

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of all covariates tested
- ☒ ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Ex-dSTORM and conventional widefield images were collected with a custom-built setup based on a commercial inverted microscope (Eclipse Ti2-E, Nikon) detailed in the manuscript (Methods section). The microscopy system, mainly consisting of a microscope body, laser module, and camera, is integrated by MetaMorph (version 7.10.4.407, Molecular Devices). Superresolution dSTORM imaging was rendered with the Superresolution Module (Wavetracer, version 1.50, Molecular Devices).
Data analysis	Ex-dSTORM data were processed using a homemade code based on Labview (version 14.0.1), Matlab (version R2021b), and ImageJ (version 1.52) for in-situ drift correction. The position of the individual single-molecule peak was then localized using MetaMorph Superresolution Module (version 7.10.4.407, Molecular Devices) based on a wavelet segmentation algorithm (version 1.50, Molecular Devices). The superresolution images were cleaned with the gaussian filter using ImageJ (version 1.52 p). The quantitative analyses were performed by ImageJ and Prism (version 9.4.1, GraphPad).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All data generated in this study, including source data, are provided in the article and supplementary information files.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender n/a

Reporting on race, ethnicity, or other socially relevant groupings n/a

Population characteristics n/a

Recruitment n/a

Ethics oversight n/a

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size Sample sizes were determined based on experience from previous, similar analyses and based on methods used in related published studies (e.g., Yang, T. Tony, et al. Nature communications 9.1 (2018): 1-11)

Data exclusions Data from failed experiments, such as failed in-situ drift correction or centrioles with wrong orientation, were excluded. The criteria of exclusion were predetermined and applied equally..

Replication All presented data were replicated in multiple independent experiments and all attempts at replication were successful. The number of replications were provided in figure legends.

Randomization For quantifying the relative centriole size, only centrioles with the lateral-view orientation were examined. Otherwise, cells or samples were randomly selected for data collection in all experiments.

Blinding Investigators could not be blinded to group allocations as groups could be identified by differences in phenotypic or immunofluorescence staining patterns in most experiments. For loss-of-function studies, only efficient knockdown cells were selected for phenotypic analysis as the RNAi effect on cells was variable within the population (not 100%).

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

Methods

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Primary antibodies:

-rabbit polyclonal anti-Acetyl- α -tubulin (1/50 for UExM, 5335S, Cell Signaling)
 -mouse monoclonal anti-Acetylated Tubulin (1/500 for UExM, T7451-200UL, Sigma-Aldrich)
 -mouse monoclonal anti-Acetylated α -tubulin Alexa Fluor 488 (1/50 for UExM, sc-23950 AF488, Santa Cruz)
 -rabbit polyclonal anti-ALMS1 (1/2000 for IF, WB and PLA, 1/1000 for UExM, A301-815A, Bethyl Laboratories)
 -rabbit polyclonal anti-ALMS1 (1/30 for IP, 27231-1-AP, Proteintech)
 -rabbit polyclonal anti-ALMS1 (1/1000 for UExM, A301-816A, Bethyl Laboratories)
 -goat polyclonal anti-ALMS1(1/100 for UExM, TA302859, OriGene)
 -rabbit monoclonal anti-CDT1 (1/200 for IF, 8064T, Cell Signaling)
 -mouse monoclonal anti-centrin (1/1000 for IF, 04-1624, Millipore-Sigma)
 -rabbit polyclonal anti-CEP63 (1/1000 for IF and WB, 1/200-500 for UExM, 1/125 for IP, 16268-1-AP, Proteintech)
 -mouse monoclonal anti-CEP63 (1/50-100 for UExM, 66996-1-Ig, Proteintech)
 -mouse monoclonal anti-CEP63 (1/200 for IF, 1/50 for UExM, TA809289, OriGene)
 -mouse monoclonal anti-CEP63 (1/200 for IF and PLA, 1/50 for UExM, TA809290, OriGene)
 -rabbit polyclonal anti-CEP83 (1/100 for UExM, HPA038161, Sigma-Aldrich)
 -rabbit polyclonal anti-CEP128 (1/100 for UExM, HPA001116, Sigma-Aldrich)
 -rabbit polyclonal anti-CEP135 (1/50 for UExM, ab75005, Abcam)
 -rabbit polyclonal anti-CEP152 (1/200 for IF, 1/100-200 for UExM, HPA039408, Sigma-Aldrich)
 -rabbit polyclonal anti-CEP152 (1/400 for UExM, A302-480A, Bethyl Laboratories)
 -mouse monoclonal anti-CEP152 (1/1000 for IF, WB and PLA, 1/50-1/200 for UExM, GTX631486, GeneTex)
 -rabbit polyclonal anti-CEP192 (1/50 for UExM, A302-324A, Bethyl Laboratories)
 -rabbit polyclonal anti-C-Nap1 (1/50 for UExM, Tsou and Stearns, 2006, Nature 442(7105):947-51)
 -rabbit polyclonal anti-CNTROB (1/100 for UExM, 26880-1-AP, Proteintech)
 -rabbit polyclonal anti-CP110 (1/200 for IF, 1/100 for UExM, 12780-1-AP, Proteintech)
 -mouse monoclonal anti-CyclinB1 (1/200 for IF, 4135T, Cell Signaling)
 -rat monoclonal anti-BrdU (1/500 for IF, MCA2060, Bio-Rad)
 -mouse monoclonal anti- γ -tubulin (1/500 for IF, 1/50 for UExM, sc-51715, Santa Cruz)
 -mouse monoclonal anti-HA (1/500 for IF, 1/50 for UExM, 901501, BioLegend)
 -rabbit polyclonal anti-ODF2 (1/100 for UExM, HPA001874, Sigma-Aldrich)
 -rabbit polyclonal anti-PLK4 (1/10 for UExM, gift from Dr. Andrew Holland)
 -mouse monoclonal anti-PLK4 (1/200 for IF, 1/50 for UExM, TA810562, OriGene)
 -mouse monoclonal anti-PLK4 (1/200 for IF, 1/50 for UExM, TA810507, OriGene)
 -rabbit polyclonal anti-Pericentrin (1/1000 for IF, 1/1000 for WB, ab4448, Abcam)
 -mouse monoclonal anti-Pericentrin (1/200 for IF, 1/50 for UExM, ab28144, Abcam)
 -mouse monoclonal anti-SAS-6 (1/200 for IF, 1/50 for UExM, sc-81431, Santa Cruz)
 -mouse monoclonal anti-SAS-6 (1/200 for IF, 1/50 for UExM, sc-376836, Santa Cruz)
 -mouse monoclonal anti-SAS-6 Alexa Fluor 594 (1/50 for UExM, sc-81431 AF594, Santa Cruz)
 -mouse monoclonal anti-TUBA4A (TUBA1) (1/60000 for WB, T6199, Sigma-Aldrich)

Secondary antibodies:

-goat anti-rabbit IgG(H+L) Alexa Fluor 405 (1/100 for UExM, A31556, Thermo Fisher)
 -goat anti-rabbit IgG(H+L) Alexa Fluor 488 (1/500 for IF, 1/100 for UExM, A11034, Thermo Fisher)
 -goat anti-rabbit IgG(H+L) Alexa Fluor 594 (1/500 for IF, 1/100 for UExM, A11037, Thermo Fisher)
 -goat anti-rabbit IgG(H+L) Alexa Fluor 680 (1/500 for IF, A21109, Thermo Fisher)
 -goat anti-rabbit IgG(H+L) ATTO647N (1/100 for UExM, 611-156-122, Rockland)
 -goat anti-rabbit IgG(H+L) CF647 (1/100 for UExM, SAB4600185-50UL, Sigma-Aldrich)
 -goat anti-rabbit IgG(H+L) HRP (1/2000 for WB, AP307P, Sigma-Aldrich)
 -goat anti-mouse IgG(H+L) Alexa Fluor Plus 488 (1/500 for IF, A32723, Thermo Fisher)
 -goat anti-mouse IgG(H+L) Alexa Fluor 594 (1/500 for IF, A11032, Thermo Fisher)
 -goat anti-mouse IgG1(γ 1) Alexa Fluor 488 (1/500 for IF, 1/100 for UExM, A21121, Thermo Fisher)
 -goat anti-mouse IgG1(γ 1) Alexa Fluor 594 (1/500 for IF, 1/100 for UExM, A21125, Thermo Fisher)
 -goat anti-mouse IgG1(γ 1) Alexa Fluor 647 (1/500 for IF, A21240, Thermo Fisher)
 -goat anti-mouse IgG1(γ 1) CF647 (1/100 for UExM, SAB4600353-50UL, Sigma-Aldrich)
 -goat anti-mouse IgG2a(γ 2a) Alexa Fluor 488 (1/500 for IF, A21131, Thermo Fisher)
 -goat anti-mouse IgG2b(γ 2b) Alexa Fluor 594 (1/500 for IF, 1/100 for UExM, A21145, Thermo Fisher)
 -goat anti-mouse IgG2b(γ 2b) Alexa Fluor 647 (1/500 for IF, A21242, Thermo Fisher)
 -goat anti-mouse IgG2b(γ 2b) CF647 (1/100 for UExM, SAB4600357-50UL, Sigma-Aldrich)
 -goat anti-mouse Immunoglobulins/HRP (1/2000 for WB, P0447, Agilent Dako)

