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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Fluorescent images were collected using SoftWorx 6.0 software (Applied Precision/GE Healthcare) and Nikon Imaging Software (NIS-Elements AR 5.51.00). Western blot signals were detected using an ImageQuant LAS 4000 (GE Healthcare) imager and its control software. Electron microscopy images were collected using Tecnai Imaging & Analysis 4.7 (Thermo Scientific, Eindhoven). Mass-spectrometry data was collected using Thermo Scientific Xcalibur processing and its instrument control software. SEC data was collected using the UNICORN software (GE healthcare). Single molecule EM images were collected using JEOL-1230 and its instrument control software. SEC-MALLS: Scattering data collected using miniDAWN Tristar detector (Wyatt Technologies) and ASTRA V 5.3.2.17. SPR: Biacore T200 (GE Healthcare) and Biacore T200 Control Software Version: 2.0.1. SAXS: Synchrotron X-ray data collected on Pilatus 1M detector and BsxCuBE (doi:10.1107/S0909049513010431).
Data analysis	Prism 6 and 7 and Microsoft Excel 2017 were used for statistical analysis and graphical data presentation (https://www.graphpad.com/). Image J v1.50e (https://imagej.nih.gov/ij/) and Fiji v2.0.0-rc-43/1.51h (https://fiji.sc/) were used to analyze image data. Proteome Discoverer™ Software v2.2 (ThermoFisher Scientific) was used to analyze MS data. CRYO-EM image processing was performed using the Scipion platform (http://scipion.cnb.csic.es). SAXS data analysis was performed using ScÅtter (http://www.bioisis.net/tutorial). The SEC-MALLS data were analysed by ASTRA (Wyatt Technologies) software. Conserved Feature Extraction was performed with IUpred, MARCOIL, MEME algorithm, MAFFT-LINSI, HMMER package.

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Field-specific reporting

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

☒ Life sciences   ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

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

Sample size	No statistical method was used to predetermine the sample size. Sample size was chosen based on previous experience and standards in the field. Sample size and number of independent experiments are stated in the figure legend or in the Methods section. Three to more independent results were used to perform statistical analyses. If less, no statistics were performed.
Data exclusions	No data is excluded if the experiments were successfully performed.
Replication	All experiments were reliably reproduced.
Randomization	Electron microscopy pictures of cells were randomized
Blinding	Electron microscopy pictures of cells were blinded and independently analyzed by two investigators.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☐	☒ Unique materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Research animals
☒	☐ Human research participants

Unique materials

Obtaining unique materials	Cpd-5 was obtained from Rene Medema lab (DOI:10.1038/onc.2015.319). Relevant plasmids used in this study are available upon reasonable request.

Antibodies

Antibodies used	Information of antibodies, RRIDs, including species and dilution ratio are described in Supplementary Table 2
Validation	Validations are based on the datasheets from the manufacturers (RRIDs of the antibodies are provided). We additionally validated the following antibodies by the use of siRNA- treated samples as negative control: ZW10, p150Glued, HEC1, KNL1, BUB1, CENP-F, MAD1, CENP-E, ROD, Spindly, Zwilch.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HeLa Flp-in cells provided by the S. Taylor lab, University of Manchester, England, UK. The Tnao38 cells come from (Hashimoto et al, 2012. BMC Biotechnol. 12:12. doi:10.1186/1472-6750-12-12)
Authentication	Cell lines were not authenticated by ourselves.
Mycoplasma contamination	Cell lines were tested multiple times over the study for eliminating possible mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell line was used

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ Magnetic resonance imaging