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Corresponding author(s): Helder Maiato

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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	Western Blot images were detected in a Bio-Rad Chemidoc XRS system, with Image Lab software. Immunofluorescence images were acquired with an Axio imager Z1 (Carl Zeiss) equipped with an ORCA-R2 pre-cooled charge-coupled device (CCD; Hamamatsu), using an immersion oil 63× 1.46 NA planapochromatic objective lens, controlled by Zen software. For Coherent-Hybrid STED (CH-STED) imaging, an Abberior 'Expert Line' gated-STED microscope was used, equipped with a Nikon Lambda Plan-Apo 1.4 NA 60x objective lens. CH-STED was implemented as described in Material and Methods. All acquisition channels (confocal and CH-STED) were performed using a 0.8 Airy unit pinhole. A time-gate threshold of 500 ps was applied to the STED channel to avoid residual confocal-resolution signal contribution. Fixed-cell images were acquired using excitation wavelengths at 561 nm and 640 nm. Excited volumes were doughnut-depleted with a single laser at 775 nm Time-lapse imaging was performed in a heated chamber (37°C) using a 60× oil immersion 1.40 NA plan-apochromatic objective mounted on an inverted microscope (Eclipse TE2000U; Nikon) equipped with a CSU-X1 spinning-disk confocal head (Yokogawa Corporation of America) controlled by NIS-Elements software and with two laser lines (488 nm, 561 nm). Images were detected with an iXonEM+ EM-CCD camera (Andor Technology). Protein identification and quantification by Mass Spec analysis were performed by nanoLC-MS/MS. This equipment is composed of an Ultimate 3000 liquid chromatography system coupled to a Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany); Data acquisition was controlled by Xcalibur 4.0 and Tune 2.11 software (Thermo Scientific, Bremen, Germany). In vitro experiments were performed by TIRF microscopy as described in Material and Methods
Data analysis	Western Blot images were analyzed with Image Lab software and mageJ software 3D deconvolution was performed using AutoQuant X Image Deconvolution software (Media Cybernetics).

Immunofluoresce quantifications, histogram adjustments and panel construction were performed with ImageJ software, Adobe Photoshop CS6 and Adobe Illustrator CS5 (Adobe Systems)
Live cell Imaging quantifications, histogram adjustments and panel construction were performed with NIS Viewer (Nikon), ImageJ software, Adobe Photoshop CS6 and Adobe Illustrator CS5 (Adobe Systems)
Statistical analysis was performed with Graphpad Prism, version 9.
Mass Spec raw data was processed using the Proteome Discoverer 2.5.0.400 software (Thermo Scientific)
In vitro data was analysed with a custom-made program written in Mathematica program, as described in Material and Methods
Kinetochore tracking data was analyzed by an automated Kinetochore tracking code written in MATLAB 2013b (The Math Works, Natic, USA). The code is available under https://github.com/cmcb-warwick

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

Data are available via ProteomeXchange with identifier PXD047133.

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	U2OS cells used in this study were derived from a female osteosarcoma patient of unclear gender and sexual orientation
Reporting on race, ethnicity, or other socially relevant groupings	U2OS cells derive from a white Caucasian individual
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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

Life sciences study design

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

Sample size	Sample sizes were not pre-determined but were approximately obtained based on current practices in the field. Sample sizes and number of independent experiments are indicated in the figure legends.
Data exclusions	No data were excluded from analyses.
Replication	All experiments were independently replicated as described in the figure legends. P-values were derived from measurements obtained from experiments conducted independently at least three times. Figures with representative images were reproduced and obtained from at least two or more independent experiments with similar results.
Randomization	NA
Blinding	Kinetochore tracking analyses were made blind by the investigators. The remaining analysis in this work was performed without blinding.

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

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

Methods

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

Antibodies

Antibodies used

Mouse anti-Vasohibin-1 (C-6) (Santa Cruz Biotechnology, Inc.) Cat# sc-365541
Mouse monoclonal FLAG M2 (Sigma-Aldrich) Cat# F1804-200UG
Rabbit anti-detyrosinated tubulin (kind gift from Marin Barisic, Liao et al., 2019)
Rat anti-tyrosinated tubulin (BioRad) Cat# MCA77G
Mouse anti-TTL (Proteintech) Cat# 66076-1-Ig
Mouse monoclonal anti-α-tubulin B-512 (Sigma-Aldrich) Cat# T5168
Mouse anti-GAPDH (Proteintech) Cat# 60004-1-Ig
Rabbit anti-β-tubulin (Abcam) Cat# T5201
Rat monoclonal anti-CLASP2 (Pereira et al., 2009)
Mouse monoclonal anti-Hec1/NDC80 (Abcam) Cat# ab3613
Mouse anti-Mad1 clone BB3-8 (Millipore) Cat# MABE867
Human anti-centromere antiserum (ACA; Fitzgerald) Cat# 90C-CS1058
Goat Anti-rat HRP (Jackson ImmunoResearch)Cat# 112-035-003
Goat anti-rabbit HRP (Jackson ImmunoResearch)Cat# 111-005-003
Goat anti-mouse HRP (Jackson ImmunoResearch)Cat# 115-035-003
Alexa Fluor Goat anti-mouse 568 (Invitrogen) Cat# A-11031
Alexa Fluor Goat anti-mouse 488 (Invitrogen) Cat# A-11029
Alexa Fluor Goat anti-rat 568 (Invitrogen) Catalog # A-11077
Alexa Fluor Goat anti-rat 488 (Invitrogen) Catalog # A-11006
Alexa Fluor Goat anti-human 405 (Invitrogen) Catalog # A48275
Alexa Fluor Goat anti-rabbit 488 (Invitrogen) Cat# A-11008
Alexa Fluor Goat anti-human 647 (Invitrogen) Cat # A-21445
Alexa Fluor Goat anti-rat 647 (Invitrogen) Cat # A-21247
Goat Anti-mouse STAR 580 (Abberior Instruments) Cat# 2-0002-005-1
Goat anti-rabbit STAR 635 (Abberior Instruments) Cat# 2-0012-007-2

Validation

The antibodies used in this work were purchased from reputable sources validated by the respective manufacters, as described in their websites, with the exception of:
Anti-Rabbit anti-detyrosinated tubulin - Liao et al., 2019 - https://www.nature.com/articles/s41422-019-0187-y
Anti-Rat monoclonal anti-CLASP2 - Pereira et al., 2009 - https://link.springer.com/protocol/10.1007/978-1-60327-993-2_9

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Parental U20S cells were obtained from Stephen Geley (Innsbruck, Austria)
Authentication	The cell line was authenticated by genotyping.
Mycoplasma contamination	All cell lines were tested negative for Mycoplasma using PCR amplification with primers MGSO1/GPO1
Commonly misidentified lines (See ICLAC register)	NA

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA