

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,

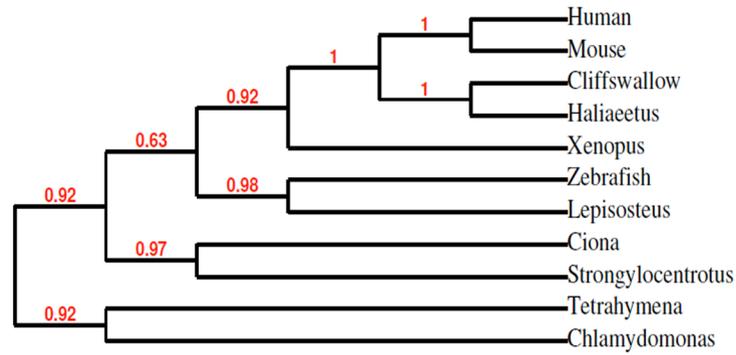
Ccdc11 or Cep290 (green). Cells were also stained for centrioles (Centrin, red) and nuclei (DAPI, blue). Depletion of PCM-1 causes dispersal of Ccdc11 and Cep290 from centriolar satellites, but not the centrosome. Insets are magnified images of the centrosome region. Scale bar = 10 μ m. **(B)** siRNA-mediated 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 μ m.

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 μ m. 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 μ m.

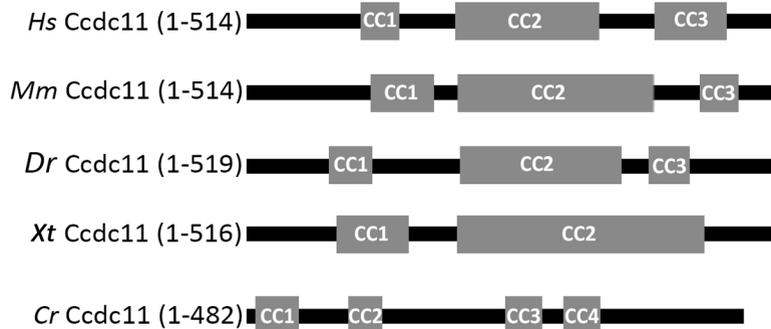
Supplementary Figure 5. (A) Validation of the Ccdc11 translation (ATG)-blocking morpholino in *Xenopus* embryos. 100 pg of GFP-CCDC11 or GFP-Centrin mRNA were injected into 2-cell stage embryos, together with 25 ng of either control or CCDC11 morpholino. Lysates from embryos was prepared