abcam

ab27074

Anti-gamma Tubulin antibody [TU-30] - Centrosome Marker

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.4 Preservative: 0.097% Sodium azide
Form	Liquid
Clonality	Monoclonal
Immunogen	Synthetic Peptide within Human TUBG1 aa 400-450 conjugated to Keyhole Limpet Haemocyanin. The exact immunogen used to generate this antibody is proprietary information. Database link P23258
Clone number	TU-30
Purification technique	Affinity purification Protein A
Specificity	This antibody recognises gamma tubulin expressed on stomach, kidney, liver, lung, muscle, ovary, heart, colon, thymus, brain.
Epitope	This antibody reacts with an epitope located in the C terminal region of gamma tubulin.
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	This antibody was purified from hybridoma culture supernatant by affinity chromatography. Purity is > 95% (by SDS-PAGE).

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	1-2 µg/mL
Notes	Note: Fix cells for 10 min in methanol at -20°C and for 6 min in acetone at -20°C. Alternatively, fix cells directly in methanol for 10 min at -20°C or in acetone for 10 min at -20°C. After fixing cells, incubate for 45 minutes at room temperature. This antibody will only stain fixed cells.

Species	Mouse
Dilution info	1-2 µg/mL
Notes	Note: Fix cells for 10 min in methanol at -20°C and for 6 min in acetone at -20°C. Alternatively, fix cells directly in methanol for 10 min at -20°C or in acetone for 10 min at -20°C. After fixing cells, incubate for 45 minutes at room temperature. This antibody will only stain fixed cells.

Species	Rat
Dilution info	1-2 µg/mL
Notes	Note: Fix cells for 10 min in methanol at -20°C and for 6 min in acetone at -20°C. Alternatively, fix cells directly in methanol for 10 min at -20°C or in acetone for 10 min at -20°C. After fixing cells, incubate for 45 minutes at room temperature. This antibody will only stain fixed cells.

Predicted

Species	Chicken, Cow, Pig, Plants
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1-2 µg/mL
Notes	reducing conditions.

Expected

Species	Mouse
Dilution info	1-2 µg/mL
Notes	reducing conditions.

Species	Rat
Dilution info	1-2 µg/mL
Notes	reducing conditions.

Predicted

Species	Chicken, Cow, Pig, Plants
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1 µg for 10^6 Cells
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Chicken, Cow, Pig, Plants
Dilution info	-
Notes	-

Target data

See full target information TUBG2

Function	Tubulin is the major constituent of microtubules, protein filaments consisting of alpha- and beta-tubulin heterodimers (PubMed:38305685). Gamma-tubulin is a key component of the gamma-tubulin ring complex (gTuRC) which mediates microtubule nucleation (PubMed:38305685). The gTuRC regulates the minus-end nucleation of alpha-beta tubulin heterodimers that grow into microtubule protafilaments, a critical step in centrosome duplication and spindle formation (PubMed:38305685).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Gamma-tubulin an important component in the microtubule nucleation process is also known as Gtub or γ-tub. It weighs approximately 48 kDa. Gamma-tubulin is a significant protein inside the centrosome where it plays a role in the formation of microtubule organizing centers. Scientists often find and express it in abundance within rapidly dividing cells both in humans and other eukaryotes. Researchers employ gamma-tubulin staining techniques to visualize its location and understand its function within the centrosome.
Biological function summary	Gamma-tubulin is part of the gamma-tubulin ring complex also known as gamma-TuRC which anchors the microtubule minus ends influencing microtubule stability and organization. This complex functions as a nucleating template for microtubules which are critical for various cell processes including intracellular transport and chromosome separation during cell division. Gamma-tubulin plays an indispensable role in maintaining the correct function of the centrosome an important cellular organelle in cell cycle progression.
Pathways	Gamma-tubulin participates in processes involving cell cycle regulation and mitotic spindle formation. These processes depend heavily on microtubule dynamics and

	organization. Gamma-tubulin interacts closely with proteins like centrins and pericentrin within these pathways. Such interactions ensure accurate chromosomal segregation during mitosis highlighting its importance in cellular reproduction and stability.
Associated diseases and disorders	Abnormal gamma-tubulin expression and function relate to conditions like cancer and neurological disorders. Changes in gamma-tubulin levels can affect the centrosome's function and microtubule dynamics leading to cell cycle dysregulation and tumorigenesis. Moreover it interacts with mutated tumor suppressor proteins like p53 further contributing to cancer progression. Scientists continue to study gamma-tubulin's role in these diseases providing insights into new therapeutic targets.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

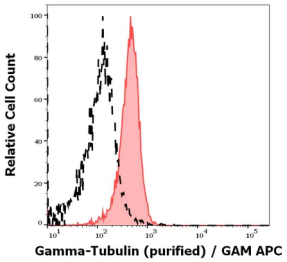
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

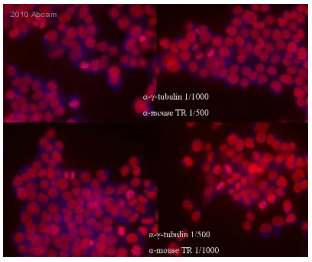
15 product images



Flow Cytometry (Intracellular) - Anti-gamma Tubulin antibody [TU-30] - Centrosome Marker (ab27074)

Gamma Tubulin Flow Cytometry (Intracellular) staining using mouse Anti-gamma Tubulin antibody

Separation of MCF-7 cells stained using ab27074 (concentration in sample 9 µg/ml, GAM APC, red-filled) from MCF-7 cells unstained by primary antibody (GAM APC, black-dashed) in flow cytometry analysis (intracellular staining).

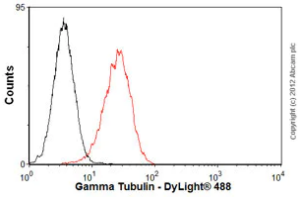


This image is courtesy of an anonymous customer review

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Gamma Tubulin Immunocytochemistry/ Immunofluorescence staining using mouse Anti-gamma Tubulin antibody

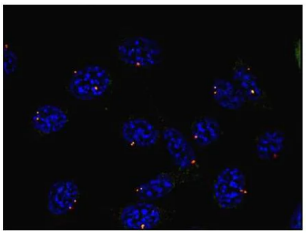
ab27074 staining gamma tubulin in the Human HEK293T cells by ICC. Cells were fixed with paraformaldehyde, permeabilized with methanol (-20°C 20 min) and blocked with 1% BSA for 1 hour at room temperature. Samples were incubated with primary antibody (1/500 or 1/1000 in blocking solution) for 45 minutes. A Texas Red®-conjugated Goat anti-mouse polyclonal (1/500) was used as the secondary antibody.



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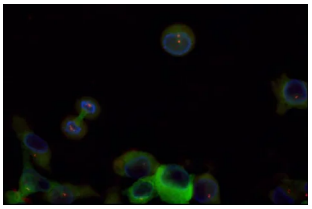
Overlay histogram showing HeLa cells stained with ab27074 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab27074, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



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Gamma Tubulin Immunocytochemistry/ Immunofluorescence staining using mouse Anti-gamma Tubulin antibody

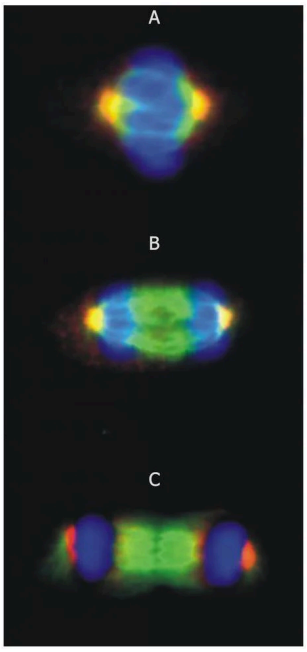
Immunocytochemisrty analysis of murine fibroblasts labeling gamma Tubulin with ab27074 at 1 ug/ml (red). Nuclei were stained with DAPI (blue).



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Gamma Tubulin Immunocytochemistry/ Immunofluorescence staining using mouse Anti-gamma Tubulin antibody

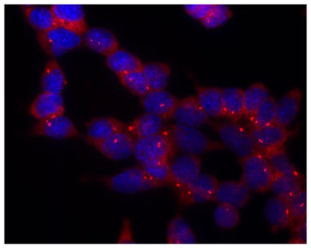
ab27074 staining gamma tubulin (red) in rat prostate carcinoma cells by ICC. The cells were also transfected with beta-tubulin GFP which stained green.



