Precise Control of Microtubule Disassembly in Living Cells

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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.)
Thank you for submitting your manuscript reporting an approach for microtubule disassembly in living cells to The EMBO Journal. We have now received three referee reports on your study, which are included below for your information. In light of the referees’ comments, we would like to invite you to prepare and submit a revised manuscript.

As you will see, the reviewers are overall positive and appreciate the methodology you develop. However, they also raise a number of points that should be addressed before the study can be considered further for publication as a resource article. All referees find that additional controls and experiments are needed to further define the system, in particular for the constructs using rapamycin. Please carefully consider all referee comments and add experiments and revise the manuscript and figures as needed. Please also remember to provide a detailed response to each comment.

Please note that it is our policy to allow only a single round of major revision. Acceptance depends on a positive outcome of a second round of review and therefore on the completeness of your responses included in the next, final version of the manuscript. I encourage you to review the referees’ comments and contact me to discuss a preliminary revision plan in case there are any uncertainties regarding specific points or the revision in general.
Referee #1:

In the manuscript, Lui et al developed and functionally tested a novel tool that allows to remove microtubules in living cells in a targeted and precisely controlled manner. Microtubules are key cytoskeletal elements in every eukaryotic cell, and are of central importance to many physiological functions such as cell division, cell motility, cell architecture and intracellular transport. To fulfill the wide spectrum of functions, the dynamics and stability of microtubules is tightly regulated and adapted to their specific functions. Complete loss of microtubules in cells, which can be obtained by the use of polymerization-blocking drugs, or overexpression of depolymerising enzymes, perturbs the entire spectrum of microtubule functions, and thus does not allow to investigate specific microtubule functions. Tools allowing to depolymerise microtubules in a controlled manner, i.e. at a given time, locally restricted, or restricted to selected microtubule subspecies in cells would allow to study microtubule functions in a much more precise way. The current manuscript proposes a set of tools that allows to disassemble microtubules in cells following (i) chemical induction, (ii) light-induction, and (iii) to target the microtubule-severing activity to specific subsets of microtubules in the cell, such as cilia, or to microtubules carrying specific posttranslational modifications.

The approach the authors chose is to engineer the microtubule-severing enzymes spastin in a way that it can be attracted by different adapter-proteins, which in turn target to specific microtubules in the cell. Given that spastin is an efficient severing enzyme, those microtubules are then removed relatively rapidly. The great advantage of this new system over the so-called tubulin-depolymerising drugs such as nocodazole or colchicine is that spastin actively removes microtubules (importantly, as the authors show, stable as well as dynamic microtubules), whereas the drugs are in reality polymerisation inhibitors that only prevent microtubule re-polymerisation. Therefore, those drugs cannot affect stable microtubules, which is the huge advantage of the here-presented spastin-based system that does so.

The authors demonstrate the immediate effects of this controlled microtubule removal from cells by monitoring the altered arrangement and behaviour of cell organelles, the behaviour of actin, as well as the impact of microtubule loss on cell division. This provides a comprehensive demonstration of the possible applications of their new tools, and unambiguously demonstrate their high potential to become key tools for a broad spectrum of cell biologists.

The paper is well-written, and figures and videos are of high quality. Most experiments have been thoroughly performed and reported, which makes the reading of the manuscript a pleasant experience. However, there are a number of important points listed below that must be addressed before the paper can be further considered.

Major points:

1) The microtubule targeting of spastin followed by microtubule disruption is exhaustively demonstrated in this manuscript, and appropriate controls have been performed to ensure the effect is specific. However, the authors have not characterised the dynamics of microtubule disassembly in the absence of rapamycin. All measurements shown the manuscript start shortly before the addition of the drug, making it impossible to judge what happens before. However, given that the system is genetically encoded, it requires prior expression in the cells at least for several hours - what happens during this time?

A related question that is not discussed in the manuscript is the heterogeneity of expression: do all cells behave like the cells shown and analysed in the manuscript? Or are there cells that express great amounts of spastin constructs in which microtubules already disassemble without any microtubule targeting of the overexpressed spastin? Did the authors need to select low-expressing cells to obtain the described induced microtubule severing? These details are essential to clarify, as they will provide a clearer picture of the versatility of the method, and, most importantly, of its limits.

2) Expression of EMTB-CFP, the microtubule adapter protein, clearly bundles microtubules. This is visible in all micrographs showing EMTB-expressing cells throughout the manuscript, which clearly show the wavy thick microtubules that are characteristic for cells overexpressing a MAP. It thus appears that before targeting spastin to the microtubule to remove them,
the system stabilises microtubules, and most likely alters their dynamics. This stands in a certain contrast to the claim of this work, which is that microtubules can be removed on command without major side effects. The aspect of microtubule bundling and potential stabilisation must be addressed in this manuscript to highlight the potential limitations of the method. The authors should address two key issues: i) the degree of microtubule bundling and stabilisation, and ii) the heterogeneity of this phenomenon between different cells depending on EMTB expression levels. They might want to connect this with the below-suggested analyses of the effect of microtubule drugs on microtubule disassembly in the absence and presence of EMTB.

3) Targeting of spastin with CFP-RFB-MAP4m: the authors show a specific localisation of this construct to primary cilia, mitotic spindle and midbody in Fig. S11. However, along the lines discussed in the previous two points, there is no information on the reproducibility of these specific localisations at different expression levels of this targeting construct. Without this information, it is impossible to judge the broad applicability of the approach.

Minor points:

1) The authors present their tool as a new way of addressing defined microtubule subspecies in cells. However, what they demonstrate only two ways of removing specific microtubules: tyrosinated microtubules and microtubules that can be targeted with CFP-RFB-MAP4m. They should adapt sections in the introduction and the abstract to reflect this fact, as otherwise the reader might expect a tool to disassemble any type of microtubule, which the authors do not provide.

2) The authors make a strong point about the rapidity with which they are able to disassemble microtubules with their system as compared to nocodazole and colchicine (Fig. S3). These data are convincing, however there is a caveat that the authors should address: as mentioned above in major point 2), the expression of EMTB clearly bundles, and thus stabilises microtubules. As both drugs do not actively disassemble microtubules, but rather prevent microtubule assembly, they have no impact on stabilised microtubules. The authors should compare the disassembly rates they get with their spastin-based system with the impact of nocodazole and colchicine on cells that do not express EMTB. This would give them a better comparison of their method with drug-based approaches, as nobody would express EMTB in cells before treating them with nocodazole or colchicine.

