# nature portfolio

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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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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

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n/a	infirmed
	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.
x	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)
x	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> 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 <i>d</i> , Pearson's <i>r</i> ), 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

NIS-Elements was used to acquire TIRF data. TIA was used to collect negative stain data. EPU was used to acquire cryo-EM data.

Data analysis

 $ImageJ\ 2.3.0/1.53f\ was\ used\ to\ process\ TIRF\ data.\ Relion\ 3.0.6\ was\ used\ to\ process\ regative\ stain\ data.\ CryoSPARC\ v3.3.2+220824\ was\ used\ to\ process\ cryo-EM\ data.$ 

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 <u>data availability statement</u>. 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 <u>policy</u>

The reconstructed electron density map can be obtained from the EMDB using accession code EMD-28981. The rigid-body fit structural model associated with this map can be obtained from the PDB using accession code 8FCK.

Human rese	arch part	icipants		
Policy information	about <u>studies i</u>	involving human research participants and Sex and Gender in Research.		
Reporting on sex a	nd gender	N/A		
Population charact	eristics	N/A		
Recruitment		N/A		
Ethics oversight N/A		N/A		
Note that full informa	ation on the app	roval of the study protocol must also be provided in the manuscript.		
Field-spe	ecific re	eporting		
Please select the o	ne below that i	is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.		
<b>x</b> Life sciences		Behavioural & social sciences Ecological, evolutionary & environmental sciences		
For a reference copy of	the document with	all sections, see <a href="nature.com/documents/nr-reporting-summary-flat.pdf">nature.com/documents/nr-reporting-summary-flat.pdf</a>		
		udy design		
All studies must dis		points even when the disclosure is negative.		
Sample size	_	rain and cryo-EM data collection, sample size (ie number of particles) was determined by intended final structural resolution.  The listed in the Methods section and Supplementary Figure 2a.		
Data exclusions	_	rain and cryo-EM data processing, particles were excluded based on automated sorting into low resolution 2D and 3D classes. lata exclusion is diagrammed in Supplementary Figure 2a.		
Replication	with different l	For reconstitution of branching MT nucleation, experiments were replicated twice (technical replicates performed on different days are the state of augmin and $\gamma$ -TuRC). For cryo-EM, two different data sets were collected on the same sample, as detailed in technical replicate.		
Randomization	For determinat	r determination of cryo-EM resolution, particles were randomly assigned to half-map A or B by cryoSPARC and processed independently.		
Blinding	It is standard in the field of structural biology and biochemistry that blinding is not relevant.			
We require informati system or method lis	ion from authors ted is relevant to perimental s	·		
n/a Involved in th	•	n/a   Involved in the study		
Antibodies    K   Eukaryotic cell lines		ChIP-seq Flow cytometry		
Palaeontology and archaeology				

Animals and other organisms

Dual use research of concern

Clinical data

## Eukaryotic cell lines

Policy information about <u>cell lines and Sex and Gender in Research</u>

Cell line source(s) Sf9 cells were purchased from ATCC

Authentication The cell line was not authenticated subsequent to receipt

Mycoplasma contamination The cell line was not tested for mycoplasma contamination subsequent to receipt

Commonly misidentified lines (See <u>ICLAC</u> register)

No commonly misidentified lines were used in this research

### Animals and other research organisms

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

Laboratory animals	Adult, female Xenopus laevis frogs were used in the study	
Wild animals	The study did not involve wild animals	
Reporting on sex	Only female frogs were used, as unfertilized Xenopus laevis eggs were required for generation of meiotic Xenopus egg extract	
Field-collected samples	The study did not involve samples collected in the field	
Ethics oversight	The Princeton University Institutional Animal Care and Use Committee provided guidance and approved the use of Xenopus laevis frogs for this study (protocol number 1941).	

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