Supporting Information

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SI Materials and Methods

DNA Constructs for Transfection. *C2cd3, Bbs4, Ift88, Ift52, Sclt1, Cep89*, and *Fbf1* cDNAs were cloned through RT-PCR. *Pcm1, Rab8a, p50/Dynamitin/Dctn2*, and *Ccdc41* cDNAs were purchased from Open Biosystems. FLAG- and GFP-tagged constructs were assembled by subcloning the above genes into pcDNA3-FLAG-PRMT5 (a gift from Y. Wang, The Pennsylvania State University, University Park, PA), pFLAG–CMV2 (Sigma), or pEGFP (Clontech).

A *GFP–Ttbk2* construct was a gift from K. V. Anderson (Sloan–Kettering Institute, New York). *GFP–Cep290* (Addgene plasmid 27379) (deposited by J. Gleeson) (1) and *GFP–Smo* (Addgene plasmid 25395) (deposited by P. Beachy) (2) were obtained from Addgene (www.addgene.org).

Antibodies for Immunofluorescence. Mouse α-acetylated α-tubulin (Sigma; T7451) at 1:1,000, mouse α-γ-tubulin (Sigma; T5326) at 1:500, rabbit α-GFP (Life Technologies; A11122) at 1:500, mouse α-GFP (Life Technologies; A11120) at 1:50, rabbit α-PCM1 (Santa Cruz Biotechnology; sc-67204) at 1:1,000, rabbit α-PCM1 (a gift from A. Merdes, Université de Toulouse, Toulouse, France) at 1:100,000, rabbit α-FLAG (Sigma; F7425) at 1:100, rabbit α-CEP164 (Sigma; SAB3500022) at 1:200, rabbit α-ninein (a gift from J. E. Sillibourne, Institut Curie, Paris) at 1:5,000, rabbit α-Cp110 (Proteintech; 12780-1-AP) at 1:300, rabbit α-Ift88 (3) at 1:10,000, mouse α-β-tubulin (Sigma; T4026) at 1:200, rabbit α-Ofd1 at 1:100 (4), and rabbit α-pericentrin (Abcam; ab4448) at 1:500. An antibody against mouse C2cd3 was generated by immunizing rabbits with a 400-aa peptide corresponding to the N terminus of mouse C2cd3 protein, and was used at 1:1,000.

Antibodies for Western Blot and Coimmunoprecipitation. Mouse α-FLAG (Sigma; F1804) at 1:2,000, rabbit α-GFP (Life Technologies; A11122) at 1:2,000, rabbit α-Ift88 (3) at 1:50,000, and mouse α-β-tubulin (Sigma; T4026) at 1:5,000 were used.

1. Valente EM, et al. (2006) Mutations in CEP290, which encodes a centrosomal protein, cause pleiotropic forms of Joubert syndrome. *Nat Genet* 38(6):623–625.
2. Chen JK, Taipale J, Cooper MK, Beachy PA (2002) Inhibition of Hedgehog signaling by direct binding of cyclopamine to Smoothened. *Genes Dev* 16(21):2743–2748.
3. Jia J, et al. (2009) Suppressor of Fused inhibits mammalian Hedgehog signaling in the absence of cilia. *Dev Biol* 330(2):452–460.
4. Singla V, Romaguera-Ros M, Garcia-Verdugo JM, Reiter JF (2010) Ofd1, a human disease gene, regulates the length and distal structure of centrioles. *Dev Cell* 18(3):410–424.

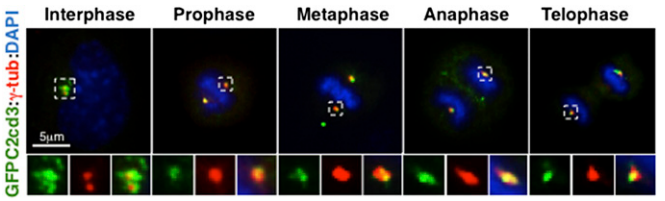

Fig. S1. Dynamic subcellular localization of GFP–C2cd3. GFP–C2cd3 is localized to punctate structures around the centrosome in interphase. In mitosis (prophase, metaphase, anaphase, and telophase), GFP–C2cd3 is localized to the spindle poles. γ-Tubulin labels the centrosome and spindle poles. DAPI stains DNA.