3) In Fig. S11, the authors show how CFP-RFB-MAP4m specifically localises to primary cilia, spindle microtubules and the midbody. While the absence of CFP-RFB-MAP4m from cytosolic microtubules in panel A (primary cilium) and C (midbody) is quite clear, there is no clear preferential localisation visible in panel B (mitotic spindle) - the construct basically localises to the spindle microtubules. The authors should explain what specific advantage of this construct they expect in mitosis.

4) The authors use a tyrosinated-tubulin binding biosensor to specifically target spastin to tyrosinated microtubules in cells. The goal is to specifically remove tyrosinated microtubules from cells, while keeping detyrosinated microtubules intact. While the idea is a great starting point to specifically explore the role of posttranslationally divergent microtubules inside cells, it has a caveat: most microtubules in cells are assembled from a mix of tyrosinated and detyrosinated tubulin, and might thus attract the biosensor irrespective of the percentage of tyrosinated tubulin within a given microtubule. In other words, only fully detyrosinated microtubules, which are very rate in nature, would be protected from disassembly by this system. This issue is not addressed in the manuscript, and again, as in all previous points, there is little information about the behaviour of the system in a population of cells. The authors should also explain in which cell type they expect to apply their system - would it work in neurons where microtubules are very little tyrosinated?

5) A question to the authors: is the light-inducible system reversible? If yes, could it be used to synchronise cells in G2 phase, and then release them at once into mitosis as usually done with nocodazole? If so, they might want to discuss this exciting potential application of their system.

6) The discussion chapter is relatively long. If the journal format allows, subtitles would be great to facilitate reading. Another possibility would be do shift some of the detailed discussions of experiments like organelle localisation, impact on transport etc. into the according result chapters, and restrict the final discussion chapter for a detailed discussion of the new approach.

Referee #2:

General Summary:

The manuscript by Liu et al. describes the use of engineered tools to disassemble microtubules and several microtubule-based structures such as primary cilia, mitotic spindles, and intercellular bridges. Recent advances in cellular optogenetics have been reported to spatially control microtubule dynamics using either photo-controlled microtubule-associated proteins (Adikes et al., J Cell Biol, 2017; Haren et al., Nat. Cell Bio., 2018) or microtubule-severing enzymes (Lu et al., Elife, 2020; Meiring et al., bioRxiv, 2021), underlining the broad interest in the cell biology field to develop such tools. The authors convincingly demonstrate the system that recruits Spastin onto targeted microtubule subtypes and microtubule-based structures and provides insights to distinguish between microtubule-dependent and independent events in the cell. Moreover, the study confirms the role of...
Microtubules in the movement of Golgi vesicles and lysosomes, mitochondrial division cycle, and lamellipodia formation. The authors point out plausible mechanisms for their observations in the discussion section.

The work is novel and of great importance, given the vast potential for such tools in the studies of cytoskeleton biology.

Comments:

Major comments:

1. As a control for cell fitness, it is important to check if the microtubule-depolymerizing effect of the Spastin constructs can be reversed on rapamycin washout (similar to MT reassembly after photoactivation in respective experiments).

2. The authors did not explain why the dNSpastinCD construct did not show any severing activity on the addition of rapamycin.

3. The co-localization of TagRFP-FRB-A1AY1 with tyrosinated and detyrosinated microtubules in Figure 3 need to be evaluated statistically.

4. In Figure 7C, how did the authors measure the duration of cell survival? Did the authors observe any cell death after 30 h?

5. It was surprising that the authors did not observe an increase in cell death even after persistent microtubule disassembly (Figure 7D and E). Does the effect of microtubule depolymerization on apoptosis be rather cell-line specific? It would be interesting to confirm a similar effect in other cell lines.

6. Were the transfected cells arrested in a particular cell-cycle phase in the absence of microtubules? What was the cell-cycle distribution of the transfected cells?

7. In Figure S9, the western blot for α-tubulin intensity with respect to GAPDH intensity needs to be quantified in each case.

8. The images for the Mitotracker Red panels in Figure 5G for 50% and 0% are the same. Please correct.

Minor comments:

9. In Figure 7E, the cleaved caspase-3 positive cells (%) for staurosporine doesn't show any error bar.

10. For Figure S1, the degree of translocation of EMTB-CFP-FRB on microtubules needs to be evaluated with statistics.

11. In Figure S10C, the units mentioned in the corresponding text for microtubule polymerization rate is in m/min, whereas in the figure, it is m/sec.

12. For Figure S15, the authors could show the circled area as an enlarged panel for better understanding.

13. Technically, tyrosinated MTs cannot be called as carrying a PTM. Please rephrase.

14. There are many typos throughout the figure panels that needs to be corrected:
   i. 'Normalized' in Figures 1C, 1E, 3D, 3F, S2C, S3B, S4C, S5B, S6B, S7B, S8B, S10B
   ii. 'Acetylated' in Figure 1G
   iii. 'Intensity' in Figure 5G

Referee #3:

The manuscript entitled "Precise Control of Microtubule Disassembly in Living Cells" by Liu et al describes a methodology to target spastin (microtubule severing enzyme) to microtubules using MAPs and nanobodies against a specific modification via chemical induced and photo-activatable dimerization. This allows the authors to disassemble microtubule structures in cells within a specified time period, spatial distribution and also sub-population of microtubules. Since the spastin mediated microtubule disassembly targets almost all of the microtubules, this technology is advantageous compared to the existing generic microtubule drug mediated disassembly commonly used in studies. The authors have also demonstrated the applicability of their tool in targeting various microtubule structures and the fate of sub-cellular organelles after precisely disassembling the microtubules. The imaging and quantification of data appear of good technical quality. The manuscript and the tools presented
in this study will be a great value to other researchers in the community. To this reviewer following are the essential points that need to be addressed before publication.

1. Since the authors claim that the reversibility of their microtubule disassembly in the abstract it will be good to describe this with the chemical inducible disassembly also. The authors have performed reversible experiments with their light inducible system. Rapamycin inducible dimerization is also reversible. Have the authors tried washing out the rapamycin?
2. In the force measurement experiments, the authors should provide more information about the surface medium used for the AFM experiments. Similarly, does the microtubule disassembly is enhanced or reduced upon a treating the cells with actin depolymerizing drugs should be addressed.
3. A great advantage from this tool is the temporal control of spastin microtubule severing activity. Spastin severing activity against different microtubule PTMs has been shown in vitro, therefore it will enhance the study if the authors express different PTM enzymes in the cells and compare the severing activity.
4. Another added advantage compared to the photo-switchable microtubule inhibitors is the complete disassembly of drug-resistant stable microtubules. Either the authors should directly compare their tool with such photo-switchable microtubule inhibitors or should be discussed in detail in the discussion section.
5. On similar lines the, introduction and discussion will benefit adding information about the microtubule sub-types and how targeting their inducible microtubule disassembly tool to specific microtubule sub-population will open new avenues in understanding the tubulin isotype/PTM function.
6. Minor comment: I recommend avoiding abbreviating microtubules as MT. The CRY2 induced disassembly shown in Figure 4 will benefit with a cartoon representation as shown in Figure 1A.
We would like to thank the reviewers and the editors for the positive notes and critical suggestions. We have revised the manuscript accordingly, and have provided a separate file with tracked changes. Significant changes are itemized below, followed by our point-by-point responses that address the reviewers’ concerns (in blue).

