Supplemental Materials

Molecular Biology of the Cell

Bärenz et al.

Supplemental Figure Legends

Figure S1. Ccdc61 is a centrosome associated protein and characterization of the Ccdc61 antibody. (A) Localization of transiently expressed Ccdc61 in interphase RPE1 cells. GFP or FLAG tagged Ccdc61 (green) was transiently expressed and co-stained with γ-tubulin, pericentrin or PCM1 (red); DNA was co-stained with Hoechst33258 (blue). Magnified panels (magn.) show enlarged views of the boxed regions. Bar, 10 µm. (B) HeLa-TDS-mCcdc61-GFP stable cell line was used to visualize the co-localization of Ccdc61 (green) with γ-tubulin or pericentrin (red) in interphase or mitotic cells. Ccdc61 was visualized with GFP or the endogenous Ccdc61 antibody (green); DNA was co-stained with Hoechst33258 (blue). Magnified panels (magn.) show enlarged views of the boxed regions. Bar, 10 µm. (C) IF analysis of U2OS cells after treatment with control siRNA (ct), Oligo1 siRNA (O1) or Oligo2 siRNA (O2). Endogenous Ccdc61 was visualized with monoclonal Ccdc61 antibody (green), centrosomes by pericentrin (red) and chromatin by Hoechst33258 staining (blue). Magnified panels (magn.) show enlarged views of the boxed regions. Bar, 10 µm. Line scans of endogenous Ccdc61 (upper) signals from representative cells demonstrate the reduction of Ccdc61 levels after siRNA treatment. Line scans of endogenous pericentrin (lower) signals from representative cells indicate no change in level after Ccdc61 siRNA treatment. (D) Recombinantly purified 6xHis-Ccdc61 was analyzed by a SDS-PAGE at different concentrations (as indicated) and via Western blotting using Ccdc61 monoclonal antibody (upper left). As Ccdc61 was not detectable in crude cell lysates we used HEK293T cells to immunoprecipitate endogenous Ccdc61 with the monoclonal Ccdc61 antibody that was coupled to Protein G Sepharose beads. The enriched endogenous Ccdc61 protein was then detected in Western blotting using the same antibody. The heavy chain of the antibody is marked with an asterisk (lower left). GFP-Ccdc61 or FLAG-Ccdc61 was transiently expressed in HeLa or U2OS cells. Cell lysates were analyzed by Western blotting using Ccdc61 monoclonal antibody (right). Empty vector (EV) was transfected as a control.

Figure S2. The centrosomal localization of Ccdc61 requires properly arranged MTs. (A) Co-staining of endogenous Ccdc61 (green) with pericentrin or PCM1 (red) in HeLa cells. Chromatin is visualized by Hoechst33258 staining (blue). Magnified panels (magn.) show enlarged views of the boxed regions. Bar, 10 µm. (B) U2OS cells were subjected to nocodazole (noco) to depolymerize MTs. After nocodazole washout, cells were harvested at the indicated time points and stained with antibodies directed against Ccdc61 (green), PCM1 (red) and γ-tubulin (grey). Control (ct): cells without

nocodazole treatment. Magnified panels (magn.) show enlarged views of the boxed areas. Bar, 10 µm. (C) U2OS cells were treated with control (ct) or PCM1 siRNA and the PCM1 levels around the centrosome analyzed. Representative images show staining of U2OS cells with antibodies directed against Ccdc61 (green) and PCM1 (red); DNA was co-stained with Hoechst33258 (blue). Magnified panels (magn.) show enlarged views of the boxed regions. Bar, 10 µm. Scattered dot plot shows Ccdc61 intensity quantified in a 25 μm^2 circle around the centrosome normalized to the control (ct). Data represent mean ± s.d. from three independent experiments, n>122 cells. ns: not significant (unpaired Student´s t-test). Western blot shows downregulation efficiency of PCM1 siRNA analyzed by PCM1 antibody. Actin served as a loading control.

Figure S3. Downregulation of Ccdc61 leads to spindle formation defects. (A) left: Western blot shows the efficiency of indicated Ccdc61 Oligo that was tested by its ability to deplete transiently transfected FLAG-Ccdc61 or siRNA-resistant FLAG-Ccdc61 (O2R). right: Western blot shows the efficiency of indicated Ccdc61 Oligos that was tested by their ability to deplete transiently transfected FLAG-Ccdc61. α-tubulin served as a loading control. (B) HeLa cells stably expressing α-tubulin-mEGFP and H2B-RFP were arrested at the G1/S phase transition by the addition of 2 mM thymidine for 19 h, released for 6 h and then imaged for 16 h at 5 min time intervals after siRNA depletion of Ccdc61 (Oligo1 and 2) for 72 h. Quantification shows mitotic duration (mitosis: prophase-cytokinesis) and time from correctly assembled metaphase plate until the beginning of anaphase onset (metaphase-anaphase). Representative images from the movies were cropped out from the original time series (time in min). (C, D) U2OS cells were transfected with the indicated siRNAs or siRNA/plasmid combinations and stained with an antibody directed against Mad2 (left) or α-tubulin (right; green) and co-stained with CREST (red) and Hoechst33258 to visualize the DNA (blue). Mad2 staining is used as a marker of an active SAC. Co-staining of α-tubulin and CREST indicates whether MTs are properly attached to kinetochores. Magnified panels (magn.) show enlarged views of the boxed regions. Bar,10 µm.