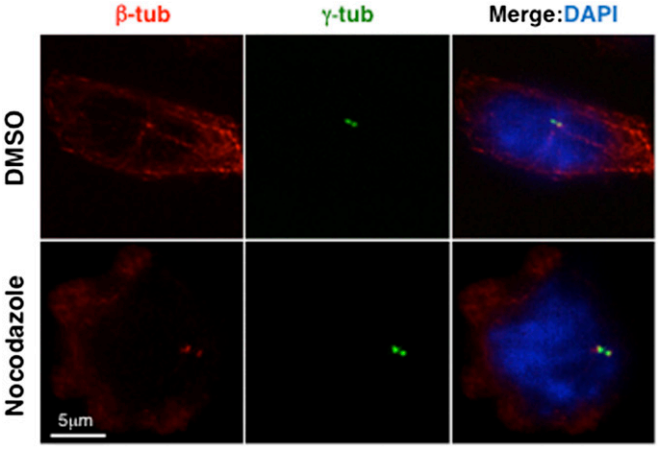

Fig. S2. Nocodazole treatment disrupted the microtubule network. β-Tubulin staining in wild-type mouse embryonic fibroblasts (MEFs) treated with DMSO shows microtubules originated from the centrosomes labeled with γ-tubulin. In contrast, a well-organized microtubule network does not exist in cells treated with nocodazole. The nucleus was visualized by DAPI.

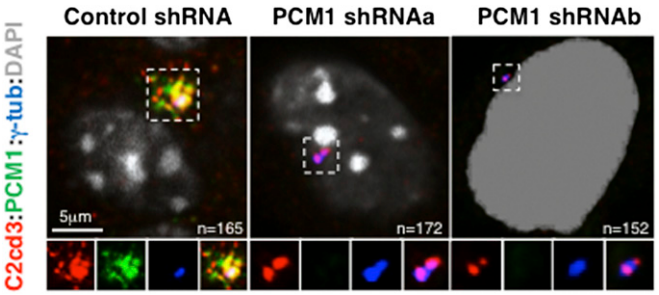

Fig. S3. The centriolar satellite localization of C2cd3 is dependent on Pcm1. A control plasmid expressing shRNA against GFP does not disrupt the centriolar satellite localization of Pcm1 and C2cd3, whereas two plasmids expressing shRNAs (shRNAa and shRNAb) against two different regions of the Pcm1 transcript greatly reduce the levels of Pcm1 protein and disrupt C2cd3 localization to centriolar satellites. Note that C2cd3 localization to the centrioles is not affected. γ-Tubulin labels the centrosomes. DAPI stains DNA.