at the 15-cell 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 *BsmA1* 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 *BsmA1*-digested amplicon encompassing *CCDC11* TALEN target sequence from genomic DNA. The uncut

amplicon is 576 bp in size, while *BsmA1* 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.

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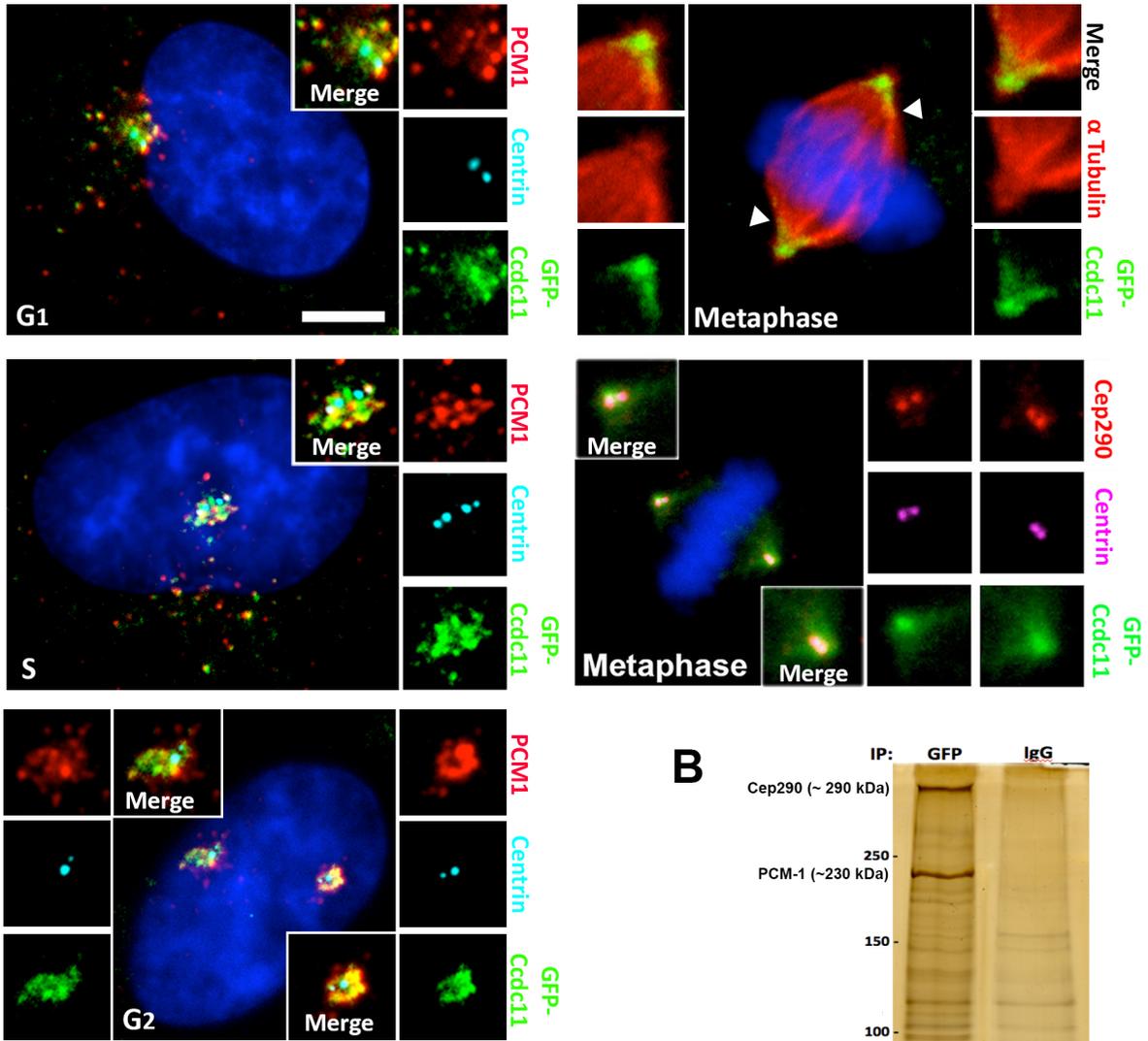


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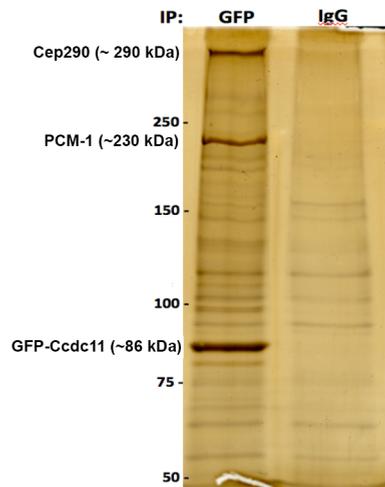
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| Mouse | 1 | MY-SQQFGTVPREFKGPTPKAVIIRAKPPKAQRAEQHLKRIQRSYHYHTT LASKSNEENRLKCD | 65 |
| Zebrafish | 1 | MLTAQRSRIRCREITGPA-HSVALKARPP LSRDIDDIFVRRRKQEA TRGEVLEFTKDOSSCDVRMR | 65 |
| Xenopus | 1 | MLYSQRSRPR LREVTPQPHSVAVVAKFPSSRPIDFAI LERRRYEEAREHMLTFKONTTTERVNE | 66 |
| Chlamydomonas | 1 | MN-KSRA-----PP-----DARIQKMRELEERLANL KADRVEQKMVVAE | 40 |
