Supplemental Materials

Molecular Biology of the Cell

Silva et al.

Supplementary Figure 1. **(A)** Phylogenetic analysis of the full-length amino acid sequences of putative Ccdc11 homologs generated using Phylogeny.fr (http://www.phylogeny.fr/). Numbers indicate bootstrap values. **(B)** Domain analysis of indicated Ccdc11 homologs generated using the Simple Modular Architecture Research Tool (SMART; http://smart.embl.de/). NCBI accession numbers: *Homo sapiens* Ccdc11 (NP_659457), *Mus musculus* Ccdc11 (NP_083224), *Danio reiro* Ccdc11 (NP_001038595), *Xenopus tropicalis* Ccdc11 (NP_001120281), *Chlamydomonas reinhardtii* FAP53 (XP_001691996.1). **(C)** Pairwise amino acid sequence alignments of selected putative homologs performed using Tree based Consistency Objective Function For AlignmeEnt Evaluation program (T-Coffee; http://www.ebi.ac.uk/Tools/msa/tcoffee/). Red color denotes perfect pairwise alignment between the input sequences, while blue regions have poor similarity.

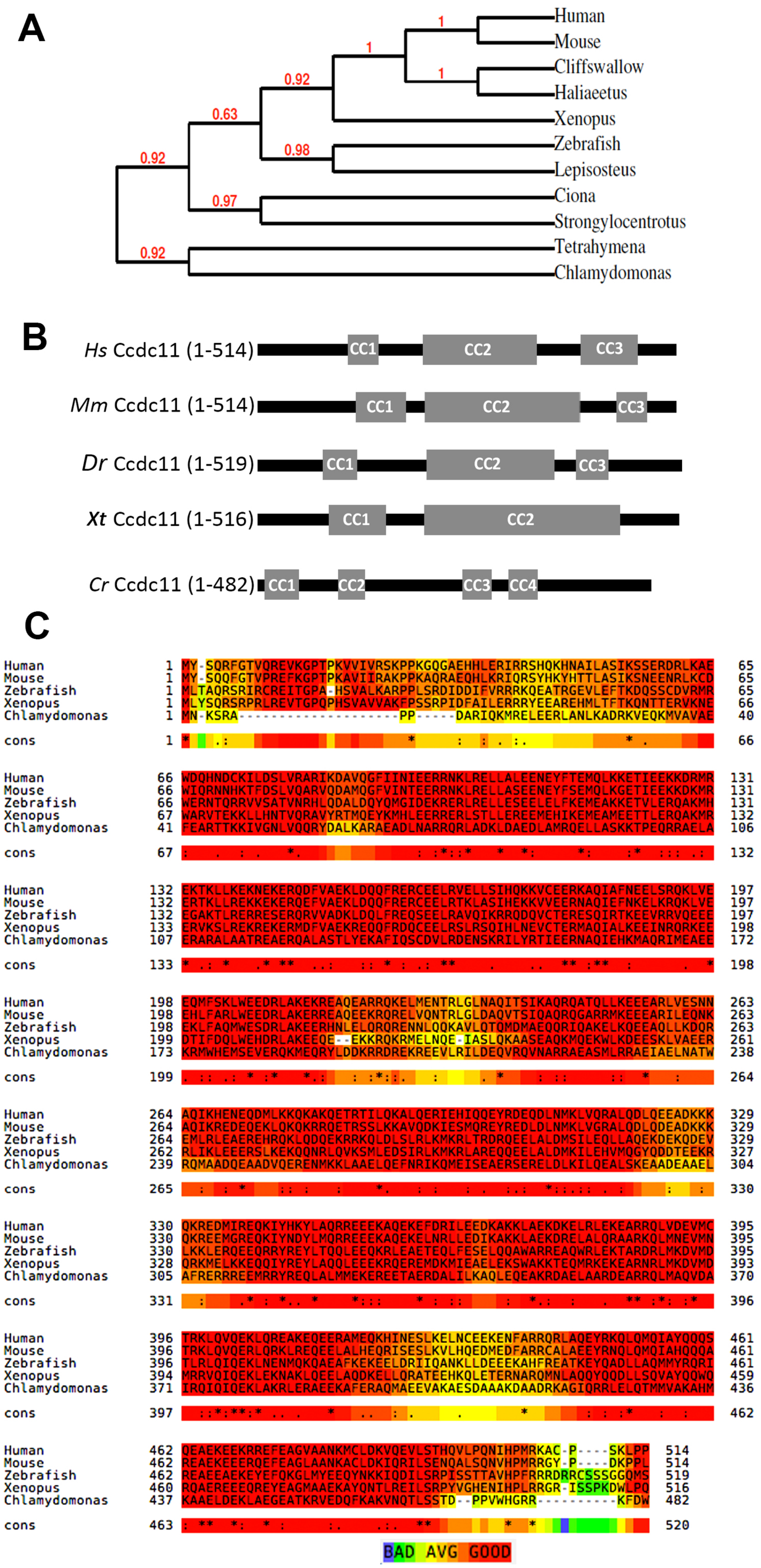
Supplementary Figure 2. **(A)** Ccdc11 colocalizes with centriolar satellite proteins PCM-1 and Cep290 throughout the cell cycle. Asynchronously growing RPE::GFP-Ccdc11 cells were fixed and stained using antibodies against GFP, endogenous PCM-1 or Cep290 (to mark satellites), Centrin (to highlight centrioles), acetylated tubulin (to mark cilia) and DNA (DAPI). The centriolar satellite distribution of Ccdc11 is conserved through G1, S phase, G2, and mitosis. Insets are magnified images of the centrosome region. Scale bar = 5μm. **(B)** Immunoprecipitation of GFP-Ccdc11. Lysates were prepared from asynchronously dividing RPE::GFP-Ccdc11 cells, and GFP-Ccdc11-containing complexes were purified using anti-GFP antibody (or control IgG) coupled to magnetic beads. Eluates were resolved on 4%–15% SDS-PAGE gels and silver stained. Numbers indicate molecular mass of markers in kilodaltons. **(C)** Localization of truncated versions of Ccdc11. RPE-1 cells were transfected with the indicated Myc-tagged Ccdc11 deletion constructs, fixed and immunostained with antibodies against Myc, Centrin (to mark centrioles), and DAPI. Scale bar = 5μm. **(D)** Expression of Ccdc11 (PM) in RPE-1 cells acts in a dominant negative manner, causing a significant reduction in the percentage of cells with organized satellites, and disrupts ciliogenesis. N = 300 cells for each sample, from 3 independent experiments; ** denotes $p<0.05$.

