

Golgi-associated tethers capture vesicles from specific membrane recycling pathways

Adam Krahn, Natalie Johnson, Jotham Austin II, and Benjamin Glick

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Dear Ben,

Thank you again for the submission of your manuscript entitled "Functional assignment of Golgi-associated vesicle tethers to specific membrane recycling pathways" and for your patience during the review process. We have now received reports from three referees, which I copy below.

As you can see from their comments, all stated that your manuscript represents a valuable and timely development to our understanding of trafficking in the Golgi. Some comments and suggestions are made, I would be grateful if you would consider these carefully. In addition, Referee #2 recommends the inclusion of a control that would test potential dominant-negative effects of the Imh1-GFP and Sgm1-GFP proteins.

Based on these reports, I would like to invite you to address the comments of referees #2 and #3 in a revised version of the manuscript. I should add that it is The EMBO Journal policy to allow only a single major round of revision and that it is therefore important to resolve the main concerns at this stage. I believe the concerns of the referees are reasonable and addressable, but please contact me if you have any questions, need further input on the referee comments or if you anticipate any problems in addressing any of their points. Please, follow the instructions below when preparing your manuscript for resubmission.

I would also like to point out that as a matter of policy, competing manuscripts published during this period will not be taken into consideration in our assessment of the novelty presented by your study ("scooping" protection). We have extended this 'scooping protection policy' beyond the usual 3 month revision timeline to cover the period required for a full revision to address the essential experimental issues. Please contact me if you see a paper with related content published elsewhere to discuss the appropriate course of action.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: <https://link.springer.com/journal/44318/submission-guidelines#cms-Revised-submissions>

Again, please contact me at any time during revision if you need any help or have further questions.

Thank you very much again for the opportunity to consider your work for publication. I look forward to your revision.

Best regards,

William

William Teale, Ph.D.
Editor
The EMBO Journal

When submitting your revised manuscript, please carefully review the instructions below and include the following items:

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point response to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (<https://link.springer.com/journal/44318/submission-guidelines#cms-Revised-submissions>). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript.

6) We require a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public (see

<https://link.springer.com/partners/embo-press/editorial-policies#Data%20deposition>). If no data deposition in external databases is needed for this paper, please then state in this section: This study includes no data deposited in external repositories. Note that the Data Availability Section is restricted to new primary data that are part of this study.

Note - All links should resolve to a page where the data can be accessed.

7) When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:

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Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

8) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.).

9) We would also encourage you to include the source data for figure panels that show essential data. Numerical data can be provided as individual .xls or .csv files (including a tab describing the data). For 'blots' or microscopy, uncropped images should be submitted (using a zip archive or a single pdf per main figure if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available at

<https://link.springer.com/journal/44318/submission-guidelines#cms-Source-data>

10) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online (see examples in <https://www.embopress.org/doi/10.15252/embj.201695874>). A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2' etc. in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: < <https://link.springer.com/journal/44318/submission-guidelines#cms-Expanded-View-data> >.

- Additional Tables/Datasets should be labelled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

11) At EMBO Press we ask authors to provide source data for the main manuscript figures. Our source data coordinator will contact you to discuss which figure panels we would need source data for and will also provide you with helpful tips on how to upload and organize the files.

12) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at < <https://link.springer.com/journal/44318/submission-guidelines#cms-Reference-guidelines> >.

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IMPORTANT: When you send the revision we will require

- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist
- Expanded View files (replacing Supplementary Information)
- a Reagents and Tools Table as part of the Methods section

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We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (29th Sep 2026). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

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Referee #1:

EMBOJ-2026-124768

The maturation of Golgi cisternae is one of the paradigmatic fields of study in membrane biology. In groundbreaking work, the team of Benjamin Glick has previously devised one of the strongest experimental approaches to study this maturation, using *S. cerevisiae* as a model system. In the currently submitted manuscript, Krahn and colleagues have undertaken the assignment of the golgins Sgm1 and Imh1 and of the multi-subunit GARP complex to specific membrane recycling pathways.

The manuscript is highly accessible, arguments are carefully developed, the experimental approach is elegant, and data are cleanly presented. The theme of Golgi maturation remains timely and of high interest to a general molecular and cellular biology readership. The results of the current manuscript make an important contribution to the overall effort of obtaining a holistic picture of Golgi dynamics.

Referee #2:

The manuscript by Krahn and colleagues is a tour de force to establish molecular details of vesicle targeting in the yeast Golgi. This is an intriguing topic with relevance to biotechnological and biomedical applications, where the yeast model system shows clear advantages and good level of conservation to make the results interesting and important for the whole of the molecular bioscience community. The authors establish the molecular identities of tethers for three different vesicular carrier types at the Golgi, with one protein, the GRIP domain protein Imh1 being involved in the tethering of two distinct vesicle populations. The story is very well developed with nice data, and should make a great addition to the EMBO Journal once a few details are clarified in a revised version of the manuscript as detailed below.

Major comments:

1. The presented experiments convincingly show that the investigated tethers are sufficient for tethering the different vesicle populations, but not whether they are essential for these tethering events. I understand the arguments made most clearly in the discussion regarding the unsuitability of knock out strains for this purpose (especially for GARP, although see below for Imh1 and Sgm1) and the used quantifications with kinetic and vesicle tethering data is indeed a sound approach. However, conclusive evidence using this approach can only be reached once all potential tethers are evaluated, as we cannot rule out different levels of redundancy in the system. This needs to be acknowledged more in the paper I would think.

Related to this, a weakness in the authors' logic regarding suitability of knock-outs is when tethers are targeted to the bud neck without apparent negative consequences on Golgi trafficking or cell health. This implies that a knock-out of these tethers should be interpretable?

2. For kinetic mapping of Imh1 and Sgm1 the WT copy was kept intact and a second non-functional copy of these proteins with a

GFP tag was used, which could follow the same trafficking itinerary. The authors argue that the WT copy could perform all the necessary functions, however, there could be dominant effects of the GFP constructs if the GRIP domain "receptor" for Imh1 or Ypt6 for Sgm1 are saturated. Unperturbed localisation of relevant cargo needs to be verified in the presence of the GFP constructs.

Minor comments:

1. Why does Imh1 leave before Sys1 if Sys1 is responsible for its recruitment? Similar is observed for Sgm1 departing before Ypt6 - is this a general theme for golgin tethers?
2. Fig 1C legend should say that this is a rapamycin treated sample.
3. Repeated references are made to work from the Munro group regarding their pioneering approach to capture Golgi vesicles on ectopically localised golgins in the mammalian system. An earlier study has already used this approach for non-golgin tethers in the mammalian system - DOI: 10.1038/ncomms2535 - and should be considered for citation.

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This manuscript from Krahn, Johnson et al. investigates the function of tethering proteins in Golgi trafficking using the budding yeast model system. Budding yeast is the most powerful model for studying Golgi maturation, which is now widely believed to occur in mammalian cells as well (for example, see Taylor, Briggs, Munro et al. *Science Advances* 2025). In this manuscript, the primary approaches involve timelapse imaging of Golgi maturation and a variation of their recently developed bud-neck relocalization (vesicle capture) assay. The vesicle-capture assay used in this work, in which Golgi tethering proteins are ectopically localized to the bud neck, is reminiscent of an approach previously used by the Munro lab to identify the roles of tethering proteins in mammalian cells by ectopically localizing them to mitochondria. This assay is quite powerful because it interrogates the sufficiency of specific tethers, and is helpful in cases where redundancy complicates experiments testing whether a tether is necessary for a particular pathway.

The authors begin by replacing the C-terminal GRIP domain of the golgin protein Imh1 with FRB, and use this to induce ectopic localization of Imh1 to the bud neck by addition of rapamycin. Using cryo-ET, they confirm that this leads to an accumulation of ~50nm vesicles near the septin ring. They then determined whether a specific GFP-tagged Golgi cargos were relocalized to the bud neck. They saw strongest capture of Kex2, Ste13, Vps10, Nhx1, and Sys1. This indicates that Imh1 captures vesicles from the AP-1/Ent5 and endosomally-derived pathways.

They next seek additional experimental data to distinguish the itinerary of Kex2 and Ste13 from that of Vps10 and Nhx1, as their prior work has led to the hypothesis that Kex2 and Ste13 are recycled via AP-1/Ent5 vesicles at the TGN, whereas Vps10 and Nhx1 first transit to endosomes in GGA/Ent3 vesicles before being returned via endosomally-derived vesicles. They confirm these assignments by examining the kinetic signatures of these cargos and use of the vesicle co-capture assay.

They next focus on GARP, a tethering complex known to function in tethering endosomally-derived vesicles. They first establish the maturation timing of GARP and find its kinetic signature closely resembles that of Vps10, appearing slightly after Imh1 and slightly before Kex2. They relocalize GARP to the bud-neck and find that the AP-1/Ent5 vesicle cargos Kex2 and Ste13 are also relocalized. As expected, the COPI cargo Vrg4 was not captured. contrast, the endosomally-derived vesicle cargos Vps10 and Nhx1 were also not captured, challenging the prevailing view that GARP is directly responsible for tethering these vesicles. The authors provide a convincing explanation for why previous studies likely arrived at an incorrect conclusion regarding the precise function of GARP, as GARP mutants perturb Golgi trafficking and may prevent proper Imh1 function.

Interestingly, by observing that relocalization of Kex2 vesicles did not lead to relocalization of GARP, they conclude that GARP is likely recruited directly to Golgi compartments rather than being delivered to the Golgi by first binding to AP-1/Ent5 vesicles.

Finally, using a similar analysis, they determine that the golgin protein Sgm1 tethers COPI vesicles belonging to the 'intermediate' (COPI-b') pathway, which contain Tmn1, Gnt1, and Sys1. Interestingly, Sys1 relocalization in particular was especially significant.

Overall I found this to be a rigorous and carefully interpreted study. The findings are very important for our understanding of Golgi trafficking and how the several different trafficking pathways are organized and orchestrated. This study provides additional evidence in support of previous findings and significantly extends our understanding by mapping the key tethering factors that are used by the cell to capture specific types of vesicles. As I find the data to be compelling and I agree with the authors interpretations, I have no significant requests for improvement.

A minor point is with regards to the introduction, where the authors state "Studies of ectopically localized tethers in animal cells suggested that different tethers function in distinct recycling pathways (Gillingham & Munro, 2019; Wong & Munro, 2014), but the identities of those recycling pathways were unknown". I think this understates the information gained from Wong & Munro, in which they determined distinct cargos captured by each tether. I suggest the authors modify this statement, perhaps they can be

more specific about exactly what was or was not learned in that previous study?

We thank the reviewers for their supportive and constructive comments. Detailed responses are provided below, and the corresponding changes in the manuscript are marked in red.

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using this approach can only be reached once all potential tethers are evaluated, as we cannot rule out different levels of redundancy in the system. This needs to be acknowledged more in the paper I would think.

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To address these two comments, we have added a control experiment to document that genetic manipulations of Imh1 and Sgm1 did not substantially perturb Golgi operation. These data are presented in a new paragraph at the end of the Results section and in the new Figure EV6. The approach was straightforward: in a variety of golgin mutant strains, we examined the fluorescence pattern of tagged Kex2, which follows a recycling pathway involving Imh1, as well as the fluorescence pattern of tagged Tmn1, which follows a recycling pathway involving Sgm1. This experiment achieved two goals. First, we documented that the knockouts of Imh1 and Sgm1 confer phenotypes that are too mild to be reliably observed with our available methods. By contrast, the vesicle tethering assay is informative because it directly measures tether function rather than assessing the consequences of tether loss. Second, we confirmed that expression of N-terminally tagged Imh1 or Sgm1 as a second copy does not exert a notable dominant negative effect. This issue was a potential concern because expression of a nonfunctional N-terminally tagged golgin could inhibit the activity of the untagged golgin via heterodimerization, and because elevated golgin levels could sequester the relevant GTPase and thereby inhibit other effectors of the GTPase. Our analysis suggests that any such effects are minor. Thanks to the reviewer for prompting us to characterize our experimental system more thoroughly.

Regarding the point about the importance of evaluating all potential tethers: we agree, and an obvious missing piece of the puzzle is the role of the multi-subunit COG tether in the pathways examined here. We are currently attempting to study yeast COG by ectopic localization. This avenue for future investigation is now mentioned at the end of the Discussion.

Minor comments:

1. Why does Imh1 leave before Sys1 if Sys1 is responsible for its recruitment? Similar is observed for Sgm1 departing before Ypt6 - is this a general theme for golgin tethers?

The finding that Imh1 departs before Sys1 is indeed interesting and a bit surprising. We had debated whether to comment on this observation, and now we do so in the Results section. Our guess is that the GTPase activating protein (GAP) for Arl1 arrives before Sys1 departs, thereby inactivating Arl1 even in the ongoing presence of Sys1. Based on work from other labs, the

putative GAP for Arl1 is Gcs1, and Gcs1 arrives at an intermediate stage of cisternal maturation, so this scenario is plausible. Moreover, as described in our earlier paper (Krahn et al., JCB 2026), tagged Sys1 partitions between the COPI-dependent intermediate intra-Golgi recycling pathway and the AP-1/Ent5-dependent late intra-Golgi recycling pathway, but this partitioning might result from increased downstream leakage of Sys1 due to the fluorescent tag. If so, then untagged Sys1 would depart earlier, in which case there would be no competition with the GAP. This reasoning is summarized in the revised text.

The finding that Sgm1 departs before Ypt6 is also interesting. In the text, we speculated that a second factor cooperates with Ypt6 to recruit Sgm1, and that this second factor recycles synchronously with Vrg4. After the original submission, we learned that Boyan Zhang in Matthew Freeman's lab has identified mammalian TMEM115 as a transmembrane protein that cooperates with Rab6A to recruit TMF, the mammalian ortholog of Sgm1 (<https://www.biorxiv.org/content/10.64898/2026.09.07.749849v1>). We are now collaborating with Boyan to study the yeast ortholog of TMEM115. Our preliminary data indicate that this yeast protein is important for Sgm1 recruitment and that it recycles synchronously with Vrg4, so the two-factor mechanism of golgin recruitment is likely conserved for TMF/Sgm1. If our experiments are finished in time, we will add them to Boyan's manuscript during the revision process. If not, we will publish a follow-up study. This development is exciting not only because of the evolutionary conservation, but also because the yeast ortholog of TMEM115 is apparently part of the molecular logic circuit for Golgi maturation, i.e., this yeast protein follows an intra-Golgi recycling pathway to recruit a tether that captures vesicles in the subsequent intra-Golgi recycling pathway. Despite the new insights, we have decided to preserve the original language in the current manuscript to avoid a digression that would be distracting in this context.

2. Fig 1C legend should say that this is a rapamycin treated sample.

The legend to Figure 1C states that the cells were treated with rapamycin for 10 min. We therefore left the text as is.

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Thanks to the reviewer for pointing out this oversight—we were too focused on golgins. That reference has now been added in the appropriate locations in the text. We also added a couple of additional references to papers that include relevant data.

Referee #3:

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We agree that it is appropriate to acknowledge the important contributions of the earlier work while explaining how our analysis has advanced the field. The penultimate paragraph of the Introduction has been expanded accordingly.

Dear Ben,

We have now received a re-review report from Referee #2, which I have included below. As you will see, you have addressed all concerns satisfactorily. Before I can finally accept the manuscript, there are some remaining editorial points which need to be addressed. In this regard would you please:

- remove main, EV figures and movie snapshots from the main manuscript, the figure legends should stay at the end of the main manuscript text,
- remove "Reagents and guidance are available from the corresponding author upon reasonable request." from the data availability statement,
- remove the AC/CrediT section from the text,
- remove both references to 'unpublished data',
- check whether NIH-funded Cancer Center Support Grant P30 CA014599 needs to be acknowledged in our online submission as a separate funder,
- rename Table EV1 as Dataset EV1 in all places (source file name, title in the submission system, callouts in the manuscript),
- remove the abbreviations section from the manuscript and define abbreviations in brackets after their first mention in the text,
- remove movie legends and snapshots from the main manuscript, instead each legend should be provided in a readme file and then zipped up together with its movie file so that in the end, we have 5 zip movie folders Movie EV1-Movie EV5,
- place Table 1 between main and EV figure legends; it should be in black and white,
- define the annotated p values */**/** and indicate the statistical test used; provide the exact p-values for the same in the legend of figures 2D,E as appropriate,
- state exact p values in the legends of 1B; 4A-C; 5B; 6A; 7C; 8A; EV-2E; EV-4B,
- indicate the statistical test used for data analysis in the legends of 1B; 2B,C; 4A-C; 5B; 6A-C; 7C; 8A,B; EV-2E; EV-4B; EV-5A,B, and
- define the white and red arrows in the legend of EV-2B,C.

We include a synopsis of the paper (see <http://emboj.embopress.org/>). Please provide suggestions for a short 'blurb' text prefacing and summing up the conceptual aspect of the study in two sentences (max. 250 characters), followed by 3-5 one-sentence 'bullet points' with brief factual statements of key results of the paper; they will form the basis of an editor-written 'Synopsis' accompanying the online version of the article. The synopsis image should be in .jpg or .png format, exactly 550 pixels wide and 300 pixels high. Please ensure that the text is legible at the final size and that the synopsis image has appropriate proportions.

I am looking forward to receiving your revised manuscript.

EMBO Press is an editorially independent publishing platform for the development of EMBO scientific publications.

Best wishes,

William

William Teale, PhD
Editor
The EMBO Journal
w.teale@embojournal.org

Instructions for preparing your revised manuscript:

Read our guidance for manuscript revisions and related editorial policies: <https://link.springer.com/journal/44318/submission-guidelines#cms-Revised-submissions>

Please also check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

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<https://media.springernature.com/original/springer-cms/rest/v1/content/27825798/data/v1>

IMPORTANT: When you send the revision we will require

- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).

- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist
- Expanded View files (replacing Supplementary Information)
- a Reagents and Tools Table as part of the Methods section

Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (31st Dec 2026). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

<https://emboj.msubmit.net/cgi-bin/main.plex>

Referee #2:

The authors have addressed all questions raised in my previous review. I am happy for the current version of the manuscript to be published.

All editorial and formatting issues were resolved by the authors.

Dear Ben,

I am pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal.

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If you have any questions, please do not hesitate to contact the Editorial Office. Thank you for your contribution to The EMBO Journal.

Best wishes,

William

William Teale, PhD
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Reporting Checklist for Life Science Articles (updated January

This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: [10.31222/osf.io/9sm4x](#)). Please follow the journal's guidelines in preparing your

Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
 - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ^2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
 - are tests one-sided or two-sided?
 - are there adjustments for multiple comparisons?
 - exact statistical test results, e.g., P values = x but not P values < x;
 - definition of 'center values' as median or average;
 - definition of error bars as s.d. or s.e.m.

Please complete ALL of the questions below.

Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Yes	Reagents and Tools Table, Methods
Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For antibodies provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Not Applicable	
DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Reagents and Tools Table
Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository OR supplier name, catalog number, clone number, and OR RRID.	Not Applicable	
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Not Applicable	
Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID.	Not Applicable	
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail housing and husbandry conditions .	Not Applicable	
Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Yes	Methods
Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	
Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknowledgments

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If study protocol has been pre-registered , provide DOI in the manuscript . For clinical trials, provide the trial registration number OR cite DOI.	Not Applicable	
Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if external detailed step-by-step protocols are available.	Not Applicable	

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about sample size estimate even if no statistical methods were used.	Not Applicable	
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, have they been described?	Yes	Methods
Include a statement about blinding even if no blinding was done.	Yes	Cells were chosen for analysis without examining the fluorescence channel that included the tethering data.
Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Yes	Cells were excluded from analysis if subtraction of the blue channel (Golgi) mask removed 65% or more of the area from the red channel (septin) mask. This criterion was pre-established, and it was used because cells in which Golgi cisternae obscured the septin ring were not useful for analysis.
If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.		
For every figure, are statistical tests justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	The statistical tests were carefully chosen and validated, as described in a section in Methods.

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was replicated in laboratory.	Yes	This information is in the figures and accompanying legends.
In the figure legends: define whether data describe technical or biological replicates .	Yes	Both technical and biological replicates are described.

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving human participants : State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	Not Applicable	
Studies involving human participants : Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Not Applicable	
Studies involving human participants : For publication of patient photos , include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental animals : State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Not Applicable	
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Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of select agents and toxins (CDC): https://www.selectagents.gov/sat/list.htm .	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the authority granting approval and reference number for the regulatory approval provided in the manuscript?	Not Applicable	

Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., ICMJE, MIBBI, ARRIVE, PRISMA) have been followed or provided.	Not Applicable	
For tumor marker prognostic studies , we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not Applicable	
For phase II and III randomized controlled trials , please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have primary datasets been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	Plasmids have been deposited with Addgene and are described in the Reagents and Tools Table.
Were human clinical and genomic datasets deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are computational models that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective data citations in the reference list .	Not Applicable	