| cons | 1 | | 66 |
| Human | 66 | WDQHNDCIKLDSLVRARIKDAVQGFIIIEERRNKLRELLALEENEYPTMQLKKEITIEKKDRMR | 131 |
| Mouse | 66 | WIQRNNHKTFDLSVQARVQDAMQGFVINTERRNKLRELLASEENEYFSEMQLKGETIEEKKDKMR | 131 |
| Zebrafish | 66 | WERNTRRVVSATVNRHLQDALDQYQMGIDEKRERLRELLSEELFKEMEAKKETV LEROAKMH | 131 |
| Xenopus | 67 | HARVTEKLLHNTVQRAVYRTMQEYKMLLEERRERLSTLLEREEHEH IKEMEAMEETT LEROAKMR | 132 |
| Chlamydomonas | 41 | FEARTTKKIVGNLVQQRVDAL KARA EADLNARRORLADK L DAEDLAMRQEL LASKKTPERRAELA | 106 |
| cons | 67 | | 132 |
| Human | 132 | EKTKLLKEKNEKERQDFVAEKLDQDFRERCEELRVELLSIHQKVC EERKAQIAFN EELSROK LVE | 197 |
| Mouse | 132 | ERTKLLREKKEKERQDFVAEKLDQDFRERCEELRTKLASIH EKKVVEERNAQIEFNKELKROK LVE | 197 |
| Zebrafish | 132 | EGAKTLRERRESEQRQVADKLDQLFREOSEELRAVQIKRRQDVCTERESQIRTKEEVRRVQEE | 197 |
| Xenopus | 133 | ERVKSLREKREKERMD FVAEKREQDFRDC EELRSLRSQIHLNEVCTERMAQIALKEEINRORKEE | 198 |
| Chlamydomonas | 107 | ERARALAAATRAERQALASTLYEKAFIQSCDVL RDENSKRILYRTIEERNAQIEHKMAQRIMEAE | 172 |
| cons | 133 | | 198 |
| Human | 198 | EQMFSKLWEEDRLAKEKRAEQEARROKELM ENTRLGLNAQITSIKAQRQATOLLKEEEARLVESNN | 263 |
| Mouse | 198 | EHLFARLWEEDRLAKERRAEQEEKRQREL VONTRLGLDAQVTSIQAQROGARRMKEEEARILEQNK | 263 |
| Zebrafish | 198 | EKLFAQMWESDRLAKEERHNL LQRQRENNLQOKVALQTDMDMAEQQRIOAKELQKEEAQLKQDR | 263 |
| Xenopus | 199 | DTIFDQLWEHDLAKEEQE--EKKRKRMLNQE-IASLQKAASEAQKMQEKVLKQDESKLVAEER | 261 |
| Chlamydomonas | 173 | KRMWHMSEVERQKMEQRYLDDKRRDRKREEVLRILDEQVRQV NARRAEASMLRRAEIAELNATW | 238 |
| cons | 199 | | 264 |
| Human | 264 | AQIKHNEQDMLKKQAKQETRTILQKALQERIEHIIQEYRDEQDLNMKLVORALQDLQEEADKKK | 329 |
| Mouse | 264 | AQIKREDEQELQKQKRQETRS LKKA VQDKIESMQREYREDLDMNMLVGRALQDLQEEADKKK | 329 |
| Zebrafish | 264 | EMLRLEAEREHRQLQDQEKRRKLDLSLR LKMKRLTRDROEELALDMSILEQLAQEKDEKQDEV | 329 |
| Xenopus | 262 | RLIKLEERSLKEKQONRLQVKSML EDSIRLKMKRLAREQOEE LALDMKIL EHVMOGYODDTTEKR | 327 |
| Chlamydomonas | 239 | RQMAADQEAADVQERENMKL LAELQEFNR IKQMEISEAERSERELDKILQEALSKEAADAEL | 304 |
| cons | 265 | | 330 |
| Human | 330 | QKREDMIREQKIYHKYLAQRREEEKAQEKFDRILEEDKAKKLA EKDKLRLKEARRQLVDEVMC | 395 |
| Mouse | 330 | QKREEMGREQKIYNDYLMORREEEKAQEKENLRLLEDIKAKKLA EKDKRELA LORAARQLMNEVMN | 395 |
| Zebrafish | 330 | LKKLERQEEQRRYREYLTQQL EEQKRLAETEQLFESE LQOAWARREAOQWLEKTARDR LMKDVMD | 395 |
| Xenopus | 328 | DRKMLKKEQIYREYLAQQL EEEKRQEREMDKMIEAELEKSWAKKTEQMRKEKARNRLMKDVMD | 393 |
| Chlamydomonas | 305 | AFRERRREEMRRYREQLALMMEKEREE TAERDALILKAQLEQEA KRDAELARDEARRQLMAQVDA | 370 |
| cons | 331 | | 396 |
| Human | 396 | TRKLQVQELQREAKEQERAMEQKHINESL KELNCEEKENFARRQRLAQEYRKLQMQIAYQ00S | 461 |
| Mouse | 396 | TRKLQVQERLQRKLRQEELALHEQRIS ESKVLVHQEDMEDFARRCALAE EYRNQ LQMQIAHQ00A | 461 |
| Zebrafish | 396 | TLRLQIQEKL NENMQQAEAFKEKEELDRITQANKLLD EEEKAHFREATKEYQADL LAAQMYRQRI | 461 |