Supplementary Figure 3. **(A)** RPE-1 cells transfected with either non-targeting control siRNA or siRNA against PCM-1. Cells were fixed and immunostained with antibodies against PCM-1, Ccdc11 or Cep290 (green). Cells were also stained for centrioles (Centrin, red) and nuclei (DAPI, blue). Depletion of PCM-1 causes dispersal of Ccdc11 and Cep290 from centriolar satellites, but not the centrosome. Insets are magnified images of the centrosome region. Scale bar = 10 μm. **(B)** siRNA-mediated depletion of PCM-1 causes a significant reduction in the percentage of cells with Ccdc11 and Cep290 at satellites. N = 300 cells for each sample, from 3 independent experiments; ** denotes $p<0.05$. **(C-D)** Control and PCM-1-depleted RPE-1 cells were serum starved, fixed, and immunostained for PCM-1, centrin (centrioles) and acetylated tubulin to label primary cilia. As expected, depletion of PCM-1 significantly reduced the percentage of cells with cilia. N = 300 cells for each sample, from 3 independent experiments; ** denotes $p<0.05$. Scale bar = 10 μm.

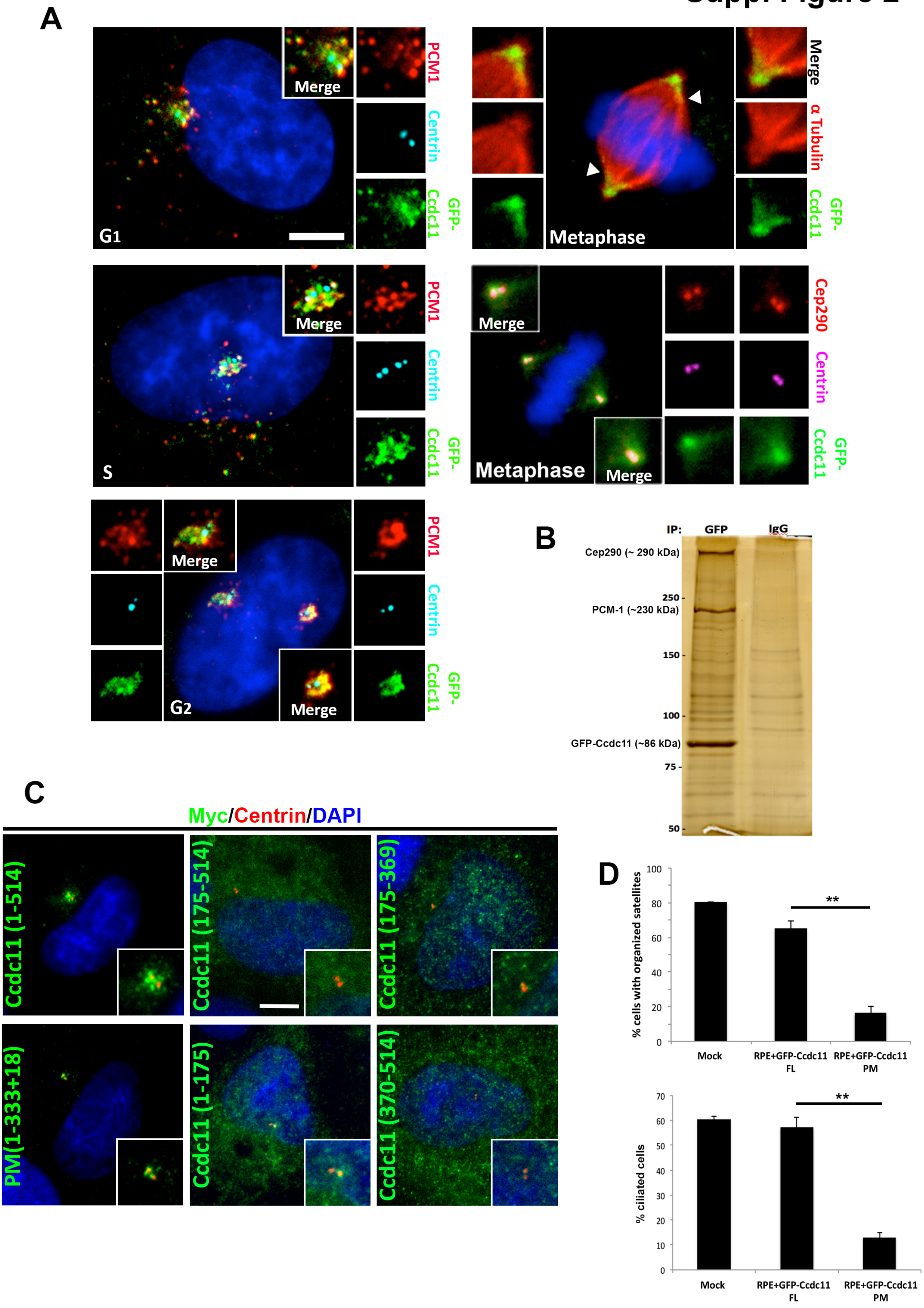
Supplementary Figure 4. **(A)** Depletion of Ccdc11 causes dispersal of satellites and disrupts primary cilium assembly in hTEC. Progenitor cells were infected with lentivirus expressing either control shRNA or shRNA targeting *CCDC11*. Infected cells were selected using puromycin and grown until confluent. Samples were fixed and stained with antibodies against Ccdc11, PCM-1, acetylated tubulin and ZO1. Scale bar = 5 μm. N = 200 cells for each sample, from 2 independent experiments; ** denotes $p<0.05$. **(B)** Block of multiciliated cell formation in Ccdc11-depleted hTEC. Progenitor cells were infected with Ccdc11 shRNA or control shRNA lentivirus 4-6 days before establishing ALI, fixed on ALI day 21 and stained for Ccdc11 (magenta), centrioles (Centrin, green), cilia (acetylated tubulin, red) and DNA (DAPI, blue). Scale bar = 20 μm.

Supplementary Figure 5. **(A)** Validation of the Ccdc11 translation (ATG)-blocking morpholino in *Xenopus* embryos. 100 pg of GFP-CCDC11 or GFP-Centrin mRNA were injected into 2-cell stage embryos, together with 25 ng of either control or CCDC11 morpholino. Lysates from embryos was prepared at the 15-cell stage and analyzed by immunoblotting with anti-GFP antibody. **(B)** Overview of *CCDC11* knockout strategy in zebrafish, depicting the position of TALEN binding regions relative to the *Bsm*A1 restriction site within exon 2. The location of sequencing primers used for genotyping is also indicated. **(C)** Genotyping analysis of zebrafish mutants by PCR and restriction enzyme analysis. Each lane represents a *Bsm*A1-digested amplicon encompassing *CCDC11* TALEN target sequence from genomic DNA. The uncut amplicon is 576 bp in size, while *Bsm*A1 digestion yields fragments of 388 bp and 188 bp. **(D)** Sequencing of independent strains of TALEN-mutagenized zebrafish, indicating the deleted base pairs and the position of the resulting stop codons. **(E)** RT-PCR of cDNA (transcribed from mRNA) from wild-type and *CCDC11* mutant embryos. Primers targeting exons 3-4 and exons 4-5 of *CCDC11* were used. Primers targeting the *β-actin* gene were employed as loading control.