Figure S4. MT EB3-tracking measurements reveal a significant decrease in spindle biorientation in the absence of Ccdc61. (A) HeLa cells transiently transfected with EGFP-EB3 and SiR-DNA were imaged for 1 min at a time resolution of 2 s. Representative mitotic figures from each treatment were time projected. (B) Projection of a time-lapse image series along the direction of time with time-based color-coding (blue: early-time, white: mid-time, and red: late-time). The directions of the observed tracks

(growing MT tips; irrespective of their positions and sizes) can be quantified according to this common definition of angle (right). Here, three exemplary tracks are outlined (white circles) and magnified to show their angular orientation (yellow lines and numbers). (C) The histogram of the so-obtained growth directions of MT tips is used to quantify the directionality and the symmetry of mitosis with two indices. The histogram of calculated orientations is used to determine the "dominant" orientation, which is the angle with the largest area under the histogram curve (i.e., number of links) compared to the area under the other half of the histogram. Once the dominant angle is found, two figures of merit are defined: *Orientation Index* (directionality) is defined as the area under the histogram around the dominant orientation down to the baseline (A_1) divided by the corresponding area under the baseline (A_0). *Biorientation Index* (biorientation/asymmetry) is defined as the number of links growing along the dominant orientation (A_1) divided by the number of the rest of the links (A_2). Please not that the same histogram as presented here is used in Figure 2 of the manuscript in the ArXiv manuscript (arXiv:1703.02611v1).

Figure S5. Ccdc61 BioID approach to identify novel interaction partners. (A) Representative images of HEK293T FLAG-Ccdc61-BirA* cells treated with doxycycline to induce expression (for 24 h) in the presence (+) or absence of 50 µM biotin. Biotinylated proteins visualized with streptavidin (green), centrosomes with pericentrin (red) and chromatin by Hoechst33258 staining (blue). Magnified panels (magn.) show enlarged views of the boxed regions. Bar, 10 µm. (B) Upper panel: HEK293T cells stably expressing FLAG-Ccdc61-BirA* upon doxycycline (Dox) induction were used for BioID to identify novel Ccdc61 interaction partners in the presence or absence of 50 µM biotin. Cells in the presence of biotin were either arrested in mitosis by the addition of 100 nM nocodazole for 16 h or blocked in G1/S phase by the addition of 2 mM thymidine for 16 h. BioID samples were separated by SDS-PAGE and stained with colloidal Coomassie. Lower panel: Input samples from BioID were subjected to Western blot analysis to determine the cell cycle stage of the sample. Plk1 served as a mitotic marker and α-tubulin as a loading control. (C) Tet-inducible FLAG-Ccdc61-BirA* HEK293T T-Rex cells were used to pull down Ccdc61 by using streptavidin Sepharose (BioID) in the presence or absence of FLAG-Ccdc61-BirA* (+ Dox; 1 µg/ml; 24 h) and biotin (50 µM; 24 h). Western blots were performed on input samples from total cell extracts (TCL) and corresponding BioID and analyzed with antibodies directed against streptavidin, Cep290 and Cep170. (D) Tables highlight mass spectrometry-identified candidates from the FLAG-Ccdc61-BirA* performed BioID from nocodazole (left) or thymidine (right) blocked cells. The first 20 specifically identified candidates are listed

according to their score. The bait protein Ccdc61 is highlighted in red; proteins analyzed (Cep290, TBK1, Cep170, PCM1) are additionally indicated in bold. Listed are name, score (standard Mascot score: summed score for the individual peptides), number of peptide matches (prot matches), number of significant peptide matches (sign prot matches), number of distinct sequences (prot sequences), number of significant distinct sequences (sign prot sequences) and protein sequence coverage (%).

Figure S6: Ccdc61 loss disrupts centrosomal localization of Cep170. (A, B) Co-staining of Ccdc61 (green) with either ODF-2 (left) or C-Nap1 (right; red) in U2OS or RPE1 cells. DNA was visualized with Hoechst33258 staining (blue). Magnified panels (magn.) show enlarged views of the boxed regions. Bar, 10 µm. (C) IF analysis of mitotic HeLa cells after knockdown of Ccdc61 with siRNA O1 and O2. Cells were stained with antibodies directed against γ-tubulin (green) and Cep170 (red); DNA was co-stained with Hoechst33258 (blue). Magnified panels (magn.) show enlarged views of the boxed regions. Bar, 10 µm. Quantification shows the analysis of 20 mitotic cells in each of three independent experiments that were visually inspected for a centrosomal localization, the loss of the centrosomal signal (no signal) and the cytoplasmic dispersal of Cep170. The significance was calculated by a two-way ANOVA test and scored as follows: **p<0.01; ***p<0.001; ****p<0.0001. (D) A 6.25 μm^2-ring around the mitotic, centrosomal Cep170 signal of 20 U2OS images was cropped out of each individual image for following maximum projection. γ-tubulin signals were used to determine the centrosomal Cep170 signals in the middle of the ring. (E) U2OS cells were transfected with the indicated siRNAs or siRNA/plasmid combination and stained for Cep170. Cell peripheries are marked with yellow lines. Boxed regions show enlarged views of the boxed regions. Bar, 10 µm. (F) Western blot of U2OS cells shows Cep170 protein levels and illustrates the efficiency of indicated Ccdc61 Oligos that was tested by their ability to deplete transiently transfected FLAG-Ccdc61. α-tubulin served as a loading control.