Major changes we have made, including new items:
1) Fig 1A: The information of light-induced Cry2/CIBN dimerization and GA3-AM-induced GAIs/mGID1 dimerization.
2) Fig 3C: The Pearson’s Correlation Coefficients for TagRFP-FRB-A1AY1 and indicated tubulin.
3) Fig 6D, right: The effect of Blebbistatin on cell rigidity.
4) Fig EV1D: The correlation of microtubule disruption efficiency and EMTB/dNSpastin3Q expression level.
5) Fig EV3: Disassembly rates of detyrosinated and hyperglutamylated microtubules.
6) Appendix Fig S1C: The Pearson’s correlation coefficients for EMTB-CFP-FRB and α-tubulin
7) Appendix Fig S1D: The normalized level of microtubules in non-transfected cells and EMTB-CFP-FRB-transfected cells after addition of nocodazole or colchicine.
8) Appendix Fig S8C: The normalized protein level of α-tubulin in cells before and after microtubule disruption.
9) Appendix Fig S9B,D,F: The Pearson’s correlation coefficients for CFP-FRB-MAP4m and Glu-tubulin or α-tubulin.
10) Appendix Fig S9C: A new figure of CFP-FRB-MAP4m and mitotic spindles in metaphase cells.
11) Appendix Fig S13: The enlarged images of light-illuminated regions.
12) Appendix Fig S14: The duration of cell survival in three cell types before and after microtubule disruption.
13) Appendix Fig S15: Microtubule disruption arrests cells at G2/M phase.
14) Source data Fig. 1: The uncropped image of western blot data in Appendix Fig S8B.
15) Movie EV10: Expression of EMTB and dNSpastin3Q does not induce adverse effects.
16) Movie EV17: Microtubule disruption of detyrosinated and hyperglutamylated microtubules.
17) Synopsis: A schematic diagram of our microtubule disruption system.

Referee #1:

In the manuscript, Lui et al developed and functionally tested a novel tool that allows to remove microtubules in living cells in a targeted and precisely controlled manner. Microtubules are key cytoskeletal elements in every eukaryotic cell, and are of central importance to many physiological functions such as cell division, cell motility, cell architecture and intracellular transport. To fulfill the wide spectrum of functions, the dynamics and stability of microtubules is tightly regulated and adapted to their specific functions. Complete loss of microtubules in cells, which can be obtained by the use of polymerization-blocking drugs, or overexpression of depolymerising enzymes, perturbs the entire spectrum of microtubule functions, and thus does not allow to investigate specific microtubule functions. Tools allowing to depolymerise microtubules in a controlled manner, i.e. at a given time, locally restricted, or restricted to selected microtubule subspecies in cells would allow to study microtubule functions in a much more precise way. The current manuscript proposes a set of tools that allows to disassemble microtubules in cells following (i) chemical induction, (ii) light-induction, and (iii) to target the microtubule-severing activity to specific subsets of microtubules in the cell, such as cilia, or to microtubules carrying specific posttranslational modifications. The approach the authors chose is to engineer the microtubule-severing enzymes spastin in a way that it can be attracted by different adapter-proteins, which in turn target to specific microtubules in the cell. Given that spastin is an efficient severing enzyme, those microtubules are then removed...
relatively rapidly. The great advantage of this new system over the so-called tubulin-depolymerising drugs such as nocodazole or colchicine is that spastin actively removes microtubules (importantly, as the authors show, stable as well as dynamic microtubules), whereas the drugs are in reality polymerisation inhibitors that only prevent microtubule re-polymerisation. Therefore, those drugs cannot affect stable microtubules, which is the huge advantage of the here-presented spastin-based system that does so.

The authors demonstrate the immediate effects of this controlled microtubule removal from cells by monitoring the altered arrangement and behaviour of cell organelles, the behaviour of actin, as well as the impact of microtubule loss on cell division. This provides a comprehensive demonstration of the possible applications of their new tools, and unambiguously demonstrate their high potential to become key tools for a broad spectrum of cell biologists.

The paper is well-written, and figures and videos are of high quality. Most experiments have been thoroughly performed and reported, which makes the reading of the manuscript a pleasant experience. However, there are a number of important points listed below that must be addressed before the paper can be further considered.

We are thankful for the positive comments and suggestions from the reviewer.

Major points:

1) The microtubule targeting of spastin followed by microtubule disruption is exhaustively demonstrated in this manuscript, and appropriate controls have been performed to ensure the effect is specific. However, the authors have not characterised the dynamics of microtubule disassembly in the absence of rapamycin. All measurements shown the manuscript start shortly before the addition of the drug, making it impossible to judge what happens before. However, given that the system is genetically encoded, it requires prior expression in the cells at least for several hours - what happens during this time?

We have monitored the morphology of cells transfected with EMTB-CFP-FRB and dNSpastin3Q-YFP-FKBP in the absence of rapamycin for ~30 hours (Movie EV10). Our results show that transfected cells exhibited normal morphology of microtubules and could properly undergo cell division, suggesting the expression of these components does not induce adverse effects in cells in the absence of protein dimerization (Movie EV10).

We have included this result in the revised manuscript.

A related question that is not discussed in the manuscript is the heterogeneity of expression: do all cells behave like the cells shown and analysed in the manuscript? Or are there cells that express great amounts of spastin constructs in which microtubules already disassemble without any microtubule targeting of the overexpressed spastin? Did the authors need to select low-expressing cells to obtain the described induced microtubule severing? These details are essential to clarify, as they will provide a clearer picture of the versatility of the method, and, most importantly, of its limits.

We have measured the level of EMTB-CFP-FRB, dNSpastin3Q-YFP-FKBP, and microtubule in cells before and after rapamycin treatment (Fig EV1D). Although variation in microtubule level naturally exists in different cells, microtubules level was 40% higher in most transfected cells than in those non-transfected cells without rapamycin treatment. And there was no strong correlation between microtubule level and EMTB/dNSpastin3Q level (Fig EV1D, Left). Rapamycin treatment robustly decreased microtubule level in cells expressing either low or high levels of EMTB/dNSpastin3Q (Fig EV1D, Right). In summary, our system efficiently triggers microtubule disruption regardless of the expression level of EMTB/dNSpastin3Q.

