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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	The EM data were collected automatically using EPU software (Thermo Fisher Scientific)
Data analysis	CryoSPARC v.4.6, FREALIGN v9.11, Relion v4.0, Coot v0.9.8, Chimera v1.17, ChimeraX 1.7.1, and Phenix v.1.20.1 software were used to process and display EM data. The deep-learning based software ModelAngelo and Coot v0.9.8 was used to determine the protein identity of the cryo-EM map. Python scripts needed for the new CryoSPARC- and FREALIGN-based MT data processing pipeline is available at https://github.com/rui--zhang/Microtubule. For fluorescence microscopy image analyses, ImageJ software (Version 1.54f) was utilized with built-in plugins (StackReg and KymoResliceWide) to correct for drift and create kymographs for analysis. Statistical tests were performed with GraphPad Prism (version 10.0.3 for Windows). For mass spectrometric analysis of HURP and TPX2 purifications, Scaffold 5.3.3 software was used by the Mass Spectrometry Core at Princeton University.

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

The single-particle cryoEM structure of HURP65-174-decorated GMPCCP-MT has been deposited in the Electron Microscopy Data Bank (EMDB) with accession code EMD-47173 (https://www.ebi.ac.uk/emdb/EMD-47173). The refined atomic model for a patch (3x3) of tubulin dimers with HURP decoration has been deposited in the Protein Data Bank (PDB) with ID code 9DUQ (https://doi.org/10.2210/pdb9duq/pdb). Source data underlying Figs. 1-5 and Supplementary Figs. 1, 4, and Supplementary Figs. 7-11 are presented in the Source Data file provided with this paper.

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	No human participants or human data were used in this study.
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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.

[x] 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	No statistical method was used to predetermine sample size. In all experiments, every sample (individual microtubule) was measured and included in the analyses. For non-dynamic microtubule assays, 9 fields of view were imaged at once to ensure a representative sample was taken and that microtubule distribution was uniform. This allowed for >100 microtubules to be analyzed per replicate. For dynamic microtubule assays, all microtubules in the field of view over replicates was measured and included in the analyses. This allowed for >14 microtubules to be measured in each experiment. Reproducible results over 3-4 replicates allowed us to determine that the number of replicates was sufficient. Co-condensate assay produced the same result three times, using varying levels of TPX2, which allowed us to determine that as a sufficient number of replicates. For the TPX2 phase assay, two field of views were taken per experimental replicate to ensure that the field of view was representative of a larger area. In Xenopus laevis egg extract experiments, the sample sizes are described as the number of biologically distinct replicates (i.e., extract from different frogs laid on different days). Extract experiments were imaged over the course of 30 minutes (due to limits of the extract lifetime, which is ~20-25 minutes). After extract experiments were imaged, a 3x3 field of view was taken to ensure that the imaged field of view was representative of the whole reaction.
Data exclusions	No relevant data were excluded.
Replication	Xenopus egg extract data were done over multiple distinct biological replicates to ensure reproducibility against biological variation. Similar results were seen across all replicates. In all in vitro microtubule nucleation and localization assays where statistics are reported, the experiment was repeated at least three times with similar results. All pull-down experiments, except for the optimization of pulldown

	conditions seen in Supp Fig 8b, were repeated three times all with similar results. All phase co-condensate assays were repeated at least three times with varying levels of condensed TPX2. All resulted in similar results.
Randomization	N/A, none of the experiments performed required the separation of distinct samples into experimental groups
Blinding	Investigators were not blinded in any data collection, as it is not possible for an investigator to prepare a condition and live-image it in a blinded manner. Blinding was performed for fluorescence image data analysis either by automated analyses tools or numbering of microscopy image files to conceal the condition.

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	1:1000 γ-tubulin (Sigma: T6557), 1:2000 chTOG (Abcam: ab86073), 4 microgram/mL custom TPX2 antibody (Alfaro Aco et al 2017), 3.6 microgram/mL custom HAUS1 antibody (Song et al 2018), 1:1000 α-tubulin (Invitrogen: 62204), 2 micrograms/mL of Alexa Fluor 647-conjugate γ-tubulin (XenC) antibody, 4 microgram/mL custom HURP antibody (this study), 1:2000 Mouse-IgG HRP linked secondary antibody (Amersham: NA931-1ML), 1:2000 Rabbit-IgG HRP linked secondary antibody (Amersham: NA934-1ML), KPNB1 antibody (ABclonal: A8610), HURP antibody (proteintech: 12038-1-AP), 1:1000 GFP antibody (Abcam: AB290), 1:1000 GST antibody (Abcam: ab92), and 1:1000 GCP5 antibody (Santa Cruz Biotechnology: sc-365837)
Validation	γ-tubulin (Sigma: T6557), chTOG (Abcam: ab86073), TPX2 (Alfaro Aco et al 2017), HAUS1, (Song et al 2018), and α-tubulin (Invitrogen: 62204) antibodies were validated by western blot detection of the endogenous protein in Xenopus egg extract in blots presented in Figure 1a of this manuscript. The GCP5 antibody (Santa Cruz Biotechnology: sc-365837) was validated by western blot detection of purified protein in Supplemental Figure 10. Alexa Fluor 647-conjugated γ-tubulin (XenC) antibody was validated in Supplementary Table 1 of Thawani et al 2018 (PMID: 29695792). The custom HURP antibody (this study), KPNB1 antibody (ABclonal: A8610), and commercial HURP antibody (proteintech: 12038-1-AP) were validated in detecting the Xenopus ortholog of their protein targets via western blotting in Fig 1a, Fig S8b, and Fig S8b, respectively. The GFP antibody (Abcam: AB290) and GST antibody (Abcam: ab92) were both validated by detection of purified protein in western blots provided in Fig 8a. Uncropped images of all blots are provided in the source data file of the manuscript.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	We use wild-type adult (mature) female Xenopus laevis animals for our studies. These animals were sourced from Nasco/Xenopus One. The use of these animals is to obtain unfertilized eggs, with which we perform our assays.
Wild animals	N/A, this study did not involve wild animals
Reporting on sex	N/A. this This information is not relevant to our Xenopus egg extract studies and is not possible to obtain (egg extract is made from unfertilized eggs).
Field-collected samples	N/A, this study did not involve samples collected from the field
Ethics oversight	Xenopus laevis husbandry was done in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the approved Institutional Animal Care and Use Committee (IACUC) protocol 1941 of Princeton University.

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

Plants

Seed stocks

N//A, no plants were used in this study

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.