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Experimental design

1. Sample size

Describe how sample size was determined.

No sample size calculation were performed and sample sizes were chosen with the following rationale for individual experiments.

For measuring nucleation in Xenopus egg extract, a number of samples were compared across each other. Each reaction was performed and observed for 15-20 minutes once in an experimental set due to limited lifetime of egg extract. During one reaction, a large field of view, roughly 140x140 um, was imaged that encompassed multiple branching networks containing 1000's of microtubules or 100's of de novo nucleated microtubules. The large numbers of microtubules measured in one field of view led to robust measurements above any noise. Before and after the experiment, the entire slide was scanned, which showed homogeneous distribution of networks across the slide. Therefore choosing one field of view for each reaction was representative and sufficient such that clear differences between samples were observed by imaging these large fields of view. The entire time-series was reported. The experimental results and trends were reproducible exactly, as indicated in respective figure legends.

For in vitro microtubule nucleation assays, a large coverslip was prepared with the sample. All fields of view (132x132um) were homogeneous and measurements from one field of view with another were consistent. 10-20 fields of view were imaged for each reaction to provide sufficient confidence in the measurements and results showed small error bars (plotted in respective figures) and clear patterns across various reactions performed. Therefore, 10-20 fields of sample size was sufficient to account for variation across fields and ensure reproducibility. The experimental results and trends were reproducible exactly from multiple experimental sets. Data from all experiments was pooled and reported.

For in vitro polymerization experiments, a large field of view (132x132um) was imaged for 15 minutes, usually containing more than 20 microtubule templates. Growth speed from more than 20 microtubule templates was obtained for every concentration of each protein construct. Before and after the experiment, the entire slide was scanned, which showed homogeneous distribution of microtubules and growths across the slide. The experiments performed on different days with different batches of purified protein, which resulted in the exact agreement in measurements across each other. The measurements were reproducible, and therefore 20 measurements for each reaction was sufficient. Data from all experiments was pooled and reported.

For chromatography, each run was performed once in an experimental set and shift of proteins due to binding was observed. Clear shift of g-tubulin or alpha/beta-tubulin signal was observed in each experiment, showing clear interaction or no interaction. In some experimental sets, repeat of one reaction was performed and gave the same results. Therefore, one reaction for each condition for every experiment was sufficient. Sets of chromatography runs were repeated on different days using different protein preparations and freshly made buffers. The repeats resulted in an exact agreement.

For immunoprecipitation experiments in vitro and in Xenopus egg extracts, the set of precipitations indicated in the figures were performed simultaneously and at the same concentrations across samples. Clear signal of prey protein(s) was observed using immunoblots in each experiment, showing clear binding or no binding. In certain experiments, the same immunoprecipitation reaction was repeated and gave the same result, therefore, one sample for each reaction within an experimental set was sufficient. The experimental set was repeated and similar results were observed, as described in figure

legends.

All experiments performed in this work were repeated at least three times, unless indicated otherwise in individual figure legends. All repeats performed resulted in exact agreement or similar results.

2. Data exclusions

Describe any data exclusions.

No data was excluded in experiments or analyses.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

For experiments performed in Xenopus egg extract, each experimental set was repeated with at least 3 independent extract preparations, unless indicated otherwise. Each experiment was performed from independent extract preparations, i.e. on different days using freshly laid eggs from different animals and with freshly made buffers. Similar results or exact agreement was seen across all repeats.

For in vitro microtubule nucleation assays with purified g-TuRC, fresh g-TuRC protein was prepared using freshly made buffers and Xenopus egg extracts obtained from different animals for each experimental set. These experiments were repeated at least two-three times as indicated in respective figure legends, with additional supporting experimental sets. Similar results were seen across all g-TuRC preparations.

For in vitro polymerization, two sets of experiments were performed on different days with fresh protein purifications and fresh buffers for each experiments. Multiple replicates were performed on individual purifications as well in one day. No difference was observed between any repeats.