-donkey anti-mouse IgG(H+L) Alexa Fluor 488 (1/100 for UExM, A21202, Thermo Fisher)
 -goat anti-rat IgG(H+L) Alexa Fluor 350 (1/500 for IF, A21093, Thermo Fisher)
 -goat anti-rat IgG(H+L) Alexa Fluor 594 (1/500 for IF, A11007, Thermo Fisher)
 -donkey anti-goat IgG(H+L) Alexa Fluor Plus 594 (1/100 for UExM, A32758, Thermo Fisher)

Validation

For all antibodies, staining patterns in fixed cells matched the expected/published characteristics, and band sizes in western blots were as expected.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

U2OS (ATCC)
 hTERT-RPE1 (ATCC)
 hTERT-RPE1 p53^{-/-} (Izquierdo et. al., 2014, Cell Rep., 8(4):957-65)
 hTERT-RPE1 p53^{-/-}; SAS6^{-/-} (Wang et al., 2015, Elife, 4:e10586)
 hTERT-RPE1 p53^{-/-}; PLK4^{-/-} (Kim et al., 2016, Cell Rep., 16(5):1195-1203)
 hTERT-RPE1 p53^{-/-}; STIL^{-/-} (Kim et al., 2016, Cell Rep., 16(5):1195-1203)
 hTERT-RPE1 p53^{-/-}; PLK4^{-/-}; tet-PLK4as (Kim et al., 2016, Cell Rep., 16(5):1195-1203)
 hTERT-RPE1 PLK4as knock-in; p53^{-/-} (Moyer et al., 2015, JCB, 209:863–878; Fong et al., 2016, Elife, 5:e16270)
 hTERT-RPE1 p53^{-/-}; CEP295^{-/-} (Izquierdo et. al., 2014, Cell Rep., 8(4):957-65)
 hTERT-RPE1 RPE1 p53^{-/-}; ALMS1delta-exon8 (this study)
 hTERT-RPE1 p53^{-/-}; NF2^{-/-} (this study)
 hTERT-RPE1 RPE1 p53^{-/-}; NF2^{-/-}; ALMS1delta-exon8 (this study)
 hTERT-RPE1 p53^{-/-}; NF2^{-/-}; STIL^{-/-} (this study)
 hTERT-RPE1 p53^{-/-}; NF2^{-/-}; CEP152^{-/-} (this study)
 hTERT-RPE1 p53^{-/-}; NF2^{-/-}; ishALMS-lentiviral insertion (this study)
 hTERT-RPE1 p53^{-/-}; NF2^{-/-}; ishALMS1-AAVS1 knock-in (this study)
 hTERT-RPE1 p53^{-/-}; NF2^{-/-}; AAVS1-ishALMS1; SAS6^{-/-} (this study)
 hTERT-RPE1 p53^{-/-}; NF2^{-/-}; HA:ALMS1 knock-in (this study)
 hTERT-RPE1 p53^{-/-}; NF2^{-/-}; HA:ALMS1delta-exon8 (this study)
 hTERT-RPE1 p53^{-/-}; NF2^{-/-}; ishALMS1_80-23-26-8-44 (tiny centrioles, this study)

Authentication

Cell lines were not further authenticated

Mycoplasma contamination

All cell lines tested negative for mycoplasma contamination

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell line was used

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a