

Fig. S7. IFT20 depletion does not affect the integrity of the Golgi complex, and CCDC41 depletion does not affect IFT88 recruitment to the centriole. (*A*) RPE1 cells were transfected with FLAG-IFT20 for 16 h and double-stained with anti-FLAG and anti-Giantin antibodies. (*B* and *C*) RPE1 cells were transfected with siRNAs for 3 d and stained with indicated antibodies. (*D*) CCDC41 depletion does not affect IFT88 recruitment to the centriole. RPE1 cells were transfected with siRNAs for 3 d (cells were serum-starved for the final 24 h) and double-stained with anti-IFT88 and anti–glu-Tub antibodies. Arrows indicate IFT88 accumulation at the distal tip of cilia. (Scale bars: *A*, 10 μm; *B*–*D*, 20 μm.)

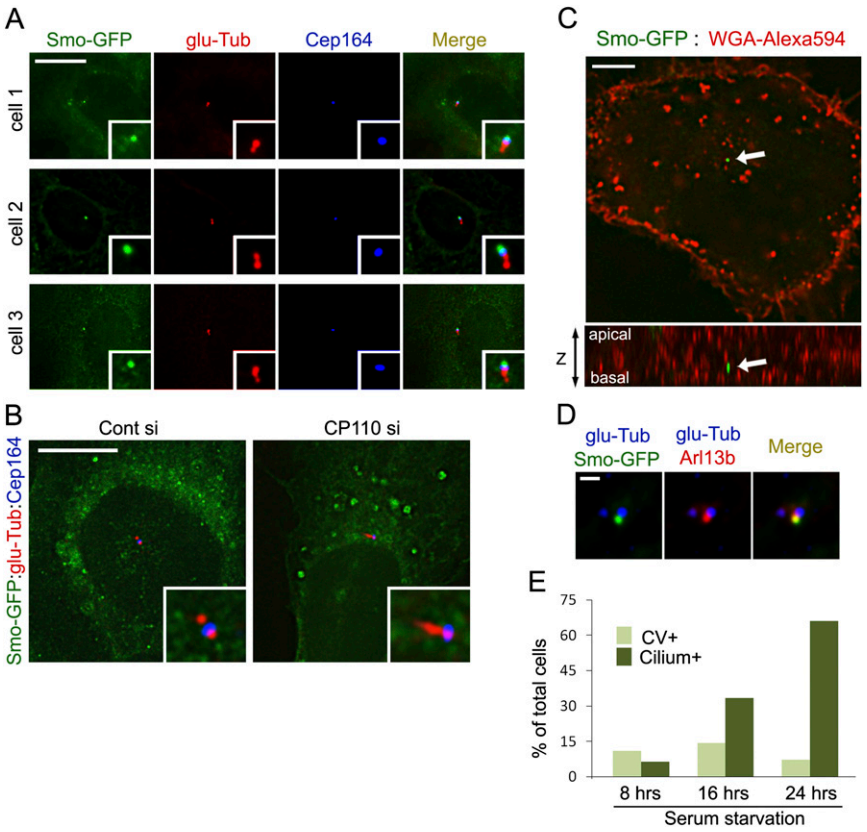

Fig. S8. Detection of the primary ciliary vesicle using Smo-GFP as a marker. (*A*) RPE1-SmoGFP cells were serum-starved for 16 h and double-stained with anti-Cep164 and anti–glu-Tub antibodies. (*B*) U2OS cells stably expressing Smo-GFP were transfected with CP110 siRNA for 3 d (cells were serum-starved for the final 24 h) and double-stained with anti-Cep164 and anti–glu-Tub antibodies. (*C*) Living RPE1-SmoGFP cells were labeled with WGA-Alexa 594 (5 μg/mL) for 10 min, washed with PBS solution, and imaged without fixation. Image stacks were collected with a *z*-step size of 0.2 μm and deconvolved, and 3D rotation was performed with DeltaVision software. Arrow indicates the presumptive primary ciliary vesicle exhibiting Smo-GFP fluorescence. (*D*) RPE1-SmoGFP cells were serum-starved for 16 h and double-stained with anti-Arl13b and anti–glu-Tub antibodies. (*E*) RPE1-SmoGFP cells were serum starved for the indicated hours, and stained with anti–glu-Tub antibody. More than 300 cells from each time point were analyzed. (Scale bars: *A* and *B*, 10 μm; *C*, 5 μm; *D*, 1 μm.)

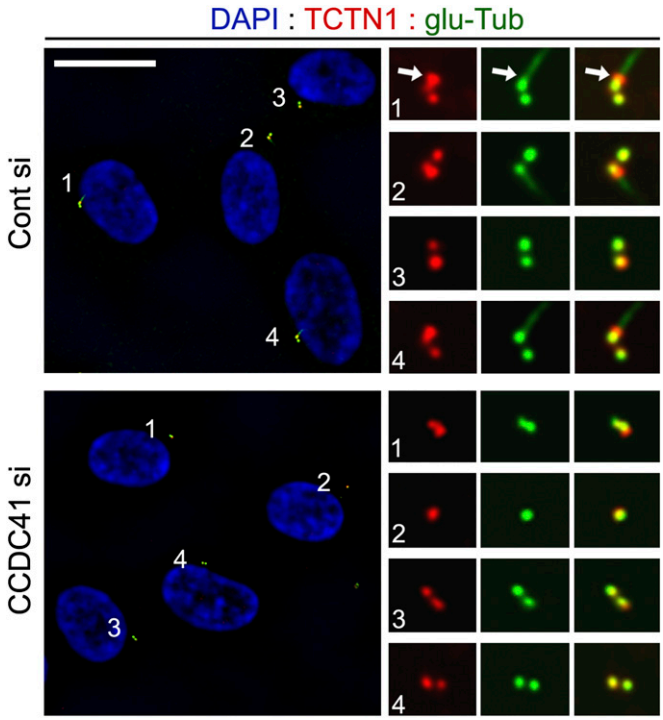

Fig. S9. CCDC41 is required for transition zone formation. RPE1 cells were transfected with siRNAs for 3 d (cells were serum-starved for the final 24 h) and double-stained with anti-TCTN1 and anti–glu-Tub antibodies. Arrows indicate the transition zone of cilia. (Scale bar: 20 μm.)