For size exclusion chromatography with gamma-tubulin, at least 2 independent runs were performed on different days with each XMAP215 construct, using freshly made buffers. Multiple supporting runs were also performed with multiple protein preparations, as described in individual figure legends. For size exclusion chromatography with alpha/beta-tubulin, 1 experimental run at the reported buffer and protein concentrations was performed and multiple supporting experiments were performed on different days with fresh buffers and multiple protein purifications, as described in respective figure legends. Exact agreement was seen across all repeats.

Immunoprecipitation experiments were repeated on different days, with freshly prepared egg extracts or buffers (independent experiments) two to four times as indicated in individual figure legends. Similar results were seen across all repeats.

All experimental repeats resulted in exact agreement or similar results across all experiments. Extensive details on statistics and reproducibility are reported in respective figure legends and "Statistics and Reproducibility" section of Methods section.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Randomization was not relevant for this study, as samples were never needed to be distributed into experimental groups. Experiments in Xenopus egg extracts were performed on one tube of Xenopus egg extract prepared fresh on the day and no allocation of sample was needed. For all other experiments, purified proteins were used that were from the same batch of purification for every experimental set, and no allocation was required. Therefore, no randomization was performed.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Investigators were not blinded to data collection and analyses.

DATA COLLECTION
With Xenopus egg extracts, on one tube of Xenopus egg extract prepared fresh on the day and purified proteins either at different concentrations or different protein constructs were added. The imaging was performed by the investigators during the experiment, therefore blinding was not possible. For in vitro polymerization, again, purified proteins either at different concentrations or different protein constructs were added. The imaging was performed by the investigators during the experiment, therefore blinding was not possible. For in vitro nucleation and size exclusion chromatography, the experiments were performed in a series and their data was collected in a batch, either through microscopy or SDS-PAGE or immunoblot analysis on a later day, therefore blinding during the experimentation was not required. For the in vitro nucleation reactions, the difference between absence or presence of g-TuRC was so dramatic that blinding to the sample was not possible during image acquisition. Similarly, during data collection for chromatography, the presence or absence of interaction was very clear, and blinding to the sample was not possible during acquisition.

DATA ANALYSES
Most analyses performed in Xenopus egg extracts was done via automated image analyses software for nucleation measurements and growth speed without investigators' interference.

Therefore, blinding was not required. Similarly, total microtubule mass measurement in g-TuRC nucleation assay was performed via automated MATLAB scripts and blinding was not required. For counting microtubules in vitro (S4C, S6C) or Xenopus egg extracts (1F, 2B), all microtubules were meticulously counted by the investigators and blinding was not performed.

Thus, for this study, blinding was either not required during the experiments or analyses, or not performed since data was collected by the investigators.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☒	☐ 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 any assumptions or corrections, such as an adjustment for multiple comparisons
☒	☐ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

MATLAB-based custom-built software in conjunction with open source software (Plus-tip tracker in uTrack 2.1.3, Danuser group) were used for detecting and tracking EB1 particles in Xenopus egg extract. For other measurements and data analysis, MATLAB-based custom-built scripts were used. All codes with detailed instructions on their use are available from the authors upon request.

For building kymographs for growth speed measurements (Fig. 6A and Supp Fig. 2D), ImageJ software was used with inbuilt multi-kymograph plugin.

For mass spectrometric analysis of g-TuRC purification, Proteome Discoverer 2.1 and Scaffold 4.7.5 softwares were used by the Mass Spectometry Core at Princeton University.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

All materials along with detailed instructions on their use are available from the authors upon request.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Detailed information on antibodies used in this study can be found in Supplementary Table 1. Commercial antibodies were validated as noted on manufacturer's website and with our tests performed in the lab, as described in the Supplementary Table 1.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No cell lines were used in this manuscript.

b. Describe the method of cell line authentication used.

As no cell lines were used, no cell line authentication was needed or performed.

c. Report whether the cell lines were tested for mycoplasma contamination.

Because no cell lines were used, cell lines were not tested for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No cell lines were used in this study, so no cell lines were found in the database of commonly misidentified cell lines that is maintained by ICLAC and NCBI Biosample.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

Mitotic extracts were prepared from Xenopus laevis oocytes. Mature, female frogs (age 2-7 years) were used. All experiments were performed in compliance with IACUC protocols and guidelines.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.