Figure S7: Ccdc61 affects the correct centrosomal anchorage of Cep290 and PCM1. (A) HeLa cells were treated as indicated and γ-tubulin was used to visualize centrosomes (green), PCM1 visualized in red, and Hoechst33258 to stain DNA (blue). Magnified panels (magn.) show enlarged views of the boxed regions. Bar, 10 µm. 20 mitotic cells in each of three independent experiments were visually inspected for a centrosomal localization, the loss of the centrosomal signal (no signal) and the cytoplasmic dispersal of the PCM1 signal. γ-tubulin signals were used to determine the centrosomal localization. The significance was calculated by a two-way ANOVA test and scored as follows: *p<0.05, ***p<0.001, ****p<0.0001. (B) Representative images to Figure 6F. U2OS cells were treated

as indicate and stained for Cep290 (green) and γ-tubulin (red) to visualize centrosomes (green); co-staining of DNA with Hoechst33258 (blue). Magnified panels (magn.) show enlarged views of the boxed regions. Bar, 10 μm. (C, D) HeLa cells were treated as indicated and γ-tubulin was used to visualize centrosomes (green), Cep290 visualized in red, and Hoechst33258 to stain DNA (blue). Magnified panels (magn.) show enlarged views of the boxed regions. Bar, 10 μm. 20 mitotic cells in each of three independent experiments were visually inspected for a centrosomal localization, the loss of the centrosomal signal (no signal) and the cytoplasmic dispersal of the Cep290 signal. γ-tubulin signals were used to determine the centrosomal localization. The significance was calculated by a two-way ANOVA test and scored as follows: ****$p<0.0001$. (E) Western bot to control downregulation efficiency of Cep170 siRNA using an antibody directed against Cep170. Actin served as a loading control. (F) FRAP analysis in HeLa cells expressing full-length GFP-Cep170 after knockdown of Ccdc61 with siRNA O1 and O2 for 72 h. Recovery profiles of bleached GFP centrosome signal are plotted over 80 s.

Movie 1 and 2. Time lapse imaging of 2 control (ct) siRNA treated U2OS H2B-GFP α-tubulin-mCherry cells. Imaging was performed every 5 min on an inverted microscope (Observer.Z1; Zeiss) using a 20× objective lens. Time is indicated in h:min. Bar, 10 μm.

Movie 3 and 4. Time lapse imaging of 2 Ccdc61 Oligo2 siRNA treated U2OS H2B-GFP α-tubulin-mCherry cells. Imaging was performed every 5 min on an inverted microscope (Observer.Z1; Zeiss) using a 20× objective lens. Time is indicated in h:min. Bar, 10 μm.

Movie 5 and 6. Time lapse imaging of 2 U2OS H2B-GFP α-tubulin-mCherry cells treated with Ccdc61 Oligo2 siRNA and FLAG-Ccdc61 siRNA-resistant construct. Imaging was performed every 5 min on an inverted microscope (Observer.Z1; Zeiss) using a 20× objective lens. Time is indicated in h:min. Bar, 10 μm.

Figure S1

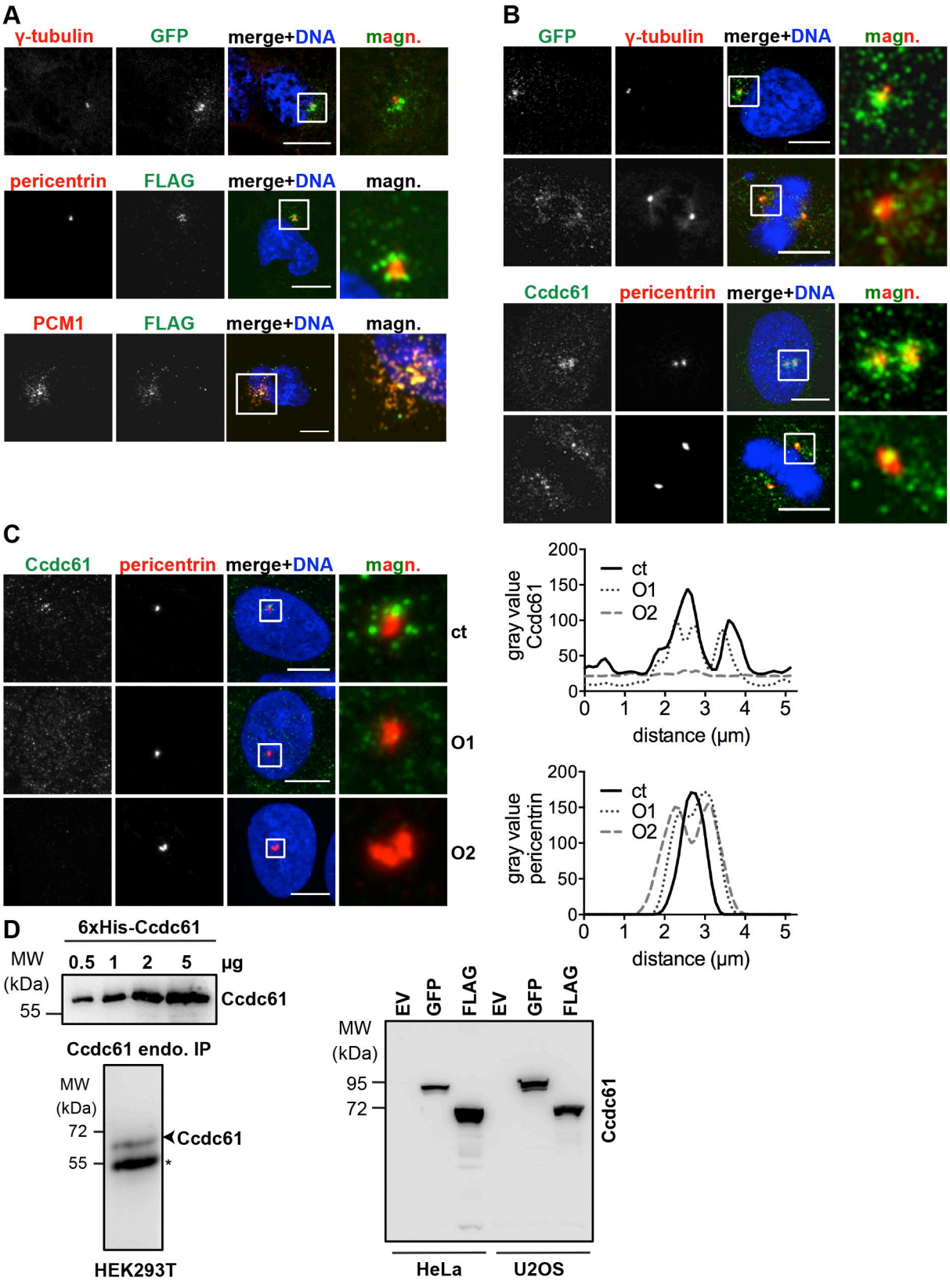
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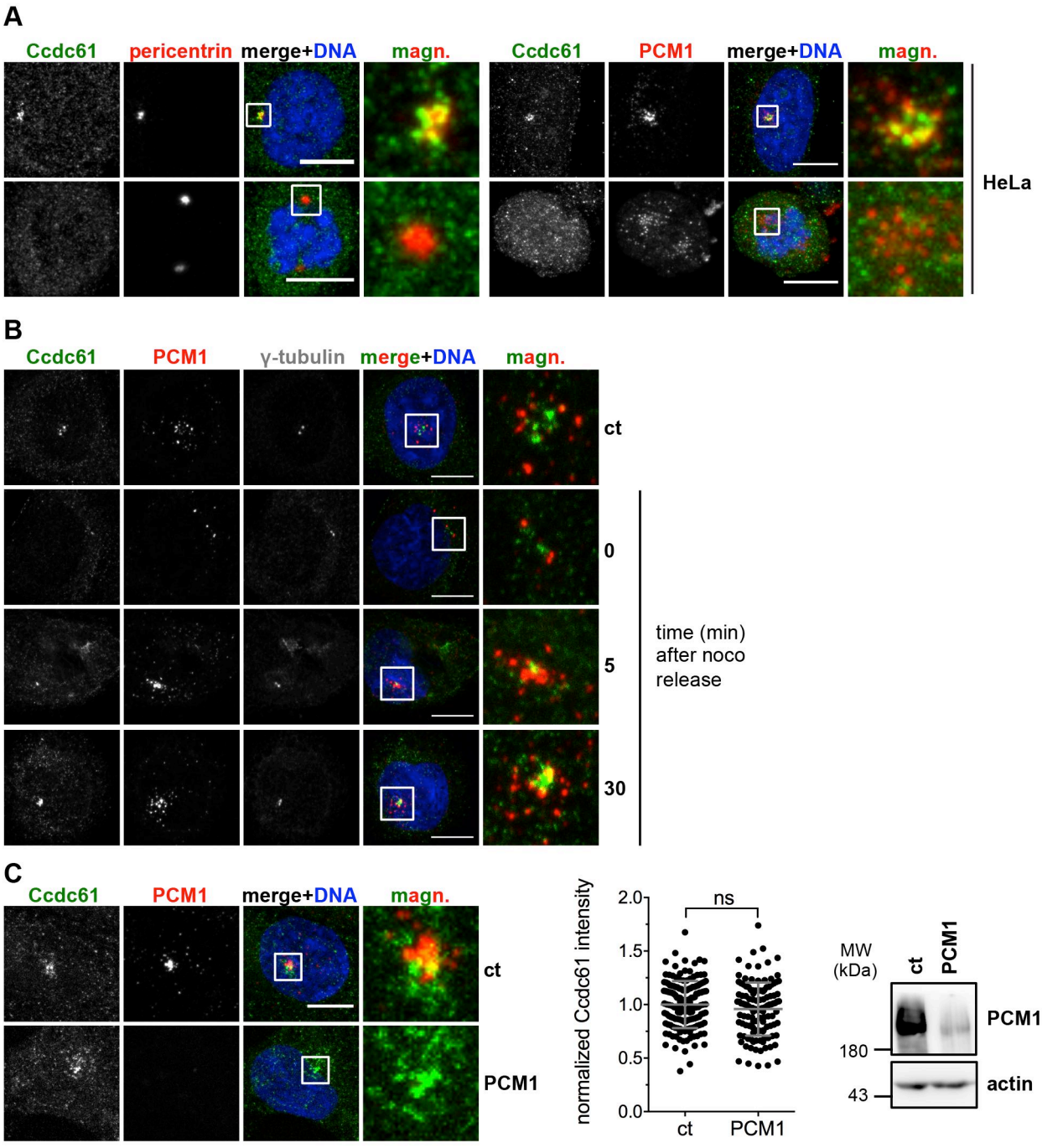
Figure S2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time (min)
after noco
release
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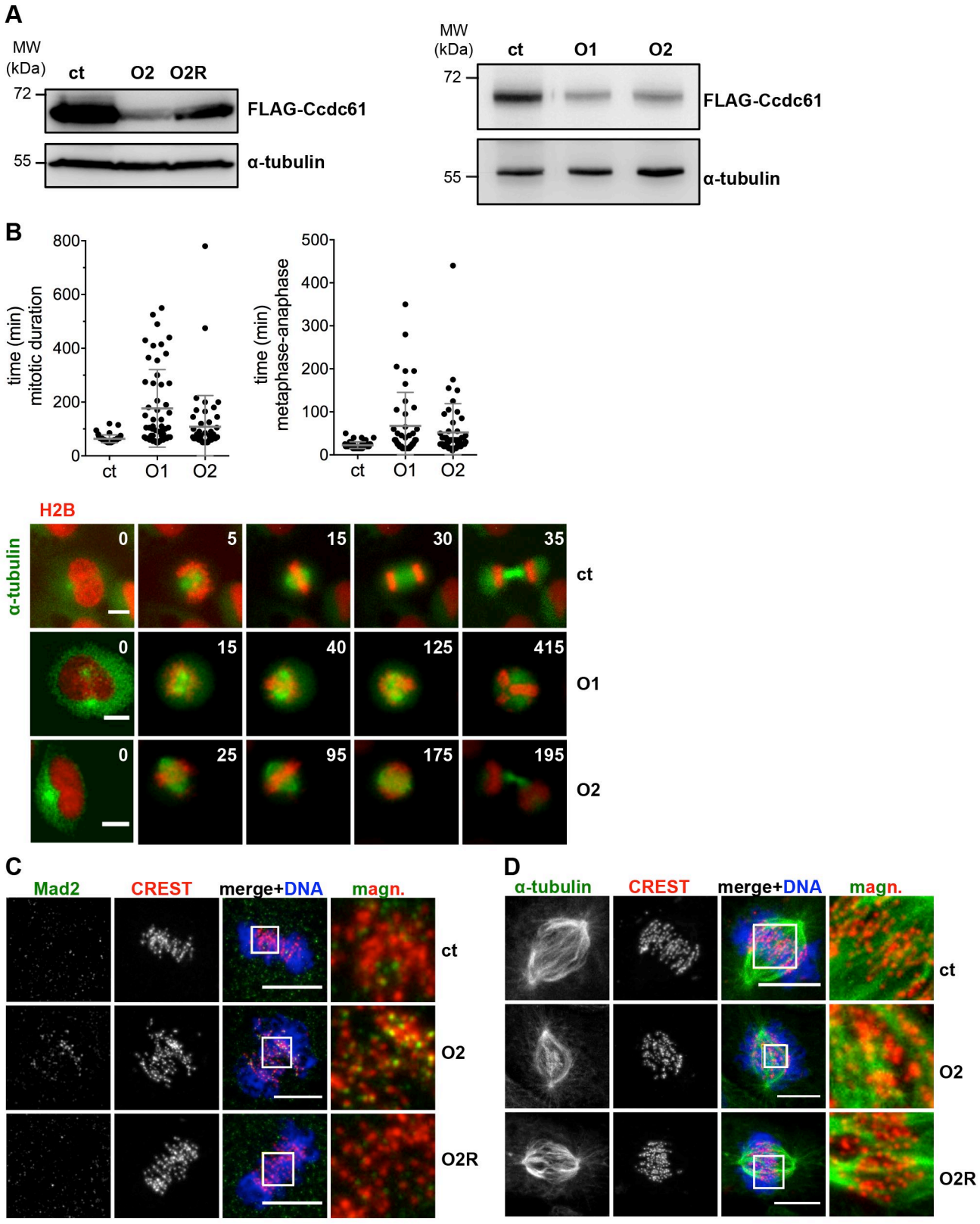
Figure S3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α-tubulin
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600