| Xenopus | 394 | MRRVQIQEKLKNAK LQEE LAQDKELQRATEEHKQLETERNARQMNLAQOYQD LLSQVAYQ0W | 459 |
| Chlamydomonas | 371 | TRQIQIQELAKRLERAEEKAFERAQMAEEVAKAESDAAAKDAADRKAGIORRELEQTMVYAKAHM | 436 |
| cons | 397 | | 462 |
| Human | 462 | QEAEEKEKRRFEAGVAANKMCLDKVQEV LSTHQVLPONIHPMRKAC-P---SKLPP | 514 |
| Mouse | 462 | REAEKEERQEF EAGLAANKAC LDKTORILSENQALSONVHPMRRGY-P---DKPLP | 514 |
| Zebrafish | 462 | REAEAEAEKEYEFQKGLMYEEQYNNK IODLSRPSISSTTAVHPFRRRDRRCS5SGG0MS | 519 |
| Xenopus | 460 | ROAEREQEQREY EAGMAAEKAYQNTLREILSRPYVGHENIHLPRGR-ISSPKDWLQ | 516 |
| Chlamydomonas | 437 | KAAELDEKLAEGEATKRVEDQFKAVNQ T LSSTD--PPVWHGR-----KFDW | 482 |
| cons | 463 | | 520 |

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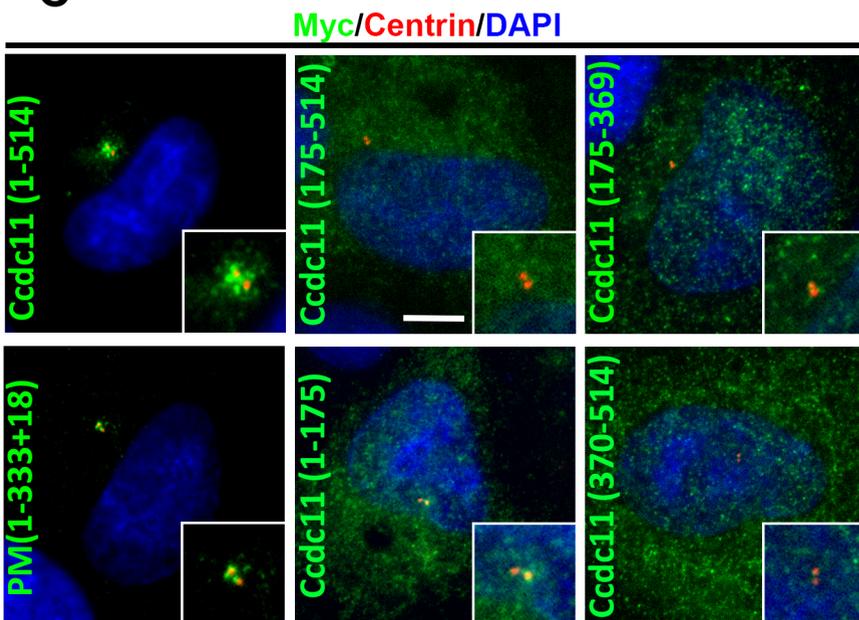
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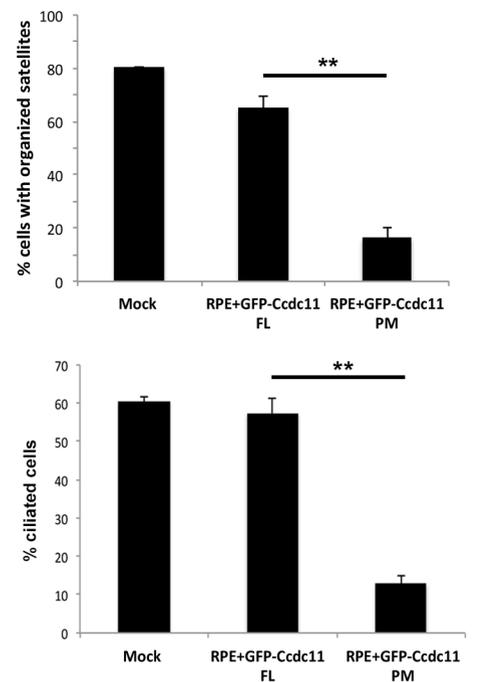
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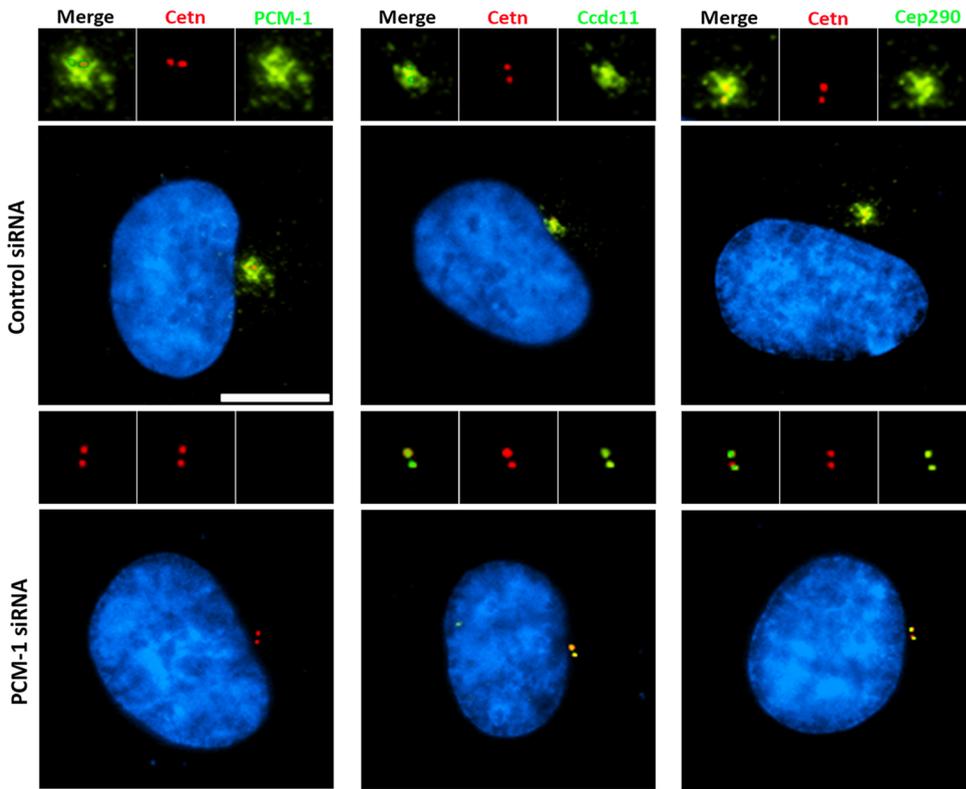