Immunocytochemistry/ Immunofluorescence - Anti-gamma Tubulin antibody [TU-30] - Centrosome Marker (ab27074)

Gamma Tubulin Immunocytochemistry/ Immunofluorescence staining using mouse Anti-gamma Tubulin antibody

Immunocytochemisrty analysis of 3T3 mouse fibroblasts in metaphase (A), anaphase (B) and telophase (C), labeling gamma Tubulin with ab27074 at 1 ug/ml (red). Cells were costained for alpha-tubulin (green). Nuclei were stained with DAPI (blue).



Immunocytochemistry/ Immunofluorescence - Anti-gamma Tubulin antibody [TU-30] - Centrosome Marker (ab27074)

Gamma Tubulin Immunocytochemistry/ Immunofluorescence staining using mouse Anti-gamma Tubulin antibody

Immunocytochemistry staining of P19X1 mouse embryonal carcinoma cell line using anti-gamma-tubulin (TU-30) (detection by secondary antibody Goat anti-mouse Cy3). Nuclei were stained with DAPI (blue).

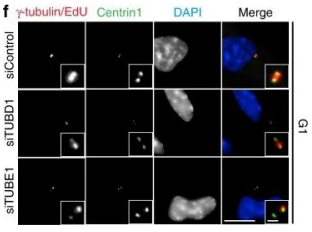


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Immunocytochemistry-immunofluorescence using Anti-gamma Tubulin antibody [TU-30], ab27074. Publication image from Atorino, E. S. et al., 2020, Nat Commun, 32060285. Legend direct from paper.

Structural integrity of the centrioles ensures their conversion to centrosomes.a–c EM images of turned and tilted sections of proximal region of daughter centrosomes in G1. The two values on the top of the image give the turning and tilting degree of the sample, respectively. Red arrowheads indicate open structural defects (A–C linker absent), while yellow ones highlight defects of triplets. The side table (right) shows quantification of the number of cells showing these defects. While in a G1 siControl cells had no structural defects, in b siTUBD1 and c siTUBE1 G1 dCs showed defects in triplet formation (yellow arrow). b siTUBD1 G1 cell with defect in the A–C linker formation on top of the triplet formation of dCs and lack of triplet MTs (red arrow). d Depletion of TUBD1 and TUBE1 proteins also generated reduced glutamylation of the centrioles as judged by GT335 staining (36.7 ± 2.2% of G1 cells, Cenp-F in Supplementary Fig. 12a) with <2 GT335 foci in siTUBE1 sample; 23.5 ± 2.0% for siTUBD1). e Quantification of d. f In absence TUBE1 or TUBD1 many centrioles in G1 cells (Cenp-F in Supplementary Fig. 12b) did not efficiently acquire PCM (<2 γ-tubulin defined foci) to convert to centrosomes. g Quantification of f. 33.1 ± 1.3% of cells with

<2 γ-tubulin foci in G1 for siTUBE1; 26.2 ± 4.1% for siTUBD1. (a–c, scale bars: 100 nm; d, f scale bars: 10 µm, magnification scale bars: 1 µm; e, g data are presented as mean ± s.d., all statistics were derived from two-tail unpaired t-test analysis of n = 6 biologically independent experiments and source data are provided as a Source Data file). h Model of the dependency of CCC mechanism on the correct centriolar wall maturation. See Discussion for description.

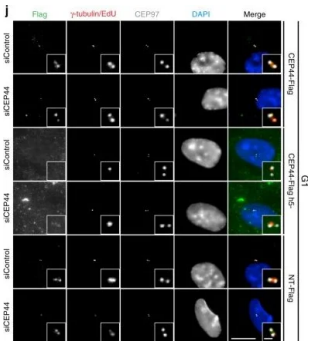


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Gamma Tubulin Immunocytochemistry/ Immunofluorescence staining using mouse Anti-gamma Tubulin antibody