400
200
0
ct
O1
O2
time (min) metaphase-anaphase
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400
300
200
100
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ct
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O2
H2B
α-tubulin
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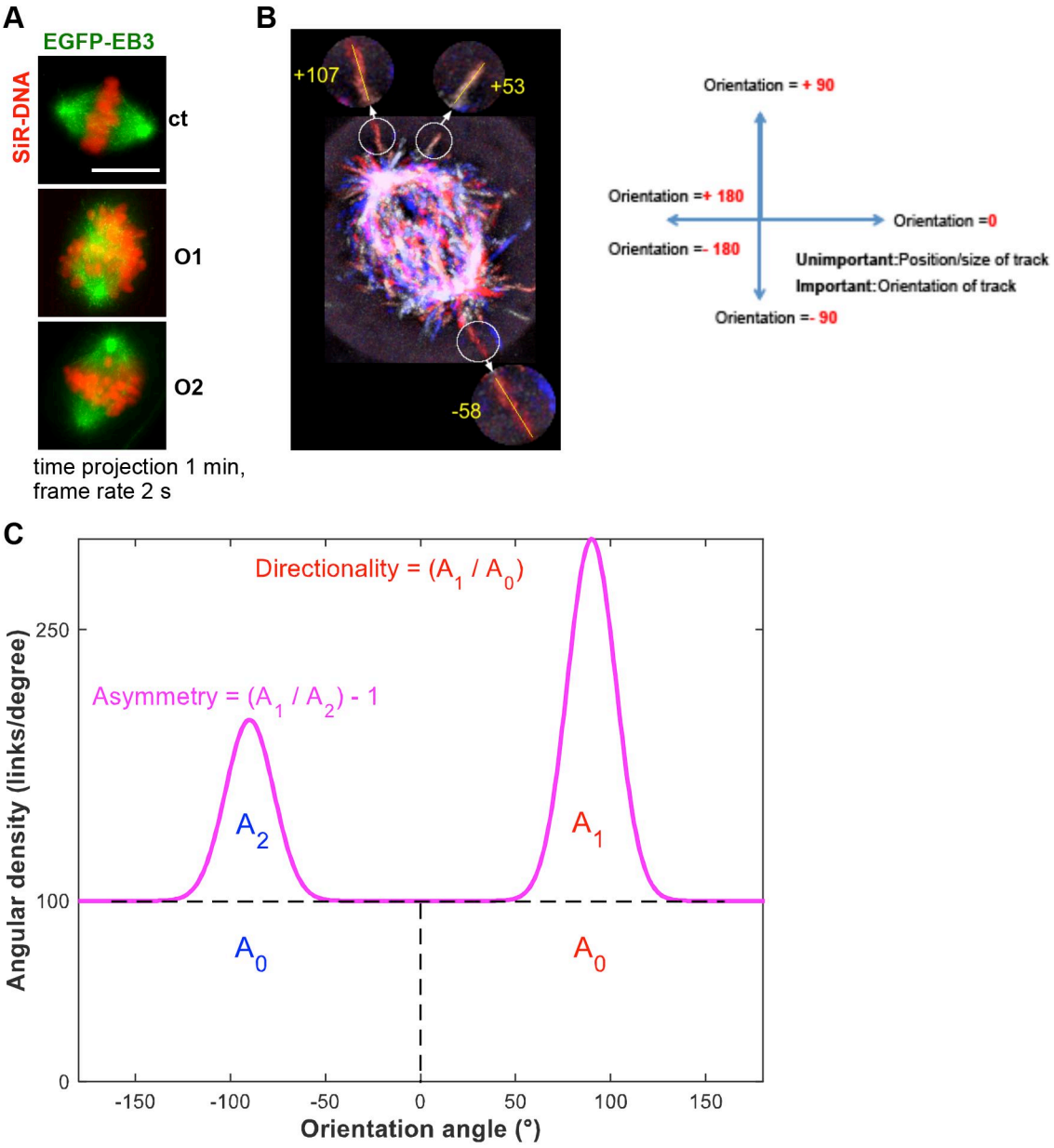
Figure S4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+53
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Orientation =0