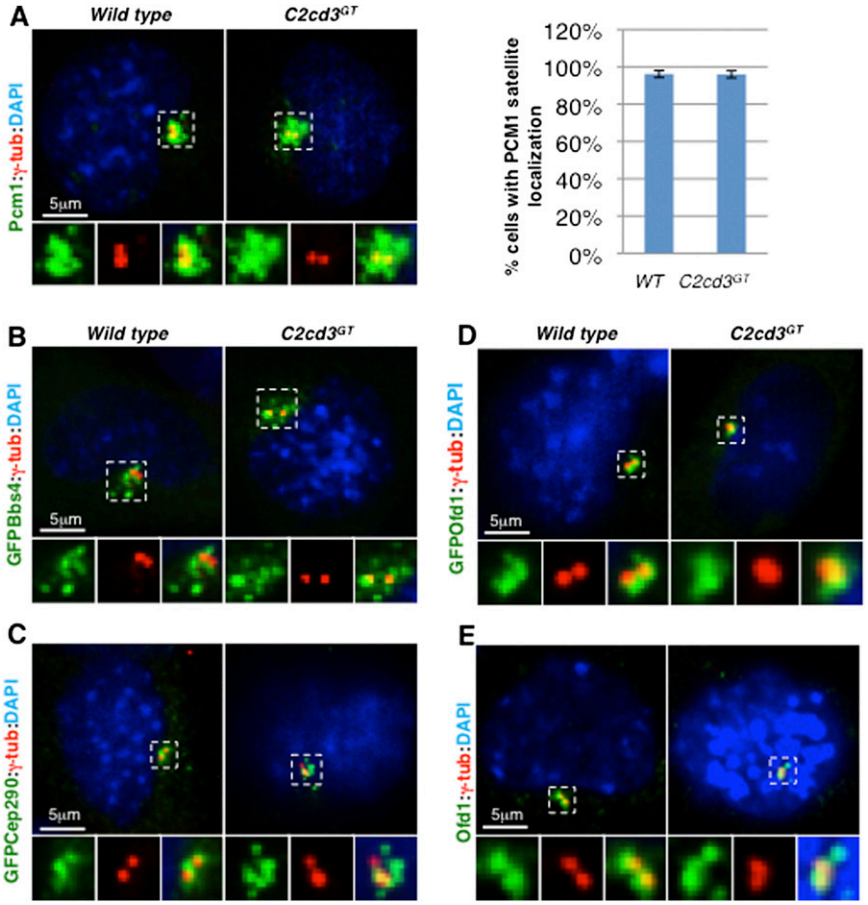

Fig. S4. C2cd3 is dispensable for centriolar satellite integrity. (*A*) Pcm1, (*B*) GFP–Bbs4, (*C*) GFP–Cep290, (*D*) GFP–Ofd1, and (*E*) endogenous Ofd1 are all localized to centriolar satellites around the centrosome in both wild-type and *C2cd3*^{GT} MEFs. γ-Tubulin labels the centrosomes. DAPI stains DNA. For quantitative analyses, SD is indicated.

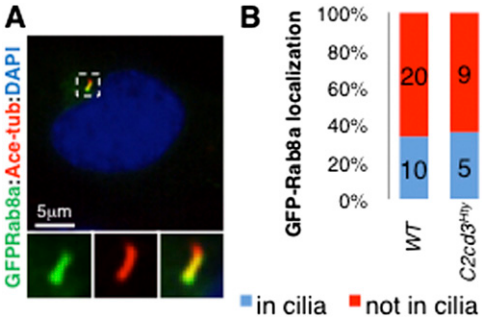

Fig. S5. C2cd3 is not required for Rab8 ciliary localization. (*A*) GFP–Rab8a is localized to the primary cilium. The cilium was labeled with acetylated α-tubulin. The nucleus was visualized by DAPI. (*B*) Quantitative analysis showed that a similar percentage of cilia were GFP–Rab8a positive in wild-type and *C2cd3*^{Hty} mutant cells.

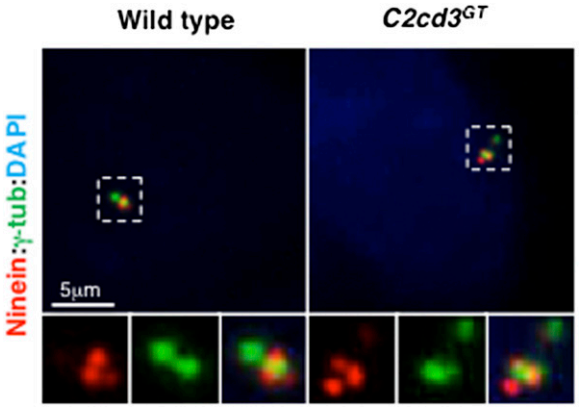

Fig. S6. The absence of C2cd3 does not disrupt ninein localization. Ninein is localized to the centrosome labeled with γ-tubulin in both wild-type and *C2cd3*^{GT} MEFs. The nucleus was visualized by DAPI.

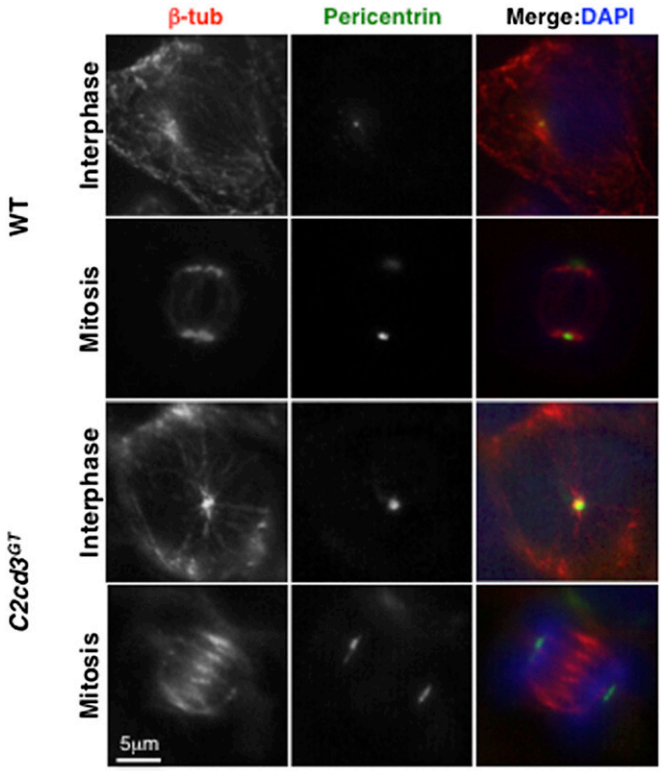

Fig. S7. The microtubule network and spindle apparatus are not disrupted in the absence of C2cd3. Microtubules were labeled with β-tubulin. Centrosomes/spindle poles were labeled with pericentrin. The nucleus was visualized by DAPI.