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CEP44 localises to the centriole lumen via its MT-binding affinity.a–c 2D-SIM images of G1 centrioles (α-tubulin) and corresponding normalised intensity profiles of centrosomes positioned perpendicularly to the imaging plane (white arrows). a CEP44 localised in the centriole lumen as POC1B (b). c CEP295 decorates the outer centriolar wall. d 2D-SIM of centrosomes with duplicated centriole pairs co-stained withα-tubulin, CEP44 and POC1B. e Intensity profiles of dC cross-section. CEP44 (left) and POC1B (right) reside in the dC lumen. f (left) 2D-SIM of centrosomes with duplicated centriole pairs co-stained withα-tubulin and CEP295. f (right) Intensity profiles of dC cross-section. g CEP44 immuno-gold labelling in purified centrosomes (left). Red arrows indicate 10 nm gold particles. g (right) Distance of the gold particles from A-, B- and C-tubule of the same triplet respectively 21.9 ± 3.1 nm, 32.0 ± 4.7 nm, 43.1 ± 7.5 nm (all cases, n = 23 particles, data present mean ± s.d.). h Binding assay of recombinant CEP44-Flag and h5- mutant purified from E.coli to MTs. GST-Flag was used as control. Proteins were incubated with soluble polymerised tubulin. MTs with bound proteins were sedimented by centrifugation. The supernatant (S) and pellet (P) were analysed by IB forα-tubulin and Flag. Supplementary Fig. 8f shows Coomassie blue stain gel of purified proteins. i Schematic representation of CEP44 domain organisation. (Bottom) Comparison of CEP44 domain predicted secondary structure organisation with the MT-binding domain of EB1 and EB3 proteins. j The h5- and the NT-fragment could not rescue the CCC defect vs. CEP44-Flag. Constructs were mildly expressed by the addition of 2 ng/ml doxycycline. k Quantification of j and Supplementary Fig. 9c. While the CT-Flag was unable to rescue the loss of PCM (63.9 ± 3.2% of cells with <2 γ-tubulin foci) and the NT only partially (32.6 ± 3.8%), the h5-mutant generated a CCC defect even in the siControl (27.6 ± 2.3%) and a stronger CCC phenotype in the siCEP44 (76.1 ± 2.6%). Data presented as mean ± s.d., all statistics derived from two-tail unpaired t-test analysis of n = 6 biologically independent experiments. (a, b, c, d, f, scale bars: 1 µm; g, scale bar: 100 nm; j, scale bar 10 µm, magnification scale bar: 1 µm). (a–c, e–g, k) Source data are provided as a Source Data file.

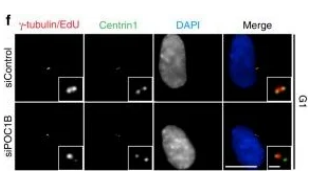


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The CEP44-POC1B complex is needed to convert centrioles to centrosomes.a Anti-Flag IP using CEP44-Flag from RPE1 cells was analysed for POC1B, POC1A and CEP295 by immuno-blotting (IB). GAPDH was used as input control. b Coomassie Blue stained gel of in vitro binding between

purified, recombinant CEP44-Flag and purified, recombinant POC1B-HA. See Supplementary Fig. 5b for IB and 5c for Coomassie Blue stained gels of purified proteins used in the experiment. c Schematic representation of CEP44 protein sequence identity in vertebrata (referred to Supplementary Fig. 5a). d CEP44-Flag constructs that were designed based on c and used in e. e IB of input and eluted samples from RPE1 IPs using CEP44-Flag constructs as outlined in d. The CEP44-Flag IPs were tested for the presence of POC1B. GAPDH was used as loading control for the input. f, g 39.2 ± 2.8% of G1 cells in which POC1B was depleted show <2 γ-tubulin defined foci (Cenp-F in Supplementary Fig. 5g). h–j Loss of either CEP44 or POC1B in response to siRNAs depletion by one of them. h, i CEP44 loss upon CEP44 siRNA has a similar impact on POC1B loss from dCs. i Quantification of h. h, j CEP44 delocalisation was less severe than POC1B loss upon POC1B siRNA. j Quantification of h. Upon siPOC1B depletion, CEP44 delocalised (18.9 ± 4.3% of G1 cells) less sever than POC1B (45.5 ± 2.3%). k Schematic representation of the functional interdependency between the conversion molecules in the CCC mechanism. (f, h, scale bars: 10 µm, magnification scale bars: 1 µm; g, i, j data are presented as mean ± s.d., all statistics were derived from two-tail unpaired t-test analysis of n = 6 biologically independent experiments and source data are provided as a Source Data file).