We have included this result in the revised manuscript.
2) Expression of EMTB-CFP, the microtubule adapter protein, clearly bundles microtubules. This is visible in all micrographs showing EMTB-expressing cells throughout the manuscript, which clearly show the wavy thick microtubules that are characteristic for cells overexpressing a MAP. It thus appears that before targeting spastin to the microtubule to remove them, the system stabilises microtubules, and most likely alters their dynamics. This stands in a certain contrast to the claim of this work, which is that microtubules can be removed on command without major side effects. The aspect of microtubule bundling and potential stabilisation must be addressed in this manuscript to highlight the potential limitations of the method. The authors should address two key issues: i) the degree of microtubule bundling and stabilisation, and ii) the heterogeneity of this phenomenon between different cells depending on EMTB expression levels. They might want to connect this with the below-suggested analyses of the effect of microtubule drugs on microtubule disassembly in the absence and presence of EMTB.

We have evaluated the effects of nocodazole and colchicine on microtubule disruption in non-transfected cells and EMTB-CFP-FRB-transfected cells (Appendix Fig S1D). Indeed, compared with non-transfected cells, there was a marginal increase in microtubule level under expression of EMTB. However, both nocodazole and colchicine slowly disassembled microtubules in cells with or without EMTB expression, indicating its modest stabilizing effect on microtubules (Appendix Fig S1D). Compared to these conditions, our microtubule disruption system can more efficiently and rapidly disrupt microtubules (Figs 1E, G, and Appendix Fig S3). We have included this result in the revised manuscript.

3) Targeting of spastin with CFP-RFB-MAP4m: the authors show a specific localisation of this construct to primary cilia, mitotic spindle and midbody in Fig. S11. However, along the lines discussed in the previous two points, there is no information on the reproducibility of these specific localisations at different expression levels of this targeting construct. Without this information, it is impossible to judge the broad applicability of the approach.

We have evaluated the colocation of CFP-FRB-MAP4m with markers of primary cilia, mitotic spindles, and intercellular bridges, respectively, by calculating Pearson’s correlation coefficients (Appendix Fig S9B, S9D, S9F). The analysis from 137 cells expressing different levels of CFP-FRB-MAP4m shows similar Pearson’s correlation coefficients, indicating that localization of CFP-FRB-MAP4m at the indicated sites is repeatable.

Minor points:

1) The authors present their tool as a new way of addressing defined microtubule subspecies in cells. However, what they demonstrate only two ways of removing specific microtubules: tyrosinated microtubules and microtubules that can be targeted with CFP-RFB-MAP4m. They should adapt sections in the introduction and the abstract to reflect this fact, as otherwise the reader might expect a tool to disassembly any type of microtubule, which the authors do not provide.

We thank reviewer for pointing it out. We have mentioned that “we have used chemogenetics and optogenetics to disassemble specific microtubule subtypes including tyrosinated microtubules, primary cilia, mitotic spindles, and intercellular bridges,...” in the abstract; “we have developed a new and easy-to-use system that enables precise disassembly of targeted microtubule subtypes or several microtubule-based structures including tyrosinated microtubules, primary cilia, mitotic spindles, and intercellular bridges,...” in the introduction.
2) The authors make a strong point about the rapidity with which they are able to disassemble microtubules with their system as compared to nocodazole and colchicine (Fig. S3). These data are convincing, however there is a caveat that the authors should address: as mentioned above in major point 2), the expression of EMTB clearly bundles, and thus stabilises microtubules. As both drugs do not actively disassemble microtubules, but rather prevent microtubule assembly, they have no impact on stabilised microtubules. The authors should compare the disassembly rates they get with their spastin-based system with the impact of nocodazole and colchicine on cells that do not express EMTB. This would give them a better comparison of their method with drug-based approaches, as nobody would express EMTB in cells before treating them with nocodazole or colchicine.

We have measured the effects of nocodazole and colchicine on microtubule disruption in both non-transfected or EMTB-CFP-FRB-transfected cells (Appendix Fig S1D). Compared with non-transfected cells, there was a marginal increase in microtubule level under expression of EMTB. However, both nocodazole and colchicine slowly disassembled microtubules in cells with or without EMTB expression, indicating its modest stabilizing effect on microtubules (Appendix Fig S1D). Compared to these conditions, our microtubule disruption system can more efficiently and rapidly disrupt microtubules (Figs 1E, G, and Appendix Fig S3).

3) In Fig. S11, the authors show how CFP-RFB-MAP4m specifically localises to primary cilia, spindle microtubules and the midbody. While the absence of CFP-RFB-MAP4m from cytosolic microtubules in panel A (primary cilium) and C (midbody) is quite clear, there is no clear preferential localisation visible in panel B (mitotic spindle) - the construct basically localises to the spindle microtubules. The authors should explain what specific advantage of this construct they expect in mitosis.

We have imaged the CFP-RFB-MAP4m and α-tubulin in metaphase cells by multiple-z-stacks imaging. CFP-RFB-MAP4m preferentially localizes to mitotic spindles but not aster microtubules (Appendix Fig S9C). Recruitment of dNSpastin3Q-YFP-FKBP onto MAP4m-labeled microtubule subtypes in metaphase cells can spatiotemporally disrupt mitotic spindles. We have updated the images.

4) The authors use a tyrosinated-tubulin binding biosensor to specifically target spastin to tyrosinated microtubules in cells. The goal is to specifically remove tyrosinated microtubules from cells, while keeping detyrosinated microtubules intact. While the idea is a great starting point to specifically explore the role of posttranslationally divergent microtubules inside cells, it has a caveat: most microtubules in cells are assembled from a mix of tyrosinated and detyrosinated tubulin, and might thus attract the biosensor irrespective of the percentage of tyrosinated tubulin within a given microtubule. In other words, only fully detyrosinated microtubules, which are very rare in nature, would be protected from disassembly by this system. This issue is not addressed in the manuscript, and again, as in all previous points, there is little information about the behaviour of the system in a population of cells. The authors should also explain in which cell type they expect to apply their system - would it work in neurons where microtubules are very little tyrosinated?

We agree with the reviewer that our microtubule disruption system cannot specifically disrupt tyrosinated microtubules due to the natural mix of tyrosinated/detyrosinated tubulin and the limited specificity of biosensors. We have rephrased it as below: Our approach is able to rapidly reduce the level of highly tyrosinated microtubules. Since the tyrosinated microtubules are abundant in growth cones of neuronal axons (Janke & Magiera, 2020), our system serves as a promising tool to disturb tyrosinated microtubule level and uncover its roles in neuronal structure and functions. We have updated the description in the manuscript.
Reference:
Janke C, Magiera MM (2020) The tubulin code and its role in controlling microtubule properties and functions. Nat Rev Mol Cell Biol 21: 307–326

5) A question to the authors: is the light-inducible system reversible? If yes, could it be used to synchronise cells in G2 phase, and then release them at once into mitosis as usually done with nocodazole? If so, they might want to discuss this exciting potential application of their system.

Yes, our light-inducible microtubule disruption is reversible (Fig 4 and Movie EV19). Although this optogenetic tool can be potentially used to synchronize cells in G2 phase, continuous light illumination may induce severe phototoxicity since 16~18 hr of microtubule disassembly is usually required for cell synchronization.

6) The discussion chapter is relatively long. If the journal format allows, subtitles would be great to facilitate reading. Another possibility would be do shift some of the detailed discussions of experiments like organelle localisation, impact on transport etc. into the according result chapters, and restrict the final discussion chapter for a detailed discussion of the new approach.

We have relocated and shortened the discussion on organelle localization, impact on transport to the according result chapters.

Referee #2:

General Summary:

The manuscript by Liu et al. describes the use of engineered tools to disassemble microtubules and several microtubule-based structures such as primary cilia, mitotic spindles, and intercellular bridges. Recent advances in cellular optogenetics have been reported to spatially control microtubule dynamics using either photo-controlled microtubule-associated proteins (Adikes et al., J Cell Biol, 2017; Haren et al., Nat. Cell Bio., 2018) or microtubule-severing enzymes (Lu et al., Elife, 2020; Meiring et al., bioRxiv, 2021), underlining the broad interest in the cell biology field to develop such tools. The authors convincingly demonstrate the system that recruits Spastin onto targeted microtubule subtypes and microtubule-based structures and provides insights to distinguish between microtubule-dependent and independent events in the cell. Moreover, the study confirms the role of microtubules in the movement of Golgi vesicles and lysosomes, mitochondrial division cycle, and lamellipodia formation. The authors point out plausible mechanisms for their observations in the discussion section.

The work is novel and of great importance, given the vast potential for such tools in the studies of cytoskeleton biology.

Thank you for your positive comments.

Comments:

Major comments:
1. As a control for cell fitness, it is important to check if the microtubule-depolymerizing effect of the Spastin constructs can be reversed on rapamycin washout (similar to MT reassembly after photoactivation in respective experiments).
We thank reviewer for pointing it out. Rapamycin-inducible dimerization has been widely considered an irreversible process as the clearance of rapamycin from cells is very slow (~30 hr) due to extremely high affinity between rapamycin and FKBP (Lin et al., Angew Chem Int Ed Engl, 2013; Voß et al., Curr Opin Chem Biol, 2015; Putyrski and Schultz, FEBS Lett. 2012). We have emphasized this aspect in the revised manuscript.

Reference:
1. Yu-Chun Lin, Yuta Nihongaki, Tzu-Yu Liu, Shiva Razavi, Moritoshi Sato, Takanari Inoue. Rapidly reversible manipulation of molecular activity with dual chemical dimerizers. Angew Chem Int Ed Engl. 2013, 52(25):6450-4.
2. Stephanie Voß, Laura Klewer, Yao-Wen Wu. Chemically induced dimerization: reversible and spatiotemporal control of protein function in cells. Curr Opin Chem Biol. 2015, 28:194-201.
3. Mateusz Putyrski, Carsten Schultz. Protein translocation as a tool: The current rapamycin story. FEBS Lett. 2012, 586(15):2097-105.

2. The authors did not explain why the dNSpastinCD construct did not show any severing activity on the addition of rapamycin.

We have provided explanation as below:
dNSpastinCD-YFP-FKBP did not show severing activity which is consistent with a previous report, suggesting that dNSpastinCD needs to cowork with other domains to perform microtubule severing (Fig EV1B and C)(White et al, 2007).

Reference:
Susan Roehl White, Katia J. Evans, Jeffrey Lary, James L. Cole, Brett Lauring. Recognition of C-terminal amino acids in tubulin by pore loops in Spastin is important for microtubule severing. J Cell Biol. 2007, 176(7):995-1005.

3. The co-localization of TagRFP-FRB-A1AY1 with tyrosinated and detyrosinated microtubules in Figure 3 need to be evaluated statistically.

We have statistically analyzed the colocalization of TagRFP-FRB-A1AY1 with tyrosinated and detyrosinated microtubules using Pearson’s correlation coefficients (Fig 3C).

4. In Figure 7C, how did the authors measure the duration of cell survival? Did the authors observe any cell death after 30 h?

Transfected cells were incubated with Propidium iodide-containing medium and imaged for 30 h by time-lapse microscopy. Propidium iodide serves as a cell death indicator in this experiment. The duration of cell death occurred during imaging were calculated. We have checked the cell fate by 60 hr time-lapse imaging in another experiment which still showed no significant increase in cell death after microtubule disruption (Appendix Fig S14).

5. It was surprising that the authors did not observe an increase in cell death even after persistent microtubule disassembly (Figure 7D and E). Does the effect of microtubule depolymerization on apoptosis be rather cell-line specific? It would be interesting to confirm a similar effect in other cell lines.
We have evaluated the effect of persistent microtubule disassembly on cell survival in three cell types including HeLa cells, COS7 cells, and 293T cells. As expected, the duration of cell survival was cell type-specific (Appendix Fig S14). However, at least in these three cell lines, there is no significant increase in cell death after microtubule disruption (Appendix Fig S14).

6. Were the transfected cells arrested in a particular cell-cycle phase in the absence of microtubules? What was the cell-cycle distribution of the transfected cells?

We have applied persistent microtubule disruption for 18 hr and checked the cell-cycle phase of cells in the absence of microtubules. Our results showed that many cells were arrested in G2/M phase as evidence by the elevation of a G2/mitotic-specific marker, cyclin B1 level (Appendix Fig S15), indicating that microtubule disruption by our system arrests cells in G2/M phase.

We have included this result in revised manuscript.

7. In Figure S9, the western blot for α-tubulin intensity with respect to GAPDH intensity needs to be quantified in each case.

We have quantitatively analyzed the α-tubulin intensity (Appendix Fig S8C).

8. The images for the Mitotracker Red panels in Figure 5G for 50% and 0% are the same. Please correct.

We have corrected the image of mitotracker red (Fig 5G).

Minor comments:

9. In Figure 7E, the cleaved caspase-3 positive cells (%) for staurosporine doesn't show any error bar.

The error of that condition was small (0.09%).

The raw data of this condition from three independent experiment was listed below:
Cleaved caspase3 positive cells/total cells: 1st exp: 23/54=42.59%; 2nd exp: 43/104=41.35%; 3rd exp: 19/40=47.5%; Mean ± S.E.M. = 43.06 ± 0.09 %

10. For Figure S1, the degree of translocation of EMTB-CFP-FRB on microtubules needs to be evaluated with statistics.

We have analyzed the colocalization of EMTB-CFP-FRB and α-tubulin using Pearson’s correlation coefficients (Appendix Fig S1C).

11. In Figure S10C, the units mentioned in the corresponding text for microtubule polymerization rate is in μm/min, whereas in the figure, it is μm/sec.

We have corrected the unit in the figure (Fig EV2C).

12. For Figure S15, the authors could show the circled area as an enlarged panel for better understanding.
We have added the enlarged images of the circled area (Appendix Fig S13).

13. Technically, tyrosinated MTs cannot be called as carrying a PTM. Please rephrase.

We have rephrased it.

14. There are many typos throughout the figure panels that needs to be corrected:
   i. 'Normalized' in Figures 1C, 1E, 3D, 3F, S2C, S3B, S4C, S5B, S6B, S7B, S8B, S10B
   ii. 'Acetylated' in Figure 1G
   iii. 'Intensity' in Figure 5G

We have corrected these.

Referee #3:

The manuscript entitled "Precise Control of Microtubule Disassembly in Living Cells" by Liu et al describes a methodology to target spastin (microtubule severing enzyme) to microtubules using MAPs and nanobodies against a specific modification via chemical induced and photo-activable dimerization. This allows the authors to disassemble microtubule structures in cells within a specified time period, spatial distribution and also sub-population of microtubules. Since the spastin mediated microtubule disassembly targets almost all of the microtubules, this technology is advantageous compared to the existing generic microtubule drug mediated disassembly commonly used in studies. The authors have also demonstrated the applicability of their tool in targeting various microtubule structures and the fate of sub-cellular organelles after precisely disassembling the microtubules. The imaging and quantification of data appear of good technical quality. The manuscript and the tools presented in this study will be a great value to other researchers in the community. To this reviewer following are the essential points that need to be addressed before publication.

Thank you for your positive comments and suggestions.

1. Since the authors claim that the reversibility of their microtubule disassembly in the abstract it will be good to describe this with the chemical inducible disassembly also. The authors have performed reversible experiments with their light inducible system. Rapamycin inducible dimerization is also reversible. Have the authors tried washing out the rapamycin?

We thank reviewer for pointing it out. Rapamycin-inducible dimerization has been widely considered an irreversible process as the clearance of rapamycin from cells is very slow (~30 hr) due to extremely high affinity between rapamycin and FKBP (Lin et al., Angew Chem Int Ed Engl, 2013; Voß et al., Curr Opin Chem Biol, 2015; Putyrski and Schultz, FEBS Lett. 2012).

We have emphasized this aspect in the revised manuscript.

Reference:

1. Yu-Chun Lin, Yuta Nihongaki, Tzu-Yu Liu, Shiva Razavi, Moritoshi Sato, Takanari Inoue. Rapidly reversible manipulation of molecular activity with dual chemical dimerizers. Angew Chem Int Ed Engl. 2013, 52(25):6450-4.
2. Stephanie Voß, Laura Klewer, Yao-Wen Wu. Chemically induced dimerization: reversible and spatiotemporal control of protein function in cells. Curr Opin Chem Biol. 2015, 28:194-201.
3. Mateusz Putyrski, Carsten Schultz. Protein translocation as a tool: The current rapamycin story. FEBS Lett. 2012, 586(15):2097-105.

2. In the force measurement experiments, the authors should provide more information about the surface medium used for the AFM experiments. Similarly, does the microtubule disassembly is enhanced or reduced upon a treating the cells with actin depolymerizing drugs should be addressed.

We have provided detailed information about the medium used in the AFM experiments in Material and Methods.

We have perturbed the contractile stress fiber formation by Blebbistatin treatment (Dou et al., 2007). Upon the treatment of Blebbistatin, an inhibitor of myosin II and contractile stress fiber formation (Dou et al., 2007), Spastin-mediated microtubule disruption can not increase cell rigidity (Fig 6C, right). This data confirm that microtubules indirectly modulate cell rigidity by providing support against contractile stress fibers.

Reference:
Ying Dou, er Arlock, Anders Arner. Blebbistatin specifically inhibits actin-myosin interaction in mouse cardiac muscle. Am J Physiol Cell Physiol. 2007, 293(3):C1148-53.

3. A great advantage from this tool is the temporal control of spastin microtubule severing activity. Spastin severing activity against different microtubule PTMs has been shown in vitro, therefore it will enhance the study if the authors express different PTM enzymes in the cells and compare the severing activity.

We have performed the experiments below to address reviewer’s suggestion accordingly.

In result section:
We tested the disruption efficiency on different modified microtubules. Overexpression of a detyrosination protein complex, VASH1(Vasohibin 1)/SVBP (Small vasohibin binding protein) complex (Aillaud et al, 2017), and a truncated glutamylase, TTLL4C639 (The C639 fragment of TTLL4)(van Dijk et al, 2007), significantly increased the level of detyrosinated and glutamylated microtubules, respectively (Fig EV3A-D). Intriguingly, disruption of glutamylated microtubules was slower than that of unmodified microtubules (Fig EV3E-G and Movie EV17). There was no significant difference in microtubule disruption rates between detyrosinated and unmodified microtubules (Fig EV3E-G and Movie EV17). These data indicate the glutamylation but not detyrosination slows down the Spastin-mediated microtubule severing.

In discussion section:
PTMs of microtubules may also regulate the severing activity of Spastin. Indeed, our results demonstrated that microtubule hyperglutamylation induced by TTLL4C639 overexpression prevents modified microtubules from Spastin-mediated severing (Fig EV3 and Movie EV17). The effects of other PTMs on Spastin-dependent microtubule severing are worthy of comprehensive evaluation.

We have included the results and descriptions in revised manuscript.

References:
1. Aillaud C, Bosc C, Peris L, Bosson A, Heemeryck P, Van Dijk J, Le Friec J, Boulan B, Vossier F, Sanman LE et al (2017) Vasohibins/SVBP are tubulin carboxypeptidases (TCPs) that regulate neuron differentiation. Science 358: 1448-1453

2. van Dijk J, Rogowski K, Miro J, Lacroix B, Eddé B, Janke C (2007) A targeted multienzyme mechanism for selective microtubule polyglutamylation. Mol Cell 26: 437–448

4. Another added advantage compared to the photo-switchable microtubule inhibitors is the complete disassembly of drug-resistant stable microtubules. Either the authors should directly
compare their tool with such photo-switchable microtubule inhibitors or should be discussed in detail in the discussion section.

We have added the comparison of photo-switchable microtubule inhibitor and our system as below in the revised manuscript.

To precisely control microtubule properties, many photoswitchable MTAs have been developed to manipulate microtubules under the control of light illumination (Borowiak et al, 2015; Müller-Deku et al, 2020). These chemicals can be easily introduced into target cells without genetic modification. However, thus far, the photoswitchable MTAs against specific microtubule subtypes are still sparse. In contrast, our genetically-encoded system can be driven by tissue-specific promoters. Together with microtubule subtype-specific biosensors or associated proteins (Hong et al, 2018; Kesarwani et al, 2020), our approaches are promising to precisely perturb specific microtubule subpopulations in target cells in vitro and in vivo.

References:

1. Borowiak M, Nahaboo W, Reynders M, Nekolla K, Jalinot P, Hasserodt J, Rehberg M, Delattre M, Zahler S, Vollmar A et al (2015) Photoswitchable Inhibitors of Microtubule Dynamics Optically Control Mitosis and Cell Death. Cell 162: 403–411

2. Müller-Deku A, Meiring JCM, Loy K, Kraus Y, Heise C, Bingham R, Jansen KI, Qu X, Bartolini F, Kapitein LC et al (2020) Photoswitchable paclitaxel-based microtubule stabilisers allow optical control over the microtubule cytoskeleton. Nat Commun 11: 4640

3. Hong SR, Wang CL, Huang YS, Chang YC, Chang YC, Pusapati GV, Lin CY, Hsu N, Cheng HC, Chiang YC et al (2018) Spatiotemporal manipulation of ciliary glutamylation reveals its roles in intraciliary trafficking and Hedgehog signaling. Nat Commun 9: 1–13

4. Kesarwani S, Lama P, Chandra A, Reddy PP, Jijumon AS, Bodakuntla S, Rao BM, Janke C, Das R, Sirajuddin M (2020) Genetically encoded live-cell sensor for tyrosinated microtubules. J Cell Biol 219: e201912107

5. On similar lines the, introduction and discussion will benefit adding information about the microtubule sub-types and how targeting their inducible microtubule disassembly tool to specific microtubule sub-population will open new avenues in understanding the tubulin isotype/PTM function.

We appreciate the comment from the reviewer and have added this aspect to the discussion.

6. Minor comment: I recommend avoiding abbreviating microtubules as MT. The CRY2 induced disassembly shown in Figure 4 will benefit with a cartoon representation as shown in Figure 1A.

We have removed the abbreviation and added the Cry2/CIBN system in Figure 1A.
Thank you for submitting your revised manuscript. We have now received comments from the initial referees (please see below) and I am pleased to say that they now in principle support publication. Two referees have some remaining issues, which should be addressed in the final revised version of the manuscript. In addition, I would ask you to address a number of editorial issues that are listed in detail below in a final revised version of the manuscript. Please make all changes *only* to the document provided by our data editors (please see below for more information) and upload this as the main manuscript file when submitting the final revision.
4. In the results section "Rapid disruption of specific microtubule-based structures' first sentence, 'In addition to cytosol microtubules...' what are cytosol microtubules?

Referee #1:

The authors have carefully responded to all referees' comments. Therefore, the revised manuscript could now be considered for publications.

I would like to comment on the answer of the authors to referee #3, point 3. The authors report that modification of microtubules with the glutamylase TTLL4 does abolishes the severing of microtubules in their system, while overexpression of VASH1/SVBP, a tubulin detyrosinase, did not. As a possible explanation, they suggest that the known impact of tubulin PTMs on spastin severing needed to be more carefully studied.

I would like to suggest an alternative explanation: the tyr-tubulin sensor that is used to target spastin to tyrosinated microtubules does not bind to microtubules modified by TTLL4, as shown in Kesarwani et al 2020 (Fig. 4). It is likely that this is the predominant reason for the lack of severing in cells that express TTLL4, rather than an inhibitory effect of polyglutamylation on microtubule severing (it has been shown in Lacroix et al 2010 that in vitro, TTLL4-glutamylated microtubules are severed more rapidly than non-modified microtubules).

Kesarwani et al 2020 further demonstrate that only the isoform of VASH2-X1, but not VASH1, are able to fully detyrosinate microtubules in cells upon overexpression (Fig. S4). Given that the authors use VASH1, they might simply not have fully detyrosinated their microtubules, and hence the tyr-tubulin sensor is still binding to microtubules and thus inducing severing.

The authors might consider discussing this in the paper.

Referee #2:

All concerns of this reviewer were addressed in revision. The manuscript in recommended for publication

Referee #3:

Controlled sub-cellular targeting of a protein of interest using chemical or photo inducible dimerization modules has been extensively used in cell biology studies. The authors from the manuscript by Liu et al entitled "Precise Control of Microtubule Disassembly in Living Cells" have coupled the inducible dimerization method to localize microtubule severing enzyme, which precisely targets sub-population of microtubule structures involved in different cellular process. Using this tool the authors have demonstrated that diverse microtubule structures can be targeted with spatial-temporal precision. Additionally, this tool can be used to study the sub-cellular organelle dynamics and the interplay of actin-microtubule cytoskeleton in cell shape, after precisely disassembling the microtubules. This method also offers more advantages over existing tools, such as generic and photoswitchable microtubule depolymerizing drugs, which are often slow and can leave stable microtubules intact. Regarding this, in the revised manuscript the authors have addressed this reviewer comment by adding this detail in the discussion section. On similar lines, the authors have also added experimental data on the application of their tool on differentially modified microtubule. It is interesting to note that the glutamylated microtubules by TTLL4 show decreased microtubule severing (Fig EV3E-G and Movie EV17), which will excite and encourage others to use their tool to understand tubulin PTMs. The authors have also satisfactorily addressed other concerns; I recommend the manuscript for publication after addressing the following minor points.