Supp. Figure 1



Supp. Figure 2



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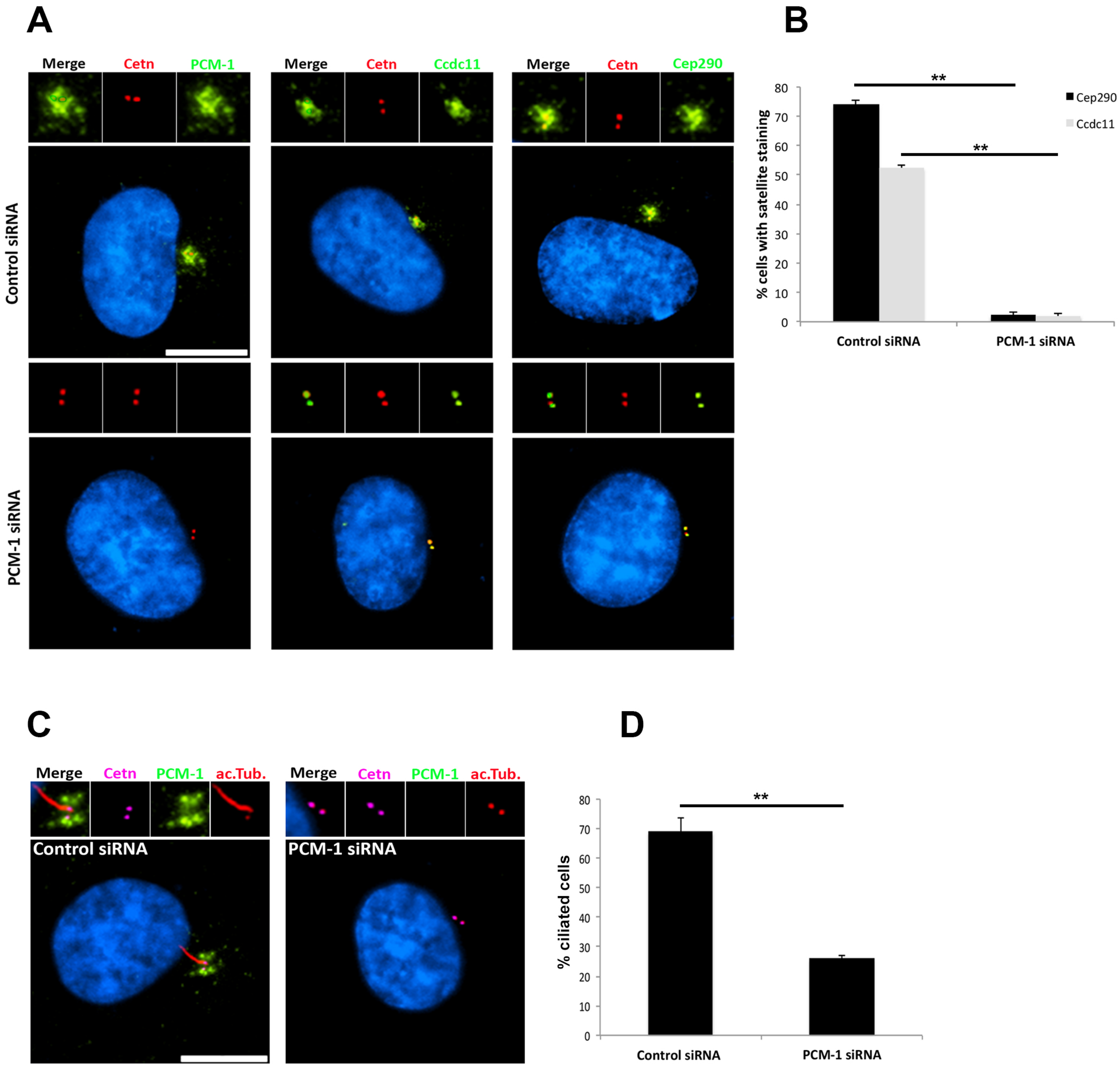
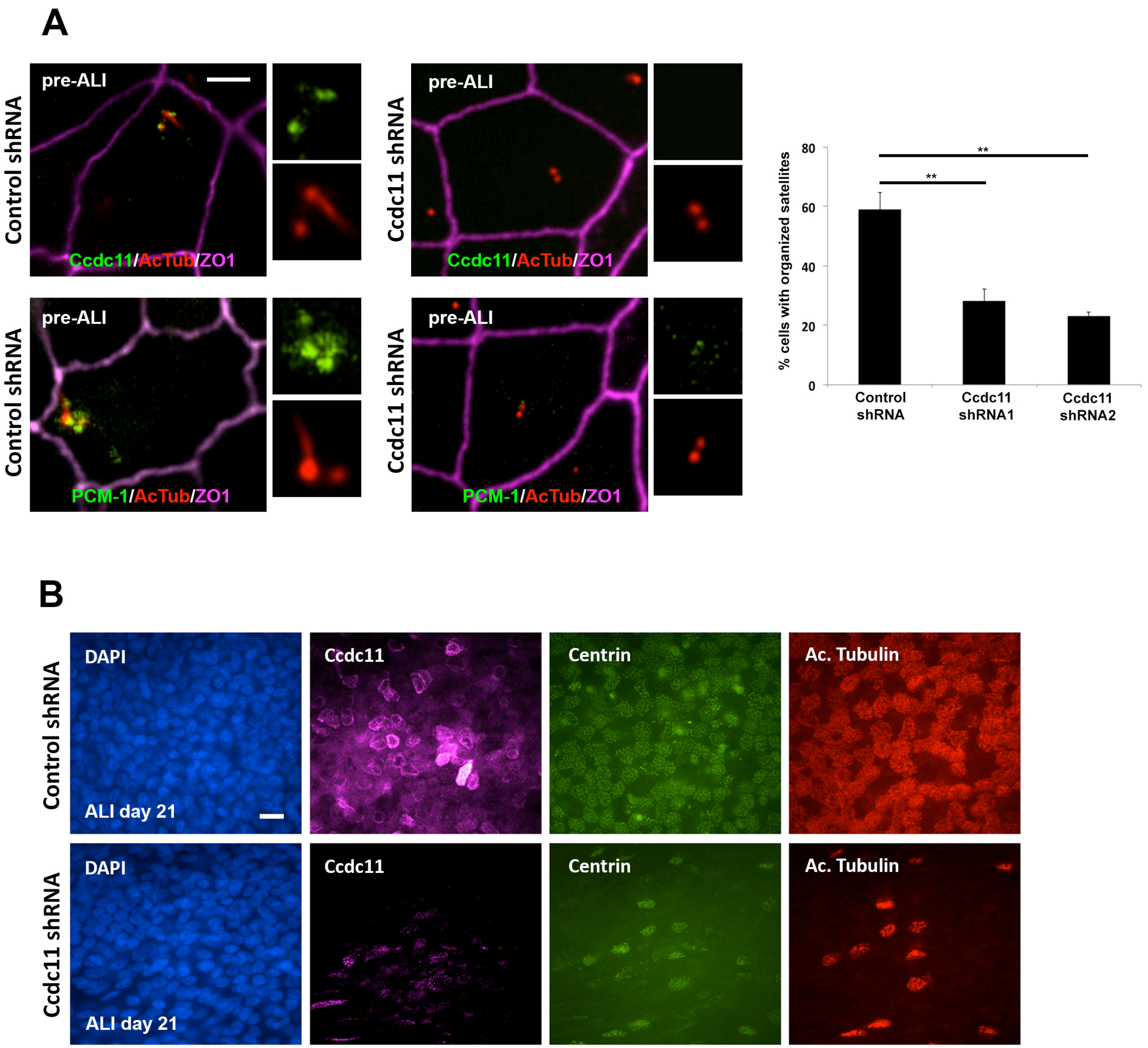


Supp. Figure 4



Supp. Figure 5