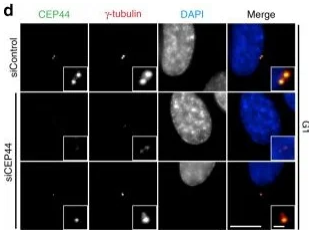


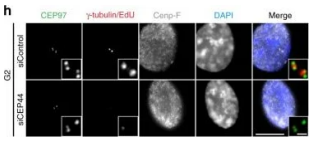
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CEP44 is a centriolar protein necessary for the CCC mechanism.a IF of cycling RPE1 cells showing that CEP44 binds to the new dCs during G2. While S phase cells were detected by EdU stain, G1 and G2 cells were discerned by the lack of EdU stain and the number of centrin1 signals (centrioles). b IF of cells after 72 h of depletion. In the siCEP44 sample (lower panel) G1 cells contained less centrosomes as judged by the number of γ-tubulin foci (Cenp-F in Supplementary Fig. 1b). c Quantification of b. 80.4 ± 5.0% of G1 cells contained <2 centrosomes. d IF of 60 h siRNA treated RPE1 cells in G1. While the G1 control cells contained 2 defined PCM foci (γ-tubulin) accompanied by equal number of CEP44 foci, in the siCEP44 sample the loss of CEP44 correlated with inefficient PCM (γ-tubulin) recruitment to only one (bottom panel) or both centrosomes (middle panel) (Cenp-F, Supplementary Fig. 1g). e Quantification of CEP44 loss in d. 65.1 ± 7.3% of G1 cells contained <2 CEP44 foci. f Quantification of γ-tubulin defined foci in d. 60.7 ± 4.2% of G1 cells contained <2 defined γ-tubulin signals. g, h G2 CEP44-depleted cells after 60 h of CEP44 depletion showed a mild centriole duplication defect as judged by the counting of CEP97 foci (<4). g Quantification of h. 30.3 ± 4.1% of CEP44-depleted cells contained <4 centrioles. i MT regrowth assay in RPE1 C-Nap1 KO cells. The non-converted daughter centrosome without CEP164 staining regrew lower numbers of MTs (5.1 ± 2.7 MTs/centrosome) than the siControl daughter centrosomes (10.3 ± 3.0 MTs/centrosome) upon cold treatment and MT regrowth. j Quantification of i. k Loss of CEP44 leads to misalignment of mitotic spindles (>65%) generating either bipolar asymmetric spindles (51.3 ± 4.7%) or mono-/multipolar ones (14.3 ± 4.0). l Quantification of k. (a, b, d, h, i, k, scale bars: 10 µm, magnification scale bars: 1 µm; c, e, f, g, j and l data are presented as mean ± s.d., all statistics were derived from two-tail unpaired t-test analysis of n = 6 biologically independent experiments and source data are provided as a Source Data file).



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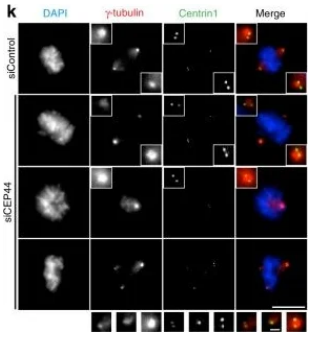