1. In the previous comment I recommended adding a cartoon representation for the CRY2 induced disassembly shown in Figure 4, which is still missing.
2. In the results section "Engineering microtubule-severing enzymes for precise microtubule disruption' in the newly added text "...dNSpastinCD needs to cowork...." reads awkwardly.
3. In the same section, the last paragraph discussing the potential concerns of the system seems out of its place, perhaps this detail should be in the first section of results or in the discussion.
4. In the results section "Rapid disruption of specific microtubule-based structures' first sentence, 'In addition to cytosol microtubules...' what are cytosol microtubules?
We thank the reviewers and the editors for the positive notes and constructive suggestions. We have addressed the editorial issues and reviewers' comments accordingly. The reviewers' comments are itemized below, followed by our point-by-point responses (in blue).

Referee #1:

The authors have carefully responded to all referees' comments. Therefore, the revised manuscript could now be considered for publications.

We greatly thank the reviewer for the positive comments.

I would like to comment on the answer of the authors to referee #3, point 3. The authors report that modification of microtubules with the glutamylase TTLL4 does abolishes the severing of microtubules in their system, while overexpression of VASH1/SVBP, a tubulin detyrosinase, did not. As a possible explanation, they suggest that the known impact of tubulin PTMs on spastin severing needed to be more carefully studied.

I would like to suggest an alternative explanation: the tyr-tubulin sensor that is used to target spastin to tyrosinated microtubules does not bind to microtubules modified by TTLL4, as shown in Kesarwani et al 2020 (Fig. 4). It is likely that this is the predominant reason for the lack of severing in cells that express TTLL4, rather than an inhibitory effect of polyglutamylation on microtubule severing (it has been shown in Lacroix et al 2010 that in vitro, TTLL4-glutamylated microtubules are severed more rapidly than non-modified microtubules).

Kesarwani et al 2020 further demonstrate that only the isoform of VASH2-X1, but not VASH1, are able to fully detyrosinate microtubules in cells upon overexpression (Fig. S4). Given that the authors use VASH1, they might simply not have fully detyrosinated their microtubules, and hence the tyr-tubulin sensor is still binding to microtubules and thus inducing severing.

The authors might consider discussing this in the paper.

We thank the reviewer for pointing this out. In Fig. EV3E, EMTB-CFP-FRB efficiently bound to unmodified, detyrosinated, and glutamylated microtubules in YFP alone, VASH1-YFP/SVBP, and TTLL4C639-YFP-transfected cells, respectively (Fig EV3E). We therefore used EMTB-CFP-FRB to recruit dNSpastin3Q-mCh-FKBP onto unmodified and modified microtubules and compared their severing activities (Fig EV3E).

We did not use tyr-tubulin sensor in this experiment. To be clearer, we have added the description above to the manuscript.

Referee #2:

All concerns of this reviewer were addressed in revision. The manuscript is recommended for publication.

We thank the reviewer for the positive comments.
Referee #3:

Controlled sub-cellular targeting of a protein of interest using chemical or photo inducible dimerization modules has been extensively used in cell biology studies. The authors from the manuscript by Liu et al entitled “Precise Control of Microtubule Disassembly in Living Cells” have coupled the inducible dimerization method to localize microtubule severing enzyme, which precisely targets sub-population of microtubule structures involved in different cellular process. Using this tool the authors have demonstrated that diverse microtubule structures can be targeted with spatial-temporal precision. Additionally, this tool can be used to study the sub-cellular organelle dynamics and the interplay of actin-microtubule cytoskeleton in cell shape, after precisely disassembling the microtubules. This method also offers more advantages over existing tools, such as generic and photoswitchable microtubule depolymerizing drugs, which are often slow and can leave stable microtubules intact. Regarding this, in the revised manuscript the authors have added this reviewer comment by adding this detail in the discussion section. On similar lines, the authors have also added experimental data on the application of their tool on differentially modified microtubule. It is interesting to note that the glutamylated microtubules by TTLL4 show decreased microtubule severing (Fig EV3E-G and Movie EV17), which will excite and encourage others to use their tool to understand tubulin PTMs. The authors have also satisfactorily addressed other concerns; I recommend the manuscript for publication after addressing the following minor points.

We thank the reviewer for the positive comments.

1. In the previous comment I recommended adding a cartoon representation for the CRY2 induced disassembly shown in Figure 4, which is still missing.

We thank the reviewer’s constructive suggestion and previously have added a cartoon representation for the Cry2 dependent system in Figure 1A as below.

2. In the results section "Engineering microtubule-severing enzymes for precise microtubule disruption' in the newly added text "...dNSpastinCD needs to cowork...." reads awkwardly.

We thank for the reviewer’s comments and have edited this sentence to “dNSpastinCD-YFP-FKBP did not show severing activity which is consistent with a previous report, suggesting other domains of spastin may also be important to perform microtubule severing (Fig EV1B and C)”.

3. In the same section, the last paragraph discussing the potential concerns of the system seems out of its place, perhaps this detail should be in the first section of results or in the discussion.

We appreciate the suggestion and have moved that paragraph to the discussion.
4. In the results section "Rapid disruption of specific microtubule-based structures' first sentence, 'In addition to cytosol microtubules...' what are cytosol microtubules?

To prevent any confusion, we have removed the sentence “In addition to cytosol microtubules”.
Thank you again for submitting the final revised version of your manuscript and addressing the remaining points. I am pleased to inform you that we have now accepted it for publication in The EMBO Journal.
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Corresponding Author Name: Yu-Chieh Lin
Journal Submitted to: EMBO Journal
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The data shown in figures should satisfy the following conditions:
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Each figure caption should contain the following information, for each panel where they are relevant:
- a specification of the experimental system investigated (e.g. cell line, species name).
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- an explicit mention of the biological and chemical entities that are being measured.
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- are tests one-sided or two-sided?
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- exact statistical test results, e.g., P values = x but not P values < x;
- definition of 'center values' as median or average;
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Materials

Newly Created Materials

Information included in the manuscript? Yes
In which section is the information available? The DNA constructs used in this study are available from the corresponding author upon reasonable request.

Antibodies

Information included in the manuscript? Yes
In which section is the information available? The detailed information of antibodies was provided in Material and Methods.

DNA and RNA sequences

Information included in the manuscript? Yes
In which section is the information available? The protein sequences of the constructs used in the study were provided in Appendix.

Cell lines

Information included in the manuscript? Yes
In which section is the information available? The detailed information of cell lines used in this study was provided in Material and Methods.

Primary cultures: Provide species, strain, sex of origin, genetic modifications, authenticated status.

Report if the cell lines were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.

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Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in secondary OR supplier name, catalog number, clone number, and OR RRD.

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Not Applicable

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In which section is the information available? Not Applicable

If collected and with the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.

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