Orientation =- 180
Orientation =- 90
Unimportant:Position/size of track
Important:Orientation of track
C
Directionality = (A1 / A0)
Asymmetry = (A1 / A2) - 1
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A1
A0
A0
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-100
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0
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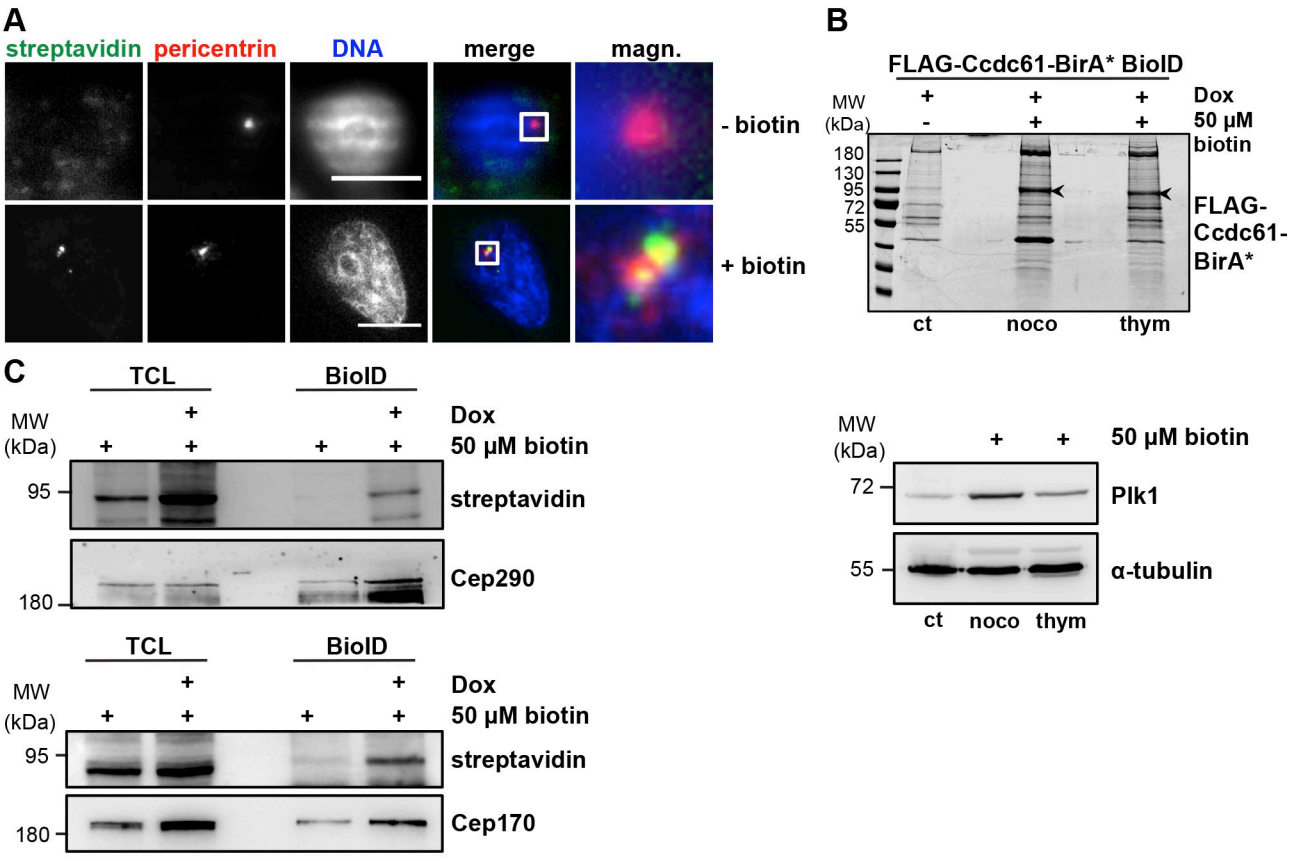
Orientation angle (°)