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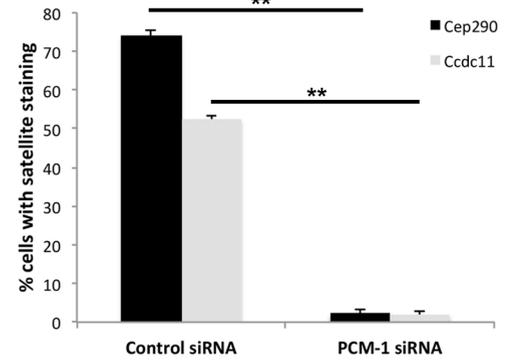
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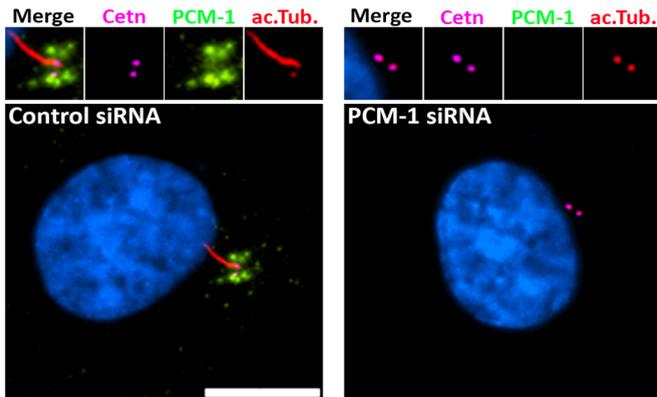
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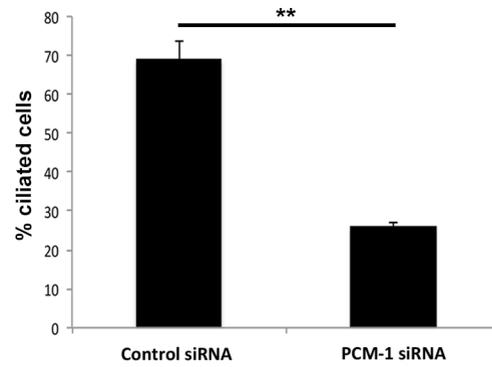
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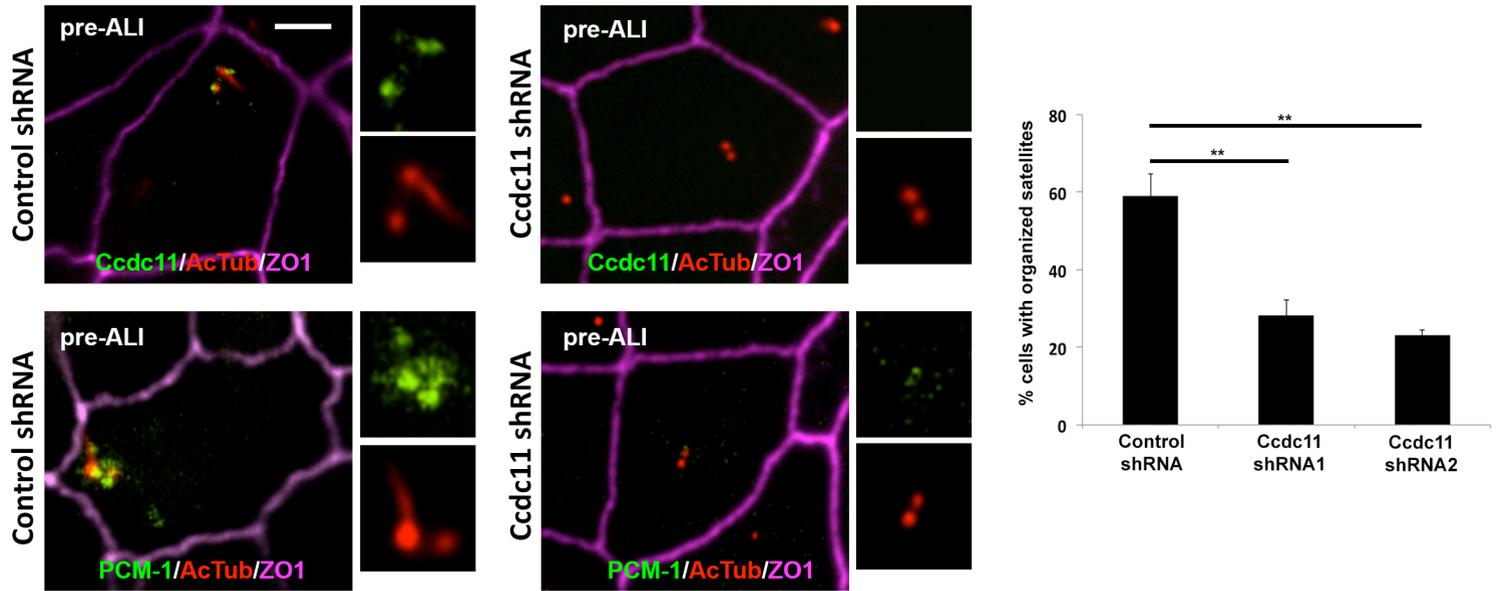
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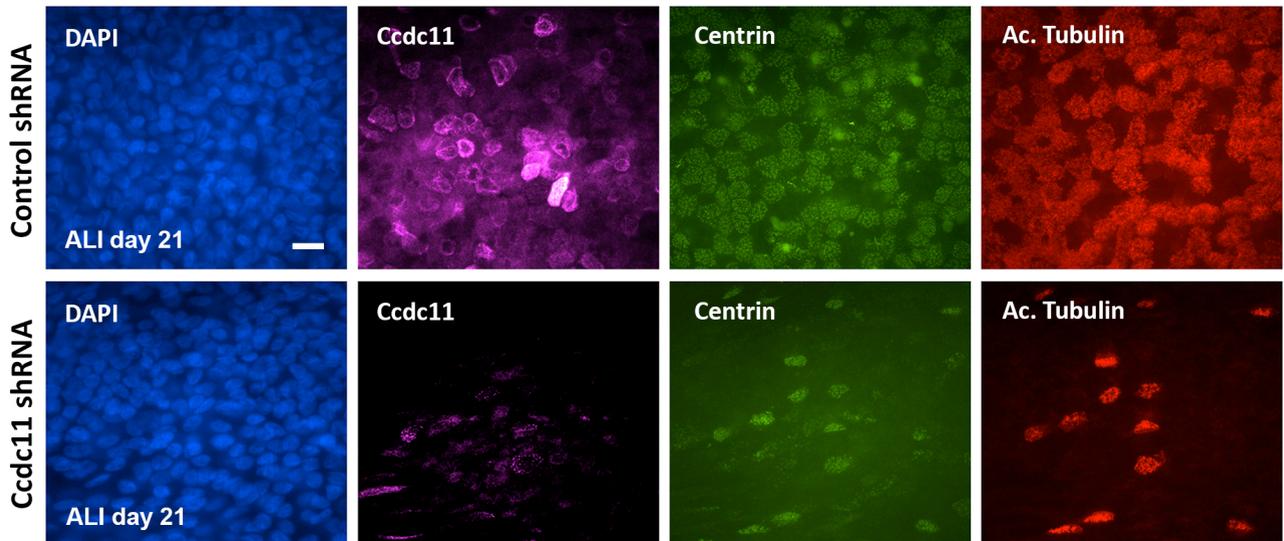
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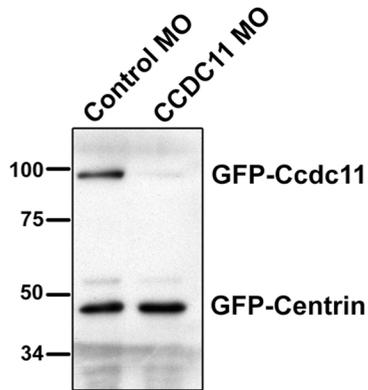
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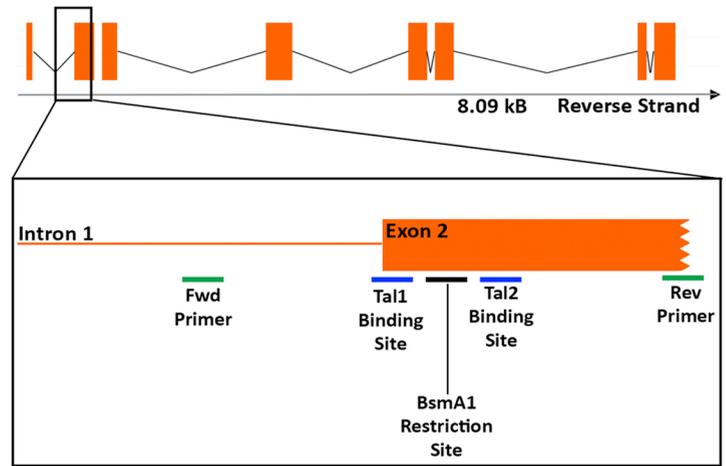
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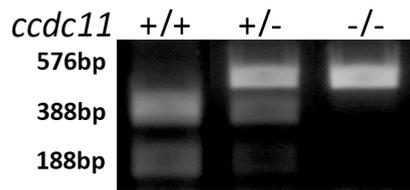
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Residue 26 37

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R P P L S R H R **STOP**

E