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CEP44 is a centriolar protein necessary for the CCC mechanism.a IF of cycling RPE1 cells showing that CEP44 binds to the new dCs during G2. While S phase cells were detected by EdU stain, G1 and G2 cells were discerned by the lack of EdU stain and the number of centrin1 signals (centrioles). b IF of cells after 72 h of depletion. In the siCEP44 sample (lower panel) G1 cells contained less centrosomes as judged by the number of γ-tubulin foci (Cenp-F in Supplementary Fig. 1b). c Quantification of b. 80.4 ± 5.0% of G1 cells contained <2 centrosomes. d IF of 60 h siRNA treated RPE1 cells in G1. While the G1 control cells contained 2 defined PCM foci (γ-tubulin) accompanied by equal number of CEP44 foci, in the siCEP44 sample the loss of CEP44 correlated with inefficient PCM (γ-tubulin) recruitment to only one (bottom panel) or both centrosomes (middle panel) (Cenp-F, Supplementary Fig. 1g). e Quantification of CEP44 loss in d. 65.1 ± 7.3% of G1 cells contained <2 CEP44 foci. f Quantification of γ-tubulin defined foci in d. 60.7 ± 4.2% of G1 cells contained <2 defined γ-tubulin signals. g, h G2 CEP44-depleted cells after 60 h of CEP44 depletion showed a mild centriole duplication defect as judged by the counting of CEP97 foci (<4). g Quantification of h. 30.3 ± 4.1% of CEP44-depleted cells contained <4 centrioles. i MT regrowth assay in RPE1 C-Nap1 KO cells. The non-converted daughter centrosome without CEP164 staining regrew lower numbers of MTs (5.1 ± 2.7 MTs/centrosome) than the siControl daughter centrosomes (10.3 ± 3.0 MTs/centrosome) upon cold treatment and MT regrowth. j Quantification of i. k Loss of CEP44 leads to misalignment of mitotic spindles (>65%) generating either bipolar asymmetric spindles (51.3 ± 4.7%) or mono-/multipolar ones (14.3 ± 4.0). l Quantification of k. (a, b, d, h, i, k, scale bars: 10 µm, magnification scale bars: 1 µm; c, e, f, g, j and l data are presented as

mean ± s.d., all statistics were derived from two-tail unpaired t-test analysis of n = 6 biologically independent experiments and source data are provided as a Source Data file).

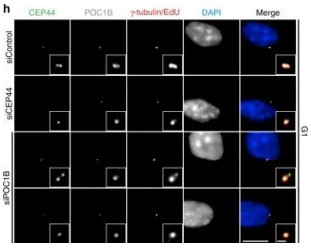


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Immunocytochemistry/ Immunofluorescence - Anti-gamma Tubulin antibody [TU-30] - Centrosome Marker (ab27074)

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The CEP44-POC1B complex is needed to convert centrioles to centrosomes.a Anti-Flag IP using CEP44-Flag from RPE1 cells was analysed for POC1B, POC1A and CEP295 by immuno-blotting (IB). GAPDH was used as input control. b Coomassie Blue stained gel of in vitro binding between purified, recombinant CEP44-Flag and purified, recombinant POC1B-HA. See Supplementary Fig. 5b for IB and 5c for Coomassie Blue stained gels of purified proteins used in the experiment. c Schematic representation of CEP44 protein sequence identity in vertebrata (referred to Supplementary Fig. 5a). d CEP44-Flag constructs that were designed based on c and used in e. e IB of input and eluted samples from RPE1 IPs using CEP44-Flag constructs as outlined in d. The CEP44-Flag IPs were tested for the presence of POC1B. GAPDH was used as loading control for the input. f, g 39.2 ± 2.8% of G1 cells in which POC1B was depleted show <2 γ-tubulin defined foci (Cenp-F in Supplementary Fig. 5g). h–j Loss of either CEP44 or POC1B in response to siRNAs depletion by one of them. h, i CEP44 loss upon CEP44 siRNA has a similar impact on POC1B loss from dCs. i Quantification of h. h, j CEP44 delocalisation was less severe than POC1B loss upon POC1B siRNA. j Quantification of h. Upon siPOC1B depletion, CEP44 delocalised (18.9 ± 4.3% of G1 cells) less sever than POC1B (45.5 ± 2.3%). k Schematic representation of the functional interdependency between the conversion molecules in the CCC mechanism. (f, h, scale bars: 10 µm, magnification scale bars: 1 µm; g, i, j data are presented as mean ± s.d., all statistics were derived from two-tail unpaired t-test analysis of n = 6 biologically independent experiments and source data are provided as a Source Data file).

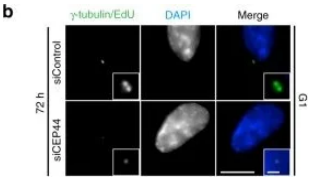


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