Figure S5



D Ccdc61 BioID nocodazole block

name	score	prot matches	sign prot matches	prot sequences	sign prot sequences	coverage [%]
CCDC61	1930	450	396	32	27	55.7
MYH9	10135	887	809	153	143	69
FLNA	3100	111	95	51	45	28.2
FAS	1600	51	47	28	26	13.7
TCPD	1393	41	35	23	22	59
RUVB2	965	27	27	15	15	38.4
PSMD2	915	26	22	15	15	20.6
TCPQ	901	25	25	12	12	22.3
TPM4	894	39	31	18	14	51.2
ACTN1	858	29	26	16	15	19.6
RAGP1	856	24	21	12	12	23.5
TCPZ	790	27	21	13	11	28.4
MYO1E	753	16	15	12	12	12.4
PP1A	694	22	21	13	12	42.4
CDK1	674	27	23	13	11	42.4
COR1C	669	22	21	11	10	28.3
KIF11	650	16	14	11	10	13
PSMD2	639	24	17	14	12	17.4
PSD12	607	11	10	9	8	23
PRS7	606	15	14	11	10	27.9
RUVB1	588	13	13	9	9	26.8
CEP290	**80**	**5**	**4**	**2**	**2**	**0.5**
TBK1	**67**	**1**	**1**	**1**	**1**	**1.6**

Ccdc61 BioID thymidine block

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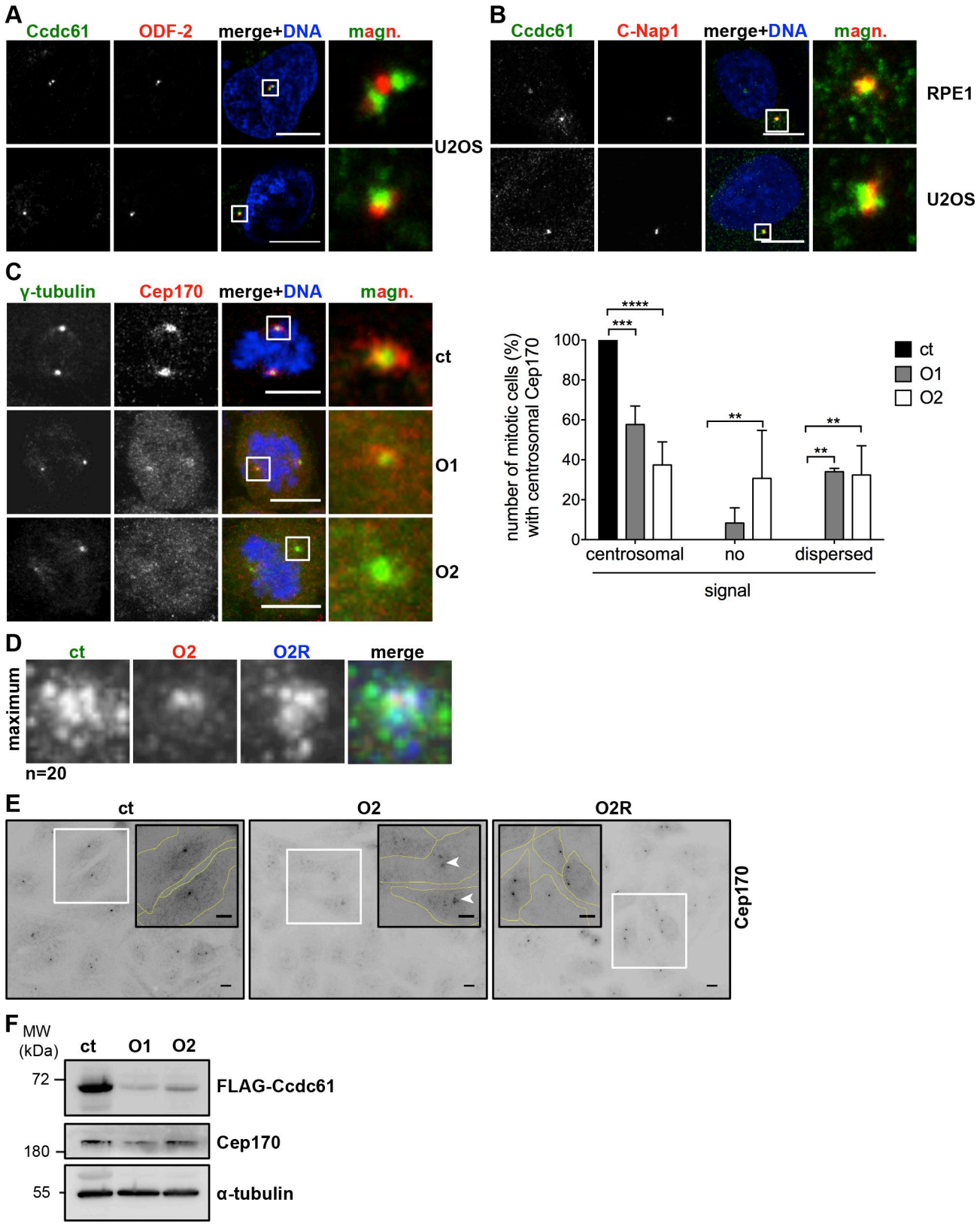
FAS	5219	376	345	79	74	39.7
TCPD	1898	71	67	28	27	68.5
RBP2	1889	54	50	33	30	13.6
KIF11	1851	72	60	30	27	32.1
TCPQ	1661	65	62	23	21	45.4
RUVB2	1616	65	60	22	21	60
CKAP5	1586	51	48	26	25	14.6
KINH	1553	50	47	25	23	31.6
TCPE	1506	73	69	26	26	53.4
RAGP1	1407	52	47	19	16	35.9
PSMD1	1305	44	37	21	19	33.3
PSMD2	1247	72	51	24	22	29.7
NEK9	1240	57	52	20	19	20.4
TCPZ	1237	79	72	20	19	39.9
PSD11	1210	50	49	19	19	51.7
COPB2	1192	37	36	19	19	26.5
LUZP1	1137	28	26	20	19	22
CSK21	1097	72	58	19	17	55.5
RUVB1	1090	35	35	15	15	44.3
TBK1	**493**	**10**	**10**	**7**	**7**	**11.9**
CEP170	**363**	**10**	**9**	**6**	**5**	**4.2**
PCM1	**96**	**7**	**1**	**2**	**1**	**0.9**
CEP290	**75**	**10**	**7**	**2**	**2**	**0.5**

Figure S6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U2OS
C
γ-tubulin
Cep170
merge+DNA
magn.
ct
O1
O2
number of mitotic cells (%) with centrosomal Cep170
****
***
**
**
**
100
80
60
40
20
0
centrosomal
no
dispersed
signal
ct
O1
O2
D
ct
O2
O2R
merge
maximum
n=20
E
ct
O2
O2R
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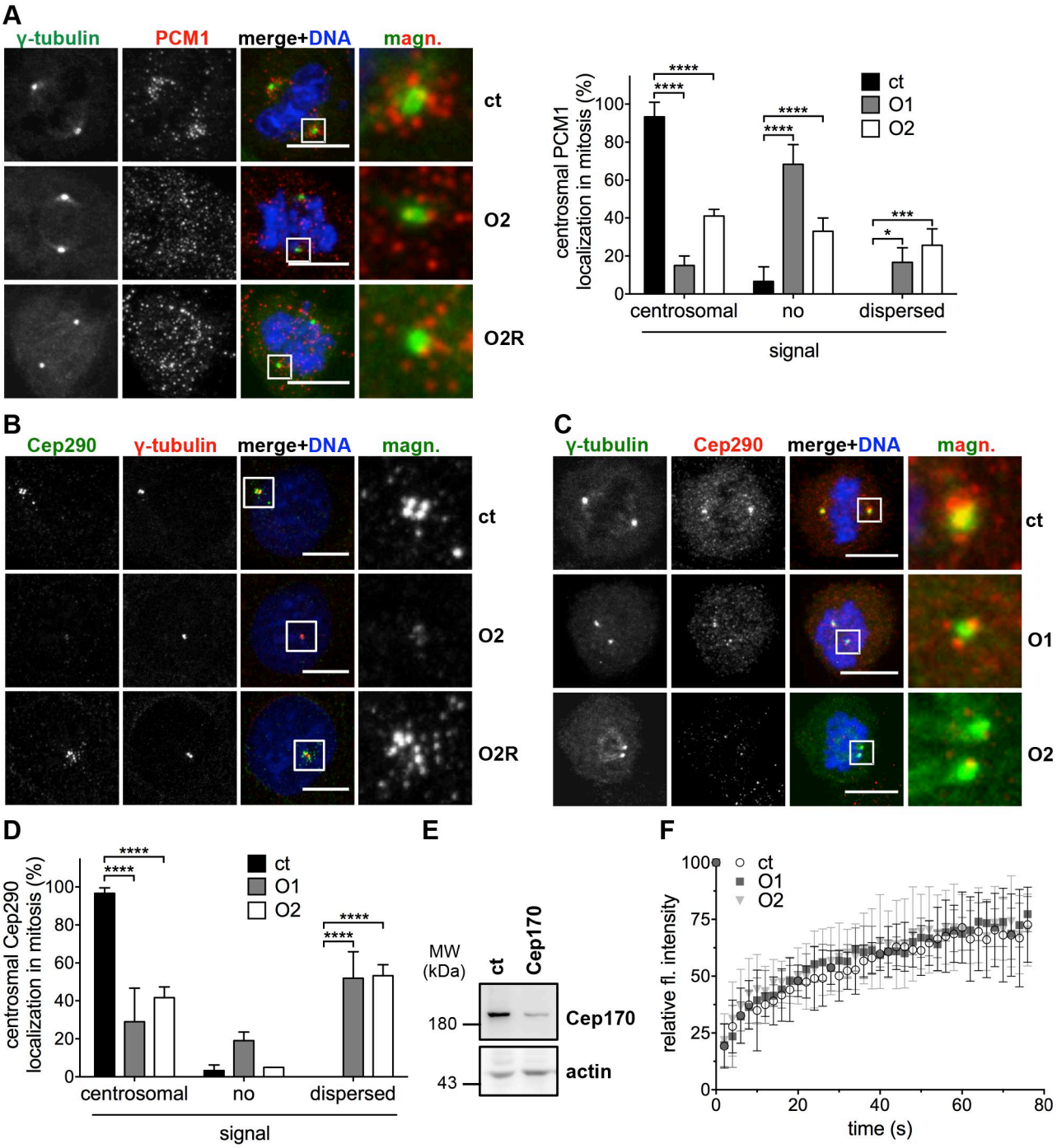
α-tubulin

Figure S7


A
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no
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signal
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γ-tubulin
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ct
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C
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magn.
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O2
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centrosmal Cep290 localization in mitosis (%)
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signal
E
MW (kDa)
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Cep170
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actin
F
relative fl. intensity
ct
O1
O2
time (s)